

Molecular mechanisms in allergy and clinical immunology

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Understanding exocytosis in immune and inflammatory cells: The molecular basis of mediator secretion

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Inflammatory cells secrete proteins from intracellular vesicles or granules by a process referred to either as *exocytosis* or as *degranulation*, which is common to all cell types. *Exocytosis* is a precise term that describes the process of granule or vesicular fusion with the plasma membrane and is accompanied by release of granule/vesicle contents to the cell exterior. This process is of particular significance with respect to tissue damage and remodeling in inflammatory diseases, inasmuch as these changes are the consequences of inflammatory cell activation and mediator elaboration. Despite its unifying importance to all inflammatory cell types, little is known about the precise molecular and intracellular mechanisms that regulate mobilization of secretory granules/vesicles and, ultimately, secretion of mediators from immune and inflammatory cells. This article reviews the mechanisms and molecules currently implicated at distal stages of exocytosis from eosinophils, neutrophils, mast cells, platelets, and macrophages. Conserved molecules identified among inflammatory cell types indicate a convergence of pathways leading to mediator secretion. The identification of essential molecules in the cascade of events leading to exocytosis is critical in the search for novel therapeutic targets aimed at modulating mediator secretion from these cell types. (*J Allergy Clin Immunol* 2003;111:923-32.)

Key words: *Botulinum, eosinophils, exocytosis, munc18, neutrophils, SNARE, tetanus, Rab*

The release of stored mediators from inflammatory cells has been reported to occur by at least 4 patterns or modes (Fig 1, A and Table I). Classical exocytosis involves the extrusion of single secretory granules, by discrete fusion events, to the cell exterior after stimulation. Compound exocytosis is characterized by multiple intracellular granule-granule fusions that precede their

Abbreviations used

BoNT:	Botulinum neurotoxin
cdk-5:	Cyclin-dependent kinase 5
CG:	Crystalloid granule (eosinophil)
Munc:	Mammalian homolog of unc
NSF:	N-ethylmaleimide-sensitive factor
PKC:	Protein kinase C
PMD:	Piecemeal degranulation
RPMC:	Rat peritoneal mast cell
SNAP:	Soluble NSF attachment protein [unrelated to SNAP-23/25]
SNAP-23/-25:	Synaptosome-associated protein of molecular weight 23 kd/25 kd
SNARE:	SNAP receptor
SM:	Sec1/Munc18
TeNT:	Tetanus neurotoxin
VAMP:	Vesicle-associated membrane protein

secretion in a focused manner (single fusion pore) onto the target surface at the site of cell adherence.^{1,2} Compound exocytosis is exhibited by mast cells,^{3,4} eosinophils activated in response to helminth infection,² neutrophils,⁵ and platelets.⁶ Piecemeal degranulation (PMD) has been identified as the predominant mode of secretion from eosinophils localized to sites of allergic inflammation.⁷⁻⁹ Our studies have suggested that PMD is regulated by selective mobilization of cytoplasmic secretory vesicles containing mediators, with the characteristic presence of intact cytoplasmic crystalloid granules (CGs) containing partially eroded core components.^{10,11} It is postulated that these vesicles function as transport conduits for the selective release of granule-derived mediators (Fig 2, A). PMD has also been reported as a mode of exocytosis utilized by mast cells.^{12,13} Cytolysis (also known as *necrosis* or *total granule extrusion*), which we believe is not a mode of exocytosis, is associated with the deposition of intact granules in the tissue that coincides with cell death. Cytolysis has been identified as an important mode for deposition of granules from tissue eosinophils, secondary to PMD.^{7,14}

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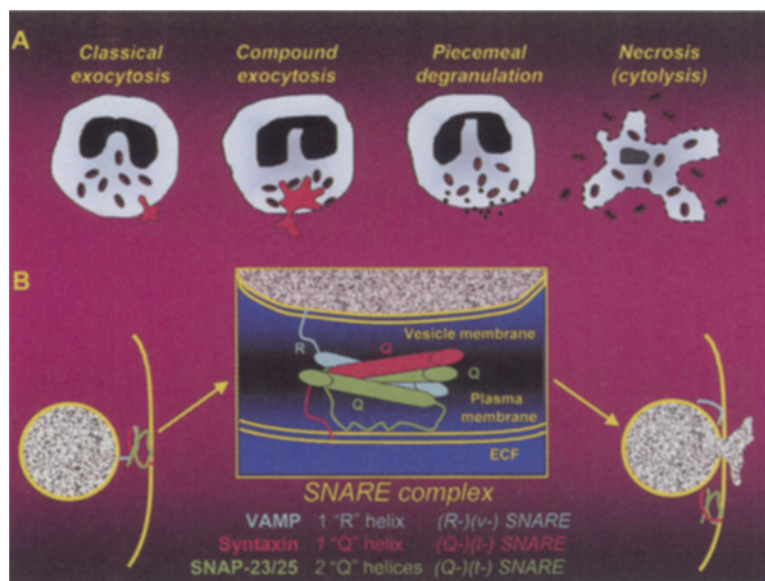


