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A little sugar goes a long way: The cell biology of O-GlcNAc

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Unlike the complex glycans decorating the cell surface, the O-linked β-*N*-acetyl glucosamine (O-GlcNAc) modification is a simple intracellular Ser/Thr-linked monosaccharide that is important for disease-relevant signaling and enzyme regulation. O-GlcNAcylation requires uridine diphosphate–GlcNAc, a precursor responsive to nutrient status and other environmental cues. Alternative splicing of the genes encoding the O-GlcNAc cycling enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) yields isoforms targeted to discrete sites in the nucleus, cytoplasm, and mitochondria. OGT and OGA also partner with cellular effectors and act in tandem with other posttranslational modifications. The enzymes of O-GlcNAc cycling act preferentially on intrinsically disordered domains of target proteins impacting transcription, metabolism, apoptosis, organelle biogenesis, and transport.

Protein modification by O-linked β-*N*-acetyl glucosamine (O-GlcNAc) has rapidly emerged as a major cellular signaling mechanism rivaling protein phosphorylation in terms of the number of modified targets (Fig. 1 A). O-GlcNAc is an uncharged acetylated hexosamine sugar attached through a glycosyl linkage to hydroxyl-containing amino acids that adds bulk to modified amino acid side chains. O-GlcNAc is added to and removed from nucleocytoplasmic and mitochondrial target proteins by the intracellular enzymes O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). These O-GlcNAc cycling enzymes are distinct from the glycosyltransferases and glycosidases sequestered within the lumen of endomembrane organelles or routed to the cell surface. The enzymatic machinery catalyzing O-GlcNAc addition and removal bear some structural resemblance to nucleocytoplasmic kinases and phosphatases. In fact, there are many parallels between phosphorylation and O-GlcNAcylation: O-GlcNAc is added to Ser and Thr residues; the modification rapidly cycles on and off modified proteins at a rate faster than protein turnover; and like kinases and phosphatases, OGT and OGA are phosphorylated (Fig. 1 B; Butkinaree et al., 2010; Hanover et al., 2010). Many target proteins are modified by both O-GlcNAc and phosphate at exposed regions, suggesting the presence of shared or coexisting recognition motifs. However, although the sites of protein phosphorylation can often be identified by primary sequence alone, O-GlcNAcylation is not associated with a clear consensus motif.

OGT uses UDP-GlcNAc, a nucleotide sugar derived from the nutrient-dependent hexosamine biosynthetic pathway (HBP), to catalyze O-GlcNAc addition (Fig. 2 A). Concentrations of UDP-GlcNAc and its epimer UDP-GalNAc are directly influenced by nutrient availability (Fig. 2, B and C). The mammalian enzymes of O-GlcNAc cycling are ubiquitously expressed with a wide tissue distribution. Transcripts encoding O-GlcNAc cycling are most highly expressed in immune cells including circulating lymphocytes, B cells, and macrophages (NCBI public database). Other tissues with relatively high expression of OGT and OGA include the brain and pancreas. Consistent with the role of this posttranslational modification in numerous human diseases, O-GlcNAc cycling has been genetically linked to systemic lupus erythematosus, Alzheimer's disease, autism, and X-linked Parkinson dystonia (Bond and Hanover, 2013). The OGA gene (MGEA5; Heckel et al., 1998) has been identified as a susceptibility locus for noninsulin-dependent diabetes mellitus and obesity. In this review, we focus on how the enzymatic machinery of hexosamine synthesis and O-GlcNAc cycling work together to modulate properties such as the activity, localization, and stability of targets.

O-GlcNAc addition and removal

OGT. OGT is the single enzyme that catalyzes the addition of O-GlcNAc to its ∼4,000 targets (Ma and Hart, 2014) and is regulated through: (a) splice variants (Lazarus et al., 2006), (b) tissue- and organelle-specific expression, (c) substrate interaction via multiple domains (Yang et al., 2008; Lazarus et al., 2011), (d) its own posttranslational modifications (Kreppel et al.,

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Abbreviations used in this paper: AMPK, AMP-activated protein kinase; CTD, C-terminal domain; GFAT, glutamine:fructose-6-phosphate-amidotransferase; HAT, histone acetyl transferase; HBP, hexosamine biosynthetic pathway; HCF-1, host cell factor-1; ID, intrinsically disordered; mER-β, murine estrogen receptor β; mOGT, mitochondrial OGT; ncOGT, nucleocytoplasmic OGT; NPC, nuclear pore complex; NUP, nuclear pore protein; OGA, O-GlcNAcase; O-GlcNAc, O-linked β-*N*-acetyl glucosamine; OGT, O-GlcNAc transferase; Ph, polyhomeotic; TET, ten eleven translocation; TPR, tetratricopeptide repeat.

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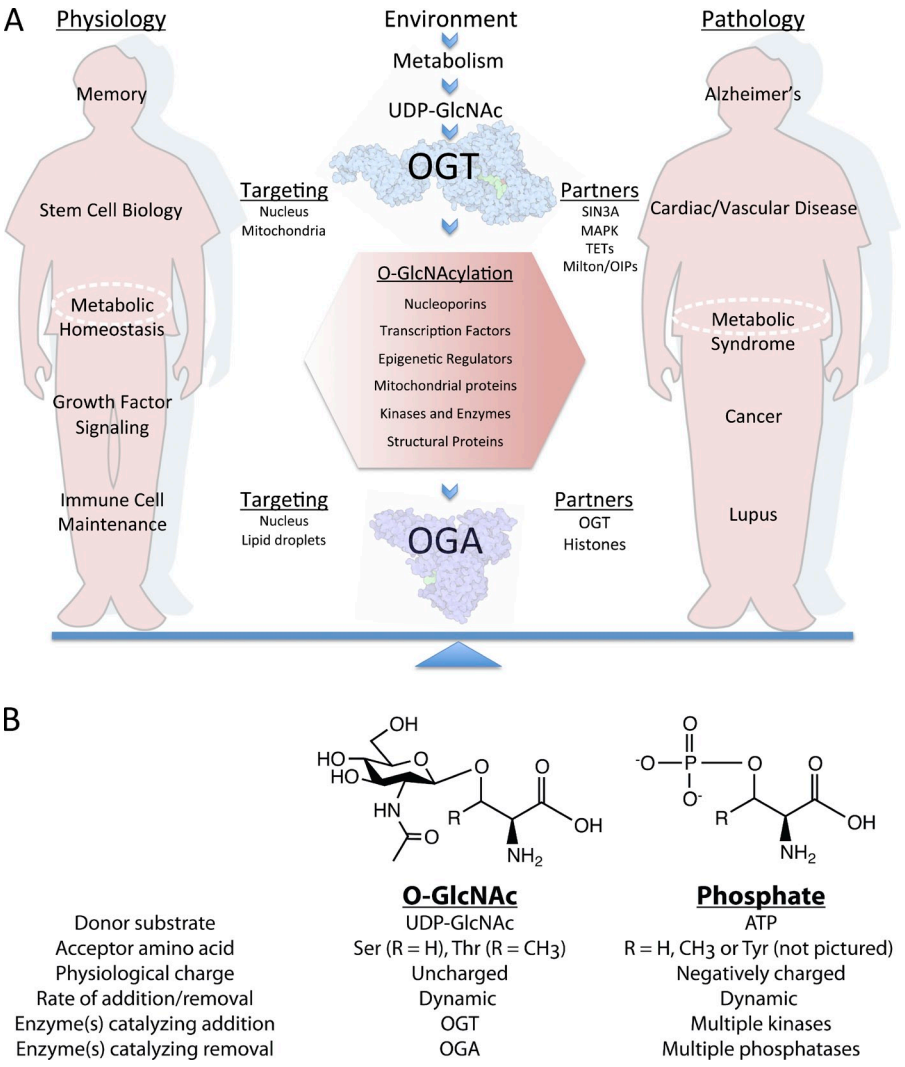

