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Regulation of secretory vesicle traffic by Rab small GTPases

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Abstract. Secretion is a fundamental biological activity of all eukaryotic cells by which they release certain substances in the extracellular space. It is considered a specialized mode of membrane trafficking that is achieved by docking and fusion of secretory vesicles to the plasma membrane (i.e., exocytosis). Secretory vesicle traffic is thought to be regulated by a family of Rab small GTPases, which are regulators of membrane traffic that are common to all eukaryotic cells. Classically, mammalian Rab3 subfamily members were thought to be critical regulators of secretory vesicle exocytosis in neurons and endocrine cells, but recent genetic and proteomic studies indicate that Rab3 is not the sole Rab isoform that regulates secretory vesicle traffic. Rather, additional Rab isoforms, especially Rab27 subfamily members, are required for this process. In this article I review the current literature on the function of Rab isoforms and their effectors in regulated secretory vesicle traffic. (Part of a Multi-author Review)

Keywords. Docking, exocytosis, Rab3, Rab27, Rab effector, rabphilin, secretory vesicle, synaptotagmin-like protein.

Introduction

Rab small GTPases constitute the largest family of the known membrane trafficking proteins [e.g., soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) and coat proteins] that are common to all eukaryotic cells (reviewed in [1–3]). The same as other monomeric Ras-like GTPases, Rab functions as a molecular switch by cycling between two nucleotide-bound states, a GDP-bound inactive state and a GTP-bound active state, and the cycling is controlled by two regulatory enzymes, guanine nucleotide exchange factor (GEF) and GTPase-activating protein (GAP). The GTP-bound active form of Rab is recruited to transport vesicles/organelles, and it promotes their trafficking (such as vesicle budding, vesicle motility, vesicle docking to specific membranes, and/or vesicle fusion) by interacting with specific effector molecules (Fig. 1A) [1–3]. The number of Rab isoforms varies from species to species, ranging from 11 in budding yeasts to 29 in *Caenorhabditis elegans* and *Drosophila melanogaster*, and to more than 60 in humans and mice [4–6] (Fig. 1B). Only five Rab isoforms, Rab1/Ypt1, Rab5/Ypt5, Rab6/Ypt6, Rab7/Ypt7, and Rab11/Ypt31, presumably 'housekeeping Rabs' (blue in Fig. 1B), are conserved from yeasts to humans, and 17 Rab isoforms are shared by *Caenorhabditis elegans*, *Drosophila*, and humans (magenta in Fig. 1B). By contrast, most other Rab isoforms are vertebrate- or mammalian-specific (green in Fig. 1B), and they may have unique roles in cell-type-specific or tissue-specific membrane trafficking events, i.e., be specialized for membrane trafficking pathways in specialized cell types in higher eukaryotes; however, their functions are poorly understood.

The large number of Rab isoforms in mammals has often been considered a major obstacle to identifying the Rab isoforms involved in specific types of membrane trafficking events, and until recently analysis of even the well-known regulated secretory pathway in which secretory vesicles are docked and fused to the plasma membrane in response to stimulation has been limited to specific Rab isoforms, i.e., members of the Rab3 subfamily (reviewed in [7, 8]). Although more than 400 papers have been published on the vesicle

Figure 1. Rab family members in mammals. (*A*) Schematic representation of the general role of Rab protein and its effector in membrane traffic (see text for details). (*B*) Phylogenetic trees of mouse and human Rab family members [5, 74]. Rabs conserved from yeasts to humans are shown against a blue background, and Rabs shared by *C. elegans*, *Drosophila*, mice, and humans are shown against a magenta background. Rabs shown against a green background are presumed to be vertebrate- or mammalian-specific [5]. Red branches indicate Rab isoforms involved in regulated secretory vesicle traffic, indicating that they contain a Rab functional group specialized for secretory vesicle traffic (i.e., 'secretory Rabs', including Rab3, Rab26, Rab27, and Rab37; dotted box) [5, 6]. Five Rabs are known to be associated with human and/or mouse (parentheses) diseases (indicated by arrows), but regulated secretion defects have only been reported in type 2 Griscelli syndrome patients and the corresponding mouse model *ashen* [16, 17, 21, 44–46]. The phylogenetic tree was drawn by using the ClustalW program (available at http://clustalw.ddbj.nig.ac.jp/top-e.html) set at the default parameters.

