学位論文審査の要旨

博士の専攻分野の名称　　博士（農学）　　氏　名　　Sheliang Wang

審査担当者　主　査　准教授　尾之内　均
副　査　教授　　木村　淳夫
副　査　教授　　伴戸　久徳
副　査　教授　　内藤　　哲（生命科学院）
副　査　助教　　高野　順平

学位論文題名

UDP-D-galactose-dependent organization of the *trans*-Golgi network &
Mechanisms for polar localization of boric acid channels in *Arabidopsis thaliana*
(シロイヌナズナにおける UDP-D-ガラクトースに依存したトランスゴルジ網の構造維持およびホウ酸チャネルの細胞膜内偏在機構)

This thesis is composed of 71 text pages, 28 figures, 1 table, and attached with 2 related papers.

Boron (B) is essential for plant development but is toxic when presents in excess. In *Arabidopsis thaliana*, a boric acid channel NIP5;1 is required for B uptake under B deficiency. NIP5;1 is expressed in outermost cell layers of roots and shows polar localization toward the soil-side in the plasma membrane (PM). The polar localization is assumed to be important for the efficient uptake of boric acid from soil solution. However, the molecular mechanisms of PM targeting and polar localization of NIP5;1 and their physiological importance were not established.

In this study, the nature of a mutant developing intracellular aggregates containing GFP-tagged NIP5;1 was investigated. In addition, the mechanism of polar localization of NIP5;1 and its physiological importance on plant growth were elucidated.

1) UDP-D-galactose synthesis by UDP-glucose 4-epimerase 4 is required for organization of the trans-Golgi network/early endosome in *Arabidopsis thaliana* root epidermal cells

Previously, a fluorescence-imaging based screening isolated an Arabidopsis mutant developing intracellular aggregates containing GFP-NIP5;1 in epidermal cells of the root elongation zone. This mutant was identified as an allele of *UDP-glucose 4-epimerase 4* (*uge4*) mutant which has defects in UDP-D-galactose synthesis and channeling into the Golgi.

Here, the author further investigated the nature of intracellular aggregates in the *uge4* mutants. Live-cell imaging indicated that the markers of multi-vesicular bodies/late endosomes (MVB/LEs) and PM were strongly accumulated in the intracellular aggregates

in the *uge4* mutant. Furthermore, electron microscopy analysis revealed accumulation of abnormal high-electron-density vesicles in elongating epidermal cells. The abnormal vesicles were often associated or interconnected with *trans*-Golgi network/early endosomes (TGN/EEs) and contained ADP-ribosylation factor 1, which is usually localized to the Golgi and the TGN/EEs. Together, these results indicate the importance of UDP-D-galactose synthesis by UGE4 for the organization and function of endomembranes, especially TGN/EEs, which are a sorting station of the secretory and vacuolar pathways.

2) Polar localization of a boric acid channel is mediated by phosphorylation and plays a crucial role in growth of *Arabidopsis thaliana* under low boron conditions

To elucidate the mechanism of the polar localization of NIP5;1, the author performed structure-localization analysis of NIP5;1 by expression of GFP-used homologs and chimera proteins in *Arabidopsis thaliana.* The N-terminal region of NIP5;1 was identified to be required for the polar localization and have the ability to target non-polar aquaporin proteins to the soli-side PM domain. Further analysis revealed that the conserved threonine residues in the N-terminal region of NIP5;1 were phosphorylated and required for the polar localization.

The author then investigated the physiological importance of the polar localization using transgenic Arabidopsis lines expressing a non-polar NIP5;1 variant. Although the non-polar variant showed comparable boric acid transport activity in *Xenopus* oocytes, the transgenic lines with the variant developed shorter roots than those with the wild-type NIP5;1 under low B conditions.

These results revealed that the polar localization of NIP5;1 is mediated by phosphorylation, and plays a crucial role in growth of *Arabidopsis thaliana* under low boron conditions probably by improving the efficiency of boric acid uptake in the roots.

These studies improved understanding of mechanisms underlying endomembrane organization and intracellular trafficking of membrane proteins in plant cells, and demonstrated the importance of the polar localization of a transport protein in plant nutrition for the first time.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.