O-GlcNAc and the Epigenetic Regulation of Gene Expression*

Published, JBC Papers in Press, October 21, 2014, DOI 10.1074/jbc.R114.595439
Brian A. Lewis1 and John A. Hanover†2
From the 1NCI and 2NIDDK, National Institutes of Health, Bethesda, Maryland 20892

O-GlcNAcylation is an abundant nutrient-driven modification linked to cellular signaling and regulation of gene expression. Utilizing precursors derived from metabolic flux, O-GlcNAc functions as a homeostatic regulator. The enzymes of O-GlcNAc cycling, OGT and O-GlcNACase, act in mitochondria, the cytoplasm, and the nucleus in association with epigenetic “writers” and “erasers” of the histone code. Both O-GlcNAc and O-phosphate modify repeats within the RNA polymerase II C-terminal domain (CTD). By communicating with the histone and CTD codes, O-GlcNAc cycling provides a link between cellular metabolic status and the epigenetic machinery. Thus, O-GlcNAcylation is poised to influence trans-generational epigenetic inheritance.

Intermediary metabolism, the process by which nutrients are converted into cellular biomass, is an interwoven network of biochemical reactions allowing reproduction, development, and response to the environment. The intermediary metabolic network is highly conserved and includes every cellular process ranging from DNA replication to transcription and translation to enzyme regulation. Epigenetics, the study of how genes may alter phenotypes beyond their ability to genetically encode information, is ultimately linked to intermediary metabolism (1). Many enzymes that participate in epigenetic gene regulation depend upon co-substrates produced by cellular metabolism, thus providing a potential link between metabolism and gene regulation (2). Mitochondria, key players in these metabolic inter-conversions, exhibit a pattern of cytoplasmic inheritance distinct from Mendelian inheritance of genes encoded in the nucleus (3). O-GlcNAcylation is a key integrator of cellular nutritional status and occurs in the nucleus, cytoplasm, and mitochondrion. O-GlcNAc transferase (OGT)3 utilizes UDP-GlcNAc to catalyze the addition of O-GlcNAc to target proteins. UDP-GlcNAc is the end product of the hexosamine biosynthetic pathway (HSP), a series of enzymatic reactions requiring key metabolites, including glucose, glutamine, ATP, and acetyl-CoA (4). The nutrient-derived precursors render the synthesis of UDP-GlcNAc and subsequent O-GlcNAc addition by OGT nutrient-responsive. The O-GlcNAcase (OGA; MGEA5) removes the O-GlcNAc modification, and evidence suggests that its transcription and activity is highly regulated. Both enzymes of O-GlcNAc cycling contain domains that allow them to bind to epigenetic modifiers (5, 6). As illustrated in Fig. 1, UDP-GlcNAc is central both to the formation of O-GlcNAc but also to the synthesis of membrane and secretory glycoproteins that perform essential roles in extracellular signaling. The intracellular O-GlcNAc modification plays a role in signaling, leading to growth and apoptosis (7–9), metabolism (10, 11), and the cell cycle (12–14). In addition, O-GlcNAc has been implicated in translation (15), circadian rhythm (16), the establishment of molecular memory in neurons (17), and calmodulin-kinase signaling (16).

The focus of this review is the role of O-GlcNAc in transcription and epigenetics (5, 13, 18–20). As shown in Fig. 1A, O-GlcNAc modifies transcription factors (21), epigenetic modulators (5), and RNA polymerase II (18–20). These targets suggest that O-GlcNAc may be poised to be a metabolically sensitive effector of gene expression and may function as an epigenetic modifier (Fig. 1B). Fig. 1B highlights the metabolic inter-convolutions required for the synthesis of UDP-GlcNAc that involve a dynamic interplay between cytoplasm, mitochondria, and the nucleus. Second, the figure illustrates dynamic modification of proteins both in the nucleus and mitochondria by differentially localized forms of OGT. Finally, we describe those epigenetic effectors, including transcription factors, histones, TET1–2, and RNA polymerase II, that are modified by O-GlcNAc to influence their function. These diverse functions of O-GlcNAc cycling suggest that the pathway plays a key role in human diseases of aging such as metabolic syndrome, diabetes, Alzheimer disease, heart disease, and cancer (16, 22, 23). Indeed, the O-GlcNAc gene is a susceptibility locus for obesity and type II diabetes (24). Growing evidence also suggests that OGT may play a key role in the intrauterine environment (5, 22, 25, 26). Thus, the impact of O-GlcNAc cycling in trans-generational epigenetic phenomena is an emerging area of interest.

The Enzymes of O-GlcNAc Cycling

O-GlcNAc is added by the enzyme OGT to serine and threonine of target proteins in the cytoplasm, nucleus, and mitochondrion (27, 28). The single mammalian OGT gene is located on the human X chromosome near the Xist locus and encodes multiple alternatively spliced isoforms of OGT targeted to these different intracellular locations (27, 28). The three major deacetylase; pol II, polymerase II; PTM, post-translational modifications; TCA, tricarboxylic acid.

* This work was supported, in whole or in part, by National Institutes of Health intramural grants from the NCI (to B. A. L.) and NIDDK (to J. A. H.). This is the third article in the Minireview Series on the Thirtieth Anniversary of Research on O-GlcNAcylation of Nuclear and Cytoplasmic Proteins: Nutrient Regulation of Cellular Metabolism and Physiology by O-GlcNAcylation.

