J. Biochem. **137**, 9–16 (2005)
DOI: 10.1093/jb/mvi002

Versatile Role of Rab27 in Membrane Trafficking: Focus on the Rab27 Effector Families

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Received August 18, 2004; accepted September 3, 2004

Rab27A was the only Rab protein whose dysfunction was found to cause human immunodeficiency. Since Griscelli syndrome patients (*i.e.*, Rab27A-deficient) exhibit silvery hair color (*i.e.*, pigmentary dilution) in addition to loss of cytotoxic killing activity by cytotoxic T lymphocytes, and Rab27A protein is expressed in a wide variety of secretory cells, Rab27A (or its closely related isoform Rab27B) has been implicated in the regulation of different types of membrane trafficking, including melanosome transport and various regulated secretion events. How does Rab27 protein regulate these different types of membrane trafficking? Recent discoveries of three different families of Rab27-binding proteins (a total of eleven distinct proteins) have supplied an important clue to the answer of this question: different types of Rab27 effectors function in different cell types. In this review I describe the literature on the identification of Rab27-binding proteins (*i.e.*, the synaptotagmin-like protein (Slp) family with tandem C2 Ca^{2+}-binding motifs, the Slac2 family without any C2 motifs, and Munc13-4, a putative priming factor for exocytosis) and the current state of our understanding of the molecular mechanism of the Rab27-dependent membrane trafficking.

Key words: Griscelli syndrome, Munc13-4, Rab27 effector, Slac2, synaptotagmin-like protein.

Abbreviations: ABD, actin-binding domain; GS, Griscelli syndrome; MBD, myosin-binding domain; RPE, retinal pigment epithelium; SHD, Slp homology domain; Slac2, Slp homologue lacking C2 domains; Slp, synaptotagmin-like protein.

Structures of putative Rab27 effector families

The Rab small GTP-binding protein family is widely believed to control intracellular membrane trafficking in eukaryotic cells in concert with specific effector molecules that bind to the GTP-bound activated form of Rab (reviewed in Ref. *1*). More than 60 distinct Rab proteins have been identified in mice and humans, and these proteins appear to regulate various types (or various steps) of membrane trafficking (*1*). Rab27A/ram was the first Rab protein to be closely associated with several human diseases, including Griscelli syndrome (GS), Hermansky-Pudlak syndrome, and choroideremia (reviewed in Ref. *2*). Mutations in the *RAB27A* gene directly cause hemophagocytic syndrome (Griscelli syndrome) in humans (unless otherwise specified, "GS" refers to *RAB27A* mutations throughout the text) (*3*, *4*) and in the corresponding mouse model *ashen* (*5*). GS is a rare autosomal recessive disorder, and GS patients exhibit hypopigmentation and loss of cytotoxic killing activity by cytotoxic T lymphocytes (*3*, *6*). Although Rab27A protein has been shown to be involved in the control of melanosome transport in melanocytes (*7–9*) as well as lytic granule exocytosis in cytotoxic T lymphocytes (*10*, *11*), the molecular mechanisms by which Rab27A protein controls two different types of membrane trafficking was unclear until early 2002, when the first Rab27A/B-binding proteins were reported (*12*).

The first Rab27-binding proteins identified were members of the synaptotagmin-like protein (Slp) family (Fig. 1A) (*13*), and to date, five distinct Slp isoforms (Slp1–5) have been reported in mammals (*14–16*), a solitary isoform (Dm-Slp/Btsz) in *Drosophila* (*17*), and no homologues at all in *Caenorhabditis elegans* (Table 1). All Slp members basically share an N-terminal Slp homology domain (SHD) (*15*) and C-terminal tandem C2 domains (named the C2A domain and the C2B domain) (*14*), and the SHD and C2 domains are separated by a linker sequence of various length (Fig. 1A). Because of alternative splicing, however, some of the Slp proteins lack one of these domains (*e.g.*, Slp2-b, Slp-3-b, Slp4-b, and Btsz1-3; see Table 1). The SHD usually consists of two conserved potential α-helical regions (named SHD1 and SHD2) separated by two zinc-finger motifs (referred to as type I SHD), but the SHDs of Slp1 and Slp2-a lack such zinc-finger motifs, and the two SHDs are linked together directly (referred to as type II SHD) (*13*) (Fig. 1A). Interestingly, the SHD is also found in another protein family, whose members do not contain tandem C2 domains at the C terminus (named Slac2; Slp homologue lacking C2 domains) (Slac2-a/melanophilin, Slac2-b, and Slac2-c/MyRIP; Fig. 1C and Table 1) (*12*, *13*, *18–20*).

