



Three decades of research on O-GlcNAcylation – a major nutrient sensor that regulates signaling, transcription and cellular metabolism

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Even though the dynamic modification of polypeptides by the monosaccharide, O-linked N-acetylglucosamine (O-GlcNAcylation) was discovered over 30 years ago, its physiological significance as a major nutrient sensor that regulates myriad cellular processes has only recently been more widely appreciated. O-GlcNAcylation, either on its own or by its interplay with other post-translational modifications, such as phosphorylation, ubiquitination, and others, modulates the activities of signaling proteins, regulates most components of the transcription machinery, affects cell cycle progression and regulates the targeting/turnover or functions of myriad other regulatory proteins, in response to nutrients. Acute increases in O-GlcNAcylation protect cells from stress-induced injury, while chronic deregulation of O-GlcNAc cycling contributes to the etiology of major human diseases of aging, such as diabetes, cancer, and neurodegeneration. Recent advances in tools to study O-GlcNAcylation at the individual site level and specific inhibitors of O-GlcNAc cycling have allowed more rapid progress toward elucidating the specific functions of O-GlcNAcylation in essential cellular processes.

Keywords: O-GlcNAcylation, O-GlcNAc transferase, O-GlcNAcase, signaling, transcription, diabetes, cancer, Alzheimer's disease

EARLY HISTORY

O-GlcNAcylation was discovered in the early 1980s when bovine milk galactosyltransferase was used as an enzymatic probe of terminal N-acetylglucosamine moieties in cells of the murine immune system (1). Later studies established O-GlcNAc's surprising nucleocytoplasmic subcellular localization and distribution at a time when dogma stated that protein glycosylation only occurs within the secretory pathway or extracellular compartments (2). O-GlcNAcylation was shown to be highly abundant within the nucleus and particularly enriched at the nuclear envelope and on nuclear pore proteins (3–5). However, O-GlcNAcylation was also found to be abundant on cytoskeletal proteins of human erythrocytes, which lack a nucleus (6). Viruses were also found to contain O-GlcNAcylated proteins, which occur on proteins surrounding their nucleic acid cores, rather than on their capsids, where other forms of “classical” protein glycosylation are found (7). O-GlcNAcylation was subsequently found to be highly enriched on proteins associated with chromatin in *Drosophila* (8), and O-GlcNAc was shown to not only be a major modifier of transcription factors (9), but also a major modification of the C-terminal domain (CTD) of RNA polymerase II itself (10). Early studies in lymphocytes showed that cellular activation resulted in rapid changes, suggesting that O-GlcNAc cycled like phosphorylation and could be a regulatory modification (11), which was later confirmed by the sugar's rapid cycling on small heat shock proteins, shown by classical pulse-chase analyses (12). An assay for O-GlcNAc transferase (OGT), based upon tritiated UDP-GlcNAc as the donor and synthetic peptide acceptors, was developed and

OGT activity was identified and characterized (13). OGT was subsequently purified to apparent homogeneity by brute-force biochemical approaches combined with nucleotide affinity chromatography (14). O-GlcNAcase was originally purified from rat spleen cytosol (15) and was found to be similar to hexosaminidase C (16, 17), which was known but had not been purified to homogeneity. Based upon polypeptide sequencing, in conjunction with PCR cloning, the OGT cDNA from rat (18), *C. elegans* and human (19) were cloned. OGT was found to be a very highly conserved protein with no homology to other known glycosyltransferases. OGT was also found to have two distinct domains, a catalytic domain and a protein–protein interaction domain consisting of over 11 tetratricopeptide (TPR) repeats separated by a linker region. Likewise, O-GlcNAcase was purified from bovine brain and the protein was sequenced by mass spectrometry, and used to clone the enzyme from a human library (20). The OGA gene was found to be identical to MGEA5 a putative hyaluronidase associated with meningioma (21). Early studies identified O-GlcNAc on nuclear receptors, tau protein in the brain, intermediate filament proteins, nuclear oncogenes and tumor suppressors, and many other proteins with a wide-range of functions [reviewed in Ref. (22)].

MORE RECENT FINDINGS

As the tools for the detection and analysis of O-GlcNAcylation improved, it became apparent that this post-translational modification is much more abundant than previously expected [reviewed in Ref. (23, 24)]. In addition, it was soon realized that not only was

the donor for O-GlcNAcylation, UDP-GlcNAc, a major node of metabolism, but also that O-GlcNAc has extensive interplay with protein phosphorylation [reviewed in Ref. (23)]. Gene deletion studies have shown that both OGT and O-GlcNAcase are essential genes in mammals and plants (25–27).