1 To whom correspondence may be addressed: Lymphoid Malignancies Branch, NCI, National Institutes of Health, Bldg. 10, Rm. 6B05, 10 Center Dr., Bethesda MD, 20814. Tel: 301-435-8323; E-mail: lewisbr@mail.nih.gov.

2 To whom correspondence may be addressed: Laboratory of Cell and Molecular Biology, NIDDK, Bldg. 8, Rm. B127, National Institutes of Health, Bethesda, MD 20892-0851. Tel: 301-496-0943; Fax: 301-496-9431; E-mail: jah@helix.nih.gov.

3 The abbreviations used are: OGT, O-GlcNAc transferase; OGA, O-GlcNACase; mOGT, mitochondrial OGT; nOGT, nucleocytoplasmic OGT; sOGT, short OGT; HSP, hexosamine signaling pathway; HBP, hexosamine biosynthetic pathway; CTD, C-terminal domain; TPR, tetratricopeptide repeats; AdoMet, S-adenosylmethionine; TET, Ten-eleven translocation; HDAC, histone deacetylase; pol II, polymerase II; PTM, post-translational modifications; TCA, tricarboxylic acid.
isoforms are termed mOGT (mitochondrial OGT), ncOGT (nucleocytoplasmic OGT), and sOGT (short OGT) (Fig. 1B). The mOGT isoform is targeted to the mitochondrial matrix and is proapoptotic (28, 29). The sOGT isoform, present in the nucleus and cytoplasm, is antiapoptotic (28, 30) and may inhibit the action of the other isoforms (29, 31). The longest isoform, ncOGT, is present in the nucleus and cytoplasm and contains 13 complete tetratricopeptide repeats (TPR) (32–36). The mOGT and sOGT variants contain 9 and 2 TPR, respectively (37). The TPR mediate interaction with a large number of effector proteins that target or regulate OGT (38, 39). It is likely that the TPR also serve a scaffolding function, distinct from any role in enzymatic activity (38). All three isoforms share a common catalytic domain with a deep UDP-GlcNAc binding pocket and a groove for target peptide binding (40).

The O-GlcNAcase is encoded by a single gene on human chromosome 10 (26). Two major O-GlcNAcase isoforms are produced by alternative splicing of the O-GlcNAcase gene. One of these contains a domain with similarities to a histone acetyltransferase domain but lacking the critical residues for catalytic activity. This has been termed a pseudoHAT domain (41). The other major O-GlcNAcase isoform has a 14-amino extension that serves to target it to lipid droplets. This O-GlcNAcase isoform is involved in the remodeling of lipid droplet surface proteins by local activation of proteasomes on the lipid droplet surface (38, 42). This study suggested a nexus between the regulation of lipid storage and hexosamine signaling through O-GlcNAc cycling (42).

**The Synthesis of UDP-GlcNAc as a Metabolic Sensor Linked to O-GlcNAcylation**

The levels of both enzyme substrate and product are critical regulators of the activities of most of the enzymes of intermediary metabolism. This is in contrast to the enzymes mediating intracellular signaling, which are not typically regulated in this way. Protein kinases, which use ATP as a substrate, recognize and phosphorylate target proteins, but with the sole exception of the AMP-regulated protein AMPK (43, 44), kinases do not respond to ATP levels. Protein kinases bind to ATP with an affinity that is saturated by normal levels of intracellular ATP. This effectively isolates kinase signaling from normal intracellular metabolism. O-GlcNAcylation appears to be regulated quite differently. The synthesis of UDP-GlcNAc is a tightly regulated process requiring precursors derived from glucose, glutamine, acetyl-CoA, ATP, and uridine (4)(Fig. 1B). Precursor levels drive the flux through this pathway, but the overall output is limited by substrate inhibition of the key rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). UDP-GlcNAc inhibits this enzyme, creating a feedback loop that serves to limit the levels of total UDP-GlcNAc (4). However, the situation is much more complex in a cellular context. UDP-GlcNAc levels are also limited by epimerization to UDP-GalNAc for the synthesis of O-linked glycoproteins in the endomembrane system. Other inter-conversions can occur within nucleotide sugar pools themselves to form CMP-sialic acid. UDP-GlcNAc is transported into the endoplasmic reticulum and Golgi for glycosyltransferase reactions for complex oligosaccharide assembly in those organelles (45). In certain cell types, this pool of UDP-GlcNAc may be released extracellularly, where it may activate the broadly distributed P2X and P2Y purinergic receptors (46). Taking into account the known volumes occupied by intracellular organelles, enrichment of UDP-GlcNAc in the endomembrane system (~30-fold) can result in cytoplasmic levels that are in the range expected from the known K_m values for OGT (~2–5 μM) and UDP-GlcNAc transporters (~7 μM) (47). Finally, the mOGT isoform com-
petes with ncOGT in the nucleus for UDP-GlcNAc (28) (Fig. 1B). Although total UDP-GlcNAc levels have been estimated to be as high as 1 mM, the concentrations of the cytoplasmic and mitochondrial pool are likely to be much lower. Utilization of UDP-GlcNAc for hyaluronan synthesis has been proposed to limit cytoplasmic O-GlcNAc levels (48). Thus, the pool of UDP-GlcNAc available for O-GlcNAcylation is influenced by many factors including metabolic flux. At the level of metabolism, other reactions contribute to, and compete for, precursors to the hexosamine biosynthetic pathway. Glutamine is one of these key metabolites. Glutamine can be converted to glutamate in mitochondria for the production of α-ketoglutarate and entry into the tricarboxylic acid cycle (2). In addition, the regulated entry of glutamine may be influenced by the synthesis of UDP-GlcNAc (2). Another precursor of UDP-GlcNAc, acetyl-CoA, is primarily produced by the conversion of pyruvate into acetyl-CoA via the mitochondrial pyruvate dehydrogenase complex during the oxidation of glucose and the β-oxidation of fatty acids (49). There is growing evidence in both yeast and mammalian cells that acetyl-CoA levels can fluctuate to influence gene expression (44). These many factors influencing the levels of UDP-GlcNAc are summarized in Fig. 1B.

