Preface to the special issue “Stem cell reformation in plants”

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Introduction: Plant stem cells as ‘founder’ for plant biotechnology

Plant tissue culture is the basis of plant biotechnology, and it has long been used to increase the number of elite plant lines or rare species, to generate new varieties through culture mutation or transformation, and to produce valuable metabolites by culturing callus and tissues (Kumar and Loh 2012; Tabei and Muranaka 2020).

Plant tissue culture is, in other words, a technology for utilizing stem cells which are defined as cells with abilities for self-renewal and pluripotency (Hall and Watt 1989). Both plants and animals generate tissues/organs via stem cells during development. Therefore, uncovering the secrets of stem cell-ness will pave the way for establishing efficient methods for biotechnology. In the plant science field, with the increasing demand for genome editing for plant breeding, the importance of plant regeneration via tissue culture is increasing more than ever.

Molecular biology approaches in recent decades have begun to uncover essential factors related to stem cell formation and maintenance and allow us to induce pluripotent cells applicable to tissue culture engineering. As one specific example in plant science, overexpression of transcription factors involved in shoot apical meristem maintenance and embryogenesis greatly enhanced monocot regeneration and transformation efficiency (Lowe et al. 2016). Even today, however, there are many plant species and cultivars which are recalcitrant to tissue culture engineering because of their reduced ability to regenerate. This is partly due to the lack of knowledge of how plants generate and maintain stem cells throughout their life and how plants reform stem cells in response to various stimuli.

Tackling to understand plant stem cells

To explore deep inside of plant stem cells, a project entitled “Principles of Pluripotent Stem Cells Underlying Plant Vitality” was launched in 2017, supported by the Ministry of Education, Culture, Sports, Science and Technology’s Grant-in-Aid for Scientific Research on Innovative Areas (http://www.plant-stem-cells.jp/en/). This project aims to understand the persistence and vitality of plants from the perspective of stem cells that enable sustainable organogenesis and regeneration. Researchers who participate in this project are from different disciplines and have been investigating molecular mechanisms of stem cell proliferation and maintenance using several plant species (Umeda et al. 2020). This special issue introduces parts of our recent findings related to regulation of plant stem cells. We believe that this special issue will provide the latest information to researchers working on plant biotechnology. Our achievements in the plant stem cell project are summarized in the web page (http://www.plant-stem-cells.jp/en/achievement).

Alteration of cell division pattern leads to stem cell formation

Asymmetric cell division is critical for the generation and maintenance of plant stem cells. In Marchantia polymorpha, the first asymmetric cell division after spore germination generates stem cells for the thallus and the rhizoid formation. In this special issue, Sakai et al. (page 5–12) observed microtubule dynamics with live-cell imaging during the first asymmetric division and found that the specific pattern of microtubule structure contributes to the generation of asymmetricity and that actin filaments have critical roles in the occurrence of asymmetric division. This work provides a novel insight into cell-autonomous asymmetric division in plants.

Alteration of cell division pattern triggered by stress enhances stem cell reformation in another moss, Pyscomitrium patens. Hiroguchi et al. (page 13–17) focused on the formation of vegetative diaspore, or brood cells, as one of the de novo stem-cell generation patterns after ABA treatment. They found that the cell division asymmetricity of stem cells in the chloronema tip is...
shifted to symmetry after the ABA treatment, and it triggers brood cell formation. The ABA treatment alters the patterns of actin microfilaments deposition in the tip stem cells and causes fewer polarities than the normal condition. The brood cells induce cell protrusion and chloronema regeneration after removal of ABA; thus, the authors propose that the symmetrical/asymmetrical cell division controlled by the ABA-induced redistribution of actin filaments switches the chloronema cell to the brood cell bidirectionally.

Molecular pathways for formation and maintenance of plant stem cells

The shoot apical meristems (SAMs) in flowering plants contain multipotent stem cells, enabling the plants to achieve sustainable growth. The review article in this issue by Shimotohno (page 19–28) summarizes recent research advances in the molecular networks that function in SAM homeostasis, especially the homeodomain transcription factor WUSCHEL (WUS), known as a master regulator of shoot development as well as regeneration. Understanding how plants utilize WUS will be helpful information for manipulating plant stem cells.

Lateral root formation is one of the clear examples of stem cell reformation in normal development, and the pericycle cells are known to be founder cells to generate stem cells. Zhang et al. (page 29–36) summarize the molecular basis of how auxin activates pericycle cell division in the lateral root formation, vascular/cork cambium initiation, and hormone-induced callus formation. The authors recently identified crucial transcription factors to regulate ‘pericycle cell-ness’ (Zhang et al. 2021), and here they also discuss the possible involvement of the transcription factors in each developmental context.

Importance of endogenous auxin biosynthesis

Accumulating evidences suggest that de novo biosynthesis of the phytohormone auxin is essential for proper SAM formation/maintenance during embryogenesis and regeneration. Yamada et al. (page 37–42) found that the expression levels of YUC1 and YUC4, genes for auxin biosynthesis, are decreased in the embryo of the Arabidopsis mutant defective for CUC1 and CUC3 transcription factors. Furthermore, the cuc1cuc3 double mutant shows cup-shaped cotyledon phenotype due to abnormality of the boundary formation which affects embryonic SAM formation (Hibara et al. 2006), together suggesting that CUC genes play an important role in the regulation of auxin biosynthetic gene expression during embryogenesis.

