Summary of Doctoral Dissertation

Degree requested Doctor of Life Science Applicant's name SINGH PRERNA

Title of Doctoral Dissertation

Identification of novel factors involved in polarized cell expansion and development in the moss, *Physcomitrium patens*

(コケ植物 *Physcomitrium patens* における極性細胞の伸長と発生に関与する新規因子の同定)

In multicellular eukaryotes, cell polarity crucial for various cellular, tissue, and organism-level functions and physiology, including responses to the external environment. These properties are significant for developmental processes, such as establishing the body plan and forming organizational systems. The establishment and maintenance of cell polarity represent a highly conserved and intricate mechanism that relies on the precise delivery of intracellular signals and materials. The moss, *Physcomitrium patens,* spends a majority of its gametophytic life stage in the juvenile, protonemal form, where the protonemata display distinct unidirectional and anisotropic growth patterns by undergoing tip growth via polarized cell expansion and asymmetric cell division. Actin is vital for tip growth in bryophytes like *P. patens*. Pharmacological inhibition of actin cytoskeleton or mutants defective in the function actin binding proteins or actin nucleating factors exhibit a loss of polarized growth. Microtubules help in establishing the tip growth direction in *P. patens* while also intersecting with the polarization of actin clusters in the apical cells, facilitated by myosin VIII and kinesin proteins. Furthermore, polarized growth is also influenced by exocytosis and specific cell wall proteins along with their modifications.

One of the key signaling molecules involved in the regulation of cell growth and polarization in tip-growing cells of moss protonemata is the CDC42/RHO/RAC-like small GTPases known as RHO-related GTPases from plants (ROPs). In *P. patens protonema*, ROP polarizes in a compact apical gradient in the plasma membrane of the tip-growing cells, while maintaining a diffused plasma membrane localization in other cells of the protonemata. Loss-of-function mutants of ROP and its effectors compromises polarized tip growth *in P. patens*. The polarized distribution of ROP is mediated through interactions with lipids facilitated by the polybasic hypervariable region and a carboxyl-terminal CAAX prenylation motif. In root hair cells, the disruption of the activity of PHOSPHATIDYLINOSITOL PHOSPHATE 5-KINASE 3 (PIP5K3), an enzyme responsible for converting phosphatidylinositol-4-phosphate (PI4P) to PI(4,5)P2, leads to a reduction in root hair growthand disruption of PI(4,5)P2 causes mislocalization of ROP2/ROP, indicating that the polarized accumulation of ROP is dependent on the interaction of ROP with the anionic phospholipids to form nanodomains. While the importance of the prenylation signal in proper localization of ROP in *P. patens* and regulation of tip growth by PpPIPK1/2 is known, the mechanisms underlying ROP-membrane interaction *in P. patens* are not fully understood. I aimed to identify factors that could possibly work in regulating such interactions.

Over the last two decades, classical genetics has served as the primary means to unravel the mechanisms underlying cell polarity. Although this approach has yielded valuable insights, it does come with its limitations. For instance, plant genomes often exhibit high genetic redundancy, resulting in loss-of-function

mutants displaying phenotypes similar to the wild type. Additionally, creating a complete collection of loss-of-function mutants for essential genes may lead to lethality. Both redundancy and lethality are frequently encountered in genes that regulate essential cellular functions. Chemical genetics offers a promising alternative to address these challenges. The cellular responses can be finely tuned by applying small molecules in a spatially and temporally controlled manner. In this study, I employed a novel chemical called F4 to explore the mechanisms behind polarized cell expansion in the moss *P. patens*. F4 disrupts actin organization and leads to a loss of apical localization of ROP small GTPases. The unique structure of this compound, which has not been previously associated with any biological activity, suggests that F4 may interact with novel components or pathways related to cell polarity. To identify Reagent F4's mechanism of action, I performed transcriptomic analyses, and my results suggest that Reagent F4 might be involved in the maintenance of lipid asymmetry via flippase activity. Lipid flippases have been implicated in regulating pollen tube growth as well as PIN polarity. With my data, I hope to propose a new factor involved in tip growth in *P. patens*, utilizing the novel chemical Reagent F4.