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REVIEW

The Roles of Rab27 and Its Effectors in the Regulated Secretory Pathways

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ABSTRACT. **Regulated secretory pathways are highly developed in multicellular organisms as a means of intercellular communication. Each of these pathways harbors unique store organelles, such as granules in endocrine and exocrine tissues and melanosomes in melanocytes. It has recently been shown that the monomeric GTPase Rab27 subfamily regulates the exocytosis of these cell-specific store organelles. Furthermore, genetic alterations of Rab27a cause Griscelli syndrome in humans that manifests as pigmentary dilution of the skin and the hair and variable immunodeficiency due to defects in the transport of melanosomes in melanocytes and lytic granules in cytotoxic T-lymphocytes. Rab27 acts through organelle-specific effector proteins, such as granuphilin in pancreatic beta cells and melanophilin in melanocytes. The Rab27 and effector complex then interacts with proteins that are essential for membrane transport and fusion, such as syntaxin 1a and Munc18-1 for granuphilin and myosin Va for melanophilin. Genome information suggests that other putative Rab27 effector proteins, tentatively termed as exophilins or Slp/Slac2, are predicted to exist because these proteins share the conserved N-terminal Rab27-binding domain and show Rab27-binding activity *in vitro* or when overexpressed in cell lines. These findings suggest that the Rab27 subfamily regulates various exocytotic pathways using multiple organelle-specific effector proteins.**

Key words: regulated secretion/Rab27/granuphilin/melanophilin/secretory granule/lysosome-related organelle

1. Regulated secretory pathways

Secretion of bioactive substances is a means of intercellular communication by which multicellular organisms coordinate the activities of their constituent cells and thereby function as an integrated unit. The secretory pathways can be divided into two types: constitutive and regulated secretion (Burgess and Kelly, 1987). In the constitutive secretory pathway, proteins are continuously secreted depending on the amount synthesized. The transcription and translation steps are the limiting steps in this type of pathway, and these take at least several hours. By contrast, in the regulated secretory pathway, synthesized products are first stored in organelles and released only when cells are stimulated by an appropriate extracellular secretagogue. The secretagogue in turn changes the level of an intracellular second messenger, such as Ca^{2+}. Differentiation of store organelles in this pathway enables rapid and quantitative intercellular communications. In neuronal cells, synaptic vesicles mediate membrane fusion in periods as short as 60 μs after Ca^{2+} entry (Sabatini and Regehr, 1996), and undergo extensive exocytic-endocytic recycling. Granules in endocrine and exocrine cells, however, show longer latencies (5–100 ms) between Ca^{2+} entry and membrane fusion (Chow *et al.*, 1992), but secrete a larger amount of hormones or enzymes that will be diluted out in circulation or digestive tracts. Other lysosome-related organelles, such as melanosomes containing the pigment melanin, are translocated and secreted by poorly characterized mechanisms; three modes of melanosome transfer from melanocytes to adjacent keratinocytes have thus far been proposed (Marks and Seabra, 2001). Thus, each store organelle is exocytosed at a distinct speed and kinetics in accordance with the biological function of the secretory products. Although the basic machinery for vesicle transport is believed to be conserved from yeast to humans and in all intracellular pathways, the regulated secretory pathways should be endowed with additional components. These include a constraint that prevents spon-

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Abbreviations: SM, Sec1/Munc18; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor.

taneous secretion in the absence of a secretagogue, a sensor that detects the arrival of secretagogue-dependent signals such as an increase in intracellular Ca^{2+}, and a modifier that amplifies or reduces the efficiency of exocytotic events. In fact, biochemical studies have identified numbers of proteins that associate with the store organelle and release site, and that finely tune each pathway, as in the case of synaptic vesicles (Südhof, 1995) and presynaptic active zones (Garner *et al.*, 2000). Genetic approaches have also uncovered the genes whose mutations specifically affect the transport of melanosomes and other related organelles in several monogenic diseases (Marks and Seabra, 2001).

The present study reviewed recent progress in the understanding of the roles of Rab27 and its effector proteins in the regulated secretory pathways. Rab proteins are monomeric GTPases of the Ras superfamily and regulate various transport pathways of intracellular vesicles. Many Rab proteins have been described to show specific patterns of subcellular localization and tissue distribution. The number of RAB genes is expanded to 60 in humans compared with 11 corresponding Ypt genes in budding yeast *S. cerevisiae* (Bock *et al.*, 2001). Robust expansion of Rab proteins in higher eukaryotes reflects the increased complexity and variability of intracellular compartments, particularly the uniquely differentiated store organelles in the regulated secretory pathways. Until recently, however, Rab3 was the only Rab subfamily to be investigated in the regulated secretory pathways, although its exact role remains enigmatic (Jahn and Südhof, 1999) (see later discussion). The Rab27 subfamily, which consists of Rab27a and b, has recently been studied extensively, and despite its relatively short history of investigation, it has been demonstrated to function in several regulated secretory pathways.

