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Review

O-GlcNAcylation at promoters, nutrient sensors, and transcriptional regulation

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ABSTRACT

Post-translational modifications play important roles in transcriptional regulation. Among the less understood PTMs is O-GlcNAcylation. Nevertheless, O-GlcNAcylation in the nucleus is found on hundreds of transcription factors and coactivators and is often found in a mutually exclusive ying–yang relationship with phosphorylation. O-GlcNAcylation also links cellular metabolism directly to the proteome, serving as a conduit of metabolic information to the nucleus. This review serves as a brief introduction to O-GlcNAcylation, emphasizing its important thematic roles in transcriptional regulation, and highlights several recent and important additions to the literature that illustrate the connections between O-GlcNAc and transcription.

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1. Introduction

The modification of proteins with N-acetylglucosamine, or O-GlcNAc, is a vastly underappreciated post-translational modification, despite its discovery nearly 30 years ago. It is nearly as common as phosphorylation and is often found as part of a ying–yang state: many serine and threonine residues are either O-GlcNAcylated or phosphorylated [1]. In contrast to the large number of protein kinases and phosphatases in the cell, there are only two enzymes directly involved in O-GlcNAcylation [1]. The O-GlcNAc transferase (OGT) adds O-GlcNAc to S/T residues using UDP-GlcNAc as the nucleotide-sugar donor. β-D-N-glucosaminidase (OGA) is responsible for removing O-GlcNAc from proteins. The regulation of OGT and OGA is not well understood but a variety of evidence indicates that O-GlcNAcylation blocks phosphorylation, ubiquitination, and directly controls target enzyme activity and specificity. It is the dynamics of these events in concert with other post-translational modifications that create the regulatory functions of OGT and OGA.

A study of O-GlcNAcylation requires that one view this post-translational modification differently than, for example, phosphorylation. Most kinases are tightly regulated at least by their substrate specificity and activation, and their relatively narrow application to a relatively small number of processes. In contrast, OGT itself is responsible for several hundred targets. Substrate specificity, as far as we know it, is not the main thematic regulatory idea here; instead, the function of O-GlcNAcylation in the cell must be considered separately for each system utilizing it and is likely mediated by protein–protein interactions to recruit OGT and OGA to the necessary sites of action [2,3].

The substrate for OGT is UDP-GlcNAc and its synthesis occurs via a little-known pathway, the hexosamine biosynthetic pathway or HBP [4]. The HBP uses fructose-6-phosphate (F6P) as its starting material and thus splits off from the glycolytic pathway at this point. About 2–5% of glucose flux into the cell is shunted into the HBP [5]. The rate-limiting step is the next enzyme in the pathway, glutamine–fructose aminotransferase, which uses F6P and glutamine to synthesize glucosamine-6-P [4,6]. Thereafter, acetyl CoA and uridine are brought in to synthesize UDP-GlcNAc. As a result of these influxes of metabolic intermediates, it has long been thought that O-GlcNAc is a metabolic sensor of the nutrient state of the cell [4,7,8].

That the flux of glucose into the cell is reflected in the [UDP-GlcNAc] suggested that insulin-resistance and type II diabetes might be causally related to perturbations in the HBP or OGT and OGA activity [9]. There is much causal data to support this hypothesis. Overexpression of OGT in muscle and adipocytes led to insulin resistance and GFAT overexpression also resulted in insulin resistance in mice. Type II diabetes patients also exhibit elevated O-GlcNAc levels and an influx of glucosamine will induce insulin-resistance [9–11]. Experiments from several groups show that insulin signaling is regulated by OGT [12–14], that O-GlcNAcylation of Foxo1, presumably by an OGT/PGC-1a recruitment mechanism, increased gluconeogenesis-specific expression in the liver [15,16], and that the CREB coactivator, CRTC2, is O-GlcNAcylated and results in increased gluconeogenic gene expression [17]. Furthermore, the link between O-GlcNAc and insulin resistance has been extensively developed using genetics in *Caenorhabditis elegans* and *Drosophila* by Hanover and colleagues [18–21].

