Chapter 1: Introduction to the Role of Glycans in the Nervous System[1]

Introduction

The cell surface displays a complex array of oligosaccharides, glycoproteins, and glycolipids. This diverse mixture of glycans contains a wealth of information, modulating a wide range of processes such as cell migration, proliferation, transcriptional regulation, and differentiation.[1-7] Glycosylation is one of the most ubiquitous forms of post-translational modification, with more than 50 percent of the human proteome estimated to be glycosylated.[8] Glycosylation adds another facet to the complexity of cellular signaling and expands the ability of a cell to modulate protein function. The astonishingly varied structural complexity of glycan modifications ranges from the addition of a single monosaccharide unit to polysaccharides containing hundreds of sugars in branched or linear arrays.[9] This chemical diversity enables glycans to impart a vast array of functions, including structural stability, proteolytic protection, protein recognition, cell migration, neurite outgrowth and fasciculation, and modulation of cell signaling networks.[3, 9-13]

Emerging evidence suggests a pivotal role for glycans in regulating nervous system development and function. For instance, glycosylation influences various neuronal processes, such as neurite outgrowth and morphology, and contributes to the molecular events that govern synaptic plasticity, a neurochemical model of learning and memory.[9, 14, 15] Glycosylation is an efficient modulator of cell signaling and has been

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implicated in memory consolidation pathways.[16-18] Genetic ablation of glycosylation enzymes often leads to developmental defects and can influence various organismal behaviors such as stress and cognition.[19-21] Thus, the complexity of glycan functions help to orchestrate proper neuronal development during embryogenesis, as well as influence adult behaviors.

The importance of glycosylation is further highlighted by defects in glycan structures that often lead to human disease, as exhibited by the congenital disorders of glycosylation (CDG).[22-26] These are usually inherited disorders resulting from defects in glycan biosynthesis, which are accompanied by severe developmental abnormalities, mental retardation, and difficulties with motor coordination. Such disorders highlight the importance of glycan biosynthesis in human health and development. As therapeutic treatments are currently limited, investigations into the structure-activity relationships of glycans, as well as disease-associated alterations to glycan structure, are crucial to find new therapeutic targets to treat the pathological conditions associated with GDGs. For

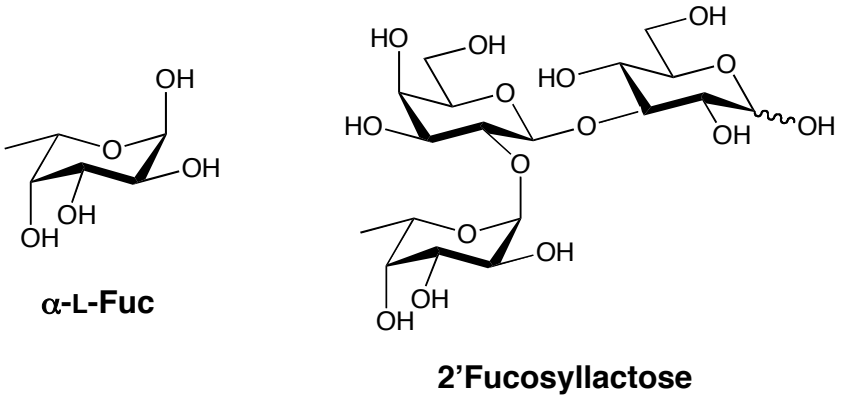

Figure 1.1. Chemical structure of α-L-Fucose and 2'fucosyllactose.

example, current treatments in the congenital disorder that has a defective enzyme that

produces a substrate for mannosyltransferate I is treated orally with mannose.[9] Another congenital disorder that leads to a reduction in fucose glycoconjugates can also be treated orally with fucose, highlighting the importance of understanding glycan biology to develop the proper treatment for these diseases.

