# Regulated Airway Goblet Cell Mucin Secretion

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## Key Words

secretion, exocytosis, mucus, mucociliary clearance

## Abstract

Major advances in understanding regulated mucin secretion from airway goblet cells have been made in the past decade in the areas of pharmacology and basic cell biology. For instance, it is now appreciated that nucleotide agonists acting locally through P2Y purinoceptors on apical membranes of surface goblet cells provide the major regulatory system for mucin secretion. Similarly, Clara cells, the primary secretory cell in the mouse airways (and human small airways), are now recognized as major mucin-secreting cells. In Clara cells, the relative lack of staining for mucosubstances reflects essentially equal baseline rates of mucin synthesis and secretion, with little to no accumulation of mucin granules in storage pools. During mucous metaplasia induced under inflammatory conditions, mucin synthesis is massively upregulated in Clara cells, and stored mucin granules come to dominate the secretory cell phenotype. More importantly, we have seen a transition in the past few years from a pharmacological focus on regulated mucin secretion to a more molecular mechanistic focus that has great promise going forward. In part, these advances are occurring through the use of well-differentiated primary human bronchial epithelial cell cultures, but recent work in mouse models perhaps has had the most important impact. Emerging data from Munc13-2- and synaptotagmin 2–deficient mouse models represent the first direct, molecular-level manipulations of proteins involved in regulated secretory cell mucin secretion. These new data indicate that Munc13-2 is responsible for regulating a baseline mucin secretory pathway in the airways and is not essential for purinergic agonist–induced mucin secretion. In contrast, synaptotagmin 2, a fast $Ca^{2+}$ sensor for the SNARE complex, is essential for regulated secretion. Interestingly, these early results suggest that there are two pathways for excocytic mucin release from goblet cells.

## INTRODUCTION

**Baseline:** specifies (*a*) steady-state polymeric mucin production in the absence of cytokine stimulation and (*b*) steady-state secretory activity

**Regulated secretion (or pathway):** exocytic release of cargo from vesicles or secretory granules in response to an increase in $Ca^{2+}$, generally associated with agonist stimulation of the cell

Mucin-secreting surface goblet cells are widely distributed through the mammalian alimentary, reproductive, and airway tracts, where they function in hydrating, lubricating, and clearing particulates and pathogens from the underlying epithelium. Goblet cells in the lung are the principal secretory cell in the superficial, or surface, epithelium of the upper airways. In humans, this population would generally be the cartilaginous airways, those with diameters greater than approximately 3–4 mm. The terminal bronchioles, with diameters <~2 mm, lack goblet cells under normal conditions; their principal epithelial secretory cell is the Clara cell. In the mouse, submucosal glands are restricted to nasal cavity and the first couple of tracheal rings, depending upon the mouse strain. Surface goblet cells are rare to absent; the Clara cell is the predominant secretory cell throughout the tracheobronchial tree. Goblet cells differentiate from basal cells in pseudostratified airways epithelium in humans, which extends to the terminal bronchioles. In the mouse, pseudostratified epithelium extends from the nasal cavity to the upper bronchus, but goblet cells, plentiful in nasal septum and nasopharynx epithelia, are rare to absent in the upper airways, again depending on the strain. The developmental and morphological aspects of these cells have been reviewed extensively in recent years (e.g., References 1–3) and are considered only briefly here. Also not considered in detail are many aspects of the regulation of mucin secretion and mucin biophysics, which pertinent reviews in the recent past in this series (4–6) and elsewhere (7–9) have discussed. In this review, we treat recent work in mouse airways, delineating the metaplastic transformation of Clara cells to goblet-like cells as part of a broader consideration of the mucin secretory pathway. By focusing on the secretory pathway, using information from nonmucin secretory cells where necessary, we identify those many areas requiring significant attention in the future from mucin-oriented investigators.

## MUCOUS METAPLASIA IN SMALL AIRWAYS

Surprisingly, the surface epithelium of the intrapulmonary airways of mice and the distal airways of humans shows few, or no, identifiable goblet cells under baseline conditions, although numerous AB/PAS+ goblet cells are seen after allergic, fungal, or viral inflammatory stimulation (10, 11). Recent work has established that the polymeric mucin Muc5b is produced at baseline by the resident apical secretory cell of the airways, the Clara cell, but is promptly secreted so that it does not accumulate intracellularly (12, 13). With inflammatory stimulation, abundant rough endoplasmic reticulum (ER) appears in the Clara cell cytoplasm, Muc5b production increases modestly (2–5-fold), and Muc5ac production increases dramatically (40–100-fold); the combined production of these polymeric mucins exceeds the rate of baseline mucin secretion, so they accumulate intracellularly (12, 14; see **Figure 1**). This accumulation leads to AB/PAS+ histochemical staining and the presence of large electron-lucent secretory granules (SGs) characteristic of goblet cells in a process termed mucous metaplasia. These accumulated intracellular mucin secretory granules (MSGs) can be rapidly secreted in response to external stimuli (13, 15) in a process of regulated exocytosis that is the primary subject of this review.

## VESICLE TRAFFICKING ALONG THE SECRETORY PATHWAY

Although intestinal goblet cells were important to studies in the 1960s and 1970s defining the Golgi apparatus as the site of protein *O*-glycosylation (e.g., see Reference 16), there has been little work since relevant to the trafficking of mucins through the secretory pathway. This is perhaps understandable, given the size and complexity of mucins [see companion reviews by Hattrup & Gendler (16a) and Thornton et al. (16b) in this volume], but it is also unfortunate because, in secreting both

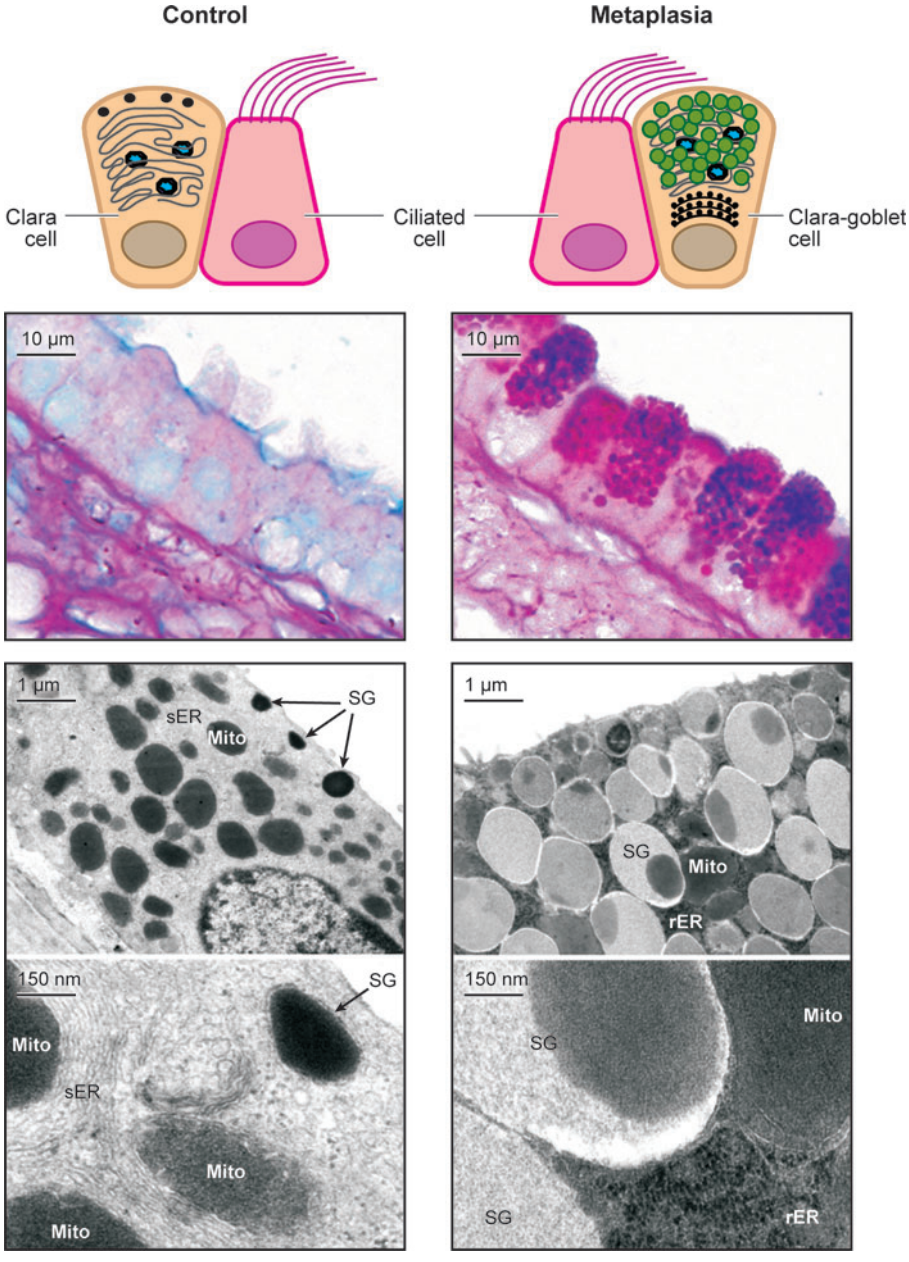


**Figure 1**

Mucous metaplasia in the mouse airways: Clara cells transform phenotypically to Clara-goblet cells. Under baseline conditions (*left*), mouse bronchial epithelium shows little or no AB-PAS staining (photomicrograph), and Clara cells show abundant mitochondria (Mito) and smooth endoplasmic reticulum (sER) at the apical pole with small electron-dense secretory granules (SG) just below the plasma membrane (electron micrographs). During inflammatory mucous metaplasia (*right*), the apical cytoplasm of Clara cells fills with large mucin-containing SG that stain with AB-PAS (photomicrograph) and are electron-lucent with dense cores (electron micrographs). Mitochondria are now seen interspersed among the SG, and the ER is now mostly rough (rER). These changes are schematized in the cartoons at top, with Clara cell SG shown in black, mucin SG in green, and mitochondria in blue. After Reference 10.

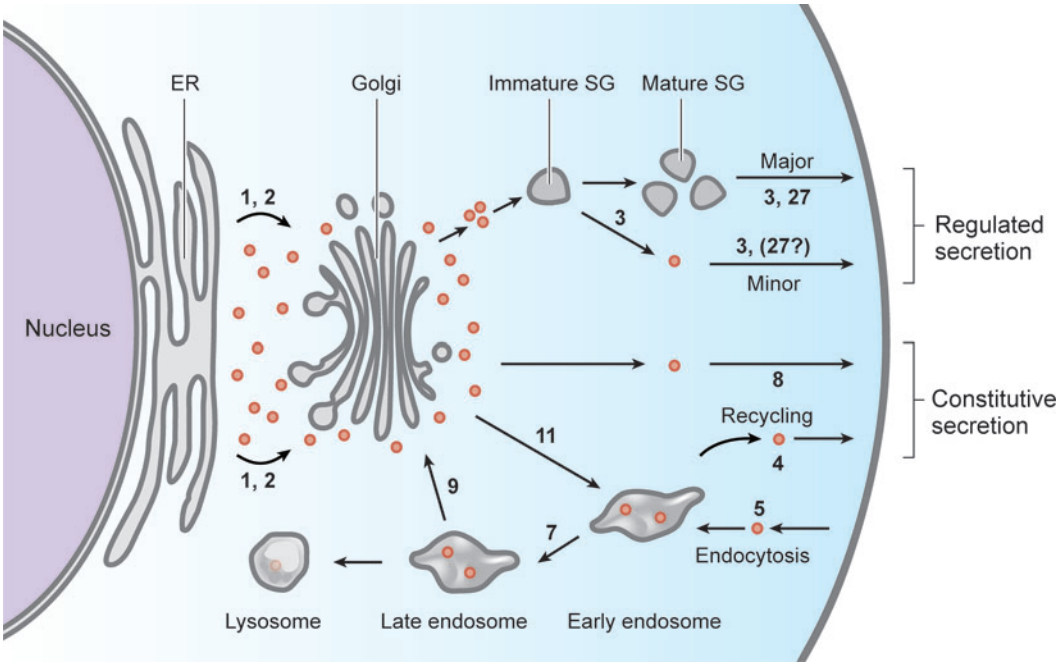


**Figure 2**

Secretory pathway showing vesicle trafficking along its primary subdivisions. The subdivisions of the secretory pathway are defined by the Rab proteins (numbered) that regulate the trafficking of the vesicles and granules from compartment to compartment, in part by controlling the targeting of the vesicles and granules. Major, major regulated secretory pathway; minor, minor regulated secretory pathway; SG, secretory granule.

