Rab27 and its effectors in secretory granule exocytosis: a novel docking machinery composed of a Rab27-effector complex

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Abstract

A small GTPase Rab27 is present on secretory granules in a wide variety of secretory cells and on melanosomes in melanocytes, and it is involved in controlling the trafficking of these organelles through interaction with a cell-type- or tissue-specific Rab27 effector(s). Slps (synaptotagmin-like proteins) and rabphilin contain an N-terminal Rab27-binding domain and C-terminal tandem C2 domains, and some of the Rab27-binding proteins have recently been shown to promote docking of Rab27-bound organelles to the plasma membrane. This mini-review presents a model for how the Rab27-effector complex controls the docking step in the trafficking of Rab27-bound organelles. Our results indicate that Slp2-a, Slp4-a/granuphilin-a and rabphilin are capable of interacting with the plasma membrane directly or indirectly, and thus that these Rab27 effectors form a bridge between Rab27-bound organelles and the plasma membrane.

Introduction

Cells in multicellular organisms secrete a variety of molecules, including neurotransmitters, hormones and enzymes, by exocytosis (i.e. fusion of secretory vesicles with the plasma membrane) in response to extracellular stimuli. Vesicular exocytosis generally consists of three distinct steps: docking/tethering of a transport vesicle to the plasma membrane, ATP-dependent priming of the vesicle and actual fusion of the vesicle with the plasma membrane (reviewed in [1]). Various molecules (so-called exocytotic proteins) have been reported to be involved in the control of vesicular exocytosis [1]. Rab GTPases constitute the largest family of exocytotic proteins and they are commonly found in all eukaryotic cells (from yeasts to mammals) (reviewed in [2,3]). More than 60 Rab isoforms (Rab1–Rab43) are present in humans and mice [4–6], and our recent systematic analysis has shown that members of the two Rab subfamilies, i.e. the Rab3 subfamily (Rab3A/B/C/D) and the Rab27 subfamily (Rab27A/B), but not of other Rab subfamilies, are specifically targeted to dense-core vesicles in neuroendocrine PC12 cells and control their exocytosis [7]. Both Rab3A and Rab27A are involved in the docking step of dense-core vesicles to the plasma membrane in PC12 cells, and they are likely to function in the vesicle-docking step of exocytosis in a co-operative manner.

Key words: docking machinery, melanosome transport, Rab27 effector, rabphilin, secretory granule, synaptotagmin-like protein

Abbreviations used: PS, phosphatidylserine; RBD, Rab-binding domain; SHD, Slp homology domain; Slac2, Slp homologue lacking C2 domains; Slp, synaptotagmin-like protein; SNAP-25, synaptosome-associated protein of 25 kDa; SNAP25, soluble N-ethylmaleimide-sensitive factor attachment protein receptor

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Analysis of rab27a-deficient ashen mice (a model for human Griscelli syndrome [8]) has revealed involvement of Rab27A in the vesicle-docking step in other secretory cells, including cytotoxic T-lymphocytes [9,10] and pancreatic β-cells [11].

Rab proteins are generally believed to promote membrane trafficking through interaction of the GTP-bound form of Rab with a specific effector molecule(s) [2,3]. To date, three groups of Rab27 effectors (a total of 11 Rab27A effectors) have been identified in humans and mice: Slp (synaptotagmin-like protein)/rabphilin, Slac2 (Slp homologue lacking C2 domains)/Noc2 and Munc13-4 [12–15]. All of them except for Munc13-4 share a common N-terminal Rab27A-binding domain (RBD27; also called SHD (Slp homology domain)), which consists of two potential α-helical regions [16,17]. The Slp family proteins (Slp1/JFC1, Slp2-a, Slp3-a, Slp4/granuphilin and Slp5) [12,18] and rabphilin [19] contain C-terminal tandem C2 domains (putative phospholipid-binding sites [20]; known as the C2A domain and C2B domain), whereas the Slac2 family proteins (Slac2-a/melanophilin, Slac2-b and Slac2-c/MyRIP) and Noc2 lack such domains [12–14]. The Rab-binding specificity of Slps, Slac2s, rabphilin and Noc2 has been thoroughly investigated by co-immunoprecipitation assays and GST (glutathione S-transferase) pull-down assays [12,21] using ‘collections of 60 different Rabs’ [6]. Slp1, Slp2-a, Slp3-a, Slp5 and Slac2-a–c specifically interact with Rab27A/B (lanes 40 and 41 in the middle panel of Figure 1), and do not interact with any of the other 58 Rabs, including Rab3, a closely related isoform of Rab27. In contrast, Slp4-a, rabphilin and Noc2 are capable of interacting with two additional Rabs, Rab3 and Rab8, in vitro, although they prefer to interact with Rab27 to Rab3 (or Rab8) in vivo [12,22–25]. Recent studies have
Figure 1  Specific interaction between Slp2-a and Rab27A/B
Agarose beads coupled with 17-Slp2-a were incubated with COS-7 cell lysates containing each of the 60 different Rabs [6] tagged with FLAG (input: top panel) as described previously [12,21], and proteins bound to the beads were analysed by SDS/10% PAGE followed by immunoblotting with anti-FLAG (DYKDDDDK) tag antibody (blot: anti-FLAG; middle panel) and anti-T7 tag antibody (blot: anti-T7; bottom panel). The positions of the molecular mass markers (×10^{-3}) are shown on the left in kDa. Note that Slp2-a specifically interacted with Rab27 isoforms, but not with any of the other Rab isoforms (lanes 40 and 41) [12,21].

