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Role of mucins in lung homeostasis: regulated expression and biosynthesis in health and disease

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Abstract

In humans and mice, the first line of innate defense against inhaled pathogens and particles in the respiratory tract is airway mucus. The primary solid components of the mucus layer are the mucins MUC5AC and MUC5B, polymeric glycoproteins whose changes in abundance and structure can dramatically affect airway defense. Accordingly, *MUC5AC/Muc5ac* and *MUC5B/Muc5b* are tightly regulated at a transcriptional level by tissue-specific transcription factors in homeostasis and in response to injurious and inflammatory triggers. In addition to modulated levels of mucin gene transcription, translational and post-translational biosynthetic processes also exert significant influence upon mucin function. Mucins are massive macromolecules with numerous functional domains that contribute to their structural composition and biophysical properties. Single MUC5AC and MUC5B apoproteins have molecular masses of >400 kDa, and von Willebrand factor D-like as well as other cysteine-rich domain segments contribute to mucin polymerization and flexibility, thus increasing apoprotein length and complexity. Additional domains serve as sites for O-glycosylation, which increase further mucin mass several-fold. Glycosylation is a defining process for mucins that is specific with respect to additions of glycans to mucin apoprotein backbones, and glycan additions influence the physical properties of the mucins via structural modifications as well as charge interactions. Ultimately, through their tight regulation and complex assembly, airway mucins follow the biological rule of ‘form fits function’ in that their structural organization influences their role in lung homeostatic mechanisms.

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Author Contribution

B.A.S., A.L.S., C.M.M., and C.M.E. wrote the manuscript. B.A.S. and C.M.E. oversaw editorial management and submission of the manuscript.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

Mucus: an essential first line of defense

Each day, respiratory tissues are exposed to billions of particles. Because inhaled particles include potentially infectious, injurious, and thus inflammatory stimuli, homeostatic mechanisms have evolved to reduce the inherent risks associated with their exposures in the lungs. The initial sites of particle exposure, the conducting airways, are lined by a barrier that constitutes the first line of innate defense. Defense is conferred by a mucus gel that traps particles and a ciliary escalator that transports mucus contents towards the oropharynx for ultimate elimination by expectoration or swallowing. This process is called mucociliary clearance (MCC) [1–3]. For MCC-mediated innate defense, two cell types are essential: ciliated cells and mucous cells [4–6].

In this review, we focus on mucous cells, and specifically, the mucin glycoproteins they produce, as critical mediators of the first line of innate defense. We use the term mucous cells to broadly define two mucin-producing cell types found in tracheobronchial submucosal glands (SMGs) and on airway surfaces. SMG mucous cells are interspersed among heterogeneous ciliated and secretory cell populations within glands and gland ducts in the large airways (trachea and bronchi) of humans and large mammals such as swine [7], but in mice these cells are present only in the trachea [8]. Surface mucous cells, often called goblet cells based on their chalice-shaped morphologies, are present on conducting airway surfaces, predominantly within the central tracheal and bronchial airways. Surface mucous cells are also present in small bronchiolar airways, but mucous cell numbers are lower in these peripheral air-spaces. Both mucous cell types produce secreted polymeric mucin glycoproteins that are released by regulated exocytosis and then become hydrated in the airway lumen to form the macromolecular matrix of the mucus gel [9–11].

Mucins are extremely large, heavily-glycosylated proteins with molecular masses ranging from 10 to 40 MDa. Over 20 different mucin genes are expressed in humans, each in a tissue-specific manner [12]. The lungs contain the membrane-bound mucins MUC1, MUC4, and MUC16, secreted mucin MUC7, and also the secreted polymeric mucins MUC5AC and MUC5B [13,14]. MUC5AC and MUC5B play dominant roles in the formation of viscoelastic mucus gels [15,16].

Mucin and mucus functions

Mucus is a complex mixture of salts, macromolecules, cells, and cellular debris that are contained in a hydrogel formed by polymeric mucins and water [17–21]. Mucus serves two important homeostatic functions in the lungs: innate airway defense and airway surface hydration. Mucus is critical for protection from inhaled particles and pathogens, as well as for the clearance of recruited leukocytes, dead cells, and endogenous debris [5]. Mucus also plays an important role in the maintenance of airway surface liquid equilibrium. In conjunction with the regulated transport of ions and water across membranes, mucus facilitates hydration of the underlying epithelium by modulating airway surface liquid osmotic gradients, thereby maintaining epithelial tonicity and responses to mechanical stress [22,23]. Hydration of mucus is also essential for maintaining the stability and function of cilia [24]. Accordingly, mucus dysfunction can result in, or worsen, disease states [2–4,25–

28]. Maintaining healthy and effective mucociliary function thus requires tight regulation of the mucus gel.