membrane-tethered and polymeric mucins, goblet cells are likely to offer valuable lessons on the mechanisms of directing large, heavily glycosylated proteins into different subpaths. The secretory pathway, as it has been defined from work on other cells, is comprised of vesicles carrying cargo from a donor compartment to a target compartment, through the sequential actions of multiple protein complexes, under the regulation of Rab GTPases (see References 17–19; **Figure 2**). Transport vesicles form at the donor compartment when soluble and membrane-associated cargo macromolecules are concentrated at the site of a nascent vesicle, generally through interactions with cytoplasmic coat proteins. The coat proteins, acting to bend the donor membrane, also assist the process of vesicle budding. After pinching off as a fully formed transport vesicle, the protein coat dissociates, and the vesicle travels to a target compartment, either by diffusion or by directed transport along cytoskeletal elements. At the target membrane, the vesicle initially becomes loosely tethered by large protein complexes, then becomes firmly docked through initial interactions of the SNARE proteins, and finally fuses when the SNARE proteins fully coil to pull the membranes into contact with one another for the fusion event (see below and side bar on SNAREs). Examination of the same step of traffic across species from yeast to humans reveals the participation of closely related orthologs, whereas comparison of different trafficking steps within a single species reveals the participation of more distantly related paralogs. Together, this structural conservation indicates the retention of a fundamental mechanism throughout the evolution of interorganellar traffic in eukaryotic

**Rab:** a family of small (20–29 kDa) monomeric GTPases that ubiquitously regulate secretory and endosomal vesicular traffic

**Coat proteins:** the formation of transport vesicles often occurs with the structural support and adapter function of an overlying spherical lattice formed by the polymerization of monomers of either clathrin (clathrin-coated vesicles) or coatomer (COPI and COPII vesicles)

organisms. Macromolecules such as mucins, destined for exocytic membrane insertion or secretion, undergo several rounds of intercompartmental vesicular trafficking beginning with transport from the ER, where they are synthesized; to the *cis*-Golgi, where they undergo core glycosylation; then through the Golgi, where they undergo further posttranslational processing and sorting; and finally from the *trans*-Golgi to the cell surface (**Figure 2**).

The constitutive pathway is a conduit for vesicles budding from the *trans*-Golgi network that are destined for immediate release by $Ca^{2+}$-independent exocytic release (20, 21). (Note: Constitutive is often used in the physiological literature to mean a baseline activity, a meaning studiously avoided in this review to prevent confusion.) Tethered mucins likely utilize the constitutive pathway for exocytic insertion into the plasma membrane, and they may be processed on occasion by recycling endosomal trafficking, which also utilizes a constitutive exocytic pathway.

## Secretory Granule Biogenesis

Vesicles carrying the primary secretory product of the cell also bud from the *trans*-Golgi network, but little is known about the sorting of polymeric mucins into nascent secretory vesicles in the *trans*-Golgi network, the budding of secretory vesicles, or SG maturation in airway goblet cells. In other cell types, macromolecules destined for regulated secretion are aggregated in the *trans*-Golgi by a variety of mechanisms, including specific interactions with chaperones, segregation into lipid domains, and precipitation (22). Budding of secretory vesicles from the *trans*-Golgi network is mediated either by a clathrin coat in cooperation with membrane vesiculating and scission proteins, including amphiphysin, endophilin, and dynamin, or by the packaging of bulky cargo molecules such as collagen fibrils into elongated tubules protruding from the *trans*-Golgi whose scission is induced by local production of diacylglycerol (DAG) (23, 24).

### SNAREs: THE CORE MACHINERY OF MEMBRANE FUSION

SNARE proteins appear to function in every membrane fusion event in eukaryotic cells. They comprise a superfamily of 25 proteins in the yeast *Saccharomyces cerevisiae* and 54 proteins in mammals that have conserved coiled-coil domains (α-helical regions that intertwine with other α-helices to form a rope-like structure). In membrane fusion, four SNARE proteins come together in a core complex, most commonly contributed by one SNARE protein anchored in the vesicle membrane and three SNARE proteins in the target membrane. The coiling of the SNAREs provides much of the energy and some of the specificity of vesicle traffic (143, 145, 149). The SNARE coiled-coil domain is 60–70 amino acids in length, with a central charged amino acid and 7–8 stacked hydrophobic heptad repeats on each side. The coiled-coil domains align in a parallel orientation in the core complex, and most SNARE proteins are anchored by a C-terminal transmembrane domain. The SNAREs were originally classified as v-SNAREs (vesicle membrane SNAREs) or t-SNAREs (target membrane SNAREs). However, some SNAREs function in multiple transport steps with different roles at each step. Further structural and functional studies led to a reclassification as Q- and R-SNAREs on the basis of whether glutamine or arginine is the central charged amino acid, with t-SNAREs generally being synonymous with Q-SNAREs and v-SNAREs with R-SNAREs. Each core complex is comprised of three Q-SNAREs and one R-SNARE. The Q-SNAREs known as Syntaxins are the central components of core complexes: their SNARE domains are structured when the proteins occur in isolation, whereas the structures of the other SNARE proteins become ordered only after interaction with Syntaxins. Syntaxins contain three other coiled-coil domains besides the SNARE domain, and these other domains form an intramolecular four-helix bundle prior to interaction with other SNAREs. In regulated exocytosis, the opening of the closed Syntaxin structure to allow the formation of the core complex is a critical event regulated by Munc13 and Munc18 proteins through activation by second messengers released in response to extracellular signals.

We have observed amorphous electron-lucent material, consistent with mucin, in tubules budding from the *trans*-Golgi network of airway goblet cells, suggesting that the latter mechanism is used for polymeric mucin

export (A.H. Rossi & C.W. Davis, unpublished observations). Post-Golgi secretory vesicles fuse homotypically to form larger immature SGs, which then mature and shrink via the budding of clathrin-coated vesicles that remove membrane proteins and lipids and lumenal proteins that are not destined for secretion (22). Some of the vesicles budding from immature SGs may also carry secretory cargo for exocytic release via the minor regulated pathway for secretion at baseline, in a $Ca^{2+}$-dependent fashion, under the tonic control of an agonist (21, 25, 26).

**Exocytic insertion:** the transfer of membrane-tethered mucins, or other proteins, from the membrane of a vesicle to the plasma membrane following vesicle fusion

**Vesicle traffic or vesicular transport:** the process by which membrane lipids and proteins and lumenal contents of membrane-bound organelles are concentrated, are formed into transport vesicles, and then travel to and fuse with other membrane-bound organelles

**Constitutive secretion:** exocytic release of cargo from vesicles immediately following their budding from the *trans*-Golgi network in a $Ca^{2+}$-independent manner

**Exocytic release or secretion:** the release of materials from a secretory granule lumen to the outside of the cells following granule fusion with the plasma membrane

**DAG:** diacylglycerol

## Secretory Granule Transport

Mature secretory granules move from the *trans*-Golgi region to the cell periphery along microtubules. This movement has been best studied in melanocytes, whose pigment granules are modified lysosomes, but it has also been observed in exocrine, endocrine, and hematopoietic cells, although not goblet cells (23, 27, 28). Near the cell periphery, SGs transition from microtubules to actin filaments through the actions of a tripartite complex of a Rab27 GTPase, an atypical, or nonconventional, myosin, and a granulophilin (27, 28). Rab27a is found in high abundance in airway goblet cells (29), but *ashen* mice lacking Rab27a have no defects in airway mucin secretion (O. Williams & B.F. Dickey, unpublished observations). Rab27b is also expressed in the lungs (30), and its expression increases and functionally compensates for the absence of Rab27a in other tissues (31). In addition, Rab3 proteins share upstream and downstream interactions with Rab27 proteins (27, 28), and both Rab3b and Rab3d are expressed in airway secretory cells (10), with Rab3d localized on MSGs (15). Thus, redundant or compensatory Rab3/27 function may obscure the roles of individual Rab proteins in granule transport.

Class V atypical myosins are required for polarized secretion in yeast, and myosin Va transcripts are expressed in the lung (32), but *dilute* mice lacking myosin Va have no defect in airway mucin secretion (O. Williams & B.F. Dickey, unpublished observations). Myosin Vb and Vc transcripts are also expressed in the lungs, and myosin Vc protein is expressed in the lungs and in secretory epithelia of other tissues at high levels, making it a good candidate to mediate airway MSG transport (32).

The granulophilin Slp2a colocalizes with apical MSGs in gastric mucosa of mice and physically interacts with Rab27 proteins (33). Its disruption by homologous recombination results in larger MSGs but a reduction in granule number, impaired granule apposition to the apical plasma membrane, and reduced baseline mucin secretion. Together, these findings suggest abnormalities in granule maturation and transport. Slp2a is also expressed in the lung (33), but knockout mice show no apparent defect in mucin secretion (M.J. Tuvim & B.F. Dickey, unpublished observations).

The net result of MSG synthesis, maturation, and transport is the formation and maintenance of a stored pool of MSGs for secretion as needed. The MSGs are released exocytically via the $Ca^{2+}$-dependent major regulated secretory pathway (21, 26, 34; see **Figure 2**), following an acute presentation of agonist to the goblet cell.

## RECEPTOR ACTIVATION OF MUCIN SECRETION

During the 1970s to 1980s, as the regulation of submucosal gland secretions was being defined (see Reference 8), the regulation of mucin secretion from goblet cells in the superficial epithelium of the airways remained largely unknown. It was not until the discovery of purinergic receptors and the recognition in the 1990s that nucleotides and nucleosides had extracellular signaling activities in the airways that goblet cells were demonstrated to be regulated at their apical plasma membranes by ATP and UTP (35, 36). The P2Y purinoceptors are now recognized as a major G protein–coupled receptor (GPCR) family (37) involved in the paracrine

or autocrine control of many physiological functions in the body. In addition to mucin secretion in the airways, ATP and UTP also stimulate $Cl^-$ and fluid secretion (see References 38 and 39), ciliary activity (40), and, importantly, mucociliary clearance (41). $P2Y_2$-R was cloned from the airways (42) and appears to be the major purinoceptor regulating mucociliary clearance in the airways, including mucin release from goblet cells. ATP and UTP, the primary $P2Y_2$-R agonists, elicit maximal secretion from goblet cells in all vertebrate species examined (e.g., see References 9 and 43), including human (44, 45), the receptor mRNA has been identified in two goblet cell models (46, 47), and the tracheal mucin secretory response to ATP is severely compromised in the $P2Y_2$-R-deficient mouse (48).

The only $P2Y_2$-R agonist other than ATP/UTP with consistent, positive mucin secretory actions on goblet cells across species is platelet-activating factor (e.g., Reference 49); unfortunately, interest in this receptor system in goblet cells has waned. Neutrophil elastase reportedly has nonagonist effects in promoting goblet cell metaplasia through diverse pathways (see Reference 50) and in stimulating mucin secretion (50, 51). Importantly, the cellular messengers underlying the secretagogue effects of elastase are unknown but do not appear to be associated with classical pathways (52).

Purinergic agonist effects stimulating mucin secretion have also been observed for goblet cell basolateral membrane exposures (36, 53). However, neither the molecular identity of the receptor(s) nor the associated cellular messengers have been established.

## Muscarinic Regulation of Goblet Cell Mucin Secretion?

The submucosal glands are richly innervated by parasympathetic, sympathetic, and nonadrenergic, noncholinergic nervous (NANC) systems (8, 54). These glands are densely populated by $M_3$ muscarinic receptors (see Reference 55), and there is a variety of experimental evidence that acetylcholine (ACh) stimulates a copious secretion of mucus from glands onto the airway surface (e.g., Reference 56). The superficial epithelium, in contrast, especially in the human airways, is poorly innervated (see Reference 8), and there is "little specific labeling" of muscarinic receptors when probed with radiolabeled agonist (57). Although the supply of parasympathetic nerves to the superficial epithelium is sparse, ACh may serve as a local mediator: For example, there is evidence of ACh synthesis in, and release via, an organic cation transporter from nonneuronal cells, including airway epithelium (see Reference 58). Regardless of the source, however, whether or not muscarinic agonists stimulate mucin secretion from goblet cells in the superficial epithelium is a major unanswered question.

