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The O-GlcNAc modification on kinases

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Abstract

O-Linked N-acetyl glucosamine (O-GlcNAc) is a protein modification found on thousands of nuclear, cytosolic, and mitochondrial proteins. Many O-GlcNAc sites occur in close proximity to protein sites that are likewise modified by phosphorylation. While several studies have uncovered crosstalk between these two signaling modifications on individual proteins and pathways, an understanding of the role of O-GlcNAc in regulating kinases, the enzymes that install the phosphate modification, is still emerging. Here we review recent methods to profile the O-GlcNAc modification on a global scale that have revealed over 100 kinases as modified by O-GlcNAc, and highlight existing studies about regulation of these kinases by O-GlcNAc. Continuing efforts to profile the O-GlcNAc proteome and understand the role of O-GlcNAc on kinases will reveal new mechanisms of regulation and potential avenues for manipulation of the signaling mechanisms at the intersection of O-GlcNAc and phosphorylation.

Keywords

O-GlcNAc; glycosylation; kinase; phosphatase; phosphorylation; glycoproteomics; crosstalk; post-translational modification; PTM crosstalk; signaling

Introduction

O-Linked *N*-acetyl glucosamine (O-GlcNAc) is one of the major chemical codes used for cellular signaling. O-GlcNAc was first discovered in mammalian cells in 1984[1] and has now been found across species on serine or threonine residues of thousands of proteins in the nuclear, cytosolic, and mitochondrial compartments (Figure 1A).[2, 3] Due to the occurrence of O-GlcNAc on proteins in these intracellular compartments and the fact that O-GlcNAc is a biosynthetic product culminating from glycolysis, amino acid synthesis, nucleotide levels, and fatty acid levels, the modification is commonly thought to act as a nutrient sensor for the proteome.[3] The O-GlcNAc modification is found across species. The O-GlcNAc modification modifies thousands of proteins in animals and plants,[4] and has now been identified on proteins in fungi[5] and the tailoring enzymes has been found in bacteria.[6] Notably, the recent discovery of intracellular O-fucose in the plant proteome[7] and O-mannose in the yeast proteome[8] points to the potentially central role for a sugar-based nutrient sensing mechanism across organisms. The function of O-GlcNAc in nutrient sensing and the impact of its dysregulation on specific diseases have been previously and extensively reviewed,[9–17] which highlights a rapidly growing focus on deciphering the O-GlcNAc code.

The intersection between O-GlcNAc and phosphorylation signaling was first posited with the discovery of O-GlcNAc on serine and threonine residues that also serve as modification sites for phosphorylation.[18] This discovery sparked multiple inquiries aimed at investigating the crosstalk between these two modifications. The interface of O-GlcNAc and phosphorylation has since proven to be an important pillar of cellular signaling arising from these modifications, particularly as many regions of O-GlcNAcylation are also substrates for phosphorylation. O-GlcNAc, like phosphorylation, is a dynamic modification that is rapidly cycled on protein substrates.[15] However, in contrast to phosphorylation for which over 500 kinases and phosphatases execute the enzymatic introduction and removal of the phosphate group,[19] O-GlcNAc is tailored on thousands of substrates by only two enzymes: O-GlcNAc transferase (OGT) installs and O-GlcNAcase (OGA) removes O-GlcNAc from proteins (Figure 1B). Evidence for the functional significance of O-GlcNAc cycling was obtained when early work demonstrated that both OGT and OGA are essential for organismal development. OGT is required for normal human neurological development[20] and deletion of OGT leads to embryonic lethality in mice,[21] while deletion of OGA leads to neonatal lethality with developmental delay in mouse embryos.[22] Furthermore, conditional deletion of OGT in numerous cell types leads to senescence and apoptosis.[23] Numerous functional outcomes of protein O-GlcNAcylation have been described, including alteration of protein–protein interactions, subcellular localization, enzymatic activity, and protein stability.[15, 24] The dysregulation and abnormal levels of O-GlcNAc have been linked to various diseases, including diabetes,[25] cancer,[26, 27] immune disorders[28] and neurodegeneration,[29, 30] which have inspired efforts to pursue the O-GlcNAc modification as a potential therapeutic target.[31, 32]

Signaling between O-GlcNAc and phosphorylation pathways may be broadly categorized into two major models: that of crosstalk on a protein substrate, or through post-translational regulation of the PTM-installing enzymes themselves (Figure 2). In the first model, a protein substrate may act as a scaffold for O-GlcNAc or phosphorylation via competitive modification or cooperative modification (Figure 2A). In instances of competitive modification, a protein substrate may be alternatively glycosylated or phosphorylated, which inhibits the subsequent modification of the protein at the same or proximal amino acid residues. The competitive modification of a protein by O-GlcNAc or phosphorylation has been described for the tumor suppressor p53,[33] the oncoprotein c-Myc,[34] and the Alzheimer's associated-protein tau,[35] among others. For example, the structural effects of modification on a region of tau revealed that the addition of phosphate drove helical formation, while the addition of O-GlcNAc opposed helical formation by NMR solution structure.[36] Conversely, modification of the murine estrogen receptor with O-GlcNAc increased the helical turn propensity while phosphorylation decreased helicity.[37] In instances of cooperative modification, the O-GlcNAcylation or phosphorylation of a protein substrate promotes subsequent modification of the protein substrate. For example, mutation of the three O-GlcNAc sites of cyclin-dependent kinase inhibitor p27(Kip1) to alanine significantly decreased phosphorylation at S10; conversely, a S10A mutation decreased O-GlcNAcylation of p27(Kip1), while the S10E mutation acted as a phosphomimetic that increased O-GlcNAcylation of p27(Kip1).[38] Recently, the 14-3-3 proteins were identified as receptors for the O-GlcNAc modification, pointing to the potential integration of O-GlcNAc and

phosphorylation signaling pathways through a common protein "reader".[39] Thus, tuning a protein surface with O-GlcNAc, phosphorylation, or a combination thereof results in distinct conformational changes that drive downstream functional effects; these have been previously reviewed.[10, 40, 41]

Here, we aim to highlight the intersection of O-GlcNAc and phosphorylation from studies on the systems level and focus on an additional model arising from the system-wide profiling data, where the direct modification of kinases by O-GlcNAc results in regulation of substrate scope, kinase activity, and ultimately drives changes to downstream signaling (Figure 2B).

