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O-GlcNAc Cycling: A Link Between Metabolism and Chronic Disease

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Abstract

To maintain homeostasis under variable nutrient conditions, cells rapidly and robustly respond to fluctuations through adaptable signaling networks. Evidence suggests that the *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) posttranslational modification of serine and threonine residues functions as a critical regulator of intracellular signaling cascades in response to nutrient changes. *O*-GlcNAc is a highly regulated, reversible modification poised to integrate metabolic signals and acts to influence many cellular processes, including cellular signaling, protein stability, and transcription. This review describes the role *O*-GlcNAc plays in governing both integrated cellular processes and the activity of individual proteins in response to nutrient levels. Moreover, we discuss the ways in which cellular changes in *O*-GlcNAc status may be linked to chronic diseases such as type 2 diabetes, neurodegeneration, and cancers, providing a unique window through which to identify and treat disease conditions.

Keywords

O-GlcNAc transferase (OGT); *O*-GlcNAcase (OGA); uridine diphospho-*N*-acetylglucosamine (UDP-GlcNAc); hexosamine biosynthetic pathway (HBP)

INTRODUCTION

The complex interplay between nutrient-sensing mechanisms and the cellular machinery responsible for energy production and utilization ensures cellular energy homeostasis. An integrated, evolutionarily conserved set of pathways is required for maintaining metabolite levels, pathogen sensing, and the immune response (56). Metabolite homeostasis, including the maintenance of amino acid, fatty acid, and sugar levels, is governed in part by the posttranslational modification (PTM) *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) through a series of conserved cellular mechanisms triggered by changes in nutrition and other stressors (Figure 1). Recent evidence illustrates a multifaceted pattern of *O*-GlcNAc regulation,

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allowing for complex modulation and integration of nutritional cues and cues of stressors. Herein, we describe the role *O*-GlcNAcylation plays in responding deftly to changes in nutritional status by altering cell signaling and transcriptional regulation. We not only discuss the implications of the nutritional load in the context of protein *O*-GlcNAc status, but we also outline the consequences that changes in *O*-GlcNAcylation have on disease progression and prognosis.

O-GLCNAC CYCLING IS REQUIRED FOR NUTRIENT SENSING

Organisms maintain a delicate network of cellular machinery in order to appropriately process nutrients ranging from amino acids and fatty acids to sugars and nucleotides. A central player in this machinery is the hexosamine biosynthetic pathway (HBP). This metabolically driven pathway is key for regulating nutrient processing, as its ultimate product, UDP-*N*-acetylglucosamine (UDP-GlcNAc), is synthesized based on nutrient concentration and the availability of nutrient-processing enzymes (110) (Figure 1). The activated sugar-nucleotide UDP-GlcNAc is utilized to produce not only complex glycan structures in the endoplasmic reticulum and Golgi but also the small yet potent nucleocytoplasmic modification, *O*-GlcNAc. As the resulting product of the HBP, *O*-GlcNAc is a nutrient-sensitive modification poised to dynamically integrate metabolic signals.

O-GlcNAc is added to and removed from protein serine and threonine residues by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), respectively (Figure 1). The cycling of this PTM results from a variety of cellular cues including those from extracellular stimuli, nutrient levels, stressors, cell cycle changes, and development. Changes in protein *O*-GlcNAc status yield alterations in protein folding, cellular localization, and catalytic activity (for review, see 145 and references therein), all of which can actively influence the downstream biological processes associated with those proteins (Figure 2) and can play critical roles in chronic disease onset and progression.

Mounting evidence supports that *O*-GlcNAc plays a role in nutrient sensing at both the cellular (86) and whole-body (130) levels, with genetic disruption of *O*-GlcNAc addition or removal resulting in either developmental delay (142) or lethality (118). The critical nature of *O*-GlcNAcylation likely lies in its ubiquity and the singularity of the enzymes encoded for each step in dynamic *O*-GlcNAc cycling. Given its sensitivity to nutrient conditions, rapid turnover, and universal presence, *O*-GlcNAc is equipped to be a critical, nutrient-sensitive signaling molecule cuing metabolic changes in important biological processes by its regulation of diverse targets.

CELLULAR TARGETS OF *O*-GLCNAC

Among the more than 1,000 substrates for OGT are nuclear and cytoplasmic proteins involved in transcription (44), translation, signal transduction, cell cycle progression, and synaptic plasticity (reviewed in 9, 48, 49, and 51 and outlined in Table 1). Interestingly, Sakaidani et al. recently demonstrated that *O*-GlcNAc also modifies at least one extracellular protein (114). The diverse set of proteins modified by *O*-GlcNAc includes chaperones [heat

shock protein 90 (HSP90)], transcription factors [neurogenic differentiation 1 (NeuroD1)], RNA polymerase II (RNA Pol II), nuclear pore proteins, RNA-binding proteins, kinases (AKT), adaptor proteins [insulin receptor substrate 1 (IRS1)], cytoskeletal proteins [amyloid beta precursor protein (APP) and tau], and others [endothelial nitric oxide synthase (eNOS), OGT, and the proteasome] (reviewed in 9, 48, 49, 51). The diversity of OGT's target population underscores the importance of this small macronutrient sensor.

The variety and large number of proteins modified by *O*-GlcNAc in addition to the role of the modification in frequent signaling draw comparisons to the phosphorylation of serine/threonine residues, which has also been shown to play a vital role in many biological processes. However, unlike the phosphate modification, which is governed by a whole host of cellular enzymes, only two enzymes govern the addition and removal of *O*-GlcNAc. This fact entreats questions such as, How does *O*-GlcNAcylation influence key downstream biological processes, and how does *O*-GlcNAc dynamically link nutrient levels with practical cellular signaling? Although these questions have been previously addressed to some degree (49, 51), here we focus on data that highlight the universality of the *O*-GlcNAc modification and imply that its deregulation will have both broad and acute effects on cellular processes. Broad effects (i.e., the knockout of OGT or OGA) may result in organismal lethality, whereas acute effects may trigger the onset and/or progression of a chronic disease [i.e., diabetes mellitus type 2 (DMII)] (Figure 3) (80). In the next sections, we discuss several *O*-GlcNAcylated proteins that play key roles in nutrient sensing and/or signaling cascades related to changes in nutrient status. We then provide an overview of how *O*-GlcNAc influences both physiological and pathological metabolic states related to nutrient-sensing and nutrient-managing networks in disease.

Protein Signaling and Targeting Are Influenced by *O*-GlcNAcylation

A protein's potential interaction partners are directly influenced by its folding and localization: Alterations in protein folding can dramatically change the residues available for interaction, whereas cellular localization may eliminate potential interactions. Interestingly, NMR studies demonstrate that *O*-GlcNAcylation can directly influence the polypeptide backbone shape, thereby affecting accessibility to particular residues in vivo (20, 121). In addition, protein *O*-GlcNAcylation has been shown to influence cellular localization and access to discrete cellular compartments. As a consequence of OGT's interaction with target proteins in a UDP-GlcNAc concentration-dependent manner (67), *O*-GlcNAcylation of protein targets such as AKT (protein kinase B) (125) and protein 53 (p53) (139) perturb the protein's targeting, degradation, and/or function.

Glucose is a critical product of digestion and an essential energy source in metabolism. Nutrient flux is responsible for directly triggering the insulin signaling pathway, resulting in cellular glucose uptake and processing. Briefly, upon binding of insulin to the insulin receptor, the insulin receptor phosphorylates its substrate proteins including insulin receptor substrate 1 (IRS1), which in turn binds to phosphoinositide 3-kinase (PI3K). PI3K activates pyruvate dehydrogenase kinase isozyme 1 (PDK1) through phosphoinositide phosphorylation, and PDK1 activates AKT (B) through phosphorylation as well. Importantly, after insulin induction, phosphatidylinositol-3,4,5-triphosphate [$PI(3,4,5)P_3$]

recruits OGT to the plasma membrane, where the enzyme is responsible for *O*-GlcNAcylating insulin-signaling pathway members (e.g., IRS1 and AKT), yielding changes in their phosphorylation status and ultimately resulting in signaling changes (Figure 2a). Indeed, increased *O*-GlcNAcylation correlates with both a decreased AKT activation and decreased IRS1 phosphorylation (135). Upon further examination of AKT *O*-GlcNAcylation in hepatocytes, researchers determined that decreased levels of AKT *O*-GlcNAc correlated with increased downstream target phosphorylation and changed mRNA levels for more than one AKT substrate (125). Moreover, as this kinase is known for its roles in glucose transport, gene transcription, and cell survival, perturbation of this signaling cascade demonstrates the intimate link between nutrient levels, *O*-GlcNAc, and signaling.

In addition to modulating changes in signaling, *O*-GlcNAcylation of individual proteins can directly affect their targeting to the proteasome (Figure 2b). The 26S proteasome is a critical multisubunit complex responsible for the degradation of proteins in the cytoplasm and nucleus, thus controlling protein half-life, maintenance of metabolic proteins, and elimination of damaged proteins. Upon polyubiquitination, proteins are targeted to the proteasome, and their recognition by the 19S subunit is required for degradation. The transcription factor specificity protein 1 (Sp1) is degraded by the proteasome in a nutrient-dependent manner: At times of low nutrition, Sp1 is degraded more rapidly than at times of nutrient excess (44). Moreover, this increased degradation correlates with an increase in *O*-GlcNAcylation, potentially serving to use Sp1 as a nutritional checkpoint by generally reducing transcription. More recently, Yang et al. demonstrated that ubiquitination of p53, the most extensively studied tumor suppressor, and its degradation by the proteasome is blocked upon p53 *O*-GlcNAcylation, which is also correlated with a decrease in p53 phosphorylation (139). Likewise, phosphorylation and *O*-GlcNAcylation modulate proteasomal degradation of estrogen receptor beta (ER-β) (13). Although studies of p53 and ER-β do not directly interrogate nutrient changes affecting the *O*-GlcNAc status of p53 or ER-β, it has been demonstrated that both proteins play a role in glucose metabolism: p53 is crucial for resisting the increased glycolysis in cancer cells as well as helping cells survive during times of limited metabolic stress (14), and ER-β affects energy balance and metabolism (108). This cross talk between phosphate and *O*-GlcNAc highlights the complexity of regulating protein turnover during times of metabolic flux. Together, these data support that *O*-GlcNAc modulates protein degradation and is crucial for appropriate function during times of metabolic stress.

O-GlcNAc is also critical for the effective mechanics of cellular machinery including the function of the proteasome (Figure 2b). Interestingly, the cell's general metabolic state is coupled with 19S subunit *O*-GlcNAcylation and the proteasome's relative activity: Increased *O*-GlcNAc modification of the 19S regulatory subcomplex is correlated with decreased proteasome activity (148). Organismal starvation often correlates with globally decreased *O*-GlcNAcylation, suggesting that the *O*-GlcNAc modification of the proteasome, its activity, and subsequent protein degradation are directly linked. Indeed, muscle fiber digestion is often stimulated during times of nutrient deprivation, when stored energy must, consequently, be released through proteasome activity. In this way, the *O*-GlcNAc status of the proteasome reflects the cell's nutrient status and perhaps allows an organism to directly

control amino acid availability and the presence of regulatory proteins, thereby influencing downstream processes (148).

Dramatic changes in protein activity and targeting during nutrient-rich and nutrient-poor conditions may yield fruitful protection of a cell during times of limited metabolic stress or upon exposure to a stressor. During times of prolonged nutrient excess or stress and/or inappropriate or continued signaling [as seen in chronic diseases (rather than the cyclical signaling expected under normal nutritional conditions)], deleterious effects can result, including insulin resistance and cardiovascular complications. Similarly, extended nutrient-replete conditions that yield altered *O*-GlcNAc levels and altered protein degradation would also be globally detrimental. Together, these findings imply that there is a continuum upon which *O*-GlcNAc can appropriately modulate nutritional cues, but prolonged, dramatic changes in nutrient load may forever change the way in which an organism responds (Figure 3).