Biochemical characterization of the SHD of Slp and Slac2 has indicated that all SHDs (except for the Slp4-SHD) function as a specific Rab27A/B-binding domain (*12*, *16*, *19–29*). SHD1 is a central Rab27-binding site,

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Table 1. **Putative Rab27 effectors in vertebrates and invertebrates.**

Name	Alternative spliced isoforms	Rab binding specificity	GTP/GDP-dependency	Tissue distribution and subcellular localization	Putative functions
Slp1/JFC1		Rab27A/B	GTP	ND[a]	PIP_3- and NADPH oxidase–binding protein (*63*).
Slp2	Slp2-a/b/c/d, +SI–SIII	Rab27A/B	GTP	Melanosomes in melanocytes (*12*, *64*).	Melanosome-associated protein (*12*, *64*).
Slp3	Slp3-a/b	Rab27A/B	GTP	ND	Ca^{2+}/phospholipid binding protein that promotes regulated exocytosis in PC12 cells (*27*, *29*).
Slp4/ granuphilin	Slp4-a/b	Rab27A/B, Rab3A/B/C/D, Rab8A/B	GTP/GDP	Dense-core vesicles in endocrine cells (PC12, AtT20, pancreatic β-cells) (*25*, *27*, *59*). Secretory granules in parotid acinar cells (*55*).	Inhibition of dense-core vesicle exocytosis, possibly through interaction with GDP-Rab27A in PC12 cells (*29*). Munc18-1- (*29*, *59*) and syntaxin 1A–binding protein (*57*, *58*).
Slp5	+SI	Rab27A/B	GTP	Pancreatic β-cells (*49*).	Ca^{2+}/phospholipid binding protein that promotes regulated exocytosis in PC12 cells (*29*).
Dm-Slp/Btsz	Btsz1-3	Dm-Rab27	GTP[b]	Apical plasma membrane localization of Dm-Slp/Btsz mRNA in polarized epithelial cells (*17*).	Dm-Slp/Btsz mutants develop slowly due to a reduced cell size and number (*17*).
rabphilin		Rab27A/B, Rab3A/B/C/D, Rab8A	GTP	Synaptic vesicles in neurons (*30*). Dense-core vesicles in endocrine cells.	Modulation of secretory vesicle exocytosis through interaction with both Rab3A and Rab27A (*35*).
Dm/ Ce-rabphilin		Dm/Ce-Rab27	GTP[b]	Synaptic vesicles in neurons (*37*).	Rabphilin functions independently of Rab3 and potentiates SNARE function (*37*).
Slac2-a/ melanophilin		Rab27A/B	GTP	Melanosomes in melanocytes (*21–23*).	Linker protein between Rab27A and myosin Va that regulates melanosome transport (*21–24*, *43*, *44*). *leaden* gene or *GS3* gene product (*4*, *18*).
Slac2-b		Rab27A/B	GTP	ND	Unknown function (*12*).
Slac2-c/MyRIP		Rab27A/B	GTP	Retinal melanosomes in RPE cells. Dense-core vesicles in endocrine cells (PC12, chromaffin, pancreatic β-cells) (*49*, *50*). Secretory granules in parotid acinar cells (*55*).	Linker protein between Rab27A and myosin VIIa (*19*, *20*). Modulation of secretory vesicle exocytosis in endocrine cells (*49*, *50*). Modulation of secretory vesicle exocytosis in exocrine cells through interaction with actin filaments (*55*).
Noc2		Rab27A/B, Rab3A/B/C/D, Rab8A	GTP	Dense-core vesicles in endocrine cells (PC12, pancreatic β-cells) (32–34). Secretory granules in exocrine cells (*61*).	Modulation of dense-core vesicle exocytosis through interaction with Rab27A rather than Rab3A (*33*, *34*). Accumulation of secretory granules with increased size and irregular shape in some exocrine tissues in Noc2 null mutant mice (*61*).
Munc13-4		Rab27A/B	GTP	Cytotoxic T lymphocytes (*62*), platelets (*38*). Cytosolic and membrane-bound.	A putative priming factor that promotes dense granule secretion from platelets (*38*). Human familial hemophagocytic lymphohistiocytosis (FHL3) gene product (*62*).