Glycan Biosynthesis

The wide range of glycan structures can be attached to either proteins or lipids. This diversity of structures is specific to cell type and is developmentally regulated. The carbohydrate compositions can change in the monosaccharide content and linkages within oligosaccharide chains. The monosaccharides composing glycan chains include glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), fucose (Fuc), sialic acid (Neu5Ac), mannose (Man), glucuronic acid, and xylose (Xyl). These monosaccharides can be linked in different glycosidic bonds at different hydroxyl groups of the monosaccharides, creating significant chemical diversity in oligosaccharide structures.

Oligosaccharides are biosynthesized in the endoplasmic reticulum (ER) and Golgi compartments of the cell and can be either *N*-linked to Asn in the consensus sequence Asn-X-Ser/Thr, (where X cannot be proline), or *O*-linked to Ser and Thr residues. [9] To date, there is no consensus sequence of *O*-linked glycosylation. *N*-linked glycans are formed by the addition of a core structure synthesized on the lipid dolichol in the ER. This core oligosaccharide is formed by the sequential addition of three Glc, nine Man, and two GlcNAc residues in different linkages where it is transferred to the core Asn on the nascent protein chain. This structure is then trimmed of the Glc residues and one

Man, and the glycoprotein then moves to the Golgi apparatus for terminal processing. This carbohydrate structure gets trimmed and the addition of new monosaccharides are added which leads to the great diversity in composition and chain length of *N*-linked glycans. Glycans in the brain are characterized by core α(1-6)-fucosylation, bisecting GlcNAc residues, and outer-arm α(1-3)-fucosylation.

In contrast to *N*-linked glycosylation, *O*-glycosylation occurs in the Golgi apparatus with the attachment of either GalNAc or Man to Ser/Thr residues to nascent proteins.[9] The monosaccharides are then elongated with GlcNAc, Gal, Fuc, or Neu5Ac in different structures with different linkages creating great diversity in glycan composition. *O*-linked oligosaccharides tend to be much smaller than *N*-linked glycans. However, the structural diversity in both *N*- and *O*-linked glycoconjugates is unfathomable, due to the hundreds of combinations of chain length, composition, and sugar linkages that can be present in each oligo- or polysaccharide.

α-L-Fucose

α-L-Fucose (6-deoxy-L-galactose) is generally expressed as a terminal monosaccharide on *N*- and *O*-linked glycoproteins and glycolipids. As such, it often serves as an important molecular recognition element for proteins. Fucose is distinct from other naturally occurring sugars in that it is a deoxyhexose sugar and exists exclusively in the L-configuration in nature (Figure 1.1). At least 13 human fucosyltransferases have been identified, which are responsible for the synthesis of a structurally diverse array of fucosylated glycans (Figure 1.2). Fucose is often linked to the C-2, C-3, of C-4 positions of the penultimate galactose in glycoconjugates or to the

C-6 position of the core GlcNAc residue of *N*-linked glycans.[1] *O*-Fucosylation, the direct modification of serine and threonine residues by α-L-Fuc, has also been observed on epidermal growth factor (EGF) repeats of glycoproteins such as Notch, a protein involved in cell growth and differentiation.[27] While Fuc is not elongated in *N*-linked and *O*-linked glycans, *O*-Fuc can be elongated by other sugars.[1]

Given the structural diversity of fucosylated glycans, it is perhaps not surprising that more than a dozen different human enzymes are involved in the formation of Fuc linkages, most of which exist in the terminal Golgi compartments.[1]. Two enzymes,