Protein Solubility and Stability Are Affected by *O*-GlcNAc Modification

Filamentous and cytoskeletal proteins require an appropriate pattern of PTMs to maintain both stability and activity (Figure 2c). Indeed, proteins involved in chronic neurodegenerative diseases such as Alzheimer's disease (AD) and tauopathies are known to be *O*-GlcNAcylated. Specifically, APP and tau as well as cytokeratins rely on *O*-GlcNAcylation for modulating changes in solubility and stability. For example, increased *O*-GlcNAcylation of tau is associated with a decrease in its aggregation and a subsequent improvement in neurodegenerative pathologies in mice (144). Likewise, an increase of *O*-GlcNAcylation on keratins 8 and 18 (K8/18), intermediate filament proteins, has been shown to positively correlate with their solubility and stability, likely resulting in regulation of their function (126). Perhaps the abnormal stability of K8/18 upon decreased *O*-GlcNAcylation increases aggregates and decreases appropriate protein disposal similar to that seen with tau in neurons (78). It will be important to clearly define the role glucose plays in the stability of filamentous and cytoskeletal proteins in a variety of neurodegenerative diseases and how *O*-GlcNAc directly influences their pathogenesis.

Acute stress is often accompanied by a significant increase in protein *O*-GlcNAcylation as well as activation of HSPs, which are responsible for appropriate protein folding and suppression of protein aggregation. Many chaperones including HSP27 (43), α-crystallin (25), and HSP90 (134) are known to be *O*-GlcNAc modified. Interestingly, HSP90 is required for appropriate downstream *O*-GlcNAcylation of OGT targets (31), and the high-glucose-induced increase in global *O*-GlcNAc levels is attenuated with the inhibition of HSP90, suggesting a nutrient-responsive regulation of OGT activity through the chaperone, adding to the complex nature of the modification. These findings support the idea that *O*-GlcNAc may be a key component of the stress response, specifically relating to proteotoxicity prevention, resistance to oxidative stress (102), and ultraviolet stress tolerance (79).

With perturbations of glucose levels occurring during times of cellular stress, the cell likely utilizes its nutrient sensor *O*-GlcNAc to signal a change in overall nutritional status. Importantly, often associated with an increase in nutrient flux, *O*-GlcNAc levels also

rapidly elevate upon exposure to stressors and generally decrease upon stressor removal (147). Without normalization of glucose levels, inappropriate protein solubility and stability yield downstream consequences. If a change in *O*-GlcNAcylation initially acts to protect and maintain critical cellular processes, a sustained increase in nutrients (i.e., induced hyperglycemia caused by prolonged stress) will likely be damaging to the cell. Examples of this are seen with insulin resistance and glucose toxicity whereby cellular systems shut down specific functions when *O*-GlcNAc is maintained at too high a level on discrete targets in both DMII and AD (71, 127). A prolonged increase or decrease of *O*-GlcNAcylation is deleterious to cellular homeostasis because the pattern of PTMs required for protein stability and activity is thereby perturbed (Figure 3).

O-GlcNAc Regulates Transcription and Genome Replication

Alterations in nutritional load correlate with transcriptional changes, and the resulting inappropriate gene expression is linked with chronic disease. Moreover, extensive work has demonstrated that *O*-GlcNAcylation plays a key role in both directly and indirectly influencing transcription (Figure 2d). Not only is the nuclear localization of transcription factors influenced by *O*-GlcNAcylation, but the modification also directly affects downstream changes in individual transcript production. Indeed, increased *O*-GlcNAc modification of multiple transcription factors increases their binding to the insulin gene promoter linking them to metabolic regulation at the transcriptional level (3, 40). Thus, simple changes in nutritional status may alter the efficiencies of downstream cellular processes including metabolism, protein degradation, and enzyme activity.

A link between nutrient increase and protein localization has been demonstrated: Nutritional cues dictating an increase or decrease in *O*-GlcNAcylation appear to directly change the way in which proteins, including OGT and OGA, interact with cellular targets, thereby signaling changes in nutritional status to cellular networks. Indeed, a variety of cellular cues are known to influence OGT's interaction partners, altering the enzyme's target substrates. For example, hyperglycemia yields increased *O*-GlcNAcylation of NeuroD1, a transcription factor that plays a critical role in insulin gene transcription and pancreatic beta-cell function. Upon *O*-GlcNAcylation, NeuroD1 is translocated into the nucleus, and its exit to the cytoplasm is contingent upon a decrease in glucose levels (3, 4). In another example, the localization of the transcriptional coactivator cAMP response element–binding protein (CREB)-regulated transcription coactivator 2 (CRTC2) is directly linked to its *O*-GlcNAc status via nutritional load (27). Animals exposed to chronic hyperglycemia exhibit translocation of CRTC2 to the nucleus as a consequence of its *O*-GlcNAcylation. Subsequently, CRTC2 interacts with CREB, yielding elevation of glucose-6-phosphatase (G6PC) and other gluconeogenic gene product expression (94 and references therein). Together, these data demonstrate that nutrient-level alterations not only influence cellular localization of *O*-GlcNAc-modified proteins but also set in place a cascade of events that changes protein function and physiological outputs such as gluconeogenesis.

Changes in *O*-GlcNAcylation for many of OGT's targets can influence additional properties including target activation. One of OGT's targets, the mammalian transcription factor forkhead box protein O1 (FoxO1), is activated in response to cellular treatment with

glucose or glucosamine (57). However, there is likely a complicated regulatory mechanism, as cellular treatment with insulin results in decreased *O*-GlcNAcylated FoxO1 levels. Hyperglycemia yields an increase in UDP-GlcNAc in rat liver that correlates with increased FoxO1 *O*-GlcNAcylation, as is also seen in DMII (57). Importantly, activation of FoxO1 is correlated with its modification by *O*-GlcNAc, resulting in the upregulation of phosphoenolpyruvate carboxykinase and G6PC, two proteins critical for appropriate gluconeogenesis. That *O*-GlcNAcylation of FoxO1 directly influences gluconeogenic and oxidative stress response gene expression in a nutrient-sensitive manner implies that its regulation influences downstream metabolism efficiency as well as the deleterious effects of a poor metabolism. Another transcription factor, pancreatic and duodenal homeobox 1 (PDX1), is also a key player in gene expression upon changes of cellular glucose concentration. PDX1 dynamically alters the downstream regulation of insulin secretion by altering the gene expression of G protein–coupled free fatty acid receptor-1 upon an increase of glucose levels (63). Interestingly, elevated glucose levels correlate with increased PDX1 *O*-GlcNAcylation as well as its increased DNA binding. In this way, beyond being contingent upon the upstream interaction between OGT and $PI(3,4,5)P_3$, the activity of PDX1 is directly linked to its *O*-GlcNAc status. The data above clearly demonstrate that through *O*-GlcNAc, glucose metabolism directly regulates transcription factor function and thereby influences gene expression.

Gene expression is influenced by not only by *O*-GlcNAc-modified transcription factor activities but also by RNA Pol II, which is dynamically modified by a myriad of PTMs including *O*-GlcNAc. RNA Pol II is responsible for catalyzing DNA transcription, and this suggests that *O*-GlcNAc is among the signaling cues playing a role either in regulating transcriptional elongation or in the stability of RNA Pol II itself. In a recent paper, Ranuncolo and colleagues described that the C-terminal domain of RNA Pol II is *O*-GlcNAcylated in a dynamic fashion either before or during preinitiation complex assembly. In fact, perturbation of the modification using OGT and OGA inhibitors completely abrogates transcription, supporting the hypothesis that appropriate removal of *O*-GlcNAc from RNA Pol II is required for transcription (103). Another group, using the model organism *Caenorhabditis elegans*, used chromatin immunoprecipitation to demonstrate that substantial overlap exists between genes marked by RNA Pol II and *O*-GlcNAc on promoters (79). The correlation between the occupancy seen for both is striking, and in addition, there is a significant enrichment of genes involved in nutrient response. Together, these data suggest that *O*-GlcNAc may mediate transcriptional effects on genes involved in responding to nutritional cues directly through RNA Pol II.

Nutrient status can affect both gene expression and genome replication. Changes in glucose levels have been shown to directly affect histone 2B (H2B) and histone 3 (H3) modifications. In 2011, researchers described that the *O*-GlcNAcylation of H2B at serine 112 promoted its monoubiquitination and that this modification was frequently located near transcribed genes, suggesting that his-tone *O*-GlcNAcylation facilitates gene transcription (38). In addition, recent work from several laboratories has identified that ten-eleven translocation (TET) proteins, OGT, and *O*-GlcNAcylated H2B Ser112 are colocalized at sites corresponding to promoters of transcriptionally active genes (11, 28, 129). These data are supported by work that elucidates that OGT is a key coactivator of transcriptional output

(16, 27, 37, 60), that many *O*-GlcNAcylated factors are involved in transcriptional repression and gene silencing (39, 141), and that *O*-GlcNAc plays an active role in nutrient-responsive gene transcription (49, 51, 103).

A burgeoning amount of data also supports the notion that *O*-GlcNAc is a key player in modulating the cell cycle (112, 123, 124, 132). Research published in 2012 demonstrates that *O*-GlcNAc is likely a critical link between histone modification and the G2-M transition checkpoint of the cell cycle (35) (Figure 2e). Appropriate phosphorylation of H3 is required for cells to enter mitosis and the *O*-GlcNAc modification of H3 hinders cell division. Though these findings are not in line with evidence from a 2011 paper in which the authors observe an increase in *O*-GlcNAc on H3 during mitosis as compared with interphase (149), it is likely that experimental differences account for these discrepancies. Importantly, the studies above highlight the complexity of *O*-GlcNAc as part of the histone code and the processing of cellular information. We suggest that *O*-GlcNAc and other PTMs act as rheostats fine-tuning biological processes rather than as molecular on/off switches, as they are often categorized. Importantly, the data clearly show that PTMs influence the way in which DNA is “read” and that *O*-GlcNAc is a nutrient-sensitive component of the histone code that is significant for both general biological processes and chronic disease.

That OGT interacts with transcription factors, protein production machinery, and transcriptional corepressors suggests that *O*-GlcNAcylation influences gene activation and silencing in a nutrient-dependent manner at several levels. It is interesting to think that the simple readout of cellular nutrition status through *O*-GlcNAcylation may profoundly change cellular protein production. We suggest that the above data describing the role of *O*-GlcNAc in regulating gene transcription and the cell cycle implies that *O*-GlcNAc directly affects gene expression by integrating cues indicating the cell’s nutritional status, resulting in an appropriate cellular response. Inappropriate *O*-GlcNAcylation as a response to either hypernutrition or starvation likely triggers changes in transcription and the cell cycle that, if temporary, are reversible, but if the changes are prolonged they may result in transgenerational problems regulated, in part, by an epigenetic *O*-GlcNAc modification [this topic is more thoroughly discussed below and by Love and colleagues (81)] (Figure 2f). Indeed, understanding *O*-GlcNAc is likely to have profound implications for our understanding of how nutrition and metabolism directly affect gene transcription and the overall genetic code.

ABERRANT *O*-GLCNAC CYCLING IS IMPLICATED IN CHRONIC DISEASES

Changes in nutritional status and cellular processing of nutrients affect chronic diseases beyond those considered to be typical metabolic disorders. DMII, neurodegenerative diseases, and cancers all require a convergence of PTMs, signaling cascades, and transcriptional regulation for either combating or succumbing to the disease. The aforementioned diseases along with others are all either directly or indirectly influenced by the nutritional status of the organism. In the next section, we explore how *O*-GlcNAc contributes to the etiology of a variety of chronic conditions.

O-GlcNAc Is Associated with Insulin Regulation

Evolutionarily conserved insulin signaling is influenced by the flux of the HBP. Given that the two enzymes governing *O*-GlcNAc cycling are known to be involved in insulin signaling (OGT) (36, 136, 140) and reside at a DMII susceptibility locus (OGA) (72), it is not surprising that the HBP product *O*-GlcNAc is implicated in insulin resistance (36, 140). Insulin resistance not only plays a critical role in DMII but is also associated with other health complications including heart disease, obesity, and dyslipidemia (22). The influence that *O*-GlcNAc has on cellular processes associated with insulin signaling is just beginning to be revealed. For example, it has been shown that *O*-GlcNAc is associated with direct inhibition of insulin signaling in several cell lines (33, 98, 135), though its role in DMII may be specific to certain cell types [summarized by Whelan and coworkers (135)]. Paradoxically, both high-fat and low-calorie supplementation can yield increases in *O*-GlcNAcylation in cell culture, suggesting that *O*-GlcNAc acts to fine-tune biological processes, with an appropriate amount of the modification required for proper signaling (Figure 3).

