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Post-Translational Modification by O-GlcNAc: Another Way to Change Protein Function

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Abstract Modification of intracellular proteins by the β-linkage of the monosaccharide, N-acetylglucosamine to serine or threonine hydroxyls (O-GlcNAc) is abundant and reversible. Although many proteins bear this post-translational covalent modification, the changes in function of the proteins as a result of this modification are only starting to be understood. In this article, we describe how aspects of the flux from the glucose backbone to this modification are modified and how the cellular activity and content of the GC-box binding transcription factor, Sp1, is altered by O-glycosylation. The association of the enzyme that puts on the O-GlcNAc modification with the bi-functional enzyme that removes this modification is discussed relative to the transition between transcriptional repression and activation. J. Cell. Biochem. 98: 1062–1075, 2006.

Key words: diabetes; streptozotocin (STZ); gene repression; gene activation; proteasome; transcription

Protein functions are changed as a result of post-translational modifications. One such modification is the covalent addition of the monosaccharide, N-acetylglucosamine, to serine or threonine residues (O-GlcNAc) by the enzyme, O-GlcNAc transferase (OGT). The modification is reversible, in that the sugar can be removed by the enzyme, O-GlcNAc-selective N-acetyl-D-glucosaminidase (O-GlcNAcase). This intracellular modification was discovered in Dr. G.W. Hart's laboratory in 1984 [Torres and Hart, 1984], and since that time, he and others have added about 200 proteins to the list of those modified [Wells et al., 2003]. The modification is highly dynamic [Zachara et al., 2004], responding to various cell stimuli. Although the list of proteins itself gives tantalizing evidence for the importance of the modification, so far relatively little is known about the functional changes induced by this modification.

Jackson and Tjian [1988] found that the transcriptional activator, Sp1, is modified by O-glycosylation. Wheat germ agglutinin, which binds to O-GlcNAc, and can be used in the purification of Sp1, inhibited transcription activation in a cell-free system. At that time, it was assumed that Sp1 was the only relevant protein in the extract that was modified by O-GlcNAc in this assay and they therefore deduced that the modification activated Sp1-mediated transcription. Covering up the modification with this lectin would inhibit the activation. Since then we have learned that RNA polymerase II itself [Kelly et al., 1993] is O-GlcNAcylated, making the interpretation of this experiment problematical. The inhibition of transcription could have resulted from the interaction of the lectin with vital components of the trancriptional apparatus besides Sp1.

At about the same time, we were studying the effect of glucose and glucosamine on the expression of the transforming growth factor-α (TGFα) gene in vascular smooth muscle cells [Mueller et al., 1990; McClain et al., 1992]. Studies had shown that transcription of the TGFα gene is very dependent on the Sp1 transcriptional activator [Shin et al., 1992] and it is upregulated by exposure to supra-physiological glucose concentrations [McClain et al., 1992]. The carbohydrate effect was mapped in the gene to the region containing the Sp1 binding sites. This

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effect of glucose could be mimicked by exposing the cells to glucosamine (GlcN). Indeed, GlcN had a much greater effect on TGFα gene transcription than did glucose. The stimulatory effects of glucose and GlcN required several hours of incubation while short incubations resulted in decreased transcription. This biphasic response we now think results from direct early and indirect late effects of GlcN on transcription factors that will be explained later.

GLUTAMINE: FRUCTOSE-6-PHOSPHATE AMIDOTRANSFERASE AND ITS REGULATION

To solve the problem of how the TGFα gene is regulated by glucose/glucosamine, we studied the pathway from glucose to GlcN in some detail. GlcN is synthesized from fructose-6-phosphate (F-6-P) as a result of the transfer of the amide group from glutamine to the phosphosugar by the enzyme, glutamine: fructose-6-phosphate amidotransferase (GFAT) (Fig. 1, GlcN synthesis). GFAT is the rate-limiting step in GlcN synthesis and there are two genes that encode this enzyme [McKnight et al., 1992; Oki et al., 1999]. We used an antisense strategy and a chemical enzyme inhibitor to block GFAT expression or activity. Those cells that expressed the antisense construct or were exposed to the inhibitor exhibited a marked decrease in the ability of glucose to activate TGFα gene transcription [Roos et al., 1996; Sayeski and Kudlow, 1996]. These experiments demonstrated that GlcN could not only mimic the action of glucose on the transcription of this gene, but glucose metabolism to GlcN was necessary for this effect of glucose on TGFα gene transcription. The expression of other genes, such as the leptin [Wang et al., 1998], TGFβ [Kolm-Litty et al., 1998] and plasminogen activator inhibitor [Du et al., 2000] genes have also been proposed to be regulated by GlcN.

The GFAT enzyme is tightly regulated in eukaryotic cells. It has been estimated that only 2–5% of the F-6-P generated at an early step in glycolysis is diverted into the synthesis of GlcN [Hassell et al., 1986]. One reason that GlcN synthesis is limited, is that both eukaryotic forms of GFAT are feedback inhibited by a

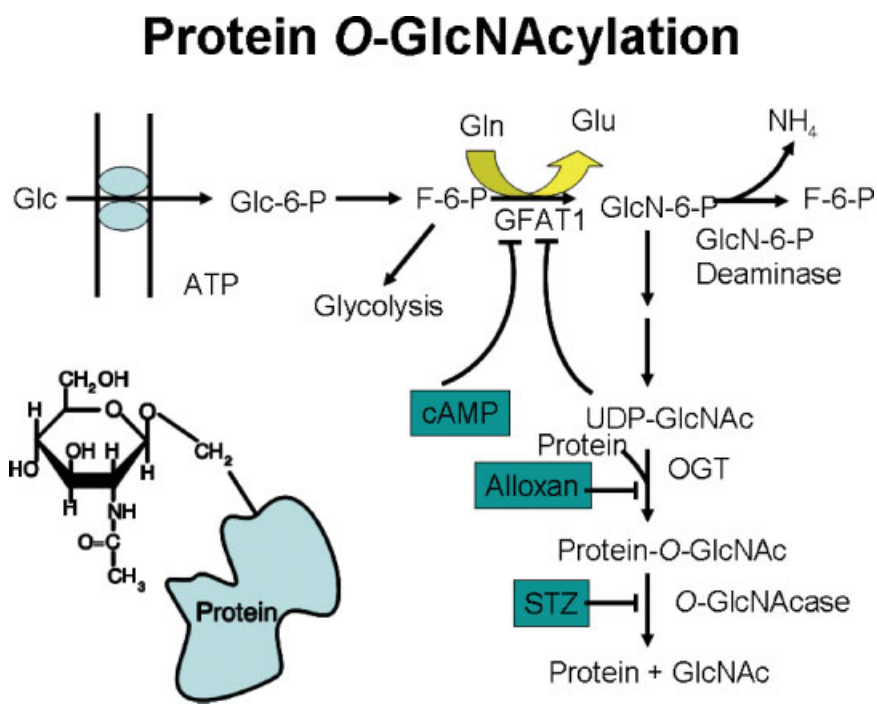

Fig. 1. Glucose enters the cell through the respective glucose transporter and ATP is consumed to make glucose-6-phosphate catalyzed by a specialized hexokinase. The phosphorylated glucose is isomerized to fructose-6-phosphate (F-6-P), most of which is utilized in energy production. Up to 5% of the F-6-P is converted to glucosamine-6-phosphate by GFAT. The glucosamine is acetylated and charged with UDP where some of it is used by O-GlcNAc transferase (OGT) for protein modification. The inhibitors of this latter pathway are shown.

downstream product of GlcN, UDP-GlcNAc [Kornfeld, 1967; McKnight et al., 1992; Hu et al., 2004]. UDP-GlcNAc is ultimately used as the substrate of glycosyltransferases for the synthesis of glycoproteins and glycosaminoglycans. Since the intracellular concentration of UDP-GlcNAc is controlled both by its rate of synthesis and its rate of consumption, the negative feedback on GFAT activity by UDP-GlcNAc in eukaryotes is then regulated in a manner that corresponds to the metabolic needs of the cell for UDP-GlcNAc. For a rapidly anabolic cell, where glycoprotein synthesis is required for cell growth, the activity of GFAT can be turned on as a result of the consumption of UDP-GlcNAc. We have shown also that the expression of the GFAT1 gene can be increased in the cell under conditions of growth factor stimulation. Epidermal growth factor (EGF) upregulates transcription of the GFAT1 gene [Paterson and Kudlow, 1995; Sayeski et al., 1997]. These results suggest that GFAT1 gene transcription may be regulated in a cell growth-dependent fashion such that the amount of enzyme protein is coupled to the level of macromolecular synthesis in the cell.