shown that some of these Rab27 effectors are involved in the docking of secretory vesicles to the plasma membrane in concert with Rab27 and that they directly or indirectly interact with a plasma membrane protein [26–29]. In this review, I describe a model for how Rab27 effectors anchor the Rab27-bound organelles to the plasma membrane in secretory cells or melanocytes.

Slp4-a increases the number of ‘inert’ dense-core vesicles docked to the plasma membrane in PC12 cells
Slp4-a is the only member of the Slp family that is endogenously expressed on the dense-core vesicles of PC12 cells [30]. When recombinant Slp4-a is expressed in PC12 cells, it is also targeted to dense-core vesicles [28], where Rab3A and Rab27A are abundant [7,30], and dense-core vesicle localization of Slp4-a is completely dependent on its Rab27-binding ability. Total internal reflection fluorescence microscopy observations have revealed that expression of Slp4-a, but not other Slps, significantly increases the number of dense-core vesicles docked to the plasma membrane before stimulation [28]. Nevertheless, Slp4-a expression in PC12 cells dramatically decreases the number of exocytotic events and the probability of exocytosis (i.e. inhibited hormone secretion) without altering the kinetics of individual vesicle fusion events, indicating that Slp4-a increases the number of ‘inert docked vesicles’ at the plasma membrane. Consistent with these findings, silencing of endogenous Slp4-a in PC12 cells with specific siRNA decreased the number of vesicles docked at the plasma membrane and increased the number of exocytotic events (i.e. enhanced hormone secretion). Similar observations have been reported with regard to insulin secretion by pancreatic β-cells in Slp4-a/granuphilin-a knockout mice [26]: a decrease in the number of plasma membrane-docked insulin-containing vesicles and enhancement of insulin secretion.

How does Slp4-a anchor dense-core vesicles to the plasma membrane? Biochemical analysis has shown that Slp4-a, but not other Slps or rabphilin, directly interacts with Munc18-1 or Munc18-1·syntaxin-1a complex via the linker domain between the SHD and C2A domain [23,28,31,32] (but see [33], Slp4-a directly interacts with syntaxin-1a via the N-terminal SHD in a Rab27-dependent manner). In addition, we found that recombinant Slp4-a co-localizes with endogenous Munc18-1 on dense-core vesicles and partially co-localizes with endogenous syntaxin-1a just beneath the plasma membrane [28]. I have therefore proposed the following model for the docking machinery composed of Rab27, Slp4-a, Munc18-1 and syntaxin-1a (Figure 2C, top): (i) Slp4-a is first recruited to Rab27 on dense-core vesicles via the N-terminal SHD; (ii) Slp4-a interacts with Munc18-1 and a tripartite protein complex (Rab27·Slp4-a·Munc18-1) is formed on dense-core vesicles; (iii) the Munc18-1 in the tripartite complex then interacts with the closed conformation of syntaxin-1a in the plasma membrane, which does not contribute to SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex formation; and (iv) the resulting quadripartite protein complex (Rab27·Slp4-a·Munc18-1·syntaxin-1a) forms a bridge between the dense-core vesicle and plasma membrane.