In some situations it is clear that muscarinic agonists do stimulate mucin secretion. For example, injection of methacholine intraperitoneally in the mouse (59) or exposure of isolated ferret trachea to muscarinic agonists (55, 60) results in the secretion of measurable amounts of mucins, and/or in the loss of mucin stores, from the airway epithelium. In all such cases, however, mucin secretion was detected from intact airway tissues in vitro or laboratory mammals in vivo, leaving open the possibilities of (*a*) the detected mucins emanating from submucosal glands and not from the superficial epithelium, (*b*) indirect effects of other, secreted local mediators (e.g., ATP) acting on the superficial epithelium, and/or (*c*) significant species differences in muscarinic receptor localization to goblet cells. Using primary cell cultures or cell lines derived from superficial epithelia or isolated epithelium of canine, rat, or human origin, our laboratory has not observed a mucin secretory response to muscarinic stimulation, even though the same preparations respond robustly to purinergic agonist when applied subsequently as an internal control (C.W. Davis, unpublished observations). The argument has been made that the $M_3$ receptors that would normally be present are labile

**Minor regulated secretion (or pathway):** exocytic release of cargo from vesicles following their budding from immature secretory granules by a $Ca^{2+}$-dependent process; generally, forms the baseline secretory activity of the cell, which may be under the tonic control of agonist

**Atypical myosins:** members of the myosin superfamily other than skeletal muscle myosin II, typically having one globular head instead of two and involved in a wide variety of actin-based cellular functions

**Granulophilin:** a family of synaptotagmin-like (Slp and Slac) and unrelated proteins that interact with Rab GTPases and atypical myosins to mediate actin-based vesicular transport

***ashen* and *dilute*:** spontaneously derived mutant mouse strains that are hypopigmented owing to defects in the transport of melanosomes, the secretory granules of melanocytes, to the cell periphery for secretion and uptake by keratinocytes

**Major regulated secretion (or pathway):** the exocytic release of cargo from mature secretory granules by a $Ca^{2+}$-dependent process, generally for acute release in response to the transient release of agonist

**NHBE:** normal human bronchial epithelial (cell)

and may be lost from the cultured preparations (see Group Discussion section following references in Reference 61). This possibility seems remote, however, because primary cultures of ovine ciliated cells respond robustly to muscarinic agonists (42) whereas human cells respond poorly or not at all (63; M. Salathe, personal communication). On balance, the data suggest that goblet cells in the superficial epithelium, at least of human airways, lack significant expression of muscarinic receptors. Given the apparent importance of muscarinic signaling in asthma and chronic obstructive pulmonary disease (COPD) (e.g., Reference 64), however, a carefully planned study of the direct effects of muscarinic agonists on human ciliated and goblet cells of the superficial epithelium would appear prudent.

## Cellular Messengers in Purinergic Signaling

$P2Y_2$-R typically couples to phospholipase C (PLC) (37), and the existing data are consistent in suggesting inositol 1,4,5-trisphosphate ($IP_3$) and DAG as the major cellular messengers underlying regulated mucin secretion. The PLC-specific inhibitor U73122 and loading cells with the calcium chelator BAPTA inhibit agonist-stimulated secretion (45, 65). In contrast, secretion is stimulated by (*a*) the DAG mimic PMA, (*b*) increasing intracellular $Ca^{2+}$ with the ionophore ionomycin, (*c*) permeabilizing the cells into an extracellular $Ca^{2+}$ EGTA-based buffer with Streptolysin-O, and (*d*) applying $IP_3$ to permeabilized cells (9, 44, 66–68). Recent data show, additionally, that intracellular $Ca^{2+}$ is mobilized in agonist-stimulated human goblet cells with a classic peak-and-plateau waveform and that this mobilization is inhibited by U73122 or by BAPTA loading (68).

Classically, mucin secretion has been considered to be under the control of GPCRs, which couple to PLC-β to initiate a cellular messenger cascade. Mucin hypersecretion is characteristic of inflammatory environments in many airways diseases, however, which raises the possibility of alternative signaling mechanisms. We used the tyrosine phosphatase inhibitor pervanadate to increase the level of tyrosine phosphorylation in goblet cells and found that mucin secretion was stimulated to levels similar to those of purinergic agonist exposures. Significantly, U73122 reversed the effects of pervanadate, suggesting that PLC-γ also mediates the effects of inflammatory mediators in goblet cells (65). Likely, PLC mediates the effects of pervanadate (69); however, given that PLC is a family of genes subdivided into at least 13 isoforms in 6 subfamilies, the participation of other isoforms cannot be ruled out (70). Nonetheless, that PLC should play such a central role in regulated mucin secretion is interesting in potentially simplifying the scheme of control: Multiple inputs (purinergic agonists and inflammatory mediators) converge via PLC to generate just two cellular messengers, DAG and $Ca^{2+}$, that then lead to exocytic mucin release by common regulatory pathways.

DAG typically activates conventional and novel PKC isoforms, and airway goblet cells express cPKCα, nPKCδ, nPKCε, and nPKCη (51, 71). Originally, we reported that nPKCδ appeared to be the isoform mediating the effects of agonist to stimulate mucin secretion from SPOC1 cells, because this was the only isoform to translocate to the membrane fraction in an agonist-concentration-dependent manner (71). nPKCδ was also implicated in mediating the effects of neutrophil elastase on NHBE (normal human bronchial epithelial) cells (51). The use of retroviral infection vectors with SPOC1 cells, however, indicated that overexpression of cPKCα, nPKCδ, and nPKCη was without effect, whereas overexpression of nPKCε caused an increase in agonist-induced mucin secretion. Additionally, in perfused mouse tracheas the mucin secretory response to agonist was blunted in the nPKCε-deficient mouse, but not the nPKCδ-deficient mouse (48). Hence, nPKCε appears to be the isoform mediating agonist effects in goblet cells. That it does so without a classic cytosol-to-membrane translocation suggests

a local activation within the membrane fraction, which may be facilitated by the unique actin filament–binding motif found in nPKCε (see Reference 48).

It is important to recognize, however, that DAG signaling is not simple. DAG has multiple synthetic sources and metabolic fates, many of which have other signaling activities, and it has targets other than PKC. DAG is synthesized from phosphotidylinositol lipids by PLC and from phosphatidylcholine by the sequential actions of phospholipase D (PLD) and phosphatidate phosphohydrolase, and it can be phosphorylated by DAG kinase to produce phosphatidic acid or converted to arachidonic acid by DAG lipase. DAG and PMA attract proteins to the plasma membrane by binding to C1 domains. These domains were defined originally for PKC but are now recognized to be incorporated into many proteins, including some, such as DAG kinase and Munc13, that have known actions in secretory cells (72, 73). Interestingly, PMA has PKC-independent effects in regulated mucin secretion, and its major stimulatory activities are elicited at concentrations of 300–1000 nM, well above the 10–30 nM necessary to activate PKC fully (44, 66, 67, 71). Because these other C1 domain proteins lie downstream of PKC in their actions to regulate mucin secretion, they are covered in more detail in the sections below.

## REGULATED MUCIN SECRETORY GRANULE EXOCYTOSIS

Direct observation of goblet cell MSG exocytosis showed that there is a lag of several seconds (5–15 s) between the application of agonist and the first exocytotic event (36), suggesting that a ready releasable pool of SGs, as described for neuronal synapses and excitatory secretory cells (e.g., Reference 74), is lacking or is very small in goblet cells. Apparently, after agonist stimulation those MSGs destined for secretion transit from the storage pool to the apical plasma membrane for exocytic release in a series of steps that can be broken down categorically as actin filament disruption and remodeling, MSG positioning, tethering/docking, priming, and exocytosis (**Figure 3**).

**Priming:** an activity inferred from the lower number of synaptic vesicles competent for calcium-dependent release than are docked morphologically, and the submillisecond kinetics of release ruling out multiple biochemical steps

## REGULATION OF THE CORTICAL ACTIN CYTOSKELETON

### Actin Filament Disruption

The cortical actin cytoskeleton is well known for its scaffolding functions, serving to anchor and regulate membrane proteins involved in many cell signaling events. It also serves the simple task of separating cytoplasmic organelles from the plasma membrane and the more complex task of regulating vesicle trafficking to and from the plasma membrane. In goblet cells, for example, β-actin and γ-actin filaments form a cortical mesh immediately beneath the apical membrane, appearing as a cap over large stores of MSGs (75); the MSGs must transit through this mesh to gain access to the plasma membrane. The actin cytoskeleton is often called a barrier because of its general appearance, because it is often visibly disrupted during secretion, and because experimental disruption frequently accelerates the rate of exocytic (76) and endocytotic (77) events. The apical actin barrier in SPOC1 cells is disrupted fully during agonist-induced mucin secretion. Furthermore, experimental manipulations to disrupt the barrier with latrunculin accelerate mucin secretion under baseline and agonist-stimulated conditions, whereas mucin secretion is inhibited by either stabilizing actin filaments with jasplakinolide or supplementing the barrier through the overexpression of β- or γ-actin (75).

In other secretory cells, however, disruption of the actin barrier with cytochalasin or latrunculin has no effect, exocytosis is inhibited, or the effect is complex, with stimulatory effects at low doses but inhibitory effects at high doses (see Reference 76). In primary

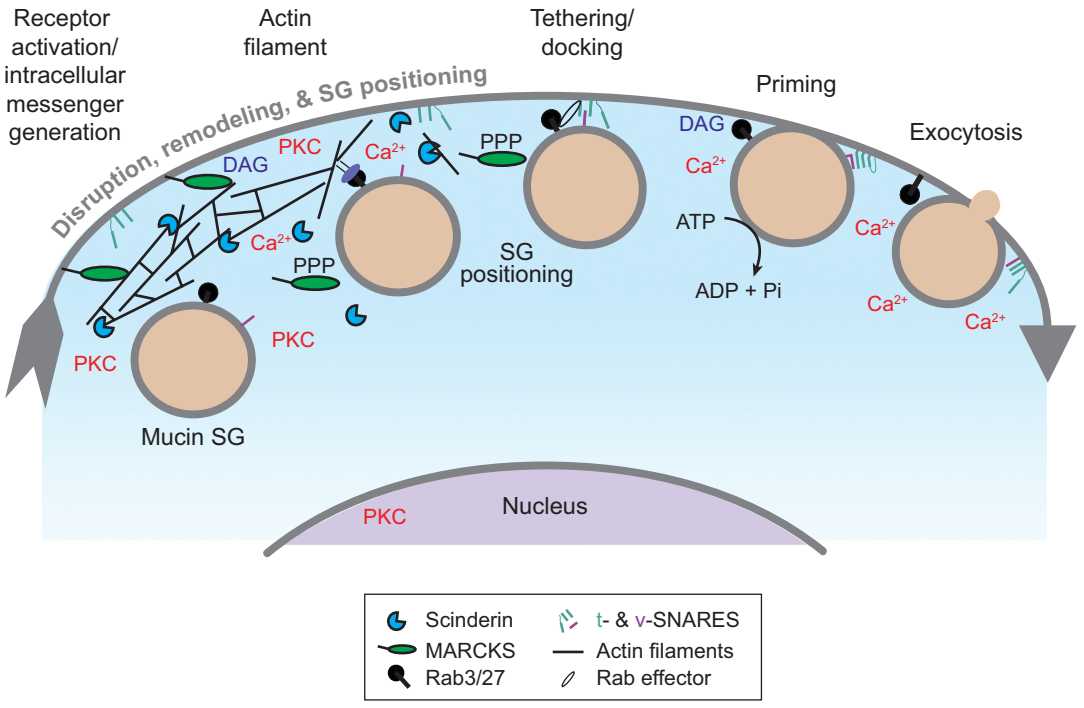


**Figure 3**

Primary events of regulated mucin granule exocytosis. The mucin secretory granules (SGs) are arrayed along a timeline corepresenting the plasma membrane and are depicted at different stages of the exocytic process. From left to right: Following receptor activation, the mucin SGs transit through the actin cytoskeleton; dock with exocytic sites on the plasma membrane; are primed through assembly of the SNARE complex; and, following an appropriate $Ca^{2+}$ signal, are then exocytosed through fusion of the granule and plasma membranes.

cultures of NHBE cells, in contrast to SPOC1 cells, latrunculin-A or cytochalasin D, applied separately or in combination, has no effect on mucin secretion over a wide range of concentrations (L.H. Abdullah & C.W. Davis, unpublished results). Hence, even though the mobility of SGs relative to the plasma membrane is two orders of magnitude less than it is in other directions within the near-membrane environment (78), the concept of an actin barrier to exocytosis is insufficient to explain the role of actin filaments in regulating exocytic mucin release. Rather, the cortical actin matrix is dynamically disrupted and remodeled in such a way as to propel granules to the plasma membrane and to regulate the traffic (78–80).

