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O-GlcNAc cycling: Implications for Neurodegenerative disorders

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Abstract

The dynamic post-translational modification of proteins by O-linked *N*-Acetylglucosamine (O-GlcNAc), termed O-GlcNAcylation, is an important mechanism for modulating cellular signaling pathways. O-GlcNAcylation impacts transcription, translation, organelle trafficking, proteasomal degradation and apoptosis. O-GlcNAcylation has been implicated in the etiology of several human diseases including type-2 diabetes and neurodegeneration. This review describes the pair of enzymes responsible for the cycling of this post-translational modification: O-GlcNAc transferase (OGT) and β -*N*-acetylglucosaminidase (OGA), with a focus on the function of their structural domains. We will also highlight the important processes and substrates regulated by these enzymes, with an emphasis on the role of O-GlcNAc as a nutrient sensor impacting insulin signaling and the cellular stress response. Finally, we will focus attention on the many ways by which O-GlcNAc cycling may affect the cellular machinery in the neuroendocrine and central nervous systems.

Keywords

Glycosylation; O-GlcNAc transferase; O-GlcNAcase

Introduction

The addition and removal of O-GlcNAc on serine and threonine residues of proteins is an important regulatory modification that is comparable to the addition and removal of phosphate by protein kinases and phosphatases. This post-translational modification was discovered over twenty years ago, however its function and importance as a method of protein regulation have only recently begun to be clearly understood (Love and Hanover, 2005; Slawson et al., 2006). Evidence from many laboratories suggests that this modification integrates the nutrient status of the cell and transmits this information to a diverse array of substrates via direct interaction with substrates and/or adapters (Fig. 1). Some of the molecular details of the “O-GlcNAc code” from generation and maintenance to deregulation and disease will be covered in this review. The focus of the review will be on the enzymes of O-GlcNAc cycling, their interactions with adapters and substrates and how O-GlcNAc may impact physiology, particularly in the central nervous system.

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Early studies of protein O-GlcNAcylation suggested that the primary intracellular location of O-GlcNAc was at the nuclear envelope and that the nuclear pore proteins were the most abundant substrates (Miller and Hanover, 1994; Love and Hanover, 2005; Slawson et al., 2006; Hart et al., 2007). Consequently, this led to speculation that O-GlcNAc may play a role in nuclear transport, however later research suggested that this is unlikely, and it is still unclear what the function of nucleoporin O-GlcNAcylation may be. While nucleoporins are easily detected by an abundance of O-GlcNAc, more modestly modified substrates have been identified with the advent of proteomics technology. To date, over five hundred cytoplasmic and nuclear O-GlcNAc-modified proteins have been identified and a majority of these proteins are also phosphorylated (Copeland et al., 2008). These substrates are involved in a myriad of cellular process including, transcription, translation, the stress response, metabolism, protein processing and cellular structure (reviewed in Love and Hanover, 2005; Slawson et al., 2006). The addition of O-GlcNAcylation to the post-translational repertoire exponentially increases the complexity of protein regulation (Hart et al., 2007). Therefore, teasing out the scope and regulation of O-GlcNAc post-translational modification is essential to fully understand the regulation of these cellular processes.

O-GlcNAc and the enzymes that regulate its addition and removal are present in many mammalian tissues. Disrupting this tightly controlled form of protein modification can affect tissues in different ways. Disrupting O-GlcNAc cycling impacts the insulin signaling pathway. The link between irregular O-GlcNAc cycling and insulin resistance has been shown in several animal models by over-expression or deletion of OGT or OGA (McClain et al., 2002; Hanover et al., 2005; Forsythe et al., 2006). In humans, disruption of O-GlcNAc cycling in glucose sensing in tissues such as the liver, pancreas, skeletal muscle or fat may contribute to insulin resistance and type-2 diabetes (reviewed in Akimoto et al., 2005). In addition, aberrant O-GlcNAcylation in the brain is linked to the etiology of neurodegeneration and the group of diseases collectively known as tauopathies (Dias and Hart, 2007). There is also evidence that O-GlcNAcylation is altered in response to aging (Fulop et al., 2008) and heart disease (Clark et al., 2003; Liu et al., 2007).

O-GlcNAc as a modulator of nutrient sensing

One of the core functions of O-GlcNAc cycling may be to serve as a nutrient sensor of glucose flux through the hexosamine biosynthetic pathway (Marshall, 2006). As glucose enters the cell, it is transformed into glucose-6-phosphate that can then either participate in glycogen synthesis, or be converted to fructose-6-phosphate and enter the hexosamine biosynthetic pathway. Fructose-6-phosphate is converted to glucosamine-6-phosphate by the rate-limiting enzyme, L-glutamine:D-fructose-6-phosphate amidotransferase and then into uridine 5'-diphospho-*N*-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is the terminal step in the hexosamine biosynthetic pathway and is utilized by OGT to O-GlcNAcyate proteins, UDP-GlcNAc also negatively regulates the activity of L-glutamine:D-fructose-6-phosphate amidotransferase in a feedback loop to blunt further production of UDP-GlcNAc (Kornfeld, 1967).

Fluctuations in cellular levels of UDP-GlcNAc are caused by changes in the level of nutrients entering the cell. This information is transmitted to target proteins via the enzymes of O-GlcNAc cycling (OGT and OGA). Early studies by Marshall and colleagues linked hexosamine flux and insulin signaling (Marshall et al., 1991). In 1997 we proposed that OGT might play a role in diabetes based on the molecular features of the enzyme (Lubas et al., 1997). We tested this hypothesis by over expressing OGT in the muscle and fat of transgenic mice; the resulting mice were highly insulin resistant (McClain et al., 2002). Determining how the insulin signaling pathway is connected to nutrient excess and the mechanism by which insulin resistance develops is a key step in understanding and preventing type-2 diabetes. O-GlcNAc cycling is

thought to play an important roll in these processes, as it provides a link between sensing nutrient excess in the cell and regulation of the insulin-signaling pathway that controls the cells response to nutrients.