FIG 1. Modes of mediator release and SNARE complex assembly. **A**, Major modes of mediator release from hematopoietic cells. **B**, In mammals, the SNARE complex is a 4-helix bundle: 1 helix is contributed from a VAMP isoform, 1 helix from a syntaxin isoform, and 2 helices from SNAP-23, SNAP-25, or SNAP-29. Complexed SNAREs are resistant to proteolytic cleavage from tetanus and botulinum toxins. Cleavage of free, uncomplexed SNAREs impairs their assembly and prevents membrane fusion and mediator release.

TABLE I. Granule populations and patterns of exocytosis in hematopoietic cells

Cell	Mode(s) of exocytosis	Granule(s)	Granule/vesicle		Plasma membrane* Q-SNARE(s)
			R-SNARE(s)	Q-SNARE(s)	
Eosinophil	Classical, PMD, compound	Crystalloid granules	(—) VAMP-2 ¹¹	ND	SNAP-23 ³⁷
		Small granules	ND	ND	Syntaxin 4 ³⁷
		Small vesicles	VAMP-2 ¹¹	ND	
Neutrophil	Classical, compound	Azurophilic granules	(—) VAMP-2 ⁴⁴	ND	SNAP-23 ⁴⁵
		Secondary granules	VAMP-2 ^{43,44}	SNAP-25, -23 ^{45,51}	Syntaxin 4 ⁴⁴
		Tertiary granules	VAMP-2 ^{43,44}	SNAP-23 ⁴⁵	Syntaxin 6 ⁴⁵
		Small vesicles	VAMP-2 ^{43,44}	ND	
Mast cell	Classical, PMD, compound	Secretory granules	VAMP-2, 3, 7, 8 ⁵²⁻⁵⁴	SNAP-23 ⁵²	SNAP-23 ⁵²
		Small vesicles	VAMP-2, 3, 7 ⁵²⁻⁵⁴	ND	Syntaxin 2,3,4 ^{52,53}
Macrophage	Classical	Small vesicles	VAMP-2, 3 ⁷²⁻⁷⁴	Syntaxin 4 ⁷⁹	Syntaxin 2, 3, 4 ⁷⁷⁸
		Phagosomes	VAMP-2, 3 ⁷²⁻⁷⁴	Syntaxin 2, 3, 4 ⁷⁸	
Platelet	Classical, compound	α-granules	VAMP-3 ⁶⁵	SNAP-23, syntaxin 2, 4 ^{65,69}	Syntaxin 4 ⁶⁹
		Dense granules	VAMP-8 ⁷⁶⁶	4 ^{65,69}	SNAP-23 ⁶⁵
		Secretory lysosome	VAMP-3?	ND	Syntaxin 2 ^{65,69}
		Small vesicles	ND	Syntaxin 2, 4 ⁶⁹	

(—) denotes absence.

ND, Not determined.

*Entries for Q-SNARE(s) in the plasma membrane column are unrelated to the entries in the granule/vesicle columns.

SNAREs IN VESICLE/GRANULE DOCKING: SPECIFICITY FOR FUSION?

Exocytosis of secretory vesicles/granules occurs by a highly regulated series of events. These include (a) mobilization or translocation of the vesicle/granule to the cell periphery, (b) tethering of the granule to the plasma membrane, and (c) docking between the vesicle/granule and plasma membrane, which is a prerequisite for (d)

membrane fusion and mediator release. For the past decade, investigations have focused intently on a highly homologous set of membrane-associated proteins known as *SNAP receptors* (SNAREs), which are critically involved in granule/vesicle docking. Isoforms of SNAREs are classified on the basis of whether they contribute a conserved arginine (R) or glutamine (Q) residue to the central core region of a conserved docking (SNARE) complex.¹⁵ This classification is a suggested