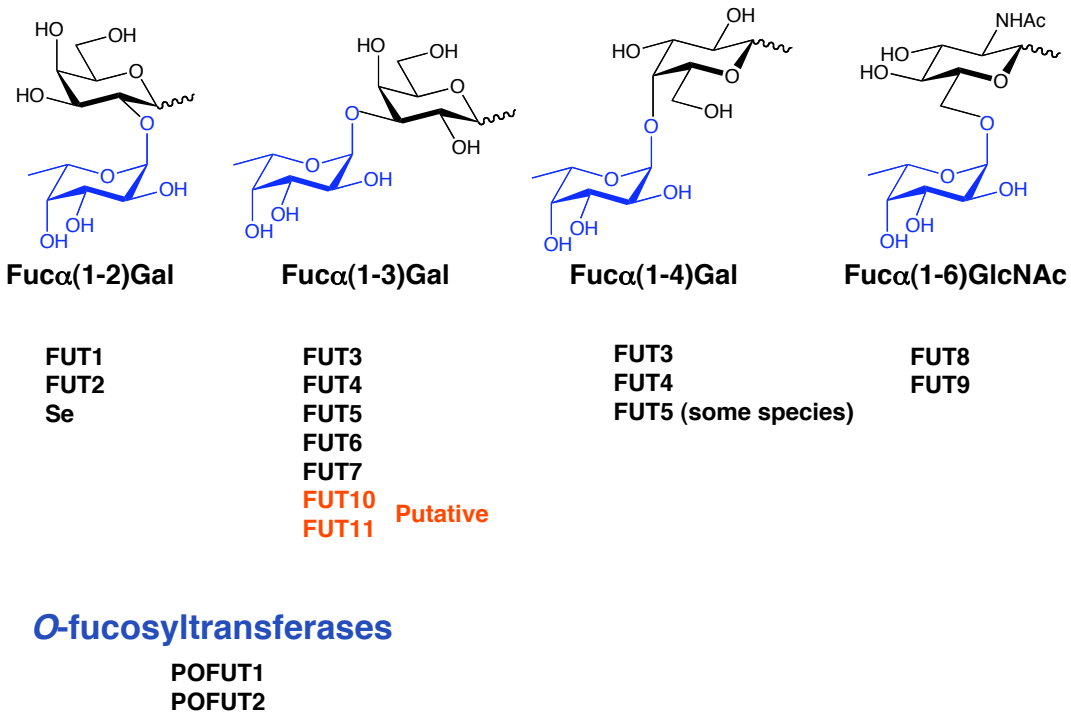

Figure 1.2. Fucosyltransferases catalyze diverse fucose structures. Fuc is in blue and Gal or GalNAc is in black. Enzymes known to catalyze the structures are written below. FUT10 and FUT11 are putative α(1-3)fucosylatransferases.

FUT1 and FUT2, are dedicated to the synthesis of Fucα(1-2)Gal glycans, an epitope

found on the ABO blood group antigens (Figure 1.2)[28-30] that has also been implicated in synaptic plasticity.[14, 31, 32] A gene homologous to FUT1 and FUT2, called Sec1, contains translational frameshifts and stop codons that interrupt potential open reading frames and thus appears to be a pseudogene.[28] FUT3 catalyzes the synthesis of both α(1-3)- and α(1-4)-fucosylated glycans and can transfer fucose to both Gal and GlcNAc in an oligosaccharide chain, whereas FUT4-7 form only α(1-3)-fucosylated glycans.[33, 34] FUT8 and FUT9 generate Fucα(1-6)GlcNAc structures, with FUT8 generally catalyzing attachment of this structure to the core Asn residue of *N*-linked oligosaccharides[35] and FUT9 catalyzing its attachment to a distal GlcNAc of polylactosamine chains.[36] FUT10 and FUT11 are putative fucosyltransferases that are reported to synthesize α(1-3)-fucosylated glycans based on sequence homology, although no functional studies have yet been performed.[1] Finally, POFUT1 and POFUT2, also known as *O*-fucosyltransferase 1 and *O*-fucosyltransferase 2, catalyze the direct fucosylation of

