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Mucus hypersecretion in asthma: causes and effects

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Abstract

Purpose of review—Airway mucus plugging has long been recognized as a principal cause of death in asthma. However, molecular mechanisms of mucin overproduction and secretion have not been understood until recently. These mechanisms are reviewed together with ongoing investigations relating them to lung pathophysiology.

Recent findings—Of the five secreted gel-forming mucins in mammals, only MUC5AC and MUC5B are produced in significant quantities in intrapulmonary airways. MUC5B is the principal gel-forming mucin at baseline in small airways of humans and mice, and therefore likely performs most homeostatic clearance functions. MUC5AC is the principal gel-forming mucin upregulated in airway inflammation and is under negative control by forkhead box a2 and positive control by hypoxia inducible factor-1. Mucin secretion is regulated separately from production, principally by extracellular triphosphate nucleotides that bind $P2Y_2$ receptors on the lumenal surface of airway secretory cells, generating intracellular second messengers that activate the exocytic proteins, Munc13-2 and synaptotagmin-2.

Summary—Markedly upregulated production of MUC5AC together with stimulated secretion leads to airflow obstruction in asthma. As MUC5B appears to mediate homeostatic functions, it may be possible to selectively inhibit MUC5AC production without impairing airway function. The precise roles of mucin hypersecretion in asthma symptoms such as dyspnea and cough and in physiologic phenomena such as airway hyperresponsiveness remain to be defined.

Keywords

airway; asthma; mucin; mucous; mucus

Introduction

The central role of mucus hypersecretion in severe asthma has been known since pathologic studies in the late nineteenth and early twentieth centuries. However, exactly what constitutes mucus hypersecretion and how it connects with disease manifestations other than asphyxiation has been poorly understood. This review will present recent data that clarify the relations among mucous metaplasia, mucin production, mucin secretion, and mucus hypersecretion. The connections between these molecular and cellular processes and the symptoms, signs, and pathophysiology of asthma are increasingly studied and will also be reviewed.

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Mucus hypersecretion

To begin, a few definitions are warranted. The term ‘mucin’ denotes very large (typically >1000kDa), heavily glycosylated (typically >70% carbohydrate) proteins that contain characteristic domains, including at least one region rich in serines and threonines that are the sites of O-glycosylation [1,2••]. There are approximately 20 mucin and mucin-like genes encoded in mammalian genomes, and these are designated by the letters MUC (all capitals in humans, first letter only capitalized in mice) followed by a number [3,4]. Mucins can be divided into those that are anchored in the plasma membrane and those that are secreted. The secreted mucins can be further divided into those that contain sulfhydryl groups near their termini, which allow cross-linking, and those that do not; the former are primarily responsible for the structure of the mucus gel layer and are often referred to as polymeric or gel-forming mucins. ‘Mucus’ refers to the extracellular mixture of mucins that have been secreted or released by hydrolysis of a membrane anchor, other macromolecules such as proteoglycans and antimicrobial proteins, water, ions, and cellular debris. ‘Mucous’ is an adjective referring to an association with mucin or mucus, as in ‘mucous metaplasia’ or ‘mucous cell’.

The airway epithelium is normally covered by a mucus gel layer approximately 5–50μm thick that overlies a liquid layer approximately 7μm in depth [5–7]. Cilia beat within the liquid layer, propelling the gel layer proximally to the pharynx where it is swallowed together with entrapped particles and pathogens. Under pathological conditions, airway mucins may be produced and secreted in greatly increased quantities (‘mucus hypersecretion’). This can be observed pathologically as an increase in intracellular mucins (‘mucous metaplasia’) or an increase in mucus in the airway lumen. Excessive lumenal mucus may become impacted and lead to airway closure. In this circumstance, cough clearance can complement ciliary clearance. However, the volume of expectorated mucus (sputum) correlates only weakly with distal airway mucus hypersecretion for the following reasons. Mucus produced in submucosal glands in large airways can lead to large amounts of sputum with little distal airway obstruction in diseases such as chronic bronchitis or bronchiectasis. Conversely, distal airways may be impacted with mucus produced by surface epithelium with minimal sputum in diseases such as asthma, bronchiolitis, or emphysema-predominant chronic obstructive pulmonary disease [3].