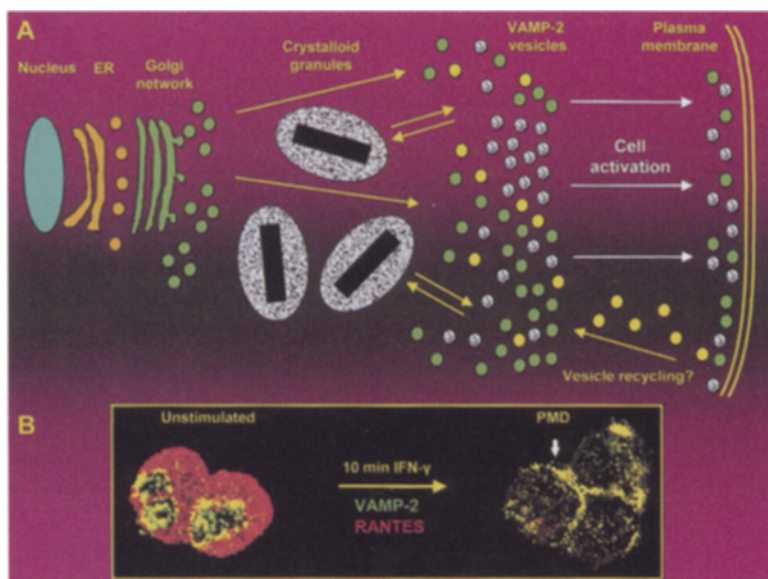


FIG 2. Model of SNARE-mediated docking in eosinophil PMD. **A**, Post-Golgi-derived VAMP-2⁺ cytoplasmic vesicles are postulated to act as exchange vessels with large CGs. Receptor-coupled stimulation causes rapid mobilization of cytoplasmic vesicles to the plasma membrane, SNARE complex assembly (VAMP-2/SNAP-23/syntaxin 4), and mediator release. After their recycling, small vesicles might further act as transport vehicles for the selective release of CG-derived mediators in further rounds of exocytosis. **B**, Double-labeled confocal microscopy. RANTES⁺ and VAMP-2 are colocalized to cytoplasmic vesicles (yellow). IFN- γ -induced PMD exhibited a rapid shift in immunoreactivity of VAMP-2 to the cell periphery, which coincided with RANTES mobilization and content release.¹¹

alternative to the nomenclature of v- (vesicle) and t- (target membrane) SNAREs, which categorized isoforms according to their cellular localization. In mammals, the 4-helix SNARE complex is formed from the interaction of 1 R-SNARE coil, from a vesicle-associated membrane protein (VAMP) isoform, with 3 Q-SNARE coils—1 coil from a syntaxin isoform and 2 coils from either synaptosome-associated protein of 23, 25, or 29 kd (SNAP-23, SNAP-25, or SNAP-29; Fig 1, B).^{16,17} The cytosolic proteins soluble NSF attachment protein (SNAP; this is unrelated to the Q-SNAREs [SNAP-23, SNAP-25, and SNAP-29]) and N-ethylmaleimide-sensitive factor (NSF) are recruited to the SNARE complex and are involved in the rapid disassembly and recycling of SNAREs to their original compartments.¹⁸⁻²⁰

It has been demonstrated in a variety of cell types that formation of SNARE complexes is a critical event preceding membrane fusion and mediator release. This has been elegantly demonstrated by a number of studies that have examined the inhibitory activity of tetanus neurotoxin (TeNT) and botulinum neurotoxin. These neurotoxins specifically target and cleave free SNAREs, preventing both their assembly into docking complexes and the subsequent fusion of granule/vesicle and plasma membranes.^{21,22} The potency and specificity of these toxins has recently received much attention for their clinical use in cosmetic applications and treatment of spasticity and headaches. Injection of Botox (botulinum neurotoxin A) into specific peripheral nerves induces nonpermanent, but often long-lasting, paralysis of muscular activity.²³⁻²⁵

An intriguing hypothesis, originally proposed by Rothman et al,²⁶ stated that interactions between v(R)- and t(Q)-SNAREs are a critical step that confers specificity to the trafficking of all distinct vesicle/granule populations to their target membranes. That is, SNAREs localized to specific microenvironments are anticipated to form complexes with each other (cognate SNAREs) but not with SNAREs localized to other regions (noncognate SNAREs). To date, this concept remains controversial. The diversity of SNARE isoforms suggests that they are likely to be sufficient in number to be involved in this task. However, noncognate SNAREs have been reported to readily associate *in vitro*,²⁷⁻³¹ and studies in yeast have supported that an individual R-SNARE can interact with multiple syntaxins at different cellular localizations *in vivo*.^{32,33} Biochemical studies performed by Rothman and colleagues³⁴⁻³⁶ have demonstrated that complex formation does require a correct stoichiometry of helices: 1 “R” coil and 3 “Q” coils. However, multiple R-SNAREs equally promoted efficient fusion with plasma membrane Q-SNAREs,³⁴ suggesting that isolated SNAREs cannot by themselves account for specificity.

