CHAPTER 5

Masterclass with Rab3 and Rab27: Orchestrating Regulated Secretion

François Darchen* **and Claire Desnos**

CNRS/Université Paris Descartes, France

Abstract: A subset of Rab GTPases have instrumental roles in the biogenesis, trafficking, docking and exocytosis of secretory granules, secretory lysosomes and synaptic vesicles. The four Rab3 isoforms and the two Rab27 isoforms are the main members of this family of "secretory Rabs." Redundancy between the isoforms and between the Rab3 and Rab27 proteins has made the functional characterization of these proteins difficult. Data collected from different cell types suggest that the main role of Rab27 is to promote the recruitment of secretory vesicles at the release sites, while that of Rab3 is to control the number of ready-to-fuse vesicles. However, many observations that cannot be incorporated into this simplified scheme suggest that Rab3 and Rab27 have overlapping functions at different stages of the "life cycle" of secretory vesicles. Consistent with this, while some effector molecules are specific for Rab3 or Rab27, several interact with both of them.

Keywords: Rab3, Rab27, Exocytosis, Secretory Vesicle, Membrane Traffic.

1. INTRODUCTION

All eukaryotic cells have developed a secretory pathway that targets proteins synthesized in the endoplasmic reticulum and shuttles them through the Golgi apparatus to the plasma membrane (PM) or external milieu. In neurons and cells of the endocrine and exocrine glands, this secretory pathway has evolved to achieve the spatially and temporally controlled release of secretory products. In "regulated secretion", a multi-step process, the release of secretory products is triggered by a signal, generally calcium entry into the cell. In endocrine cells, for instance, water-soluble hormones are packaged into secretory granules (SGs) in the *trans*-Golgi network (TGN). SGs are transported along the microtubules to the cell periphery and interact with the actin-rich cortex until they attach to the PM (docking). Then, in a process called priming, Soluble NSF Attachment Protein Receptor (SNARE) complexes are formed between the fusing membranes and makes SGs ready to fuse with the PM. The process of regulated secretion is arrested at this stage until exocytosis is triggered by an elevation in calcium levels (Fig. **1A**) [1-3]. Synaptic vesicles (SVs) are formed at the synapse and undergo cycles of exocytosis and endocytosis (Fig. **1B**).

The secretory process highlights several points at which regulation is required. Specifically, how can a vesicle with a selective set of components be created? How is a vesicle transported to its release site? Finally, how is the vesicle attached to its target membrane, and how is exocytosis coupled with the stimulus? The Rab GTPases appear to be essential for these processes. By sequentially recruiting diverse effectors, they drive secretory vesicles from their biogenesis to their final exocytosis, while other Rabs are involved in the vesicle recycling steps.

Rab GTPases constitute a large family of membrane trafficking regulators (with approximately 60 isoforms in humans). Within this family, a subset of phylogenetically related Rab proteins have been linked to regulated secretion [4]. These "secretory Rabs" include Rab3a, b, c and d; Rab27a and b; Rab4a; Rab11b; Rab26; and Rab37. In this chapter, we review the data on Rab3 and Rab27, secretory Rabs that are widely distributed and that have been well characterized.

***Address correspondence to François Darchen:** CNRS/Université Paris Descartes UMR 8192, Centre Universitaire des Saints-Peres, 47 rue des Saints-Peres, 75006 Paris, France; Tel: +33-1-70-64-99-16; Fax: +33-1-70-64-99-13 ; E-mail: francois.darchen@parisdescartes.fr

Guangpu Li and Nava Segev (Eds)

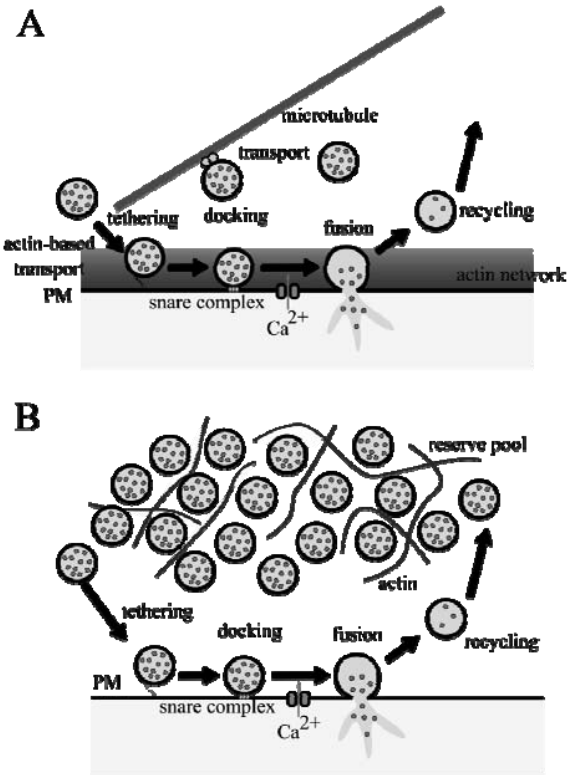


Figure 1: The final steps of the regulated secretory process. (**A**) In endocrine cells, secretory granules (SGs) are transported along microtubules from the TGN to the cell periphery. The subplasmalemmal actin meshwork is depicted in burgundy. Actin-binding proteins, including myosin Va, promote the dissociation of the SGs from the microtubules and the transport of the SGs to the plasma membrane (PM). Tethering factors capture SGs at the PM before they are stably docked. A priming reaction, during which SNAREs assemble, makes SGs ready to fuse. Exocytosis is triggered by stimulus-dependent calcium entry. (**B**) At the synapse, synaptic vesicles (SVs) undergo local cycling through exocytosis and endocytosis. At the active zone, clusters of SVs are stabilized *via* interactions with actin filaments and synapsins and form a reserve pool of vesicles. For clarity, an SV cluster is depicted at a non-proportional distance from the PM. The SVs undergo reactions similar to those of the SGs before fusing with the presynaptic membrane to release neurotransmitters. Tight temporal coupling between the stimulus and the neurotransmitter release is mediated by the close apposition of the SVs to the calcium channels.

2. RAB3 AND RAB27 ARE IMPLICATED IN REGULATED SECRETION

2.1. Multiple Rabs are Present on Secretory Vesicles

Four highly homologous *Rab3* genes are found in mammals [5]. Rab3a and c are mainly expressed in the brain and neuroendocrine cells. Rab3b is present at low levels in neurons but is abundant in the pituitary gland and epithelial cells [6-8]. Rab3d is expressed at very low levels in the brain but is enriched in adipocytes, muscle, lungs and in the exocrine pancreas [9, 10]. Despite these differences in tissue distribution, several isoforms of Rab3 are often simultaneously expressed in single cells [7, 11].

Rab27a is also expressed in a broad range of secretory cells of endocrine and exocrine glands, in immune cells and in melanocytes [4]. Using mice expressing *LacZ* under the control of the *Rab27b* promoter, Gomi and collaborators showed that the Rab27b expression pattern differs from that of Rab27a [12] but is still restricted to specialized secretory cells. For instance, in the pancreas, the expression of Rab27a is dominant in the islets and that of Rab27b is dominant in acinar cells. Notably, Rab27b, but not Rab27a, is expressed in neurons from many regions of the brain [13]. Rab27b is also expressed in cells (such as those of the skin, esophageal epithelium and bladder) that increase their surface area, most likely by exocytosis, in response to mechanical stress. The reason for this diversity is unknown, and this diversity is surprising because

knockout studies have revealed that Rab isoforms can generally compensate for the lack of the other isoforms (see below).

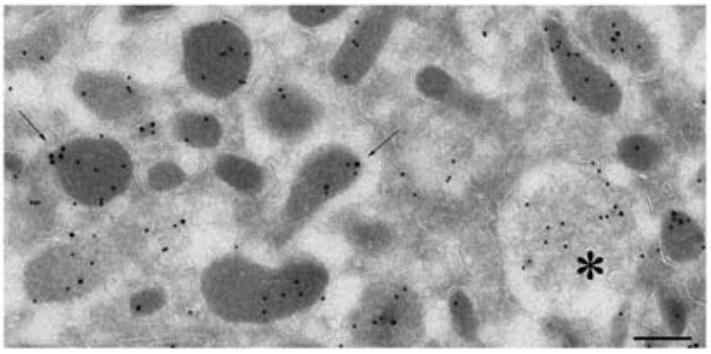


Figure 2: The association of Rab27a with secretory granules. Ultrathin cryosections of adrenal chromaffin cells were double immunogold-labeled for Rab27a (15-nm gold particles) and chromogranin A/B (10-nm gold particles), a component of the granule matrix. Rab27A localizes to the chromogranin-positive dense core granules (arrows) and to immature granules (star). Bar, 200 nm. Originally published in [19].

Many studies have identified the localization of Rab3 and Rab27 to secretory vesicles (Fig. **2**). Rab3a and Rab3c are associated with synaptic vesicles in neurons [14-16], while Rab3a, Rab3b, Rab3c, Rab27a and Rab27b are found on SGs in neuroendocrine cells [13, 17-22]. Rab3d is associated with the granules of exocrine cells [10, 23-25]. Rab27 is also found on secretory organelles such as melanosomes [26], secretory lysosomes [27, 28] multi-vesicular bodies [29] and Weibel-Palade bodies [30]. In *Caenorhabditis elegans*, Rab27 colocalizes with Rab3 in neurons. Rab27 is mislocalized in *C. elegans aex-3* mutants (which are defective in the exchange factor for Rab3 and Rab27) and in animals deficient in the kinesin UNC104, suggesting that Rab27 is associated with SVs [31]. The association of Rab27b with synaptic vesicles has been recently demonstrated [32, 33]. Fukuda and colleagues fused a collection of Rabs to GFP and expressed them in neuroendocrine PC12 cells. Only Rab3 (a-d), Rab27 (a, b) and Rab37 showed a specific association with SGs [34].

2.2. Rab3 and Rab27 Regulate Secretion

2.2.1. Rab3

Many studies using antisense oligonucleotides or overexpressed Rab3 constructs have implicated Rab3 in regulated exocytosis [6, 7, 21, 22, 35-41]. Positive and negative roles for Rab3 in secretion have been reported, and no consensus on its role has emerged from these studies. Redundancy between Rabs is certainly one of the reasons for the difficulty in solving the "Rab3 issue". Another reason is that these studies were performed in different cell types using different methods, and each study highlighted one aspect of Rab3 function.

Knockout (KO) mouse lines for each of the four *RAB3* genes have been generated. The single Rab3 KO mice are viable and fertile [42, 43], and only mild phenotypes were found in these mice. The circadian period of locomotor activity is shortened in Rab3a KO mice and in *earlybird* mice, which contain mutations in *RAB3a* [44]. Rab3a KO mice perform well in memory tasks and display only mildly increased locomotor activity, suggesting a role for this gene in behavioral stability [45]. In the hippocampal neurons of Rab3a KO mice, the amplitude of the excitatory postsynaptic potential (EPSP, a measure of the postsynaptic response to released neurotransmitters) is similar to [46, 47] or even greater than [48] that found in control animals. These data suggest that, despite the status of Rab3a as the most abundant Rab protein on SVs, Rab3a is not essential for synaptic vesicle exocytosis but rather is a modulator of the release process. Consistent with this, several forms of short-term or long-term synaptic plasticity are affected by Rab3a deletion: paired-pulse facilitation is increased in cultured hippocampal neurons [48], there is a modest synaptic depression of hippocampal CA1 synapses during the repetitive stimulation of Schaffer collaterals [47], and long-term potentiation (LTP) is abolished in hippocampal CA3-mossy fiber synapses [46].

The inactivation of a single *RAB3* gene is well tolerated. However, quadruple Rab3 KO mice die immediately after birth because they cannot breathe [43]. Viability is restored by a single Rab3a allele or by two Rab3b or Rab3c alleles and is partially restored by two Rab3d alleles. These observations clearly indicate that the Rab3 isoforms can compensate, at least partially, for the lack of the others. Strikingly, only mild synaptic defects have been observed in Rab3 abcd quadruple KO mouse embryos. The amplitude of the evoked response was reduced by only 30% in neuronal cultures, and spontaneous release events (miniatures) were not significantly modified [42, 43, 48]. In *C. elegans*, in which a single *RAB3* gene exists, *RAB3* deletion also causes relatively minor synaptic defects [31, 49].

Although Rab3 is not mandatory for exocytosis in some cells, it seems to be essential in others. For example, reducing Rab3b expression in anterior pituitary cells [6] or Rab3a in intermediate pituitary cells [7] with antisense oligonucleotides severely inhibited secretion. Furthermore, the inability of the quadruple Rab3 KO mice to breathe [43] suggests that exocytosis is severely impaired at the neuromuscular junction. The simplest explanation would be that another Rab GTPase has redundant activity that can compensate, at least partially, for the lack of Rab3. The following findings support this possibility: (i) mutations in *GDI1,* which encodes αGDI, a factor that delivers Rabs:GDP to the membranes and extract them for recycling (Box 1), are more severe than the deletion of Rab3 [50-52]; and (ii) deletion of the Rab3 exchange factor, Rab3-GEF (Box 1), causes a marked reduction in the evoked neurotransmitter release in mice [53, 54] and *C. elegans* [55]. These findings suggest that these Rab3 exchange factors activate another Rab. Several lines of research indicate that this other Rab is Rab27.