localization of Rab3 subfamily members (Rab3A/B/C/D in humans and mice) and their involvement in regulated secretion [7, 8], complete absence of all four Rab3s, but not of a single isoform, results in only a 30 % reduction in the probability of Ca^{2+}-triggered neurotransmitter release (however, quadruple knockout mice die shortly after birth) [9–11], suggesting that additional Rab isoforms are also involved in regulated secretory vesicle traffic. Consistent with this notion, recent proteomic analysis of several types of secretory vesicles has revealed the presence of an unexpectedly large number of Rab isoforms on secretory vesicles (e.g., >28 Rabs on neuronal synaptic vesicles [12], >20 on natural killer (NK) cell secretory lysosomes [13], 14 Rabs on pancreatic β-cell insulin secretory granules [14], and >16 Rabs on pancreatic acinar zymogen granules [15]), although the number of Rab isoforms involved in the control of

secretory vesicle traffic is still unclear. Among the Rab isoforms associated with secretory vesicles, considerable attention has recently been paid to Rab27 subfamily members, i.e., Rab27A/B in humans and mice, for the following reasons. (i) Rab27-deficient animals display clear secretion defects in certain secretory cells [16–21]. (ii) Rab27A/B proteins are present on a variety of secretory vesicles in mice [18, 20–22], and (iii) Rab27 is the closest isoform of Rab3 in the phylogenetic tree (Fig. 1B) and has been retained from *C. elegans* to humans [5, 23]. In this article I provide an overview of the function of Rab proteins on secretory vesicle traffic, with a special focus on recent advances (later than 2000) in research on the molecular mechanisms of regulated secretory vesicle traffic mediated by Rab3/27 and their regulators.

Rab proteins associated with secretory vesicles

As mentioned above, a large number of Rab isoforms (more than 10 different Rabs) are usually present on isolated secretory vesicles, but the majority of them are not exclusively present on secretory vesicles, and well-characterized Golgi-resident Rabs (e.g., Rab1 and Rab2) and endosomal Rabs (e.g., Rab5 and Rab21) have also been identified on secretory vesicles by recent proteomic studies [12–15]. The latter Rabs are probably traces of immature vesicles that emerged from the Golgi and/or endosomes, and they may not be involved in regulated secretion itself. Although proteomic analysis of secretory vesicles by tandem mass spectroscopy is a powerful method of determining how many Rab isoforms are present on them, it is virtually impossible to determine which of the Rab isoforms identified are specifically localized on secretory vesicles and actually control regulated secretion. Systematic screening for Rab isoforms that are specifically targeted to secretory vesicles by expressing fluorescently labeled Rabs has recently been employed to overcome this problem [24]. Expression of green fluorescent protein (GFP)-tagged Rab1–43 in neuroendocrine PC12 cells has indicated that only seven Rab isoforms (Rab3A/B/C/D, Rab27A/B, and Rab37) are specifically targeted to dense-core vesicles (some other Rab isoforms, e.g., Rab33A, are also targeted to dense-core vesicles, but they are present too on other organelles, e.g., the Golgi apparatus). It should be noted that the Rab isoforms identified by this screening method were also identified by proteomic analysis of secretory vesicles: Rab3A/B/C, Rab26 and Rab27B on synaptic vesicles [12]; Rab3A/C/D and Rab37 on insulin secretory granules [14]; Rab3A, Rab26, and Rab27A on pancreatic acinar zymogen granules [15]; and Rab27A and Rab37 on NK cell secretory lysosomes [13].

To date, 11 Rab isoforms (Rab3A/B/C/D, Rab4A, Rab8B, Rab11B, Rab26, Rab27A/B, and Rab37; summarized in Table 1) have been shown to be involved in regulated secretion by certain secretory cells. Three of them, Rab4A (endosome-resident), Rab8B (Golgi-resident), and Rab11B (endosome-resident), are unlikely to be general regulators of stimulated secretion and may be involved in a specific type of secretion (e.g., involvement of Rab4A in α-granule secretion by platelets) [25–27], whereas the other Rabs (Rab3A/B/C/D, Rab26, Rab27A/B, and Rab37) are predominantly present on 'mature' secretory vesicles and form a small branch on the phylogenetic tree (red branch in Fig. 1B) [5, 6]. Since these phylogenetically similar Rab isoforms are not conserved in yeasts, which possess only a constitutive secretion pathway and do not have any regulated secretion pathways, they have been classified as specialized Rabs that control regulated secretion in animals and are referred to as 'secretory Rabs' below (dotted box in Fig. 1B). As summarized in Table 1, both Rab3 and Rab27 subfamily members are present on a variety of secretory vesicles, including neuronal, endocrine, exocrine, and immune cell secretory vesicles ([7, 8, 20, 22, 28] and references therein), whereas Rab26 and Rab37 seem to be present on secretory vesicles in specialized cell types [14, 29–31]. Although overexpression of Rab3 or Rab27 protein has previously been reported to have an inhibitory role in regulated secretion [32–36] (but see [37–40], which report a positive role of Rab27), endogenous Rab3 and Rab27 proteins should play positive roles in regulated secretion, because both Rab3- and Rab27-mutant animals display clear secretion defects [10, 11, 16–20]. For example, mutations in the *RAB27A* gene cause a human hereditary disease (type 2 Griscelli syndrome; Fig. 1B), and the corresponding murine model *ashen*, and both type 2 Griscelli syndrome patients and *ashen* mice have defects in lytic granule exocytosis in cytotoxic T lymphocytes (CTLs) [16, 17]. Rab27B knockout (KO) mice and Rab27A/B double-knockout (DKO) mice also exhibit secretion defects in some secretory cell types, although DKO mice are viable [18–20]. In addition, reduced expression of Rab3A and Rab27A has been suggested to be associated with defective insulin release in type 2 diabetes [41].

