Published in final edited form as:

Biochim Biophys Acta. 2010 February ; 1800(2): 80. doi:10.1016/j.bbagen.2009.07.017.

The Hexosamine Signaling Pathway: O-GlcNAc cycling in feast or famine

John A. Hanover^{*}, Michael W. Krause, and Dona C. Love

Laboratory of Cell Biochemistry and Biology and Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892

Abstract

The enzymes of O-GlcNAc cycling couple the nutrient-dependent synthesis of UDP-GlcNAc to O-GlcNAc modification of Ser/Thr residues of key nuclear and cytoplasmic targets. This series of reactions culminating in O-GlcNAcylation of targets has been termed the Hexosamine Signaling Pathway (HSP). The evolutionarily ancient enzymes of O-GlcNAc cycling have co-evolved with other signaling effector molecules; they are recruited to their targets by many of the same mechanisms used to organize canonic kinase-dependent signaling pathways. This co-recruitment of the enzymes of O-GlcNAc cycling drives a binary switch impacting pathways of anabolism and growth (nutrient uptake) and catabolic pathways (nutrient sparing and salvage). The Hexosamine Signaling Pathway (HSP) has thus emerged as a versatile cellular regulator modulating numerous cellular signaling cascades influencing growth, metabolism, cellular stress, circadian rhythm, and host-pathogen interactions. In mammals, the nutrient-sensing HSP has been harnessed to regulate such cell-specific functions as neutrophil migration, and activation of B-cells and T-cells. This review summarizes the diverse approaches being used to examine O-GlcNAc cycling. It will emphasize the impact O-GlcNAcylation has upon signaling pathways that may become deregulated in diseases of the immune system, diabetes mellitus, cancer, cardiovascular disease, and neurodegenerative diseases.

Introduction

Organisms have evolved a robust network of signaling pathways allowing them to distinguish sources of food from pathogens (immunity), regulate the uptake and utilization of food (metabolism), and adapt to nutrient availability (gene expression). One of the most evolutionarily ancient of these pathways is the nutrient-sensing addition of O-GlcNAc to target proteins [1-5]. Many reviews have appeared in the past few years detailing many aspects of O-GlcNAc metabolism [1,2,5-7]. In addition, several excellent reviews have focused on methods of detection of O-GlcNAc [8,9]. This review will focus on the genetic, molecular genetic and chemical genomic dissection of the HSP in an attempt to expose the molecular logic linking the many intracellular signaling pathways influenced by O-GlcNAc cycling.

O-GlcNAc metabolism and Human Disease

Before embarking on a detailed analysis of mechanism, the likely involvement of O-GlcNAc cycling in human disease must be emphasized. Based on large-scale genetic associations, the

^{*}To Whom All Correspondence should be addressed: John A Hanover Rm B127 Bldg 8, NIDDK, National Institutes of Health Bethesda MD 20892-0851.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

gene encoding the enzyme that removes the O-GlcNAc modification has recently been shown to be a susceptibility locus for type-2 diabetes in the Mexican-American population [10]. Additionally, O-GlcNAc transferase (OGT) is encoded on chromosome X and has emerged as a candidate gene for X-linked Dystonia Parkinsonism [11,12]. Numerous other studies have linked O-GlcNAc cycling to the proteotoxicity associated with neurodegeneration [5,13,14, 14-19], type-2 diabetes mellitus [20-24], and cardiovascular disease [25-30]. It is likely that alterations in O-GlcNAc cycling may also contribute to the pathology of other human diseases, including cancer [1,2,5], and diseases of immune function [31,32], thus making the HSP a potentially important drug target.

The Enzymes of O-GlcNAc Cycling

In animals, two highly conserved enzymes are responsible for the cycling of O-GlcNAc on Ser/Thr residues of target proteins. The addition of O-GlcNAc is mediated by the glycosyltransferase, OGT. This enzyme was first identified in human, rat and *C. elegans* [22, 33]. In addition, the transferases from *Arabidopsis* and *Giardia* have also been identified [4, 34]. The enzyme mediating O-GlcNAc removal is the O-GlcNAcase, encoded in mammals by the MGEA5 locus [35,36]. This enzyme has now been characterized in human, mouse, rat, and *C. elegans*. In all animals studied to date, single genes encode OGT and the O-GlcNAcase. *Arabidopsis* appear to be an exception and contains two genes encoding OGT. The following sections will detail the protein expression, localization, chemical and structural biology of these enzymes of O-GlcNAc cycling.

O-linked GlcNAc Transferase

In mammals, OGT is encoded on chromosome X (Xq13) in close proximity to the Xist locus involved in X-inactivation [37-39]. From publicly available databases (such as BioGPS), it is now clear that the transcripts encoding mammalian OGT gene are very highly expressed in T-cells, B-cells, and macrophages. It is also present in moderately high levels in pancreatic β cells and in the central nervous system. Low level, ubiquitous expression is found in other organs. The gene encodes a number of splice variants producing protein products with distinct amino termini targeting them either to the nucleus, cytoplasm or mitochondrion [40] (See Fig 1A, left panel). These variants range in molecular mass from about 70 kDa to about 110 kDa, and contain between 2 and 13 tetratricopeptide (TPR) repeats at their amino terminus. The enzyme variants all have a common catalytic domain (Fig 1A). Extensive mutagenesis analysis of the catalytic domain identified both activating and inactivating mutations [3,15,41,42]. A number of mutations have also been molecularly identified in the *Arabidopsis* OGT homologs *Spindly* and *Secret Agent* by forward genetic approaches [43,44]. However, the divergent coding sequences between the plant and animal OGT make comparison of catalytically important residues difficult. The other domains of OGT have come under similar scrutiny. When the structure of the TPR region of the nucleocytoplasmic form of OGT was solved, it revealed that the enzyme exists as a superhelical assembly that dimerizes using important contacts within the TPR repeats [45] (Fig 1B, right panel). Mutagenesis of this dimerization motif inhibited enzyme activity [45]. These findings likely suggest that formation of oligomers of OGT may be required for full catalytic activity.

The structure of the catalytic domain of a bacterial homolog of OGT has recently been solved by two groups [46,47], now allowing a model of human OGT to be produced by combining the human TPR structure [45] with the bacterial OGT homolog structures [46,47] (Fig 1B). It should be noted that the bacterial structure lacks the “linker region” separating the catalytic domains in human OGT [3], and is catalytically inactive with respect to macromolecular substrates. Although this linker could have significance for the regulation of OGT, the overall fold is unlikely to be altered by its omission. The structure of the bacterial OGT has revealed

a number of important clues as to how OGT may function to recognize substrate (Fig 1B). The catalytic domain adopts the glycosyltransferase-B fold-a metal independent catalytic center [47]. The catalytic center is indicated between the two lobes of the catalytic domain in Fig 1B, as indicated in the two projections of the structure (right and left, Fig 1B.). The TPR residues form a binding groove interacting with the nucleotide sugar-binding site. The structure confirms previous speculations that the TPR residues were key components of the substrate-binding pocket [42,48]. It extends these observations by pointing to the central importance of the arrangement of catalytic and TPR domains. The superhelical TPR repeats were shown to have an asparagine ladder motif similar to that present in the nuclear import receptor, importin α [45]. This asparagine ladder is likely to serve as a binding surface for the interaction of target peptides. The TPR repeats are also the site of interaction with many of the binding partners of OGT discussed in later sections (Fig 1B, left).

The other significant finding emerging from the crystal structure of OGT was the localization of the C-terminal PIP3 binding domain to a helix at the 'top' of the head of the bi-lobed catalytic domain (Fig 1B, right and left). Since it is present in a solvent exposed volume, it is now rather easy to imagine this domain interacting with membrane inositol-phosphate (PIP3) lipids, temporarily anchoring the OGT catalytic domain to the plane of the PIP3-rich membrane. Recruitment of OGT would enhance access to the key substrates similarly recruited to the cell surface by the activation of tyrosine kinase receptors (see below). A more detailed analysis of OGT structure-function can be found in the article by Davies in this volume.

O-GlcNAcase

When first identified as a neutral pH hexosaminidase [35], it became clear that O-GlcNAcase had been previously cloned as an autoantigen associated with meningioma designated MGEA5 [5,36]. Like OGT, transcripts derived from the O-GlcNAcase gene are highly expressed in immune cells and in the central nervous system. It is also widely expressed at lower levels in other tissues, including endocrine tissues. The gene encodes at least two alternatively spliced (an probably many more) isoforms varying in molecular mass from about 72 kDa to over 100 kDa. The longer of these variants has, at its C-terminus, a histone acetyltransferase (HAT) domain [5,35,36](Fig. 2 A, long OGA). The shorter variant has a much smaller C-terminal domain (Fig. 2 A, short OGA). Based on similarity to hyaluronidases, the catalytic domain was proposed to be restricted to the N-terminal domain [5]. This conclusion was initially questioned [49], but was subsequently confirmed directly by mutagenesis [50,51]. The HAT domain has been suggested to be enzymatically active, but histone acetyltransferase activity has not yet been independently verified [50,52]. Each of the isoforms contains a domain which has been shown to interact with OGT as indicated in Fig. 2A [53].

Recent progress in understanding the catalytic mechanism of the O-GlcNAcase active site was greatly facilitated by the solution of the crystal structure of putative O-GlcNAcase homologs from *Clostridium perfringens* and the human gut endosymbiont, *Bacteroides thetaiotaomicron* [54,55] (Fig 2B). The bacterial O-GlcNAcase homolog is organized as 'TIM-barrels' with a deep active site pocket consistent with binding of O-GlcNAc-modified peptides. Mutagenesis and kinetics studies on these homologs show that the bacterial enzymes behave similarly to their human counterparts, operating via a previously proposed 'substrate-assisted' catalytic mechanism involving the acetamide group of GlcNAc [51,56]. A central domain of human O-GlcNAcase has been shown to interact with human OGT (indicated as OGT binding) [53]. The solved bacterial structures lack this 'OGT binding domain' as well as the 'HAT' domain that are features of the human enzyme. It has been shown that the HAT domain of O-GlcNAcase interacts with histone tails (H3 and H4), and as mentioned previously, has been suggested to serve as a HAT under these conditions. However, the inability of some laboratories to demonstrate intrinsic HAT activity using recombinant O-GlcNAcase suggests a

contaminating enzyme could account for the observed activity. The HAT-like domain is modeled in Fig. 2B using the related structure of *Tetrahymena* Gcn5 histone acetyltransferase (PDB: 5GCN) [57].

Subcellular targeting of the Enzymes of O-GlcNAc cycling

Although O-GlcNAc was originally identified on the surface of T-lymphocytes [58], it shortly became clear that most of the O-GlcNAc in cells resides in intracellular compartments [58-62]. In mammals, the intracellular targeting of the enzymes of O-GlcNAc cycling appears to be a critically important aspect of the diverse biological processes in which they are involved.

The nucleus was particularly enriched in the O-GlcNAc modification and the nuclear pores were the most obvious of the glycosylated substrates [58-62]. For example, high levels of O-GlcNAc found on the nucleoporin, Nup62, results in this protein standing out as the immunodominant band in cellular extracts probed with O-GlcNAc-specific antibodies. The antibodies raised against O-GlcNAc vary slightly in their binding avidities and their requirement for polypeptide backbone. GlcNAc will compete for binding of some of the antibodies with their targets (eg., CTD 110.6), while it does not compete for binding of others (eg., RL2). Thus, competition controls should be used, when possible, in studies employing antibodies to avoid false positives in target identification. A large number of cytoplasmic proteins are now known to be substrates. These include structural proteins such as the cytokeratins, myosin signaling kinases, transcription factors, and ribosomal proteins. More recent analysis of OGT splice variants has revealed a larger isoform of OGT, termed ncOGT, and the short isoform (sOGT) are both distributed between the nucleus and cytoplasm (See Fig 1A) [40,63]. Although a sequence in the central portion of human OGT was suggested to be its nuclear targeting signal, this short basic sequence has not been demonstrated to be essential for nuclear targeting of OGT [22]. The longer form of the O-GlcNAcase has a nuclear targeting domain in its C-terminal 'HAT' domain. Since O-GlcNAcase and OGT interact, it is formally possible that they enter the nucleus in a complex. OGT might also 'piggyback' into the nucleus bound to partners such as p38 MAPK. Alternatively, OGT may use an alternative nuclear transport pathway. OGT exhibits structural similarities to importin α [45], and like importin α , OGT could directly interact with an importin β for nuclear import.

Early studies showed that mitochondria contained few, if any, O-GlcNAc modified substrates [58-62]. However, one of the isoforms of OGT (mOGT) is targeted to the mitochondrion by a unique N-terminal sequence [40,63] (See Fig 1A). On the basis of these findings, the mitochondrial form of OGT was proposed to regulate mitochondrial function, including apoptosis. Interestingly, the shortest isoform of OGT, which is nuclear and cytoplasmic (Fig. 1 A) has been shown to be an anti-apoptotic protein in a screen for anti-apoptotic genes using leukemia cells [64]. Recent work suggests that O-GlcNAc metabolism in the mitochondria may be of great importance in many cell types including the heart and cardiovascular system [29,30,40,65-69]. The effects of the O-GlcNAc modification on the physiology and pathology of the heart will be discussed in another of the articles in this volume. It is not yet clear if the O-GlcNAcase becomes localized to the mitochondrion since no obvious mitochondrial signal is present in either isoform of O-GlcNAcase. The shorter isoform of O-GlcNAcase (Fig. 2, B) was originally thought to be a nuclear isoform[70]. It has more recently been found to be associated with the endoplasmic reticulum and lipid droplets [51], and could potentially enter the mitochondria by an unusual vesicular route.