SNARE ISOFORMS IMPLICATED IN EXOCYTOSIS FROM HEMATOPOETIC CELLS

Eosinophils and neutrophils

We have recently demonstrated that human eosinophils express the Q-SNAREs SNAP-23 and syntaxin 4, which are predominantly localized to the plasma mem-

brane.³⁷ SNAP-23 additionally exhibited substantial immunoreactivity in the Golgi, which has similarly been reported for transfected HeLa cells.³⁸ Our confocal microscopy and subcellular fractionation studies showed that VAMP-2 is predominantly localized to cytoplasmic vesicles.¹¹ Interestingly, we found negligible immunoreactivity for VAMP-2 in CG-enriched fractions, which has been supported by the findings of 2 other separate groups.^{39,40} Previous investigations from our laboratory¹⁰ and others⁴¹ have supported the concept that stimulation with IFN- γ coincides with mobilization of RANTES⁺ secretory vesicles to the cell periphery and PMD from human eosinophils. VAMP-2 exhibited significant colocalization with RANTES⁺ secretory vesicles and was similarly mobilized to the cell periphery on stimulation with IFN- γ (Fig 2, B).¹¹ Our recent experiments suggest that these SNARE isoforms are critically involved in granule-derived mediator secretion from human eosinophils.⁴²

A similar pattern of localization of VAMP-2 has been reported for human neutrophils. Brumell et al⁴³ first reported the localization of VAMP-2 predominantly within small vesicles/granules, with negligible amounts localized to large azurophilic granules. Neither the VAMP-1 nor the VAMP-3 (cellubrevin) isoform was detected by Western blot analysis. Electron microscopy studies by Mollinedo et al⁴⁴ recently confirmed the localization of VAMP-2 to both lactoferrin- and gelatinase-containing compartments, markers for secondary and tertiary granules, respectively. Separate studies by Brumell et al⁴³ and Mollinedo and colleagues^{44,45} indicate that syntaxin 4 and syntaxin 6 are similarly localized to the plasma membrane. Syntaxin 6 appears to be a promiscuous SNARE involved in trans-Golgi or post-Golgi trafficking in fibroblasts⁴⁶⁻⁴⁸ and neuroendocrine cells⁴⁶ but is rather exclusively localized to the plasma membrane in human neutrophils.⁴⁵ In contrast to eosinophils, substantial immunoreactivity for SNAP-23 in neutrophils was detected in secondary/tertiary granules, in addition to the plasma membrane.⁴⁵ Both PMA and ionomycin-induced neutrophil activation have been associated with significant shifts in immunoreactivity for VAMP-2^{43,44} and SNAP-23⁴⁵ from cytoplasmic regions to the cell periphery. Syntaxin 4 and VAMP-2 are increasingly associated with each other after PMA-induced activation.⁴⁴ Similarly, SNAP-23 increasingly coprecipitates with syntaxin 6 after activation.⁴⁵ In electroporabilized neutrophils stimulated with Ca²⁺ and GTP γ S, preincubation with TeNT or antibodies directed to VAMP-2, syntaxin 4, syntaxin 6, or SNAP-23 are all effective agents to impair CD66b surface upregulation.^{44,45} CD66b (formerly CD67) was recently demonstrated to be a protein marker for both secondary and tertiary granules.⁴⁴ Surface upregulation of CD63, a tetraspanin localized to azurophilic granules, was impaired by anti-syntaxin 6 but not anti-SNAP-23 antibodies.⁴⁵ Other inhibitors were not examined for their effects on CD63 upregulation.

