Chapter 2: The Roles of *O*-GlcNAc Glycosylation in the Brain

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***O*-GlcNAc glycosylation is a unique, dynamic form of glycosylation found on intracellular proteins of all multicellular organisms. Studies suggest that *O*-GlcNAc represents a key regulatory modification in the brain, contributing to transcriptional regulation, neuronal communication, and neurodegenerative disease. Here, we highlight some of the emerging roles for *O*-GlcNAc in the nervous system and describe the challenges in understanding and studying the biology behind *O*-GlcNAc.**

O-GlcNAc glycosylation, the covalent attachment of β-*N*-acetyl-D-glucosamine to serine or threonine residues of proteins, is an unusual form of protein glycosylation (**Fig. 1**)[1]. Unlike other types of glycosylation, this single sugar modification occurs on intracellular proteins and is not elaborated further into complex glycans. The *O*-GlcNAc transferase (OGT) enzyme is a soluble protein that is found in the cytosol, nucleus, and mitochondria[2] rather than in the endoplasmic reticulum or Golgi. The dynamics of *O*-GlcNAc are also unique among sugar modifications, being cycled on a time scale shorter than protein turnover[3]. Thus, in many respects *O*-GlcNAc is more akin to phosphorylation than to conventional forms of glycosylation.

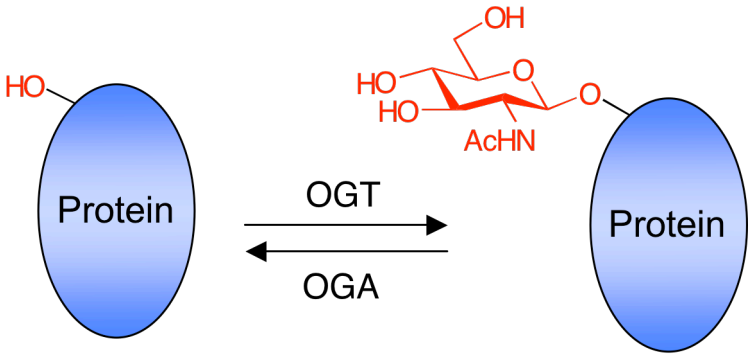

Figure 1: *O*-GlcNAc glycosylation is the addition of β-*N*-acetylglucosamine to serine or threonine residues of proteins

Several reviews have described the roles of *O*-

GlcNAc in cellular processes, such as transcription[2,4], the stress response[5,6], apoptosis[7,8], signal transduction[2,9], glucose-sensing[5,10], and proteasomal degradation[5]. Only a few reviews have highlighted the importance of *O*-GlcNAc glycosylation in the nervous system, and those reports have focused on its potential impact on neurodegenerative diseases[11,12]. However, multiple lines of evidence suggest that *O*-GlcNAc plays critical roles in both neuronal function and dysfunction. The enzymes responsible for the modification are most highly expressed in the brain[13,14] and are enriched at neuronal synapses[15,16]. Neuron-specific deletion of the OGT gene in mice leads to locomotor defects and neuronal dysfunction, resulting in neonatal death[17]. The *O*-GlcNAc modification is abundant in the brain and present on many proteins important for transcription, neuronal signaling, and synaptic plasticity, such as cAMP-responsive element binding protein (CREB)[18], synucleins[19], and β-amyloid precursor protein (APP)[20]. An intriguing interplay between *O*-GlcNAc and phosphorylation has been observed in cerebellar neurons, wherein activation of certain kinase pathways reduced *O*-GlcNAc levels on cytoskeletal-associated proteins[21]. Finally, recent studies suggest that *O*-GlcNAc can modulate calcium signaling and affect long-term potentiation[22,23].

Here we will describe emerging functions for *O*-GlcNAc glycosylation in the nervous system.

