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Post-translational regulation of signaling mucins

Paul J Cullen

Signaling mucins are large transmembrane glycoproteins that regulate signal transduction pathways. Recent advances have shown that two major types of post-translational modifications, protein glycosylation and proteolytic processing, play important and unexpected roles in regulating signaling mucin function. New O-glycosyltransferases and proteases have been identified, and the structure of the domain that undergoes auto-proteolysis has been solved. A picture is beginning to emerge where specific glycosyl modifications and regulated processing control the signaling and adherence properties of signaling glycoproteins and contribute to the routing of signals to specific pathways.

Address
Department of Biological Sciences, State University of New York at Buffalo, 341 Cooke Hall, Buffalo, NY 14260-1300, United States

Corresponding author: Cullen, Paul J (pjcullen@buffalo.edu)

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Introduction

Signaling mucins are high molecular weight transmembrane glycoproteins that regulate signal transduction pathways. These molecules have gained widespread popularity as markers for many different types of cancers and because of their prominent roles in regulating a wide variety of signal transduction pathways [1,2]. Signaling mucins are regulated by two major types of post-translational modifications, protein glycosylation and proteolytic processing. This review highlights what the precise modifications are, the enzymes that carry out those modifications, and the consequences of protein modification on signaling mucin function and regulation. Other signaling glycoproteins are discussed in the context of signaling mucin regulation.

Although generally variable in primary amino acid sequence, signaling mucins have a number of common features and the same overall topology (Figure 1). Signaling mucins are single-pass cell-surface glycoproteins with a rod-like extracellular domain that is connected to a cytosolic C-terminal domain by a transmembrane α helix. The cytosolic signaling domain distinguishes signaling-type mucins from nonsignaling mucins (like MUC2), which do not contain cytosolic domains. A defining feature of mucin glycoproteins is the presence of tandem repeats rich in proline, threonine, and serine residues (PTS domain) in the extracellular domain (Figure 1).

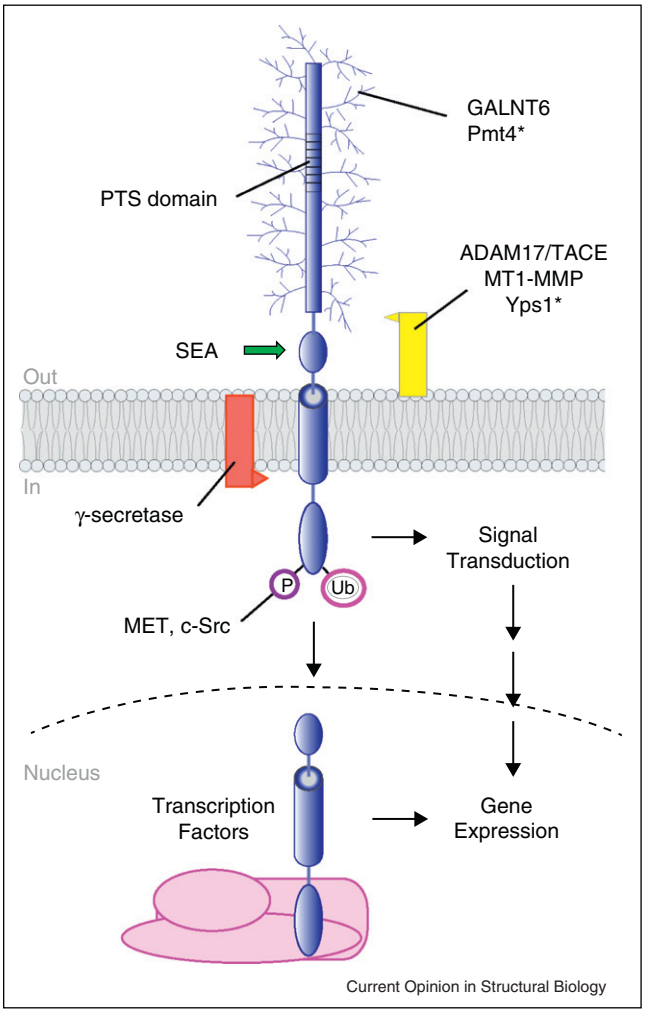
The most extensively studied signaling mucin is MUC1, which regulates the RAS-MEK-ERK mitogen-activated protein kinase (MAPK) pathway [3] and many other signaling pathways [1,2]. Other signaling mucins including MUC4 and MUC12 have also been characterized. Mucin-like glycoproteins also exist in other organisms including genetically tractable model systems like the baker's yeast *Saccharomyces cerevisiae*. In yeast, two signaling glycoproteins have been characterized, Msb2 [4] and Hkr1 [5], which like their mammalian counterparts contain PTS domains and regulate MAPK pathways. Msb2 regulates MAPK signaling by associating with the ubiquitous polarity Ras homology (Rho) GTPase Cdc42 [6] and might represent a functional homolog of human MUC12 [7]. Nonsignaling glycoproteins have also been studied in yeast such as the adhesion flocculin Flo11 [8].