## Remodeling of Actin and Positioning of Secretory Granules

Experiments tracking the movements of individual SGs in the near-membrane environment of chromaffin and PC12 cells have shown that directed movement to the membrane increases following cell stimulation and slows or ceases when actin filaments are disrupted by latrunculin or disassembled by ATP depletion (78–82). These movements are generally attributed to a conventional and an unconventional myosin, myosins II and V, respectively (83), although whether these myosins propel separate populations of granules along actin filaments or act cooperatively on the same granule population is

unresolved. For example, mysosin II has been reported to associate with granules (84, 85); however, other reports suggest that myosin V, but not myosin II, does so (86). In addition to myosin II, a plethora of studies have demonstrated myosin light-chain kinase (MLCK) involvement in exocytic secretion of amylase, gonadotropin-releasing hormone (GnRH), insulin, tear proteins, and epinephrine (85, 87–90). In goblet cells, inhibition of mucin secretion by wortmannin, but not LY-294002, is consistent with MLCK participation in mucin secretion (65) and suggests the involvement of myosin II. The participation of myosin V in regulated secretion has been known for some time (e.g., see Reference 83); however, the association of myosin V with Rab27a in the genetic diseases Griscelli syndrome and hemophagocytic syndrome was key to the realization that Rab27 couples vesicles/granules to myosin V (91, 92). This relationship is presently understood to be widespread in secretory cells (see above). Other than the report of wortmannin effects on mucin secretion cited above, there have been no studies of MSG trafficking related to secretion.

In addition to actin disruption and actomyosin involvement, actin filaments are dynamically remodeled by polymerization during exocytosis (76). In part, this phenomenon was elucidated initially from the findings that actin filament polymerization is activated during mast cell secretion, under the control of Rho and Rac (93), and that pancreatic acinar zymogen granules are coated with actin during exocytosis (94). Recent studies in PC12 and chromaffin cells have shown that Cdc42 triggers filament polymerization and is essential for secretion (95, 96) and that N-WASP and Arp2/3 mediate actin nucleation on SGs (95, 97, 98). The principal function of this SG-associated actin filament polymerization is not fully understood, and it may differ by cell type. In different cells, such polymerization may be essential for the alignment of granules to docking sites, the regulation of fusion pore kinetics (99), the compression of granules after fusion to drive the expulsion of secretory cargo (100), or the stabilization of fused granules undergoing compound exocytosis (101).

## Regulation of Actin Disruption and Remodeling by Scinderin and MARCKS

**MARCKS:** myristolated, alanine-rich C-kinase substrate

To allow the passage of micrometer-sized SGs, the cortical maze is disrupted by the actions of scinderin, a $Ca^{2+}$-activated, actin filament–severing and –capping enzyme closely related to gelsolin that is enriched in many secretory tissues, e.g., the adrenal cortex (102–104). Like many secretory tissues, airway goblet cells express scinderin, but not gelsolin (75), and in mouse airways scinderin is upregulated significantly during IL-13-mediated mucous metaplasia (14) as Clara cells are transformed to mucin-secreting "goblet" cells (10, 12). Inhibition or knockdown of scinderin in all secretory cells examined, including goblet cells (75), results in the inhibition of exocytic activity (103, 105).

Scinderin, like other members of the gelsolin family, is a complicated enzyme whose functionality is not fully understood. It severs actin filaments when $Ca^{2+}$ levels are elevated, and it caps and nucleates filament + ends when $Ca^{2+}$ is low. Additionally, scinderin activity is also modulated by the inhibitory actions of phosphatidylinositol bisphosphate ($PIP_2$), which may be effected by membrane sequestration (76, 103, 106, 107).

MARCKS (myristolated, alanine-rich C-kinase substrate) is a novel protein, well established as participating in the regulation of the cortical actin cytoskeleton; however, its exact mode of action in this regulation is highly controversial. Significantly, Adler and associates have shown that a myristolated synthetic peptide derived from the N-terminal sequence of MARCKS is a potent inhibitor of mucin secretion in the airways (59, 108, 109). MARCKS is a randomly coiled protein whose properties are dominated by its effector or phosphorylation site domain (PSD), a region of 25 residues containing 13 basic residues,

6 hydrophobic residues, and 4 serine residues (3 of these serine residues are phosphorylated by PKC) (110, 111). Under control conditions, the PSD is unphosphorylated, and MARCKS associates with the plasma membrane via its myristolated N terminus and the PSD. Following phosphorylation, the partial charge neutralization of the PSD is sufficient to release MARCKS from the plasma membrane to translocate to the cytosol (see Reference 111). Consistent with this behavior, MARCKS in goblet cells translocates to the cytosol following agonist activation (75, 108).

nPKCε effects MARCKS phosphorylation in many secretory cells. Overexpression of nPKCε in GH4 cells leads to increased phosphorylation of MARCKS as well as increased TRH secretion (112). In contrast, expression of dominant negative nPKCε constructions or nPKCε knockdown with RNAi reduces MARCKS phosphorylation and secretion in chromaffin cells (113) and lacrimal gland acinar cells (114). Notably, nPKCε appears to be the isoform active in agonist-induced goblet cell mucin secretion (48), consistent with its participation in the regulation of MARCKS activity and secretion in these other secretory cells.

The molecular mechanism of MARCKS actions in exocytosis is controversial. One school of thought holds that MARCKS regulates cortical actin cytoskeletion directly: Unphosphorylated MARCKS is postulated to bind actin filaments through its highly positively charged PSD, thereby tethering filaments to the plasma membrane (115–118). A second school holds that MARCKS effects are indirect, that the unphosphorylated PSD sequesters $PIP_2$ (119–122), which in a resting cell would have the effect of minimizing actin turnover (106). When PKC phosphorylates the MARCKS PSD, MARCKS translocates to the cytosol, which, depending on the school of thought, either disrupts actin filaments by destroying their plasma membrane attachment sites (104) or frees $PIP_2$ to activate actin filament remodeling and polymerization (106). Recently, phosphorylated MARCKS has also been proposed to bind to goblet cell MSGs in the cytosol, secondary to its translocation, to provide actin filament attachment sites for transport to the plasma membrane (108). MARCKS appears to have broad functions in cell and developmental biology, none of which have been well defined (111, 122). That a MARCKS-deficient mouse is perinatally lethal, owing to defects in closure of the neural tube (123), underscores both the importance of this protein in the body and our need to understand the molecular mechanism by which it affects and regulates the actin cytoskeleton.

## TETHERING AND DOCKING

After transport to the vicinity of a target membrane, trafficking secretory vesicles and granules bind to and interact with the membrane in sequential steps (**Figure 4**). These include relatively loose initial binding, often termed tethering, that is initiated by activated Rab GTPases anchored on the vesicle surface. Rab3/27 proteins may provide a link between actin-mediated peripheral transport of MSGs and their tethering to the plasma membrane in goblet cells, similar to the role of their homolog Sec4 in yeast (124). Tethering is mediated by at least two classes of proteins: (*a*) large, elongated coiled-coil proteins such as EEA1, which acts in endosome fusion, or p115 and GM130/GRASP-65, which interact in ER-to-Golgi traffic, and (*b*) multisubunit protein complexes such as TRAPP, which act in intra-Golgi traffic, or the exocyst, which acts in constitutive exocytosis (124). Subsequent interactions lead to the initial formation of the core complex, which is a tight form of binding that draws the membranes into close apposition and is often called docking. Investigators have used the terms tethering and docking variously for distinct biochemical, genetic, and morphological phenomena at diverse steps of vesicle traffic, so their meanings can be ambiguous. In any case, the proteins and activities involved in tethering and

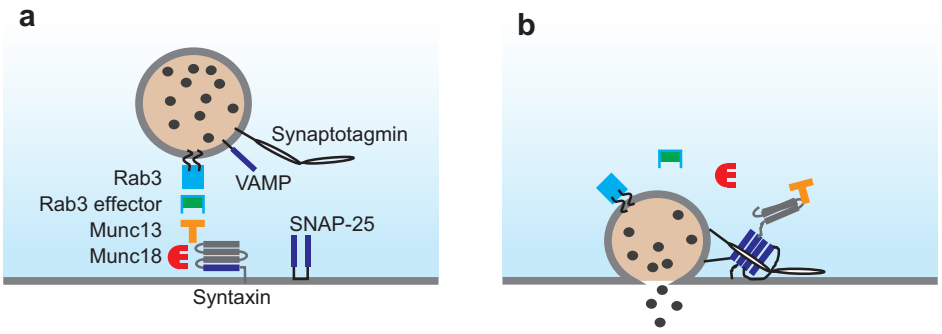


**Figure 4**

Proteins effecting and regulating exocytosis. (*a*) Munc13, after interacting with a Rab3 effector or Rab27 directly, displaces Munc18 from its syntaxin binding site, thereby enabling SNARE assembly, a step that constitutes priming and readies the complex for the exocytic event. (*b*) Following activation of synaptotagmin by a local elevation of $Ca^{2+}$, exocytosis is activated.



docking MSGs and other nonneuronal SGs are not well studied.

In contrast, the octameric protein complex that mediates the tethering of constitutive SGs, the exocyst, is well studied in yeast, revealing many of the molecular details of tethering and associated interactions (124). In neurons, scaffolding proteins, including RIM, Bassoon, Piccolo, and CASK, hold synaptic vesicles in close proximity to the presynaptic membrane (125), but whether these proteins or paralogs function in nonneuronal regulated secretion is not known. The cochaperones cysteine string protein and Hsc70 have been found on airway MSGs and are suggested to interact with MARCKS protein (126), but whether they also interact with tethering/docking proteins in goblet cells has not yet been studied. Noc2 is another candidate docking protein in goblet cells because of its expression in the lung and SPOC1 cells and its widespread roles in regulated secretion in endocrine and exocrine cells, although no abnormalities were found in the morphology of goblet cells of the gastrointestinal tract in knockout mice (71, 127, 128).

## PRIMING

In parallel with tethering and docking, a critical interaction occurs at all steps of vesicle traffic between an SM (Sec1/Munc18) protein and the SNARE machinery to initiate formation of the core complex (129, 130; **Figure 4**). In many steps of traffic, components of tethering complexes promote this interaction directly (124). In regulated exocytosis, however, there is an intermediate requirement for the activation of the regulatory protein Munc13 in a process termed priming. At most steps of vesicle traffic, the SM protein binds only to the open conformation of its cognate syntaxin, alone and in complex with its SNARE partners. In regulated exocytosis, however, the SM protein first binds the closed conformation of syntaxin, preventing interaction of syntaxin with other SNAREs until a Munc13 protein opens the Syntaxin conformation and allows core complex assembly to proceed (131–133). This provides a key point of control of regulated secretion by the second messengers DAG and $Ca^{2+}$ (see sections above and below).