Mucins are components of mucus whose regulated production and secretion are strictly controlled. Their co-ordinated regulation is tissue- and cell-specific, resulting in mucus gels whose physical properties are tailored to their tissue environment. Healthy airway mucus is comprised mainly of water (>95%), and mucins constitute over 80% of the mass of solid materials within the gel. Increasing the mass of mucins in mucus can have dramatic effects on gel transport. Accordingly, MUC5AC and MUC5B are tightly regulated at the levels of expression, polymerization, and glycosylation [29–31].

Transcriptional regulation of mucin expression

MUC5AC/*Muc5ac* and *MUC5B*/*Muc5b* (per standard rules of nomenclature, all letters are capitalized for the human gene; first letter capitalized with all following letters lowercase for mouse gene) are located on chromosome 11p15.5 in humans and 7F5 in mice [32–34]. Under normal conditions in human lungs, the *MUC5AC* gene is expressed in central airway tracheobronchial surface epithelial goblet cells at low levels, whereas *MUC5B* is primarily expressed in SMGs found throughout the central airways. *MUC5B* is also expressed in goblet cells in more peripheral bronchiolar airways, where glands are absent [35–39]. In mice, *Muc5ac* is hardly expressed at baseline, and *Muc5b* gene expression predominates within surface secretory cells [13,14,40,41]. Since mice lack submucosal glands in intrapulmonary airways, the patterns of gene expression for mouse airway mucins appear to resemble human bronchioles. *MUC5AC*/*Muc5ac* expression increases dramatically in lung diseases and in mouse models of lung disease. On the other hand, *MUC5B*/*Muc5b* expression remains relatively stable, or in some cases, its expression actually decreases [14,42–44].

With these patterns of gene expression, it is generally accepted that the two mucins serve separate functions: MUC5B is employed for MCC in regular maintenance of a healthy respiratory tract, and MUC5AC is primarily produced in response to acute respiratory inflammation for reasons that are not entirely clear. The consensus favoring a homeostatic role for *MUC5B*/*Muc5b* expression at baseline stems from findings that it is essential in the mouse respiratory tract to facilitate MCC and thus prevent infection [45,46]. On the other hand, induced expression of *MUC5AC*/*Muc5ac* in inflamed and diseased lungs is considered largely to be a detrimental factor, since it has been found to be overproduced in respiratory diseases such as asthma, where mucus obstruction plays an important role in pathophysiology [47]. To achieve these baseline and induced levels, mucin expression is broadly regulated by a network of transcription factors that are involved in secretory differentiation and inflammation signaling.

Mucin expression at baseline

In healthy lungs, baseline *MUC5B*/*Muc5b* gene expression is regulated by transcriptional mechanisms that are still poorly understood. The high levels of *MUC5B*/*Muc5b* transcripts expressed at baseline are linked to several gene regulatory programs that are tied to lung development and secretory epithelial cell lineage specification. Histological experiments and

western blots demonstrate the prevalence of Muc5b in airway epithelium even in the absence of allergic response [41,48,49]. During embryonic lung development, the transcription factor Nkx2-1 represses *Muc5b* gene expression [50–53], whereas GATA-6 activates *Muc5b* expression [53,54]. Forkhead box a2 (Foxa2) is required for normal differentiation of the airway epithelium, and it also suppresses *Muc5ac* transcription, whereas selective deletion of *Foxa2* results in increased Muc5ac mRNA and immunohistochemically detectable protein [55,56], suggesting that this key developmental transcription factor plays an important role in maintaing low levels of *Muc5ac* expression relative to abundant *Muc5b* transcripts.