GFAT enzymatic activity is regulated by cAMP [Chang et al., 2000; Hu et al., 2004]. Recombinant human GFAT1 was shown to be phosphorylated at two serine residues, ser^{205} and ser^{231}, by cAMP-dependent protein kinase (A-kinase) in vitro [Chang et al., 2000]. GFAT2 is also phosphorylated by A-kinase at ser^{202} [Hu et al., 2004]. This latter site is homologous to ser^{205} in GFAT1 but GFAT2 does not have the other homologous site to ser^{231} in GFAT1. In GFAT1, ser^{205} phosphorylation is inhibitory to the GFAT enzymatic activity [Chang et al., 2000] whereas phosphorylation of GFAT2 at ser^{202} is stimulatory [Hu et al., 2004]. The modification of ser^{231} in GFAT1 does not affect the GFAT activity [Chang et al., 2000]. Since the tissue distribution of the two isoenzymes differs, the effects of cAMP on this pathway of glucosamine metabolism will be different depending on which enzyme is expressed [Hu et al., 2004]. This result implies that GFAT activity can be regulated by hormones through cAMP.

Our studies on the role of GlcN in TGFα gene transcription suggested that GlcN might be impinging on the cellular mechanisms that control transcription. The majority of the GlcN synthesized in the cell is destined for glycoprotein synthesis. Quantitatively, most glycosylation occurs on those proteins destined for export to the cell surface. This type of glycosylation is initiated as the protein is being translated in the rough endoplasmic reticulum mostly through the N-linkage of complex sugar groups to asparagine residues in the protein. These N-linked complex sugar chains are then modified further in the Golgi prior to the trafficking of these proteins to the plasma membrane or to exocytotic vesicles. While it is possible to propose a number of indirect mechanisms by which GlcN might influence gene transcription by its effects on cell surface glycoproteins, our laboratory focused on the glycosylation of Sp1, uncovered by Jackson and Tjian [1988]. This type of modification might have a much more direct influence on gene transcription.

O-GLYCOSYLATION

In the cells of plants [Robertson et al., 1998; Hartweck et al., 2006] and animals [Comer and Hart, 1999] (for reviews, see [Comer and Hart, 1999; Wells et al., 2001, 2003; Vosseller et al., 2002a; Wells and Hart, 2003]), there is also a form of glycosylation that involves the O-linkage of the monosaccharide, GlcNAc to serine or threonine residues in the protein backbone. The modification is catalyzed by OGT. The OGT cDNA has been cloned [Kreppel et al., 1997; Lubas et al., 1997] and the expression of this enzyme is ubiquitous, although it appears to be most highly expressed in the pancreas, brain [Lubas et al., 1997] and pituitary [Liu et al., 2002]. In humans and mice, the gene encoding OGT is unique, on the X chromosome and vital for life. Knock-out of the gene encoding this enzyme is lethal at the embryonic stem cell stage [Shafi et al., 2000; O'Donnell et al., 2004]. The domain structure of OGT has been investigated and described [Kreppel et al., 1997; Lubas et al., 1997; Wrabl and Grishin, 2001; Lazarus et al., 2005]. Near the N-terminus, there are 11 tetratricopeptide repeats (TPR), a motif that is involved in protein–protein interactions. The crystal structure for another TPR has been solved [Das et al., 1998] and the TPRs in OGT have been solved more recently [Jinek et al., 2004]. In the C-terminal half of the molecule are the domains that bind the UDP and sugar moieties. OGT is localized in both the cytoplasm and nucleus and hence, the O-GlcNAc modification can be found on both

nuclear and cytoplasmic proteins. This modification also can occur co-translationally [Starr and Hanover, 1990], but is highly dynamic. For some proteins, it has been shown that the half-life of the O-GlcNAc is shorter than the half-life of the protein [Hart, 1997], implying that the O-GlcNAc can be removed and added to proteins post-translationally, in a manner similar to phosphorylation. Extracellular signals [Kearse and Hart, 1991] and the cell cycle [Haltiwanger and Philipsberg, 1997; Slawson et al., 2005] have been shown to alter the pattern of proteins modified by O-GlcNAc.

As reviewed earlier, while OGT catalyzes the addition of this amino-sugar modification, the opposing reaction is catalyzed by the O-GlcNAc-selective N-acetyl-β-D-glucosaminidase (O-Glc NAcase). First cloned as a hyaluronidase and named MGEA5 [Heckel et al., 1998], Hart's group showed that this protein, also encoded by a unique gene (10q24 in humans [Gao et al., 2001]) and near the telemere of chromosome 1 in rats [Van Tine et al., 2003], (accession NM_131904), is in fact the O-GlcNAcase. This region of the genome is a hot spot for type 2 diabetes [Van Tine et al., 2003; Lehman et al., 2005] and late-onset Alzheimer's disease [Bertram et al., 2000; Liu et al., 2004]. The domain structure of the protein and its enzymatic activity revealed that in fact it has two domains. The N-terminus of the protein contains the O-GlcNAcase enzyme domain [Toleman et al., 2006] while the C-terminus contains a histone acetyltransferase (HAT) domain, but activation is required [Toleman et al., 2004]. We have placed mutations in the active sites of the O-GlcNAcase and HAT. By sequence homology, the O-GlcNAcase contains a TIM barrel with β-pleated sheets so that the two active-site aspartic acids, predicted to be in the β region, require a spacer amino acid so that they both point in the same direction, into the active site of the enzyme. The presence of these two domains has caused us to rename the protein again to nuclear cytoplamic O-GlcNAcase and acetyltransferase (NCOAT) [Toleman et al., 2004]. The putative significance of these two domain will become evident.

STREPTOZOTOCIN AND THE INHIBITION OF THE O-GlcNAcase

Our laboratory [Roos et al., 1998] and others [Roos et al., 1998] have shown that the O-GlcNAcase part of NCOAT, which is in the amino-terminal part of the molecule, is inhibited by the GlcNAc analog, streptozoticin (STZ) (Fig. 2). STZ has been used extensively to cause experimental diabetes and in chemotherapy for neuroendocrine tumors but its action as an O-GlcNAcase inhibitor was discovered 40 years [Roos et al., 1998] after the discovery of the drug. Much interest into the mode of action of STZ in causing diabetes and other conditions [Liu

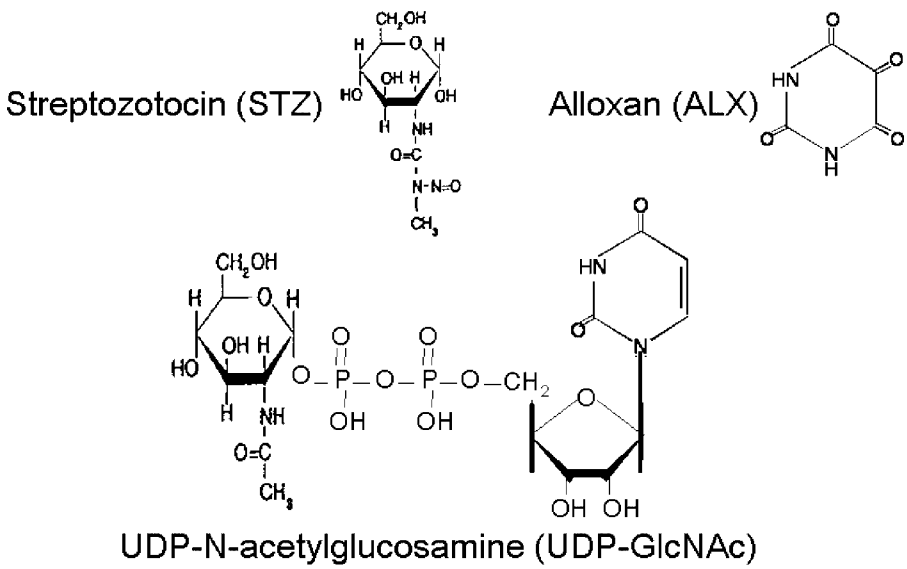

Fig. 2. The structural similarities of UDP-GlcNAc and the inhibitors of OGT and the O-GlcNAcase part of NCOAT are shown. Streptozotocin (STZ) has a structure resembling N-acetylglucosamine (GlcNAc) and inhibits O-GlcNAcase. Alloxan is structurally similar to the uracil moiety and inhibits OGT.

