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Nutrient regulation of signaling and transcription

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In the early 1980s, while using purified glycosyltransferases to probe glycan structures on surfaces of living cells in the murine immune system, we discovered a novel form of serine/threonine protein glycosylation (*O*-linked β-GlcNAc; *O*-GlcNAc) that occurs on thousands of proteins within the nucleus, cytoplasm, and mitochondria. Prior to this discovery, it was dogma that protein glycosylation was restricted to the luminal compartments of the secretory pathway and on extracellular domains of membrane and secretory proteins. Work in the last 3 decades from several laboratories has shown that *O*-GlcNAc cycling serves as a nutrient sensor to regulate signaling, transcription, mitochondrial activity, and cytoskeletal functions. *O*-GlcNAc also has extensive cross-talk with phosphorylation, not only at the same or proximal sites on polypeptides, but also by regulating each other's enzymes that catalyze cycling of the modifications. *O*-GlcNAc is generally not elongated or modified. It cycles on and off polypeptides in a time scale similar to phosphorylation, and both the enzyme that adds *O*-GlcNAc, the *O*-GlcNAc transferase (OGT), and the enzyme that removes *O*-GlcNAc, *O*-GlcNAcase (OGA), are highly conserved from *C. elegans* to humans. Both *O*-GlcNAc cycling enzymes are essential in mammals and plants. Due to *O*-GlcNAc's fundamental roles as a nutrient and stress sensor, it plays an important role in the etiologies of chronic diseases of aging, including diabetes, cancer, and neurodegenerative disease. This review will present an overview of our current understanding of *O*-GlcNAc's regulation, functions, and roles in chronic diseases of aging.

O-GlcNAc was discovered when bovine milk galactosyltransferase and UDP-[^{3}H]galactose were used to probe the surfaces of living cells of the murine immune system for terminal GlcNAc moieties (1). Surprisingly, further analyses showed that nearly all of the incorporated [^{3}H]galactose was added to single β-*O*-linked GlcNAc moieties attached to Ser(Thr) residues on polypeptides. Follow-up experiments showed that *O*-GlcNAc is highly enriched within the nucleus and cytoplasm (2), particularly on nuclear envelope and chromatin proteins. Thus, the labeling in initial experiments (1) detected *O*-GlcNAcylated proteins on the small percentage of lysed or damaged cells in the cultures, indicating that *O*-GlcNAc is quite abundant on nucleocytoplasmic proteins. Cytosolic localization of *O*-GlcNAc was further confirmed by identification of *O*-GlcNAc on cytoplasmic proteins in human erythrocytes (3). Subsequently, *O*-GlcNAc was found to be particularly enriched on the nuclear and cytosolic faces of the nuclear pore complex (4–6). Similar to its unusual localization in cells, *O*-GlcNAc was found to be on proteins associated with nucleic acids in viruses (7, 8) and also to be abundant on RNA polymerase II transcription factors (9, 10), some of which are well-known oncogenic factors (11, 12) or tumor suppressor proteins (13). The IIa (nonphosphorylated) form of RNA polymerase II is heavily *O*-GlcNAcylated on its C-terminal domain (CTD),[2] in a region that reciprocally becomes heavily phosphorylated during the elongation phase of transcription (14). Early studies also suggested that *O*-GlcNAc is involved in the regulation of protein translation (15). Analyses of *Drosophila* polytene chromosomes showed that *O*-GlcNAc is particularly abundant on chromatin, especially at sites of active gene transcription (16). Pulse–chase studies found that *O*-GlcNAc cycles rapidly on the HSP27 family of heat shock proteins (17). *O*-GlcNAc was also shown to be highly dynamic on lymphocyte proteins, cycling rapidly in response to activation (18).

Using a synthetic peptide as a substrate, an assay for the *O*-GlcNAc transferase (OGT) was developed, and properties of the enzyme from rabbit reticulocytes were defined (19). Using the peptide substrate assay, combined with conventional and affinity chromatography, OGT was purified over 30,000-fold from rat liver and found to be a large multimeric enzyme with high affinity for its donor substrate, UDP-GlcNAc (20). An assay for *O*-GlcNAcase (OGA; the enzyme that removes *O*-GlcNAc) was also developed, and *O*-GlcNAcase was purified 22,000-fold from rat spleen (21) and shown to have enzymatic properties similar to those of previously described crude preparations of hexosaminidase C (22, 23). Subsequently, the OGT was cloned and sequenced from both rats and humans (24, 25) and was shown to be a unique enzyme with multiple tetratricopeptide repeats (protein-docking domains). OGT was shown to be an X-linked gene located near the centromere, and its

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[2] The abbreviations used are: CTD, C-terminal domain; OGT, *O*-GlcNAc transferase; mOGT, mitochondrial isoform of OGT; OGA, *O*-GlcNAcase; eNOS, endothelial nitric-oxide synthase; CaMKII and CaMKIV, calcium/calmodulin kinase II and IV, respectively; TAK1, transforming growth factor-β–activated kinase; CDK, cyclin-dependent kinase; HBP, hexosamine biosynthetic pathway; AD, Alzheimer's disease; PFK1, phosphofructokinase 1; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor; PDAC, pancreatic ductal adenocarcinoma.

sequence is very highly conserved from *C. elegans* to humans (26). Knockout of OGT showed that it is essential to the viability of murine embryonic stem cells and is required for embryonic development (27). Bovine brain OGA was purified, partially sequenced, and used to clone and sequence human OGA (28). OGA was shown to be identical to a previously cloned gene (*MGEA5*) associated with meningiomas and postulated to be a hyaluronidase (29). The *O*-GlcNAcase gene is also unique. It is located on human chromosome 10, is essential in mammals and plants, and is also very highly conserved from *C. elegans* to humans. Recent structural analyses of OGT have not only led to a much better understanding of its enzymatic mechanism, but also, these studies have helped us to understand how a single catalytic subunit can specifically target thousands of different substrates. These investigations have also led to the development of useful inhibitors (30–39) (for a recent review, see Ref. 40). Surprisingly, OGT was also recently shown to use a novel mechanism, involving UDP-GlcNAc at its active site, to proteolytically cleave the important transcription factor, host cell factor 1 (41, 42), into its active forms.

Whereas less is known about how OGA is targeted to it substrates, several recent studies have defined its detailed structure. These studies have also elucidated the molecular mechanisms of the enzyme, and they have led to the development of highly specific and potent OGA inhibitors that work in living cells (43–53).

Perhaps the greatest impediment to understanding the functions of *O*-GlcNAcylation is the enormous difficulty in detecting and mapping the sites of *O*-GlcNAc on proteins (54). Despite its abundance within the nucleus and cytoplasm, *O*-GlcNAc remained undetected until 1983 for many reasons. 1) Generally, the presence or absence of *O*-GlcNAc does *not* alter the electrophoretic migration of a polypeptide, even in two-dimensional electrophoresis; 2) *O*-GlcNAc is very labile at the source and in the gas phase in MS, making detection of *O*-GlcNAc peptides and site mapping very difficult (55, 56); 3) in mixtures, ion suppression of *O*-GlcNAc peptides by unmodified peptides in MS masks the presence of the *O*-GlcNAcylated species. Fortunately, pan-specific *O*-GlcNAc monoclonal antibodies have greatly improved methods for detection of *O*-GlcNAc (5, 57, 58), and specific enrichment methods have been developed to circumvent the ion suppression problem (55, 59). Perhaps the most important breakthrough in site mapping for *O*-GlcNAc on polypeptides has been the development of electron transfer dissociation fragmentation MS, which does not result in the cleavage of the very labile *O*-GlcNAc glycosidic linkage to serine or threonine (60, 61). Like other post-translational modifications, the functions of *O*-GlcNAc must be understood at the individual site level, making site mapping a key first step to elucidate its biological functions. Whereas inhibitors of OGT and OGA and genetic knockout experiments of OGA or OGT have allowed us to make great strides in understanding the functions of *O*-GlcNAc, the lack of methods to alter *O*-GlcNAcylation levels on a single protein or at a single site has greatly limited progress in this area. Site-directed mutagenesis of *O*-GlcNAc sites to alanine is useful, but effects are difficult to interpret if the same or proximal site is also subjected to phosphorylation or other modifications.

