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Review article

Protein O-linked β-N-acetylglucosamine: A novel effector of cardiomyocyte metabolism and function

Victor M. Darley-Usmar [a], Lauren E. Ball [b], John C. Chatham [a,*]

[a] Division of Molecular and Cellular Pathology, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA
[b] Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, SC, USA

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ABSTRACT

The post-translational modification of serine and threonine residues of nuclear and cytoplasmic proteins by the O-linked attachment of the monosaccharide β-N-acetyl-glucosamine (O-GlcNAc) is emerging as an important mechanism for the regulation of numerous biological processes critical for normal cell function. Active synthesis of O-GlcNAc is essential for cell viability and acute activation of pathways resulting in increased protein O-GlcNAc levels improves the tolerance of cells to a wide range of stress stimuli. Conversely sustained increases in O-GlcNAc levels have been implicated in numerous chronic disease states, especially as a pathogenic contributor to diabetic complications. There has been increasing interest in the role of O-GlcNAc in the heart and vascular system and acute activation of O-GlcNAc levels have been shown to reduce ischemia/reperfusion injury, attenuate vascular injury responses as well mediate some of the detrimental effects of diabetes and hypertension on cardiac and vascular function. Here we provide an overview of our current understanding of pathways regulating protein O-GlcNAcylation, summarize the different methodologies for identifying and characterizing O-GlcNAcylated proteins and subsequently focus on two emerging areas: 1) the role of O-GlcNAc as a potential regulator of cardiac metabolism and 2) the cross talk between O-GlcNAc and reactive oxygen species. This article is part of a Special Section entitled "Post-translational Modification."

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1. Introduction

Classical protein glycosylation involves the synthesis of complex elongated oligosaccharide structures via N-linkage on asparagine and O-linkage on the hydroxy amino acids serine (Ser) and threonine (Thr), as well as hydroxyproline, hydroxylysine, and tyrosine residues

* Corresponding author at: Division of Molecular and Cellular Pathology, Department of Pathology, University of Alabama at Birmingham, 1670 University Boulevard, Volker Hall G001 Birmingham, AL 35294-0019, USA. Tel.: +1 205 934 0240.
E-mail address: jchatham@uab.edu (J.C. Chatham).

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of proteins that become secreted or membrane component glycoproteins [1]. Importantly these glycosylation reactions occur exclusively in the ER and Golgi; however, in 1984 a novel glycosylation modification was first reported in which a single β-N-acetyl-glucosamine moiety was attached via an O-linkage to serine (Ser) and threonine (Thr) residues of cytoplasmic and nuclear proteins [2]. This modification, now commonly known as O-GlcNAc, has been shown to be present in over 1000 nuclear, cytoplasmic and mitochondrial proteins and has been found in all metazoans, as well as in some bacteria, protozoa, viruses and fungi [3–7]. O-GlcNAcylation is distinct from traditional glycosylation in that it is restricted to the cytoplasm, nucleus and mitochondria and it is not extended into complex elongated structures; it also exhibits parallels with protein phosphorylation, in that it responds to acute stimuli, alters protein function and enzyme activity and modifies the same or proximal Ser/Thr residues as phosphorylation [3–7].

It has become increasingly apparent that O-GlcNAc modification of proteins is an important mechanism for the regulation of numerous biological processes critical for normal cell function such as signal transduction [8–11], proteasome activity [12,13], apoptosis [14,15], nuclear transport [16], translation and transcription [17]. Most of our understanding of the impact of alterations in O-GlcNAc levels on cell function has come from studies of chronic disease models such as cancer [18–20], senescence [21–23], neurodegeneration [11,14,24,25] as well as diabetes and diabetic complications [26,27]. However, a number of studies have demonstrated that O-GlcNAc is essential for cell viability [28–30] and that acute activation of pathways resulting in increased protein O-GlcNAc levels improves the tolerance of cells to a wide range of stress stimuli [29].

There has been increasing interest in the role of O-GlcNAc in the heart and vascular system and acute activation of O-GlcNAc levels has been shown to reduce ischemia/reperfusion injury [31–41], attenuate vascular injury responses [42] as well mediate some of the detrimental effects of diabetes [21,43–49] and hypertension [50–53] on cardiac and vascular function. The growing awareness of the effects of altered O-GlcNAc on cardiovascular function is reflected in part by a relatively large number of reviews on this subject over the past few years [37,52–58]. Therefore, in light of these and other recent comprehensive reviews on O-GlcNAc biology [3–7], we provide a brief summary on the regulation of O-GlcNAc synthesis, followed by a practical overview regarding the 1) modulation of cellular O-GlcNAc levels and 2) the characterization and identification of O-GlcNAcylated proteins. We then focus on two emerging areas, namely the role of O-GlcNAc as a potential regulator of cardiac metabolism and the cross talk between O-GlcNAc and reactive oxygen species, with an emphasis on the role of mitochondria in mediating this interaction.

