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VIEWPOINT

Glycosylation of Nucleocytoplasmic Proteins: Signal Transduction and O-GlcNAc

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The dynamic glycosylation of serine or threonine residues on nuclear and cytosolic proteins by O-linked β-*N*-acetylglucosamine (O-GlcNAc) is abundant in all multicellular eukaryotes. On several proteins, O-GlcNAc and O-phosphate alternatively occupy the same or adjacent sites, leading to the hypothesis that one function of this saccharide is to transiently block phosphorylation. The diversity of proteins modified by O-GlcNAc implies its importance in many basic cellular and disease processes. Here we systematically examine the current data implicating O-GlcNAc as a regulatory modification important to signal transduction cascades.

Cells respond to their environment through dynamic posttranslational modification of their existing proteins. There are more than 20 posttranslational modifications that occur on eukaryotic proteins (*1*). Several of these modifications, with phosphorylation being the hallmark, participate in signal transduction. Generally, glycosylation is not thought to participate directly in signaling. Complex N- and O-linked glycosylation occurs on membrane-bound or secreted proteins that are synthesized in the endoplasmic reticulum and Golgi apparatus. The lumenal or extracellular localization of these glycans restricts their potential for dynamic responsiveness to signals. In contrast, O-GlcNAc is a simple monosaccharide modification that is abundant on serine or threonine residues of nucleocytoplasmic proteins (*2*, *3*). An O-GlcNAc site consensus motif has not yet been identified. However, many attachment sites are identical to those used by serine/threonine) kinases, and a neural network program has been developed to predict O-GlcNAc sites (*4*). Unlike phosphorylation, O-GlcNAc modification of tyrosine residues has yet to be observed. Many proteins have been identified that carry this modification, including transcription factors, cytoskeletal proteins, nuclear pore proteins, oncogene products, and tumor suppressors (*5*–*7*). O-GlcNAc appears to modify a large number of nucleocytoplasmic proteins (Fig. 1). The attributes of O-GlcNAc, which are distinct from those of complex carbohydrates, predict that it plays an important role in signaling.

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O-GlcNAc Meets the Requirements for a Signal Transduction Modification

In order for a protein modification to play an active role in signal transduction, it needs to have certain key features. First, the modification needs to be dynamic. For the proteins that have been examined to date, the O-GlcNAc half-life is much shorter than that of the modified polypeptide chain (*8*). Second, the removal or attachment of the modification should be inducible by certain stimuli. O-GlcNAc modification of certain proteins is known to change in response to T cell activation, insulin signaling, glucose metabolism, and cell cycle progression (*6*). Thus, O-GlcNAc displays features essential for a role in signal transduction.

Consistent with O-GlcNAc being dynamic and inducible, regulated nucleocytoplasmic enzymes for the attachment [O-GlcNAc transferase (OGT)] and for the removal (O-GlcNAcase) of the modification have been purified, characterized, and cloned (*9*–*12*). The OGT enzyme is modified by both O-GlcNAc and tyrosine phosphorylation and has 11 protein-protein interaction domains known as tetratricopeptide (TPR) repeats (*13*). OGT specifically interacts with a variety of other proteins. Because the transferase has a variety of binding partners and is itself Viewpointposttranslationally modified, OGT's substrate specificity, localization, and/or activity are likely to be regulated by signal transduction cascades. Furthermore, the activity of this enzyme is exquisitely responsive to intracellular uridine 5′-diphosphate (UDP)–GlcNAc and UDP concentrations, which are in turn highly sensitive to glucose concentrations and are known to fluctuate in response to a number of stimuli (*14*). Disruption of OGT activity is lethal in mouse embryonic stem cells, underscoring the importance of this modification (*15*). O-GlcNAcase, which specifically catalyzes the removal of O-GlcNAc from proteins, is a cytosolic neutral β-*N*-acetylglucosaminidase, unlike the general acidic lysosomal hexosaminidases. Both OGT and O-GlcNAcase are highly conserved from *Caenorhabditis elegans* to humans (*9*, *10*, *12*). Regulation of O-GlcNAc levels by these two enzymes may be analogous to the regulation of phosphorylation by kinases and phosphatases.