Recently, it was revealed that the single-nucleotide polymorphism (SNP) rs35705950 in the promoter region of the human *MUC5B* gene strongly regulates its expression [39,57,58]. The presence of the rs35705950 minor allele enhances transcription of *MUC5B*, increasing mucin mRNA production, especially in the distal airways where SNP genotype correlates with MUC5B protein levels in the bronchioles *in vivo* and with promoter activity *in vitro* [3,39]. Importantly, rs35705950 strongly affects expression at baseline, with healthy subjects carrying either one or two copies of the minor allele expressing ~40-fold higher levels of MUC5B than major allele homozygotes [58]. The rs35705950 SNP is part of an active enhancer containing a FOXA2-binding site, suggesting that repression of *MUC5B* transcription by FOXA2 is lost when the gain-of-function SNP site is in an inactive state that was recently shown to potentially involve epigenetic control via methylation [59]. Since rs35705950 does regulate *MUC5B* expression under homeostatic conditions, it is important to note that the very high levels of expression imparted by rs35705950 may be detrimental in the long term. Chronic overexpression of *MUC5B* driven by rs35705950 is the single greatest risk factor for the development of idiopathic pulmonary fibrosis [60]. Paradoxically, though rs35705950 is a risk factor for developing the disease, patients who carry the minor allele variant actually live longer, on average, than their major allele-carrying counterparts [61]. Taken together, these data highlight the complexities underlying the regulation of the *MUC5B* gene and the long-term effects of *MUC5B* expression.

Mucin overexpression in inflammation and disease

Selective induction of *MUC5AC* gene expression is characteristic of numerous respiratory and inflammatory disease states including asthma, cystic fibrosis, and chronic obstructive pulmonary disease, as well as transient infectious or injurious responses [14,16,49,62,63]. Accordingly, a large number of cytokine and growth factor signaling pathways have been shown to drive MUC5AC expression. In innate inflammation, tumor necrosis factor (TNF) activates mitogen-activated protein kinases ERK and P38, which in turn activate cyclic AMP-responsive element-binding protein (CREB) downstream. CREB binds to a *cis* region of the *MUC5AC* promoter, resulting in increased transcriptional activation [64,65]. Epidermal growth factor receptor (EGFR) signaling has also been shown to drive *MUC5AC* expression. EGFR-mediated *MUC5AC* transcriptional regulation may utilize some of the same signal transduction pathways implicated above for TNF [66–68].

EGFR signaling in mouse lung epithelial cells up-regulates *Muc5ac* via hypoxia inducible factor-1 (HIF-1)-binding site, which is conserved in mammalian *MUC5AC* orthologs and is present in the core promoter [14]. This effect of EGFR was also observed with the

type 2 inflammatory cytokine IL-13 [42]. IL-13 and EGFR are drivers of mucin expression in classic type 2 inflammation settings such as allergy and asthma, and it has also been implicated in MUC5AC/Muc5ac responses to some viral infections [42,69–73]. Like EGFR, IL-13 also induces HIF-1 binding and *Muc5ac* promoter activation [14]. In addition to HIF-1, IL-13 has also been shown to induce *Muc5ac* expression via up-regulation of SAM-pointed domain-containing ETS transcription factor, which in turn inhibits Foxa2-mediated *Muc5ac* repression during inflammation [56,74]. This loss of repressor function, along with the induction of activating transcription factors, has been proposed as a mechanism for tightly controlling *Muc5ac* expression levels and localization [75,76].

Lastly, some core promoter elements include sites for specificity protein 1 (Sp1) and nuclear factor κ-B (NFκB), which are present in both the *MUC5AC* and *MUC5B* genes [77–81]. The extents to which these regulate baseline versus inducible expression are still being deciphered. Ultimately, while mucin expression levels are tightly regulated by transcription, the functions of those expressed are also significantly affected by protein biosynthesis and post-translational modifications.

Form fits function: airway mucin function depends on protein structure and formation

Structurally, mucins are huge macromolecules with apoprotein lengths ranging from 3000 to >5000 amino acids. MUC5AC and MUC5B monomers are ~500 nm long, and they form homopolymeric chains of up to 100s of μm in length [82,83]. Given the size of these sequences, it is no surprise that each mucin contains multiple functional domains that contribute to their structure and their function (Figure 1). The primary amino acid sequences of MUC5AC and MUC5B contain multiple cysteine-rich von Willebrand factor D (vWD)-like domains at their N-terminal and C-terminal regions. These vWD domains mediate mucin polymerization via disulfide bonding (described below) and thus contribute to the overall assembly and ultimate size of mature mucins [34,84–86]. In addition, MUC5AC and MUC5B have internal glycosylated domains, separated by short, cysteine-rich domain (CysD) segments, which demonstrate high intra- and interspecies conservation in structure, albeit with variation in their frequencies. This structural homology within CysDs suggests that those segments contribute to mucus gel formation [84,87,88]. This is supported by a recent functional study, showing that increasing the number of CysD residues in intestinal mucins increases the strength of the intestinal mucus layer and reduces bacterial infection in mice [89]. The glycosylated domains of mucins are rich in proline, threonine, and serine residues, and are thus referred to as ‘PTS’ domains. The frequencies and specific sequences of PTS domains vary across animal species, among mucins isoforms, and even between alleles of individual mucin genes, affording unique structural and functional modifications for specialization purposes [90–95].