Glycosyl modifications in the extracellular domain

Signaling mucins are rich in serine and threonine residues (>40% of the total residues). Many of these residues are thought to be modified by O-linked glycosylation, and the PTS domain has been shown to be extensively O-glycosylated [9]. As a result, oligosaccharides can more than double the molecular mass of the proteins. Signaling mucins are modified by different sugars including galactose, N-acetylgalactosamine (GalNAc), fucose, and/or sialic acid, which can influence the size and overall charge of the protein. MUC1 for example is heavily sialylated, and this modification becomes more extensive after the protein has been internalized from the cell surface and recycled back to the plasma membrane through the Golgi apparatus [10]. Glycosylation can stabilize mucins at the cell surface, by limiting their endocytosis [11,12] and by protecting the polypeptide chain from degradation by extracellular proteases [13].

What enzymes glycosylate signaling mucins? Mucin-type O-glycosylation is initiated by members of the polypeptide N-acetylglucosaminyltransferase family (gene name GALNT for mammals, PGANT for Drosophila). There are at least 15 GALNT genes in the human genome [14], and several of these enzymes likely participate in O-GalNAc addition to MUC1 depending on the cell and

Figure 1



Post-translational modifications of signaling mucins. A schematic representation of a signaling mucin is shown. Proteins that modify signaling mucins include O-glycosyltransferases, like GALNT6 and Pmt4, and proteases γ-secretase, Yps1, ADAM17/TACE, and MT1-MMP. Autocatalytic processing by the SEA domain is shown by a green arrow. The cytosolic domain is modified by ubiquitin (Ub), and phosphate (P) moieties. The cytosolic and transmembrane domains of MUC1 can translocate to the nucleus and associate with transcription factors to regulate gene expression. Modifiers of yeast mucin-like proteins are marked with an asterisk. Not all signaling mucins undergo all the modifications shown. The extracellular domain is not to scale and can be much larger than shown.

