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O-GlcNAc Cycling: How a Single Sugar Post-Translational Modification Is Changing the Way We Think About Signaling Networks

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Abstract O-GlcNAc is an ubiquitous post-translational protein modification consisting of a single *N*-acetylglucosamine moiety linked to serine or threonine residues on nuclear and cytoplasmic proteins. Recent work has begun to uncover the functional roles of O-GlcNAc in cellular processes. O-GlcNAc modified proteins are involved in sensing the nutrient status of the surrounding cellular environment and adjusting the activity of cellular proteins accordingly. O-GlcNAc regulates cellular responses to hormones such as insulin, initiates a protective response to stress, modulates a cell's capacity to grow and divide, and regulates gene transcription. This review will focus on recent work involving O-GlcNAc in sensing the environment and regulating signaling cascades. J. Cell. Biochem. 97: 71–83, 2006. © 2005 Wiley-Liss, Inc.

Key words: O-GlcNAc; O-GlcNAc transferase; O-GlcNAcase; diabetes; stress; cell cycle; transcription; signaling; phosphorylation

THE ROAD NOT TAKEN: THE DYNAMIC CYCLING OF O-GlcNAc

The field of glycobiology was turned inside-out more than 20 years ago by the discovery of nuclear and cytoplasmic glycoproteins containing a single N-acetylglucosamine moiety linked to serine and threonine hydroxyl residues [Torres and Hart, 1984]. This was a startling discovery for two reasons. At this time, glycosylated proteins were thought to exist only in luminal compartments and on the cell surface. In addition, unlike extracellular complex glycans, which are static, O-GlcNAc rapidly cycles on and off proteins on a time scale similar to that for phosphorylation/dephosphorylation. Several laboratories have now demonstrated O-GlcNAc to be a highly dynamic process responsive to the extracellular environment [Kearse and Hart, 1991; Vosseller et al., 2002; Zachara et al., 2004; Slawson et al., 2005]. O-GlcNAc is similar to a protein phosphorylation in that both modifications are found on serine and threonine residues, both are dynamically added and removed from proteins in response to cellular signals, and both alter the functions and associations of the modified protein. Many phosphorylation sites are also known glycosylation sites [Chou et al., 1995a,b]. However, the view that O-GlcNAc is simply reciprocal to phosphorylation is an overly simplistic model, since several proteins can be concomitantly phosphorylated and O-GlcNAcylated. Additionally, adjacent phosphorylation and glycosylation sites can regulate the addition of either moiety [Kamemura et al., 2002]. A multitude of kinases and phosphatases exist in cells; however, only one catalytic subunit for the addition (O-GlcNAc transferase) or removal (O-GlcNAcase) of O-GlcNAc has been thus far found in mammals (Fig. 1) [Haltiwanger et al., 1990, Kreppel et al., 1997; Dong and Hart, 1994; Lubas et al., 1997].

O-GlcNAc Transferase

O-GlcNAc transferase (uridine diphospho-N-acetylglucosamine: polypeptide β-N-acetylglucosaminyltransferase) is responsible for catalyzing the addition of N-acetylglucosamine

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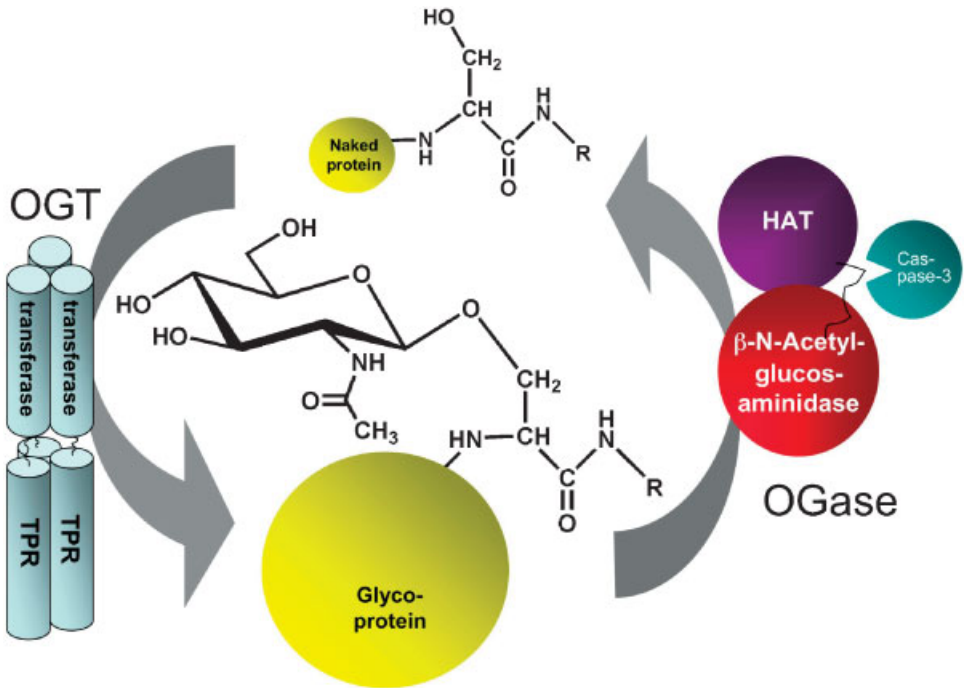

Fig. 1. O-GlcNAc is dynamically added to and removed from serine and threonine residues by O-GlcNAc transferase (OGT) and O-GlcNAcase, respectively. The human OGT trimer consists of tetratricopeptide repeats (TPR) as well as the transferase domain. O-GlcNAcase consists of a β-N-acetylglucosaminidase domain and a HAT domain with a caspase-3 cleavage site between them.

to the hydroxyl group of serine or threonine residues of a target protein [Haltiwanger et al., 1990]. All metazoans studied to date from *C. elegans* to humans, including plants, contain a highly conserved O-GlcNAc transferase (OGT) [Kreppel et al., 1997; Lubas et al., 1997]. Surprisingly, neither enzyme nor O-GlcNAc itself is found in prokaryotes or budding yeast, suggesting that O-GlcNAc evolved with multicellular organisms.