Table 1. Rab isoforms that control regulated secretion.

Names	Cell types/types of secretion or membrane trafficking	Putative Rab effectors or binding proteins	Human diseases or mutant mice	GEF or GAP
Rab3A	neurons/neurotransmitter release	rabphilin, Rim1/2, synapsin I		
	endocrine cells/hormone secretion			
	chromaffin and PC12 cells	rabphilin,Noc2,Rim1/2 Munc18-1, Rabin3	*earlybird* or Rab3A KO mice	Rab3-GAP Rab3-GEP/AEX-3,
	pituitary and AtT-20 cells	rabphilin	Rab3A/B/C/D KO mice	GRAB
	pancreatic β cells and cell lines	Slp4, rabphilin, Noc2, Rim1/2, calmodulin		
	sperm cells/acrosome reaction	ND		
	eggs/cortical granule exocytosis	rabphilin		
Rab3B	pituitary cells/gonadotrophin release	ND		
	chromaffin and PC12 cells/hormone secretion	rabphilin, PI3K		Rab3-GAP
	platelets/granule exocytosis	calmodulin	Rab3A/B/C/D KO mice	Rab3-GEP/AEX-3
	epithelial cells/basolateral transport of LDLR	Noc2		
	MDCK cells/transport of pIgR	pIgR		
Rab3C	neurons/neurotransmitter release	rabphilin		Rab3-GAP
	chromaffin and PC12 cells	rabphilin	Rab3A/B/C/D KO mice	Rab3-GEP/AEX-3
Rab3D	chromaffin and PC12 cells	rabphilin		
	pancreatic acinar cells/zymogen granule exocytosis	ND		
	parotid acinar cells/amylase release	ND	Rab3D KO or	Rab3-GAP
	AtT-20 cells/ACTH release	ND	Rab3A/B/C/D KO mice	Rab3-GEP/AEX-3
	mast cells/histamine release	ND		
	endothelial cells/vWF secretion	ND		
	intestinal goblet cells/Golgi trafficking	rabphilin		
	osteoclasts/osteoclastic bone resorption	ND		
Rab4A	platelets/α-granule secretion	ND		
	pancreatic acinar cells/amylase release	ND		GAP-CenA
	3T3-L1 adipocytes/Glut4 transport	kinesin?		
Rab8B	AtT-20 cells/ACTH release	TRIP8b		ND
Rab11B	neurons/neurotransmitter release	ND		ND
	PC12 cells/hormone secretion	ND		
Rab26	parotid acinar cells/amylase release	ND		ND
Rab27A	endocrine cells/hormone secretion			
	chromaffin and PC12 cells	Slp4, rabphilin, Slac2-c, Noc2		
	pituitary cells	Slp4		
	pancreatic β cells and cell lines	Slp4, Slp5, Slac2-c, Noc2		
	pancreatic α cells and their cell lines	Slp2-a		
	gastric surface mucous cells/mucus secretion	Slp2-a		
	melanocytes/melanosome anchoring	Slp2-a		AEX-3
	platelets/dense granule exocytosis	Munc13-4	human type 2 Griscelli	Rab27A-GAPα/EPI64
	CTLs/cytotoxic granule exocytosis	Munc13-4	syndrome	Rab27A-GAPβ/
	mast cells/histamine release	Munc13-4	*ashen* or Rab27A/B KO mice	FLJ13130
	granulocytes/azurophilic granule exocytosis	ND		
	endothelial cells/Weibel-Palade body exocytosis	ND		
	prostate carcinoma cells/prostate-specific marker secretion	Slp1		
	epithelial cells/epithelial sodium channel transport	Slp5, Munc13-4		

Table 1 *(Continued)*

Names	Cell types/types of secretion or membrane trafficking	Putative Rab effectors or binding proteins	Human diseases or mutant mice	GEF or GAP
Rab27B	pituitary and AtT-20 cells	Slp4		
	mast cells/histamine release	ND	Rab27B KO	
	parotid acinar cells/amylase release	Slp4-a, Slac2-c, Noc2	or Rab27A/B KO mice	ND
	pancreatic acinar cells/zymogen granule exocytosis	ND		
	platelets/dense granule exocytosis	Munc13-4		
Rab37	pancreatic β cells and cell lines/insulin secretion	ND		ND
	mast cells/secretory granule exocytosis	ND		

Rab3/27 isoforms are known to be expressed in a variety of secretory cells, but some secretory cell types have not been included in this table. The details concerning expression of Rab27A and Rab27B on secretory vesicles were reported in [20, 22]. ND, not determined.