Figure 1. **O-GlcNAc dynamically impacts biological homeostasis and disease pathologies by integrating environmental and genetic information.** (A) The ubiquitous and essential modification of protein serine and threonine residues with O-GlcNAc modulates cellular biology by responding to variable nutrient conditions and integrating cellular programs to respond through nutrient-sensing and -managing networks. By targeting OGT and OGA to discrete intracellular sites, O-GlcNAcylation of diverse proteins (pink hexagon) influences the physiology of processes including memory, metabolism, and immunity. Aberrant O-GlcNAc modification is implicated in pathologies of metabolic and neurodegenertive diseases as well as cancers and autoimmunity. OIP, OGT interacting protein. (B) The O-GlcNAc and O-phosphate modifications share some characteristics but differ in others.

1997; Ryu and Do, 2011), (e) the substrates' posttranslational modifications (Kreppel et al., 1997; Shen et al., 2012), and (f) nutrient flux (Fig. 2; Haltiwanger et al., 1992).

hOGT encodes three splice variants whose products vary only in the number of N-terminal tetratricopeptide repeat (TPR) motifs (Fig. 2 A). The longest OGT isoform, nucleocytoplasmic OGT (ncOGT), is localized in the nucleus and cytoplasm (Kreppel et al., 1997; Lubas et al., 1997). ncOGT is linked to transcriptional repression (Comer and Hart, 2001), proteasomal inhibition (Zhang et al., 2003, 2007), and stress tolerance (Zachara et al., 2004). A unique start site in the fourth intron generates a mitochondrial OGT (mOGT) that is thought to be proapoptotic (Hanover et al., 2003; Love et al., 2003; Shin et al., 2011). The shortest isoform (sOGT) derives from a longer transcript and has also been linked to apoptosis (Hanover et al., 2003; Love et al., 2003; Shin et al., 2011).

The variable TPR number alters the amphipathic groove created by the domains' superhelical structure, yielding a region that accommodates different sequences to modulate substrate specificity (Blatch and Lässle, 1999; Jínek et al., 2004; Kim et al., 2014). With partners and substrates interacting with OGT via multiple domains (Yang et al., 2008; Lazarus et al., 2011), it is likely that interactions influence OGT's local activity, localization, and other interaction partners. OGT interacts with Trak1 (Iyer et al., 2003), OGA (Whisenhunt et al., 2006), and p38 MAPK (Cheung and Hart, 2008) as well as several other proteins (Yang et al., 2002; März et al., 2006; Riu et al., 2008) that influence the enzyme's recruitment toward targets including RNA polymerase II, transcription complexes, and neurofilament H.

Structures and enzymology favor the bi–bi mechanism in which OGT first binds UDP-GlcNAc followed by the protein substrate (Janetzko and Walker, 2014; Kim et al., 2014). Interestingly, OGT has a wide range of affinities for protein substrates depending on the localized concentration of UDP-GlcNAc (Shen et al., 2012). In addition, protein modifications, such as phosphorylation on CaMKIV (calcium/calmodulin-dependent protein kinase type IV), are also thought to change the affinity hOGT has for individual substrates (Shen et al., 2012). Thus, it is likely that some of OGT's protein substrates are constitutively modified at physiological UDP-GlcNAc concentrations, whereas the modification level for others varies widely (Shen et al., 2012).

Recently, OGT was found to catalyze site-specific proteolysis: host cell factor 1 (HCF-1) is a transcriptional coregulator