In liver, OGT consists of a heterotrimer of two 110 kDa subunits and one 78 kDa subunit [Haltiwanger et al., 1992]. The 78 kDa subunit is differentially expressed in certain tissue with detectable levels only in kidney, liver, and muscle while the 110 kDa band is detectable in all tissue examined and exists as a homotrimer [Kreppel et al., 1997]. Additionally, a mitochondrial form of OGT exists. This isoform is enriched in a 103 kDa species that contains a unique mitochondrial targeting sequence normally found in plants [Love et al., 2003]. Localization studies indicate that this protein is targeted to the inner mitochrondrial membrane, and while the species appears catalytically active in vitro, few O-GlcNAc modified proteins are found within this compartment [Love et al., 2003].

The 110 kDa subunit is composed of two distinct domains, an N-terminal protein interaction domain containing multiple tetratricopeptide repeats (TPR), a catalytic C-terminal domain, and bridging the two domains is a nuclear localization sequence [Kreppel et al., 1997; Lubas et al., 1997]. The TPR domains appear to modulate the interactions of OGT and its protein substrates, giving OGT its unique substrate specificity. The crystal structure of the human TPR domain of OGT shows a stacking of antiparallel α-helices into a long cylindrical tube [Jinek et al., 2004]. α-Helices comprise the TPR repeat domains and dimerize forming the inner and outer faces, which in turn give rise to a continuous hydrophobic core [Jinek et al., 2004]. From the crystal structure data, the TPR domain clearly forms an elongated, flexible scaffold of numerous electrostatic surfaces, which allow multiple protein–protein contacts and facilitates a wide range of protein interactions.

Regulation of OGT activity and substrate specificity is complex and not yet fully understood.

The TPR domains control the enzyme's affinity for targeting protein subunits and substrates, but additional protein–protein interactions and post-translational protein modifications (PTM) greatly increase the complexity of OGT regulation. OGT is modified by O-GlcNAc and is recognized by anti-phosphotyrosine antibodies, but the PTM sites have not been mapped and how the PTMs affect enzymatic activity is largely unknown [Kreppel et al., 1997]. Recent data suggest that tyrosine phosphorylation activates OGT [Whelan et al., unpublished]. The key high-energy donor substrate for OGT is UDP-GlcNAc. Amazingly, the enzyme appears to have multiple apparent Km values for UDP-GlcNAc, when a peptide is the acceptor substrate [Kreppel and Hart, 1999]. Furthermore, activity toward peptide substrates increases as UDP-GlcNAc increases, and in kinetic studies using a peptide derived from known O-GlcNAc modified proteins (the RNA polymerase II CTD10mer) [Kelly et al., 1993], the enzyme was never saturated with UDP-GlcNAc at very high concentrations well beyond the physiological range [Comer and Hart, 2001]. A low apparent Km (~500 nM) gives the enzyme a competitive advantage compared to UDP-GlcNAc transporters in the ER and Golgi, which rapidly move UDP-GlcNAc from the cytosol to the lumen [Haltiwanger et al., 1990]. Consequently, cellular activity of OGT toward protein substrates is intimately dependent on the levels of UDP-GlcNAc, which unlike other sugar nucleotides rapidly changes in concentration in response to a multitude of nutrient and environmental factors [Haltiwanger et al., 1990]. Therefore, OGT is able to sense changes in UDP-GlcNAc concentration and in turn, transduce this information to protein regulatory networks.

OGT activity toward peptide and protein substrates is also dependent on the presence of other post-translational modifications. Studies using a peptide developed from the C-terminal domain of RNA polymerase II demonstrated a reciprocal activity of OGT and CTD kinase toward modified substrates [Comer and Hart, 2001]. When a CTD10mer was phosphorylated in vitro with CTD kinase, OGT was unable to glycosylate the peptide [Comer and Hart, 2001]. Further analysis of this peptide, revealed a substoichiometric amount of incorporated phosphate near the N-terminus. OGT appears to bind sequentially to the CTD10mer, which is disrupted by phosphorylation [Comer and Hart, 2001]. When a CTD5mer was synthesized with an O-GlcNAc on Threonine 4 of each CTD repeat, CTD kinase was unable to phosphorylate this peptide. Since the major phosphorylation sites are Serines 2 and 5 of the CTD repeat, these data indicate that the affect of the glycopeptide is not due to capping the reactive sites, but to disrupting the physical reaction of CTD kinase with the peptide [Comer and Hart, 2001].

Since OGT has a vast number of cellular substrates, OGT target regulation is paramount. Apart from UDP-GlcNAc levels, cellular mechanisms exist to recruit O-GlcNAc transferase to specific target proteins. Initially identified through yeast two-hybrid analysis, a novel OGT binding protein was found named OGT interacting protein, OIP106 [Iyer et al., 2003]. This protein is a homolog of the $GABA_A$ receptor associated protein, GRIF-1 with ~40% sequence homology [Beck et al., 2002; Iyer et al., 2003]. Both of these proteins immunopurify with OGT and specifically interact with the TPR domains of OGT as determined by blot overlay interaction studies [Iyer et al., 2003]. These proteins are modified by O-GlcNAc, but the O-GlcNAc appears to have no effect on binding [Iyer and Hart, 2003; Iyer et al., 2003]. Both proteins appear to target OGT to specific complexes within the cell further increasing the potential regulation of OGT.