2. Rab27 and lysosome-related organelles

The epoch-making study of Rab27 was the result of a discovery that mutations of the *RAB27A* gene cause Griscelli syndrome in humans (Ménasché *et al.*, 2000). Rab27 is the first example of a Rab specifically implicated in a human genetic disease (Table I). Griscelli syndrome, a rare autosomal recessive disorder, results in pigmentary dilution of the skin and the hair and variable immunodeficiency. It is characterized by the presence of large clumps of pigment in hair shafts and the accumulation of mature melanosomes in melanocytes with reduced pigmentation of adjacent keratinocytes, and is accompanied by an uncontrolled activity of the T-lymphocytes and macrophages triggered by infections known as haemophagocytotic syndrome (Kleine *et al.*, 1994). A mutation of the mouse ortholog *Rab27a* is responsible for a lightened coat color in *ashen* mice (Wilson *et al.*, 2000). Subsequent analyses demonstrated that Rab27a is

Table I. PHENOTYPES DUE TO RAB MUTATION AND DELETION *IN VIVO*

Rab	Expression	Human disease *Mouse mutant*	Phenotypes	Function	References
Rab3a	Nerve cells Endocrine cells	Rab3a knockout	More exocytotic events, an enhanced rundown, and lack of LTP (hippocampal mossy fibre synapses) Decreased secretagogue-induced insulin release	Inhibition of fusion?	Geppert *et al.*, 1994, 1997 Castillo *et al.*, 1997 Lonart *et al.*, 1998 Kapfhamer *et al.*, 2002 Yaekura *et al.*, 2003
		earlybird (Rab3a D77G)	Shortened circadian period of locomotor activity	Circadian period and sleep homeostasis	
Rab3d	Mast cells Exocrine cells Adipocytes	Rab3d knockout	Increased size of granules Normal secretion	Granule maturation (preventing homotypic fusion?)	Riedel *et al.*, 2002
Rab7	Ubiquitous	**Charcot-Marie-Tooth type 2B (CMT2B) neuropathy**	Ulceromutilating neuropathies (muscle weakness, foot ulcers and infection)	Transport between late endosomes and lysosomes (late endocytotic transport)	Verhoeven *et al.*, 2003
Rab23	Nerve cells	*open brain* (*opb*)	Embryonic lethal Open neural tubes (head and spinal cord)	Neural patterning (translocation of vesicles to the plasma membrane?)	Eggenschwiler *et al.*, 2001
Rab27a	Endocrine cells Melanocytes Hemopoietic cells	**Griscelli syndrome (type 1)** *ashen* (*ash*)	Partial albinism Haemophagocytic syndrome	Movement of melanosomes Cytotoxic T lymphocyte granule release	Ménasché *et al.*, 2000 Wilson *et al.*, 2000
Rab38	Melanocytes	*chocolate* (*cht*)	Partial albinism	Sorting of TYRP1 (tyrosinase -related protein)	Loftus *et al.*, 2002

In vivo function of Rab proteins manifested in human disease or revealed by the phenotypes of natural mutant or gene-targeted mice. Bold, name of human disease; Italics, name of natural mutant mouse.

colocalized with melanosomes in melanocytes (Bahadoran *et al.*, 2001; Hume *et al.*, 2001) and lytic granules in cytotoxic T-lymphocytes (Haddad *et al.*, 2001). In melanocytes of Griscelli patients and *ashen* mice, melanosomes are clumped in perinuclear regions, and reexpression of wild-type Rab27a protein restores melanosome transport to dendrite tips (Bahadoran *et al.*, 2001; Hume *et al.*, 2001; Wilson *et al.*, 2000). Rab27a-deficient T-lymphocytes exhibit drastically reduced cytotoxicity and lytic granule exocytosis (Haddad *et al.*, 2001; Ménasché *et al.*, 2000; Stinchcombe *et al.*, 2001). Consistently, *ashen* lytic granules do not reach the plasma membrane at the immunological synapse, although they are polarized to the target cell interface (Stinchcombe *et al.*, 2001). While the presence of a bleeding tendency in *ashen* mice is controversial, the defect in platelet dense granules is either dependent on genetic background (Novak *et al.*, 2002; Wilson *et al.*, 2000) or compensated for by coexisting Rab27b (Barral *et al.*, 2002). These findings indicate that Rab27a regulates the exocytosis of organelles generically named as secretory lysosomes (Blott and Griffiths, 2002) or lysosome-related organelles (Dell'Angelica *et al.*, 2000). Melanosomes in melanocytes, lytic granules in T-lymphocytes, and platelet dense granules share mixed characteristics of lysosomes and secretory granules. They harbor lysosomal marker proteins, although they specifically perform exocytotic functions unrelated to degradation.