The subcellular localization of VAMP-2 in both human neutrophils and eosinophils suggests that it might be

exclusively involved in mediator release from small granules/vesicles.^{11,44} It has been proposed that membrane "budding" of CGs might be a mechanism for PMD and might contribute in part to the cytoplasmic vesicle pool in eosinophils.^{8,49} Given that cytoplasmic CGs remain approximately uniform in size even under conditions in which depletion of matrix content has occurred,⁷⁻⁹ CG "budding" would require the replacement of "lost" granule membrane during PMD. It is undetermined whether there are common SNAREs localized to both CG and cytoplasmic vesicles in eosinophils. However, the observation that VAMP-2 is not localized to CGs suggests that CG "budding" does not significantly contribute to the formation of the cytoplasmic vesicle pool. We postulate that cytoplasmic vesicles function as a rapidly mobilizable pool for mediator secretion, independent of CG docking. It is conceivable that these vesicles can later act as transport vesicles for selective CG mediator release after their recycling from the plasma membrane (Fig 2, A).

Mollinedo et al⁴⁴ proposed a hypothetical model in which 2 ternary SNARE complexes are required for exocytosis of secondary/tertiary granules: (a) VAMP-2/SNAP-23/syntaxin 4 and (b) VAMP-2/SNAP-23/syntaxin 6. In accord with this model, it is generally accepted that multiple SNARE complexes would be required to induce fusion of vesicle/granule membrane with its target membrane.¹⁶ Whether these multiple SNARE complexes are homotypic or heterotypic awaits further testing. It is unclear which SNARE(s) localize to eosinophil CG, and the same is true for neutrophil azurophilic granules. In addition to the neutrophil SNAREs mentioned above, Mollinedo and colleagues have reported intracellular immunoreactivity for syntaxin 1A⁵⁰ and localization of SNAP-25 to secondary granules.⁵¹ In addition, several syntaxin isoforms are expressed at the mRNA level in neutrophils.⁵⁰ The role of these isoforms in neutrophil membrane trafficking events has not been adequately examined.

Mast cells

Recruitment of SNAP-23 to granule membranes in rat peritoneal mast cells (RPMCs) has been implicated as an essential prerequisite for mediator release from mast cells. In resting mast cells, SNAP-23 is concentrated in distinct focal points of lamellipodia-like projections of the cell membrane. On cell activation, SNAP-23 is relocated to intracellular granules and is a prerequisite step secretion of the granule protein β -hexosaminidase. Introduction of antibodies to SNAP-23 in streptolysin-*O*-permeabilized RPMCs prevented this relocation and impaired β -hexosaminidase secretion. It is postulated that relocation of SNAP-23 is essential in the process of enabling a "fusion-ready" state for cytoplasmic granule-granule and subsequent granule-plasma membrane fusions during compound exocytosis in these cells. In this study, VAMP-2 exhibited a punctate intracellular staining pattern by immunofluorescence microscopy in RPMCs.⁵² Several R-SNARE isoforms, including VAMP-2, VAMP-3, VAMP-8 (endobrevin), and VAMP-7 (tetanus-insensitive VAMP) are localized to granules

and/or secretory vesicles in the rat mast cell line, RBL-2H3.^(53,54) Unlike VAMP-2 and VAMP-3,⁵⁵ the isoforms VAMP-7 and VAMP-8 are both resistant to proteolytic cleavage by neurotoxins.^{53,56-58} VAMP-7 and VAMP-8 are reported to localize to endocytic compartments in non-neuronal cells.⁵⁸⁻⁶⁰ A recent study has indicated that VAMP-7 colocalized with CD63⁺ compartments in PC12 neuronal cells,⁶¹ which could suggest a potential role for this isoform in exocytosis of secretory lysosomes from hematopoietic cells.⁶²⁻⁶⁴ It is presently unclear which of the identified VAMP isoforms are preferentially involved in mast cell granule docking to the plasma membrane.

Platelets

Studies by separate groups have indicated that VAMP-3^{55,65,66} and VAMP-8,⁶⁶ but not VAMP-2,^{55,67,68} are expressed in human platelets. Electron microscopy studies by Feng et al⁶⁵ indicate that VAMP-3 is predominantly localized to α -granules, though not all cytoplasmic granules exhibited VAMP-3 immunoreactivity. Functional analyses of secretion in streptolysin-*O*-permeabilized platelets utilizing neutralizing antibodies,⁶⁵ TeNT,⁶⁸ and peptide analogs,⁶⁶ however, have supported that VAMP-3 is involved in mediator release from both α -granules and dense granules. A study by Polgar et al⁶⁶ supported that VAMP-8 is implicated in dense granule secretion but does not appear to be involved in mediator release from α -granules. The authors suggest that both R-SNAREs might be critically involved in compound exocytosis of cytoplasmic α -granules and dense granules,⁶⁶ which has been observed after thrombin-induced activation.⁶ Alternatively, it is possible that VAMP-3 is a common R-SNARE utilized by both granule populations.