Several lines of evidence suggest that *O*-GlcNAcylation is critical for maintaining a metabolic balance at both the cellular and the whole-organism levels. When cells are chronically exposed to elevated glucose concentrations, *O*-GlcNAc is likely to contribute to the alterations in insulin signaling observed in diabetic patients, as hyperglycemia is associated with an increase in UDP-GlcNAc as well as an increase in *O*-GlcNAcylation (115, 130, 143). In particular, *O*-GlcNAcylation appears to contribute to hyperglycemia with excessive glucose production by the liver (68) and deterioration of beta-cell pancreatic function (58), resulting in glucotoxicity. In addition, increased *O*-GlcNAcylation of two transcription factors, NeuroD1 and PDX1, increases their binding to the insulin gene promoter, linking them to metabolic regulation at the transcriptional level (3, 40). Finally, researchers have demonstrated that increased cellular *O*-GlcNAcylation reduces the phosphorylation of IRS1 Tyr608, which is correlated with a decrease in AKT activation and subsequent decrease in glucose transporter type 4 glucose uptake (135). In these ways, the nutritional status of the cell is intimately linked with the level of *O*-GlcNAcylation, nutrient processing, and insulin signaling.

The research described above highlights that changes resulting in attenuation of insulin signaling due to high *O*-GlcNAc levels are linked with hypernutrition and/or DMII-induced hyperglycemia (140). Moreover, *O*-GlcNAcylation is directly coupled to several complications associated with DMII, including problems with regulation of vascular tone, atherosclerosis, and angiogenesis (59). Increased plasma glucose and cardiovascular disease rates are also correlated with increased morbidity and mortality rates (104, 111). Indeed, it has been shown that with high levels of glucose, there is an increase of the *O*-GlcNAc modification on a variety of proteins including eNOS, a protein responsible for generating nitric oxide and regulating vascular tone. Increased glycosylation of eNOS correlates with its decreased phosphorylation, decreased activity, and decreased NO production, yielding endothelial cells with micro- and macrovascular complications (31). Likewise, *O*-GlcNAcylation activates the expression of profibrotic and antifibrinolytic factors, contributing to vascular and renal dysfunctions. Impaired insulin signaling, insulin

resistance, and complications relating thereof are all correlated with excess caloric intake and are likely to be associated with changes in *O*-GlcNAcylation.

Nearly instantaneous signaling cascades govern stress and inflammation after exposure to a particular stressor such as increased or decreased nutritional load. This signaling, which may initially act in a protective fashion, may result in deleterious cellular effects if prolonged (due to continued starvation or excess caloric intake) (Figure 3). Utilizing *O*-GlcNAc as a determinant of stressor level or disease progression may be possible if the right target protein is identified in association with insulin resistance. The direct role that *O*-GlcNAc plays in DMII and the complications associated with the disease has implications for developing unexplored therapeutic interventions associated with nutritional changes. Excessive flux through the HBP affects insulin signaling in a variety of cell types as well as in whole-animal models. Therefore, it is likely that the *O*-GlcNAcylation of signaling proteins alters signal transduction and subsequently changes the effectiveness of the insulin-signaling pathway.

Changes in OGT and OGA Expression Are Correlated with Cancer Prognosis

As has been previously described, *O*-GlcNAcylated proteins maintain roles in many biological processes (reviewed in 9, 48, 49, 51) that are misregulated during tumorigenesis, suggesting a correlation between *O*-GlcNAc levels and cancer onset and/or progression. Interestingly, changes in OGT and OGA levels as well as higher and lower levels of *O*-GlcNAcylation are correlated with cancer metastasis and prognosis. Although many cancer proteins [p53 (139), IkappaB (62), c-myc (17), and Snail (96)] are known to be *O*-GlcNAcylated, the mechanism by which *O*-GlcNAc influences biological processes related to these proteins remains undefined. OGT directly modifies core histones, transcriptional machinery, and other proteins that are directly related to cellular growth and expression in cancers. Given the altered metabolic state found in cancer cells, *O*-GlcNAc couples metabolism, the nutrient state of the cell, and gene regulation at the level of DNA. Moreover, activities of transcriptional machinery, including activating and repressive complexes and transcription factors, are influenced by direct *O*-GlcNAc modification: *O*-GlcNAc can change the activity and/or localization of these proteins. With these simple alterations, critical biological processes (i.e., proliferation, cell cycle, and degradation) can be altered, thereby affecting the metastatic potential of cells. Finally, *O*-GlcNAcylation may affect cellular growth and expression by playing a regulatory role in the onset and/or progression of cancer. Evidence from primary tumor samples demonstrates that our understanding of *O*-GlcNAc as a regulator of cancers needs to be expanded beyond the recent, interesting findings described below.

With increased glucose consumption and processing in cancer cells (21, 66, 133), there is a correlated increase in *O*-GlcNAcylation in many cancer types as well as an increase in OGT expression level (and in some cases a decrease in OGA expression). Specifically, recent findings have demonstrated that increases in OGT and *O*-GlcNAc are generally tumor promoting for both breast (10) and prostate (84) cancers. As such, decreases of OGT both in vitro and in vivo block breast cancer growth (10), breast cancer metastasis in a mouse model (42), and prostate cancer invasiveness (84). Although increased OGT

and *O*-GlcNAc levels correlate with tumor-promoting properties in breast and prostate cancers, the exact mechanism of cancer regulation by *O*-GlcNAc remains unclear. It is likely that the *O*-GlcNAc "rheostat" plays different roles depending on the cancer type, as there are variations in how different oncogenic cells respond to manipulations of *O*-GlcNAc levels. Importantly, all of these data imply that OGT may be a good therapeutic target for cancer treatment, and the recently published crystal structure of OGT (69) will allow structure-based, selective inhibitors to be designed for use in therapeutics. Likewise, a better understanding of the way in which cancer cells utilize the increased glucose load could yield insights about which proteins may be ideal to target as a therapeutic on the basis of *O*-GlcNAcylation status.

Interestingly, some cases exist in which increased levels of *O*-GlcNAc are correlated with improved cancer prognosis (119). Pathways associated with rapid cancer cell proliferation in leukemic lymphocytes appear to be blocked by high levels of *O*-GlcNAcylation (124). In fact, patients with the highest level of *O*-GlcNAcylation in cancerous lymphocyte tissue had better prognosis (119). This stark contrast with the correlation of recovery in breast and prostate cancers highlights that both increases and decreases in *O*-GlcNAc yield changes in cancer prognosis and demonstrates that our understanding of the way in which *O*-GlcNAc influences cancer is incomplete. We speculate that it is the appropriate *O*-GlcNAcylation of key proteins in each cancer type that most profoundly alters metabolism, cellular processes, and eventual disease prognosis.

Interestingly, many proteins found to be deregulated in cancers are modified by both *O*-GlcNAc and phosphate. Modifying proteins to their appropriately posttranslationally decorated state utilizing current drug technology is one approach in treatment and has been explored with kinases as the target of many anticancer therapies. This suggests that it would be useful to consider both specific OGT target proteins and OGT/OGA themselves as reasonable targets for cancer drugs. Certainly, better understanding the role that OGT and OGA play in altering transcriptional machinery and chromatin will yield a set of tools to answer questions about the regulation of cancer by *O*-GlcNAc and potentially give researchers a foothold for interpreting gene profiles in cancers.

Neurodegenerative Diseases Are Associated with Altered *O*-GlcNAc Status

Metabolic impairment and improper glucose processing are linked to many neurodegenerative diseases including AD, Huntington's disease, and Parkinson's disease. Indeed, an early hallmark of AD is impaired glucose metabolism (53), and this poor glucose processing correlates with the severity of cognitive problems found in patients (30, 89). OGA meningioma-expressed antigen 5 (MGEA5) maps to chromosome 10q24.1, a locus associated with late-onset AD (26), whereas OGT maps to a locus on the X-chromosome associated with Parkinson's disease and X-linked Parkinson's dystonia (human Xq13.1) (118). Accumulating evidence links obesity, the dysfunction of insulin and glucose metabolism in the brain, and AD. These correlations have led some researchers to suggest that AD should be considered type 3 diabetes (71, 127). Interestingly, individuals with DMII have a greater than twofold-increased risk of developing AD compared to matched control individuals without DMII (5). Together, these findings suggest that nutritional conditions,

appropriate processing of glucose, and correct *O*-GlcNAcylation are likely to be intimately linked with neurodegenerative disease onset or development.

That appropriate glucose metabolism is closely linked with neurodegenerative diseases also suggests a link with the nutrient-responsive modification *O*-GlcNAc. Proteins such as APP and tau that are involved in neurodegenerative pathologies (i.e., the accumulation of neurofibrillary tangles) are known to be *O*-GlcNAcylated. The consequence of APP and tau *O*-GlcNAcylation remains to be further explored but there is some exciting new evidence: APP is modified by *O*-GlcNAc at a position that likely affects its degradation. *O*-GlcNAcylation of APP appears to correlate with its increased nonamyloidogenic α-secretase processing, a consequence of which is a decrease in pathological neurofibrillary tangles (61). Likewise, the *O*-GlcNAcylation of tau in a glucose-dependent manner decreases tau aggregation and subsequent neuronal cell loss in mouse models (144). Finally, the increased *O*-GlcNAcylation of an additional protein, transforming growth factor β-activated kinase 1, was found to decrease its thermally induced aggregation, suggesting that *O*-GlcNAc may play a basic biochemical role in preventing protein aggregation (144). We propose that these data collectively imply that the disruption of glucose metabolism, and therefore *O*-GlcNAcylation, will likely affect amyloid and tau pathology. In fact, an increase in *O*-GlcNAcylation generally correlates with an improved neuronal outcome, suggesting that inhibition of OGA (yielding increased *O*-GlcNAc) could be a promising therapeutic target for hindering neurodegenerative disease progression.

Although it has been established that *O*-GlcNAcylation at a single site on an individual protein can have significant biological consequences, it was unclear how, mechanistically, such a small chemical modification contributes to critical neuronal functions and thereby influences neurodegenerative diseases. Recently, using *C. elegans* mutants in *O*-GlcNAc cycling, several distinct neurodegenerative human disease models including Huntington's disease, frontotemporal dementia with Parkinsonism-17 tauopathy, and AD were shown to be dependent upon *O*-GlcNAc cycling (131). Moreover, the Hseih-Wilson laboratory studied the implications of *O*-GlcNAc modification to CREB, a key brain transcription factor (105). Researchers determined that Ser40 *O*-GlcNAcylation inhibits basal- and activity-induced CREB-mediated transcription by impairing its interaction with its transcriptional coactivator, CRTC2. Resulting changes from blocking glycosylation yield activity-dependent gene transcriptional changes, axonal and dendritic growth increase, and long-term memory consolidation enhancement. The implications of these findings for memory and neurodegeneration are profound: If small changes in *O*-GlcNAc levels can perturb complex, higher-order brain functions, it is likely that the nutritional load of the cell can actively and functionally influence neurite outgrowth and long-term memory formation.

Stress response pathways have been shown to be responsive to *O*-GlcNAcylation. Indeed, perturbation of protein degradation pathways in nutrient-replete or nutrient-rich conditions may directly affect the ability of the proteasome to function properly and, in neurons, could lead to protein aggregation. Likewise, changes in *O*-GlcNAcylation can influence the structural shape of the protein backbone, affecting protein interactions as well as oligomerization (12, 20, 121). Finally, *O*-GlcNAcylation on individual proteins can have significant effects on transcription of memory-related genes. Together, these findings suggest

that—in the context of neurons—*O*-GlcNAcylation likely influences not only memory formation but also a cell's ability to deal with proteotoxicity. Importantly, *O*-GlcNAc likely acts on a continuum to influence fundamental biological processes, and profiling *O*-GlcNAcylated proteins may yield insight into which protein(s) will most directly affect neurological disease outcomes.

Glyco-Epigenetics: Can *O*-GlcNAc Influence the Epigenetics of Human Disease?

