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## Diverse exocytic pathways for mast cell mediators

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### Summary

Mast cells play pivotal roles in innate and adaptive immunities but are also culprits in allergy, autoimmunity and cardiovascular diseases. Mast cells respond environmental changes by initiating regulated exocytosis/secretion of various biologically active compounds called mediators (e.g., proteases, amines and cytokines). Many of these mediators are stored in granules/lysosomes and rely on an intricate degranulation process for release. Mast cell stabilizers (such as sodium cromoglicate) which prevent such degranulation process have therefore been clinically approved to treat asthma and allergic rhinitis. However, it has become increasingly clear that different mast cell diseases often involve multiple mediators, which seem to rely on overlapping but distinct mechanisms for release. This review highlights the evidence for diverse exocytic pathways and discusses strategies to identify unique molecular components in these pathways which could serve as new drug targets for more effective and specific treatments against mast cell-related diseases.

### Keywords

membrane fusion; exocytosis; SNARE; Munc18; mast cell; degranulation

### Introduction

Mast cells are best known for their ability to release a diverse range of biologically active compounds (aka mediators) when the cell surface receptors bind to their specific ligands [1]. The eventual targets of signaling cascades include the transcriptional machinery (i.e., to produce cytokines etc.), the cytoskeleton network (i.e., to facilitate granule translocation) and the membrane fusion machinery (i.e., to promote vesicular/granular fusion) [2, 3]. Much progress has been made in understanding the upstream events in the intricate signaling cascades [4, 5]. This review, however, intends to draw readers' attention to the interface between signaling and the exocytic fusion machinery, because different modes of activation at the cell surface are shown to generate different secretory profiles (Fig. 1) [6, 7]. This has

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important implication to mast cell-related diseases that involve different mediators as well as different modes of activation.

## Mast cell mediators in health and disease

Mast cell mediators can be divided into two groups, preformed (including histamine, tryptase and  $\beta$ -hexosaminidase, etc.) and newly synthesized (cytokines, chemokines, prostaglandin and leukotriens, etc.). Some (such as TNF $\alpha$ ) belong to both categories. For detailed description of different mediators please consult recent reviews [8, 9]. It is important to note that the *de novo* synthesized cytokines and chemokines are thought to undergo constitutive secretion [10], which means their release is regulated at the transcription level, contrary to the preformed the mediators.

### Native immunity

Mast cells from human and rodent sources have the capacity to directly respond to the challenge of pathogens and their products by releasing preformed mediators, newly synthesized mediators, or both [11]. For example, *E.coli* induces the secretion of mediators in both categories [12–15] but *S.pneumonia* only elicits the release of preformed mediators [16, 17]. Meanwhile, HIV [18, 19], Dengue virus [18, 19], and cholera toxin [18, 19] seem to exclusively impact the newly synthesized mediators. Thus far, it is not clear whether specific subsets of the mediators within either group are selectively released to combat against different pathogens.

### Allergic inflammation

In respiratory disorders, mast cells are known for their accidental or mistaken activation via cross-linking of surface-bound IgE which leads to rapid degranulation, mediator release (e.g., histamine, P<sub>DT</sub>2, tryptase, Cys-LTs) and manifestation of an acute phase allergic reaction [20]. Apart from their pro-inflammatory actions, mast cells have an impressive capability to down-regulate immunological responses, by releasing the anti-inflammatory cytokine IL-10 [21]. Another anti-inflammatory action is through the release of mast cell granule proteases to degrade and neutralize key cytokines such as TNF $\alpha$ , IL-4, IL-13 and IL-33 [22, 23]. Thus, mast cells act as local immune modulators which coordinate the delicate balance between pro- and anti-inflammatory responses of the host.

### Autoimmunity

Mast cells are associated with a variety of autoimmune diseases ranging from multiple sclerosis (MS), rheumatoid arthritis (RA), to bullous pemphigoid (BP) [24]. Studies of murine models of MS (EAE, or Experimental autoimmune encephalomyelitis), RA and BP have revealed common underlying mechanisms of mast cell influence on these diseases [25]. For instance, in primary progressive EAE, mast cell-derived TNF $\alpha$  and tryptase are intimately associated with disease onset and development [26–30]. Similarly in BP, the exocytosis of preformed mediators including tryptase, histamine, and TNF $\alpha$  from skin mast cells result in an accumulation of neutrophils and skin blistering [31–35]. In RA, the *de novo* synthesis of TNF $\alpha$  by mast cells results in IL-1 $\beta$  release from macrophages, and subsequent increase in inflammatory cell infiltration in synovial joints [36]. Synovial inflammation can

also be augmented by mast cell-derived tryptase that promotes synovial fibroblasts to express neutrophil-recruiting chemokines [37].