In neurons, Munc13-1 is found at baseline mostly in cytoplasmic and cytoskeletal pools. Upon activation by DAG, it translocates to the plasma membrane to form a tripartite complex with Rab3a on tethered vesicles and RIM on the plasma membrane. Airway goblet cells express ubMunc13-2 (13, 71), which is closely related to Munc13-1 in overall structure and 78% identical in amino acid

**Cysteine string protein:** a cochaperone with Hsc70 that is anchored to synaptic vesicles by palmitoylation and is thought to function in recruiting other components of the exocytic machinery. Nonneuronal isoforms have also been identified

sequence, so it likely functions similarly. Airway goblet cells express Rab3b and Rab3d rather than Rab3a (10), but expression of RIM proteins has not been analyzed, so the precise molecular details of these interactions in goblet cells remain unknown. Nonetheless, airway goblet cells of mice lacking Munc13-2 (134) have been analyzed and show striking phenotypes in baseline and stimulated mucin secretion (13). In contrast to wild-type mice, which have few or no goblet cells in intrapulmonary airways at baseline, the mutant mice show intracellular accumulation of polymeric mucins in the absence of inflammation. This finding demonstrated for the first time that the secretory (Clara) cells in the intrapulmonary airways of mice produce polymeric mucins at baseline, despite the paucity of morphological evidence, and implicated a secretory pathway regulated by Munc13-2 in their baseline export. These data further suggested that the rate of export fully matches the rate of mucin production at baseline in wild-type mice, such that polymeric mucins do not accumulate intracellularly and that baseline mucin release occurs through tonic activity of a regulated secretory pathway. When stimulated with extracellular ATP, the mucin-containing secretory cells of Munc13-2 mutant mice show partial release of the intracellular mucin, indicating a secretory defect even in the presence of a strong stimulus. When polymeric mucin production is induced by allergic inflammation and secretion is then stimulated with extracellular ATP, mucin release is approximately half that of wild-type mice. Munc13-2 null mice also show accumulation of intracellular mucin in goblet cells of salivary glands, nasal mucosa, and intestine, suggesting that Munc13-2 has a conserved function in the regulation of mucin secretion in diverse tissues (13).

A good candidate for the residual priming function in airway goblet cells of Munc13-2 null mice is Munc13-4. This distantly related member of the Munc13 family, with only 24–26% sequence identity to the other isoforms, is strongly expressed in lungs, and the protein has been localized to airway goblet cells (71, 135). A conditional mutant mouse has been generated, although its phenotype in the airway has not yet been described (B.F. Dickey, unpublished observations). In hematopoietic cells, Munc13-4 can be localized to SGs by activated Rab27, and its deficiency results in defects in stimulated secretion from platelets and cytolytic T cells (136). The capacity of Rab27 proteins to interact both with granulophilin/myosin V and with Munc13-4 suggests sequential interactions leading directly from granule transport to exocytic priming. Whether Munc13-2 and Munc13-4 function on the same granule in response to different signals or regulate distinct SG populations is currently unknown, although the observation that secretory cells in the Munc13-2-deficient mouse possess a population of MSGs resistant to agonist stimulation suggests the latter possibility.

## EXOCYTOSIS

Despite the role of the exocytic core complex of airway goblet cells as the essential fusion machinery of vesicle traffic, the molecular composition of this complex is not known. Some clues are given by the specificity of interactions with SM proteins because these have been studied in polarized epithelia, including the airway (**Figure 4**). The three Munc18 proteins comprise a subset of SM proteins that appear to be specialized for secretion (129, 130, 137). Munc18a is expressed primarily in neurons, where it is required for synaptic vesicle release (138); Munc18b is expressed in secretory epithelia of the lungs, kidney, and other tissues and regulates apical traffic in polarized epithelial cell lines (139, 140); and Munc18c is expressed ubiquitously and appears to mediate basolateral secretion (137). We have found that Munc18b is localized to the apical membrane of murine airway secretory cells, that it regulates mucin secretion, and that in adenocarcinoma cell lines it coimmunoprecipiates with Syntaxins 2 and 3 (B.L. Scott & B.F. Dickey, unpublished

observations). Therefore, Syntaxins 2 and 3 are candidate Qa-SNAREs in MSG exocytosis, consistent with the localization of these Syntaxins on the apical plasma membrane of other polarized epithelial cells (141 and references therein). Another candidate Qa-SNARE is Syntaxin 11, which phenocopies Munc13-4 in hematopoietic cells when mutated (142). The Qb- and Qc-SNAREs in yeast exocytosis are provided by a single protein (Sec9), in distinction from all other steps of vesicle traffic in yeast (143–145). Similarly, Qb- and Qc-SNAREs are provided by a single protein of the orthologous SNAP-25 family in all known examples of metazoan-regulated secretion. Thus, it is likely that a member of the SNAP-25 family plays this role in airway goblet cells, although expression, localization, and functional studies have not yet been reported. VAMP-8 functions broadly in exocrine secretion (146, 147) and is therefore a candidate R-SNARE in airway goblet cells.

In neurons, the primed and partially coiled core complex is prevented from full coiling by complexin, which is displaced by a synaptotagmin (Syt) in response to depolarization-induced calcium entry (148). This is thought to be the final event in synaptic vesicle fusion and results in vesicle content release with submillisecond kinetics (143, 149). Complexins have not been studied in airway goblet cells, but the kinetics of MSG exocytosis, at least as they are observed by microscopy and transepithelial capacitance measurements, are slower by several orders of magnitude (36, 53). In addition, the large size of MSGs precludes the close apposition of more than a small fraction of granules to the plasma membrane at one time. Together, these facts suggest that formation of the core complex is not initiated in advance of secretory signaling but that granule priming and fusion occur acutely in response to the second messengers DAG and $Ca^{2+}$. Despite these differences, a low-affinity, fast calcium-sensing Syt is required for airway mucin secretion as it is in neurons (15). Syt-2 null mice were generated by knocking the β-galactosidase gene into the Syt-2 locus,

## MUNC PROTEINS: CRITICAL REGULATORS OF EXOCYTOSIS

Sydney Brenner originally identified the *unc* genes in a chemical mutagenesis screen in *Caenorhabditis elegans* for uncoordinated mutants in 1974, and many of these genes were found to encode proteins that mediate synaptic vesicle release. The mammalian orthologs of unc13 and unc18 were not separately discovered and given other names as had occurred for most other orthologs, so they came to be known as Munc proteins.

Munc13 comprises a family of five proteins derived from four genes. Munc13-2 is expressed in two splice variants: a ubiquitous isoform that is expressed widely outside the nervous system (ubMun13-2) and a brain isoform whose expression is mostly confined to neurons (bMunc13-2). All Munc13 proteins except Munc13-4 contain a C1 domain that binds diacylglycerol (DAG); this site is the major target of phorbol esters in pharmacologically stimulated secretion (157). All Munc13 proteins contain multiple C2 domains, some that bind calcium and phospholipids and others that are specialized for protein-protein interactions (158). The MUN domain is an α-helical region that interacts with Syntaxin either directly or indirectly, and this interaction is essential for Munc13 function (159).

Munc18 is a family of three proteins that is a subset of the larger family of seven vertebrate SM (Sec1/Munc18) proteins. Sec1 was the first of a series of genes encoding proteins that mediate secretion and were identified in a chemical mutagenesis screen in yeast by Peter Novick and Randy Schekman in 1980. Sec1, which functions in ER-to-Golgi traffic, was later recognized as being distantly related to Munc18, which functions in regulated exocytosis, but the proteins were found to function in fundamentally different ways. Therefore, the term SM proteins was adopted to include all members of the evolutionarily related family, with four members in yeast and seven in mammals. An SM protein functions at every step of vesicle traffic, so some SM proteins function at more than one step. Loss of SM protein function causes a profound defect in the corresponding trafficking event (129, 130, 137). All SM proteins interact with Syntaxins, but the critical function they perform and whether it exclusively reflects their interactions with Syntaxins are unknown.

allowing the expression of Syt-2 to be analyzed by lacZ staining (150). This revealed that Syt-2 is expressed in airway secretory cells of mice as well as in neurons of caudal brain regions. Syt-2 null mice with IL-13-induced mucous metaplasia display a severe defect in ATP-induced mucin. However, unlike Munc13-2 null mice, which accumulate intracellular mucin in the absence of inflammation-induced mucin gene upregulation, Syt-2 null mice do not accumulate intracellular mucin at baseline. This is consistent with the increase in spontaneous neurotransmitter release and the lack of change in total neurotransmitter release despite a slowing of the kinetics of evoked release in the neurons of Syt-2 null mice (150), although other explanations are possible.

**SNAP-25 (synaptosome-associated protein of 25 kDa):** a Q-SNARE (see side bar on SNAREs) containing two coiled-coil domains. SNAP-23 and SNAP-27 are closely related nonneuronal family members, and like SNAP-25, also contribute two coiled-coil domains to their cognate exocytic core complexes

**Complexins:** a family of four soluble 13-kDa proteins with coiled-coil structures that are capable of associating with partially formed exocytic SNARE complexes and preventing complete coiling

**Synaptotagmin (Syt):** sometimes called tagmin; a family of at least 13 genes in vertebrates that serve as calcium sensors in vesicle traffic and are composed of a short intravesicular N terminus, single membrane-spanning domain, and two homologous C2 domains

## LOCAL SIGNALING IN EXOCYTOSIS

Time and distance in regulated secretion have different scales that apply to its different processes; none are likely to be as fast and short as the final steps of exocytic fusion. The distances are in nanometers, and time is in milliseconds. The best time estimate for the exocytosis of the entire contents of a single MSG is <100 ms (36); likely, formation of the fusion pore is on the order of a few milliseconds (see Reference 151). Regulation of such small-scale events requires signaling on equally local scales.

### Local $Ca^{2+}$ Signaling

There is very good evidence for localized $Ca^{2+}$ signaling relevant to exocytosis (see Reference 152). In nerve terminals, for instance, voltage-sensitive $Ca^{2+}$ channels located within tens of nanometers of primed synaptic vesicle supply peak $Ca^{2+}$ concentrations of hundreds of micromolar in hundreds of microseconds to drive exocytosis. In many nonexcitable secretory cells, including goblet cells, however, $Ca^{2+}$ is supplied from stores in the endoplasmic reticulum (68); hence, a close juxtaposition of intracellular $Ca^{2+}$ stores and apical membrane exocytic sites is indicated. Unfortunately, epithelia are much more difficult to work with experimentally than are single cells, especially when the former need to be studied as native tissues or primary cultures for clinical relevance. Hence, the advanced biophysical techniques available to the cellular biophysicists, e.g., capacitance and amperometry measurements, have not been applied to the study of regulated mucin secretion. Nonetheless, more indirect observations have yielded some progress.

One can estimate distances and times for $Ca^{2+}$ effects by taking advantage of the 1000-fold-faster binding kinetics of BAPTA relative to EGTA; BAPTA and EGTA otherwise have similar affinities for $Ca^{2+}$. Model calculations, for instance, suggest that for cellular ionic conditions BAPTA buffers a step 10-μM $Ca^{2+}$ increase to <1 μM, out to ~50 nm of the membrane surface within ~2 μs, whereas with EGTA the $Ca^{2+}$ remains >7.5 μM, out to ~125 nm, for >100 ms. In experiments with SLO-permeabilized SPOC1 cells, we found that BAPTA essentially quenched the $IP_3$-mediated mucin release allowed by EGTA, suggesting that the $IP_3$-sensitive $Ca^{2+}$ release sites on the ER are located very close to the plasma membrane (67). An ongoing controversy in secretory biology concerns the possibility that $IP_3$ receptors are located on SGs, including MSGs (153, 154). Although this is an attractive idea, we recently reported that $IP_3$ receptors colocalize with the ER, not MSG, in human goblet cells. Additionally, electron microscopy revealed that the ER extends throughout the apical pole of the goblet cells, right up to the plasma membrane (15; **Figure 1**). Hence, these data are consistent with the ER as the source of $Ca^{2+}$ for regulated mucin secretion, as it appears to be for other secretory cells as well (see discussion in Reference 15). Minimally, $Ca^{2+}$ that is released locally to the apical plasma membrane from the goblet cell ER activates actin filament disruption and remodeling (scinderin), priming (Munc13), and exocytic fusion (Syt).

One hopes that future technical advances applied to the study of goblet cells can determine whether these events are controlled globally or by sequential, independent regulatory events.

## Local Lipid Signaling in Exocytosis

In addition to local $Ca^{2+}$ signaling, local phosphatidic acid production by PLD is important in secretory cell exocytic release (see Reference 155). The phosphatidic acid produced by PLD can affect exocytosis indirectly, by being metabolized to DAG, and/or it acts to promote membrane curvature and fusion (e.g., see Reference 156). There has been no investigation into the potential roles of either PLD or phosphatidic acid in regulated mucin secretion, indicating an area that needs the attention of the field.

