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Nutrient regulation of transcription and signalling by O-GlcNAcylation☆

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Summary The cycling (addition and removal) of O-linked N-acetylglucosamine (O-GlcNAc) on serine or threonine residues of nuclear and cytoplasmic proteins serves as a nutrient sensor via the hexosamine biosynthetic pathway's production of UDP-GlcNAc, the donor for the O-GlcNAc transferase (OGT). OGT is exquisitely sensitive both in terms of its catalytic activity and by its specificity to the levels of this nucleotide sugar. UDP-GlcNAc is a major node of metabolism whose levels are coupled to flux through the major metabolic pathways of the cell. O-GlcNAcylation has extensive crosstalk with protein phosphorylation to regulate signalling pathways in response to flux through glucose, amino acid, fatty acid, energy and nucleotide metabolism. Not only does O-GlcNAcylation compete for phosphorylation sites on proteins, but also over one-half of all kinases appear to be O-GlcNAcylated, and many are regulated by O-GlcNAcylation. O-GlcNAcylation is also fundamentally important to nutrient regulation of gene expression. OGT is a polycomb gene. Nearly all RNA polymerase II transcription factors are O-GlcNAcylated, and the sugar regulates their activities in many different ways, depending upon the transcription factor and even upon the specific O-GlcNAc site on the protein. O-GlcNAc is part of the histone code, and the sugar affects the modification of histones by other epigenetic marks. O-GlcNAcylation regulates DNA methylation by the TET family of proteins. O-GlcNAc modification of the basal transcription machinery is required for assembly of the pre-initiation complex in the transcription cycle. Dysregulated O-GlcNAcylation is directly involved in the aetiology of the major chronic diseases associated with ageing.

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Introduction

The modification of nuclear and cytoplasmic proteins by O-linked N-acetylglucosamine (O-GlcNAc) was discovered about thirty years ago (Torres and Hart, 1984; Holt and Hart, 1986). Until then, it was widely regarded not only in textbooks, but also by glycobiology experts, that protein glycosylation only occurred in the lumenal or extracellular compartments of cells. Early studies established that O-GlcNAcylation is particularly abundant within the nucleus (Holt and Hart, 1986; Holt et al., 1987a; Park et al., 1987; Kelly and Hart, 1989), but also occurs on many cytoskeletal and other cytosolic proteins (Holt et al., 1987b; Hart et al., 1988). Studies also showed that O-GlcNAc cycles rapidly on proteins in response to stimuli, suggesting that it is a regulatory modification analogous to protein phosphorylation (Kearse and Hart, 1991; Roquemore et al., 1992). Work from several laboratories in the past three decades have shown that not only is O-GlcNAc amongst the most abundant and wide-spread of post-translational modifications, but also that the cycling sugar serves as a nutrient sensor to regulate nearly all aspects of cellular physiology (for recent reviews: Hart et al., 2011; Slawson and Hart, 2011; Hardiville and Hart, 2014) (Fig. 1). OGT is essential in mammals and plants, even at the single cell level (Shafi et al., 2000; Hartweck et al., 2002; O'Donnell et al., 2004; Olszewski et al., 2010). Highlights of its many functions, which depend upon protein and even upon the sites on the protein to which the sugar is attached, include: (1) It is essential for both B- and T-lymphocyte activation (Golks et al., 2007). (2) It regulates many protein—protein interactions (Lim and Chang, 2009a,b; Roos et al., 1997; Hiromura et al., 2003; Wells et al., 2011). (3) Nutrients regulate our circadian clocks via the cycling of O-GlcNAc on transcription factors (Durgan et al., 2011; Kim et al., 2012; Hart, 2013; Kaasik et al., 2013). (4) Multiple subunits of the proteasome are O-GlcNAcylated and the sugar regulates the activity of this degradation complex (Zhang et al., 2003; Liu et al., 2004; Bowe et al., 2006). (5) O-GlcNAc has a dynamic interplay with phosphorylation, ubiquitination and other key regulatory protein modifications, allowing nutrients to regulate known signalling pathways (Wang et al., 2010a; Zeidan and Hart, 2010; Trinidad et al., 2012; Ruan et al., 2013). (6) O-GlcNAcylation plays a direct role in neuronal functions, including learning and memory and synaptic vesicle trafficking (Trinidad et al., 2012; Cole and Hart, 1999, 2001; Lagerlof and Hart, 2014; Vosseller et al., 2006; Francisco et al., 2009; Tallent et al., 2009; Skorobogatko et al., 2014; Trinidad et al., 2013). (7) O-GlcNAc cycling regulates growth hormone signalling in plants (Scott et al., 2006). (8) Increased O-GlcNAcylation protects cells from acute stresses, such as heat, high salt, ultraviolet light and hypoxia, among others (Zachara et al., 2004; Slawson et al., 2006; Cheung and Hart, 2008; Zachara, 2012). (9) O-GlcNAcylation also regulates translation and ribosome biogenesis (Ohn et al., 2008; Zeidan et al., 2010), but much more work in this area is needed. (10) Nutrients regulate transition through the cell cycle by O-GlcNAcylation and abnormal increases in OGT lead to polyploidy, a common feature of cancer cells (Wang et al., 2010a,b; Slawson et al., 2005, 2008). (11) O-GlcNAcylation is up-regulated in all cancers examined to date, and in chronic lymphocytic leukaemia the extent of O-GlcNAcylation of the leukocytes correlates with patient prognosis (Slawson and Hart, 2011; Chou and Hart, 2001; Caldwell et al., 2010; Slawson et al., 2010; Yi et al., 2012; Ma and Vosseller, 2013; Shi et al., 2010; Lynch et al., 2012; Ma and Vosseller, 2014). (12) Decreased O-GlcNAcylation, due to reduced glucose utilization in the brain, is directly involved in the aetiology of Alzheimer's disease, and inhibitors of O-GlcNAcase show promise as therapeutics (Yuzwa and Vocadlo, 2014; Zhu et al., 2014; Dias and Hart, 2007; Arnold et al., 1996). This list represents only a few of the many functions of this ubiquitous protein modification, which thus far has been reported on over four thousand proteins. However, the number of nuclear and cytoplasmic proteins modified is likely much larger. In this short review, I will focus on the fundamental importance of O-GlcNAcylation in transcription and also on its ubiquitous importance to nutrient modulation of cellular signalling.

O-GlcNAcylation is fundamentally important to nutrient regulation of transcription

Some of the earliest work on O-GlcNAcylation showed that it is highly enriched in chromatin and visualization of *Drosophila* polytene chromosomes showed that the sugar is localized at active sites of transcription (Kelly and Hart, 1989). The IIa form of RNA polymerase II, which is the form of the enzyme involved in initiation of transcription, was found to be extensively O-GlcNAcylated on its C-terminal (CTD) domain (Kelly et al., 1993). O-GlcNAcylation of the CTD is mutually exclusive to phosphorylation of the CTD, which produces the RNA polymerase IIo isoform, the form of the enzyme involved in elongation (Comer and Hart, 2000, 2001). Using more modern methods, like high-throughput ChIP analyses in both mammalian cells and *Caenorhabditis elegans*, both cycling enzymes, OGT and OGA, as well as O-GlcNAc itself, are found to be highly localized at the start sites of thousands of genes (Lewis and Hanover, 2014; Ranuncolo et al., 2012). In a B lymphocyte cell line, partial knock down of OGT by shRNA, strikingly reduces the