Like phosphorylation and ubiquitination, O-GlcNAcylation regulates many different cellular processes. O-GlcNAcylation is essential in the process of lymphocyte activation in both B- and T-lymphocytes (28). There are several examples where the glycan regulates protein:protein interactions [e.g., Ref. (29, 30)]. Nutrients fine-tune circadian clocks via O-GlcNAcylation (31–34). O-GlcNAc modulates the activity of the proteasome (35–39), and also has interplay with ubiquitination (40, 41). Recent studies indicate that O-GlcNAcylation is very important to neuronal and brain functions, including synaptic plasticity, synaptic vesicle trafficking, and axonal branching (42–46). O-GlcNAcylation also regulates growth hormone signaling in plants (27), protects cells from acute stresses (47), and modulates transition through the cell cycle (48). Even though O-GlcNAcylation has not yet been documented to occur in yeast, such as *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*, O-GlcNAc does occur in some of oldest known eukaryotes (49), including in some important human parasites (50–52). In certain bacteria, O-GlcNAcylation regulates flagellar motility (53), and in *Streptococcus pneumoniae*, O-GlcNAcylation of an adhesion plays a role in infection and pathogenesis (54). However, the bacterial OGT involved in each case is quite different from the eukaryotic enzyme.

As a key nutrient sensor, O-GlcNAcylation is fundamentally important to the regulation of transcription at nearly all levels, including regulation of the functions of RNA polymerase II itself (55, 56), modulating the activities of nearly all transcription factors (30), regulating both histone and DNA methylation (57–61), crosstalking with other epigenetic modifications (62), and serving as an integral part of the histone code (63). Not only does O-GlcNAcylation have extensive crosstalk with protein phosphorylation at the protein site level but also the sugar modifies many kinases and regulates their activities or specificity (64–69).

Given the myriad functions associated with O-GlcNAcylation, it is not surprising that this nutrient sensor plays a fundamental role in the etiology of diabetes and glucose toxicity (70–72). O-GlcNAcylation is elevated in all cancers studied to date and appears to play a role in tumor cell progression (24, 73, 74), and in patient prognosis (75). Given O-GlcNAcylation's abundance and presence on hundreds of proteins in the brain, it is also a major mechanism contributing to neurodegeneration (76–78). After 30-years of research on O-GlcNAcylation, it is now not only more apparent than ever that this post-translation modification plays a central role in the nutrient regulation of cellular physiology but also it is clear that we have a long way to go to fully understand the importance of O-GlcNAcylation in most cellular and disease processes.

FUTURE DIRECTIONS

Many important questions remain with respect to O-GlcNAcylation. (1) How does O-GlcNAc cycling achieve substrate specificity with only two known genes in mammals, OGT and OGA (*MGEA5*)? Clearly, several different mechanisms are

involved. *In vitro*, OGT has remarkable specificity for peptide substrates, which appears to change with UDP-GlcNAc concentrations (79). Most importantly, both enzymes function as part of transient holoenzyme complexes, which number in the hundreds, are cell type specific and serve to target the enzymes to their specific substrates. A key question is how is the formation of these holoenzyme complexes regulated by nutrients and other signals? (2) How are kinases regulated by O-GlcNAcylation? Many kinases are dynamically O-GlcNAcyated, and thus far, those studied are regulated by the glycan. How does this observation alter our view of signaling and system biological studies of cellular physiology? (3) How does O-GlcNAcylation play a role in neuronal functions and in learning and memory? O-GlcNAcylation is incredibly abundant in the mammalian brain, and in neurons, particularly at the synapse and in dendritic spines (42, 45, 80). Elucidation of O-GlcNAc's roles in normal neuronal functions and in brain biology will become a huge area of future research. (4) What are the specific roles of O-GlcNAcylation in nutrient regulation of transcription? While it is now clear that O-GlcNAcylation is fundamentally important in nearly every aspect of transcription, we currently know almost nothing with respect to its protein-specific or site-specific roles on individual transcription regulatory proteins. This area will remain an enormous challenge for some time to come.

Finally, while the tools to study O-GlcNAcylation have advanced substantially in the last three decades, there remains an acute need to develop better methods and approaches that can be applied by biologists. These include: (1) The development of many site-specific O-GlcNAc antibodies; (2) A molecular biology approach to either mimic O-GlcNAcylation or to generate site-specific O-GlcNAcylation on proteins; (3) Methods are needed that can raise or lower O-GlcNAcylation on individual proteins or at individual sites to evaluate functions. Unfortunately, current methods either based upon inhibitors or genetic approaches to alter O-GlcNAcylation, all act globally. (5) There continues to be a need for better methods to both detect and site-map O-GlcNAc on proteins. The challenges in this field are large but so is the pay off for our understanding of cellular physiology and chronic disease.

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