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Involvement of Rab27 in antigen-induced histamine release from rat basophilic leukemia 2H3 cells

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Abstract

The Rab family small G proteins regulate discrete steps in vesicular transport pathways. Recent studies indicate that one member of the Rab family, Rab27A, regulates the transport of lysosome-related organelles, such as melanosome distribution in melanocytes, lytic granule release in cytotoxic T cells, and dense granule release in platelets. Here, we have examined the involvement of Rab27A in the exocytic transport of another lysosome-related organelle, the basophilic secretory granule, in basophils. We have found that Rab27A locates on basophilic secretory granules containing histamine in rat basophilic leukemia (RBL) 2H3 cells. In addition, exogenous expression of dominant active Rab27A reduces antigen-induced histamine release from the cells. We have moreover identified Munc13-4 as a Rab27A target using a CytoTrap system and found that exogenous expression of Munc13-4 affects antigen-induced histamine release from RBL-2H3 cells. These results demonstrate that Rab27A plays a crucial role in antigen-induced histamine release from RBL-2H3 cells.

Keywords: Rab27A; Histamine release; Basophil; Lentiviral vector; Munc13-4

The Rab family small G proteins, which comprise more than 60 members in mammalian cells, regulate discrete steps in vesicular transport pathways [1]. Each Rab family member is localized to a distinct compartment in the exocytic or endocytic pathway and functions as a molecular switch, cycling between GTP-bound and GDP-bound conformations, at the compartment where it resides. Rab27 is a member of the Rab family and consists of two isoforms, Rab27A and Rab27B [2]. These proteins share 71% amino acid sequence similarity. Rab27A is widely expressed, whereas the expression of Rab27B is restricted to platelets, the pituitary gland, and the digestive tract [3–5]. Rab27A has been shown to regulate the transport of melanosomes in melanocytes, the exocytosis of lytic granules in cytotoxic T lymphocytes, and the exocytosis of dense granules in platelets [5–12]. In humans, mutations in Rab27A cause Griscelli syndrome, an autosomal recessive disorder characterized by pigment dilution of the hair and an uncontrolled T lymphocyte [6,12,13]. At the cellular level, this disorder reflects the dysfunction of melanosomes and lytic granules.

It has been shown that melanosomes and lytic granules share several biochemical properties with those of lysosomes. These organelles contain the lysosomal proteins Lamp-1, Lamp-2, and CD63/Lamp-3, and their lumenal pH is acidic [14]. The basophilic secretory granules also contain these lysosomal proteins and are regarded to be lysosome-related organelles [15,16]. The basophilic secretory granules also store various inflammatory mediators including histamine [17], and the

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activation of the high affinity immunoglobulin E (IgE) receptor induces exocytosis of the granules, resulting in histamine release. While various intracellular events are induced by the cross-linking of the high affinity IgE receptor [17], the involvement of Rab27A in antigen-induced histamine release from basophils has not been described.

We now report that Rab27A and histamine show a remarkably similar distribution in rat basophilic leukemia (RBL) 2H3 cells and that antigen-induced histamine release is reduced in dominant active Rab27A-transduced RBL-2H3 cells. Moreover, we identified Munc13-4 as a Rab27A target and found that this protein also affects antigen-induced histamine release from the cells.

Materials and methods

Fluorescence microscopy and antibodies. RBL-2H3 cells cultured on cover glasses were fixed with 3.7% formaldehyde in PBS for 20 min at room temperature, washed three times with PBS, and treated with 0.1% Triton X-100 in PBS for 5 min at room temperature. The cells were then washed three times with PBS and treated with 100 mM NH_4Cl in PBS for 5 min at room temperature. After blocking with 1% bovine serum albumin (BSA) in PBS, the cells were incubated in primary antibody overnight at 4 °C, washed three times with PBS, and were incubated in secondary antibody for 1 h at room temperature. The cells were washed three times with PBS and were mounted on slides.

Fluorescence was visualized through a Bio-Rad Radiance 2000 confocal laser-scanning microscope. *Z* axial sections were collected at a 0.85 μm step and the projected images were generated.