# CONCLUSIONS

In recent years, the study of airway goblet cell physiology and pharmacology has transcended a focus on important signaling molecules and cellular messengers to see the beginnings of more mechanistic studies oriented toward events along the secretory pathway. To a major degree, this transition has been helped through the use of genetically manipulated mice; however, we have also learned that mouse airway "goblet cells" are really Clara cells that have undergone mucous metaplasia, suggesting cautious comparisons to true goblet cells of human larger airways, which have different cellular origins. Mucin SGs exist wholly in a storage pool (i.e., there is no ready releasable pool, or it is very small) and must transit the cortical actin cytoskeleton to reach exocytic sites on the goblet cell apical membrane. Disruption of the actin barrier is achieved through the activation of scinderin by $Ca^{2+}$ and, likely, of MARCKS by nPKCε. Transit through the actin mesh probably is accomplished by interactions of MSGs with myosin II and V, with regulation by $Ca^{2+}$ and MLCK, by dynamic actin remodeling, and possibly with MARCKS; however, there has been little work in this area. Interactions of MSGs with the proteins and protein complexes that effect tethering, docking, priming, and fusion are in their preliminary stages of understanding, and the relevant proteins are being identified: Rab3d and 27 have been identified as the important targeting GTPases, cysteine string protein and Hsc70 as potential tethering/docking proteins, Munc13-2 and Munc13-4 as the priming proteins, Munc18b as the important SM protein, and syntaxins 2, 3, and 11 and VAMP-8 as candidate SNARE proteins. Lastly, Syt-2 is an important $Ca^{2+}$ sensor and fusion activator for the SNARE complex, although other isoforms likely are involved. Following the completion of this inventory phase, we can look forward to many years of functional studies before the definitive review that describes fully the series of events that comprise MSG exocytosis can be written.

### SUMMARY POINTS

1. Polymeric mucin secretion by airway epithelial cells occurs exclusively through regulated exocytic pathways. The absence of accumulated intracellular mucin in mouse airway secretory (Clara) cells at baseline despite constitutive polymeric mucin synthesis indicates that this secretory pathway has a baseline level of activity sufficient to rapidly secrete all newly produced mucin.
2. With inflammatory stimulation of increased polymeric mucin production, intracellular mucin accumulates in mouse secretory cells in a process termed mucous metaplasia. It is currently unknown whether mucous metaplasia simply reflects an increased rate

of mucin production that exceeds the baseline rate of mucin secretion, or whether inflammatory stimuli also reduce the baseline rate of secretion. In any case, further activation of phospholipase C by extracellular agonists with the generation of the second messengers DAG and $IP_3$ results in the rapid secretion of accumulated mucins.

3. Munc13-2, Syt-2, and MARCKS are important mediators of regulated mucin secretion, but the identities and precise roles of many other molecular components of the goblet cell secretory pathway remain to be defined. Until the constituent proteins along the entire MSG exocytic pathway are defined and the functions of each component understood, it is useful to use the knowledge of these exocytic proteins derived from other secretory cells to build a working model.
4. In view of the roles of apical extracellular nucleotides in the regulation of airway surface liquid volume and ciliary beat frequency, their regulation of the rate of polymeric mucin secretion would provide a mechanism to couple these three key components of mucociliary clearance.

## DISCLOSURE STATEMENT

B.F.D. serves on the Scientific Advisory Board of BioMarck Pharmaceuticals, which is developing for clinical use a compound that inhibits mucin secretion.

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## LITERATURE CITED

1. Randell SH. 2006. Airway epithelial stem cells and the pathophysiology of chronic obstructive pulmonary disease. *Proc. Am. Thorac. Soc.* 3:718–25
2. Jeffery PK, Gaillard D, Moret S. 1992. Human airway secretory cells during development and in mature airway epithelium. *Eur. Respir. J.* 5:93–104
3. Davis CW, Randell SH. 2001. Airway goblet and mucous cells: identical, similar, or different? In *Cilia and Mucus: From Development to Respiratory Disease*, ed. M Salathe, pp. 195–210. NY: Marcel-Dekker
4. Verdugo P. 1990. Goblet cells secretion and mucogenesis. *Annu. Rev. Physiol.* 52:157–76
5. Bansil R, Stanley E, LaMont JT. 1995. Mucin biophysics. *Annu. Rev. Physiol.* 57:635–57
6. Forstner G. 1995. Signal transduction, packaging and secretion of mucins. *Annu. Rev. Physiol.* 57:585–605
7. Verdugo P. 1991. Mucin exocytosis. *Am. Rev. Respir. Dis.* 144:S33–37
8. Davis CW. 1997. Goblet cells: physiology and pharmacology. In *Airway Mucus: Basic Mechanisms and Clinical Perspectives*, ed. DF Rogers, MI Lethem, pp. 150–77. Basel: Berkhauser
9. Kim KC, Hisatsune A, Kim DJ, Miyata T. 2003. Pharmacology of airway goblet cell mucin release. *J. Pharmacol. Sci.* 92:301–7

10. Evans CM, Williams OW, Tuvim MJ, Nigam R, Mixides GP, et al. 2004. Mucin is produced by Clara cells in the proximal airways of antigen-challenged mice. *Am. J. Respir. Cell Mol. Biol.* 31:382–94
11. Williams OW, Sharafkhaneh A, Kim V, Dickey BF, Evans CM. 2006. Airway mucus: from production to secretion. *Am. J. Respir. Cell Mol. Biol.* 34:527–36
12. Young HW, Williams OW, Chandra D, Bellinghausen LK, Perez G, et al. 2007. Central role of Muc5ac expression in mucous metaplasia and its regulation by conserved 5′ elements. *Am. J. Respir. Cell Mol. Biol.* 37:273–90
13. Zhu Y, Ehre C, Abdullah LH, Sheehan JK, Dickey BF, Davis CW. 2007. Munc13-2 deficient mice reveal Clara cells as the source of polymeric mucins in the lung. *J. Physiol.* Submitted
14. Zhen G, Park SW, Nguyenvu LT, Rodriguez MW, Barbeau R, et al. 2007. IL-13 and epidermal growth factor receptor have critical but distinct roles in epithelial cell mucin production. *Am. J. Respir. Cell Mol. Biol.* 36:244–53
15. Rossi AH, Mohler PJ, Burns KA, Chua M, Davis CW, Dickey BF. 2007. $IP_3$ receptors and synaptotagmin 2 couple $Ca^{2+}$ signaling to mucin granule exocytosis from airway goblet cells. *Nat. Cell Biol.* Submitted
16. Peterson M, Leblond CP. 1964. Synthesis of complex carbohydrates in the Golgi region, as shown by radioautography after injection of labeled glucose. *J. Cell Biol.* 21:143–48
16a. Hattrup CL, Gendler SJ. 2008. Structure and function of the cell surface (tethered) mucins. *Annu. Rev. Physiol.* 70:431–57
16b. Thornton DJ, Rousseau K, McGuckin MA. 2008. Structure and function of the polymeric mucins in airways mucus. *Annu. Rev. Physiol.* 70:459–86
17. Tuvim MJ, Adachi R, Hoffenberg S, Dickey BF. 2001. Traffic control: Rab GTPases and the regulation of interorganellar transport. *News Physiol. Sci.* 16:56–61
18. Deneka M, Neeft M, van der Sluijs P. 2003. Regulation of membrane transport by Rab GTPases. *Crit Rev. Biochem. Mol. Biol.* 38:121–42
19. Collins RN. 2003. Rab and ARF GTPase regulation of exocytosis. *Mol. Membr. Biol.* 20:105–15
20. Miller SG, Moore HP. 1991. Reconstitution of constitutive secretion using semi-intact cells: regulation by GTP but not calcium. *J. Cell Biol.* 112:39–54
21. Gorr SU, Venkatesh SG, Darling DS. 2005. Parotid secretory granules: crossroads of secretory pathways and protein storage. *J. Dent. Res.* 84:500–9
22. Tooze SA, Martens GJ, Huttner WB. 2001. Secretory granule biogenesis: rafting to the SNARE. *Trends Cell Biol.* 11:116–22
23. Bard F, Malhotra V. 2006. The formation of TGN-to-plasma-membrane transport carriers. *Annu. Rev. Cell Dev. Biol.* 22:439–55
24. McNiven MA, Thompson HM. 2006. Vesicle formation at the plasma membrane and trans-Golgi network: the same but different. *Science* 313:1591–94
25. Castle AM, Huang AY, Castle JD. 2002. The minor regulated pathway, a rapid component of salivary secretion, may provide docking/fusion sites for granule exocytosis at the apical surface of acinar cells. *J. Cell Sci.* 115:2963–73
26. Castle D, Castle A. 1998. Intracellular transport and secretion of salivary proteins. *Crit. Rev. Oral Biol. Med.* 9:4–22
27. Fukuda M. 2005. Versatile role of Rab27 in membrane trafficking: focus on the Rab27 effector families. *J. Biochem.* 137:9–16
28. Seabra MC, Coudrier E. 2004. Rab GTPases and myosin motors in organelle motility. *Traffic* 5:393–99

29. Tolmachova T, Anders R, Stinchcombe J, Bossi G, Griffiths GM, et al. 2004. A general role for Rab27a in secretory cells. *Mol. Biol. Cell* 15:332–44
30. Barral DC, Ramalho JS, Anders R, Hume AN, Knapton HJ, et al. 2002. Functional redundancy of Rab27 proteins and the pathogenesis of Griscelli syndrome. *J. Clin. Invest.* 110:247–57
31. Westbroek W, Lambert J, De Schepper S, Kleta R, Van Den Bossche K, et al. 2004. Rab27b is up-regulated in human Griscelli syndrome type II melanocytes and linked to the actin cytoskeleton via exon F-Myosin Va transcripts. *Pigment Cell Res.* 17:498–505
32. Rodriguez OC, Cheney RE. 2002. Human myosin-Vc is a novel class V myosin expressed in epithelial cells. *J. Cell Sci.* 115:991–1004
33. Saegusa C, Tanaka T, Tani S, Itohara S, Mikoshiba K, Fukuda M. 2006. Decreased basal mucus secretion by Slp2-a-deficient gastric surface mucous cells. *Genes Cells* 11:623–31
34. Miller SG, Moore HP. 1990. Regulated secretion. *Curr. Opin. Cell Biol.* 2:642–47
35. Kim KC, Lee BC. 1991. P2 purinoceptor regulation of mucin release by airway goblet cells in primary culture. *Br. J. Pharmacol.* 103:1053–56
36. Davis CW, Dowell ML, Lethem M, Van Scott M. 1992. Goblet cell degranulation in isolated canine tracheal epithelium: response to exogenous ATP, ADP, and adenosine. *Am. J. Physiol.* 262:C1313–23
37. von Kugelgen I. 2006. Pharmacological profiles of cloned mammalian P2Y-receptor subtypes. *Pharmacol. Ther.* 110:415–32
38. Boucher RC. 1994. Human airway ion transport. Part one. *Am. J. Respir. Crit. Care Med.* 150:271–81
39. Boucher RC. 1994. Human airway ion transport. Part two. *Am. J. Respir. Crit. Care Med.* 150:581–93
40. Salathe M. 2007. Regulation of mammalian ciliary beating. *Annu. Rev. Physiol.* 69:401–22
41. Olivier KN, Bennett WD, Hohneker KW, Zeman KL, Edwards LJ, et al. 1996. Acute safety and effects on mucociliary clearance of aerosolized uridine 5′-triphosphate +/− amiloride in normal human adults. *Am. J. Respir. Crit. Care Med.* 154:217–23
42. Parr CE, Sullivan DM, Paradiso AM, Lazarowski ER, Burch LH, et al. 1994. Cloning and expression of a human $P_{2U}$ nucleotide receptor, a target for cystic fibrosis pharmacotherapy. *Proc. Natl. Acad. Sci. USA* 91:3275–79
43. Davis CW, Abdullah LH. 1997. In vitro models for airways mucin secretion. *Pulm. Pharmacol. Ther.* 10:145–55
44. Conway JD, Bartolotta T, Abdullah LH, Davis CW. 2003. Regulation of mucin secretion from human bronchial epithelial cells grown in murine hosted xenografts. *Am. J. Physiol. Lung Cell Mol. Physiol.* 284:L945–54
45. Kemp PA, Sugar RA, Jackson AD. 2004. Nucleotide-mediated mucin secretion from differentiated human bronchial epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 31:446–55
46. Abdullah LH, Davis SW, Burch L, Yamauchi M, Randell SH, et al. 1996. $P_{2u}$ purinoceptor regulation of mucin secretion in SPOC1 cells, a goblet cell line from the airways. *Biochem. J.* 316(Pt. 3):943–51
47. Kim KC, Park HR, Shin CY, Akiyama T, Ko KH. 1996. Nucleotide-induced mucin release from primary hamster tracheal surface epithelial cells involves the $P_{2u}$ purinoceptor. *Eur. Respir. J.* 9:542–48
48. Ehre C, Zhu Y, Abdullah LH, Olsen JC, Nakayama KI, et al. 2007. nPKC$\varepsilon$, a P2Y2-R downstream effector in regulated mucin secretion from airway goblet cells. *Am. J. Physiol. Cell Physiol.* 293:C1445–54