3. Rab27 and secretory granules

A completely different and independent approach revealed that the Rab27 subfamily also regulates the exocytosis of classical secretory granules. In 1999, a novel gene was discovered that is specifically expressed in pancreatic beta cells and pituitary tissue (Wang *et al.*, 1999). The domain structure of the protein product named granuphilin is similar to that of rabphilin3, a Rab3 effector protein (Shirataki *et al.*, 1993), although the overall identity of the primary sequences only amounts to 22%. It was subsequently shown that granuphilin physiologically interacts with Rab27a in pancreatic beta cells, although it also has an affinity to Rab3a *in vitro* (Yi *et al.*, 2002). Rab27a is localized on the membrane of insulin granules, and its overexpression enhances high K^+-stimulated insulin secretion. Furthermore, Rab27b is also expressed in pituitary endocrine cells including corticotrophs and intermediate lobe cells, and the expression of the inactive mutant of Rab27b inhibits ACTH secretion from AtT20 cells (Zhao *et al.*, 2002). These findings suggest that Rab27a and Rab27b have positive roles in the exocytosis of endocrine secretory granules, although, in contrast to the case of lysosome-related organelles, genetic evidence is lacking. In any case, we recently found that the exocytosis of endocrine granules is actually affected in *ashen* mice (our unpublished observations).

4. Multiple putative Rab27 effectors

A search of the genome using granuphilin as a prototype indicated that similar proteins probably exist. These proteins are tentatively termed as exophilins (exocytosis-associated rabphilin3/granuphilin-like proteins) (Nagashima *et al.*, 2002) or Slp (synaptotagmin-like protein)/Slac2 (Slp homologus lacking C2 domains) (Fukuda and Mikoshiba, 2001; Kuroda *et al.*, 2002a) (Fig. 1). They share highly conserved amino acid sequences at the N-termini (Fig. 2), including the SGAWFF motif that in rabphilin3 may play a critical role in binding to Rab3a-GTP (Ostermeier and Brunger, 1999). Although these putative Rab-binding regions are often inserted by a zinc finger domain harboring a series of conserved cysteine residues, this domain is missing in exophilins 4, 5, and 7, and is shown to be dispensable for Rab binding in some exophilins (Fukuda, 2002; Nagashima *et al.*, 2002; our unpublished observations), which suggests that it may have other roles. The zinc finger region may confer higher affinity to Rab, similar to that of rabphilin3 to Rab3a as seen in surface plasmon resonance and pull-down experiments (Wang *et al.*, 2001).

In addition to granuphilin, another protein termed melanophilin (exophilin3, Slac2-a) has been found to function as a Rab27a effector. The gene encoding melanophilin is mutated in *leaden* coat-color mutant mice (Matesic *et al.*, 2001) that are genetically correlated with *ashen* mice. Although all exophilins can form a complex with Rab27a or Rab27b *in vitro* or when overexpressed in non-secretory cells (Kuroda *et al.*, 2002a; our unpublished observations), it should be noted that the existence of endogenous complexes has not been demonstrated in most cases. Furthermore, some of these proteins have been shown to have affinities to other Rab proteins (Kuroda *et al.*, 2002a; Yi *et al.*, 2002). Thus, it remains to be clarified whether they physiologically represent Rab27 effectors, except for melanophilin and granuphilin whose physiological interactions with Rab27a have been demonstrated (see below).

In contrast to the N-termini, the C-termini of these proteins bear unique sequences (Fig. 1). Several have two tandem C2-domains at the C-termini. This feature is consistent with their roles in membrane traffic, because the C2-domain in general has affinities to phospholipids in either a Ca^{2+}-dependent or a Ca^{2+}-independent manner (Rizo and Südhof, 1998). Almost all of the C2 domains of exophilins, however, partly lack the aspartate, glutamate, or serine residues that are conserved and involved in Ca^{2+} binding in synaptotagmins and rabphilin3 (Rizo and Südhof, 1998), placing in doubt whether they actually bind Ca^{2+}. Exophilins 3, 5, and 8 lack C2 domains, and exophilins 3 and 8 have been shown to interact with unconventional myosin Va and VIIa, respectively, through the predicted coiled-coil C-terminal domains (see below). The existence of multiple putative effector proteins suggests that the roles of Rab27 vary in different cells, depending on the effector protein