The enzymes OGT and OGA

OGT and β-*N*-acetylglucosaminidase (OGA or *O*-GlcNAcase) catalyze the reversible addition and removal of *O*-GlcNAc, respectively. Both enzymes are most highly expressed in the brain and exist as multiple different isoforms[2,24]. Three distinct

isoforms of OGT have been identified, including a 110-kDa and 78-kDa isoform that can assemble into a multimer[25,26], and a smaller mitochondrial isoform. Each isoform contains the C-terminal catalytic domain, but differs in the number of tetratricopeptide repeats (TPRs) within its N-terminal domain. The TPRs serve as protein-protein interaction modules that appear to target OGT to accessory proteins and potential substrates, such as the $GABA_A$ receptor interacting factor-1 (GRIF-1)[27] and the related *O*-GlcNAc transferase interacting protein (OIP106)[27], which have been implicated in mitochondrial trafficking to synapses[28,29], and the transcriptional repressor complex mSin3A-histone deacetylase 1 (HDAC1)[30]. In addition, OGT forms a complex with protein phosphatase-1 (PP1) in the brain[31]. The association between OGT and PP1 is particularly intriguing as it may provide a direct mechanism to couple *O*-GlcNAc glycosylation to dephosphorylation of specific substrates. Although OGT is found in the nucleus, cytosol, and mitochondria, it is particularly enriched in the nucleus[15] and the soluble synaptic compartment[16].

Like OGT, OGA appears to be highly active at neuronal synapses[16], and it is also found in the nucleus and cytosol[32]. OGA contains an N-terminal glycosidase domain and a putative C-terminal histone acetyltransferase (HAT) domain[33]. Two distinct isoforms of OGA exist, a 130-kDa and 75-kDa variant, which share the same catalytic domain but differ in their C-terminus[34]. The potential HAT activity of OGA may provide an intriguing mechanism for coupling deglycosylation of nuclear proteins to transcriptional activation. As with OGT, OGA has been shown to interact with specific proteins, including calcineurin/protein phosphatase-2B, amphiphysin, and dihydropyrimidinase-related protein 2 (DRP-2)[32].

Transcriptional regulation

Early studies revealed that the *O*-GlcNAc modification is enriched on chromatin[35] and is found on RNA polymerase II and a large number of its transcription factors[36]. As described in several reviews[2,4,37], *O*-GlcNAc glycosylation has been shown both to enhance and suppress the activity of transcription factors. *O*-GlcNAc can function to disrupt protein-protein interactions, as in the case of Sp1, whose glycosylation represses transcription at Sp1-driven promoters[38 39]. In other cases, it can promote protein-protein interactions, as in the case of STAT5A, whose glycosylation enhances its activity by recruiting the transcriptional coactivator CREB-binding protein (CBP)[40]. *O*-GlcNAc may also play a more general role in transcriptional repression through a mechanism involving the targeting of OGT to an HDAC1 complex by the corepressor mSin3A[30]. In addition to altering protein-protein interactions, *O*-GlcNAc can affect posttranslational modifications. For instance, glycosylation stabilized the tumor suppressor protein p53 by decreasing its phosphorylation and subsequent degradation by the proteasome[41].

Much less information is known about the roles of *O*-GlcNAc in regulating transcription in the brain. However, CREB, a transcription factor important for neuronal survival, long-term memory storage, and drug addiction[42,43], was shown to be *O*-GlcNAc glycosylated in the rodent brain[18]. Glycosylation occurred at two major sites within the Q2 transactivation domain of CREB and disrupted binding of CREB to $TAF_{II}130$, a component of the basal transcriptional machinery. As a result, glycosylation repressed the transcription of CRE-mediated genes both *in vitro* and in cells[18]. It will be interesting to investigate whether glycosylation of CREB is dynamically regulated in neurons and

whether it down-regulates specific genes associated with memory storage and cell survival.