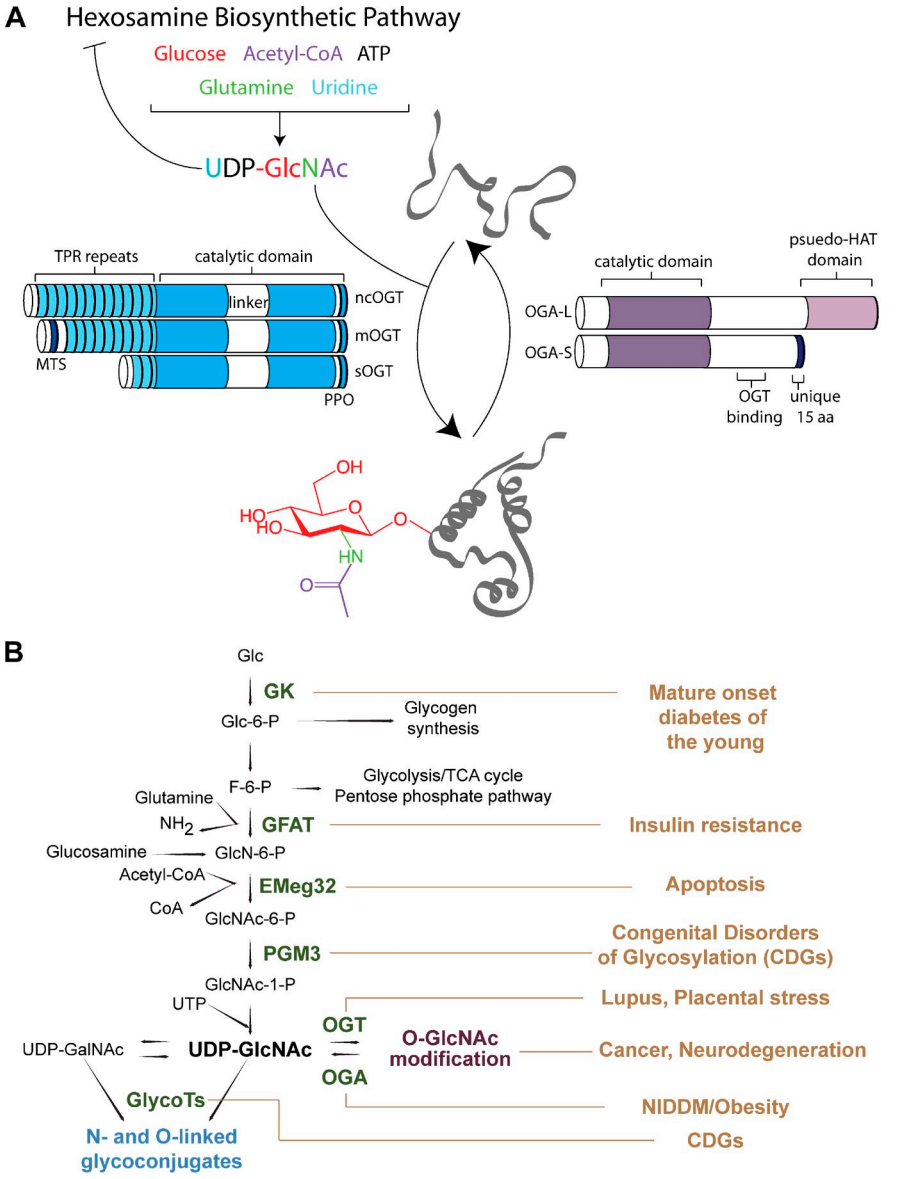

Figure 2. **The nucleotide sugar UDP-GlcNAc resides at the nexus of protein and lipid glycosylation.** (A) Dynamic, nutrient-sensitive O-GlcNAc cycling is modulated by metabolite and enzyme availability. Glucose, acetyl-CoA, ATP, glutamine, and uridine are required for the synthesis of UDP-GlcNAc, the ultimate product of the hexosamine biosynthetic pathway (HBP). OGT uses the nucleotide sugar to modify proteins, whereas OGA catalyzes the modification's removal. *hOGT* and *hOGA* splice variants are depicted. OGT and OGA isoforms have unique subcellular distributions and interaction partners, including one another. The O-GlcNAcylation of ID protein domains is known to influence protein secondary structure. MTS, mitochondrial targeting sequence; PPO, phosphoinositide binding domain. (B) Although most cellular glucose (Glc) is metabolized by glycolysis, 2–3% enters the HBP (Marshall et al., 1991a) and is phosphorylated and isomerized in two enzymatic steps to yield fructose-6-phosphate (F-6-P; Aguilera and Zimmermann, 1986; Stachelek et al., 1986). Next, glutamine:fructose-6-phosphate-amidotransferase (GFAT) acts to convert fructose-6-phosphate to glucosamine-6-phosphate (GlcN-6-P) in the HBP's rate-limiting step (Watzele and Tanner, 1989; Marshall et al., 1991a,b). The installation of an acetyl group (Boehmelt et al., 2000a) is followed by a second isomerization by phosphoglucomutase 3 (PGM3), yielding GlcNAc-1-phosphate (GlcNAc-1-P; Hofmann et al., 1994). In the final step, UDP-GlcNAc pyrophosphorylase utilizes UTP and GlcNAc-1-phosphate to produce UDP-GlcNAc (Mio et al., 1998). Importantly, along with other mechanisms of regulation, GFAT is sensitive to UDP-GlcNAc inhibition, thereby modulating the cellular UDP-GlcNAc available to glycosyltransferases (GlycoTs) at any given time within the cell (McKnight et al., 1992). Salvage pathways can also introduce glucosamine and GlcNAc to enter the HBP directly bypassing GFAT (Bueding and MacKinnon, 1955; Hinderlich et al., 2000). Cells can rapidly take up exogenous glucosamine via the glucose transporter, which can be fully processed to UDP-GlcNAc (Schleicher and Weigert, 2000). Cellular GlcNAc from lysosomal degradation or the degradation of nutritional constituents can be converted by GlcNAc kinase to GlcNAc-6-phosphate (GlcNAc-6-P) and then converted to UDP-GlcNAc (Hinderlich et al., 2000). The diseases associated with deregulation of each HBP enzyme (left) are indicated on the right side of the figure, connected to the enzyme by a line. Glc-6-P, glucose-6-phosphate; GlcNAc-6-P, *N*-acetylglucosamine-6-phosphate; GK, glucokinase; EMeg32, glucosamine-6-phosphate acetyltransferase; TCA, tricarboxylic acid; NIDDM, noninsulin-dependent diabetes mellitus. (C) The activated nucleotide sugar UDP-GlcNAc is used by concentration-sensitive enzymes in the nucleus, cytoplasm, and on the plasma membrane to glycosylate substrates. Nucleotide sugar transporters actively transport UDP-GlcNAc into cellular organelles including the ER and Golgi. The relative cellular volumes of these organelles that differ in their permeability characteristics make estimates of cytoplasmic and nuclear concentrations of UDP-GlcNAc complicated. The relative abundance of O-GlcNAc is roughly inversely related to the abundance of more complex glycans.

that is bound by OGT. HCF-1 sits in the active site of OGT where UDP-GlcNAc resides, and the nucleotide sugar is required for HCF-1's cleavage (Capotosti et al., 2011). Crystal structures defining the manner in which OGT interacts with HCF-1 will better define the implications of this new biological role. Furthermore, future insight about OGT's mechanisms of action may be augmented by in silico modeling (Kumari et al., 2015).

OGA. Evolutionarily conserved OGA is the single enzyme responsible for removing O-GlcNAc. hOGA exists as two major splice variants: the long and short isoforms (OGA-L and OGA-S) have identical glycoside hydrolase domains at the

N terminus (Fig. 2 A). OGA-L is localized to the cytosol and nucleus (Comtesse et al., 2001; Gao et al., 2001; Wells et al., 2002), whereas OGA-S resides primarily in lipid droplets (Comtesse et al., 2001; Keembiyehetty et al., 2011). The OGA-L C-terminal region has predicted similarity to GCN5-related histone acetyl transferases (HATs; Schultz and Pils, 2002). Despite early in vitro work that suggested HAT activity (Toleman et al., 2004), other studies do not support these findings (Butkinaree et al., 2008) and suggest that the region should, thus, be termed a "pseudo-HAT" (He et al., 2014). The second splice variant, OGA-S, has a unique 15–amino acid C-terminal extension and lacks the pseudo-HAT domain. Intriguingly, OGA-S shows lower in vitro activity for select substrates (Kim et al., 2006; Macauley and Vocadlo, 2009), suggesting that cellular cofactors or the C-terminal region may be important for targeting, proper folding, or activity. The region between OGA's N- and C-terminal domains (CTDs) is linked by an apoptosis-induced capsase-3 cleavage site, which is processed during programmed cell death. Interestingly, this cleavage yields two truncated fragments that only recapitulate full enzyme activity when associated (Butkinaree et al., 2008).