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There are several hundred transcription factors that are O-GlcNAcylated [3]. Among them are the p65 subunit of NF-kB, Sp1, CREB and CRCT2, p53, and myc. OGT is also in a complex with the histone deacetylase mSin3A, and OGT/mSin3A work in concert to repress transcription [22]. Lastly, OGT is super sex combs (sxc), a member of the polycomb group genes, and polycomb response elements contain O-GlcNAcylated proteins [23–25]. Furthermore, ogt mutants lost polycomb-dependent repression. It is clear from these studies and others that O-GlcNAcylation of transcription factors can affect transcription in many ways: nuclear localization, protein stability, prevention of phosphorylation, and increased transactivation potential [3,26].

This review will focus on advances in understanding the O-GlcNAcylation events that occur in the nucleus, how these events reflect the nutrient state of the cell and influence transcriptional output, and attempt to synthesize this material under a unifying thematic idea. The reader is referred to several more detailed and recent reviews of the literature for further reading [2,4,26–29].

2. TET family members and OGT

The Tet proteins are responsible for converting 5-methylcytosine (5mC) to 5-hydroxymethyl cytosine (5hmC). These enzymes have important functions in regulating the amount of 5mC in the cell and show that this too is a dynamic epigenetic mark. MLL family members are histone methyltransferases and often found as breakpoint fusion proteins in mixed-lineage leukemia (MLL). MLL-Tet fusions have been shown to be involved in several hematopoietic malignancies and appear to maintain the genome in a more pluripotent stem cell state [30]. The Tet proteins are not O-GlcNAcylated themselves but all three associate with OGT [31–33]. Genomically, these proteins localize to CpG islands and promoters and significantly overlap with H3 K4me3, which is a histone mark of actively transcribed genes and is predominantly localized to promoters [32]. Tet2 appears necessary for recruitment of OGT as a Tet2 shRNA abolished chromatin-associated OGT [31]. OGT and Tet2 ChIP-seq showed that they are both heavily concentrated at transcriptional start sites (TSS), as is O-GlcNAc [31–33]. Tet2 was also necessary for the H3 K4me3 events and Tet2 KO showed global decreases in O-GlcNAc [32]. Interestingly, treatment of cells with alloxan (an OGT and OGA inhibitor, [34,35]) also showed a decrease in H3K4me3 [32]. The interaction between Tet proteins and OGT illustrates a rather complex interrelationship between DNA methylation, H3 K4me3, and O-GlcNAcylation. Further elucidation of these connections should prove to be very interesting.

3. O-GlcNAc and chromatin

The Tet proteins are not the only chromatin-associated proteins associated with O-GlcNAcylation. O-GlcNAcylation of MLL5 histone methyltransferase augments retinoic-acid induced differentiation of HL60 macrophage cell line via its targeting histone H3 K4 residues for trimethylation [36]. MLL5's methylation activity increased several-fold after O-GlcNAcylation. Additionally, OGT is part of the MLL5 complex [36]. A second set of experiments by the same group showed that histone H2B was O-GlcNAcylated at S112 and this then stimulated histone H2B K120 monoubiquitylation, which is prevalent in transcriptionally active loci. Inhibition of GFAT in the HBP blocked this ubiquitinylation [37].

A second chromatin modifier, CARM1, is O-GlcNAcylated during the cell cycle. Increased O-GlcNAcylation from OGT overexpression resulted in a decrease of H3 R27 methylation by CARM1 suggesting that OGT plays important roles in the regulation of histone modifications [38].