Mouse monoclonal anti-Rab27 antibody was purchased from BD Transduction Laboratories (Lexington, KY, USA). Rabbit anti-histamine antibody and mouse monoclonal anti-FLAG M2 antibody were purchased from Sigma (St. Louis, MO, USA). Rabbit anti-cathepsin D antibody was purchased from WAKO (Osaka, Japan). Alexa488- and Texas Red-conjugated secondary antibodies were purchased from Molecular Probes (Eugene, OR, USA). Horseradish peroxidase-linked antibodies were purchased from Bio-Rad (Hercules, CA, USA).

Construction and preparation of the lentiviral vectors. The cDNAs encoding the full open reading frames of murine Rab27A and Rab8 were amplified from a cDNA library from the murine immature B cell line, WEHI231, by PCR. The PCR products were purified from an agarose gel and were directly inserted into the pGEM-T Easy vector (Promega, Madison, WI, USA). Site-directed mutagenesis was carried out using a PCR-based method. The cDNAs of Rab27A and Rab8 mutants (Rab27AQ78L, Rab27AT23N, Rab8Q67L, and Rab8T22N) used in this study have been previously described elsewhere [3,4,12,18]. To 3×FLAG tag Rab27A and Rab8 at the N terminus, the cDNAs of the Rab27A and Rab8 mutants were subcloned into p3×FLAG/CMV-7.1 (Sigma, St. Louis, MO, USA). The cDNA of Munc13-4 was also subcloned into p3×FLAG/CMV-7.1. Entry clones (pENTR/FLAG-Rab27AQ78L, pENTR/FLAG-Rab27T23N, pENTR/FLAG-Rab8Q67L, pENTR/FLAG-Rab8T22N, and pENTR/FLAG-Munc13-4) were prepared by using the pENTR Directional TOPO Cloning Kit (Invitrogen, Carlsbad, CA, USA). The pSos/Rab27A plasmids were made by subcloning the Rab27Awt, Q78L, and T23N cDNA into pSos (Stratagene, La Jolla, CA, USA). All expression plasmids were sequenced using an Applied Biosystems automated DNA sequencer 377.

Lentiviral vectors were prepared with the ViraPower Lentiviral Expression System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Briefly, in vitro recombination was promoted between the pENTR and pLenti4/V5-DEST (Invitrogen, Carlsbad, CA, USA) in order to generate the pLenti4/FLAG-Rab27AQ78L, pENTR/FLAG-Rab27T23N, pENTR/FLAG-Rab8Q67L, pENTR/FLAG-Rab8T22N, and pENTR/FLAG-Munc13-4. Next, the pLenti4 constructs were co-transfected with pLP1, pLP2, and pLP/VSVG into 293FT cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The cells were grown in DMEM containing 10% FCS, 2 mM L-glutamine, 0.1 mM MEM non-essential amino acids, 100 μg/ml penicillin, and 100 U/ml streptomycin. Lentiviral supernatants were harvested at 48 and 72 h after co-transfection, spun down at 800*g*, and passed through 0.45 μm filters before aliquots were frozen at −80 °C. To further increase the titers, the supernatants were centrifuged at 6000*g* for 16 h and the lentiviral vectors were collected in the precipitates.

Cell culture, transduction, and histamine release assay. RBL-2H3 cells were maintained in EMEM containing 10% FCS, 100 μg/ml penicillin, and 100 U/ml streptomycin. For the histamine release assay, RBL-2H3 cells were seeded at 1×10^5 cells/well in 12-well culture dishes. On the following day, the cells were transduced with lentiviral supernatants in the presence of 5 μg/ml polybrene (Sigma, St. Louis, MO, USA) at a multiplicity of infection (MOI) of 1. The cells were incubated at 37 °C overnight and the medium was changed. After 72 h, the cells were washed twice in Pipes buffer (140 mM NaCl, 5 mM KCl, 0.6 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM Pipes [pH 7.4], and 5.5 mM glucose) and sensitized for 1 h by 0.5 μg/ml anti-dinitrophenyl (DNP) mouse monoclonal IgE (Yamasa, Tokyo, Japan). Next, the cells were washed twice with Pipes buffer and stimulated with DNP-BSA (LSL, Tokyo, Japan). Following antigen-stimulation, the medium was collected and treated with perchloric acid. Intracellular histamine remaining in the cells was released by perchloric acid treatment. The histamine content was measured by the *o*-phthalaldehyde fluorometric procedure [19]. The fluorescent intensity was measured with a F-3010 spectrofluorometer (Hitachi, Tokyo, Japan). Histamine release was expressed as the percentage of total histamine content.

