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Nutrient Regulation of Gene Expression by O-GlcNAcylation of Chromatin

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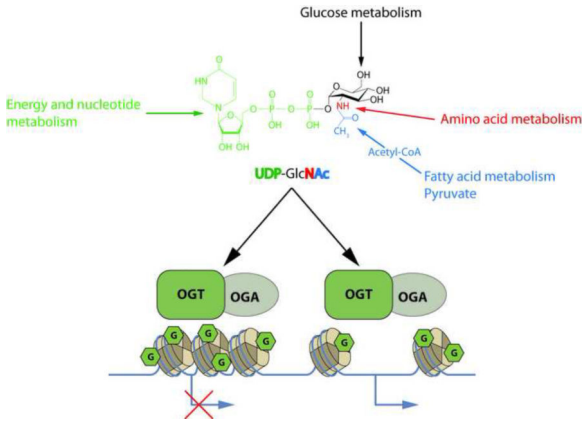
Abstract

O-GlcNAcylation is a dynamic post-translational modification that is responsive to nutrient availably *via* the hexosamine biosynthetic pathway and its endproduct UDP-GlcNAc. O-GlcNAcylation serves as a nutrient sensor to regulate the activities of many proteins involved in nearly all biological processes. Within the last decade, OGT, OGA and O-GlcNAcylation have been shown to be at the nexus of epigenetic marks controlling gene expression during embryonic development, cell differentiation, in the maintenance of epigenetic states and in the etiology of epigenetic related diseases. OGT O-GlcNAcylates histones and epigenetic writers/erasers, and regulates gene activation, as well as gene repression. Here, we highlight recent work documenting the important roles O-GlcNAcylation and its cycling enzymes play in the nutrient regulation of epigenetic partners controlling gene expression.

Graphical Abstract



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Introduction

Can what we eat change our genetics? Beside toxic compounds that have the ability to induce mutations, our eating habits don't change the nucleotide sequence of our genes. However, organisms with a predefined set of genes have to maintain proper homeostasis and adapt to their environment, especially to adapt to nutrient availability. Phenotype adaptation is a long-term adjustment to the environment and can be done by heritable encoded information on DNA without changes in the gene sequence [1]. This second layer of information is called epigenetics and includes DNA methylation, post-translational modifications (PTMs) of histones and chromatin remodeling. Epigenetics is also an important feature of embryogenesis and cell fate, controlling and defining transcriptional pattern crucial for cellular lineage.

The first evidence that link O-GlcNAcylation to chromatin and transcription was found in Drosophila [2]. O-GlcNAcylation is a versatile PTM controlled by two non-redundant enzymes: the O-GlcNAc transferase (OGT) transfers the GlcNAc moiety from UDP-GlcNAc to a serine or a threonine residue, while the O-GlcNAcase (OGA) removes the modification. UDP-GlcNAc is a main cellular nutrient sensor since its synthesis through the hexosamine biosynthetic pathway (HBP) depends on flux through every major metabolic pathway (graphical abstract). Since OGT's enzymatic activity and substrate specificity varies according UDP-GlcNAc concentration, variation in metabolism that feed the HBP have profound effects on protein O-GlcNAcylation [3]. Within the last decade, studies have defined O-GlcNAcylation as an epigenetic mark and linked its cycle to the regulation of chromatin modifications.

Multiple roles of histone O-GlcNAcylation

The histone code is written by molecular complexes that add or remove part of the code in response to various cellular stimuli or metabolism. Although a recent paper called into question histone O-GlcNAcylation [4], the presence of the sugar on each subunit of the nucleosome has been reported independently by many laboratories and some sites have been mapped (reviewed in [2]). Some of the site-specific functions have been documented (Figure 1).