et al., 2004] was expended prior to the appreciation of the O-GlcNAc pathway. Hence, many actions of STZ were attributed to the compound [LeDoux et al., 1986; Turk et al., 1993; Schnedl et al., 1994; Pieper et al., 1999]. These other actions of STZ may indeed happen. For example, the donation of NO by STZ may occur but, by itself, is insufficient to cause cell death [Konrad et al., 2001a]. To summarize our studies, inhibition of O-GlcNAcase by STZ is associated with apoptosis in β-cells [Liu et al., 2000], pituitary [Liu et al., 2002] and the hippocampus of the brain [Liu et al., 2004]. All these cell types express high levels of OGT. We have shown that these cells with more OGT modify proteins with O-GlcNAc most rapidly [Liu et al., 2000, 2002, 2004; Konrad et al., 2001b]. In the presence of STZ, OGT is unopposed therefore the removal of this modification cannot occur [Liu et al., 2000]. The inhibition of the O-GlcNAcase requires the active enzyme to convert STZ [Toleman et al., 2006a] to a stable transition-state analog causing sustained inactivation of the one and only known O-GlcNAcase. Why programmed cell death occurs in association with O-GlcNAc maintenance is not entirely established, but we have presented some compelling evidence [Zhang et al., 2003] about the proteasome, an organelle that, among other things, clears oxidized proteins from the cell. Perhaps the oxidative damage caused by STZ cannot be cleared by the proteasome, which is irreversibly blocked by O-GlcNAc, which also cannot be removed because of O-GlcNAcase blockade by STZ. Protein oxidation and O-GlcNAcase inhibition may act together to give STZ its toxicity. Unfortunately, STZ is a poor chemotherapeutic agent. It blocks the one and only known O-GlcNAcase, which is ubiquitous so its action is non-specific. While some specificity results from cells expressing high levels of OGT, and for cells where STZ can enter [Schnedl et al., 1994], these properties of STZ make it a very poor candidate for cancer therapy in general. Whether a derivative of STZ might hold promise cannot be ruled out.

ANTIBODIES TO DETECT O-GlcNAc

The original means of detecting this O-GlcNAc modification on proteins was through the use of bovine milk galactosyltransferase using labeled UDP-[^{3}H]-galactose as a substrate [Torres and Hart, 1984]. This enzyme labels terminal GlcNAc residues. While this technique is very valuable for the mapping of O-GlcNAc protein modification sites [Roos et al., 1997; Yang et al., 2001], my laboratory has also made extensive use of a monoclonal antibody, RL-2 [Snow et al., 1987], although other O-GlcNAc-specific antibodies have also been described [Turner et al., 1990; Jung et al., 1997; Comer et al., 2001]. The binding of RL-2 to such proteins is dependent on the GlcNAc modification and this binding can be blocked by the incubation of the antibody with free GlcNAc [Roos et al., 1996; Konrad et al., 2000; Liu et al., 2000, 2002]. N-acetylgalactosamine and even unacetylated GlcN itself are unable to block the binding of RL-2 to proteins on Western blots [Roos et al., 1996; Konrad et al., 2000; Liu et al., 2002] or on tissue sections [Liu et al., 2000].

RL-2 immuno-staining studies of cells in culture [Sayeski and Kudlow, 1996], or of intact tissues and cell fractionation studies using the galactosyltransferase method have both shown that the majority of O-GlcNAc is on proteins either in the nucleus or on cytoskeletal proteins [Hart, 1997]. So far, many proteins in which O-GlcNAc sites have been mapped turn out to be transcription factors [Hart, 1997; Comer and Hart, 1999, 2000; Wells et al., 2001]. Indeed, as outlined above, the first transcription factor shown to contain O-GlcNAc was Sp1 [Jackson and Tjian, 1988]. However, the serum response factor [Reason et al., 1992], c-myc [Chou et al., 1995], estrogen receptors [Cheng and Hart, 2000, 2001] and RNA polymerase II itself [Kelly et al., 1993; Comer and Hart, 2001] are now among the growing list of transcription factors known to contain this modification [Comer and Hart, 1999]. That transcription factors are modified by O-GlcNAc and that the modifications often occur in the transcriptional activation domain suggests that this modification may have a role in the function of these proteins in the control of transcription.

THE EFFECTS OF O-GlcNAc ON THE Sp1 TRANSCRIPTION FACTOR

We embarked on studies of the role of O-Glc NAc in the Sp1 transcription factor because it had been shown to be vital for TGFα gene transcription [Shin et al., 1992] and the region of the gene that we had mapped to be the glucose/glucosamine response element [McClain et al., 1992] turned out to contain three Sp1

binding sites as identified by DNAase I foot printing [Shin et al., 1992]. Also, the Sp1 molecule was known to contain at least eight O-Glc NAc modifications [Jackson and Tjian, 1988].

THE PROTEASOME AND O-GlcNAc

Our studies on Sp1 have uncovered two mechanisms of regulation. The amount of Sp1, like other regulatory proteins, is controlled by both its rate of synthesis and degradation by the proteasome, the organelle in the cell that is primarily responsible for nuclear and cytoplasmic protein proteolysis. In the original study, we showed that cells exposed to conditions of low glucose and cAMP accumulation, display a rapid depletion of Sp1 DNA-binding activity and protein [Han and Kudlow, 1997]. This loss of Sp1 could be blocked by the highly specific inhibitors of proteasomes, MG132 and lactacystin and also by LLnL, suggesting that Sp1 was subject to proteasome degradation. In experiments where proteasome degradation of Sp1 was blocked by lactacystin, we could also show that the treatment with cAMP and low glucose resulted in the near total loss of the O-GlcNAc residues from the Sp1 protein and other proteins in the cell. This study therefore correlated the stability of Sp1 against proteasome degradation with the level of Sp1 modification by O-GlcNAc.

The depletion of O-GlcNAc on Sp1 and other proteins appears to have resulted both from the glucose starvation and from the cAMP. The glucose starvation probably lowered F-6-P levels while the cAMP blocked the activity of GFAT1 [Chang et al., 2000; Hu et al., 2004]. When the cells were provided GlcN following glucose starvation and forskolin, Sp1 O-glycosylation was increased and degradation decreased.

These findings suggested that the proteasomal degradation of Sp1 might be part of a nutritional sensing system. That is, when cells are under nutritional stress (glucose starvation), Sp1, which controls the transcription of most housekeeping genes, is degraded. The consequence of Sp1 degradation by the proteasome might be to block macromolecular synthesis to conserve nutrients under nutritional stress situations. We have since shown that the ATPase activity of the 19S proteasome cap is inhibited by the post-translation modification of the Rpt2 ATPase by OGT using UDP-GlcNAc as a substrate. The degradation of Sp1 was found to be independent of Sp1's level of glycosylation. Rather, this modification inhibits proteasome function directly [Zhang et al., 2003] and results in the accumulation of Sp1 and other proteins [Zhang et al., 2003; Liu et al., 2004]. While OGT enzymatic modification inhibits proteasomes, removal of the O-GlcNAc modification by the O-GlcNAcase domain of NCOAT activates proteasomes [Zhang et al., 2003], making the process reversible.