More recent findings have strengthened the link between aberrant O-GlcNAc cycling and insulin resistance, specifically these reports identified key regulators of the insulin signaling pathway that are regulated by O-GlcNAc. Increasing the level of O-GlcNAc using either a chemical inhibitor of OGA, *O*-(2-acetamido-2-deoxy-D-glycopyranosylidene)amino-*N*-phenylcarbamate (PUGNAc), or elevating O-GlcNAc levels with glucosamine induces insulin resistance in 3T3-L1 adipocytes (Vosseller et al., 2002). Insulin resistance in this model was associated with increased O-GlcNAcylation of Insulin Receptor Substrate 1 and β -catenin, while phosphorylation of Akt and glycogen synthase kinase 3 β was decreased. In similar models others have shown that increasing O-GlcNAcylation with PUGNAc inhibits the translocation of the insulin-regulated glucose transporter, GLUT4, to the cell surface (Park et al., 2005; Yang et al., 2008). Also noted was a decrease in phosphorylation of the Insulin Receptor Substrate-1 and glycogen synthase kinase 3 β (GSK3 β) (Park et al., 2005), which subsequently blunts downstream effectors of the insulin signaling pathway and also prevents translocation of the insulin-regulated glucose transporter (GLUT4) to the cell surface. PUGNAc inhibits OGA as well as lysosomal hexosaminidases. Recently, an OGA-selective inhibitor was used on 3T3-L1 adipocytes to increase O-GlcNAc. Surprisingly, this treatment did not induce insulin resistance as measured by 2-deoxyglucose uptake (Macauley et al., 2008). It will be important to use these OGA-selective inhibitors in other models of insulin resistance such as translocation of GLUT4 to the plasma membrane to understand what role OGA plays in insulin resistance.

In addition to regulating proteins in the insulin-signaling pathway, O-GlcNAc also regulates insulin-responsive transcription factors. One of the first to be characterized was Specificity protein 1 (Sp1). It is glycosylated by OGT in its transcriptional activation domain and this modification represses the ability of Sp1 to activate transcription (Yang et al., 2001). O-GlcNAcylation also inhibits the association of Sp1 with its binding partner, TAF110, and may play a role in preventing untimely protein-protein interactions (Roos et al., 1997). Furthermore, there is evidence that O-GlcNAc modification of Sp1 may play a role as a nutritional checkpoint, as hypoglycosylation occurs under conditions of glucose starvation, which leads to cyclic AMP (cAMP)-mediated degradation of Sp1 by the proteasome (Han and Kudlow, 1997). Treatment of cells with high levels of glucosamine or glucose increases the level of O-GlcNAcylation on Sp1 and protects it from proteasomal degradation suggesting that down regulation of transcription during starvation may help the cell to conserve nutrients. Another transcription factor important for regulation of glucose homeostasis, pancreatic and duodenal homeobox 1 (pdx-1), is also a substrate of OGT. Pdx-1 plays an important role in pancreatic function and development, and an increase in glucose has been known to increase its DNA binding affinity (Rafiq et al., 2000). In β -cells, Pdx-1 was found to contain two sites of O-GlcNAc modification (Gao et al., 2003), and the increase in DNA binding affinity in response to increased glucose was correlated with an increase in global O-GlcNAcylation. In addition, the increase in O-GlcNAcylation corresponded to an increase in insulin secretion. NeuroD1 regulates insulin gene expression and is O-GlcNAcylated. Its nuclear/cytoplasmic localization appears to be regulated by the cycling of O-GlcNAc (Andrali et al., 2007). More recently, O-GlcNAc modification of hepatic FoxO1, facilitated by PGC1 α , is increased in diabetes and this modification was associated with the enhanced expression of gluconeogenic enzymes (Housley et al., 2008a; Housley et al., 2008b). In summary, O-GlcNAc cycling has a multi-faceted role in controlling cellular responses to glucose. Its influence is felt in the initial receptor activation, through the intermediate kinases, to the activation of insulin-responsive transcription factors.

The enzymes regulating O-GlcNAc cycling

The two enzymes required for the addition and removal of O-GlcNAc on proteins, are OGT and OGA, respectively (Fig. 2 and 3) (Love and Hanover, 2005). These enzymes are highly conserved throughout evolution and are essential for life in higher eukaryotes. In humans and other higher eukaryotes, OGT and OGA are found in all cells, but are most abundant in the brain and pancreas, both glucose-sensing organs. Both OGT and OGA are expressed as multiple isoforms. These isoforms have differential targeting and activity towards various substrates and these characteristics may serve to explain how a single set of enzymes can control O-GlcNAc cycling in a number of cell types and targets.

Functional domains of OGA

The gene encoding OGA was originally identified as meningioma-expressed antigen 5, thus the gene for OGA is called *MGEA5* and maps to human chromosome 10 (Heckel et al., 1998). The gene encodes two alternatively spliced isoforms of OGA that are expressed in almost all mammalian tissues (Comtesse et al., 2001) (Fig. 2). The longest form of OGA has two distinct domains, an O-GlcNAcase domain at the N-terminus that shows high homology with bacterial hyaluronidases and a putative acetyltransferase domain at the C-terminus (Schultz and Pils, 2002). The acetyltransferase domain may have histone acetyltransferase (HAT) activity (Toleman et al., 2004) but its sequence is dissimilar to other, canonical HAT domains. Interestingly, OGA contains a caspase-3 cleavage site between these domains (Wells et al., 2002). During apoptosis, OGA is cleaved and remains active (Butkinaree et al., 2008). O-GlcNAc has been implicated in the processes of chromatin remodeling and transcriptional repression via its interaction with chromatin associated proteins (Comer and Hart, 2001) and the mSin3A repression complex (Yang et al., 2002), thus it is likely that a HAT domain may be involved. The shorter form of OGA contains only the N-terminal O-GlcNAcase domain, suggesting this domain is responsible for catalyzing the removal of O-GlcNAc (Hanover, 2001). OGA is also glycosylated by OGT (Lazarus et al., 2006) suggesting a possible regulatory feedback loop between these two enzymes and in fact, they have been found to associate together in transcriptional co repression complexes with histone deacetylases (Whisenhunt et al., 2006).

OGA belongs to the family 84 glycoside hydrolases. Hydrolysis by OGA is accomplished through an unusual substrate-assisted mechanism (Macauley et al., 2005). While human OGA has resisted crystallization, the structure of two bacterial hydrolases from the same family has been solved (Dennis et al., 2006; Rao et al., 2006). These homologs display almost perfect identity in the catalytic site and are active against human substrates and therefore are suitable as models for human OGA. The OGA catalytic site contains a deep pocket that accommodates the glycosylated protein. In two other human hexosaminidases (lysosomal hexosaminidase A and B) the floor of the catalytic site is restricted by the presence of a tryptophan residue. In contrast, human OGA and the bacterial OGA contain a pocket that is not constrained by this residue and is much more "open". This difference was exploited to synthesize a specific substrate, and specific inhibitors of OGA (Macauley et al., 2005; Dorfmueller et al., 2006; Kim et al., 2006a; Kim et al., 2006b; Kim et al., 2007). These tools will be key in elucidating the role of OGA in nutrient sensing.