Regulation of O-GlcNAc by O-GlcNAc transferase and O-GlcNAcase

Like the O-GlcNAc modification itself, the enzymes OGT and OGA that install O-GlcNAc perform multifaceted roles that are still under investigation. OGT is a glycosyltransferase that is expressed in all mammalian tissues and possesses a tetratricopeptide repeat (TPR) domain and a catalytic domain (Figure 3). OGT is expressed in three isoforms that vary by the length of the TPR domain, termed the nucleocytoplasmic or full-length OGT (ncOGT, 13.5 TPRs), the mitochondrial isoform (mOGT, 9 TPRs), and the short isoform (sOGT, 2.5 TPRs) (Figure 3A).[42] The TPR domain mediates protein–protein interactions (PPIs) of OGT with substrate proteins, which is only beginning to be understood.[24, 43–45] Structurally, the TPR domain forms a series of stacked alpha-helical domains that form a coiled tube-like structure that funnels polypeptide sequences to the catalytic domain primarily through associations with asparagine and aspartate residues lining the domain.[43, 46] The catalytic domain transfers the sugar from a UDP-GlcNAc donor to serine or threonine residues that are positioned by the TPR domain (Figure 3B). Mutation of H508 or K852 reduce binding of UDP-GlcNAc and thus impair catalytic activity (highlighted in red, Figure 3B). The catalytic domain catalyzes additional chemistries, including transfer of UDP-GlcNAc to cysteine residues,[47] transfer of glucose,[48] proteolysis,[49] and deamidation.[50] In cells, OGT forms dimers and trimers through associations in the TPR domain that also alter UDP-GlcNAc binding constants.[51, 52] OGT possesses three of its own O-GlcNAc sites and is modified by other PTMs, including phosphorylation, ubiquitinylation, and sumoylation.[53] OGT appears to require accessory proteins to modify protein substrates efficiently, as reduction or removal of the TPR domain reduces activity with full length proteins, but retains catalytic activity with synthetic peptides in vitro.[42, 46] Further exploration of the substrate selection mechanisms for OGT will improve the understanding of the regulatory role of O-GlcNAc and its dynamic cycling.

O-GlcNAc is catalytically removed from proteins by OGA. Similar to OGT, the mechanisms of how OGA selects from numerous O-GlcNAc substrates and the functions of the separate domains of OGA are still emerging. OGA is composed of three domains: a catalytic domain and a histone acetyltransferase (HAT)-like domain connected by a stalk domain (Figure 4A).[54] The stalk domain is interspersed with an unstructured region that forms a binding interaction with OGT.[55] OGA is encoded by a single gene that is expressed as two main isoforms in vertebrates, the full length isoform, OGA(I), and a short isoform lacking the C-terminal HAT-like domain, OGA(II).[56] Full-length OGA(I) is a nucleocytoplasmic enzyme,

while OGA(II) is found predominantly in the nucleus and in lipid droplets.[57] The crystal structure of human OGA had eluded definition until very recently.[58–60] These structures of OGA(II) revealed a remarkable homodimer, wherein the stalk domain of one monomer covers the catalytic domain of the other monomer to create a substrate-binding cleft.[59] A series of contacts between the stalk domains stabilize the homodimer. OGA is regulated at least in part by an O-GlcNAc-related feedback mechanism that triggers gene expression; inhibition of OGA by Thiamet G treatment causes a compensatory increase in OGA expression and decrease in OGT expression.[61] Large-scale profiling studies have revealed several sites for ubiquitination and phosphorylation of OGA, including an O-GlcNAc site at S405, indicating possible regulation by OGT.[62] The biochemical characterization of these modification sites may illuminate additional mechanisms of OGA regulation.

Methods to detect and map O-GlcNAc on the systems-level

The O-GlcNAc modification is relatively difficult to detect and quantify in the proteome.[2] O-GlcNAc was discovered in 1984 when sugar radiolabeling was measured in the nucleocytoplasmic space,[1] several decades after other PTMs like phosphorylation were discovered on the same proteins. The discovery of O-GlcNAc may have been delayed due to difficulty in detection since generally changes in O-GlcNAc levels do not affect glycoprotein migration during gel electrophoresis, and O-GlcNAc is enzymatically labile and rapidly removed from proteins when the cell is damaged or lysed. Furthermore, O-GlcNAc is also chemically labile to common mapping techniques to analyze PTMs, such as mass spectrometry (MS).[63] Analysis of O-GlcNAc by MS pushes detection limits due to occurrence of O-GlcNAc at substoichiometric levels on the protein, ion suppression of the glycopeptide in the presence of unmodified peptides, and ready fragmentation of the glycan from the peptide during ionization processes in the mass spectrometer.[64, 65] Recently, advances in chemical glycoproteomics have drastically accelerated the mapping of the modification site in the global proteome; these advances have recently been reviewed.[66] Here we highlight specifically advances in the large-scale profiling of O-GlcNAc and summarize the O-GlcNAcome characterized from large-scale complex glycoproteomic studies to date.

Although O-GlcNAc is found widely throughout the nucleocytoplasmic proteome, its substoichiometric modification site occupancy necessitates the combination of an efficient enrichment method coupled to a sensitive analytical detection method. The enrichment of O-GlcNAc has been achieved by several means. Lectin weak affinity column chromatography using wheat germ agglutinin (WGA) enables the enrichment of O-GlcNAc and other sugars after multiple rounds of enrichment. Alternatively, the introduction of bioorthogonal handles via metabolic labeling[67, 68] or chemoenzymatic labeling[69] results in the selective installation of an azido-sugar as a reporter for O-GlcNAc on proteins, enabling the further functionalization with a variety of reporting strategies (e.g., fluorescence microscopy, anti-biotin Western blot). Metabolic labeling involves the addition of a sugar carrying a bioorthogonal handle, such as an azido-sugar, to living systems that metabolically incorporate the azido-sugar to protein substrates. Several sugar reporter molecules for O-GlcNAc have been developed, including Ac_4GalNAz or Ac_4GlcNAz, or O-GlcNAc-specific reporters 6AlkGlcNAc,[70] 6AzGlcNAc,[71] 1,3-Ac_2GalNAz, and 1,3-Pr_2GalNAz.[72] The latter