vWD domains

In one sense, mucins follow the classic model for protein assembly from primary through quaternary structuring (Figure 2). MUC5AC and MUC5B form three-dimensional conformations through intramolecular interactions at their globular ends, solidified with

tertiary interactions between (and within) the molecular functional domains. These mucins also utilize disulfide intra- and intermolecular linkages to conduct quaternary protein assembly that results in complex homo-oligomers that begin with dimers attached via the cysteine-rich segments.

Cysteine-mediated polymerization is essential for mucus viscoelasticity. These segments are stabilized by C-terminal-to-C-terminal disulfide linkages formed inside the endoplasmic reticulum (ER) via protein disulfide isomerase (PDI) enzymes [96]. Anterior gradient homolog (AGR)2, a disulfide isomerase-like protein, is essential for production of MUC2 in intestines, as well as MUC5AC and MUC5B overproduction in an asthma model [97,98]. Originally suggested as being involved in disulfide-mediated assembly, the role of AGR2 is now less clear [99]. AGR2 can be induced by XBP-1, a transcription factor, which in turn is activated by inositol-requiring enzyme (IRE)1β. IRE1β is specifically expressed in mouse and human respiratory mucosal cells and is required for mucin production [100].

The dimers formed in the ER are further connected via N-terminal-to-N-terminal linkage within the Golgi, thus establishing the mucin polymer in a manner similar to von Willebrand factor assembly [85,101–103]. It was recently observed that in the acidic, calcium-rich conditions of the Golgi, these disulfide bridges pull the D3 domains of MUC5B in close proximity to cross-link (non-covalently) into tetrad-like structures, which link in turn to form dense linear constructs for condensation of MUC5B in secretory granules [103,104]. Thus, MUC5AC and MUC5B proteins, which are already significant in size upon translation, become even larger as the result of disulfide-mediated polymerization in the ER and Golgi. In addition to this process of polymeric assembly, within the Golgi, MUC5AC and MUC5B become even more massive through glycosylation.

PTS domains

Glycosylation is the ultimate determining factor of mucin function, as these huge molecules are actually comprised 50–90% of carbohydrates [87]. Ultimately, glycosylation increases mucin size 3–5-fold [3,34], and glycans can dramatically affect the functions of mucins within the mucus gels that they form [12,105,106]. The composition of this dense mass of protein is highly variable between each mucin type, and because of its structural influences, greatly affects the function of individual mucins. The PTS tandem repeats in MUC5AC and MUC5B serve as the sites for this O-glycosylation, accommodating the addition of hundreds of *O*-glycans to each molecule [87].

Glycosylation

Inside the Golgi, O-glycosylation is initiated by the transfer of *N*-acetylgalactosamine (GalNAc) from UDP-GalNAc to serine and threonine amino acids within the mucin PTS domains by a polypeptide GalNAc-transferase (GALNT). In humans, 20 distinct GALNT family members have been identified with some GALNTs expressed ubiquitously, and others with restricted expression across tissue types. The addition of *O*-GalNAc to a serine/threonine amino acid in a PTS domain is affected by both the sequence of amino acids immediately flanking (−3 to +3) and longer-range interaction with neighboring glycosylated residues (6–17 residues away) [107,108]. This addition of GalNAc to the apomucin results