Both the long and short form of OGA contain identical active sites, yet for several years no activity could be detected in the short isoform (Wells et al., 2002). The advent of a sensitive fluorogenic substrate revealed that both isoforms are active *in vitro* (Kim et al., 2006a). Additionally, both isoforms were active against cellular extracts, suggesting that both isoforms are physiologically relevant *in vivo*. It will be essential to understand how each isoform contributes to the balance of O-GlcNAc cycling within the cell.

Functional domains of OGT

OGT is involved in a myriad of cell pathways and glycosylates many substrate proteins, whereas kinases and other enzymes that create post-translational modifications normally only have one, or possibly a few substrates. One of the key questions regarding the function of OGT is how it interacts with so many substrates. To date OGT is known to glycosylate over 500 substrates and this is due, at least in part, to its structure and expression. There are three known isoforms of OGT, all of which share an identical catalytic domain, but differ in the number of tetratricopeptide repeat motifs found at the N-terminus (Fig. 3). The longest form of OGT is found in the nucleus and cytoplasm (ncOGT; 116kD), with the next longest isoform found in the mitochondria (mOGT; 103kD). The shortest form of OGT (sOGT; 70kD) is also nuclear/cytoplasmic. In addition to the differential targeting of these isoforms, the level of expression varies depending on the target tissue. OGT is highly expressed in the pancreatic β cells and the brain (Lubas et al., 1997; Hanover et al., 1999).

Unique N-terminal Domain of the multiple isoforms of OGT

Each of the three OGT isoforms has a unique N-terminus that is thought to contribute to their sub-cellular localization. The human OGT gene is comprised of 23 exons with 2 putative start codons. The unique N-terminus of ncOGT is produced by exons 1 to 4 and ncOGT is found in the nucleus and cytoplasm. This is the most abundant isoform and it has been implicated in proteasomal inhibition, transcriptional repression and stress tolerance (Comer and Hart, 2001; Zhang et al., 2003; Zachara et al., 2004; Zhang et al., 2007). The mOGT isoform is generated by an alternative start site located in exon 5 and produces an N-terminal domain that contains a unique mitochondrial targeting sequence, followed by a membrane-spanning α -helix that localizes the enzyme to the inner leaflet of the mitochondrial membrane (Love et al., 2003). Indeed, ncOGT and mOGT have been shown to have different specificity for substrate proteins (Lazarus et al., 2006) and mOGT may be associated with activation of transcription factor Sp1 by mitochondrial superoxide overproduction (Du et al., 2001). The shortest isoform of OGT is generated by an internal start codon in exon 10 and it does not appear to have any specific targeting information in the sequence. Like ncOGT, sOGT is found in the nucleus and cytoplasm, and the only function attributed to this isoform to date is from a screen for anti-apoptotic factors that identified sOGT as a protein that could protect cells from growth factor withdrawal-mediated apoptosis (Fletcher et al., 2002).

Tetratricopeptide Repeat Domain

Tetratricopeptide repeat (TPR) motifs are an ancient form of repeat that are found in proteins from eukarya, bacteria and archaea (Andrade et al., 2001). They are highly conserved 34-amino acid repeats that are arranged as two α -helices which pack together to form a right handed super-helix with a large groove available for protein and ligand binding (Sikorski et al., 1990). This domain allows OGT to bind many different substrate proteins and each isoform of OGT has a varying number of TPR motifs (Fig 3A) with which to bind a specific set of substrates. The longest form of OGT (ncOGT) has 11.5 TPR motifs, whereas the mitochondrial form (mOGT) has 9.5 TPR motifs. The shortest form of OGT (sOGT) has 2.5 TPR motifs.

The TPR domain of ncOGT has been crystallized and adopts a fold similar to other TPR-containing proteins, such as protein phosphatase 5. Contrary to previously published reports, ncOGT exists as a homo-dimer, with each monomer consisting of 23 anti-parallel α -helices that form a right-handed superhelix. Each superhelix packs together at an angle of approximately 80° (Fig. 3B) (Jinek et al., 2004). The elongated nature of these superhelices may confer an inherent flexibility to this domain of OGT, allowing it to adopt different overall conformations when interacting with different substrate partners, similar to the helical HEAT-repeat domain found in importin- β (Cingolani et al., 1999; Lee et al., 2003). Furthermore, a

striking similarity was observed between the structure of the OGT and importin- α protein interaction domain (Jinek et al., 2004). Importin- α is a protein critical for nuclear import and like OGT, is capable of binding a variety of different proteins. The 10 armadillo repeat motifs found in importin- α form an elongated superhelix. The concave surface of the superhelix contains an unusual conserved asparagine ladder (Lange et al., 2007). In Importin- α , these asparagines are thought to form bidentate hydrogen bonds with the substrate peptide backbone and it is thought that OGT may use a similar mechanism of protein-protein recognition (Jinek et al., 2004). This would also help to account for the ability of OGT to recognize a wide range of physiological substrates. It may also provide another level of regulation for determining the specificity of protein-protein interactions, as multiple isoforms of OGT have different numbers of TPR motifs. Differences in TPR number would change the number of asparagines found in the superhelix structure and could account for differences in substrate specificity between the isoforms. Such differences have previously been observed in *in vitro* experiments (Lazarus et al., 2006).

Requirements of the TPR-substrate protein interaction have been identified for a few proteins. The first to be examined was nucleoporin 62 (Nup62). Removing three TPR repeats did not alter OGT catalytic activity against Nup62, but removing more than three TPR repeats was not tolerated. Interestingly, this truncated OGT containing only three TPRs was capable of autoglycosylation, indicating that substrate recognition was compromised by the deletions, not catalytic activity (Lubas and Hanover, 2000). The binding of OGT to mSin3A, an adaptor protein that is part of a co-repressor complex involved in transcriptional repression, was determined in a similar fashion (see below for further discussion) (Yang et al., 2002). Removal of two TPR motifs abolished binding. These data also suggest that mOGT would not interact with mSin3A as it is missing the first two TPR motifs that are found in ncOGT. Most of the binding interactions between OGT and substrate proteins occur through the TPR repeats, although there are a few reports of binding via the catalytic domain and those will be described below. By modulating the number of TPR motifs, each OGT isoform has numerous, yet distinct binding partners.