two were validated to reduce background S-glycosylation from other metabolic reporters.[73] Chemoenzymatic labeling uses a mutant GalT1 enzyme that accepts azido- or keto-sugars for enzymatic labeling of the O-GlcNAc residue itself.[74] The azide introduces a handle that is selectively tagged with reporter molecules using copper-catalyzed azide–alkyne cycloaddition (CuAAC) chemistry. Reaction of the azide groups with cleavable biotin tags and isolation of the O-GlcNAc peptide enables maps of O-GlcNAc modification sites throughout the proteome. Both metabolic labeling and chemoenzymatic labeling may produce off-target labeling products due to the addition of reactive azido-sugar intermediates or promiscuity of the labeling for additional glycan types.[73, 75] Thus, the assignment of O-GlcNAcylated proteins is best performed at glycosite-level resolution.

Analysis of the O-GlcNAc modification is commonly achieved by Western blot or MS-based proteomics. Visualization of O-GlcNAcylated proteins by Western blot is commonly performed using the O-GlcNAc CTD110.6, RL2, or 18B10.C7 antibodies. If the glycoprotein is labeled by an azido-sugar, a mass shift assay using a 5-kilodalton PEG mass tag carrying an alkynyl functional group may be performed to determine the stoichiometry of the O-GlcNAc modification on individual proteins.[76] The intensity of the shifted bands relative to the unshifted band allows for determination of O-GlcNAc stoichiometry. To characterize the protein glycosite(s), mass spectrometry-based proteomics has emerged as the primary mechanism for site-specific mapping of the O-GlcNAc modification site on individual proteins to the complex proteome. However, due to the chemical lability of the O-linked glycosidic bond from the peptide backbone by collision induced dissociation (CID) or higher-energy CID (HCD) resulting in altered fragmentation mechanisms for glycopeptides, the assignment of the glycopeptide species is challenging, and in cases of successful identification of the glycopeptide the glycosite may only be localized to the serine and threonine residues in the peptide sequence. Solutions to this challenge included determination of the modification site using a sequence of induced beta-elimination of O-GlcNAc from the peptide backbone, followed by controlled Michael addition of dithiothreitol as a reporter for the glycosite, yielding early insight to the O-GlcNAc proteome.[77] The further development of ETD and electron-transfer higher energy collision induced dissociation (EthCD) methods on high resolution mass spectrometers enabled the detection of O-GlcNAcylated peptides via a fragmentation method that leaves the glycosidic bond intact. Chemical glycoproteomics methods, such as Isotope Targeted Glycoproteomics (IsoTaG), combine metabolic labeling with enrichment to map exactly when and where O-GlcNAc is modifying the protein network (Figure 5).[78] The development of efficient enrichment methods for O-GlcNAc coupled to advances in MS technology have drastically increased the number of O-GlcNAc sites that have been identified from the whole proteome of multiple species.[28, 62, 79, 80]

Intersection of O-GlcNAc and phosphorylation on the systems-level

The cell integrates glycosylation, phosphorylation, and the myriad of other chemical modifications on a protein into a functional signaling output. Through MS-based proteomics, the ability to map a diversity of modification sites has enhanced significantly over the past decade, concomitantly increasing the depth of maps of the O-GlcNAc proteome.[66] In particular, the crosstalk between O-GlcNAc and phosphorylation has long

fascinated the field. The possibility of O-GlcNAc blocking proximal phosphosites was first suggested by Hart and coworkers in 1987.[18] In 2008, a large-scale phosphosite profiling study in mouse fibroblasts revealed roughly half of the 711 mapped phosphosites changed in abundance in response to a global increase in O-GlcNAc glycosylation via chemical inhibition of OGA.[81] Furthermore, inhibition of phosphatase by okadaic acid decreased global O-GlcNAc levels by Western blot in NIH/3T3 cells.[82, 83] In primary human T cells, 45% of O-GlcNAc sites occur in close proximity to a previously mapped phosphorylation site (within 10 amino acids).[84] However, conflicting evidence for crosstalk between the O-GlcNAc modification and phosphorylation exists. In 2012, clustering of a profile of 1,750 glycosites and 16,500 phosphosites mapped from the murine synaptosome was statistically independent, implying that crosstalk between glycosites and phosphosites at a static state was insignificant.[85] In contrast, evidence for reciprocal regulation between O-GlcNAc and phosphorylation was recently demonstrated in vitro on a specific four-amino acid consensus sequence: *N*-S/T, P, V/A/T, S/T-*C*.[40] Phosphorylation of the sequence at the N-terminal S/T resulted in inhibition of glycosylation at the C-terminal S/T, while glycosylation of the N-terminal S/T resulted in inhibition of phosphorylation at the C-terminal S/T. The observed competitive modification model with this consensus sequence was subsequently evaluated on synthetic peptides derived from ten proteins naturally containing the consensus sequence. All possible permutations of this four-residue sequence are enriched in the human proteome compared to randomly-selected four-residue sequences, potentially indicative of an evolutionary selection for this consensus sequence. Further evaluation of O-GlcNAc and phosphorylation maps from cellular or in vivo systems may reveal instances of PTM crosstalk on a global scale.

O-GlcNAc regulation of kinases

Crosstalk between O-GlcNAc and phosphorylation additionally occurs through the direct O-GlcNAcylation of the kinome, thus regulating downstream phosphorylation events. While crosstalk between O-GlcNAc and phosphorylation is most commonly studied from the perspective of regulation at an individual protein substrate, emerging examples demonstrate that O-GlcNAcylation of the kinase influences substrate selection and enzymatic activity.[86, 87] Both OGT and OGA have been immunoprecipitated in protein complexes containing kinases (and phosphatases, see below).[88, 89] In vitro glycosylation of a kinase microarray with OGT found that approximately 39% of these kinases are substrates.[90] Our analysis of the O-GlcNAc literature and glycoproteomics datasets found over 100 kinases possessing a mapped O-GlcNAc site to date. We review the current O-GlcNAcylated kinome based on large-scale O-GlcNAc maps[78, 84, 91–98] and the several examples of the O-GlcNAc modification regulating kinase activity, thereby highlighting the central role of O-GlcNAc in regulating phosphorylation on protein substrates and on the kinases themselves (Figure 6, Table 1).