Mast cell activation in autoimmune diseases such as RA likely involves several pathways, including autoantibodies, Toll-Like Receptor ligands and cytokines, each via a distinct cell surface receptor [38]. These pathways are thought to cooperate to create the pro-inflammatory environment which eventually results in tissue destruction. The development of biologic agents that target various immune mediators and their receptors has dramatically improved the patient prognosis. To date, established and approved therapies for rheumatoid arthritis are designed specifically to block cytokine responses toward TNF $\alpha$  and IL-6 [39].

### Cardiovascular diseases

Cardiac mast cell activation/infiltration has been reported in a number of cardiac conditions including idiopathic cardiomyopathy [40], atherosclerosis [41], myocarditis [42] and ischemic heart disease [40]. The release of mast cell mediators (histamine, TNF $\alpha$ , IL-6, platelet activating factor and reactive oxygen species, etc.) leads to an inflammatory cascade that is detrimental to myocardial contractile function, tissue integrity and electrophysiological activity, and as expected, treatment with mast cell stabilizers has been shown to reduce the extent of cellular injury [43]. Interestingly, both cardiovascular disease risk factor endothelin-1[44] and cardioprotector adrenomedullin [45] were shown to induce cardiac mast cell degranulation [43]. Whether these two peptides impose opposite effects on cardiovascular diseases by eliciting distinct degranulation secretory pathways is currently not known.

### Cancer

Mast cells promote tumorigenesis and tumor progression via a number of mechanisms. Mast cells may stimulate tumor expansion by releasing cytokines and growth factors (e.g., FGF-2, NGF, PDGF, IL-10 and IL-8) in the tumor stroma[46]. Mast cells also provide histamine, which induces tumor cell proliferation through H1 receptors while suppressing the immune system through H2 receptors [47]. Additionally, mast cell-derived angiogenic factors [48, 49] and matrix metalloproteinases [50, 51] induce tumor vascularization and metastasis. Furthermore, mast cells cause immunosuppression through the release of IL-10 and TNF $\alpha$  [52, 53], which facilitates tumorigenesis and progression. By contrast, mast cells play inhibitory roles in tumor cell growth, apoptosis, and inflammation by releasing cytokines such as IL-1, IL-4, IL-6, MCP-3, MCP-4, TGF, and chymase [46]. Other anti-cancer mediators include chondroitin sulphate which inhibits tumor cell diffusion, and tryptase which causes tumor cell disruption [54]. To control the differential release of these pro- and anti- cancer mediators may become strategically important in the fight against cancer.

### Fusion machinery underlying mast cell exocytosis

Like other eukaryotic fusion events along the secretory and endocytic pathways, mast cell exocytosis depends on the specific interaction of SNAREs that are conserved from yeast to human. Anchored to apposed membranes, cognate SNAREs form a fusogenic 4-helical bundle, the so-called trans-SNARE complex [55–57]. SNAREs can be sorted into the Q- and

R- classes, depending on whether they contribute a glutamyl (Q) or an arginyl (R) side chain at the center of the helical bundle [58, 59]. A functional/fusogenic trans-SNARE complex is typically formed by three Q-SNAREs (Qa, b, c) that are emanated from one membrane and one R-SNARE emanated from the other [55]. It is largely accepted a different set of SNAREs are required for distinct fusion events, and that the specificity of vesicular transport is in part encoded by the specific interaction of the cognate SNAREs.

In mast cells at least seven exocytic SNAREs have been identified [60–68], which include two Qa-SNAREs (syntaxin3, 4), one Qb,c-SNARE (SNAP23; contributing two helices), and four R-SNAREs (VAMP2, 3, 7, 8). Our lab has recently reported that these Q- and R-SNAREs could form 8 distinct fusogenic trans-SNARE complexes in reconstitution [69], suggesting that mast cells could exploit distinct exocytic fusion machineries to release its mediators.