Linker Domain

A flexible, 120-residue linker-region is found between the two halves that form the catalytic domain of OGT (Lubas et al., 1997; Roos and Hanover, 2000). This linker is not conserved in the crystallized bacterial glycosyltransferases and appears to be found only in metazoan OGTs (Clarke et al., 2008; Martinez-Fleites et al., 2008). The flexibility of this region has likely contributed to the failure to crystallize the OGT active site in higher metazoans. Understanding how this region contributes to OGT activity is key to fully understanding the regulation of OGT.

Catalytic Domain

The catalytic domain of OGT is 100% conserved across all isoforms and molecular modeling predicts that it is comprised of two Rossmann-like folds, placing it into the GT-B superfamily (Roos and Hanover, 2000). Both ncOGT and mOGT exist as a homodimer (Jinek et al., 2004) and there is some evidence that at least mOGT may be capable of autoglycosylation.

Site-specific mutations indicate that amino acid residues important for catalytic activity are clustered around the UDP-GlcNAc binding pocket (Lazarus et al., 2005). Interestingly, some of the most direct evidence for OGT as a sensor of nutrient levels and its ability to detect the cellular levels of UDP-GlcNAc comes from this study where single point mutations were made in the catalytic domain of mOGT (Lazarus et al., 2005). In this study, over 30 single point mutations were made, many of which caused inactivation of the enzyme. Three mutations, however, were mapped close to the sugar nucleotide-binding site and caused an increase in the

catalytic activity of OGT toward Nup62. These results show that a single point mutation in the catalytic domain of OGT is capable of changing the kinetics of the enzyme with respect to UDP-GlcNAc, which may explain how the enzyme could become de-regulated in human disease and possibly lead to phenotypes such as insulin resistance or even neurodegeneration. To date, none of the known single nucleotide polymorphisms in the OGT catalytic domain map to the single point mutations in this particular study, however, all mutations in the study had an effect on enzyme kinetics, suggesting that the possibility of catalytic domain single nucleotide polymorphisms causing a change in OGT kinetics is very high. The resulting effect on nutrient sensing and/or other pathways regulated by OGT may potentially lead to insulin resistance or other human diseases.

Crystal structures of bacterial OGT largely confirm the modeling of the human OGT (Clarke et al., 2008; Martinez-Fleites et al., 2008). Amino acid residues conserved between the bacterial and human protein cluster around the sugar nucleotide-binding site (Fig. 3B). Mutating histidine 558 inactivates the enzyme. It is likely that this histidine residue forms the general base to deprotonate the serine/threonine hydroxyl group of the acceptor. The deprotonated acceptor, through a nucleophilic attack, displaces the UDP moiety, covalently transferring GlcNAc to the protein acceptor. This mechanism of sugar transfer is conserved in the GT-B superfamily (Lairson et al., 2008). A composite of the catalytic domain from *Xanthomonas* and the human TPRs is shown (Fig 3B).

The catalytic domain is also important for protein partner interactions. A yeast two-hybrid screen identified p38 mitogen-activated protein kinase (p38 MAPK) as a binding partner (Li et al., 2004). The interacting domain was narrowed down to amino acids 979-998 of ncOGT (Cheung and Hart, 2008). Under conditions of limiting glucose, activated p38 MAPK binds better to OGT and is thought to regulate OGT activity by directly facilitating target interactions, as it does not phosphorylate OGT. Interestingly, this region also targets OGT to the plasma membrane during insulin stimulation (see below).

PIP binding activity of OGT

The p38 MAPK binding region contains a recently identified PIP-binding domain or PPO domain (Yang et al., 2008). Specifically, this region of OGT shows a strong interaction with PI(3,4,5)P₃, which targets OGT to the plasma membrane in response to the activation of phosphatidylinositol-3-OH kinase by insulin. Once there, OGT can glycosylate proteins involved in the insulin-signaling pathway, such as Akt and Insulin receptor substrate 1 (Yang et al., 2008). The interaction between OGT and phosphatidylinositol 3,4,5-triphosphate does not appear to alter OGT catalytic activity, suggesting this is not a regulatory modification, but a means to target it to a discrete site of activation within the cell. In addition to relocating OGT to the plasma membrane, insulin stimulation correlates with OGT tyrosine phosphorylation and increased enzymatic activity (Whelan et al., 2008). These are important observations given OGT's suggested role as a nutrient sensor and its role in regulating the insulin signaling pathway.

OGT and neurodegeneration

The number one risk factor for developing a neurodegenerative disease is aging. Additionally, obesity and type-2 diabetes have also been shown to increase the likelihood of developing a neurodegenerative disease. Insulin signaling modulates all of these risk factors. Invertebrate models of neurodegeneration genetically link life span, insulin signaling and neurodegeneration. Specifically, lowered insulin signaling correlated with enhanced longevity and a lowered burden of neurotoxicity (Morley et al., 2002; Hsu et al., 2003). In mammals the paradigm is more complicated, as various tissues are differentially affected by insulin signaling. For example, lowered insulin signaling in the periphery is associated with insulin resistance

and type-2 diabetes, whereas lowered insulin signaling in the brain may extend life span be protective against neurodegeneration (reviewed (Cohen and Dillin, 2008). Caloric restriction in mammals also extends life span and protects against age-related diseases. While it is widely accepted that O-GlcNAcylation plays some role in the pathogenesis of neurodegeneration, its exact function in this role is unclear. Paradoxically, both high fat treatment and caloric restriction increase O-GlcNAcylation in cell culture (Li et al., 2005; Taylor et al., 2008). Studies that have examined changes in O-GlcNAc modified proteins in the aging brain are equivocal (Rex-Mathes et al., 2001; Fulop et al., 2008). What is clear is that OGT is highly enriched in the brain and that proteins in the brain are O-GlcNAcylated. In addition to altering the phosphorylation status of neuronal proteins directly, O-GlcNAc cycling influences a whole host of cellular processes (Fig. 4). These include: transcriptional regulation, signaling, proteasomal degradation, and organelle trafficking. Alterations in any of these pathways could lead neuronal dysfunction. The role of O-GlcNAc cycling in each of these cellular processes will be discussed.