[a]ND, not determined; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; and SNARE, soluble NSF (*N*-ethylmaleimide–sensitive factor) attachment protein receptor. [b]M. Fukuda, unpublished observations.

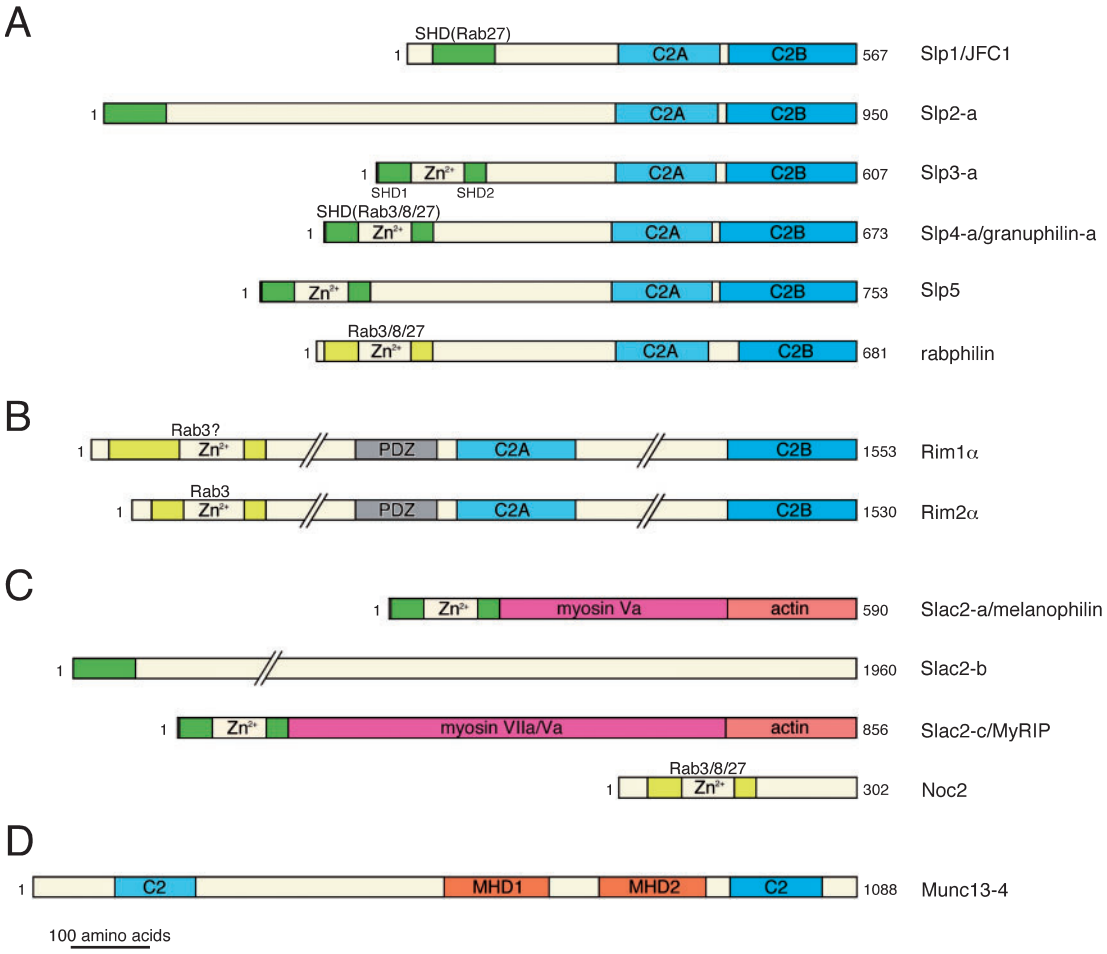

Fig. 1. **Structure of putative Rab27 effector proteins of mice and rats.** (A) Schematic representation of mouse Rab27-binding proteins having an N-terminal SHD (green boxes) and C-terminal tandem C2 domains (blue boxes) (mouse Slp1/JFC1, Slp2-a, Slp3-a, Slp4-a/granuphilin-a, Slp5, and rabphilin). Both Slp4 and rabphilin interact with three Rab families (Rab3/8/27) *in vitro* (*12*, *25*, *27*, *28*, *34*), whereas the others specifically bind Rab27A/B (*12*, *28*). (B) Schematic representation of mouse Rim1α and Rim2α, putative Rab3 effectors. Although Rim contains a Rab-binding domain similar to rabphilin, Rim-RBDs do not bind Rab27 (*28*, *36*). (C) Schematic representation of mouse Rab27-binding proteins that do not contain C-terminal tandem C2 domains (mouse Slac2-a/melanophilin, Slac2-b, Slac2-c/MyRIP, and Noc2). Slac2-a and Slac2-c contain a myosin-binding domain in the middle region (magenta boxes) and an actin-binding domain at the C terminus (red boxes) (*19*, *21*, *43*). (D) Schematic representation of rat Munc13-4. Amino acid numbers are given on both sides.