PROTEASOMES AND APOPTOSIS

Considerable progress has been made in the general understanding of the pathways leading to apoptosis. Several reviews of the subject have appeared (e.g., [Green and Reed, 1998; Hengartner, 2001; Stergiou and Hengartner, 2004]). Briefly, apoptosis can be induced through the activation of 'death-receptors' on the cell surface or as a result of internal protective mechanisms such as the p53 response to DNA damage. Conversely, pro-growth signals such as growth factor receptor activation can block apoptosis thought the PI3 kinase-AKT-forkhead pathway [Tran et al., 2003] while relief of growth signals can result in apoptosis. Central to apoptosis is the mitochondrion. The apoptotic signals largely appear to converge on the mitochondrion, resulting in the release of cytochrome c and other molecules. The release of cytochrome c is stimulated by a p53 target gene, Bax, a member of the Bcl family. The cytochrome c release stimulates the assembly of the 'apoptosome' which cleaves procaspase-3 to activated caspase-3. The activated caspase-3 then becomes the major trigger of the multitude of events leading to the disassembly of the cell. With respect to proteasomes: (1) Proapoptotic factors such as p53 and bax are degraded by the proteasome [Chen et al., 2000; Li and Dou, 2000; Seluanov et al., 2001; Sun et al., 2001]. (2) Proteasome inhibitors are being used as an adjunct to cancer chemotherapy because these inhibitors promote apoptosis [Cusack et al., 2001; Kim, 2001; Milligan and Nopajaroonsri, 2001; Zavrski et al., 2003] presumably because these pro-apoptotic factors accumulate. (3) Proteasome function is inhibited O-GlcNAc [Han and Kudlow, 1997; Su et al., 1999, 2000; Zhang et al., 2003]; O-GlcNAc may also inhibit the proteolysis of pro-apoptotic factors. (4) O-GlcNAc accumulation as a result of irreversible chemical O-GlcNAcase inhibition (STZ) results

in β-cell [Liu et al., 2000], pituitary [Liu et al., 2002] and neuronal [Liu et al., 2004] apoptosis. Taken together, these data suggest that the inhibition of the proteasome by O-GlcNAc may result in apoptosis in susceptible cells.

DIRECT EFFECT OF O-GlcNAc MODIFICATION ON TRANSCRIPTION

Transcriptional activation essentially results from DNA-directed protein–protein interactions and protein complex formation. Such interactions had been worked out for Sp1. Domain B of Sp1 had been shown to confer the ability of Sp1 to homo-multimerize [Kadonaga et al., 1988; Pascal and Tjian, 1991; Hoey et al., 1993; Gill et al., 1994] and to interact with the TATA-binding protein associated factor, termed TAF110 [Gill et al., 1994]. Using a portion of Sp1 that confers these interactions that we called SpE, we performed direct pull-down experiments with glycosylated and unglycosylated Sp1 peptide [Roos et al., 1997]. We showed that the interactions involving this Sp1 peptide were blocked by O-GlcNAc. Subsequently, we have confirmed that the in vivo interaction between holo-Sp1 and the SpE peptide of Sp1 is blocked by O-GlcNAc [Yang et al., 2001].

As predicted from these interaction studies, we have gone on to show that the glycosylation of one of the Sp1 transcriptional activation domain blocks transcriptional activation in nuclear extracts using in vitro transcription assays [Yang et al., 2001]. That is, unmodified recombinant SpE was much more potent than O-GlcNAc modified SpE at stimulating transcription. Indeed, overexpression of OGT in cells suppresses transcriptional activation stimulated by holo-Sp1 and a Gal4-SpE fusion protein [Yang et al., 2001]. This difference in transcriptional activation can also be observed in pancreatic β-cells, a cell type that naturally overexpresses OGT [Roos et al., 1998; Yang et al., 2001]. However, because Sp1 is multiply O-glycosylated, it still remains possible that glycosylation at a different site in perhaps a different cell type or on a different promoter, might stimulate transcription [Goldberg et al., 2005]. Going back to TGFα, we observed a biphasic response. In the first few hours, TGFα expression was decreased, probably as a result of Sp1 O-GlcNAc modification. However, at the same time, Sp1 accumulated because it degradation was blocked by modification of the proteasome [Zhang et al., 2003]. Later, we think that as the modification of the now abundant Sp1 decreased, TGFα gene expression increased because of its dependence the Sp1 transcription factor [Shin et al., 1992].

Because the O-GlcNAc modification blocks Sp1-mediated gene activation, and Sp1 plays so broad a role in transcription, we investigated the notion that OGT may be part of a co-repressor complex, hence playing a very general role in the repression of multiple genes ([Yang et al., 2002], reviewed by Hart and co-workers [Vosseller et al., 2002a]). The blocking of Sp1-mediated transcription would affect many genes and thus would have no specificity. However, targeting OGT to specific genes would overcome this problem by bringing the enzyme to specific genes needing repression. This targeting would allow OGT to modify the transcription apparatus specifically of repressed genes. To accomplish this targeting, OGT associates with mSin3A [Yang et al., 2002]. We propose that mSin3A targets OGT to specifically repressed promoters to inactivate transcription factors and RNA polymerase II by O-GlcNAc modification, which acts in concert with histone deacetylation to promote gene silencing in an efficient, concerted and specific manner [Yang et al., 2002]. These two independent modifications, removal of acetates from histones and O-GlcNAcylation act synergistically in the repression process. This profound difference in behavior of a transcriptional activation domain depending on whether the domain is modified by O-GlcNAc or not, is the first direct demonstration that O-GlcNAc can directly change the function of a transcription factor and that this change of function is not related to phosphorylation (MALDI mass spectoscopy indicated that the Sp1 activation domain under study is never phosphorylated, only glycosylated [Yang et al., 2001]). Based on findings so far that the OGT TPR motif recognizes hydrophobic or amphipathic domains in proteins [Yang et al., 2002], it is likely that modification by O-GlcNAc of these hydrophobic regions in OGT substrates results in a change in the degree of hydrophobicity in the protein interaction domain, thus disrupting protein–protein interactions.

These studies suggest that if OGT is involved in co-repression, then the converse enzyme, O-GlcNAcase may be involved in the activation of gene expression. O-GlcNAcase has been detected in nuclear extract [Dong and Hart, 1994],

however, there are no studies to show how this activity is controlled. The cloning of the O-GlcNAcase has been reported [Heckel et al., 1998; Comtesse et al., 2001; Gao et al., 2001] and we have independently cloned the rat and mouse cDNAs and splice variants (accession AY039679).

O-GlcNAczyme

We provided evidence that OGT, through its first six TPR domains, associates with mSin3A in co-repressor complexes associated with unliganded estrogen receptors [Yang et al., 2002]. Furthermore, ChIP assays showed that proteins in repressed chromatin contains more O-GlcNAc than chromatin proteins following estrogen-stimulation. This and other evidence [Yang et al., 2002] implied that the enzymatic activity of OGT was utilized to catalyze the placement of O-GlcNAc onto proteins like Sp1 and act in concert with HDAC, which removes acetate groups from histones, to silence estrogen-responsive genes. Since NCOAT has both O-GlcNAcase activity to remove inhibitory sugars and HAT activity to acetylate histones, it was reasonable for us to suppose that NCOAT was part of the co-activator complex just as OGT was part of the co-repressor complex. Our experiments did not agree.

NCOAT, with very high affinity, associates with OGT [Whisenhunt et al., 2006]. For OGT, the N-terminus and first six TPR domains are required. For NCOAT, a domain between the O-GlcNAcase and HAT domains is necessary and sufficient for this binding (Fig. 3).

ESTROGEN RECEPTORS AND SIGNALING

The classical estrogen receptor is actually a DNA-binding transcription factor. Like other nuclear hormone receptors, an active ligand, binding to the receptor, turns on DNA transcription. This positive transcriptional effect requires that the liganded receptor recruit an activation complex which includes ATP-dependent chromatin remodeling factors, the HAT activity of CBP and interacting molecules that aid the receptor in contacting the core transcription machinery (see [Hanstein et al., 1996; Freedman, 1999; Glass and Rosenfeld, 2000; Yang et al., 2002]).

