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Chromatin Regulation and Degradation by Molecules of Metabolism; Implications for Cell Function and Disease.

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ABSTRACT

The cellular concentrations of several of the intermediary metabolites fluctuate as a result of functional changes in the metabolic status of the cell, the activity of the chromatin regulators may change as a function of metabolic status and thereby transduce a homeostatic transcriptional response. There is now compelling evidence which has accumulated in recent years in support of this hypothesis. In this review the emerging model that is the chromatin-associated enzymes sense intermediary metabolism products and process this information into dynamic chromatin post translational modifications. These chromatin modifications, in turn, help to coordinate homeostatic or adaptive transcriptional responses. In many other situations, sensing of metabolic signals can also drive the activity of gene networks that control fundamental cell fate. In this regard, the monosaccharide O-linked N-acetylglucosaminyl is linked to serine or threonine residues of a protein by the UDP-GlcNAc-peptide-β-GlcNAc transferase (OGT) using UDP-GlcNAc as a sugar donor and can be removed by the N-acetyl-β-D-glucosaminidase. This review evaluates the role of this monosaccharide in relation to chromatin function and degradation and evaluates possible implications for cell function and disease.

Keywords: Chromatin, degradation, metabolic, β-N-acetylglucosamine, skeletal, muscle

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INTRODUCTION

A complex set of cellular regulatory mechanisms are known to determine the genes which are activated by transcription factors at a given time and in a specific cellular context. The packaging of DNA and histones into chromatin is an important aspect of gene regulation, allowing the access of transcription complexes to DNA to be regulated and for histones to participate in the regulatory process [1,2]. The modifications occurring in the chromatin in response to metabolic signals are dynamic or stable and might even be inherited transgenerationally [3]. In fact, almost all chromatin-modifying enzymes utilize co-factors or substrates that are crucial metabolites in critical pathways of intermediary metabolism. These metabolites include acetyl-CoA, uridine diphosphate (UDP)-glucose, α-ketoglutarate (α-KG), nicotinamide adenine dinucleotide (NAD+), flavin adenine dinucleotide (FAD), ATP or *S*-adenosylmethionine (SAM). The cellular concentrations of several of these metabolites fluctuate as a function of the metabolic status of the cell, the activity of the chromatin regulators may change as a consequence of metabolic status and thereby transduce a homeostatic transcriptional response [4]. Post translational modifications (PTM) are important events which include acetylation, ubiquitylation and glycosylation. These are important for cellular function and response to external stress. Chromatin function and its possible degradation may be altered by sensors which predict changes in key metabolites of the intermediary metabolism for e.g O-linked GlcNAc. This article evaluates the reciprocal interaction between chromatin function and intermediary metabolites, with a focus on O-linked GlcNAc.

Skeletal muscle contraction and O-linked β-N-acetylglucosamine

The muscular contraction phenomenon is both dependent on glucose metabolism and is highly regulated by the phosphorylation/dephosphorylation processes. Moreover, mammalian skeletal muscle fibers display a great potential of adaptation that results from the ability of muscle fibers to adjust their functional, molecular, and metabolic properties in response to modified functional demands, such as changes in neuromuscular activity or mechanical loading [5]. Indeed, it has previously been shown that slow-to-fast functional transitions induced by hindlimb unloading that encompassed slow-to-fast transitions in the isoform composition of myosin heavy chain as well as in other key proteins involved in the muscle contraction. These transitions were associated with aerobic-oxidative to glycolytic metabolic changes that involves both variations in the glucose metabolism and phosphorylation/dephosphorylation processes. Moreover, the hind limb muscular manouveres when applied for 14 days on the slow-twitch anti-gravitational soleus muscle caused an atrophy and a decrease in calcium sensitivity of skinned fibers. As described previously, UDP-GlcNAc has been demonstrated to be sensitive to glucose levels; moreover, correlations between the glycogen content and the *O*-GlcNAc levels have been measured in the skeletal muscle .

O-linked β-N-acetylglucosamine and its role in metabolic processes

Among the important cellular events, post-translational modifications such as acetylation, phosphorylation or glycosylation, provide additional levels of functional complexity to the cell's proteome. Carbohydrates share a real structural diversity and are attached to proteins through two main types of linkage, respectively asparagine (*N*-) or serine/threonine (*O*-) residues. The structural diversity of the carbohydrates allows them to ensure very specific and selective interactions with other molecules [6]. *O*-linked *N*-acetylglucosaminyl moieties (*O*-GlcNAc) constitute an abundant and dynamic reversible form of glycosylation for numerous cytoplasmic and nuclear proteins [7]. The monosaccharide *N*-acetylglucosamine is linked to serine or threonine residues of a protein by the UDP-GlcNAc-peptide-β-GlcNAc transferase (OGT) using UDP-GlcNAc as a sugar donor and can be removed by the *N*-acetyl-β-D-glucosaminidase (*O*-GlcNAcase) as shown in Fig.1. The concentration of UDP-GlcNAc is highly sensitive to glucose levels and depends on the hexosamine pathway. OGT and *O*-GlcNAcase appear to regulate the attachment and removal of *O*-GlcNAc and could be compared with the kinase/phosphatase system in phosphorylation process [8]. Indeed, phosphorylation and *O*-GlcNAc modification are often reciprocal at the same or at neighboring hydroxyl moieties, and *O*-GlcNAc appears as a regulatory modification that has a complex dynamic interplay with phosphorylation. This relationship between *O*-GlcNAc and *O*-phosphate, called the "Yin-Yang" process, has been demonstrated on the total level of cellular proteins but also on isolated proteins [9]. Many *O*-GlcNAc proteins have been identified to date: they belong to various classes of proteins including cytoskeletal components , hormone receptors, transcriptional factors, kinases, signaling molecules, nuclear pore proteins, and microbial proteins, suggesting that*O*-GlcNAc may be implicated in several key cellular systems such as transcription, nuclear