and SHD2 and the zinc finger motifs are required for higher affinity Rab27 binding and stability of the SHD structure (*26*, *29*). Slp4 exceptionally binds eight Rabs (Rab3A/B/C/D, Rab8A/B, and Rab27A/B) *in vitro*, but it preferentially interacts with Rab27 isoforms in living cells (*25*, *27*, *29*). Another unexpected and surprising finding was that rabphilin (*30*) and Noc2 (*31*, *32*), previously characterized as Rab3 effectors, function as Rab27-binding proteins both *in vitro* and in living cells (*12*, *28*, *33*–*35*) (Fig. 1, A and C), although other putative Rab3 effectors, Rim1α and Rim2α, do not bind Rab27 (Fig. 1B) (*28*, *36*). Since rabphilin and Noc2 bind Rab27A with more than 3 times and more than 10 times, respectively, higher affinity than they bind Rab3A (*34*, *35*), they are both likely to function as a Rab27 effector under physiological conditions (Table 1). Actually, rabphilin and Noc2 mutants lacking Rab3A binding activity are still targeted to dense-core vesicles and modulate their exocytosis, whereas rabphilin and Noc2 mutants lacking Rab27A binding activity are present in the cytoplasm and have no effect on dense-core vesicle exocytosis (*33*, *34*). It should be noted that the rabphilin·Rab27 interaction, but not the rabphilin·Rab3 interaction, has been retained during evolution (*34*, *35*, *37*): *C. elegans* and *Drosophila* rabphilin specifically interact with Rab27, but not with Rab3 or Rab8.