Western blotting. For Western blotting, RBL-2H3 cells grown in 12-well plates were washed twice in phosphate buffered saline and solubilized in 2× SDS sample buffer. Samples were loaded on polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After blocking, the membranes were treated with the anti-FLAG or anti-Rab27 antibody and immunoreactive proteins were detected using the enhanced chemiluminescence (ECL) protocol (Amersham Biosciences, Piscataway, NJ, USA) with horseradish peroxidase-linked secondary antibody (Bio-Rad, Hercules, CA, USA).

Strain, media, and yeast transformation. The yeast strain cdc25Hα has the following genotype: *MAT*α *ura3*, *lys2*, *leu2*, *trp1*, *his200*, *ade101*, *cdc25-2*, *GAL*+ [20,21]. A point mutation of the *cdc25* gene in cdc25Hα prevents host growth at a restrictive temperature (37 °C), but not at a permissive temperature (24 °C). This strain was grown in YPAD medium or Burkholder's minimum media (BMM) with appropriate supplements at 24 °C and was transformed by the standard lithium acetate method [22]. For selection of transformants, glucose base BMM lacking leucine and uracil (BMM/glucose -leu, ura) was used. For induction of protein expression from pMry (Stratagene), galactose base BMM lacking leucine and uracil (BMM/galactose -leu, ura) was used.

CytoTrap screening. The CytoTrap screening was performed as described previously [23]. Briefly, the yeast containing pSos/Rab27AQ78L was transformed with a WEHI231 cDNA library. The transformants were grown on a BMM/galactose -leu, ura plate at 24 °C for 3 days and then grown at 37 °C. The colonies grown at 37 °C were picked and tested for growth on BMM/glucose -leu, ura and BMM/galactose -leu, ura plates at 37 °C. The library plasmids were isolated from the clones that exhibited galactose-dependent growth at 37 °C and were re-transformed into the cdc25Hα cells either with pSos/Rab27AQ78L or with pSos/Collagenase IV as a negative

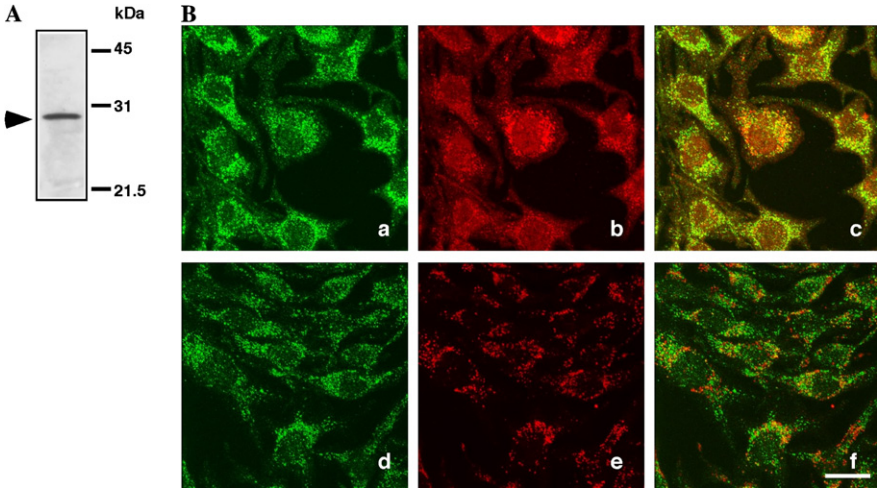

Fig. 1. (A) Expression of Rab27A in RBL-2H3 cells. RBL-2H3 cells were solubilized with 2× SDS sample buffer. The solubilized samples were loaded on polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After blocking, the membrane was treated with anti-Rab27 antibody and immunoreactive proteins were detected by ECL. The arrowhead indicates the position of endogenous Rab27A. (B) Intracellular localization of Rab27A in RBL-2H3 cells. RBL-2H3 cells were double-immunostained with anti-Rab27 antibody (a and d) and anti-histamine (b) or anti-cathepsin D antibody (e). Merged images are also shown (c and f). Bar, 30 μm.