The O-GlcNAc/phosphorylation interplay on histone H3 is essential for mitosis. Overexpression of OGT reduces phosphorylation of $H3^{S10}$ and leads to errors in chromosomal segregation, while OGA inhibition impairs G2-M transition [5,6]. The $H2B^{S112O\text{-}GlcNAc}$ mark is associated with DNA damage response and genomic stability [7]. O-GlcNAcylation at $H2B^{S112}$ is increased at DNA double strand breaks. Down-regulation of OGT or $H2B^{S112A}$ mutant over-expression impairs homologous repair (HR) and non-homologous end joining. Since $H2B^{S112O\text{-}GlcNAc}$ stimulates $H2BK^{120}$ ubiquitination that activates the ring finger protein 20 [8], OGT and O-GlcNAcylation could be key initiators for the recruitment of the HR complex in response to DNA damage.

Histone O-GlcNAcylation is linked to gene transcription. The sugar at T101 of H2A destabilized H2A/H2B dimmers in the nucleosome, promoting an open chromatin state [9].

This suggests that O-GlcNAcylation at $H2A^{T101}$ would lower the barrier for RNA polymerase passage and hence increase transcription. H2B O-GlcNAcylation at S112 has been reported to have multiple roles. In HepG2 cells, activated AMPK phosphorylates OGT, which lowers $H2B^{S112}$ O-GlcNAcylation and inhibits expression of genes regulated by $H2B^{S112O\text{-}GlcNAc}$ [10]. In HeLa cells, $H2B^{S112}$ O-GlcNAcylation co-localizes with $H2B^{K120Ub}$ mark. The $H2B^{K120Ub}$ mark acts as a platform for the SET1/COMPASS complex that stimulates $H3^{K4}$ trimethylation and gene transcription. Conversely, H2B O-GlcNAcylation is a stable chromatin landmark during adipocyte differentiation [11]. Ronningen *et al.* identified long $H2B^{S112O\text{-}GlcNAc}$ enriched domains, called GADs, ranging from 60kb to about 10Mb. At the early stage of adipogenesis, lamin-associated domains rearrange following GADs pattern, releasing the repression of genes mainly related to metabolic processes, but repressing genes within GADs [11], suggesting a repressive role for $H2B^{S112O\text{-}GlcNAc}$ in cell fate.

While yeast apparently lack O-GlcNAcylation and O-GlcNAcylation enzymes, it was recently reported that O-Man glycosylation of nuclear and cytoplasmic proteins mirror mammals O-GlcNAcylation [12]. A peptide covering the K123 of yH2B (ubiquitination of $yH2B^{K123}$ is homologue of the mammalian $H2B^{K120Ub}$) is O-Man glycosylated. Considering that glucose metabolism increases both mammalian $H2B^{K120ub}$ and yeast $H2B^{K123Ub}$ [13,14], O-Man glycosylation of yH2B could mimic the molecular mechanism of $H2B^{K120}$ ubiquitination mediated by $H2B^{S112O\text{-}GlcNAc}$ observed in mammals.

As mentioned above, discrepancies and lack of reproducibility regarding Histone O-GlcNAcylation have been observed recently [4]. These issues could arise because investigations have used different models and different techniques to assess histone O-GlcNAcylation. As different studies have suggested, some of if not all histone O-GlcNAcylation could be cell type specific, highly dynamic and cell cycle stage specific and/or restricted to a specific developmental stage [5,6,7,11]. Although the $H2B^{S112O\text{-}GlcNAc}$ site-specific antibody is commercially available, some concerns have been raised regarding its specificity [2,4]. Re-analysis of five randomly picked loci in the top 20 hits of the $H2B^{S112\text{-}O\text{-}GlcNAc}$ ChIP-seq data from Fujiki et al., using this antibody by Gambetta et al., has pointed out that, except for the locus of *GSK3B*, the signal over background of theses specific loci is low and might not be of sufficient quality to justify the authors' conclusions. In general, the conditions to work with O-GlcNAc site-specific antibodies, which often have low affinities, are very precise and specific due to the nature of the antigen and the lack of counter selection (i.e. antibody must recognize both peptide backbone and GlcNAc moiety but none of them separately). It is essential when developing and using O-GlcNAc site-specific antibodies, that the specificity be well documented and as many details as possible should be provided in the methods section when publishing with these antibodies in order to ensure reproducibility.

OGA, a histone acetyltranferase?