Phosphorylation and O-GlcNAcylation of neuronal proteins

OGT is localized in the nucleus, perikaryon and dendrites of neuronal cells and is also found at pre-synaptic nerve terminals and around microtubules (Akimoto et al., 2003). Some of the proteins known to be glycosylated by OGT include β -amyloid precursor protein (Griffith and Schmitz, 1995), microtubule associated proteins (Arnold et al., 1996; Ding and Vandre, 1996), synapsins (Cole and Hart, 1999), neurofilaments (Dong et al., 1996; Al-Chalabi and Miller, 2003), synaptopodin (Khidekel et al., 2004) and clathrin assembly protein-3 (Murphy et al., 1994). The normal function of the O-GlcNAc modification on most of these proteins is yet to be determined. Both OGT and OGA map to chromosomal regions associated with neurodegenerative disorders. OGT is located on the X chromosome at position Xq13.1, which is very close to the locus responsible for dystonia-parkinsonism syndrome, also found at position Xq13.1 (Haberhausen et al., 1995). OGA is found on chromosome 10 and maps close to a region suggested to be a candidate locus for late-onset Alzheimer's Disease (AD) (10q24.1-q24.3) (Bertram et al., 2000).

One of the first studies to indicate a role for O-GlcNAc modification in AD was performed by Griffith *et al*, showing that O-GlcNAc was up regulated in AD brains compared with age-matched controls (Griffith and Schmitz, 1995). A number of studies then went on to show that alterations in protein O-GlcNAc levels, either by starvation (Liu et al., 2004), over-expression of okadaic acid (Lefebvre et al., 2003). A conditional knockout of OGT (O'Donnell et al., 2004) also effected the level of protein phosphorylation on neurodegenerative disease related proteins, tau and β -amyloid precursor protein. Protein aggregation is one of the characteristic features of neurodegenerative disorders and in AD, aggregates of hyperphosphorylated β -amyloid peptide and tau are a hallmark of this disease (Ross and Poirier, 2004). Aggregation of hyperphosphorylated tau into paired helical filaments is also seen in a number of other neurodegenerative disorders that are collectively known as tauopathies. This group of diseases includes AD, frontotemporal dementia with parkinsonism (FTDP-17), Pick's disease and Amyotrophic lateral sclerosis (Lou Gehrig's disease), reviewed in (Lee et al., 2001). Many believe that hyperphosphorylation of the tau protein may be affected by its O-GlcNAcylation status and that the accumulation of hyperphosphorylated tau may be a causative agent for many tauopathies. However, the pathology of many tauopathies, AD in particular, is so complicated that there has been no agreement in the research field about the role of protein aggregation and whether or not it is a cause of neuronal degradation and death, or a downstream effect of some other pathological event such as a deficiency in neurotransmission, synaptic vesicle recycling, axonal transport or protein degradation by the proteasome (reviewed in Avila et al., 2006). It is clear, however, that AD, and probably other tauopathies, are caused by a disruption of the

cell's machinery. OGT could play a role in this disruption and some of the possible areas where it may be involved are discussed below.

As mentioned above, much emphasis has been placed on the ability of OGT to alter the phosphorylation status of proteins, especially tau. In normal adult human brain, tau is known to have at least 12 O-GlcNAcylation sites (Liu et al., 2004). When knocking out OGT in mouse neurons, the amount of tau phosphorylation was found to increase (O'Donnell et al., 2004), whilst reducing O-GlcNAcylation levels in mice by starvation also caused an increase in tau phosphorylation (Liu et al., 2004; Li et al., 2006). In contrast to the study by Griffith *et al* (1995) mentioned above, where AD brains were found to have an increase in O-GlcNAc, Robertson and colleagues suggested that tissue taken from AD brains had a decrease in O-GlcNAc levels and that this also correlated with an increase in tau phosphorylation (Robertson et al., 2004). Taken together, the results from these studies strongly suggest that there is a relationship between O-GlcNAcylation and phosphorylation on tau, however the nature of this relationship and its function in tau activity and/or regulation is yet to be determined. There are at least 30 known sites of phosphorylation on tau under physiological conditions (reviewed in Mazanetz and Fischer, 2007), thus making it improbable for O-GlcNAc to be regulating all of these sites. There are also discrepancies between which phosphorylation sites of tau are affected by changes in O-GlcNAcylation (reviewed in Lefebvre et al., 2005). However, studies that increase O-GlcNAc using an OGA-specific inhibitor, in PC-12 cells and in a whole animal model, show a decrease in endogenous tau phosphorylation at several physiologically relevant sites (Yuzwa et al., 2008). While it is tempting to speculate that increasing O-GlcNAcylation by an OGA-specific inhibitor could prevent tau hyperphosphorylation and perhaps the formation of tauopathies, these types of experiments will have to be confirmed in several specific models of neurodegeneration.

It is possible that the effect of O-GlcNAcylation on the amount of tau phosphorylation is due to modification of the protein kinases that phosphorylate tau. Many kinases that are known to phosphorylate tau (reviewed in Buee et al., 2000), such as GSK3 β and cdk5, are also known to be substrates of OGT. While the exact function of O-GlcNAcylation on these kinases is unknown, one could speculate that it has a regulatory function such as inhibition or activation. In addition, OGT has been implicated in transcriptional regulation and any potential role in the regulation of kinases may also occur at the level of transcription.

Transcriptional regulation

The first hints that O-GlcNAc cycling could impact transcription came with the identification of O-GlcNAc sites on chromatin (Hanover et al., 1987; Holt et al., 1987; Kelly and Hart, 1989). Shortly thereafter, the C-terminal domain of RNA polymerase II was shown to be a substrate for O-GlcNAc suggesting that O-GlcNAcylation could play a regulatory role in this process (Kelly et al., 1993).

Recently, more mechanistic studies have attempted to approach the problem of what O-GlcNAcylation might be doing to the transcription machinery. Mutational analysis of the ncOGT TPR domain was used to determine that the first six TPR motifs are absolutely required for mSin3A binding and that removing just the first TPR motif can abolish this binding (Yang et al., 2002). These results would also suggest that mOGT, which is missing the first two TPR motifs, would be unable to interact with mSin3A. In the same study, it was discovered that the paired alpha helical 4 domain of mSin3A was sufficient to bind to the OGT TPR motifs and that co-immunoprecipitation of OGT *in vivo* showed a complex forming between mSin3A and HDAC1 (Yang et al., 2002). In addition, OGA was also found to be present in this complex, suggesting that a complex mechanism of glycosylation and de-glycosylation is involved in coordinating transcriptional repression and activation (Whisenhunt et al., 2006). As with

mSin3A, the interaction between OGT and OGA occurs through the first six TPR motifs, however there is also a requirement for the N-terminus region as well (Whisenhunt et al., 2006). The region of OGA that interacts with the TPR motifs and N-terminus of OGT is found in the middle region of the enzyme, between the N-terminal O-GlcNAcase domain and the C-terminal putative HAT domain. The functional significance of this transcriptional co-repression complex was demonstrated using estrogen-responsive genes, where recruitment of the OGT/OGA/mSin3A/HDAC1 complex to the promoter regions of the *CatD* and *CAD* genes caused an increase in O-GlcNAcylation and a complete block of transcription. In whole mice this translated to a reduction in the amount of mammary ductal side branching and less mammary ducts (Whisenhunt et al., 2006). The presence of more O-GlcNAcylation at the promoter regions of genes that have been repressed would indicate that OGA in these complexes has somehow been inactivated and this is likely to occur via a post-translational modification. The exact mechanism for such a form of regulation has yet to be discovered, but one possibility may be in the form of phosphorylation, although one could also speculate that glycosylation by OGT may also play a role.