Potential Roles of O-GlcNAc Addition

Although O-GlcNAc meets all of the requirements of a signal transduction modification and is apparently essential for life at the cellular level, the clear functional consequences of this modification are only beginning to be elucidated. Most signal-induced modifications affect a protein's ability to associate with other proteins or affect its activity directly. In some recent examples, O-GlcNAc is implicated in both contexts (Fig. 2). The ubiquitous transcription factor Sp1 is extensively modified by O-GlcNAc (*16*). Sp1 becomes hyperglycosylated in response to hyperglycemia or elevated glucosamine (*17*). Increased O-GlcNAc modification of Sp1 prevents its degradation via the proteasome (*18*). Also, O-GlcNAc modification is able to inhibit the interaction of an Sp1 peptide with TATA-binding protein–associated factor (TAF110) or holo-Sp1 (*19*). Finally, the glycosylation state of Sp1 is highly correlated with its ability to transactivate genes important in diabetes (*17*). Other examples include the eukaryotic peptide chain iniation factor 2 (eIF-2)–associated 67-kD polypeptide (p67) that upon deglycosylation is degraded and no longer protects eIF-2 from phosphorylation (*20*). Phosphorylated eIF-2 is then able to inhibit protein translation. O-GlcNAc modification of the tumor suppressor p53 has been reported to increase its DNA binding, presumably by disrupting an intramolecular interaction (*21*). Modification of estrogen receptor β by O-GlcNAc appears to stabilize the protein while decreasing its transactivation capability (*22*). Finally, several proteins that are thought to be involved in Alzheimer's disease, including tau, AP3, and the β-amyloid precursor protein, are modified by O-GlcNAc, and there is some evidence that O-GlcNAc levels are perturbed in this disease state (*5*). Thus, O-GlcNAc is implicated in transcription, translation, cancer, neuronal pathology, and other biological processes.

O-GlcNAc and O-Phosphorylation

Several groups have documented an apparent reciprocity between O-GlcNAc and O-phosphorylation (*5*, *7*). This so-called "yin-yang" relationship has been shown at both the global cellular protein level and at specific sites on particular proteins. For example, treatment of cells with okadaic acid (a broad-spectrum phosphatase inhibitor), phorbol esters (activator of protein kinase C), or adenosine 3′,5′-monophosphate (an activator of protein kinase A) leads to global decreases in O-GlcNAc–modified protein levels (*23*). Conversely, inhibitors of kinases, such as staurosporine, increase the overall level of O-GlcNAc–modified proteins. There are specific examples of a reciprocal relationship, in which the sites of glycosylation and phosphorylation have been mapped to the same residue, such as Ser^{16} on estrogen receptor β (*24*). The multifunctional COOH-terminal domain (CTD) of RNA polymerase II is another example of reciprocity (*25*). Phosphorylation of the CTD is required for transcriptional elongation. Glycosylation of CTD in vitro inhibits its phosphorylation and vice versa (*26*). The protooncogene product c-Myc is alternatively modified at Thr^{58}, a known mutational hot spot in lymphomas, by both O-GlcNAc and phosphate (*27*). Thus, a given serine/threonine may exist in three states: glycosylated, phosphorylated, or unmodified. This raises the possibility of additional control of signaling beyond the binary model (phosphorylation on/off) and complicates the interpretation of functional analyses of phosphorylation based solely on site-directed mutants, which prevent both modifications.

Putative Role of O-GlcNAc as a Glucose Sensor

There is growing evidence of a link between aberrant O-GlcNAc modification and diabetes (*14*). One of the hallmarks of type II diabetes is the hyperglycemia associated with an inability of insulin to trigger appropriate glucose uptake (insulin resistance). Glucose flux through the hexosamine pathway has been linked to the onset of insulin resistance (*28*). Concentrations of UDP-GlcNAc (the end product of the hexosamine pathway) are highly sensitive to ambient glucose levels (*29*). Because OGT uses this donor sugar nucleotide in catalyzing the attachment of O-GlcNAc to proteins, increased levels of extracellular glucose and glucosamine lead to elevated intracellular O-GlcNAc modification of proteins in skeletal muscle (*30*) and in pancreatic beta cells (*31*). In the case of muscle cells, several postreceptor insulin signaling events are dampened under hyperglycemic conditions, and reduced insulin receptor substrate (IRS) -1 and -2 signaling are associated with their increased O-GlcNAc modification and decreased phosphorylation (*32*). Thus, several groups have proposed that hyperglycemia-induced O-GlcNAc modifications perturb normal signaling events required for insulin-mediated homeostasis (Fig. 2).