control. Only plasmids that suppressed the cdc25Hα phenotype in the presence of pSos/Rab27AQ78L were sequenced. DNA sequencing was performed on an Applied Biosystems automated DNA sequencer 377.

Results

Rab27A localizes to histamine-containing vesicles in RBL-2H3 cells

To determine the expression of Rab27 in rat basophilic leukemia (RBL) 2H3 cells, Western blot analysis was performed using the anti-Rab27 antibody (Fig. 1A). It has been reported that the anti-Rab27 antibody used for this study specifically recognizes Rab27A but not Rab27B [4]. As shown in Fig. 1A, this anti-Rab27 antibody specifically detected a single band in RBL-2H3 cell lysates. The size of the band (29 kDa) was similar to the size estimated from the amino acid sequence of Rab27A ($M_r = 25{,}017$). This result indicates that Rab27A is expressed in RBL-2H3 cells.

To determine the intracellular localization of Rab27A in RBL-2H3 cells, we next performed immunohistochemical analysis in RBL-2H3 cells (Fig. 1B). Immunostaining with the anti-Rab27 antibody indicated that Rab27A localized in a punctate pattern throughout the cytoplasm (Figs. 1B, a and d). A similar immunostaining pattern was observed for histamine in RBL-2H3 cells (Fig. 1B, b). These signals overlapped significantly (Fig. 1B, c). These results suggest that Rab27A is associated with basophilic secretory granules containing histamine in RBL-2H3 cells. In contrast, cathepsin D, a lysosomal protein, showed a punctate pattern different from that of Rab27A (Fig. 1B, d–f). This result indicates that cathepsin D is

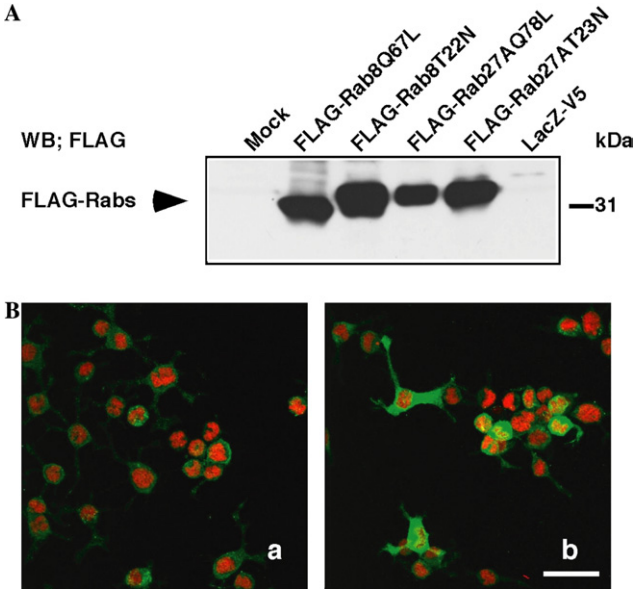

Fig. 2. (A) Exogenous expression of Rab27A and Rab8 mutants in RBL-2H3 cells. RBL-2H3 cells transduced with various lentiviral vectors (FLAG-Rab8Q67L, FLAG-Rab8T22N, FLAG-Rab27AQ78L, FLAG-Rab27AT23N, and LacZ-V5) were solubilized with 2× SDS sample buffer. The solubilized samples were loaded on polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After blocking, the membrane was treated with the anti-FLAG M2 antibody and immunoreactive proteins were detected by ECL. The positions of the various FLAG-Rabs are indicated. (B) Efficiency of transduction of Rab27AQ78L in RBL-2H3 cells. Mock-transduced (a) or FLAG-Rab27AQ78L-transduced (b) RBL-2H3 cells were immunostained with the anti-FLAG M2 antibody (green). Nuclear DNA was also stained with propidium iodide (red). Bar, 30 μm.

distributed at vesicles other than those that store histamine.