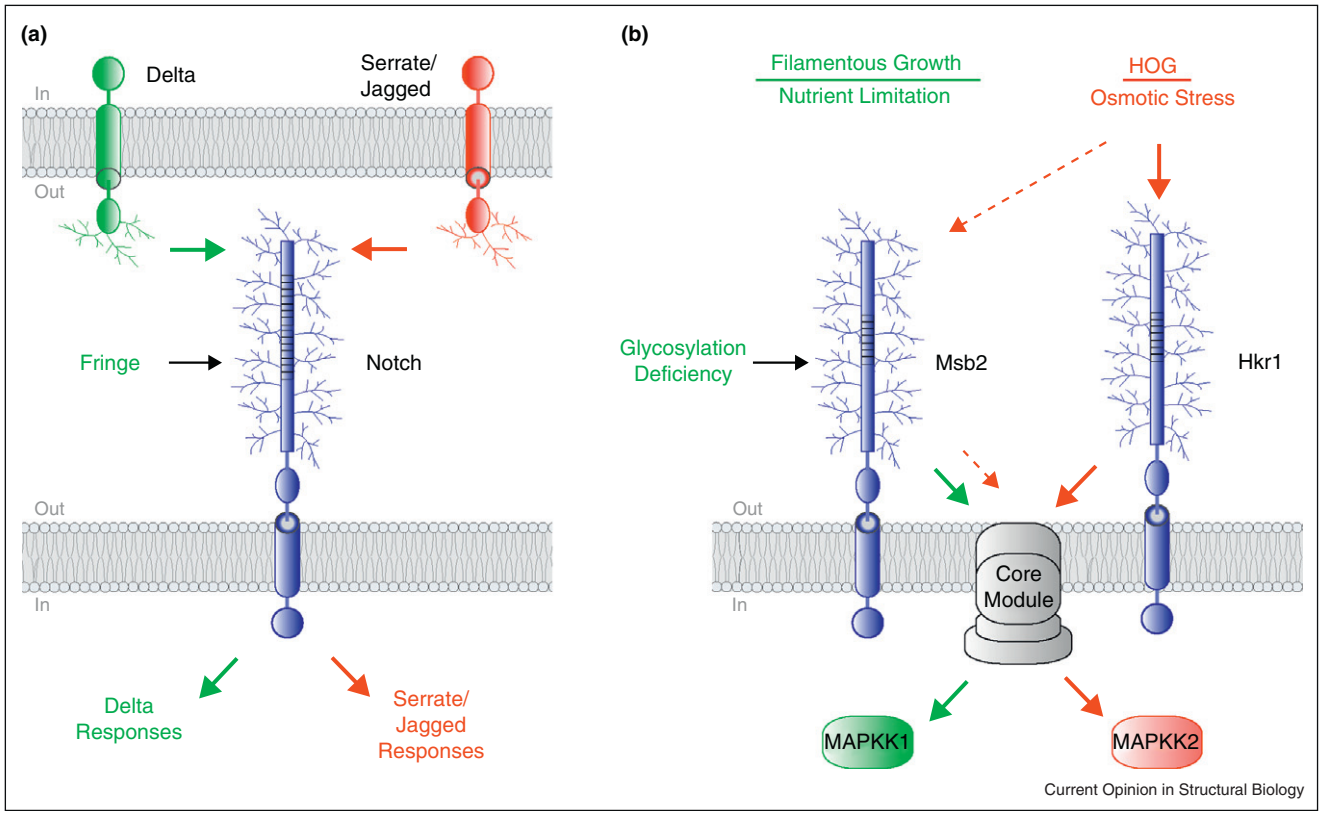
tissue type. Other glycosyltransferases may add various sugars subsequent to O-GalNAc addition [14].

Recently, a specific GALNT, GALNT6, was found to modify MUC1 in breast cancer cells [15•]. The expression of the GALNT6 gene is elevated in cancer cells, which also express high levels of MUC1. Down-regulation of GALNT6 expression by small interfering RNAs suppressed the growth of breast cancer cells [15•]. The idea that different GALNTs specifically glycosylate mucins in different contexts is supported by studies in model organisms. A specific yeast O-mannosyltransferase, Pmt4, among a large family of Pmt proteins, plays the major role in Msb2 glycosylation [16••]. Similarly, in the fruit fly, *Drosophila melanogaster*, loss of different PGANT genes results in different phenotypes, which suggests that different PGANTs glycosylate nonoverlapping targets [17•]. An implication of these findings is that individual glycosyltransferases selectively modify signaling mucins to precisely regulate their function and activity.

What role does protein glycosylation play in regulating signaling mucin function? An exciting possibility is to regulate signaling pathway specificity. Signaling pathways typically function in web-like networks composed of many different proteins. In these networks, signals are routed through common protein modules to induce selective responses. How signals are faithfully transmitted along one of many possible paths is not clear. A role for glycosylation in influencing signaling specificity was demonstrated by studies of Notch, a transmembrane glycoprotein which is similar to signaling mucins and which regulates many developmental processes [18]. Notch is activated by binding to proteins expressed on adjacent cells, Delta and Serrate/Jagged. Fringe is a glycosyltransferase (specifically an N-acetylglucosaminyltransferase), that modifies Notch [19] by elongating O-fucosyl residues through the addition of N-acetylglucosamine. The glycosyl modification by Fringe has the effect of potentiating signaling from Delta while inhibiting signaling from the Serrate/Jagged family of ligands (Figure 2a). Thus, the selective glycosylation of Notch by Fringe can evoke a pathway-specific response.