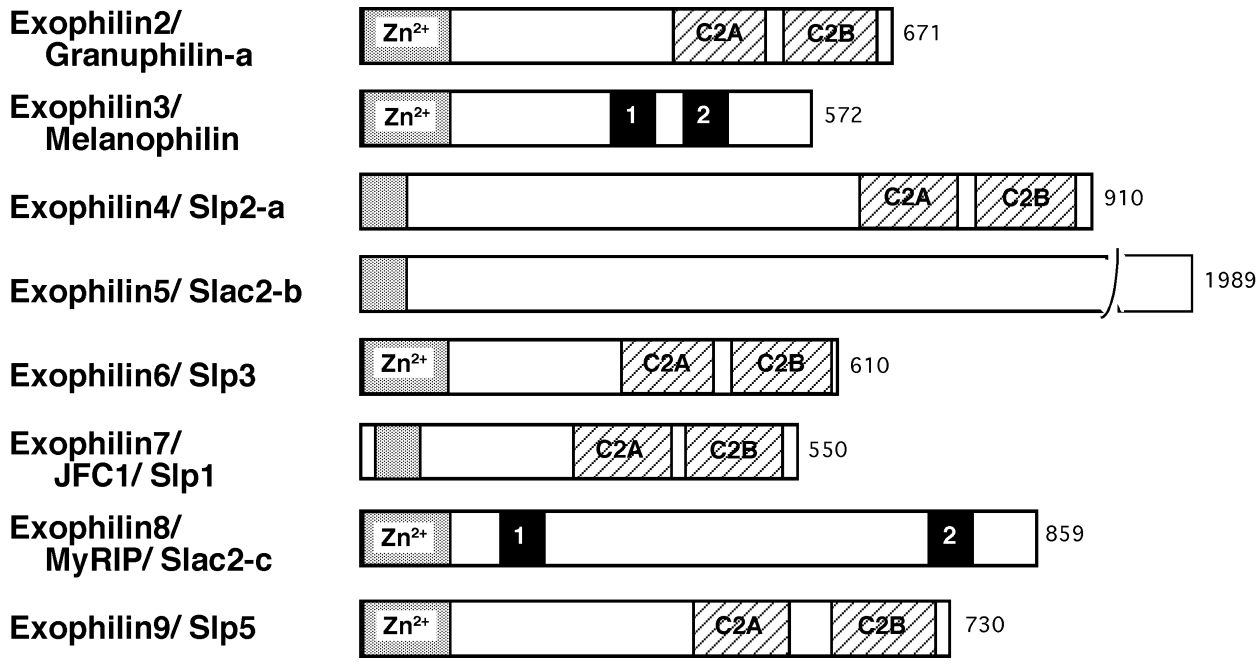

Fig. 1. Domain structures of putative Rab27 effector proteins (Modified from Nagashima *et al*., 2002). The domain structures of putative Rab27 effector proteins are schematically represented. The amino acid numbers shown on the right are those of human genes. Gray box, N-terminal homologous Rab-binding region; Zn^{2+}, zinc-finger motif; dashed box, C2 domain; black box, coiled-coils.

employed.

5. Granuphilin (Slp4)

Granuphilin is the first Rab27 effector whose amino acid sequence is completely determined. It was identified by mRNA differential display as a gene that is preferentially expressed in pancreatic beta versus alpha cell lines (Wang *et al.*, 1999). The larger isoform, granuphilin-a, has two C2 domains, whereas the smaller one, granuphilin-b, contains only the first C2 domain. Granuphilins have a restricted tissue distribution, being specifically expressed in pancreatic beta cells and pituitary tissue, but not in pancreatic alpha cells, the adrenal gland, or other major organs including the brain. They are localized on the membrane of insulin granules in pancreatic beta cells (Yi *et al.*, 2002), and their overexpression is inhibitory for K^+-induced insulin secretion (Coppola *et al.*, 2002; Torii *et al.*, 2002), although it significantly enhances basal secretion (Torii *et al.*, 2002). Although Rab3a has an ability to interact with the N-terminal region of granuphilin *in vitro* and when overexpressed in cells (Coppola *et al.*, 2002; Yi *et al.*, 2002), Rab27a is considered a principal Rab partner of granuphilin, at least in a beta cell line MIN6, for the following reasons (Yi *et al.*, 2002). First, endogenous Rab27a, but not Rab3a, significantly forms a complex with granuphilin. Second, Rab27a, but not Rab3a, has been shown to be cofractionated with granuphilin in an analysis of sucrose density gradient subcellular fractionation. Third, the tissue distributions of granuphilin and Rab27a are remarkably similar. Neither protein is expressed in brain where Rab3a is specifically and abundantly expressed. It is, however, possible that a portion of Rab3a interacts with granuphilin and is involved in a distinct exocytotic step of insulin granules because pancreatic beta cells express members of the Rab3 subfamily (Regazzi *et al.*, 1996).

Although the mechanism of granuphilin activity in the exocytotic pathway remains unknown, it is known to directly bind to syntaxin 1a (Torii *et al.*, 2002) and Munc18-1 (Coppola *et al.*, 2002), both of which are essential for membrane fusion (Fig. 3, left). Similar interactions of Rab effectors with either syntaxins or Sec1/Munc18 (SM) proteins are found in other transport pathways, including those in yeast where there is supporting genetic evidence (Segev, 2001; Zerial and McBride, 2001). For example, a Rab5 effector EEA1 interacts with syntaxin 6 and syntaxin 13, whereas a Rab1 effector p115 forms a complex including syntaxin 5. Interactions with SM proteins are exemplified by a Ypt51 effector Vac1 or a Rab5 effector rabenosyn to Vps45 and by a Ypt7 effector complex HOPS to Vps33. These findings suggest a model in which Rab recruits an effector and then, in turn, interacts with syntaxin, a component of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE), and/or a SM protein, which has a direct role in SNARE complex formation and in membrane fusion. Therefore, the interaction of granuphilin with syntaxin 1a and/or Munc18-1 may have an important role in the tethering and fusion of granules to plasma