Because O-GlcNAc levels on proteins appear to be sensitive to flux through the hexosamine biosynthetic pathway, a role as a general sensor of energy (glucose) availability can be hypothesized for O-GlcNAc. In this model, the state and stoichiometry of O-GlcNAc modification are based largely on UDP-GlcNAc availability and would represent a "set point" reflecting the nutritional state of the cell. If O-GlcNAc levels globally increased or decreased, there would be a profound effect on a cell's ability to respond to specific extracellular stimuli. In this context, O-GlcNAc can be viewed as an intracellular signal that generally determines how a cell is poised to respond to extracellular stimuli by blocking certain phosphorylation signaling events and/or through an independent mechanism, such as the modulation of protein-protein interactions.

Future Prospects

Many of the tools required for the study of O-GlcNAc have only become available in the past few years. The ability to overexpress, localize, and characterize OGT and O-GlcNAcase should help us to better understand the function of O-GlcNAc modification on proteins. Furthermore, an antibody that is specific for O-GlcNAc–modified proteins is now available (*33*), and O-GlcNAc site-specific antibodies for particular proteins are being generated. Other detection methods, including tandem mass spectrometry approaches (*34*), have only recently been refined or are still under development.

Testing whether O-GlcNAc is serving as a glucose sensor will be one of the primary challenges of the future. Determining how O-GlcNAc fits into existing models of transcription, translation, and signaling (Fig. 2), as well as understanding the role of this modification in disease states such as cancer, diabetes, and Alzheimer's disease, will also be important. Determining the impact of glycosylation on protein-protein interactions and whether O-GlcNAc,

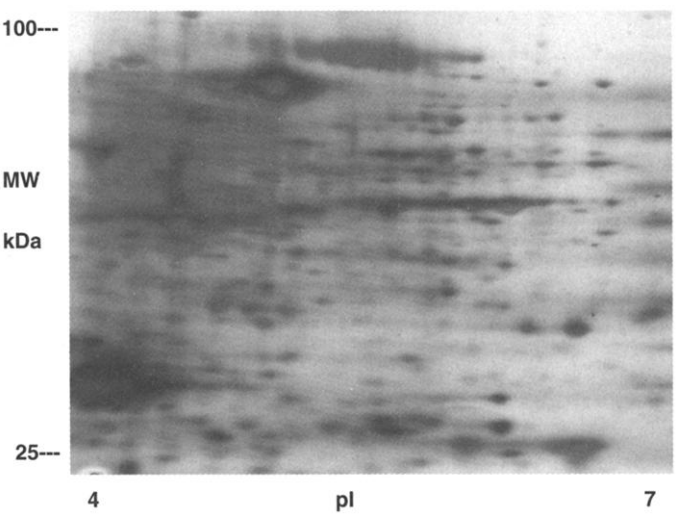

Fig. 1. O-GlcNAc is an abundant modification of nucleocytoplasmic proteins. Nucleocytoplasmic proteins from HeLaS3 cells were immunopurified with an O-GlcNAc–specific antibody (*33*) and stringently washed, and the O-GlcNAc–containing proteins were specifically eluted with free GlcNAc. The resulting proteins were separated on two-dimensional gels and visualized by silver staining. pI, isoelectric point; MW, molecular weight.