AGC

The AGC kinase family contains Ser/Thr protein kinases named after three representative families, the cAMP-dependent protein kinase (PKA), the cGMP-dependent protein kinase (PKG) and the protein kinase C (PKC) families. The AGC family contains more than 60

human protein kinases. To our knowledge, seven members of this kinase family have been identified as O-GlcNAc modified.

PKA plays a role in CREB signaling and additionally modifies tau. O-GlcNAcylation of PKA subunit PKAcα and PKAcβ alters their subcellular localization and enhances their kinase activity.[99] Protein kinase B (AKT) is a serine/threonine kinase involved in multiple cellular processes, including the insulin response, apoptosis, and cell migration. AKT was found to be modified by O-GlcNAc in 2006.[100] Enrichment of AKT by wheat germ agglutinin increased under high glucose conditions or when dosing the OGA inhibitor PUGNAc, indicative of higher O-GlcNAc levels on AKT. Follow up experiments demonstrated that elevated global O-GlcNAc levels correlate with AKT translocation from the cytoplasm to the nucleus. Two separate laboratories later reported distinct glycosites on AKT.[101, 102] Hart and co-workers used tyrosine as a glyco-mimetic mutation to show that O-GlcNAcylation at T305 and/or T312 on AKT inhibits phosphorylation at T308, a residue in the activation loop of AKT whose phosphorylation is necessary for AKT activation.[103] These studies implicated glycosylation as a mechanism to downregulate AKT activity using an in vitro AKT activity assay.[101] Gong and co-workers later report that increased O-GlcNAcylation on AKT positively correlates with markers for apoptosis and overexpression of AKT alleviates this phenotype.[102] AKT2 has also been identified as O-GlcNAc modified.[104] Treatment of rat adipocytes with the OGA inhibitor PUGNAc increased glycosylation and decreased insulin-induced phosphorylation of AKT2 by Western blot.

O-GlcNAc may be linked to regulation of cancer cell migration via another member of the AGC kinase family, ROCK.[105] Chemical inhibition of OGA resulted in accelerated migration that was found to be mediated by the RhoA/ROCK/MLC (myosin light chain) interaction in SKOV3 and 59M ovarian cancer cells. Knockout RhoA or inhibition of ROCK eliminates the change in cell migration caused by OGA inhibition. While direct O-GlcNAcylation of RhoA or ROCK was not established in this study, a glycosite on ROCK has recently been identified in a large-scale glycoproteomics study.[84] Biochemical confirmation and characterization of this glycosite will lead to further conclusions about the role of O-GlcNAc in regulating ROCK and cell migration.

CMGC

The CMGC family of kinases contains the cyclin-dependent kinases (CDK), mitogen-activated protein kinases (MAPK), glycogen synthase kinases, and the CDC-like kinases (CLK). These kinases are involved in cell-cycle regulation and signaling, cell communication, and cell growth.

GSK3β regulates OGT via phosphorylation and is likewise regulated by OGT through O-GlcNAc. Inhibition of GSK3β alters the abundance of several O-GlcNAc sites on GSK3β in the mouse and monkey proteomes, a result that could be attributed to the loss of phosphorylation on OGT or other GSK3β substrates.[106, 107] In addition, the O-GlcNAc modification on GSK3β results in regulation of the molecular chaperones that are stably expressed under heat-shock conditions.[108] Specifically, knockout of OGT in MEF cells

results in altered expression of HSP72, a heat shock protein that is governed by the GSK3β-substrate HSF1.

Several studies additionally report regulation of CMGC complexes by O-GlcNAc. In these examples, members of the CMGC kinase complex are glycosylated, leading to alteration of the kinase function and substrate selectivity that affect essential cellular processes, including motility and cell cycle progression. For example, p27 is a tumor suppressor gene that functions by inhibiting CDK2/Cyclin E. Shen and co-workers mapped glycosites on p27 at Ser2, Ser106, Ser110, Thr157, and Thr198.[109] O-GlcNAcylation of Ser2 destabilizes p27 and works synergistically with phosphorylation of Ser10 to move the cell cycle forward. Furthermore, Western blot and immunohistochemical analyses of hepatocellular carcinoma tissues and their corresponding nontumorous tissues were performed, and revealed that elevated O-GlcNAc on p27 correlates with increased cell proliferation. Together, these results indicate that the dynamic interplay between O-GlcNAcylation and phosphorylation on p27 in complex with CDK2 controls CDK2 activity via regulation of p27 stability.

CAMK

The calcium/calmodulin-dependent kinases (CAMK) are a family of enzymes stimulated by calmodulin, a protein that is activated in response to increased intracellular calcium concentrations. As many CAMK substrates are transcription factors, this kinase family is known for being closely tied to regulation of gene expression. A number of CAMK subunits are modified by OGT, with the effects on CAMKIV being best studied.[86] CAMKIV is O-GlcNAcylated at several sites. Modification of CAMKIV with O-GlcNAc at the active site reduces the level of stimulatory phosphorylation at T200 and results in inhibition of the kinase activity.

Unc-51-like-kinase 1 (ULK1) is an important gatekeeper of the autophagy pathway. ULK1 is glycosylated at T754. Glycosylation of ULK1 can only occur once ULK1 has been dephosphorylated by PP1 to remove a phosphosite installed by mTOR.[110] O-GlcNAcylation of ULK1 at T754 promotes binding to substrate ATG14L, which results in phosphatidylinositol-(3)-phosphate production and initiation of autophagy. In this example, dephosphorylation by PP1 represents a gatekeeping step for subsequent O-GlcNAcylation, and illustrates key regulatory mechanisms by O-GlcNAc in the autophagy pathway.