2. Regulation of O-GlcNAc synthesis and turnover

The primary components involved in regulating O-GlcNAc synthesis and turnover are summarized in Fig. 1. Uridine-diphosphate-N-acetylglucosamine (UDP-GlcNAc) is the sugar nucleotide donor for the synthesis of O-GlcNAc modified proteins, by O-GlcNAc transferase (OGT). OGT has a high affinity for UDP-GlcNAc, which provides it with a competitive advantage over nucleotide transporters in the endoplasmic reticulum and Golgi that compete for cytoplasmic UDP-GlcNAc, but also makes it highly sensitive to changes in UDP-GlcNAc concentrations [59]. Thus, the rate of O-GlcNAc synthesis and overall levels of O-GlcNAc protein modification are highly dependent on the flux through hexosamine biosynthesis pathway (HBP). HBP flux is regulated largely by L-glutamine-D-fructose 6-phosphate amidotransferase (GFAT), which converts fructose-6-phosphate to glucosamine-6-phosphate with glutamine as the amine donor [60].

Cell culture studies suggest that ~2–5% of total glucose entering the cell is metabolized via GFAT [61]; however, the extent to which this holds true for a highly metabolic organ, such as the heart is unknown.

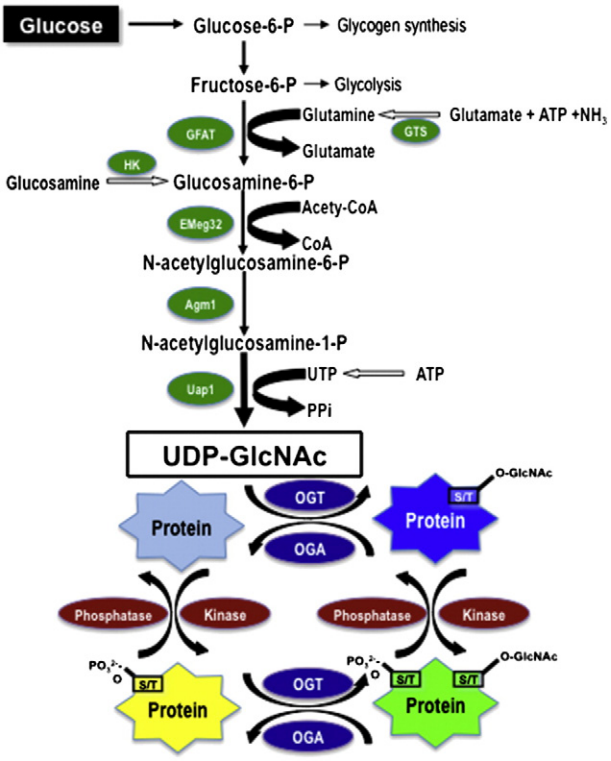

Fig. 1. The hexosamine biosynthesis pathway (HBP) and protein O-GlcNAcylation. Glucose imported into the cells is rapidly phosphorylated to glucose-6-phosphate and converted to fructose-6-phosphate, which is subsequently metabolized to glucosamine-6-phosphate by L-glutamine-D-fructose 6-phosphate amidotransferase (GFAT). Synthesis of glucosamine-6-phosphate is dependent on availability of glutamine, which is formed by glutamine synthetase (GTS). Glucosamine-6-phosphate is subsequently metabolized by glucosamine 6-phosphate N-acetyltransferase (Emeg32) to N-acetylglucosamine-6-phosphate, which is converted to N-acetylglucosamine-1-phosphate by phosphoacetylglucosamine mutase (Agm1) and the synthesis of UDP-uridine-diphosphate-N-acetylglucosamine (UDP-GlcNAc) is catalyzed by UDP-N-acetylglucosamine pyrophosphorylase (Uap1). Flux through the HBP can be increased with glucosamine, which is phosphorylated by hexokinase (HK) to form glucosamine 6-phosphate thereby bypassing GFAT. UDP-GlcNAc is the obligatory substrate for OGT (uridine-diphospho-N-acetylglucosamine: polypeptide β-N-acetylglucosaminyltransferase) leading to the formation of O-linked β-N-acetylglucosamine (O-GlcNAc)-modified proteins. β-N-acetylglucosaminidase (OGA) catalyzes the removal of O-GlcNAc from the proteins.

GFAT is considered to be the primary rate-determining step in the HBP and exists in two isoforms (GFAT1 and GFAT2), transcribed from separate genes. In the heart GFAT2 is the most abundant isoform [62] and in rodent models of aging [23] and pressure overload hypertrophy [63] there were significant increases in GFAT2 mRNA levels, but little or no change in GFAT1 mRNA levels. In both studies there were also increased UDP-GlcNAc levels, consistent with an increase in HBP flux, suggesting that GFAT expression and HBP flux in the heart are subject to regulation in response to chronic stress. It is also of note that both GFAT1 and GFAT2 are subject to phosphorylation by cAMP-dependent protein kinase [64,65]; in addition both AMPK and CaMKII lead to phosphorylation of Ser243 of human GFAT1 resulting in increased enzyme activity [66]. While our understanding of the regulation of GFAT activity remains poorly understood, the fact that it appears to be a target for AMPK, raises the possibility that it is a key link between AMPK, the HBP and O-GlcNAc signaling.

Following its synthesis by GFAT, glucosamine-6-phosphate is metabolized via glucosamine 6-phosphate N-acetyltransferase (Emeg32), phosphoacetylglucosamine mutase (Agm1) and finally UDP-N-acetylglucosamine pyrophosphorylase (Uap1) to UDP-GlcNAc (Fig. 1). The HBP is often referred to as a "nutrient-sensing" pathway, and this highlighted by the fact that in addition to glucose availability, UDP-GlcNAc synthesis is dependent on amino acid metabolism, specifically

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