Proteomic studies of *O*-GlcNAc modified-proteins from the brain have also underscored the importance of *O*-GlcNAc in regulating transcription. Approximately one-quarter of the neuronal *O*-GlcNAc proteins known to date are transcriptional regulatory proteins (**Fig. 2**)[44]. This includes numerous transcription factors (e.g., Sox2, ATF-2), as well as transcriptional coactivators (SRC-1), repressors (MeCP2[19], p66β, BHC80) and corepressors (TLE-4, CCR4-NOT). For instance, Sox2 is a member of the high mobility group box (HMG) superfamily of minor groove DNA-binding proteins, and it functions to regulate transcription on different promoters depending on its interactions with different protein partners[45]. Sox2 interacts with proteins through its highly conserved HMG DNA-binding domain, which also contains its *O*-GlcNAc modification site[44]. One of the well-established functions of Sox2 is its critical role in the maintenance of embryonic stem cell pluripotency in partnership with OCT3/4[46]. In the adult rat brain, Sox2 expression has been reported to occur in actively dividing adult neuronal precursor cells and in neurogenic astrogoglia[47]. Another example of the expanding role of *O*-GlcNAc in transcription is the modification of two proteins (including a ubiquitin ligase) in carbon catabolite repression 4-negative on TATA-less (CCR4-NOT)[44], a large protein complex involved in mRNA metabolism and the global control of gene expression[48]. Together with earlier studies demonstrating glycosylation of RNA polymerase II, these findings suggest that *O*-GlcNAc may participate in regulating multiple aspects of transcription.

Synaptic proteins and neuronal communication

Consistent with the observation that OGT and OGA are highly active at synapses, proteomic studies have uncovered a significant number of synaptic proteins in the *O*-GlcNAc proteome (**Fig. 2**)[44,49-51]. Many of these proteins are enriched in the postsynaptic density where they participate in the regulation of dendritic spine morphology and associate with the cytoskeleton. For instance, synaptopodin[44], SH3 and multiple ankyrin repeat domains protein 2 (shank2)[49] are critical for the normal formation of dendritic spine apparatuses[52-54]. Synaptopodin and δ-catenin have been shown to play important roles in learning and memory[52,55].

O-GlcNAc modifications are also highly abundant in presynaptic terminals. Several proteins involved in neurotransmitter release or synaptic vesicle endocytosis, such as bassoon[44], piccolo[49], synapsin[49], and clathrin assembly protein (AP180)[56], are *O*-

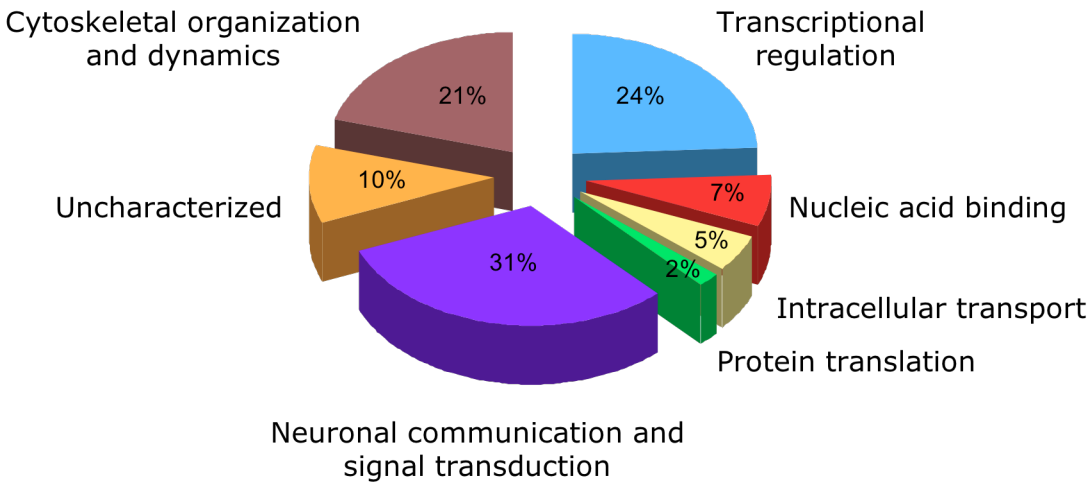

Figure 2: *O*-GlcNAc proteome from rodent brain. Approximately 24% of the known *O*-GlcNAc proteins participate in transcriptional regulation, 31% are involved in neuronal communication and signaling, and 21% are associated with forming cytoskeletal structures. Proteins were classified according to categories described by Schoof et al.[119]

GlcNAc glycosylated. The *O*-GlcNAc-modified protein, collapsin response mediator protein 2 (CRMP-2)[16], plays key roles in axon formation, elongation, and branching[57]. Moreover, many cytoskeletal proteins themselves are known to be glycosylated, including tau[58], the neurofilament proteins NF-H[59], NF-L[49] and NF-M[49], and the microtubule-associated proteins MAP1B[44] and 2B[44].

