*Journal of Experimental Botany*, Vol. 66, No. 1 pp. 213–224, 2015
doi:10.1093/jxb/eru412 Advance Access publication 14 October, 2014
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Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

# Rab geranylgeranyl transferase β subunit is essential for male fertility and tip growth in *Arabidopsis*

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Received 23 July 2014; Revised 9 September 2014; Accepted 10 September 2014

## Abstract

**Rab proteins, key players in vesicular transport in all eukaryotic cells, are post-translationally modified by lipid moieties. Two geranylgeranyl groups are attached to the Rab protein by the heterodimeric enzyme Rab geranylgeranyl transferase (RGT) αβ. Partial impairment in this enzyme activity in *Arabidopsis*, by disruption of the *AtRGTB1* gene, is known to influence plant stature and disturb gravitropic and light responses. Here it is shown that mutations in each of the *RGTB* genes cause a tip growth defect, visible as root hair and pollen tube deformations. Moreover, FM 1–43 styryl dye endocytosis and recycling are affected in the mutant root hairs. Finally, it is demonstrated that the double mutant, with both *AtRGTB* genes disrupted, is non-viable due to absolute male sterility. Doubly mutated pollen is shrunken, has an abnormal exine structure, and shows strong disorganization of internal membranes, particularly of the endoplasmic reticulum system.**

**Key words:** *Arabidopsis*, male fertility, pollen, pollen tube, protein prenylation, Rab geranylgeranyl transferase, Rab protein, root hair, tip growth.

## Introduction

Eukaryotic cells are dependent on vesicular transport, employing protein machinery that is conserved throughout all Eukarya. Crucial players in this process are small GTPases, especially Rab proteins. Rabs possess a GTP-binding site and low intrinsic GTPase activity. In the yeast *Saccharomyces cerevisiae* the Rab protein family has only seven members, but in higher eukaryotes the diversification of Rab functions in specialized tissues and cell types results in as many as 57 Rabs in *Arabidopsis* and 60 in mammals (Pereira-Leal and Seabra, 2001). In plants the Rab family is divided into eight subfamilies, depending on the protein localization and general function in the cell (Rutherford and Moore, 2002).

All Rab proteins comprise a globular domain and an unstructured C-terminal tail of ~35 amino acids (Pfeffer, 2005). Structural motifs of the globular domain determine the Rab specificity towards interacting proteins. At the very end of the C-terminal tail lies a double cysteine motif, which serves as the target for geranylgeranylation by Rab geranylgeranyl transferase (RGT) (Rak *et al.*, 2004; Wu *et al.*, 2009). This modification with two 20-carbon isoprenoid chains enables anchoring of Rab proteins to membranes. A lack of this modification (due to mutations of these particular cysteines) has a disastrous effect on Rab function, since unprenylated Rabs are soluble and therefore cannot play their roles in vesicle formation, transport, and delivery (Gomes *et al.*, 2003).

RGT is a heterodimeric enzyme built of α and β subunits (RGTA and RGTB) (Guo *et al.*, 2008). The RGT substrate, a newly synthesized Rab protein, is initially recognized by