## Heterogeneity of exocytic fusion in mast cell - from a theoretical perspective

A close look at the different type of exocytic events in mast cell helps explain the requirement for different sets of SNAREs. Both constitutive and regulated secretions are known to occur in mast cells [10]. Constitutive secretion depends on Golgi-derived vesicles that fuse directly with the plasma membrane. Newly synthesized cytokines are thought to get released via this route, and probably require at least one unique set of SNAREs. Regulated secretion applies to cargos which are pre-stored in granules. In response to extracellular cues, mast cells may undergo signaling-dependent anaphylactic degranulation (AND) [70], in which granules fuse homotypically with each other and heterotypically to the plasma membrane. These two types of fusion may require different molecular machineries. In fact, localization studies of resting murine mast cells indicate that secretory granules are decorated with R-SNAREs VAMP2, 3, 7, 8 [61, 62, 67, 68, 71, 72] and Qa-SNARE syntaxin3 [62, 67, 68, 71], whereas the plasma membrane is enriched with Qa-SNARE syntaxin4 [68, 73] and Qb,c-SNARE SNAP23 [61, 68]. During mast cell compound degranulation, syntaxin3 relocates from the granules to the plasma membrane [64], whereas SNAP23 relocates from the plasma membrane to the secretory granules [68]. These observations implicate syntaxin3 in homotypic fusion and syntaxin4 in heterotypic fusion. In contrast to AND described above, piecemeal degranulation (PMD) has been observed via ultra-structural studies [74]. In PMD, granule-associated materials are packaged into small vesicles that subsequently travel to and fuse with cell surface. Interestingly, both AND and PMD could happen within the same mast cell [75].

What further confounds our understanding of the regulation of mast cell exocytosis is the existence of granule subpopulations. At the ultrastructural level, human mast cell granules display scrolls, crystalline arrays, particles, or variable mixtures of any combination of two or all of these patterns [76]. Mast cells with these granule substructural patterns can be found in any human tissue [77]. Other granule patterns occur much less frequently, which include completely homogeneously dense, finely granular and reticular/beaded granules [76, 77]. Meanwhile, granules can also be classified into different groups based on their protein

contents and accessibility to different tracers [78]. Moon et al [10] recently proposed three types of secretory granules in mast cells: type I granules contain MHC class II,  $\beta$ -hexosaminidase, LAMP-1, LAMP-2, and M6PR but not serotonin (resembling a classical lysosome); type II granules contain the same set as mentioned above plus serotonin (perhaps a late secretory lysosome); type III granules contain  $\beta$ -hexosaminidase and serotonin but not MHC class II. Although mast cell degranulation is a term often linked to type II granules, the extent of participation of the other two types of granules in mast cell exocytosis has not been resolved.

Finally, it is important to note that even in PMD, different types of vesicles might be formed, containing different cargo and SNAREs. These different types of vesicles may form and fuse in response to different regulatory signals. This could be yet another mechanism used in the differential release of mast cell mediators, a topic to be discussed in the next section.

## Functional Evidence for distinct degranulation pathways

### Distinct pathways for newly synthesized mediators

The traditional assumption which has over relied on the release of  $\beta$ -hexosaminidase (which is present in all three types of granules [10, 78, 79]) as the hallmark for degranulation [80], has delayed the comparative investigation of the selective discharge of key mediators. However, pioneering studies by Blank and colleagues using bone marrow-derived mast cells (BMMCs) isolated from VAMP8 null mice indicate that preformed mediators (e.g., histamine and  $\beta$ -hexosaminidase) and newly synthesized cytokines and chemokines (IL-6, MIP-1 $\alpha$ ) exploit distinct set of SNAREs for IgE/Fc $\epsilon$ RI-mediated release [62].

More recently, human mast cells (isolated from intestine) were used to examine the IgE/Fc $\epsilon$ RI-dependent release of *de novo* synthesized chemokines (e.g., CXCL8, CCL2, CCL3, and CCL4) [81]. Partially permeabilized cells were incubated with antibodies to inhibit individual SNAREs before stimulation. While inhibition of syntaxin4 (Qa-SNARE) or VAMP8 (R-SNARE) resulted in a reduced release of CXCL8 (but not of CCL2, CCL3, or CCL4), inhibition of syntaxin6 (Qc-SNARE) attenuated the release of CXCL8 and CCL2, and inhibition of VAMP7 (R-SNARE) that of CCL3. In contrast, syntaxin3 (Qa-SNARE) and SNAP23 (Qbc-SNARE) are crucial for the release of all 4 chemokines, which suggests, in accordance with the 3Q: 1R rule, certain chemokines (e.g., CXCL8 and CCL2) involve at least 2 sets of SNAREs for release. How these 2 sets of SNAREs are differentially regulated in a signaling-dependent fashion is not well understood. Nevertheless, these observations are consistent with the finding that eosinophils – granulocytes related to mast cells – are capable of releasing different cytokines in a highly selective manner [82, 83].