Which step in secretory vesicle traffic is regulated by Rab proteins?

Regulated secretion consists of at least four distinct steps: recruitment of secretory vesicles to the release site, docking of secretory vesicles to the plasma membrane, priming, and stimulus-dependent fusion of secretory vesicles to the plasma membrane. Although the alterations of the level of expression of mammalian Rab proteins by overexpression or knockdown shown in Table 1 have been demonstrated to increase or decrease stimulated secretion, the involvement of the proteins in specific steps of secretory vesicle traffic is still poorly understood and is a matter of controversy (e.g., different roles of Rab3A have been reported in the past [42, 43]). However, recent imaging techniques that use total internal reflection fluorescence (TIRF) microscopy have made it possible to directly analyze the involvement of Rab proteins in each step of the secretory vesicle exocytosis that occurs just beneath the plasma membrane. As a result, Rab3A and Rab27A proteins have been shown to be involved in the recruitment/docking of secretory vesicles to the plasma membrane, rather than in the priming/fusion step [24, 44]. For example, the results showed that siRNA-mediated knockdown of either Rab3A or Rab27A protein in PC12 cells reduced the number of plasma membrane-docked dense-core vesicles and stimulated hormone secretion [24]. Similar docking defects have been observed at the electron microscopic level in CTLs from Rab27A-defective *ashen* mice [45, 46] and in pituitary cells from Rab27A/B DKO mice [20]. In contrast to Rab27 mutant mice, no clear docking defects have been observed in Rab3 mutant mice, even at the electron microscopic level [9–11] (but see [47]; Rab3A deletion reduced vesicle docking at the neuromuscular junction), and Rab3 mutant animals seem to lack activity-dependent recruitment of synaptic vesicles to the active zone [48, 49].

In addition to the role of Rab3 and Rab27 proteins in the recruitment and/or docking step of secretory vesicle traffic, they are also likely to be involved in the formation and/or maturation of secretory vesicles. For instance, the secretory vesicles in both the exocrine pancreas and parotid gland of Rab3D KO mice are significantly larger [9], and the number of dense granules in the platelets of Rab27B KO mice is significantly smaller [18]. Similar abnormalities in the size and number of secretory vesicles have been observed in granuphilin/Slp4 KO mice [50] and Slp2-a KO mice [51]. Since Rab27A, and possibly Rab27B and Rab3 subfamily members, tend to be localized on mature secretory vesicles, they may also function as a maturation sensor during the formation of fusion-competent mature secretory vesicles [52] (but see [53], according to which both Rab3A and Rab27A are rapidly recruited to newly synthesized immature secretory vesicles in PC12 cells).

Domain structure of Rab3 and Rab27 effectors in vertebrates and invertebrates

Identifying specific Rab effector molecules is one of the most important steps toward understanding the role of Rab protein in specific steps of regulated secretion at the molecular level (Fig. 1A), and the domain structures of candidate Rab3 and Rab27 effector molecules that have been identified thus far are summarized in Fig. 2A and Table 1 (precise structures are described in [28]), which include synaptotagmin-like proteins (Slp1–5) [54–58], rabphilin [6, 59], Slp homologue lacking C2 domains (Slac2-a-c) [55, 60–63], Noc2 [6, 23, 64–66], Munc13-4 [67–69], and Rim [6, 70, 71]. All Rab3/27-binding proteins in Fig. 2A except Munc13-4 contain a conserved Rab-binding domain (RBD) at their N terminus (often referred to as the Slp homology domain (SHD) for Slps and Slac2s [72]), and their Rab-