Mucous metaplasia

In small airways of humans (<2mm lumenal diameter) and all intrapulmonary airways of mice, there are few or no visible ‘mucous’ or ‘goblet’ cells by histochemical staining under baseline conditions. In allergic inflammation [8,9••,10], a rapid and dramatic increase in epithelial staining for carbohydrates (e.g., alcian blue and/or periodic acid Shiff, AB—PAS) occurs that is traditionally termed ‘mucous metaplasia’ (Fig. 1aFig. 1 [10,11•]). Metaplasia denotes a change in cellular phenotype, and examples include the differentiation of proximal airway epithelium from a mucociliary into a squamous phenotype as a result of cigarette smoke exposure or the differentiation of distal esophageal cells from a squamous to a mucous phenotype as a result of gastric reflux. However, recent work has revealed that airway secretory (Clara) cells of mice produce gel-forming mucins even at baseline but secrete them so rapidly that there is insufficient intracellular mucin to be visible by insensitive histochemical techniques. More sensitive immunohistochemical staining utilizing sandwich amplification techniques reveals intracellular Muc5b at baseline [11•,12••], and mice defective in airway epithelial apical secretion accumulate sufficient Muc5b at baseline to be visible even by simple histochemical staining [12••]. Thus, the amount of intracellular mucin in airway epithelial cells reflects the relative balance between the rates of mucin production and secretion.

The secretory capacity of airway secretory cells does not appear to change substantially during allergic mucous metaplasia, as Clara cells themselves are capable of vigorous regulated secretion, and the expression of molecular components of the secretory machinery does not change during metaplasia [10,13•]. Neither does the rate of activity of the secretory machinery appear to change, as mucous cells in allergic metaplasia are highly responsive to secretagogues present in the inflammatory milieu [10,14•,15]. Rather, the accumulation of intracellular mucin in asthma primarily reflects a greatly increased production of Muc5ac along with a modest increase in Muc5b [9••,16••]. The simple upregulation of secreted products without a fundamental change in underlying secretory function of Clara/mucous cells does not warrant the designation 'metaplasia' any more than lymphocytes that upregulate cytokine production or endocrine cells that upregulate hormone production. Nonetheless, we retain the term because of its widespread use. Caution must be used in diagnosing mucous metaplasia histologically, as even cells with greatly increased mucin production can be stimulated to rapidly secrete intracellular mucins and lose their histochemical staining (Fig. 1). Measurement of the expression of Muc5ac and coordinately expressed genes such as calcium-activated chloride channel provides a molecular definition of mucous metaplasia that is not susceptible to the complex kinetics of intracellular mucin accumulation [9••,16••,17,18•,19,20•].

Control of polymeric mucin production

The induction of Muc5ac in allergically inflamed mice is dependent upon two important signaling pathways: the IL-13/IL-4 receptor-α complex [21–23] and the epidermal growth factor receptor (EGFR) [16••,24]. The functional dominance of these signaling pathways, however, does not translate into a simple intracellular pathway for Muc5ac gene activation. The principal signaling molecule activated by IL-13 is signal transducer and activator of transcription 6 (STAT6). STAT6 signaling in mouse airway Clara cells is necessary and sufficient for Muc5ac induction (mucous metaplasia) and airway hyperreactivity in response to IL-13 [22]. STAT6 binds to a canonical motif, 5′-TTCN_4GAA-3′, but this motif is not present in the conserved promoter regions of any mammalian MUC5AC orthologs [9••]. One indirect mechanism that may explain IL-13-mediated Muc5ac promoter activation is STAT6-dependent downregulation of forkhead box a2 (Foxa2) (Fig. 2Fig. 2). Foxa2 is a critical negative regulator of Muc5ac expression, and genetic deletion of Foxa2 in mice leads to constitutive Muc5ac overproduction resembling mucous metaplasia [25].