A loose parallel can be drawn for yeast mucin-like proteins, in that glycosylation can differentially influence the activity of one MAPK pathway over another. Two yeast glycoproteins, Msb2 and Hkr1, regulate two MAPK pathways (Figure 2b [20]). The pathways sense different stimuli and induce nonoverlapping responses but share a common protein module (Figure 2b [21]). In fact, one of the mucin-like glycoproteins, Msb2, can itself function in both pathways (Figure 2b [5]). One difference between the pathways is that they require different downstream kinases (Figure 2b, MAPKK). In a recent landmark study, glycosylation deficiency was shown to activate Msb2 in one of the pathways but not the other (Figure 2b [16]). This unexpected discovery, together with other evidence [22], suggests that Msb2 and Hkr1 function preferentially in different pathways. It remains unclear how the two proteins activate different MAPKKs through a shared module. Possibly, pathway-specific proteins that remain to be identified differentially associate with the C-termini of the two mucins, which are dissimilar. Therefore, the selective glycosylation of transmembrane glycoproteins is becoming an emerging paradigm for how pathway specificity is achieved.

Figure 2



Protein glycosylation can regulate signaling specificity. **(a)** Notch can bind to transmembrane ligands of the Delta and Serrate/Jagged families. Fringe is a glycosyltransferase that modifies Notch and enhances a Delta-specific response (green arrows) while inhibiting Serrate/Jagged-specific responses (red arrows). **(b)** Two yeast MAPK pathways that share components. Nutrient limitation activates the filamentous growth pathway through Msb2 (green arrows). Osmotic stress activates the HOG pathway through Msb2 and Hkr1 (red arrows). Reduced glycosylation of Msb2 specifically triggers the filamentous growth pathway through a core module composed of proteins that are shared between the two pathways, shown in grey. The lighter dashed red arrows designate a function for Msb2 in the HOG pathway.

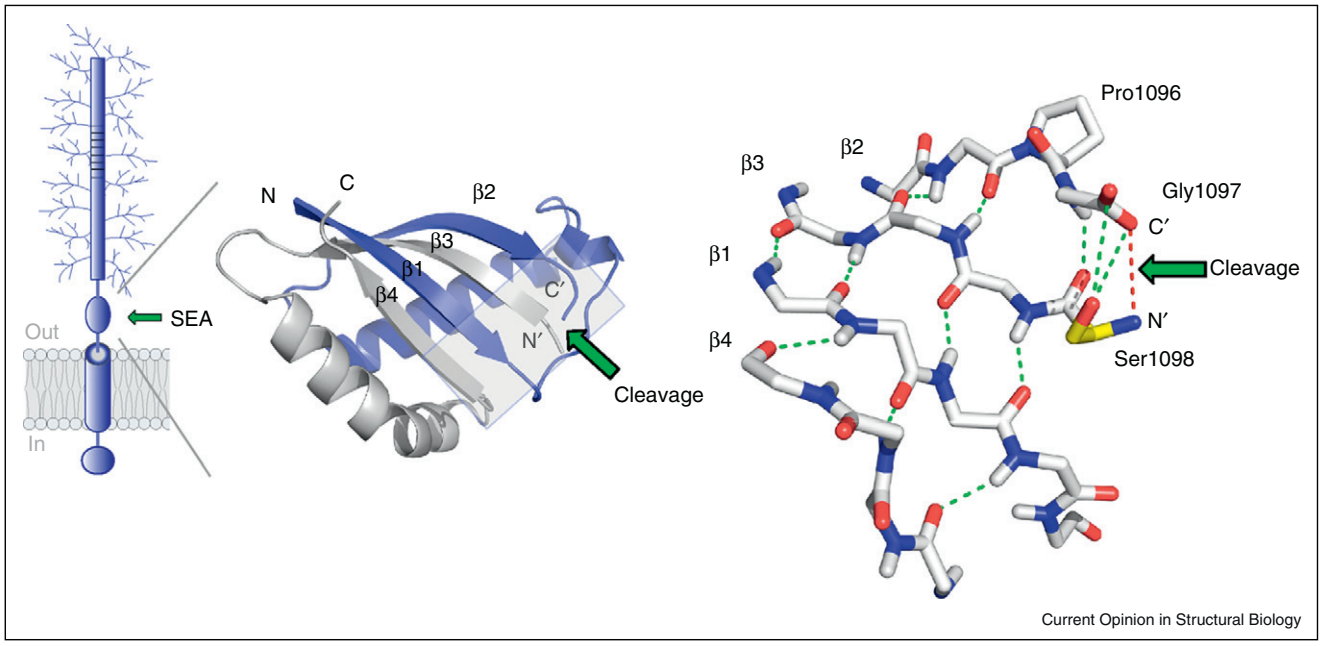
Processing and release of the extracellular domain