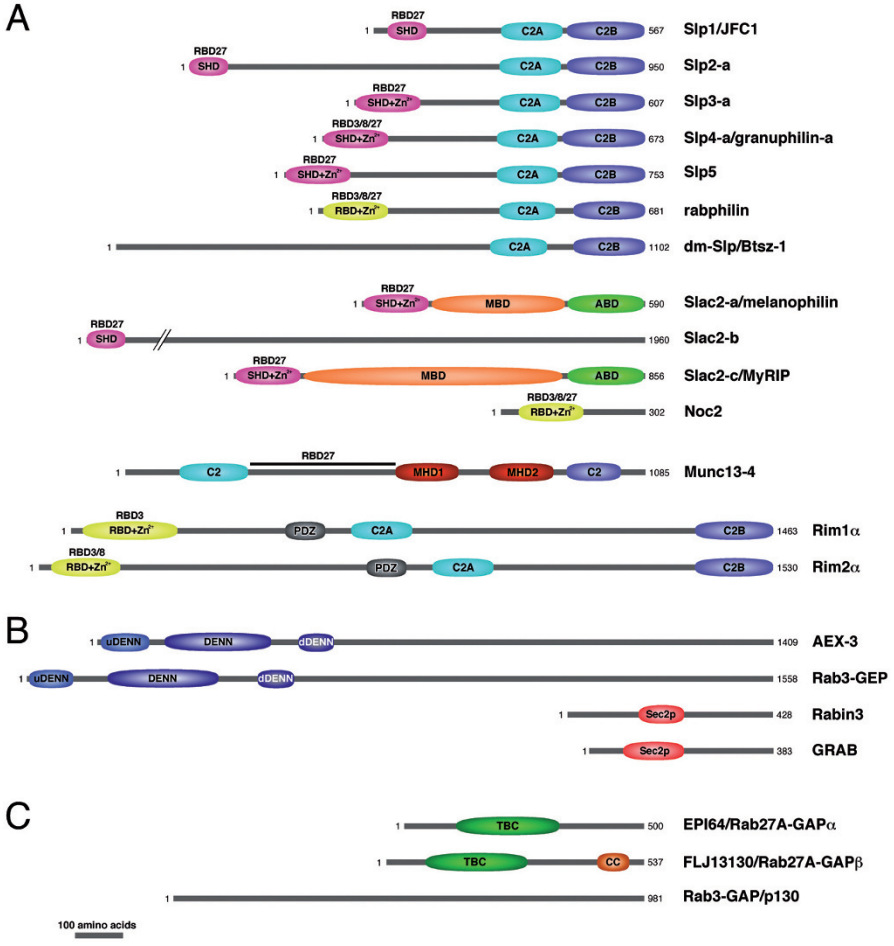

Figure 2. Structure of proteins that function as Rab effectors or regulators. (*A*) Rab3 and/or Rab27 effectors previously reported. All of them except Munc13-4 contain a conserved N-terminal Rab-binding domain (RBD), and most of them contain zinc finger motifs (Zn^{2+}). The Rab27-binding site of Munc13-4 has been mapped to the region between the C2 domain and MHD1 [69]. The RBD of the Slp and Slac2 family members (sometimes called exophilins) is often referred to as Slp homology domain (SHD) [28]. MBD, myosin Va/VIIa-binding domain; ABD, actin-binding domain; and MHD, Munc13 homology domain. (*B*) Rab3 and/or Rab27 GEF previously reported. (*C*) Rab3 and/or Rab27 GAP previously reported. All proteins except dm-Slp (from *Drosophila)* and AEX-3 (from *C. elegans*) are from mice.

binding specificity has been determined *in vitro* by using 'Rab panels' [6, 73, 74] (their Rab-binding specificity is shown in Fig. 2A). Other Rab3-binding proteins, including synapsin I [75], Munc18-1 [76], Rabin3 [77], calmodulin [78], and phosphoinositide 3-kinase (PI3K) [79], have been reported, but their Rab-binding specificity has never been thoroughly investigated. Specific effector molecules for other secretory-vesicle-associated Rabs, including Rab26 and Rab37, have not yet been elucidated.

Since Rab3 and Rab27 are found in lower invertebrates, e.g., *C. elegans* [5, 23, 80], their effector molecules were expected to have been conserved during evolution. Surprisingly, however, no homologues of most of the Rab27 effectors (e.g., Slps and Slac2s) have been found in *C. elegans* or *Drosophila*, and only one *Drosophila* Slp homologue Btsz (bite-size) has been reported, although it lacks an N-terminal RBD (or SHD) [81, 82] (Fig. 2A). A single isoform of rabphilin and Rim has been found in *C. elegans* and *Drosophila* [71, 81, 83–85], although the *Drosophila* Rim protein lacks a putative RBD [83] and the invertebrate rabphilin interacts with Rab27 alone, and not with Rab3, even when assessed in an *in vitro* overexpression study [23]. Thus, in contrast to mammalian rabphilin, *C. elegans* rabphilin is likely to function as an *in vivo* Rab27 effector, and not as a Rab3 effector [80].

Rab effectors that control docking of secretory vesicles to the plasma membrane

Three Rab effectors, Slp2-a, Slp4-a/granuphilin-a, and rabphilin, have been found to be involved in the docking process of secretory vesicle traffic in neuroendocrine cells and gastric surface mucous cells (Fig. 3) (reviewed in [73]). Single knockdown of either Rab3A or Rab27A in PC12 cells reduced both the number of dense-core vesicles docked to the plasma membrane and hormone secretion, and simultaneous knockdown of both Rabs further reduced the number of plasma membrane-docked vesicles, indicating that Rab3A and Rab27A cooperatively regulate the docking of dense-core vesicles to the plasma membrane [24]. This finding suggests that Rab3A and