in the formation of a ‘Tn’ antigen that functions as a scaffold for the further elaboration of mucin glycans [109]. Neutral sugar(s) can then be attached to *O*-GalNAc, which allows for the formation of the *O*-glycan ‘cores’ [12,110–112]. Core 1 structures are formed by the addition of galactose (Gal) β1–3 onto GalNAc-*O*-Ser/Thr, which is catalyzed by the glycosyltransferase C1GalT1 and which also requires the chaperone C1CALT1C1/Cosmc. The core 2 structure is formed by the addition of a second neutral sugar, *N*-acetylglucosamine (GlcNAc), β1–6 to the core 1 structure by core 2 synthases GCNT1/3. Core 3 structures are formed by the addition of a single GlcNAc in a β1–3 linkage to the GalNAc-*O*-Ser/Thr catalyzed by B3GNT6. Lastly, core 4 structures are formed by the further branching of a core 3 *O*-glycan via the addition of a second GlcNAc-linked β1–6 to the GalNAc-*O*-Ser/Thr catalyzed is GCNT3 [12,87,109]. These cores ultimately form a central glycosylation backbone off of which the rest of the glycan complexes are constructed.

After core glycosylation, the further addition of Gals and GlcNAcs proceeds to lengthen core 1- and 3-anchored strands, and core 2 and 4 bi-antennary structures. In addition, cores 2 and 4 can become further branched leading to tri-antennary and tetra-antennary structures on mucins [113]. These chains are extended with highly variable combinations of glycans. Additionally, glycan chains may be modified by the addition of fucose by fucosyltransferases including FUT2 [109,114]. Ultimately, these elaborated structures terminate in either Gal or GlcNAc to form ‘uncapped’ glycan structures, or they can be ‘capped’ by sulfate (SO_4) or sialic acid (NeuNac). In general, the addition of terminal sialic acid prevents further elaboration of the glycan chain [109,115–117]. The heterogeneity of glycan structures is what endows each mucin with unique physical properties. Sialic acid and sulfates, for example, confer negative charges to molecules, which introduce steric repulsion between glycosylated side chains that, in turn, confer rigidity to mucins [12,105,106,118]. In contrast, fucose can confer more neutrally charged attributes [12,87,105,106,119]. These can contribute to the viscous gel properties of mucins by regulating how many water molecules (or other charged ions) can interact with them.

CysD domains

Beyond glycosylation of the PTS domains, the CysD domains of mucins serve as sites that affect physical properties conferred by glycans. It has been suggested that the interruption of PTS domains in MUC5AC and MUC5B by CysD domains may confer flexibility within the molecules to allow for intermolecular interactions [87]. In addition, CysD’s also potentially serve as mannosylation sites, where a single α-mannose residue can be covalently added to the second carbon of tryptophan in WxxW motifs within CysDs. MUC5AC and MUC5B contain nine and seven potential mannosylation sites, respectively [12,88,120]. CysD mannosylation may be essential to the maturation of secreted mucins, as lack of mannosylation in MUC5AC and MUC5B arrests these proteins in the ER in a cell culture model of mucin synthesis [121]. While CysD mannosylation function is incompletely understood, it is thought to be related to intracellular trafficking, subcellular localization, and protein folding.

Transglutamination

One further potential post-translational modification to mucins is cross-linking via transglutamination. Transglutaminase 2 has been shown to mediate cross-linking of MUC2 via the second CysD domain as part of mucus formation [122,123]. Inhibition of this enzyme in a mouse model of asthma leads to decreased airway hyperresponsiveness, decreased number of inflammatory and goblet cells, and decreased expression of TNF, NFκB, and *Muc5ac* transcripts, indicating that transglutamination plays a key role in asthma-induced inflammation and mucin production [124]. In a somewhat conflicting study, overexpression of transglutaminase 1 in a mouse model of dry eye led to a decrease in goblet cells and Muc5ac [125]. Thus, there is still much to learn about transglutamination in mucin production and formation. It is possible that different transglutaminases serve unique functions in mucin regulation and structure, depending upon which tissues they are expressed in.

Summary and implications

For all their size and complexity, the synthesis of polymeric mucins is remarkably efficient. Polymeric mucins are translated and dimers are assembled within the endoplasmic reticulum in ~20 min [101,102]. Processing through the Golgi takes 90–120 min to yield a fully constructed mucin glycopolymer [102]. Upon completion, the mucins bud from the trans-Golgi and undergo homotypic fusion to form large secretory granules (SGs) that are calcium-rich vesicles and have low pHs [12,126]. Large SG's mature can be stored for long periods of time, awaiting a secretory stimulus that mobilizes SGs towards apical plasma membrane docking sites where they fuse and release their contents via regulated exocytosis [40,127,128]. There is also emerging evidence that some mucins are secreted via small vesicles in a tonic fashion to maintain steady-state levels of secreted mucins in the mucus gel [41,129].