Box 1: The life cycles of Rab3 and Rab27

Rabs oscillate between GDP- and GTP-bound forms. GDP-bound Rabs are mostly found in the cytosol in complex with Rab-GDI (guanine nucleotide dissociation inhibitor), whereas Rab-GTP complexes are anchored in membranes *via* a geranylgeranyl moiety. Rab-GDI is instrumental in the "life cycle" of Rabs: it can extract or deliver Rab:GDP to membranes [175, 176]. Mutations in *GDI1*, which encodes GDIα, the most abundant form of GDI in the brain, cause X-linked nonspecific mental retardation [50]. *GDI1* KO mice have impaired short-term memory and display altered social behavior [51]. The relatively severe synaptic defects caused by GDIα deletion may be related to improper recycling or delivery of Rab3 and Rab27 or may be due to the inhibition of endocytic Rabs, as suggested by the reduced numbers of synaptic vesicles found in nerve terminals [177]. Park and colleagues [178] found that calmodulin (CaM) is able to extract Rab3 from synaptosomal membranes in a Ca^{2+}-dependent manner, but the physiological consequences of this interaction are unclear. The delivery of Rabs to membranes is facilitated by GDI displacement factors (GDFs) [175]. In the case of Rab3, the only known molecule with such activity is synapsin, which competes with Rab-GDI for Rab3 binding [127, 128].

The equilibrium between Rab:GDP and Rab:GTP depends on the intrinsic GTPase activity of the Rab proteins, which is low in the case of Rab3 and Rab27 [179, 180], and on the respective activities of Rab-GEF (which catalyses the exchange of GTP for GDP) and Rab-GAP (which catalyses the hydrolysis of GTP). Rab3-GEF (also called DENN/MADD) is also active against Rab27, but not against other Rabs [181, 182]. Rab3A, but not Rab27A, cycles rapidly between the granule membrane and the cytosol [74] and readily dissociates from membranes during active secretion [176, 183]. Rab27 may be stably bound to GTP and rapidly reactivated by a GEF or more resistant to GDI-mediated extraction [184]. In an attempt to correlate the nucleotide status of Rab3a in synaptosomes with the secretory process, Stahl *et al.* observed a marked increase in the GDP/GTP ratio on Rab3a after strong stimulation of exocytosis [124]. These results indicate that GTP hydrolysis by Rab3 and Rab27 is somehow coupled to the secretory activity.

Rab3- and Rab27-specific GTPase activating proteins (Rab3-GAP and EPI64/Rab27-GAP) have been described [180, 185, 186]. Rab3-GAP consists of two subunits, the catalytic subunit p130 and the noncatalytic subunit p150. Mutations in the gene encoding p130 cause Warburg Micro syndrome, which is characterized by ocular and neurodevelopmental defects and severe mental retardation [187]. P130 null mice are viable and fertile [188]. Although no obvious defect in basal neurotransmission has been reported, glutamate release from synaptosomes is severely impaired in these animals. The data indicate that GTP hydrolysis by Rab3 is rate-limiting at some stage in the secretory process. How Rab3-GAP activity is regulated during secretion is not known. Calcium ions do not

Box 1: cont….

change the activity of p130 [179]. The Rab3-GAP binding site involves the switch I region of Rab3 and overlaps with the rabphilin-binding domain. Dissociation of the Rab3-effector interaction upon calcium entry may thus allow Rab3-GAP to trigger GTP hydrolysis.

Although GEFs and GAPs are thought to function by regulating the nucleotide cycle of the Rabs, they may exert other functions. For instance, Rab3-GEF interacts with the kinesins Kif1Bb and Kif1A, regulates axonal transport of synaptic vesicle precursors [189] and is involved in neuroprotection [190].

2.2.2. Rab27

RAB27a was identified as the gene mutated in Griscelli syndrome type II [27, 56] and in the coat-color mutant *ashen* [57]. Griscelli syndrome (GS; MIM 214450) type II is an often fatal disease combining albinism and hemophagocytic syndrome, an uncontrolled activation of lymphocytes and macrophages [58]. At the cellular level, Rab27a deletion induces defects in melanosome transport and lysosome exocytosis in cytotoxic T lymphocytes [27, 28, 59] (see also the chapter by J. Hammer in this eBook). Dendritic cells from *ashen* mice demonstrate a deficiency in antigen cross-presentation caused by the premature acidification of the phagosomes [60]. The impaired delivery of lysosome-related organelles containing the NADPH oxidase Nox2 to the phagosome is responsible for this defect. In addition to these defects, moderate glucose intolerance has been found in *ashen* mice, which correlates with decreased glucose-induced insulin secretion [61]. Despite the expression of Rab27b in many secretory cells and neurons [12], the only phenotype caused by Rab27b deletion is a hemorrhagic tendency, which has been attributed to the reduced density and exocytosis of the dense granules in platelets [62]. The phenotype caused by *RAB27A* and *RAB27B* inactivation simply combines the defects found in the single KOs. Again, redundancy between the Rabs and especially between Rab3 and Rab27 may explain the mild phenotype of *RAB27* deletion. Nevertheless, interfering with Rab27a or Rab27b function impairs stimulus-dependent secretion in neuroendocrine cells [12, 13, 19, 34, 63, 64], platelets [62, 65], pancreatic acinar cells [66] and neutrophils [67-69]. RNA silencing of Rab27a or Rab27b also impairs the secretion of exosomes, the luminal vesicles contained in multi-vesicular bodies [29]. Conversely, overexpression of Rab27a promotes secretion in endocrine cells [13, 20] and the secretion of prostate-specific markers [70].

2.2.3. Synergy between Rab3 and Rab27

Rab27b is expressed in neurons [12] and is associated with synaptic vesicles [32, 33]. In agreement with a role for Rab27 in SV exocytosis, it was found that an anti-Rab27 antibody inhibited neurotransmitter release at the squid giant synapse and impaired the recovery of the releasable pool of SVs after the stimulus-induced exhaustion of this pool [71]. In *C. elegans*, the inactivation of *RAB27* in the *aex-6* mutant causes minor synaptic defects, as do mutations in *RAB3* [49]. However, both spontaneous and evoked neurotransmission are more severely impaired in the *RAB3*; *RAB27* double mutant than in the *RAB3* or *RAB27* single mutants [31]. The authors of this study also found that the exchange factor aex-3 activates both Rab3 and Rab27 and that inactivating *aex-3* causes a severe phenotype similar to that of the *RAB3*; *RAB27* double mutant. These data suggest that Rab3 and Rab27 have overlapping functions in secretion, and one may compensate for the lack of the other. Thus, it will be interesting to cross Rab3- and Rab27-deficient mice to analyze the resulting effects in neurons and other secretory cells.

3. REGULATION OF SECRETION STEPS BY RAB3, RAB27 AND THEIR EFFECTORS

3.1. Biogenesis and Maturation of Secretory Vesicles

Secretory granules are formed in the *trans*-Golgi network as immature SGs. They are converted to mature SGs through a process that combines membrane fusion and fission, matrix condensation and the retrieval of various components [72]. A 50% reduction in the number of SGs has been observed in the adrenal chromaffin cells of the quadruple Rab3 KO mice [73], suggesting a role for Rab3 in granule biogenesis. However, an increased homotypic fusion of immature SGs or uncontrolled constitutive exocytosis may also account for the observed reduction in the number of SGs, and there is no direct evidence that Rab3 controls the budding of immature

SGs from the *trans*-Golgi network. Both Rab3 and Rab27 are found on immature SGs [19, 74]. However, live-cell imaging has revealed that GFP-Rab3a and GFP-Rab27a associate with immature SGs only after a lag period of approximately 20 min after the release of these proteins from the *trans*-Golgi network [74], arguing against their involvement in granule formation. Rab3 and Rab27 may nevertheless contribute to granule maturation. Indeed, Rab3d deletion causes an increase in the size of SGs in pancreatic acinar cells and in the parotid gland, with the volume being doubled [75]. Similar results were obtained in PC12 cells upon expression of dominant negative Rab3a or Rab3d mutants [76] and in Rab27b-deficient platelets [62]. The size of the multivesicular endosomes was also increased in HeLa B6HA cells upon RNA silencing of Rab27a or its effector, granuphilin [29], perhaps as a result of increased homotypic fusion. Myosin Va, probably recruited by Rab27 and its effector MyRIP (myosin- and Rab-interacting protein; also called Slac2c), participates in SG maturation [77]. The proposed mechanism is the facilitation, by the tethering of the vesicle membrane to actin, of the retrieval of components pulled by microtubule-based motors.

3.2. Recruitment of Secretory Vesicles at Release Sites

3.2.1. A Role for Rab3 and Rab27 in Targeting Secretory Vesicles to Release Sites

In endocrine cells, SGs are transported along microtubules from the *trans*-Golgi network (TGN) toward the cell periphery [78], where they accumulate in the actin-rich cortex [79]. To become available for release, SGs must cross the actin cortex and interact with the plasma membrane (Fig. **1A**). The actin-rich cortex has long been viewed as a barrier to fusion [80], but it is also important for vesicle recruitment at release sites [81]. Indeed, impairing the interaction of SGs with actin (by interfering with Rab27, MyRIP or myosin Va; see below) induces a redistribution of SGs from the actin-rich cell cortex to the perinuclear area [82] (Fig. **3**). Most likely, this is due to the fact that without the help of actin-binding proteins and actin-based motor molecules such as myosin Va, minus-end directed microtubule-based motors have an advantage over plus-end directed ones and drive SGs away from the PM because of the polarity of the microtubule network [81]. Presynaptic terminals also contain an actin-rich cytomatrix that controls the mobility of SVs. SVs interact with F-actin *via* synapsin, and these interactions are thought to mediate the formation of a reserve pool of SVs and its availability for release (Fig. **1B**). Indeed, deletion of synapsin reduces the number of vesicles found at the synapse [83, 84].

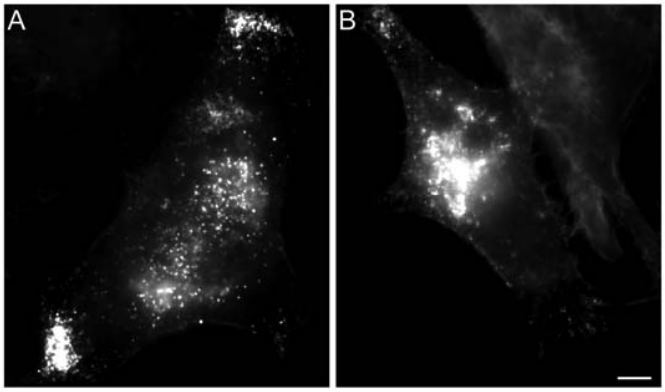


Figure 3: MyRIP RNA silencing impairs secretory granule distribution. Enterochromaffin BON cells were transfected with a control siRNA duplex (**A**) or with siRNAs direted to MyRIP (**B**) and with GFP-tagged neuropeptide-Y, a secretory granule marker. In control cells, SGs display a marked enrichment at the cell periphery, particularly in cell extensions. However, in cells treated with MyRIP siRNAs, SGs accumulate in the perinuclear region. Bar, 3 μM.

Rab3, Rab27 and several of their effectors have been implicated in the recruitment of secretory vesicles at the cell periphery [34, 49, 69, 82]. Notably, several of these effectors (MyRIP, rabphilin and synapsin) interact with actin or actin-binding proteins. Controlling the association of vesicles with actin filaments to maintain them in the vicinity of the release sites, to control their availability for release or to power vesicle movement along actin tracks may be a common theme of those effectors.

Attaching vesicles to the plasma membrane, referred to as docking, is another important step in the secretory process. Secretory vesicle docking is impaired in Rab27-deficient cytotoxic T lymphocytes [28, 85], pancreatic β-cells [64], pituitary cells [12] and in squid synapses loaded with anti-Rab27 antibodies [71]. The docking of multivesicular endosomes is also impaired by the knockdown of the Rab27a or Rab27b genes [29]. There is also evidence that Rab3 is involved in secretory vesicle docking. SG docking is increased upon Rab3 overexpression [76, 86] and decreased in PC12 cells expressing Rab3a or Rab3d dominant negative mutants [76]. Docking is unchanged, however, in the chromaffin cells of quadruple Rab3 KO mice [73], which may be accounted for by the remaining expression of Rab27. Although SV docking is not modified in hippocampal neurons from Rab3a null mice, the increase in SV docking that normally follows calcium elevation is abolished in these cells [87]. Moreover, SV docking is reduced in the neuromuscular junctions of Rab3a null mice [88]. Taken together, the data suggest that Rab3 facilitates SV attachment to the PM. The direct interaction between Rab3 and munc18-1 has been proposed to mediate this effect, but the association of Rab3 with wild-type munc18-1 is weak and independent of the nucleotide-bound status of Rab3 [89].