In model systems ranging from yeast to man, *O*-GlcNAc cycling has been shown to play an important role in modulating developmental plasticity (48, 81). As mentioned previously, *O*-GlcNAc may be involved in histone remodeling by modifying histones; *O*-GlcNAc is also essential for polycomb repression, a key player in stem cell differentiation (34, 39, 46, 82, 113, 122). *O*-GlcNAcylation alters key cellular signaling pathways implicated in morphogenesis including the insulin, fibroblast growth factor, and transforming growth factor-β pathways and also modifies key morphogenic factors such as Snail, Notch, β-catenin, and p53 (9, 48, 81, 120). OGT is essential for stem cell viability and is tightly regulated by X-inactivation (50, 77, 118). In addition, OGT physically interacts with the methyltransferases MLL5, CARM1, and MMSET, each implicated in epigenetic regulation (15, 37, 92). However, direct analysis of the transcriptional complexes involved in the maintenance of stem cell pluripotency points to an even more direct role for *O*-GlcNAcylation in stem cell biology. The OGT-binding partner host cell factor 1 also combines with Ronin (Thap11), an essential factor for the self-renewal of ES cells (23, 24), and OGT physically interacts with Oct4, Nanog, Sox2, and Myc in the Oct4-centered network, maintaining stem cell pluripotency (73, 95, 128). Moreover, the OGA gene is present in the polycomb-regulated NK homeobox cluster, and its promoter is co-occupied by Sox2, Oct4, and Nanog, allowing expression in ES cells (8, 70, 81). Excessive *O*-GlcNAcylation suppresses ES cell differentiation into cardiomyocytes (65). Finally, *O*-GlcNAc is involved in mammalian polycomb repression in mouse embryonic stem cells wherein a number of *O*-GlcNAcylation sites were mapped to pluripotency factors such as SOX2 (91). The results suggest that OGT may regulate both the SOX2/OCT4 and Nanog pathways. Therefore, altered function of these pluripotency factors may contribute to the nonviability of OGT mutant ES cell lines, demonstrating that the levels and activities of OGT and OGA are critical for the maintenance of stem cell pluripotency and the modulation of developmental plasticity.

Several lines of evidence suggest that *O*-GlcNAcylation plays a role in mammalian dosage compensation. In mammals, including humans, OGT is present on the X-chromosome at Xq13.1, quite close to the Xist locus and the X-inactivation center (50, 93, 118). Indeed, the levels of OGT appear to be quite tightly regulated during the process of X-inactivation in mice (77). This is of great interest because OGT has been found to be a component of the dosage compensation complex (human MOF) in mammals (88). In addition, growing evidence suggests that X-inactivation and cell differentiation are tightly coupled, as blocking one process compromises the other, and dedifferentiation of somatic cells to induced pluripotent stem cells is accompanied by X chromosome reactivation. Oct4 and CTCF play essential roles in the process of X-inactivation, and as mentioned above, OGT is a component part of this pluripotency network (29, 73, 95, 128). The process

of mammalian X-inactivation shares many characteristics with genomic imprinting, and incomplete X-inactivation has been linked to human disorders such as autoimmunity (54, 55, 106).

The human OGA, encoded by MGEA5, is part of a highly conserved gene linkage region containing one of the four NK homeobox clusters (137). The presence of MGEA5 in this cluster was observed for a variety of vertebrate species ranging from pufferfish to human. The NK homeobox genes are important regulators of developmental processes including those in muscle, heart, central nervous system, and sensory organ. Remarkably, the *Drosophila* OGA gene is also present within the single NK cluster of homeotic genes (also called the 93DE cluster) on chromosome 3 of the fly (81). Thus, despite the huge evolutionary distance between fly and man, the OGA gene has remained associated with this important cluster of homeobox genes involved in mesoderm development. It has been suggested that this conserved linkage of genes likely reflects conserved features of the chromatin landscape required for expression of such tightly linked genes (137). The NK cluster is flanked by highly conserved noncoding regulatory elements, suggesting that shared regulatory regions may have resulted in evolutionary pressure to maintain these linkages. Consistent with this, these genes have largely overlapping expression patterns (137).

One interpretation of these findings is that the genes encoding the *O*-GlcNAc cycling enzymes are subject to very tight regulation by higher-order chromatin structure. Homeostatic mechanisms frequently use feedback loops to maintain the delicate balance that must exist between expression of the enzymes catalyzing *O*-GlcNAc addition and removal. Evidence suggests that deregulation of these pathways is associated with a number of human disorders including those influenced by intergenerational epigenetic phenomena. Establishment of the links between these epigenetic phenomena and human disease susceptibility is one of the great challenges facing the *O*-GlcNAc field.

EVOLUTIONARILY CONSERVED *O*-GLCNAC HAS IMPLICATIONS FOR DISEASE TREATMENT: CONCLUSIONS AND PERSPECTIVES

Metabolism and the *O*-GlcNAc PTM are undoubtedly linked. The many ways in which *O*-GlcNAc influences cell physiology are summarized in Figure 2. Indeed, perturbed *O*-GlcNAcylation is intimately connected to pathologies of chronic diseases that are associated with changes in glucose consumption and processing. Considering the modification's involvement in diseases ranging from DMII and cancers to neurodegenerative disorders, OGT and OGA have become attractive candidates for pharmacological targeting. In addition, the proteins with which the *O*-GlcNAc cycling enzymes associate (including transcription factors, signaling proteins, etc.) are also considered appealing for biological manipulation. These interaction partners may, in fact, be better targets given the ubiquity of *O*-GlcNAcylation in biological processes: If the global level of *O*-GlcNAcylation is altered to ameliorate a single set of symptoms, another cellular process may unwittingly and disastrously be perturbed.

The ubiquity of the *O*-GlcNAc modification, its conserved status evolutionarily, and its function in integrating metabolic cues underscore the importance of the modification's role

in a nutritional context. With the current intense focus on diseases in which nutrient status is directly affected, we expect some of the most important research linking nutrition and *O*-GlcNAc to include:

1. **Comprehensive profiling of *O*GlcNAcylated proteins under normal and perturbed conditions.** Despite the apparent simplicity of this research domain, advances will require improvement in technologies and the integration of chemistries with biology given the limitation of detecting *O*-GlcNAcylation in large, complex biological samples. Restrictions in *O*-GlcNAc detection and site definition lie not only in the chemistry of the modification but also in the available bioinformatics power associated with current mass spectrometry technology. The research of several groups is focused toward improving the chemistries that can be used for *O*-GlcNAc sample isolation, and others concentrate efforts on improving algorithms for accurately and robustly sorting large mass spectra data sets. Data resulting from the improved comprehensive profiling of *O*-GlcNAcylated proteins will not only yield information about the ubiquity of the modification but will also reveal potential targets for downstream research and pharmacological intervention. In addition, we hope the data will suggest a specific subset of proteins that could be used as a disease state marker or disease progression indicator.

2. **Further elucidation of the key players (e.g., transcription factors) whose *O*-GlcNAcylation status directly influences downstream gene expression profile changes.** Defining the proteins that are responsible for modulating gene expression changes depending on nutritional load and disease state will provide a key link between *O*-GlcNAc and potential therapeutic targets. With critical changes in gene expression influencing not only disease onset but also disease prognosis, it is vital to determine how *O*-GlcNAc modulates gene expression changes. Once identified, the desired substrates of OGT that directly influence the disease state could be targeted through pharmaceutical intervention. Alternatively, a protein's *O*-GlcNAcylation status could be directly perturbed (by modulating the activity and/or availability of OGT and OGA) to influence downstream gene expression changes.

3. **Investigation of the impact of dietary supplementation with glucosamine on hexosamine signaling and chronic disease.** Glucosamine is now widely used as a supplement in food and some beverages (101). It has been argued to have therapeutic value, particularly with respect to joint pain and inflammation (53a). However, because glucosamine bypasses many of the regulatory steps in hexosamine synthesis, there may be real concern for the dietary consequences of long-term consumption of glucosamine. Reports to date are conflicting on the dangers of glucosamine supplementation (1, 2, 6, 7, 100, 116). Whereas some reports suggest there is no effect on glucose metabolism (1, 6, 7, 116), more recent findings suggest that adults with poor insulin sensitivity may be at risk upon glucosamine consumption (100). Knowledge of the biochemical and epigenetic impact of *O*-GlcNAc cycling may provide a means of addressing how long-term exposure to glucosamine may impact human health.

4. **Identification of characteristics of nutritional load or nutrient processing that contribute to the physical and pathological changes associated with alterations in *O*-GlcNAcylation.** Certainly, fluctuation in nutritional levels is expected regardless of the nutrient-consuming organism or the environment in which it resides. However, dramatic changes in nutrient load (prolonged nutrient excess or starvation) clearly link *O*-GlcNAc cycling to metabolic syndrome and its associated complications (87). Defining how this occurs mechanistically will be an important advance for our understanding of conditions including obesity and DMII. We expect that advances here will require the increased use of model organisms such as *C. elegans* and *D. melanogaster* (36, 39, 47, 81, 90, 117, 122). The use of the nematode, for example, allows for profiling evolutionarily conserved processes as well as rapid data generation in fields ranging from metabolic disease profiling to research in neurodegeneration and innate immunity (36, 47, 81, 90, 102, 131). Answering mechanistic questions will require genetically teasing apart the pathway components that are affected during changes in nutrient availability on a reasonable time scale, and model organisms will continue to facilitate this research.

5. **Establishment of *O*-GlcNAc as a potential therapeutic target influencing circadian rhythm and other factors linked to the obesity epidemic.** DMII, cardiovascular complications, and dyslipidemia are among the obesity-associated complications that have increased in penetrance and are a growing burden on our healthcare system. Although the obesity epidemic likely has many contributing factors, hypernutrition, poor nutrition, and abnormal sleeping patterns are among the likely culprits. Indeed, it has been proposed that dietary shifts, including increased food and sugar-sweetened beverage consumption, correlate with the growing US waistline (85). Increased chronic flux through the HBP due to hypernutrition generally correlates with an increase in target *O*-GlcNAcylation and deleterious results (Figure 3). Moreover, epidemiological studies demonstrate that shift workers have a higher incidence of obesity and its comorbidities, which are generally attributed to abnormal sleeping and eating patterns (41). Recent evidence links *O*-GlcNAc with the tightly controlled circadian clock that mammalian cells use to run in synchrony with environmental cues (32, 64, 74). The covalent *O*-GlcNAcylation of core clock components directly couples glucose availability and the metabolic entrainment of the circadian clock, suggesting that food-derived signals are critical for a healthy circadian rhythm. Data support that OGT, OGA, or proteins modified by these enzymes are likely good drug targets for treating disorders of nutrient metabolism as well as circadian rhythm. A detailed understanding of obesity in the current nutritional environment could lead to obesity prevention and health complication interventions, yielding an improvement in this national public health concern.

6. **Definition of the way in which *O*GlcNAc and other epigenetic changes work together to directly influence not only metabolism but also disease susceptibility.** We expect that, given its role as a nutrient sensor and given

that it is known to directly affect gene expression, *O*-GlcNAc is poised to act as an indicator of external environmental influences even at the level of prenatal nutrient exposure. Data indicate that *O*-GlcNAc impacts polycomb group function, likely early in development (81), and in utero nutrient exposure clearly correlates with DMII (99) as evidenced by offspring of individuals conceived during the Dutch Hunger Winter (52). Results from studies expanding on the research outlined above will not only advance our understanding of *O*-GlcNAc as an epigenetic modulator but also yield information about preventing and treating nutrient-related chronic diseases that may or may not have an underlying epigenetic foundation.

The suggested research avenues described above are critical to broaden our basic scientific understanding of the *O*-GlcNAc modification and to deepen understanding of the direct mechanistic influence that the modification has on biological processes. Given that cells must rapidly and robustly respond to changes in their environment, including changes in nutrient status, *O*-GlcNAc is the requisite PTM poised to function as a regulator of intracellular signaling cascades in response to nutrient flux. Developing therapeutics to combat a broad range of chronic diseases that are influenced by *O*-GlcNAc will require a careful identification of OGT's target proteins as well as the resulting effects of the presence or absence of the modification.