Gene transduction into RBL-2H3 cells using lentiviral vectors

To examine the involvement of Rab27A in the exocytosis of basophilic secretory granules, we attempted to express dominant active (Rab27AQ78L) and dominant negative (Rab27AT23N) mutants of Rab27A in RBL-2H3 cells. In this study, we used lentiviral vectors to transduce FLAG-tagged Rab27A mutants into RBL-2H3 cells. We also used a lentiviral vector to express FLAG-tagged dominant active (Rab8Q67L) and negative (Rab8T22N) mutants of Rab8, which has been shown to regulate exocytic pathways [18,24]. Western blot analysis with anti-FLAG antibody demonstrated that the lentiviral vectors sufficiently transduced the Rab27A and Rab8 mutants into RBL-2H3 cells (Fig. 2A). In addition, the expression levels of these exogenous FLAG-tagged Rab proteins were similar. The expression of FLAG-Rab27AQ78L was also confirmed by immunostaining with anti-FLAG antibody (Fig. 2B). Approximately 30% of RBL-2H3 cells strongly expressed FLAG-Rab27AQ78L after lentiviral vector transduction (Fig. 2B). Other vectors showed similar transduction efficiency (data not shown).

Exogenous expression of Rab27AQ78L reduced antigen-induced histamine release from RBL-2H3 cells

We next examined the effects of exogenous expression of Rab27A mutants on histamine release from RBL-2H3 cells. We used DNP-BSA as an antigen and measured histamine release from RBL-2H3 cells sensitized with anti-DNP IgE. Histamine release was critically dependent on antigen stimulation (Fig. 3A), as has been described previously [17]. DNP-BSA (30 ng/ml) caused histamine release from mock-transduced RBL-2H3 cells in a time-dependent manner. Antigen-induced histamine release reached a maximal level within 20 min and a 10-fold increment in release was observed. In addition, DNP-BSA induced histamine release in a concentration-dependent manner with a maximal effective concentration of 30–100 ng/ml in mock-transduced RBL-2H3 cells (Fig. 3B). In FLAG-Rab27AQ78L-transduced cells, antigen-induced histamine release also reached a maximal level within 20 min and a similar concentration-dependence was observed (Figs. 3A and B). However, the maximal level of antigen-induced histamine release was reduced significantly compared with the level released from mock-transduced cells. In contrast, other transgenes including FLAG-Rab27AT23N, FLAG-Rab8Q67L, and FLAG-Rab8T22N did not affect antigen-induced histamine release (data not shown).