The third Rab27-binding protein is Munc13-4, a putative priming factor for exocytosis that was purified from the platelet cytosolic fraction with GTP-Rab27A–immobilized beads (*38*). Mun13-4 is a member of the Munc13 family, and all members consist of two separate C2 domains and two Munc13 homology domains (named MHD1 and MHD2) (Fig. 1D). Unlike other members, however, Munc13-4 lacks an N-terminal phorbol ester–binding C1 domain. Since Munc13-4 does not contain an SHD, Munc13-4 must contain a novel Rab27-binding domain, although nothing is known about the Rab27-binding site in Munc13-4 as yet. Since the fundamental

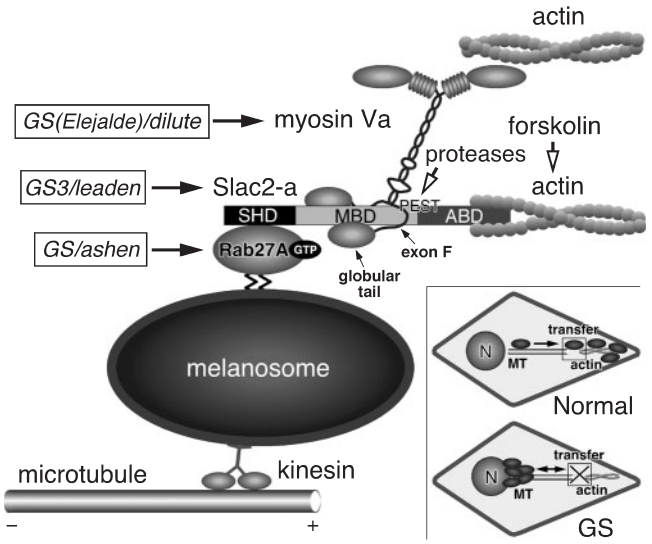

Fig. 2. **Role of the tripartite protein complex (Rab27A·Slac2-a·myosin Va) in melanosome transfer from microtubules to actin filaments.** After microtubule-dependent movement driven by the kinesin motor(s), the melanosome is transferred from microtubules to actin filaments by the tripartite protein complex formed by Rab27A, Slac2-a, and myosin Va (*21–24*, *43*). Slac2-a is first recruited to melanosomes through direct interaction of the SHD with Rab27A on melanosomes, and then myosin Va is recruited to the Rab27A·Slac2-a complex through direct interaction with the middle domain of Slac2-a (MBD). Slac2-a binds myosin Va *via* two distinct domains (exon F and globular tail) (*44*). The C-terminal actin-binding domain (ABD) is also essential for the melanosome transfer step (*43*), and forskolin rapidly stimulates the Slac2-a·actin interaction (*45*). Loss of one of the components results in GS or the coat color phenotype. The inset shows normal (top: "peripheral melanosome distribution") and GS-derived melanocytes (bottom: "perinuclear aggregation of melanosomes"). GS with the *MYO5A* mutation is also called Elejalde syndrome, which is characterized by hypopigmentation and a neurological disorder (without immunodeficiency). "PEST" between MBD and ABD represents potential signals for rapid protein degradation (*46*). N, nucleus; and MT, microtubule.

structures of Munc13s are quite similar, it would be interesting to determine whether other members of the Munc13 family also interact with Rab27. A future study will soon answer this question.