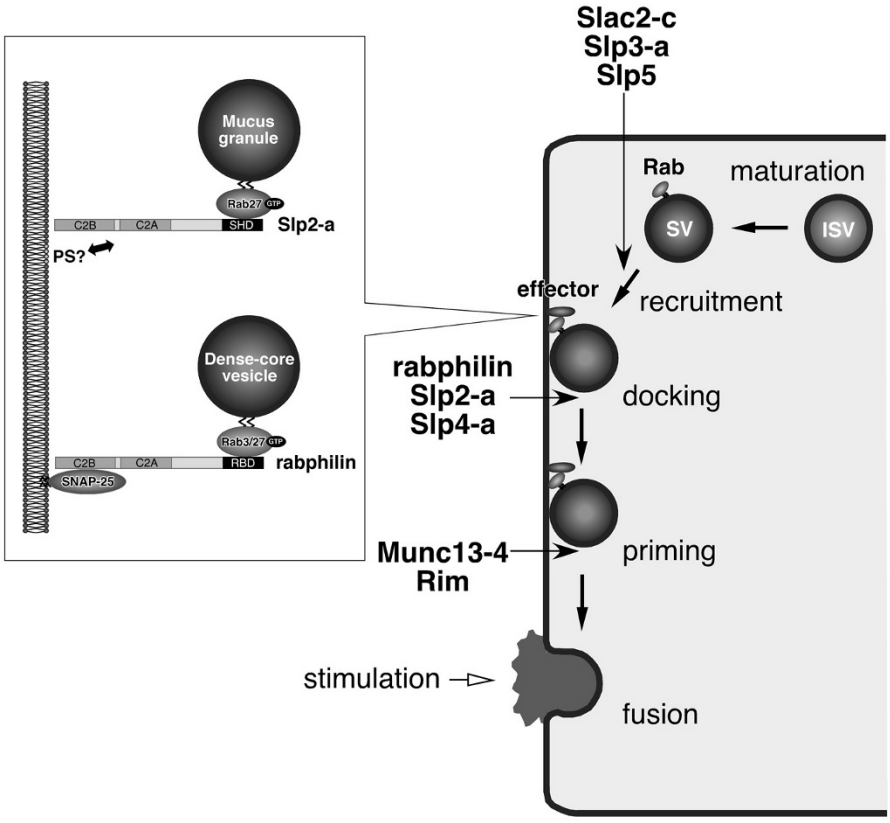

Figure 3. Proposed function of Rab·effector complex in regulated secretory vesicle traffic. After the formation and maturation of immature secretory vesicles (ISV), mature secretory vesicles (SV) are recruited to the release site (recruitment step) and docked to the plasma membrane (docking step). After an ATP-dependent priming step, secretory vesicles fuse to the plasma membrane in response to stimulation (fusion step). Possible involvement of Rab3/27 effectors in each step of secretory vesicle traffic is indicated by thin arrows (see text for details). Inset shows the proposed model of the docking machinery composed of Rab3/27 and Slp2-a or rabphilin [51, 87, 90]. These Rab effectors function as linker proteins between Rab3/27 on secretory vesicles and proteins/lipids in the plasma membrane [73].

Rab27A share some of the same effector molecules. Actually, rabphilin, Noc2, and Slp4-a/granuphilin-a bind both Rab3A and Rab27A *in vitro*, although Noc2 and Slp4-a prefer Rab27A to Rab3A *in vivo* [6, 23, 37, 66, 86]. All three proteins are endogenously expressed in PC12 cells, but only rabphilin expression promotes both dense-core vesicle-docking to the plasma membrane and hormone secretion [87]. This effect is mediated by the intact C2B domain of rabphilin [87, 88], which directly interacts with t-SNARE SNAP-25 (synaptosome-associated protein of 25 kDa) on the plasma membrane (Fig. 3, inset) [87, 89]. The rabphilin·SNAP-25 interaction is increased by Ca^{2+} ions, and it is likely to mediate dense-core vesicle-docking to the plasma membrane in PC12 cells [90, 91]. Rabphilin also regulates neurotransmitter release in a SNARE-dependent manner [85, 89], especially at the refilling step of the readily releasable pool of synaptic vesicles, when the pool has been exhausted. Slp4-a/granuphilin-a is expressed in certain endocrine cells, most abundantly in pancreatic β-cells [37, 38, 86], and promotes dense-core vesicle-docking to the plasma membrane [50, 92, 93]. Unlike rabphilin, however, Slp4-a/granuphilin-a functions as a negative regulator of dense-core vesicle exocytosis [37, 38, 50, 86, 92–95], because deletion of Slp4-a/granuphilin-a increases hormone (e.g., insulin) secretion despite reducing the number of docked dense-core vesicles [50, 93]. The mechanism by which Slp4-a/granuphilin-a increases the number of inert plasma membrane-docked dense-core vesicles at the molecular level is a matter of controversy [86, 92–96], but it is likely to regulate hormone secretion through interaction with Munc18-1·syntaxin-1a complex.