Despite numerous attempts to crystallize full-length hOGA, the structure has remained elusive, as it likely samples several conformations to interact with its binding partners and substrates. Much work has been done with OGA homologues to determine the manner in which OGA recognizes its substrates (Dorfmueller et al., 2006; Rao et al., 2006; Schimpl et al., 2010, 2012; Kim et al., 2014) and to define the key catalytic residues (Çetinbas et al., 2006). Interestingly, catalytic efficiency (K(cat)/K(m)) values are similar for hOGA regardless of the peptide substrate, suggesting that O-GlcNAc itself, rather than the backbone or amino acid side chains modulate OGA's specificity (Shen et al., 2012). OGA processing is likely to be influenced by differential expression in cellular organelles, pH, secondary structure, and electrostatics (Nagel and Ball, 2014, and references therein).

Hexosamine biosynthetic pathway

Synthesized by a series of enzymes in the HBP (Fig. 2 B), UDP-GlcNAc is the activated nucleotide sugar donor used by OGT to glycosylate protein serine and threonine residues. This pathway integrates the metabolism of carbohydrates, amino acids, fat, and nucleotides to produce UDP-GlcNAc, rendering it sensitive to nutrient flux, metabolite availability, and enzyme activities (Figs. 1 and 2).

Perturbation of the HBP is linked with modulation of several biological processes including glucose-induced insulin resistance and changes in cell growth (Fig. 2 B; Einstein et al., 2005). Furthermore, globally decreased UDP-GlcNAc has been shown to yield profound phenotypes in mammals including immune system dysfunction (Schwartz et al., 1996; Sassi et al., 2014), diabetic nephropathy (Leehey et al., 2000), and embryonic lethality (Fig. 2 B; Boehmelt et al., 2000b). Recent work has also demonstrated that elimination of the *Caenorhabditis elegans* OGT homologue alters the expression of HBP members as well as other enzymes responsible for metabolic processing of cellular components, supporting an intimate relationship between the levels of O-GlcNAc and the HBP (Ghosh et al., 2014).

Another enzyme that plays a key role in de novo UDP-GlcNAc synthesis is GlcNAc-6-phosphate acetyltransferase EMeg32 (Fig. 2 B; Boehmelt et al., 2000a). During embryonic development, *Emeg32* is expressed in only a subset of organs, but nearing birth, the expression pattern is ubiquitous. Indeed, early embryonic lethality is characteristic of homozygous mutant EMeg32 embryos, and mouse embryonic fibroblasts deficient for EMeg32 show proliferation and adhesion defects (Boehmelt et al., 2000b). Interestingly, reduced O-GlcNAcylation of cytosolic and nuclear proteins was the predominant effect of reduced UDP-GlcNAc levels in these studies. Nutritional restoration of intracellular UDP-GlcNAc levels restored normal properties to mouse embryonic fibroblasts. These findings suggest that decreased intracellular UDP-GlcNAc, and therefore decreased O-GlcNAcylation of proteins, influences both cellular proliferation and apoptosis.

Another level of regulation of O-GlcNAc cycling downstream of the HBP is the transport and utilization of UDP-GlcNAc in various membrane-bound organelles. Current findings suggest that the cytoplasm, nucleus, and mitochondria may be exposed to a "privileged pool" of UDP-GlcNAc. The nuclear and cytoplasmic concentrations of UDP-GlcNAc are influenced by membrane permeability and utilization within individual organelles (Fig. 2 C). Although the ER and Golgi concentrate UDP-GlcNAc for endomembrane glycosylation, other organelles are impermeable to the nucleotide sugar. Still others, including the nucleus and mitochondria, are largely permeable to UDP-GlcNAc but do not concentrate it. From the values shown in Fig. 2 C, rough calculations suggest that if the ER and Golgi concentrate UDP-GlcNAc from 10–30-fold, the concentration of UDP-GlcNAc in the cytoplasm, nucleus, and mitochondria is in the range of 2–30 µM, near the known Michaelis constants for OGT and the UDP-GlcNAc transporters. The relative abundance of O-GlcNAc and complex glycans in these organelles reflects this pattern of nucleotide sugar transport and utilization by the O-GlcNAc cycling enzymes and the enzymatic machinery catalyzing complex glycan biosynthesis, respectively (Fig. 2 C, right column).

Intracellular targets of O-GlcNAcylation

Although identification of O-GlcNAc sites has historically presented technical challenges, modern mass spectrometry methods have accelerated this research. A recent global analysis of synapse O-GlcNAc and O-phosphate sites has yielded insight into the relative abundance and sites of these posttranslational modifications (Trinidad et al., 2012). Protein target abundance has little to no effect on the phosphorylation stoichiometry and only a modest impact on the extent of O-GlcNAcylation. In each abundance class, 10–20% of all proteins are O-GlcNAcylated, and 40–60% are phosphorylated with the most abundant proteins containing the highest number of modifications. Although the motifs associated with sites of O-GlcNAc are enriched in small side chain amino acids as well as proline and differ little from phosphorylation sites, there is no obvious consensus motif for O-GlcNAcylation. O-GlcNAcylation and

O-phosphorylation are rarely present in helical or β-sheet regions of proteins appearing more often on loops and intrinsically disordered (ID) regions (Nishikawa et al., 2010). Indeed, it has been estimated that >70% of the nuclear O-GlcNAc resides in ID regions. We have previously argued that this preference for ID regions may be a property of OGT whose TPR domain is very similar in structure to the importins that cross the highly disordered FG-rich domains of nucleoporins in the nuclear pore complex (NPC; Jínek et al., 2004). This topic will be discussed more extensively in the next sections.

Relationship with phosphorylation/kinases. The complex interaction between phosphorylation and O-GlcNAcylation has been hard to study in a global fashion. *C. elegans* mutants defective in both the addition and removal of O-GlcNAc have altered phosphorylation patterns (Forsythe et al., 2006). Mammalian cells subjected to elevated O-GlcNAcylation show altered phosphate stoichiometry with 70% of sites affected (Wang et al., 2008). The ∼400 known proteins modified by both post-translational modifications include chaperones, cytoskeletal and regulatory proteins, metabolic enzymes, kinases, transcription factors, and RNA-processing proteins (Wang et al., 2008). The large number of kinases modified by O-GlcNAc suggests that O-GlcNAc participates in kinase-dependent signaling cascades; in a later section, we discuss the direct impact of O-GlcNAc on the activity of multiple kinases.

