論文内容の要旨

論文題目：Study on characteristics of xylogen-type proteins (Xylogen 型タンパク質の特徴に関する研究)

氏名：小林　裕樹

In higher plants, many extracellular proteins are involved in developmental processes, including cell-cell signaling and cell wall construction. Xylogen is an extracellular arabinogalactan protein (AGP) isolated from *Zinnia elegans* xylogenic culture medium, which promotes xylem cell differentiation. Xylogen has a unique structure, containing a nonspecific lipid transfer protein (nsLTP) domain and AGP domains. AGP is a kind of glycoproteins and characterized by complex arabinogalactan glycosylation, and nsLTP is characterized by about 100 amino acid length sequence harboring eight cysteine motif and lipid binding ability in vitro. However, no functional protein containing AGP and nsLTP other than xylogen has been reported.

In this thesis, I searched for xylogen-type genes in the genomes of land plants, including *Arabidopsis thaliana,* to further our knowledge of xylogen-type genes as functional extracellular proteins in plants. I revealed that many xylogen-type genes comprise a gene family in land plants, including *Arabidopsis thaliana*, *Populus trichocarpa*, *Vitis vinifera*, *Lotus japonicus*, *Oryza sativa*,

Selaginella moellendorffii and *Physcomitrella patens*. The genes shared an N-terminal signal peptide sequence, a distinct nsLTP domain, one or more AGP domains, and a glycosylphosphatidylinositol (GPI) anchored sequence. Using transgenic plants harboring *promoter::GUS* constructs, I examined the expression of 13 xylogen-type genes in *A. thaliana*, and indicated that a diversity of gene family members exhibited their own expression patterns such as anther-specific and endodermis/pericycle-specific expression patterns. Based on these results, I concluded that xylogen-type genes, which may have diverse functions, form a novel chimeric AGP gene family with a distinct nsLTP domain. I also revealed that *AtXYP2* is the best candidate for the Arabidopsis counterpart of the *Zinnia* xylogen gene.

Xylogen and most xylogen-type proteins are GPI-anchored at C-terminus. Therefore I investigated the behavior of xylogen and plant xylogen-type GPI-anchored protein in its secretory process with AtXYP2 as a model. I constructed *pAtXYP2::AtXYP2-GFP* and introduced it to form stable transgenic plants. The comparison of the expression patterns between *pAtXYP2::AtXYP2-GFP, pAtXYP2::SP-GFP* and *pAtXYP2::GUS* suggested that the expression of *AtXYP2* may be regulated by not only transcriptional but also post-transcriptional levels. The secretion of AtXYP2-GFP depended on vesicle transport, but differed from that of auxin flux carrier PIN1. In the arabidopsis root, the expression of AtXYP2 first appeared in immature xylem cell lines, and then shifted to procambium. AtXYP2-GFP was membrane-bound in the premature side and apoplastic in the mature side of the differentiation zone of roots. This fact is consistent with zinnia xylogen functions as a soluble intercellular signal between differentiating and non-differentiating cells. All together, my results suggested the differentiation stage-dependent solubilization of GPI-AP to function as a signaling molecule promoting xylem differentiation.