**O-GlcNAc as an Integrator of Metabolite-driven Post-translational Modifications and Transcription**

Fig. 2 provides a brief overview of how nutrition, metabolism, and environmental cues may result in a change in metabolites critical for the epigenetic regulation of gene expression. These homeostatic transcriptional responses have been the subject of several excellent recent reviews (49, 50); here we highlight the role of O-GlcNAcylation in transcriptional control. A number of key metabolites (Fig. 2) may fluctuate in response to nutrients or such cues as circadian period. These metabolites either serve as direct donors (S-adenosylmethionine (AdoMet), acetyl-CoA) or contribute to the synthesis of other key metabolites (ATP, NAD, β-hydroxybutyrate, and α-ketoglutarate) (49). The metabolites then act through key enzymes such as kinases, lysine methyltransferases, histone acetyltransferases, or O-GlcNAc transferase to modify chromatin-associated factors. The DNA methyltransferases (DNMTs) are dependent upon the methyl donor AdoMet to modify cytosine residues of DNA. The Krebs cycle intermediate α-ketoglutarate modulates the activity of the TET (Ten-eleven translocation) proteins (see below). These key enzymes of addition are often referred to as “writers” of an epigenetic code (49). In turn, the modifications are removed by phosphatases, lysine demethylases, histone deacetylases, and O-GlcNAcase. DNA demethylation is thought to occur both passively and through the action of the TET enzymes. Thus, the enzymes catalyzing removal are frequently termed “erasers” (49). The writers and erasers act on effector molecules that serve to maintain transcriptional homeostasis: DNA, histones, transcription factors, and RNA polymerase II. We note that O-GlcNAc cycling plays a key role in most of these phenomena that maintain homeostasis. In the following sections, we will discuss the potential role of O-GlcNAc on these effectors including the “histone code,” DNA methylation, DNA demethylation, and the RNA polymerase II “CTD code.”

**O-GlcNAc and Epigenetic Regulation of Transcription by the Histone Code**

As illustrated in Figs. 1 and 2, the enzymes of O-GlcNAc cycling have also been shown to physically and genetically interact with a number of transcriptional regulators with established roles in epigenetics (5, 23, 26). OGT modifies many kinases and is thought to interact with numerous kinase signaling cascades both at the level of kinase regulation and through competition for acceptor serine and threonine residues (51, 52). O-GlcNAc has been shown to directly modify histones including the mitosis-specific H3 Ser-10 modification, which is conditionally phosphorylated (6, 12, 14, 53). Other sites of O-GlcNAcylation occur on histones H2A and H2B that may be linked to histone exchange (45, 53). It is noteworthy that the known sites of O-GlcNAcylation of histones are sites distinct from H3 and H4 tail modifications more directly linked to the regulation of gene expression. Perhaps the best established role for OGT in transcriptional regulation is involvement in polycomb repression (26, 54, 55). In *Drosophila*, OGT is allelic with *Sxc*, a gene initially characterized as a homeotic mutant (56). OGT is essential for polycomb repression in *Drosophila* (55).
and may play a similar role in mammals (5, 26, 57). Polycomb repression is linked to the trimethylation of histone H3 at Lys-27 (58). Polycomb is best known for its role in regulating the Hox (homeotic) genes in Drosophila. The Hox gene clusters from Caenorhabditis elegans to humans. In mammals, polycomb repression is involved in maintenance of pluripotency, differentiation, and imprinting (26, 54, 55). There is also evidence that OGT may interact with members of the trithorax group of epigenetic regulators (reviewed in Refs. 5 and 26). The trithorax group is associated with “activating histone modifications” such as histone H3 Lys-4 and Lys-36 methylation (59, 60) and may involve O-GlcNAcylation as part of the regulatory network (26). In general terms, the trithorax group acts as an anti-repressor that counters the action of the polycomb repressive complex. In addition to its role in polycomb repression, OGT has been shown to interact with mSin3A, a transcriptional repression complex associated with histone deacetylases (HDAC1, HDAC2) (61). The HDACs remove the acetyl residue laid down by the numerous histone acetyltransferases, which use acetyl-CoA as a precursor (1, 5). The OGT-mSin3A complex is also found in association with HCF-1, a protein that is cleaved by OGT in a process linked to mitotic cell cycle progression (45, 62–64). OGT is also a central hub in the Oct4 network and associates with other factors maintaining stem cell pluripotency (26). O-GlcNAc modifies many, if not most, RNA polymerase II transcription factors (21). In addition to these numerous interactions, OGT binds to the TET class of DNA cytosine demethylases that may serve as 5-methylcytosine erasers (65–67). TET proteins were first discovered through their numerous interactions, OGT binds to the TET class of DNA demethylases that may serve as 5-methylcytosine erasers (68). The TET proteins are a member of a family of DNA methyltransferases that are dependent upon both α-ketoglutarate and iron(II) for their activities (38, 67, 69, 70). Nutrient-dependent O-GlcNAcylation does not seem to play a direct role in regulating TET demethylation activity but may influence its nuclear residency (71).