The tumor suppressor protein p53 is another important substrate of OGT that is involved in the maintenance of DNA integrity and is mutated in approximately half of all human tumors (Hollstein et al., 1994; Vousden, 2000). The site of O-GlcNAcylation on p53 was mapped to serine 149 (Ser149) and the presence of this modification caused the inhibition of phosphorylation at threonine 155 (Yang et al., 2006). In addition, the decrease in phosphorylation at threonine 155 causes a decrease in p53 ubiquitination that protects the protein from ubiquitin-mediated proteasomal degradation and leads to p53 accumulation. Interestingly, mutations of serine 149 to proline and phenylalanine have been found in some human cancers and these mutants would be expected to exhibit altered stability or activity (Licitra et al., 2004; Hsu et al., 1999). Furthermore, O-GlcNAcylation of the carboxyl-terminus of p53 causes an increase in its ability to bind DNA and subsequent transcriptional activation (Shaw et al., 1996).

The oncoprotein c-Myc contains multiple O-GlcNAcylation sites within its N-terminal transactivation domain, a region of the protein that is known to be involved in mediating neoplastic transformation (Stone et al., 1987), inhibition of cellular differentiation (Freytag et al., 1990) and induction of apoptosis (Evan et al., 1992). One of the main sites of glycosylation was reported to be on threonine 58 and this site can also be phosphorylated. Phosphorylation at this position is known to target c-Myc for ubiquitin-mediated degradation by the proteasome and reciprocal O-GlcNAcylation at threonine 58 may also play a role in regulating the stability and turnover rate of c-Myc.

Many other transcription factors are known to be modified (Comer and Hart, 1999; Love and Hanover, 2005), however functions of these modifications are only known for a few, including Stat5A (Gewinner et al., 2004), Elf-1 (Tsokos et al., 2003), NeuroD (Andrali et al., 2007), HMGN-1 (Ahmad et al., 2008), runx2 (Kim et al., 2007), Oct-2 (Ahmad et al., 2006), HIC1 (Lefebvre et al., 2004), CREB (Lamarre-Vincent and Hsieh-Wilson, 2003), FoxO1 (Housley et al., 2008a), and CRTC2 (Dentin et al., 2008). From the information gathered to date, it is clear that O-GlcNAcylation is an important functional modification of a number of transcription factors. This type of post-translational modification allows another level of complexity in the regulation of the transcriptional machinery.

Proteasomal regulation

Many neurodegenerative disorders, and particularly tauopathies, are characterized by the presence of aberrant protein aggregates, and many studies have investigated the cause of aggregation and how this affects the degradation of proteins by the proteasome (reviewed in

Avila et al., 2006). The tau protein has limited ordered structure in solution and is considered a natively unfolded protein (Schweers et al., 1994). Its unordered structure makes it highly prone to aggregation (Pawar et al., 2005) and in the aggregated state, tau is resistant to degradation by the proteasome (David et al., 2002). In fact, it has been suggested that the degradation of tau by the proteasome is regulated by posttranslational modifications such as phosphorylation (Johnson et al., 1989) and does not have an absolute requirement for ubiquitinylation (David et al., 2002). A study in *Drosophila* found that inhibition of the proteasome led to an accumulation of hyperphosphorylated tau and that the proteasome was capable of degrading tau that had been hyperphosphorylated by GSK3 β (Blard et al., 2006). Furthermore, the authors show that inhibition of GSK3 β led to a huge accumulation of non-degradable, hyperphosphorylated tau, suggesting that GSK3 β inhibition allows other kinases to hyperphosphorylate tau in a manner that causes it to be resistant to proteasomal degradation (Blard et al., 2006). As mentioned earlier, GSK3 β is a substrate of OGT and O-GlcNAcylation of this kinase could potentially have a role in regulating its enzymatic activity.

Further evidence for the role of proteasomal degradation in removing hyperphosphorylated tau came from a study by Elliott *et al* (Elliott et al., 2007), where they showed that tau interacts with Bcl2-associated athanogene-1 (BAG-1) in a Hsc70-dependent manner and that this interaction may regulate tau degradation by the proteasome. The authors show that over expression of BAG-1 causes an increase in tau levels due to inhibition of proteasomal degradation, whereas inhibition of BAG-1 causes a decrease in the levels of tau and results in tau hyperphosphorylation (Elliott et al., 2007).

It is possible that OGT and OGA play a direct role in regulating the proteasome and that this may have an effect on the degradation of tau and other proteins involved in disorders of aggregation, such as Huntington's disease. OGT was found to directly modify and inhibit the 19S regulatory particle of the 26S proteasomal subunit, as well as the ATPase, rpt2 and this leads to an overall decrease in proteasomal function (Zhang et al., 2003; Liu et al., 2004). In contrast, knockdown of OGT with siRNA, and overexpression of OGA results in higher levels of protein degradation, suggesting that this is a reversible regulatory reaction (Zhang et al., 2003). These results would suggest that disruption of either OGT or OGA function may interfere with the normal regulation of proteasomal function and in the neuron this may lead to the aggregation of proteins such as tau, A β peptide, synucleins, ataxin or Huntington.

The Cellular Stress Response

In addition to the documented changes in proteasomal function, there is growing evidence that O-GlcNAcylation may be a key player in the general cellular response to stress (Sohn et al., 2004; Zachara et al., 2004; Hanover et al., 2005; Forsythe et al., 2006). This area has been the subject of a number of recent reviews, so we will limit our remarks to those that might be important for neurotoxicity and neurodegeneration. Recently, the formation of stress granules was shown to be regulated by O-GlcNAcylation. Stress granules, in association with P-granules, are involved in mRNA triage, and are thought to play a key role in regulating the stress response (Ohn et al., 2008). It is very likely that the other known stress response pathways (enzymes of detoxication, chaperone induction, ER stress, autophagy, etc) may be similarly responsive to O-GlcNAc modification. Alterations in these pathways by O-GlcNAcylation would clearly alter the ability of neurons to deal with the proteotoxicity associated with neurodegenerative diseases.