transport, and cytoskeletal structure [10,11]. Numerous studies suggest the importance of *O*-GlcNAc in many pathologies including diabetes, cancer, neurodegenerative diseases, and also in cardioprotection, underlying its crucial role in cell life [12].

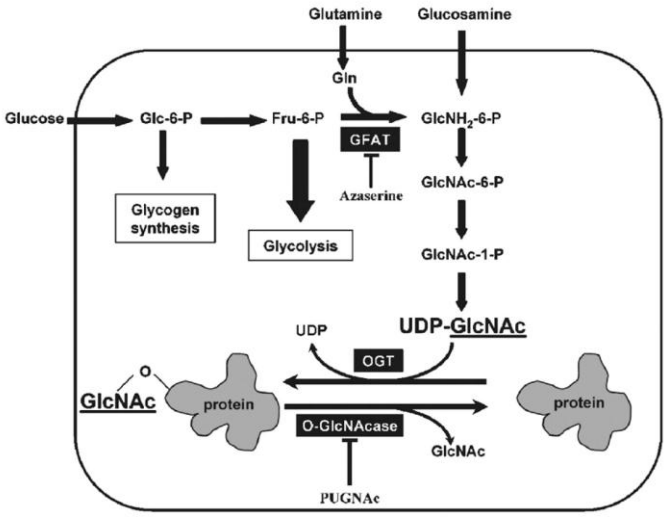

Figure 1: Courtesy J C Chatham *et al* , Cardiovasc Res. 2007 Jan 15;73(2):288-97

Mitochondria as an integrator of redox and O-GlcNAc signaling

It is now well established that mitochondria plays a central role in integrating cellular energy metabolism, redox signaling and cell survival pathways; however, the role of O-GlcNAcylation in regulating these processes is not clearly understood [13]. The assumption that mitochondrial proteins might be potential targets for O-GlcNAc modification was first raised by the identification of a specific mitochondrial isoform of OGT (mOGT), which is localized to the inner membrane of mitochondria and contains mitochondrial targeting sequence [14]. While initial reports suggested that mOGT had either limited catalytic activity or that substrates for OGT in the mitochondria were very limited, however recently, a number of studies have suggested that O-GlcNAcylation of mitochondria proteins contributes both to acute cardioprotection as well as mitochondrial dysfunction and increased programmed cell death. There are reports of interaction of mitochondrial sirtuins and elements of PTMs [15,16].

Chromatin and intermediary metabolism

In the recent development in cell function research, posttranslational modification (PTMs) of histone proteins are emerging as a significant mechanism to rapidly alter DNA–chromatin interactions in response to intracellular signals, thereby modulating gene regulation. PTMs are covalent, reversible chemical modifications of amino acid residues within a protein that change the functional aspects of the protein, its stability, its subcellular localization or its interaction with other proteins [17]. The best-studied PTMs are phosphorylation of threonine or serine residues, as well as ubiquitination and acetylation of lysine residues. In the case of acetylation, it has recently emerged that places the PTM of enzymes of intermediary metabolism as a key regulatory mechanism that rapidly adapts metabolite flux to changes in a cell's energetic state.

Another example where significant insight has been revealed for a novel histone PTM is with the serine/threonine sugar modification O-linked β-N-acetylglucosamine (O-GlcNAc). Studies have found that O-GlcNAc transferase (OGT) and the hydrolase O-GlcNAcase (OGA) are the enzymes that inscribe and erase this PTM, both on histones and non-histone proteins [18,19]. The nucleotide sugar donor used by OGT (UDP-GlcNAc) is highly regulated by nutrient levels and is rate limiting in the cell. Studies therefore suggest that O-GlcNAc is a sensor of nutrient status, controlling gene expression programs in response to changing nutrient conditions and environmental stress. In mammals, O-GlcNAcylation is a modification that occurs on each of the core histones and is regulated in a cell cycle-dependent manner. Peak levels of O-GlcNAc occur during G1 and drop in S-phase, followed by a rise occurring through G2 and M phase [20,21]. O-GlcNAc was found to occur at H3S10, posing an interesting question as to whether O-GlcNAcylation is functionally antagonistic with H3S10 phosphorylation [22,23]. Since O-GlcNAc levels are highest in G1, it is plausible that H3S10 O-GlcNAcylation