Enzymes of O-GlcNAc cycling: Recombinant Expression and Chemical Biology

The enzymes of O-GlcNAc cycling, unlike many carbohydrate-acting enzymes, are soluble proteins, and are particularly amenable to production *in vitro* using *E. coli* expression systems. Recombinant enzymes have been used for structural work, enzymology, and chemical biology approaches. The isoforms of OGT were expressed individually, and the two longest of these (termed ncOGT, and mOGT, Fig. 1A) were shown to be enzymatically active [15,41,45]. The shorter isoform (sOGT, Fig. 1A) did not glycosylate any known substrate but showed nucleotide hydrolase activity and was a dominant negative inhibitor of the other two enzymes *in vitro*. The other isoforms of OGT show differential abilities to glycosylate substrates. For example, O-GlcNAcase and Tau are substrates for ncOGT as expected. Surprisingly, however, the src-family Yes tyrosine kinase was a substrate only for the mOGT [15]. The shorter isoform sOGT was recently used in a high throughput screen, based on fluorescence anisotropy, which identified some initial lead compounds that function as inhibitors of OGT [71]. These inhibitors are moderately complex heterocyclic compounds with no obvious 'pharmacophore' emerging. Obviously, additional high potency inhibitors are essential for moving forward on the function of OGT in higher metazoans where genetics are not feasible. Further high throughput assays are underway in a number of laboratories.

Bacterial expression of the O-GlcNAcase has also led to studies that have been quite fruitful. The human, and *C. elegans* enzymes have each been expressed in active, recombinant form [5,35,50-53,72-75]. Both isoforms of the mammalian O-GlcNAcase are enzymatically active (Fig. 2, A) [51,76]. The O-GlcNAcase activity has been detected using a number of assays including p-Nitrophenyl- β -D-GlcNAc, and more recently, using a sensitive fluorescent derivative fluorescein di-(N-acetyl-beta-D-glucosaminide) (FDGlcNAc). This latter substrate was devised and synthesized in efforts to increase sensitivity of O-GlcNAcase detection. Extension of the acetyl-moiety of this probe, also allowed the production of an O-GlcNAcase-specific substrate [77]. This general principle pioneered in a study with GlcNAc-thiazolines [56], and was subsequently used by a number of laboratories for inhibitor design, producing a number of selective agents [56,72,78-84]. These approaches will be further detailed in other articles in this issue.

OGT and O-GlcNAcase: Physical Interactions mediating enzyme targeting

Identification of the enzymes of O-GlcNAc cycling has led to the identification of protein binding partners. The physical interactions of OGT that are the best characterized and are summarized in Figure 1B. However since O-GlcNAcase can associate with, and is modified by OGT [15, 53, 85], both activities may, under certain circumstances, be recruited by the OGT binding partners. For OGT, two-hybrid screens have identified numerous proteins that interact with the TPR region [85, 86]. The proteins identified in these screens include OIP106 and GRIF-1. These proteins are members of a family of proteins with the so-called 'HAP' (Huntington Associated Protein) motif. Proteins containing this motif are associated with vesicle and organelle movement in neurons. The *Drosophila* Milton protein, involved in mitochondrial movement, is another member of this class of proteins. OGT was also shown to interact with mSin3A [87], HCF-1 [88], and the human dosage compensation complex [89] consistent with a role for OGT in the control of gene expression. OGT directly binds to several classes of enzymes. For example, OGT interacts with protein phosphatase PP1 [90], a regulatory subunit of PP1, MYPT1 (a myosin phosphatase), as well as the histone methyltransferases CARM1 [85] and MLL5 [91]. The interaction of OGT with this diverse and numerous list of factors reflects the growing awareness that OGT is involved in complex and robust regulatory paradigms, touching many aspects of cellular physiology.

Other probable interacting partners for OGT have arisen from two-hybrid analysis in invertebrates. In *C. elegans*, two-hybrid analysis indicates an interaction of the C-terminus of OGT-1 with the MAPK, PMK-1 [92]. The interaction of the C-terminus of OGT with p38 MAPK has now been shown in mammals [93]. Intriguingly, this interaction increases with p38 MAPK activation during glucose deprivation. The catalytic activity of OGT is not altered by glucose deprivation, but p38 MAPK serves to recruit OGT to specific targets, including neurofilament H. In *Drosophila*, OGT has been shown to interact with 26 other proteins by two-hybrid analysis [94]. These interacting partners include park (parkin), eyg (eye-gone), Stam (signal transducing adaptor molecule, CSN4, (COP9 signalosome subunit 4), sns (sticks-and-stones), dys (dystrophin-like protein 1), and the ubiquitin-ligase ara-2 (ariadne 2). As seen in other systems, these probable interactions suggest that OGT may be involved in a number of unexplored signal transduction pathways altering numerous biological processes in the fly. Verification of these interactions and continued development of reagents to examine the *Drosophila* HSP will enable these approaches.

Upstream of O-GlcNAc: Hexosamine Biosynthesis and Signaling

The hexosamine biosynthetic pathway, as it is now understood, is shown in Figure 3; the enzymes listed are given for both mammals and the genes of *C. elegans* (in parenthesis). Historically, Marshall and his colleagues first described the impact of hexosamine biosynthesis on desensitization of the insulin-signaling pathway [95-97]. These studies revealed that desensitization of insulin-responsive glucose transporters in adipocytes required glucose, insulin and glutamine. The authors speculated that hexosamine biosynthesis was the cause, and confirmed this by using inhibitors of the rate-limiting enzyme in hexosamine synthesis, glutamine: fructose-6-phosphate amidotransferase (GFAT). Desensitization was also induced by glucosamine, a metabolite bypassing GFAT, and causing increased production of UDP-GlcNAc [95,96]. Of course, the products of the hexosamine biosynthetic pathway were known to be used by many intracellular enzymes, and reside in many intracellular compartments (See Figure 3). The challenge facing the field then became deciding which of the many metabolic byproducts of UDP-GlcNAc were responsible for altering insulin sensitivity.

When OGT was first molecularly identified [22,33], similarities emerged with known signaling molecules including yeast SSN6 and the *Arabidopsis* protein *Spindly* [34], involved in gibberelin signaling. Based on these similarities and the nutrient sensing potential for OGT, O-GlcNAc was proposed to play a key role in diabetes and insulin resistance [22]. An ongoing discussion about the role of the hexosamine biosynthetic pathway in signaling still continues; two broad theories have been advanced. The first suggests that altered insulin signaling is the result of changes in O-GlcNAc cycling (shown in Fig. 3) [23,24]; the second theory suggests that altered N-glycosylation can impact insulin signaling [98,99]. These theories are not mutually exclusive, of course, and it is probable that both pathways contribute to insulin resistance *in vitro*. For the purposes of this review, the role of O-GlcNAc cycling in altering signaling will be emphasized, but any discussion of the findings must also take into account the complexities of cellular sugar nucleotide utilization.

Following the initial observation suggesting that O-GlcNAc may play a key role in insulin resistance [23,24], much support for this idea has emerged [20,100-109]. Many of the key substrates in the insulin-signaling pathway are O-GlcNAc modified, yet the mechanism of O-GlcNAc-mediated insulin resistance is still not precisely known.

Mouse Models of Hexosamine Signaling

The mouse has proven to be a useful model for understanding the physiological impact of the HSP in mammals. Among the first experiments to address the problem in the context of a whole animal were studies involving overexpression of GFAT, the rate-limiting enzyme in hexosamine

synthesis in several target tissues [110-113]. These mouse studies showed that over expression of GFAT lead to peripheral insulin resistance, and leptin secretion in a manner predicted by the early studies on preadipocytes. A direct link to O-GlcNAc metabolism was made in a study in which O-GlcNAc transferase (OGT) was over expressed in muscle and fat of transgenic mice [23]. These transgenic animals exhibited elevated insulin and leptin levels, and were insulin resistant as evidenced by the hyperinsulinemic-euglycemic clamp procedure. Parallel studies in rat L1-3T3 preadipocytes demonstrated that inhibition of the O-GlcNAcase also induced insulin resistance [24]. Taken together, these experiments strongly suggested a link between the hexosamine biosynthetic pathway, O-GlcNAc cycling, and mammalian insulin resistance. More recent evidence has questioned this link since specific inhibitors of O-GlcNAc cycling may not produce the insulin resistance phenotype [80]. Thus, genetic loss and gain of function studies to address this key issue have become essential.

The enzymes of hexosamine synthesis have yet to be carefully examined in mouse loss of function models except for a knockout of EMeg32, the acetyltransferase involved in assembly of the nucleotide sugar UDP-GlcNAc [114] (See Figure 3). Homozygous null embryos lacking EMeg32 die at embryonic day 7.5 and are developmentally delayed. *In vitro* differentiated EMeg32^{-/-} ES cells show reduced proliferation and mouse embryonic fibroblasts (MEFs) derived from knockout embryos exhibit defects in proliferation and adhesiveness. These defects could be reversed by stable expression of EMeg32 or by nutritional restoration of intracellular UDP-GlcNAc levels. The knockout results in reduced O-GlcNAc modifications of cytosolic and nuclear proteins. Interestingly, the EMeg32 knockout MEFs exhibited activated PKB/AKT and are resistant to apoptosis. Thus, EMeg32-dependent UDP-GlcNAc levels influence cell cycle progression and susceptibility to apoptotic stimuli [114]. The impact of the knockout was attributed to the decreased levels of O-GlcNAc addition.

Mouse knockouts of the enzymes of O-GlcNAc cycling have been more problematic. OGT is an X-linked gene, and since most embryonic stem cells are XY, early attempts to generate knockout mice resulted in stem cell lethality [38,63]. Subsequent studies circumvented this problem by using Cre-recombinase technology, but knocking out OGT in early development is embryonic lethal. Tissue-specific knockouts of OGT have been generated in a variety of cell lineages including thymocytes, neurons and fibroblasts [115], resulting in T-cell apoptosis, neuronal tau hyperphosphorylation, and fibroblast growth arrest, respectively. However, the general cytotoxic effects observed in the targeted tissues make interpretation of these results difficult. Efforts are underway to generate hypomorphic alleles of OGT that might alter O-GlcNAc levels but not lead to embryonic lethality. A conditional knockout of O-GlcNAcase has not yet been reported.

O-GlcNAc cycling mutants in invertebrate model organisms

The pathway of insulin signaling leading to transcriptional programming is now well understood, in large part, because of genetic analysis carried out in the nematode *Caenorhabditis elegans* and the fruitfly, *Drosophila melanogaster* [14,14,74,74,116-122]. Genetic analysis in these organisms allowed an ordering of the series of reactions following binding of insulin-like peptides to the insulin receptor. These pathways are summarized in Figure 4. Much of what was learned in the worm, and the fly, directly translates to the mammalian signaling pathways. For example, in *C. elegans*, the insulin-signaling pathway has been genetically linked to the dauer diapause, longevity, innate immunity and the cellular stress response (Fig. 4) [118-122]. The mammalian insulin signaling pathway is now known to be highly similar to those present in *C. elegans* and *D. melanogaster* [14,74,123,124].

As an approach to understanding the role of O-GlcNAc cycling in mammalian insulin resistance, the HSP has recently been examined in the worm and fly. In *Drosophila*, the OGT

locus was recently demonstrated to correspond to a previously identified gene termed 'Super Sex Combs' (Sxc) [125]. This locus was identified in 1984 as a regulator of homeotic gene expression. Several Sxc alleles displayed anterior and posterior transformations [126]. Loss of Ogt/Sxc function suggests a role in Polycomb-dependent transcriptional repression (See later section). The O-GlcNAcase of the fly has been disrupted using P-element insertion proximal to its promoter [127]. This fly strain is viable and fertile. These genetic and molecular tools should prove invaluable for sorting out the role of hexosamine signaling and insulin signaling in *Drosophila*. The study of the *Drosophila melanogaster* HSP is currently ongoing in several laboratories.

A *C. elegans* model of Insulin-Resistance and Type 2 diabetes

Unlike the knockouts of OGT and O-GlcNAcase in mammals, null alleles of these enzymes in *C. elegans* are viable and fertile, facilitating genetic analysis. In addition, the *C. elegans* system is amenable to genetic analysis for signaling pathways such as insulin-like signaling (See Fig. 4), TGF- β signaling, and MAPK signaling using a large collection of previously identified mutants and reverse genetics [121,128,129].

Knockout of the O-GlcNAc transferase (encoded by *ogt-1*) in *C. elegans* is viable and fertile providing a biological platform to study the impact of the modification on cell signaling and metabolism [14,74]. Interestingly, the nuclear pore proteins, normally highly decorated with O-GlcNAc, completely lacked O-GlcNAc, yet no impact on nuclear transport of key transcription factors was observed in these null animals. As summarized in Figure 4, metabolic changes were detected in the *ogt-1(ok430)* knockout animals including 3-fold increases in glycogen and trehalose, and a dramatic (3 fold) reduction in lipid stores. Pathways regulated by insulin-like signaling in the worm, dauer (diapause) and longevity were also examined in these studies. The *C. elegans* dauer is a nutritionally responsive diapause induced upon crowding and starvation and triggered by a released pheromone termed daumone. The dauer pathway requires the concerted action of the *C. elegans* insulin-signaling pathway (Fig. 4), and TGF- β -like pathways. The study employed a temperature sensitive allele of the insulin-like receptor (*daf-2(e1370)*) (Fig. 4) that allows controlled inactivation of insulin signaling, scored as the extent of dauer larvae formation. By generating a double mutant harboring *daf-2(e1370)* and *ogt-1(ok430)*, the impact of O-GlcNAc modification on insulin-like signaling was examined. The *ogt-1* knockout was shown to greatly diminish dauer larvae formation in the sensitized *daf-2* mutant background suggesting that the wild-type function of the *ogt-1* gene is inhibition of insulin signaling downstream of *daf-2*. Inactivation of the *ts* allele of *daf-2* normally greatly extends the lifespan of *C. elegans*, but the *ogt-1(ok430)* knockout suppresses this lifespan extension (in preparation), once again arguing that the normal function of OGT is to blunt insulin signaling [14]. The *ogt-1* knockout strain was also more susceptible to UV and osmotic stress than the wildtype strains (Love and Hanover, *in preparation*). These findings are summarized in Fig. 4, *bottom right*. Thus, the *C. elegans* studies of OGT have shown that OGT plays a role in regulating the process of dauer formation, stress and longevity, processes intimately linked to intracellular signaling and nutrient status.