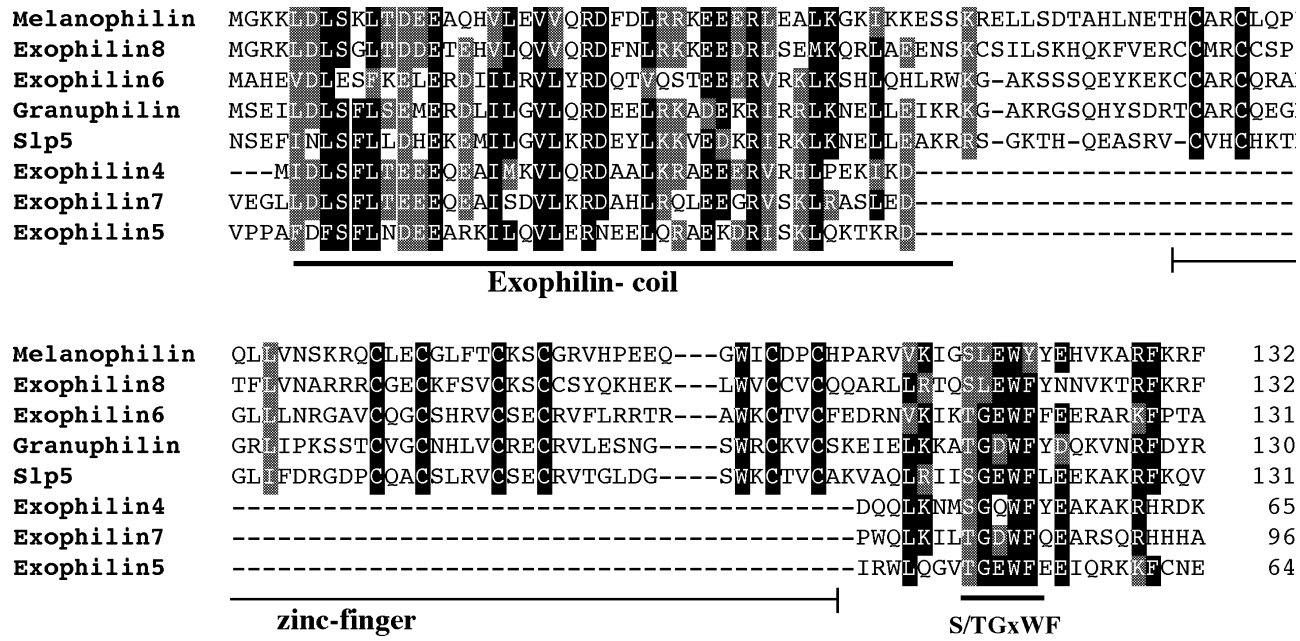

Fig. 2. Amino acid alignments of N-terminal sequences of exophilins (Modified from Nagashima *et al*., 2002). The N-terminal Rab27-binding region of putative Rab27 effector proteins is aligned. The putative Rab-binding coiled-coil sequences are shown. Residues conserved in at least five members are shown in dark boxes. The sequences of granuphilin, exophilins 6–8, and Slp5 are derived from mouse genes, whereas those of melanophilin, and exophilins 4 and 5 are from human genes.

membrane.

It should be noted that most SM proteins have a high binding affinity for syntaxins, as originally found between Munc18-1 and syntaxin 1a in neurons (Hata *et al.*, 1993). Munc18-1 shows an affinity exclusively to a closed form of syntaxin 1a that is not compatible with the SNARE assembly (Dulubova *et al.*, 1999). This mode of interaction seems to represent a specialization in the regulated secretory pathway because in other pathways SM proteins interact with syntaxins in a way that does not prevent SNARE complex formation (Rizo and Südhof, 2002). Therefore, Munc18-1 must be dissociated from syntaxin 1a before membrane fusion, which may reflect the nature of the regulated secretory pathway where membrane fusion should not occur until an appropriate extracellular secretagogue arrives. Granuphilin can directly and separately bind to syntaxin 1a and Munc18-1 *in vitro*. Because syntaxin 1a and Munc18-1 also forms a complex, it is currently unclear whether granuphilin forms a complex with either protein simultaneously or separately *in vivo*. In any case, since granuphilin shows preference to a closed form of syntaxin 1a like Munc18-1 (Torii *et al.*, 2002), granuphilin and Munc18-1 must somehow be dissociated from syntaxin 1a to allow syntaxin 1a to form an open conformation and thus core-complex with other SNARE proteins. An inhibitory effect of overexpressed granuphilin on high K^+-induced insulin secretion may be related to its ability to trap syntaxin 1a in a closed conformation. Future studies should focus on how the complex formation involving granuphilin, syntaxin 1a, and Munc18-1 is regulated.