The formation of mucin glycopolymers is a complex and highly regulated process. Disulfide bonding and glycosylation are heterogeneous between different mucin types, tissues, and disease states versus homeostasis [12,130–132]. In healthy states, the production of hydrated and easily transported mucus is critical for maintaining airway homeostasis and MCC. In diseases such as asthma, the production of tenacious mucus plugs is clearly detrimental in the context of contemporary clinical settings. Emerging techniques have been utilized to produce glycopeptide polymers through direct polymerization of glycosylated monomers or post-polymerization glycosylation of reactive polypeptides [133]. These new synthetic routes not only begin to mimic the assembly process described on the cellular level, but also lead to polymers that recapitulate the structure and function of natural glycoproteins [134,135]. Delivery of glycopeptide polymers has immense potential to alleviate the effects of changes in natural mucus viscosity caused by disease or inflammation and help to return airways to homeostasis.

However, mucus overproduction in disease states may also reflect an evolutionarily guided mechanism for controlling the spread and transport of bacteria, viruses, and nematodes [136–140]. Recent data have demonstrated novel mechanisms through which mucins steer both innate and adaptive immune responses across many phases: mediating antigen

presentation during early phases, driving inflammosuppression during persistent exposures during chronic phases, and inducing apoptosis of potentially toxic immune effector cells during late phases [113,141–144]. As data emerge in the field, it has become clear that mucus is more than just a sticky substance. Rather, it is a crucial component of an integrated system that is designed to maintain health and to do so in by employing diverse ways to promote health while limiting the detrimental effects of foreign and host-derived challenges.

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Abbreviations

AGR2	anterior gradient homolog
CGNT	core glycosyltransferase
CREB	cAMP response element-binding protein
CysD	cysteine-rich domain
EGFR	epidermal growth factor receptor
ER	endoplasmic reticulum
Foxa2	forkhead box a2
Gal	galactose
GalNAc	*N*-acetylgalactosamine
GALNT	GalNAc-transferase
GalT	βGal transferase
GlcNAc	*N*-acetylglucosamine
GlcNAcT	GlcNAc transferase
HIF-1	hypoxia inducible factor-1
IRE1β	inositol-requiring enzyme (IRE)1β
kDa	kilodalton
MCC	mucociliary clearance
NeuNAc	sialic acid

NFκB	nuclear factor κ-B
PDI	protein disulfide isomerase
PTS	proline-, serine-, threonine-rich sequence
SG	secretory granule
SMG	submucosal gland
SNP	single-nucleotide polymorphism
SO_4	sulfate
TNF	tumor necrosis factor
vWD	von Willebrand factor D