Recent evidence suggests that a loose mode of vesicle attachment, referred to as tethering, precedes docking. The transition between the two states has been visualized by total internal reflection fluorescence microscopy (TIRFM) as a 20-nm move toward the PM that occurs a few seconds before exocytosis [90]. The SNARE proteins syntaxin 1 and SNAP-25 are anchored in the PM and, with the help of Munc18 and Munc13, can assemble with synaptotagmin-1 on the vesicle [91-93]. This complex is likely to mediate docking (rather than tethering), given the small size of SNAREs. Consistently, SVs are not docked in Munc13-deficient neurons but remain attached to the PM by small filaments [94]. It is unknown whether Rab3 and Rab27 are involved in SNARE-mediated docking or in tethering. Large proteins act as tethering factors in the secretory and endocytic pathways [95]. The Rab effectors MyRIP, rabphilin and granuphilin (see Box 2) may have a similar function in SG tethering.

Box 2: Rab3 and Rab27 Partnership

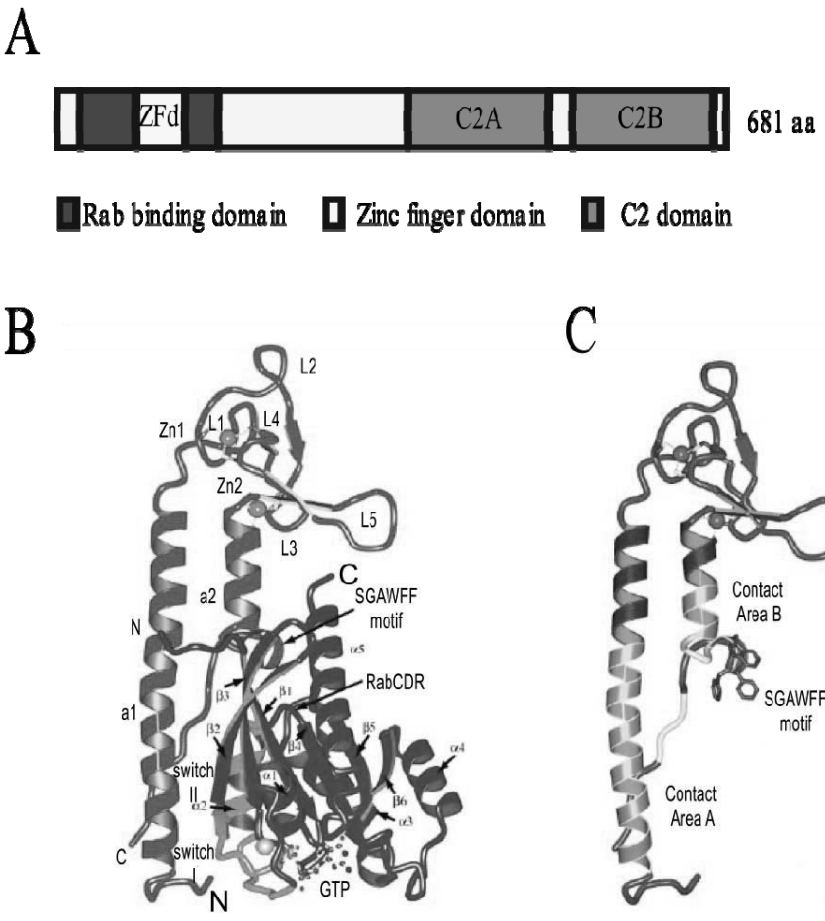
The family of Rab3 and Rab27 effectors is comprised of a dozen members. With the exception of Munc13-4, the effectors contain a helical Rab-binding domain. Some of them contain, in addition, a zinc finger and an aromatic motif (SGAWFF in rabphilin) that contribute to the interaction. Most of the effectors interact with Rab27, but only a subset bind to Rab3. The C2 domains, which differ between the different partners, and other regions allow the effectors to interact with different molecules. The main features are summarized in the table below without distinction between the Rab isoforms (see [4, 191]).

Name	Rab3 binding	Rab27 binding	Helical RBD	Zinc finger	C2 domains	Interactions	References
Rabphilin	‡	‡	+	+	+	Ca2+, phospholipids, SNAP25, a-actinin, rabaptin 5	[47, 121, 122]
Rim	+		+	+	+	calcium channels, liprins, RimBP, Munc13, CAST, piccolo, ELKS, synaptotagmin, SNAP-25, Scrapper	[156, 192, 193]
Slp1/JFC1		‡	+		+	NADPH oxidase, PI(3,4,5)P3	[67]
Slp2-a		‡	+		+	PS, PIP2	[120, 194, 195]
Slp3-a		+	+	+	+		[196]
Slp4a/granuphilin	+	‡	+	+	+	syntaxin-1a, Munc18, PS, PIP2	[13, 20, 116]

Box: 2 cont….

Slp5		+	+	+	+	[197]
Slac2-a/melanophilin		‡	+	+	myosin Va, actin, EB1	[97-99, 198]
Slac2-b		+	+			[199]
MyRIP		‡	+	+	myosin Va, myosin VIIa, actin, sec6, sec8, AKAP*	[104, 105, 107]
Noc2	+	‡	+	+		[142, 144, 192]
Munc13-4		+		+		[65, 148, 200]
Synapsin	‡				Actin	[127, 128]
Munc18-1	+				syntaxin-1, granuphilin	[89]

* A-kinase anchoring proteins; ‡ Interaction demonstrated with endogenous proteins



Rabphilin is the prototypical Rab3 and Rab27 effector. It was the first Rab3 effector to be identified [121], and it contains several features that are also found in many other effectors. Rabphilin consists of an N-terminal helical Rab-binding domain (RBD), which contacts Rab3 or Rab27, a zinc finger motif, a proline-rich linker region, and two C2 domains (Panel A). The C2B domain binds calcium ions [201] and phospholipids (phosphatidylserine and phosphatidylinositol 4,5-bisphosphate) with high Ca^{2+} affinity [134, 202]. It also associates with the SNARE protein SNAP-25 in a calcium-insensitive manner [134, 135, 137, 202]. The structure of rabphilin complexed with Rab3a has been characterized at a 2.6-Å resolution [203]. In panel B (reprinted from

Box: 2 cont….

Ostermeier and Brunger [203] with permission), residues 40 to 170 of rabphilin are shown in red, and Rab3a is shown in blue. The Rab3a:GTP structure is similar to the catalytic core of other G proteins, with a central six-stranded ß sheet surrounded by α–helices. The switch I and switch II regions of Rab3a (whose conformations change upon GTP hydrolysis; in green in panel B) span residues 49 to 57 and 80 to 95, respectively. The N-terminal domain of rabphilin consists of two antiparallel α–helices separated by a subdomain with two Zn2+-binding sites and several interspersed loops. The second, shorter α-helix ends in a turn composed of a SGAWFF motif (conserved in the sequences of rabphilin, Noc2, Rim and MyRIP) and is followed by an extended polypeptide segment. The structure reveals two distinct interfaces between Rab3a and rabphilin (in yellow in panel C, also reprinted from Ref. [203]). Rabphilin contacts the switch regions of Rab3a through part of its long α-helix α1 and its C-terminal extended segment. The second contact area involves the C-terminal end of rabphilin α–helix α2, the adjacent SGAWFF motif, and regions of Rab3a called RabCDRs (complementarity determining regions), which consist of residues 19 to 22, 94 to 96 (the α2β4 loop), 124 to 128 (the α3β5 loop), and 182 to 193 (the C-terminal half of helix α5). In contrast to the switch regions, RabCDRs exhibit a high degree of sequence variability within the Rab family. RabCDRs could be primarily involved in providing binding specificity, whereas the switch regions might function as a Rab conformation sensor.

3.2.2. Rab Effectors in the Recruitment of Secretory Vesicles to Release Sites

MyRIP. Rab27 can recruit myosin Va *via* melanophilin, and this tripartite complex mediates the interaction of melanosomes with actin, the retention of melanosomes in actin-rich areas of the cell and their transfer to keratinocytes [96-99]. Disruption of this complex is responsible for the partial albinism observed in Griscelli syndrome and in *ashen*, *leaden* and *dilute* coat-color mutants [27, 57, 100-103]. *Via* MyRIP, which has strong similarity to melanophilin, Rab27 can also recruit actin-based motors onto SGs, retinal melanosomes and Weibel-Palade bodies [19, 63, 82, 104-106]. MyRIP interacts with Rab27:GTP *via* an N-terminal helix and with myosin VIIa or myosin Va *via* a central region [19, 104, 105, 107-110]. In addition, MyRIP interacts directly with actin *via* its C-terminal region. This region restricts SG mobility within the actin cortex [19] and may contribute to SG docking (CD and FD, unpublished data). The interactions of MyRIP with sec6 and sec8, two components of the exocyst complex, and the A-kinase-anchoring protein (AKAP) have also been reported [111].

Impairing the function of MyRIP or myosin Va inhibits secretion [19, 82, 112-114, 204]. This inhibition is largely due to a reduction in SG recruitment to release sites (Fig. **3**). Indeed, lowering the levels of Rab27, MyRIP or myosin Va reduces the density of SGs near the PM as observed by TIRFM [34, 69, 82, 204]. Different mechanisms may contribute to this effect. (i) MyRIP and myosin Va promote the capture of SGs in the actin-rich cortex by competing with microtubule-based motors [82, 110]. (ii) MyRIP and myosin Va may power the directed motion of SGs along actin tracks toward the PM. Although there is no direct evidence for the myosin Va-driven movement of SGs through the actin cortex, myosin V motors have been shown to propel organelles along actin cables [81]. (iii) MyRIP and myosin Va promote SG tethering at the PM. Analysis of the trajectories of single SGs imaged by TIRFM revealed that impairing myosin Va or MyRIP activity reduced the occurrence of long-lasting (>10 s) immobilization events, which likely reflects the attachment of SGs to the PM [82, 204]. Strikingly, only a MyRIP knockdown reduces the characteristic time of immobilization. Taken together, these data suggest that (i) myosin Va promotes the retention of SGs in the actin cortex and powers their motion toward the PM, where they can find a docking platform, and that (ii) MyRIP promotes docking not only by recruiting myosin Va but also by stabilizing the attachment of SGs to the PM.

Granuphilin. Granuphilin is preferentially expressed in pancreatic β-cells [115] and pituitary cells [12] but is not expressed in neurons. It interacts with Rab27a and Rab3 [13, 20, 64, 116]. In addition, it directly binds to the PM-anchored SNARE protein syntaxin-1 [117, 118] and to Munc18-1 [116], which participate in SG docking [91-93, 116]. Overexpression of granuphilin redistributes insulin granules to the cell periphery [117], whereas deletion of granuphilin severely reduces the number of docked SGs, as measured by electron microscopy [12, 64].

Despite the severe reduction in SG docking caused by granuphilin deletion, there is no defect in SG secretion [12, 64, 117, 119]; rather, SG secretion is increased. Consistent with these results, overexpressed granuphilin inhibits secretion in several cell lines [19, 20, 116, 118]. Similar observations have been made for the Rab27 effector Slp2-a in pancreatic α-cells [120]. In addition, spontaneous SG secretion is increased

by the deletion of granuphilin [119]. Granuphilin thus increases the threshold for secretion and tightens the coupling between stimulation and secretion. Notably, granuphilin interacts with the closed conformation of syntaxin, which cannot pair with other SNAREs [118]. By preventing the assembly of a productive SNARE complex, granuphilin may function as a fusion clamp. However, granuphilin also exerts positive effects on secretion. For instance, in HeLa B6H4 cells, a granuphilin gene knockdown inhibits exosome secretion [29]. How the positive and negative effects of granuphilin are coordinated is unclear. One possibility is that fusion-incompetent granuphilin/syntaxin complexes recruit vesicles to the PM and are replaced by fusion-competent SNARE complexes upon stimulation of the secretory activity.

Rabphilin. Rabphilin (see Box 2) binds to Rab3:GTP [121] and Rab27:GTP [31, 122], and both Rab3 and Rab27 recruit rabphilin onto secretory vesicles [31, 122-124]. Overexpression of rabphilin in neuroendocrine PC12 cells increases the density of SGs detected in the vicinity of the plasma membrane by TIRFM [34], suggesting that rabphilin is involved in vesicle docking at the plasma membrane. However, secretory vesicles that are not physically attached to the PM have been observed by TIRFM [19, 34, 82]. Therefore, the role of rabphilin in docking must be further tested. Alternatively, rabphilin may promote the retention of vesicles in the actin-rich cell cortex. In support of this possibility, rabphilin interacts with α-actinin and ß-adducin, promotes the actin-bundling activity of α-actinin and stimulates the association of SGs with F-actin in the presence of α-actinin [125, 126].

Synapsin. An interaction between Rab3 and synapsin has been reported [127, 128]. Rab3 interferes with several properties of synapsin: its ability to bind actin filaments and induce their bundling as well as its ability to aggregate phospholipid vesicles. The interaction between synapsin and Rab3 likely occurs *in vivo* because the amount of Rab3a associated with SVs is reduced in synapsin I, synapsin II and synapsin I/II KO mice [127]. By reducing the attachment of SVs to actin-bound clusters, Rab3 may increase the availability of SVs for exocytosis. The functional consequences of the Rab3-synapsin interaction have not yet been formally tested. The decrease in the activity-dependent recruitment of SVs to the releasable pool observed in the Rab3a null cells might be accounted for by the Rab3-synapsin interaction [47]. However, an analysis of neuromuscular junctions from *Rab3a* and *synapsin II* double KO mice has suggested that Rab3a controls SV docking, whereas synapsin II regulates the size of the reserve pool of SVs [83].