AMPK is a heterotrimeric kinase that has a protective function from cellular metabolic stress. AMPK activity is strongly associated with depleted cellular energy levels as the kinase is activated by 5’-AMP and ADP, but inhibited by ATP. Stimulation with 5’-AMP or ADP yields a net upregulation of catabolic and downregulation of anabolic processes. The kinase complex is further activated by phosphorylation of T172 in the AMPK alpha subunit. During differentiation of C2C12 mouse skeletal muscle myotubes, AMPK activity is closely associated with OGT translocation to the nucleus. The altered localization of OGT results in increased O-GlcNAcylation of nuclear proteins and H3K9 acetylation[111] and results in phosphorylation of OGT at T444. Phosphorylation of OGT by AMPK alters the O-GlcNAc landscape. All α and γ subunits of AMPK substrates for OGT, and active AMPK shows increased O-GlcNAcylation of the γ1 subunit.

STE

The homologs of yeast Sterile 7, 11, and 20 kinase family contains many kinases involved in cell growth, differentiation, oxidative damage, and apoptosis, including many MAP and serine/threonine protein kinases.[112, 113] PAK, GCK, MEK, and MKK kinases are also part of the STE family. Prominent O-GlcNAcylated members of this family include WNK1–3, PAK1–2, TAO1–2, SLK, OSR1, and several MAP3K proteins, all revealed in large-scale proteomics experiments. The functional outcomes of glycosites on kinases in the STE family have yet to be biochemically characterized.

CK1

Despite the limited number of kinases in the CK1 family, kinases in the CK1 family are involved in regulation of membrane transport, cell division, DNA repair, and nuclear localization. CK1δ and CK2α have been discovered to be O-GlcNAc modified to date. The O-GlcNAcylation of CK1δ was discovered in a large-scale murine synaptosome proteomics study at multiple potential sites that remain to be functionally characterized.[91] Glycosylation of human CK2α was evaluated by Cole and coworkers.[87] The mapped glycosite S347 was found proximal to multiple known phosphosites on CK2α. By semi-synthesis, O-GlcNAcylation of CK2α was found to inhibit phosphorylation at T344, which decreased the interaction of CK2α with Pin1 and produced a net destabilization of CK2α.

TK/TKL

The protein tyrosine kinase family can be subdivided into two main groups: cytosolic tyrosine kinases (CTKs) (*e.g.*, Src, JAK, Abl) and receptor tyrosine kinases (RTKs) (*e.g.*, EGFR, VEGFR, FLT3). Receptor tyrosine kinases are transmembrane proteins that are activated by the binding of an extracellular ligand that induces dimerization and subsequent autophosphorylation of two RTK monomers, followed by phosphorylation of downstream signaling proteins. Since tyrosine kinases regulate many key processes including cell growth and survival, their dysregulation has been found in the development and progression of a wide range of cancers. The tyrosine kinase-like family (TKL) is closely related to TK, but its members are serine/threonine kinases instead (e.g. Raf). Diverse members of this kinase family are shown to be glycosylated, including those involved in cell differentiation (BMPR2).[30, 84, 91] Regulatory functions for the O-GlcNAc sites on kinases in this family await biochemical characterization.

Other kinases

Kinases that phosphorylate non-protein targets are also privy to regulation by O-GlcNAc. GNE is an epimerase/kinase responsible for converting UDP-GlcNAc to ManNAc-6P.[114] A GNE point mutation at M743T, commonly observed in GNE myopathy, results in significantly higher O-GlcNAcylation of GNE than its wildtype counterpart. Elevated O-GlcNAcylation on GNE was found to inhibit the epimerase activity of both the wildtype enzyme and the M743T mutant. One hypothesis for why GNE is regulated by O-GlcNAc is

due to its role in the consumption of UDP-GlcNAc, the donor sugar used by OGT from the hexosamine biosynthesis pathway.

OGT modifies kinases involved in sugar metabolism and glycolysis in addition to GNE, including pyruvate kinase M2 and phosphofructokinase 1.[115, 116] Phosphofructokinase 1 (PFK1) is an enzyme in glycolysis responsible for converting fructose-6-phosphate to fructose-1,6-bisphosphate, a committal step that sends the product through the rest of the glycolytic pathway as compared to the hexosamine biosynthetic pathway. OGT installs O-GlcNAc at S529 of PFK1, attenuating its kinase activity.[116] This modification increases in abundance under hypoxic conditions, leading to the redirection of glycolytic flux from the glycolysis pathway toward the pentose phosphate pathway, a glucose-consuming metabolic pathway necessary for synthesis of nucleotides and other sugars.

Phosphatases

Protein phosphatases are a family of approximately 200 enzymes that remove phosphorylation from the protein substrate. Several examples of interplay between O-GlcNAc and dephosphorylation have been reported, including the identification of a functional complex between OGT and protein phosphatase 1.[117] For example, priming phosphorylation of folliculin-interacting protein 1 (FNIP1) at S938 by CK2 leads to many subsequent phosphorylation events of FNIP1, ultimately resulting in binding to Hsp90 to inhibit its ATPase activity. If this priming phosphorylation does not occur, OGT can glycosylate FNIP1 at S938, blocking subsequent phosphorylation steps, and consequently lead FNIP1 to be ubiquitinated and degraded.[118] Activation of the transcription factor Sp1 is enhanced on dephosphorylation by phosphatase 2A and may be additionally controlled by reciprocal O-GlcNAc and phosphate modification.[119]

A number of phosphatases are also privy to modification by O-GlcNAc (e.g., MYPT1, PPFIA2–4, PPP6R2, PTPN6, PTPN7, PTPRC, TNS2, SIRPA).[84, 85, 92, 93] Direct regulation of phosphatase activity by O-GlcNAc has been reported in a few instances. O-GlcNAcylation of protein tyrosine phosphatase 1B (PTP1B) at S104, S201, and S386 inhibits PTP1B activity, which leads to an increase in AKT and GSK3β activity and therefore insulin response in HepG2 cells.[120] Human small CTD phosphatase 1 (hSCP1) was identified as O-GlcNAc modified by Western blot, and its glycosite at S41 was confirmed by Q-TOF MS and site-directed mutagenesis.[121] Additionally, the phosphatase myosin phosphatase target subunit 1 (MYPT1) may regulate the substrate specificity of OGT.[89] MYPT1 and OGT can be co-immunoprecipitated, MYPT1 is modified by O-GlcNAc, and depletion of MYPT1 alters OGT substrate selectivity in Neuro-2a neuroblastoma cells. These studies highlight additional mechanisms of cellular integration of the O-GlcNAc modification and phosphorylation signaling and a significant opportunity for further study.