### Distinct pathways for preformed mediators

To-date there has been a lack of systematic effort to characterize the different degranulation pathways for preformed mediators. Published studies of human mast cells initially showed that histamine release requires syntaxin4 (Qa-SNARE), SNAP23 (Qbc-SNARE), and both VAMP7 (R-SNARE) and VAMP8 (R-SNARE) [65]. However, whether VAMP8 indeed plays a crucial role in histamine release from rodent cells has become controversial because

BMMCs isolated from VAMP8 knockout mice demonstrated modest reduction of histamine release in one study [62] while no reduction at all in another [72]. Furthermore, depletion of VAMP8 using siRNA, which inhibits  $\beta$ -hexosaminidase release, fails to affect histamine release from RBL-2H3 cells [60]. We favor a model which suggests that VAMP8 is required for the regulated release of serotonin rather than histamine because i) serotonin secretion is completely blocked in VAMP8 knockout mast cells [72]; ii) histamine and serotonin are enriched in distinct populations of granules (with only 20% overlap) [72]; and iii) regulated secretion of serotonin without comparable histamine release has been observed in rat mast cells [84, 85]. TNF $\alpha$  secretion on the other hand, is processed via a VAMP8-independent pathway [62, 72]. Based on the co-localization data [62] as well as functional studies of TNF $\alpha$  secretion from human synovial sarcoma cells [86], we propose the degranulation pathway for TNF $\alpha$  utilizes a distinctive set of SNAREs that includes VAMP3.

## Differential regulation of distinct pathways

### Addition fusion factors in exocytosis

Although SNAREs are at the core of exocytic fusion, exocytosis is an intricate process that required multiple factors to regulate three successive steps: 1) docking, 2) priming, and 3) merger of lipid bilayers [87–89]. Docking refers to the retention of transport vesicles to the target membrane (e.g., plasma membrane) [90], which is facilitated by membrane-tethering factor exocyst [91]. Exocyst is a multi-subunit protein complex that brings vesicles and target membranes into proximity by exploiting interactions with lipids and/or membrane-anchored proteins (e.g., Rab GTPase) [92]. Priming renders docked vesicles competent for Ca<sup>2+</sup>-triggered exocytosis, through the activities of Munc13/CAPS and other factors including NSF [87, 93–95]. Priming results in partially assembled trans-SNARE complexes. In the final step of exocytosis, Ca<sup>2+</sup> influx is thought to activate synaptotagmin, which relieves the inhibition imposed by complexin while cooperating with the trans-SNARE complex to merge the apposed lipid bilayers [96, 97]. The activities of all these factors could be modulated via signaling-dependent modification (e.g., reversible phosphorylation) to provide temporarily regulation for mediator release. However, emerging evidence suggests that the differential release of mast cell mediators is likely controlled by the different sets of SNAREs and through their interactions with Munc18 proteins [62, 65, 69, 72, 81].

Munc18 proteins act at various steps of exocytic fusion by exploiting different modes of association with the fusion machinery [98]. All three Munc18 isoforms specific for regulated exocytosis in mammals are implicated in mast cell degranulation, particularly Munc18b. Munc18b knockdown or overexpression in RBL-2H3 cells inhibited IgE/Fc $\epsilon$ RI-mediated  $\beta$ -hexosaminidase release [73, 99]. Co-immunoprecipitation and immunofluorescence studies showed that Munc18b associated with syntaxin3 on both granules and the plasma membrane [73, 99, 100]. Munc18c, on the other hand, appears to interact specifically with syntaxin4 on the plasma membrane [99], although the functional relevance of the interaction has yet to be established. Munc18a was thought to function mainly in neurotransmission, but has now been identified in non-neuronal tissues [101–103]. Recently, a double knockdown of Munc18a and Munc18b in RBL cells was found to eliminate  $\beta$ -hexosaminidase release, yet the phenotype was effectively rescued by reintroducing Munc18a alone [104]. Using



reconstitution, we and others have shown that Munc18a and Munc18c effectively and specifically promote VAMP2- and VAMP3- mediated fusion reactions [69, 105], but the cognate Munc18 proteins for VAMP7- or VAMP8- based reactions have yet to be identified. We propose that either SNARE and/or Munc18 modification might be necessary to kick start VAMP7- and VAMP8- mediated exocytosis in activated mast cells.

