

論文の内容の要旨

Studies on functions of ANTH domain proteins in *Arabidopsis thaliana*

(シロイヌナズナ ANTH ドメインタンパク質の機能に関する研究)

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Eukaryotic cells internalize nutrients, proteins, and membrane materials via multiple endocytic pathways. Clathrin-mediated endocytosis (CME) is the best-characterized pathway and is responsible for a number of major endocytic activities in plant cells. In mammals, epsin N-terminal homology (ENTH) and AP180 N-terminal homology (ANTH) domain-containing proteins are proposed to function in initiation of clathrin-coated pit formation, bridging coat components and cargo proteins to donor membranes. The *Arabidopsis thaliana* genome encodes eighteen ANTH domain proteins whereas metazoa and fungi have much fewer ANTH domain proteins, which implies that plant cell ANTH proteins have more divergent and significant functions than non-plant systems. However, despite their assumed importance, little is known about the physiological significance of this family in plants.

First, I examined the phenotypes of multiple mutants of ANTH domain proteins. I found that double mutant plants of *PICALM5a* (aka ECA2, At1g03050) and *PICALM5b* (At4g02650) exhibited reduced fertility (Figure 1A). The double mutant plants had short siliques, and the seed number in these siliques was significantly smaller than that of the wild-type plants (Figure 1B). Reciprocal cross-pollination revealed that mutant pollen was responsible for the reduced fertility. The double mutant was defective in pollen tube growth both *in vivo* and *in vitro* (Figure 1A).

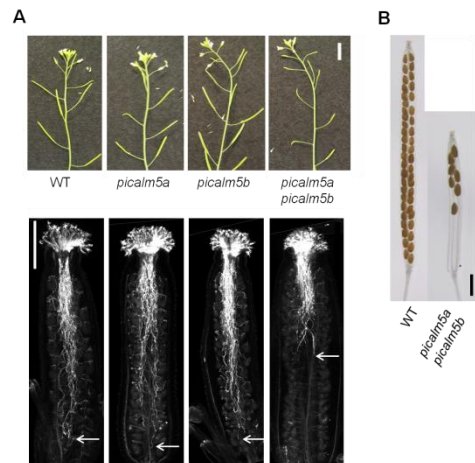


Figure 1. Phenotypes of *picalm5* mutants. (A) bar = 1 cm (top) and 500 μ m (bottom). (B) bar = 1 mm.

Next I examined the functions of *PICALM5a* and *PICALM5b*. Both GFP-fused *PICALM5a* and *PICALM5b* were localized to the subapical region plasma membrane (PM) of growing pollen tubes. Clathrin coated vesicles are reported to be formed mainly in this region. So I examined whether clathrin light chain 1 (CLC1) and *PICALM5a* were co-localized (Figure 2). CLC1-GFP was localized to the punctate cytoplasmic structures and the subapical PM. It was also co-localized with *PICALM5a*-mRFP on the subapical PM, indicating the functions of *PICALM5* proteins in CME.

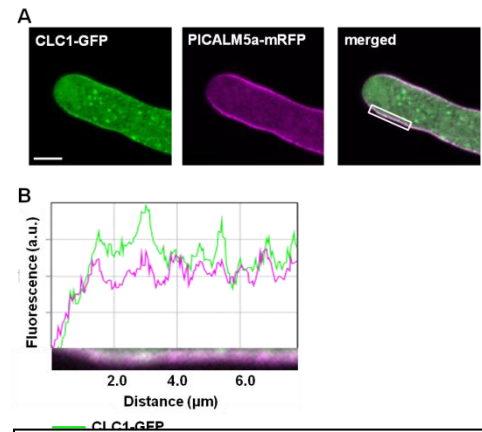


Figure 2. (A) Subcellular localizations of CLC1-GFP and *PICALM5a*-mRFP in growing pollen tube. bar = 5 μ m. (B) Fluorescence intensity profiles for the white box shown in (A).

In search of the cargo proteins of *PICALM5a/5b* whose mislocalization lead to the pollen tube growth defect, I examined functional relationship between *PICALM5a/5b* and the proteins

reported to be involved in the regulation of pollen tube growth. I generated transgenic plants expressing GFP-fused SYP124, SYP125, SYP131, ANX1 or ANX2 in the wild-type or *picalm5a picalm5b* double mutant background. Although subcellular localizations of tip-localized SNARE proteins (SYP124, SYP125 and SYP131) were not affected by the double mutation, the tip localizations of ANX1 and ANX2 receptor-like kinases were severely impaired in *picalm5a picalm5b* pollen tubes, which indicated compromised recycling of ANX1 and ANX2 (Figure 3). Subcellular localization of ANX1 and ANX2 was not affected in *picalm5a* or *picalm5b* single mutant

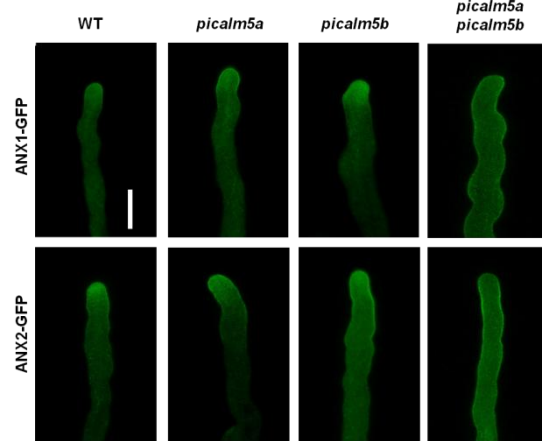


Figure 3. Subcellular localizations of ANX1-GFP and ANX2-GFP in the growing pollen tubes of WT and *picalm* mutants. bar = 10 μ m.

pollen tubes, indicating that PICALM5a and PICALM5b have overlapping function in regulating the localizations of ANX1 and ANX2. Mislocalization of ANX2-GFP in *picalm5a picalm5b* pollen tubes was rescued by the expression of PICALM5a-mRFP, which further indicated that PICALM5 proteins are responsible for the proper localization of ANX receptor-like kinases as well as the functional redundancy between PICALM5 proteins (Figure 4).

In growing pollen tubes, secretory vesicles are delivered to the tip region, where accumulated vesicles

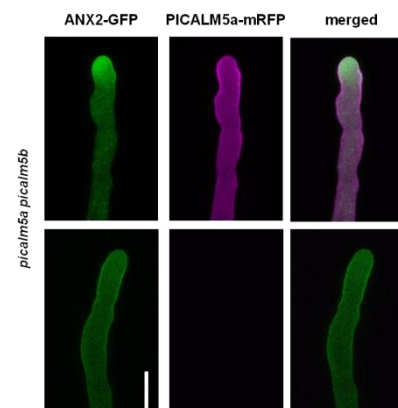


Figure 4. Subcellular localizations of ANX2-GFP in the growing pollen tubes of *picalm5a picalm5b* double mutants expressed with or without *PICALM5a-mRFP*. bar = 10 μ m.

form inverted cone-shaped region, and supply cell wall and PM material. On the other hand, excessive PM materials are recycled via endocytosis. Studies on endocytosis in growing pollen tubes

suggested that most of the endocytosed vesicles are rapidly recycled to the secretory pathway. The disturbed tip localization of ANX1 and ANX2 in the *picalm5a picalm5b* double mutant pollen tube indicates, together with the subapical localization of PICALM5a and PICALM5b, that PICALM5a and PICALM5b have a redundant role in CME in pollen tubes, loading specific cargo proteins such as ANX1 and ANX2 into vesicles.