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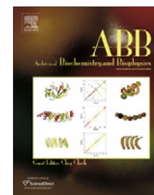

Expression of Rab27B-binding protein Slp1 in pancreatic acinar cells and its involvement in amylase secretion

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Slp1 is a putative Rab27 effector protein and implicated in intracellular membrane transport; however, the precise tissue distribution and function of Slp1 protein remain largely unknown. In this study we investigated the tissue distribution of Slp1 in mice and found that Slp1 is abundantly expressed in the pancreas, especially in the apical region of pancreatic acinar cells. Slp1 interacted with Rab27B *in vivo* and both proteins were co-localized on zymogen granules. Morphological analysis of fasted Slp1 knockout mice showed an increased number of zymogen granules in the pancreatic acinar cells, indicating that Slp1 is part of the machinery of amylase secretion by the exocrine pancreas.

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Small GTPase Rab is generally thought to control intracellular membrane transport by mediating the interaction between vesicular carriers and their specific acceptor compartments [1]. Rab27A, a member of the Rab family, is expressed in a cell-type specific manner, and its involvement in melanosome transport in melanocytes and granule exocytosis by cytotoxic T lymphocytes has been well characterized by analysis of Rab27A-deficient *ashen* mice [2–6]. In addition, mutations in the *RAB27A* gene cause human type II Griscelli syndrome, which is characterized by pigment dilution and immunodeficiency [7]. Rab27B, a closely related isoform of Rab27A, has also been shown to control membrane transport in pituitary cells [8,9], amylase-secreting cells in the parotid gland [10], platelets [11], mast cells [12], and urothelial umbrella cells [13]. Chen et al. have recently demonstrated that Rab27B is present on zymogen granules in rat pancreatic acinar cells and that overexpression of a dominant negative mutant of Rab27B significantly inhibited cholecystokinin-induced amylase secretion [14], while overexpression of an active mutant enhanced it. The molecular mechanism by which Rab27B controls zymogen granule exocytosis (e.g., identification of a Rab27B effector), however, remains to be achieved.

To date, 11 putative Rab27 effectors, including synaptotagmin-like proteins (Slps), Slac2s, rabphilin, Noc2, and Munc13-4, have been reported and suggested to regulate multiple membrane transport events (reviewed in Ref. [15]). Noc2 is a candidate for Rab27 effectors in pancreatic acinar cells because it binds Rab27A/B *in vitro* [16] and in neuroendocrine cells [17,18], and because marked accumulation of zymogen granules is observed in the pancreatic acinar cells of Noc2-deficient mice and there is no amylase response to stimuli [19]. However, the subcellular localization of Noc2 protein in pancreatic acinar cells and its interaction with Rab27 in the pancreas has never been elucidated. Other candidate Rab27 effectors in pancreatic acinar cells are the Slp family members (Slp1-5), all of which have an N-terminal Rab27-binding domain (named the Slp homology domain) and C-terminal tandem C2 domains [20]. One of the Slp family members, Slp2-a, is involved in the peripheral melanosome distribution of melan-a cells [21], in mucus secretion by surface mucous cells in mouse stomach [22], in transport of glucagon granules in pancreatic α-cell lines [23], and secretion by cytotoxic T lymphocytes [24]. Slp4-a/granuphilin-a plays a role in the docking of insulin granules in mouse pancreatic β-cells [25] and of dense-core vesicles in PC12 cells [26]. However, no Slp protein that functions in pancreatic acinar cells has ever been identified.

In this study we investigated the pattern of Slp1/JFC1 protein expression in mice and found that it is most abundantly expressed

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in the murine pancreas and stomach. We then examined the physiological function of Slp1 protein in mouse pancreas by analysis of mutant mice with a functional disruption of the *slp1* gene. Possible functions of Slp1 protein in amylase secretion by pancreatic acinar cells are discussed based on our results.