Recently *O*-GlcNAc has been shown to regulate and modify processes important for neuronal communication. Inducing glycosylation by inhibiting OGA decreases the number of axonal filopodia whereas decreasing glycosylation by overexpressing OGA increases the number of filopodia as well as the percentage of neurons exhibiting axon branching in cultured primary chicken forebrain neurons[60]. Furthermore the *O*-GlcNAc modification on neuronal inositol 1,4,5-trisphosphate receptor type 1 decrease channel activity[23]. Finally elevation or reduction of *O*-GlcNAc levels enhances or blocks long term potentiation in acute hippocampal slices[22].

Additional functional studies are needed to define the mechanisms by which *O*-GlcNAc regulates these proteins. Nonetheless, the prevalence of *O*-GlcNAc on proteins intricately involved in neurotransmitter release and cytoskeletal rearrangements underlying synaptic plasticity suggests roles for the modification in regulating key neuronal functions. As described below, emerging evidence indicates that *O*-GlcNAc levels can be dynamically modulated in response to neuronal stimuli. Moreover, the potential interplay between *O*-GlcNAc and kinase pathways in neurons may provide a powerful means to control protein function and modulate neuronal communication processes.

Neurodegenerative disease

O-GlcNAc glycosylation has been implicated in several neurodegenerative diseases, such as Alzheimer's[58,61,62] and amyotrophic lateral sclerosis (ALS)[63]. The genes encoding OGA and OGT map to chromosomal regions associated with late-onset Alzheimer's disease[64] and dystonia-Parkinsonism syndrome[65], respectively. Moreover, *O*-GlcNAc levels are abnormally altered in the brains of Alzheimer's disease patients, although the magnitude and direction of the change appears to depend on the subcellular protein fraction[61,62].

In the pathology of Alzheimer's disease, the microtubule protein tau becomes hyperphosphorylated, which in turn, causes it to aggregate and form neurofibrillary tangles that are hallmarks of the disease[66]. Tau is extensively *O*-GlcNAc glycosylated in the adult rat brain, although the estimated 12 or more modification sites have yet to be mapped[58]. Importantly, several studies suggest that *O*-GlcNAc glycosylation of tau negatively regulates its ability to be phosphorylated. For instance, inducing tau glycosylation with OGA inhibitors or by overexpression of OGT decreases tau phosphorylation at specific sites[62,67,68]. Conversely, stimulation of hyperphosphorylated tau using the phosphatase inhibitor okadaic acid leads to hypoglycosylated tau in human neuroblastoma cells[69]. Neuron-specific deletion of the OGT gene in mice[17] or inhibition of *O*-GlcNAc biosynthesis in rats[70] induces hyperphosphorylated tau similar to that found in Alzheimer's disease. As impaired glucose uptake/metabolism has been linked to Alzheimer's disease and appears to worsen as the disease progresses[71], one theory is that tau glycosylation becomes reduced in Alzheimer's patients and leads to hyperphosphorylated tau. Consistent with this view, mouse models of starvation that

mimic this impaired glucose metabolism display reduced tau glycosylation and a corresponding increase in tau phosphorylation at specific sites[62,72].

Abnormal *O*-GlcNAc glycosylation may also contribute to neurodegenerative diseases in more diverse ways. The amyloid precursor protein (APP), which forms the β-amyloid plaques characteristic of the disease, is both *O*-GlcNAc glycosylated and phosphorylated[20]. In an animal model of ALS, the *O*-GlcNAc levels of neurofilament protein M are decreased at the same time as its phosphorylation levels are increased[63]. Finally, *O*-GlcNAc glycosylation has been demonstrated to inhibit the proteasome[73], thus providing a mechanism to couple ubiquitin-mediated protein degradation to the general metabolic state of the cell. Blocking the removal of *O*-GlcNAc from the proteasome leads to increased protein ubiquitination[73] and possibly neuronal apoptosis[74]. Proteasomal dysfunction and ubiquitinated inclusion bodies are found in the diseased tissue of ALS, Parkinson's, Huntington's, and Alzheimer's disease patients[75]. Thus, aberrations in glucose metabolism and the *O*-GlcNAc glycosylation of specific proteins have been associated with several neurodegenerative disorders. It will be important in the future to determine the extent to which these changes are critical to the development and progression of such diseases.