EGFR signaling is also required for mucous metaplasia in a wide variety of animal and in-vitro models [24,26•,16••]. Transcription factors downstream of EGFR include Sp1 [27] and hypoxia inducible factor-1 (HIF-1) [9••]. Sp1 is a ubiquitous transcription factor that binds to a wide variety of promoters where it acts as a transactivator through interactions with other proteins [28], and its activity can be modulated by phosphorylation [29]. A role for HIF-1 was suggested when a consensus-binding motif was found in the core promotors of all presently sequenced mammalian Muc5ac orthologs [9••]. Its mutation dramatically reduces promoter activity, and its binding is induced by IL-13 and EGF stimulation [9••]. A critical role for HIF-1 in the regulation of Muc5ac in allergic mucous metaplasia is further attractive as it contains a conserved STAT6 motif in its promoter. Because HIF-1 is acutely responsive to a wide variety of inflammatory signals [30,31], its activation may be a central regulator of mucous metaplasia in the diverse inflammatory responses to allergens, fungi, viruses, and toxicants. EGFR signaling also downregulates Foxa2 expression [16••]. Nuclear factor-κB (NF-κB) consensus-binding sites have been identified in the human MUC5AC promoter [32], but these are not conserved in other mammalian genomes, and animal models do not support an important role for NF-kB activation in Muc5ac transcription and mucous metaplasia [9••,23,33,34•].

A required permissive role for β_2-adrenoceptor signaling in mucous metaplasia has recently been identified. Inverse agonists that reduce β_2-adrenoceptor signaling to less than its

unliganded level markedly reduce intracellular mucin accumulation and Muc5ac gene expression in a mouse model of allergic mucous metaplasia [11•]. This is due to the loss of physiological β_2-adrenoceptor signaling rather than the activation of a novel signaling pathway by these artificial ligands (biased agonism) as the β_2-adrenoceptor knockout mouse phenocopies the exposure to inverse agonists (Richard A. Bond, personal communication). Increased β_2-adrenoceptor signaling from agonist stimulation did not augment mucin accumulation in this model [11•], although it did (two-fold) in another model [35]. Together, these results suggest that the high density of β_2-adrenoceptors in airway epithelium provides sufficient signaling from empty receptors or receptors bound by endogenous ligands to fully support mucous metaplasia induced by strong inflammatory stimuli, but that exogenous β-agonists might augment mucous metaplasia induced by weak stimuli. Whether signaling through a G-protein, β-arrestin, or some other pathway mediates this effect is not yet known.

Control of polymeric mucin secretion

Mucin secretion is controlled separately from mucin production, with triphosphate nucleotides such as ATP and UTP playing central roles in the regulation of secretion [10,13•,36•]. Extracellular nucleotides also play important roles in the regulation of airway surface liquid depth and ciliary beat frequency, suggesting that multiple aspects of mucociliary clearance are coordinately regulated by nucleotides released into the airway surface liquid layer [36•]. Other ligands such as cholinergic agonists can also induce polymeric mucin secretion *in vivo* [14•, 15], but whether this is indirect through the release of extracellular nucleotides induced by smooth muscle contraction is not yet known. Extracellular nucleotides bind to $P2Y_2$ receptors on the apical surface of airway secretory cells to generate intracellular second messengers that activate the exocytic machinery, leading to the rapid release of polymeric mucins into the airway lumen (Fig. 3aFig. 3 [12•,13•,37•,38,39•,40–42]).

Analysis of the phenotypes of mice with null mutations in exocytic proteins has been highly informative of the molecular composition of the secretory machinery and its mechanism of function [13•]. Munc13-2 null mice accumulate mucin in the absence of inflammation or increased mucin production, suggesting that baseline mucin secretion is mediated by a low baseline rate of activity of a regulated exocytic machine as Munc13 proteins function exclusively in regulated and not in constitutive exocytosis [12••]. In addition, these mice are defective in stimulated secretion, supporting the notion that a single machine mediates both baseline and stimulated secretion (Fig. 3b). The profound defect in stimulated mucin secretion in Syt-2 null mice but the absence of mucin accumulation under baseline conditions [13•] can be explained by loss of the positive role of Syt-2 at high cytoplasmic Ca^{2+} concentrations and loss of its inhibitory role at low cytoplasmic Ca^{2+} concentrations [43]. Together, these results lead to a parsimonious model in which a single population of mucin granules is released at a low tonic rate under baseline conditions and a high phasic rate upon exocytic stimulation (Fig. 3). Additional analyses in mutant mice will further test this model.