6. Melanophilin (exophilin3, Slac2-a)

Melanophilin is a single protein that has been genetically proven to function as a Rab27a effector. The gene coding melanophilin has been discovered to be mutated in *leaden* coat-color mutant mice (Matesic *et al.*, 2001). The *leaden* product was previously shown to function in the same pathway as Rab27a (*ashen*) and myosin Va (*dilute*), because all three mutations are suppressed by the cell autonomous, semidominant *dilute suppressor*, *dsu* (Moore *et al.*, 1988). Because melanophilin shares a putative N-terminal Rab27a binding domain with granuphilin, it was predicted to be a Rab27a effector protein (Matesic *et al.*, 2001). This proposition was proven to be correct by subsequent biochemical analyses showing that melanophilin directly and nucleotide-dependently binds to Rab27a *in vitro* (Fukuda *et al.*, 2002; Nagashima *et al.*, 2002; Strom *et al.*, 2002; Wu *et al.*, 2002). Moreover, it directly binds to myosin Va through its C-terminal coiled-coil region-1 (Fukuda and Kuroda, 2002; Nagashima *et al.*, 2002; Strom *et al.*, 2002) (Fig. 1). The binding domain in myosin Va requires the presence of exon F, an alternatively spliced exon expressed in melanocytes but not in neuroendocrine cells (Nagashima *et al.*, 2002; Seperack *et al.*, 1995; Wu *et al.*, 2002). Genetic evidence and these biochemical studies suggest that melanophilin