Role of Rab27A effectors in melanosome transport in melanocytes

Melanosomes are melanin-containing organelles that belong to a group of lysosome-related organelles and are responsible for the protection of our bodies from ultraviolet radiation. Melanosomes are produced around the nucleus of melanocytes, and mature melanosomes are transported from their cell body to the tips of their dendrites by two sequential motors, a microtubule-dependent motor (bidirectional movements) and an actin-dependent motor (unidirectional movement) (Fig. 2, inset and reviewed in Ref. *39*). The process of melanosome transfer from the microtubules to the actin filaments is a crucial step in melanosome transport in melanocytes, because defects in this step are responsible for the "silvery hair" in GS patients and the corresponding coat-color of the mutant mouse *ashen* (*2*, *3*, *5*) (compare the normal melanocyte with "peripheral melanosomes" and the GS melanocyte with "aggregated melanosomes" in the inset in Fig. 2). Discovery of the tripartite protein complex formed by Rab27A (*GS/ashen* gene product), Slac2-a/melanophilin (*GS3/leaden* gene product), and myosin Va (*GS(Elejalde)/dilute* gene product) in 2002 revealed the molecular mechanism of the melanosome transfer from the microtubules to the actin filaments in melanocytes (*4*, *21–24*, *40–44*). As summarized in Fig. 2, Rab27A is present on mature melanosomes via the C-terminal geranylgeranylation motifs. The Rab27A effector Slac2-a is first recruited to the GTP-bound form of Rab27A on the melanosome via the N-terminal SHD. The actin-based motor protein myosin Va is then recruited to the Rab27A·Slac2-a complex *via* direct interaction of the tail domain with the middle region of Slac2-a (MBD, myosin Va-binding domain). Myosin Va interacts with Slac2-a *via* two distinct sites: melanocyte-specific exon F and the globular tail (*44*). The resulting tripartite protein complex mediates melanosome transfer from the microtubules to the actin filaments. Interaction of actin with the C-terminal actin-binding domain (ABD) of Slac2-a is also required for the melanosome transfer step (*20*, *43*), and this interaction is promoted by cAMP, which induces rapid accumulation of the melanosomes at the ends of the dendrites of melanocytes. (*45*). Loss of any one of the components of the tripartite protein complex causes the same silvery hair phenotype because of inefficient melanosome transfer between the two motors (*3–5*). Several missense mutations and a deletion have been found in each component in GS patients and coat-color mutant mice. As examples, a W73G mutation of Rab27A in GS patients abrogates interaction with Slac2-a (*41*, *42*), and a R35W mutation of Slac2-a in GS3 patients and seven amino acid deletion (REEERLQ in the SHD1) of Slac2-a in *leaden* mice abrogate interaction with Rab27A (*4*, *18*, *26*). Missense mutations (I1510N, M1513K, and D1519G) in the globular tail of myosin Va in *dilute* mice partially impair Slac2-a binding activity (*44*). Expression of a mutant Slac2-a lacking one of the ligand binding activities (Rab27A, myosin Va, or actin) in wild-type melanocytes induces perinuclear aggregation of melanosomes, possibly by a dominant negative effect (*43*).

Although the importance of the tripartite protein complex in melanosome transport is well documented, very little is known about the disassembly of the complex after actin-based melanosome transport (or the transfer of melanosomes to actin filaments). Phosphorylation of Slac2-a may be involved in the detachment of myosin Va from melanosomes, but the phosphorylation of the myosin Va globular tail (Ser-1650) has no effect on interaction with Slac2-a (*44*). Alternatively, certain GTPase-activating proteins specific for Rab27A may be involved in this process through conversion of GTP-Rab27A to GDP-Rab27A, which induces dissociation of Slac2-a from Rab27A. Slac2-a has very recently been shown to contain multiple PEST-like sequences (potential signals for rapid protein degradation) at the C terminus, and endogenous Slac2-a molecules, but not Rab27A or myosin Va, in melanocytes selectively degraded by Ca^{2+}-dependent calpains (*46*). A mutant Slac2-a protein lacking one of the PEST-like sequences (ΔPEST) is expressed more stably than the wild-type protein in melanocytes and often accu-

mulates on the peripheral actin filaments. Excess amounts of Slac2-a on actin filaments seem to inhibit melanosome transfer from microtubules to actin filaments, possibly by promoting excess actin bundles (*46*). These observations strongly suggest that degradation of Slac2-a is important for normal melanosome distribution, the same as has been shown for the regulated degradation of yeast Vac17p (a vacuole-specific Myo2p, class V myosin, receptor) in vacuole transport (*47*). Further investigation is needed to determine whether the regulated degradation machinery for the Slac2-a molecule is present in melanocytes.