49. Adler KB, Akley NJ, Glasgow WC. 1992. Platelet-activating factor provokes release of mucin-like glycoproteins from guinea pig respiratory epithelial cells via a lipoxygenase-dependent mechanism. *Am. J. Respir. Cell Mol. Biol.* 6:550–56
50. Kim S, Nadel JA. 2004. Role of neutrophils in mucus hypersecretion in COPD and implications for therapy. *Treat. Respir. Med.* 3:147–59
51. Park JA, He F, Martin LD, Li Y, Chorley BN, Adler KB. 2005. Human neutrophil elastase induces hypersecretion of mucin from well-differentiated human bronchial epithelial cells in vitro via a protein kinase Cδ-mediated mechanism. *Am. J. Pathol.* 167:651–61
52. Sommerhoff CP, Fang KC, Nadel JA, Caughey GH. 1996. Classical second messengers are not involved in proteinase-induced degranulation of airway gland cells. *Am. J. Physiol.* 271:L796–803
53. Danahay H, Atherton HC, Jackson AD, Kreindler JL, Poll CT, Bridges RJ. 2006. Membrane capacitance and conductance changes parallel mucin secretion in the human airway epithelium. *Am. J. Physiol. Lung Cell Mol. Physiol.* 290:L558–69
54. Rogers DF. 2002. Pharmacological regulation of the neuronal control of airway mucus secretion. *Curr. Opin. Pharmacol.* 2:249–55
55. Rogers DF. 2001. Motor control of airway goblet cells and glands. *Respir. Physiol.* 125:129–44
56. Joo NS, Saenz Y, Krouse ME, Wine JJ. 2002. Mucus secretion from single submucosal glands of pig. Stimulation by carbachol and vasoactive intestinal peptide. *J. Biol. Chem.* 277:28167–75
57. Barnes PJ, Nadel JA, Roberts JM, Basbaum CB. 1982. Muscarinic receptors in lung and trachea: autoradiographic localization using [3H]quinuclidinyl benzilate. *Eur. J. Pharmacol.* 86:103–6
58. Racke K, Matthiesen S. 2004. The airway cholinergic system: physiology and pharmacology. *Pulm. Pharmacol. Ther.* 17:181–98
59. Agrawal A, Rengarajan S, Adler KB, Ram A, Ghosh B, et al. 2007. Inhibition of mucin secretion with MARCKS-related peptide improves airway obstruction in a mouse model of asthma. *J. Appl. Physiol.* 102:399–405
60. Kishioka C, Okamoto K, Kim J, Rubin BK. 2001. Regulation of secretion from mucous and serous cells in the excised ferret trachea. *Respir. Physiol.* 126:163–71
61. Davis CW. 2002. Regulation of mucin secretion from in vitro cellular models. *Novartis Found. Symp.* 248:113–25
62. Salathe M, Lipson EJ, Ivonnet PI, Bookman RJ. 1997. Muscarinic signaling in ciliated tracheal epithelial cells: dual effects on $Ca^{2+}$ and ciliary beating. *Am. J. Physiol.* 272:L301–10
63. Lieb T, Frei CW, Frohock JI, Bookman RJ, Salathe M. 2002. Prolonged increase in ciliary beat frequency after short-term purinergic stimulation in human airway epithelial cells. *J. Physiol.* 538:633–46
64. Gosens R, Zaagsma J, Meurs H, Halayko AJ. 2006. Muscarinic receptor signaling in the pathophysiology of asthma and COPD. *Respir. Res.* 7:73
65. Abdullah LH, Davis CW. 2007. Regulation of airway goblet cell mucin secretion by tyrosine phosphorylation signaling pathways. *Am. J. Physiol. Lung Cell Mol. Physiol.* 293:L591–99
66. Abdullah LH, Conway JD, Cohn JA, Davis CW. 1997. Protein kinase C and $Ca^{2+}$ activation of mucin secretion in airway goblet cells. *Am. J. Physiol.* 273:L201–10
67. Rossi AH, Sears PR, Davis CW. 2004. $Ca^{2+}$ dependency of '$Ca^{2+}$-independent' exocytosis in SPOC1 airway goblet cells. *J. Physiol.* 559:555–65

68. Rossi AH, Salmon WC, Chua M, Davis CW. 2007. Calcium signaling in human airway goblet cells following purinergic activation. *Am. J. Physiol. Lung Cell Mol. Physiol.* 292:L92–98
69. Katan M, Rodriguez R, Matsuda M, Newbatt YM, Aherne GW. 2003. Structural and mechanistic aspects of phospholipase Cγ regulation. *Adv. Enzyme Regul.* 43:77–85
70. Harden TK, Sondek J. 2006. Regulation of phospholipase C isozymes by Ras superfamily GTPases. *Annu. Rev. Pharmacol. Toxicol.* 46:355–79
71. Abdullah LH, Bundy JT, Ehre C, Davis CW. 2003. Mucin secretion and PKC isoforms in SPOC1 goblet cells: differential activation by purinergic agonist and PMA. *Am. J. Physiol. Lung Cell Mol. Physiol.* 285:L149–60
72. Brose N, Rosenmund C. 2002. Move over protein kinase C, you've got company: alternative cellular effectors of diacylglycerol and phorbol esters. *J. Cell Sci.* 115:4399–411
73. Kazanietz MG. 2005. Targeting protein kinase C and "nonkinase" phorbol ester receptors: emerging concepts and therapeutic implications. *Biochim. Biophys. Acta* 1754:296–304
74. Steyer JA, Horstmann H, Almers W. 1997. Transport, docking and exocytosis of single secretory granules in live chromaffin cells. *Nature* 388:474–78
75. Ehre C, Rossi AH, Abdullah LH, De Pestel K, Hill S, et al. 2005. Barrier role of actin filaments in regulated mucin secretion from airway goblet cells. *Am. J. Physiol. Cell Physiol.* 288:C46–56
76. Malacombe M, Bader MF, Gasman S. 2006. Exocytosis in neuroendocrine cells: new tasks for actin. *Biochim. Biophys. Acta* 1763:1175–83
77. da Costa SR, Okamoto CT, Hamm-Alvarez SF. 2003. Actin microfilaments et al.—the many components, effectors and regulators of epithelial cell endocytosis. *Adv. Drug Deliv. Rev.* 55:1359–83
78. Johns LM, Levitan ES, Shelden EA, Holz RW, Axelrod D. 2001. Restriction of secretory granule motion near the plasma membrane of chromaffin cells. *J. Cell Biol.* 153:177–90
79. Lang T, Wacker I, Wunderlich I, Rohrbach A, Giese G, et al. 2000. Role of actin cortex in the subplasmalemmal transport of secretory granules in PC-12 cells. *Biophys. J.* 78:2863–77
80. Giner D, Neco P, Frances MM, Lopez I, Viniegra S, Gutierrez LM. 2005. Real-time dynamics of the F-actin cytoskeleton during secretion from chromaffin cells. *J. Cell Sci.* 118:2871–80
81. Li D, Xiong J, Qu A, Xu T. 2004. Three-dimensional tracking of single secretory granules in live PC12 cells. *Biophys. J.* 87:1991–2001
82. Oheim M. 2004. A deeper look into single-secretory vesicle dynamics. *Biophys. J.* 87:1403–5
83. DePina AS, Langford GM. 1999. Vesicle transport: the role of actin filaments and myosin motors. *Microsc. Res. Tech.* 47:93–106
84. Neco P, Giner D, Viniegra S, Borges R, Villarroel A, Gutierrez LM. 2004. New roles of myosin II during vesicle transport and fusion in chromaffin cells. *J. Biol. Chem.* 279:27450–57
85. Jerdeva GV, Wu K, Yarber FA, Rhodes CJ, Kalman D, et al. 2005. Actin and nonmuscle myosin II facilitate apical exocytosis of tear proteins in rabbit lacrimal acinar epithelial cells. *J. Cell Sci.* 118:4797–812
86. Rose SD, Lejen T, Casaletti L, Larson RE, Pene TD, Trifaro JM. 2003. Myosins II and V in chromaffin cells: Myosin V is a chromaffin vesicle molecular motor involved in secretion. *J. Neurochem.* 85:287–98

87. Mizuno N, Naruse S, Kitagawa M, Ishiguro H, Hayakawa T. 2000. Effects of an inhibitor of myosin light chain kinase on amylase secretion from rat pancreatic acini. *Biochem. Biophys. Res. Commun.* 269:792–97
88. Rao K, Paik WY, Zheng L, Jobin RM, Tomic M, et al. 1997. Wortmannin-sensitive and -insensitive steps in calcium-controlled exocytosis in pituitary gonadotrophs: evidence that myosin light chain kinase mediates calcium-dependent and wortmannin-sensitive gonadotropin secretion. *Endocrinology* 138:1440–49
89. Yu W, Niwa T, Fukasawa T, Hidaka H, Senda T, et al. 2000. Synergism of protein kinase A, protein kinase C, and myosin light-chain kinase in the secretory cascade of the pancreatic beta-cell. *Diabetes* 49:945–52
90. Matsumura C, Kuwashima H, Kimura T. 1999. Myosin light chain kinase inhibitors and calmodulin antagonist inhibit $Ca^{2+}$- and ATP-dependent catecholamine secretion from bovine adrenal chromaffin cells. *J. Auton. Pharmacol.* 19:115–21
91. Menasche G, Pastural E, Feldmann J, Certain S, Ersoy F, et al. 2000. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nat. Genet.* 25:173–76
92. Langford GM. 2002. Myosin-V, a versatile motor for short-range vesicle transport. *Traffic* 3:859–65
93. Norman JC, Price LS, Ridley AJ, Hall A, Koffer A. 1994. Actin filament organization in activated mast cells is regulated by heterotrimeric and small GTP-binding proteins. *J. Cell Biol.* 126:1005–15
94. Valentijn JA, Valentijn K, Pastore LM, Jamieson JD. 2000. Actin coating of secretory granules during regulated exocytosis correlates with the release of rab3D. *Proc. Natl. Acad. Sci. USA* 97:1091–95
95. Gasman S, Chasserot-Golaz S, Malacombe M, Way M, Bader MF. 2004. Regulated exocytosis in neuroendocrine cells: a role for subplasmalemmal Cdc42/N-WASP-induced actin filaments. *Mol. Biol. Cell* 15:520–31
96. Bader MF, Doussau F, Chasserot-Golaz S, Vitale N, Gasman S. 2004. Coupling actin and membrane dynamics during calcium-regulated exocytosis: a role for Rho and ARF GTPases. *Biochim. Biophys. Acta* 1742:37–49
97. Taunton J. 2001. Actin filament nucleation by endosomes, lysosomes and secretory vesicles. *Curr. Opin. Cell Biol.* 13:85–91
98. Frantz C, Coppola T, Regazzi R. 2002. Involvement of Rho GTPases and their effectors in the secretory process of PC12 cells. *Exp. Cell Res.* 273:119–26
99. Alberts P, Rudge R, Irinopoulou T, Danglot L, Gauthier-Rouviere C, Galli T. 2006. Cdc42 and actin control polarized expression of TI-VAMP vesicles to neuronal growth cones and their fusion with the plasma membrane. *Mol. Biol. Cell* 17:1194–203
100. Sokac AM, Co C, Taunton J, Bement W. 2003. Cdc42-dependent actin polymerization during compensatory endocytosis in *Xenopus* eggs. *Nat. Cell Biol.* 5:727–32
101. Nemoto T, Kojima T, Oshima A, Bito H, Kasai H. 2004. Stabilization of exocytosis by dynamic F-actin coating of zymogen granules in pancreatic acini. *J. Biol. Chem.* 279:37544–50
102. Tchakarov L, Vitale ML, Jeyapragasan M, Rodriguez Del Castillo A, Trifaro JM. 1990. Expression of scinderin, an actin filament-severing protein, in different tissues. *FEBS Lett.* 268:209–12
103. Trifaro JM, Rose SD, Marcu MG. 2000. Scinderin, a $Ca^{2+}$-dependent actin filament severing protein that controls cortical actin network dynamics during secretion. *Neurochem. Res.* 25:133–44