These observations were extended when a null allele of the O-GlcNAcase (*oga-1(ok1207)*) was characterized in *C. elegans* [74]. This null allele was shown to be enzymatically inactive *in vitro* and has a similar metabolic phenotype as the *ogt-1* knockout animals. Namely, the worms have elevated trehalose and glycogen levels and decreased neutral lipid stores. When insulin signaling was examined by the assays described above, the *oga-1(ok1207)* knockout showed an insulin-resistant phenotype, suggesting that the normal function of the O-GlcNAcase is to relieve the desensitization of insulin signaling created by O-GlcNAc transferase. Other phenotypes of the O-GlcNAc cycling mutants included changes in

phosphorylation profiles, and strikingly increased levels of glycogen synthase kinase 3 (GSK3) [74].

Other Genetic Interactions of *C. elegans* O-GlcNAc cycling mutants

Identification of O-GlcNAc cycling mutants in *C. elegans* has also led to the inclusion of *ogt-1* and *oga-1* in unbiased high-throughput screening efforts using RNA interference to create genetic interaction networks. One such study examined some of the most important signaling pathways in *C. elegans* including growth factor signaling, Ras-signaling, Wnt signaling, Notch signaling and DNA damage repair [130]. This screen was restricted to *C. elegans* LGIII (Chromosome III) fortuitously the chromosomal location of *ogt-1*. The findings of that global interaction study are consistent with a role for *ogt-1* in pathways distinct from the insulin-like signaling pathway. In particular, genetic interactions of *ogt-1* with the β -catenin-encoding gene *bar-1* and the FGF, encoded by *let-756*, were detected. The interaction module associated with *bar-1* and *ogt-1* is of particular interest since it was confirmed to play a role in the regulation of fat storage and metabolism. This finding is also consistent with earlier observations that *ogt-1* mutants have dramatically lower neutral lipid stores than do wild-type animals (See also Fig. 4)[14]. In mammals, β -catenin has been shown to be O-GlcNAc modified, and the function of the protein is altered by the modification. [24,131]. β -catenin is a key transcriptional modulator interacting with the TCF family of architectural transcription factors and triggering key developmental switches including those involved in triggering formation of T-cells and B-cells in adaptive immunity[132].

The genetic interaction of *ogt-1* with *let-756*, encoding a FGF homolog, further suggests that O-GlcNAc cycling may participate in a similar module involved in the regulation of growth. The *let-756* gene is involved in numerous genetic interactions in *C. elegans* and, like the interaction of *ogt-1* and *bar-1*, the interaction of *ogt-1* with *let-756* is likely to be quite complex. Although the insulin-like pathway was examined in this high throughput screen (*daf-2*), no genetic interaction was observed with *ogt-1* as measured by a slow growth phenotype. This demonstrates the limitations of specific assays and highlights the need for multiple approaches in dissecting and defining the many roles of O-GlcNAc cycling.

O-GlcNAc and Nutrient-Sensing kinase cascades: Insulin-AKT, MAPK, mTOR, and AMPK

One way of visualizing the nutrient-sensing HSP is to view it as the calm 'eye' in the violent 'storm' of intracellular signaling. The HSP sensor 'calmly' detects nutrient availability communicating that information to the signaling 'storm' circling about it. The combined forces of the signaling cascades that respond to nutrient status in metazoans are not unlike a revolving storm, constantly moving, yet adapting to a changing environment. Some nutrient-response systems respond to specific signals, while others react to more general nutrient stimuli. For example, AMPK responds to the AMP/ATP ratio, while mTOR responds to both amino acid levels and insulin signaling. Perhaps the most general sensor of cellular nutritional status is the nutrient-integrating HSP and the enzymes of O-GlcNAc cycling. For this reason, the HSP may be placed at the center, or 'eye', of a swirling nutrient-sensing 'storm' (Figure 5). It is clear that significant crosstalk occurs between phosphorylation and O-GlcNAc addition. A recent study examined 711 phosphopeptides and found that approximately half of the sites of phosphorylation cycle; of these, elevated GlcNAcylation resulted in lower phosphorylation at 280 sites and caused increased phosphorylation at 148 sites [133]. Thus the interplay between these signaling modifications is complex. A coherent model of how O-GlcNAc cycling may serve to modulate intracellular signaling in such a complex system must take into account a number of variables. These include the nutrient-sensing capabilities of hexosamine synthesis, the intracellular localization and interactions of the O-GlcNAc cycling enzymes, the molecular

targets, other homeostatic mechanisms and signaling cascades, and the final biological output. In the robust signaling networks in which O-GlcNAc cycling is likely to act, this is a daunting task. However, as is clear from the evidence presented here, some generalizations emerge. These generalizations are summarized in Figure 5. The HSP appears to be a central regulator of the cellular response to feast and famine.

A highly simplified diagram of the complex interaction between the Insulin-AKT, MAPK, mTOR and AMPK pathways is shown in Figure 5. OGT is likely to interact with these canonic nutrient-sensing pathways at many points as indicated below. First, there is growing evidence that tyrosine kinase receptors may directly interact with OGT. OGT itself is tyrosine phosphorylated [33] and auto-glycosylated [22,33,42]. In addition, insulin treatment of 3T3-L1 adipocytes stimulates both the tyrosine phosphorylation and the catalytic activity of OGT. This study also demonstrated that a subset of OGT coimmunoprecipitates with the insulin receptor. OGT also interacts with other tyrosine kinases including the Src kinase family member, Yes [15]. A recent bioinformatic analysis of known O-GlcNAc modification sites and known tyrosine phosphorylation sites revealed potential association between these two post-translational modifications in various proteins including the protein prohibitin. This has led to speculation that O-GlcNAc modification and tyrosine phosphorylation may act as a novel and previously unidentified binary switch adding additional complexity to cell signaling pathways [134].

In addition to directly interacting with tyrosine kinases, OGT is recruited to sites of PIP3 formation during growth factor signaling, suggesting that the many signaling cascades triggered by PI3K are likely to be influenced by O-GlcNAc signaling [109]. Growth factor receptors are key activators of PI3K. At the cell surface, growth factor receptor occupancy would trigger PI3K leading to PIP3 formation and OGT recruitment (Fig. 5). Factors co-recruited to PIP3, such as AKT and PDK, would actively compete with OGT for the modification of key Ser/Thr residues, thus competition with OGT could effectively modulate the action of these kinase cascades. The nutrient sensing capabilities of the OGT catalytic domain would allow real-time monitoring of nutrient status to be directly communicated to the canonic growth factor signaling cascade. Activated AKT modifies many transcription factors whose nuclear concentrations may be modulated by interaction with the 14-3-3 family of binding proteins. By blocking phosphorylation at AKT sites, OGT can oppose the cytoplasmic sequestration leading to transcriptional activation. Notable examples of this kind of regulation are the insulin-responsive Foxo family transcription factors [135] and the gluconeogenic regulator CRCT2 [136]. *In vitro* studies have demonstrated the capacity of OGT to function over a wide range of UDP-GlcNAc concentrations making the enzyme ideally suited for a role as a nutrient sensor [42,48].

Another critically important interaction occurs between OGT and p38 MAPK (Fig 5). The MAP kinases are responsive to stress stimuli, including cytokines, ultraviolet irradiation, heat shock and osmotic shock; the enzymes are, therefore, involved in many aspects of cellular physiology and apoptosis. The interaction of MAPK p38 with the C-terminus of OGT occurs at a site overlapping the PIP-interacting domain, or PPO (PIP-binding activity of OGT) [93]. It is likely that the binding of p38 MAPK kinase would block interaction of PIP3 with the PPO binding site. From a regulatory perspective, this makes sense. The cellular stress response is triggered by numerous environmental insults including heat shock, oxidative stress, starvation, and proteotoxicity. The MAPK signaling cascade is critical for these responses and typically acts counter to growth signals. A balance exists between growth and response to stress in organisms from yeast to man. Recruitment of OGT to different sites of action, depending on the activation state of either PI3K or MAP kinase, may act as a kind of binary switch, providing a nutrient-sensitive balance between growth control and the cellular stress response (Fig. 5).

AMPK is a highly conserved sensor of cellular energy status that becomes activated by AMP under conditions where intracellular ATP levels drop. AMPK responds to this energy stress by blunting growth and biosynthetic pathways, partly through its inhibition of the rapamycin-sensitive *mTOR* (*mTORC1*) pathway (Fig. 5) [137,138]. The mammalian TOR (*mTOR*) pathway is also a key regulator of cell growth and proliferation and its deregulation is associated with human diseases, including cancer and diabetes [139]. The *mTOR* pathway serves to integrate the status of nutrients and cellular energy, and growth factors. In response to these upstream effectors, the *mTOR* pathway regulates autophagy, ribosome biogenesis, translation and metabolism. Recent work has identified two distinct *mTOR*-containing complexes termed *mTORC1* (containing raptor) and *mTORC2* (containing rictor). These complexes, along with TSC1/2, Rheb, and AMPK, function as upstream regulators of *mTOR* (See below). Growth factors like insulin, act through both AKT-dependent and extracellular signal-regulated kinase (ERK)-kinase dependent phosphorylation to suppress the GTPase activity of the TSC complex. This suppression leads to full activation of GTP-Rheb, which is the principal activator of the *mTOR*-raptor complex. Nutrients and growth factors act in concert to activate this pathway, which is, in turn, essential for conveying pro-growth and pro-survival signals (Fig. 5). In contrast, depletion of nutrients or energy stores inhibits *mTOR* through independent activation of the TSC either by direct phosphorylation by AMP kinase (AMPK) in response to falling ATP or via REDD1 induction during hypoxia [140]. Potential cross talk between *mTOR* activation and the HSP could modulate many of the regulatory functions of this signaling pathway.

The interaction of OGT with AMPK is also complex and has great potential importance in signaling (Fig 5). AMPK-dependent processes up-regulate the levels of OGT transcript upon glucose deprivation [93]. In addition, evidence has been presented that OGT may directly modify AMPK [141] leading to activation of AMPK activity in response to hexosamine flux. While this has not been directly examined in *C. elegans*, it is intriguing that AMPK (*aak-1*) mutants blunt dauer formation in manner very similar to *ogt-1* mutants [14,74,142]. These findings all point to a concerted role for the nutrient-sensing AMPK and OGT-dependent pathways in the regulation of dauer formation and insulin-like signaling.

In single-cell eukaryotes and in *C. elegans*, TOR is the dominant regulator of overall mRNA translation (Fig. 5). It has become clear that in higher metazoans, *mTOR* controls the expression of mRNAs that are important to cell growth. Although there are no reports of direct interactions between OGT and *mTOR* to date, similarities certainly exist in the manner in which they may interact with the cellular translational machinery. The net result of *mTOR* regulation is either to stimulate protein synthesis and growth when activated or to blunt translation in response to stress and starvation. *mTOR* regulates translation by altering ribosome biogenesis, S6 kinase activation, activation of eIF4G and depressing the activity of eIF2. OGT has been also shown to impact the translational machinery at several different levels. Many ribosomal proteins are modified by O-GlcNAc [143]. O-GlcNAc modifies an eIF-2 associated 67-kDa protein (p67) that protects the eIF-2 alpha-subunit from eIF-2 kinase, thus promoting protein synthesis [144]. Both eIF-2 and eIF5 were identified as O-GlcNAc modified in a proteomics screen for O-GlcNAc modified proteins [145] using azido-GlcNAc probes [73]. These probes allow protein identification, but not stoichiometry to be directly determined, and much work remains to understand the extent of modification of these key substrates. Undoubtedly, many other translation factors are modified by O-GlcNAc at levels that are below the sensitivity of detection in these preliminary studies.

Protein translation may also be regulated by mRNA triage by specialized structures termed stress granules (SG). Recent findings also point to a role for O-GlcNAcylation in mRNA triage and translation arrest by SGs [146]. These authors showed that OGT and upstream components of hexosamine signaling are required for SG and processing body (PB) assembly. O-GlcNAc-

modified proteins were found to be components of SGs, but not PBs, including numerous ribosomal proteins. These findings strongly suggest that O-GlcNAc modification of the translational machinery is required for aggregation of untranslated messenger ribonucleoproteins into SGs. Thus, the regulation of translation in response to stress is mediated both by the mTOR and hexosamine signaling pathways. Finally, as will be detailed below, OGT may play a role in modulating autophagy, a process also regulated by mTOR in response to stress and starvation.

OGT and the sirtuins: calorie restriction and the extension of lifespan

Sirtuins, or so-called class III histone deacetylases (HDACs), are protein deacetylases/ADP ribosyltransferases acting on a wide range of cellular targets [147-150]. These target proteins reside in the nucleus, cytoplasm, and mitochondria and are post-translationally modified by either acetylation (*via* SIRT1, -2, -3 and -5) or by ADP ribosylation (*via* SIRT4 and -6) [149]. Sirtuins play a key role in regulating lifespan extension in *Drosophila* and *C. elegans* and also are regulators of lifespan extension via calorie restriction in mammals. Finally, the sirtuins may regulate cellular responses to stress and prevent propagation of damaged DNA.

Where it has been directly examined, O-GlcNAc addition often seems to oppose the action of the sirtuins, like SirT1, on protein function (Fig. 5). An example of this is the action of the enzymes on the key glucagon-responsive transcription factor CRCT2. CRCT2 was shown to be O-GlcNAc modified at sites normally phosphorylated to keep CRCT2 in the cytoplasm. Decreasing amounts of O-GlcNAc on CRCT2, by overexpression of the deglycosylating enzyme O-GlcNAcase, blocked the effects of glucose on gluconeogenesis [136]. In a parallel study, this laboratory showed that the histone acetyltransferase p300 and sirtuin 1 (SirT1) act on CRCT2 to modulate its activity. p300 increased hepatic CRCT2 activity by acetylating it; these effects were attenuated during late fasting, when CRCT2 was down regulated by SIRT1-mediated deacetylation. Disrupting SIRT1 activity, by liver-specific knockout of the Sirt1 gene or by administration of a SIRT1 antagonist, increased CRCT2 activity and glucose output, whereas exposure to SIRT1 agonists reduced them [151]. A similar series of observations have been made with the enzyme endothelial nitric oxide synthase (eNOS). This enzyme is O-GlcNAcylated at a critical AKT site (serine 177) that is normally phosphorylated to activate the enzyme. The presence of O-GlcNAc at this site is associated with decreased NO production in hyperglycemic diabetic patients and is suggested to play a key role in triggering erectile dysfunction in this patient population [152,153]. In contrast, SIRT1 acts to deacetylate eNOS, leading to a stimulation of eNOS activity and increasing endothelial NO. These studies, and others, suggest that OGT-mediated glycosylation and sirtuin-dependent deacetylation may play opposing roles in situations of nutrient excess or starvation.