O-GlcNAcylation at ID regions of proteins. Many proteins modified by phosphate and O-GlcNAc contain ID domains (Chen et al., 2006; Yang et al., 2006; Xie et al., 2007a,b). The impact of O-GlcNAcylation on such domains has been explored in some cases. For example, Ser16 on the N-terminal ID domain of murine estrogen receptor β (mER-β) can be both O-GlcNAcylated and O-phosphorylated. Nuclear magnetic resonance, circular dichroism, and molecular dynamics simulations were used to examine the structure of synthetic peptides corresponding to the ID region of mER-β and suggest that O-phosphorylation and O-GlcNAcylation discourages and promotes β-turn formation in this region, respectively (Chen et al., 2006). Thus, the varied local structure in the N terminus of mER-β may directly influence the global ID region, which is related to its modulatory activity.

Direct activation/inhibition of enzyme activity. In some instances, O-GlcNAcylation appears to be directly involved in the regulation of enzymatic activity. The endothelial nitric acid synthase is normally activated by AKT-dependent phosphorylation, but in diabetic patients, a significant fraction of the enzyme is O-GlcNAcylated and inactive (Federici et al., 2002; Musicki et al., 2005). Indeed, the activity of AKT itself may also be regulated by its O-GlcNAcylation (Luo et al., 2008; Wang et al., 2012b). CaMKII (calmodulin kinase II) is regulated in a similar way; acute hyperglycemia increases O-GlcNAcylation of CaMKII and creates a kind of molecular memory of activation, even after Ca^{+2} levels decline (Erickson et al., 2013).

O-GlcNAc modification of PFK1 (phosphofructokinase 1), an enzyme responsible for glycolysis regulation, modulates the enzyme's activity, yielding increased glucose flux through the pentose phosphate pathway, thereby conferring a growth advantage to cancer cells (Yi et al., 2012). Recent work suggests a reciprocal relationship between OGT and AMP-activated protein kinase (AMPK) in which the subcellular localization of OGT is strongly correlated with its phosphorylation by AMPK on Thr-444. To define the cross talk between these enzymes, the researchers note that several AMPK subunits are dynamically modified by O-GlcNAc and suggest that AMPK activation is influenced by its state of O-GlcNAcylation (Bullen et al., 2014).

Regulation of epigenetics, transcription, translation, protein stability, and protein degradation. O-GlcNAc has emerged as a key regulator of gene expression through its influence on higher-order chromatin structure, transcription, and modulation of RNA polymerase II (Hanover et al., 2012 and references therein as well as the nuclear functions section). O-GlcNAc is known to modify all four core histone proteins and is thought to compete with or promote modifications including phosphorylation and ubiquitination, which are associated with activation and repression of transcription (Sakabe et al., 2010; Lewis and Hanover, 2014). In addition, O-GlcNAc modulates the stability of transcriptional activator β-catenin in a nutrient-dependent manner, potentially acting as the link between glucose metabolism with the development of cancers (Olivier-Van Stichelen et al., 2014). Interestingly, although the O-GlcNAc modification of CARM1 (coactivator-associated arginine methyltransferase 1) does not change its stability, dimerization, or cellular localization, it does influence the enzyme's substrate specificity (Charoensuksai et al., 2015).

Through modification of HIF-1α (hypoxia-inducible factor 1) and its transcriptional target GLUT1, O-GlcNAc regulates glycolysis in cancer cells and activates ER stress. Indeed, poor patient outcome is correlated with breast cancers in which OGT expression is increased, and levels of HIF-1α are elevated (Ferrer et al., 2014). Furthermore, in other studies, increased O-GlcNAc modification correlated with attenuated activation of the unfolded protein response and ER stress (Ngoh et al., 2009).

Along with modulating the stability of individual proteins, O-GlcNAc has been linked to regulating lysosomal protein degradation (autophagy) in a nutrient-dependent fashion (Wang et al., 2012a; Guo et al., 2014). Most recently, researchers discovered that O-GlcNAc modification of SNARE protein SNAP-29 correlates with inhibition of autophagosome–lysosome fusion. Interestingly, in the *C. elegans* whole animal model, animals lacking *ogt-1* exhibit a modest increase in proteasome activity and a reduction in proteotoxicity (Wang et al., 2012a). Other researchers have also suggested roles for O-GlcNAc in autophagy, but the exact mechanisms of action remains to be seen (Marsh et al., 2013; Kumar et al., 2014).

Subcellular localization of targets. O-GlcNAc influences the subcellular localization of its targets. Recently, the O-GlcNAcylation of both a ten eleven translocation (TET) dioxygenase and β-catenin were demonstrated to influence not only localization but also activity (Sayat et al., 2008; Ha et al., 2014; Zhang et al., 2014). TETs are responsible for the conversion of 5mc (5-methylcytosine) to 5hmC (5-hydroxymethylcytosine)