### SNARE phosphorylation in exocytosis

The importance of SNARE phosphorylation in exocytosis has been recognized for decades [106, 107]. Biochemically, these modifications either regulate SNARE interactions with their binding partners including cognate SNAREs or Munc18 proteins (Table 1). In mast cells, site-specific phosphorylation of exocytic SNAREs has been reported for VAMP8, SNAP23 and syntaxin3. PKC-dependent phosphorylation of VAMP8 targets residues inside the SNARE domain, and appears to reduce both reconstituted fusion and the regulated release of  $\beta$ -hexosaminidase from RBL-2H3 cells [108]. IKK2-dependent phosphorylation of SNAP23 at Ser95 and Ser120 increases SNAP23 binding to syntaxin4 and VAMP2 *in vitro*, and is required for optimal degranulation *in vivo* [66]. On the other hand, CaMKII-dependent phosphorylation of syntaxin3 at Thr14 negatively regulates mast cell exocytosis, likely by inhibiting syntaxin3-Munc18b interaction [109]. Meanwhile, we have preliminary data suggesting that both VAMP7 and syntaxin4 are also phosphorylated in RBL-2H3 cells (Xu, unpublished). It is rather conceivable that these phosphorylation events play key roles in the differential regulation of mediator release.

### Munc18 phosphorylation in exocytosis

Reversible phosphorylation of Munc18 has been widely exploited to connect signaling cascades with the fusion apparatus. In neurotransmission and chromaffin cell exocytosis, PKC-dependent phosphorylation of Munc18a at Ser306 and Ser313 reduces its affinity for syntaxin1 and changes the kinetics of transmitter/vesicle release [110, 111]. In contrast, Dyrk1A-dependent phosphorylation of Munc18a at Thr479 in embryonic kidney cells enhances Munc18a binding to syntaxin1 [112]. Polarized secretion in rabbit gastric parietal cells requires CDK5-dependent phosphorylation of Munc18b at Thr572 (Thr573 in rat), which promotes the assembly of the Munc18b/VAMP2/syntaxin3/SNAP25 tetra-complex [113]. For GLUT4 exocytosis in adipocytes and muscle cells, Insulin receptor-mediated Munc18c phosphorylation at Y521 appears to facilitate SNARE complex formation between VAMP2, syntaxin4, and SNAP23 [114]. Collectively, these studies indicate that i) signaling-dependent Munc18 phosphorylation is a prevalent regulatory mechanism in exocytosis and ii) Munc18 phosphorylation drives conformational changes to modulate their affinity for binding partners. Besides these site-specific studies, proteomic discovery-mode mass spectrometry has uncovered scores of Munc18 phosphorylation sites from various tissues and cell lines (Fig. 2). However, the functional significance of these sites in mast cell exocytosis has yet to be clarified.

To-date there is no direct evidence connecting Munc18 phosphorylation to mast cell exocytosis. However, mast cell degranulation requires activated PKC pathways [115–118], which are shown to phosphorylate Munc18a [110, 111] and Munc18c [119–121] in a variety of secretory cells. In addition, cGMP-dependent protein kinase (PKG) has been found



critical for degranulation, targeting a number of fusion components including Munc18c [122]. We hypothesize that signaling-dependent phosphorylation of Munc18 proteins provide an important way to regulate the functions of these Munc18 isoforms in distinct exocytic fusion events in activated mast cells.

## Future Perspective

Tremendous progress has been made over the decades in understanding the complexity of mast cell exocytosis, and the diverse roles of mast cell mediators in health and disease. The increasing appreciation of the differential release of mast cell mediators will likely lead to more focused investigation on the specific regulation of individual mediators, particularly at the interface between signaling and exocytic fusion. It may also lead to better integration of cell-based secretion assays with “omics” studies, which will generate global insights on the diverse secretory pathways in mast cells. We expect the combination of these approaches to accelerate the development of “smart” drugs to treat mast cell related diseases (e.g., inhibiting the release of pro-inflammatory mediators while promoting the release of anti-inflammatory mediators) in the near future.