O-GlcNAc as a sensor of nutrients and stress

The concentration of UDP-GlcNAc (the donor substrate for OGT) in cells is highly responsive to nutrients and flux through the major metabolic pathways via their connectivity to the hexosamine biosynthetic pathway, including glucose metabolism, nitrogen metabolism, nucleotide metabolism, and fatty acid metabolism (Fig. 1) (62, 63). Both the activity of OGT and its substrate selectivity are highly responsive to UDP-GlcNAc concentrations across a large range (24, 64), indicating that *O*-GlcNAcylation at specific sites on polypeptides is highly responsive to the metabolic state of the cell. *O*-GlcNAcylation of nuclear pore proteins (65) and *O*-GlcNAcylation of many polypeptides within β-cells of the pancreas (66) are very responsive to extracellular glucose concentrations. Hyperglycemia qualitatively and quantitatively alters the *O*-GlcNAcylation or expression of many *O*-GlcNAc–modified proteins within the nucleus of rat aorta or smooth muscle cells (67). Hyperglycemia also inhibits vascular endothelial nitric-oxide synthase (eNOS) by *O*-GlcNAcylation, blocking its key regulatory AKT phosphorylation site (68). Elevated glucose and insulin both stimulate increased *O*-GlcNAcylation in L6 myotubes (a model of skeletal muscle) (69). Hyperglycemia-induced *O*-GlcNAcylation mediates plasminogen activator inhibitor-1 gene expression and Sp1 transcriptional activity in glomerular mesangial cells (70).

O-GlcNAcylation of a large subset of proteins increases rapidly in response to almost any type of cellular stress, and this increased *O*-GlcNAcylation protects cells from cellular damage (71), including regulating DNA damage/repair (72). Paradoxically, several laboratories have shown that whereas short-term elevation of *O*-GlcNAcylation is cardioprotective (73–80), prolonged elevation of *O*-GlcNAcylation, as occurs in diabetes, contributes to cardiomyopathy and heart failure (81–86) (for a review, see Ref. 87).

Nutrient regulation of gene expression

Cells must closely regulate gene expression in response to their metabolic state and the availability of building blocks and fuels. It is now clear that *O*-GlcNAcylation plays key roles in nutrient regulation of transcription, yet we know very little about the molecular mechanisms involved. Recent studies indicate that *O*-GlcNAcylation affects nearly every step of transcription (Fig. 2) (for reviews, see Refs. 88–96). Nearly all RNA polymerase II transcription factors are *O*-GlcNAcylated, often at multiple sites, and the functions of the modification depend not only on the specific transcription factor but also on the specific sites to which the sugar is attached (13, 96–143). OGT is an essential polycomb gene, which regulates expression of homeotic genes that control major morphogenetic events in development (144–147). Assembly of the preinitiation complex in the transcription cycle requires *O*-GlcNAcylation of the C-terminal domain of RNA polymerase II (95, 148, 149). OGA is a transcription elongation factor, which is required to remove *O*-GlcNAc from RNA polymerase II prior to the phosphorylation of the CTD (150). Phosphorylation of the CTD is required and precedes elongation (151). *O*-GlcNAc is part of the histone code (152–159). Whereas several *O*-GlcNAc sites on histones are in the tail regions, along with other epigenetic marks, some

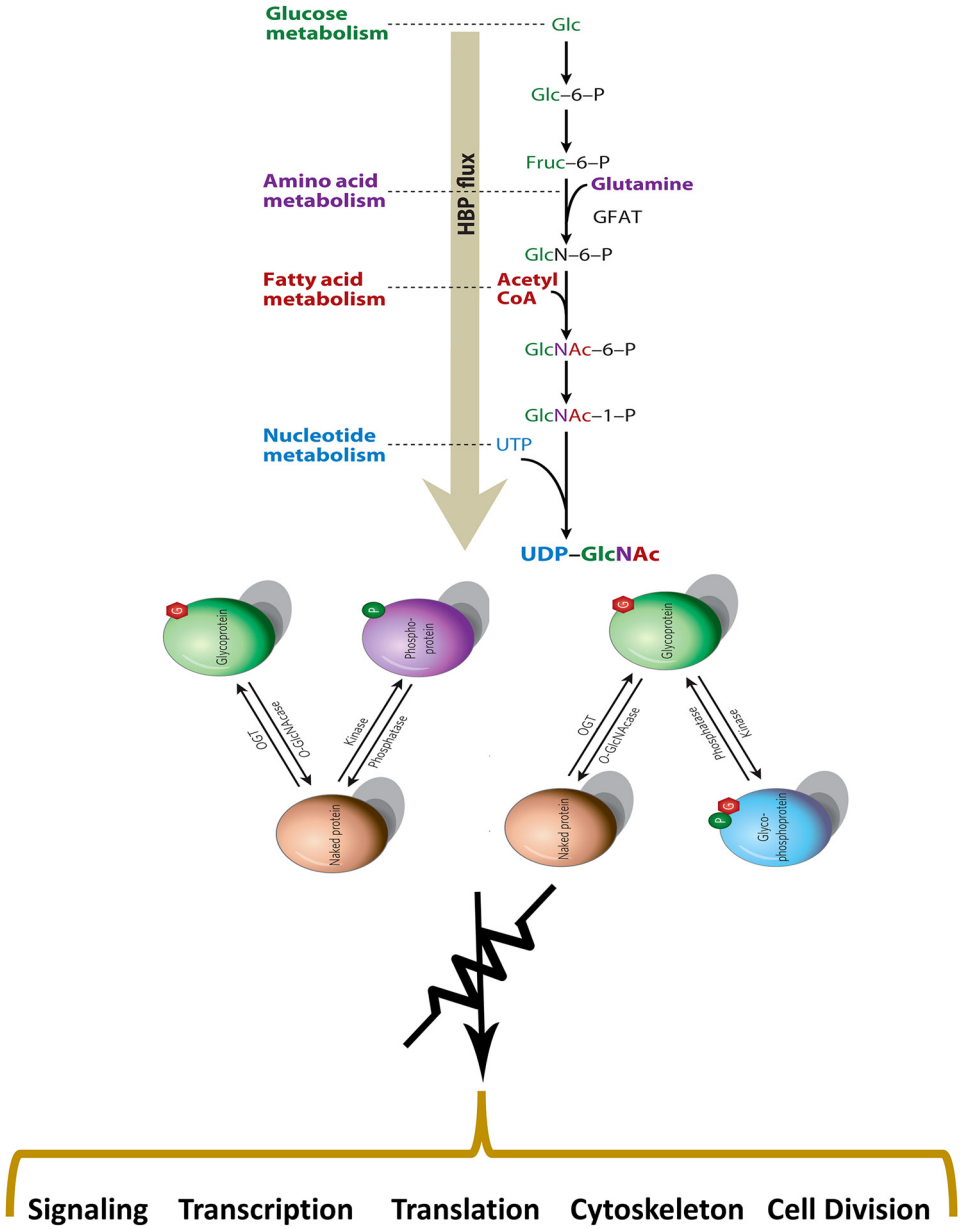

Figure 1. The HBP links flux through major metabolic pathways, allowing *O*-GlcNAcylation to serve as a "rheostat" that modulates most cellular processes in response to nutrients. The biosynthesis of UDP-GlcNAc, the donor for the OGT, is directly coupled to flux through glucose, amino acid, fatty acid, and nucleotide metabolic pathways. OGT is highly sensitive to UDP-GlcNAc concentrations, both in terms of activity and selectivity. *O*-GlcNAcylation has extensive cross-talk with phosphorylation. Shown is the universal symbol for a rheostat, indicating that unlike phosphorylation, which is more analogous to a switch, *O*-GlcNAc serves in a more analog fashion as a rheostat to modulate processes in response to nutrients and stress. *GFAT*, glutamine:fructose-6-phosphate amidotransferase. Modified from Refs. 63 and 341. This research was originally published in Annual Review of Biochemistry. Hart, G. W., Slawson, C., Ramirez-Correa, G., and Lagerlof, O. Cross talk between *O*-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu. Rev. Biochem.* 2011; 80:825–858 © Annual Reviews and Nature. Hart, G. W., Housley, M. P., and Slawson, C. Cycling of *O*-linked *β*-*N*-acetylglucosamine on nucleocytoplasmic proteins. *Nature* 2007; 446:1017–1022 © Springer Nature.

O-GlcNAc moieties are located at the histone:DNA interface. *O*-GlcNAc regulates both ubiquitination and methylation of histones (159–161). OGT and *O*-GlcNAc also regulate DNA methylation via their interactions and via regulation and modification of the TET proteins. Very recent studies have shown that the TATA-binding protein's (TBP's) cycling on and off of DNA is regulated by its *O*-GlcNAcylation.[3] When bound to DNA, TBP is *O*-GlcNAcylated, and this modification reduces its interaction with the TFIID complex, specifically preventing binding to the BTAF1 subunit. Reducing the BTAF1:TBP interaction increases TBP's residence time at promoters, increasing overall promoter occupancy on several promoters and resulting in major changes in the expression of many metabolic enzymes.