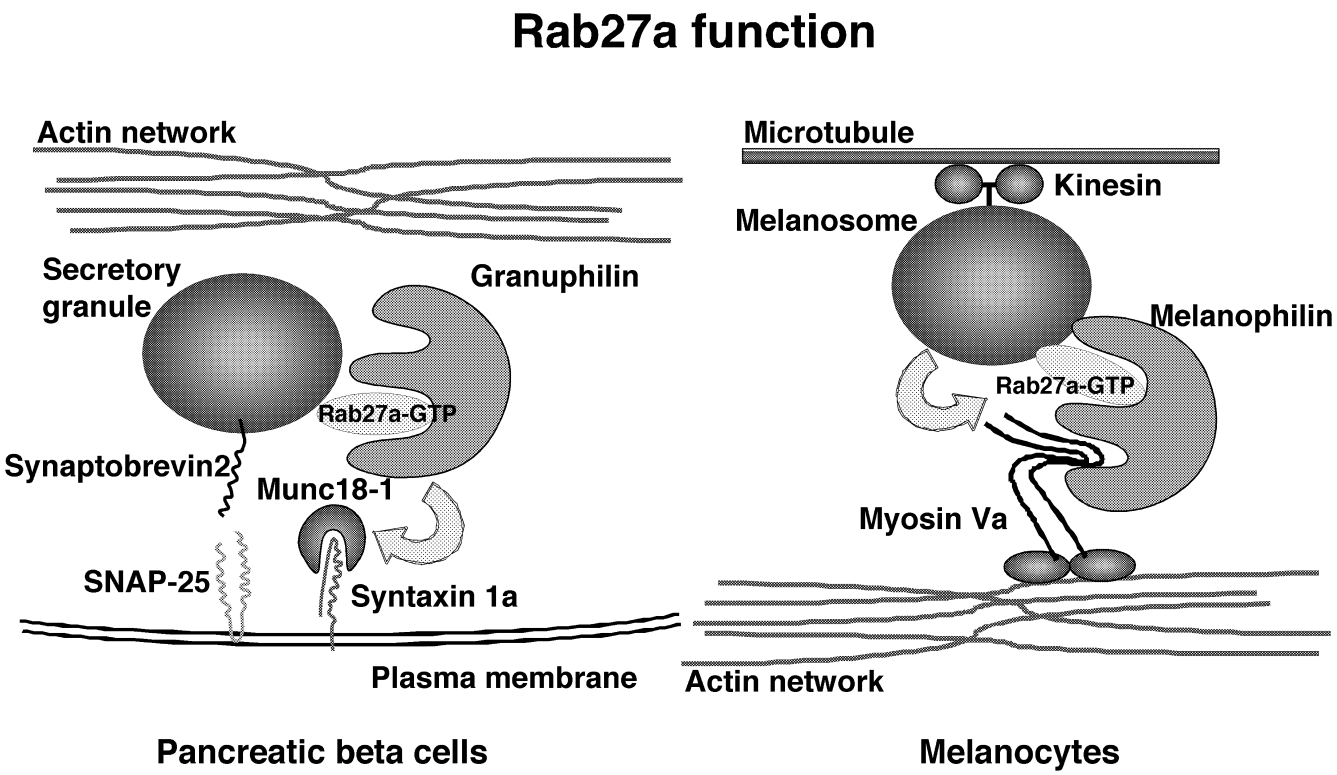

Fig. 3. Modes of action of granuphilin and melanophilin. Rab27a is located on endocrine granules or lysosome-related organelles such as melanosomes, and is involved in various steps of the transport of these granule-like organelles. GTP-bound, active Rab27a forms a complex with organelle-specific effector proteins, such as granuphilin and melanophilin. *Left*, in pancreatic beta cells, granuphilin binds Rab27a on insulin granules and tether the granules to plasma membrane possibly through the interaction with syntaxin 1a and/or Munc18-1. Granuphilin complexes with syntaxin 1a and Munc18-1 may also regulate subsequent fusion events. *Right*, in melanocytes, melanophilin links Rab27a on melanosomes and myosin Va on actin filaments, and traps melanosomes in actin-rich peripheral areas to be ready for the transfer of melanosomes to adjacent keratinocytes.

links Rab27a on melanosomes and myosin Va on actin filaments (Fig. 3, right). Melanosomes are transported by fast, long-range, bi-directional, microtubule-dependent movements along the length of dendrites and by local movement on to actin filaments within distal regions of the dendrite (Wu *et al.*, 1998). Without capture of melanosomes on actin networks through melanophilin, melanosomes are not retained in the periphery but are clustered near the perikaryotic regions, and thereby cannot be transferred to neighboring keratinocytes. The mode of melanophilin action is probably the clearest example of how Rab effector proteins act on a molecular level.

7. Other putative effectors

Other putative Rab27a effector proteins have not been characterized in detail and their physiological functions are unknown, although they are likely to regulate other secretory pathways in differentiated cells such as cytotoxic T-lymphocytes (Fig. 1).

(1) MyRIP (exophilin8, Slac2-c)

MyRIP was identified as a protein interacting with the tail of myosin VIIa from a retinal two-hybrid cDNA library (El-Amraoui *et al.*, 2002). It directly binds to Rab27a by the N-terminus and myosin VIIa through the middle region *in vitro*. MyRIP is localized on melanosomes in microvilli of retinal pigment epithelial cells that surround the tips of photoreceptor outer segments. It shows marked similarity to melanophilin in that it connects Rab27a and unconventional myosin in cells harboring melanosomes. MyRIP is expressed in a variety of tissues, including the synaptic area of retinal photoreceptor cells and inner ear hair cells, suggesting that it may be involved in the trafficking of other organelles, such as synaptic vesicles in specialized sensor cells.

(2) JFC1 (exophilin7, Slp1)

JFC1 was identified by a yeast two-hybrid system as a protein that interacts with regulatory components of the NADPH oxidase, $p67^{phox}$ (Berkowitz *et al.*, 2001). Although it is abundantly expressed in leukocytes where the NADPH oxidase is restricted, it is widely expressed in other tissues. *In vitro*, JFC1 binds to Rab27a (Kuroda *et al.*, 2002a), but it was not cofractionated with azurophil granules (Berkowitz *et al.*, 2001), the lysosome-related organelles in neutrophils.

Thus, the physiological function of JFC1 is unknown. The first C2A domain of the two tandem C2 domains, which is homologous to the C2B domain of synaptotagmin 1, possesses 3'-phosphoinositide binding activity. When this domain is expressed in cells, it shows the ability to bind to the plasma membrane in a Ca^{2+}-inhibitory manner (Catz *et al.*, 2002).

(3) Others

Other proteins that have a putative Rab27-binding domain are reported, including exophilin4-6 (Slp2, Slac2-b, Slp3) and Slp5 (Fukuda and Mikoshiba, 2001; Kuroda *et al.*, 2002a; Kuroda *et al.*, 2002b; Nagashima *et al.*, 2002), although they remain poorly characterized except for the sequence information and the ability to bind Rab27a *in vitro* or when overexpressed in cell lines. It remains to be seen whether they actually represent Rab27 effectors.

8. Rab27 vs. Rab3

Members of the Rab3 subfamily (Rab3a, b, c, and d) have been proven to be specifically associated with various secretory vesicles (Jahn and Südhof, 1999; Takai *et al.*, 2001). Overexpression of Rab3 proteins in various cell lines inhibits secretion in most cases, although Rabs are generally thought to positively promote vesicle transport. Rab3a, its regulators such as guanine nucleotide exchange factor and GTPase activating protein, and its effectors such as rabphilin3 and Rims, have been intensively characterized and are thought to be involved in the exocytosis of various secretory vesicles. Despite numerous studies, the function of Rab3 proteins has yet to be determined at the molecular level. One reason for this is that there is no clear phenotype in Rab3-knockout mice. For example, neither the size nor the refilling rate of the readily releasable pool was changed in Rab3a-deficient synapses. Instead, Ca^{2+}-triggered fusion was increased, which resulted in an enhanced rundown of synaptic transmission (Geppert *et al.*, 1997). These findings suggest that Rab3a is not involved in vesicle tethering, but rather plays an inhibitory role in vesicle fusion. Similarly, the only phenotype detected to date in Rab3d-deleted mice showed a slight enlargement of the granule size in exocrine cells (Riedel *et al.*, 2002). The lack of clear phenotypes cannot be simply explained by functional complementation by coexisting Rab3 isoforms because the situation is similar even in cells that do not express other isoforms.