The histone acetyltransferase (HAT) activity of OGA has been controvertial. Its putative HAT domain is located in the C-terminal domain, while the O-GlcNAcase activity resides in the N-terminal domain of the molecule. Although our lab was not able to observe this

activity *in vitro* [15], others have reported it in different publications. Toleman *et al.* have shown that mouse OGA acetylates histones *in vitro* and mutations within the C-terminal domain lead to substantial loss of its HAT activity [16]. Hayakawa *et al.* over-expressed OGA in emybryonic stem cells (ESC) and observed a decreased HAT activity toward $H3^{K14}$ and $H4^{K8}$ upon Thiamet-G treatment or with the expression of OGA^{D175A}, which also showed a significant decrease in O-GlcNAcase activity [17], suggesting that O-GlcNAcase activity is required for HAT activity *in vivo*. Conversely, a structural study of the HAT domain of human OGA has shown that it shouldn't be able to bind Acetyl-CoA [18]. Since recombinant OGA purified from bacteria acetylated $H3^{K14}$ and $H4^{K8}$ but only when pre-incubated with mammalian cell lysates [16], it is then possible that the observed HAT activity could be due to interaction and activation with a third HAT. Nevertheless the HAT domain appears to be important and could act as a scaffold for HAT interactions and OGA function.

OGA function is fundamental during embryogenesis since its genetic disruption leads to nearly complete neonatal lethality with developmental delay [19,20]. At the cellular level, OGA KO induces increased O-GlcNAcylation and leads to mitotic defects associated with cytokinesis failure and binucleation, accompanied by increased lagging chromosome and micronuclei formation [19]. The antagonistic relationship between OGT and OGA toward the histone code is likely important for the regulation and the maintenance of genomic stability, especially considering that $H2B^{S112}$ O-GlcNAcylation is associated with and facilitates DNA homologous recombination, as well as the essential de-GlcNAcylation of H3 for proper mitosis [5,6].

OGT and O-GlcNAcylation regulate epigenetic marks

Much evidence strongly suggest that nutrients impact epigenetic modifications of chromatin [21] and recent publications have highlighted the important role of the nutrient sensors OGT and O-GlcNAcylation modulating chromatin marks.

Nutrient and energy sensor interplay

The energy sensor 5'-AMP-Activated Protein Kinase (AMPK), phosphorylates OGT at T444 and targets it to the nucleus, which is correlated with increased $H3^{K9Ac}$ in C2C12 myoblast cells. $H3^{K9Ac}$ levels are significantly decreased with nutrient/growth factor deprivation and increased with an AMPK activator [22]. Phosphorylation of OGT inhibits OGT:chromatin association, histone O-GlcNAcylation and gene transcription. Using a KO/KI strategy, OGT phosphorylation by AMPK was shown to impair $H2B^{S112}$ O-GlcNAcylation [10]. Phosphorylation of H2B at S36 by AMPK is essential for transcription and survival in response to metabolic stress [23]. Since $H2B^{S36}$ is also a target of OGT [24], O-GlcNAc/phosphorylation interplay may occur at $H2B^{S36}$ in a responsive to nutrient/energy availability manner. Also, since phosphorylation of OGT at T444 by AMPK affects OGT's substrate selectivity [22] and O-GlcNAcylation of AMPK positively regulates its activity [10], the crosstalk between both enzymes is not limited to site-specific competition but also results from a regulatory feedback loop (figure 1).

Coactivator-associated arginine methyltransferase 1 (CARM1) is regulated by O-GlcNAcylation (Figure 1). CARM1 catalyzes the methylation of the histone H3 at R2, R17 and R36. In diabetes, CARM1 activates expression of key genes involved in gluconeogenesis and glycogen metabolism. Over-expression of OGT decreases $H3^{R17me2}$ [5], suggesting that O-GlcNAcylation interplays with histone marks *via* CARM1. Although O-GlcNAcylation does not appear to regulate the methyltransferase activity of CARM1, it does appear to control its substrate specificity since enriched O-GlcNAcylated CARM1 isoform methylates a different set of targets compared to the non-O-GlcNAcylated enzyme [25].