What is now clear is that these receptors are actively repressed when they are depleted of their ligand. It is not just the absence of activation that allows repression to occur, it is the exchange of co-activation complexes for co-repression complexes that is required. These co-repressor complexes are nucleated by unliganded receptor and contain some of the opposite enzymes from the activation complex. The co-repressor complex recruits mSin3A and other proteins. Part of the co-repression is mediated by histone deacetylases (HDAC), which remove acetyl groups from histones (HDAC) so that the closed nucleasomal structures of chromatin can be formed as part of the repression process [Glass and Rosenfeld, 2000; Shang et al., 2000; Wen et al., 2000; Perissi et al., 2004]. By showing that OGT also binds to mSin3A [Yang et al., 2002], we have added O-GlcNAc-modification of the transcription apparatus to the mechanism of repression. The enzymatic modification by O-GlcNAc of positive-acting transciptional factors like Sp1 turns off transcription in concert with the modification of chromatin structure. By cooperating, deacetylation and O-GlcNAcylation ensure that the repressed gene is thoroughly shut off. The targeting of OGT to unliganded genes provides

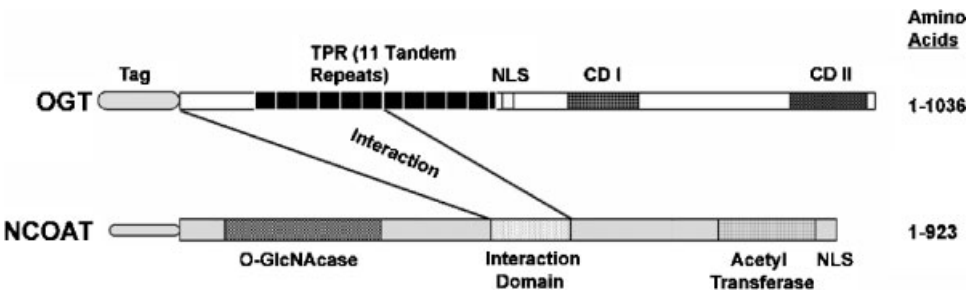

Fig. 3. OGT and NCOAT associate to form the O-GlcNAczyme. This association was shown to exist in vivo using antibodies to the proteins and in vitro by pull-down assays. The interaction domains are shown diagrammatically. OGT interacts with NCOAT through the N-terminus and first six TPRs in OGT. NCOAT uses a domain between the two enzymes. The pull-down assays indicate that the association of these proteins is very strong. The putative nuclear localization signals (NLS) in each protein are shown.

the required specificity to the modification process. Even if OGT is generally off, the specificity of the enzyme for a particular gene is conferred by its recruitment to that gene.

ESCAPE FROM REPRESSION

As alluded to above, NCOAT associates with OGT. Therefore, NCOAT accompanies OGT in the cell. The O-GlcNAczyme that is formed by the combination of these proteins contains the enzymes that both places O-GlcNAc on proteins and removes this modification. However, even though NCOAT is resident with OGT in the repression complex, the OGT dominates during repression. This can be deduced, because ChIP assays show that proteins on the DNA of repressed genes are O-GlcNAcylated [Yang et al., 2002; Whisenhunt et al., 2006]. This dominance of OGT over NCOAT during repression of gene transcription prevents a futile cycle of modification and de-modification. In addition, the O-GlcNAczyme contains HAT activity to oppose the action of HDAC [Toleman et al., 2004, 2006b], which is recruited along with OGT by mSin3A [Yang et al., 2002; Whisenhunt et al., 2006] (see Fig. 4). But the HAT activity of NCOAT which would oppose the HDAC activity also requires activation [Toleman et al., 2004]. The HAT of NCOAT made recombinantly in bacteria (unmodified) is inactive unless exposed to a mammalian extract [Toleman et al., 2004]. We deduce that this default activity of OGT and HDAC switches when the signal for activation is received. During activation, NCOAT itself is somehow activated and this activation can be deduced again, at least for the O-GlcNAcase, because activated genes have less O-GlcNAc. This activation process begins with the switching from a repression to an activation complex. NCOAT, by reversing both enzymatic processes, removal of sugars (O-GlcNAcase) and addition of acetates (HAT), plays a central role in the gene's escape from repression (Fig. 4).

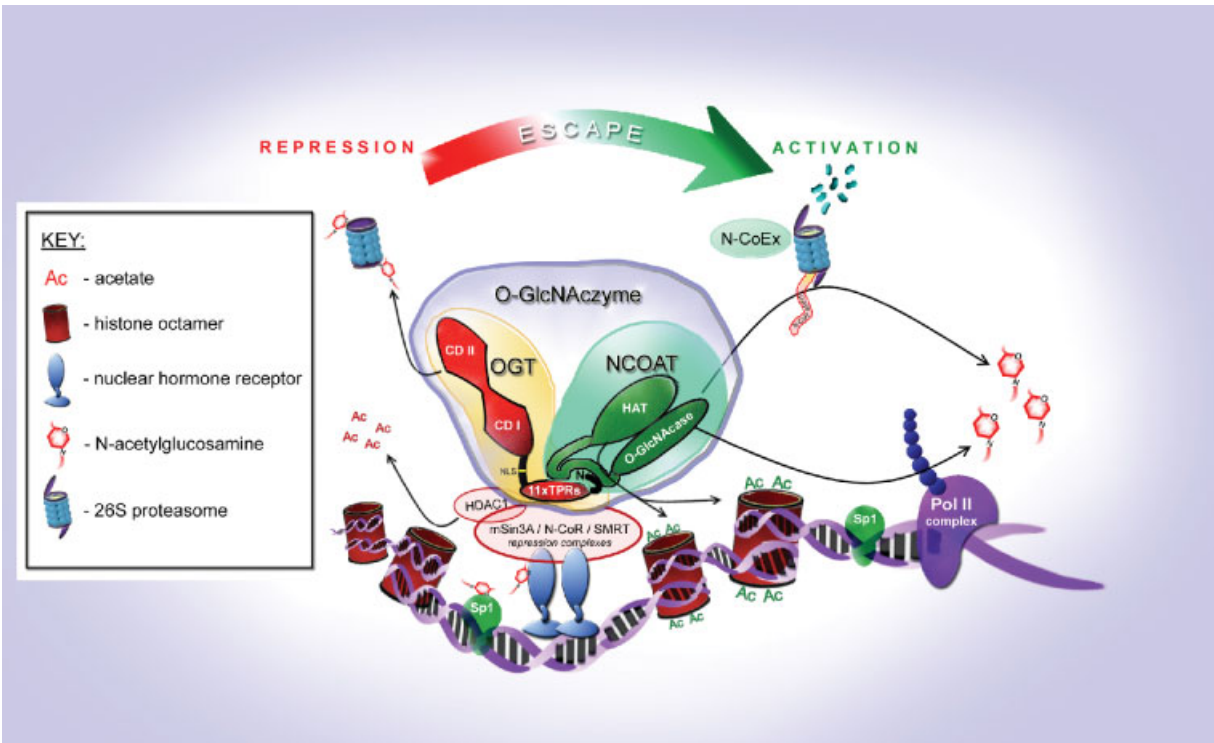

Fig. 4. A cartoon depicting on unoccupied nuclear receptor, the O-GlcNAczyme, composed of OGT and NCOAT. This complex might regulate the transition between repression and activation when the receptor becomes occupied. Repression is depicted as red and activation as green. When a gene is repressed, OGT dominates as the active enzyme, modifying transcriptional activators with O-GlcNAc. OGT cooperates with HDAC to turn off expression of the gene. However, NCOAT is required to transition from repression to activation when hormone is present. The activities of NCOAT, that remove the O-GlcNAc modifications on the activators while replacing the acetates on the histones, are turned on to allow this transition, but how the NCOAT enzymes are activated remains unknown.

Because NCOAT resides in the co-repression complex of proteins, it is already in position to initiate activation. Therefore, only post-translational modification(s) is required, making the transition rapid. Furthermore, proteins in the repression complex are degraded by the proteasome [Li et al., 2003]. Since the O-GlcNAcase in NCOAT activates proteasome function [Zhang et al., 2003], activated NCOAT, through the proteasome, plays a role in assuring that gene activation ensues because the repression complex in the locality of the gene is proteasomally degraded. By residing in complex with OGT, NCOAT is also gene-specific. The exact positions and the nature of these modifications of NCOAT that activate it are not known, nor are the enzymes that perform these changes.