Additional mucin secretory proteins have been identified and studied functionally. The myristoylated alanine-rich C-kinase substrate (MARCKS) protein plays roles in positioning secretory granules near the plasma membrane and in remodeling the cortical actin cytoskeleton to allow membrane apposition [13•]. A lipidated peptide that enters cells and interferes with MARCKS function markedly reduced mucin secretion in cultured human airway cells and in mice in a model of allergic mucous metaplasia [15]. This allows pharmacologic manipulation of mucin secretion in pathophysiologic models (see below) and is being developed as a therapeutic [44]. In addition, the cysteine string protein [45] and Munc18b (Kyubo Kim and Michael J. Tuvim, personal communication) regulate mucin secretion.

Mucus hypersecretion in airflow obstruction and airway hyperresponsiveness

Autopsy studies in Germany in the 1880s first identified widespread airway mucus plugging as a central cause of death from asthma [46]. These findings have been repeatedly confirmed [3], and a recent quantitative study of fatal asthma found more than 98% of airways occluded to some extent by mucus [47]. However, the contribution of mucus hypersecretion to airflow obstruction in nonfatal asthma relative to other contributors to airway closure such as extravasated plasma and to airway narrowing from smooth muscle contraction and wall thickening remains to be determined [48,49]. The increase in baseline airway and lung tissue resistance in Foxa2 knockout mice with spontaneous Muc5ac overproduction supports a substantial role for mucus [25,25].

Similarly, the role of mucus hypersecretion in airway hyperresponsiveness relative to closure from other causes and airway narrowing remains to be fully defined. Although it might seem that lumenal occlusion by secreted mucins would be offset by an equal reduction in airway epithelial cell volume with no net change in the aerated lumen cross-sectional area, this is not the case as mucins swell approximately 500-fold after secretion because of hydration [50]. Pharmacological inhibition of mucin secretion with a MARCKS peptide blocked methacholine-induced airway resistance increases by approximately 80% in a mouse model of allergic asthma [14•], suggesting a major contribution by mucus hypersecretion at least under some circumstances. Future studies examining animals deficient in mucin production and secretion and humans treated with inhibitors of mucus hypersecretion will further clarify the role of mucus in baseline airflow obstruction and in airway hyperresponsiveness.

An evolutionary perspective on mucus hypersecretion

The resemblance between allergic asthma and parasitic worm (helminth) infestation of the lungs has been frequently noted. Symptoms of cough and wheezing, airway infiltration by immune cells such as eosinophils and type 2 helper T (Th2) lymphocytes, and elevated Th2 cytokines are prominent in both disorders. This suggests that asthma is an atavistic response to a misperceived pulmonary worm infestation. Insight into how the misperception might occur has been provided by the recent identification of chitin, which is found in helminths and common triggers of asthma such as dust mites and fungi, as a powerful innate stimulus of type 2 inflammation [51,52••]. Similarly, secreted proteases are abundant in helminths as well as in fungi, the feces of dust mites, and the saliva of cats and may serve as another innate stimulus of type 2 inflammation [53••,54••]. Residence of dust mites in homes, domestication of cats, and other factors that increase exposure to type 2 stimuli, in concert with decreased exposure to type 1 and type 17 stimuli (the ‘hygiene hypothesis’), may explain the rise in asthma prevalence in modern societies.