Rab27A and myosin VIIa are also involved in retinal melanosome transport in mammalian retinal pigment epithelium (RPE) cells, because abnormal melanosome distribution is observed in *ashen* and *shaker-1* mice (mutation in the *myo7a* gene) (*48*). In contrast to skin melanocytes, however, it has been suggested that RPE cells use a different Rab27A effector, Slac2-c (also identified as MyRIP, myosin VIIa- and Rab-interacting protein), for retinal melanosome transport (*19*). Slac2-c is a homologue of Slac2-a (Fig. 1C), and both proteins share the same domain structures (SHD, MBD, and ABD) and simultaneously link Rab27A and myosin *in vitro* (*19*, *20*). Although Slac2-c binds both myosin Va and VIIa *in vitro*, it preferentially binds myosin VIIa under physiological conditions (*48–50*). Further work is necessary to determine whether the Rab27A·Slac2-c·myosin VIIa complex actually regulates retinal melanosome transport.

Role of Rab27 effectors in regulated exocytosis in secretory cells

Although Rab27A was initially proposed to be involved in the control of the maturation or secretion of lysosome-related organelles, such as the lytic granules in cytotoxic T lymphocytes, dense granules in platelets, and Weibel-Palade bodies in endothelial cells (*5*, *10*, *11*, *51–53*), both Rab27A and Rab27B proteins are now known to be expressed in various types of secretory cells that exhibit regulated secretion (*25*, *27*, *54–58*). Unlike melanosome transport in melanocytes, however, different Rab27 effectors other than Slac2-a have been found in secretory cells. For example, two Rab27 effectors, Slp4-a/granuphilin-a and Slac2-c, have been found to be present on dense-core vesicles in some endocrine cells (*e.g.*, pancreatic β-cells, chromaffin cells, and PC12 cells) (*25*, *27*, *49*, *50*, *59*) and amylase-containing granules in parotid acinar cells (*55*), suggesting that these endocrine and exocrine cells use the same Rab27·effector complexes for regulated secretion. Overexpression of Slp4-a in endocrine cells strongly attenuates dense-core vesicle exocytosis (*25*, *27*, *29*, *59*), whereas other members of the Slp family are not inhibitory and instead promote dense-core vesicle exocytosis when expressed in PC12 cells (*27*, *29*). Slp4-a also exhibits several unique biochemical properties not found in other Slp members. First, Slp4-a binds Rab3 and Rab8, in addition to Rab27, both *in vitro* and in cultured cells (*12*, *27–29*, *59*), although the Rab27 binding affinity of Slp4-a is much higher than the Rab3 or Rab8 binding affinity (Table 1). Second, Slp4-a interacts with both Rab27A(Q78L) (constitutive active form that mimics the GTP-bound form of Rab27A) and Rab27A(T23N) (constitutive negative form that mimics the GDP-bound form of Rab27A), whereas other Slps specifically recognize Rab27A(Q78L) (Table 1) (*29*). The latter property seems to be directly related to the inhibition of dense-core vesicle exocytosis by Slp4-a, because a mutant Slp4-a lacking Rab27A(T23N) binding activity fails to inhibit dense-core vesicle exocytosis in PC12 cells (*29*). Interaction of Slp4-a with syntaxin 1A (not with syntaxin 3) has been proposed to promote docking of insulin-containing granules to the plasma membrane of pancreatic β-cells (*58*) (but see Refs. *29* and *59*; Slp4-a does not directly interact with syntaxin 1A). However, this mechanism is unlikely to be used in amylase secretion from exocrine parotid acinar cells, because neither syntaxin 1A nor 1B are expressed in these cells (*60*). Slp4-a also interacts with Munc18-1, a syntaxin 1A-binding protein, via the C-terminal domain (*29*, *59*), although the physiological significance of this interaction is currently unknown.

Slac2-c, originally described as a linker protein between myosin Va/VIIa and Rab27A (*19*, *20*), is also involved in the control of dense-core vesicle exocytosis in some endocrine cells (*49*, *50*) and of exocytosis of amylase-containing granules in parotid acinar cells (*55*) through interaction with Rab27A/B. Unlike RPE cells, however, Slac2-c does not interact with myosin Va/VIIa in endocrine cells (*49*, *50*) or in parotid acinar cells (*55*). Slac2-c promotes secretory granule exocytosis, possibly by tethering secretory granules with peripheral actin filaments via the C-terminal actin-binding domain (*20*, *49*, *55*). Knockdown of Slac2-c by RNA interference in pancreatic β-cells (*49*) or introduction of functionally blocking antibodies against Slac2-c or Slac2-c-ABD fragment into parotid acinar cells causes a reduction of regulated secretion (*55*) (but see Ref. *50*; overexpression of Slac2-c attenuates dense-core vesicle exocytosis). Further study is necessary to determine which exocytotic step(s) (transport, docking, priming, or fusion of secretory vesicles) Slac2-c regulates.