Nutrient acquisition, Mitochondrial Movement, and Autophagy

As mentioned previously, OGT interacts with components of the machinery involved in mitochondrial movement in neurons, binding to the protein GRIF1, the mouse homolog of Milton in *Drosophila* [154-156,156-160]. The transport of mitochondria to specific neuronal locations is critical for maintaining cellular energy demands and for buffering calcium. It is not yet clear whether the nutrient-sensing capabilities of OGT are brought to bear on regulating the axonal transport of mitochondria, but its presence in those complexes is provocative. Coupled with the finding that an isoform of OGT may be targeted to mitochondria [40,63], these data suggest that the hexosamine signaling pathway may directly influence such critical mitochondrial functions as energy metabolism, fission and fusion, and apoptosis (Fig. 6).

A recent proteomics study has identified OGT as an integral part of the *Drosophila* phagosome [161]. Phagocytic cells have critical functions in remodeling tissues during embryogenesis and are central effectors of immune defenses. During phagocytosis, large objects are internalized

into 'phagosomes'. These are the organelles in which immune processes such as microbial destruction and antigen presentation are initiated. A related process occurs when intracellular components are engulfed, the resulting structure is called an autophagosome and the process is dubbed 'autophagy.' This is a process regulated by nutrient availability; activity of AKT and mTOR inhibit the formation of autophagosomes. However, stress and starvation induce the formation of autophagosomes through a process involving mTOR inhibition and activation of a type III PI-3 kinase. First, by analogy from its recruitment to PIP3 in the plasma membrane, OGT may also be directly recruited to the forming autophagosome. This interaction of OGT with the preautophagosome is expected to occur with newly formed PIP3 resulting from Type III PI3K activation (Fig. 6). Second, by limiting AKT activation OGT may serve to blunt mTOR activation—a critical step in autophagy. In this way, the nutrient sensing hexosamine-signaling pathway has at least two potential means to directly communicate with the forming autophagy machinery.

OGT is also potentially a component of the machinery regulating endosome trafficking [162, 163](Fig. 6). GRIF1 and Trak1, two OGT interacting proteins, interact with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs), an essential component of the endosomal sorting machinery. Although a direct role for OGT in modulating endosome to lysosome trafficking via these Trak1/GRIF1 complexes has yet to be shown, such regulation by a nutrient sensor is likely. Given the central role of O-GlcNAc cycling as a cellular nutrient sensor, a role in processes essential for nutrient acquisition might well be expected.

Regulation of the Proteasome

In addition to endocytosis, phagocytosis and autophagy, the other major cellular degradation pathway is the ubiquitin-ligase-proteasome system (Fig 6). The main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis. A large body of evidence has accumulated suggesting that the HSP modulates the 26S proteasome [164-170]. These data suggest that O-GlcNAc normally serves to blunt the action of the proteasome, either through direct modification of the AAA ATPase rpt2, [167,170] or possibly by interfering with activating kinases [169]. There is also some recent evidence suggesting that protein ubiquitination is modulated by O-GlcNAc and that the ubiquitin-activating enzyme E1 is O-GlcNAc modified [165]. Modulation of proteasomal activity is yet another example of how the nutrient-sensing hexosamine pathway may be coupled to modulate anabolic and catabolic metabolism intracellularly.

Transcription, Nuclear Transport and mRNA stability

Even the earliest studies identifying O-GlcNAc targets hinted at a role for O-GlcNAc cycling in modulating gene expression [58,59,61]. Some of the sites at which O-GlcNAc may be important for gene expression are summarized Fig. 6. A more extensive treatment of this intriguing topic is presented in the article by Hart in this volume. The high relative concentration of O-GlcNAc in the nucleus and on nuclear pores suggested a regulatory role. In addition, components of transcription complexes were shown to bear O-GlcNAc residues [171,172] and O-GlcNAc was shown to decorate *Drosophila* polytene chromosomes [173]. A more detailed study then showed that the CTD domain of RNA polymerase II was modified and a model was proposed which suggested that O-GlcNAc might regulate transcription directly [174-176]. Since these early findings, a large number of papers have appeared highlighting the role of O-GlcNAc in transcription [1,53,87,89,131,135,177-181]. A detailed discussion of these many recent advances is beyond the scope of this review, but they point to a central role for O-GlcNAc in the regulation of transcription. Several models are in place to examine this. In *C. elegans* the impact of null alleles of the O-GlcNAc cycling enzymes can be examined with respect to their roles in altering chromatin structure and transcription in a

living organism. In *Drosophila*, the Sxc/Ogt locus plays an essential role in regulating Polycomb-dependent transcriptional repression[125].

O-GlcNAc may also alter chromatin structure, mRNA splicing and the nuclear export machinery. Some evidence suggests that RNA binding proteins bear O-GlcNAc but no mechanistic studies have emerged testing this hypothesis [143,145]. It is clear that OGT and nuclear pore components are part of the dosage compensation complex involved in X-inactivation in mammals [89]. X-inactivation is triggered by an RNA dependent mechanism involving an X-inactive specific transcript (Xist) that encodes a large non-coding RNA responsible for mediating the specific silencing of the mammalian X chromosome from which it is transcribed [182]. The dosage compensation complex, which includes OGT, and the acetyltransferase hMOF (human homolog of Male on First), is then recruited to silence chromatin on the inactive X. The role, if any, of OGT in mediating this process is currently under further study. The impact of O-GlcNAc on mRNA export has not been directly examined, but the modification has not emerged as a central player in this process. However, O-GlcNAc is likely to play a role in mRNA stability for some transcripts. As mentioned above, recent evidence points to a role for OGT in mediating the formation of SGs and PBs [146]. These intracellular structures cooperatively regulate the translation and decay of messenger RNA.

O-GlcNAc may also have an impact on gene expression post-transcriptionally at the level of nuclear import, RNA export and mRNA stability (Fig. 6). Although the nuclear pore is a major site of O-GlcNAc addition, the role of the modification on nuclear protein import remains unclear. Using *Xenopus laevis*, this has been examined directly; the findings suggest that nuclear pore proteins in which O-GlcNAc was altered by addition of terminal galactose still supported nuclear import [183]. In addition, *C. elegans* strains lacking O-GlcNAc cycling enzymes do not appear to have changes in the rate or extent of transcription factor nuclear localization [14,74]. Other studies have pointed to a role for O-GlcNAc in triggering nuclear import of target proteins in response to O-GlcNAcylation[184-186]. Whether this is a primary function of the modification or a secondary consequence of O-GlcNAc cycling is an open question. Alternatively, the presence of O-GlcNAc on nuclear pores may simply reflect the recruitment of OGT to the Pore Complex during the formation of perinuclear heterochromatin associated with transcriptional repression.

Evolution of the Hexosamine Signaling Pathway

It has been suggested that chitin, a polymer of GlcNAc, is the most abundant biological polymer on earth, surpassing even plant-derived cellulose. Hexosamine biosynthesis itself appears to be highly evolutionarily conserved (See Figure 3). GlcNAc and glucosamine are features of the cell wall of both gram-negative and gram-positive bacteria and amino sugars feature prominently in the glycans of protists, plants and animal species. In animals, the synthesis of UDP-GlcNAc from biosynthetic precursors by the enzymes EMeg32 and GFAT (Figure 3) is a highly regulated process. GFAT is feedback inhibited by the end product UDP-GlcNAc, suggesting tight metabolic control of hexosamine biosynthesis and UDP-HexNAc levels [187]. In budding yeast, where a chitin-rich cell wall must be synthesized using UDP-GlcNAc, GFAT does not appear to be feedback inhibited by the product of hexosamine synthesis. Presumably, the hexosamine signaling pathway has become deregulated in yeast to allow for the formation of the chitin-rich cell wall. Curiously many yeast appear to lack OGT coding sequences (see below).

Bioinformatic inspection of known genomes and sequences suggests that the TPR domain of OGT bears some similarity to an essential domain in the yeast glucose repressor SSN6, but the yeast protein apparently lacks a catalytic domain. It has also become clear from these analyses that the Arabidopsis *spindly* protein is highly related to animal OGT, placing OGT in both

plant and animal kingdoms. This will be discussed in greater detail in the Article by Olszewski in this volume. The animal and plant sequences, coupled with subsequent genome sequencing efforts, have allowed inspection of the likely evolution of the HSP. The sophistication of that evolutionary analysis itself continuously evolves, as more and more genomes are sequenced. Previous studies have considered the evolution of the enzymes of O-GlcNAc cycling, and sequences from certain bacteria and fungi were related in primary sequence to the OGT from human and *C. elegans* [3]. In this early study, it was suggested that either OGT arose quite early in evolution and was selectively lost or that lateral transfer could explain the appearance of the enzyme in such divergent forms of life. Following this suggestion, the pathogenic bacterium *Listeria* was shown to encode a bifunctional OGT (GmaR) that regulates flagellar motility through anti-repression [188]. This is a particularly interesting example, since bacterial flagella are important both for bacterial colonization of the host and for host recognition of the bacteria by the innate immune system. Many pathogens negatively regulate flagella production after infection, presumably as a defense against surveillance by the host. The *Listeria* GmaR protein O-GlcNAc modifies the flagellum, while also serving as a temperature-dependent repressor of flagella production by an O-GlcNAc-independent mechanism. This may represent an example of convergent evolution, where O-GlcNAc addition is catalyzed by an enzyme with distinct properties from the norm. This suggests that bacteria have used the OGT coding modules in a unique way to circumvent host defense mechanisms.

A recent detailed analysis of OGT evolution suggests that OGT-related proteins appear in many forms of bacteria, fungi, protists, plants, and of course, invertebrate and vertebrate animals [4]. These recent findings suggest that lateral transfer is unlikely to account for these many occurrences, and points toward a more evolutionarily ancient origin of the enzyme with selective loss in some current species.

The evolution of the O-GlcNAcase is somewhat more puzzling. Enzymes clearly related to O-GlcNAcase are found in most animals and homologs are suggested to occur in some bacteria, but have yet to be identified in many organisms with homologs of OGT. The most notable absence is the lack of a clear O-GlcNAcase homolog in higher plants where two OGT-like enzymes exist. Of course, the O-GlcNAcase catalytic domain has many similarities with chitinases and hyaluronidases making unambiguous identification of more remotely related orthologs difficult. However, the suggestion that the two enzymes may not always coexist as cycling partners is an intriguing finding. Particularly in bacteria, this phenomenon may be partially explained by the use of the enzymes as toxins. Toxins are the subject of another article in the volume by Aktories. The bacterial OGT-related enzyme that has been structurally characterized (see above) has only 2 full TPR repeats and is, therefore, similar to the inactive sOGT human isoform which is a dominant negative inhibitor of human OGT and a known anti-apoptotic gene product in humans [15,64]. If OGT-related and O-GlcNAcase-related bacterial toxins evolved from common ancestors of O-GlcNAcase and OGT, selective loss of one or both could be tolerated. They may be retained in bacteria only because of their usefulness in dealing with animal and plant hosts where O-GlcNAc cycling does occur. Alternatively, organisms may have evolved independent means of removing O-GlcNAc modified protein other than cycling, such as selective proteolysis. This might explain why OGT appears to be more widespread in evolution than O-GlcNAcase. Do these evolutionary relationships reflect a kind of host-pathogen 'Arms-Race,' in the use of O-GlcNAc cycling modules? Answers to this important question must await a more detailed analysis of the significance of O-GlcNAc cycling in divergent forms of life.

Summary and Future Directions

What is the molecular logic dictating the targets and effects of O-GlcNAc cycling? The cellular response to feast or famine is mediated by the concerted action of a variety of key signaling

pathways including the Sirtuins, AMP-kinase, mTOR, and Hexosamine Signaling Pathways (Figures 5 and 6). In turn, these pathways interact with, and serve to modulate, homeostatic mechanisms such as the Insulin signaling, and MAP kinase-signaling cascades (Figs. 4-6). The HSP is of particular interest since it is responsive to cellular levels of amino acids, sugars and ATP (Fig. 3). The HSP is an evolutionarily ancient pathway that has presumably coevolved with other canonic nutrient-sensing modules (Fig 4-6). Growing evidence suggests that O-GlcNAc, utilizing nutrient responsive levels of UDP-GlcNAc, modulates intracellular signaling by its covalent attachment to key components of kinase-dependent signaling cascades (Figs. 4-6). The enzymes of O-GlcNAc cycling are recruited to their sites of action by the same activation mechanism (PI-3 kinase) triggering insulin and many other signaling cascades (Figures 4-6). Thus, the HSP impacts insulin signaling and other pathways by directly responding to nutrient availability. Genetic evidence further suggests that HSP, via O-GlcNAc, serves as a modulator of transcription, translation, and protein stability (Figure 6). The two key enzymes in this process, OGT and O-GlcNAcase, have thus emerged as promising drug targets. The pathways impacted by the nutrient-responsive HSP modulate key physiological processes deregulated in metabolic syndrome (stress, innate immunity, and metabolism). In fact, the O-GlcNAcase gene is a known diabetes susceptibility locus in Mexican Americans. A ‘vicious cycle’ exists in such populations; children of mothers with diabetes show increased risk for developing the disease due to unknown epigenetic factors in the intrauterine environment. One of the current hypotheses currently being pursued is that O-GlcNAc cycling integrates metabolic information, potentially leading to epigenetic reprogramming in the intrauterine environment. The HSP appears to be a central node in a complex web regulating of pathways of nutrient acquisition, stress response, immunity and metabolism. Future work in many laboratories will be required to understand the global effects of the hexosamine-signaling pathway and its role in human disease.

