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Rab27

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Version 2.0, April 24, 2015 [DOI: 10.3998/panc.2015.12]

Gene Symbols: Rab27A, Rab27B

1. General Function:

Rab proteins are monomeric Ras-like small GTPases and constitute the largest family of the known membrane trafficking proteins. Rabs act as molecular switches cycling between GTP-bound active and GDP-bound inactive conformations (19, 82). Among the Rab proteins, Rab27 proteins, along with Rab3 and Rab26, play important roles in regulation of various regulated secretion events (68). There are two isoforms of Rab27, Rab27A and Rab27B, which were originally cloned from human melanoma cells, melanocytes and platelet cytosol (8, 57). Human Rab27A and Rab27B share 66% identity at the nucleotide level in their open reading frames (ORFs) and 71% identity in amino acid sequence **(Figure 1)**. Variation is greatest in the carboxyl terminal. It is not clear whether the two Rab27 isoforms mediate different actions or are expressed in different cell types or both. It appears that Rab27A and 27B have been fully divided from each other since amphibians, as the two Rab27 isoforms of Xenopus similarly show 73% identity in amino acid sequence. **Appendix 1** shows the protein sequences of Rab27A and Rab27B from all reported species. Zebrafish, Drosophila and C. elegans have only one form of Rab27, which is most similar to human Rab27A.

Rab proteins require prenylation to properly exert their function. Rab27 proteins bear Cys-X-Cys at the C terminal; geranylgeranyl residues can be attached to the two cysteins by Rab geranylgeranyl transferase (RGGT), also called type II geranylgeranyl transferase (GGT II) (62). The newly synthesized Rab proteins are first recognized and bound by Rab escort protein (REP) and then are presented to RGGT for the posttranslational modification (61). In **Figure 1**, the REP and RGGT recognition motifs of human Rab27A and 27B are highlighted.

Rab27A

Mutations in the Rab27A gene cause type 2 Griscelli Syndrome (GS2), a rare, autosomal recessive disorder that results in pigmentary dilution of the skin and hair with the presence of large clumps of pigment in hair shafts and an accumulation of melanosomes in melanocytes. Most patients also develop an uncontrolled T-lymphocyte and macrophage activation syndrome known as haemophagocytic syndrome (52). A mutation in the mouse ortholog Rab27a is responsible for the phenotypes in *ashen* mice, including uneven release of pigment into the hair bulb and a lightened coat color, as well as the reduction in the number of platelet dense granules.

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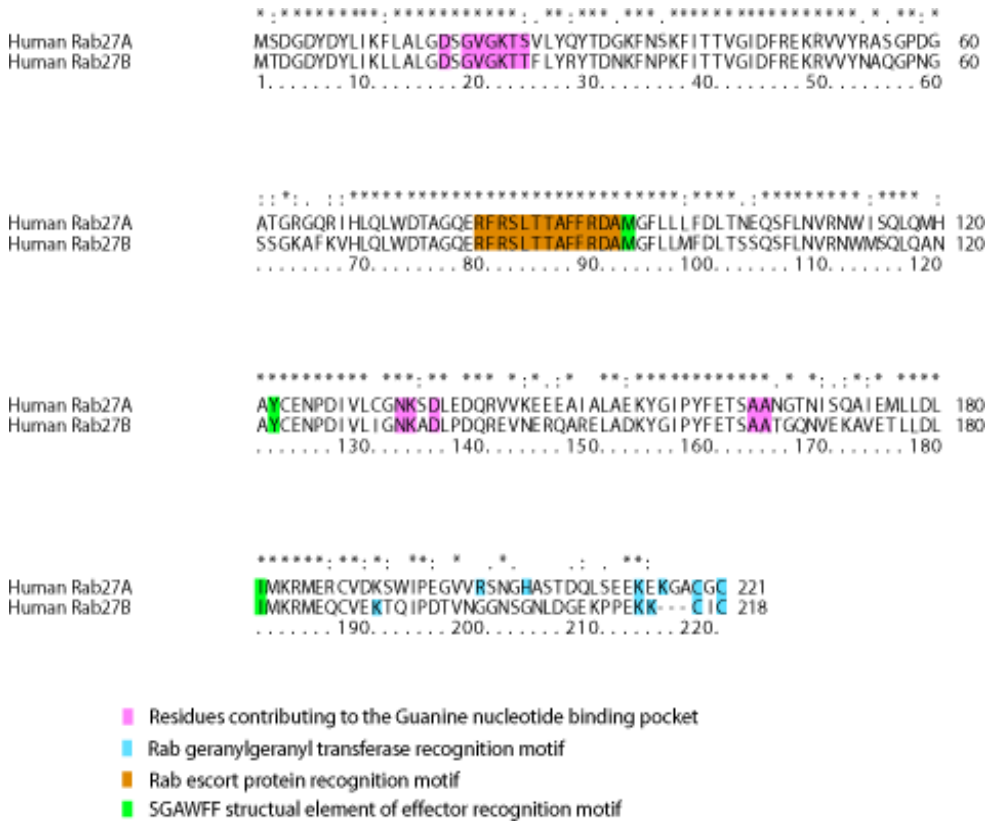

