REI-1, a Novel Rab11 GEF with a SH3BP5 domain

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ABSTRACT

The small GTPase Rab proteins are key regulators of membrane trafficking. Rab11 is one of the best-characterized molecules among the Rab family proteins and it plays multiple roles in endocytic recycling, exocytosis, and cytokinesis. However, it remains unclear how Rab11 is activated at a precise timing and location and regulates its diverse functions. Specifically, our knowledge of the upstream regulatory factors that activate Rab11 is limited. In this regard, we have identified the RAB-11-interacting protein-1 (REI-1) as a novel guanine nucleotide exchange factor (GEF) for RAB-11 in Caenorhabditis elegans (C. elegans). REI-1 family proteins are conserved among metazoans, and its human homolog, SH3BP5, also exhibits strong GEF activity toward human Rab11. In C. elegans, REI-1 is expressed in the germline and co-localizes with RAB-11 on late-Golgi membranes. The loss of REI-1 impaired the targeting of RAB-11 to the late-Golgi compartment, as well as the recycling endosomes in embryos and further reduced the recruitment of RAB-11 to the cleavage furrow, resulting in the delay of cytokinesis. We suggest that REI-1 is the GEF responsible for regulating RAB-11 localization and function in early embryos.

REI-1 family proteins are conserved GEFs for Rab11

We have previously shown that C. elegans RAB-11.1, a homolog of human Rab11a, dynamically changes its localization and performs essential functions during the oocyte-to-embryo transition. To investigate the molecular mechanism by which RAB-11.1 is activated, we searched for RAB-11.1 binding proteins using a mutant form of RAB-11.1 (S25N), which mimics GDP-bound RAB-11.1, and successfully identified RAB-11-interacting protein-1 (REI-1) in C. elegans (Fig. 1A). The C. elegans genome also contains a rei-1 homolog, namely rei-2, and its gene product, REI-2, was also found to interact with RAB-11.1 (Fig. 1A). REI-1 and REI-2 also strongly interacted with a nucleotide-free mutant of RAB-11.1 (N124I). These binding patterns were reminiscent of the properties of known Rab GEF proteins. Based on this, we hypothesized that REI-1 is a GEF for RAB-11.1. Consistent with this hypothesis, REI-1 showed a strong GDP-GTP exchange activity toward RAB-11.1 in vitro. Importantly, REI-1 possessed GEF activity only in the presence of liposomes. We also found that REI-1 has the ability to bind liposomes, suggesting that the interaction of REI-1 with membranes modulates its GEF activity.

Since REI-1 and REI-2 form a protein family that is well conserved in metazoans, including Drosophila and human (Fig. 1A), we further examined whether the
molecular function of REI-1 is conserved in its mammalian homolog, namely SH3-binding protein 5 (SH3BP5). As expected, SH3BP5 interacted with the GDP-bound and nucleotide-free forms of human Rab11a and possessed a strong GEF activity toward human Rab11a. Interestingly, the REI-1 family proteins do not possess any domains with sequence similarity to known Rab-GEF domains, such as the DENN and Vps9 domains. However, REI-2 and SH3BP5 do exhibit a sequence similarity to BAR and F-BAR domains, respectively. These domains potentially function as modules for dimerization, lipid binding, and membrane-curvature sensing. In fact, REI-1 interacts with itself in a homophilic manner and directly binds to membranes. These observations suggest that the BAR/F-BAR domains may function to target REI-1 family proteins to a specific compartment where they activate Rab11.

**REI-1 regulates RAB-11.1 localization and function in *C. elegans* embryos**

We further addressed the mechanism by which REI-1 regulates the diverse functions of RAB-11.1 *in vivo*. In growing oocytes, RAB-11.1 is found to localize on REs as well as on the Golgi and it regulates yolk receptor recycling. When oocytes mature, RAB-11.1 is targeted to the cortical granules (CGs) and regulates CG exocytosis after fertilization, contributing to proper egg shell formation. In embryos, RAB-11.1 redistributes to the Golgi and RE. Our results show that REI-1 is expressed in germline cells and co-localizes with RAB-11.1 on late-Golgi membranes. The deletion of the *rei-1* and *rei-2* genes did not strongly affect RAB-11.1 localization in oocytes, yolk uptake by oocytes or CG exocytosis in zygotes. In contrast, in early embryos, targeting of RAB-11.1 to the late-Golgi compartment and recycling endosomes was significantly impaired in *rei-1* mutants and this defect was enhanced by *rei-2* mutation. These results suggest that REI-1 and REI-2 specifically regulate RAB-11.1 localization in early embryos.

In *C. elegans* embryos, RAB-11.1 also localizes to the cleavage furrow during cell division, and plays an essential role in cytokinesis, a phenomenon that has also been previously shown in mammals. **RNAi**-treated *rab-11.1* embryos fail to complete cytokinesis. In *rei-1* mutant embryos, ingress occurred but RAB-11.1 was not targeted to the late-Golgi or the cleavage furrow, which resulted in delayed cytokinesis, and this defect was enhanced by *rei-2* mutation. In wild-type embryos, REI-1 itself and a late-Golgi marker SYN-16 were not recruited to the cleavage furrow. Based on this, we propose a model whereby REI-1-dependent activation of RAB-11.1 on the late-Golgi compartment is a prerequisite for the targeting of RAB-11.1 to the cleavage furrow thereby facilitating its involvement in cytokinesis (Fig. 1B).

Our results also indicate that RAB-11.1 is able to bind membranes even in the absence of its GEFs, REI-1 and REI-2. This is consistent with a previous report on a yeast Rab-GEF mutant. This observation could potentially explain why the phenotypes of *rei-1* and *rei-2* mutants are milder than that of the *rab-11.1* RNAi. Alternatively, another unidentified potential GEF could partially activate RAB-11.1 even in *rei-1* and *rei-2* mutants. Additional analysis using a deletion series of the REI-1 protein suggests that almost full-length of REI-1 is required for its Rab11-binding ability (Fig. 1A).

It has been reported that human Rab11 is involved in a variety of disease settings including cancer progression. Therefore, as REI-1 family proteins regulate the activation of Rab11, the actions of these GEFs may present novel targets for therapeutic invention. More extensive studies of the REI/SH3BP5 family proteins could uncover molecular mechanisms of tissue-specific and spatiotemporal regulation of Rab11 and will likely prompt further research in the field of membrane trafficking.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.
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