Slp2-a is most abundantly expressed in the exocrine surface mucous cells of the mouse stomach, and deletion of Slp2-a reduces both the total number of mucus granules and the number of plasma membrane-docked granules [51]. Slp2-a is also expressed in pancreatic α-cells, and overexpression of Slp2-a promotes docking of glucagon granules to the plasma membrane [97]. In addition, Slp2-a has been found to be expressed on mature melanosomes in cultured melanocytes [60], and it promotes melanosome-anchoring to the plasma membrane through interaction of the C2A domain with phosphatidylserine [98].

Rab effectors associated with other steps in secretory vesicle traffic

Expression or deletion of certain other Rab3/27 effector molecules also affects secretory vesicle traffic, but the molecular mechanisms underlying the proc-

esses largely remain unknown. For example, deletion or functional ablation of Noc2 reduced stimulated secretion both in endocrine and exocrine cells [66, 99, 100]. Failure to secrete amylase and marked accumulation of secretory vesicles have been observed in the exocrine pancreas of Noc2 mutant mice [99]. MyRIP (myosin-VIIa- and Rab-interacting protein), also identified as the third member of the Slac2 family (Slac2-c) [62, 63], is abundantly expressed on secretory vesicles in neurons, some endocrine cells, and parotid acinar cells, and it positively regulates stimulated secretion [101, 102] (but see ref. [36], which reports that overexpression of MyRIP attenuates hormone secretion). By analogy to the function of Slac2-a/melanophilin in melanosome transport in melanocytes [28, 103–105] and of Slac2-c/MyRIP in melanosome transport in retinal pigment epithelial cells [62, 106–108], Slac2-c has been hypothesized to regulate recruitment and/or tethering of secretory vesicles to the release site through interaction with myosin Va [109], actin [36, 101, 102], and/or Sec6/8 [110]. Rab27A-binding protein Munc13-4, mutations of which cause familial hemophagocytic lymphohistiocytosis subtype 3 (FHL3) [111], controls lysosome secretion in hematopoietic cells [25, 68, 69]. In contrast to the docking defects observed in the CTLs of *ashen* mice (i.e., Rab27A-defective), lytic granules are clearly docked to the plasma membrane in Munc13-4-deficient CTLs [111], suggesting that Munc13-4 functions at a step after the docking step (presumably a priming step or fusion step). Expression of either Slp3-a or Slp5 (i.e., Ca^{2+}-dependent Slps [56, 112], but not of other Slps, in PC12 cells promotes stimulated hormone secretion by increasing the recruitment of dense-core vesicles to the plasma membrane after stimulation, not by increasing the number of plasma membrane-docked vesicles [93, 95], although the precise tissue distribution and function of Slp3-a and Slp5 protein remain largely unknown.

Rim1α and Rim2α have been established to be active zone proteins that regulate neurotransmitter release and synaptic plasticity through interaction with a variety of presynaptic active zone proteins, including Munc13-1 and CAST (reviewed in [113, 114]). Although Rim1α was originally described as an *in vitro* Rab3A-binding protein [70], Rim1α and Rab3A are distinctively localized at presynaptic active zone membranes and at synaptic vesicles, respectively, in contrast to the synaptic vesicle-localization of rabphilin described above. Curiously, interaction between endogenous Rim1α and Rab3A molecules *in vivo* has never been reported, and all the binding experiments have been performed by using recombinant proteins [6, 70, 71, 115–117]. In addition, expression of the Rab3A-binding-deficient mutant of Rim1 is still capable of increasing stimulated hormone secretion by chromaffin cells [115] and PC12 cells [71], the same as the wild-type protein, suggesting that Rim1 modulates hormone secretion independent of Rab3A. Although Rim actually plays an important role in synaptic vesicle traffic [84, 118], it will be necessary to determine whether the Rab3A·Rim1/2α interaction itself is important to the regulation of neurotransmitter release in a future study (e.g., whether Rim1α/Rim2α DKO mice will be rescued by a Rab3A-binding deficient mutant of Rim).