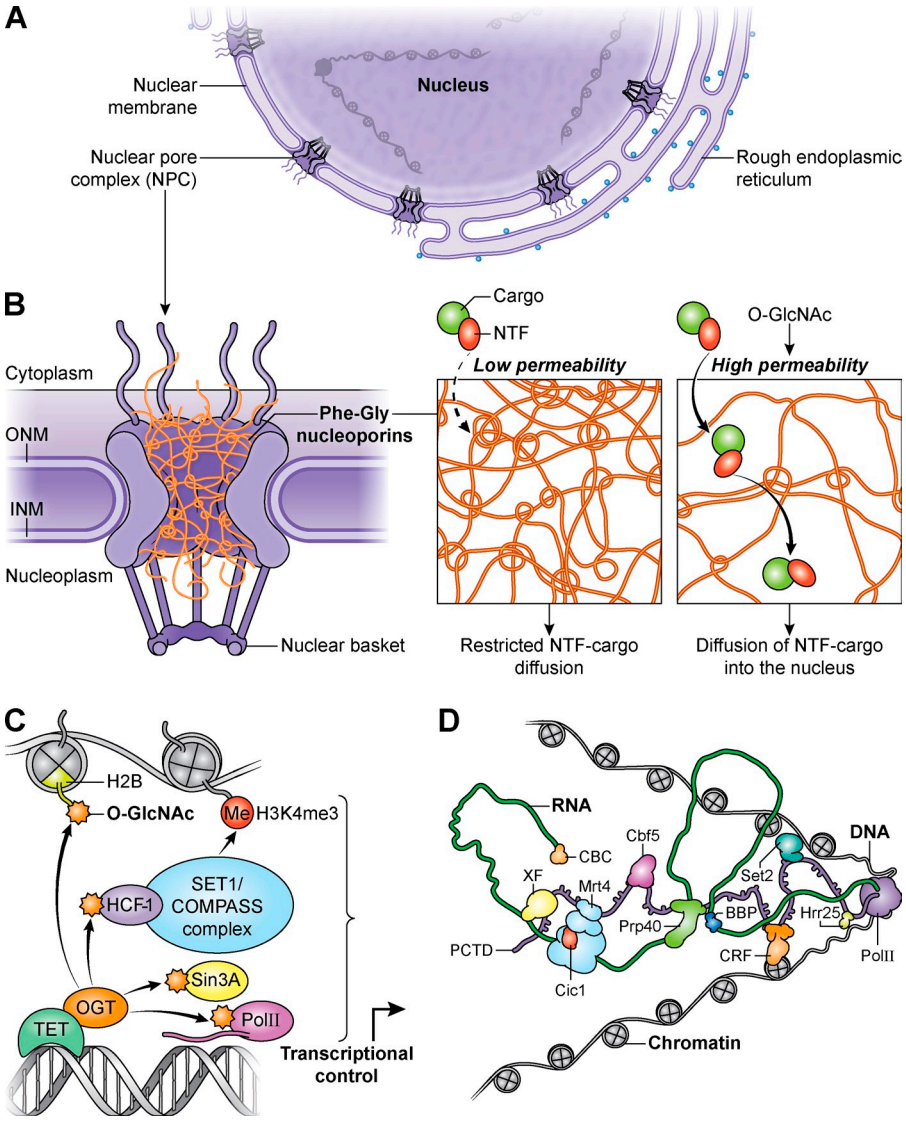

Figure 3. **O-GlcNAc modulates nuclear processes.** (A) The nuclear envelope is contiguous with the rough ER and serves to separate the transcriptional machinery in the nucleus from the translation machinery associated with the rough ER and cytoplasm. Among the most heavily O-GlcNAcylated proteins are the nucleoporins (NUPs), 30 of which form the nuclear pore complex (NPC). (B) The NPC is responsible for the exchange of molecules across the nuclear envelope (NE) comprised of the inner and outer nuclear membranes (INM and ONM, respectively). The interaction between NUPs' phenylalanine-glycine (FG) repeats and nuclear transport factors (NTFs) is required for the transport of cargo through NPCs. In vitro work has suggested that O-GlcNAc addition to FG repeats is critical for nuclear transport events through these "gateways." (C) O-GlcNAc is implicated in higher-order chromatin structure through its modification of histone tails. In addition, nuclear machinery associated with transcription including transcription factors, transcriptional comodulators (SIN3A), ten eleven translocation proteins (TETs), and the host cell factor 1 (HCF-1) are known to associate with OGT or be O-GlcNAc modified. (D) O-GlcNAc is thought to modulate transcriptional initiation through its modification of the CTD of RNA polymerase II (PolII), which is known to act as a scaffold to recruit numerous transcriptional effectors. Panel D is redrawn and adapted from Phatnani and Greenleaf (2006). BBP, branch-point-binding protein; PCTD, phosphocarboxy terminal domain; CRF, chromatin remodeling factor; XF, processing/export factor.

and the functional relevance of TET3 O-GlcNAcylation directly links epigenetics with glucose metabolism (Zhang et al., 2014). Furthermore, site-specific O-GlcNAcylation of β-catenin is responsible for regulating its localization and transactivator function (Ha et al., 2014). However, the implications of β-catenin O-GlcNAc modification in a whole animal remains to be clearly defined as OGA may act independent of Wnt/β-catenin signaling in some cases (Yang et al., 2014).

Nuclear functions of O-GlcNAcylation. The nucleus is separated from the cytoplasm by the nuclear envelope, a structure contiguous with the rough ER, a major site for endomembrane glycosylation (Fig. 3 A). Embedded in the nuclear envelope is the NPC through which nucleocytoplasmic exchange occurs (Fig. 3, A and B). Nuclear pore proteins (NUPs) were the first O-GlcNAcylated targets identified and remain one of the most widely studied (Hanover et al., 1987; Holt et al., 1987; Park et al., 1987; Snow et al., 1987; D'Onofrio et al., 1988; Starr and Hanover, 1990, 1991; Starr et al., 1990). Nuclear pores are assembled from ∼30 unique proteins in multiple copies totaling nearly 500/pore. Roughly half of these proteins can be classified as solenoid protein domains (α solenoid or β propeller), and the other half contain domains that are lacking ordered secondary structure or are "ID." These proteins are the FG nucleoporins that associate with the interior channel of the nuclear pore (Fig. 3 B) and contain the canonic phenylalanine-glycine (FG) signature motif (Starr and Hanover, 1991). Many of the FG NUPs, including Nup62 and Nup98, are modified by O-GlcNAc in the FG domains' TTPST sequence (Starr et al., 1990). Recently, it has been shown that O-GlcNAcylation of the FG repeat region of Nup98 allows hydrogels generated from this protein to accommodate passage of very large cargoes (Labokha et al., 2013). Thus, it would appear that one function of the O-GlcNAc modification of nucleoporins is to allow fine adjustment of the permeability characteristics of the nuclear pore (Fig. 3 B, right). Intriguingly, these same NUPs (Nup62, Nup88, Nup98, and Nup214) are involved in tumorigenesis (Simon and Rout, 2014). What role O-GlcNAcylation may play in this process is currently under active investigation, although early data suggest it may be involved in cell cycle progression (Fang and Miller, 2001).