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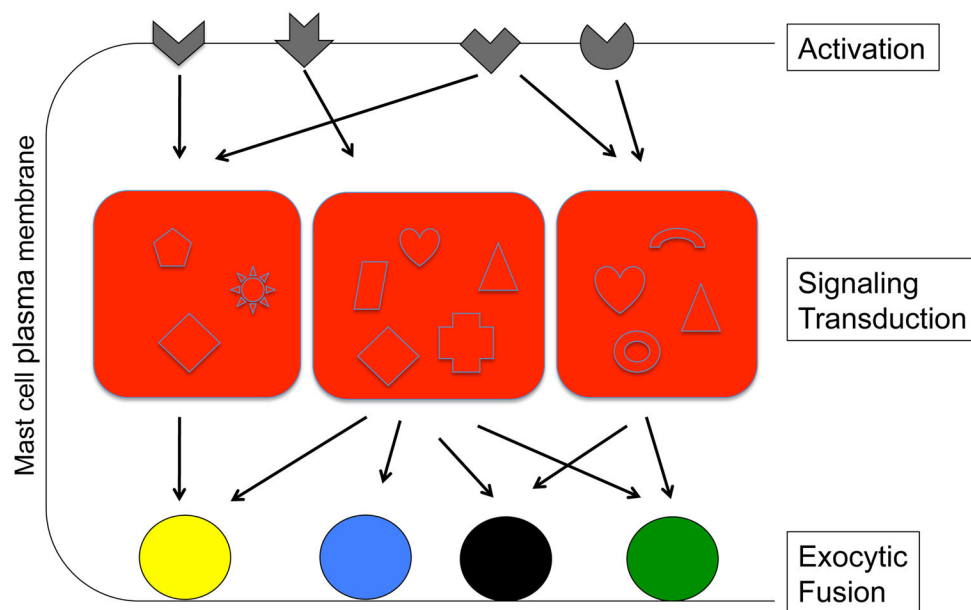


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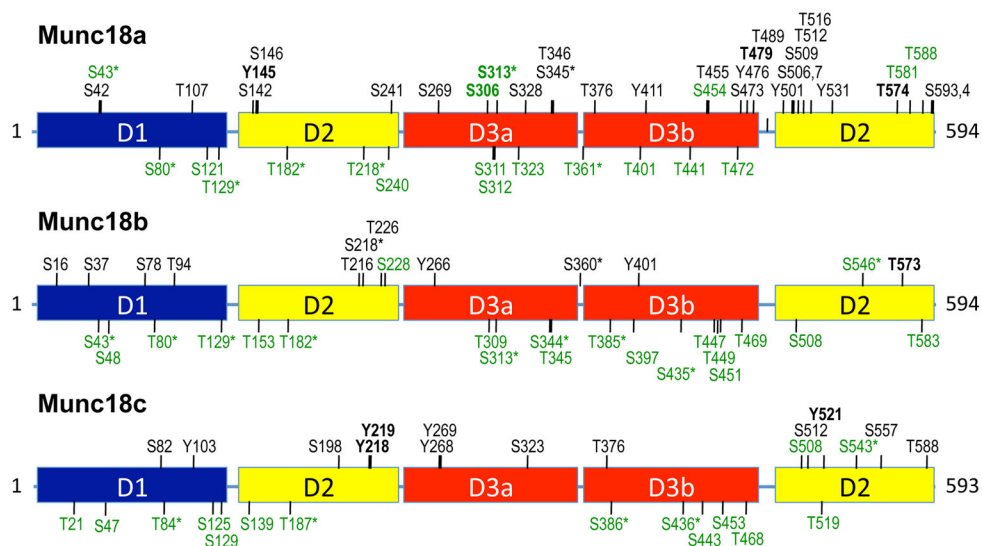


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**Fig. 1. Differentiation regulation of mast cell exocytosis**

Different modes of mast cell activation exploit distinct cell surface receptors which can be broadly categorized into three groups: i) FcRI receptors (including FcεRI and FcγRI, ii) IL-1/TLR/ST2 family of receptors and pathogen recognition receptors (including toll-like receptors, the IL-33 receptor, and NOD-like receptors), and iii) G protein coupled receptors (including adenosine receptors, Prostaglandin E<sub>2</sub> receptors, and sphingosine 1-phosphate receptors, C3A and chemokine receptors, neuropeptide receptors, and antimicrobial peptide receptors) [Gilfillan and Beaven (2011)]. Different receptors at mast cell surface seem to rely on distinct signaling transduction mechanisms (in red) to target exocytic fusion events. These different signaling transduction mechanisms may have overlapping signaling transducers.