Taken together, the interactions of these effector molecules (Fig. 2) with the enzymes of O-GlcNAc cycling and other mediators of epigenetic programming suggest that O-GlcNAcylation integrates metabolic information. Small molecule metabolites are central players in the regulation of these enzymes. These metabolites show complex interactions and inter-conversions providing links between diverse metabolic pathways. The changes in these metabolites from environmental influences such as circadian rhythms, nutritional flux, infection, and stress may contribute to the maintenance of homeostasis depicted in Fig. 2. From this figure, it is clear that O-GlcNAc is a central player in these processes. Although all of the effectors depicted in Fig. 2 are potentially important, we have chosen to focus this review on the impact of RNA pol II O-GlcNAcylation. pol II is downstream of all of the complex epigenetic regulatory paradigms described above in which O-GlcNAc has been implicated.

**RNA Polymerase II O-GlcNAcylation and Nutrient Sensing: The Differing Forms of RNA Polymerase II**

In humans, the pol II CTD consists of 52 heptad repeats of predominantly YSPTSPS. This “consensus repeat” is an accurate description of the yeast CTD and the first 26 repeats of the human CTD. However, this sequence diverges in human heptads 27–52. Notably, there are eight lysine residues (replacing serine 7), one arginine residue, and conservative switches between serine and threonine residues and asparagine and glutamic acids; all are at position 7 of the CTD repeat (72–74).

RNA polymerase II exists in many different forms dictated by the post-translational modifications on its C-terminal domain (CTD). These modifications are summarized in Fig. 3. The literature refers predominantly to two different species, pol IIA and pol IIO. pol IIA is thought to be the species required for promoter binding and initiation (75, 76). It is unclear whether this species is completely unmodified or whether it is more accurately considered hypo-phosphorylated. In contrast, pol IIO is thought to be heavily phosphorylated. A third distinct species of pol II is defined by the O-GlcNAcylation of the CTD (pol IIγ) (19, 20). pol IIγ was found within the pol IIA population, and in fact, because of the mixing of pol IIA and pol IIγ, it has never been clear whether pol IIA or pol IIγ is the initiation-specific species of pol II. Regardless, upon initiation, pol II is phosphorylated, first at serines 5 and 7, and subsequently at serine 2 (72–74).

**CTD Modifications and Enzymes**

The enzymes responsible for RNA pol II phosphorylation are quite numerous and have been discussed in detail elsewhere, especially in comparing yeast and human transcription systems (72, 77). CDK7 of the TFIH complex phosphorylates serine 5 and 7 residues. The phosphorylation of serine 2 is much less clear; CDK8, -9, -12, and -13 and Brd4 have all been suggested as serine 2 kinases (72, 77–79). It is clear that in vitro, these kinases can modify the CTD. However, in vivo, only CDK9, -12, and -13 have definitively been shown to be CTD kinases. Additionally, both threonine 4 and tyrosine 1 can be phosphorylated (80–82). Two kinases are capable of modifying Thr-4, whereas c-Abl is thought to be the Tyr-1 kinase (81, 83–86). There are also several CTD phosphatases: FCP1 (serine 2), SCP (serine 5),
MINIREVIEW: O-GlcNAc and Epigenetics

and RPAP2 (serine 5) (72). Pin1 (Ess1 in yeast) is unique among the CTD modification enzymes as it is a prolyl isomerase, converting proline from a predominantly trans-conformation to a cis-conformation that alters the substrate specificity of the CTD (for example, FCP1 requires Pin1 activity for CTD substrate binding) (87). Acetylation by p300 occurs on the lysine residues in the C-terminal half of the CTD (88). Lastly, the one arginine residue in the human CTD is a substrate for CARM1 arginine methyltransferase (89).

CTD O-GlcNAcylation was originally found on calf thymus RNA pol II and more recently on human pol II (19, 20). Edman degradation of calf thymus pol II indicated that both Thr-4 and Ser-5 of the CTD are O-GlcNAcylated (19), whereas serine to alanine substitutions (20) and mass spectrometry indicated that Ser-5 was O-GlcNAcylated. Additionally, O-GlcNAcylation of Ser-5 can block subsequent phosphorylation, suggesting that these are mutually exclusive events (18, 20); these data argued that pol II y exists either before pol I I0 or afterward (i.e. it is a pre-initiation species or a post-elongation species). Focus centered on pol II y acting before initiation after finding that OGT and OGA catalytic activity are necessary for transcription in vitro and that inhibition of these enzymes blocks transcription during pre-initiation complex assembly (20). OGT can be detected at promoters and immunoprecipitated with pol II (20). In vivo, shRNA inhibition of OGT decreased pol II occupancy at several B-cell-specific promoters (20), and both OGT and O-GlcNAc localize almost exclusively at transcription start sites (65). Similar results were obtained in C. elegans ChIP-chip analysis where OGT mutants are viable, thus providing robust controls in the ChIP-chip and ChIP-Seq experiments (13).

