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Review

Functional significance of O-GlcNAc modification in regulating neuronal properties

Hongik Hwang [a], Hyewhon Rhim [a,b,*]

[a] Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea
[b] Division of Bio-Medical Science & Technology, KIST School, Korea University of Science and Technology, Seoul 02792, Republic of Korea

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ABSTRACT

Post-translational modifications (PTMs) covalently modify proteins and diversify protein functions. Along with protein phosphorylation, another common PTM is the addition of O-linked β-*N*-acetylglucosamine (O-GlcNAc) to serine and/or threonine residues. O-GlcNAc modification is similar to phosphorylation in that it occurs to serine and threonine residues and cycles on and off with a similar time scale. However, a striking difference is that the addition and removal of the O-GlcNAc moiety on all substrates are mediated by the two enzymes regardless of proteins, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. O-GlcNAcylation can interact or potentially compete with phosphorylation on serine and threonine residues, and thus serves as an important molecular mechanism to modulate protein functions and activation. However, it has been challenging to address the role of O-GlcNAc modification in regulating protein functions at the molecular level due to the lack of convenient tools to determine the sites and degrees of O-GlcNAcylation. Studies in this field have only begun to expand significantly thanks to the recent advances in detection and manipulation methods such as quantitative proteomics and highly selective small-molecule inhibitors for OGT and OGA. Interestingly, multiple brain regions, especially hippocampus, express high levels of both OGT and OGA, and a number of neuron-specific proteins have been reported to undergo O-GlcNAcylation. This review aims to discuss the recent updates concerning the impacts of O-GlcNAc modification on neuronal functions at multiple levels ranging from intrinsic neuronal properties to synaptic plasticity and animal behaviors.

Contents

Abbreviations: PTM, post-translational modification; OGT, O-GlcNAc transferase; OGA, O-GlcNAcase; O-GlcNAc, O-linked β-*N*-acetylglucosamine; UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Gal, galactose; PSD, postsynaptic density; NFT, neurofibrillary tangle; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CREB, cyclic AMP-response element binding protein; CRE, cAMP response element; CBP, CREB-binding protein; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NMDAR, N-methyl-D-aspartate receptor; BZX2, benzoxazolinone; PVN, paraventricular nucleus; AgRP, agouti-related peptide; MAPK, mitogen-activated protein kinase; T2DM, type 2 diabetes mellitus; AD, Alzheimer's disease; Aβ, β-amyloid; APP, β-amyloid precursor protein; cKO, conditional knockout; DG, dentate gyrus; SC, Schaffer collateral; PPF, paired-pulse facilitation; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; LTP, long-term potentiation; LTD, long-term depression; STP, short-term potentiation; GlcN, glucosamine; PKC, protein kinase C; HFS, high-frequency stimulation; LFS, low-frequency stimulation; CFC, contextual fear conditioning; NOR, novel object recognition; NOP, novel object placement; mESC, mouse embryonic stem cell; $InsP_3R$-I, inositol 1,4,5-trisphosphate receptor type I; NF-M, neurofilament protein M; SOX2, sex determining region Y-box 2; PS1, presenilin-1; GFAT1, glutamine-fructose-6-phosphate amidotransferase 1.

* Corresponding author at: Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul 02792, Republic of Korea.
E-mail address: hrhim@kist.re.kr (H. Rhim).

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1. Introduction

Post-translational modifications (PTMs) are covalent modifications occurred on a protein during or following protein translation, and play a significant role in regulating cellular processes by increasing proteome complexity. Common PTMs include phosphorylation, ubiquitination, glycosylation, lipidation, methylation, nitrosylation, acetylation, and each modification differently modulates protein functions, ranging from protein localization to enzymatic activity and protein–protein interaction [1,2]. Of these PTMs, glycosylation refers to the covalent attachment of a carbohydrate or glycans to proteins, and is mainly divided into N-linked and O-linked glycosylation depending on which functional group of side chains is modified. In N-linked glycosylation, glycans are linked to a protein through a nitrogen atom of asparagine or arginine residues, which mainly occurs in the endoplasmic reticulum and the Golgi apparatus [3]. N-linked glycans usually form a complex and branched structure composed of oligosaccharide and are known to contribute to protein folding and stabilization [4,5]. In contrast, O-linked glycans are attached to the hydroxyl oxygen of side chains, including serine, threonine and tyrosine, and tend to have less branching compared to N-glycans. The most common O-linked glycosylation is the initial addition of N-acetylgalactosamine (GalNAc) to serine or threonine residues which can then be further extended with galactose (Gal) or N-acetylglucosamine (GlcNAc) [6]. Another common example of O-linked glycosylation is the addition of a single GlcNAc to hydroxyl moieties of serine or threonine residues [7,8], known as O-GlcNAcylation (Fig. 1). However, O-GlcNAcylation is distinct from all other types of protein glycosylation in that it is not elongated to form more complex structures with branching, and that the added O-GlcNAc cycles on and off multiple times unlike stable N- and O-glycans [9]. In this regard, O-GlcNAc modification is considered to behave similar to phosphorylation, but a striking difference is that the addition and removal of an O-GlcNAc moiety is entirely mediated by the two enzymes regardless of substrates, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. Notably, OGT knockout animals die during embryogenesis and OGA knockout also results in death within a day after birth [10–12], which together implies the requirement of adequate levels of protein O-GlcNAcylation in maintaining survival.