A second major post-translational modification of signaling mucins is proteolytic processing. Signaling mucins can be processed outside the cell in their extracellular domains and inside the cell in their cytosolic domains. These processing events have important consequences on mucin function and regulation. MUC1 undergoes autoproteolytic cleavage [23], which maps between the glycine and serine residues (G↑SVVV) at position 1097 in the extracellular domain of the protein [24,25]. The cleavage site and surrounding region is also found (almost exclusively) in other O-glycosylated proteins and is referred to as the sea-urchin sperm protein, enterokinase, and agrin (SEA) module [26]. The structure of the SEA module has been determined by NMR spectroscopy, which has shed light on the mechanism of cleavage (Figure 3 [27]). Structural analysis of the SEA domain has also provided detailed energetic and thermodynamic information about self-cleavage reaction [28,29].

Signaling mucins are also processed by other proteases. The protease that processes MUC4 remains to be identified [30]. The yeast glycoprotein Msb2 is processed by Yps1 [31], a member of an evolutionary conserved family of glycophosphatidylinositol (GPI)-anchored aspartyl proteases [32]. The secreted rat mucin, Muc2 [33], and the yeast flocculin Flo11 [34••] are processed by furin (Kex2 in yeast), a member of an evolutionarily conserved family of pro-protein convertases [35].

Release of MUC1 from the cell surface requires additional processing, because the two fragments generated by auto-proteolysis remain associated by noncovalent interactions. Two 'sheddases' are required for the release of MUC1 from cells. One is tumor necrosis factor-alpha converting enzyme (TACE), also called A Disintegrin And Metalloprotease domain-containing protein 17 (ADAM17) [36], and the other is a membrane-type 1-matrix metalloprotease (MT1-MMP) [37]. ADAM-type sheddases are themselves highly

Figure 3



Structure of the self-cleaving SEA domain of MUC1. The structure is based on NMR spectroscopy data ([27], PDB number 2ACM). At left is shown the folding topology and secondary structure of the cleaved SEA heterodimer with the two intertwined subunits colored in blue and grey, respectively. Autoproteolytic cleavage occurs at the edge of a four-stranded β-sheet to generate novel N′ and C′ peptide termini, as indicated. The structure on the right shows a detailed view of the peptide backbone at the cleavage site (boxed area in figure to the left). Autoproteolytic cleavage has occurred between glycine and serine in the tight turn Pro1096-Gly1097-Ser1098 as a result of conformational strain generated by the β-sheet structure (hydrogen bonds in green) and the nucleophilic action of the serine hydroxyl (Ser1098 side chain in yellow). The location of the former glycine–serine peptide bond is illustrated by a red line. The site of cleavage is denoted by a green arrow.

regulated at the transcriptional and post-transcriptional levels and regulate the shedding of many different types of glycoproteins (Figure 4 [38]).