CTD Code and Function

The concept of a code on the CTD comprising the various PTMs was first proposed by Buratowski (90). In general, there are three ways that a code might manifest itself. The first is simply a binary code, consisting of either a modified or unmodified residue. The second is a combinatorial code, where two different residues are modified, either with the same or with different PTMs. The third is a sequential code, where one PTM establishes a second modification. There is evidence of all three (phospho-Ser-5; phospho-Ser-2; phospho-Ser-5/phospho-Ser-7; phospho-Thr-4; arginine-methyl; phospho-Tyr-1; lysine-acetyl) (72–74, 88). It is clear that specific combinations of phosphoserines in the CTD are binding sites for the functional output of the protein in question. Examples of protein must be dynamically regulated by either OGT and/or O-GlcNAc levels on proteins have been suggested to reflect the nutrient state of the cell (6, 23, 50). It is also critically important to consider the proteins that are O-GlcNAcylated and not just UDP-GlcNAc levels. For an O-GlcNAcylated protein to be considered a nutrient sensor, it should satisfy several criteria. The first is that the protein-O-GlcNAc levels must reflect changing nutrient conditions. For example, nuclear pore proteins are not likely to be nutrient sensors as their O-GlcNAc levels are very stable (49). Secondly, the O-GlcNAc levels of a protein must be dynamically regulated by either OGT and/or OGA. Finally, these changing levels of O-GlcNAc should alter the functional output of the protein in question. Examples of likely nutrient sensor proteins (NSPs) include CRTC2, RelA, and c-Rel NF-κB family members, and FoxoO1 (21, 97, 98).

The CTD as a Paradigm for Nutrient Sensing via O-GlcNAc

O-GlcNAc levels on proteins have been suggested to reflect the nutrient state of the cell (6, 23, 50). It is also critically important to consider the proteins that are O-GlcNAcylated and not just UDP-GlcNAc levels. For an O-GlcNAcylated protein to be considered a nutrient sensor, it should satisfy several criteria. The first is that the protein-O-GlcNAc levels must reflect changing nutrient conditions. For example, nuclear pore proteins are not likely to be nutrient sensors as their O-GlcNAc levels are very stable (49). Secondly, the O-GlcNAc levels of a protein must be dynamically regulated by either OGT and/or OGA. Finally, these changing levels of O-GlcNAc should alter the functional output of the protein in question. Examples of likely nutrient sensor proteins (NSPs) include CRTC2, RelA, and c-Rel NF-κB family members, and FoxoO1 (21, 97, 98).

CTD as a Scaffold for Nutrient-dependent Histone Modification Enzymes

The CTD is modified by O-GlcNAcylation, phosphorylation, and acetylation that are all dependent on intracellular nutrient-sensitive pools. However, we would like to suggest a second function of the CTD; it serves as a nexus for the nutrient-dependent histone methylation enzymes. These enzymes, such as MLL1/2, SET2, and Dot1, bind different phospho-CTD marks and deposit methyl groups onto Lys-4, Lys-36, and Lys-79 residues of the H3 histone tail, respectively (Fig. 3) (95). These enzymes are dependent on the AdoMet methyl donor (this
The figure illustrates the numerous connections synthetic pathway. We have explored the RNA pol II CTD modification by interaction with other CTD post-translational modifications (65–67, 69). OGT binds to the TET proteins involved in recognizing methylation (68). It is a critical regulator of cell signaling, mitochondrial step in the cascade of effectors implicated in epigenetic regulation (49, 99)).

Acknowledgments—We thank members of the Lewis, Hanover, and Krause laboratories for helpful comments in preparing this manuscript.

REFERENCES

1. Heard, E., and Martienssen, R. (2014) Transgenerational epigenetic inheritance: myths and mechanisms. Cell 157, 95–109
2. Wellen, K. E., and Thompson, C. B. (2012) A two-way street: reciprocal regulation of metabolism and signalling. Nat. Rev. Mol. Cell Biol. 13, 270–276
3. Hagemann, R. (2010) The foundation of extranuclear inheritance: plastid and mitochondrial genetics. Mol. Genet. Genomics 283, 199–209
4. Kornfeld, S., Kornfeld, R., Neufeld, E. F., and O’Brien, P. J. (1964) The feedback control of sugar nucleotide biosynthesis in liver. Proc. Natl. Acad. Sci. U.S.A. 52, 371–379
5. Hanover, J. A., Krause, M. W., and Love, D. C. (2012) Bittersweet memories: linking metabolism to epigenetics through O-GlcNacylation. Nat. Rev. Mol. Cell Biol. 13, 312–321
6. Hanover, J. A., Krause, M. W., and Love, D. C. (2010) The hexosamine signaling pathway: O-GlcNac cycling in feast or famine. Biochim. Biophys. Acta 1800, 80–95
7. Kang, E. S., Han, D., Park, J., Kwak, T. K., Oh, M. A., Lee, S. A., Choi, S., Park, Z. Y., Kim, Y., and Lee, J. W. (2008) O-GlcNac modification at Akt1 Ser473 correlates with apoptosis of murine pancreatic ß cells. Exp. Cell Res. 314, 2238–2248
8. Kearse, K. P., and Hart, G. W. (1991) Lymphocyte activation induces rapid changes in nuclear and cytoplasmic glycoproteins. Proc. Natl. Acad. Sci. U.S.A. 88, 1701–1705
9. Ito, S., Shen, L., Dai, Q., Wu, S. C., Collins, L. B., Swenberg, J. A., He, C., and Zhang, Y. (2011) Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxycytosine. Science 333, 1300–1303
10. McClain, D. A., Lubas, W. A., Cooksey, R. C., Hazel, M., Parker, G. I., Love, D. C., and Hanover, J. A. (2002) Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proc. Natl. Acad. Sci. U.S.A. 99, 10695–10699
11. Vosseller, K., Wells, L., Lane, M. D., and Hart, G. W. (2002) Elevated nucleocytoplasmic glycosylation by O-GlcNac results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. Proc. Natl. Acad. Sci. U.S.A. 99, 5313–5318
12. Fong, J. J., Nguyen, B. L., Bridger, R., Medrano, E. E., Wells, L., Pan, S., and Sifers, R. N. (2012) ß-N-Acetylglucosamine (O-GlcNac) is a novel regulator of mitosis-specific phosphorylations on histone H3. J. Biol. Chem. 287, 12195–12203
MINIREVIEW: O-GlcNAc and Epigenetics