Materials and methods

Antibodies

Anti-Slp1 rabbit (or guinea pig) polyclonal antibody was raised against GST (glutathione *S*-transferase)-Slp1-linker (amino acid residues 117–267 of mouse Slp1) as described previously [20]. Anti-Rab27A, anti-Rab27B, and anti-Slp1-C2B antibodies were prepared as described elsewhere [10,20]. Anti-amylase antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Rab3 and anti-syntaxin 4 antibodies were from BD Transduction Laboratories (Lexington, KY). Anti-lipase antibody was from Abcam (Cambridge, UK). Anti-syntaxin 2 and anti-syntaxin 3 antibodies were from Merck Biosciences Calbiochem (Darmstadt, Germany).

Production of Slp1 mutant mice

To isolate the mouse *slp1* gene, a mouse 129/Sv genomic library (Stratagene, La Jolla, CA) was screened with mouse Slp1 cDNA as a probe. In the targeting vector, the exon encoding the first methionine and Rab27-binding domain was replaced with a neomycin resistance gene (Fig. 3A). Linearized targeting vector was introduced into E14 embryonic stem cells by electroporation. Homologous recombinants were identified by Southern blot and PCR analyses. Germline transmission was obtained for three independent ES clones, and no apparent differences in phenotype among the three lines of mice were observed. The mice were maintained at the Research Resource Center of the RIKEN Brain Science Institute. All animal experiments were carried out according to the guidelines for animal experimentation at RIKEN.

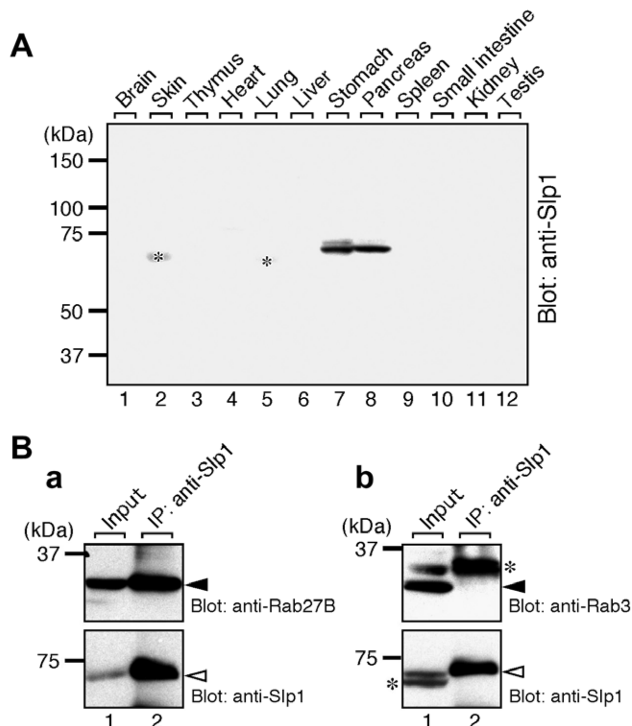

Fig. 1. Slp1 is a Rab27B-binding protein in mouse pancreas. (A) Tissue distribution of mouse Slp1 protein as determined by immunoblot analysis. Equal amounts of total protein (125 μg) from the mouse tissue homogenates indicated were analyzed by 7.5% SDS–PAGE and immunoblotted with anti-Slp1 antibody (2.5 μg/ml). Note the presence of strong Slp1 signals in mouse stomach and pancreas. Asterisks correspond to the non-specific bands of secondary antibody. The positions of the molecular mass markers ($\times 10^{-3}$) are shown on the left. (B) Interaction between Slp1 and Rab27B analyzed by immunoprecipitation. Lysate from mouse pancreas was immunoprecipitated with anti-Slp1-C2B antibody, and the immunoprecipitates were immunoblotted with anti-Rab27B antibody (closed arrowhead, top panel in (a)) or anti-Rab3 antibody (b). The same blots were then immunoblotted with anti-Slp-linker antibody (open arrowheads in bottom panels). The asterisk indicates the non-specific bands of IgG.