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LITERATURE CITED

1. Albert SG, Oiknine RF, Parseghian S, Mooradian AD, Haas MJ, McPherson T. 2007. The effect of glucosamine on serum HDL cholesterol and apolipoprotein AI levels in people with diabetes. Diabetes Care 30:2800–3 [PubMed: 17682119]
2. Anderson JW, Nicolosi RJ, Borzelleca JF. 2005. Glucosamine effects in humans: a review of effects on glucose metabolism, side effects, safety considerations and efficacy. Food Chem. Toxicol 43:187–201 [PubMed: 15621331]
3. Andrali SS, Qian Q, Ozcan S. 2007. Glucose mediates the translocation of NeuroD1 by *O*-linked glycosylation. J. Biol. Chem 282:15589–96 [PubMed: 17403669]
4. Andrali SS, Sampley ML, Vanderford NL, Özcan S. 2008. Glucose regulation of insulin gene expression in pancreatic β-cells. Biochem. J 415:1–10 [PubMed: 18778246]
5. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P. 2006. Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol. 5:64–74 [PubMed: 16361024]
6. Biggee BA, Blinn CM, McAlindon TE, Nuite M, Silbert JE. 2006. Low levels of human serum glucosamine after ingestion of glucosamine sulphate relative to capability for peripheral effectiveness. Ann. Rheum. Dis 65:222–26 [PubMed: 16079170]
7. Biggee BA, Blinn CM, Nuite M, Silbert JE, McAlindon TE. 2007. Effects of oral glucosamine sulphate on serum glucose and insulin during an oral glucose tolerance test of subjects with osteoarthritis. Ann. Rheum. Dis 66:260–62 [PubMed: 16818461]
8. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, et al. 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 122:947–56 [PubMed: 16153702]
9. Butkinaree C, Park K, Hart GW. 2010. O-linked β-N-acetylglucosamine (O-GlcNAc): extensive crosstalk with phosphorylation to regulate signaling and transcription in response to nutrients and stress. Biochim. Biophys. Acta 1800:96–106 [PubMed: 19647786]

10. Caldwell SA, Jackson SR, Shahriari KS, Lynch TP, Sethi G, et al. 2010. Nutrient sensor *O*-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. Oncogene 29:2831–42 [PubMed: 20190804]
11. Chen Q, Chen Y, Bian C, Fujiki R, Yu X. 2013. TET2 promotes histone *O*-GlcNAcylation during gene transcription. Nature 493:561–64 [PubMed: 23222540]
12. Chen YX, Du JT, Zhou LX, Liu XH, Zhao YF, et al. 2006. Alternative *O*-GlcNAcylation/*O*-phosphorylation of Ser16 induce different conformational disturbances to the N terminus of murine estrogen receptor beta. Chem. Biol 13:937–44 [PubMed: 16984883]
13. Cheng X, Hart GW. 2001. Alternative *O*-glycosylation/*O*-phosphorylation of serine-16 in murine estrogen receptor β: post-translational regulation of turnover and transactivation activity. J. Biol. Chem 276:10570–75 [PubMed: 11150304]
14. Cheung EC, Vousden KH. 2010. The role of p53 in glucose metabolism. Curr. Opin. Cell Biol 22:186–91 [PubMed: 20061129]
15. Cheung WD, Sakabe K, Housley MP, Dias WB, Hart GW. 2008. *O*-linked β-*N*-acetylglucosaminyltransferase substrate specificity is regulated by myosin phosphatase targeting and other interacting proteins. J. Biol. Chem 283:33935–41 [PubMed: 18840611]
16. Chikanishi T, Fujiki R, Hashiba W, Sekine H, Yokoyama A, Kato S. 2010. Glucose-induced expression of *MIP-1* genes requires *O*-GlcNAc transferase in monocytes. Biochem. Biophys. Res. Commun 394:865–70 [PubMed: 20206135]
17. Chou TY, Dang CV, Hart GW. 1995. Glycosylation of the c-Myc transactivation domain. Proc. Natl. Acad. Sci. USA 92:4417–21 [PubMed: 7753821]
18. Clark RJ, McDonough PM, Swanson E, Trost SU, Suzuki M, et al. 2003. Diabetes and the accompanying hyperglycemia impairs cardiomyocyte calcium cycling through increased nuclear *O*-GlcNAcylation. J. Biol. Chem 278:44230–37 [PubMed: 12941958]
19. Comer FI, Hart GW. 2001. Reciprocity between *O*-GlcNAc and *O*-phosphate on the carboxyl terminal domain of RNA polymerase II. Biochemistry 40:7845–52 [PubMed: 11425311]
20. Daly NL, Hoffmann R, Otvos LJ, Craik DJ. 2000. Role of phosphorylation in the conformation of τ peptides implicated in Alzheimer's disease. Biochemistry 39:9039–46 [PubMed: 10913317]
21. Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB. 2008. Brick by brick: metabolism and tumor cell growth. Curr. Opin. Genet. Dev 18:54–61 [PubMed: 18387799]
22. DeFronzo RA, Ferrannini E. 1991. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–94 [PubMed: 2044434]
23. Dejosez M, Krumenacker JS, Zitur LJ, Passeri M, Chu LF, et al. 2008. Ronin is essential for embryogenesis and the pluripotency of mouse embryonic stem cells. Cell 133:1162–74 [PubMed: 18585351]
24. Dejosez M, Levine SS, Frampton GM, Whyte WA, Stratton SA, et al. 2010. Ronin/Hcf-1 binds to a hyperconserved enhancer element and regulates genes involved in the growth of embryonic stem cells. Genes Dev. 24:1479–84 [PubMed: 20581084]
25. Dell A 1992. Vertebrate lens alpha-crystallins are modified by *O*-linked *N*-acetylglucosamine. J. Biol. Chem 267:555–63 [PubMed: 1730617]
26. Deng Y, Li B, Liu Y, Iqbal K, Grundke-Iqbal I, Gong CX. 2009. Dysregulation of insulin signaling, glucose transporters, *O*-GlcNAcylation, and phosphorylation of tau and neurofilaments in the brain: implication for Alzheimer's disease. Am. J. Pathol 175:2089–98 [PubMed: 19815707]
27. Dentin R, Hedrick S, Xie J, Yates J 3rd, Montminy M. 2008. Hepatic glucose sensing via the CREB coactivator CRTC2. Science 319:1402–5 [PubMed: 18323454]
28. Deplus R, Delatte B, Schwinn MK, Defrance M, Mendez J, et al. 2013. TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. EMBO J. 32:645–55 [PubMed: 23353889]
29. Donohoe ME, Silva SS, Pinter SF, Xu N, Lee JT. 2009. The pluripotency factor Oct4 interacts with Ctcf and also controls X-chromosome pairing and counting. Nature 460:128–32 [PubMed: 19536159]
30. Drzezga A, Lautenschlager N, Siebner H, Riemenschneider M, Willoch F, et al. 2003. Cerebral metabolic changes accompanying conversion of mild cognitive impairment into Alzheimer's

disease: a PET follow-up study. Eur. J. Nucl. Med. Mol. Imaging 30:1104–13 [PubMed: 12764551]

31. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. 2001. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J. Clin. Invest 108:1341–48 [PubMed: 11696579]
32. Durgan DJ, Pat BM, Laczy B, Bradley JA, Tsai JY, et al. 2011. *O*-GlcNAcylation, novel post-translational modification linking myocardial metabolism and cardiomyocyte circadian clock. J. Biol. Chem 286:44606–19 [PubMed: 22069332]
33. Federici M, Menghini R, Mauriello A, Hribal ML, Ferrelli F, et al. 2002. Insulin-dependent activation of endothelial nitric oxide synthase is impaired by *O*-linked glycosylation modification of signaling proteins in human coronary endothelial cells. Circulation 106:466–72 [PubMed: 12135947]
34. Fleming AB, Pennings S. 2001. Antagonistic remodelling by Swi-Snf and Tup1-Ssn6 of an extensive chromatin region forms the background for *FLO1* gene regulation. EMBO J. 20:5219–31 [PubMed: 11566885]
35. Fong JJ, Nguyen BL, Bridger R, Medrano EE, Wells L, et al. 2012. β-N-acetylglucosamine (O-GlcNAc) is a novel regulator of mitosis-specific phosphorylations on histone H3. J. Biol. Chem 287:12195–203 [PubMed: 22371497]
36. Forsythe ME, Love DC, Lazarus BD, Kim EJ, Prinz WA, et al. 2006. Caenorhabditis elegans ortholog of a diabetes susceptibility locus: oga-1 (O-GlcNAcase) knockout impacts *O*-GlcNAc cycling, metabolism, and dauer. Proc. Natl. Acad. Sci. USA 103:11952–57 [PubMed: 16882729]
37. Fujiki R, Chikanishi T, Hashiba W, Ito H, Takada I, et al. 2009. GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. Nature 459:455–59 [PubMed: 19377461]
38. Fujiki R, Hashiba W, Sekine H, Yokoyama A, Chikanishi T, et al. 2011. GlcNAcylation of histone H2B facilitates its monoubiquitination. Nature 480:557–60 [PubMed: 22121020]
39. Gambetta MC, Oktaba K, Müller J. 2009. Essential role of the glycosyltransferase sxc/Ogt in polycomb repression. Science 325:93–96 [PubMed: 19478141]
40. Gao Y, Miyazaki J, Hart GW. 2003. The transcription factor PDX-1 is post-translationally modified by *O*-linked *N*-acetylglucosamine and this modification is correlated with its DNA binding activity and insulin secretion in min6 β-cells. Arch. Biochem. Biophys 415:155–63 [PubMed: 12831837]
41. Garaulet M, Madrid JA. 2010. Chronobiological aspects of nutrition, metabolic syndrome and obesity. Adv. Drug Deliv. Rev 62:967–78 [PubMed: 20580916]
42. Gu Y, Mi W, Ge Y, Liu H, Fan Q, et al. 2010. GlcNAcylation plays an essential role in breast cancer metastasis. Cancer Res. 70:6344–51 [PubMed: 20610629]
43. Guo K, Gan L, Zhang S, Cui FJ, Cun W, et al. 2012. Translocation of HSP27 into liver cancer cell nucleus may be associated with phosphorylation and *O*-GlcNAc glycosylation. Oncol. Rep 28:494–500 [PubMed: 22664592]
44. Han I, Kudlow JE. 1997. Reduced *O* glycosylation of Sp1 is associated with increased proteasome susceptibility. Mol. Cell. Biol 17:2550–58 [PubMed: 9111324]
45. Han I, Roos MD, Kudlow JE. 1998. Interaction of the transcription factor Sp1 with the nuclear pore protein p62 requires the C-terminal domain of p62. J. Cell. Biochem 68:50–61 [PubMed: 9407313]
46. Hanover JA. 2010. Epigenetics gets sweeter: O-GlcNAc joins the “histone code.” Chem. Biol 17:1272–74 [PubMed: 21168762]
47. Hanover JA, Forsythe ME, Hennessey PT, Brodigan TM, Love DC, et al. 2005. A *Caenorhabditis elegans* model of insulin resistance: altered macronutrient storage and dauer formation in an OGT-1 knockout. Proc. Natl. Acad. Sci. USA 102:11266–71 [PubMed: 16051707]
48. Hanover JA, Krause MW, Love DC. 2010. The hexosamine signaling pathway: *O*-GlcNAc cycling in feast or famine. Biochim. Biophys. Acta 1800:80–95 [PubMed: 19647043]
49. Hanover JA, Krause MW, Love DC. 2012. Bittersweet memories: linking metabolism to epigenetics through *O*-GlcNAcylation. Nat. Rev. Mol. Cell. Biol 13:312–21 [PubMed: 22522719]