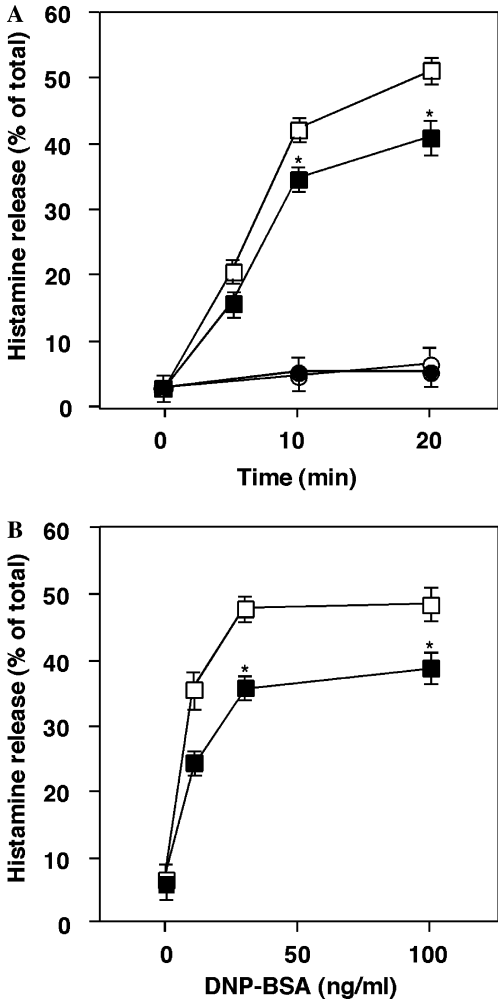

Fig. 3. Effects of FLAG-Rab27AQ78L transduction on antigen-induced histamine release from RBL-2H3 cells. (A) Time course of antigen-induced histamine release from RBL-2H3 cells. Mock-transduced (open) or FLAG-Rab27AQ78L-transduced (closed) RBL-2H3 cells were sensitized for 1 h with 0.5 μg/ml anti-DNP IgE and stimulated with (square) or without (circle) 30 ng/ml DNP-BSA for the indicated periods. (B) Dose-dependent effects of antigen on histamine release from RBL-2H3 cells. Mock-transduced (open) or FLAG-Rab27AQ78L-transduced (closed) RBL-2H3 cells were sensitized for 1 h with 0.5 μg/ml anti-DNP IgE and stimulated with the indicated concentrations of DNP-BSA for 20 min. The histamine was measured by the *o*-phthalaldehyde fluorometric procedure. Histamine release was expressed as the percentage of the total histamine content. The data shown are means ± SEM of three independent experiments. Student's *t* test was used to test the statistical significance of the data (*$p < 0.01$ vs. histamine release from corresponding cells transduced with mock).

Identification of Munc13-4 as a Rab27A target protein

To understand the mode of action of Rab27A on histamine release from RBL-2H3 cells, we next tried to identify Rab27A target proteins. For this purpose, we performed a CytoTrap screening from a *murine* immature B cell (WEHI231) cDNA library. Human

Ras GEP, Sos-tagged dominant active mutant of Rab27A, Rab27AQ78L, was used as bait. Expression of Sos-Rab27AQ78L was confirmed by Western blot with the anti-Sos antibody (data not shown). The yeast cells expressing Sos-Rab27AQ78L were subsequently transformed with a WEHI231 cDNA library. Approximately 4×10^5 transformants were screened. Finally, this screen resulted in the isolation of nine clones that specifically interacted with Rab27AQ78L but not with Collagenase IV. The library plasmids rescued from the clones were sequenced and 1 clone was identified as a *murine* Munc13-4. Recently, Munc13-4 was identified as a Rab27A target protein by GTP-bound Rab27A affinity chromatography using platelet cytosol [25]. Consistent with this report, Munc13-4 interacted with the wild-type Rab27A (Rab27Awt) and Rab27AQ78L but not with Rab27AT23N in the CytoTrap system (Fig. 4A). Exogenous expression of EGFP-Rab27Awt and FLAG-Munc13-4 showed a similar punctate pattern of immunostaining in baby hamster kidney (BHK) cells and both signals overlapped significantly (Fig. 4B). Finally, we examined the effect of Munc13-4 on antigen-induced histamine release from RBL-2H3 cells. In FLAG-Munc13-4-transduced RBL-2H3 cells, antigen-induced histamine release was increased significantly compared with the level released from mock-transduced cells