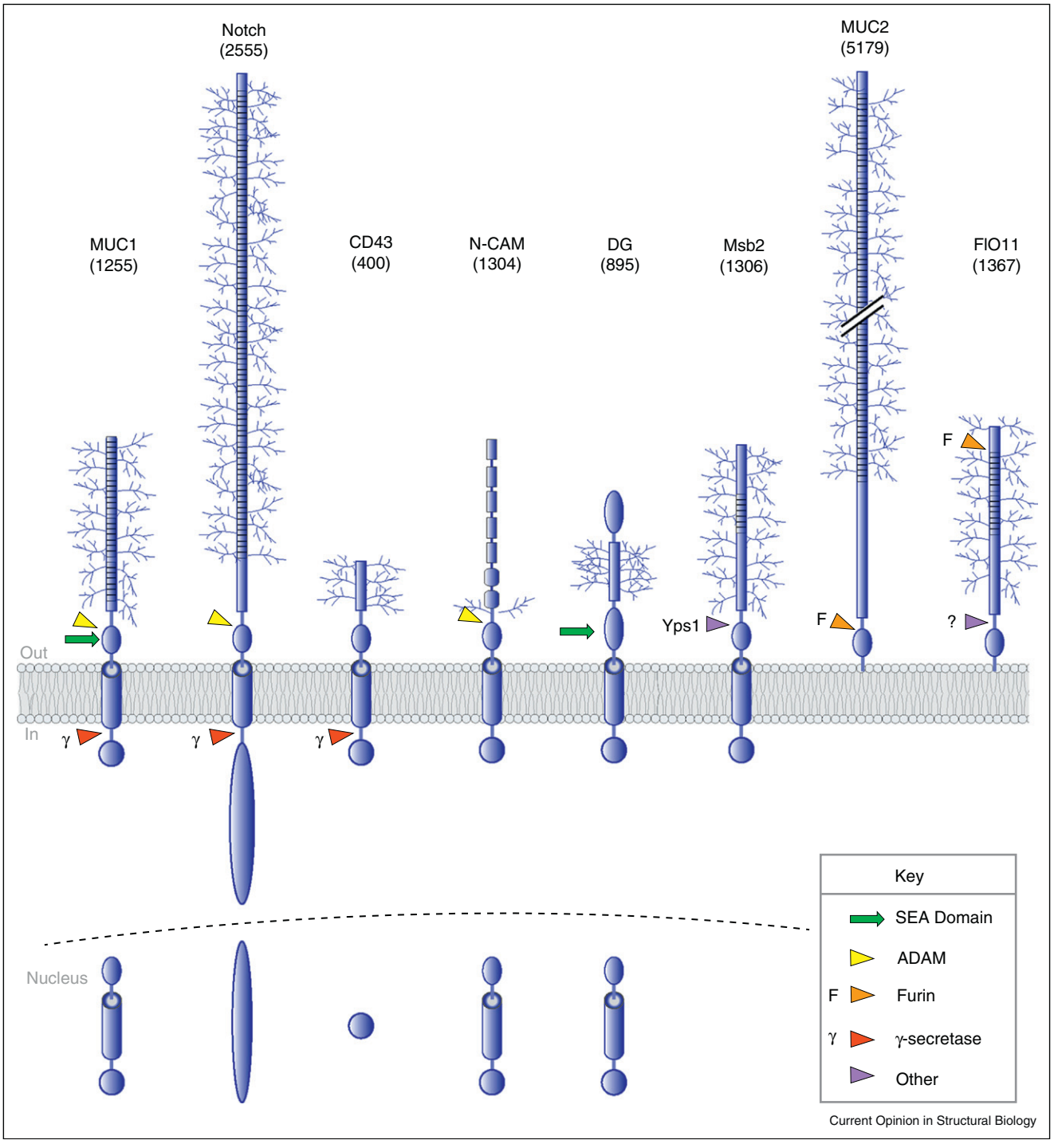
What effect does processing have on signaling mucin function? A possible insight has emerged from studies of Msb2, through observations that were at first perplexing. Initial observations showed that a version of Msb2 lacking the PTS domain hyper-activates the MAPK pathway, which suggested an inhibitory function for that domain [4]. Subsequent experiments demonstrated that the extracellular inhibitory domain is released from cells as a result of processing [31]. Together these findings suggest a cleavage-dependent activation mechanism [31]. Notch similarly has a large inhibitory domain that is processed and released from cells as part of its activation mechanism [39]. Cleavage-dependent activation may underlie the regulation of other signaling glycoproteins, such as MUC1, although this possibility has not been explicitly tested.