3.3. Accumulation of a Pool of Ready-to-Fuse Vesicles

3.3.1. Rab3 and Rab27 in Vesicle Priming

At the plasma membrane, vesicles undergo a priming reaction that makes them ready to fuse upon the elevation of calcium levels. In molecular terms, priming most likely corresponds to the assembly of SNARE complexes, the core of the fusion machinery [2, 129]. The number of primed (ready-to-fuse) vesicles can be measured by combining the UV photolysis of caged calcium and time-resolved membrane capacitance measurements [130]. Upon UV delivery, the calcium concentration rises almost instantaneously from resting to saturation levels (Fig. **4A**), and the different kinetic components of the secretory response can be resolved. An exocytotic burst that corresponds to the fusion of primed vesicles and lasts approximately one second precedes a slower, sustained phase of release that corresponds to the sequential priming and fusion of the vesicles that were not primed at the time the stimulus was given.

Using this approach, it was found that Rab3 controls the number of primed vesicles: in adrenal chromaffin cells from quadruple Rab3 KO mice, the fast exocytotic burst was severely reduced, whereas the sustained phase was unaffected [73] (Fig. **4A**). Similar results were obtained in Rab3a null pancreatic β-cells [131]. The lack of change in the sustained component of the release demonstrates that Rab3 is not essential for exocytosis *per se*. In the absence of Rab3, SGs can reach the plasma membrane, undergo priming and exocytose, provided that calcium is elevated. If priming proceeds normally, the severe reduction of the exocytotic burst suggests that SGs cannot remain in the primed state. Therefore, Rab3 would be needed to stabilize priming. This represents a specific and important function, especially in neurons or neuroendocrine cells, in which the stimulus is transient and can operate only on primed vesicles to trigger exocytosis in less than a millisecond.

Other possibilities can be envisioned for Rab3 function. (i) Priming may proceed normally at high but not low calcium concentrations in the absence of Rab3. This possibility is unlikely because lowering Rab3a levels using antisense oligonucleotides increased rather than decreased priming at low calcium levels [37] (Fig. **4B**). (ii) A Rab3 deficiency may increase spontaneous release, leading to the continuous consumption of the pool of primed vesicles. This possibility is also unlikely because the frequency of miniatures was unchanged in quadruple Rab3 KO mice [43] and because the time constant of the fusion reaction was not modified in cells lacking Rab3 [73]. However, it would be worth testing this possibility carefully.

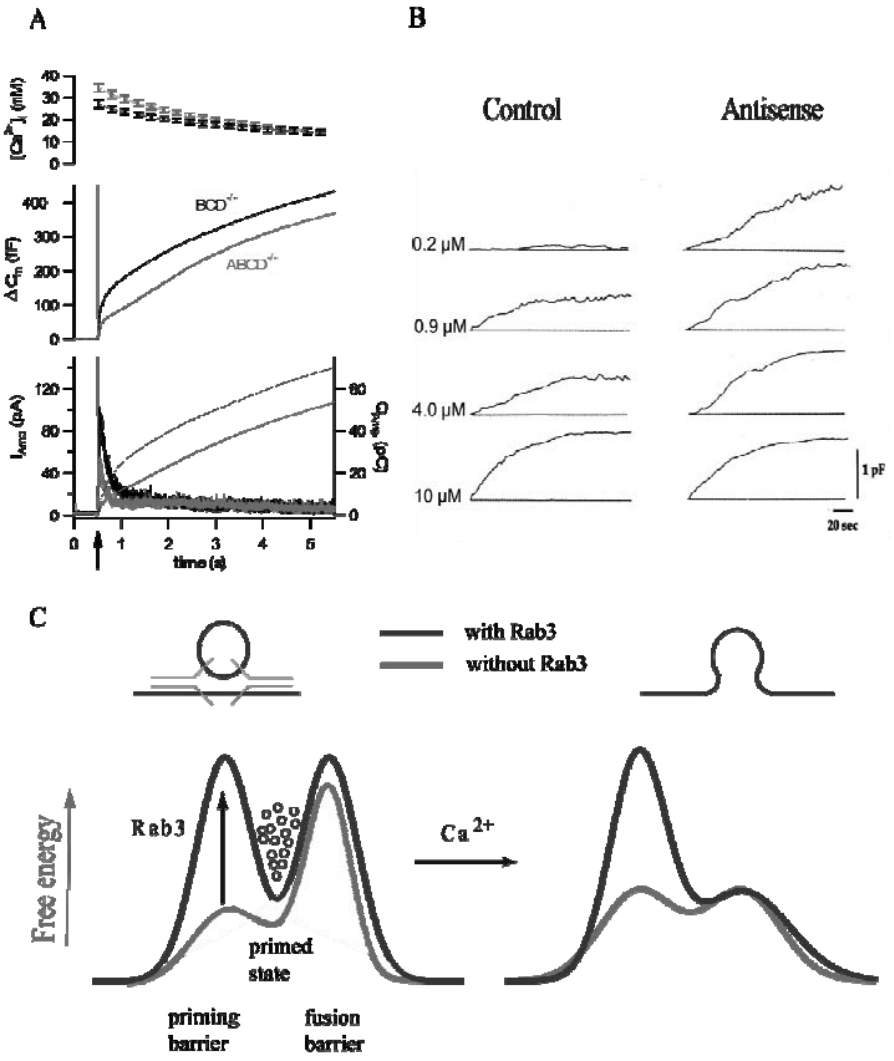


Figure 4: Rab3 controls the number of ready-to-fuse vesicles. (**A**) Deletion of Rab3 reduces the exocytotic burst but does not affect the sustained phase of release. Secretion was triggered in adrenal chromaffin cells by UV-flash-induced Ca^{2+} uncaging (at 500 ms; arrow). The secretory response was monitored by membrane capacitance recording (middle panel), and catecholamine oxidation was measured by amperometry (the lowest panel shows the amperometric current and the integrated trace). Intracellular Ca^{2+} levels were monitored using ratiometric fluorescence measurement of Fura dyes (upper panel). Analysis of the responses from control cells ($A^{+/+}BCD^{-/-}$ or $A^{+/-}BCD^{-/-}$, denoted here as $BCD^{-/-}$ and shown in black) and quadruple Rab3 KO cells ($ABCD^{-/-}$, shown in red) following Ca^{2+} uncaging shows that the size of the burst phase is strongly compromised in the quadruple KO, whereas the sustained phase is not dramatically modified. Reprinted from [73] with permission. (**B**) Lowering Rab3a levels increases the secretory response of adrenal chromaffin cells monitored by measuring changes in membrane capacitance (Cm). Exocytosis was elicited by dialyzing the cells through the patch pipette with solutions buffered at various Ca^{2+} concentrations (shown on the left). The Cm

traces obtained after injection into chromaffin cells of control (left) or antisense (right) oligonucleotides directed to Rab3a mRNA are shown. Whole-cell recordings were performed 5 days after microinjection. Reprinted from [37]. (**C**) The energy landscape of exocytosis. Two energy barriers must be overcome before exocytosis can proceed (blue line). The first barrier corresponds to the priming reaction and the second to membrane fusion. Primed vesicles are contained between these two peaks in a metastable state and are endowed with assembled SNARE complexes (depicted in the upper left panel). Calcium elevation lowers the energy barrier for fusion (downward arrow in the right panel), allowing primed vesicles to fuse (upper right drawing). Deletion of Rab3 (red line) is proposed to lower the priming energy barrier. This mechanism would account for the reduced exocytotic burst (panel A) due to the accumulation of fewer vesicles in the primed state in the absence of Rab3 because they undergo unpriming before the arrival of the stimulus. This mechanism would also explain the increase in secretory activity observed during sustained calcium elevation (panel B); in that case, vesicles could fuse once they were primed, and there would be no need for stable priming.

In the exocytosis energy landscape depicted in Fig. **4C**, the primed vesicles sit between the two peaks corresponding to the priming and fusion reactions. The barrier to fusion is lowered by the entry of calcium ions, which are thought to act on synaptotagmins [1-3, 129]. The increased unpriming rate found in Rab3 null mice suggests that the priming barrier is reduced in the absence of Rab3. A similar phenotype (unstable priming) was found in snapin KO mice [132]. Snapin binds to the SNARE protein SNAP-25 and may help to recruit synaptotagmin to the SNARE complex. Rab3 may thus cooperate with snapin to stabilize the fusion machinery. Priming would be facilitated if the priming barrier were reduced. Consistent with this, inhibiting Rab3a expression in chromaffin cells increased the responses to long-lasting calcium elevation (calcium dialysis *via* use of a patch pipette, a procedure that primarily probes the priming reaction) and repetitive stimulation [37, 38] (Fig. **4B**). Conversely, increased Rab3 levels should inhibit priming. Indeed, overexpression of Rab3 or GTPase-deficient Rab3 constructs inhibited secretion in several endocrine cell types and in neurons [22, 35-38, 40]. The strong inhibitory effect of GTPase-deficient Rab3 suggests that GTP hydrolysis by Rab3 may be rate limiting in the priming reaction and may be mandatory to terminate the priming reaction. Accordingly, GTP hydrolysis has been proposed to occur downstream of SNARE protein assembly because toxin-insensitive SNARE complexes accumulated in Aplysia neurons injected with GTPase-deficient Rab3 proteins [39].

The data supporting a role for Rab27 in priming are less compelling. Glucose-dependent insulin secretion was reduced in Rab27a-deficient *ashen* β-cells [61, 119], but the pool of ready-to-fuse insulin granules was not affected. Additionally, the initial response to a depolarization-induced Ca^{2+} influx was increased in Rab27a null cells, arguing against a role for Rab27 in priming [131]. The fact that the replenishment of the readily releasable pool (RRP) of SGs was slower in Rab27a-deficient cells than in control ones, may rather suggest a role for Rab27 in the priming reaction [131]. But because Rab27 deficiency impairs the recruitment and docking of insulin granules at the plasma membrane [119], priming may be reduced simply by mass action.

3.3.2. Rab Effectors in Secretory Vesicle Priming

Rabphilin. Rab3 and Rab27 have many effectors (see Box 2). However, it is currently unclear which of these effectors can mediate their priming effect. In endocrine cells, secretion is increased upon overexpression of rabphilin, whereas it is inhibited when rabphilin is impaired [133-135]. Rabphilin-deficient nematodes are lethargic, indicating a mild locomotor defect [136]. However, the rabphilin phenotype is more severe in the Rab3 null background, indicating that Rab27 and rabphilin function synergistically with Rab3 at the synapse [31]. There is also a synergistic interaction with SNARE proteins containing hypomorphic mutations, which suggests that the physical coupling of rabphilin and SNAP-25 is physiologically relevant [135, 137]. Inactivation of rabphilin in mice does not induce any detectable phenotype [138]. Basal neurotransmission, short-term synaptic plasticity and long-term potentiation are unchanged in the hippocampal synapses of rabphilin-deficient mice [138]. However, in cultured neurons from rabphilin null mice, an increase in the recovery rate of synaptic responses after depletion of the releasable pool of vesicles has been observed [137]. Altogether, the data suggest a role for rabphilin in recruiting vesicles to the releasable pool, but the molecular mechanisms of this recruitment are still unclear. Other studies have suggested a role for rabphilin in the coupling between exocytosis and endocytosis [139], which is eventually mediated by the interaction between rabphilin and the Rab5 effector Rabaptin-5 [140, 141].

Noc2. Like rabphilin, Noc2 binds to both Rab3 and Rab27, but it lacks the C2 domains (hence its name; see Box 2) [122]. Overexpression studies have demonstrated both positive and negative effects of Noc2 on exocytosis [142-144]. Inactivation of the *NOC2* gene in mice causes glucose intolerance, but only in animals stressed by water immersion [145]. This defect in insulin secretion is apparently due to an upregulation of Go or Gi signaling and cannot be taken as evidence for Noc2 being involved in the release mechanism. Noc2 deficiency has more profound effects in exocrine glands, causing an accumulation of SGs and a defect in amylase secretion, but the step at which secretion is arrested is not known [146]. An *in vitro* interaction between Noc2 and Munc13 has been reported [142], which suggests that Noc2 may function in priming because Munc13 is an important priming factor [147].

Munc13. *Munc13-4* mutations cause familial hemophagocytic lymphohistiocytosis type 3 [85]. As in Griscelli syndrome, an uncontrolled activation of macrophages and T cells results from a defect in secretory lysosome exocytosis. However, in contrast to *Rab27a* mutations, *Munc13-4* mutations do not impair cytolytic granule docking at the plasma membrane [85]. Munc13-4 thus functions at a post-docking stage, most likely in priming, similar to other members of the Munc13 family [147]. This effect on priming is probably dependent on Rab27a, which directly interacts with Munc13-4 [65, 148] and recruits it onto secretory lysosomes [149]. Munc13-4 also has a Rab27-independent role in the maturation of cytolytic granules [150]. The function of Munc13-4 is not restricted to T cells; it also promotes the secretion of secretory lysosomes in platelets, mast cells and neutrophils [65, 148, 151, 152]. Concerning Rab3, an interaction with Munc13-1 *via* Rim have been described [153] and this complex promotes the priming of synaptic vesicles within the active zone [154].