104. Trifaro JM, Lejen T, Rose SD, Pene TD, Barkar ND, Seward EP. 2002. Pathways that control cortical F-actin dynamics during secretion. *Neurochem. Res.* 27:1371–85
105. Abouzahr S, Bismuth G, Gaudin C, Caroll O, Van Endert P, et al. 2006. Identification of target actin content and polymerization status as a mechanism of tumor resistance after cytolytic T lymphocyte pressure. *Proc. Natl. Acad. Sci. USA* 103:1428–33
106. Yin HL, Janmey PA. 2003. Phosphoinositide regulation of the actin cytoskeleton. *Annu. Rev. Physiol.* 65:761–89
107. Dumitrescu PT, Rose SD, Lejen T, Marcu MG, Trifaro JM. 2005. Expression of various scinderin domains in chromaffin cells indicates that this protein acts as a molecular switch in the control of actin filament dynamics and exocytosis. *J. Neurochem.* 92:780–89
108. Li Y, Martin LD, Spizz G, Adler KB. 2001. MARCKS protein is a key molecule regulating mucin secretion by human airway epithelial cells in vitro. *J. Biol. Chem.* 276:40982–90
109. Singer M, Martin LD, Vargaftig BB, Park J, Gruber AD, et al. 2004. A MARCKS-related peptide blocks mucus hypersecretion in a mouse model of asthma. *Nat. Med.* 10:193–96
110. Blackshear PJ. 1993. The MARCKS family of cellular protein kinase C substrates. *J. Biol. Chem.* 268:1501–4
111. Arbuzova A, Schmitz AA, Vergeres G. 2002. Cross-talk unfolded: MARCKS proteins. *Biochem. J.* 362:1–12
112. Akita Y, Kawasaki H, Ohno S, Suzuki K, Kawashima S. 2000. Involvement of protein kinase Cε in thyrotropin-releasing hormone-stimulated phosphorylation of the myristoylated alanine-rich C kinase substrate in rat pituitary clonal cells. *Electrophoresis* 21:452–59
113. Park YS, Hur EM, Choi BH, Kwak E, Jun DJ, et al. 2006. Involvement of protein kinase C-ε in activity-dependent potentiation of large dense-core vesicle exocytosis in chromaffin cells. *J. Neurosci.* 26:8999–9005
114. Jerdeva GV, Yarber FA, Trousdale MD, Rhodes CJ, Okamoto CT, et al. 2005. Dominant-negative PKC-ε impairs apical actin remodeling in parallel with inhibition of carbachol-stimulated secretion in rabbit lacrimal acini. *Am. J. Physiol. Cell Physiol.* 289:C1052–68
115. Hartwig JH, Thelen M, Rosen A, Janmey PA, Nairn AC, Aderem A. 1992. MARCKS is an actin filament crosslinking protein regulated by protein kinase C and calcium-calmodulin. *Nature* 356:618–22
116. Aderem A. 1992. The MARCKS brothers: a family of protein kinase C substrates. *Cell* 71:713–16
117. Tang JX, Janmey PA. 1996. The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. *J. Biol. Chem.* 271:8556–63
118. Tapp H, Al-Naggar IM, Yarmola EG, Harrison A, Shaw G, et al. 2005. MARCKS is a natively unfolded protein with an inaccessible actin-binding site: evidence for long-range intramolecular interactions. *J. Biol. Chem.* 280:9946–56
119. Arbuzova A, Wang L, Wang J, Hangyas-Mihalyne G, Murray D, et al. 2000. Membrane binding of peptides containing both basic and aromatic residues. Experimental studies with peptides corresponding to the scaffolding region of caveolin and the effector region of MARCKS. *Biochemistry* 39:10330–39
120. Laux T, Fukami K, Thelen M, Golub T, Frey D, Caroni P. 2000. GAP43, MARCKS, and CAP23 modulate $PI(4,5)P_2$ at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. *J. Cell Biol.* 149:1455–72
121. McLaughlin S, Murray D. 2005. Plasma membrane phosphoinositide organization by protein electrostatics. *Nature* 438:605–11
122. Mosevitsky MI. 2005. Nerve ending "signal" proteins GAP-43, MARCKS, and BASP1. *Int. Rev. Cytol.* 245:245–325

123. Swierczynski SL, Siddhanti SR, Tuttle JS, Blackshear PJ. 1996. Nonmyristoylated MARCKS complements some but not all of the developmental defects associated with MARCKS deficiency in mice. *Dev. Biol.* 179:135–47
124. Grosshans BL, Ortiz D, Novick P. 2006. Rabs and their effectors: achieving specificity in membrane traffic. *Proc. Natl. Acad. Sci. USA* 103:11821–27
125. Schoch S, Gundelfinger ED. 2006. Molecular organization of the presynaptic active zone. *Cell Tissue Res.* 326:379–91
126. Park J, Fang S, Adler KB. 2006. Regulation of airway mucin secretion by MARCKS protein involves the chaperones heat shock protein 70 and cysteine string protein. *Proc. Am. Thorac. Soc.* 3:493
127. Cheviet S, Waselle L, Regazzi R. 2004. Noc-king out exocrine and endocrine secretion. *Trends Cell Biol.* 14:525–28
128. Teramae H, Fujimoto W, Seino S, Iwanaga T. 2007. Cellular expression of Noc2, a Rab effector protein, in endocrine and exocrine tissues in the mouse. *Histochem. Cell Biol.* 127:1–11
129. Burgoyne RD, Morgan A. 2007. Membrane trafficking: three steps to fusion. *Curr. Biol.* 17:R255–58
130. Rizo J, Sudhof TC. 2002. Snares and Munc18 in synaptic vesicle fusion. *Nat. Rev. Neurosci.* 3:641–53
131. Brose N, Rosenmund C, Rettig J. 2000. Regulation of transmitter release by Unc-13 and its homologues. *Curr. Opin. Neurobiol.* 10:303–11
132. Brose N, Betz A, Wegmeyer H. 2004. Divergent and convergent signaling by the diacylglycerol second messenger pathway in mammals. *Curr. Opin. Neurobiol.* 14:328–40
133. Martin TF. 2002. Prime movers of synaptic vesicle exocytosis. *Neuron* 34:9–12
134. Varoqueaux F, Sigler A, Rhee JS, Brose N, Enk C, et al. 2002. Total arrest of spontaneous and evoked synaptic transmission but normal synaptogenesis in the absence of Munc13-mediated vesicle priming. *Proc. Natl. Acad. Sci. USA* 99:9037–42
135. Koch H, Hofmann K, Brose N. 2000. Definition of Munc13-homology-domains and characterization of a novel ubiquitously expressed Munc13 isoform. *Biochem. J.* 349:247–53
136. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, et al. 2003. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). *Cell* 115:461–73
137. Toonen RF, Verhage M. 2003. Vesicle trafficking: pleasure and pain from SM genes. *Trends Cell Biol.* 13:177–86
138. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, et al. 2000. Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science* 287:864–69
139. Riento K, Kauppi M, Keranen S, Olkkonen VM. 2000. Munc18-2, a functional partner of syntaxin 3, controls apical membrane trafficking in epithelial cells. *J. Biol. Chem.* 275:13476–83
140. Riento K, Jantti J, Jansson S, Hielm S, Lehtonen E, et al. 1996. A Sec1-related vesicle-transport protein that is expressed predominantly in epithelial cells. *Eur. J. Biochem.* 239:638–46
141. ter Beest MB, Chapin SJ, Avrahami D, Mostov KE. 2005. The role of syntaxins in the specificity of vesicle targeting in polarized epithelial cells. *Mol. Biol. Cell* 16:5784–92
142. Zur SU, Beutel K, Kolberg S, Schneppenheim R, Kabisch H, et al. 2006. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: molecular and functional analyses of PRF1, UNC13D, STX11, and RAB27A. *Hum. Mutat.* 27:62–68

143. Brunger AT. 2005. Structure and function of SNARE and SNARE-interacting proteins. *Q. Rev. Biophys.* 38:1–47
144. Jahn R, Lang T, Sudhof TC. 2003. Membrane fusion. *Cell* 112:519–33
145. Jahn R, Scheller RH. 2006. SNAREs—engines for membrane fusion. *Nat. Rev. Mol. Cell Biol.* 7:631–43
146. Wang CC, Shi H, Guo K, Ng CP, Li J, et al. 2007. VAMP8/endobrevin as a general vesicular SNARE for regulated exocytosis of the exocrine system. *Mol. Biol. Cell* 18:1056–63
147. Weng N, Thomas DD, Groblewski GE. 2007. Pancreatic acinar cells express vesicle-associated membrane protein 2- and 8-specific populations of zymogen granules with distinct and overlapping roles in secretion. *J. Biol. Chem.* 282:9635–45
148. Tang J, Maximov A, Shin OH, Dai H, Rizo J, Sudhof TC. 2006. A complexin/synaptotagmin 1 switch controls fast synaptic vesicle exocytosis. *Cell* 126:1175–87
149. Rizo J, Chen X, Arac D. 2006. Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. *Trends Cell Biol.* 16:339–50
150. Pang ZP, Melicoff E, Padgett D, Liu Y, Teich AF, et al. 2006. Synaptotagmin-2 is essential for survival and contributes to $Ca^{2+}$ triggering of neurotransmitter release in central and neuromuscular synapses. *J. Neurosci.* 26:13493–504
151. Jackson MB, Chapman ER. 2006. Fusion pores and fusion machines in $Ca^{2+}$-triggered exocytosis. *Annu. Rev. Biophys. Biomol. Struct.* 35:135–60
152. Oheim M, Kirchhoff F, Stuhmer W. 2006. Calcium microdomains in regulated exocytosis. *Cell Calcium* 40:423–39
153. Petersen OH. 1996. Can $Ca^{2+}$ be released from secretory granules or synaptic vesicles? *Trends Neurosci.* 19:411–13
154. Nguyen T, Chin WC, Verdugo P. 1998. Role of $Ca^{2+}/K^{+}$ ion exchange in intracellular storage and release of $Ca^{2+}$. *Nature* 395:908–12
155. Cazzolli R, Shemon AN, Fang MQ, Hughes WE. 2006. Phospholipid signalling through phospholipase D and phosphatidic acid. *IUBMB Life* 58:457–61
156. Vicogne J, Vollenweider D, Smith JR, Huang P, Frohman MA, Pessin JE. 2006. Asymmetric phospholipid distribution drives in vitro reconstituted SNARE-dependent membrane fusion. *Proc. Natl. Acad. Sci. USA* 103:14761–66
157. Rhee JS, Betz A, Pyott S, Reim K, Varoqueaux F, et al. 2002. β phorbol ester- and diacylglycerol-induced augmentation of transmitter release is mediated by Munc13s and not by PKCs. *Cell* 108:121–33
158. Lu J, Machius M, Dulubova I, Dai H, Sudhof TC, et al. 2006. Structural basis for a Munc13-1 homodimer to Munc13-1/RIM heterodimer switch. *PLoS Biol.* 4:e192
159. Basu J, Shen N, Dulubova I, Lu J, Guan R, et al. 2005. A minimal domain responsible for Munc13 activity. *Nat. Struct. Mol. Biol.* 12:1017–18

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