Collectively, separate studies indicate that platelet Q-SNAREs are likely involved in multiple docking events from different granule populations. Electron microscopy studies indicate that SNAP-23, syntaxin 2, and syntaxin 4 are localized to multiple membranes, including storage granules, membrane channels, and the plasma membrane.^{65,69} Syntaxin 4 can functionally associate with SNAP-25,⁷⁰ VAMP-3, and VAMP-8,⁶⁶ whereas syntaxin 2 has been reported to associate with SNAP-23.⁷¹ Targeted inhibition of syntaxin 4 was reported to impair mediator release from both α -granules⁶⁸ and lysosomes,⁶⁹ but not dense granule secretion.⁷¹ Both SNAP-23 and syntaxin 2 are functionally involved in mediator release from dense granules and lysosomes.^{69,71} It is unclear whether syntaxin 7 participates in platelet secretion, inasmuch as targeted inhibition of this isoform did not significantly affect mediator release from either dense granules⁷¹ or lysosomes.⁶⁹

Macrophages

VAMP-2 and VAMP-3 have been implicated in exocytosis of secretory vesicles, which is a prerequisite for phagocytosis in murine macrophages to maintain plasma membrane homeostasis.^{72,73} Hackam et al⁷² reported that treatment with TeNT impaired phagocytosis in murine J774 macrophages. Clustering of VAMP-3 was found at the site

of phagosome formation and was additionally incorporated into early phagosomes, suggesting its involvement in phagosomal maturation events.⁷³ A study by Allen et al,⁷⁴ however, demonstrated that macrophages derived from VAMP-3 knockout mice did not exhibit impaired phagocytosis or phagosome maturation, regardless of the type of receptor engagement or particle load.⁷⁴ They suggest that R-SNARE isoforms, VAMP-2, VAMP-3, and VAMP-8, all of which have been localized to recycling endosomes,⁷⁵⁻⁷⁷ might perform redundant roles and/or be able to compensate for each other in phagocytic processes. Docking partners for macrophage R-SNAREs include syntaxins 2, 3, and 4, all of which have been localized to both plasma membrane and isolated phagosomes.⁷⁸ Syntaxin 4 was recently identified as an important component involved in LPS-induced cytokine secretion in J774 macrophages.⁷⁹

REGULATION OF VESICLE/GRANULE DOCKING THROUGH ACCESSORY MOLECULES

Sec1/Munc18 proteins

A family of conserved syntaxin-binding proteins, the Sec1/Munc18 (SM) proteins, has been directly implicated in regulation of SNARE complex assembly. SM proteins were initially identified as critical vesicular transport proteins in yeast (Sec1) and *Caenorhabditis elegans* (unc-18).^{80,81} Three mammalian homologs of unc-18 (Munc-18) have been identified: Munc18-a (or -1), Munc18-b (or -2) and Munc18-c (or -3).⁽¹⁶⁾ Munc18-c is the most broadly expressed isoform and has been identified in non-neuronal tissues.^{70,82-86} Like SNAREs, SM proteins have been demonstrated to be essential for secretion. Knockout mutants in *C. elegans* (unc18),⁸⁰ *Drosophila* (Rop),^{87,88} and mice (Munc18-a)⁸⁹ are all paralyzed and devoid of secretion from neuronal tissues.

SM proteins selectively bind to a "closed" conformation of syntaxin. In this conformation, syntaxin's amino terminal region is folded over its SNARE-binding region. Thus, when bound to Munc18, syntaxin has an impaired ability to associate with other SNAREs and form docking complexes.⁹⁰⁻⁹² Houg et al⁸⁶ recently reported that human platelets express all 3 mammalian SM isoforms (Munc18-a, Munc18-b, and Munc18-c), which were detected in membrane and cytosolic fractions. Their observations support that the disassembly of the Munc18-syntaxin complex is associated with mediator release in platelets. In their study, administration of peptides or neutralizing antibodies that interfere with the assembly of Munc18-syntaxin complexes resulted in a potentiation of Ca²⁺-induced secretion.⁸⁶ SM proteins have also been critically implicated in mediator secretion from mast cells.⁹³

Similar to the reported promiscuous interaction of SNARE isoforms, Munc18 isoforms can bind multiple syntaxins, at least in vitro. Munc18-a and Munc18-b exhibit similar binding to syntaxins 1A, 2, and 3,⁹⁴⁻⁹⁶ and Munc18-c is capable of interacting with syntaxins 2 and 4.⁹⁴ These observations lend further support to the