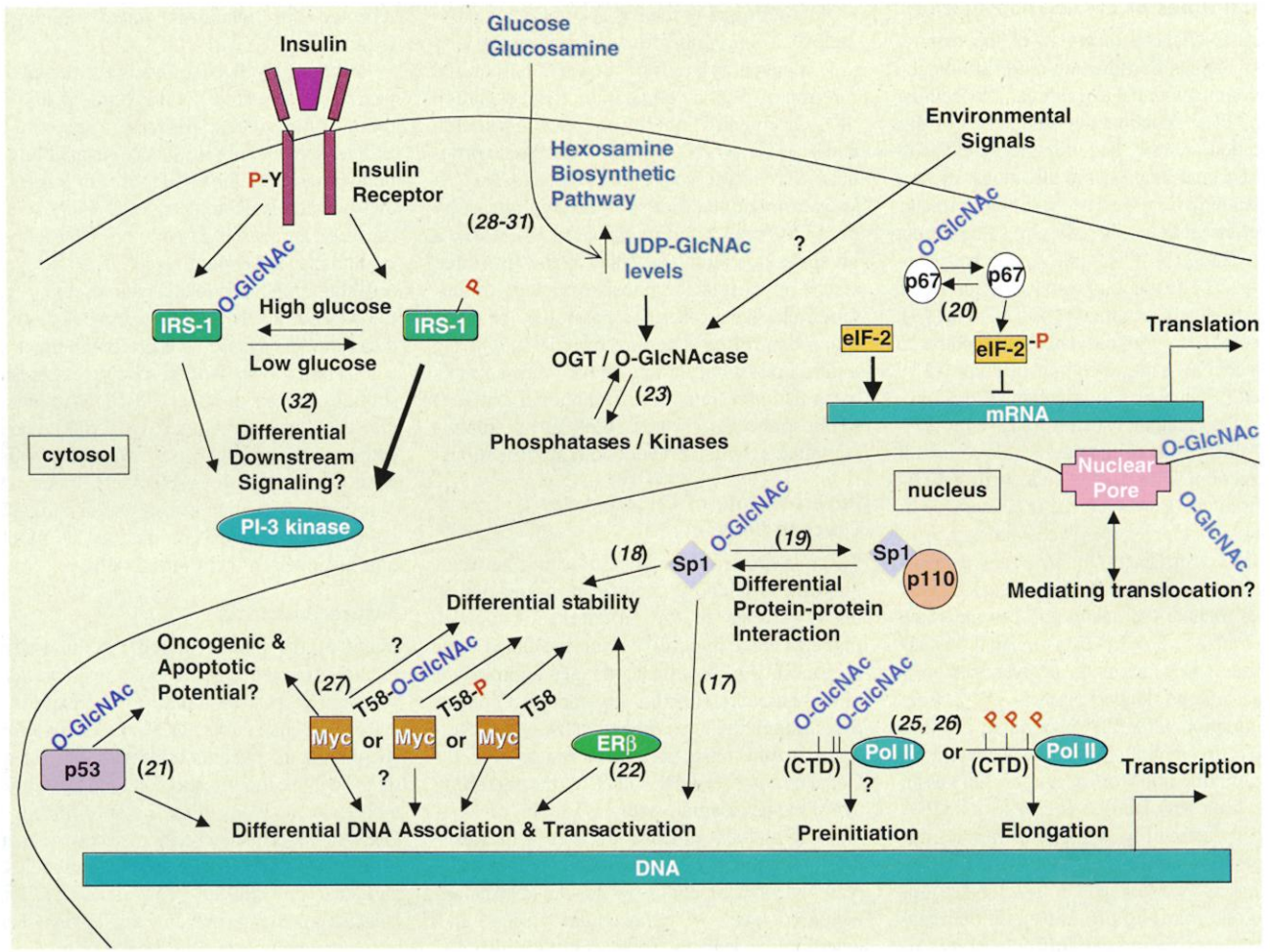

Fig. 2. Cellular processes in which O-GlcNAc has been implicated. Abbreviations not specified in the text include phosphatidylinositol 3-kinase (PI-3 kinase), TATA-binding protein–associated factor (p110), c-myc (myc), estrogen receptor β (ERβ), and RNA polymerase II (Pol II). Numbers in parentheses are reference numbers. Question marks represent unpublished work and/or speculation on the part of the authors.

like phosphate, creates binding domains for certain proteins is another aim of current and future research. In addition, comparative proteomics can now be used to determine what proteins are undergoing dynamic glycosylation in response to various stimuli and to study the interplay between glycosylation and phosphorylation (*35*).

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