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Rabex-5 determines the neurite localization of its downstream Rab proteins in hippocampal neurons

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Rab family small GTPases function as molecular switches in the regulation of membrane traffic, and their activity is thought to be controlled by guanine nucleotide exchange factors (GEFs). However, the role of GEFs in targeting Rab proteins to specific membrane compartments is poorly understood. We have recently reported finding that Rabex-5, originally described as a Rab5-GEF, also functions as a Rab17-GEF in mouse hippocampal neurons. The Rab17 in developing hippocampal neurons is specifically targeted to their dendrites and not to their axons, and the GEF activity of Rabex-5 is required for translocation of Rab17 from the cell body to the dendrites. Interestingly, Rabex-5 is also required for the axon and dendrite localization of Rab5 and Rab21 in hippocampal neurons. Our findings indicate that Rabex-5 determines the targeting of its downstream Rab proteins to the dendrites (Rab17) or to both the axon and dendrites (Rab5 and Rab21).

Keywords: axon, dendrite, guanine nucleotide exchange factor (GEF), hippocampal neurons, polarized trafficking, Rab17, Rabex-5, Rab-type small GTPase

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Rabex-5 Functions as a Rab17-GEF that Determines the Targeting of Rab17 to the Dendrites of Developing Hippocampal Neurons

Rab-type small GTPases are conserved membrane trafficking proteins in all eukaryotes, and they mediate various steps in membrane trafficking, including vesicle budding, vesicle movement along cytoskeletons, vesicle docking to specific membranes, and vesicle fusion.[1,2] Rabs function as a molecular switch by cycling between two nucleotide-bound states, a GDP-bound inactive state ("OFF" state) and a GTP-bound active state ("ON" state). Rabs are activated by specific guanine nucleotide exchange factors (GEFs), which promote the release of GDP from Rab and binding of GTP to Rab, and the activated Rabs are then inactivated by GTPase-activating proteins (GAPs) or spontaneously inactivated by their intrinsic GTPase activity.[3] Thus, investigation of Rab-GEFs is crucial to understanding the spatio-temporal regulation of Rab GTPase activation. Although a large number of putative Rab-GEFs, including DENN-domain-containing proteins, have recently been reported,[3,4] very little is known about their in vivo roles in Rab targeting to specific membrane compartments.

Rab17 was originally described as an epithelial cell-specific Rab isoform that regulates polarized trafficking[5-7] but was subsequently found to be expressed in melanocytes[8] and human breast adenocarcinoma,[9] and more recently we demonstrated that Rab17 is also expressed in mouse brain. Localization of Rab17 in mouse hippocampal neurons is unique, because Rab17 is the only Rab isoform that is specifically targeted to the dendrites and is not targeted to the axon.[10] The targeting of Rab17 to the dendrites is neuronal differentiation stage-dependent: at an early stage (3 d of in vitro culture) it localizes only in the cell body, whereas at a later stage (11 d of in vitro culture) some of the Rab17 is translocated from the cell body to the dendrites. By contrast, other

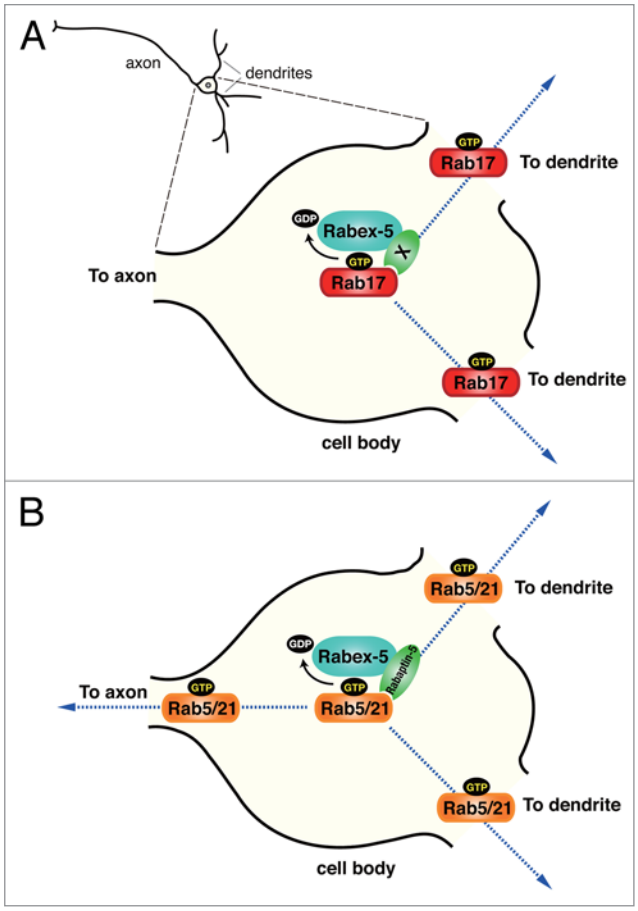

Figure 1. Proposed model of Rabex-5-mediated translocation of its downstream Rabs to the neurites of developing hippocampal neurons. Rabex-5 functions not only as a Rab17-GEF (**A**) but also as a Rab5/21-GEF (**B**), and it determines dendrite targeting of Rab17 and axon/dendrite targeting of Rab5/21, respectively (dotted arrows). The solid arrows indicate the GEF function of Rabex-5 that promotes release of bound GDP from Rab5/17/21 in exchange for GTP. Because of the different sorting functions of Rabex-5, Rab17 specifically regulates dendrite morphogenesis, whereas Rabex-5 and Rab5 are involved in neurite morphogenesis in general. Factor "X" in (**A**) is a putative Rab17-specific effector that may also interact with Rabex-5 and support dendrite targeting of Rab17. Rabaptin-5 is known to interact with both Rab5/21 and Rabex-5 at early endosomes,[13,18] but whether Rabaptin-5 is involved in neurite outgrowth remains to be determined (**B**).