3.4. Triggering Fusion: Coupling Synaptic Vesicles to Calcium Channels

At the active zone, some SVs are attached to the plasma membrane, and a subset of these docked vesicles are physically coupled to Ca^{2+} channels. This spatial organization of the nerve terminals contributes to the extremely fast kinetics and efficacy of neurotransmitter release. Ca^{2+} gradients are sharp, and vesicles can experience high amounts of Ca^{2+} (approximately 100 μM) near the mouth of open channels and a submicromolar Ca^{2+} concentration a few microns away. Therefore, the ability to fuse is not sufficient to ensure a rapid neurotransmitter release. Wadel and colleagues [155] demonstrated that rapid release can be triggered by calcium uncaging after depletion of the pool of vesicles responsible for the synchronous release triggered by action potentials. These authors concluded that the recruitment of synaptic vesicles to sites where Ca^{2+} channels cluster is a rate-limiting step for rapid neurotransmitter release.

At neuromuscular junctions in Rab3a knockout mice, the defect in evoked release is more pronounced at low calcium concentrations than at physiological calcium concentrations, suggesting reduced calcium sensitivity [88]. Conversely, calcium elevation partially rescues Rab3a deficiency [156]. Because synchronous, but not asynchronous, release was found to be Rab3a-dependent, the data suggest that Rab3 mediates the recruitment of SVs to calcium channel clusters. The regulation of Rab3 effects by neuronal activity was also observed in the cholinergic neurons of *Aplysia californica* [35]. In these cells, the inhibitory effect of a GTPase-deficient Rab3 protein was potentiated by the prior injection of a Ca^{2+} chelator but reduced by a train of electrical stimuli, a treatment known to increase the Ca^{2+} concentration in the nerve terminal. Facilitation was also increased in cultured autaptic hippocampal neurons from quadruple Rab3 KO mice [42], which suggests that calcium elevation overcomes Rab3 deficiency. These studies are consistent with a role for Rab3 in targeting synaptic vesicles to calcium channels.

Rim. The Rab3 effector Rim [157] interacts with calcium channels and could thus mediate the Rab3-dependent tethering of synaptic vesicles to calcium channels. Rim interacts with Rab3 [158] but not with Rab27. The Rim family includes seven members encoded by four genes (*RIM1* through *4*). The extensive alternative splicing of Rim1 and Rim2 produces many Rim variants, but little is known about their function. Only Rim1α and Rim2α include the zinc finger and the SGAWFF motif that confer Rab3 binding activity (see Box 2). The other Rim domains consist of a central PDZ domain and two C-terminal atypical C2 domains that do not associate with phospholipids [159].

Rim is concentrated at the active zone (the region of the presynaptic compartment where synaptic vesicles are docked) and interacts with other components of the active zone protein network such as CAST, Munc13, Piccolo, Bassoon and ELKS [157, 160-162]. Rab3 and Munc13 can bind simultaneously to Rim [163] (see also [162] for further discussion). Calcium-dependent associations of the Rim C2A domain with the SNARE protein SNAP-25, synaptotagmin [164] (see also [165] for contrasting evidence) and the alpha subunits of voltage-gated calcium channels [164] have also been described. The Rim C2B domains interact with Liprin-α [166], synaptotagmin 1 [164, 166], the ubiquitin ligase Scrapper [167] and the β-subunits of voltage-gated calcium channels [168]. Finally, Rim binds to Rim-binding proteins (RBP 1 and 2) *via* a proline-rich region located between the two C2 domains [169]. RBP1 and 2 are connected to the α-subunits of calcium channels and thus can also physically link Rim to calcium channels.

Inactivation of the *RIM* gene in *C. elegans* [170] provokes an uncoordinated phenotype more severe than the *RAB3* phenotype. Both spontaneous neurotransmitter release and evoked responses are greatly reduced. Using high pressure freezing to maintain the architecture of the synapse, Weimer *et al.* [171] highlighted a selective decrease in the number of SVs close to dense projections (specialized areas enriched in calcium channels that contain Liprin-α proteins), while the overall number of SVs in the synaptic boutons remained unaffected.

In mammals, functional studies of Rim function have been complicated by the existence of several Rim genes. Rim1α null mice are viable and fertile. They have difficulties in spatial learning and fear conditioning and have a defect in maternal behavior. Their basal neurotransmission is not modified, but their short-term and long-term synaptic plasticity are affected [166, 172]. Mice lacking both Rim1α and Rim2α die immediately after birth because they cannot breathe. At the neuromuscular junction, spontaneous release is normal, indicating that the membrane fusion mechanism is not impaired, but evoked responses are severely reduced [173]. Altogether, the data suggest that the main function of Rim is to position SVs near calcium channels where the fusion machinery can ideally sense changes in calcium concentration upon the arrival of a stimulus. This positioning may be mediated by the direct interaction of Rim with calcium channels or interaction *via* liprins or RBP. In the absence of Rim1α, the levels of Munc13 are reduced. Because Munc13 has an important role in vesicle priming, some of the effects of Rim inactivation may be accounted for by this Munc13 effect.

4. CONCLUSIONS AND PERSPECTIVES

A large number of studies have been devoted to Rab proteins since their discovery in 1987 [174]. Even when study of these proteins is limited to their roles in secretion, Rab functions are complex. One source of complexity is the redundancy between the Rab isoforms and between Rab3 and Rab27. Clearly, Rab3 and Rab27 have specific roles and effectors, but there is also some overlap between their functions. Another source of complexity comes from the time courses of regulated secretion in various types of secretory cells. In neurons, research emphasis is put on time-resolved techniques because the relevant unit of time for neurotransmission is the millisecond. Hence, much importance has been placed on the priming reaction, which is largely controlled by Rab3. In endocrine or exocrine cells, given the slower kinetics of the secretory responses, the emphasis is put on techniques that measure the sustained components of release and on the recruitment of vesicles at release sites, a process largely controlled by Rab27.

By recruiting multiple effector molecules, Rab3 and Rab27 orchestrate multiple events that proceed from the biogenesis of a secretory vesicle to its exocytosis. Further work is needed to precisely delineate the functions of these effectors and to determine the chronology of the above events. How the recruitment of different effectors is regulated in time and space and coordinated with the progress of the vesicle along the secretory process is an intriguing issue.

ACKNOWLEDGEMENTS

We thank O. Jouannot and I. Fanget for their help in preparing the figures and S. O'Regan for revising the manuscript. This work was supported by the Centre National de la Recherche Scientifique and the Agence Nationale de la Recherche. FD is supported by INSERM.

CONFLICT OF INTEREST

There is no conflict of interest from any of the authors.

REFERENCES

[1] Rizo J, Rosenmund C. Synaptic vesicle fusion. Nat Struct Mol Biol 2008 Jul;15(7):665-74.
[2] Sorensen JB. Conflicting views on the membrane fusion machinery and the fusion pore. Annual Rev Cell Dev Biol 2009;25:513-37.
[3] Südhof TC. Neurotransmitter release. Handbook Exp Pharm 2008(184):1-21.
[4] Fukuda M. Regulation of secretory vesicle traffic by Rab small GTPases. Cell Mol Life Sci 2008 Sep;65(18):2801-13.
[5] Darchen F, Goud B. Multiple aspects of Rab protein action in the secretory pathway: focus on Rab3 and Rab6. Biochimie 2000 Apr;82(4):375-84.
[6] Lledo PM, Vernier P, Vincent JD, Mason WT, Zorec R. Inhibition of Rab3B expression attenuates Ca(2+)-dependent exocytosis in rat anterior pituitary cells. Nature 1993;364(6437):540-4.
[7] Rupnik M, Kreft M, Nothias F, *et al.* Distinct role of Rab3A and Rab3B in secretory activity of rat melanotrophs. Am J Physiol Cell Physiol 2007 Jan;292(1):C98-105.
[8] Weber E, Berta G, Tousson A, *et al.* Expression and polarized targeting of a rab3 isoform in epithelial cells. J Cell Biol 1994;125(3):583-94.
[9] Baldini G, Hohl T, Lin HY, Lodish HF. Cloning of a Rab3 isotype predominantly expressed in adipocytes. Proc Natl Acad Sci USA 1992;89(11):5049-52.
[10] Valentijn JA, Gumkowski FD, Jamieson JD. The expression pattern of rab3D in the developing rat exocrine pancreas coincides with the acquisition of regulated exocytosis. Eur J Cell Biol 1996;71(2):129-36.
[11] Stettler O, Nothias F, Tavitian B, Vernier P. Double *in situ* hybridization reveals overlapping neuronal populations expressing the low molecular weight GTPases Rab3a and Rab3b in Rat brain. Eur J Neurosci 1995;7(4):702-13.
[12] Gomi H, Mori K, Itohara S, Izumi T. Rab27b is expressed in a wide range of exocytic cells and involved in the delivery of secretory granules near the plasma membrane. Mol Biol Cell 2007 Nov;18(11):4377-86.
[13] Yi Z, Yokota H, Torii S, *et al.* The Rab27a/granuphilin complex regulates the exocytosis of insulin-containing dense-core granules. Mol Cell Biol 2002 Mar;22(6):1858-67.
[14] Fischer von Mollard G, Mignery GA, Baumert M, *et al.* rab3 is a small GTP-binding protein exclusively localized to synaptic vesicles. Proc Natl Acad Sci USA 1990;87(5):1988-92.
[15] Fischer von Mollard G, Stahl B, Khokhlatchev A, Sudhof TC, Jahn R. Rab3C is a synaptic vesicle protein that dissociates from synaptic vesicles after stimulation of exocytosis. J Biol Chem 1994;269(15):10971-4.
[16] Takamori S, Rhee JS, Rosenmund C, Jahn R. Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. Nature 2000;407(6801):189-94.
[17] Darchen F, Senyshyn J, Brondyk WH, *et al.* The GTPase Rab3a is associated with large dense core vesicles in bovine chromaffin cells and rat PC12 cells. J Cell Sci 1995;108(Pt 4):1639-49.
[18] Darchen F, Zahraoui A, Hammel F, Monteils MP, Tavitian A, Scherman D. Association of the GTP-binding protein Rab3A with bovine adrenal chromaffin granules. Proc Natl Acad Sci USA 1990;87(15):5692-6.
[19] Desnos C, Schonn JS, Huet S, *et al.* Rab27A and its effector MyRIP link secretory granules to F-actin and control their motion towards release sites. J Cell Biol 2003 Nov 10;163(3):559-70.
[20] Fukuda M, Kanno E, Saegusa C, Ogata Y, Kuroda TS. Slp4-a/Granuphilin-a Regulates Dense-core Vesicle Exocytosis in PC12 Cells. J Biol Chem 2002;277(42):39673-8.
[21] Iezzi M, Escher G, Meda P, *et al.* Subcellular distribution and function of Rab3A, B, C, and D isoforms in insulin-secreting cells. Mol Endocrinol 1999;13(2):202-12.
[22] Regazzi R, Ravazzola M, Iezzi M, *et al.* Expression, localization and functional role of small GTPases of the Rab3 family in insulin-secreting cells. J Cell Sci 1996;109(Pt 9):2265-73.
[23] Baldini G, Wang G, Weber M, *et al.* Expression of Rab3D N135I inhibits regulated secretion of ACTH in AtT- 20 cells. J Cell Biol 1998;140(2):305-13.
[24] Ohnishi H, Ernst SA, Wys N, McNiven M, Williams JA. Rab3D localizes to zymogen granules in rat pancreatic acini and other exocrine glands. Am J Physiol 1996;271(3 Pt 1):G531-8.
[25] Tang LH, Gumkowski FD, Sengupta D, Modlin IM, Jamieson JD. rab3D protein is a specific marker for zymogen granules in gastric chief cells of rats and rabbits. Gastroenterol 1996;110(3):809-20.