Conclusion

The O-GlcNAc modification has emerged a prominent regulator of phosphorylation during cellular signaling via tuning kinase activity in addition to crosstalk between O-GlcNAc and

phosphorylation on protein substrates. Regulation of kinases by the O-GlcNAc modification may enable cells to manage resources in disparate pathways according to nutrient availability, and thus finely tune signaling pathways through other modifications like phosphorylation. With the convergence of methods to study and engineer O-GlcNAc on the systems scale and on individual proteins emerging, the increasing evaluation of the functions for O-GlcNAc on kinases and the enzymes that install it will yield a wealth of insights to regulatory mechanisms cells use to integrate these pathways. In particular, the role of O-GlcNAcylation in the STE, TK, and TKL kinase families awaits elucidation. Due to the global nature of O-GlcNAc in cells, further illumination of the functions of O-GlcNAc on kinases will lead to important discoveries in cellular regulation and dysregulation relevant to all areas of biology under normal physiology or disease.

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Keywords

O-linked N-acetyl glucosamine (O-GlcNAc): a carbohydrate that is installed on serine/threonine residues of nuclear/cytosolic proteins by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA)

Glycosylation: the enzymatic addition of a sugar molecule to another biomolecule

Kinase: an enzyme that catalyzes covalent attachment of a phosphate group to its substrate

Phosphatase: an enzyme that catalyzes the removal of a phosphate group from its substrate

Phosphorylation: the enzymatic addition of a phosphate group to another molecule

Glycoproteomics: the identification and characterization of carbohydrate-modified proteins from a biological sample in the whole proteome via a profiling method (e.g., mass spectrometry)

Crosstalk: the phenomenon where changes in one biological pathway directly affects signaling in another biological pathway

Post-Translational Modification (PTM): the chemical modification of proteins after protein biosynthesis, often catalyzed by enzymes

PTM Crosstalk: the presence of one PTM affecting the substitution pattern of another PTM

Signaling: the transduction of a signal via non-covalent or covalent associations of biological molecules within a pathway

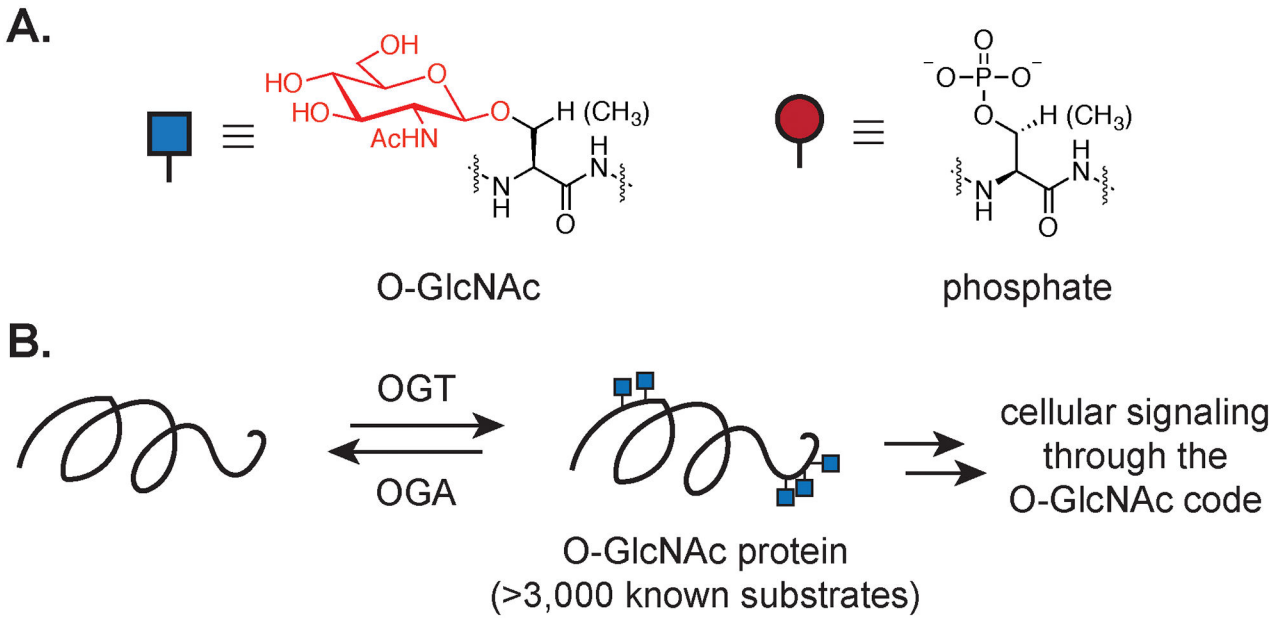

Figure 1.

The essential O-GlcNAc modification of proteins. **A.** Structure of O-GlcNAc (highlighted in red) and phosphate appended to a serine or threonine amino acid. **B.** O-GlcNAc is installed by OGT and removed by OGA to over 3,000 known nuclear and cytoplasmic proteins.