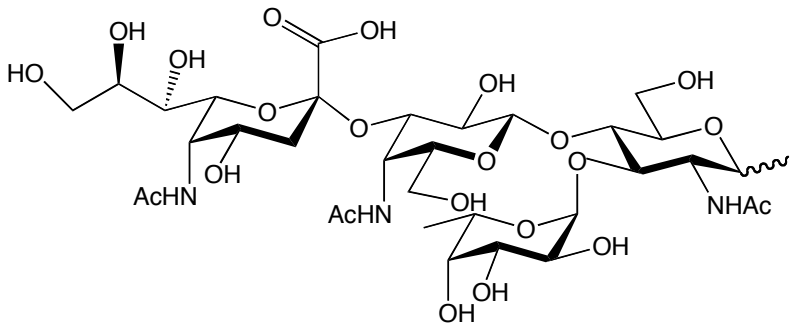

Figure 1.3. Structure of sialyl Lewis^X, an important ligand for selectin interactions.

serine/threonine residues within epidermal growth factor repeats and exist in the ER.[37, 38]

Neurobiological functions

Fucosylated glycans play pivotal roles in various physiological and pathological processes, including leukocyte adhesion,[39, 40] host-microbe interactions,[41, 42] and neuronal development.[43, 44] They are prevalent on the glycolipids of erythrocytes, where they form the ABO blood group antigens that distinguish specific blood types.[30] Aberrant expression of fucosylated glycoconjugates has been associated with cancer,[45-48] inflammation,[39, 49-51] and neoplastic processes.[52, 53] For instance, the α(1-3)-fucosylated antigens, sialyl Lewis^X (Figure 1.3), sialyl Lewis^Y, and sialyl Lewis^B, are up-regulated in certain cancers and have been associated with advanced tumor progression and poor clinical prognosis.[54-57] Moreover, the α(1-3)-fucosylated Lewis^X serves as a marker for neural stem cells[58] and radial glia cells that differentiate into mainly astrocytes and a small number of cortical neurons.[59] Furthermore, deficiency in fucose leads to a congenital disorder of glycosylation type IIc in humans, also known as leukocyte adhesion deficiency type II (LAD II). This disorder results in the impairment of leukocyte-vascular epithelium interactions and is characterized by immunodeficiency, developmental abnormalities, psychomotor difficulties, and deficits in mental capabilities.[60]

α-L-Fucose in Neuronal Development

Although their roles in the brain are less well understood, fucosylated glycans have been implicated in neural development, learning, and memory. *O*-Fucosylation is essential for the activity of Notch, a transmembrane receptor protein that controls a broad range of cell-fate decisions during development.[18, 61-65] Studies suggest that fucose modulates Notch signaling either by inducing a conformational change in the protein or

by interacting directly with Notch ligands.[64, 66] Notch signaling is believed to be involved in neuronal progenitor maintenance, and governs the cell-fate decision between neuronal and glial lineages. Notch signaling may also contribute to the behavior of differentiated neurons and neuronal migration.[67] Genetic deletion of the POFUT1 gene is embryonic lethal in mice and causes developmental defects similar to those observed upon deletion of Notch receptors, including abnormal vasculogenesis, somitogenensis, and neurogenesis.[68, 69] These studies demonstrate the importance of fucose in proper neuronal development and implicate Notch fucosylation as an important mediator of these events.

In addition to Notch, the Lewis^X epitope is reported to play roles in neurite outgrowth and neuronal migration during development of the central nervous system. The Lewis^X epitope is involved in neurite outgrowth of Xenopus tadpoles[70] and participates in several cell-cell interactions in the early neural development of chicks and rats.[71, 72] Deletion of the FUT9 gene, which synthesizes the Lewis^X epitope, leads to anxiety-related behaviors in mice, suggesting that abnormal neuronal development may have phenotypic effects in the adult organism.[73, 74]

