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A novel plant-specific *Arabidopsis* VEC1 protein, a membranous C2 domain protein, is associated with vesicular trafficking

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Objectives

We tried to elucidate a novel plant-specific *Arabidopsis* VEC1 protein associated with vesicular trafficking.

Materials and Methods

1. Material

Plant *Arabidopsis thaliana* Col-0

FM4-64 as an endocytic tracer

2. Methods

We made transgenic *Arabidopsis* by floral dipping. RT-PCR and GUS assay were employed to show expression of VEC1. *Arabidopsis* mesophyll protoplast transformation by PEG-mediated method was performed to see the subcellular localization of VEC1.

Results and Discussion

We surveyed the C2 domain proteins in *Arabidopsis* genome and found 82 C2 domain proteins in which most of the animal counterparts and extra plant-specific ones with peculiar primary structure are included. Among them *Arabidopsis* VEC1(Vesicular trafficking-associated C2 domain protein) is a novel plant-specific protein with a unique small size and primary structure. It is composed of a C2 domain at N-terminus, a GYP-rich region, a TMD, and a D-rich region at the very C-terminus.With histochemical GUS assay of pVEC1::GUS transgenic plants and RT-PCR method, we found that the expression in leaves increased by aging and wounding. Public GeneChip data and previous AFLP data report that VEC1 is induced by cold, drought, and pathogen infection, which, along with our data, tells the involvement of VEC1 in stress-response. With recombinant VEC1TMH and VEC1C2TMH proteins, protein-phospholipid overlay assay was performed in the presence of Ca^{2+} or EGTA. VEC1TMH bound to PI(3)P, PI(4)P, and PI(5)P only in the presence of Ca^{2+}. The fact that VEC1 is localized at PM and endosomes was confirmed from partial- or complete- co-localization among VEC1, VEC1C2, and VEC1C2TM, spontaneous re-location of VEC1 from PM to motile vesicles, partial co-localization of VEC1C2 with EBD, and co-localization with FM4-64.

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