Less structural information is known about the C-terminal domain of OGT. C-terminal deletions of OGT lead to complete loss of activity toward both protein and peptide substrates suggesting the catalytic core of the enzyme is located in the C-terminus [Lubas and Hanover, 2000]. Through molecular modeling techniques, the C-terminal domain shows structural similarity to the glycogen phosphorylase superfamily of carbohydrate active enzymes [Wrabl and Grishin, 2001]. Molecular models predict that the C-terminal domain adopts a fold consisting of two Rossmann-like domains, and the presence of a conserved acidic residue in helix 4 of the second domain identifies this region to be the putative UDP-GlcNAc binding site [Wrabl and Grishin, 2001].

In humans, the *OGT* gene is mapped to the X chromosome region, q13 [Shafi et al., 2000]. When the *OGT* gene was knocked-out in mice, the loss of OGT function led to embryonic lethality [Shafi et al., 2000]. Newborn mice with

a neuron-specific knockout of OGT, developed using the Syn1-Cre transgene that restricts Cre recombinase expression to neurons, failed to develop normal locomotion and were smaller than their littermates. These animals rarely nursed and died by day 10 [O'Donnell et al., 2004]. Examination of brain and spinal cord tissue of these mice demonstrated an increase in the production of microtubule-associated protein, tau, along with an increase in the levels of hyperphosphorylated tau [O'Donnell et al., 2004].

A similar approach in T-cells leads to apoptosis; therefore, an inducible OGT knockout fibroblast cell line was generated in order to facilitate more detailed biochemical studies [O'Donnell et al., 2004]. Cre retrovirus induced a loss of OGT in fibroblasts; these cells showed signs of growth senescence and die by day 12 [O'Donnell et al., 2004]. Although total protein levels appear unaffected by the loss of OGT, Sp1 levels are markedly decreased while cyclin-dependent kinase inhibitor, p27 is increased. These cells also show a failure to respond to serum after serum deprivation [O'Donnell et al., 2004]. OGT is essential for proper cellular function including growth and response to extracellular stimulus.

O-GlcNAcase

O-GlcNAcase was first characterized as a neutral hexosaminidase, hexosaminidase C and often compared to the lysosomal acidic hexosaminidases A and B. However, analysis and purification of hexosaminidase C identified it as a specific β-N-acetylglucosaminidase, O-Glc NAcase [Dong and Hart, 1994]. O-GlcNAcase was subsequently purified from cow brain, found in a tightly associated complex with several other soluble proteins, and cloned based upon MS/MS sequencing [Gao et al., 2001]. Upon cloning and sequencing, O-GlcNAcase was found to be identical to MGEA5, a putative hyaluronidase cloned based upon its association with meningioma [Heckel et al., 1998].

The N-terminal portion of O-GlcNAcase is weakly homologous to a hyaluronidase, while the C-terminus contains a putative acetyltransferase domain [Heckel et al., 1998; Gao et al., 2001; Schultz and Pils, 2002]. Secondary structural alignment studies demonstrate a significant congruence between O-GlcNAcase and the GCN5-related family of acetyltransferases [Schultz and Pils, 2002]. Enzymatic in vitro studies of O-GlcNAcase confirmed the inherent acetyltransferase activity of the enzyme [Toleman et al., 2004]. Specifically, activity was mapped to the C-terminal domain of the enzyme [Toleman et al., 2004]. Interestingly, the 75 kDa splice variant lacking this domain localizes to the nucleus, so the possibility exists that this variant could act as a dominate negative enzyme regulating some aspect of full length O-GlcNAcase's acetyltransferase activity.

Additionally, between the two different catalytic domains is a caspase-3 cleavage site [Wells et al., 2002]. During an apoptotic response, caspase-3 cleaves O-GlcNAcase, but the enzyme still retains full β-N-acetylglucosaminidase activity. However, it remains unclear how the cleaved enzyme is regulated or what occurs to the acetyltransferase domain. A scenario can be envisioned in which O-GlcNAcase is cleaved, and the β-N-acetylglucosaminidase portion begins to remove O-GlcNAc residues in an unregulated manner, and the acetyltransferase portion moves into the nucleus either acetylating proteins or interfering with normal GCN-5 acetyltransferase activity.

Clearly, regulation of O-GlcNAc is dependent upon the actions of the O-GlcNAc processing enzymes, O-GlcNAc transferase and O-GlcNAcase. Although our understanding of each enzyme has grown over the past few years, there is still much to learn. Full-length crystal structures are needed for each enzyme, which would provide detailed analysis of the catalytic domains and facilitate the production of more specific inhibitors. More understanding of how phosphorylation and glycosylation regulate activity and cellular location is needed. Finally, more detailed studies are needed of the regulation of specific protein–protein interactions that control these O-GlcNAc processing enzymes.

SENSING THE ENVIRONMENT: REGULATION OF SIGNALING NETWORKS

Unlike the static nature of extracellular glycosylation, O-GlcNAc cycles dynamically by the combined actions of OGT and O-GlcNAcase in response to the extracellular environment. When T lymphocytes were treated with the mitogenic lectin concanavalin A, cytoplasmic O-GlcNAc levels decreased while nuclear O-GlcNAc levels increased rapidly [Kearse and

Hart, 1991]. Neutrophils also rapidly modulate their O-GlcNAc levels dynamically in response to a chemotactic agent [Kneass and Marchase, 2004].

Interactions With Signaling Molecules

Recent work on the signal transducer and activator of transcription (Stat) family proteins has provided insight into how O-GlcNAc can regulate a signaling network. When Stat 5 is hormonally activated, the protein becomes phosphorylated, dimerizes, translocates into the nucleus, and activates target gene transcription. Stat 5 is modified by O-GlcNAc after hormonal induction, but only after entry into the nucleus [Gewinner et al., 2004]. O-GlcNAcylation of Stat 5 is required for Stat 5 binding to CREB [Gewinner et al., 2004]. These data strongly suggest that O-GlcNAc is a necessary requirement for proper function of Stat 5 after a signaling event. Other signaling molecules also appear to be regulated by O-GlcNAc levels. Cytolocalization of PKC-α and PKC-ε changes in response to elevated O-GlcNAc [Matthews et al., 2005]. Less PKC-α and PKC-ε were located at the membrane where PKC activation occurs, which in turn dampens PKC signaling. The resulting change in PKC localization has the potential to alter a cells ability to respond to an extracellular signal potentially having a profound effect on cell signaling.