References

1. Love DC, Hanover JA. The hexosamine signaling pathway: deciphering the “O-GlcNAc code”. *Sci STKE* 2005;2005:re13. [PubMed: 16317114]
2. Hart GW, Housley MP, Slawson C. Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. *Nature* 2007;446:1017–1022. [PubMed: 17460662]
3. Roos MD, Hanover JA. Structure of O-linked GlcNAc transferase: mediator of glycan-dependent signaling. *Biochem Biophys Res Commun* 2000;271:275–280. [PubMed: 10799287]
4. Banerjee S, Robbins PW, Samuelson J. Molecular characterization of nucleocytosolic O-GlcNAc transferases of *Giardia lamblia* and *Cryptosporidium parvum*. *Glycobiology* 2009;19:331–336. [PubMed: 18948359]
5. Hanover JA. Glycan-dependent signaling: O-linked N-acetylglucosamine. *FASEB J* 2001;15:1865–1876. [PubMed: 11532966]
6. Kamemura K, Hart GW. Dynamic interplay between O-glycosylation and O-phosphorylation of nucleocytoplasmic proteins: a new paradigm for metabolic control of signal transduction and transcription. *Prog Nucleic Acid Res Mol Biol* 2003;73:107–136. [PubMed: 12882516]
7. Vosseller K, Wells L, Hart GW. Nucleocytoplasmic O-glycosylation: O-GlcNAc and functional proteomics. *Biochimie* 2001;83:575–581. [PubMed: 11522385]
8. Whelan SA, Hart GW. Identification of O-GlcNAc sites on proteins. *Methods Enzymol* 2006;415:113–133. [PubMed: 17116471]
9. Zachara NE. Detecting the “O-GlcNAc-ome”; Detection, Purification, and Analysis of O-GlcNAc Modified Proteins. *Methods Mol Biol* 2009;534:1–30.
10. Lehman DM, Fu DJ, Freeman AB, Hunt KJ, Leach RJ, Johnson-Pais T, Hamlington J, Dyer TD, Arya R, Abboud H, Goring HH, Duggirala R, Blangero J, Konrad RJ, Stern MP. 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* 2005;54:1214–1221. [PubMed: 15793264]

11. Haberhausen G, Schmitt I, Kohler A, Peters U, Rider S, Chelly J, Terwilliger JD, Monaco AP, Muller U. Assignment of the dystonia-parkinsonism syndrome locus, DYT3, to a small region within a 1.8-Mb YAC contig of Xq13.1. *Am J Hum Genet* 1995;57:644–650. [PubMed: 7668293]
12. Muller U, Haberhausen G, Wagner T, Fairweather ND, Chelly J, Monaco AP. DXS106 and DXS559 flank the X-linked dystonia-parkinsonism syndrome locus (DYT3). *Genomics* 1994;23:114–117. [PubMed: 7829058]
13. Cole RN, Hart GW. Cytosolic O-glycosylation is abundant in nerve terminals. *J Neurochem* 2001;79:1080–1089. [PubMed: 11739622]
14. Hanover JA, Forsythe ME, Hennessey PT, Brodigan TM, Love DC, Ashwell G, Krause M. A *Caenorhabditis elegans* model of insulin resistance: altered macronutrient storage and dauer formation in an OGT-1 knockout. *Proc Natl Acad Sci U S A* 2005;102:11266–11271. [PubMed: 16051707]
15. Lazarus BD, Love DC, Hanover JA. Recombinant O-GlcNAc transferase isoforms: identification of O-GlcNAcase, yes tyrosine kinase, and tau as isoform-specific substrates. *Glycobiology* 2006;16:415–421. [PubMed: 16434389]
16. Lefebvre T, Guinez C, Dehennaut V, Beseme-Dekeyser O, Morelle W, Michalski JC. Does O-GlcNAc play a role in neurodegenerative diseases? *Expert Rev Proteomics* 2005;2:265–275. [PubMed: 15892570]
17. Marz P, Stetefeld J, Bendfeldt K, Nitsch C, Reinstein J, Shoeman RL, Dimitriadis-Schmutz B, Schwager M, Leiser D, Ozcan S, Otten U, Ozbek S. Ataxin-10 interacts with O-linked beta-N-acetylglucosamine transferase in the brain. *J Biol Chem* 2006;281:20263–20270. [PubMed: 16714295]
18. Okuyama R, Marshall S. UDP-N-acetylglucosaminyl transferase (OGT) in brain tissue: temperature sensitivity and subcellular distribution of cytosolic and nuclear enzyme. *J Neurochem* 2003;86:1271–1280. [PubMed: 12911634]
19. Rexach JE, Clark PM, Hsieh-Wilson LC. Chemical approaches to understanding O-GlcNAc glycosylation in the brain. *Nat Chem Biol* 2008;4:97–106. [PubMed: 18202679]
20. Akimoto Y, Hart GW, Hirano H, Kawakami H. O-GlcNAc modification of nucleocytoplasmic proteins and diabetes. *Med Mol Morphol* 2005;38:84–91. [PubMed: 15944815]
21. Akimoto Y, Kawakami H, Yamamoto K, Munetomo E, Hida T, Hirano H. Elevated expression of O-GlcNAc-modified proteins and O-GlcNAc transferase in corneas of diabetic Goto-Kakizaki rats. *Invest Ophthalmol Vis Sci* 2003;44:3802–3809. [PubMed: 12939295]
22. Lubas WA, Frank DW, Krause M, Hanover JA. O-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. *J Biol Chem* 1997;272:9316–9324. [PubMed: 9083068]
23. McClain DA, Lubas WA, Cooksey RC, Hazel M, Parker GJ, Love DC, Hanover JA. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. *Proc Natl Acad Sci U S A* 2002;99:10695–10699. [PubMed: 12136128]
24. Vosseller K, Wells L, Lane MD, Hart GW. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. *Proc Natl Acad Sci U S A* 2002;99:5313–5318. [PubMed: 11959983]
25. Chatham JC, Not LG, Fulop N, Marchase RB. Hexosamine biosynthesis and protein O-glycosylation: the first line of defense against stress, ischemia, and trauma. *Shock* 2008;29:431–440. [PubMed: 17909453]
26. Fulop N, Marchase RB, Chatham JC. Role of protein O-linked N-acetylglucosamine in mediating cell function and survival in the cardiovascular system. *Cardiovasc Res* 2007;73:288–297. [PubMed: 16970929]
27. Lacy B, Hill BG, Wang K, Paterson AJ, White CR, Xing D, Chen YF, Darley-Usmar V, Oparil S, Chatham JC. Protein O-GlcNAcylation: a new signaling paradigm for the cardiovascular system. *Am J Physiol Heart Circ Physiol* 2009;296:H13–28. [PubMed: 19028792]
28. Clark RJ, McDonough PM, Swanson E, Trost SU, Suzuki M, Fukuda M, Dillmann WH. Diabetes and the accompanying hyperglycemia impairs cardiomyocyte calcium cycling through increased nuclear O-GlcNAcylation. *J Biol Chem* 2003;278:44230–44237. [PubMed: 12941958]

29. Ngoh GA, Facundo HT, Hamid T, Dillmann W, Zachara NE, Jones SP. Unique hexosaminidase reduces metabolic survival signal and sensitizes cardiac myocytes to hypoxia/reoxygenation injury. *Circ Res* 2009;104:41–49. [PubMed: 19023128]
30. Ngoh GA, Watson LJ, Facundo HT, Dillmann W, Jones SP. Non-canonical glycosyltransferase modulates post-hypoxic cardiac myocyte death and mitochondrial permeability transition. *J Mol Cell Cardiol* 2008;45:313–325. [PubMed: 18539296]
31. Golks A, Guerini D. The O-linked N-acetylglucosamine modification in cellular signalling and the immune system. 'Protein modifications: beyond the usual suspects' review series. *EMBO Rep* 2008;9:748–753. [PubMed: 18617890]
32. Golks A, Tran TT, Goetschy JF, Guerini D. Requirement for O-linked N-acetylglucosaminyltransferase in lymphocytes activation. *EMBO J* 2007;26:4368–4379. [PubMed: 17882263]
33. Kreppel LK, Blomberg MA, Hart GW. Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. *J Biol Chem* 1997;272:9308–9315. [PubMed: 9083067]
34. Jacobsen SE, Binkowski KA, Olszewski NE. SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in Arabidopsis. *Proc Natl Acad Sci U S A* 1996;93:9292–9296. [PubMed: 8799194]
35. Gao Y, Wells L, Comer FI, Parker GJ, Hart GW. Dynamic O-glycosylation of nuclear and cytosolic proteins: cloning and characterization of a neutral, cytosolic beta-N-acetylglucosaminidase from human brain. *J Biol Chem* 2001;276:9838–9845. [PubMed: 11148210]
36. Heckel D, Comtesse N, Brass N, Blin N, Zang KD, Meese E. Novel immunogenic antigen homologous to hyaluronidase in meningioma. *Hum Mol Genet* 1998;7:1859–1872. [PubMed: 9811929]
37. Nolte D, Muller U. Human O-GlcNAc transferase (OGT): genomic structure, analysis of splice variants, fine mapping in Xq13.1. *Mamm Genome* 2002;13:62–64. [PubMed: 11773972]
38. Shafi R, Iyer SP, Ellies LG, O'Donnell N, Marek KW, Chui D, Hart GW, Marth JD. 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 U S A* 2000;97:5735–5739. [PubMed: 10801981]
39. Hanover JA, Lai Z, Lee G, Lubas WA, Sato SM. Elevated O-linked N-acetylglucosamine metabolism in pancreatic beta-cells. *Arch Biochem Biophys* 1999;362:38–45. [PubMed: 9917327]
40. Love DC, Kochan J, Cathey RL, Shin SH, Hanover JA. Mitochondrial and nucleocytoplasmic targeting of O-linked GlcNAc transferase. *J Cell Sci* 2003;116:647–654. [PubMed: 12538765]
41. Lazarus BD, Roos MD, Hanover JA. Mutational analysis of the catalytic domain of O-linked N-acetylglucosaminyl transferase. *J Biol Chem* 2005;280:35537–35544. [PubMed: 16105839]
42. Lubas WA, Hanover JA. Functional expression of O-linked GlcNAc transferase. Domain structure and substrate specificity. *J Biol Chem* 2000;275:10983–10988. [PubMed: 10753899]
43. Hartweck LM, Genger RK, Grey WM, Olszewski NE. SECRET AGENT and SPINDLY have overlapping roles in the development of Arabidopsis thaliana L. Heyn. *J Exp Bot* 2006;57:865–875. [PubMed: 16473894]
44. Hartweck LM, Scott CL, Olszewski NE. Two O-linked N-acetylglucosamine transferase genes of Arabidopsis thaliana L. Heynh. have overlapping functions necessary for gamete and seed development. *Genetics* 2002;161:1279–1291. [PubMed: 12136030]
45. Jinek M, Rehwinkel J, Lazarus BD, Izaurre E, Hanover JA, Conti E. The superhelical TPR-repeat domain of O-linked GlcNAc transferase exhibits structural similarities to importin alpha. *Nat Struct Mol Biol* 2004;11:1001–1007. [PubMed: 15361863]
46. Martinez-Fleites C, Macauley MS, He Y, Shen DL, Vocadlo DJ, Davies GJ. Structure of an O-GlcNAc transferase homolog provides insight into intracellular glycosylation. *Nat Struct Mol Biol* 2008;15:764–765. [PubMed: 18536723]
47. Clarke AJ, Hurtado-Guerrero R, Pathak S, Schuttelkopf AW, Borodkin V, Shepherd SM, Ibrahim AF, van Aalten DM. Structural insights into mechanism and specificity of O-GlcNAc transferase. *EMBO J* 2008;27:2780–2788. [PubMed: 18818698]
48. Kreppel LK, Hart GW. Regulation of a cytosolic and nuclear O-GlcNAc transferase. Role of the tetratricopeptide repeats. *J Biol Chem* 1999;274:32015–32022. [PubMed: 10542233]