Why are the plasma membrane-docked vesicles anchored by the quadripartite protein complex insensitive to high-KCl stimulation? Unique biochemical properties of Slp4-a seem to be involved in the ‘inert docking machinery’. For example, the SHD of Slp4-a, but not other SHDs, is capable of interacting with the constitutive negative mutant of Rab27(T23N) (mimics the GDP form) in addition to the constitutive active mutant of Rab27(Q78L) (mimics the GTP form) [31], and the C2A domain of Slp4-a does not show...
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Figure 2 | Proposed model for the docking mechanism mediated by Slps and rabphilin

(A) The GTP-bound activated form of Rab27 is present on a Rab27-bound organelle (e.g. secretory granule or melanosome). A Rab27A effector is first recruited to the Rab27-bound organelle via the N-terminal Rab27-binding domain (RBD or SHD) and functions as a linker between the Rab27-bound organelle and the plasma membrane by simultaneously interacting with Rab27 and a plasma membrane protein (or phospholipid). Proposed docking mechanism mediated by (B) rabphilin in PC12 cells [25,27], (C) Slp4-a complex in PC12 cells [28,31] and parotid acinar cells [32] and (D) Slp2-a in mammalian skin melanocytes [37] and gastric surface mucous cells [29].

Ca\(^{2+}\)-dependent phospholipid-binding activity, in contrast with the C2A domain of Slp3-a and Slp5 [20], both of which promote hormone secretion when expressed in PC12 cells [28,31]. Domain-swapping analysis between Slp4-a and Slp5 has indicated that replacement of the SHD, linker domain and C2A domain of Slp4-a by the SHD, linker domain and the C2A domain of Slp5 respectively dramatically reduces the docking activity of Slp4-a mutant proteins [28], suggesting that all of the domains of Slp4-a are required for full docking activity of the Slp4-a molecule. Although disassembly of the quadripartite protein complex must be necessary for dense-core vesicle exocytosis to occur, when and how the quadripartite protein complex disassembles remains completely unknown.

In addition to being present on dense-core vesicles in neuroendocrine cells, Slp4-a is present on the secretory granules of rat parotid acinar cells and has been shown to control isoproterenol (isoproterenone)-stimulated amylase release from parotid acinar cells [32,34]. Although Munc18-1 and syntaxin-1a are not expressed in parotid acinar cells, their closely related isoforms (Munc18-2 and syntaxin-2/3) are expressed at the apical plasma membrane of parotid acinar cells, where secretory granules are docked and fused. Interestingly, both in vitro and in rat parotid glands, the Slp4-a linker domain interacts with the closed conformation of syntaxin-2/3 in a Munc18-2-dependent manner, but not with syntaxin-2/3 or Munc18-2 alone. Thus the Rab27 · Slp4-a · syntaxin-2/3 tripartite protein complex may mediate the docking of amylase-containing granules to the apical plasma membrane of parotid acinar cells [32] (Figure 2C, bottom). However, it is unknown whether the apical plasma membrane-docked granules anchored by the Rab27 · Slp4-a · syntaxin-2/3 tripartite protein complex are ‘inert vesicles’ or ‘releasable vesicles’. Further study is necessary to address this issue.

Rabphilin increases the number of ‘releasable’ dense-core vesicles docked to the plasma membrane in PC12 cells

Rabphilin was originally described as a specific Rab3 effector in the brain [19], and it has recently been shown to function as a Rab27 effector on dense-core vesicles in PC12 cells [25] and on synaptic vesicles in Caenorhabditis elegans [35]. The same as Slp4-a, expression of rabphilin in PC12 cells significantly increased the number of vesicles docked to the plasma membrane, but unlike Slp4-a, rabphilin greatly enhanced high-KCl-dependent hormone secretion without altering the kinetics of vesicle fusion [27], indicating that rabphilin increases the number of ‘releasable docked vesicles’ at the plasma membrane. Since deletion of the C2B domain of rabphilin (rabphilin-AC2B) decreased the number of plasma membrane-docked vesicles and exocytotic events [27], rabphilin should anchor dense-core vesicles to the plasma membrane via the C2B domain. Biochemical analysis has shown that the C2B domain of rabphilin, but not of Slps, interacts with t-SNARE (target-membrane-associated SNARE) SNAP-25 (synaptosome-associated protein of 25 kDa) and that this interaction is enhanced by the presence of Ca\(^{2+}\) [27]. Thus, in the most plausible model, rabphilin forms a bridge between Rab27 on the dense-core vesicle and SNAP-25 on the plasma membrane (Figure 2B). Consistent with this model, genetic interactions between

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Rab27 and rabphilin and between rabphilin and SNAP-25 have recently been reported for synaptic vesicle trafficking in *C. elegans* [35], although no abnormalities in synaptic vesicle docking have been reported in rabphilin knockout mice [36]. It is possible that other docking machineries compensate for the function of the Rab27 · rabphilin · SNAP-25 system in rabphilin knockout mice, or alternatively, that the contribution of the Rab27 · rabphilin · SNAP-25 complex to the synaptic vesicle-docking step is much smaller in mice than in *C. elegans.*