In a signal transduction process, it is useful that a step is in signaling is determined to be upstream or downstream of another step. As far as nuclear hormone signaling, this question becomes whether NCOAT is upstream or downstream of estrogen signaling. If NCOAT were downstream of the estrogen signal, then cell expressing a dominant-negative NCOAT would fail to respond to estrogen. To test this idea, MCF-7 human breast cancer cells were transfected with a construct of NCOAT that behaved as a positional dominant negative. The plasmid encoded the interaction domain of NCOAT but not it enzymatic activities. Because the creation of the O-GlcNAczyme involves saturable binding of NCOAT to OGT [Whisenhunt et al., 2006], enough of the interaction domain protein would displace normal enzymatically active NCOAT from the complex. The displaced full-length NCOAT still has its enzymatic activities but is relatively inactive because it is not targeted by its partner, OGT. The cells were then treated with estrogen. A northern blot was performed on two classical estrogen responsive genes [Garcia et al., 1996; Khan et al., 2003], cathepsin D and cad. In both cases, the effect of estrogen was blocked by the interaction domain of NCOAT, but not NCOAT lacking the interaction domain (Fig. 6) [Whisenhunt et al., 2006]. The implication of this study is that NCOAT is downstream of estrogen signals. As a corollary, it can be inferred that the enzymes that activate NCOAT must act downstream of estrogen as well because they act upon NCOAT. Thus, these enzymes could confer hormone-independence if they could modify NCOAT to activate its enzymatic activities without an estrogen (hormone) signal. Because the gene repression by the O-GlcNAczyme is downstream of estrogen, potential for a new therapeutic approach to hormone unresponsive tumors may arise. Which of the two activities of NCOAT is more important, the HAT or the O-GlcNAcase cannot be stated with certainty. While there are many HATs, the O-GlcNAcase of NCOAT is unique [Gao et al., 2001; Van Tine et al., 2003], so one might guess that the latter is more important but experimental verification awaits. The generality of gene regulation by the O-GlcNAczyme has not been established. Since the O-Glc NAczyme binds to very general co-repressor molecules like mSin3A [Yang et al., 2002; Whisenhunt et al., 2006], the likelihood is high that this enzyme system is also involved with many other categories of genes. While repressing many genes, the OGT in the O-GlcNAczyme may be involved with the activation of other genes. For example, p53 may be activated by O-GlcNAc [Shaw et al., 1996]. Expression of the targets of p53 would allow gene repairs while the expression of temporally expendable gene targets might be repressed.

Speculating that to O-GlcNAczyme mechanism is very general, perhaps this mechanism is also involved in the PPARγ response because the PPARγ receptor is also a nuclear receptor. To sensitize type 2 diabetic's fat cells to insulin, activating analogs, thiazolidinediones, are now in common therapeutic use. Thus, part of the desensitization to insulin seen with glucosamine [McClain et al., 2002; Vosseller et al., 2002b] might result from greater creation of the UDP-GlcNAc substrate for OGT repression of PPARγ-responsive genes. One prediction of this hypothesis would be that these analogs might overcome the glucosamine-induced desensitization.

REFERENCES

Bertram L, Blacker D, Mullin K, Keeney D, Jones J, Basu S, Yhu S, McInnis MG, Go RC, Vekrellis K, Selkoe DJ, Saunders AJ, Tanzi RE. 2000. Evidence for genetic linkage of Alzheimer's disease to chromosome 10q. Science 290:2302–2303.

Chang Q, Su K, Baker JR, Yang X, Paterson AJ, Kudlow JE. 2000. Phosphorylation of human glutamine: Fructose-6-phosphate amidotransferase by cAMP-dependent protein kinase at serine 205 blocks the enzyme activity. J Biol Chem 275:21981–21987.

Chen F, Chang D, Goh M, Klibanov SA, Ljungman M. 2000. Role of p53 in cell cycle regulation and apoptosis following exposure to proteasome inhibitors. Cell Growth Differ 11:239–246.

Cheng X, Hart GW. 2000. Glycosylation of the murine estrogen receptor-alpha. J Steroid Biochem Mol Biol 75: 147–158.

Cheng X, Hart GW. 2001. Alternative O-glycosylation/O-phosphorylation of serine-16 in murine estrogen receptor beta: Post-translational regulation of turnover and transactivation activity. J Biol Chem 276:10570–10575.

Chou TY, Hart GW, Dang CV. 1995. c-Myc is glycosylated at threonine 58, a known phosphorylation site and a mutational hot spot in lymphomas. J Biol Chem 270: 18961–18965.

Comer FI, Hart GW. 1999. O-GlcNAc and the control of gene expression. Biochim Biophys Acta 1473:161–171.

Comer FI, Hart GW. 2000. O-Glycosylation of nuclear and cytosolic proteins. Dynamic interplay between O-GlcNAc and O-phosphate. J Biol Chem 275:29179–29182.

Comer FI, Hart GW. 2001. Reciprocity between O-GlcNAc and O-phosphate on the carboxyl terminal domain of RNA polymerase II. Biochemistry 40:7845–7852.

Comer FI, Vosseller K, Wells L, Accavitti MA, Hart GW. 2001. Characterization of a mouse monoclonal antibody specific for O-linked N-acetylglucosamine. Anal Biochem 293:169–177.

Comtesse N, Maldener E, Meese E. 2001. Identification of a nuclear variant of MGEA5, a cytoplasmic hyaluronidase and a beta-N-acetylglucosaminidase. Biochem Biophys Res Commun 283:634–640.

Cusack JC Jr., Liu R, Houston M, Abendroth K, Elliott PJ, Adams J, Baldwin AS Jr. 2001. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: Implications for systemic nuclear factor-kappaB inhibition. Cancer Res 61:3535–3540.

Das AK, Cohen PW, Barford D. 1998. The structure of the tetratricopeptide repeats of protein phosphatase 5: Implications for TPR-mediated protein-protein interactions. Embo J 17:1192–1199.

Dong DL, Hart GW. 1994. Purification and characterization of an O-GlcNAc selective N-acetyl-beta-D-glucosaminidase from rat spleen cytosol. J Biol Chem 269:19321–19330.

Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M. 2000. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proc Natl Acad Sci USA 97:12222–12226.

Freedman LP. 1999. Increasing the complexity of coactivation in nuclear receptor signaling. Cell 97:5–8.

Gao Y, Wells L, Comer FI, Parker GJ, Hart GW. 2001. Dynamic O-glycosylation of nuclear and cytosolic proteins: Cloning and characterization of a neutral, cytosolic beta-N-acetylglucosaminidase from human brain. J Biol Chem 276:9838–9845.

Garcia M, Platet N, Liaudet E, Laurent V, Derocq D, Brouillet JP, Rochefort H. 1996. Biological and clinical significance of cathepsin D in breast cancer metastasis. Stem Cells 14:642–650.

Gill G, Pascal E, Tseng ZH, Tjian R. 1994. A glutamine-rich hydrophobic patch in transcription factor Sp1 contacts the dTAFII110 component of the Drosophila TFIID complex and mediates transcriptional activation. Proc Natl Acad Sci USA 91:192–196.

Glass CK, Rosenfeld MG. 2000. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 14:121–141.

Goldberg HJ, Whiteside CI, Hart GW, Fantus IG. 2006. Posttranslational, reversible O-glycosylation is stimulated by high glucose and mediates plasminogen activator inhibitor-1 (PAI-1) gene expression and Sp1 transcriptional activity in glomerular mesangial cells. Endocrinology 147:222–231.

Green DR, Reed JC. 1998. Mitochondria and apoptosis. Science 281:1309–1312.

Haltiwanger RS, Philipsberg GA. 1997. Mitotic arrest with nocodazole induces selective changes in the level of O-linked N-acetylglucosamine and accumulation of incompletely processed N-glycans on proteins from HT29 cells. J Biol Chem 272:8752–8758.

Han I, Kudlow JE. 1997. Reduced O glycosylation of Sp1 is associated with increased proteasome susceptibility. Mol Cell Biol 17:2550–2558.

Hanstein B, Eckner R, DiRenzo J, Halachmi S, Liu H, Searcy B, Kurokawa R, Brown M. 1996. p300 is a component of an estrogen receptor coactivator complex. Proc Natl Acad Sci USA 93:11540–11545.

Hart GW. 1997. Dynamic O-linked glycosylation of nuclear and cytoskeletal proteins. Annu Rev Biochem 66:315–335.

Hartweck LM, Genger RK, Grey WM, Olszewski NE. 2006. SECRET AGENT and SPINDLY have overlapping roles in the development of Arabidopsis thaliana L. Heyn. J Exp Bot 57:865–875.

Hassell JR, Kimura JH, Hascall VC. 1986. Proteoglycan core protein families. Annu Rev Biochem 55:539–567.

Heckel D, Comtesse N, Brass N, Blin N, Zang KD, Meese E. 1998. Novel immunogenic antigen homologous to hyaluronidase in meningioma. Hum Mol Genet 7:1859–1872.

Hengartner MO. 2001. Apoptosis: Corralling the corpses. Cell 104:325–328.

Hoey T, Weinzierl RO, Gill G, Chen JL, Dynlacht BD, Tjian R. 1993. Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of coactivators. Cell 72:247–260.