A second function for glycoprotein shedding may be to regulate adhesion. Many cell-surface glycoproteins have adhesive (or anti-adhesive) functions, by associating with proteins on the surface of other cells or in the extracellular matrix. Studies on the yeast flocculin Flo11, a large glycoprotein that is attached to cells by a GPI anchor [8], have shown that the protein is released from cells. Release of Flo11 from cells provides a mechanism for attenuating adherence [33]. The regulated processing of O-glycoproteins therefore represents a means to regulate the signaling and adherence properties of these proteins.

Processing and modification of the cytoplasmic domain

Many transmembrane O-glycosylated proteins including MUC1 [40], Notch [41,42], and other proteins (Figure 4) are processed in their cytosolic domains. For most proteins that have been examined, the membrane-embedded protease complex γ-secretase [43] is responsible for processing at the cytosol–transmembrane boundary. The cytoplasmic domain of MUC1 can enter the nucleus [44] and associate with transcription factors to directly modulate gene expression. At first glance, one might expect that processing of MUC1 by γ-secretase results in nuclear entry. This may be an oversimplification, however, because nuclear forms of MUC1 include the transmembrane domain [45]. How versions of MUC1 that contain the transmembrane domain are liberated from the plasma membrane and trafficked to the nucleus is not clear. Nevertheless, this unconventional trafficking

Figure 4



Post-translational processing of cell-surface O-glycosylated proteins. Processing and nuclear entry of proteins are based on reports for MUC1 [40,54•], Notch [55], dystroglycan (DG) [45,56], N-CAM [57], and CD43 [58]. Notch is processed by ADAM10 and ADAM17. Arrows refer to sites of cleavage: red, γ secretase (γ); green, SEA domain; yellow, ADAM; orange, furin (F); and purple, processing by other protease(s). For some proteins, the exact site of cleavage is not known. In parentheses is the predicted molecular weight of the proteins shown. Only O-glycan (not N-glycan) modifications are depicted.

route appears to underlie the nuclear localization other cell-surface glycoproteins such as β-dystroglycan and N-CAM (Figure 4). The cytoplasmic domain of MUC1 may function in other organelles such as the mitochondria. The localization of MUC1 to the mitochondria occurs in an HSP90-dependent manner [46].

The cytosolic domain of MUC1 associates with many different signaling proteins [1,2] and is extensively regulated by multiple post-translational modifications. For example, the C-terminus of MUC1 is phosphorylated by protein kinases including MET [47] and SRC [48]. Phosphorylation of MUC1 by MET induces interaction with p53 [47], and phosphorylation by SRC induces interaction with HSP90 [46]. The cytosolic domain can also be ubiquitinated. Ubiquitination of MUC4 targets the protein for turnover [49].

Conclusions and future directions

Signaling mucins are regulated by many different post-translational modifications. These modifications modulate the charge, stability, and activity of signaling mucins and consequently regulate the strength, duration, and specificity of the signals generated. A future challenge will be to understand which precise glycosyl modifications regulate signaling mucin function. The extensive glycosylation of mucin proteins complicates structural and compositional analysis. However, recent advances in the synthesis of glycosylated peptides [50,51], the construction of glycopeptide arrays [52], and the utilization of genomic approaches [53] may facilitate future studies. Future studies on signaling mucins and other glycoproteins will continue to deepen our appreciation of these interesting molecules and assist in the design of effective therapies in the treatment of human disease.

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