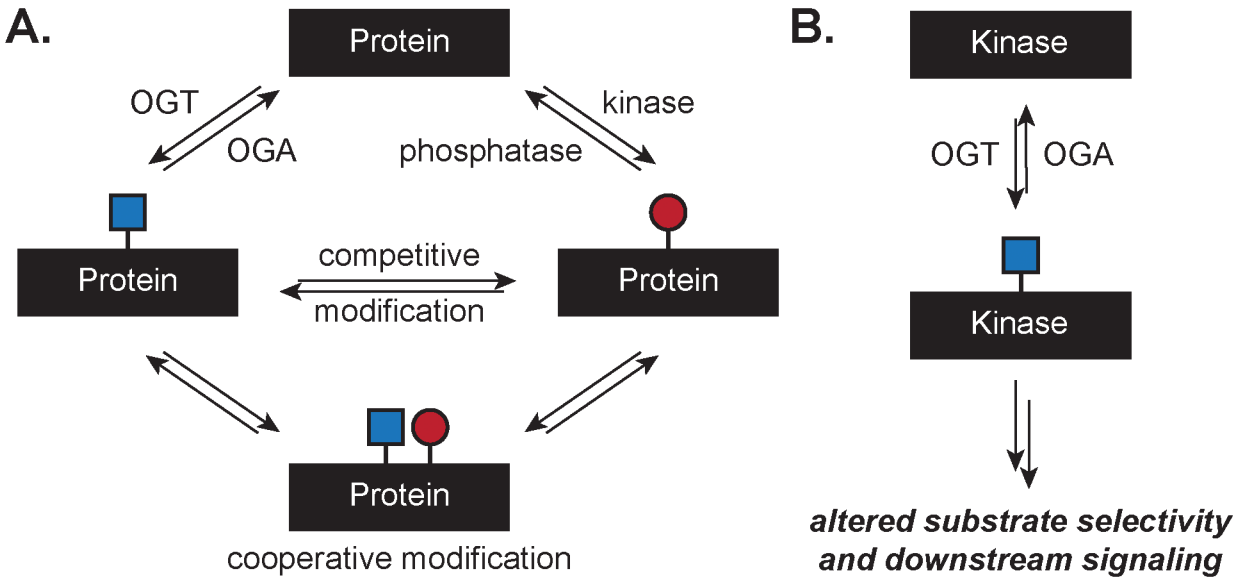

Figure 2.

Modes of crosstalk between O-GlcNAc and phosphorylation. **A.** Post-translational modification of a protein with O-GlcNAc or phosphorylation may be competitive, where one modification precludes the other, or cooperative, where multiple modifications propagate specific regulatory outcomes. **B.** Modification of a kinase with O-GlcNAc can alter downstream substrate selection and signaling through phosphorylation.

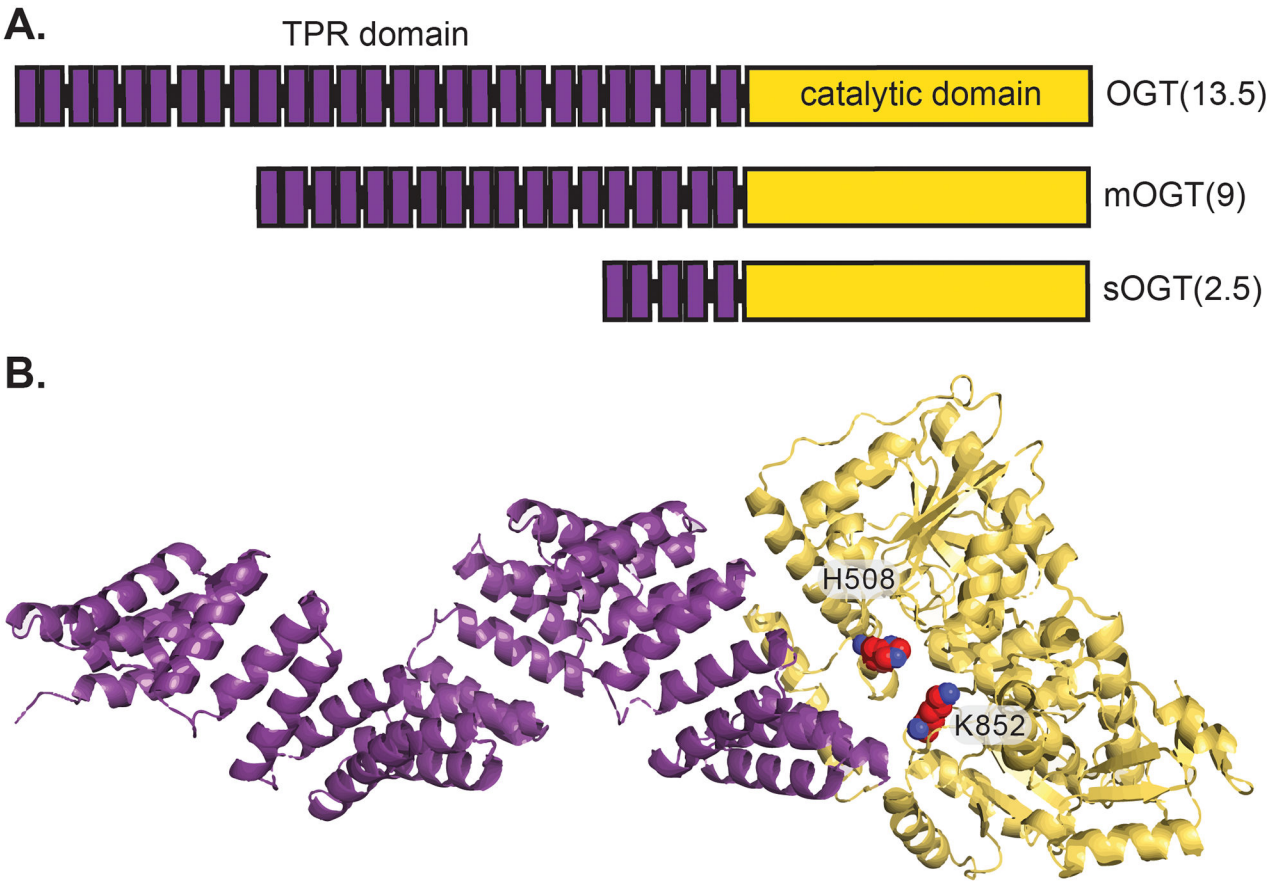

Figure 3.

Structure of OGT. **A.** Linear representation of full length OGT(13.5), mOGT(9), and sOGT(2.5). **B.** Model of the TPR domain (purple) and catalytic domain of OGT (yellow).[43] Point mutations at H508 and K852 reduce catalytic activity (highlighted in red).