Figure 1 Alignment of protein sequences of Rab27A and Rab27B from human origin. Sequences were aligned by ClustalX 2.0.12 (downloaded from www.clustal.org). The designation of the specific regions is taken from Ref. (7) and the analysis generated by online program PrePS (http://mendel.imp.ac.at/sat/PrePS/index.html). *--- identical residues; :--- conserved substitutions; .--- semi-conserved substitutions.

This single point mutation prevents the proper splicing of Rab27A transcripts, leading to Rab27A deficiency (78). Subsequent studies showed that Rab27A colocalizes with melanosomes and regulates melanosome transport in melanocytes (1, 30) as well as being involved in the regulated granule exocytosis in cytotoxic T lymphocytes (CTLs) (25, 69). Pigment granules in melanocytes from ashen mice and GS patients showed perinuclear clustering. Reexpression of Rab27A in GS melanocytes restored the normal distribution of melanosomes (1, 30). CTLs from *ashen* mice and GS patients are unable to kill target cells or to secrete granzyme A and hexosaminidase, although these CTLs showed normal levels of perforin and granzymes A and B and normal-appearing perforin-positive granules (25, 52, 69), indicating that Rab27A is required for a late step in granule exocytosis in CTLs.

In melanocytes, Rab27A binds to melanosomes and then recruits its effector melanophilin/Slac2-a, which in turn recruits myosin-Va, an actin-based molecular motor. Thus, melanophilin/Slac2-a acts as a linker between Rab27B and myosin-Va. It binds to to Rab27a in a GTP-dependent fashion through its amino terminus, and to myosin-Va through its carboxy terminus (22, 56, 70, 79). Mutations in the myosin-Va gene (MYO5A) cause type 1 Griscelli Syndrome (GS1) (59); the corresponding coat-color mutant mouse *dilute* exhibits a defect in melanosome transport and also bears missense mutations in the globular tail of myosin-Va or has F-exon deletion in the myosin-Va gene (21, 51). The third type of Griscelli syndrome (GS3) was found to be caused by the mutation in the melanophilin/Slac2-a (51). The mutation of melanophilin/Slac2-a leads to the partial albinism in *leaden* color mutant mice (50). However, recently it has been further

demonstrated that the GTPase activity of Rab27A is required for its melanosome localization but is not required for melanosome transport (36).

It was recently shown that the general endo-lysosomal secretory pathway is dependent on Rab27A (48). Another study further highlighted that upregulation of Rab27A-dependent trafficking and secretory mechanisms improves lysosomal trafficking and secretion, as well as reduces lysosomal overload in cystinosis (40). The Munc 13-4-Rab27A complex was identified to be responsible in regulating the lysosomal secretory pathway in cytotoxic T lymphocytes (17) and neutrophils (39).

Rab27A and its effector MyRIP (also known as Slac2-c) were reported to be associated with large dense core granules in adrenal chromaffin cells and pheochromocytoma PC12 cells and to control the secretory activity in a manner that depends on the state of the actin cortex (16). Overexpression of Rab27A in PC12 cells promoted high KCl-dependent secretion of neuropeptide Y (20). Rab27A was also found to be expressed on dense-core vesicles and play a key role in the docking step of dense-core vesicle exocytosis in PC12 cells (74). Silencing of Rab27A significantly decreased the number of dense-core vesicles docked to the plasma membrane without altering the kinetics of individual exocytotic events (74).

