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Abstract of Doctoral Dissertation

Degree requested Doctor of Life Science Junling Ren

Title of Doctoral Dissertation

Characterization of the Rho-related Rop/RAC GTPase and a plasma membrane protein in cell polarity regulation in the moss *Physcomitrella patens*

(細胞極性制御に関わるヒメツリガネゴケRop/Rac G タンパク質と膜タンパク質の解析)

Polarity is an intrinsic feature for all life forms ranging from single-cell to the complex multicellular organisms. Correct control of cell polarity is essential for their development. Although all eukaryotes employ the basic set of evolutionarily conserved core mechanisms to regulate cell polarity including the small GTPases, cytoskeleton, targeted membrane delivery and so on, yet in plants, it is still largely unknown including how the polar domain of membrane was established, what kind of targeted membrane delivery occurred. The moss *Physcomitrella patens* has been used as a good model system owing to its advantages such as easy observation, the completion of genome sequence, and high frequency of gene targeting. Also, apical cells of the moss show polar tip growth and the brood cells which were formed under stress lose cell polarity. Thus, using the moss as a model system, I conducted the following experiments.

Conserved function of Rho-related Rop/RAC GTPase signaling in regulation of cell polarity in *Physcomitrella patens*

Flowering plants have a distinct group of Rho GTPases that regulate polarity in polarized cell growth. Rop/RAC signaling module components have also been identified in non-flowering plants, but their roles remain unclear. To understand the importance and evolution of Rop/RAC signaling in polarity regulation in land plants, we examined the functions of PpRop and PpRopGEF in protonemal apical cells of the

moss *Physcomitrella patens*. Inducible overexpression of PpRop2 or PpRopGEF3 caused depolarized growth of tip-growing apical cells. PpRop2 overexpression also caused aberrant cross wall formation. Fluorescent protein-tagged PpRop2 localized to the plasma membrane, including the cross wall membrane, and fluorescent-tagged PpRopGEF3 showed polarized localization to the tip region in apical cells. Thus, these results suggest common functions of PpRop and PpRopGEF in the tip-growing apical cells and the importance of a conserved Rop/RAC signaling module in the control of cell polarity in land plants.

An evenly distributed protein control cell polarity by affecting the number, position and growth direction of the protrusion or branch

To identify novel cell polarity regulators in the moss *P. patens*, the full-length cDNA library of the moss *P. patens* was screened by taking advantage of cell polarity lost in brood cells; and the novel gene called *n1p04* was obtained. *n1p04* was evenly distributed at the plasma membrane and inducible overexpression of *n1p04* or its paralogous gene, *sp4f05*, caused ectopic protrusions or branches with variable growth directions. RNA-Seq data showed that overexpression of *n1p04* altered the expression level of LRR (Leucine-Rich Repeat) receptor kinase, PpRopGEF and cell wall loosening proteins. However no obviously defective phenotype was found in *n1p04* and *sp4f05* double knock-out (dKO) lines. There might be other functional homologs which can complement the defective of dKO phenotype. Thus, these results suggested that n1p04, an evenly localized plasma membrane and a putative glycoprotein, was a novel cell polarity player which might work together with LRR receptor kinase and RopGEF to affect the small GTPase signaling, and regulate the local accumulation of cell wall loosening protein expansins, to control the branch positioning of protonema in the moss *P. patens*. This study also highlighted the idea that the evenly distributed protein also can contribute to the cell polarity regulation.