The interaction of the O-GlcNAc cycling enzymes with signaling molecules is another factor regulating cellular responses. OGT is found in a tight complex with protein phosphatase, 1β and 1γ [Wells et al., 2004]. This exciting finding lends credence to the purposed "yin-yang" complex of reciprocal O-phosphate/O-GlcNAc on proteins. The yin-yang hypothesis states that O-GlcNAc and O-phosphate are reciprocal to each other on a given amino acid and work together to modulate protein function (Fig. 2). The OGT/PP1c complex potentially removes a phosphate from a serine or threonine, and then adds back to that amino acid an O-GlcNAc moiety. When co-purified together, the OGT/PP1c complex has both O-GlcNAc transferase and phosphatase activity [Wells et al., 2004]. Additionally, when phosphopeptide substrates are used in enzyme assays, the phosphatase removes the phosphate and OGT concomitantly adds a N-acetylglucosamine residue back [Wells et al., 2004].

Insulin Signaling and Diabetes

Is O-GlcNAc just a placeholder to prevent site-specific phosphorylation? Unlikely, the regulatory strength of O-GlcNAc is that the N-acetylglucosamine molecule is ideally suited to act as a nutrient sensor. When glucose enters the cell, roughly 2–5% is diverted into the hexosamine biosynthetic pathway [Marshall et al., 1991], which makes the high energy UDP-GlcNAc substrate for OGT. Since OGT is exquisitely sensitive to the levels of UDP-GlcNAc, cellular O-GlcNAc levels are respondent to flux through the hexosamine pathway. A clear example of O-GlcNAc nutrient sensing is demonstrated in the onset of type II diabetes.

Flux through the hexosamine biosynthetic pathway either through increased glucose uptake or glucosamine treatment, which is distal to glutamine: fructose-6-phosphate transferase (GFAT) the rate-limiting enzyme of the pathway, causes insulin resistance in cultured adipocytes [Marshall et al., 1991]. The actions of O-GlcNAc appear to be mediated through a dampening of the insulin-signaling cascade [Vosseller et al., 2002]. Elevated O-GlcNAc dampens the phosphorylation and subsequent activation of AKT. Reduced AKT phosphorylation appears to be partially mediated by increased glycosylation of the insulin receptor substrate 1 (IRS-1) [Vosseller et al., 2002]. Not only has this been seen in adipocyte models but also in human coronary artery endothelial cells. IRS-1, IRS-2, and the p85 subunit of PI-3 kinase have elevated O-GlcNAcylation after increased flux through the hexosamine pathway [Federici et al., 2002]. Attenuating insulin signaling severely impaired a cell's ability to uptake glucose [Vosseller et al., 2002]. These affects appear to be mediated by reduced translocation of Glut-4 to the membrane [Park et al., 2005]. Since Glut-4 is also modified by O-GlcNAc [Park et al., 2005] and many proteins in Glut-4 transport vesicles are O-GlcNAc modified [Buse et al., 2002], it is unclear whether the reduction in translocation is due to O-GlcNAc blocking of Glut-4 phosphorylation, AKT activation, or the O-GlcNAcylation of vesicular trafficking proteins [Cole and Hart, 2001].

Another key insulin responsive metabolic pathway affected by O-GlcNAc is glycogen metabolism. Glycogen synthase, which converts activated glucose into glycogen, is modified by O-GlcNAc in NIH 3T3-L1 adipocytes [Parker