Despite many descriptive studies of the roles of *O*-GlcNAc in transcription, there remain many questions about the functions of OGT, OGA, and *O*-GlcNAc in transcription. For example, 1) there are limited data suggesting that *O*-GlcNAcylation of transcription factors may affect their interactions with other components of the transcription machinery and alter their pro-

[3] S. Hardivillé, P. S. Banerjee, E. S. Selen Alpergin, G. Han, J. Ma, C. C. Talbot, Jr., P. Hu, M. J. Wolfgang, and G. W. Hart, submitted for publication.

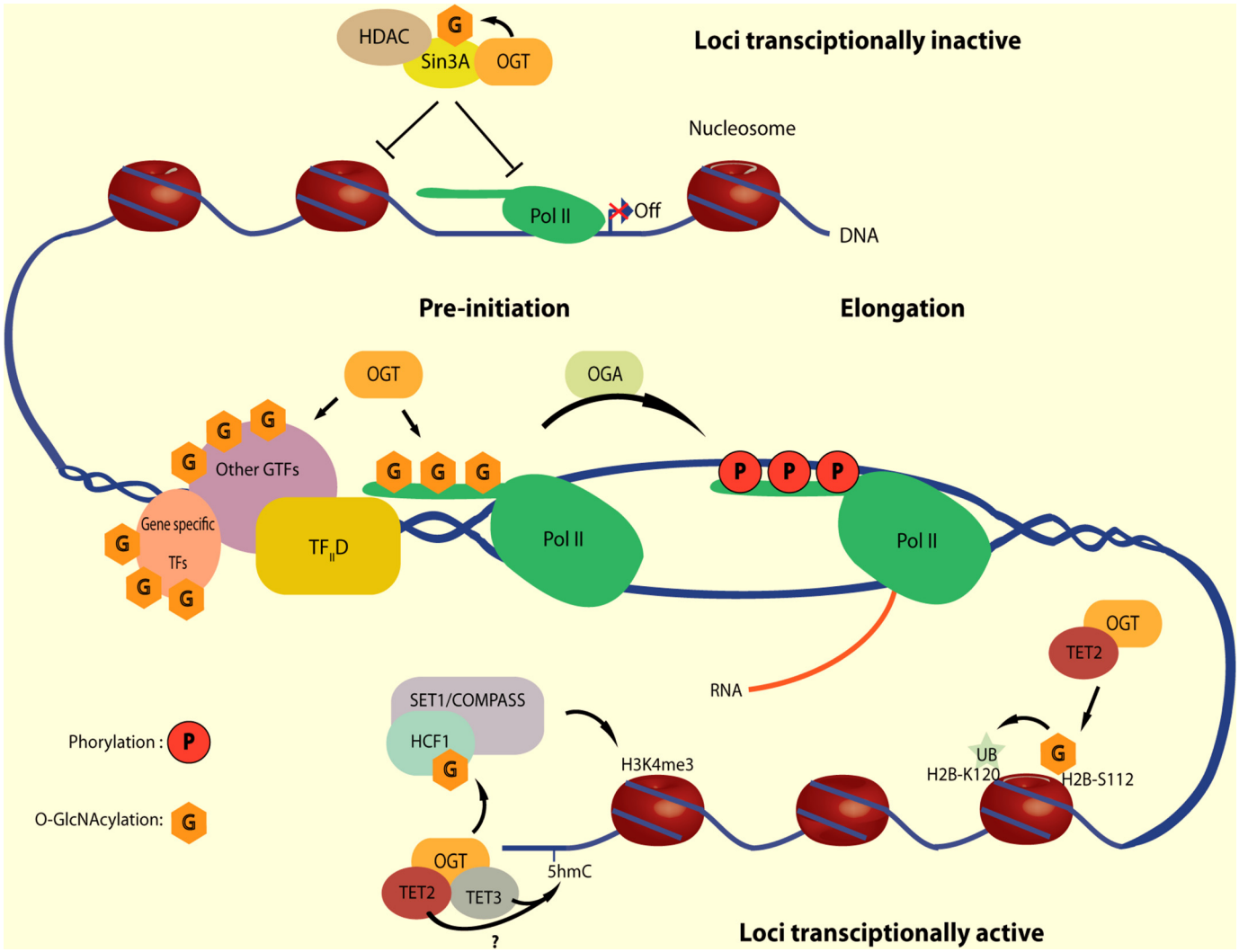

Figure 2. *O*-GlcNAcylation serves as a nutrient sensor to modulate nearly every step in transcription. Nearly every transcription factor is *O*-GlcNAcylated, often at multiple sites. OGT is a polycomb gene. Assembly of the preinitiation complex requires *O*-GlcNAcylation of RNA polymerase II. Elongation of mRNA requires removal of *O*-GlcNAc from RNA polymerase II. *O*-GlcNAc is part of the histone code. *O*-GlcNAc regulates ubiquitinylation and methylation of histones. *O*-GlcNAc regulates DNA methylation by the TET proteins. TATA-binding protein's residence time at promoters is regulated by *O*-GlcNAcylation. *HDAC*, histone deacetylase; *Pol*, polymerase; *TF*, transcription factor; *H3K4me3*, histone H3 Lys-4 trimethylation. Modified from Ref. 94. This research was originally published in Cell Metabolism. Hardivillé, S., and Hart, G. W. Nutrient regulation of signaling, transcription, and cell physiology by *O*-GlcNAcylation. *Cell Metab.* 2014; 20:208–213. © Cell Press.

moter specificity, but detailed studies of this possibility are lacking. This topic is of particular importance to molecular mechanisms underlying glucose toxicity, where abnormal gene expression occurs in many tissues exposed to prolonged hyperglycemia. 2) How *O*-GlcNAcylation regulates the basal machinery and the transcription cycle is not understood. 3) Are so-called "housekeeping transcription factors" *O*-GlcNAcylated differently in different cell types or in different metabolic states of the same cell type? These are but a few of the questions that need study. Clearly, how nutrients regulate transcription will be a fertile area for future research that will impact our understanding of disease etiologies.

We know even less about how dynamic *O*-GlcNAcylation regulates protein translation. However, data are emerging from several studies suggesting that nutrients also regulate protein synthesis via *O*-GlcNAcylation. Early studies suggested that *O*-GlcNAcylation of p67 protein plays a required role in p67's regulation of phosphorylation of the eIF-2 α subunit (15). Reticulocyte extracts, which are often used for the study of *in vitro* protein translation, are very efficient at *O*-GlcNAcylation of nascent polypeptides (162). It was proposed that OGT and *O*-GlcNAcylation protect proteins from aggregation during heat stress (163, 164). An unbiased RNA-mediated interference-based screen showed extensive *O*-GlcNAcylation of stress granules, which are ribonucleoprotein granules that regulate translation and mRNA decay, during cellular stress. *O*-GlcNAcylation of the translational machinery is required for aggregation of untranslated messenger ribonucleoproteins in the formation of stress granules (165). Gycomic analyses identified many *O*-GlcNAcylated translation factors and ribosome proteins (166). Over 20 core ribosome proteins are *O*-GlcNAcylated, and both OGT and OGA are tightly bound to purified ribosomal preparations. Even though most OGT is nuclear in dividing cells, the transferase is completely excluded from the nucleolus, the site of ribosome biogenesis (166). A mild overexpression of OGT causes some of the enzyme to

"leak" into the nucleolus, resulting in profound disruption of nucleolar structure and an accumulation of 60S subunits and 80S monosomes. Upon inhibition of the proteasome, both OGT and OGA become very tightly bound to ribosomes. *O*-GlcNAcylation of many ribosome-associated proteins dramatically increases, and protein synthesis stops for a period of time. The significance of these observations needs further investigation. Recently, *O*-GlcNAcylation was found to be more extensive on nascent polypeptide chains, presumably protecting them from premature degradation by blocking co-translational ubiquitination, suggesting that *O*-GlcNAc might play a role in the cytosolic compartment that is similar to the role of *N*-glycans in the calnexin/calreticulin system for proteins in the secretory pathway (167).