Regulators of Rabs associated with secretory vesicle traffic

In contrast to Rab effectors that function in secretory vesicle traffic, very little is known about the specific regulators (GEF and GAP) for secretory Rabs. To date, two Rab3-GEFs, Rab3-GEP [119] and GRAB, guanine nucleotide exchange factor for Rab3A [120], and one Rab3-GAP [121] have been identified in mammals (Fig. 2B and C). Rab3-GEP has also been identified as DENN/MADD [differentially expressed in normal versus neoplastic (DENN)/mitogen-activated protein kinase-activating death domain (MADD)], and the N-terminal DENN domain [122] is thought to be required for Rab3 GEF activity [123]. Consistent with the roles of Rab3 members in the recruitment and docking of secretory vesicles to the release site described above, a greatly reduced number of synaptic vesicles docked to the presynaptic plasma membrane at the neuromuscular junction has been observed in Rab3-GEP KO mice (the total number of synaptic vesicles is also reduced) [124]. In mouse hippocampal neurons, however, Rab3-GEP has been shown to act as a post-docking step in synaptic vesicle exocytosis [125]. The *C. elegans aex-3* mutant, which encodes a Rab3-GEP orthologue, also exhibits a severe synaptic transmission defect [126], and AEX-3 has recently been shown to exert GEF activity toward both *C. elegans* Rab3 and Rab27 [80]. Although no mammalian Rab27-GEF has been reported thus far (but see [127], which reports constitutive Rab27A-GEF activity in unstimulated platelets), Rab3-GEP is likely to function as a dual GEF toward Rab3 and Rab27. Although deletion of Rab3-GEP/AEX-3 causes severe secretion defects, knockdown of another Rab3A-GEF, GRAB, increases stimulated hormone secretion by PC12 cells, whereas overexpression of GRAB decreases it [120]. GRAB contains a Sec2 homology domain, a guanine nucleotide-exchange domain for the yeast Rab GTPase Sec4 [128] (Fig. 2B). Rabin3, originally identified as a Rab3A-binding protein [77], also

contains a Sec2 homology domain and has been reported as Rab8 GEF [129].

Rab3 GAP consists of two subunits, catalytic subunit p130 [121] and noncatalytic subunit p150 [130], and it is concentrated in the presynaptic terminals (especially in the synaptic soluble fraction) [131]. Interestingly, mutations in the catalytic subunit of Rab3-GAP cause Warburg Micro syndrome, which includes severe mental retardation [132], and mutations in the non-catalytic subunit cause Martsolf syndrome [133], which includes mild mental retardation. Unlike the humans with these diseases, however, p130-deficient mice are viable and fertile, and their hippocampal CA1 region exhibits increased short-term plasticity, although Ca^{2+}-stimulated secretion by the synaptosomes is greatly impaired [134]. Rab3-GAP seems to act as a specific GAP toward Rab3 subfamily members, and not to act as a GAP toward other secretory Rabs, including Rab27A [135].

It has recently been proposed that the TBC (Tre-2/Bub2/Cdc16) domain functions as a GAP domain for small GTPase Rab (reviewed in [136]). Except for the Rab3-GAP described above, all Rab GAP proteins identified thus far contain the TBC domain. More than 40 distinct TBC domain-containing proteins have been identified in humans [136], and some of them have been shown to function as a specific Rab-GAP. Two TBC proteins, EPI64 and its homologue, FLJ13130 (Fig. 2C), exhibit GAP activity toward Rab27A *in vitro* and in cultured melanocytes, and they are localized in the actin-rich cell periphery [135]. However, whether these TBC proteins are involved in regulated secretion is unknown. Although it has been pointed out that the number of TBC proteins in humans is almost the same as the number of Rab subfamilies, it remains to be determined whether TBC-domain-containing Rab3-GAP is present in mammals in addition to Rab3-GAP (p130+p150).

Concluding remarks and perspectives

Recent studies point to the existence of a functional group of Rab isoforms that are specialized for regulated secretion (i.e., 'secretory Rabs', including Rab3A/B/C/D, Rab26, Rab27A/B, and Rab37) (dotted box in Fig. 1B). Rab26 and Rab37 are presumed to be involved in specialized secretion events in specialized cell types [29–31], whereas Rab3 and Rab27 seem to function as general regulators of stimulated secretion in a variety of secreting cells [7, 8, 20, 22]. Analysis of Rab3A/B/C/D quadruple KO mice (or Rab27A/B DKO mice) has shown that some of these secretory Rabs, i.e., Rab3 subfamily members (or Rab27 subfamily members), are redundant in some forms of regulated secretion [10, 18, 20]. Since simultaneous knockdown of Rab3A and Rab27A in PC12 cells reduced stimulated hormone secretion even more than single knockdown [24] and some effectors are capable of binding both Rabs [6, 28], Rab3 and Rab27 are likely to have overlapping rather than completely redundant roles in regulated secretion. Such overlapping roles of Rab3 and Rab27 may account for the mild phenotype of Ca^{2+}-triggered neurotransmitter release by quadruple Rab3s KO mice [10] (or the absence of abnormal brain function in Rab27A/B DKO mice [18, 20]). Producing and analyzing Rab3/27-deficient animals in the future will clarify the functional overlap and diversity of two phylogenetically related Rabs, Rab3 and Rab27, in regulated secretion. Additional studies will also be needed to determine how Rab, Rab effector, and Rab regulators work together to control specific steps in regulated secretory vesicle traffic at the molecular level.

Note added in proof. While this review was being prepared for publication, possible involvement of Slp1 and Slp2-a in CTL secretion was reported (see [137] for details).

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