50. Hanover JA, Yu S, Lubas WB, Shin SH, Ragano-Caracciola M, et al. 2003. Mitochondrial and nucleocytoplasmic isoforms of *O*-linked GlcNAc transferase encoded by a single mammalian gene. Arch. Biochem. Biophys 409:287–97 [PubMed: 12504895]
51. Hart GW, Slawson C, Ramirez-Correa G, Lagerlof O. 2011. Cross talk between *O*-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. Annu. Rev. Biochem 80:825–58 [PubMed: 21391816]
52. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, et al. 2008. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc. Natl. Acad. Sci. USA 105:17046–49 [PubMed: 18955703]
53. Heiss WD, Szelies B, Kessler J, Herholz K. 1991. Abnormalities of energy metabolism in Alzheimer's disease studied with PET. Ann. N. Y. Acad. Sci 640:65–71 [PubMed: 1776760]
53a. Henrotin Y, Chevalier X, Herrero-Beaumont G, McAlindon T, Mobasheri A, et al. 2013. Physiological effects of oral glucosamine on joint health: current status and consensus on future research priorities. BMC Res. Notes 6:115 [PubMed: 23531101]
54. Hewagama A, Patel D, Yarlagadda S, Strickland FM, Richardson BC. 2009. Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis. Genes Immun. 10:509–16 [PubMed: 19279650]
55. Hewagama A, Richardson B. 2009. The genetics and epigenetics of autoimmune diseases. J. Autoimmun 33:3–11 [PubMed: 19349147]
56. Hotamisligil GS, Erbay E. 2008. Nutrient sensing and inflammation in metabolic diseases. Nat. Rev. Immunol 8:923–24 [PubMed: 19029988]
57. Housley MP, Rodgers JT, Udeshi ND, Kelly TJ, Shabanowitz J, et al. 2008. *O*-GlcNAc regulates FoxO activation in response to glucose. J. Biol. Chem 283:16283–92 [PubMed: 18420577]
58. Issad T, Kuo M. 2008. *O*-GlcNAc modification of transcription factors, glucose sensing and glucotoxicity. Trends Endocrinol. Metab 19:380–89 [PubMed: 18929495]
59. Issad T, Masson E, Pagesy P. 2010. *O*-GlcNAc modification, insulin signaling and diabetic complications. Diabetes Metab. 36:423–35 [PubMed: 21074472]
60. Jackson SP, Tjian R. 1988. *O*-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. Cell 55:125–33 [PubMed: 3139301]
61. Jacobsen KT, Iverfeldt K. 2011. *O*-GlcNAcylation increases non-amyloidogenic processing of the amyloid-beta precursor protein (APP). Biochem. Biophys. Res. Commun 404:882–86 [PubMed: 21182826]
62. Kawauchi K, Araki K, Tobiume K, Tanaka N. 2009. Loss of p53 enhances catalytic activity of IKKβ through *O*-linked β-N-acetyl glucosamine modification. Proc. Natl. Acad. Sci. USA 106:3431–36 [PubMed: 19202066]
63. Kebede M, Ferdaoussi M, Mancini A, Alquiera T, Kulkarnic RN, et al. 2012. Glucose activates free fatty acid receptor 1 gene transcription via phosphatidylinositol-3-kinase-dependent *O*-GlcNAcylation of pancreas-duodenum homeobox-1. Proc. Natl. Acad. Sci. USA 109:2376–81 [PubMed: 22308370]
64. Kim EY, Jeong EH, Park S, Jeong HJ, Edery I, Cho JW. 2012. A role for *O*-GlcNAcylation in setting circadian clock speed. Genes Dev. 26:490–502 [PubMed: 22327476]
65. Kim HS, Park SY, Choi YR, Kang JG, Joo HJ, et al. 2009. Excessive *O*-GlcNAcylation of proteins suppresses spontaneous cardiogenesis in ES cells. FEBS Lett. 583:2474–78 [PubMed: 19591829]
66. Kim JW, Dang CV. 2006. Cancer's molecular sweet tooth and the Warburg effect. Cancer Res. 66:8927–30 [PubMed: 16982728]
67. Kreppel LK, Hart GW. 1999. Regulation of a cytosolic and nuclear *O*-GlcNAc transferase: role of the tetratricopeptide repeats. J. Biol. Chem 274:32015–22 [PubMed: 10542233]
68. Kuo M, Zilberfarb V, Gangneux N, Christeff N, Issad T. 2008. *O*-GlcNAc modification of FoxO1 increases its transcriptional activity: a role in the glucotoxicity phenomenon? Biochimie 90:679–85 [PubMed: 18359296]
69. Lazarus MB, Nam Y, Jiang J, Sliz P, Walker S. 2011. Structure of human *O*-GlcNAc transferase and its complex with a peptide substrate. Nature 469:564–67 [PubMed: 21240259]
70. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, et al. 2006. Control of developmental regulators by polycomb in human embryonic stem cells. Cell 125:301–13 [PubMed: 16630818]

71. Lefebvre T, Dehennaut V, Guinez C, Olivier S, Drougat L, et al. 2010. Dysregulation of the nutrient/stress sensor *O*-GlcNAcylation is involved in the etiology of cardiovascular disorders, type-2 diabetes and Alzheimer's disease. Biochim. Biophys. Acta 1800:67–79 [PubMed: 19732809]
72. Lehman DM, Fu DJ, Freeman AB, Hunt KJ, Leach RJ, et al. 2005. A single nucleotide polymorphism in MGEA5 encoding *O*-GlcNAc-selective N-acetyl-beta-D glucosaminidase is associated with type 2 diabetes in Mexican Americans. Diabetes 54:1214–21 [PubMed: 15793264]
73. Lemischka IR. 2010. Hooking up with Oct4. Cell Stem Cell 6:291–92 [PubMed: 20362529]
74. Li MD, Ruan HB, Hughes ME, Lee JS, Singh JP, et al. 2013. *O*-GlcNAc signaling entrains the circadian clock by inhibiting BMAL1/CLOCK ubiquitination. Cell Metab. 17:303–10 [PubMed: 23395176]
75. Lima VV, Giachini FR, Carneiro FS, Carneiro ZN, Fortes ZB, et al. 2008. Increased vascular *O*-GlcNAcylation augments reactivity to constrictor stimuli—Vasoactive Peptide Symposium. J. Am. Soc. Hypertens 2:410–17 [PubMed: 19884969]
76. Lima VV, Rigsby CS, Hardy DM, Webb RC, Tostes RC. 2009. *O*-GlcNAcylation: a novel post-translational mechanism to alter vascular cellular signaling in health and disease: focus on hypertension. J. Am. Soc. Hypertens 3:374–87 [PubMed: 20409980]
77. Lin H, Gupta V, Vermilyea MD, Falciani F, Lee JT, et al. 2007. Dosage compensation in the mouse balances up-regulation and silencing of X-linked genes. PLoS Biol. 5:e326 [PubMed: 18076287]
78. Liu F, Iqbal K, Grundke-Iqbal I, Hart GW, Gong CX. 2004. *O*-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. Proc. Natl. Acad. Sci. USA 101:10804–9 [PubMed: 15249677]
79. Love DC, Ghosh S, Mondoux MA, Fukushige T, Wang P, et al. 2010. Dynamic *O*-GlcNAc cycling at promoters of *Caenorhabditis elegans* genes regulating longevity, stress, and immunity. Proc. Natl. Acad. Sci. USA 107:7413–18 [PubMed: 20368426]
80. Love DC, Hanover JA. 2005. The hexosamine signaling pathway: deciphering the "O-GlcNAc code." Sci. STKE 2005:re13 [PubMed: 16317114]
81. Love DC, Krause MW, Hanover JA. 2010. *O*-GlcNAc cycling: emerging roles in development and epigenetics. Semin. Cell Dev. Biol 21:646–54 [PubMed: 20488252]
82. Lubas WA, Frank DW, Krause M, Hanover JA. 1997. *O*-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. J. Biol. Chem 272:9316–24 [PubMed: 9083068]
83. Luo B, Soesanto Y, McClain DA. 2008. Protein modification by *O*-linked GlcNAc reduces angiogenesis by inhibiting Akt activity in endothelial cells. Arterioscler. Thromb. Vasc. Biol 28:651–57 [PubMed: 18174452]
84. Lynch TP, Ferrer CM, Jackson SR, Shahriari KS, Vosseller K, Reginato MJ. 2012. Critical role of *O*-linked β-*N*-acetylglucosamine transferase in prostate cancer invasion, angiogenesis, and metastasis. J. Biol. Chem 287:11070–81 [PubMed: 22275356]
85. Malik VS, Schulze MB, Hu FB. 2006. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am. J. Clin. Nutr 84:274–88 [PubMed: 16895873]
86. Marshall S, Bacote V, Traxinger RR. 1991. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. J. Biol. Chem 266:4706–12 [PubMed: 2002019]
87. Medford HM, Chatham JC, Marsh SA. 2012. Chronic ingestion of a Western diet increases O-linked-β-N-acetylglucosamine (O-GlcNAc) protein modification in the rat heart. Life Sci. 90:883–88 [PubMed: 22575823]
88. Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, et al. 2006. Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. Mol. Cell 21:811–23 [PubMed: 16543150]
89. Mielke R, Herholz K, Grond M, Kessler J, Heiss WD. 1994. Clinical deterioration in probable Alzheimer's disease correlates with progressive metabolic impairment of association areas. Dementia 5:36–41 [PubMed: 8156085]

90. Mondoux MA, Love DC, Ghosh SK, Fukushige T, Bond M, et al. 2011. *O*-linked-*N*-acetylglucosamine cycling and insulin signaling are required for the glucose stress response in *Caenorhabditis elegans*. Genetics 188:369–82 [PubMed: 21441213]
91. Myers SA, Panning B, Burlingame AL. 2011. Polycomb repressive complex 2 is necessary for the normal site-specific *O*-GlcNAc distribution in mouse embryonic stem cells. Proc. Natl. Acad. Sci. USA 108:9490–95 [PubMed: 21606357]
92. Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, et al. 2009. A histone H3 lysine 36 trimethyltransferase links Nkx2–5 to Wolf-Hirschhorn syndrome. Nature 460:287–91 [PubMed: 19483677]
93. O'Donnell N, Zachara NE, Hart GW, Marth JD. 2004. *Ogt*-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. Mol. Cell. Biol 24:1680–90 [PubMed: 14749383]
94. Ozcan S, Andrali SS, Cantrell JE. 2010. Modulation of transcription factor function by *O*-GlcNAc modification. Biochim. Biophys. Acta 1799:353–64 [PubMed: 20202486]
95. Pardo M, Lang B, Yu L, Prosser H, Bradley A, et al. 2010. An expanded Oct4 interaction network: implications for stem cell biology, development, and disease. Cell Stem Cell 6:382–95 [PubMed: 20362542]
96. Park SY, Kim HS, Kim NH, Ji S, Cha SY, et al. 2010. Snail1 is stabilized by *O*-GlcNAc modification in hyperglycaemic condition. EMBO J. 29:3787–96 [PubMed: 20959806]
97. Park SY, Ryu J, Lee W. 2005. *O*-GlcNAc modification on IRS-1 and Akt2 by PUGNAc inhibits their phosphorylation and induces insulin resistance in rat primary adipocytes. Exp. Mol. Med 37:220–29 [PubMed: 16000877]
98. Patti ME, Virkamaki A, Landaker EJ, Kahn CR, Yki-Jarvinen H. 1999. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. Diabetes 48:1562–71 [PubMed: 10426374]
99. Pettitt DJ, Jovanovic L. 2007. The vicious cycle of diabetes and pregnancy. Curr. Diab. Rep 7:295–97 [PubMed: 17686406]
100. Pham T, Cornea A, Blick KE, Jenkins A, Scofield RH. 2007. Oral glucosamine in doses used to treat osteoarthritis worsens insulin resistance. Am. J. Med. Sci 333:333–39 [PubMed: 17570985]
101. Ragle RL, Sawitzke AD. 2012. Nutraceuticals in the management of osteoarthritis: a critical review. Drugs Aging 29:717–31 [PubMed: 23018608]
102. Rahman MM, Stuchlick O, El-Karim EG, Stuart R, Kipreos ET, Wells L. 2010. Intracellular protein glycosylation modulates insulin mediated lifespan in *C. elegans*. Aging 2:678–90 [PubMed: 20952811]
103. Ranuncolo SM, Ghosh S, Hanover JA, Hart GW, Lewis BA. 2012. Evidence of the involvement of *O*-GlcNAc-modified human RNA polymerase II CTD in transcription in vitro and in vivo. J. Biol. Chem 287:23549–61 [PubMed: 22605332]
104. Reusch JE. 2003. Diabetes, microvascular complications, and cardiovascular complications: What is it about glucose? J. Clin. Invest 112:986–88 [PubMed: 14523035]
105. Rexach JE, Clark PM, Mason DE, Neve RL, Peters EC, Hsieh-Wilson LC. 2012. Dynamic *O*-GlcNAc modification regulates CREB-mediated gene expression and memory formation. Nat. Chem. Biol 8:253–61 [PubMed: 22267118]
106. Richardson BC. 2008. Epigenetics and autoimmunity. Overview. *Autoimmunity* 41:243–44 [PubMed: 18432404]
107. Roos MD, Su K, Baker JR, Kudlow JE. 1997. O glycosylation of an Sp1-derived peptide blocks known Sp1 protein interactions. Mol. Cell. Biol 17:6472–80 [PubMed: 9343410]
108. Roperoa AB, Alonso-Magdalenab P, Quesadaa I, Nadal A. 2008. The role of estrogen receptors in the control of energy and glucose homeostasis. Steriods 73:874–79
109. Roquemore EP, Chevrier MR, Cotter RJ, Hart GW. 1996. Dynamic *O*-GlcNAcylation of the small heat shock protein αB-crystallin. Biochemistry 35:3578–86 [PubMed: 8639509]
110. Rossetti L 2000. Perspective: hexosamines and nutrient sensing. Endocrinology 141:1922–25 [PubMed: 10830271]
111. Ruderman NB, Williamson JR, Brownlee M. 1992. Glucose and diabetic vascular disease. FASEB J. 6:2905–14 [PubMed: 1644256]