49. Schultz J, Pils B. Prediction of structure and functional residues for O-GlcNAcase, a divergent homologue of acetyltransferases. *FEBS Lett* 2002;529:179–182. [PubMed: 12372596]
50. Toleman C, Paterson AJ, Kudlow JE. Location and characterization of the O-GlcNAcase active site. *Biochim Biophys Acta* 2006;1760:829–839. [PubMed: 16517082]
51. Kim EJ, Kang DO, Love DC, Hanover JA. Enzymatic characterization of O-GlcNAcase isoforms using a fluorogenic GlcNAc substrate. *Carbohydr Res* 2006;341:971–982. [PubMed: 16584714]
52. Toleman C, Paterson AJ, Whisenhunt TR, Kudlow JE. Characterization of the histone acetyltransferase (HAT) domain of a bifunctional protein with activable O-GlcNAcase and HAT activities. *J Biol Chem* 2004;279:53665–53673. [PubMed: 15485860]
53. Whisenhunt TR, Yang X, Bowe DB, Paterson AJ, Van Tine BA, Kudlow JE. Disrupting the enzyme complex regulating O-GlcNAcylation blocks signaling and development. *Glycobiology* 2006;16:551–563. [PubMed: 16505006]
54. Rao FV, Dorfmueller HC, Villa F, Allwood M, Eggleston IM, van Aalten DM. Structural insights into the mechanism and inhibition of eukaryotic O-GlcNAc hydrolysis. *EMBO J* 2006;25:1569–1578. [PubMed: 16541109]
55. Dennis RJ, Taylor EJ, Macauley MS, Stubbs KA, Turkenburg JP, Hart SJ, Black GN, Vocadlo DJ, Davies GJ. Structure and mechanism of a bacterial beta-glucosaminidase having O-GlcNAcase activity. *Nat Struct Mol Biol* 2006;13:365–371. [PubMed: 16565725]
56. Macauley MS, Whitworth GE, Debowski AW, Chin D, Vocadlo DJ. O-GlcNAcase uses substrate-assisted catalysis: kinetic analysis and development of highly selective mechanism-inspired inhibitors. *J Biol Chem* 2005;280:25313–25322. [PubMed: 15795231]
57. Lin Y, Fletcher CM, Zhou J, Allis CD, Wagner G. Solution structure of the catalytic domain of GCN5 histone acetyltransferase bound to coenzyme A. *Nature* 1999;400:86–89. [PubMed: 10403255]
58. Holt GD, Hart GW. The subcellular distribution of terminal N-acetylglucosamine moieties. Localization of a novel protein-saccharide linkage, O-linked GlcNAc. *J Biol Chem* 1986;261:8049–8057. [PubMed: 3086323]
59. Hanover JA, Cohen CK, Willingham MC, Park MK. O-linked N-acetylglucosamine is attached to proteins of the nuclear pore. Evidence for cytoplasmic and nucleoplasmic glycoproteins. *J Biol Chem* 1987;262:9887–9894. [PubMed: 3110163]
60. Park MK, D'Onofrio M, Willingham MC, Hanover JA. A monoclonal antibody against a family of nuclear pore proteins (nucleoporins): O-linked N-acetylglucosamine is part of the immunodeterminant. *Proc Natl Acad Sci U S A* 1987;84:6462–6466. [PubMed: 2442757]
61. Holt GD, Snow CM, Senior A, Haltiwanger RS, Gerace L, Hart GW. Nuclear pore complex glycoproteins contain cytoplasmically disposed O-linked N-acetylglucosamine. *J Cell Biol* 1987;104:1157–1164. [PubMed: 3571327]
62. Snow CM, Senior A, Gerace L. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. *J Cell Biol* 1987;104:1143–1156. [PubMed: 2437126]
63. Hanover JA, Yu S, Lubas WB, Shin SH, Ragano-Caracciola M, Kochran J, Love DC. Mitochondrial and nucleocytoplasmic isoforms of O-linked GlcNAc transferase encoded by a single mammalian gene. *Arch Biochem Biophys* 2003;409:287–297. [PubMed: 12504895]
64. Fletcher BS, Dragstedt C, Notterpek L, Nolan GP. Functional cloning of SPIN-2, a nuclear anti-apoptotic protein with roles in cell cycle progression. *Leukemia* 2002;16:1507–1518. [PubMed: 12145692]
65. Arvanitis DL, Arvanitis LD, Panourias IG, Kitsoulis P, Kanavaros P. Mitochondria-rich normal, metaplastic, and neoplastic cells show overexpression of the epitope H recognized by the monoclonal antibody H. *Pathol Res Pract* 2005;201:319–324. [PubMed: 15991839]
66. Champattanachai V, Marchase RB, Chatham JC. Glucosamine protects neonatal cardiomyocytes from ischemia-reperfusion injury via increased protein O-GlcNAc and increased mitochondrial Bcl-2. *Am J Physiol Cell Physiol* 2008;294:C1509–20. [PubMed: 18367586]
67. Hu Y, Suarez J, Fricovsky E, Wang H, Scott BT, Trauger SA, Han W, Hu Y, Oyeleye MO, Dillmann WH. Increased enzymatic O-GlcNAcylation of mitochondrial proteins impairs mitochondrial function in cardiac myocytes exposed to high glucose. *J Biol Chem* 2009;284:547–555. [PubMed: 19004814]

68. Jones SP, Zachara NE, Ngoh GA, Hill BG, Teshima Y, Bhatnagar A, Hart GW, Marban E. Cardioprotection by N-acetylglucosamine linkage to cellular proteins. *Circulation* 2008;117:1172–1182. [PubMed: 18285568]
69. Suarez J, Hu Y, Makino A, Fricovsky E, Wang H, Dillmann WH. Alterations in mitochondrial function and cytosolic calcium induced by hyperglycemia are restored by mitochondrial transcription factor A in cardiomyocytes. *Am J Physiol Cell Physiol* 2008;295:C1561–8. [PubMed: 19060297]
70. Comtesse N, Maldener E, Meese E. Identification of a nuclear variant of MGEA5, a cytoplasmic hyaluronidase and a beta-N-acetylglucosaminidase. *Biochem Biophys Res Commun* 2001;283:634–640. [PubMed: 11341771]
71. Gross BJ, Kraybill BC, Walker S. Discovery of O-GlcNAc transferase inhibitors. *J Am Chem Soc* 2005;127:14588–14589. [PubMed: 16231908]
72. Kim EJ, Amorelli B, Abdo M, Thomas CJ, Love DC, Knapp S, Hanover JA. Distinctive inhibition of O-GlcNAcase isoforms by an alpha-GlcNAc thiolsulfonate. *J Am Chem Soc* 2007;129:14854–14855. [PubMed: 17994748]
73. Vocadlo DJ, Hang HC, Kim EJ, Hanover JA, Bertozzi CR. A chemical approach for identifying O-GlcNAc-modified proteins in cells. *Proc Natl Acad Sci U S A* 2003;100:9116–9121. [PubMed: 12874386]
74. Forsythe ME, Love DC, Lazarus BD, Kim EJ, Prinz WA, Ashwell G, Krause MW, Hanover JA. *Caenorhabditis elegans* ortholog of a diabetes susceptibility locus: oga-1 (O-GlcNAcase) knockout impacts O-GlcNAc cycling, metabolism, and dauer. *Proc Natl Acad Sci U S A* 2006;103:11952–11957. [PubMed: 16882729]
75. Butkinaree C, Cheung WD, Park S, Park K, Barber M, Hart GW. Characterization of beta-N-acetylglucosaminidase cleavage by caspase-3 during apoptosis. *J Biol Chem* 2008;283:23557–23566. [PubMed: 18586680]
76. Macauley MS, Vocadlo DJ. Enzymatic characterization and inhibition of the nuclear variant of human O-GlcNAcase. *Carbohydr Res* 2009;344:1079–1084. [PubMed: 19423084]
77. Kim EJ, Ferreira M, Thomas CJ, Hanover JA. An O-GlcNAcase-specific inhibitor and substrate engineered by the extension of the N-acetyl moiety. *J Am Chem Soc* 2006;128:4234–4235. [PubMed: 16568991]
78. Dehennaut V, Lefebvre T, Sellier C, Leroy Y, Gross B, Walker S, Cacan R, Michalski JC, Vilain JP, Bodart JF. O-linked N-acetylglucosaminyltransferase inhibition prevents G2/M transition in *Xenopus laevis* oocytes. *J Biol Chem* 2007;282:12527–12536. [PubMed: 17329255]
79. Lameira J, Alves CN, Moliner V, Marti S, Kanaan N, Tunon I. A quantum mechanics/molecular mechanics study of the protein-ligand interaction of two potent inhibitors of human O-GlcNAcase: PUGNAc and NAG-thiazoline. *J Phys Chem B* 2008;112:14260–14266. [PubMed: 18939790]
80. Macauley MS, Bubb AK, Martinez-Fleites C, Davies GJ, Vocadlo DJ. Elevation of global O-GlcNAc levels in 3T3-L1 adipocytes by selective inhibition of O-GlcNAcase does not induce insulin resistance. *J Biol Chem* 2008;283:34687–34695. [PubMed: 18842583]
81. Pathak S, Dorfmueller HC, Borodkin VS, van Aalten DM. Chemical dissection of the link between streptozotocin, O-GlcNAc, and pancreatic cell death. *Chem Biol* 2008;15:799–807. [PubMed: 18721751]
82. Stubbs KA, Zhang N, Vocadlo DJ. A divergent synthesis of 2-acyl derivatives of PUGNAc yields selective inhibitors of O-GlcNAcase. *Org Biomol Chem* 2006;4:839–845. [PubMed: 16493467]
83. Whitworth GE, Macauley MS, Stubbs KA, Dennis RJ, Taylor EJ, Davies GJ, Greig IR, Vocadlo DJ. Analysis of PUGNAc and NAG-thiazoline as transition state analogues for human O-GlcNAcase: mechanistic and structural insights into inhibitor selectivity and transition state poise. *J Am Chem Soc* 2007;129:635–644. [PubMed: 17227027]
84. Yuzwa SA, Macauley MS, Heinonen JE, Shan X, Dennis RJ, He Y, Whitworth GE, Stubbs KA, McEachern EJ, Davies GJ, Vocadlo DJ. A potent mechanism-inspired O-GlcNAcase inhibitor that blocks phosphorylation of tau in vivo. *Nat Chem Biol* 2008;4:483–490. [PubMed: 18587388]
85. Cheung WD, Sakabe K, Housley MP, Dias WB, Hart GW. O-linked beta-N-acetylglucosaminyltransferase substrate specificity is regulated by myosin phosphatase targeting and other interacting proteins. *J Biol Chem* 2008;283:33935–33941. [PubMed: 18840611]

86. Iyer SP, Akimoto Y, Hart GW. Identification and cloning of a novel family of coiled-coil domain proteins that interact with O-GlcNAc transferase. *J Biol Chem* 2003;278:5399–5409. [PubMed: 12435728]
87. Yang X, Zhang F, Kudlow JE. Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein O-GlcNAcylation to transcriptional repression. *Cell* 2002;110:69–80. [PubMed: 12150998]
88. Wysocka J, Myers MP, Laherty CD, Eisenman RN, Herr W. Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1. *Genes Dev* 2003;17:896–911. [PubMed: 12670868]
89. Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K, Mueller J, Wilm M, Stunnenberg HG, Saumweber H, Akhtar A. Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. *Mol Cell* 2006;21:811–823. [PubMed: 16543150]
90. Wells L, Kreppel LK, Comer FI, Wadzinski BE, Hart GW. O-GlcNAc transferase is in a functional complex with protein phosphatase 1 catalytic subunits. *J Biol Chem* 2004;279:38466–38470. [PubMed: 15247246]
91. Fujiki R, Chikanishi T, Hashiba W, Ito H, Takada I, Roeder RG, Kitagawa H, Kato S. GlcNAcylation of a histone methyltransferase in retinoic-acid-induced granulopoiesis. *Nature* 2009;459:455–459. [PubMed: 19377461]
92. Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, Vidalain PO, Han JD, Chesneau A, Hao T, Goldberg DS, Li N, Martinez M, Rual JF, Lamesch P, Xu L, Tewari M, Wong SL, Zhang LV, Berriz GF, Jacotot L, Vaglio P, Reboul J, Hirozane-Kishikawa T, Li Q, Gabel HW, Elewa A, Baumgartner B, Rose DJ, Yu H, Bosak S, Sequerra R, Fraser A, Mango SE, Saxton WM, Strome S, Van Den Heuvel S, Piano F, Vandenhaute J, Sardet C, Gerstein M, Doucette-Stamm L, Gunsalus KC, Harper JW, Cusick ME, Roth FP, Hill DE, Vidal M. A map of the interactome network of the metazoan *C. elegans*. *Science* 2004;303:540–543. [PubMed: 14704431]
93. Cheung WD, Hart GW. AMP-activated protein kinase and p38 MAPK activate O-GlcNAcylation of neuronal proteins during glucose deprivation. *J Biol Chem* 2008;283:13009–13020. [PubMed: 18353774]
94. Giot L, Bader JS, Brouwer C, Chaudhuri A, Kuang B, Li Y, Hao YL, Ooi CE, Godwin B, Vitols E, Vijayadamodar G, Pochart P, Machineni H, Welsh M, Kong Y, Zerhusen B, Malcolm R, Varrone Z, Collis A, Minto M, Burgess S, McDaniel L, Stimpson E, Spriggs F, Williams J, Neurath K, Ioime N, Agee M, Voss E, Furtak K, Renzulli R, Aanensen N, Carrola S, Bickelhaupt E, Lazovatsky Y, DaSilva A, Zhong J, Stanyon CA, Finley RLJ, White KP, Braverman M, Jarvie T, Gold S, Leach M, Knight J, Shimkets RA, McKenna MP, Chant J, Rothberg JM. A protein interaction map of *Drosophila melanogaster*. *Science* 2003;302:1727–1736. [PubMed: 14605208]
95. Marshall S, Bacote V, Traxinger RR. Complete inhibition of glucose-induced desensitization of the glucose transport system by inhibitors of mRNA synthesis. Evidence for rapid turnover of glutamine:fructose-6-phosphate amidotransferase. *J Biol Chem* 1991;266:10155–10161. [PubMed: 2037572]
96. S, Marshall; Bacote, V.; Traxinger, RR. 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* 1991;266:4706–4712. [PubMed: 2002019]
97. Marshall S, Garvey WT, Traxinger RR. New insights into the metabolic regulation of insulin action and insulin resistance: role of glucose and amino acids. *FASEB J* 1991;5:3031–3036. [PubMed: 1743436]
98. Mendelsohn R, Cheung P, Berger L, Partridge E, Lau K, Datti A, Pawling J, Dennis JW. Complex N-glycan and metabolic control in tumor cells. *Cancer Res* 2007;67:9771–9780. [PubMed: 17942907]
99. Ohtsubo K, Takamatsu S, Minowa MT, Yoshida A, Takeuchi M, Marth JD. Dietary and genetic control of glucose transporter 2 glycosylation promotes insulin secretion in suppressing diabetes. *Cell* 2005;123:1307–1321. [PubMed: 16377570]
100. Akimoto Y, Hart GW, Wells L, Vosseller K, Yamamoto K, Munetomo E, Ohara-Imaizumi M, Nishiwaki C, Nagamatsu S, Hirano H, Kawakami H. Elevation of the post-translational modification of proteins by O-linked N-acetylglucosamine leads to deterioration of the glucose-

stimulated insulin secretion in the pancreas of diabetic Goto-Kakizaki rats. *Glycobiology* 2007;17:127–140. [PubMed: 17095531]