OGT, Tet proteins and 5hmC

Ten-eleven translocation (Tet) family proteins are enzymes that catalyze oxidation of 5-methylcytosine (5mC) in DNA to produce 5-hydroxymethylcytosine (5hmC). 5hmC is predominant at transcription start sites and on promoters of genes with bivalent chromatin, harboring activating and repressive marks, or throughout the bodies of transcriptionally active genes. Simultaneous active and repressive marks, *i.e.* $H3^{K4me3}$ and $H3^{K27me3}$ respectively, are an important feature that controls gene expression supervising cell fate and differentiation. Tet1, Tet2 and Tet3 interact with OGT [3] and are extensively O-GlcNAcylated. Tet1 could have at-least 6 O-GlcNAcylation sites, while Tet2 and Tet3 could have at-least 20 O-GlcNAcylated serine or threonine moieties, some of which are in competition with phosphorylation [26].

Knockdown of OGT reduces Tet1 protein levels [27] and 5hmC on its targets [27,28], while a Tet1 mutant at the T535 putative O-GlcNAcylation site is no longer stabilized by OGT over-expression [27]. However, O-GlcNAcylation of Tet3 is responsive to increased glucose concentration, which was correlated with the nuclear export of the proteins and therefore associated with a decrease of 5hmC catalyzed by Tet3 [29], suggesting that the increased glucose effect on DNA demethylation *via* O-GlcNAcylation could be restricted to Tet1. It was also reported that, although Tet1 and Tet2 are O-GlcNAcylated, neither their localization nor their activity was affected by the sugar [29]. Since the authors of these studies have used different models, the regulation of Tet proteins' activity by O-GlcNAcylation could be cell/tissue specific.

O-GlcNAcylation associated with gene activation (figure 2)

Tet proteins target OGT to chromatin and O-GlcNAcylation of chromatin proteins is decreased upon Tet protein knockdown [30–32]. At gene specific loci, Tet3 targets OGT to GlcNAc transferase 9 (GnT-XI) promoters and the complex acts as a dock for the recruitment of NeuroD1, required for the expression of GnT-XI in a brain-specific manner [33]. Chip-seq analyses show that Tet proteins interact with OGT at the transcription start site (TSS) near CpG-rich regions and co-localized with the $H3^{k4me3}$ active mark [28,30,31]. Tet2 and Tet3 target OGT to TSS in association with Host Cell Factor C1 (HCF-1) (a member of the SETI/COMPASS complex). Both Tets and OGT activity favor integrity of SET1/COMPASS and recruitment of SET Domain containing 1A to chromatin, which favors tri-methylation of $H3^{K4}$ at target promoters [30]. Tet2 drives OGT to chromatin and enhances $H2B^{S112}$ O-GlcNAcylation [31]. O-GlcNAcylation of H2B increases its

ubiquitination at K120 that stimulates $H3^{K4}$ tri-methylation through the SETI/COMPASS complex. OGT:HCF-1 also form a complex with Lysine-(K)-Specific Methyltransferase 2E (MLL5), the enzyme that catalyses the mono- and di-methylation of $H3^{K4}$. The complex OGT:HCF-1:MLL5 is recruited to E2F transcription factor 1 (E2F1)-responsive promoters and stimulates $H3^{K4}$ trimethylation at the promoters, and promotes activation of E2F1 target genes during G1/S transition [34]. OGT stabilizes and heavily O-GlcNAcylates MLL5 [35]. Since deep sequencing data show that MLL5 preferentially binds at TSS and CpG rich regions, suggesting a link with DNA methylation, OGT could be a scaffold protein for Tet proteins, SET1/COMPASS and MLL5. In *Drosophila*, dmOGT is encoded by the polycomb group gene: *scx*, and, like in mammals, its knockout is lethal at the pharate stage. Over-expression of $dmOGT^{WT}$, as well as $dmOGT^{D955A}$ (a catalytic dead mutant), in scx^{1}/scx^{6} genetic background rescue the lethal phenotype [36], which strongly suggests that not only O-GlcNAc transferase activity is important for embryonic development but the protein itself is as well. All together, these studies indicate crucial roles of Tet proteins for targeting OGT to chromatin in order to catalyze chromatin modification favorable to transcription activation.