Of what value might mucin hypersecretion be in defense against helminths? Because increased production of MUC5AC can be induced within minutes, and mucin secretion can be induced within seconds, mucin hypersecretion together with airway smooth muscle contraction might trap worms in distal airways and allow their killing before they can ascend to the pharynx to be swallowed to complete their lifecycle in the gastrointestinal tract. Although closure of hundreds of airways from local stimulation by transiting worms does not result in serious pathophysiology, closure of millions of airways from diffuse stimulation by an airborne allergen in asthma can have serious consequences. Such derangement of an adaptive physiologic mechanism is similar to the useful localized vasoconstriction of poorly ventilated segments of lung to maintain ventilation—perfusion matching in pneumonia, but the pathophysiological diffuse vasoconstriction that leads to pulmonary hypertension upon ascent to altitude. Whether airway mucin secretion contributes significantly to defense against worm

infestation is a testable hypothesis using mutant mice defective in mucin production and secretion.

Conclusion

The role of mucin hypersecretion in death from asthma has long been recognized, but its contributions to symptoms, signs, and pathophysiology in less severe asthma are subjects of ongoing investigation. MUC5B is the principal gel-forming mucin produced and secreted in small airways under healthy conditions, and MUC5AC is the principal gel-forming mucin upregulated during asthmatic inflammation. Because MUC5AC may serve little or no important function in healthy airways, it may be possible to therapeutically suppress MUC5AC production in the airways with minimal toxicity. Strategies to suppress mucin secretion are being developed and might be helpful in acute, severe asthma.

Acknowledgments

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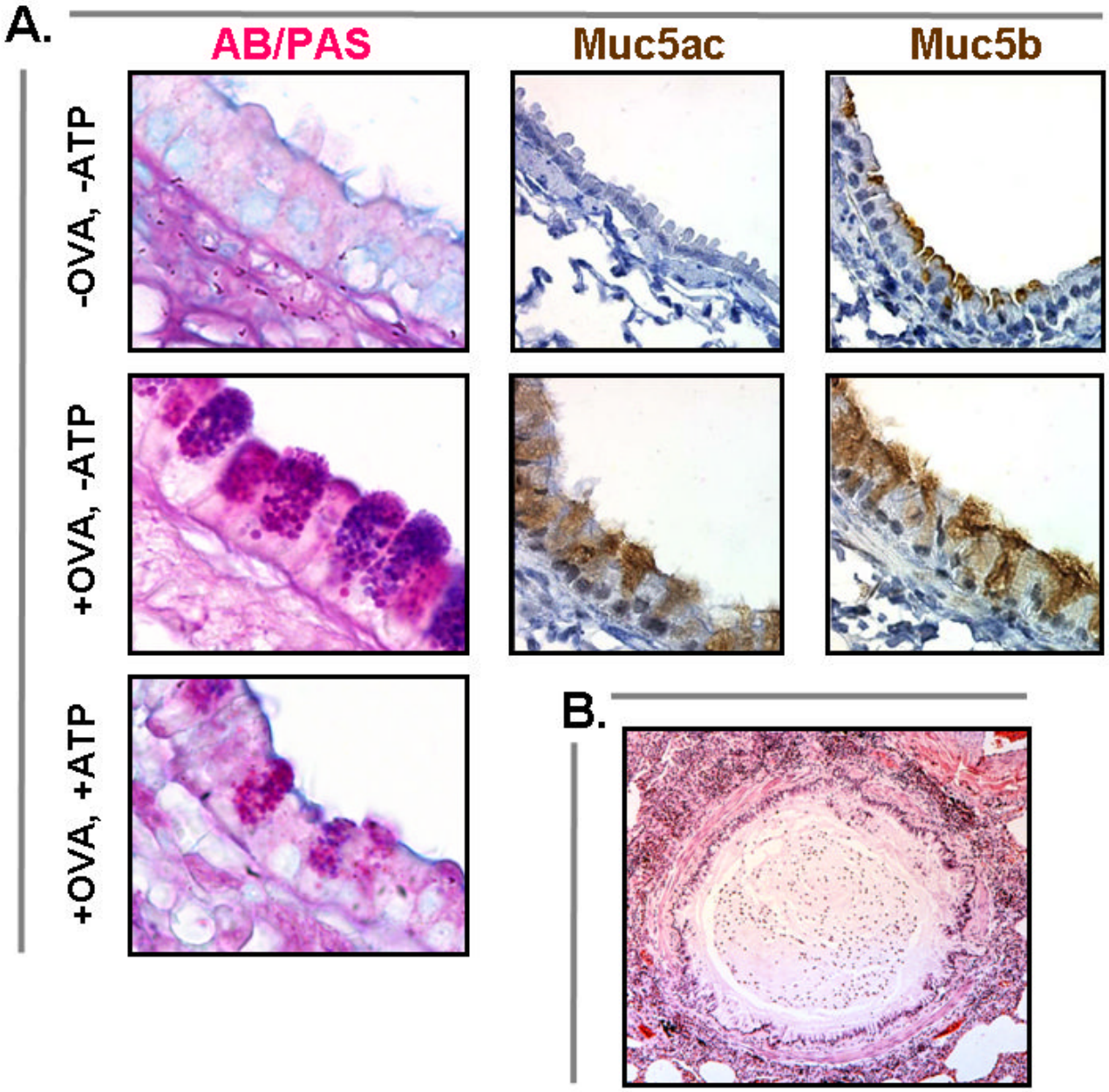