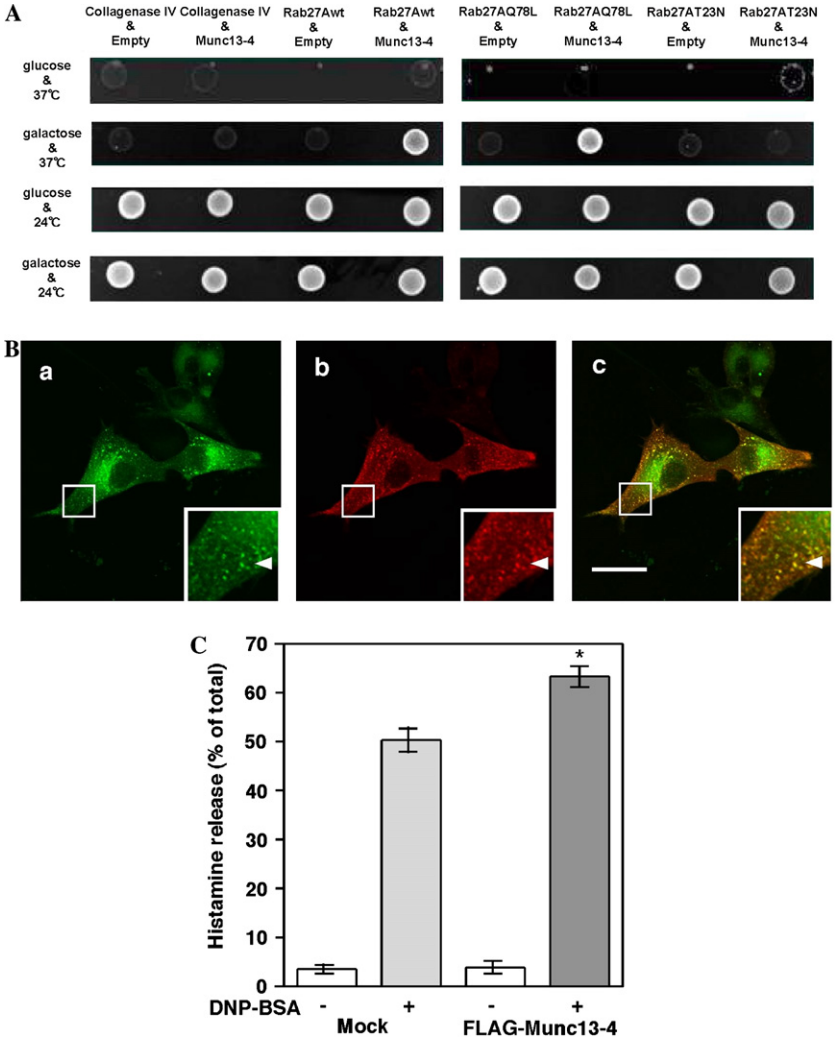

Fig. 4. Identification of Munc13-4 as a Rab27A target protein. (A) Specific interaction between Sos-Rab27AQ78L and Munc13-4 in the CytoTrap system. Yeast strain cdc25Hα was transformed with the indicated plasmids and the transformants were assayed for growth as described in Materials and methods. (B) Subcellular distribution of Munc13-4. BHK cells were transfected with pFLAG/Munc13-4 and pEGFP/Rab27Awt. After 48 h, the cells were fixed and examined by GFP fluorescence for EGFP-Rab27Awt and by immunofluorescence labeling for FLAG-Munc13-4. (a) Fluorescence images of EGFP; (b) fluorescence images of FLAG-Munc13-4; and (c) merged images. Bar, 30 μm. (C) Effects of FLAG-Munc13-4 transduction on antigen-induced histamine release. Mock- or FLAG-Munc13-4-transduced RBL-2H3 cells were sensitized for 1 h with 0.5 μg/ml anti-DNP IgE and stimulated with 30 ng/ml DNP-BSA for 20 min. The histamine was measured by the *o*-phthalaldehyde fluorometric procedure. Histamine release was expressed as the percentage of the total histamine content. The data shown are means ± SEM of three independent experiments. Student's *t* test was used to test the statistical significance of the data ($*p < 0.01$ vs. histamine release from corresponding cells transduced with mock).

(Fig. 4C). These observations suggest that Munc13-4 functions as a Rab27A target in histamine release from RBL-2H3 cells.

Discussion

Rab27A plays a pivotal role in the transport of melanosomes in melanocytes, exocytosis of lytic granules in cytotoxic T lymphocytes, and exocytosis of dense granules in platelets [5–12]. Because these organelles share several biochemical properties with lysosomes, they are considered to be lysosome-related organelles. It has been reported that Rab27A is expressed in a wide variety of cells in addition to these cells [3,5]. In this study, we have demonstrated that Rab27A is expressed in RBL-2H3 cells, and distributes to histamine-containing vesicles corresponding to basophilic secretory granules. Basophilic secretory granules are also classified as lysosome-related organelles [14]. Thus, there arose the possibility that Rab27A also regulates the exocytosis of basophilic secretory granules. Indeed, transduction of a dominant active Rab27A mutant reduced the exocytosis of basophilic secretory granules triggered by IgE cross-linking. These observations suggest that Rab27A might be involved in antigen-induced exocytosis of basophilic secretory granules.