Hu Y, Riesland L, Paterson AJ, Kudlow JE. 2004. Phosphorylation of mouse glutamine: Fructose-6-phosphate amidotransferase 2 (GFAT2) by cAMP-dependent protein kinase increases the enzyme activity. J Biol Chem 279:29988–29993.

Jackson SP, Tjian R. 1988. O-glycosylation of eukaryotic transcription factors: Implications for mechanisms of transcriptional regulation. Cell 55:125–133.

Jinek M, Rehwinkel J, Lazarus BD, Izaurralde E, Hanover JA, Conti E. 2004. The superhelical TPR-repeat domain of O-linked GlcNAc transferase exhibits structural similarities to importin alpha. Nat Struct Mol Biol 11: 1001–1007.

Jung E, Gooley AA, Packer NH, Slade MB, Williams KL, Dittrich W. 1997. An in vivo approach for the identification of acceptor sites for O-glycosyltransferases: Motifs for the addition of O-GlcNAc in Dictyostelium discoideum. Biochemistry 36:4034–4040.

Kadonaga JT, Courey AJ, Ladika J, Tjian R. 1988. Distinct regions of Sp1 modulate DNA binding and transcriptional activation. Science 242:1566–1570.

Kearse KP, Hart GW. 1991. Lymphocyte activation induces rapid changes in nuclear and cytoplasmic glycoproteins. Proc Natl Acad Sci USA 88:1701–1705.

Kelly WG, Dahmus ME, Hart GW. 1993. RNA polymerase II is a glycoprotein. Modification of the COOH-terminal domain by O-GlcNAc. J Biol Chem 268:10416–10424.

Khan S, Abdelrahim M, Samudio I, Safe S. 2003. Estrogen receptor/Sp1 complexes are required for induction of cad gene expression by 17beta-estradiol in breast cancer cells. Endocrinology 144:2325–2335.

Kim K. 2001. Proteasome inhibitors sensitize human vascular smooth muscle cells to Fas (CD95)-mediated death. Biochem Biophys Res Commun 281:305–310.

Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED. 1998. High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. J Clin Invest 101:160–169.

Konrad RJ, Janowski KM, Kudlow JE. 2000. Glucose and streptozotocin stimulate p135 O-glycosylation in pancreatic islets. Biochem Biophys Res Commun 267:26–32.

Konrad RJ, Mikolaenko I, Tolar JF, Liu K, Kudlow JE. 2001a. The potential mechanism of the diabetogenic action of streptozotocin: Inhibition of pancreatic beta-cell O-GlcNAc-selective N-acetyl-beta-D-glucosaminidase. Biochem J 356:31–41.

Konrad RJ, Tolar JF, Hale JE, Knierman MD, Becker GW, Kudlow JE. 2001b. Purification of the O-glycosylated protein p135 and identification as O-GlcNAc transferase. Biochem Biophys Res Commun 288:1136–1140.

Kornfeld R. 1967. Studies on L-glutamine D-fructose 6-phosphate amidotransferase. I. Feedback inhibition by uridine diphosphate-N-acetylglucosamine. J Biol Chem 242:3135–3141.

Kreppel LK, Blomberg MA, Hart GW. 1997. Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. J Biol Chem 272:9308–9315.

Lazarus BD, Roos MD, Hanover JA. 2005. Mutational analysis of the catalytic domain of O-linked N-acetylglucosaminyl transferase. J Biol Chem 280:35537–35544.

LeDoux SP, Woodley SE, Patton NJ, Wilson GL. 1986. Mechanisms of nitrosourea-induced beta-cell damage. Alterations in DNA. Diabetes 35:866–872.

Lehman DM, Fu DJ, Freeman AB, Hunt KJ, Leach RJ, Johnson-Pais T, Hamlington J, Dyer TD, Arya R, Abboud H, Goring HH, Duggirala R, Blangero J, Konrad RJ, Stern MP. 2005. A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective N-acetyl-beta-D glucosaminidase is associated with type 2 diabetes in Mexican Americans. Diabetes 54:1214–1221.

Li B, Dou QP. 2000. Bax degradation by the ubiquitin/proteasome-dependent pathway: Involvement in tumor survival and progression. Proc Natl Acad Sci USA 97:3850–3855.

Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK, Nigam SK, Aggarwal AK, Maas R, Rose DW, Rosenfeld MG. 2003. Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. Nature 426:247–254.

Liu K, Paterson AJ, Chin E, Kudlow JE. 2000. Glucose stimulates protein modification by O-linked GlcNAc in pancreatic beta cells: Linkage of O-linked GlcNAc to beta cell death. Proc Natl Acad Sci USA 97:2820–2825.

Liu K, Paterson AJ, Konrad RJ, Parlow AF, Jimi S, Roh M, Chin E Jr., Kudlow JE. 2002. Streptozotocin, an O-GlcNAcase inhibitor, blunts insulin and growth hormone secretion. Mol Cell Endocrinol 194:135–146.

Liu K, Paterson AJ, Zhang F, McAndrew J, Fukuchi K-I, Wyss JM, Peng L, Hu Y, Kudlow JE. 2004. Accumulation of protein *O*-GlcNAc modification inhibits proteasomes in the brain and coincides with neuronal apoptosis in brain areas with high *O*-GlcNAc metabolism. J Neurochem 89:1044–1055.

Lubas WA, Frank DW, Krause M, Hanover JA. 1997. O-Linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. J Biol Chem 272:9316–9324.

McClain DA, Paterson AJ, Roos MD, Wei X, Kudlow JE. 1992. Glucose and glucosamine regulate growth factor gene expression in vascular smooth muscle cells. Proc Natl Acad Sci USA 89:8150–8154.

McClain DA, Lubas WA, Cooksey RC, Hazel M, Parker GJ, Love DC, Hanover JA. 2002. Altered glycan-dependent signaling induces insulin resistance and hyperleptinemia. Proc Natl Acad Sci USA 99:10695–10699.

McKnight GL, Mudri SL, Mathewes SL, Traxinger RR, Marshall S, Sheppard PO, O'Hara PJ. 1992. Molecular cloning, cDNA sequence, and bacterial expression of human glutamine: Fructose-6-phosphate amidotransferase. J Biol Chem 267:25208–25212.

Milligan SA, Nopajaroonsri C. 2001. Inhibition of NF-kappa B with proteasome inhibitors enhances apoptosis in human lung adenocarcinoma cells in vitro. Anticancer Res 21:39–44.

Mueller SG, Paterson AJ, Kudlow JE. 1990. Transforming growth factor alpha in arterioles: Cell surface processing of its precursor by elastases. Mol Cell Biol 10:4596–4602.

O'Donnell N, Zachara NE, Hart GW, Marth JD. 2004. Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. Mol Cell Biol 24:1680–1690.

Oki T, Yamazaki K, Kuromitsu J, Okada M, Tanaka I. 1999. cDNA cloning and mapping of a novel subtype of glutamine: Fructose-6-phosphate amidotransferase (GFAT2) in human and mouse. Genomics 57:227–234.

Pascal E, Tjian R. 1991. Different activation domains of Sp1 govern formation of multimers and mediate transcriptional synergism. Genes Dev 5:1646–1656.

Paterson AJ, Kudlow JE. 1995. Regulation of glutamine: Fructose-6-phosphate amidotransferase gene transcription by epidermal growth factor and glucose. Endocrinology 136:2809–2816.

Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. 2004. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. Cell 116:511–526.

Pieper AA, Brat DJ, Krug DK, Watkins CC, Gupta A, Blackshaw S, Verma A, Wang ZQ, Snyder SH. 1999. Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. Proc Natl Acad Sci USA 96:3059–3064.

Reason AJ, Morris HR, Panico M, Marais R, Treisman RH, Haltiwanger RS, Hart GW, Kelly WG, Dell A. 1992. Localization of O-GlcNAc modification on the serum response transcription factor. J Biol Chem 267:16911–16921.

Robertson M, Swain SM, Chandler PM, Olszewski NE. 1998. Identification of a negative regulator of gibberellin action, HvSPY, in barley. Plant Cell 10:995–1007.

Roos MD, Han IO, Paterson AJ, Kudlow JE. 1996. Role of glucosamine synthesis in the stimulation of TGF-alpha gene transcription by glucose and EGF. Am J Physiol 270:C803–C811.