Figure 1.

Mucus hypersecretion gr1

(a) In ovalbumin-sensitized and challenged mice (+OVA, -ATP), there is a dramatic increase in the number of AB—PAS-positive mucous cells in the tracheobronchial airways (d). This increase is apparent 24h after challenge and peaks at 3–7 days. Despite the lack of AB—PAS staining at baseline (a), intracellular Muc5b (c) can be demonstrated by sensitive immunohistochemical techniques at the cell apex (-OVA, -ATP). It becomes redistributed throughout the distended cytoplasm (f) during mucous metaplasia (+OVA, -ATP). Muc5ac is not apparent immunohistochemically at baseline (b) but is strongly expressed (f) during mucous metaplasia (+OVA, -ATP). After stimulation of metaplastic mucous cells with aerosolized ATP, there is rapid secretion of most of the accumulated intracellular mucin

(+OVA, +ATP) [10,11•]. (b) Airway from a patient who died from asthma showing extensive infiltration of the airway wall and surrounding lung tissue with inflammatory cells and mucus filling the airway lumen. AB—PAS, alcian blue and/or periodic acid Shiff; OVA, ovalbumin. Reproduced with permission from [10,11•] and by courtesy of Martha Warnock, University of California at San Francisco.

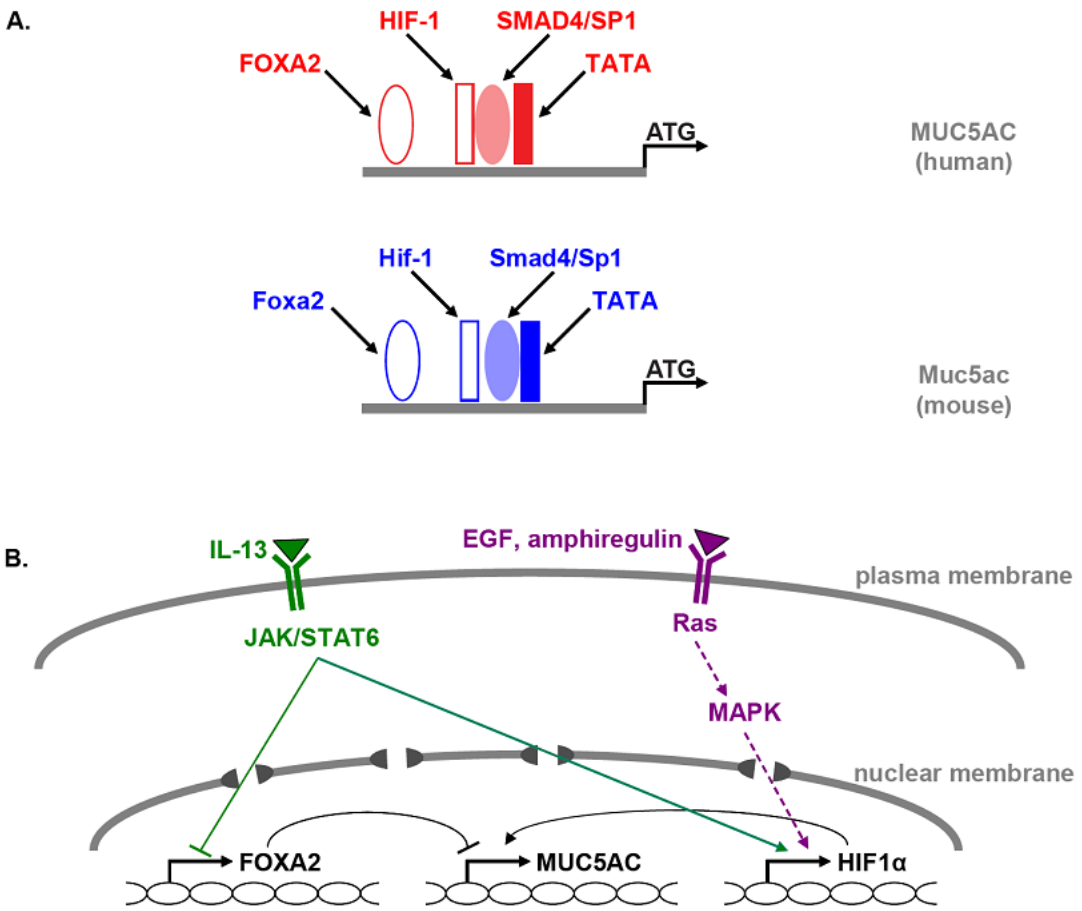

Figure 2.

Transcriptional control of Muc5ac production gr2

(a) Conserved consensus-binding sites for transcription factors in the core promoters of the human MUC5AC (red) and the mouse Muc5ac (blue) genes are indicated. (b) Known pathways for activation of MUC5AC/Muc5ac gene transcription by IL-13 (green) and EGFR ligands (violet) are illustrated. Solid lines indicate direct protein interaction with target gene (i.e., STAT6), dotted lines indicate multiple steps of interaction (i.e., EGFR pathway), arrowheads indicate positive interaction, and bars indicate inhibitory interactions (see text for citations). EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; Foxa2, forkhead box a2; HIF, hypoxia inducible factor; JAK, janus-activated kinase; MAPK, mitogen-activated protein kinase; STAT, signal transducer and activator of transcription.