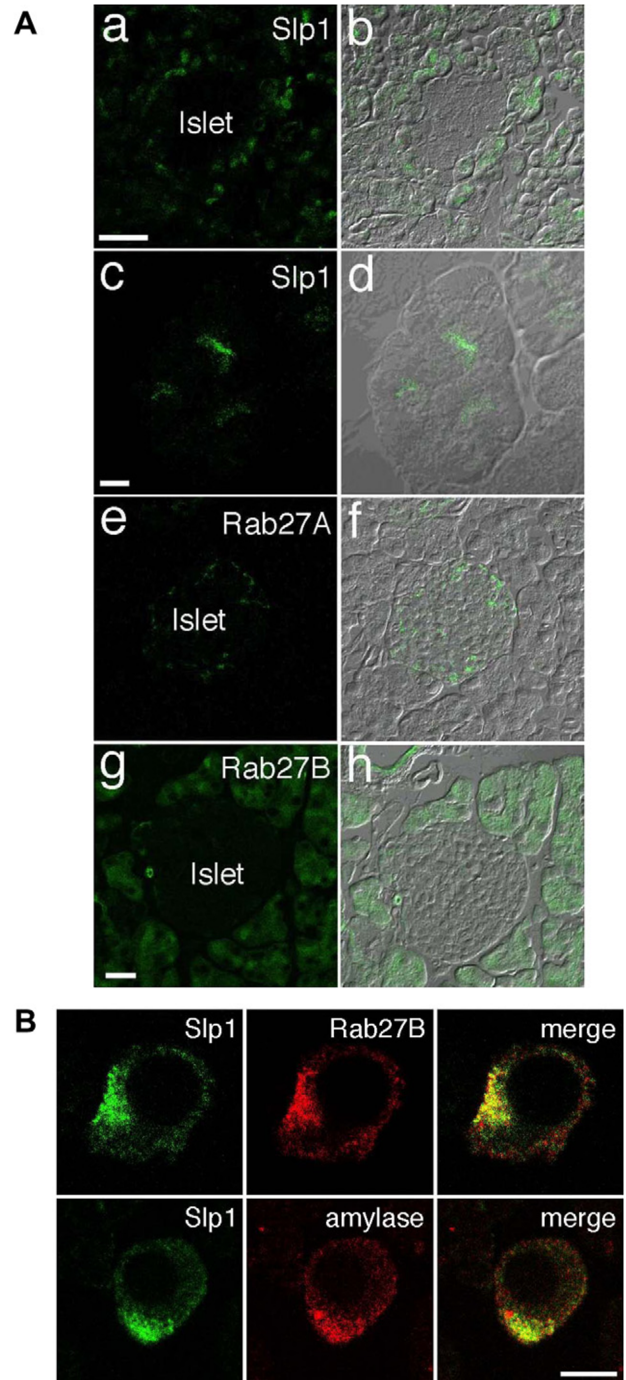

Fig. 2. Slp1 is localized on zymogen granules in pancreatic acinar cells. (A) Slp1 and Rab27B are expressed in the acinar cells of mouse pancreas. Sections of mouse pancreas were immunostained with antibody against Slp1 (green in a–d), Rab27A (green in e and f), and Rab27B (green in g and h). Note the strong Slp1 and Rab27B immunoreactivity in the apical region of the pancreatic acinar cells (c, d, g, and h). Panels b, d, f, and h are merged images of the immunostained images (a, c, e, and g) and corresponding DIC images. "Islet" in a, e, and g means islet of Langerhans. Scale bars: a, b, e–h, 50 μm; c and d, 10 μm. (B) Isolated pancreatic acinar cells were immunostained with anti-Slp1 antibody (green, left panels) and anti-Rab27B or anti-amylase antibody (red, middle panels). Right panels are merged images between left and middle panels. Note that Slp1 co-localized with Rab27B and amylase (yellow in right panels). Scale bar: 10 μm.

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