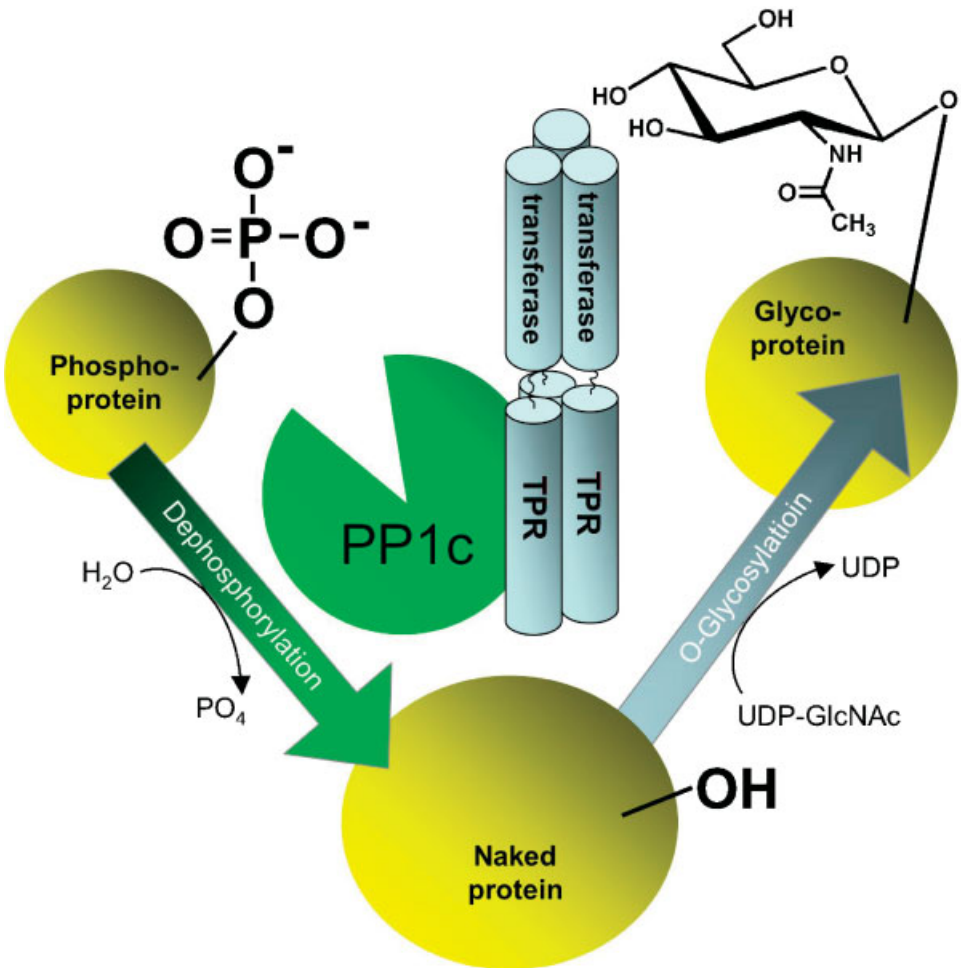

Fig. 2. The OGT/PP1 complex may act to dephosphorylate and glycosylate substrates allowing for more possibilities in target protein regulation. First, O-phosphate is hydrolyzed from a serine or threonine leaving a naked protein. This protein can then be O-GlcNAc modified at the former phosphorylation site.

et al., 2003]. The O-GlcNAc modification was increased in response to high glucose and glucosamine treatment, while enzymatic activity was impaired under the same conditions [Parker et al., 2003; Marshall et al., 2005]. Interestingly, glycogen synthase kinase 3β (GSK-3β) is a substrate for OGT, and its glycosylation could potentially influence GSK-3β activity toward glycogen synthase [Lubas and Hanover, 2000]. Animal models strengthen these data. Mice made diabetic by treatment with streptozotocin demonstrated reduced glycogen synthase activity and an increase in O-GlcNAc modified glycogen synthase [Parker et al., 2004]. The reduction in glycogen synthase could not be correlated with an increase in phosphorylation of serine 640, a key regulatory site on the protein [Parker et al., 2004]. Impairment of glycogen metabolism creates a vicious loop of increased intracellular glucose, which will elevate O-GlcNAc modified protein levels and further suppress insulin signaling.

Transgenic mice overexpressing O-GlcNAc transferase targeted to adipose, cardiac, and skeletal muscle show signs of insulin insensitivity [McClain et al., 2002]. Both muscle and adipose tissue demonstrated insulin resistance, and mice were hyperleptinemic [McClain et al., 2002]. Additionally, in genetic rat models of diabetes or streptozotocin induced diabetic rats, O-GlcNAc transferase protein expression levels are elevated in cornea [Akimoto et al., 2003], pancreas [Akimoto et al., 2000], and aortic smooth muscle cells [Akimoto et al., 2001].

Cadiomyocytes from STZ-induced diabetic mice exhibit increased O-GlcNAc and impaired

cardiac function, but when an adenovirus expressing O-GlcNAcase was delivered into the myocardium of these mice, cellular function improved [Hu et al., 2005]. Dramatic improvement in intracellular calcium transient, sarcoplasmic reticulum calcium load, and contractility with greater fractional shortening were seen. In addition, isolated perfused hearts developed internal pressure, and show changes in the expression of sarcoplasmic reticulum proteins responsible for calcium maintenance [Hu et al., 2005].

Clearly, proper function and expression of O-GlcNAcase is crucial in cellular response to nutrients. Recent genetic screens in Mexican Americans with Type II diabetes showed a decrease in O-GlcNAcase expression with age and onset of the disease [Lehman et al., 2005]. These data strongly suggest one of the main causative factors behind diabetic pathology is increased O-GlcNAc.

A potentially powerful tool has been developed in which a strain of *C. elegans* is lacking the catalytic domain of OGT [Hanover et al., 2005]. These organisms exhibit no detectable cellular O-GlcNAcylation but are viable. They do have dramatically altered sugar storage with increased glycogen and trehalose storage, but neutral lipid storage is reduced by nearly 70% [Hanover et al., 2005]. Interestingly, when crossed with a DAF2 mutant (lacking an insulin like receptor), the OGT/DAF-2 mutant did not enter into dauer formation like the DAF-2 mutant alone. These data suggests that OGT may act to suppress insulin function in *C. elegans* [Hanover et al., 2005] further supporting the data seen in tissue culture and mammalian animal models. These data strongly suggest that O-GlcNAc modifications of key enzymes in the insulin signaling cascade dampens a cell's ability to respond to increases in extracellular glucose levels, and contributes to the onset of type II diabetes (Fig. 3).

Furthermore, the damage done by high glucose to the vascular endothelia is partially due to inhibition of endothelial nitric oxide synthase (eNOS) via increased O-GlcNAc modification on eNOS [Du et al., 2001]. eNOS is an important vasodiltator, inhibits platelet aggregation and vascular smooth muscle proliferation, reduces vascular permeability and LDL oxidation [Du et al., 2001]. The changes in eNOS glycosylation appear to be mediated by flux through the hexosamine biosynthetic pathway and activation of GFAT due partially to elevated reactive oxygen species [Du et al., 2001]. Moreover, eNOS is activated by AKT phosphorylation at serine 1177 but under high glucose conditions phosphorylated eNOS at this site is decreased [Du et al., 2001]. Serine 1177 phosphorylation is also decreased in rat penis tissue under diabetic conditions where this site is blocked by O-GlcNAc [Musicki et al., 2005]. The loss of eNOS phosphorylation is correlated with diabetes-associated erectile dysfunction in these animals [Musicki et al., 2005]. Inhibition of eNOS by O-GlcNAc could lead to many of the potentially life threatening affects of diabetes such as vascular damage, heart attack, and stroke.