In the pancreatic beta-cell, Rab27A was shown to mediate the tight docking of insulin granules to the plasma membrane upon high glucose stimulation. Ashen mice showed glucose intolerance after a glucose load without signs of insulin resistance in peripheral tissues or insulin deficiency in the pancreas. The docking of insulin granules on the plasma membrane and the replenishment of docked granules during glucose stimulation were markedly reduced in *ashen* mouse pancreatic islets (42). In addition, Rab27A was shown to regulate the exocytosis of insulin-containing dense-core granules by forming a complex with granuphilin in pancreatic beta-cells (81). It seems that Rab27A regulates the tethering and docking steps of insulin-containing granules separately by binding to two different downstream effectors, Slac2-c and granuphilin (54). Another Rab27A effector protein, Slp1/JFC1/exophilin7 was also found to be expressed in pancreatic beta cells and to mediate the fusion of undocked granules through the affinity of its C2A domain toward plasma membrane phospholipids (75). It was further demonstrated that Rab27A exerts dual roles in glucose-mediated insulin granule exocytosis, facilitating refilling of releasable granule pools while also limiting the rate of release from these pools (53). Most recently, GTP/GDP nucleotide cycling of Rab27A was shown to be essential for generation of the functionally defined immediately releasable pool (IRP) and central to regulating the size of the readily releasable pool (RRP) (6). Rab27A was also found to be functional when it is in GDP-bound form and regulates endocytosis by involving coronin 3 and IQGAP1 (43-45).

Several recent studies have demonstrated that Rab27A downstream effector proteins, Slp2-a and Slp4-a are responsible for forming apical membrane in a Rab27A-binding dependent manner in Madin–Darby canine kidney II cells. First, Slp2-a specifically localizes to the luminal membrane and then targets rab27-loaded vesicles to initiate a single lumen. Vesicle tethering and fusion is mediated by Slp4-a in a Rab27/Rab3/Rab8 and syntaxin-3 binding dependent manner. Slp2-a was demonstrated to regulate Slp4-a as well as the trafficking of signaling molecule podocalyxin to the apical membrane, and potentially to regulate the expression of tight junction protein claudin (23, 80).

Rab3 GDP/GTP exchange protein (Rab3GEP), previously isolated as a guanine nucleotide exchange factor (GEF) for Rab3, was recently identified as the GEF required for the activation of Rab27A in melanocytes (18). Similar to Rab27A-deficient *ashen* melanocytes, Rab3GEP-depleted

cells show both clustering of melanosomes in the perinuclear area and loss of the Rab27a effector melanophilin. Rab27A-GTP levels are decreased in cells lacking Rab3GEP. Recombinant Rab3GEP exhibits guanine nucleotide exchange activity against Rab27A and Rab27B in vitro, in addition to its previously documented activity against Rab3 (18). These data suggest that members of related but functionally distinct Rab subfamilies can be controlled by common activators. Rab3GEP was found to be expressed in parotid acinar cells. The inhibition of Rab3GEP function resulted in decrease in isoproterenol-stimulated amylase release as well as GTP-Rab27 level (32). Rab27A has been shown to trigger a Rab-GEF cascade by binding to Rab3GEP in Rab27A-GTP form, and subsequently activate Rab3 in the large dense-core granule exocytosis from human sperm, during acrosome reaction (5).

A TBC (Tre2/Bub2/Cdc16) domain-containing protein, EPI64 was identified as a specific GTPase-activating protein (GAP) for Rab27A by a functional interaction screening (37). EPI64 showed GAP activity against Rab27A both in vivo and in vitro. In addition, a homologue of EPI64, EPI64B also exhibited Rab27A GAP activity *in vitro*. EPI64 is a physiological GAP for Rab27 in rat parotid acinar cells and down-regulation of EPI64 caused a reduction in the amount of amylase release (33). A recent study showed that EPI64 also functions as Rab27A specific GAP in a lung adenocarcinoma cell line and regulates exosome secretion. Overexpression of EPI64 enhanced exosome secretion (49).