acts as a molecular 'switch' to negatively regulate gene expression normally controlled by H3S10 phosphorylation. Intriguingly, O-GlcNAc has also been found to crosstalk with other histone PTMs, notably H2BK120 monoubiquitination [24]. The existence of a stable complex between the O-GlcNAc transferase Ogt and two components of the 5mC hydroxylase protein family, Tet1 and Tet2, linking two diverse enzymatic activities to chromatin. The fact that Ogt purification also led to the isolation of Sin3a and Hdac1 (previously identified partners of Tet1), although with a minor number of peptides in respect to Tet proteins and Hcfc1, strongly suggests the existence of a multiprotein complex containing Tet1, Tet2, Ogt, Sin3a, and Hdac1. Such complex contains diverse enzymatic activities that are associated with transcriptional repression [25].

Connection of chromatin and Ogt

The O-linked N-acetylglucosamine (O-GlcNAc) transferase Ogt is an evolutionarily conserved enzyme that catalyzes all O-linked protein glycosylation [6]. OGT is expressed in both the cytoplasm and the nucleus of cells and modifies threonine and serine residues through the attachment of an N-acetyl glucosamine moiety to their hydroxyl groups. The *Ogt* gene locus resides on the X chromosome and its activity is required for the viability of male embryonic stem cells (ESCs) [26]. Consistent with this, *Ogt* knockout (KO) mice are not viable and die during embryogenesis. Moreover, maternal loss of Ogt results in lack of viability of heterozygous embryos no later than day 5 postcoitus due to developmental defects of the extra embryonic tissues that are engaged by paternal X chromosome inactivation [27]. Over the last two decades, several different Ogt substrates have been identified, suggesting that Ogt can control different cellular functions in agreement with the severe phenotypes observed upon loss of Ogt activity [28,29]. O-linked protein glycosylation is counteracted by the activity of the O-GlcNAcase OGA that actively removes O-GlcNAc groups from O-glycosylated proteins [28]. OGT and OGA are the final executors of hexosamine signaling (HSP), a metabolic pathway that senses the nutrient state of the cells. O-GlcNAc is produced from glucose, glutamine, acetyl-CoA, uridine, and ATP, and reduced levels of O-GlcNAc inhibit proteins' O-linked glycosylation, making OGT and OGA potent nutrient sensors [30].

Effect of metabolites on Chromatin function and degradation

The histone glycosylation through O-linked *N*-acetylglucosamine (O-GlcNAc) modification of serine and threonine is a newly recognized modification [31]. It has been suggested that beta-N-acetylglucosamine is a part of the histone code and cycling of this glycosylated histone and occurs in the nucleocytoplasmic proteins. For e.g In the roundworm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, mice and humans, addition and removal of O-GlcNAc are catalysed by two enzymes, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. Because UDP-glucose (the substrate for O-GlcNAcylation) is a product of the hexosamine pathway (an alternative metabolic branch of glucose metabolism) and directly reflects changes in ambient glucose levels, this modification probably links intermediary metabolism with a unique chromatin modification [32-33]. All four core histone proteins can be glycosylated by OGT on sites that can alternatively be phosphorylated. In addition, non-histone proteins in chromatin, such as members of the Polycomb and Trithorax group complexes in *D. melanogaster,* can also be modified through O-GlcNAcylation. Despite the apparent connection between chromatin regulators and their metabolic substrates, the biological importance of this attractive concept remained largely unexplored until recently. In particular, it was not clear whether the chromatin-modifying enzyme co-factors were indeed rate limiting, and whether fluctuations of their local concentrations were sufficiently dynamic and occurred in concentrations likely to affect the enzymatic activity of their cognate enzymes [34]. At the centre of this debate is whether chromatin-regulator function is more similar to metabolic enzymes, whose activity depends on the relative abundance of their substrates and products, or more similar to protein kinases, whose activity is relatively independent of physiological fluctuations in ATP levels.

Although further work is required, recent findings suggest that chromatin-regulating proteins indeed sense intracellular co-factor levels. Histone H2B can also be modified by O-GlcNAcylation by OGT, suggesting possible competition between the two modifications for H2BS36, although the biological relevance for such competition remains to be shown experimentally. Nevertheless, this could be an excellent example of a reciprocal regulation of a chromatin mark by cellular energy sensors for low (AMPK) and high (OGT) energy levels or the Ki 67 antigen which is tightly associated with chromatin [35-37].

CONCLUSION

The identification and characterization of chromatin remodeling regulators will provide important support for the development of novel therapeutic approaches to cure or ameliorate a variety of human disorders. The recent spurt in the understanding of the role of epigenetics in cellular physiology and its link to disease and therapeutics is leading to the discovery of several small molecule modulators of chromatin enzymatic activities. It is now known that small active molecules arising from the cell intermediary metabolism such as O-linked N-acetyl glucosamine play an important role in cell function.This metabolite can themselves directly regulate the activity of nucleosome remodeling factors or can be used by other enzymes to modify other proteins regulating them. Thus modulation of gene activities utilizing drugs or small molecules can have potentially new avenues for the cure of human genetic diseases.

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