Rab isoforms are targeted to the axon alone (e.g., Rab3A) or to both the axon and dendrites (e.g., Rab5A).[10] Targeting of Rab17 to the dendrites is known to be crucial for dendrite morphogenesis and subsequent postsynapse formation, because knockdown of Rab17 has been found to result in a marked reduction in both total dendrite length and the number of dendrite branches without affecting axon morphogenesis (i.e., total axon length and the number of axon branches).[10] Although several mammalian Rab isoforms (e.g., Rab7 and Rab11) have been shown to regulate dendrite morphogenesis,[11,12] Rab17 is the first reported Rab isoform that specifically regulates dendrite morphogenesis, but not axon morphogenesis, in mammalian neurons. We therefore thought that Rab17 would be an ideal Rab isoform to use to analyze a Rab targeting mechanism at the cellular level. However, until recently the molecular basis of the specific Rab17 targeting to dendrites had completely remained unknown and no physiological Rab17-GEFs that function in hippocampal neurons had been identified.

In our latest study, we screened for Rab17-GEFs by performing yeast two-hybrid assays with a constitutive negative mutant of Rab17 as bait and succeeded in identifying Rabex-5 and ALS2, both of which were originally described as Rab5-GEFs,[13,14] as plausible candidate Rab17-GEFs in mouse hippocampal neurons.[15] It is noteworthy that overexpression of Rabex-5, but not of ALS2, increased the proportion of Rab17 in the dendrites, whereas knockdown of Rabex-5 caused a dramatic reduction in the proportion of Rab17 in the dendrites.[15] Importantly, overexpression of a GEF-activity-deficient mutant of Rabex-5 (Rabex-5-D313A) failed to increase translocation of Rab17 from the cell body to the dendrites. Based on these findings, activation of Rab17 by Rabex-5 is responsible for the stage-dependent movement of Rab17 from the cell body to the dendrites of hippocampal neurons. Actually, forced activation of Rab17, i.e., expression of a constitutive active mutant of Rab17 (Rab17-Q77L), increased the dendrite localization of Rab17 even in early stage neurons.[15] More importantly, Rabex-5 knockdown was found to cause a significant reduction in total dendrite length, the same as Rab17 knockdown did, and the reduction was partially rescued by co-expression with Rab17-Q77L.[15] These findings indicated that Rabex-5 functions as an upstream activator of Rab17 in developing hippocampal neurons (**Fig. 1A**).

Determining the molecular mechanism by which active Rab17 is translocated from the cell body to the dendrites is an important task that has yet to be achieved. Active Rab17 itself is unlikely to have the ability to target dendrites, because unlike endogenous Rab17, which specifically localizes in the dendrites, Rab17-Q77L also localizes in the axon.[15] Interestingly, overexpression of a GEF domain (i.e., VPS9 domain) of Rabex-5 alone similarly increased the translocation of Rab17 to both the axon and the dendrites,[15] whereas overexpression of full-length Rabex-5 increased endogenous Rab17 translocation to the dendrites alone. Thus, some additional domains of Rabex-5 besides its GEF domain (e.g., a zinc finger domain and/or a coiled-coil domain) must also be involved in Rab17 targeting. We speculate that Rabex-5 functionally links to dendrite-directed motors and that Rab17 activated by Rabex-5 is captured by certain motor proteins that transport it to the dendrites. Since some Rab effector molecules (or Rabs themselves) are known to directly associate with motor proteins,[16] in

the future it would be interesting to search for Rab17-specific effectors that form a link between Rabex-5 and motor proteins.

Rabex-5 Determines the Axon and Dendrite Localization of Rab5 and Rab21, Two Other Downstream Targets of Rabex-5

Our finding that Rabex-5 determines the dendrite localization of its downstream target Rab17 is highly consistent with a recent report showing that Rab-GEFs (e.g., Rabex-5 and Rabin8) are major determinants of specific Rab membrane targeting.[17] However, one puzzling result of our research is that Rab5 and Rab21, two other downstream targets of Rabex-5,[13,18] are translocated "both to the axon and to the dendrites" of developing hippocampal neurons in a Rabex-5-dependent manner (**Fig. 1B**),[15] meaning that Rabex-5 determines not only the dendrite targeting of Rab17 but the axon/dendrite targeting of Rab5 and Rab21 as well. Because of the multiple roles of Rabex-5 in Rab targeting to neurites, Rabex-5 is involved in both the axon morphogenesis and dendrite morphogenesis of developing hippocampal neurons by activating at least two downstream targets, Rab5 and Rab17.[15] However, the molecular mechanism by which Rabex-5 sorts different downstream Rab proteins into the axon and/or dendrites is completely unknown. Since Rabex-5 constitutes a GEF cascade[19] by recruiting a Rab5 effector, Rabaptin-5, to early endosomes,[13] Rabex-5 may also recruit an as yet unidentified Rab17-specific effector(s) (factor "X" in **Fig. 1A**) that does not bind Rab5 or Rab21 and support dendrite targeting of Rab17. Thus, identifying Rab17-specific effectors will be one of the most important tasks in future Rab17 research designed to understand the molecular mechanism by which Rabex-5 determines the targeting of its downstream Rab proteins to neurites at the cellular level. Since Rab17 is also expressed in epithelial cells[5-7] and melanocytes,[8] it would be interesting to investigate whether Rabex-5 also contributes to the polarized trafficking of Rab17 in other cell types.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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