Rab27B

Rab27B has been found in a large number of secretory cells **(Table 1)**. In the most complete analysis, transgenic mice, generated by replacing the Rab27B gene with reporter gene LacZ under control of the endogenous promoter, indicated that Rab27B is widely expressed in secretory cells, neurons and cells involved in surface protection and mechanical extension. The data have been largely confirmed by a later study using immnohistochemical staining on human fetal and adult tissues (24, 26). Rab27B was found to be abundantly expressed in pituitary tissue, where Rab27A and Rab27B are differentially expressed in cell types that secrete different peptide hormones (84). Rab27B also associates with secretory granules and the linker protein, granuphilin in the pituitary endocrine cell line AtT20. Furthermore, over-expression of the inactive mutant, Rab27B N133I, significantly inhibited basal and forskolin-induced ACTH secretion from AtT20 cells, indicating that Rab27B is involved in pituitary hormone secretion (84).

Rab27B is required for proplatelet formation and its expression is regulated by the transcription factor nuclear factor-erythroid 2 (NF-E2) (72). Rab27B knockout (KO) mice exhibit significant hemorrhagic disease in contrast to *ashen* mice, which do not. In vitro assays demonstrated impaired platelet aggregation with collagen and U46619 and reduced numbers and secretion of dense granules in Rab27B KO strain (73), suggesting that Rab27B is a key regulator of dense granule secretion in platelets and this regulation might be through binding to its effector Munc13-4 (63). Bone marrow derived mast cells (BMMC) from Rab27B KO mice also exhibit mild clustering of granules, indicating that Rab27B may play a crucial role in mast cell degranulation and that their action regulates the transition from microtubule to actin-based motility (55). In a more recent study, Rab27A and Rab27B were found to play opposite roles in mediating secretion in BMMC, with Rab27A negatively and Rab27B positively regulating degranulation (66).

Rab27B has been shown to be expressed in gastric parietal cells, which are responsible for producing gastric acid. Rab27B was associated with tubulovescular membrane in the parietal cells; secretagogue-treatment caused the translocation of Rab27B to the apical membrane; overexpression of dominant negative Rab27B inhibited acid secretion, suggesting Rab27B may

play a role in stimulation-associated membrane recruitment and gastric acid secretion (71).

In the urinary system, Rab27B was found to associate with the cytoplasmic face of the fusiform vesicles and to be involved in targeting uroplakins to urothelial apical membranes umbrella cells of bladder epithelium (12). Rab27B was also required for the exocytosis of type 1 fimbriated uropathogenic Escherichia coli (UPEC) from infected bladder epithelial cells (BECs) (3, 67).

Rab27B has been found to be present in lacrimal gland acinar cells and participate in aspects of secretory vesicle formation and release, in that the overexpression of constitutively active Rab27B increased the average size and enhanced the release of secretory vesicles (15). Further in detail study showed that Rab27B enrichment to in lacrimal acinar cells occurs early in the secretory vesicles formation, when the vesicles bud from a visually discernable nascent vesicle site. These results further confirm that Rab27B is involved in the formation and maturation of secretory vesicles in lacrimal acinar cells (14).

Rab27A and Rab27B deficiencies both impaired azurophilic granule exocytosis in neutrophils with the data indicating that the two Rab27 isoforms play independent roles in neutrophil exocytosis (38). Further studies showed that Rab27A and Rab27B are both involved in the regulation of neutrophil migration in response to chemokines (64, 65).

Rab27B has also been detected in melanocytes and GFP-tagged Rab27B was shown to co-localize with melanosome marker protein; transient overexpression of the dominant negative forms of Rab27B caused diminution in both numbers and length of dendrites of melanocytes (13).Transgenic Rab27B can rescue *ashen* coat color, similar to Rab27A, and melanocytes derived from transgenic mice exhibit widespread peripheral distribution of melanosomes instead of the perinuclear clumping observed in *ashen* melanocytes. Finally, transient expression in *ashen* melanocytes of Rab27A or Rab27B, but not other Rab's, restores peripheral distribution of melanosomes, indicating that Rab27B can be functionally redundant for Rab27A (2). Consistently, up-regulation of Rab27B was detected in the melanocytes from a Griscelli syndrome type II patient, which can partially compensate for the deletion of Rab27A (77).