101. Arias EB, Kim J, Cartee GD. Prolonged incubation in PUGNAc results in increased protein O-Linked glycosylation and insulin resistance in rat skeletal muscle. *Diabetes* 2004;53:921–930. [PubMed: 15047606]
102. Ball LE, Berkaw MN, Buse MG. Identification of the major site of O-linked beta-N-acetylglucosamine modification in the C terminus of insulin receptor substrate-1. *Mol Cell Proteomics* 2006;5:313–323. [PubMed: 16244361]
103. Buse MG. Hexosamines, insulin resistance, and the complications of diabetes: current status. *Am J Physiol Endocrinol Metab* 2006;290:E1–E8. [PubMed: 16339923]
104. Copeland RJ, Bullen JW, Hart GW. Cross-talk between GlcNAcylation and phosphorylation: roles in insulin resistance and glucose toxicity. *Am J Physiol Endocrinol Metab* 2008;295:E17–28. [PubMed: 18445751]
105. Matthews JA, Belof JL, Acevedo-Duncan M, Potter RL. Glucosamine-induced increase in Akt phosphorylation corresponds to increased endoplasmic reticulum stress in astroglial cells. *Mol Cell Biochem* 2007;298:109–123. [PubMed: 17136481]
106. Parker GJ, Lund KC, Taylor RP, McClain DA. Insulin resistance of glycogen synthase mediated by o-linked N-acetylglucosamine. *J Biol Chem* 2003;278:10022–10027. [PubMed: 12510058]
107. Walgren JL, Vincent TS, Schey KL, Buse MG. High glucose and insulin promote O-GlcNAc modification of proteins, including alpha-tubulin. *Am J Physiol Endocrinol Metab* 2003;284:E424–34. [PubMed: 12397027]
108. Wells L, Vosseller K, Hart GW. A role for N-acetylglucosamine as a nutrient sensor and mediator of insulin resistance. *Cell Mol Life Sci* 2003;60:222–228. [PubMed: 12678487]
109. Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, So WV, Kudlow JE, Michell RH, Olefsky JM, Field SJ, Evans RM. Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. *Nature* 2008;451:964–969. [PubMed: 18288188]
110. Hebert LFJ, Daniels MC, Zhou J, Crook ED, Turner RL, Simmons ST, Neidigh JL, Zhu JS, Baron AD, McClain DA. Overexpression of glutamine:fructose-6-phosphate amidotransferase in transgenic mice leads to insulin resistance. *J Clin Invest* 1996;98:930–936. [PubMed: 8770864]
111. McClain DA, Alexander T, Cooksey RC, Considine RV. Hexosamines stimulate leptin production in transgenic mice. *Endocrinology* 2000;141:1999–2002. [PubMed: 10830282]
112. Veerababu G, Tang J, Hoffman RT, Daniels MC, Hebert LFJ, Crook ED, Cooksey RC, McClain DA. Overexpression of glutamine: fructose-6-phosphate amidotransferase in the liver of transgenic mice results in enhanced glycogen storage, hyperlipidemia, obesity, and impaired glucose tolerance. *Diabetes* 2000;49:2070–2078. [PubMed: 11118009]
113. Cooksey RC, McClain DA. Transgenic mice overexpressing the rate-limiting enzyme for hexosamine synthesis in skeletal muscle or adipose tissue exhibit total body insulin resistance. *Ann N Y Acad Sci* 2002;967:102–111. [PubMed: 12079840]
114. Boehmelt G, Wakeham A, Elia A, Sasaki T, Plyte S, Potter J, Yang Y, Tsang E, Ruland J, Iscove NN, Dennis JW, Mak TW. Decreased UDP-GlcNAc levels abrogate proliferation control in EMeg32-deficient cells. *EMBO J* 2000;19:5092–5104. [PubMed: 11013212]
115. O'Donnell N, Zachara NE, Hart GW, Marth JD. Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. *Mol Cell Biol* 2004;24:1680–1690. [PubMed: 14749383]
116. Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, Leivers SJ, Partridge L. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* 2001;292:104–106. [PubMed: 11292874]
117. Finch CE, Ruvkun G. The genetics of aging. *Annu Rev Genomics Hum Genet* 2001;2:435–462. [PubMed: 11701657]
118. Lin K, Hsin H, Libina N, Kenyon C. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* 2001;28:139–145. [PubMed: 11381260]

119. Paradis S, Ruvkun G. Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev* 1998;12:2488–2498. [PubMed: 9716402]
120. Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, Buchman AR, Ferguson KC, Heller J, Platt DM, Pasquinelli AA, Liu LX, Doberstein SK, Ruvkun G. Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. *Genes Dev* 2001;15:672–686. [PubMed: 11274053]
121. Tissenbaum HA, Ruvkun G. An insulin-like signaling pathway affects both longevity and reproduction in Caenorhabditis elegans. *Genetics* 1998;148:703–717. [PubMed: 9504918]
122. Wolkow CA, Kimura KD, Lee MS, Ruvkun G. Regulation of C. elegans life-span by insulinlike signaling in the nervous system. *Science* 2000;290:147–150. [PubMed: 11021802]
123. Gami MS, Wolkow CA. Studies of Caenorhabditis elegans DAF-2/insulin signaling reveal targets for pharmacological manipulation of lifespan. *Aging Cell* 2006;5:31–37. [PubMed: 16441841]
124. Taguchi A, White MF. Insulin-like signaling, nutrient homeostasis, and life span. *Annu Rev Physiol* 2008;70:191–212. [PubMed: 17988211]
125. Gambetta MC, Oktaba K, Muller J. Essential Role of the Glycosyltransferase Sxc/Ogt in Polycomb Repression. *Science*. 2009
126. Ingham PW. A gene that regulates the bithorax complex differentially in larval and adult cells of Drosophila. *Cell* 1984;37:815–823. [PubMed: 6430566]
127. Bellen HJ, Levis RW, Liao G, He Y, Carlson JW, Tsang G, Evans-Holm M, Hiesinger PR, Schulze KL, Rubin GM, Hoskins RA, Spradling AC. The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. *Genetics* 2004;167:761–781. [PubMed: 15238527]
128. Patterson GI, Padgett RW. TGF beta-related pathways. Roles in Caenorhabditis elegans development. *Trends Genet* 2000;16:27–33. [PubMed: 10637628]
129. Berman K, McKay J, Avery L, Cobb M. Isolation and characterization of pmk-(1-3): three p38 homologs in Caenorhabditis elegans. *Mol Cell Biol Res Commun* 2001;4:337–344. [PubMed: 11703092]
130. Byrne AB, Weirauch MT, Wong V, Koeva M, Dixon SJ, Stuart JM, Roy PJ. A global analysis of genetic interactions in Caenorhabditis elegans. *J Biol* 2007;6:8. [PubMed: 17897480]
131. Sayat R, Leber B, Grubac V, Wiltshire L, Persad S. O-GlcNAc-glycosylation of beta-catenin regulates its nuclear localization and transcriptional activity. *Exp Cell Res* 2008;314:2774–2787. [PubMed: 18586027]
132. Hanover JA, Love DC, Prinz WA. Calmodulin-driven nuclear entry: Trigger for sex determination and terminal differentiation. *J Biol Chem*. 2009
133. Wang Z, Gucek M, Hart GW. Cross-talk between GlcNAcylation and phosphorylation: site-specific phosphorylation dynamics in response to globally elevated O-GlcNAc. *Proc Natl Acad Sci U S A* 2008;105:13793–13798. [PubMed: 18779572]
134. Ande SR, Moulik S, Mishra S. Interaction between O-GlcNAc modification and tyrosine phosphorylation of prohibitin: implication for a novel binary switch. *PLoS ONE* 2009;4:e4586. [PubMed: 19238206]
135. Housley MP, Udeshi ND, Rodgers JT, Shabanowitz J, Puigserver P, Hunt DF, Hart GW. A PGC-1 {alpha}-O-GlcNAc Transferase Complex Regulates FoxO Transcription Factor Activity in Response to Glucose. *J Biol Chem* 2009;284:5148–5157. [PubMed: 19103600]
136. Dentin R, Hedrick S, Xie J, Yates J. r. Montminy M. Hepatic glucose sensing via the CREB coactivator CRTC2. *Science* 2008;319:1402–1405. [PubMed: 18323454]
137. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE, Shaw RJ. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 2008;30:214–226. [PubMed: 18439900]
138. Hardie DG. AMPK and Raptor: matching cell growth to energy supply. *Mol Cell* 2008;30:263–265. [PubMed: 18471972]
139. Sarbassov DD, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 2005;17:596–603. [PubMed: 16226444]

140. Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 2006;441:437–443. [PubMed: 16724055]
141. Luo B, Parker GJ, Cooksey RC, Soesanto Y, Evans M, Jones D, McClain DA. Chronic hexosamine flux stimulates fatty acid oxidation by activating AMP-activated protein kinase in adipocytes. *J Biol Chem* 2007;282:7172–7180. [PubMed: 17227772]
142. Narbonne P, Roy R. Inhibition of germline proliferation during *C. elegans* dauer development requires PTEN, LKB1 and AMPK signalling. *Development* 2006;133:611–619. [PubMed: 16407400]
143. Dehennaut V, Slomianny MC, Page A, Vercoutter-Edouart AS, Jessus C, Michalski JC, Vilain JP, Bodart JF, Lefebvre T. Identification of structural and functional O-linked N-acetylglucosamine-bearing proteins in *Xenopus laevis* oocyte. *Mol Cell Proteomics* 2008;7:2229–2245. [PubMed: 18617508]
144. Chakraborty A, Saha D, Bose A, Chatterjee M, Gupta NK. Regulation of eIF-2 alpha-subunit phosphorylation in reticulocyte lysate. *Biochemistry* 1994;33:6700–6706. [PubMed: 7911326]
145. Nandi A, Sprung R, Barma DK, Zhao Y, Kim SC, Falck JR, Zhao Y. Global identification of O-GlcNAc-modified proteins. *Anal Chem* 2006;78:452–458. [PubMed: 16408927]
146. Ohn T, Kedersha N, Hickman T, Tisdale S, Anderson P. A functional RNAi screen links O-GlcNAc modification of ribosomal proteins to stress granule and processing body assembly. *Nat Cell Biol* 2008;10:1224–1231. [PubMed: 18794846]
147. Berdichevsky A, Guarente L. A stress response pathway involving sirtuins, forkheads and 14-3-3 proteins. *Cell Cycle* 2006;5:2588–2591. [PubMed: 17172829]
148. Longo VD. Linking sirtuins, IGF-I signaling, and starvation. *Exp Gerontol* 2009;44:70–74. [PubMed: 18638538]
149. Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. *Oncogene* 2007;26:5489–5504. [PubMed: 17694089]
150. Westphal CH, Dipp MA, Guarente L. A therapeutic role for sirtuins in diseases of aging? *Trends Biochem Sci* 2007;32:555–560. [PubMed: 17980602]
151. Liu Y, Dentin R, Chen D, Hedrick S, Ravnskjaer K, Schenk S, Milne J, Meyers DJ, Cole P, Yates J. r. Olefsky J, Guarente L, Montminy M. A fasting inducible switch modulates gluconeogenesis via activator/coactivator exchange. *Nature* 2008;456:269–273. [PubMed: 18849969]
152. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J Clin Invest* 2001;108:1341–1348. [PubMed: 11696579]
153. Musicki B, Kramer MF, Becker RE, Burnett AL. Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. *Proc Natl Acad Sci U S A* 2005;102:11870–11875. [PubMed: 16085713]
154. Brickley K, Smith MJ, Beck M, Stephenson FA. GRIF-1 and OIP106, members of a novel gene family of coiled-coil domain proteins: association in vivo and in vitro with kinesin. *J Biol Chem* 2005;280:14723–14732. [PubMed: 15644324]
155. MacAskill AF, Brickley K, Stephenson FA, Kittler JT. GTPase dependent recruitment of Grif-1 by Miro1 regulates mitochondrial trafficking in hippocampal neurons. *Mol Cell Neurosci* 2009;40:301–312. [PubMed: 19103291]
156. Smith MJ, Pozo K, Brickley K, Stephenson FA. Mapping the GRIF-1 binding domain of the kinesin, KIF5C, substantiates a role for GRIF-1 as an adaptor protein in the anterograde trafficking of cargoes. *J Biol Chem* 2006;281:27216–27228. [PubMed: 16835241]
157. Beck M, Brickley K, Wilkinson HL, Sharma S, Smith M, Chazot PL, Pollard S, Stephenson FA. Identification, molecular cloning, and characterization of a novel GABAA receptor-associated protein, GRIF-1. *J Biol Chem* 2002;277:30079–30090. [PubMed: 12034717]
158. Pozo K, Stephenson FA. GRIF-1-kinesin-1 interactions: a confocal microscopy study. *Biochem Soc Trans* 2006;34:48–50. [PubMed: 16417480]
159. Rice SE, Gelfand VI. Paradigm lost: mltin connects kinesin heavy chain to miro on mitochondria. *J Cell Biol* 2006;173:459–461. [PubMed: 16717123]