112. Sakabe K, Hart GW. 2010. *O*-GlcNAc transferase regulates mitotic chromatin dynamics. J. Biol. Chem 285:34460–68 [PubMed: 20805223]
113. Sakabe K, Wang Z, Hart GW. 2010. Beta-N-acetylglucosamine (O-GlcNAc) is part of the histone code. Proc. Natl. Acad. Sci. USA 107:19915–20 [PubMed: 21045127]
114. Sakaidani Y, Nomura T, Matsuura A, Ito M, Suzuki E, et al. 2011. *O*-Linked-*N*-acetylglucosamine on extracellular protein domains mediates epithelial cell–matrix interactions. Nat. Commun 13:583
115. Sayeski PP, Kudlow JE. 1996. Glucose metabolism to glucosamine is necessary for glucose stimulation of transforming growth factor-alpha gene transcription. J. Biol. Chem 271:15237–43 [PubMed: 8663078]
116. Scroggie DA, Albright A, Harris MD. 2003. The effect of glucosamine-chondroitin supplementation on glycosylated hemoglobin levels in patients with type 2 diabetes mellitus: a placebo-controlled, double-blinded, randomized clinical trial. Arch. Intern. Med 163:1587–90 [PubMed: 12860582]
117. Sekine O, Love DC, Rubenstein DS, Hanover JA. 2010. Blocking *O*-linked GlcNAc cycling in *Drosophila* insulin-producing cells perturbs glucose-insulin homeostasis. J. Biol. Chem 285:38684–91 [PubMed: 20926386]
118. Shafi R, Iyer SPN, Ellies LG, O'Donnell N, Marek KW, et al. 2000. The *O*-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc. Natl. Acad. Sci. USA 97:5735–39 [PubMed: 10801981]
119. Shi Y, Tomic J, Wen F, Shaha S, Bahlo A, et al. 2010. Aberrant *O*-GlcNAcylation characterizes chronic lymphocytic leukemia. Leukemia 24:1588–98 [PubMed: 20668475]
120. Shin SH, Love DC, Hanover JA. 2011. Elevated *O*-GlcNAc-dependent signaling through inducible mOGT expression selectively triggers apoptosis. Amino Acids 40:885–93 [PubMed: 20824293]
121. Simanek EE, Huang D-H, Pasternack L, Machajewski TD, Seitz O, et al. 1998. Glycosylation of threonine of the repeating unit of RNA polymerase II with beta-linked N-acetylglucosamine leads to a turnlike structure. J. Am. Chem. Soc 120:11567–75
122. Sinclair DA, Syrzycka M, Macauley MS, Rastgardani T, Komljenovic I, et al. 2009. Drosophila O-GlcNAc transferase (OGT) is encoded by the Polycomb group (PcG) gene, super sex combs (sxc). Proc. Natl. Acad. Sci. USA 106:13427–32 [PubMed: 19666537]
123. Slawson C, Lakshmanan T, Knapp S, Hart GW. 2008. A mitotic GlcNAcylation/phosphorylation signaling complex alters the posttranslational state of the cytoskeletal protein vimentin. Mol. Biol. Cell 19:4130–40 [PubMed: 18653473]
124. Slawson C, Zachara NE, Vosseller K, Cheung WD, Lane MD, Hart GW. 2005. Perturbations in *O*-linked beta-*N*-acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. J. Biol. Chem 280:32944–56 [PubMed: 16027160]
125. Soesanto YA, Luo B, Jones D, Taylor R, Gabrielsen JS, et al. 2008. Regulation of Akt signaling by *O*-GlcNAc in euglycemia. Am. J. Physiol. Endocrinol. Metab 295:E974–80 [PubMed: 18728220]
126. Srikanth B, Vaidya MM, Kalraiya RD. 2010. *O*-GlcNAcylation determines the solubility, filament organization, and stability of keratins 8 and 18. J. Biol. Chem 285:34062–71 [PubMed: 20729549]
127. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, et al. 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J. Alzheimers Dis 7:63–80 [PubMed: 15750215]
128. van den Berg DL, Snoek T, Mullin NP, Yates A, Bezstarosti K, et al. 2010. An Oct4-centered protein interaction network in embryonic stem cells. Cell Stem Cell 6:369–81 [PubMed: 20362541]
129. Vella P, Scelfo A, Jammula S, Chiacchiera F, Williams K, et al. 2013. TET proteins connect the *O*-linked N-acetylglucosamine transferase Ogt to chromatin in embryonic stem cells. Mol. Cell 49:645–56 [PubMed: 23352454]
130. Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393:684–88 [PubMed: 9641678]

131. Wang P, Lazarus BD, Forsythe ME, Love DC, Krause MW, Hanover JA. 2012. *O*-GlcNAc cycling mutants modulate proteotoxicity in *Caenorhabditis elegans* models of human neurodegenerative diseases. Proc. Natl. Acad. Sci. USA 109:17669–74 [PubMed: 22988095]
132. Wang Z, Udeshi ND, Slawson C, Compton PD, Sakabe K, et al. 2010. Extensive crosstalk between O-GlcNAcylation and phosphorylation regulates cytokinesis. Sci. Signal 3:ra2 [PubMed: 20068230]
133. Warburg O 1956. On respiratory impairment in cancer cells. Science 124:269–70 [PubMed: 13351639]
134. Wells L, Vosseller K, Cole RN, Cronshaw JM, Matunis MJ, Hart GW. 2002. Mapping sites of *O*-GlcNAc modification using affinity tags for serine and threonine post-translational modifications. Mol. Cell. Proteomics 1:791–804 [PubMed: 12438562]
135. Whelan SA, Dias WB, Thiruneelakantapillai L, Lane MD, Hart GW. 2010. Regulation of insulin receptor substrate 1 (IRS-1)/AKT kinase-mediated insulin signaling by O-linked beta-N-acetylglucosamine in 3T3-L1 adipocytes. J. Biol. Chem 285:5204–11 [PubMed: 20018868]
136. Whelan SA, Lane MD, Hart GW. 2008. Regulation of the *O*-linked beta-N-acetylglucosamine transferase by insulin signaling. J. Biol. Chem 283:21411–17 [PubMed: 18519567]
137. Wotton KR, Weierud FK, Juarez-Morales JL, Alvares LE, Dietrich S, Lewis KE. 2009. Conservation of gene linkage in dispersed vertebrate NK homeobox clusters. Dev. Genes Evol 219:481–96 [PubMed: 20112453]
138. Yanagisawa M, Planel E, Ishiguro K, Fujita SC. 1999. Starvation induces tau hyperphosphorylation in mouse brain: implications for Alzheimer's disease. FEBS Lett. 461:329–33 [PubMed: 10567721]
139. Yang WH, Kim JE, Nam HW, Ju JW, Kim HS, et al. 2006. Modification of p53 with *O*-linked *N*-acetylglucosamine regulates p53 activity and stability. Nat. Cell. Biol 8:1074–83 [PubMed: 16964247]
140. Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, et al. 2008. Phosphoinositide signalling links *O*-GlcNAc transferase to insulin resistance. Nature 451:964–69 [PubMed: 18288188]
141. Yang X, Zhang F, Kudlow JE. 2002. Recruitment of *O*-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein *O*-GlcNAcylation to transcriptional repression. Cell 110:69–80 [PubMed: 12150998]
142. Yang YR, Song M, Lee H, Jeon Y, Choi EJ, et al. 2012. *O*-GlcNAcase is essential for embryonic development and maintenance of genomic stability. Aging Cell 11:439–48 [PubMed: 22314054]
143. Yki-Jarvinen H, Virkamaki A, Daniels MC, McClain D, Gottschalk WK. 1998. Insulin and glucosamine infusions increase *O*-linked *N*-acetyl-glucosamine in skeletal muscle proteins in vivo. Metabolism 47:449–55 [PubMed: 9550544]
144. Yuzwa SA, Shan X, Macauley MS, Clark T, Skorobogatko Y, et al. 2012. Increasing *O*-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. Nat. Chem. Biol 8:393–99 [PubMed: 22366723]
145. Zachara NE, Hart GW. 2004. *O*-GlcNAc a sensor of cellular state: the role of nucleocytoplasmic glycosylation in modulating cellular function in response to nutrition and stress. Biochim. Biophys. Acta 1673:13–28 [PubMed: 15238246]
146. Zachara NE, Hart GW. 2004. *O*-GlcNAc modification: a nutritional sensor that modulates proteasome function. Trends Cell. Biol 14:218–21 [PubMed: 15130576]
147. Zachara NE, O'Donnell N, Cheung WD, Mercer JJ, Marth JD, Hart GW. 2004. Dynamic *O*-GlcNAc modification of nucleocytoplasmic proteins in response to stress. J. Biol. Chem 279:30133–42 [PubMed: 15138254]
148. Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE. 2003. *O*-GlcNAc modification is an endogenous inhibitor of the proteasome. Cell 115:715–25 [PubMed: 14675536]
149. Zhang S, Roche K, Nasheuer HP, Lowndes NF. 2011. Modification of histones by sugar β-N-acetylglucosamine (GlcNAc) occurs on multiple residues, including histone H3 serine 10, and is cell cycle-regulated. J. Biol. Chem 286:37483–95 [PubMed: 21896475]

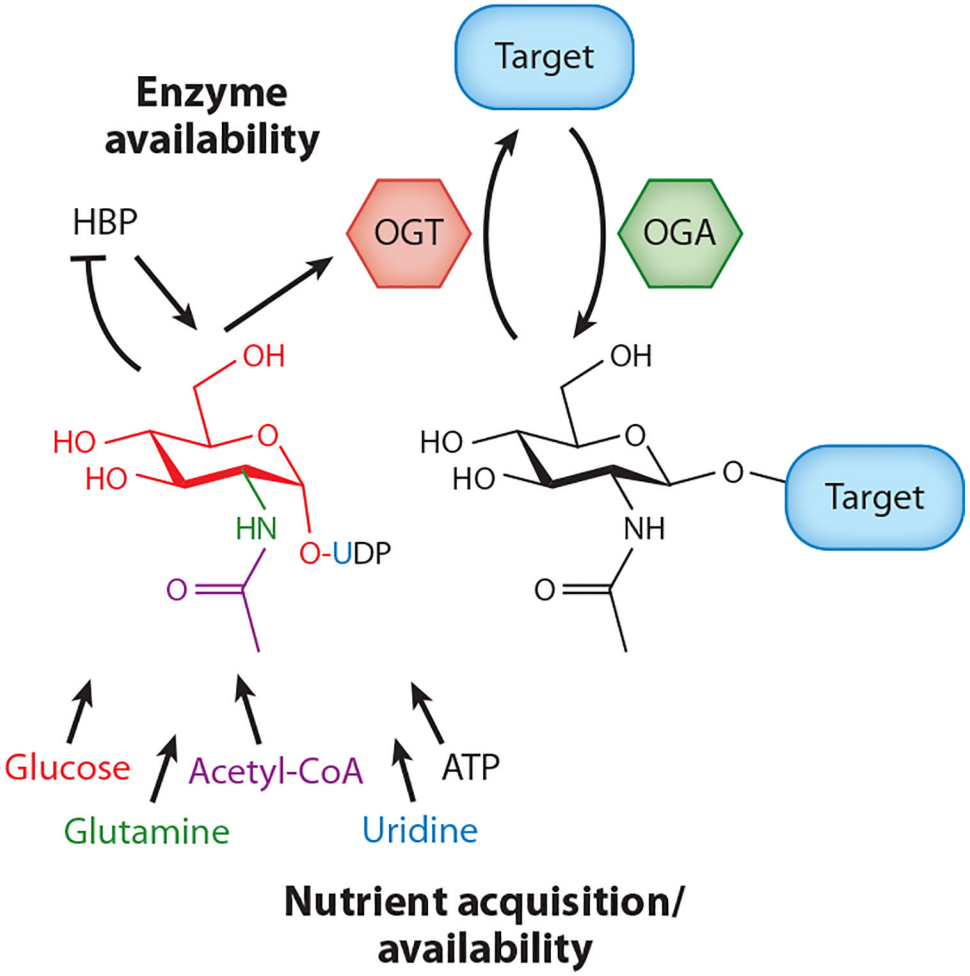

Figure 1.