Rab27 isoforms in cancer cells

Rab27 isoforms have also been implicated in the biology of cancer cells. Rab27A and 27B were found to function in multivesicular endosome (MVE) docking at the plasma membrane and to control different steps of the exosome secretion pathway (58). Blockage of Rab27A resulted in decreased primary tumor growth and metastasis (4). A recent clinical study investigated the prognostic and molecular features of glioma with Rab27A expression. Rab27A was found to be significantly associated with grade progression and high mortality in all grades of glioma, suggesting Rab27a could be used as a novel biomarker with potentially important therapeutic implications (76). Finally, Rab27B was shown to regulate invasive growth and metastasis in estrogen receptor (ER)-positive breast cancer cell lines, by mediating the secretion of a key proinvasive growth regulator, heat-shock protein 90α (27). Clinical specimens also demonstrated that presence of endogenous Rab27B mRNA and protein was associated with lymph node metastasis and differentiation grade in ER-positive human breast tumors (27). This finding has been confirmed by a recent clinical study, in which the elevated expression of Rab27B in breast cancer patients was found to be closely correlated with lymph node metastasis, advanced clinical stage, ascending pathology classification and positive ER status (83). These studies broadened the role of Rab27A and Rab27B in intracellular membrane trafficking.

Table 1 Expression of Rab27 isoforms in different secretory cells.

	Rab27A	Rab27B	References
Cytotoxic T-lymphocytes	+	-	(25, 69)
Platelets	+	+	(72)
AtT20 cells (ACTH secreting cell line)	-	+	(84)
Anterior Pituitary	+	+	(84)
PC12 cells (neuroendocine cells)	+	-	(16, 20, 74)
Pancreatic beta-cells	+	-	(42, 81)
Pancreatic acinar cells	+	+	(9-11, 29)
Pancreatic ductal cells	Not reported	+	(24, 26)
Adrenal chromaffin cells	+	-	(16)
Parotid acinar cells	+	+	(35)
Lacrimal gland acinar cells	Not reported	+	(14, 15)
Gastrics parietal cells	Not reported	+	(24)
Spermatid	+	+	(5, 26)
Bone marrow mast cells	+	+	(26, 55, 66)
Neutrophils	+	+	(38, 39, 64, 65)
Osteoblastic cells	+	+	(41)
Bladder epithelial umbrella cells	Not reported	+	(12)

2. Pancreatic Information:

Both Rab27A and 27B are present in rodent pancreas, with Rab27A primarily in islets of Langerhans (42, 81) and Rab27B in acinar cells (9, 26). Rab27B was originally identified in acinar cells, by MS/MS studies of proteins on the zymogen granule (ZG) membrane (9). This identification was further confirmed by western blot, immunofluorescence and quantitative proteomic analysis (10, 11). Protease protection studies showed that Rab27B was on the external surface of the zymogen granules (10). **Figure 2** shows the localization of Rab27B in a rat pancreatic acinus and on isolated zymogen granules. Over-expression of constitutively active Rab27B enhanced CCK- induced amylase release from isolated rat pancreatic acini, while dominant negative Rab27B inhibited amylase release (9).

These results demonstrate that Rab27b is present on ZGs and plays an important role in regulating acinar exocytosis. A recent publication showed that Rab27A is present in pancreatic acinar cells and is also required for digestive enzyme secretion (29). Expression of Rab27A in purified acini was detected by RT-PCR and Western blot; Rab27A showed partial localization with the zymogen granules by immunofluorescence **(Figure 3)**. Acini from *ashen* mouse exhibited decreased amylase release. Data presented showed that Rab27A does not act through the zymogen granule exocytosis pathway, but is mainly involved in the minor-regulated secretory pathway involving endosomal-lysosomal secretion (29).

A putative Rab27B effector protein, Synaptotagmin-like protein 1 (Slp1) was found to be abundantly expressed in the zymogen granule membranes in pancreatic acinar cells by MS and

western blotting (10). Slp1 was shown to interact with Rab27B in vivo and both proteins were co-localized on zymogen granules (60). 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 (60).