Another Rab27-binding protein, Noc2, which consists of an N-terminal Rab-binding domain alone (Fig. 1C), is also required for the control of regulated granule exocytosis in endocrine and exocrine cells (*33*, *34*, *61*). Interestingly, the exocrine cells of Noc2 null mutant mice also exhibit defects in granule transport and morphology that result in the complete loss of amylase release from pancreatic acinar cells (*61*). The mechanism(s) by which Noc2 controls both regulated secretion and granule morphology is the next issue that needs to be clarified.

Rab27A/B are also thought to be involved in the maturation and/or secretion of secretory lysosomes in some cell types [*e.g.*, cytotoxic T lymphocytes (*10*, *11*), platelets (*5*, *38*, *51*, *53*), and endothelial cells (*52*)], but very little is known about the function of Rab27 effectors in these cells. The only Rab27A/B-binding protein that has been reported to be present on secretory lysosomes is Munc13-4, which promotes dense granule secretion from platelets (*38*). Munc13-4 is presumably involved in the priming step (or downstream of the priming step before the actual fusion step) of dense granules in platelets or lytic granules in cytotoxic T lymphocytes, because lytic granules are not exocytosed in Munc13-4–deficient patients with human familial hemophagocytic lymphohistiocytosis (FHL3), even though lytic granules are attached to the plasma membrane at the immunological synapses (*62*).

By contrast, however, lytic granules are not docked to the plasma membrane at the immunological synapses in *ashen* (Rab27A-deficient)-mouse–derived cytotoxic T lymphocytes (*10*, *11*), suggesting that an additional linker protein (presumably a member of another family of Rab27A-binding protein) is required for the docking of lytic granules to the plasma membrane.

Perspectives

Based on the symptoms of human Griscelli syndrome patients (and *ashen* mice), Rab27A was previously thought to be a specific Rab that controls the maturation or transport of lysosome-related organelles, such as melanosome transport in melanocytes and lytic granule exocytosis in cytotoxic T lymphocytes. Recent discoveries, however, clearly indicate that Rab27A and its closely related isoform Rab27B are also present on non-lysosome-related organelles (specifically on various types of secretory granules that undergo regulated exocytosis) (*25*, *27*, *54*, *55*) and are likely to control a wider variety of membrane trafficking than previously thought. One of the important goals of research is identification of the Rab27·effector complex that is involved in the specific membrane trafficking (or maturation of organelles) driven by Rab27 proteins. Elucidation of the detailed tissue distribution and subcellular localization of eleven Rab27-binding proteins is required to address this issue. Another important goal of research is to determine the hierarchy of Rab27 effectors, because several Rab27-binding proteins are often expressed in the same cell type [*e.g.*, Slac2-a and Slp2-a in melanocytes (*12*, *21–23*) or Slp4-a, Slac2-c, and Noc2 in endocrine cells (*25*, *27*, *33*, *34*, *49*, *50*)]. Future functional studies will determine whether Rab27 effectors sequentially, synergistically, or redundantly function in Rab27-dependent membrane trafficking (or maturation of organelles) in the same cell type.

Note Added in Proof: After the acceptance of this paper the Rab27A effector function of Slp2-a in melanocytes was reported (see Ref. *64* for details).

I would like to thank members of the Fukuda Initiative Research Unit in RIKEN for preparing the manuscripts and for helpful discussions. This work was supported in part by grants from the Ministry of Education, Culture, Sports, and Technology of Japan (15689006 and 16044248 to M.F.).

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