Organelle trafficking

One of the most important cellular processes for maintaining homeostasis in the brain is axonal transport of both neurotransmitters and mitochondria. This has been a major area of research, as patients with AD and Parkinson's disease show a disruption in axonal transport, which is

essential for proper growth and signaling (Schindowski et al., 2008). Mitochondrial transport along axons is vital for neuronal function, as mitochondria must be moved to areas of high-energy demand along extended neuronal processes. This anterograde mitochondrial transport is dependent upon a protein complex that tethers the mitochondria to the microtubules. In *Drosophila*, this complex is composed of Miro, Milton, and kinesin heavy chain (Figure 5) (Glater et al., 2006). The mammalian homologs of Milton are OGT-interacting proteins. The interaction of these proteins with OGT is very interesting, as it raises the possibility that in addition to its catalytic activity, OGT may also function as an adaptor molecule in some complexes. Iyer *et al* were able to show that these proteins interacted specifically with the TPR domain of OGT and not the catalytic domain, but more importantly, they showed that OGT is unable to form a stable complex with one of its major substrates, Nup62 (Iyer and Hart, 2003). This indicates that whilst OGT can glycosylate a numerous proteins, it may only form stable complexes with a select subset of these proteins.

GRIF-1

GRIF-1 is a coiled-coil protein that was first identified as a highly expressed protein in rat brain and other excitable tissues such as the heart and skeletal muscle, and is proposed to function as a GABA_A receptor-trafficking factor (Beck et al., 2002). It was later identified as an OGT interacting protein through a yeast two-hybrid screen of a rat brain cDNA library, followed by co-immunoprecipitation (Iyer et al., 2003). The interaction between GRIF-1 and OGT was localized to the TPR domain of OGT however, GRIF-1 was also found to be post-translationally modified by OGT. The human orthologue of GRIF-1 is trafficking protein, kinesin binding 2 (TRAK2). The *Drosophila* orthologue is a protein called Milton, which is a kinesin associated protein required for axonal transport of mitochondria in neurons (Stowers et al., 2002), as well as early acquisition of mitochondria in oocytes (Cox and Spradling, 2006).

The interaction of GRIF-1 and OGT led to the speculation that GRIF-1 may serve to target OGT to GABA_A complexes where it could play a role in GABA signaling cascades (Iyer et al., 2003). However, more recent studies of GRIF-1 have shown that like its orthologue Milton in *Drosophila*, it plays an important role in movement of mitochondria along axonal microtubules (Smith et al., 2006). GRIF-1 binds to the cargo domain of kinesin heavy chain, which in turn allows kinesin to interact with microtubules and promote active motor transport (Smith et al., 2006). This is unusual in that most other known kinesin cargoes bind through the TPR domain of the kinesin light chains. In fact, GRIF-1 competes directly with kinesin light chains in order to bind to the heavy chain, suggesting that kinesin light chains may just be the most common cargo adapter protein that can bind to the kinesin heavy chain cargo domain (Glater et al., 2006). In addition, GRIF-1 has also been shown to directly interact with the Rho GTPase Miro (Fransson et al., 2006). Miro is an integral membrane protein found in mitochondria and is thought to be the molecule that allows the GRIF-1-Kinesin-OGT complex to tether the mitochondria for movement along microtubules (Fig. 5). To date, the functional significance of OGT binding and modification of GRIF-1 within the OGT-GRIF-1-Kinesin-Miro complex is unknown, however one could speculate that OGT is involved in regulating the GRIF-1-Kinesin-Miro interaction, or that it could be another adaptor involved in forming the complex between GRIF-1-Kinesin-Miro and the cargo molecules being transported along axons.

OIP106

OIP106 (OGT-interacting protein 106) is a homologue of GRIF-1. They share approximately 44% sequence similarity and like GRIF-1, OIP106 is highly enriched in neuronal tissue. The OIP106-OGT interaction domain was mapped to amino acid residues 639–859, close to the C-terminal end of the molecule and this domain was shown to interact with TPRs 1–6 in OGT (Iyer and Hart, 2003). OIP106 is known to interact with many of the same proteins as GRIF-1,

including kinesin heavy chains and Miro, and is therefore also thought to be involved in trafficking of mitochondria along microtubules in neurons. Some of the mechanisms for movement of mitochondria along axons, and the role of O-GlcNAcylation, were discussed earlier and involved the tethering of mitochondria to kinesin through the OGT interacting protein GRIF-1 and the Rho GTPase Miro (Brickley et al., 2005; Fransson et al., 2006). Interestingly, GRIF-1 is homologous to the human Huntingtin associated protein-1 (HAP1), which is involved in transport of synaptic vesicles along axons (Gauthier et al., 2004). HAP1 is known to interact with the p150^{glued} dynactin subunit, an essential component of the dynein/dynactin microtubule-based motor complex. It can also bind to kinesin, suggesting it is involved in both anterograde and retrograde transport of vesicles along axons. HAP1 was shown to cluster with synaptic vesicles containing brain-derived neurotrophic factor (BDNF) (Gauthier et al., 2004). These findings suggest a potential mechanism for kinesin cargo selectivity, with HAP1 specifically tethering synaptic vesicles and GRIF-1/Milton tethering the mitochondria. To date, there is no published evidence that HAP1 is an OGT interacting protein like GRIF-1, however similarities between the sequences suggest that HAP1 may potentially interact with OGT. If this were true, then disruption of the OGT signaling cascade could potentially inhibit the axonal transport of both mitochondria and synaptic vesicles, leading to neuronal dysfunction and possibly neuronal death (Fig. 5).