O-GlcNAcylation associated with gene repression (figure 3)

Catalytic subunits of polycomb repressive complex 1 and 2 (PRC1 and PRC2), which are required for maintenance of repression of homeotic genes during embryonic development, cell proliferation and differentiation, are regulated by O-GlcNAcylation. In hESC, the protein Ring Finger Protein 2 (RING1b; a subunit of the PRC1 complex that catalyzes $H2B^{K119}$ ubiquitination, a histone mark associated with transcription repression) is O-GlcNAcylated at T250 and/or T251 and S278. Non-O-GlcNAcylated RING1b complex is enriched at the promoter of genes mainly related to metabolism and cell cycle, while O-GlcNAcylated RING1b complex is enriched at promoters of genes mainly related to neural differentiation process [37], suggesting that O-GlcNAcylation targets the PRC1 complex to specific sets of genes.

During neuronal differentiation, an epigenetic switch at the locus of the orexin gene (*Hcrt*), involving DNA methylation and histone acetylation, leads to the generation of orexin producing neurons. At the locus *Hcrt*, in its inactive state, O-GlcNAcylation and OGT co-localize with Sirtuin 1 (Sirt1), with SIN3 Transcription Regulator Family Member A (Sin3A) and with Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2), a subunit of PRC2 complex that carries a $H3^{K27}$ methyltransferase activity. In contrast, OGA, E1A Binding Protein p300 (p300) and CREB Binding Protein (CBP) are present at the active *Hcrt* locus [17] (Figure 2). Interestingly, Sin3A and Histone Deacetylase 1 (HDAC1), thought to functionally repress transcription in parallel with histone deacetylation, are part of the complex formed by OGT, Tet1 and Tet3 [27,28,30]. At bivalent promoters that are considered to be poised for expression of developmental genes, Tet1 is enriched with Sin3A or with PRC2 about 39bp upstream and 455bp downstream of the TSS, respectively [38]. Considering that OGT stabilizes EZH2 by O-GlcNAcylation at S75, which promotes $H3^{K27me3}$ [39], Tet1:OGT would lead to repress transcription *via* sin3A:HDAC1:Sirt1 and PRC2:EZH2 at both upstream and downstream TSS. Remarkably, the kinase MSK phophorylates $H3^{S28}$ in the presence of $H3^{K27me3}$, which leads to the dissociation of the

PRC2 complex and gene transcription without removal of the tri-methyl mark on residue 27 of H3 [40]. Owing to the fact that the O-GlcNAcylation of $H3^{S32}$ impairs phosphorylation at S28, OGT activity could act at multiple levels to lock transcription in an “off” state at specific loci.

Conclusions and future outlook

It is clear that O-GlcNAcylation and its cycling enzymes are important regulators of epigenetics. The sugar itself is a chromatin mark and its interplay with other PTMs to histones establishes a specific pattern recognized by protein complexes to activate or repress gene expression. Deregulation of O-GlcNAcylation, observed in diabetes, cancer and neurodegenerative diseases [41], could, by altering chromatin marks and gene expression, underlie the etiology of these diseases.

As a nutrient sensor, O-GlcNAcylation is at the nexus between food intake and epigenetics. In fact, western-diet or exercise modulates OGT’s association with epigenetic writers in mice [42,43]. Since epigenetic changes can be inherited during cell division and maintained as an acquired phenotype, our eating habits and even life style have the potential to be imprinted in our genes. Many studies have highlighted the impact of nutrients or diabetes during pregnancy affecting offspring. Some of these effects are still detectable at the F3 and therefore transgenerational [44–48]. In the light that O-GlcNAcylation is deregulated under diabetes and obesity and considering its crucial role as a nutrient sensor and modulator of epigenetics, O-GlcNAcylation, as an epigenetic mark, could be one of the molecular mechanisms of nutrient- and stress-dependent non-DNA sequenced encoded inheritance phenotypes.