O-GlcNAc dynamics and cycling

A unique feature of *O*-GlcNAc glycosylation is its ability to undergo dynamic cycling in contrast to other, more static forms of protein glycosylation. Studies have shown that *O*-GlcNAc levels are altered by extracellular stimuli on a time scale similar to phosphorylation. For instance, a transient increase in glycosylation of the transcription

factor nuclear factor activated T-cells (NFAT) was observed within 5 minutes after T or B cell activation[76].

O-GlcNAc levels are highly responsive to glucose concentrations and influx through the hexosamine biosynthesis pathway (HBP) in neurons and other cell types[77,78]. Approximately 2-5% of all cellular glucose is metabolized through the HBP pathway to generate UDP-GlcNAc[79]. As OGT activity is exquisitely sensitive to UDP-GlcNAc concentrations[80], *O*-GlcNAc glycosylation may act as a sensor for the general metabolic state of the cell. Consistent with this notion, *O*-GlcNAc appears to be intricately linked to cell survival[17] and is induced by many forms of cell stress[81].

In the brain, phosphorylation serves as a central mechanism for neuronal communication by regulating ion channels, neurotransmitter receptors, gene transcription, and synaptic vesicle release[82,83]. Protein kinases and phosphatases work together to coordinate different forms of synaptic plasticity, and they are necessary for the induction and maintenance of postsynaptic long-term potentiation and long-term depression[84]. Thus, the potential interplay between *O*-GlcNAc glycosylation and phosphorylation has exciting implications for many neuronal functions. Early studies showed that activation of protein kinase C (PKC) or cAMP-dependent protein kinase (PKA) significantly decreased overall *O*-GlcNAc glycosylation levels in the cytoskeletal protein fraction of cultured cerebellar neurons[21]. Conversely, inhibition of PKC, PKA, cyclin-dependent protein kinases or S6 kinase increased overall *O*-GlcNAc levels in these fractions. A more complex relationship was observed with tyrosine kinases and phosphatases. Inhibition of tyrosine phosphatases led to a decrease in overall *O*-GlcNAc levels, while inhibition of tyrosine kinases induced both increases and decreases in *O*-

GlcNAc, depending on the protein fraction. More recent studies showed that elevation of *O*-GlcNAc levels in the brain increased in activating-phosphorylation sites on ERK 1/2 and CaMKII[22]and elevation of *O*-GlcNAc in culture affected the phosphorylation of PKA substrates in response to forskolin[60].

Together, emerging evidence suggests that *O*-GlcNAc represents a key regulatory modification in the brain. Not only is it present on a large number of functionally important neuronal proteins, it appears to be reversible, differentially regulated, and responsive to neuronal activity. Further studies are needed to elucidate the molecular mechanisms involved and how activation of specific signaling pathways leads to the regulation of OGT and OGA. Moreover, changes in *O*-GlcNAc glycosylation have been monitored only on a global level, and the specific proteins undergoing dynamic changes in glycosylation as well as how those changes affect the protein function remain largely unknown.

Conclusion and Future Challenges

Over the past decade, a surge of discoveries in *O*-GlcNAc glycosylation has revealed new roles for this modification in the nervous system. *O*-GlcNAc is abundant in the brain and present on many diverse proteins involved in transcription, neuronal signaling, and synaptic plasticity. Indeed, recent studies have begun to uncover the functional roles of *O*-GlcNAc, its complex dynamics in the brain, and the interplay between *O*-GlcNAc and phosphorylation.

Although the pace and scope of understanding *O*-GlcNAc has expanded considerably, much still remains to be discovered. Due to the challenge of studying the

modification, evidence linking *O*-GlcNAc to specific biological functions has often been indirect or correlative. This is particularly true in the brain, where the complexity of the nervous system and its unique technical challenges (e.g., post-mitotic cells, multiple cell types, blood-brain barrier, complex organization) render *O*-GlcNAc more difficult to investigate. Nonetheless, in-depth functional studies on proteins will be essential in the future to determine the roles of *O*-GlcNAc in neuronal-specific contexts.

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