Nutrient regulation of signaling

It is now clear that *O*-GlcNAcylation's interplay with phosphorylation plays a key role in modulating signaling pathways in response to nutrients and stress (168). One of the earliest studies suggesting interplay between protein phosphorylation and *O*-GlcNAcylation showed that the IIa form of RNA polymerase II is abundantly *O*-GlcNAcylated in its CTD, yet the heavily phosphorylated IIo form of RNA polymerase II CTD is completely lacking in *O*-GlcNAc moieties (14). *In vitro* assays using synthetic CTD repeats showed that *O*-GlcNAcylation and phosphorylation are mutually exclusive, with the presence of a single *O*-GlcNAc completely blocking the activity of CTD kinases and the presence of a single phosphate moiety completely blocking OGT's activity on CTD (169). *O*-GlcNAcylation at Ser-1177 of eNOS blocks its phosphorylation at this site, thus preventing its activation by AKT kinase (68). Short-term treatment of cells with the broad-spectrum phosphatase inhibitor, okadaic acid, or with phorbol esters, which activate protein kinase C, or treatment with adenosine monophosphate, which activates protein kinase A, all lead to global decreased *O*-GlcNAcylation (170). In contrast, treatment of cells with the nonspecific kinase inhibitor, staurosporine, increases global *O*-GlcNAcylation, supporting a "yin-yang" relationship between the two modifications on many proteins. One complexity in these types of studies is that if such treatments are performed at a high dose or for too long, they induce a stress response, which by itself elevates global *O*-GlcNAcylation.

Activation of protein kinase A or C in cerebellar neurons of post-natal mice results in reduced levels of *O*-GlcNAc, specifically in cytoskeletal and cytoskeleton-associated proteins, whereas inhibition of the same kinases results in increased levels of *O*-GlcNAc (171). Likewise, treatment of neuronal cells with the broad-spectrum phosphatase inhibitor, okadaic acid, which induces protein hyperphosphorylation, decreases the levels of *O*-GlcNAc in both nuclear and cytoplasmic proteins, but with a greater effect in the nuclear fraction (172). Other studies suggest that *O*-GlcNAc limits nucleoporin hyperphosphorylation during M-phase and hastens the resumption of regulated nuclear transport at the completion of cell division (173). Whereas there are numerous examples of phosphate and *O*-GlcNAc competing for the *same* hydroxyl moiety on a polypeptide (11, 12, 174–176), competition also occurs when they are located proximal to each other (100, 106, 177–185). The Stokes radius of an *O*-GlcNAc moiety is about 5 times that of a phosphate residue.

OGT occurs in a functional complex with protein phosphatases, suggesting that in some instances, the same protein complex both dephosphorylates and concomitantly *O*-GlcNAcylates polypeptides (186). Glycomic/proteomic analyses have shown that in terms of cross-talk between phosphorylation and *O*-GlcNAcylation at the site level, all possibilities exist (187, 188). In proteomic analyses of murine synaptosomes, 7% of mapped *O*-GlcNAc sites were modified reciprocally by phosphate at the same hydroxyl moiety (188). Just these two common post-translational modifications, which often occur at multiple sites on a polypeptide, greatly increase the molecular diversity of proteins. Even though both phosphorylation and *O*-GlcNAcylation are sub-stoichiometric at any single site on a polypeptide, it is likely that their competition does affect each other's cycling rates, which seems to be the most biologically relevant parameter.

Another aspect of cross-talk between protein phosphorylation and *O*-GlcNAcylation is the regulation of each other's cycling enzymes by the other modification. OGT and OGA are both regulated by phosphorylation. Calcium/calmodulin kinase IV (CaMKIV) activates OGT, and phosphorylation of OGT has an essential role in CaMKIV-dependent AP-1 activation upon depolarization of neuronal cells (189). As part of a feedback loop, OGT in turn *O*-GlcNAcylates CAMKIV in its ATP-binding pocket, inactivating the kinase (190). Insulin stimulates tyrosine phosphorylation of OGT by the insulin receptor and activates OGT's activity on many specific substrates (191). Other kinases also modify OGT and activate its catalytic activity, including Src, GSK3β, and CaMKII. OGA is phosphorylated on at least 10 different sites (Phosphosite Plus), but the functions of these modifications are unexplored.

Early studies identified at least 42 kinases that are substrates for OGT (192). Recent screens have shown that about 80% of all human kinases are substrates for OGT. To date, more than 100 kinases have been confirmed to be *O*-GlcNAcylated in living cells. Proteomic analyses found over 46 kinases modified by *O*-GlcNAc in murine synaptosomes alone (188).

Most importantly, every *O*-GlcNAcylated kinase studied to date, is regulated in some manner by the cycling sugar. *O*-GlcNAc at the ATP-binding pocket of CaMKIV completely inhibits the enzyme (190). The phosphorylated and *O*-GlcNAcylated forms of casein kinase II have different substrate selectivity (193). *O*-GlcNAc regulates the activity of the energy-sensing kinase, AMP-activated protein kinase, in skeletal muscle (194). Several members of the protein kinase C family are negatively regulated by *O*-GlcNAcylation (195). *O*-GlcNAcylation of phosphofructokinase II inhibits this key enzyme and increases flux into the pentose phosphate pathway, contributing to the "Warburg effect" in cancer cells (196). AKT (protein kinase B) is regulated by *O*-GlcNAcylation in hepatocytes (197). Activation of neurons increases *O*-GlcNAcylation of cyclin-dependent kinase 5, blocking its binding to p53 to suppress apoptosis (198). *O*-GlcNAcylation of p27 blocks cyclin/CDK–p27 binding, contributing to regulation of the cell cycle (199). *O*-GlcNAcylation of transforming growth factor-β–

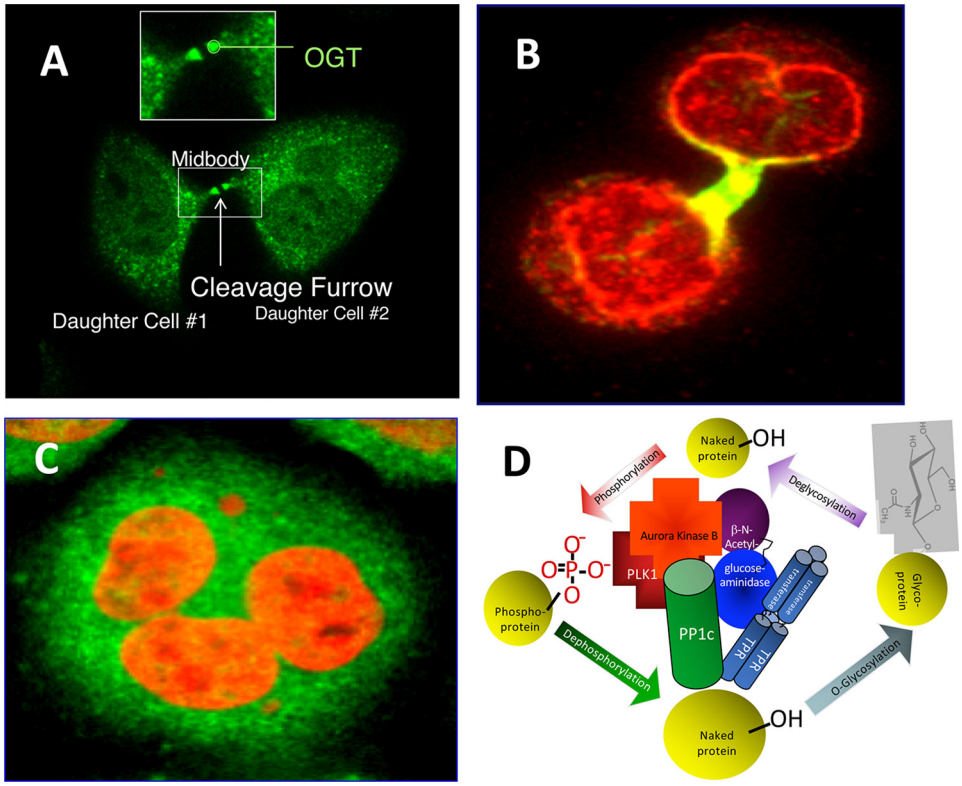

Figure 3. Nutrients regulate cytokinesis and the cell cycle by *O*-GlcNAcylation. *A*, OGT is highly concentrated at the mid-body during the late stages of cytokinesis. *B*, *O*-GlcNAcylated proteins are enriched at the midbody and at the nascent nuclear envelope during the late stages of cytokinesis. *C*, overexpression of OGT causes defective cytokinesis, resulting in polyploidy. *D*, during the late stages of cytokinesis, OGT, OGA, protein phosphatase I (*PP1c*), Polo-like kinase (*PLK1*), and Aurora kinase B (among other proteins) are in a *transient* molecular complex that modifies proteins involved in cell division.

activated kinase (TAK1) regulates pro-inflammatory activation and M1 polarization of macrophages (200). *O*-GlcNAcylation of PKAcα and PKAcβ activates the enzymes in the brain and regulates their subcellular localizations (201). Loss of *O*-GlcNAc on these PKAs leads to impaired learning and memory associated with Alzheimer's disease. Elevated *O*-GlcNAcylation, as occurs in diabetes, causes CaMKII in the heart to become constitutively active and directly contributes to diabetes-associated cardiomyopathy and arrhythmias (202). Whereas only a handful of the over 400 *O*-GlcNAcylated kinases have been studied for the effects of *O*-GlcNAcylation on their functions or on their enzymatic activities, it is already evident that nutrients regulate kinase signaling in large part by modulating their *O*-GlcNAcylation, which affects kinase functions in many different ways.