The donor carbohydrate substrate for O-GlcNAcylation is UDP-N-acetylglucosamine (UDP-GlcNAc), which is produced when a small percentage (approximately 2–5%) of glucose is metabolized through the hexosamine biosynthetic pathway (HBP, Fig. 2). The first two steps are identical to glycolysis in which glucose is converted to glucose-6-phosphate by hexokinase and then to fructose-6-phosphate by phosphor-glucose isomerase. Fructose-6-phosphate is converted to glucosamine-6-phosphate by the rate-limiting enzyme glutamine-fructose-6-phosphate amidotransferase 1 (GFAT1), which plays a key role in integrating glucose flux and amino acid metabolism. The subsequent acetylation and uridylation reactions incorporate fatty acid and nucleotide metabolism into the production of UDP-GlcNAc. Importantly, UDP-GlcNAc serves as an allosteric inhibitor of GFAT1, thus providing a negative feedback mechanism in the HBP [13]. Also, a recent study reported that nutritional stress such as glucose shortage and amino acid deprivation increases the production of GFAT1, which promotes glucose flux through the HBP and therefore protein O-GlcNAcylation [14].

Considering the fact that the biosynthesis of UDP-GlcNAc is heavily influenced by multiple metabolic pathways including carbohydrate, fatty acid, and nitrogen fluxes [15], the primary role of O-GlcNAcylation is thought to regulate cellular processes in response to the general nutrient status of the cell [16,17]. However, despite the significance of O-GlcNAcylation in modulating cellular signaling, it has been difficult to study the functions of O-GlcNAc modification at the molecular level due to the following reasons: (1) a specific function of O-GlcNAcylation varies depending on a modified protein, similar to phosphorylation, (2) a site-specific antibody detecting an O-GlcNAcylated protein is largely absent, (3) an O-GlcNAc moiety is easily lost during sample preparation for mass spectrometry, thereby making it difficult to detect and map an exact site of modified residues. Nonetheless, our understanding of cellular events affected by O-GlcNAcylation has expanded significantly for the past decade thanks to a dramatic advance in techniques available for the modulation and detection of O-GlcNAcylation. In particular, a region-specific or cell-specific study of O-GlcNAcylation modulation was made possible by transgenic animals, virus-mediated gene transfer and genome editing tools such as a CRISPR/Cas9 system [18–20]. Moreover, the rational screening/design of small molecule inhibitors for OGT and OGA led to the development of highly specific and potent inhibitors for both enzymes [21,22], thereby preventing ambiguous results produced by suboptimal inhibitors with low specificity. In addition, recent progress in sample preparation for mass spectrometry and metabolic labeling using GlcNAc analogues facilitated the high-throughput detection of O-GlcNAc-modified proteins [23–25]. Also, synthetic protein chemistry such as chemical ligation enabled the production of polypeptide with O-GlcNAc modification and the study of its functional implication in a site-specific manner [26,27].

These recent advances in chemical and biological tools for studying O-GlcNAcylation accelerated the discovery of multiple cellular functions of O-GlcNAc modification, including gene expression, signal transduction, cell cycle, mitochondrial motility, circadian clock, autophagosome maturation and proteasomal degradation [28–35]. Aberrant protein O-GlcNAcylation is also closely related with pathological conditions such as type II diabetes, cancer, cardiac hypertrophy, and neurodegenerative diseases [36–42]. However, despite the fact that OGT and OGA are highly expressed in the

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