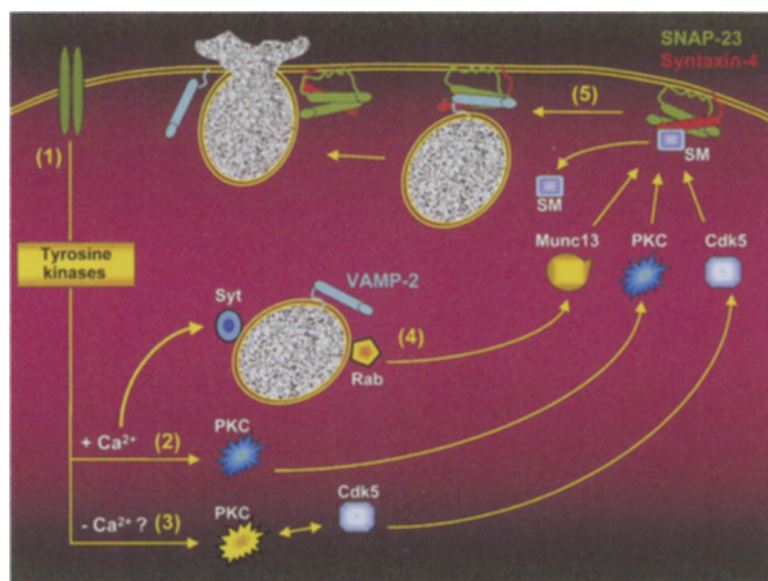


FIG 3. Model of signaling events leading to granule-vesicle docking. (1) In many hematopoietic cell types, receptor-coupled stimulation leads to increased intracellular Ca^{2+} levels via receptor-associated signaling molecules and tyrosine kinases. (2) Intracellular Ca^{2+} can recruit and activate PKC and synaptotagmins (Syt). (3) Other signaling molecules implicated in secretion include cyclin-dependent kinase 5 (*cdk5*) and nonconventional PKC isoforms, which might function in Ca^{2+} -independent signaling events. (4) Rab GTPases and their downstream effectors, such as Munc13, have also been demonstrated to be critical for granule docking. (5) PKC, cyclin-dependent kinase 5, and Munc13 are all candidate molecules involved in dissociating SM proteins from syntaxin. After dissociation of SM from syntaxin, SNAREs are assembled into a docking complex, resulting in membrane fusion and mediator release.

notion that the specificity of granule/vesicle docking is unlikely to be determined by a single family of proteins but is the result of coordinated interactions of multimeric signaling complexes.

Ca^{2+} -mediated signaling events and effector proteins

Several investigations have indicated that there are important links between early-signaling molecules activated after receptor-coupled stimulation and the late stages of exocytosis coupled to SNARE assembly. In general, diacylglycerol- and Ca^{2+} -mediated activation of protein kinase C (PKC) is regarded as a key triggering step in the coupling of stimulus to secretion in hormone and neurotransmitter release. Phorbol esters, such as phorbol myristate acetate⁹⁸ and allergen-induced mediator release from eosinophils.^{99,100} Similarly, studies in neutrophils,¹⁰¹⁻¹⁰³ mast cells,^{104,105} lymphocytes,¹⁰⁶ and platelets^{107,108} support that PKCs might be essential in regulating degranulation in hematopoietic cells.

It now appears that an important effector function of PKC is the regulation of SNARE partnering with other SNAREs and/or SNARE-associated accessory proteins.

SNAP-25, VAMP-2, and SM proteins are all readily phosphorylated by PKC *in vitro*.¹⁰⁹⁻¹¹² PKC-mediated phosphorylation of SNAREs is reported to reduce their affinity for SNARE-SNARE partnering.^{107,109} Similarly, PKC-mediated phosphorylation of SM proteins reduced their affinity for syntaxins *in vitro*.^{70,111} An attractive paradigm is that stimulus-coupled Ca^{2+} -mediated activation of PKC leads to the phosphorylation of Munc18 and/or SNAREs, leading to an "open" syntaxin conformation and SNARE assembly (Fig 3).¹¹¹

