



## Characterization of regulatory factors controlling tip growth in *Nicotiana tabacum* pollen tubes

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### Abstract

Rho GTPases constitute a family of highly conserved signaling proteins with key functions in the regulation of cellular polarization and polar cell growth. In plants, Rho GTPases are represented by a plant specific subfamily referred to as Rac/Rop GTPases. Some members of this subfamily associate with the plasma membrane specifically at the tip of pollen tubes and are important regulators of polar cell expansion. The aim of my study has been to improve our understanding of the molecular mechanisms that control the activity of pollen tube Rac/Rop, and the signaling downstream of this activity.

BiFC (Bimolecular Fluorescence Complementation) is a technique used to validate protein-protein interactions. My studies on the interactions between Nt-RhoGAP1/RhoGDI2 and Nt-Rac5 show that 35S promoter regulated BiFC works in normally growing *N. tabacum* pollen tubes and that the intracellular interaction sites between Nt-RhoGAP1/RhoGDI2 and Nt-Rac5 are found at the subapical plasma membrane and apical cytoplasm, respectively. This technique was consequently used to confirm potential interacting protein partners involved in Nt-Rac5 signaling networks (i.e. Nt-Risap and Nt-Ric), and to detect the subcellular localization of these interactions in pollen tubes.

Nt-Risap (Rac5 interacting sub-apical protein) and Ric (Rho interacting CRIB containing) were isolated as effectors of Nt-Rac5 in yeast two-hybrid screens. The two proteins were confirmed to be highly and specifically expressed in *N. tabacum* pollen tubes and to interact with Nt-Rac5 both *in vitro* and *in vivo*. Functional studies of the two proteins suggest that they behave as downstream effectors of Nt-Rac5 and are involved in the Nt-Rac5 signaling pathways that regulate pollen tube tip growth by activating specific downstream events.

*Keywords:* tip growth, Rac/Rop GTPases, *Nicotiana tabacum*, pollen tubes, BiFC, Nt-RhoGAP1, Nt-RhoGDI2, signaling pathway, Nt-Risap, Nt-Ric

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