

Targeting of a *Physcomitrella patens* Rho-GTPase (rac) gene

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Replacement targeting vectors of a low copy number *Physcomitrella patens* sequence showing homology to the *Arabidopsis thaliana* *Rop* gene sub-family have been constructed. Linear DNA fragments were transformed into *P. patens* to elucidate the target gene function. *Rop* activity, a plant specific Rho-GTPase, has been implicated in tip growth, cell polarity, the actin cytoskeleton, pathogen defence, secondary cell wall formation and meristem signalling. The signalling pathways have not yet been elucidated. Studies of the *Rop1Ps* and *Rop1A*t indicated that *Rop*-GTPases act as a central switch for the polar out growth of pollen tubes by coupling spatial control with temporal control (Zeng & Yang, 2000; Li, *et. al.*, 1998). Tip-localized calcium signalling acts downstream of *Rop* to activate tip growth and is controlled by the negative feedback loop components of which include *Rop*-GTPase-activating proteins (*Rop*GAPs) and the putative *Rop*-GTPase effector phosphatidylinositol monophosphate kinase (PtdInsPkinase). The formation of pollen tip-focused calcium gradient and the tip-localized calcium influx may therefore be regulated through the *Rop*-Ins(1,4,5) P_3 -Ca²⁺ system by hydrolysis of the PtdInsPkinase and the localized PtdIns(4,5) P_2 to Ins(1,4,5) P_3 (Zheng & Yang, 2000; Li, *et. al.*, 1999; Aspenstrom, 1999).

PCR analysis of a group of randomly selected transgenics revealed that three types of targeting incidence occurred at the targeting locus. The phototropic response to white light of all the transgenics is similar to the wild-type strain at all different light intensities examined. However, only the protonemal filaments of the targeted lines have abnormal shaped chloroplasts when cultures were treated in 2.1 mW/m² white light. Analysis of the lipid profile in the *P. patens* transgenics is discussed.

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