13. Love, D. C., Ghosh, S., Mondoux, M. A., Fukushige, T., Wang, P., Wilson, M. A., Iser, W. B., Wolkow, C. A., Krause, M. W., and Hanover, J. A. (2010) Dynamic O-GlcNAc cycling at promoters of Caenorhabditis elegans genes regulating longevity, stress, and immunity. Proc. Natl. Acad. Sci. U.S.A. 107, 7413–7418

14. Zhang, S., Roche, K., Nasheuer, H. P., and Lowndes, N. F. (2011) Modification of histones by sugar \( \beta-N\)-acytylgalactosamine (GlcNAc) occurs on multiple residues, including histone H3 serine 10, and is cell cycle-regulated. J. Biol. Chem. 286, 37483–37495

15. Zeidan, Q., Wang, Z., De Maio, A., and Hart, G. (2010) O-GlcNAc cycling enzymes associate with the translational machinery and modify core ribosomal proteins. Mol. Biol. Cell 21, 1922–1936

16. Erickson, J. R., Pereira, L., Wang, L., Han, G., Ferguson, A., Dao, K., Copeland, R. J., Despa, F., Hart, G. W., Ripplinger, C. M., and Bers, D. M. (2013) Diabetic hyperglycemia activates CaMKII and aryls bymas with \( O\)-linked glycosylation. Nature 502, 372–376

17. Rexach, J. E., Clark, P. M., Mason, D. E., Neve, R. L., Peters, E. C., and Hsieh-Wilson, L. C. (2012) Dynamic O-GlcNAc modification regulates CREB-mediated gene expression and memory formation. Nat. Chem. Biol. 8, 253–261

18. Comer, F. I., and Hart, G. W. (2001) Reciprocity between \( O\)-GlcNAc and O-phosphate on the carboxyl terminal domain of RNA polymerase II. Biochemistry 40, 7845–7852

19. Kelly, W. G., Dahmus, M. E., and Hart, G. W. (1993) RNA polymerase II is a glycoprotein: modification of the COOH-terminal domain by O-GlcNAc. J. Biol. Chem. 268, 10416–10424

20. Ranuncanlo, S. M., Ghosh, S., Hanover, J. A., Hart, G. W., and Lewis, B. A. (2012) Evidence of the involvement of O-GlcNAc-modified human RNA polymerase II CTD in transcription in vitro and in vivo. J. Biol. Chem. 287, 23549–23561

21. Ozcan, S., Andrali, S. S., and Cantrell, J. E. (2010) Modulation of transcription factor function by O-GlcNAc modification. Biochim. Biophys. Acta 1799, 353–364

22. Bond, M. R., and Hanover, J. A. (2013) O-GlcNAc cycling: a link between metabolism and chronic disease. Annu. Rev. Nutr. 33, 205–229

23. Sawlson, C., and Hart, G. W. (2011) O-GlcNAc signalling: implications for cancer cell biology. Nat. Rev. Cancer 11, 678–684

24. Lehman, D. M., Fu, D. J., Freeman, A. B., Hunt, K. J., Leach, R. J., Johnson-Narasimhan, T., Bond, M. R., and Hanover, J. A. (2013) Ataxin-10 interacts with nuclear anti-apoptotic protein with roles in metabolism and chronic disease. Annu. Rev. Nutr. 33, 262–269

25. Love, D. C., Krause, M. W., and Hanover, J. A. (2010) O-GlcNAc cycling emerging roles in development and epigenetics. Semin. Cell Dev. Biol. 21, 44–52

26. Hascll, V. C., Wang, A., Tammi, M., Oikari, S., Tammi, R., Passi, A., Vigiotti, D., Hanson, R. W., and Hart, G. W. (2014) The dynamic metabolism of hyaluronan regulates the cytosolic concentration of UDP-GlcNAc. Matrix Biol. 35, 14–17

27. Gut, P., and Verdin, E. (2013) The nexus of chromatin regulation and intermediary metabolism. Nature 502, 489–498

28. Ruan, H. B., Singh, J. P., Li, M. D., Wu, J., and Yang, X. (2013) Cracking the O-GlcNAc code in metabolism. Trends Endocrinol. Metab. 24, 301–309

29. Dias, W. B., Cheung, W. D., and Hart, G. W. (2012) O-GlcNAcylation of kinases. Biochem. Biophys. Res. Commun. 422, 224–228

30. Tarrant, M. K., Rho, H. S., Xie, Z., Jiang, Y. L., Gross, C., Culhane, J. C., Yan, G., Qian, J., Ichikawa, Y., Matsouka, T., Zachara, N., Erzkon, F. A., Hart, G. W., Jeong, S., Blackshaw, S., Zhu, H., and Cole, P. A. (2012) Regulation of CK2 by phosphorylation and O-GlcNAcylation revealed by semisynthesis. Nat. Chem. Biol. 8, 262–269