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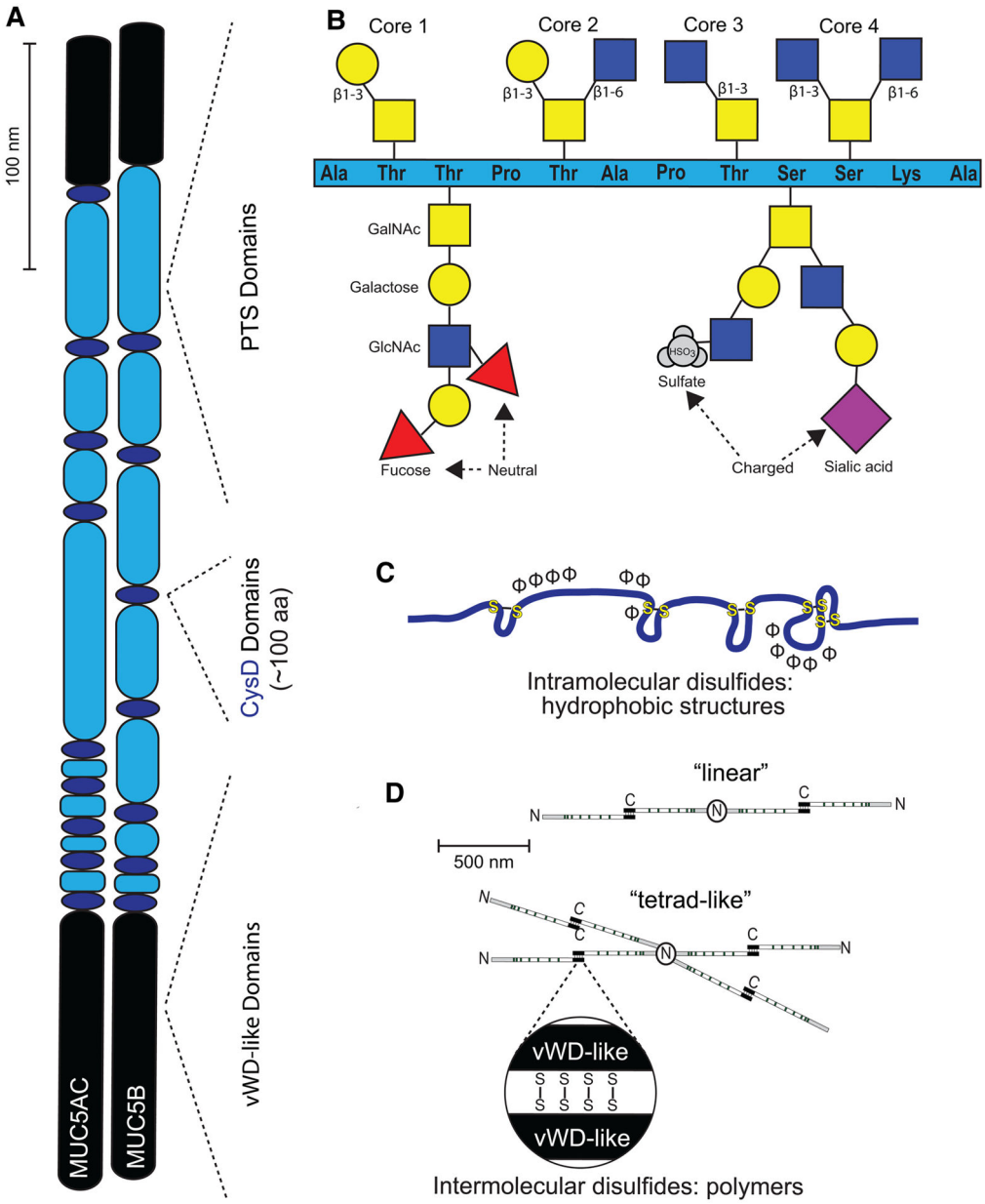

Figure 1. MUC5AC/MUC5B structural domains and molecular interactions.

(**A**) MUC5AC and MUC5B apoproteins are lengthy constructs containing PTS domains (light blue ovals) interspersed with CysD (dark blue ovals), capped with vWD (black ovals). Each of these domains contributes toward overall structure, and thus function, of these mucins in a unique way. (**B**) PTS domains serve as sites for core glycosylation. GalNAc (yellow squares) is attached to serine and threonine residues for all four core structures. This is followed by β1–3 linkage of galactose (yellow circle) to form core 1, addition of both a β1–3 linkage of galactose and a β1–6 linkage of GlcNAc (blue squares), to form core 2, a β1–3 linkage of GlcNac to form core 3, or both a β1–3 linkage of GlcNAc and a β1–6 linkage of GlcNac to form core 4. These cores can be further elongated by the addition of extra sugars, including fucose (red triangles), or capped/terminated by SO_4 (gray

circles) or NeuNAc (purple diamonds). (**C**) CysD segments reinforce tertiary structure of mucins via intramolecular disulfide linkage. (**D**) vWD-like domains assist in assembly of homo-oligomers into linear- or tetrad-like structures via intermolecular disulfide linkage.