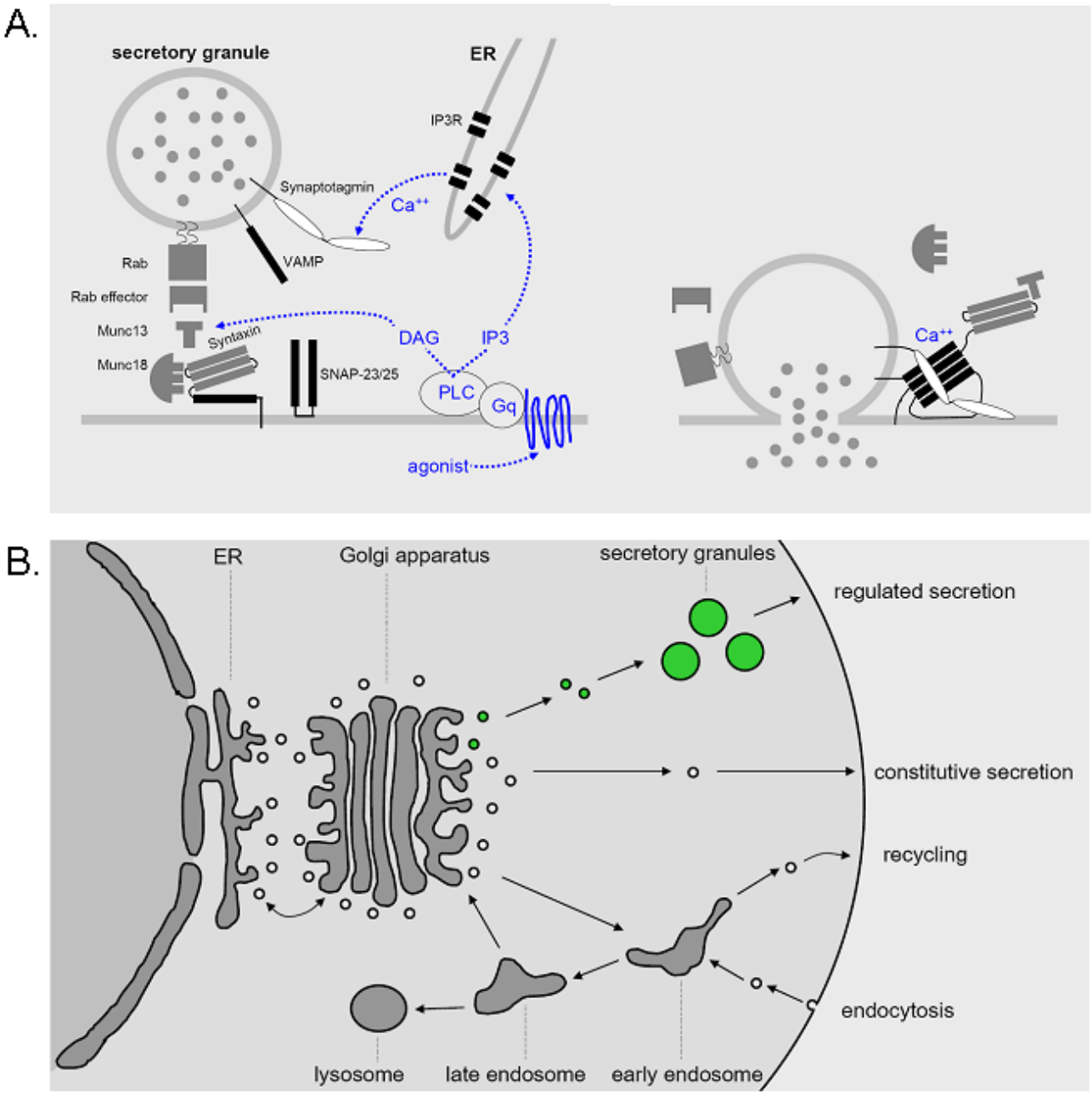

Figure 3.

Mechanism of polymeric mucin secretion gr3

(a) Extracellular nucleotides in the airway surface liquid layer bind to apical $P2Y_2$ receptors that activate the trimeric G-protein Gq, which in turn activates phospholipase C β_1, generating the intracellular second messengers, diacylglycerol and inositol trisphosphate (IP_3) [13•]. Diacylglycerol directly induces mucin granule exocytosis by activating the priming protein, Munc13-2 [12••], and indirectly regulates exocytosis by activating protein kinase Cε [37•]. IP_3 induces the release of Ca^{2+} from intracellular stores [38,39•,40,41], resulting in a rise in cytoplasmic Ca^{2+} that rapidly triggers mucin granule exocytosis through the activation of synaptotagmin-2. In airway secretory cells, IP_3 receptors are localized to endoplasmic

reticulum that lies in close apposition to mucin granules at the apical pole (C. William Davis, personal communication). The reliance of airway secretory cells on intracellular Ca^{2+} stores to activate exocytosis may reflect the instability of Ca^{2+} concentrations in airway lining fluid that is directly exposed to the external environment [42], in contrast to nonexocrine secretory cells bathed in interstitial fluid or plasma with tightly controlled Ca^{2+} concentrations. Activation of Munc13 and synaptotagmin allows formation of a four-helix bundle termed the core complex (black rectangles) that draws secretory granule and plasma membranes tightly together and induces their fusion (right). This leads to the release of granule contents, including polymeric mucins, into the airway lumen. The molecular identities of the core complex isoforms in airway secretory cells are not yet known. (b) Data from mouse genetic models suggest that both baseline and stimulated mucin secretion occur through varying rates of activity of a single regulated exocytic machinery acting on a single population of mucin secretory granules (green). DAG, diacylglycerol; ER, endoplasmic reticulum; IP_3R, inositol trisphosphate receptor; PLC, phospholipase C; VAMP, vesicle-associated membrane protein.