O-linked *N*-acetylglucosamine (*O*-GlcNAc) is a ubiquitous nucleocytoplasmic modification added to many protein targets. Two to five percent of total intracellular glucose enters the hexosamine biosynthetic pathway (HBP), and the production of its ultimate product, uridine diphospho (UDP)-GlcNAc, is governed by both enzyme and nutrient availability. *O*-GlcNAc transferase (OGT) utilizes the activated sugar nucleotide to add *O*-GlcNAc to target proteins, and *O*-GlcNAcase (OGA) removes the modification.

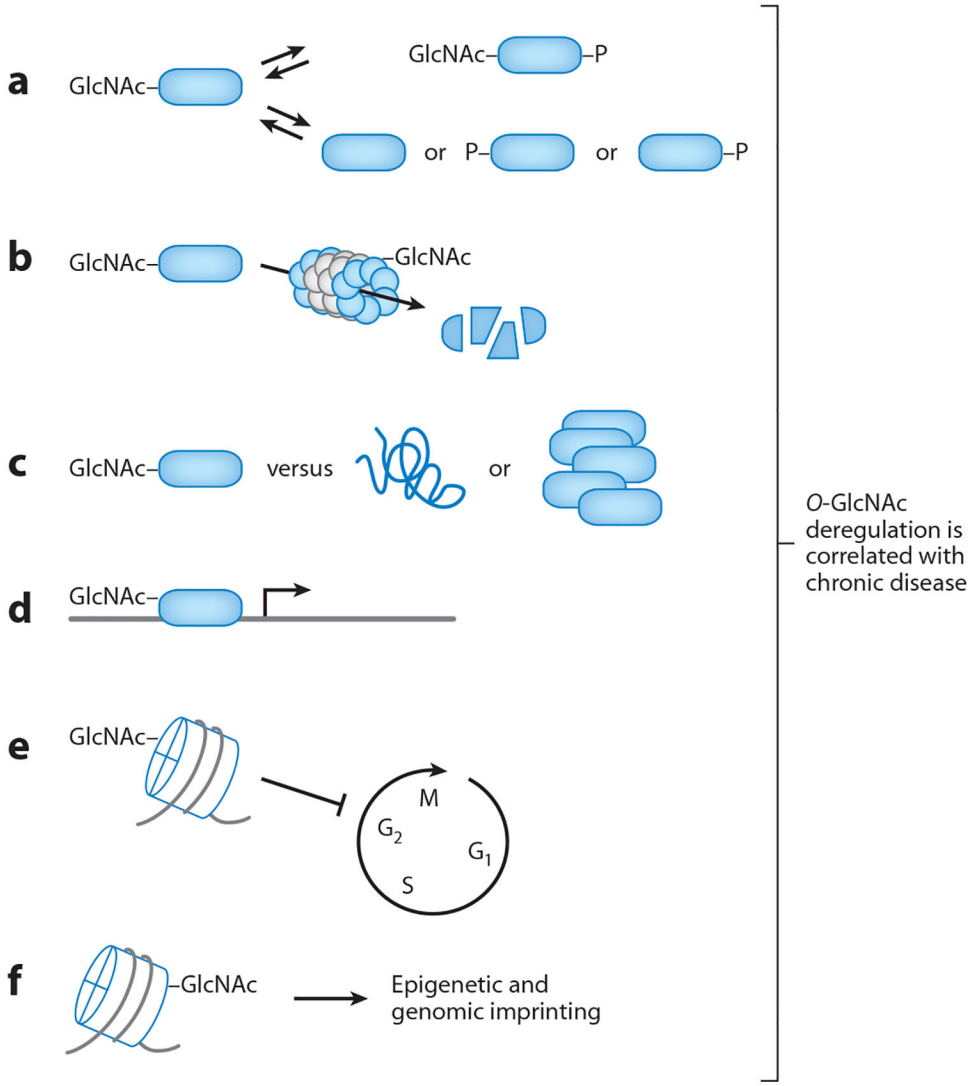

Figure 2.

O-GlcNAc acts to integrate signals and influences biological processes; its deregulation is correlated with chronic disease. More than 1,000 proteins are known to be modified by *O*-GlcNAc. *O*-GlcNAcylation affects (*a*) protein phosphorylation status as well as downstream cellular signaling and protein activity, (*b*) targeting to the proteasome as well as proteasome activity, (*c*) protein stability, (*d*) transcription, (*e*) cell cycle and survival, and (*f*) epigenetic and genomic imprinting.

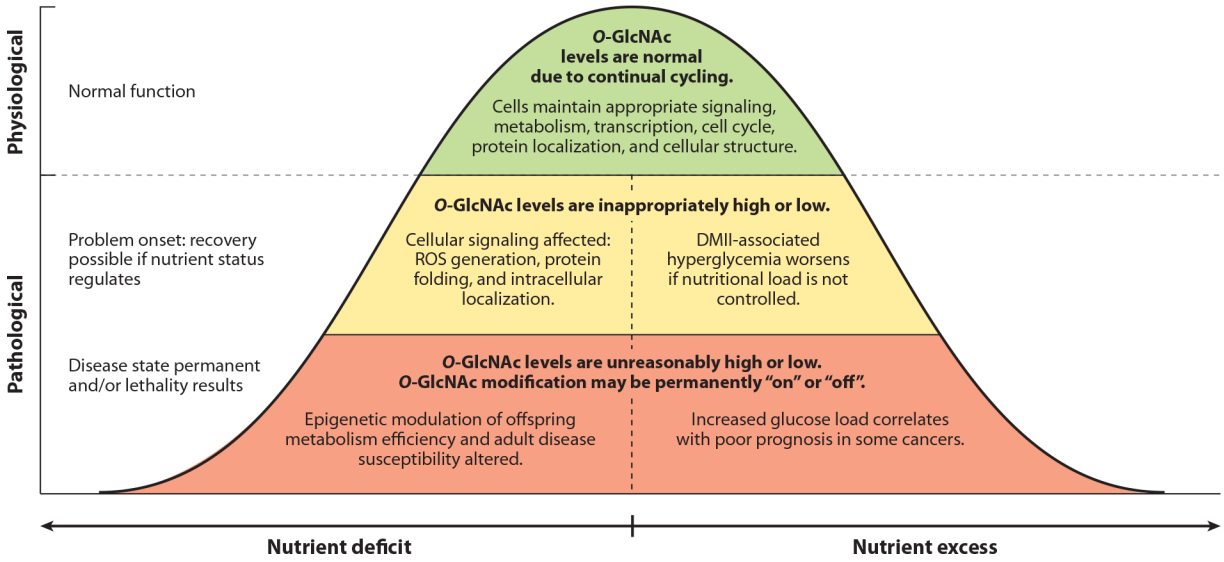

Figure 3.

The *O*-GlcNAc modification acts as a rheostat on a continuum: Inappropriately high and low levels can have deleterious effects, examples of which are discussed in more detail in the text. Abbreviations: DMII, diabetes mellitus type 2; ROS, reactive oxygen species.

Table 1

Mechanisms by which the *O*-GlcNAc posttranslational modification (PTM) can directly and indirectly influence nutrition and metabolism

O-GlcNAc-modified protein	Role of protein	Potential role of O-GlcNAc
AKT	Serine/threonine kinase required for appropriate glucose metabolism, transcription, and cell survival	Increased *O*-GlcNAc correlates with decreased phosphorylation and reduction in angiogenesis in endothelial cells[a]
α-Crystallin	Member of heat-shock protein family influencing biological regulatory control	Turnover of O-GlcNAc on protein is more rapid than protein turnover, supporting a regulatory role[b]
APP	Major component found in brain plaques of AD patients	O-GlcNAcylation increases a-secretase processing, decreasing amyloidogenic products[c]
ER- β	Nuclear receptor that works to activate transcription and acts as a tumor suppressor	O-GlcNAc regulates turnover[d]
CRTC2	Coactivator acting to regulate gluconeogenic gene expression	Nutrient levels affect *O*-GlcNAcylation and thus cellular localization, which leads to changes in gluconeogenesis[e]
eNOS	Metabolic enzyme responsible for production of nitric oxide, which is critical for vascular tone	It is proposed that the activity of eNOS is influenced by O-GlcNAcylation[f]
FoxOl	Transcription factor playing a critical role in insulin signaling	Influences transcription of genes involved in critical biological processes including those implicated in glucotoxicity[g]
H2B	Forming the nucleasome core, this histone is critical for eukaryotic chromatin structure	Acting as a handle for the H2B ubiquitin ligase, O-GlcNAc facilitates H2B ubiquitination[h]
H3	An important player in epigenetics; H3 sequence variations and PTMs influence long-term gene regulation	O-GlcNAc, through regulation of mitosis-specific phosphorylation, influences cell cycle progression[i]
HSP27	Chaperone functioning to prevent apoptotic cell death; improper expression is associated with aggressive tumors	Protein localization in liver cancer cells may be influenced by O-GlcNAcylation[j]
HSP90	A chaperone that assists in protein folding, protein stability, and protection against heat stress	Required for appropriate downstream *O*-GlcNAcylation of OGT targets[k]
IRS1	Adaptor protein that plays a key role in signal transmission from insulin and insulin-like growth factor 1 receptors to intracellular signaling pathways	O-GlcNAc modification correlates with insulin resistance in adipocytes[l]
Keratins	Filamentous, cytoskeletal proteins	O-GlcNAc influences solubility and stability[m]
NeuroD1	Transcription factor required for appropriate regulation of insulin gene	NeuroDl localization in cytosol and nucleus influenced by O-GlcNAc levels[n]
OGT	Enzyme catalyzing the addition of O-GlcNAc to protein serine and threonine residues	It is postulated that O-GlcNAc helps to regulate OGT[o]
p53	Regulates the cell cycle and acts as a tumor suppressor	p53 targeting to the proteasome is blocked upon O-GlcNAcylation[p]

O-GlcNAc-modified protein	Role of protein	Potential role of O-GlcNAc
Proteasome	Protein complex responsible for protein degradation	Unknown role; O-GlcNAc levels are increased in DMII patients[q]
PDK1	Master kinase responsible for activating other kinases such as AKT	Unknown role; activity does not appear to be modified by *O*-GlcNAc modification[r]
PDX1	Transcription factor playing a major role in insulin gene expression	*O*-GlcNAcylation correlates with DNA-binding activity[s]
RNA Pol II	Enzyme responsible for transcriptional control	It is proposed that *O*-GlcNAc regulates transcriptional elongation or ubiquitination of RNA Pol II, influencing its degradation[t]
Sp1	Transcription factor involved in organisms' early development	*O*-GlcNAc modulates turnover, transactivation, and protein-protein interactions of Sp1 that in turn influence SERCA2a expression in a DMII model[u]
tau	Cytoskeletal protein required for appropriate microtubule stabilization	*O*-GlcNAc is proposed to protect tau from hyperphosphorylation, which is also induced during starvation[v]
TET family proteins	Mediate the hydroxymethylation of DNA and mediate gene expression	Unknown role; interaction with OGT likely influences downstream transcription[w]

[a](75, 76, 83);

[b](25, 109);

[c](61);

[d](12, 13, 108);

[e](27, 94);

[f](31);

[g](68);

[h](38);

[i](35);

[j](43);

[k](134);

[l](97);

[m](126);

[n](4);

[o](67, 82);

[p](139);

[q](146, 148);

[r](140);

[s](40);

[t](19, 103);

[u](18, 44, 45, 60, 107);

[v](138);

[w](11, 28, 129).

Abbreviations: AD, Alzheimer's disease; APP, amyloid beta precursor protein; ER-β, estrogen receptor beta; CRTC2, cAMP response element–binding protein (CREB)-regulated transcription coactivator 2; DMII, diabetes mellitus type 2; eNOS, endothelial nitric oxide synthase; FoxO1, forkhead box protein O1; H2B, histone 2b; H3, histone 3; HSP27, heat shock protein 27; HSP90, heat shock protein 90; IRS1, insulin receptor substrate 1; OGT, *O*-GlcNAc transferase; p53, protein 53; PDK1, pyruvate dehydrogenase kinase isozyme 1; PDX1, pancreatic and duodenal homeobox 1; RNA Pol II, RNA polymerase II; SERCA2a, sarco/endoplasmic reticulum Ca2+-ATPase; Sp1, specificity protein 1; TET, ten-eleven translocation.