Rab27B also appears to play a similar role in other exocrine glands as well. In a recent study in salivary gland, Rab27B was demonstrated to form a complex with an effector Slac2-c on the secretory granules in rat parotid acinar cells (35). Upon isoproterenol (IPR) stimulation, Rab27B translocated from secretory granules to the subapical region, and then was released into the cytosol after longer time IPR treatment (34). The similar redistribution pattern of Rab27-specific effector Slac2-c was found in rat parotid acinar cells upon IPR stimulation (31). Blockage of Rab27B by specific antibody inhibited IPR - stimulated amylase release from streptolysin O-permeabilized parotid acinar cells (35).

Approximately half of Rab27B in pancreatic acinar cells appears to be GTP-liganded (unpublished data). A recent study has identified the presence of two potential Rab27B GAPs, EPI64 and EPI64B in mouse pancreatic acinar cells and showed that overexpression of EPI64B reduced the active form of Rab27B (28).

In conclusion, although sharing high identity and similarity in amino acid sequences, protein structure and downstream effectors, Rab27A and Rab27B are not redundant in cellular function in most of the secretory cells studied. Rab27A seems to mediate lysosomal-like granule secretion, while Rab27B is mostly responsible for the large dense core vesicles release. Rab27B plays a role in exocrine gland exocytosis, including pancreas, salivary and lacrimal gland, while Rab27A plays a role in the lysosomal minor-regulated secretion pathway in pancreatic acini.

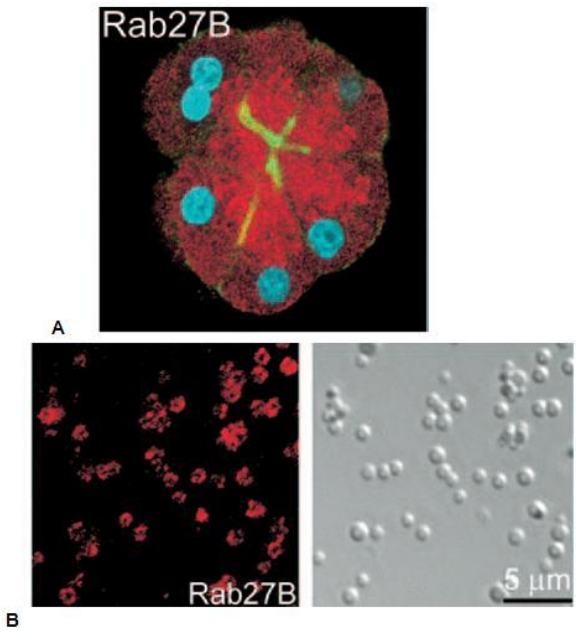

Figure 2 Immunolocalization of Rab27B in isolated pancreatic acini and zymogen granules. (A) Isolated acinus was immunostained with anti-Rab27B antibody *(red)*. The subluminal actin is stained with Oregon Green-conjugated phalloidin, and nuclei are stained with 4', 6-diamidino-2-phenylindole (DAPI) *(blue)*. (B) Purified isolated zymogen granules were immunostained with anti-Rab27B antibody *(red)*, paired with the corresponding Nomarski images. (Reproduced from Ref. (11))