Stress Response Pathways

Interestingly, cells stressed with a nutrient-poor or nutrient-excessive environment were shown to elevate O-GlcNAc levels, and this elevation promotes cell survival [Zachara et al., 2004]. Thus, not only does O-GlcNAc protein appear to be a nutrient sensor capable of responding rapidly to the changes in nutrient levels, O-GlcNAc also appears to act as a sensor of cellular stress. The change in O-GlcNAc protein modification appears to be a universal response to stress, since all forms of stress tested (osmotic, ethanolic, oxidative, and heat shock) to date rapidly raise O-GlcNAc levels [Zachara et al., 2004]. When OGT protein levels are reduced either though cre-lox knockout or RNAi, cell responsiveness to stress is lost [Zachara et al., 2004]. After a more prolonged stress response, the activity of OGT increases, suggesting that rapid induction of OGT activity after stress is protective to cells. In support of this work, a recent study showed that increased O-GlcNAc was protective under hypothermic conditions [Sohn et al., 2004].

One potential role in stress protection by O-GlcNAc is through the induction and increased stability of heat shock proteins. Cells with elevated O-GlcNAc demonstrate an increase in both heat shock protein 70 and 40 induction compared to control cells after stress [Zachara et al., 2004]. The faster induction of heat shock proteins would allow cells to stabilize their microenvironments more rapidly after a stress event. Certain heat shock proteins appear to have lectinic activity toward O-GlcNAc suggesting a further role of O-GlcNAc in stress [Guinez et al., 2004].

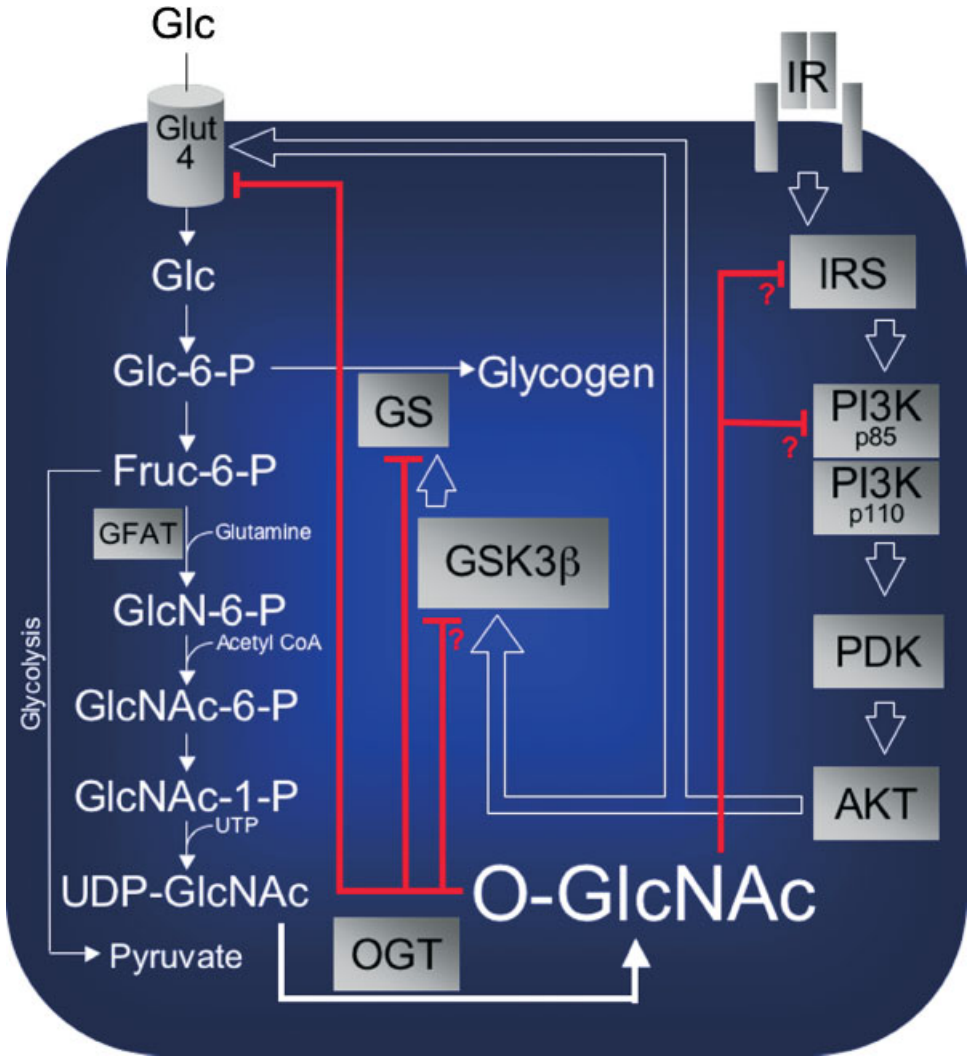

Fig. 3. The O-GlcNAc modification of several proteins involved in glucose metabolism in adipose tissue may lead to perpherial insulin resistance. Increased flux through the hexosamine biosynthetic pathway (HBP) results in greater UDP-GlcNAc levels upon which OGT is highly dependent. The increased O-GlcNAcylation of glycogen synthase (GS) reduces glycogen storage. Increased O-GlcNAcylation of IRS and PI3K leads to suppressed AKT activity and Glut4-mediated glucose uptake.

Cell Cycle Regulation

Since O-GlcNAc appears to monitor the levels of nutrients and stressors in the environment, O-GlcNAc may potentially influence cell growth and proliferation. Studies in *Xenopus laevis* oocytes suggest this. When mature oocytes were microinjected with galactosyltransferase, an enzyme which caps terminal GlcNAc residues and blocks removal of the sugar, oocytes failed to enter progesterone induced maturation [Fang and Miller, 2001]. This same technique interfered with meiosis in egg extract by disrupting aster formation [Fang and Miller, 2001]. Further oocyte studies showed that as an oocyte matures (become arrested at the second mitotic metaphase), the levels of total cellular O-GlcNAc decline [Slawson et al., 2002]. This decline was correlated with an increase in O-GlcNAcase activity. Interestingly, after progesterone-induced maturation, O-GlcNAc levels increased on a subset of cellular proteins [Lefebvre et al., 2004]. Additionally, when oocytes were either incubated in the presence of glucosamine or microinjected with glucosamine prior to progesterone-stimulated maturation, the oocytes matured slower than controls [Slawson et al., 2002; Lefebvre et al., 2004].