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Highlights

- Nearly all transcription proteins are dynamically O-GlcNAcylated
- Histone O-GlcNAcylation interplays with other histone marks
- Epigenetic writers/erasers are regulated by the sugar
- OGT and OGA are required for the transcription cycle

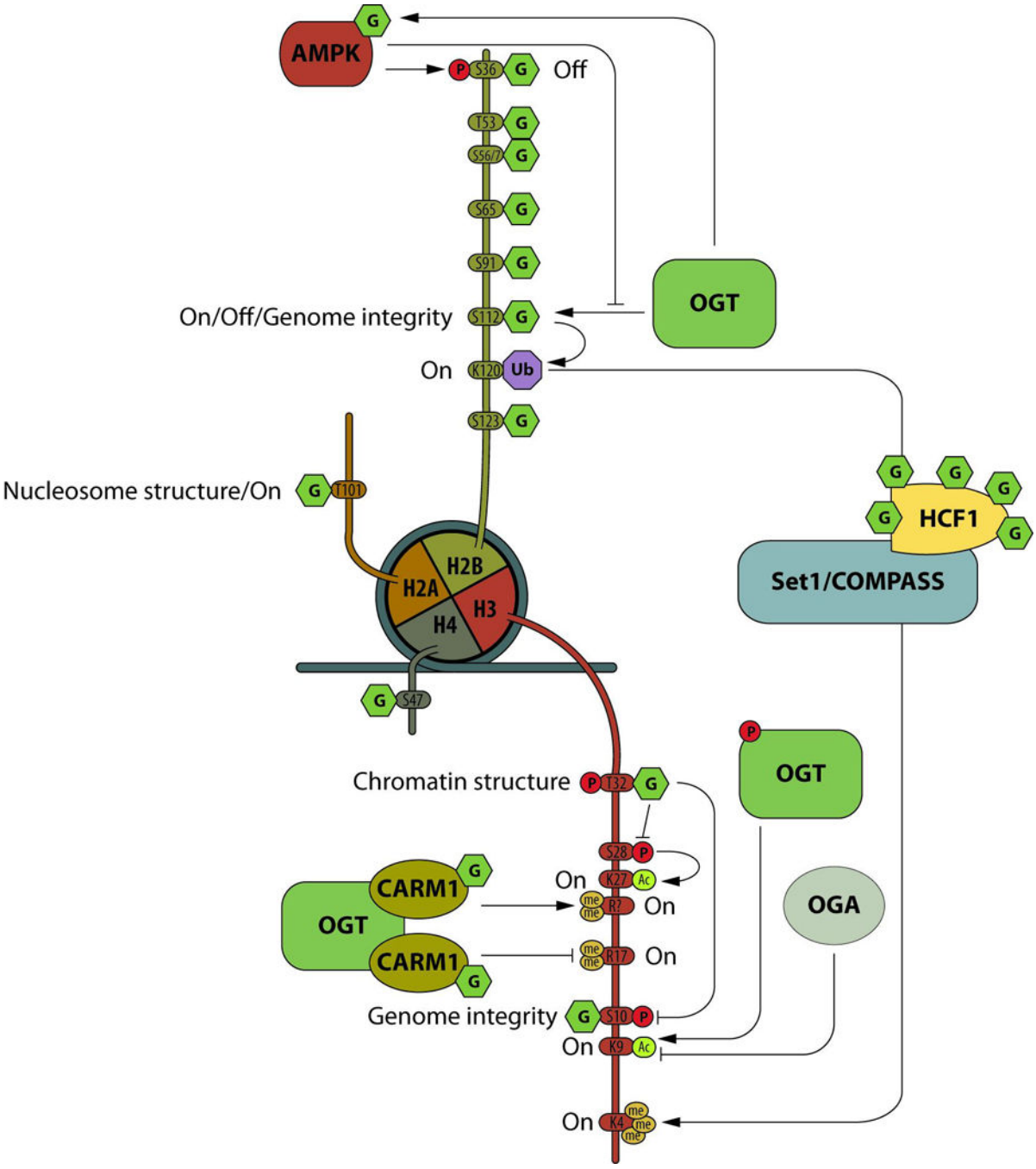

Figure 1. Nucleosome O-GlcNAcylation

The O-GlcNAcylation (G) of the histone core is extensive and interplays with other PTMs, such as phosphorylation (P), methylation (me), acetylation (Ac), or ubiquitination (Ub). All four subunits of the nucleosome are modified by the sugar, and site specific O-GlcNAcylation is involved in gene transcription activation (on) or repression (off), chromatin structure or genomic stability.