31. Sakabe, K., Wang, Z., and Hart, G. W. (2010) B-N-Acetylgalactosamine (O-GlcNAc) is part of the histone code. Proc. Natl. Acad. Sci. U.S.A. 107, 19915–19920

32. Sekine, O., Love, D. C., Rubenstein, S. D., and Hanover, J. A. (2010) Blocking O-linked GlcNAc cycling in Drosophila insulin-producing cells.
perturbs glucose-insulin homeostasis. *J. Biol. Chem.* **285**, 38684–38691

55. Sinclair, D. A., Syrzycka, M., Macauley, M. S., Rastgardani, T., Komljenovic, I., Vecdall, D. J., Brock, H. W., and Honda, B. M. (2009) *Drosophila* O-GlcNAc transferase (OGT) is encoded by the *Polycrom* group (PeG) gene, *super sex combs* (sxsc). *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13427–13432

56. Ingham, P. (1984) A gene that regulates the bithorax complex differentially in larval and adult cells of *Drosophila*. *Cell* **37**, 815–823

57. Myers, S. A., Panning, B., and Burlingame, A. L. (2011) Polycrompressive complex 2 is necessary for the normal site-specific O-GlcNAc distribution in mouse embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 9490–9495

58. Lazarus, M. B., Jiang, J., Kapuria, V., Bhuiyan, T., Janetzko, J., Zandberg, W. F., Vocadlo, D. J., Herr, W., and Walker, S. (2013) HCF-1 is cleaved in *t(10;11)(q22;q23).* *Cancer Res.* **73**, 10890–10901

59. Baskaran, R., Chiang, G. G., Myśliwiec, T., Kruh, G. D., and Wang, J. Y. J. (1997) Tyrosine Phosphorylation of RNA polymerase II carboxy-terminal domain. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6927–6932

60. Hintermair, C., Heidemann, M., Koch, F., Descotes, N., Gut, M., Gut, L., Fenouil, R., Ferrier, P., Flatley, A., Kremmer, E., Chapman, R. D., Andrau, J., C., and Eick, D. (2012) Threonine-4 of mammalian RNA polymerase II CTD is targeted by Polo-like kinase 3 and required for transcriptional elongation. *EMBO J.* **31**, 2784–2797

61. Mayer, A., Heidemann, M., Lidschreiber, M., Schreieck, A., Sun, M., Hintermair, C., Kremmer, E., Eick, D., and Cramer, P. (2012) CTD tyrosine phosphorylation impairs termination factor recruitment to RNA polymerase II. *Science* **336**, 1723–1725

62. Hsin, J., S., Sheth, A., and Manley, J. L. (2011) RNA II CTD phosphorylated on threonine-4 is required for histone mRNA 3′ end processing. *Science* **334**, 683–686

63. Baskaran, R., Chiang, G. G., and Wang, J. Y. (1996) Identification of a binding site in c-Abl tyrosine kinase for the C-terminal repeated domain of RNA polymerase II. *Mol. Cell. Biol.* **16**, 3361–3369

64. Baskaran, R., Dahmus, M. E., and Wang, J. Y. (1993) Tyrosine phosphorylation of mammalian RNA polymerase II carboxyl-terminal domain. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11167–11171

65. Baskaran, R., Escobar, S. R., and Wang, J. Y. J. (1999) Nuclear c-Abl is a atypical kinase that phosphorylates serine2 of the RNA polymerase II. *Science* **285**, 496–498

66. Baskaran, R., Takagi, T., Moore, C. R., and Buratowski, S. (1997) mRNA capping enzyme is recruited to the transcription complex by phosphorylation of the C-terminal domain of subunit Ia. *J. Biol. Chem.* **272**, 10500–10506

67. Lu, H., Flores, O., Weinmann, R., and Reinberg, D. (1991) The nonphosphorylated form of RNA polymerase II preferentially associates with the preinitiation complex. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10004–10008

68. Bartkowiak, B., and Greenleaf, A. L. (2011) Phosphorylation of RNAPII: To P-TEFb or not to P-TEFb? *Transcription* **2**, 115–119

69. Baskaran, R., Liu, P., Phatnani, H. P., Fuda, N. J., Cooper, J. J., Price, D. H., Adelman, K., Lis, J. T., and Greenleaf, A. L. (2010) CDK12 is a transcription elongation-associated CTD kinase, the metazoan ortholog of yeast Ctk1. *Genes Dev.* **24**, 2303–2316

70. Devaias, B. N., Lewis, B. A., Cherman, N., Hewitt, M. C., Albrecht, B. K., Robey, P. G., Ozato, K., Sims, R. J., 3rd, and Singer, D. S. (2012) BRD4 is an atypical kinase that phosphorylates serine2 of the RNA polymerase II carboxy-terminal domain. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 6927–6932

71. Myers, S. A., Panning, B., and Burlingame, A. L. (2011) Polycomb repression complex 2 is necessary for the normal site-specific O-GlcNAc distribution in mouse embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 9490–9495

72. Kassis, J. A., and Kennison, J. A. (2010) Recruitment of Polycomb complexes: a role for SCM. *Mol. Cell. Biol.* **30**, 2581–2583

73. Byrd, K. N., and Shearn, A. (2003) ASHI1, a *Drosophila* trithorax group protein, is required for methylation of lysine 4 residues on histone H3. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11535–11540