[26] Seabra MC, Coudrier E. Rab GTPases and myosin motors in organelle motility. Traffic 2004 Jun;5(6):393-9.
[27] Menasche G, Pastural E, Feldmann J, *et al.* Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet 2000 Jun;25(2):173-6.
[28] Stinchcombe JC, Barral DC, Mules EH, *et al.* Rab27a is required for regulated secretion in cytotoxic T lymphocytes. J Cell Biol 2001 Feb 19;152(4):825-34.
[29] Ostrowski M, Carmo NB, Krumeich S, *et al.* Rab27a and Rab27b control different steps of the exosome secretion pathway. Nat Cell Biol 2010 Jan;12(1):19-30; sup pp 1-13.
[30] Hannah MJ, Hume AN, Arribas M, *et al.* Weibel-Palade bodies recruit Rab27 by a content-driven, maturation-dependent mechanism that is independent of cell type. J Cell Sci 2003 Oct 1;116(Pt 19):3939-48.
[31] Mahoney TR, Liu Q, Itoh T, *et al.* Regulation of synaptic transmission by RAB-3 and RAB-27 in Caenorhabditis elegans. Mol Biol Cell 2006 Jun;17(6):2617-25.
[32] Pavlos NJ, Gronborg M, Riedel D, *et al.* Quantitative analysis of synaptic vesicle Rabs uncovers distinct yet overlapping roles for Rab3a and Rab27b in Ca2+-triggered exocytosis. J Neurosci 2010 Oct 6;30(40):13441-53.
[33] Takamori S, Holt M, Stenius K, *et al.* Molecular anatomy of a trafficking organelle. Cell 2006 Nov 17;127(4):831-46.
[34] Tsuboi T, Fukuda M. Rab3A and Rab27A cooperatively regulate the docking step of dense-core vesicle exocytosis in PC12 cells. J Cell Sci 2006 Jun 1;119(Pt 11):2196-203.
[35] Doussau F, Clabecq A, Henry JP, Darchen F, Poulain B. Calcium-dependent regulation of rab3 in short-term plasticity. J Neurosci 1998;18(9):3147-57.
[36] Holz RW, Brondyk WH, Senter RA, Kuizon L, Macara IG. Evidence for the involvement of Rab3A in Ca(2+)-dependent exocytosis from adrenal chromaffin cells. J Biol Chem 1994;269(14):10229-34.
[37] Johannes L, Lledo PM, Chameau P, Vincent JD, Henry JP, Darchen F. Regulation of the Ca2+ sensitivity of exocytosis by Rab3a. J Neurochem 1998;71(3):1127-33.
[38] Johannes L, Lledo PM, Roa M, Vincent JD, Henry JP, Darchen F. The GTPase Rab3a negatively controls calcium-dependent exocytosis in neuroendocrine cells. EMBO J 1994;13(9):2029-37.
[39] Johannes L, Perez F, Laran-Chich MP, Henry JP, Darchen F. Characterization of the interaction of the monomeric GTP-binding protein Rab3a with geranylgeranyl transferase II. Eur J Biochem 1996;239(2):362-8.
[40] Thiagarajan R, Tewolde T, Li Y, Becker PL, Rich MM, Engisch KL. Rab3A negatively regulates activity-dependent modulation of exocytosis in bovine adrenal chromaffin cells. J Physiol 2004 Mar 1;555(Pt 2):439-57.
[41] Yunes R, Tomes C, Michaut M, *et al.* Rab3A and calmodulin regulate acrosomal exocytosis by mechanisms that do not require a direct interaction. FEBS Lett 2002 Aug 14;525(1-3):126-30.
[42] Schlüter OM, Basu J, Sudhof TC, Rosenmund C. Rab3 superprimes synaptic vesicles for release: implications for short-term synaptic plasticity. J Neurosci 2006 Jan 25;26(4):1239-46.
[43] Schlüter OM, Schmitz F, Jahn R, Rosenmund C, Sudhof TC. A complete genetic analysis of neuronal Rab3 function. J Neurosci 2004 Jul 21;24(29):6629-37.
[44] Kapfhamer D, Valladares O, Sun Y, *et al.* Mutations in Rab3a alter circadian period and homeostatic response to sleep loss in the mouse. Nat Genet 2002;32(2):290-5.
[45] D'Adamo P, Wolfer DP, Kopp C, Tobler I, Toniolo D, Lipp HP. Mice deficient for the synaptic vesicle protein Rab3a show impaired spatial reversal learning and increased explorative activity but none of the behavioral changes shown by mice deficient for the Rab3a regulator Gdi1. Eur J Neurosci. 2004 Apr;19(7):1895-905.
[46] Castillo PE, Janz R, Südhof TC, Tzounopoulos T, Malenka RC, Nicoll RA. Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. Nature 1997;388(6642):590-3.
[47] Geppert M, Bolshakov VY, Siegelbaum SA, *et al.* The role of Rab3A in neurotransmitter release. Nature 1994 Jun 9;369(6480):493-7.
[48] Geppert M, Goda Y, Stevens CF, Südhof TC. The small GTP-binding protein Rab3A regulates a late step in synaptic vesicle fusion. Nature 1997;387(6635):810-4.
[49] Nonet ML, Staunton JE, Kilgard MP, *et al.* Caenorhabditis elegans rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. J Neurosci 1997;17(21):8061-73.
[50] D'Adamo P, Menegon A, Lo Nigro C, *et al.* Mutations in GDI1 are responsible for X-linked non-specific mental retardation [see comments] [published erratum appears in Nat Genet 1998 Jul;19(3):303]. Nat Genet. 1998;19(2):134-9.
[51] D'Adamo P, Welzl H, Papadimitriou S, *et al.* Deletion of the mental retardation gene Gdi1 impairs associative memory and alters social behavior in mice. Hum Mol Genet 2002 Oct 1;11(21):2567-80.
[52] Ishizaki H, Miyoshi J, Kamiya H, *et al.* Role of rab GDP dissociation inhibitor alpha in regulating plasticity of hippocampal neurotransmission. Proc Natl Acad Sci USA 2000;97(21):11587-92.

[53] Tanaka M, Miyoshi J, Ishizaki H, *et al.* Role of rab3 gdp/gtp exchange protein in synaptic vesicle trafficking at the mouse neuromuscular junction. Mol Biol Cell 2001;12(5):1421-30.

[54] Yamaguchi K, Tanaka M, Mizoguchi A, *et al.* A GDP/GTP exchange protein for the Rab3 small G protein family up-regulates a postdocking step of synaptic exocytosis in central synapses. Proc Natl Acad Sci USA 2002;99(22):14536-41.

[55] Iwasaki K, Staunton J, Saifee O, Nonet M, Thomas JH. aex-3 encodes a novel regulator of presynaptic activity in C. elegans. Neuron 1997;18(4):613-22.

[56] Griscelli C, Durandy A, Guy-Grand D, Daguillard F, Herzog C, Prunieras M. A syndrome associating partial albinism and immunodeficiency. Am J Med 1978 Oct;65(4):691-702.

[57] Wilson SM, Yip R, Swing DA, *et al.* A mutation in Rab27a causes the vesicle transport defects observed in ashen mice. Proc Natl Acad Sci USA 2000;97(14):7933-8.

[58] Stinchcombe J, Bossi G, Griffiths GM. Linking albinism and immunity: the secrets of secretory lysosomes. Science 2004 Jul 2;305(5680):55-9.

[59] Haddad EK, Wu X, Hammer JA, 3rd, Henkart PA. Defective granule exocytosis in Rab27a-deficient lymphocytes from Ashen mice. J Cell Biol 2001 Feb 19;152(4):835-42.

[60] Jancic C, Savina A, Wasmeier C, *et al.* Rab27a regulates phagosomal pH and NADPH oxidase recruitment to dendritic cell phagosomes. Nat Cell Biol 2007 Apr;9(4):367-78.

[61] Kasai K, Ohara-Imaizumi M, Takahashi N, *et al.* Rab27a mediates the tight docking of insulin granules onto the plasma membrane during glucose stimulation. J Clin Invest 2005 Feb;115(2):388-96.

[62] Tolmachova T, Abrink M, Futter CE, Authi KS, Seabra MC. Rab27b regulates number and secretion of platelet dense granules. Proc Natl Acad Sci USA 2007 Apr 3;104(14):5872-7.

[63] Waselle L, Coppola T, Fukuda M, *et al.* Involvement of the Rab27 binding protein Slac2c/MyRIP in insulin exocytosis. Mol Biol Cell 2003 Oct;14(10):4103-13.

[64] Gomi H, Mizutani S, Kasai K, Itohara S, Izumi T. Granuphilin molecularly docks insulin granules to the fusion machinery. J Cell Biol 2005 Oct 10;171(1):99-109.

[65] Shirakawa R, Higashi T, Tabuchi A, *et al.* Munc13-4 is a GTP-Rab27-binding protein regulating dense core granule secretion in platelets. J Biol Chem 2004 Mar 12;279(11):10730-7.

[66] Chen X, Li C, Izumi T, Ernst SA, Andrews PC, Williams JA. Rab27b localizes to zymogen granules and regulates pancreatic acinar exocytosis. Biochem Biophys Res Commun 2004 Oct 29;323(4):1157-62.

[67] Munafo DB, Johnson JL, Ellis BA, Rutschmann S, Beutler B, Catz SD. Rab27a is a key component of the secretory machinery of azurophilic granules in granulocytes. Biochem J 2007 Mar 1;402(2):229-39.

[68] Herrero-Turrion MJ, Calafat J, Janssen H, Fukuda M, Mollinedo F. Rab27a regulates exocytosis of tertiary and specific granules in human neutrophils. J Immunol 2008 Sep 15;181(6):3793-803.

[69] Johnson JL, Brzezinska AA, Tolmachova T, *et al.* Rab27a and Rab27b regulate neutrophil azurophilic granule exocytosis and NADPH oxidase activity by independent mechanisms. Traffic 2010 Apr;11(4):533-47.

[70] Johnson JL, Ellis BA, Noack D, Seabra MC, Catz SD. The Rab27a-binding protein, JFC1, regulates androgen-dependent secretion of prostate-specific antigen and prostatic-specific acid phosphatase. Biochem J 2005 Nov 1;391(Pt 3):699-710.

[71] Yu E, Kanno E, Choi S, *et al.* Role of Rab27 in synaptic transmission at the squid giant synapse. Proc Natl Acad Sci USA 2008 Oct 14;105(41):16003-8.

[72] Morvan J, Tooze SA. Discovery and progress in our understanding of the regulated secretory pathway in neuroendocrine cells. Histochem Cell Biol 2008 Mar;129(3):243-52.

[73] Schonn J-S, van Weering JRT, Mohrmann R, *et al.* Rab3 proteins involved in vesicle biogenesis and priming in embryonic mouse chromaffin cells. Traffic 2010;11:1415-8.

[74] Handley MT, Haynes LP, Burgoyne RD. Differential dynamics of Rab3A and Rab27A on secretory granules. J Cell Sci 2007 Mar 15;120(Pt 6):973-84.

[75] Riedel D, Antonin W, Fernandez-Chacon R, *et al.* Rab3D is not required for exocrine exocytosis but for maintenance of normally sized secretory granules. Mol Cell Biol 2002;22(18):6487-97.

[76] Martelli AM, Baldini G, Tabellini G, Koticha D, Bareggi R. Rab3A and Rab3D control the total granule number and the fraction of granules docked at the plasma membrane in PC12 cells. Traffic 2000;1(12):976-86.

[77] Kogel T, Rudolf R, Hodneland E, *et al.* Distinct roles of myosin Va in membrane remodeling and exocytosis of secretory granules. Traffic 2010 May;11(5):637-50.

[78] Varadi A, Tsuboi T, Johnson-Cadwell LI, Allan VJ, Rutter GA. Kinesin I and cytoplasmic dynein orchestrate glucose-stimulated insulin-containing vesicle movements in clonal MIN6 beta-cells. Biochem Biophys Res Commun 2003 Nov 14;311(2):272-82.