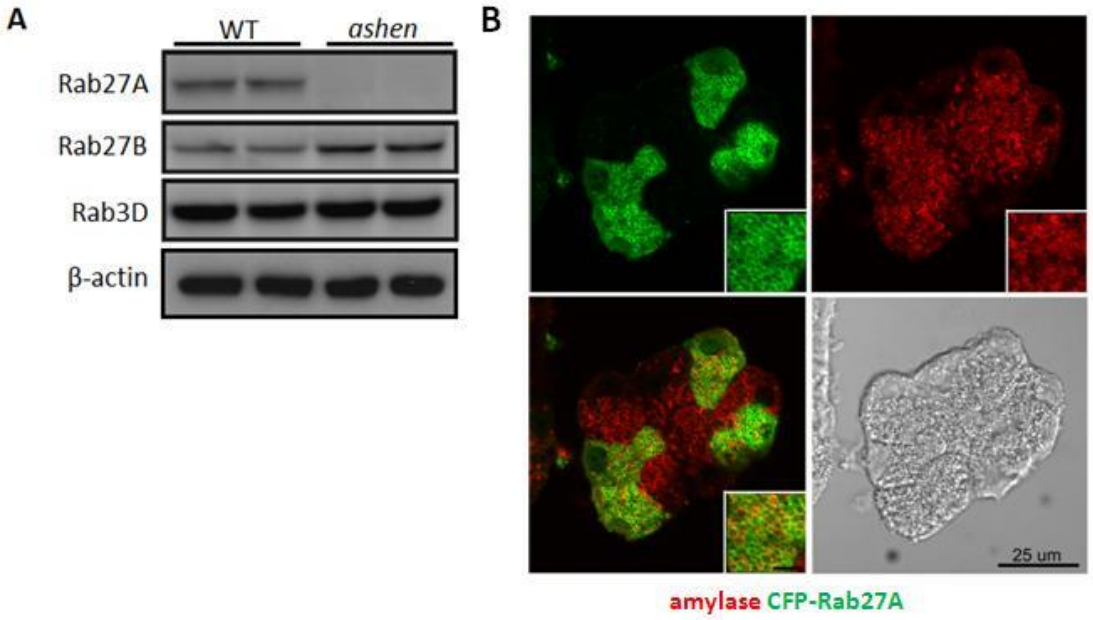

Figure 3 Expression and immunolocalization of Rab27A in isolated pancreatic acini and. (A) Total lysates of isolated pancreatic acinar cells from wild-type C3H/HeSnJ or *ashen* mice were analyzed by western blot. (B) Expression of CFP-Rab27A was mediated by adenoviral infection at a titer of 5×10^6 pfu/mL to avoid excessive expression of exogenous protein. Therefore the acinar cells were partially positive for CFP-Rab27A. Anti-GFP antibody was used to enhance the CFP-Rab27A signal and doubled stained with anti-amylase antibody. Scale bars in the insets represent 5 µm. (Reproduced from Ref. (29))

3. Tools for Study:

a. cDNA clones for human Rab27a and Rab27b in pcDNA3.1 are available from the Missoury S&T cDNA Resource (www.cdna.org). A number of investigators have published studies using constitutively active or dominant negative mutant plasmids based on mutating residues important in Ras (7, 9, 13, 47, 84).

b. Antibodies - Several antibodies against both Rab27a and 27b are available from Santa Cruz, BD and Synaptic Systems. We have had success for Western blotting and IHC using a rabbit Ab against a GST fusion protein of Rab27b (9, 11) that had been generated by Dr Tetsuro Izumi's laboratory (84). The rabbit polyclonal antibodies for Rab27A and 27B available from Synaptic Systems have worked for Western blotting, but not for IHC. We have not tested the Santa Cruz antibodies.

c. Viral vectors - Adenoviral vectors for Xpress-tagged wild type, constitutively active (Q78L) and dominant negative (N133I) Rab27b have been used in rat pancreatic acini (9). They are available from the authors with permission from Dr Tetsuro Izumi (84). The Xpress tag can be visualized for WB by use of antibody from Invitrogen.

d. Mice – Ashen mice have a naturally occurring deletion of Rab27a and are available from JAX. Mice with genetic deletion of Rab27b have been reported by two laboratories (24, 73). The Miguel Seabra group has also bred a combined ashen and Rab27b knockout mice to generate a double knockout of Rab27a

and 27b. Rab 27b KO and Rab27 double KO have impaired platelet function leading to hemorrhagic disease (73).

e. Assay for active Rab27b. GTP-bound active Rab27 isoforms preferentially bind to the synaptotagmin-like protein (Slp)-homology domain (SHD) of its specific effector, Slac2-b. By use of glutathione-Sepharose beads (Amersham Biosciences) coated with GST-SHD of Slac2-b, the active form of Rab27B will be pulled down and can then be analyzed by immunoblotting with Ab specific for Rab27B (46). We have used this assay with GST-SHD to determine active state of Rab27B in mouse acini.

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