**Fig. 2. Predicted or known phosphorylation sites in Munc18**  
 Sites above each schematic representation of the domain structure of Munc18 were identified from various tissues/cells, by either proteomic discovery-mode mass spectrometry ([www.phosphosite.org](http://www.phosphosite.org)) or site-specific methods (in **bold**). In green are identified or predicted PKC sites (<http://kinasephos2.mbc.nctu.edu.tw/>). All sites underneath the domain structure are predicted PKC sites. Conserved or semi-conserved PKC sites are indicated by \*, according to sequence alignment of rat Munc18 proteins using Clustal Omega. Note that Munc18 domain 2 is split by the insertion of domain 3.

**Table 1**

Phosphorylation of exocytic SNAREs in mammalian cells

Protein	Species	Phosphorylation site(s)	Responsible kinase(s)	Biochemical effects	Physiological effects	Ref.
Stx1	Rat	Ser14	Casein kinaseII	N/A	Regulates N-terminal interaction with Munc18-1	[123–125]
Stx1	Bovine	N/A	CaMKII	N/A	N/A	[126]
Stx1	Rat	Ser188	DAP kinase	Decreases syntaxin binding to Munc18a	N/A	[127]
Stx3	Mouse	Thr14	CaMKII	Stronger binding affinity for SNAP25	Modulates the assembly of the SNARE complex in ribbon synapses of the retina	[128]
Stx3	Rat	Thr14	CaMKII	Inhibits binding of Stx3 to Munc18b	Negatively regulates mast cell exocytosis	[129]
Stx4	Rat	N/A	Casein kinase II	Reduces interaction between SNAP25 and phosphorylated Stx4	N/A	[130]
VAMP2	Bovine	Ser61	CaMKII	N/A	N/A	[126]
VAMP7	Human	Tyr45	c-Src kinase	Promotes VAMP7 interaction with Q-SNAREs	Promotes insulin-induced exocytosis	[131]
VAMP8	Rat	Ser17, Thr47, Thr53, Ser54	PKC	Reduces SNARE complex formation	Reduces vesicle fusion <i>in vitro</i> ; suppresses secretion in living cells after vesicle docking	[132]
SNAP25	Bovine	Ser187	PKC	N/A	Enhances exocytosis in bovine chromaffin cells and INS-1 cells	[133]
SNAP25	Bovine	N/A	CaMKII	N/A	N/A	[126]
SNAP25	Rat	Ser187	PKC	Decreases binding with Stx1	Regulates subcellular localization of SNAP25, accelerates vesicle recruitment after emptying	[134–136]
SNAP25	Rat	Thr138	PKA	N/A	Maintains a large number of release-ready primed vesicles	[137]
SNAP25	Rat	Thr138	PKA	Inhibits SNARE complex formation	Inhibits functional exocytosis in PC12 cells	[138]
SNAP25	Rat	Ser187	PKC	Enhances SNARE complex formation	Enhances functional exocytosis in PC12 cells	[138]
SNAP25	Mouse	Thr138	RhoA-activated kinase	Decreases interaction between SNAP25 and syntaxin	Decreases neurotransmitter release	[139]
SNAP23	Human	Ser23, Thr24	PKC	Inhibits Stx4 interaction	Modulates SNARE complex interaction during membrane trafficking and fusion	[140]
SNAP23	Rat	Ser95, Ser120, Ser161	IKK2	Increases binding to Stx4 and VAMP2	Optimal compound exocytosis	[141, 142]
SNAP23	Human	Ser95	Pyruvate kinase type M2	Enables the formation of the SNARE complex	Promotes tumour cell exosome release	[143]
SNAP23	Rat	Ser95, Ser120, Thr102	N/A	N/A	Regulates SNAP23 internal membrane association and subsequently mast cell exocytosis	[144]