Evidence is accumulating that the GTP-bound form of Rab proteins interacts with one or more downstream target proteins and that the interaction between each Rab protein and its specific target protein is required for each step in the vesicular transport pathway [1]. In the case of Rab27A, there are multiple target proteins, Slp 1–5 (synaptotagmin-like proteins) and Slac2 a–c (Slp homologous lacking C2 domain), all of which possess a highly conserved Rab27-binding domain in the N terminus [11,26–29]. The mode of action of Rab27A and a member of the Slac2 family, Slac2-a/Melanophilin, in melanocytes has been intensively investigated [5–7,9–12,27,28]. A protein complex consisting of Rab27A, Slac2-a/Melanophilin, and Myo-Va plays an essential role in the transport of melanosomes [27,28]. We have identified Munc13-4 as another Rab27A target protein and demonstrated that antigen-induced histamine release was increased by exogenous expression of Munc13-4. This finding is consistent with the previous report, which identified Munc13-4 as a novel Rab27A target by GTP-bound Rab27A affinity chromatography using platelet cytosol and demonstrated that the addition of recombinant Munc13-4 into the permeabilized platelets increases dense core granule secretion [25].

Munc13-4 is the fourth member of the Munc13 family [30]. The other members, Munc13-1, Munc13-2, and Munc13-3, are expressed exclusively in brain, except for ubMunc13-2, a splicing variant of Munc13-2 [31,32]. In contrast, Munc13-4 is expressed in non-neuronal tissues [30]. The Munc13 family may regulate the *trans*-SNARE complex formation between the vesicle SNARE and the target membrane SNARE, which mediates the docking and fusion of the vesicle with the target membrane. Munc13-1 acts as a priming factor in neurotransmitter release [33]. From the genetic evidence that transmission defects of *unc-13* mutants in *Caenorhabditis elegans* could be rescued by expression of open form of syntaxin, a target membrane SNARE [34], Munc13-1, is suggested to promote the formation of an open conformation of syntaxin-1 and to induce the assembly of the *trans*-SNARE complex. The cooperation of Rab and SNARE proteins is believed to be a key mechanism of the regulation of vesicle docking/fusion. There have been several reports of Rab target proteins directly or indirectly interacting with the components of SNARE [35–38]. In the case of Rab27, it has been recently reported that Slp4/Granuphilin, a member of the Slp family, directly interacts with syntaxin-1 [39]. Our present results suggest that Rab27A may regulate the SNARE complex formation through Munc13-4 in basophils. Further studies are necessary to clarify the mode of action of Rab27A-Munc13-4 in the exocytosis of basophilic secretory granules.

It has been reported that mutation of Rab27A causes a human genetic disease, Griscelli syndrome [6,12]. This is a rare autosomal recessive disorder characterized by partial albinism and immunodeficiency [13]. This disorder reflects dysfunctions in the transport of melanosomes in melanocytes and in the exocytosis of lytic granules in cytotoxic T cells, both of which are regulated by Rab27A. Mutation of Munc13-4 also causes genetic desease, familial hematophagocytic lymphohistocytosis (FLH3), where the exocytosis of lytic granule in cytotoxic T lymphocytes is impaired similar to mutation in Rab27A [40]. It is known that histamine plays a role in the generation of allergic inflammation and in immediate hypersensitivity reactions, including asthma and anaphylaxis [17]. It is not clear whether the patients of these diseases suffer from allergic disorders, but there is a possibility that modification of Rab27A-Munc13-4 function may cause allergic responses.

In summary, this study is the first report that Rab27A is involved in histamine release from basophilic cells. Our experiments to directly test Rab27A function in mast cells broaden the possibility of a role for Rab27A in histamine release.

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