[79] Rudolf R, Kogel T, Kuznetsov SA, *et al.* Myosin Va facilitates the distribution of secretory granules in the F-actin rich cortex of PC12 cells. J Cell Sci 2003 Apr 1;116(Pt 7):1339-48.
[80] Malacombe M, Bader MF, Gasman S. Exocytosis in neuroendocrine cells: new tasks for actin. Biochimica et biophysica acta 2006 Nov;1763(11):1175-83.
[81] Desnos C, Huet S, Darchen F. 'Should I stay or should I go?': myosin V function in organelle trafficking. Biol Cell 2007 Aug;99(8):411-23.
[82] Desnos C, Huet S, Fanget I, *et al.* Myosin va mediates docking of secretory granules at the plasma membrane. J Neurosci 2007 Sep 26;27(39):10636-45.
[83] Fdez E, Hilfiker S. Vesicle pools and synapsins: new insights into old enigmas. Brain Cell Biol 2006 Jun;35(2-3):107-15.
[84] Fornasiero EF, Bonanomi D, Benfenati F, Valtorta F. The role of synapsins in neuronal development. Cell Mol Life Sci 2010 May;67(9):1383-96.
[85] Feldmann J, Callebaut I, Raposo G, *et al.* Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell 2003 Nov 14;115(4):461-73.
[86] van Weering JR, Toonen RF, Verhage M. The role of Rab3a in secretory vesicle docking requires association/dissociation of guanidine phosphates and Munc18-1. PLoS One 2007;2(7):e616.
[87] Leenders AG, Lopes da Silva FH, Ghijsen WE, Verhage M. Rab3a is involved in transport of synaptic vesicles to the active zone in mouse brain nerve terminals. Mol Biol Cell 2001 Oct;12(10):3095-102.
[88] Coleman WL, Bill CA, Bykhovskaia M. Rab3a deletion reduces vesicle docking and transmitter release at the mouse diaphragm synapse. Neurosci 2007 Aug 10;148(1):1-6.
[89] Graham ME, Handley MT, Barclay JW, *et al.* A gain-of-function mutant of Munc18-1 stimulates secretory granule recruitment and exocytosis and reveals a direct interaction of Munc18-1 with Rab3. Biochem J 2008 Jan 15;409(2):407-16.
[90] Karatekin E, Tran VS, Huet S, Fanget I, Cribier S, Henry JP. A 20-nm step toward the cell membrane preceding exocytosis may correspond to docking of tethered granules. Biophys J 2008 Apr 1;94(7):2891-905.
[91] de Wit H, Walter AM, Milosevic I, *et al.* Synaptotagmin-1 docks secretory vesicles to syntaxin-1/SNAP-25 acceptor complexes. Cell 2009 Sep 4;138(5):935-46.
[92] Toonen RF, Kochubey O, de Wit H, *et al.* Dissecting docking and tethering of secretory vesicles at the target membrane. EMBO J 2006 Aug 23;25(16):3725-37.
[93] Verhage M, Sorensen JB. Vesicle docking in regulated exocytosis. Traffic 2008 Sep;9(9):1414-24.
[94] Siksou L, Varoqueaux F, Pascual O, Triller A, Brose N, Marty S. A common molecular basis for membrane docking and functional priming of synaptic vesicles. Eur J Neurosci 2009 Jul;30(1):49-56.
[95] Pfeffer S. Vesicle tethering factors united. Mol Cell 2001 Oct;8(4):729-30.
[96] Fukuda M. Synaptotagmin-like Protein (Slp) Homology Domain 1 of Slac2- a/Melanophilin Is a Critical Determinant of GTP-dependent Specific Binding to Rab27A. J Biol Chem 2002;277(42):40118-24.
[97] Hume AN, Collinson LM, Hopkins CR, *et al.* The leaden gene product is required with Rab27a to recruit myosin Va to melanosomes in melanocytes. Traffic 2002;3(3):193-202.
[98] Provance DW, James TL, Mercer JA. Melanophilin, the product of the leaden locus, is required for targeting of myosin-Va to melanosomes. Traffic 2002;3(2):124-32.
[99] Wu XS, Rao K, Zhang H, *et al.* Identification of an organelle receptor for myosin-Va. Nat Cell Biol 2002;4(4):271-8.
[100] Matesic LE, Yip R, Reuss AE, *et al.* Mutations in Mlph, encoding a member of the Rab effector family, cause the melanosome transport defects observed in leaden mice. Proc Natl Acad Sci USA 2001 Aug 28;98(18):10238-43.
[101] Menasche G, Ho CH, Sanal O, *et al.* Griscelli syndrome restricted to hypopigmentation results from a melanophilin defect (GS3) or a MYO5A F-exon deletion (GS1). J Clin Invest 2003 Aug;112(3):450-6.
[102] Mercer JA, Seperack PK, Strobel MC, Copeland NG, Jenkins NA. Novel myosin heavy chain encoded by murine dilute coat colour locus. Nature 1991 Feb 21;349(6311):709-13.
[103] Pastural E, Barrat FJ, Dufourcq-Lagelouse R, *et al.* Griscelli disease maps to chromosome 15q21 and is associated with mutations in the myosin-Va gene. Nat Genet 1997 Jul;16(3):289-92.
[104] El-Amraoui A, Schonn JS, Kussel-Andermann P, *et al.* MyRIP, a novel Rab effector, enables myosin VIIa recruitment to retinal melanosomes. EMBO Rep 2002 May;3(5):463-70.
[105] Fukuda M, Kuroda TS. Slac2-c (synaptotagmin-like protein homologue lacking C2 domains-c), a novel linker protein that interacts with Rab27, myosin Va/VIIa, and actin. J Biol Chem 2002;277(45):43096-103.
[106] Nightingale TD, Pattni K, Hume AN, Seabra MC, Cutler DF. Rab27a and MyRIP regulate the amount and multimeric state of VWF released from endothelial cells. Blood 2009 May 14;113(20):5010-8.

[107] Imai A, Yoshie S, Nashida T, Shimomura H, Fukuda M. The small GTPase Rab27B regulates amylase release from rat parotid acinar cells. J Cell Sci 2004 Apr 15;117(Pt 10):1945-53.
[108] Kuroda TS, Fukuda M. Functional analysis of Slac2-c/MyRIP as a linker protein between melanosomes and myosin VIIa. J Biol Chem 2005 Jul 29;280(30):28015-22.
[109] Klomp AE, Teofilo K, Legacki E, Williams DS. Analysis of the linkage of MYRIP and MYO7A to melanosomes by RAB27A in retinal pigment epithelial cells. Cell Motil Cytoskeleton 2007 Jun;64(6):474-87.
[110] Lopes VS, Ramalho JS, Owen DM, *et al.* The ternary Rab27a-Myrip-Myosin VIIa complex regulates melanosome motility in the retinal pigment epithelium. Traffic 2007 May;8(5):486-99.
[111] Goehring AS, Pedroja BS, Hinke SA, Langeberg LK, Scott JD. MyRIP anchors protein kinase A to the exocyst complex. J Biol Chem 2007 Nov 9;282(45):33155-67.
[112] Ivarsson R, Jing X, Waselle L, Regazzi R, Renstrom E. Myosin 5a controls insulin granule recruitment during late-phase secretion. Traffic 2005 Nov;6(11):1027-35.
[113] Rose SD, Lejen T, Casaletti L, Larson RE, Pene TD, Trifaro JM. Myosins II and V in chromaffin cells: myosin V is a chromaffin vesicle molecular motor involved in secretion. J Neurochem 2003 Apr;85(2):287-98.
[114] Varadi A, Tsuboi T, Rutter GA. Myosin Va transports dense core secretory vesicles in pancreatic MIN6 beta-cells. Mol Biol Cell 2005 Jun;16(6):2670-80.
[115] Wang J, Takeuchi T, Yokota H, Izumi T. Novel rabphilin-3-like protein associates with insulin-containing granules in pancreatic beta cells. J Biol Chem 1999 Oct 1;274(40):28542-8.
[116] Coppola T, Frantz C, Perret-Menoud V, Gattesco S, Hirling H, Regazzi R. Pancreatic beta-cell protein granuphilin binds Rab3 and Munc-18 and controls exocytosis. Mol Biol Cell 2002 Jun;13(6):1906-15.
[117] Torii S, Takeuchi T, Nagamatsu S, Izumi T. Rab27 effector granuphilin promotes the plasma membrane targeting of insulin granules *via* interaction with syntaxin 1a. J Biol Chem 2004 May 21;279(21):22532-8.
[118] Torii S, Zhao S, Yi Z, Takeuchi T, Izumi T. Granuphilin modulates the exocytosis of secretory granules through interaction with syntaxin 1a. Mol Cell Biol 2002 Aug;22(15):5518-26.
[119] Kasai K, Fujita T, Gomi H, Izumi T. Docking is not a prerequisite but a temporal constraint for fusion of secretory granules. Traffic 2008 Jul;9(7):1191-203.
[120] Yu M, Kasai K, Nagashima K, *et al.* Exophilin4/Slp2-a targets glucagon granules to the plasma membrane through unique Ca2+-inhibitory phospholipid-binding activity of the C2A domain. Mol Biol Cell 2007 Feb;18(2):688-96.
[121] Shirataki H, Kaibuchi K, Sakoda T, *et al.* Rabphilin-3A, a putative target protein for smg p25A/rab3A p25 small GTP-binding protein related to synaptotagmin. Mol Cell Biol 1993 Apr;13(4):2061-8.
[122] Fukuda M, Kanno E, Yamamoto A. Rabphilin and Noc2 are recruited to dense-core vesicles through specific interaction with Rab27A in PC12 cells. J Biol Chem 2004 Mar 26;279(13):13065-75.
[123] Li C, Takei K, Geppert M, *et al.* Synaptic targeting of rabphilin-3A, a synaptic vesicle Ca2+/phospholipid-binding protein, depends on rab3A/3C. Neuron 1994;13(4):885-98.
[124] Stahl B, von Mollard GF, Walch-Solimena C, Jahn R. GTP cleavage by the small GTP-binding protein Rab3A is associated with exocytosis of synaptic vesicles induced by alpha-latrotoxin. J Biol Chem 1994;269(40):24770-6.
[125] Baldini G, Martelli AM, Tabellini G, *et al.* Rabphilin localizes with the cell actin cytoskeleton and stimulates association of granules with F-actin cross-linked by {alpha}-actinin. J Biol Chem 2005 Oct 14;280(41):34974-84.
[126] Kato M, Sasaki T, Ohya T, *et al.* Physical and functional interaction of rabphilin-3A with alpha-actinin. J Biol Chem 1996;271(50):31775-8.
[127] Giovedi S, Darchen F, Valtorta F, Greengard P, Benfenati F. Synapsin is a novel Rab3 effector protein on small synaptic vesicles. II. Functional effects of the Rab3A-synapsin I interaction. J Biol Chem 2004 Oct 15;279(42):43769-79.
[128] Giovedi S, Vaccaro P, Valtorta F, *et al.* Synapsin is a novel Rab3 effector protein on small synaptic vesicles. I. Identification and characterization of the synapsin I-Rab3 interactions *in vitro* and in intact nerve terminals. J Biol Chem 2004 Oct 15;279(42):43760-8.
[129] Wojcik SM, Brose N. Regulation of membrane fusion in synaptic excitation-secretion coupling: speed and accuracy matter. Neuron 2007 Jul 5;55(1):11-24.
[130] Rettig J, Neher E. Emerging roles of presynaptic proteins in Ca++-triggered exocytosis. Science 2002 Oct 25;298(5594):781-5.
[131] Merrins MJ, Stuenkel EL. Kinetics of Rab27a-dependent actions on vesicle docking and priming in pancreatic beta-cells. J Physiol 2008 Nov 15;586(Pt 22):5367-81.
[132] Tian JH, Wu ZX, Unzicker M, *et al.* The role of Snapin in neurosecretion: snapin knock-out mice exhibit impaired calcium-dependent exocytosis of large dense-core vesicles in chromaffin cells. J Neurosci 2005 Nov 9;25(45):10546-55.

[133] Arribas M, Regazzi R, Garcia E, Wollheim CB, Decamilli P. The Stimulatory Effect of Rabphilin 3A on Regulated Exocytosis from Insulin-Secreting Cells Does Not Require an Association-Dissociation Cycle with Membranes Mediated by Rab-3. Eur J Cell Biol 1997;74(3):209-16.

[134] Chung SH, Takai Y, Holz RW. Evidence that the Rab3a-binding protein, rabphilin3a, enhances regulated secretion. Studies in adrenal chromaffin cells. J Biol Chem 1995;270(28):16714-8.

[135] Tsuboi T, Fukuda M. The C2B domain of rabphilin directly interacts with SNAP-25 and regulates the docking step of dense core vesicle exocytosis in PC12 cells. J Biol Chem 2005 Nov 25;280(47):39253-9.

[136] Staunton J, Ganetzky B, Nonet ML. Rabphilin potentiates soluble N-ethylmaleimide sensitive factor attachment protein receptor function independently of rab3. J Neurosci 2001 Dec 1;21(23):9255-64.

[137] Deak F, Shin OH, Tang J, *et al.* Rabphilin regulates SNARE-dependent re-priming of synaptic vesicles for fusion. EMBO J 2006 Jun 21;25(12):2856-66.

[138] Schlüter OM, Schnell E, Verhage M, *et al.* Rabphilin knock-out mice reveal that rabphilin is not required for rab3 function in regulating neurotransmitter release. J Neurosci 1999 Jul 15;19(14):5834-46.

[139] Burns ME, Sasaki T, Takai Y, Augustine GJ. Rabphilin-3A - A Multifunctional Regulator of Synaptic Vesicle Traffic. J Gen Physiol 1998;111(2):243-55.

[140] Ohya T, Sasaki T, Kato M, Takai Y. Involvement of Rabphilin3 in endocytosis through interaction with Rabaptin5. J Biol Chem 1998 Jan 2;273(1):613-7.

[141] Coppola T, Hirling H, Perret-Menoud V, *et al.* Rabphilin dissociated from Rab3 promotes endocytosis through interaction with Rabaptin-5. J Cell Sci 2001 May;114(Pt 9):1757-64.

[142] Cheviet S, Coppola T, Haynes LP, Burgoyne RD, Regazzi R. The Rab-binding protein Noc2 is associated with insulin-containing secretory granules and is essential for pancreatic beta-cell exocytosis. Mol Endocrinol 2004 Jan;18(1):117-26.

[143] Haynes LP, Evans GJ, Morgan A, Burgoyne RD. A direct inhibitory role for the rab3-specific effector, noc2, in ca2+-regulated exocytosis in neuroendocrine cells. J Biol Chem 2001;276(13):9726-32.

[144] Kotake K, Ozaki N, Mizuta M, Sekiya S, Inagaki N, Seino S. Noc2, a putative zinc finger protein involved in exocytosis in endocrine cells. The J Biol Chem 1997;272(47):29407-10.

[145] Matsumoto M, Miki T, Shibasaki T, *et al.* Noc2 is essential in normal regulation of exocytosis in endocrine and exocrine cells. Proc Natl Acad Sci USA 2004 Jun 1;101(22):8313-8.

[146] Imai A, Yoshie S, Nashida T, Shimomura H, Fukuda M. Functional involvement of Noc2, a Rab27 effector, in rat parotid acinar cells. Arch Biochem Biophys 2006 Nov 15;455(2):127-35.

