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Plant PRA plays an important role in intracellular vesicular trafficking between compartments as GDF

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Rab GTPases like Ras-related monomeric GTPases are well known to regulate intracellular vesicle trafficking by cycling between membrane-bound and cytosolic states. The functions of these proteins are controlled by upstream regulators and downstream effectors. Ypt/Rabs transmit signals to downstream effectors in a GTP-dependent manner. GDP-bound Rab proteins are extracted from their target membrane by cytosolic proteins known as GDP dissociation inhibitors (GDIs), and the Rab GTPase is recruited to the membrane compartment following dissociation from the GDI by GDI displacement factor (GDF). Now, we're going to discuss the role of plant PRA concerted with Rab and GDI proteins by recycling Rab between membrane and cytosol for intracellular trafficking of cargo proteins.

Key words: GDF, GDI, PRA1, Rab, vacuolar trafficking, vesicle trafficking

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Although Rabs appear to undergo multiple cycles of GDI-mediated delivery to, and extraction from membranes,[1] the mechanisms underlying Rab membrane delivery and association by GDI and other factors remain unclear. GDP-GTP exchange occurs at the target membrane, catalyzed by a guanine nucleotide exchange factor (GEF),[2,3] and the GTP-bound Rab transmits signals to downstream effectors and associates with the membrane to ensure proper docking and fusion of transport vesicles.[4] After vesicle fusion on its target membrane, subsequently hydrolysis of GTP by the Rab is facilitated by GTPase-activating proteins (GAPs).[4] The resulting GDP-bound Rab is subsequently retrieved from the membrane by GDI, which then maintains Rab in the cytosol to complete the cycle.

Many research groups isolated PRAs, a homolog of human YIP3, in several two-hybrid screenings as interacting with multiple Rabs in their GTP- or GDP-bound form.[5,6] PRA contains two extensive hydrophobic domains which may form a membrane-spanning domain or the inner hydrophobic core of the protein.[7] PRA1 is localized to the Golgi and late endosomes,[8] and the related PRA2 is present in the endoplasmic reticulum.[9]

Recently it has been shown that the human YIP3 stimulates the rate of nucleotide binding to Rab9 when added to prenyl Rab9-GDI complex and catalyzes the dissociation of the endosomal Rab-GDI complex, indicating that YIP3 is a GDI displacement factor that recruits Rab to membranes.[10] According to the Gougeon et al. report (2002),[11] PRA1 inhibits the extraction of membrane-bound Rab3A by GDI1, suggesting that recycling of Rab depends on the opposing actions of PRA and GDI, with PRA favoring membrane retention but GDI favoring solubilization.

Moreover, mammalian PRA1 is required for vesicle formation from the Golgi complex and might influence the recruitment of Rab effectors during cargo sequestration as well as proteins required for subsequent vesicle docking and fusion.[11] This is consistent with its transport function based on interaction of yeast homologue Yip3p with proteins in the secretory pathway.[7] Yip1-Yif1p complex binds to the ER and to the Golgi SNAREs, Bos1p and Sec22p, and is required for membrane fusion machinery.[5] In addition, a role of

Yip1p had also been proposed in COPII vesicle biogenesis.[12]

To our current knowledge there is no report on the physiological role of a GDF in plants. Aims to enrich the understanding of the mechanism of Rab recycling and trafficking pathways in plant, we identified and characterized OsPRA1, a rice homolog of PRA. OsPRA1, isolated by yeast two-hybrid screening using OsRab7 as bait, localized to the prevacuolar compartment as a membrane protein.[13] Additionally, through western blot and protoplast transient assays it was confirmed that OsPRA1 has GDF activity, which dissociates the Rab7-GDI2 complex and recruits dissociated Rab from the Rab7-GDI2 complex to the donor membrane (unpublished data). When yeast two-hybrid interaction assay between OsPRA1 and OsGDI2 was performed, OsPRA1 interacted with OsGDI2 weakly (unpublished data), supporting our proposition that OsPRA1 dissociates the OsRab7-OsGDI2 complex.

Furthermore, by using yeast two-hybrid and co-immunoprecipitation assays it was demonstrated that OsPRA1 interacted with dominant negative OsRab7 (T22N) which has no GTP binding activity, but not the constitutively active OsRab7 (Q67L),[13] indicating that OsPRA1 may interact with GDP-bound OsRab7 at the donor membrane, PVC. These results support that OsPRA1 is a GDF for OsRab7.

Subsequently, in order to find its interacting proteins implicated in vesicular trafficking, such as t- or v-SNAREs, yeast two-hybrid screening using OsPRA1 as bait was performed. Interestingly, a t-SNARE, OsVam3p, homolgous to AtVam3p involved in vacuolar trafficking and localizing to both PVC and vacuole membranes in Arabidopsis,[14] was isolated (unpublished data). This suggests that OsPRA1 may be a component of the vesicle fusion machinery. To further strengthen our hypothesis, we examined whether or not mutant OsPRA1 (Y94A) and OsRab7 interact. Mutant OsPRA1 (Y94A) showed weak and no interaction with OsRab7 and OsVam3p, respectively, indicating that mutant OsPRA1 (Y94A) may lose its activity for recruiting Rab GTPase and Rab effector proteins and fusing vesicles to the vacuolar membrane. Actually, when OsPRA1 was mutated, its GDF activity was reduced to less than 50%, and its localization was changed from the PVC to the cytosol. These results are consistent with the assigned transport function of OsPRA1. Besides, our data from transient expression assay using vacuole markers suggested a direct involvement of OsPRA1 in the trafficking of vacuolar proteins.

In summary, OsPRA1, a Yip homologous protein, may function in regulating vacuolar trafficking as a GDF dissociating OsRab7-OsGDI2 complex in plant cells.

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