These data strongly suggest a dynamic cycling of O-GlcNAc during cell cycle progression, which influences the rate of maturation.

Recently, a detailed study of O-GlcNAc and the cell cycle demonstrated the sensitive control O-GlcNAc has on cell cycle progression [Slawson et al., 2005]. When both HeLa and 3T3-L1 pre-adipocytes were treated with PUGNAc (O-(2-Acetamido-3,4,6-tri-O-acetyl-d-glucopyranosylidene)amino-N-phenyl-carbamate) [Mohan and Vasella, 2000], a compound that inhibits O-GlcNAcase, these cells progressed through the cell cycle at a much slower rate than control cells The PUGNAc induced delays appeared to have the greatest affect during S-phase and G2/M progression. On the other hand, when GFAT activity was blocked using a GFAT inhibitor (6-diazo-norleucine), cells demonstrated reduced levels of O-GlcNAc and an increase in S and G2/M phase progression compared to controls [Slawson et al., 2005]. GFAT inhibited cells showed severe delays in G1 progression compared to control or PUGNAc treated cells [Slawson et al., 2005]. These data introduce the interesting hypothesis that cycling of O-GlcNAc levels is crucial for proper checkpoint progression. For example, should cells be in a nutrient poor environment, O-GlcNAc levels would be reduced, and the cells would not enter into G1 phase of the cell cycle. This is supported by the OGT knockdown work in mice embryonic fibroblast [O'Donnell et al., 2004] in which fibroblast became senescent after OGT knockdown.

Interestingly, pharmacological manipulation of O-GlcNAc levels led to a reciprocal change in the expression of OGT or O-GlcNAcase [Slawson et al., 2005]. Increased O-GlcNAc appears to cause a compensatory increase in O-GlcNAcase expression, and a decrease in O-GlcNAc leads to a compensatory increase in OGT. This data suggest that nutrients may have an impact on the expression of these enzymes. If at some point the changes in O-GlcNAc not elicit the appropriate adjustment in enzyme levels then the potential for disease increases.

Overexpression of OGT or O-GlcNAcase both disrupted normal cell cycle progression. Although the mechanism appears different, overexpression of either enzyme delayed exit through M phase. O-GlcNAcase overexpressing HeLa cells demonstrated delayed mitotic phosphorylation and a disruption of the normal expression of the cyclin proteins [Slawson et al., 2005]. In the case of O-GlcNAcase, the normal timed expression of cyclins and mitotic phosphorylations appeared delayed. Cells overexpressing OGT exhibited defects in cytokinesis leading to polyploidy [Slawson et al., 2005]. These cells also exhibited prolonged mitotic cyclin expression and disrupted proline-directed phosphorylation [Slawson et al., 2005]. Additionally, OGT appears to localize to the mitotic spindle during M phase. OGT is associated with tubulin during nascent spindle formation, but OGT then moves into and becomes concentrated in the midbody during cytokinesis (Fig. 4) [Slawson et al., 2005]. Previous work described OGT interacting with pp1-γ [Wells et al., 2004]; and pp1-γ is known to be active during M phase. Therefore, the possibility exist that OGT and pp1-γ are actively associated with each other during M phase. This association could potentially regulate numerous proteins, which undergo phosphorylation/dephosphorylation.

ACCESSING THE CODE: CONTROLLING NUCLEAR SIGNALING AND TRANSCRIPTION

One end point of signaling cascades is the alteration of gene transcription. O-GlcNAc plays a crucial role in this process. Many transcription factors and RNA polymerase II itself [Kelly et al., 1993] are modified by O-GlcNAc, and a recent list of modified transcription factors can be found in this review [Zachara and Hart, 2004]. Although numerous transcription factors are modified by O-GlcNAc, only in the past few years has a picture of how O-GlcNAc potentially regulates transcriptional complexes emerged.

One of the first O-GlcNAc modified transcription factors identified was Sp1 [Jackson and Tjian, 1989]. Sp1 is involved in regulating numerous so-called housekeeping genes, and appears to be extensively modified by O-GlcNAc [Jackson and Tjian, 1989]. The glycosylation of Sp1 has multiple affects on the function of the transcription factor. Reduction of Sp1 glycosylation led to an increase in Sp1 proteasome susceptibility [Han and Kudlow, 1997]. These data imply that conditions leading to lower O-GlcNAc levels would result in changes in Sp1 dependent transcription. Furthermore, the proteasome itself is modified by O-GlcNAc [Sumegi et al., 2003; Zhang et al., 2003]. The modification was found on several subunits of the lid, the Rpt