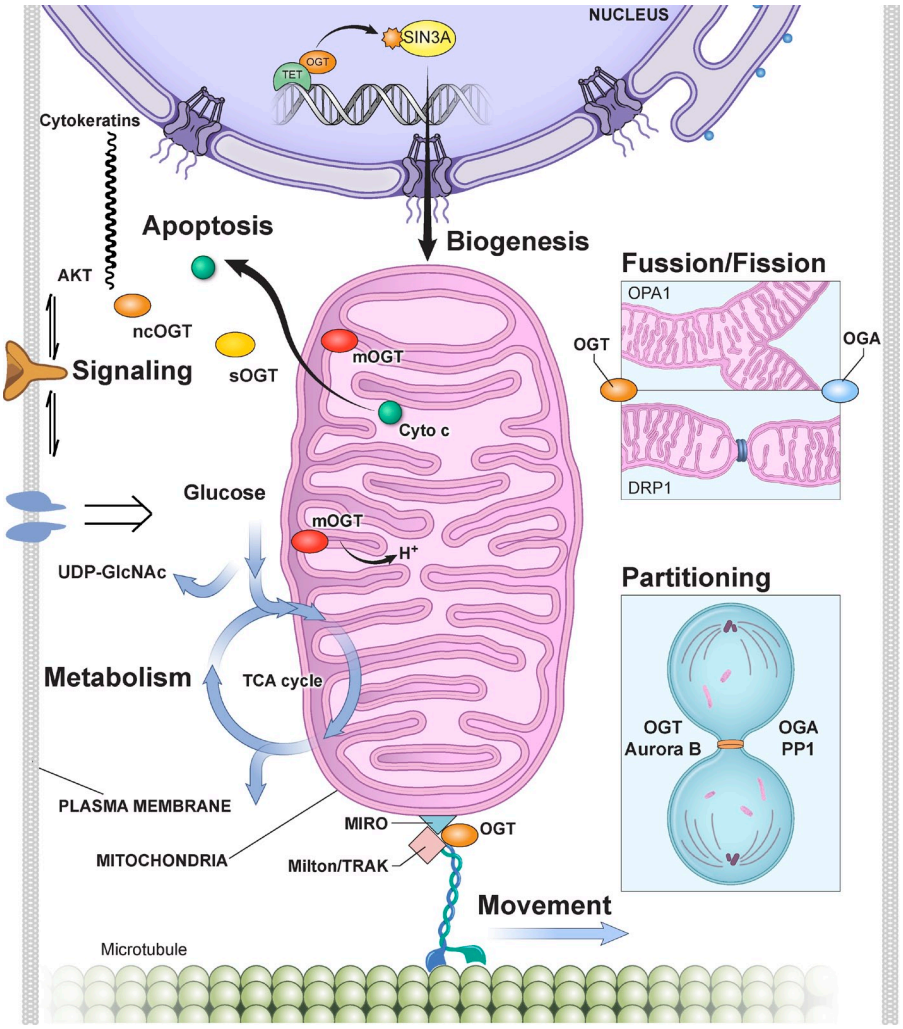

Figure 4. **O-GlcNAc modulates cellular signaling processes, metabolism, mitochondrial trafficking, and function as well as the cell cycle.** OGT is intimately involved in transcriptional regulation through its interaction with and modification of SIN3A in the histone deacetylase corepressor complex. It is possible that through this interaction, a subset of regulatory genes is suppressed in a nutrient-sensitive manner yielding downstream consequences. Outside the nucleus, O-GlcNAc-modified cytokeratins are critical mediators of stress-responsive AKT signaling, whose activity is also modulated by O-GlcNAc. O-GlcNAc plays critical roles in both the function and trafficking of mitochondria. Key modified proteins include GTPase dynamin-related protein DRP1 and optical atrophy 1 (OPA1), the consequence of which is increased mitochondrial fission. Furthermore, although it is unclear how the complex of MIRO (mitochondrial Rho GTPase), kinesin-binding protein Milton, and TRAKs (trafficking kinesin protein) regulate mitochondrial trafficking in *Drosophila*, it is clear that OGT is recruited to the same location and may modulate the movement of the organelle along actin and microtubule networks. Increased levels of O-GlcNAc are also tied to the mitigation of myocardial ischemia-associated mitochondrial Ca^{2+} and reactive oxygen species generation, loss of membrane potential, and cytochrome *c* (Cyto c) release. Cell division requires that cellular organelles, including the mitochondria, are properly segregated during mitosis. OGT and OGA are found in a transient complex with Aurora B and phosphatase PP1 during cytokinesis, although the way in which this partitioning occurs is still being studied.

In addition to nuclear pores, most transcription factors and many chromatin factors are O-GlcNAc modified (Love et al., 2010a; Hanover et al., 2012; Lewis and Hanover, 2014). Chromatin-modifying enzymes are also key targets for O-GlcNAcylation. These targets include components of the polycomb and trithorax complexes (Fig. 3 C; Hanover et al., 2012). Other targets include HCF-1, SIN3A, and the SET–COMPASS complex (Fig. 3 C). We detected O-GlcNAcylation at the promoters of *C. elegans* genes involved in stress, immunity, and longevity (Love et al., 2010a). OGT has also been shown to be essential for polycomb repression in *Drosophila melanogaster*, and its major target was the protein polyhomeotic (Ph; Gambetta et al., 2009; Sinclair et al., 2009; Gambetta and Müller, 2014). Ph forms large aggregates in the absence of O-GlcNAcylation both in vivo and in vitro, suggesting the posttranslational modification is critical to prevent nonproductive aggregation of *Drosophila* and human Ph via their C-terminal sterile α motif domains. The repressor activity of Ph requires O-GlcNAcylation of the Ser/Thr stretch. The authors propose that O-GlcNAcylation is needed for Ph to form functional, ordered assemblies via its sterile α motif domain (Gambetta and Müller, 2014).

The reversible methylation of DNA has also been linked to O-GlcNAc addition (Fig. 3 C). The TET family of demethylases binds to OGT and is O-GlcNAcylated. Although the functional significance is unclear, it has been proposed that one function of TET proteins is to recruit OGT to CpG islands, which bear the cytosine methyl mark. O-GlcNAc may also modify the stability or nuclear localization of the TET proteins (Chen et al., 2013; Deplus et al., 2013; Ito et al., 2014; Bauer et al., 2015).

Perhaps the most widely studied of these factors is the RNA polymerase II CTD domain (Kelly et al., 1993). This disordered domain contains multiple (≤52) repeats of the sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser (Fig. 3 D). A complex interplay between the O-GlcNAc and phosphate modifications of the CTD is likely to regulate gene transcription (Comer and Hart, 2001). Indeed, O-GlcNAc cycling on the CTD serine residues 5 and 2 has been shown to occur during the assembly of the preinitiation complex, underscoring a direct role of the modification in transcription (Fig. 3 D; Ranuncolo et al., 2012; Lewis and Hanover, 2014).

O-GlcNAcylation integrates mitochondrial–nuclear interactions. The discovery of a mitochondrially targeted isoform of OGT sparked interest in its mitochondrial functions (Hanover et al., 2003; Love et al., 2003). O-GlcNAcylation is involved in mitochondrial biogenesis, fission and fusion, cell cycle partitioning, metabolism, and apoptosis (Fig. 4). Mitochondrial inheritance is non-Mendelian and is intimately

tied to cytokinesis. Because the mitochondrial genome encodes some 12% of mitochondrial genes, some mechanism must allow coordination of nuclear and mitochondrial gene expression. A histone deacetylase complex (SIN3A and B) appears to play a key role in this regulation (Kadamb et al., 2013). OGT is known to interact with this complex by binding to the corepressor SIN3A in mammals at a site distinct from sites for binding histone deacetylase (Yang et al., 2002; Barnes et al., 2010; Kadamb et al., 2013). In *Drosophila* cultured cell lines, SIN3 depletion induces up-regulation of not only nuclear-encoded mitochondrial genes but also those encoded by the mitochondrial genome. In mammals, loss of OGT has been linked to alterations in mitochondrial genes (Howerton and Bale, 2014).

