ABSTRACT

Rab GTPases (Rabs) are the most populous branch of eukaryotic Ras GTPase superfamily. In active GTP-binding conformation, they serve as key instruments in defining transient membrane identity and through various effectors regulate formation, transport, conversion, and fusion of membrane vesicles. This is important for upkeep of compartmentalized structure of eukaryotic cells and for facilitating both endo- and exocytic processes. Rabs are converted into GDP-binding conformation by interactions with Rab GTPase activating proteins (Rab GAPs) that possess ability to significantly speed up weak intrinsic GTP hydrolytic activity of Rabs. Through this process, Rab GAPs can limit scope of the Rabs' activity and lay out spatiotemporal boundaries for varying Rab populations. In this thesis, I tried to characterize a Rab GAP, GAP2, seemingly necessary for standard development of thale cress plants. Besides TBC catalytic domain, GAP2 (product of At2g39280 gene) possesses a C-terminal coiled-coil motif, which was previously found to interact with Rab GTPases. Experiments aiming to complement T-DNA insertion mutant in GAP2, elucidate GAP2 intracellular localization, novel interacting partners, and character of interaction with the Rabs discovered in the pilot study were undertaken. The results suggest that GAP2 is primarily cytoplasmic and its interaction with RabA1a and RabE1d is solely through the coiled-coil motif. Y2H screen for novel interactors uncovered coiled-coil motif from a closely related Rab GAP (At3g55020) and lead to realization that GAP2 on its own can dimerize through the C-terminal coiled-coil motif. Efforts to complement the mutant were ultimately unsuccessful.

Key words: GTPase-activating protein (GAP), membrane traffic, Rab effector, Tre-2/Bub2/Cdc16 (TBC) protein, Rab GTPase, Ras superfamily, coiled-coil dimerization, exocytosis, GAP cascade, Rab cascade