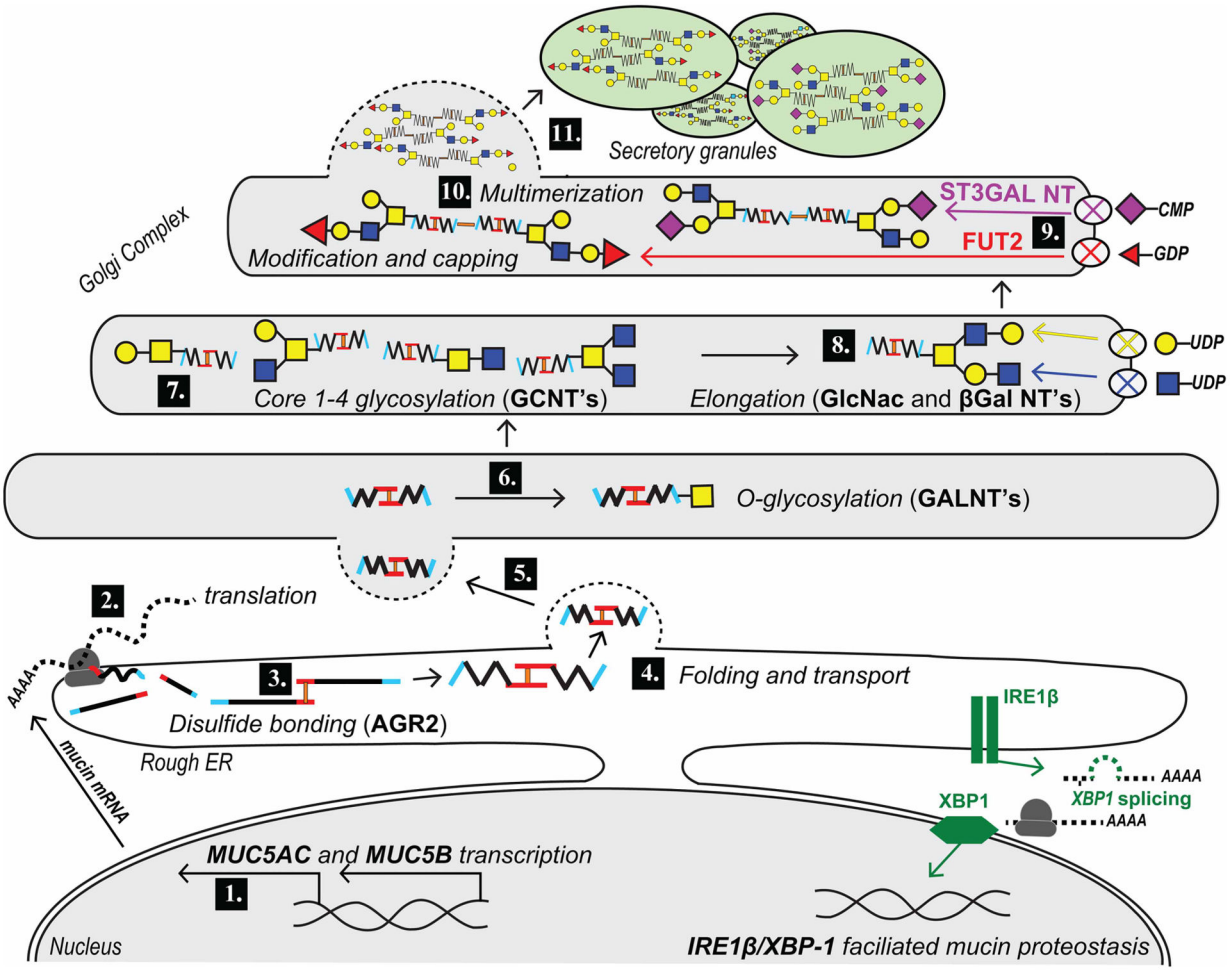

Figure 2. Pathways of mucin biosynthesis.

1. MUC5AC/MUC5B transcription in the nucleus is tightly regulated by specific transcription factors. 2. Co-translational import of mucin into the rough ER. 3. Assembly of mucin disulfide dimers is mediated by PDIs. 4 and 5. Mucin is folded and transported from ER to the Golgi complex under the guidance of chaperone and trafficking proteins. 6. In the *cis* Golgi, mucin is O-glycosylated by the addition of GalNAc (yellow squares) by GALNTs. 7. Mucin transits through the middle Golgi where additional core 1–4 structures are formed by the addition of GlcNAc (blue squares) and/or Gal (yellow circles) by CGNTs. 8. Sugars are extended by additional βGal and GlcNAc transferases (GalTs and GlcNAcTs). 9. Mucin glycosylation terminates in sialylation via α2,3-sialyltransferase or fucosylation via α1,2 fucosyltransferase enzymes ST3GAL3 and FUT2, respectively (fucose, red triangles; sialic acid, purple diamonds). 10. Mucin oligomeric subunits multimerize via N-terminal assembly. 11. Fully synthesized mucin is then transported from the *trans*-Golgi by vesicular trafficking to growing secretory granules, where it is stored for eventual release by regulated exocytosis.