Genetic studies also showed that *O*-GlcNAcylation regulates signaling in plants, especially the Gibbererellin growth hormone signaling pathway (203, 204). In *Arabidopsis*, *O*-GlcNAcylation of DELLA transcription factors, which are master growth repressors in plants, regulates and coordinates multiple signaling pathways during development (205). Plant OGT (Secret Agent) regulates flowering by activating histone methylation in *Arabidopsis* (206).

Nutrient regulation of cytokinesis and the cytoskeleton

Early studies showed that human Band 4.1, a protein that serves as a bridge joining the cytoskeleton to the inner surface of the plasma membrane in erythrocytes, is modified by *O*-GlcNAc (3). Cytokeratins 8 and 18 are *O*-GlcNAcylated at multiple sites, and pulse–chase analyses showed that the sugar is dynamically cycling on these intermediate filaments (207). Synapsin I, which anchors synaptic vesicles to the cytoskeleton at nerve terminals via a phosphorylation-regulated process, has at least seven *O*-GlcNAcylation sites clustered around its five phosphorylation sites. However, further analyses suggest that *O*-GlcNAc's roles in synapsin I's functions are more direct than simply controlling phosphorylation (178).

Several studies have shown that *O*-GlcNAcylation is involved in regulation of the cell cycle and cytokinesis (Fig. 3). Increased *O*-GlcNAcylation (induced pharmacologically or genetically) results in delayed G_2/M progression, altered mitotic phosphorylation, and altered cyclin expression. Decreasing *O*-GlcNAcylation by overexpression of OGA induces a mitotic exit phenotype accompanied by a delay in mitotic phosphorylation, altered cyclin expression, and pronounced disruption in nuclear organization (208). Overexpression of OGT results in a polyploid phenotype with faulty cytokinesis, as is often seen in cancer cells (Fig. 3). Strikingly, at M-phase, OGT is highly concentrated at the mitotic spindle and mid-body, and a significant portion of OGT is transiently in a large molecular complex with cell cycle-regulated kinases, phosphatases, and OGA. Glycomic analyses identified 141 previously unknown *O*-GlcNAc sites on proteins that function in spindle assembly and cytokinesis. Many of these *O*-GlcNAcylation sites are either identical to known phosphorylation sites or are in close proximity to them. Increased *O*-GlcNAcylation also altered the phosphorylation of key proteins associated with the mitotic spindle and midbody. Overexpression

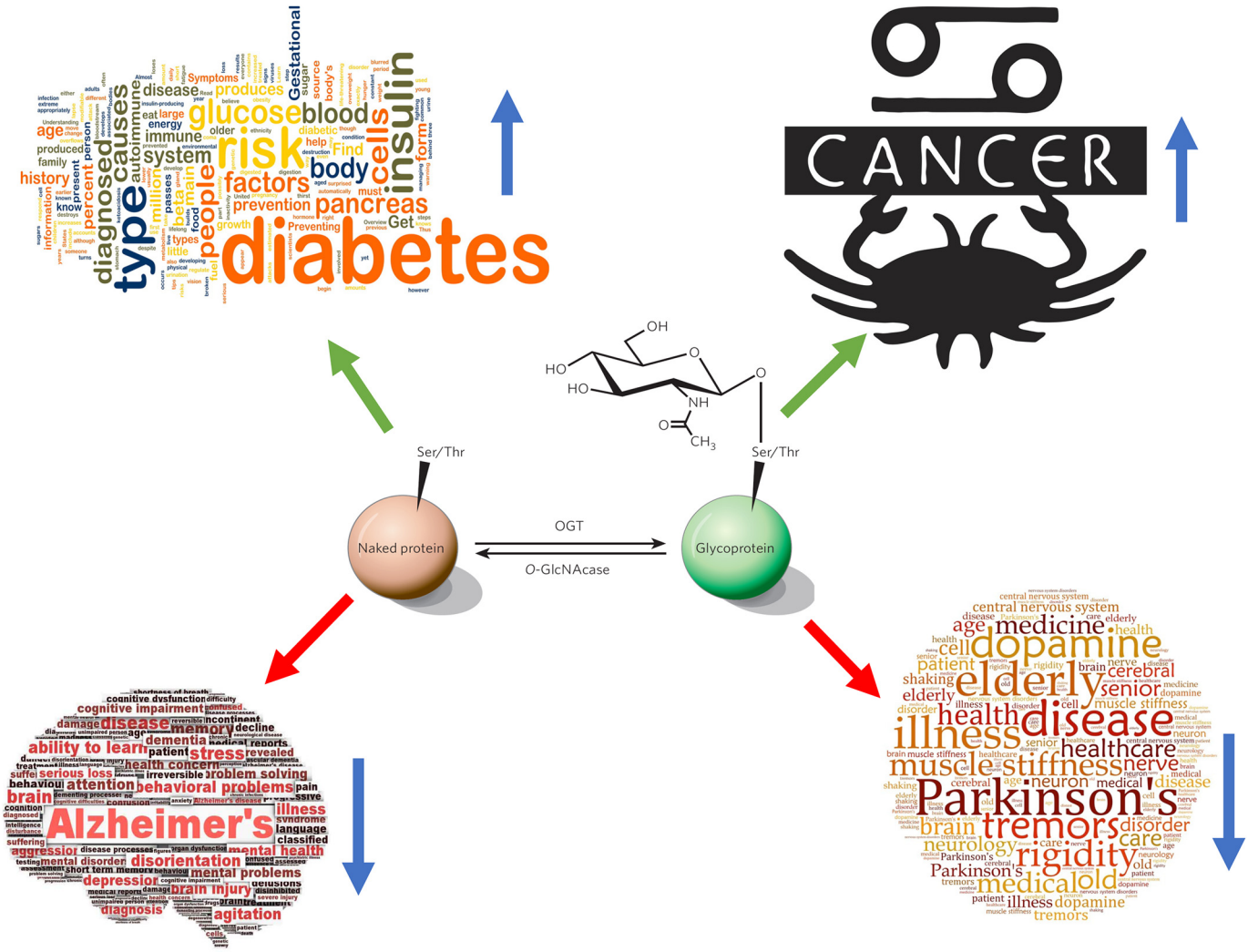

Figure 4. *O*-GlcNAcylation is directly involved in etiologies of chronic diseases associated with aging. Prolonged elevation of *O*-GlcNAcylation contributes directly to glucose toxicity, insulin resistance, and β-cell dysfunctions in diabetes. Every cancer type studied to date has elevated *O*-GlcNAc cycling, and blocking *O*-GlcNAcylation prevents cancer progression. Decreased *O*-GlcNAcylation in the brain is associated with both Alzheimer's disease and Parkinson's disease.

of OGT increased the inhibitory phosphorylation of cyclin-dependent kinase 1 (CDK1) and reduced the phosphorylation of CDK1 target proteins. Increased phosphorylation of CDK1 resulted from increased activation of its upstream kinase, MYT1, and from a concomitant reduction in transcription of CDK1 phosphatase, CDC25C. OGT overexpression also caused a reduction in both mRNA expression and protein abundance of Polo-like kinase 1, which is upstream of both MYT1 and CDC25C. Pathway analyses of these data uncovered a cascade series of events that illustrate how nutrients regulate cell division via the complex interplay between *O*-GlcNAcylation and phosphorylation (Fig. 3) (209). Collectively, these studies indicate that *O*-GlcNAc cycling is a pivotal regulatory component of nutrient regulation of the cell cycle, controlling cell cycle progression by regulating mitotic phosphorylation, cyclin expression, and cell division (208).