Roos MD, Su K, Baker JR, Kudlow JE. 1997. O glycosylation of an Sp1-derived peptide blocks known Sp1 protein interactions. Mol Cell Biol 17:6472–6480.

Roos MD, Xie W, Su K, Clark JA, Yang X, Chin E, Paterson AJ, Kudlow JE. 1998. Streptozotocin, an analog of N-acetylglucosamine, blocks the removal of O-GlcNAc from intracellular proteins. Proc Assoc Am Physicians 110: 422–432.

Sayeski PP, Kudlow JE. 1996. Glucose metabolism to glucosamine is necessary for glucose stimulation of transforming growth factor-alpha gene transcription. J Biol Chem 271:15237–15243.

Sayeski PP, Wang D, Su K, Han IO, Kudlow JE. 1997. Cloning and partial characterization of the mouse glutamine: Fructose-6-phosphate amidotransferase (GFAT) gene promoter. Nucleic Acids Res 25:1458–1466.

Schnedl WJ, Ferber S, Johnson JH, Newgard CB. 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes 43:1326–1333.

Seluanov A, Gorbunova V, Falcovitz A, Sigal A, Milyavsky M, Zurer I, Shohat G, Goldfinger N, Rotter V. 2001. Change of the death pathway in senescent human fibroblasts in response to DNA damage is caused by an inability to stabilize p53. Mol Cell Biol 21:1552–1564.

Shafi R, Iyer SP, Ellies LG, O'Donnell N, Marek KW, Chui D, Hart GW, Marth JD. 2000. The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc Natl Acad Sci USA 97:5735–5739.

Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. 2000. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. Cell 103:843–852.

Shaw P, Freeman J, Bovey R, Iggo R. 1996. Regulation of specific DNA binding by p53: Evidence for a role for O-glycosylation and charged residues at the carboxy-terminus. Oncogene 12:921–930.

Shin TH, Paterson AJ, Grant JH III, Meluch AA, Kudlow JE. 1992. 5-Azacytidine treatment of HA-A melanoma cells induces Sp1 activity and concomitant transforming growth factor alpha expression. Mol Cell Biol 12:3998–4006.

Slawson C, Zachara NE, Vosseller K, Cheung WD, Lane MD, Hart GW. 2005. Perturbations in O-linked {beta}-N-Acetylglucosamine protein modification cause severe defects in mitotic progression and cytokinesis. J Biol Chem 280:32944–32956.

Snow CM, Senior A, Gerace L. 1987. Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. J Cell Biol 104:1143–1156.

Starr CM, Hanover JA. 1990. Glycosylation of nuclear pore protein p62. Reticulocyte lysate catalyzes O-linked N-acetylglucosamine addition in vitro. J Biol Chem 265: 6868–6873.

Stergiou L, Hengartner MO. 2004. Death and more: DNA damage response pathways in the nematode *C. elegans*. Cell Death Differ 11:21–28.

Su K, Roos MD, Yang X, Han I, Paterson AJ, Kudlow JE. 1999. An N-terminal region of Sp1 targets its proteasome-dependent degradation in vitro. J Biol Chem 274: 15194–15202.

Su K, Yang X, Roos MD, Paterson AJ, Kudlow JE. 2000. Human Sug1/p45 is involved in the proteasome-dependent degradation of Sp1. Biochem J 348(Pt 2):281–289.

Sun J, Nam S, Lee CS, Li B, Coppola D, Hamilton AD, Dou QP, Sebti SM. 2001. CEP1612, a dipeptidyl proteasome inhibitor, induces p21WAF1 and p27KIP1 expression and apoptosis and inhibits the growth of the human lung adenocarcinoma A-549 in nude mice. Cancer Res 61: 1280–1284.

Toleman C, Paterson AJ, Whisenhunt TR, Kudlow JE. 2004. Characterization of the histone acetyltransferase (HAT) domain of a bifunctional protein with activable O-GlcNAcase and HAT activities. J Biol Chem 279:53665–53673.

Toleman C, Paterson AJ, Kudlow JE. 2006. Location and characterization of the O-GlcNAcase active site. Biochim Biophys Acta in press.

Toleman C, Paterson AJ, Shin R, Kudlow JE. 2006a. Streptozotocin inhibits O-GlcNAcase via the production of a transition state analog. Biochem Biophys Res Commun 340:526–534.

Toleman CA, Paterson AJ, Kudlow JE. 2006b. The histone acetyltransferase NCOAT contains a zinc finger-like motif involved in substrate recognition. J Biol Chem 281:3918–3925.

Torres CR, Hart GW. 1984. Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for O-linked GlcNAc. J Biol Chem 259:3308–3317.

Tran H, Brunet A, Griffith EC, Greenberg ME. 2003. The many forks in FOXO's road. Sci STKE 2003:RE5.

Turk J, Corbett JA, Ramanadham S, Bohrer A, McDaniel ML. 1993. Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. Biochem Biophys Res Commun 197:1458–1464.

Turner JR, Tartakoff AM, Greenspan NS. 1990. Cytologic assessment of nuclear and cytoplasmic O-linked N-acetylglucosamine distribution by using anti-streptococcal monoclonal antibodies. Proc Natl Acad Sci USA 87: 5608–5612.

Van Tine BA, Patterson AJ, Kudlow JE. 2003. Assignment of N-acetyl-D-glucosaminidase (Mgea5) to rat chromosome 1q5 by tyramide fluorescence in situ hybridization (T-FISH): Synteny between rat, mouse and human with insulin degradation enzyme (IDE). Cytogenet Genome Res 103:202B.

Vosseller K, Sakabe K, Wells L, Hart GW. 2002a. Diverse regulation of protein function by O-GlcNAc: A nuclear and cytoplasmic carbohydrate post-translational modification. Curr Opin Chem Biol 6:851–857.

Vosseller K, Wells L, Lane MD, Hart GW. 2002b. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. Proc Natl Acad Sci USA 99: 5313–5318.

Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 393:684–688.

Wells L, Hart GW. 2003. O-GlcNAc turns twenty: Functional implications for post-translational modification of nuclear and cytosolic proteins with a sugar. FEBS Lett 546:154–158.

Wells L, Vosseller K, Hart GW. 2001. Glycosylation of nucleocytoplasmic proteins: Signal transduction and O-GlcNAc. Science 291:2376–2378.

Wells L, Whalen SA, Hart GW. 2003. O-GlcNAc: A regulatory post-translational modification. Biochem Biophys Res Commun 302:435–441.

Wen YD, Perissi V, Staszewski LM, Yang WM, Krones A, Glass CK, Rosenfeld MG, Seto E. 2000. The histone deacetylase-3 complex contains nuclear receptor corepressors. Proc Natl Acad Sci USA 97:7202–7207.

Whisenhunt TR, Yang X, Bowe DB, Paterson AJ, Van Tine BA, Kudlow JE. 2006. Disrupting the enzyme complex regulating O-GlcNAcylation blocks signaling and development. Glycobiology in press.

Wrabl JO, Grishin NV. 2001. Homology between O-linked GlcNAc transferases and proteins of the glycogen phosphorylase superfamily. J Mol Biol 314:365–374.

Yang X, Su K, Roos MD, Chang Q, Paterson AJ, Kudlow JE. 2001. O-linkage of N-acetylglucosamine to Sp1 activation domain inhibits its transcriptional capability. Proc Natl Acad Sci USA 98:6611–6616.

Yang X, Zhang F, Kudlow JE. 2002. Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: Coupling protein O-GlcNAcylation to transcriptional repression. Cell 110:69–80.

Zachara NE, O'Donnell N, Cheung WD, Mercer JJ, Marth JD, Hart GW. 2004. Dynamic O-GlcNAc modification of nucleocytoplasmic proteins in response to stress. A survival response of mammalian cells. J Biol Chem 279: 30133–30142.

Zavrski I, Naujokat C, Niemoller K, Jakob C, Heider U, Langelotz C, Fleissner C, Eucker J, Possinger K, Sezer O. 2003. Proteasome inhibitors induce growth inhibition and apoptosis in myeloma cell lines and in human bone marrow myeloma cells irrespective of chromosome 13 deletion. J Cancer Res Clin Oncol 129:383–391.

Zhang F, Su K, Yang X, Bowe DB, Paterson AJ, Kudlow JE. 2003. O-GlcNAc modification is an endogenous inhibitor of the proteasome. Cell 115:715–725.