In plants, unorganized cell mass, callus, is formed at wound sites of tissue and generated from culturing-tissue in vitro. This process can lead to stem cell formation, and therefore the successful generation of callus is an important step in the utilization of stem cells in tissue culture. In this issue, Ohbayashi, Sakamoto and colleagues (page 43–50) examined the effects of auxin biosynthesis inhibitor and auxin transport inhibitor in tissue regeneration from Arabidopsis hypocotyl explants in the CIM-SIM two-step tissue culture system. They found that two auxin biosynthesis inhibitors positively affect shoot regeneration but are inhibitory for root regeneration. Similar tendencies were observed when auxin transport inhibitors were applied. Since the application of the inhibitors in the CIM condition is critical for shoot regeneration, de novo auxin biosynthesis during callus formation plays important roles in stem cell formation.

Involvement of brassinosteroid pathway for stem cell formation and maintenance

Three research papers in this special issue report relationship between molecular pathways related to brassinosteroid response and plant development through stem cell formation/maintenance. Kondo (page 59–64) reports the roles of brassinosteroid and TDIF peptide hormones in Arabidopsis xylem formation. Experiments with a reporter line revealed that brassinosteroid and TDIF are competitive for the xylem marker gene expression. The vascular cell induction culture system using Arabidopsis leaves (VISUAL) with the TDIF receptor mutant further illuminated the mutual inhibition effects between brassinosteroid and TDIF peptide hormones in Arabidopsis xylem formation. Experiments with a reporter line revealed that brassinosteroid and TDIF are competitive for the xylem marker gene expression. The vascular cell induction culture system using Arabidopsis leaves (VISUAL) with the TDIF receptor mutant further illuminated the mutual inhibition effects between brassinosteroid and TDIF peptide hormones, suggesting competitive roles of the two phytohormones in the regulation of xylem cell differentiation from cambial stem cells.

The roles of glycogen synthase kinase 3 (GSK3)-like kinases in brassinosteroid response have been well studied in Arabidopsis. The BIN2 transcription factor phosphorylated by GSK3 negatively regulates the expression of the brassinosteroid responsive gene in Arabidopsis. Furuya et al. (page 65–72, and the cover
photo of this special issue) here report the importance of GSK in *Marchantia polymorpha* cell proliferation and differentiation. Treatment with the specific inhibitor for GSK3-like kinases, bikinin, caused expansion of the meristematic region of *Marchantia*. The loss-of-function mutants of *Mpgsk* formed undifferentiated cell mass, while overexpression of *Mpgsk* reduced the size of the meristem region, suggesting that *Mpgsk* plays an important role in coordinating cell differentiation and proliferation in *M. polymorpha*.

The root tip regeneration system has helped uncovering new insights into molecular mechanisms of plant regeneration (Sena et al. 2009). In this issue, Takahashi and Umeda (page 73–78) investigated the roles of brassinosteroids in the root tip regeneration of Arabidopsis and found that brassinosteroid biosynthesis/response are activated after root tip excision. Analyses with inhibitor treatments and a signaling-defective mutant further uncovered the positive effects of brassinosteroid on cell division during root tip regeneration. The relationship between brassinosteroid and auxin responses will be one of the next important questions to unravel mechanisms underlying stem cell formation/maintenance.

**Cell cycle reentry through interaction with other organisms**

Battenberg and Hayashi (page 79–83) summarize evolutionary aspects of root nodule symbiosis (RNS), where infectious bacteria restarts the cell cycle in root cortex cells. They highlight, in the historical context, how we understand the RNS in nature, and compare and contrast three different hypotheses related to the nitrogen-fixing clade and the nitrogen-fixing plants, pointing out what we need for further research to fully grasp the evolutionary history of RNS. The authors recently reported that a key regulator for nodule formation in *Lotus japonicus*, the NODULE INCEPTION (NIN) transcription factor, directly regulates an ortholog of *LBD16*, encoding an Arabidopsis transcription factor essential for lateral root formation (Soyano et al. 2019). In this issue, they also discuss the evolutionary relationship between root nodule and lateral root formation.

**Future perspectives**

With the establishment of the Sustainable Development Goals as a common goal for human activities, we are now acknowledging the ever-growing importance of understanding plant vitality and plant biotechnology for environmental preservation, food supply, and biomass production. The key for this is to understand how plants generate and maintain stem cells during continuous development and upon stress. Further elucidation of hormonal control and regulatory networks of master transcription factors, in the context of epigenetic regulation, will help uncover the general principles underlying plant stem cell regulation. For this, studies on plant stem cells at higher resolution, such as research using single-cell/nucleus RNA-seq analysis, should be crucial for sufficient understanding of plant stem cells, and it will bring significant advances in plant biotechnology.

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