α-L-Fucose in Learning and Memory

Multiple studies have suggested a role for fucosylation in learning and memory. For instance, incorporation of fucose into glycoconjugates in the brain was significantly enhanced by task-dependent learning in both chicks and rats.[15, 75-77] Rats were trained in a brightness discrimination task, in which animals learned to enter a bright chamber while avoiding a dark one while chicks learned to avoid pecking a bitter-tasting bead. Trained animals demonstrated an increase in [^{3}H]-labeled fucose incorporation into

glycoconjugates at synapses, the specialized sites of communication between neurons.[15, 76] Moreover, exogenous application of L-fucose or 2'-fucosyllactose (Figure 1.1) enhanced long-term potentiation (LTP), an electrophysiological model for learning and memory, both *in vivo* and in hippocampal slices.[78, 79]

Fucose is highly enriched at neuronal synapses,[14, 80, 81] where the majority of the fucosylated glycoconjugates exist as complex, *N*-linked structures.[82] Studies indicate that the activity of fucosyltransferases increases during synaptogenesis[83] and upon passive-avoidance training in animals.[84] Moreover, the cellular machinery involved in protein glycosylation can be found within dendrites,[85] raising the intriguing possibility that local protein synthesis and fucosylation may be occurring at synapses in response to neuronal stimulation.

Further studies have specifically implicated Fucα(1-2)Gal (Figure 1.2) linkages in neuronal communication processes. For instance, 2-deoxy-D-galactose (2-dGal; Figure 1.4), which competes with native galactose for incorporation into glycan chains and thus prevents the formation of Fucα(1-2)Gal linkages,[86] has been shown to induce reversible amnesia in animals.[32, 86, 87] In contrast, other small molecule sugars such as 2-deoxy-D-

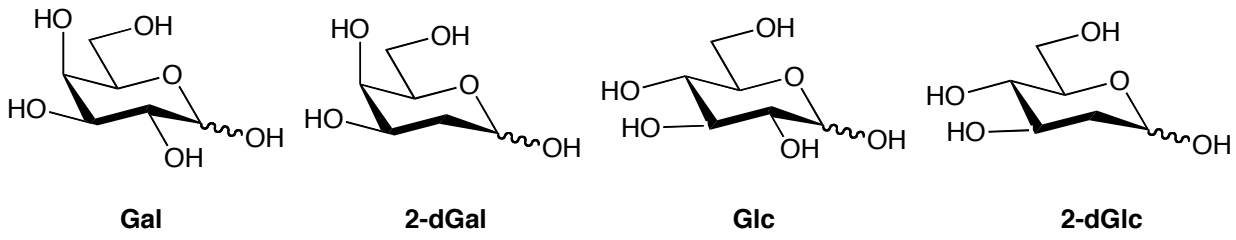

Figure 1.4. Chemical structures of D-galactose (Gal), 2-deoxy-D-galactose (2-dGal), D-glucose (Glc) and 2-deoxy-D-glucose (2-dGlc).

glucose, Gal, or Glc (Figure 1.4) had no effect, suggesting a unique function for Fucα(1-2)Gal –containing oligosaccharides. 2-dGal has also been reported to interfere with the maintenance of LTP, both *in vitro* and *in vivo*.[88, 89] Furthermore, a monoclonal antibody specific for Fucα(1-2)Gal[90] significantly impaired memory formation in animals, presumably by blocking formation of the Fucα(1-2)Gal epitope.[31]

Conclusions

Glycan structures are prevalent in the disease pathogenenesis that underlies a variety of cognitive problems associated with a range of congenital disorders. Understanding these disorders requires extensive analysis of the molecular mechanisms that perpetuate these diseases. Furthermore, the monosaccharide α-L-Fuc has also been shown to enhance memory formation, especially through the Fucα(1-2)Gal epitope suggesting that cognitive problems associated with fucose-deficiency may be due to defects in the synthesis of this disaccharide. Despite these intriguing behavioral and molecular responses for α-L-Fuc in disease and cognitive function, the precise mechanisms by which fucosyl carbohydrates exert their functional effects has been largely uncharacterized. This thesis describes our approach to elucidate these mechanisms by identifying and characterizing Fucα(1-2)Gal glycoproteins from mammalian brain.

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