Abnormal *O*-GlcNAcylation underlies the etiologies of chronic diseases associated with aging (Fig. 4)

Fundamental roles in diabetes

Data from several laboratories indicate that abnormal *O*-GlcNAcylation, such as occurs in hyperglycemia associated with diabetes, underlies molecular mechanisms of glucose toxicity, insulin resistance, mitochondrial dysfunction, and abnormal insulin synthesis and secretion by β-cells (for reviews, see Refs. 63, 94, and 210–217). Since the 1950s, there have been over 1400 papers linking the hexosamine biosynthetic pathway to the etiology of diabetes. Marshall *et al.* (218) showed that conversion of glucose to glucosamine by the hexosamine biosynthetic pathway (Fig. 1) is required for the desensitization of the insulin-responsive glucose transport system in adipocytes. Pre-exposure of isolated rat skeletal muscle to glucosamine induces insulin resistance of both glucose transport and glycogen synthesis (219). Increasing flux through the hexosamine biosynthetic pathway (HBP) in otherwise normal rats mimics the hallmarks of glucose toxicity, such as the inhibition of glucose-induced insulin secretion and reduced insulin stimulation of both glycolysis and glycogen synthesis (220). In a streptozotocin rat model of type I diabetes, prolonged hyperglycemia increased the flux through the hexosamine biosynthetic pathway, as determined by the UDP-hex/UDP-HexNAc ratio, by over 40% in skeletal muscle (221). Overexpression of glutamine: fructose-6-phosphate amidotransferase, the first and rate-lim-

iting enzyme of the HBP, in skeletal muscle and adipose tissue of mice mimics the adverse regulatory and metabolic effects of hyperglycemia, specifically with respect to insulin resistance of glucose disposal (222). Even modest transgenic overexpression of OGT in muscle and fat of mice leads to insulin resistance and hyperleptinemia (223).

OGT has a phosphoinositide-binding domain. Upon insulin stimulation, phosphatidylinositol 3,4,5-trisphosphate recruits OGT from the nucleus to the plasma membrane, where OGT catalyzes increased *O*-GlcNAcylation of the insulin signaling pathway. This increased *O*-GlcNAcylation results in the altered phosphorylation on these signaling molecules and results in attenuated insulin signaling (224). In addition, hepatic overexpression of OGT reduces the expression of insulin-responsive genes and causes insulin resistance and dyslipidemia (224). Increased *O*-GlcNAcylation of glycogen synthase results in the retention of the enzyme in a glucose 6-phosphate– dependent state and contributes to reduced activation of the enzyme associated with insulin resistance (225).

Increased flux through the hexosamine biosynthetic pathway also appears to be involved in glucose toxicity and insulin resistance in humans with diabetes (226). Nucleotide polymorphisms in OGA are associated with diabetes in Mexican Americans (227). As might be expected from the role of the HBP as a central node of metabolism (Fig. 1), fat-induced insulin resistance is also associated with increased end products of the HBP, suggesting that elevated free fatty acids induce skeletal muscle insulin resistance by increasing the flux of fructose 6-phosphate into the hexosamine pathway (228). Palmitate also activates the HBP in human myotubes (229). Expression of the *ob* gene to make leptin, a potent adipokine released by adipocytes in response to increased energy storage, is controlled by end products of the HBP (88, 230, 231).

β-Cells of the pancreas have the highest relative amounts of OGT and *O*-GlcNAc of any tissue (232, 233). Prolonged elevation of *O*-GlcNAcylation contributes to β-cell death by apoptosis in diabetes (66). Elevated *O*-GlcNAc leads to deterioration of glucose-stimulated insulin secretion by the pancreas of diabetic Goto–Kakizaki rats (234). Key transcription factors that control expression of proinsulin are dynamically regulated by *O*-GlcNAcylation. Glucose regulates the nuclear transport of NeuroD1 via its *O*-GlcNAcylation. *O*-GlcNAc regulates the DNA binding by PDX-1 (102), and glucose controls the expression of the MAF-1 transcription factor via *O*-GlcNAcylation of unknown nuclear proteins (235). Collectively, these studies indicate a direct role for *O*-GlcNAcylation in regulating the production and secretion of insulin. It has been proposed that a chronic increase in *O*-GlcNAcylation may be a major factor leading to the deterioration of β-cell function (236).

Hyperglycemia qualitatively and quantitatively alters the *O*-GlcNAcylation or expression of many proteins within the nucleus. Abnormal *O*-GlcNAcylation of transcription factors, especially Sp1, appears to play a significant role in the abnormal expression of proteins observed in diabetes (90). Hyperglycemia increases *O*-GlcNAc on Sp1 and induces expression of plasminogen activator inhibitor-1 (237), which leads to the expression of genes that contribute to the pathogenesis of diabetic complications. In contrast, activation of PPARγ, a ligand-activated nuclear receptor that increases insulin sensitivity, reduces *O*-GlcNAcylation of Sp1 (238). Enhanced *O*-GlcNAcylation is associated with insulin resistance in GLUT1-overexpressing muscles (239). Hyperglycemia, via *O*-GlcNAcylation, impairs activation of the IR/IRS/PI3K/AKT pathway, resulting in deregulation of eNOS activity (240). Elevated *O*-GlcNAc also results in insulin resistance associated with defects in AKT activation in 3T3-L1 adipocytes (241).

Increased *O*-GlcNAcylation has also been implicated in diabetic complications of the eye. The elevated expression of *O*-GlcNAc–modified proteins and *O*-GlcNAc transferase plays a causative role in the corneal epithelial disorders of diabetic GK rats (242). There is growing evidence that increased *O*-GlcNAcylation contributes to the pathogenesis of diabetic retinopathy (243), including the early loss of retinal pericytes (244).

Abnormal *O*-GlcNAc modification of nucleocytoplasmic proteins appears to also be involved in glucose toxicity in vascular tissues (67, 245). *O*-GlcNAcylation of AKT kinase promotes vascular calcification (246). Prolonged elevation of *O*-GlcNAcylation impairs cardiac myocyte function and leads to the development of diabetic cardiomyopathy (247). *O*-GlcNAcylation of cardiac mitochondrial proteins appears to play a direct role in diabetic cardiomyopathy. Several proteins, which are components of the respiratory chain, including the subunit NDUFA9 of complex I, subunits core 1 and core 2 of complex III, and the mitochondrial DNA-encoded subunit I of complex IV (COX I), are *O*-GlcNAcylated. Hyperglycemia increases mitochondrial protein *O*-GlcNAcylation. Increased mitochondrial *O*-GlcNAcylation impairs activity of complex I, III, and IV in addition to lowering mitochondrial calcium and cellular ATP content. Mitochondrial function improves when *O*-GlcNAc is reduced by OGA expression, resulting in returning the activities of complex I, III, and IV, mitochondrial calcium, and cellular ATP content to control levels (248).

Glycomic analyses of purified rat heart mitochondria from normal and streptozocin-treated diabetic rats identified 88 *O*-GlcNAcylated proteins (84, 249, 250). OGT is strikingly increased in diabetic cardiac mitochondria, whereas mitochondrial OGA is concomitantly decreased. Most importantly, OGT is mislocalized in diabetic cardiac mitochondria. In normal cardiac mitochondria, OGT is mostly localized in complex IV of the respiratory chain. However, in diabetic cardiac mitochondria, much of the OGT is now found in the matrix and in complex III, resulting in altered *O*-GlcNAcylation on many proteins. Inhibition of OGT or OGA activity within neonatal rat cardiomyocytes significantly affects energy production, mitochondrial membrane potential, and mitochondrial oxygen consumption. Therefore, not only do cardiac mitochondria have robust *O*-GlcNAc cycling, but also dysregulation of *O*-GlcNAcylation likely plays a key role in mitochondrial dysfunction associated with diabetes (84).

Increased *O*-GlcNAcylation of an important kinase, CAMKII, that helps to regulate heart contractions contributes to arrhythmias associated with diabetes (202). Acute hyperglycemia increases *O*-GlcNAcylation of CaMKII at Ser-279, which activates CaMKII autonomously, even after Ca^{2+} concentration declines. *O*-GlcNAcylation of CaMKII is increased in the

heart and brain of diabetic humans and rats. In cardiomyocytes, increased glucose concentration significantly enhances CaMKII-dependent activation of molecular processes that can contribute to cardiac mechanical dysfunction and arrhythmias. These effects are prevented by pharmacological inhibition of *O*-GlcNAc signaling or by genetic ablation of CaMKIIδ. In intact perfused hearts, arrhythmias are exacerbated by increased glucose concentration through *O*-GlcNAc– and CaMKII–dependent pathways. In diabetic animals, acute blockade of *O*-GlcNAcylation inhibited arrhythmogenesis. It was concluded that *O*-GlcNAcylation of CaMKII contributes critically to cardiac and neuronal pathophysiology in diabetes and other diseases (202).

O-GlcNAcylation also appears to play a role in diabetic nephropathy. Diabetic patients have significantly increased numbers of *O*-GlcNAc–positive cells in their glomeruli and significantly elevated staining in the tubuli (both in the nucleus and in the cytosol), suggesting that increased *O*-GlcNAcylation might contribute to the development of diabetic nephropathy (251). Increased *O*-GlcNAcylation of cytoskeletal proteins is closely associated with the morphological changes in the podocyte foot processes in the glomerulus and in microvilli of proximal tubules in the diabetic kidney (252). Hyperglycemia-induced elevation of *O*-GlcNAcylation also contributes to the progression of diabetic nephropathy via inhibition of AKT/eNOS phosphorylation and HSP72 induction (253).