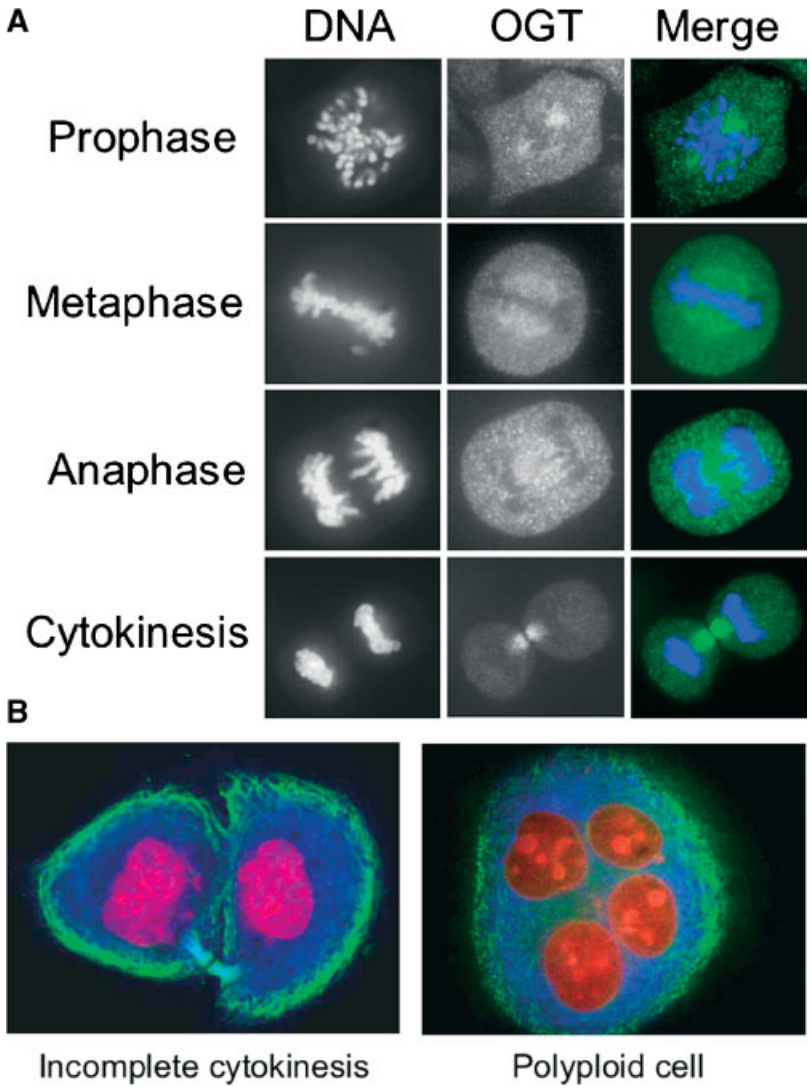

Fig. 4. **A**: OGT localizes to the mitotic spindle (DNA: blue, OGT: green). During prophase, OGT is found at the nacent spindle. OGT continues to be localized to the mitotic spindle during metaphase and is found at the central spindle assembly in anaphase. When cells undergo cytokinesis, OGT localizes to the midbody. **B**: Overexpression of OGT causes incomplete cytokinesis leading to multinucleated cells (OGT: blue, α-tubulin: green, DNA: red).

subunit in the base, and several subunits of the 20S component [Sumegi et al., 2003; Zhang et al., 2003]. Elevated levels of O-GlcNAc on the proteasome interfered with proper destruction of control peptides in in vitro assays [Zhang et al., 2003]. These data could have far reaching affects on cellular functions. Diabetic conditions could lead to impaired insulin signaling and cell cycle progression due to elevated O-GlcNAc interfering with proteasome function. Under stress conditions, proteasome activity is impaired while proteasome expression levels are static. The impairment of proteasome function after stress appears to be mediated by post-translational modifications [Glickman and Raveh, 2005], but the possible involvement of O-GlcNAc has yet to be investigated.

The glutamine rich domain of Sp1 is critical for facilitating interactions of Sp1 with the basil transcription complex (TFIID), but when a recombinant O-GlcNAcylated peptide was added to Sp1, the O-GlcNAc peptide disrupted Sp1-TFIID complex formation [Roos et al., 1997]. Additionally, increased Sp1 O-GlcNAcylation in this domain appears to reduce transcriptional activity [Yang et al., 2001]. Similar results were seen when Creb O-GlcNAcylation increased [Lamarre-Vincent and Hsieh-Wilson,

2003]. Creb binding to TAF-130 (also part of TFIID) was impaired and transcriptional activity was reduced under conditions of elevated O-GlcNAcylation [Lamarre-Vincent and Hsieh-Wilson, 2003]. YY-1 is a transcription factor that when bound to the retinoblastoma protein (pRb) is unable to bind DNA. O-GlcNAc modified YY-1 is unable to bind to pRb and, therefore, able to bind DNA and regulate transcription of target genes [Hiromura et al., 2003]. The O-GlcNAc modification of transcription factors appears to profoundly disrupt formation of transcriptional complexes resulting in transcriptional activation or repression depending upon the system.

Interestingly, OGT is localized to transcriptional regulatory complexes. OGT forms a complex with mSin3A, a transcriptional repressor protein [Yang et al., 2002]. This complex recruits histone deacetylase (HDAC) to promoters and is potentially dependent on OGT and O-GlcNAcylation [Yang et al., 2002]. Other proteins are able to recruit OGT to transcriptional complexes. OGT interacting protein, OIP-106, recruits OGT to RNA polymerase II [Iyer et al., 2003]. Once associated with RNA polymerase II, OGT can modify the enzyme and change transcriptional activity. Furthermore, O-GlcNAcase has histone acetlytransferase activity [Toleman et al., 2004]. This raises the possibility that O-GlcNAcase forms a transcriptional complex and both deglycosylates and acetylates transcription factors.

WHAT WE STILL NEED TO LEARN

O-GlcNAc is well suited to mediate protein function rapidly in response to changes in the environment. The substrate sensitivity of OGT for UDP-GlcNAc allows for rapid changes in O-GlcNAc levels in response to nutrient levels or stress. UDP-GlcNAc biosynthesis is dependent on sugar, amino acid, fatty acid, and nucleotide metabolism making O-GlcNAc a broad sensor of the cell's state and immediate environment [Zachara and Hart, 2004]. Although the cellular function of O-GlcNAc is becoming clearer, many large questions still remain. Our knowledge of OGT and O-GlcNAcase regulation is still rudimentary. Future studies will determine how O-GlcNAc contributes to the pathology of diseases such as diabetes. More research is needed to fully explain the interactions of OGT and PP1c, and how OGT affects stress response pathways, cell cycle progression, cytokinesis, and transcription. These are complicated questions but with more laboratories working toward answers, the next 20 years in O-GlcNAc research will be filled with new and exciting discoveries.

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