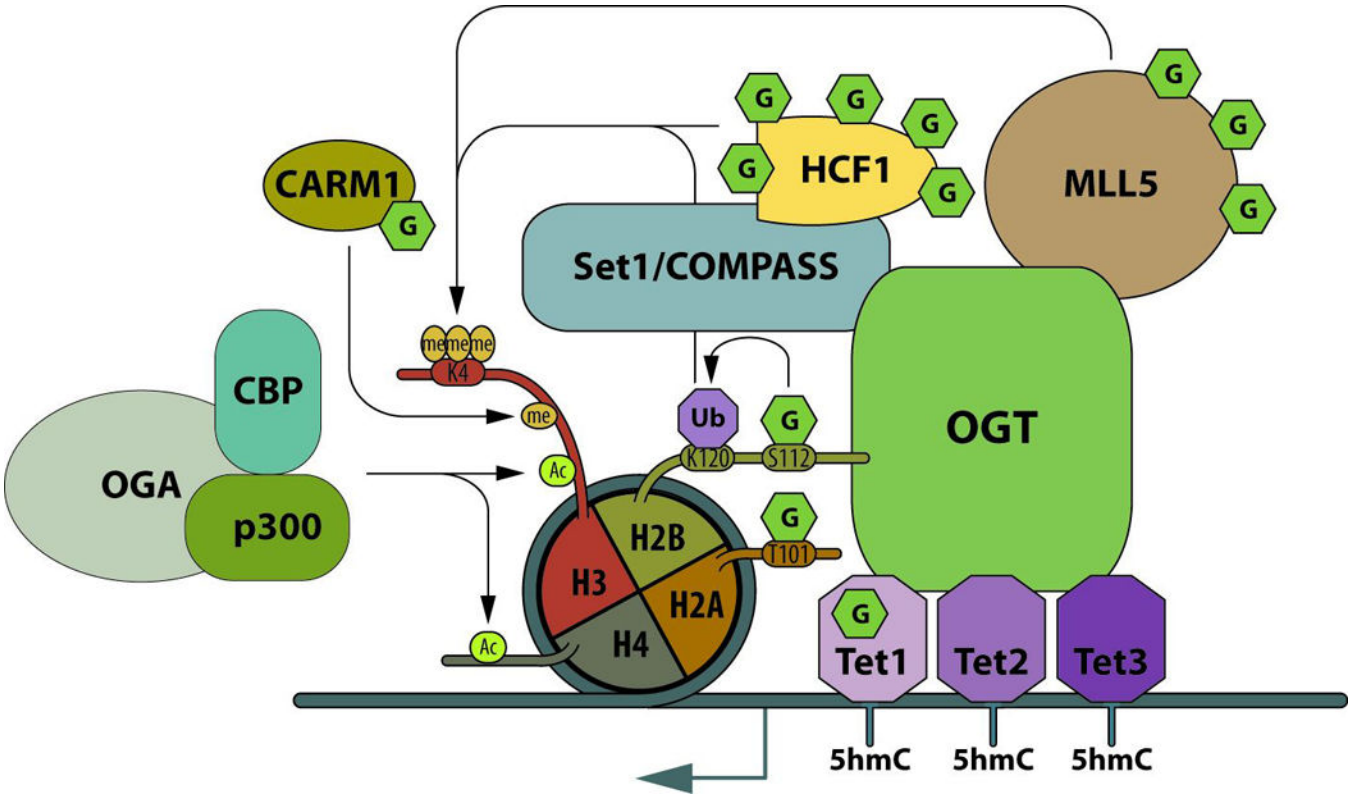

Figure 2. OGT and O-GlcNAcylation are key regulators of transcription activation

OGT is targeted to chromatin by Tet proteins, where OGT and O-GlcNAcylation regulates the conversion of 5mC to 5hmC. At promoters, OGT is a scaffold at the center of epigenetic regulators and O-GlcNAcylation of some of these proteins regulates their activity leading to gene activation. At gene specific promoters, OGA is recruited to the chromatin, where it forms a complex with the histone methyltransferases: p300 and CBP. Acetylation of H3 and H4 are mainly associated with gene activation. O-GlcNAcylation: G; methylation: me; ubiquitination: Ub; and acetylation: Ac.