Likewise, *O*-GlcNAcylation contributes to diabetic neuropathy. Hyper-*O*-GlcNAcylation of a neuronal protein, Milton, appears to contribute to diabetic neuropathy of the foot (254). To meet the high energy demands at the synapse, mitochondria need to be trafficked from the cell body to the nerve terminal. Milton serves as a bridge protein between the mitochondria and the motor protein, which carries the mitochondria along microtubules. When Milton is hyper-*O*-GlcNAcylated, as occurs in diabetes, mitochondrial motility is arrested. Because the longest axon in the body is from the spinal cord to the foot, neuropathy often manifests itself in the feet. It was proposed that OGT normally modulates mitochondrial dynamics in neurons based on nutrient availability, but when the modification becomes excessive, it abnormally arrests mitochondrial transport (254).

The relationship between Alzheimer's disease (AD) and diabetes is still unclear. However, based upon many common underlying mechanisms, some researchers refer to AD as "diabetes type 3" (255–258). Recent studies have shown that hyperglycemia in the diabetic patient appears to induce greater expression and activity of the mitochondrial isoform of OGT (mOGT). Changes in mOGT modify the structure and functionality of mitochondria in hippocampal cells, accelerate neuronal damage, and favor the early events in AD. It was proposed that mOGT activity could be a key point for AD development in patients with diabetes (259). An understanding of *O*-GlcNAc's roles in both diabetes and AD will clearly require many more detailed investigations.

Because *O*-GlcNAcylation is highly sensitive to glucose, it was postulated that monitoring *O*-GlcNAcylation of human erythrocyte proteins might serve as a biomarker for prediabetes prior to detectable changes in HbA1c. Indeed, *O*-GlcNAcylation of certain sites on human erythrocyte proteins (*e.g.* catalase) increases significantly in prediabetic patients prior to elevated HbA1c, reflecting the glycemic status of the individual. If validated on a larger clinical trial, *O*-GlcNAc site occupancy on erythrocyte proteins may eventually be useful as a diagnostic tool for the early detection of diabetes (260, 261).

O-GlcNAc and cancer

There is a rapidly growing literature suggesting that *O*-GlcNAcylation contributes to the properties and progression of cancer cells (Fig. 4) (for a review, see Refs. 262–266). Whereas it has generally been observed that *O*-GlcNAc cycling is universally elevated in cancer cells and, indeed, preventing increased *O*-GlcNAcylation can block cancer progression, it is still early days, and mechanistic details are often lacking.

Aberrant expression and activities of *O*-GlcNAc cycling enzymes, especially OGT, have been reported in all human cancers studied to date (267). Altered cellular metabolism is a major hallmark of cancer. Glucose uptake and glycolysis are accelerated in cancer cells ("Warburg Effect"), which gives cancer cells an advantage for intensive growth and proliferation. *O*-GlcNAc–dependent regulation of signaling pathways, transcription factors, enzymes, and epigenetic changes are all likely involved in metabolic reprograming of cancer (265, 266). Several researchers have proposed that inhibition of hyper-*O*-GlcNAcylation could be a potential novel therapeutic target for cancer treatment (264, 265). It has also been proposed that aberrant *O*-GlcNAcylated proteins might be novel biomarkers of cancer (268).

Recent studies suggest that *O*-GlcNAcylation plays a direct role in the Warburg effect (196). In response to hypoxia, *O*-GlcNAcylation at serine 529 of phosphofructokinase 1 (PFK1) is increased, inhibiting the enzyme and redirecting glucose flux through the pentose phosphate pathway. This metabolic switch confers a selective growth advantage on cancer cells. Blocking *O*-GlcNAcylation of PFK1 at serine 529 reduces cancer cell proliferation *in vitro* and impairs tumor formation *in vivo*. Other metabolic processes involving *O*-GlcNAc are also involved in cancer cell reprogramming. For example, *O*-GlcNAcylation also regulates glycolysis in cancer cells via hypoxia-inducible factor 1 (HIF-1α) and its transcriptional target GLUT1. Reducing *O*-GlcNAcylation in cancer cells results in endoplasmic reticulum stress and cancer cell apoptosis. Human breast cancers with high levels of HIF-1α contain elevated OGT and lower OGA levels, which correlate with poor prognosis (269). DNA methylation plays a direct role in aberrant silencing of tumor suppressor genes in cancer. Because OGT and *O*-GlcNAcylation regulate the TET proteins that hydroxymethylate DNA, it has been suggested that the cross-talk between OGT and TET proteins might play an important role in cancer cells (270).

Many oncogene and tumor suppressor gene products, such as c-Myc, SV40 large T antigen, Rb, and p53, are *O*-GlcNAcylated (271). In nongrowing cells, Thr-58, the major hot spot mutation site in Burkitt's lymphoma, is *O*-GlcNAcylated; however, in growing cells, this same site is phosphorylated. Mutation of Thr-58 to a nonhydroxy amino acid converts the transcription factor, c-Myc, into a potent oncoprotein (11, 12, 271). More recent studies have identified c-MYC as a key target of OGT's effects in prostate cancer cells

(272). The tumor suppressor HIC1 (hypermethylated in cancer 1) is *O*-GlcNAcylated (273). *O*-GlcNAcylation regulates the nuclear localization and stabilization of β-catenin, a dual-function protein that is both a transcription factor and a regulator of E-cadherin trafficking, a protein that is key to the epithelial–mesenchymal transition in cancer (114).

Reduction of *O*-GlcNAcylation by RNAi knockdown of OGT in breast cancer cells inhibits tumor growth both *in vitro* and *in vivo* and concomitantly decreases cell cycle progression. Reducing *O*-GlcNAcylation in breast cancer cells decreases expression of the transcription factor, FoxM1, resulting in lower levels of multiple FoxM1-specific targets, including Skp2 and matrix metalloproteinase-2. Reducing *O*-GlcNAcylation decreased cancer cell invasion and growth (274). In human breast cancer, poorly differentiated tumors (grade II and III) have significantly higher OGT expression than grade I tumors. In contrast, OGA transcript levels are lower in grade II and III compared with grade I tumors. Lymph node metastasis is significantly associated with decreased OGA expression (275). In breast cancer cells, *O*-GlcNAcylation at Ser-108 of cofilin, a regulator of actin assembly, is required for its proper localization to invadopodia at the leading edge during cell invasion. Loss of *O*-GlcNAcylation of cofilin leads to destabilization of invadopodia and impairs cell invasion (276). Treatments that increase *O*-GlcNAcylation in MCF-7 breast cancer cells protect them from death induced by tamoxifen. In contrast, inhibition of OGT potentiates cell killing by tamoxifen (277).

O-GlcNAcylation is also universally elevated in prostate cancer. Reducing *O*-GlcNAcylation in prostate cancer cells decreases expression of matrix metalloproteinase (MMP)-2, MMP-9, and VEGF and inhibits invasion and angiogenesis. These effects are mediated by the degradation of the transcription factor, FoxM1, a known regulator of invasion and angiogenesis. Overexpression of a degradation-resistant FoxM1 mutant abrogated OGT RNAi-mediated effects on invasion, MMP levels, angiogenesis, and VEGF expression. In a mouse model of metastasis, reduction of OGT expression blocks bone metastasis (278). Strikingly, increasing *O*-GlcNAcylation by itself induces malignant transformation of nontumorigenic prostate cells. It was proposed that inhibiting the formation of the E-cadherin/catenin/cytoskeleton complex underlies *O*-GlcNAc–induced prostate cancer progression (279).

The roles of *O*-GlcNAcylation have now been examined in many different cancer types with similar findings. Elevation of OGA activity has been reported in thyroid cancers (280). Histochemical analyses showed that OGT and *O*-GlcNAcylation are significantly elevated in lung and colon cancer tissue, relative to adjacent normal tissue. *O*-GlcNAcylation also markedly enhanced the anchorage-independent growth of lung and colon cancer cells *in vitro* (281). Studies of colorectal cancer cells support the hypothesis that metabolic disorders underlying colorectal cancer occur by up-regulation of the hexosamine biosynthetic pathway that leads to abnormally high *O*-GlcNAcylation of β-catenin (282).