74. Tanaka, Y., Katagiri, Z., Kawahashi, K., Kioussis, D., and Kitajima, S. (2007) Trithorax-group protein ASHI1 methylates histone H3 lysine 36. *Gene* **397**, 161–168

75. Chen, Q., Chen, Y., Bian, C., Fujiki, R., and Yu, X. (2013) TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* **493**, 561–564

76. Capotosti, F., Guerrier, S., Lammers, F., Wardel, P., Cai, Y., Jin, J., Conaway, J. W., Conaway, R. C., and Herr, W. (2011) O-GlcNAc transferase catalyzes site-specific proteolysis of HCF-1. *Cell* **144**, 376–388

77. Daou, S., Mashitari, N., Hammond-Martel, I., Pak, H., Yu, H., Sui, G., Vogel, J. L., Kristie, T. M., and Affar el, B. (2011) Crosstalk between O-GlcNAcylation and proteolytic cleavage regulates the host cell factor-1 maturation pathway. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 2747–2752

78. Deplus, R., Delatte, B., Schwinn, M. K., Defrance, M., Méndez, J., Lazarus, M. B., Jiang, J., Kapuria, V., Buihyan, T., Janetzko, J., Zandberg, W. F., Vecdall, D. J., Herr, W., and Walker, S. (2013) HCF-1 is cleaved in the active site of O-GlcNAc transferase. *Science* **342**, 1235–1239

79. Chen, Q., Chen, Y., Bian, C., Fujiki, R., and Yu, X. (2013) TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* **493**, 561–564

80. Ono, R., Taki, T., Taketani, T., Taniwaki, M., Kobayashi, H., and Hayashi, Y. (2002) LCX, leukemia-associated protein with a CXXC domain, is fused to Y. (2002) *Nature Cell Biol.* **8**, 397–402

81. Solary, E., Bernard, O. A., Telferi, A., Fucks, F., and Vainchenker, W. (2007) The Ten-Element-Translocation-2-TET2 gene in hematopoietic and hematopoietic diseases. *Leukemia* **21**, 485–496

82. Shi, F. T., Kim, H., Lu, W., He, Q., Liu, D., Goodell, M. A., Wan, M., and Songyang, Z. (2013) Ten-eleven translocation 1 (Tet1) is regulated by O-linked N-acetylglucosamine transferase (Ogt) for target gene repression in mouse embryonic stem cells. *J. Biol. Chem.* **288**, 20776–20784

83. Zhang, Q., Liu, X., Gao, W., Li, P., Hou, J., Li, J., and Wong, J. (2014) Differential regulation of the Ten-eleven translocation (TET) family of dioxygenases by O-linked B-N-acetylglucosamine transferase (OGT). *J. Biol. Chem.* **289**, 5986–5996

84. Corden, J. L. (2013) RNA polymerase II C-terminal domain: tethering transcription to transcript and template. *Chem. Rev.* **113**, 8423–8455

85. Eick, D., and Geyer, M. (2013) The RNA polymerase II carboxy-terminal domain (CTD) code. *Chem. Rev.* **113**, 8456–8490

86. Jeronimo, C., Bataille, A. R., and Robert, F. (2013) The writers, readers, and functions of the RNA polymerase II C-terminal domain code. *Chem. Rev.* **113**, 8491–8522

87. Cho, E. J., Takagi, T., Moore, C. R., and Buratowski, S. (1997) mRNA capping enzyme is recruited to the transcription complex by phosphorylation of the RNA polymerase II carboxy-terminal domain. *Genes Dev.* **11**, 3319–3326
94. McCracken, S., Fong, N., Rosonina, E., Yankulov, K., Brothers, G., Siderovski, D., Hessel, A., Foster, S., Shuman, S., and Bentley, D. L. (1997) 5′-Capping enzymes are targeted to pre-mRNA by binding to the phosphorylated carboxy-terminal domain of RNA polymerase II. Genes Dev. 11, 3306–3318
95. Smolle, M., and Workman, J. L. (2013) Transcription-associated histone modifications and cryptic transcription. Biochim. Biophys. Acta 1829, 84–97
96. Qian, H. (2007) Phosphorylation energy hypothesis: open chemical systems and their biological functions. Annu. Rev. Phys. Chem. 58, 113–142
97. Dentin, R., Hedrick, S., Xie, J., Yates, J., 3rd, and Montminy, M. (2008) Hepatic glucose sensing via the CREB coactivator CRTC2. Science 319, 1402–1405
98. Housley, M. P., Rodgers, J. T., Udeshi, N. D., Kelly, T. J., Shabanowitz, J., Hunt, D. F., Puigserver, P., and Hart, G. W. (2008) O-GlcNAc regulates FoxO activation in response to glucose. J. Biol. Chem. 283, 16283–16292
99. Badeaux, A. I., and Shi, Y. (2013) Emerging roles for chromatin as a signal integration and storage platform. Nat. Rev. Mol. Cell. Biol. 14, 211–224
100. Cameron, E. A., Martinez-Marignac, V. L., Chan, A., Valladares, A., Simmonds, L. V., Wacher, N., Kumate, J., McKeigue, P., Shriver, M. D., Kittles, R., Cruz, M., and Parra, E. J. (2007) MGEA5–14 polymorphism and type 2 diabetes in Mexico City. Am. J. Hum. Biol. 19, 593–596
101. Gambetta, M. C., Oktaba, K., and Müller, J. (2009) Essential role of the glycosyltransferase Sxc/Ogt in Polycomb repression. Science 325, 93–96