Ataxin-10 is known to form a stable interaction with ncOGT through the TPR motifs (Andrali et al., 2005). Ataxin-10 is a helical armadillo-like protein that is predicted to have a similar structure to the Huntingtin protein. Mutations in the gene for ataxin-10, SCA10, can cause autosomal dominant cerebellar ataxias that result from expansion of a pentanucleotide repeat (ATTCT) in intron 9 of the gene (Andrali et al., 2005). Ataxin-10 interacts with ncOGT through the dimerization motif found in TPR 6 and 7 and the complex is thought to be constitutive in neuronal cells (Marz et al., 2006). The function of this interaction is unknown at this time. Expression of recombinant TPR 6 and 7 was found to inhibit the dimerization of ncOGT and ataxin-10 and this is in agreement with findings from Jinek *et al*, who showed that recombinant expression of the ncOGT TPR domain could also inhibit the formation of ncOGT homodimers. In addition, however, Marz *et al* also showed that the short isoform of OGT, sOGT, was able to dissociate ataxin-10 from its complex with ncOGT. This is interesting because sOGT has only 2.5 TPR motifs and lacks the dimerization motif at TPRs 6 and 7. It must therefore be interacting with ncOGT either through its linker domain, or catalytic domain. In agreement with these findings, unpublished data from our laboratory has also shown that sOGT is capable of acting as a dominant negative inhibitor of both ncOGT and mOGT. These results seem unusual given that sOGT, ncOGT and mOGT are often found in the same cell, however this may also provide another level of regulation for OGT and its interaction with substrate proteins.

It is clear from the above examples, that the TPR domain of OGT is involved in recognition and binding of substrate proteins and that in some cases, these substrates can form stable complexes with OGT. This would suggest that OGT is playing an integral role in several cellular processes, such as transcriptional regulation or mitochondrial trafficking along microtubules, where a mechanism of post-translational modification via a yin-yang relationship with phosphorylation is too simplistic of a model to account for the complicated interactions of proteins within these regulatory complexes.

Other Proteins

Many other neuronal proteins are known to be O-GlcNAcylated and these include synapsin I, neurofilaments H, M and L, assembly protein 3, synaptopodin and bassoon (Lefebvre et al., 2005). In the case of synapsin I/II, increasing hippocampal O-GlcNAc levels is associated with increases in phosphorylation at several Erk1/2 and CaM kinase II sites and the activity of these enzymes is also elevated (Tallent et al., 2009). Much more research is needed in order to

determine the normal function of these modifications and the role they may play in neurodegenerative disorders such as Alzheimer's disease, FTDP and Huntington's disease.

SUMMARY AND CONCLUDING REMARKS

O-GlcNAcylation of proteins clearly has important, global effects on many signaling pathways within the cell and this review attempts to outline the importance of research into the functions and mechanisms of the enzymes involved, in addition to the many possible roles of O-GlcNAcylation in the pathology of neurodegeneration. Much of the focus in neurodegenerative research has previously been placed on the importance of the phosphorylation status of the microtubule associated protein tau and A β peptide. However, it is clear that although phosphorylation may play an important role in tau function and subsequent aggregation, many other factors such as kinase regulation, transcription, proteasomal degradation and axonal transport are equally important. In order for the role of O-GlcNAcylation in neurodegenerative disorders to be understood, a broader approach with a focus on the disruption of the cellular machinery triggered by stress and proteotoxicity may be necessary. Furthermore, a much better understanding of the enzymes OGT and OGA, and their role in regulating cellular events is necessary in order to decipher to "O-GlcNAc code" with respect to a role in the central nervous system.

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The 'O-GlcNAc code'

Fig. 1. The 'O-GlcNAc code'

In this review, we will highlight the roles of the nutrient sensing hexosamine biosynthetic pathway to generate UDP-GlcNAc, the precursor to O-GlcNAc (lower left). The enzymes of O-GlcNAc cycling, O-GlcNAc transferase and O-GlcNAcase (upper left) are differentially targeted and by interacting with adapter molecules (Trak1, HAP etc.), mediate the dynamic O-GlcNAc modification of key cellular substrates shown in the lower right.

Fig 2. Structure and function of OGA isoforms

The two main isoforms of O-GlcNAcase (long OGA and short OGA) contain a catalytic domain (blue) and the N-terminus that belongs to the family 84 of glycoside hydrolases. A crystal structure from *Clostridium perfringens* with PUGNAc in the active site is shown. Long OGA also contains a histone acetyltransferase domain at the C-terminus that is lacking in short OGA. The HAT domain from the PDB structure GCN5 is shown in green for illustrative purposes. Short OGA contains a unique 15 amino acid tail (yellow).

Fig 3. Structure and function of OGT isoforms

OGT is expressed as three main isoforms with varying numbers of tetratricopeptide repeat domains (TPR, purple). These differentially targeted isoforms all contain an identical catalytic domain (red) that is interrupted by a linker domain (gray). A phosphatidylinositol 3,4,5-triphosphate targeting domain (PPO) is found in all isoforms and directs the enzyme to the plasma membrane following PI-3 Kinase activation. mOGT contains a unique mitochondrial targeting sequence (MTS) that directs this isoform to the mitochondria. B. A composite of the TPR (purple) and catalytic domain (red) based upon the PDB files (1W3B and 2VSN, respectively) is shown. The catalytic domain is in complex with UDP. Both monomers of the homodimer are shown in their approximate orientation to each other, with one monomer (lighter opacity) in the horizontal plane and the other (red and purple) presented in the vertical plane. The PPO domain on the outer surface of the catalytic domain is illustrated in both monomers.

OGT as a cellular regulator

Fig 4. O-GlcNAc cycling and neurodegeneration: A panoply of possible mechanisms

OGT acts on numerous intracellular targets with established roles in neuronal function and the protection against proteotoxicity. Clockwise from the upper left, OGT modifies Tau and alters its phosphorylation suggesting an influence on Tau aggregation. OGT influences numerous kinase cascades including those of insulin and MAP kinase signaling which could impact the cellular response to stress. O-GlcNAc impacts the transcriptional and translational machinery by decorating chromatin and playing a key role in the formation of stress granules involved in mRNA triage. OGT is also an endogenous inhibitor of the proteasome, limiting proteasomal degradation in response to nutrient flux. Finally, OGT and its adapter molecules such as Trak1 and Trak2 (and possibly Hap) may play a key role in the organelle trafficking and axonal transport critical for neuronal function. Many of these processes are known to be dysregulated in neurodegenerative diseases such as the tauopathies and Alzheimer's disease.

Fig 5. Axonal movement of mitochondria and neurosecretory vesicles: possible involvement of O-GlcNAc transferase

OGT and organelle transport. OGT binds to the coil-coil domain of the organelle adapter GRIF1 and may regulate the interaction of this adapter with kinesin motors. This complex is tethered to mitochondria by the protein Miro. This association could allow for modulation of the trafficking of mitochondria in response to nutrient flux. Hap1 shares the same coil-coil domain; OGT could interact with Hap1 through this region and regulate synaptic vesicle movement in a similar mechanism.