Histone H3 is also O-GlcNAcylated. Using mass spectroscopy, T32 was identified as the O-GlcNAcylated residue and inhibition of OGA hindered the transition from G2 to M phase [39]. Other work showed that O-GlcNAcylation increased during G1 [38,39]. Histone phosphorylation dynamics were also susceptible to O-GlcNAcylation. For example, phospho-H3S10, a common mitotic mark, significantly decreased with OGT overexpression [38,40]. Similar results were seen by Sifers and colleagues by OGA inhibition [39], but it remains to be seen whether the MSK1/2-dependent phosphorylation of H3 S10 in interphase is also dependent on the state of O-GlcNAcylation of H3. Additional work showed that all four histones in humans are O-GlcNAcylated and LC-MS/MS was used to identify those residues: H2A T101, H2B S36, and H4 S47 [41]. The O-GlcNAcylation of the histones suggests that a further expansion of the histone code is warranted.

4. Regulation of circadian clocks

O-GlcNAcylation has recently been shown to play a significant role in the regulation of circadian clocks. This system serves as an excellent illustration of the interplay between OGT and OGA and how they both work in concert with and overlay their regulatory features upon a well-understood dynamical system. The core of the circadian clock consists of a transcriptional-translational feedback loop or TTFL. The activators BMAL1 and CLOCK bind to the promoters of the Per and Cry genes and activate their expression. Per and Cry proteins in turn repress their own expression. Once Per and Cry repress their expression, they then are degraded over time and BMAL1/CLOCK begin the cycle again. The periodicity of this loop is for the most part dependent on the half-life of the Per and Cry proteins. However, overlaying this core machinery are several post-translational modifications that further regulate and fine-tune the TTFL [42–44]. Recently, several papers have documented the role of O-GlcNAc in circadian clock regulation.

GSK3β is an important regulator of circadian clocks. Specifically, GSK3β inhibition shortens clock periodicity [45,46]. Additionally, GSK3β is a substrate of OGT [47]. Using ATP analogs and mutant GSK3β OGT was identified as a GSK3β substrate [48]. Furthermore, phosphorylation of OGT by GSK3β increased its activity. O-GlcNAc levels perturbed by OGT siRNA also shortened the clock periods while an OGA inhibitor did the opposite [48]. Similar conclusions were obtained by Kim and colleagues [49] (see also Fig. 1).

Several clock proteins were also identified as OGT substrates. O-GlcNAcylation of Per2 appears to block its phosphorylation, increasing Per2 stability, and thereby augmenting Per2 repression [48,49]. Although OGT levels did not oscillate, OGA levels did oscillate in a circadian manner, suggesting that it is the regulation of OGA that is controlling the periodicity. In a human cell line siRNA depletion of GFAT and OGT slightly decreased the expression of BMAL1 and Cry [50]. More strikingly, the GFAT inhibitor azaserine severely affected the periodicity of expression of a BMAL-luciferase reporter. OGT overexpression increased BMAL1 and CLOCK protein half-lives. Finally, alloxan (OGT inhibitor) and OGT siRNA both decrease the amplitude of the clock oscillations and shorten the period, while PUGNAc (OGA inhibitor) also decreases the amplitude lengthened the period of the oscillations [48]. Increasing OGT levels also increased the amplitude of clock oscillations. These data illustrate essential functions of OGT and OGA in the regulation of circadian rhythms, controlling both period length and transcriptional output (see also Fig. 1).

Circadian clocks are also regulated metabolically in order to entrain or reset the peripheral clock. This entrainment is potentially a problem as it shifts the metabolism of food intake away from the suprachiasmatic clock, which is controlling the wake/sleep cycle [43]. There are several ways in which metabolism can impinge on peripheral clock regulation: AMPK and NAD+ dependent deacetylases, for example [9]. Defects in the clock regulation also have severe metabolic consequences: BMAL knockouts in the liver cause hypoglycemia and Cry overexpression results in hyperglycemia [51,52]. Clearly the metabolic and circadian systems are interconnected and utilize some sort of feedback loop. The point made by the O-GlcNAc studies is that since UDP-GlcNAc levels are responsive to glucose flux into the cell, and thus is a nutrient sensor, then the utilization of O-GlcNAcylation is a clear metabolic imprint on circadian clock regulation.