Colorectal cancer SW620 metastatic clones exhibit increased *O*-GlcNAcylation and decreased OGA expression compared with primary clone cells, SW480. Increasing global *O*-GlcNAcylation by RNAi knockdown of OGA results in phenotypic alterations that include acquisition of a fibroblast-like morphology, concomitant with epithelial metastatic progression and growth retardation. OGA silencing altered the expression of about 1300 genes, mostly involved in cell movement and growth, and specifically affected metabolic pathways of lipids and carbohydrates, suggesting that *O*-GlcNAcylation serves as a link between metabolic changes and cancer (283). *O*-GlcNAcylation is increased in primary colorectal cancer tissues on proteins including, cytokeratin 18, heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1), hnRNP H, annexin A2, annexin A7, laminin-binding protein, α-tubulin, and protein DJ-1. It was proposed that aberrantly *O*-GlcNAc–modified proteins may provide novel biomarkers of cancer (284). It also has been proposed that the urinary content of OGA and OGT may be useful for bladder cancer diagnostics (285). Overexpression of OGT increases the aggressiveness of mass-forming cholangiocarcinomas (286).

O-GlcNAcylation, OGA, and OGT levels were examined in hepatocellular carcinoma (HCC) tissues of patients who underwent liver transplantation and compared with healthy liver tissues. Global *O*-GlcNAcylation levels were significantly elevated in HCC tissues more than that in healthy liver tissues. Importantly, low expression of OGA was an independent prognostic factor for predicting tumor recurrence of HCC following liver transplantation. *In vitro* assays demonstrated that *O*-GlcNAcylation plays important roles in migration, invasion, and viability of HCC cells, partly through regulating E-cadherin, MMP1, MMP2, and MMP3 expression (287).

Pancreatic cancer cells evade cell death, in part by up-regulating HSP70. A prodrug, Minnelide, down-regulates HSP70 to enhance killing of pancreatic cancer cells. Minnelide causes decreased *O*-GlcNAcylation of the transcription factor Sp1, preventing its nuclear localization and affecting its DNA binding. This in turn down-regulates prosurvival pathways in pancreatic cancer cells, allowing the drug to facilitate their killing (288). Hyper-*O*-GlcNAcylation in human pancreatic ductal adenocarcinoma (PDAC) results from elevation of OGT and reduction of OGA. Reducing hyper-*O*-GlcNAcylation has no effect on nontransformed pancreatic epithelial cell growth but inhibits PDAC cell proliferation, anchorage-independent growth, orthotopic tumor growth, and triggers apoptosis. Many of these effects appear to be mediated by *O*-GlcNAcylation of NF-κB (289).

Roles of O-GlcNAc in neurons and neurodegeneration

Except for the endocrine cells of the pancreas, *O*-GlcNAcylation, OGT, and OGA levels are the highest in the brain and in neurons. Functions ascribed to *O*-GlcNAcylation in the nervous system include modulation of circadian clocks (185, 290–293), synaptic functions and maturation (294–296), neuronal development (297–299), neuroprotection (300–302), learning and memory (303–307), neuronal apoptosis (245, 308–310), and appetite regulation (311). Mutations in OGT cause X-linked intellectual disability (312, 313). Given *O*-GlcNAc's abundance, cross-talk with phosphorylation, and involvement in many neuronal functions, it is not surprising that many researchers have reported a direct role for the cycling sugar in neurodegenerative diseases associated with aging (214, 314–317).

Although mostly indirect, the evidence that *O*-GlcNAcylation plays a major role in neurodegeneration is compelling: virtually every protein involved in AD or other forms of neurodegeneration is *O*-GlcNAcylated and phosphorylated, often reciprocally (168, 318). Glucose metabolism is impaired in AD neurons (319–321), which globally lowers *O*-GlcNAcylation. The Tau protein is normally *O*-GlcNAcylated, but *O*-GlcNAc is reduced and phosphorylation of Tau increases dramatically in AD (176). Amyloid precursor protein is *O*-GlcNAcylated, and the sugar regulates its trafficking and processing (322, 323). In addition, one of the subunits of the γ-secretase (nicastrin), which cleaves amyloid precursor protein to generate the toxic Aβ peptides, normally is *O*-GlcNAcylated. Many studies have documented the reciprocity between *O*-GlcNAcylation and phosphorylation of the Tau protein (179, 180, 324–327). Autopsies have shown reduced *O*-GlcNAcylation in patients with AD (180). The OGA gene maps to 10q24.1 coincident with the late AD locus in humans (328), and OGT maps to X13 coincident with the Parkinson dystonia locus (329). Overexpression of OGT in neurons increases *O*-GlcNAcylation at sites on Tau important to AD and concomitantly decreases protein phosphorylation at these same sites (180, 330). Cre-Lox brain targeted deletion of OGT causes hyperphosphorylation of Tau prior to neuronal death (27). Synaptic loss occurs in AD, and myriad synaptic proteins are modified by *O*-GlcNAc (188). Highly specific OGA inhibitors prevent hyperphosphorylation of Tau, decrease the production of toxic Aβ peptides, and improve memory in mice models of AD (48, 306, 331–333). It appears that one function of *O*-GlcNAcylation is to "cap" phosphorylation sites used at certain stages of development that are no longer required in the adult. We hypothesized (176) that as the brain ages, or due to vascular insufficiency, glucose utilization by certain neurons drops, leading to lower *O*-GlcNAcylation, which in turn uncovers phosphorylation sites recognized by ubiquitous kinases present in the brain. The exposure of these sites and/or the lack of *O*-GlcNAc itself causes the proteins to aggregate and form abnormal complexes and contributes to the abnormal processing of amyloid precursor protein.

Although much less studied, evidence is also emerging for *O*-GlcNAcylation's roles in Parkinson's disease (305, 317). As stated above, OGT maps to the Parkinson dystonia locus at Xq13 (329). Parkinson's disease results in degeneration of dopaminergic neurons in the substantia nigra, leading to a reduction of striatal dopamine levels. Tyrosine hydroxylase, which is the rate-limiting step in the biosynthesis of dopamine, is regulated by reciprocal *O*-GlcNAcylation/phosphorylation (334). α-Synuclein, a toxic aggregating protein involved in Parkinson's disease, is *O*-GlcNAcylated on at least eight sites, and even substoichiometric *O*-GlcNAcylation of α-synuclein affects its phosphorylation and blocks its degradation by calpain. *O*-GlcNAcylation also blocks the toxicity of α-synuclein added to cultured cells (335–338). Enzymatic *O*-GlcNAcylation by OGT also inhibits α-synuclein aggregation and promotes the formation of soluble SDS-resistant oligomers that stain negative for amyloid formation (339). However, *O*-GlcNAcylation's roles in Parkinson's disease may be much more complicated than preventing the formation of toxic protein aggregates. *O*-GlcNAcylation was found to be elevated in lysates from the post-mortem temporal cortex of Parkinson's disease patients compared with age-matched controls. Treatment of rat primary cortical neurons with a potent inhibitor of OGA significantly increased protein *O*-GlcNAcylation, activated MTOR signaling, decreased autophagic flux, and increased α-synuclein accumulation. Inhibition of MTOR by rapamycin decreased basal levels of protein *O*-GlcNAcylation, decreased AKT activation, and partially reversed the effect of the OGA inhibitor on α-synuclein monomer accumulation. It was postulated that excessive *O*-GlcNAcylation is detrimental to neurons by inhibiting autophagy and by increasing α-synuclein accumulation (340). Perhaps at moderate levels, *O*-GlcNAcylation is beneficial by preventing toxic protein aggregation, but when it is too high, the sugar modification alters other processes in a deleterious manner. Clearly, *O*-GlcNAcylation is a fertile area in which to explore new avenues for treatment of AD and other neurodegenerative diseases.

Conclusions and future directions

After more than 3 decades of research on *O*-GlcNAcylation, it is now clear that *O*-GlcNAcylation serves as a major nutrient sensor, acting like a rheostat, to modulate most cellular processes in response to the nutrient and stress status of the cell. Despite the efforts of many laboratories, this field is still in its infancy. The technological challenges of studying this protein modification remain a major hurdle, and there is an acute need for the development of new tools to study *O*-GlcNAc cycling in living cells. Tools to selectively modify *O*-GlcNAcylation on a single protein or at a specific site will be essential for elucidating molecular mechanisms underlying the sugar's physiological functions. Whereas nearly every protein involved in transcription is *O*-GlcNAcylated, and it is evident that nutrients regulate gene expression, in part, by the cycling of *O*-GlcNAc, we know very little about the molecular mechanistic roles that *O*-GlcNAcylation plays in the transcription machinery. *O*-GlcNAcylation is extraordinarily abundant in neurons and in the brain, and limited data suggest that the sugar plays major roles in neuronal functions and in neurodegeneration. However, so far, very little research with respect to the specific functions of *O*-GlcNAcylation in neuronal processes has been performed. The *O*-GlcNAcylation field is a major largely unexplored frontier that not only will have a huge effect on our understanding of fundamental biological processes, but also will impact future development of novel treatments for the chronic diseases of aging. The study of *O*-GlcNAcylation represents a wide open and exciting area for young researchers looking for a challenging yet important problem to tackle.

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