Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Characterization of Rapid Alkalinization Factors (RALFs) in *Physcomitrium patens*
(ヒメツリガネゴケにおける RALF ペプチドホルモンの解析)

Rapid Alkalinization Factors (RALFs) are small, cysteine-rich peptides known to be involved in various aspects of plant development, growth and immunity. Consistent with their diverse roles in development and stress responses, RALF peptides have been identified in dicots, monocots and gymnosperms. The *Physcomitrium patens* genome encodes only 3 copies of *RALFs* (RALF1, 2, 3), in sharp contrast to those in the vascular plants (4 in *Vitis vinifera*, 37 in *Arabidposis thaliana*). The lower number of *RALFs* in *P. patens* allow us to investigate their functional roles using loss-of-function mutants, overexpressors as well as fluorescent protein-tagged reporter lines. I thus generated *Ppralf* knock-out mutants in various combinations. Phenotypic analysis showed that single (*ralf1* and *ralf* 2) and double *ralf1 ralf2* mutans exhibited reduced chloronema cell length and delayed cell fate transition from chloronema to caulonema cell, which led to fewer gametophores. These growth defects were also observed in RALF1 and RALF2 overexpressors. The genetic evidence supports an overlapping role for *PpRALF1* and *PpRALF2* in promoting protonema tip growth and elongation. Although both PpRALFs were secreted to the plasma membrane on which PpRALF1 symmetrically localised, PpRALF2 showed a polarized localisation at the growing tip. I also investigated whether PpRALF1 is proteolytically processed *in vivo* and how this cleavage may affect its function by replacing one of the basic amino acids, Arginine, to Alanine (R73A). The analysis showed that PpRALF1 was proteolytically cleaved *in vivo* and this is necessary for the PpRALF1 function in tip growth. Finally, my study reveals that PpRALF1 and PpRALF2 have overlapping functions in promoting protonema tip growth and elongation, and proteolytic cleavage of PpRALF1 is necessary for its function.