[147] Brose N, Rosenmund C, Rettig J. Regulation of transmitter release by Unc-13 and its homologues. Curr Opin Neurobiol 2000 Jun;10(3):303-11.

[148] Neeft M, Wieffer M, de Jong AS, *et al.* Munc13-4 is an effector of rab27a and controls secretion of lysosomes in hematopoietic cells. Mol Biol Cell 2005 Feb;16(2):731-41.

[149] Wood SM, Meeths M, Chiang SC, *et al.* Different NK cell-activating receptors preferentially recruit Rab27a or Munc13-4 to perforin-containing granules for cytotoxicity. Blood 2009 Nov 5;114(19):4117-27.

[150] Menager MM, Menasche G, Romao M, *et al.* Secretory cytotoxic granule maturation and exocytosis require the effector protein hMunc13-4. Nat Immunol 2007 Mar;8(3):257-67.

[151] Brzezinska AA, Johnson JL, Munafo DB, *et al.* The Rab27a effectors JFC1/Slp1 and Munc13-4 regulate exocytosis of neutrophil granules. Traffic 2008 Dec;9(12):2151-64.

[152] Pivot-Pajot C, Varoqueaux F, de Saint Basile G, Bourgoin SG. Munc13-4 regulates granule secretion in human neutrophils. J Immunol 2008 May 15;180(10):6786-97.

[153] Dulubova I, Lou X, Lu J, *et al.* A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? EMBO J 2005 Aug 17;24(16):2839-50.

[154] Betz A, Thakur P, Junge HJ, *et al.* Functional interaction of the active zone proteins Munc13-1 and RIM1 in synaptic vesicle priming. Neuron 2001;30(1):183-96.

[155] Wadel K, Neher E, Sakaba T. The coupling between synaptic vesicles and Ca2+ channels determines fast neurotransmitter release. Neuron 2007 Feb 15;53(4):563-75.

[156] Coleman WL, Bykhovskaia M. Rab3a-mediated vesicle recruitment regulates short-term plasticity at the mouse diaphragm synapse. Mol Cell Neurosci 2009 Jun;41(2):286-96.

[157] Mittelstaedt T, Alvarez-Baron E, Schoch S. RIM proteins and their role in synapse function. Biol Chem 2010 Jun;391(6):599-606.

[158] Wang Y, Okamoto M, Schmitz F, Hofmann K, Südhof TC. Rim Is a Putative Rab3 Effector in Regulating Synaptic-Vesicle Fusion. Nature 1997;388(6642):593-8.

[159] Wang Y, Sugita S, Sudhof TC. The RIM/NIM family of neuronal C2 domain proteins. Interactions with Rab3 and a new class of Src homology 3 domain proteins. J Biol Chem 2000 Jun 30;275(26):20033-44.
[160] Takao-Rikitsu E, Mochida S, Inoue E, *et al.* Physical and functional interaction of the active zone proteins, CAST, RIM1, and Bassoon, in neurotransmitter release. J Cell Biol 2004 Jan 19;164(2):301-11.
[161] Lu J, Li H, Wang Y, Sudhof TC, Rizo J. Solution structure of the RIM1alpha PDZ domain in complex with an ELKS1b C-terminal peptide. J Mol Biol 2005 Sep 16;352(2):455-66.
[162] Betz A, Thakur P, Junge HJ, *et al.* Functional interaction of the active zone proteins Munc13-1 and RIM1 in synaptic vesicle priming. Neuron 2001;30(1):183-96.
[163] Dulubova I, Lou X, Lu J, *et al.* A Munc13/RIM/Rab3 tripartite complex: from priming to plasticity? EMBO J 2005 Aug 17;24(16):2839-50.
[164] Coppola T, Magnin-Luthi S, Perret-Menoud V, Gattesco S, Schiavo G, Regazzi R. Direct interaction of the Rab3 effector RIM with Ca2+ channels, SNAP-25, and synaptotagmin. J Biol Chem 2001 Aug 31;276(35):32756-62.
[165] Dai H, Tomchick DR, Garcia J, Sudhof TC, Machius M, Rizo J. Crystal structure of the RIM2 C2A-domain at 1.4 A resolution. Biochemistry 2005 Oct 18;44(41):13533-42.
[166] Schoch S, Castillo PE, Jo T, *et al.* RIM1alpha forms a protein scaffold for regulating neurotransmitter release at the active zone. Nature 2002 Jan 17;415(6869):321-6.
[167] Yao I, Takagi H, Ageta H, *et al.* SCRAPPER-dependent ubiquitination of active zone protein RIM1 regulates synaptic vesicle release. Cell 2007 Sep 7;130(5):943-57.
[168] Kiyonaka S, Wakamori M, Miki T, *et al.* RIM1 confers sustained activity and neurotransmitter vesicle anchoring to presynaptic Ca2+ channels. Nat Neurosci 2007 Jun;10(6):691-701.
[169] Hibino H, Pironkova R, Onwumere O, Vologodskaia M, Hudspeth AJ, Lesage F. RIM binding proteins (RBPs) couple Rab3-interacting molecules (RIMs) to voltage-gated Ca(2+) channels. Neuron 2002 Apr 25;34(3):411-23.
[170] Koushika SP, Richmond JE, Hadwiger G, Weimer RM, Jorgensen EM, Nonet ML. A post-docking role for active zone protein Rim. Nat Neurosci 2001 Oct;4(10):997-1005.
[171] Weimer RM, Gracheva EO, Meyrignac O, Miller KG, Richmond JE, Bessereau JL. UNC-13 and UNC-10/rim localize synaptic vesicles to specific membrane domains. J Neurosci 2006 Aug 2;26(31):8040-7.
[172] Castillo PE, Schoch S, Schmitz F, Sudhof TC, Malenka RC. RIM1alpha is required for presynaptic long-term potentiation. Nature 2002 Jan 17;415(6869):327-30.
[173] Schoch S, Mittelstaedt T, Kaeser PS, *et al.* Redundant functions of RIM1alpha and RIM2alpha in Ca(2+)-triggered neurotransmitter release. EMBO J 2006 Dec 13;25(24):5852-63.
[174] Touchot N, Chardin P, Tavitian A. Four additional members of the ras gene superfamily isolated by an oligonucleotide strategy: molecular cloning of YPT-related cDNAs from a rat brain library. Proc Natl Acad Sci USA 1987 Dec;84(23):8210-4.
[175] Pfeffer S. Membrane domains in the secretory and endocytic pathways. Cell 2003 Feb 21;112(4):507-17.
[176] Sakisaka T, Meerlo T, Matteson J, Plutner H, Balch WE. Rab-alphaGDI activity is regulated by a Hsp90 chaperone complex. EMBO J 2002 Nov 15;21(22):6125-35.
[177] Bianchi V, Farisello P, Baldelli P, *et al.* Cognitive impairment in Gdi1-deficient mice is associated with altered synaptic vesicle pools and short-term synaptic plasticity, and can be corrected by appropriate learning training. Hum Mol Genet 2009 Jan 1;18(1):105-17.
[178] Park JB, Farnsworth CC, Glomset JA. Ca2+/calmodulin causes Rab3A to dissociate from synaptic membranes. J Biol Chem 1997;272(33):20857-65.
[179] Clabecq A, Henry JP, Darchen F. Biochemical characterization of Rab3-GTPase-activating protein reveals a mechanism similar to that of Ras-GAP. J Biol Chem 2000 Oct 13;275(41):31786-91.
[180] Itoh T, Fukuda M. Identification of EPI64 as a GTPase-activating protein specific for Rab27A. J Biol Chem 2006 Oct 20;281(42):31823-31.
[181] Figueiredo AC, Wasmeier C, Tarafder AK, Ramalho JS, Baron RA, Seabra MC. Rab3GEP is the non-redundant guanine nucleotide exchange factor for Rab27a in melanocytes. J Biol Chem 2008 Aug 22;283(34):23209-16.
[182] Yoshimura S, Gerondopoulos A, Linford A, Rigden DJ, Barr FA. Family-wide characterization of the DENN domain Rab GDP-GTP exchange factors. J Cell Biol Oct 18;191(2):367-81.
[183] Fischer von Mollard G, Südhof TC, Jahn R. A small GTP-binding protein dissociates from synaptic vesicles during exocytosis. Nature 1991;349(6304):79-81.
[184] Kondo H, Shirakawa R, Higashi T, *et al.* Constitutive GDP/GTP exchange and secretion-dependent GTP hydrolysis activity for Rab27 in platelets. J Biol Chem 2006 Sep 29;281(39):28657-65.

[185] Fukui K, Sasaki T, Imazumi K, Matsuura Y, Nakanishi H, Takai Y. Isolation and characterization of a GTPase activating protein specific for the Rab3 subfamily of small G proteins. J Biol Chem 1997;272(8):4655-8.
[186] Nagano F, Sasaki T, Fukui K, Asakura T, Imazumi K, Takai Y. Molecular cloning and characterization of the noncatalytic subunit of the Rab3 subfamily-specific GTPase-activating protein. J Biol Chem 1998;273(38):24781-5.
[187] Aligianis IA, Johnson CA, Gissen P, *et al.* Mutations of the catalytic subunit of RAB3GAP cause Warburg Micro syndrome. Nat Genet 2005 Mar;37(3):221-3.
[188] Sakane A, Manabe S, Ishizaki H, *et al.* Rab3 GTPase-activating protein regulates synaptic transmission and plasticity through the inactivation of Rab3. Proc Natl Acad Sci USA 2006 Jun 27;103(26):10029-34.
[189] Niwa S, Tanaka Y, Hirokawa N. KIF1Bbeta- and KIF1A-mediated axonal transport of presynaptic regulator Rab3 occurs in a GTP-dependent manner through DENN/MADD. Nat Cell Biol 2008 Nov;10(11):1269-79.
[190] Miyoshi J, Takai Y. Dual role of DENN/MADD (Rab3GEP) in neurotransmission and neuroprotection. Trends Mol Med 2004 Oct;10(10):476-80.
[191] Izumi T. Physiological roles of Rab27 effectors in regulated exocytosis. Endocr J 2007 Dec;54(5):649-57.
[192] Fukuda M. Distinct Rab binding specificity of Rim1, Rim2, rabphilin, and Noc2. Identification of a critical determinant of Rab3A/Rab27A recognition by Rim2. J Biol Chem 2003 Apr 25;278(17):15373-80.
[193] Sun L, Bittner MA, Holz RW. Rab3a binding and secretion-enhancing domains in Rim1 are separate and unique. Studies in adrenal chromaffin cells. J Biol Chem 2001 Apr 20;276(16):12911-7.
[194] Holt O, Kanno E, Bossi G, *et al.* Slp1 and Slp2-a localize to the plasma membrane of CTL and contribute to secretion from the immunological synapse. Traffic 2008 Apr;9(4):446-57.
[195] Kuroda TS, Fukuda M. Rab27A-binding protein Slp2-a is required for peripheral melanosome distribution and elongated cell shape in melanocytes. Nat Cell Biol 2004 Dec;6(12):1195-203.
[196] Fukuda M. The C2A domain of synaptotagmin-like protein 3 (Slp3) is an atypical calcium-dependent phospholipid-binding machine: comparison with the C2A domain of synaptotagmin I. Biochem J 2002 Sep 1;366(Pt 2):681-7.
[197] Kuroda TS, Fukuda M, Ariga H, Mikoshiba K. Synaptotagmin-like protein 5: a novel Rab27A effector with C-terminal tandem C2 domains. Biochem Biophys Res Commun 2002 May 10;293(3):899-906.
[198] Fukuda M. The synaptotagmin-like protein (Slp) homology domain 1 of Slac2- a/melanophilin is a critical determinant of GTP-dependent, specific binding of Rab27A. J Biol Chem 2002;19:19.
[199] Kuroda TS, Fukuda M, Ariga H, Mikoshiba K. The Slp homology domain of synaptotagmin-like proteins 1-4 and Slac2 functions as a novel Rab27A binding domain. J Biol Chem 2002 Mar 15;277(11):9212-8.
[200] Goishi K, Mizuno K, Nakanishi H, Sasaki T. Involvement of Rab27 in antigen-induced histamine release from rat basophilic leukemia 2H3 cells. Biochem Biophys Res Commun 2004 Nov 5;324(1):294-301.
[201] Ubach J, Garcia J, Nittler MP, Südhof TC, Rizo J. Structure of the janus-faced C2B domain of rabphilin. Nat Cell Biol 1999;1(2):106-12.
[202] Yamaguchi T, Shirataki H, Kishida S, *et al.* Two functionally different domains of rabphilin-3A, Rab3A p25/smg p25A-binding and phospholipid- and Ca(2+)-binding domains. J Biol Chem 1993 Dec 25;268(36):27164-70.
[203] Ostermeier C, Brunger AT. Structural basis of Rab effector specificity: crystal structure of the small G protein Rab3A complexed with the effector domain of rabphilin- 3A. Cell 1999;96(3):363-74.
[204] Huet S, Fanget I, Jouannot O, *et al*. Myrip couples the capture of secretory granules by the actin-rich cell cortex and their attachment to the plasma membrane. J Neurosci 2012;32(7):2564-77.