Compared with Rab3-knockout mice, *ashen* mice lacking functional Rab27a show clearer defects in the regulated secretory pathways, as described above. In the Rab family, the Rab27 subfamily has the closest sequence similarity to the Rab3 subfamily. In addition, Rab27 and Rab3 members are associated with exocytotic vesicles, suggesting that both function as exocytotic Rab proteins. This is consistent with the finding of a recent phylogenetic analysis that showed that Rab proteins with similar sequences show a similar pattern of cellular localization and/or function (Pereira-Leal and Seabra, 2001). It is noteworthy that Rab27 and Rab3 are often coexpressed in secretory cells such as neurons (Rab3a, Rab3c, and Rab27b) (Fischer von Mollard *et al.*, 1994; our unpublished observations), pancreatic beta cells (Rab3a-d, Rab27a) (Regazzi *et al.*, 1996; Yi *et al.*, 2002), and melanocytes (Rab3a and Rab27a) (Araki *et al.*, 2000; Bahadoran *et al.*, 2001; Hume *et al.*, 2001). Interestingly, overexpression of each Rab27a and Rab3a protein in the same pancreatic beta cell line produces opposite effects on insulin secretion (Yi *et al.*, 2002). Thus, Rab3 and Rab27 may have different functions on the same secretory vesicles.

9. Future directions

Based on the findings described above, it is very likely that Rab27 regulates various exocytotic mechanisms using multiple organelle-specific effectors. Although the precise physiological roles of these putative effectors will soon be investigated in detail in differentiated cells (cell lines) or in whole mice, several important questions remain to be clarified.

First, regulators such as guanine nucleotide exchange factor, GTPase activating protein, and guanine nucleotide dissociation inhibitor are completely unknown for Rab27. Rab27a is tightly associated with the granule membrane and is merely observed in the cytosol (Yi *et al.*, 2002). Moreover, the predicted dominant-negative forms (T23N, N133I) are unstable in both transfected cell lines (Yi *et al.*, 2002) and transgenic mice (Ramalho *et al.*, 2002), whereas wild-type Rab27a is able to bind effector proteins as well as the active form (Q78L) (Yi *et al.*, 2002). These observations suggest that the regulators for Rab27a are both unique and specific. Note that Rab27a is specifically sensitive in defects that generally affect prenylation and membrane association of Rab proteins, such as choroideremia caused by mutations in the Rab escort protein-1 gene (Seabra *et al.*, 1995) or the *gunmetal* mouse that has a mutation in the Rab geranylgeranyl transferase α gene (Detter *et al.*, 2000).

Second, the tissue distributions of exophilin family proteins are relatively broad, except for granuphilin. Although melanophilin is significantly expressed in most adult tissues (Matesic *et al.*, 2001; our unpublished observations), specific cells may exist in a selected tissue; for example, melanophilin is hardly detected in whole skin but is highly expressed in melanocytes. The role of melanophilin in other tissues devoid of melanosomes is unknown. It has been shown that lytic granules kill target cells normally in *leaden* cytotoxic T-lymphocytes (Hume *et al.*, 2002). By contrast, granuphilin has a very restricted tissue distribution: it is specifically expressed in pancreatic beta cells, but not in alpha cells that are located in the same pancreatic islets (Wang *et al.*, 1999). Neighboring endocrine cells seem to employ different Rab effectors and distinct mechanisms.

Third, different putative Rab27 effectors are expressed in the same cells and show at least partially distinct distribu-

tions. For instance, melanophilin and exophilin4/Slp1 are coexpressed in melanocytes (Kuroda *et al.*, 2002a; our unpublished observations). Yet it is unknown just how these effectors discriminately bind Rab27a in distinct membrane compartments and differentially function in the same cells.

Fourth, some Rab27 effectors show affinities to other Rab proteins that belong to different subfamilies, at least *in vitro* (Kuroda *et al.*, 2002a; Yi *et al.*, 2002). It is not known whether these effector proteins can change their Rab partners in different steps of vesicle transport.

Recent advances in molecular genetics and genome identification have helped to uncover the molecular nature of genetic diseases and to identify the family of proteins that have similar primary sequences. In the field of Rab27 research, these two approaches have converged to yield promising results and continue to stimulate fruitful research. Future research should emphasize a rapid expansion of our understanding of the physiology and pathology of minor secretory vesicles differentiated in multicellular organisms, which have thus far been poorly characterized relative to that of synaptic vesicles in neurons.

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