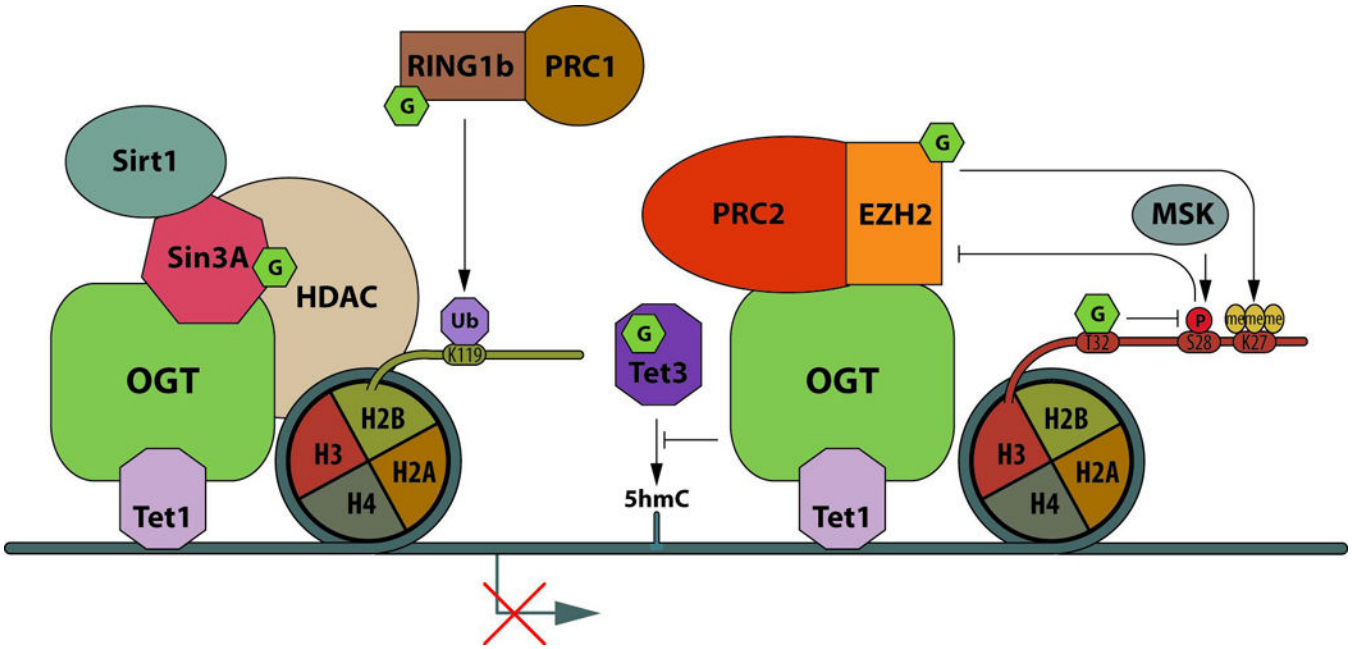

Figure 3. Gene transcription repression by OGT and O-GlcNAcylation

OGT is targeted to gene promoters by Tet1. Upstream of the TSS (around −39bp, near the area where the $TF_{II}D$ complex is recruited to DNA), OGT:Tet complex recruits the transcription repressor sin3A, as well as the histone deacetylases, HDAC1 and Sirt1. Downstream of the TSS (around +455bp), OGT:Tet1 are associated with the PRC2 complex. O-GlcNAcylation of the methyltransferase subunit of PRC2, EZH2, enhances its activity by stabilization of the protein. O-GlcNAcylation of RING1b (the catalytic subunit of the PRC1 complex) also increases its activity leading to $H2B^{K119}$ ubiquitination. All of these PMTs crosstalk and converge to result in gene transcription repression.