### Slp2-a is involved in the docking of mucus granules to the apical plasma membrane in gastric surface mucous cells and of melanosomes to the plasma membrane in melanocytes

Although Slp2-a is absent from dense-core vesicles in neuroendocrine PC12 cells and expression of Slp2-a in PC12 cells has no effect on dense-core vesicle exocytosis [28,31], Slp2-a is most abundantly expressed in the mouse stomach, specifically in the gastric surface mucous cells (i.e. exocrine cells) [29]. We found that both Slp2-a and Rab27 are predominantly localized at the apical region of gastric surface mucous cells, where mucus granules are accumulated. Analysis of Slp2-a mutant mice generated by homologous recombination has revealed a reduced number of mucus granules, a deficiency of granule docking with the apical plasma membrane in the gastric surface mucous cells and reduction of basal mucus secretion by Slp2-a-deficient gastric primary cells [29]. By analogy to the function of Slp4-a and rabphilin in the docking of dense-core vesicles to the plasma membrane (Figures 2B and 2C), Slp2-a is likely to form a bridge between Rab27 on the mucus granule and an unidentified molecule (X) that is present at the plasma membrane (Figure 2D, bottom). One of the candidates for the molecule is PS (phosphatidylserine), because the C2A domain of Slp2-a specifically interacts with PS in the plasma membrane and the Rab27 · Slp2-a · PS complex has been shown to control the anchoring of melanosomes to the plasma membrane in cultured skin melanocytes [37]. However, since mucus granules are specifically targeted to the apical plasma membrane and are not targeted to the basolateral plasma membrane, additional unidentified key molecule(s) that bind Slp2-a should be present at the apical plasma membrane.

### A new model for the docking machinery composed of the Rab27 · effector complex

Although Slp2-a, Slp4-a and rabphilin interact with distinct ligands via the linker domain or the tandem C2 domains as described above, these Rab27 effectors anchor Rab27-bound organelles (i.e. secretory vesicles or melanosomes) to the plasma membrane in a similar fashion (Figure 2A) and have a two-handed structure: one hand (i.e. SHD or RBD) grabs Rab27-bound organelles and the other, unique hand (i.e. linker domain, C2A domain, or C2B domain) grabs a specific plasma membrane molecule. How do these Rab27 effectors hold Rab27-bound organelles for a long period? A recent kinetic analysis of the Rab27 · effector interaction by surface plasmon resonance has shown that Slp2-a and Slp4-a bind Rab27 with very high affinity (~10 nM) and that the kinetic parameters of Rab27A for the Slp2-a SHD and Slp4-a SHD consist of a fast association rate constant (~4 × 10⁴ M⁻¹ · s⁻¹) and a slow dissociation rate constant (~4 × 10⁻² s⁻¹) [38]. The propensity for rapid association with the SHD by Rab27A and slow dissociation by Rab27A from the SHD, together with the low intrinsic GTPase activity of Rab27A [39], ensure the stable GTP-dependent interaction between Rab27 and its effectors (or anchoring of Rab27-bound organelles to the plasma membrane for a long period). In contrast, Slac2-a (or Slac2-c), another type of Rab27 effector that functions in the actin-based melanosome transport in melanocytes [40–42], displays relatively weak Rab27A-binding affinity (~110 nM) and has a faster dissociation constant (~2 × 10⁻³ s⁻¹), indicating that Slp2-a and Slp4-a are more suitable than Slac2-a for anchoring Rab27-bound organelles to the plasma membrane.

Based on these observations, I propose a new model for the docking machinery (Figure 2A) composed of Rab27 and its effector Slps and rabphilin. Whether this model is also applicable to uncharacterized Slps (i.e. Slp1, Slp3-a and Slp5) remains unknown, but Slp1 may promote docking of PSAP (prostatic-specific acid phosphatase)-containing granules to the plasma membrane through interaction of the C2A domain with PIP3 (phosphatidylinositol 3,4,5-trisphosphate) in prostate carcinoma cells [43], the same as Slp2-a does. Although Slp3-a and Slp5 do not promote docking of dense-core vesicles to the plasma membrane (but do promote high-KCl-dependent hormone secretion) when ectopically expressed in PC12 cells [31], Slp3-a and Slp5 may still be involved in the docking of Rab27-bound organelles to the plasma membrane in other types of cells in which Slp3-a, Slp5 and their ligands are endogenously expressed. Further study is necessary to determine whether all members of the Slp family participate in the docking machinery.

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