Studies in neuroendocrine cells indicate other signaling molecules that are recruited to facilitate SNARE assembly, which might act in concert with Ca^{2+} -mediated events or represent alternate pathways for mediator release. For example, cyclin-dependent kinase 5 phosphorylates Munc18-a in the absence of a Ca^{2+} flux in association with mediator release from neuroendocrine cells.¹¹³ Similarly, certain isoforms of PKC are recruited and activated in a Ca^{2+} -independent manner.¹¹⁴⁻¹¹⁷ Another family of molecules involved in priming of vesicles/granules for docking assembly includes Munc13 (mammalian homolog of unc13). Munc13 isoforms possess both Ca^{2+} and diacylglycerol binding domains similar to those found on Ca^{2+} -dependent PKC isoforms, indicating their potential regulation by Ca^{2+} .¹¹⁸ Munc13 proteins are implicated in dissociating SM proteins from syntaxin to promote SNARE assembly.¹¹⁹ A recent study has identified that Munc13 is a downstream effector of Rab3 activation in neurons (see the section on Rabs below) (Fig 3).¹²⁰

Although there is no compelling evidence that SNARE proteins themselves are intrinsically sensitive to calcium, calcium ions might directly influence granule/vesicle docking by binding to Ca^{2+} -binding proteins.¹²¹ Synaptotagmins are Ca^{2+} -sensing proteins associated with secretory vesicles and have been shown to be essential in docking of secretory granules and in post-docking events leading to mediator release.^{114,122} Twelve isoforms of synaptotagmins have been identified, with synaptotagmins III through XII exhibiting the widest tissue distribution.^{16,123} Baram et al^{124,125} demonstrated in separate studies that synaptotagmin I and II are expressed in RPMC and RBL-2H3 cells. Interestingly, transfection of RBL-2H3 with synaptotagmin I potentiated Ca^{2+} -induced mediator secretion,¹²⁴ whereas synaptotagmin II appears to negatively regulate lysosome secretion from these cells.¹²⁵ Synaptotagmin II has been localized to secondary granules of human neutrophils and is additionally recruited to phagosomes, suggesting a dual involvement in secretion and phagocytotic processes in these cells.¹²⁶

Rab GTPases and downstream effectors

Rabs constitute an immensely diverse family (>40 isoforms) of the Ras-related monomeric G proteins. Several studies have supported that Rab isoforms localize to membranes of distinct intracellular compartments, lending credit to the notion that this family of proteins might be particularly important in directing distinct granule/vesicle populations to their appropriate destinations.¹²⁷⁻¹²⁹ A wide variety of Rab effector proteins have been identified, and they are involved in a diverse number of roles, including Golgi vesicle budding, recruitment of cytoskeletal proteins for organelle/vesicle movement, and protein-protein tethering at granule/vesicle docking.^{128,130-132}

Recent studies have linked a variety of Rab isoforms in late stages of exocytosis and SNARE assembly in hematopoietic cells. Cytotoxic lymphocytes derived from Rab27 knockout mice are devoid of mediator secretion despite exhibiting normal polarization of granules toward the site of plasma membrane adherence to target cells. Although the precise function of Rab27 is not known, the observation that granule mobilization is not impaired suggests that Rab27 might be important for granule tethering to the plasma membrane.¹³² Rab3d is expressed in RBL-2H3 cells and recruited an unidentified kinase after antigen-induced cell activation. Rab3d was implicated in recruitment of an unidentified kinase that was capable of phosphorylating syntaxin 4 in vitro.^{133,134} A study in platelets¹³⁵ supported that inhibition of Rab4 resulted in impaired mediator release from α -granules. In addition, Rab6 was phosphorylated in a PKC-dependent manner in platelets, suggesting a potential role for this isoform in exocytosis.¹⁰⁸ Recently, it was demonstrated that Rab4 can directly interact with syntaxin 4 in vitro, suggesting that direct Rab-SNARE interactions might be an important event for tethering and/or priming for SNARE assembly.¹³⁶

CONCLUSION

Exocytosis is thus a critical event in the activation of mediator-containing and/or secretory immune and inflammatory cells. Because activation of these cell types is directly implicated in the exacerbation of allergic inflammation in diseases such as asthma, it is vital to investigate the mechanisms regulating mediator release in immune and inflammatory cells. The diversity of molecules and the complexity of signaling processes involved in exocytosis present an exciting but challenging area of research to identify critical molecules in specific cell types. Our group, for almost a decade, has focused on investigating exocytosis in a systematic, yet also translational, direction in allergy and asthma. Our ultimate goal is to identify intracellular targets for potential new therapeutic strategies aimed at modulating the distal arm of exocytosis, which we believe might prevent the clinical sequelae of mediator secretion in various dysfunctions in which exocytosis is a key proinflammatory event.

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