160. Stowers RS, Megeath LJ, Gorska-Andrzejak J, Meinertzhagen IA, Schwarz TL. Axonal transport of mitochondria to synapses depends on mltin, a novel *Drosophila* protein. *Neuron* 2002;36:1063–1077. [PubMed: 12495622]
161. Stuart LM, Boulais J, Charriere GM, Hennessy EJ, Brunet S, Jutras I, Goyette G, Rondeau C, Letarte S, Huang H, Ye P, Morales F, Kocks C, Bader JS, Desjardins M, Ezekowitz RA. A systems biology analysis of the *Drosophila* phagosome. *Nature* 2007;445:95–101. [PubMed: 17151602]
162. Kirk E, Chin LS, Li L. GRIF1 binds Hrs and is a new regulator of endosomal trafficking. *J Cell Sci* 2006;119:4689–4701. [PubMed: 17062640]
163. Webber E, Li L, Chin LS. Hypertonia-associated protein Trak1 is a novel regulator of endosome-to-lysosome trafficking. *J Mol Biol* 2008;382:638–651. [PubMed: 18675823]
164. Bowe DB, Sadlonova A, Toleman CA, Novak Z, Hu Y, Huang P, Mukherjee S, Whitsett T, Frost AR, Paterson AJ, Kudlow JE. O-GlcNAc integrates the proteasome and transcriptome to regulate nuclear hormone receptors. *Mol Cell Biol* 2006;26:8539–8550. [PubMed: 16966374]
165. Guinez C, Mir AM, Dehennaut V, Cacan R, Harduin-Lepers A, Michalski JC, Lefebvre T. Protein ubiquitination is modulated by O-GlcNAc glycosylation. *FASEB J* 2008;22:2901–2911. [PubMed: 18434435]
166. Han I, Kudlow JE. Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility. *Mol Cell Biol* 1997;17:2550–2558. [PubMed: 9111324]
167. Sumegi M, Hunyadi-Gulyas E, Medzihradszky KF, Udvardy A. 26S proteasome subunits are O-linked N-acetylglucosamine-modified in *Drosophila melanogaster*. *Biochem Biophys Res Commun* 2003;312:1284–1289. [PubMed: 14652013]
168. Zachara NE, Hart GW. O-GlcNAc modification: a nutritional sensor that modulates proteasome function. *Trends Cell Biol* 2004;14:218–221. [PubMed: 15130576]
169. Zhang F, Hu Y, Huang P, Toleman CA, Paterson AJ, Kudlow JE. Proteasome function is regulated by cyclic AMP-dependent protein kinase through phosphorylation of Rpt6. *J Biol Chem* 2007;282:22460–22471. [PubMed: 17565987]
170. Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE. O-GlcNAc modification is an endogenous inhibitor of the proteasome. *Cell* 2003;115:715–725. [PubMed: 14675536]
171. Jackson SP, Tjian R. O-glycosylation of eukaryotic transcription factors: implications for mechanisms of transcriptional regulation. *Cell* 1988;55:125–133. [PubMed: 3139301]
172. Jackson SP, Tjian R. Purification and analysis of RNA polymerase II transcription factors by using wheat germ agglutinin affinity chromatography. *Proc Natl Acad Sci U S A* 1989;86:1781–1785. [PubMed: 2648381]
173. Kelly WG, Hart GW. Glycosylation of chromosomal proteins: localization of O-linked N-acetylglucosamine in *Drosophila* chromatin. *Cell* 1989;57:243–251. [PubMed: 2495182]
174. Comer FI, Hart GW. Reciprocity between O-GlcNAc and O-phosphate on the carboxyl terminal domain of RNA polymerase II. *Biochemistry* 2001;40:7845–7852. [PubMed: 11425311]
175. Comer FI, Vosseller K, Wells L, Accavitti MA, Hart GW. Characterization of a mouse monoclonal antibody specific for O-linked N-acetylglucosamine. *Anal Biochem* 2001;293:169–177. [PubMed: 11399029]
176. Kelly WG, Dahmus ME, Hart GW. RNA polymerase II is a glycoprotein. Modification of the COOH-terminal domain by O-GlcNAc. *J Biol Chem* 1993;268:10416–10424. [PubMed: 8486697]
177. Kim SH, Kim YH, Song M, An SH, Byun HY, Heo K, Lim S, Oh YS, Ryu SH, Suh PG. O-GlcNAc modification modulates the expression of osteocalcin via OSE2 and Runx2. *Biochem Biophys Res Commun* 2007;362:325–329. [PubMed: 17707335]
178. Okuyama R, Marshall S. Potential regulation of nuclear UDP-N-acetylglucosaminyl transferase (OGT) by substrate availability: ability of chromatin protein to bind UDP-N-acetylglucosamine and reduce OGT-mediated O-Linked glycosylation. *Biol Pharm Bull* 2004;27:1293–1296. [PubMed: 15305040]
179. Reason AJ, Morris HR, Panico M, Marais R, Treisman RH, Haltiwanger RS, Hart GW, Kelly WG, Dell A. Localization of O-GlcNAc modification on the serum response transcription factor. *J Biol Chem* 1992;267:16911–16921. [PubMed: 1512232]

180. Yang X, Su K, Roos MD, Chang Q, Paterson AJ, Kudlow JE. O-linkage of N-acetylglucosamine to Sp1 activation domain inhibits its transcriptional capability. *Proc Natl Acad Sci U S A* 2001;98:6611–6616. [PubMed: 11371615]
181. Yao D, Taguchi T, Matsumura T, Pestell R, Edelstein D, Giardino I, Suske G, Ahmed N, Thornalley PJ, Sarthy VP, Hammes HP, Brownlee M. Methylglyoxal modification of mSin3A links glycolysis to angiopoietin-2 transcription. *Cell* 2006;124:275–286. [PubMed: 16413606]
182. Mendjan S, Akhtar A. The right dose for every sex. *Chromosoma* 2007;116:95–106. [PubMed: 17124606]
183. Miller MW, Hanover JA. Functional nuclear pores reconstituted with beta 1-4 galactose-modified O-linked N-acetylglucosamine glycoproteins. *J Biol Chem* 1994;269:9289–9297. [PubMed: 8132666]
184. Duverger E, Roche AC, Monsigny M. N-acetylglucosamine-dependent nuclear import of neoglycoproteins. *Glycobiology* 1996;6:381–386. [PubMed: 8842701]
185. Guinez C, Morelle W, Michalski JC, Lefebvre T. O-GlcNAc glycosylation: a signal for the nuclear transport of cytosolic proteins? *Int J Biochem Cell Biol* 2005;37:765–774. [PubMed: 15694836]
186. Park J, Han D, Kim K, Kang Y, Kim Y. O-GlcNAcylation disrupts glyceraldehyde-3-phosphate dehydrogenase homo-tetramer formation and mediates its nuclear translocation. *Biochim Biophys Acta* 2009;1794:254–262. [PubMed: 19022411]
187. Kornfeld R. Studies on L-glutamine D-fructose 6-phosphate amidotransferase. I. Feedback inhibition by uridine diphosphate-N-acetylglucosamine. *J Biol Chem* 1967;242:3135–3141. [PubMed: 4961641]
188. Shen A, Kamp HD, Grundling A, Higgins DE. A bifunctional O-GlcNAc transferase governs flagellar motility through anti-repression. *Genes Dev* 2006;20:3283–3295. [PubMed: 17158746]

Figure 1.

A. Domain structure of mammalian O-GlcNAc transferase (OGT) and its differentially-targeted isoforms derived from alternative splicing of the OGT gene. The three major isoforms of OGT encoded by the OGT gene on chromosome X (domain diagrams to the *right*), and their subcellular localization based on C-terminal GFP fusions (*on left*) [40]. The OGT isoforms differ at their amino terminus and are distinguishable from one another by their unique N-terminal targeting signals and their number of tetratricopeptide (TPR) repeats. The largest of these is the nucleocytoplasmic ncOGT with 13 TPR repeats shown in purple. The mOGT isoform is targeted to the mitochondria by a unique N-terminal mitochondrial targeting signal (MTS). mOGT has 9 TPR repeats. The shortest isoform is sOGT and contains almost 3 TPR repeats. All of the isoforms have a common catalytic domain as described in the text. Near the amino terminus of each of the isoforms is the binding site for PIP3 (*termed PPO*). The *C. elegans* and *D. melanogaster* OGTs are highly similar to ncOGT from mammals and are present in the nucleus. The worm and fly OGT transcripts are alternatively spliced but do not appear to encode differentially targeted isoforms. B.) Composite structure and interactions of higher

metazoan OGT modeled from the X-Ray structures of hOGT and bacterial homologs. The structures of human OGT (PDB: 1W3B) and bacterial *Xanthonas caepestis* (PDB: 2VSN) were combined using the NCBI VAST search and molecular alignment tools to generate a likely structure for higher metazoan OGT. The TPRs form a superhelical structure, which binds to a number of adapters as indicated on the small table (*left*). The TPRs also have an asparagines ladder motif also found in importin α (see text). The sites of PIP3 binding and UDP-GlcNAc interaction are shown by the pointers. The dimer partners are colored differently for clarity (multicolored or gold). The structure to the *right* is a different projection to show the dimer interface within the TPR motifs. ncOGT and mOGT are anticipated to form dimers while sOGT cannot. The dimer-interface and the catalytic site are indicated by the pointers.

Figure 2.

A. Domain structure of mammalian O-GlcNAcase (OGA). The domain structure of the O-GlcNAcase encoded by the MGEA5 locus on chromosome 19 in humans (Ch 10 in mice) is shown. The two main splice variants encode long OGA and short OGA that has an alternative C-terminus with 15 unique amino acids. Both isoforms of OGA have an N-terminal O-GlcNAcase domain, and a central OGT interacting domain. The long OGA has an additional histone-acetyltransferase (HAT)-like domain with an internal nuclear localization sequence (*see text*). Fly and worm OGA are similar to long OGA B. The structure of a bacterial homolog of OGT and a model of its C-terminal HAT domain. The structure (PDB: 2W4X) of an O-GlcNAcase homolog from *Bacterioides thetaiotaomicron* is shown with the position of its active site indicated. Juxtaposed to the right is the structure of a GCN5 homolog (PDB: 1QST) similar to the C-terminal HAT domain of OGA.

Figure 3. The highly conserved hexosamine biosynthetic pathway leading to O-GlcNAc cycling

The enzymes involved in the synthesis and utilization of UDP-GlcNAc from nutrient precursors are shown. The role of O-GlcNAc cycling is emphasized, but UDP-GlcNAc is also the precursor for glycolipids, O-linked GalNAc and N-linked Glycoproteins in the endomembrane system as indicated by *other* on each side of the UDP-HexNAcs (see text). The mammalian enzymes are listed with the *C. elegans* homologs in *italics*. The nutrient-derived precursors glucose, glutamine, acetyl-CoA, UTP are integrated by the pathway and UDP-GlcNAc levels reflect the nutrient status. O-GlcNAc cycling is the terminal step in the hexosamine signaling pathway.

Figure 4. The highly conserved insulin signaling pathway is influenced by O-GlcNAc cycling
 The insulin signaling pathways of mammals, *D. melanogaster* and *C. elegans*, respectively are shown from top to bottom. All of the pathways are triggered by insulin-like peptides binding to an insulin-like receptor. The binding event triggers tyrosine phosphorylation of an insulin receptor substrate (IRS, termed *chico* in *Drosophila*). This activates the PI3K leading to recruitment of PDK1, activation of AKT and phosphorylation of a Foxo protein (*daf-16* in *C. elegans*). Phosphorylation of the Foxo protein leads to cytoplasmic ‘tethering’ of the transcription factor in the cytoplasm limiting its nuclear entry and transcriptional activation. Foxo may be O-GlcNAc-modified at the canonic AKT site leading to nuclear entry and transcriptional activation. In mammals, overexpression of OGT leads to insulin resistance by acting downstream of PI3K to modify key signaling molecules. Studies in both mammals, fly and *C. elegans* indicate that O-GlcNAc cycling impacts the insulin-signaling pathway at several points downstream of PI3K signaling (indicated by the red shaded area). In *C. elegans* (bottom panel) knockouts of *ogt-1* and *oga-1*, the enzymes of O-GlcNAc cycling causes changes in macronutrient stores as indicated. Genetic analysis suggests that *ogt-1* is epistatic to *oga-1* as expected. Insulin signaling in *C. elegans* controls the dauer diapause, longevity, and stress responses, all of which are impacted by O-GlcNAc cycling (See table at bottom right).

Figure 5. O-GlcNAc cycling and crosstalk with Multiple Cellular Nutrient-responsive pathways
 The Hexosamine signaling pathway terminating in the enzymes of O-GlcNAc cycling lies at the center of many of the major nutrient sensing pathways (p38 MAPK, Insulin-AKT, mTOR, AMPK and SirT1). Broadly speaking these pathways can be considered either ‘Pro-survival’ cascades (*shown in green*), triggered by stress, DNA damage or starvation or ‘Pro-growth’ cascades (*shown in blue*) triggered by hormones, cytokines, growth factors, and nutrient excess. The pro-survival cascade limits protein synthesis and triggers nutrient salvage by proteasomal degradation and autophagy. In contrast, the pro-growth cascades enhance protein synthesis and block proteasome activation and autophagy. OGT is recruited to these two cascades by either interacting with PIP3 resulting from PI3K activation (blue double arrow) or by interacting with activated p38 MAPK (green double arrow). These mutually exclusive interactions of OGT

produce a binary switch, bringing the nutrient sensing hexosamine signaling module to bear on the two major cascades as indicated in the text. For example, by blunting the action of AKT, OGT limits the pro-growth cascade. OGT also inhibits the proteasome. In contrast, binding to activated p38 MAPK, OGT changes its substrate specificity bringing its nutrient-sensing capabilities to bear on limited stress-specific targets (see text). The net result of these interactions is either nutrient uptake or nutrient salvage and sparing.

Figure 6. Key intracellular targets of O-GlcNAc impact nutrient uptake and salvage

As a modulator of signaling, endocytosis, transcription, translation and protein stability, the HSP may coordinately regulate nutrient sparing and salvage. Some of the key intracellular targets of O-GlcNAc modification are shown with respect to their role in nutrient uptake and salvage. OGT modifies numerous signaling cascades including those triggered by PI3K, MAPK, AMPK and mTOR (For details, see figure 5). In addition, by interacting with adapters like the *D. melanogaster* protein *Milton*, OGT may modulate intracellular movement of mitochondria on microtubules. The mammalian *Milton* homolog GRIF-1 binds to OGT and may be involved in orchestrating directed organelle movement including the trafficking of neurosecretory vesicles, endosomes, lysosomes and autophagosomes. These processes are

critical for nutrient acquisition and immunity. OGT overexpression alters the movement of GLUT4 rich endosomes to the plasma membrane in response to insulin, a hallmark of mammalian insulin resistance. O-GlcNAc may also play a role in the switch from anabolic to catabolic metabolism. For example, O-GlcNAc modifies many ribosomal proteins and is required for the formation of stress granules involved in mRNA triage. O-GlcNAc also regulates the proteasome by modifying the ATP-dependent rpt2 domain as described in the text, and may directly, or indirectly regulate autophagy. O-GlcNAc also modifies nuclear pores and transcription-complexes playing an essential role in transcriptional repression [125].