In addition to these transcriptional changes, O-GlcNAc cycling participates in the process of mitochondrial division. Although recent work suggests that hyperglycemia-induced changes to cardiac myocytes' mitochondrial respiration were O-GlcNAc independent (Dassanayaka et al., 2015), elevated O-GlcNAc cycling has been shown to modulate other mitochondrial processes including the recruitment and activity of DRP1 (dynamin-related protein 1), which is involved in mitochondrial fission (Gawlowski et al., 2012). Moreover, OGT and OGA participate with Aurora B and PP1 (protein phosphatase 1) in the later stages of cell division, leading to mitochondrial partitioning into the two daughter cells (Slawson et al., 2008). The movement of mitochondria is also influenced by nutrient-driven changes in O-GlcNAcylation through a kinesin-directed mitochondrial trafficking complex (Brickley et al., 2011; Pekkurnaz et al., 2014). The mitochondrially targeted mOGT appears to play an important function in altering mitochondrial metabolism (Hanover et al., 2003; Love et al., 2003; Ngoh et al., 2008; Hu et al., 2009; Johnsen et al., 2013; Lozano et al., 2014; Tan et al., 2014). It is suggested that the effects of O-GlcNAcylation are mediated by alteration with components of the electron transport chain (Lozano et al., 2014). Finally, O-GlcNAc plays an important role in the induction of apoptosis. The mOGT isoform triggers apoptosis upon overexpression, suggesting that it is part of the machinery mediating mitochondrial apoptosis (Shin et al., 2011). The nucleocytoplasmic enzyme ncOGT also plays a role in apoptotic induction in a pathway linking cytokeratin site-specific O-GlcNAcylation to altered AKT signaling (Fig. 4; Rotty et al., 2010; Srikanth et al., 2010). Keratins 8 and 18 are expressed in simple epithelial linings such as those present in the pancreas, liver, and intestine. Indeed, keratin 18 modification by O-GlcNAc is a critical effector of stress-responsive AKT signaling in the liver and in the pancreatic islets. These diverse functions of O-GlcNAc in coordinating genome–mitochondrial functions are outlined in Fig. 4.

Summary and conclusions

Although the enzymes of O-GlcNAc cycling are highly conserved in metazoa, the pathway has been exploited in different ways during evolution (Love et al., 2010b). It is clear that in invertebrates, O-GlcNAc plays a key role in regulating signaling and the regulation of Hox gene expression. In mammals, the evidence summarized here suggests that O-GlcNAc cycling may have further evolved to coordinate mitochondrial–genome interactions. Mammalian OGT also interacts with the TET proteins involved in removing methylcytosine from DNA. Only in mammals is DNA methylation known to lead to imprinting of growth regulatory genes in a parent of origin-specific fashion. The changing genomic position of the enzymes of O-GlcNAc cycling is also illuminating. In *C. elegans*, *ogt-1* is present in the Hox gene cluster on chromosome III, whereas the gene encoding OGA (*oga-1*) is on the X chromosome in a region critical for dosage compensation. In *Drosophila*, *Sxc(Ogt)* is essential for polycomb repression and present very near the pericentric euchromatin–heterochromatin boundary on chromosome II, suggesting tight regulation by higher-order chromatin structure (Ingham, 1984). The *Oga* gene is present in the "NK cluster," a group of homeobox-containing genes regulated by polycomb repression and essential for mesoderm induction. In mammals, *OGT* is present near the *Xist* locus involved in X-inactivation (Olivier-Van Stichelen and Hanover, 2014), and *OGA* is present on human chromosome 10 in one of four NK clusters of homeotic genes analogous to the fly NK cluster (Keembiyehetty et al., 2015). Thus, the observed cosegregation of the genes encoding the enzymes of O-GlcNAc cycling with dosage compensation and homeobox regulatory modules is unlikely to have occurred by chance. That O-GlcNAc cycling and homeotic genes regulating patterning have walked arm-in-arm through evolution has important implications for intergenerational information flow, stem cell biology, development, and chronic disease.

One interesting extension of these observations is that increasing complexity of the genes encoding OGA and OGT to produce differentially targeted isoforms occurred in placental mammals. We have suggested previously (Love et al., 2010b), and recent work has confirmed, that O-GlcNAc cycling may play a key role in nutrient-dependent signaling in the intrauterine environment (Howerton et al., 2013; Howerton and Bale, 2014; Keembiyehetty et al., 2015). OGT is a placental biomarker of maternal stress and nervous system development in the fetus (Howerton et al., 2013). This is associated with mitochondrial dysfunction in the hypothalamus (Howerton and Bale, 2014). In addition, OGA knockout mice show changes in gene expression associated with the hypothalamic–pituitary axis, leading to obesity, hyperleptinemia, and insulin resistance in the offspring (Keembiyehetty et al., 2015). These findings strongly argue that O-GlcNAc may play a pivotal role in fetal reprogramming associated with human disease.

Another provocative issue emerging from work on O-GlcNAc and epigenetics is its role in stem cell biology. Like cancer cells, stem cells undergo rapid mitosis and require a stable energy source. Yet this situation must dramatically change after differentiation. Plasticity in energy metabolism allows stem cells to balance the changing demands of self-renewal and lineage specification. Stem cells rely on glycolysis to a much greater extent than differentiated cells. A switch from somatic oxidative phosphorylation to glycolysis is one of the characteristics associated with reprogramming to a pluripotent state (Folmes et al., 2012). But how is this switch coordinated in the transition from totipotency to naive pluripotency? The nutrient-sensitive O-GlcNAc posttranslational modification regulates the transcriptional activity of core pluripotency network members, including

OCT4 and SOX2 (Jang et al., 2012). Reducing O-GlcNAylation through inhibition of OGT or reducing glucose concentrations interferes with embryonic stem cell self-renewal and nuclear reprogramming. Conversely, increasing global O-GlcNAc levels inhibits differentiation, thus linking metabolic status with fundamental stem cell function (Jang et al., 2012).

Here, we have highlighted the role O-GlcNAc plays in development and cell physiology. As can be said for protein phosphorylation, there is no simple answer to the question "what does the modification do?" O-GlcNAc can counter phosphorylation, interact with other posttranslational modifications, and directly modify enzymatic function (Hanover et al., 2010, 2012; Bond and Hanover, 2013; Hardivillé and Hart, 2014; Hart, 2014). Although we have emphasized the role of O-GlcNAc in mediating genome–mitochondrial interactions, it performs functions in organelle trafficking and biogenesis. O-GlcNAc regulates autophagy, apoptosis, and proteasome-mediated protein degradation. It also regulates nearly every step in protein biogenesis, transcription, translation, and protein stability, with perturbations in these processes linking O-GlcNAc with the etiology of metabolic and neurodegenerative diseases as well as cancers (Bond and Hanover, 2013). Techniques to detect the modification are now maturing to the point at which it is possible to begin to make biological sense of these diverse functions (Ma and Hart, 2014). As a signaling molecule, O-GlcNAc is unique: it is directly nutrient responsive, allowing it to function as a real-time sensor of metabolic status. It has been harnessed to fine-tune processes critical to metazoan physiology including regulating growth, monitoring the intrauterine environment, and modulating stem cell fate. The links between O-GlcNAcylation and chronic human disease are now becoming increasingly clear, and we underscore that "a little sugar goes a long way."

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