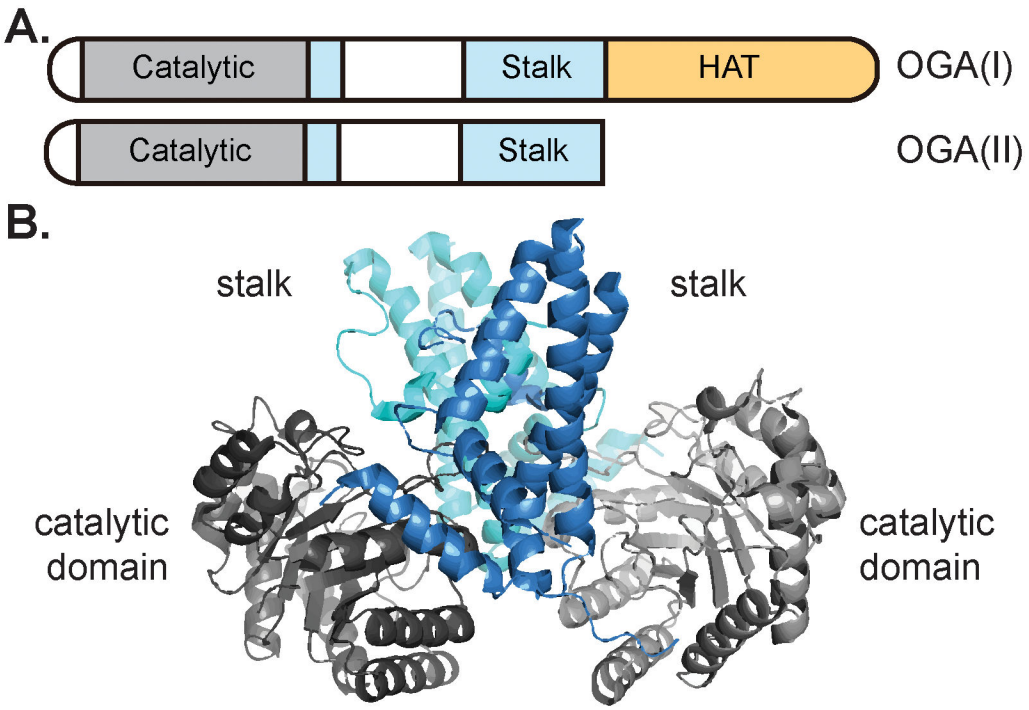

Figure 4.

Structure of OGA isoforms. **A**. Linear representation of OGA isoforms I and II. Isoform I is full length OGA. Isoform II lacks the HAT-like domain. **B.** Crystal structure of the human OGA homodimer analogous to OGA(II) from the side view (PDB: 5UN9).[59] The catalytic domain is grey and the stalk domain blue.

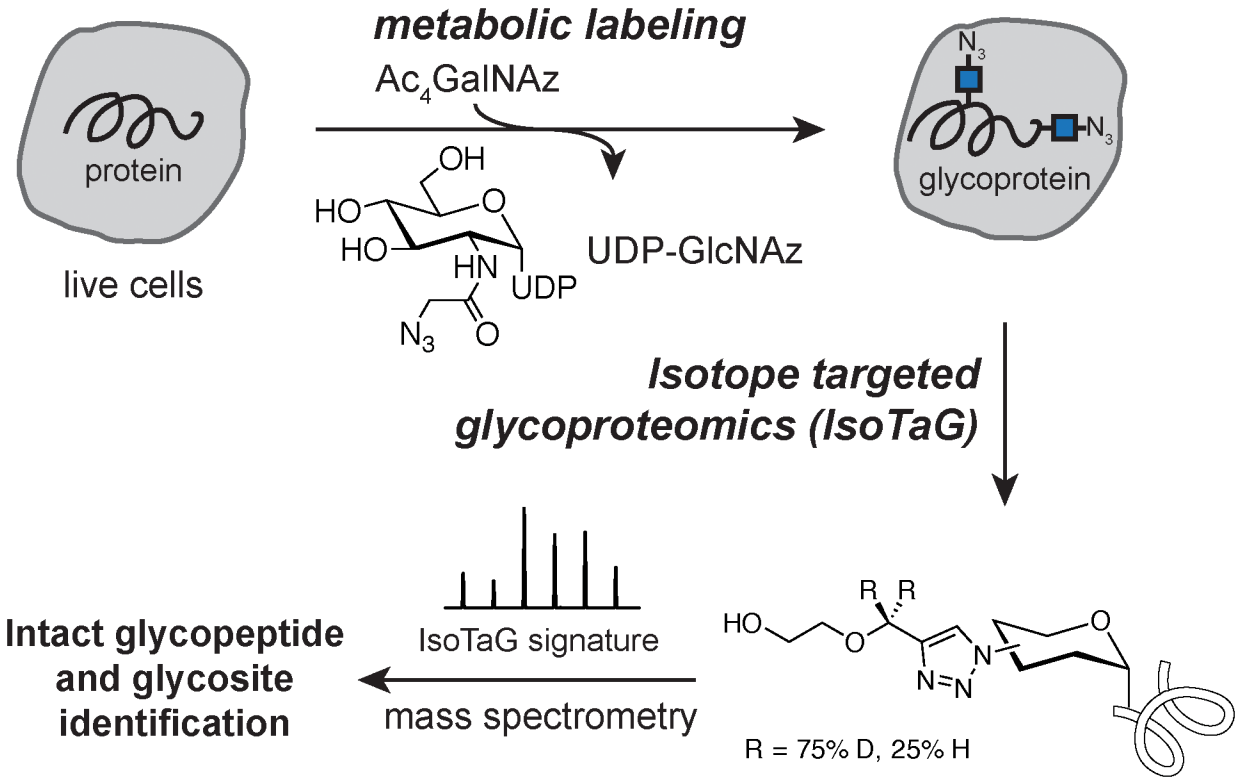

Figure 5.

Workflow for Isotope Targeted Glycoproteomics (IsoTaG). Live cells are labeled with an azidosugar (e.g., $Ac_4GalNAz$) as a reporter for the O-GlcNAc modification. Enrichment, digestion, and acid cleavage of the tag recovers the modified glycopeptide for characterization by MS.

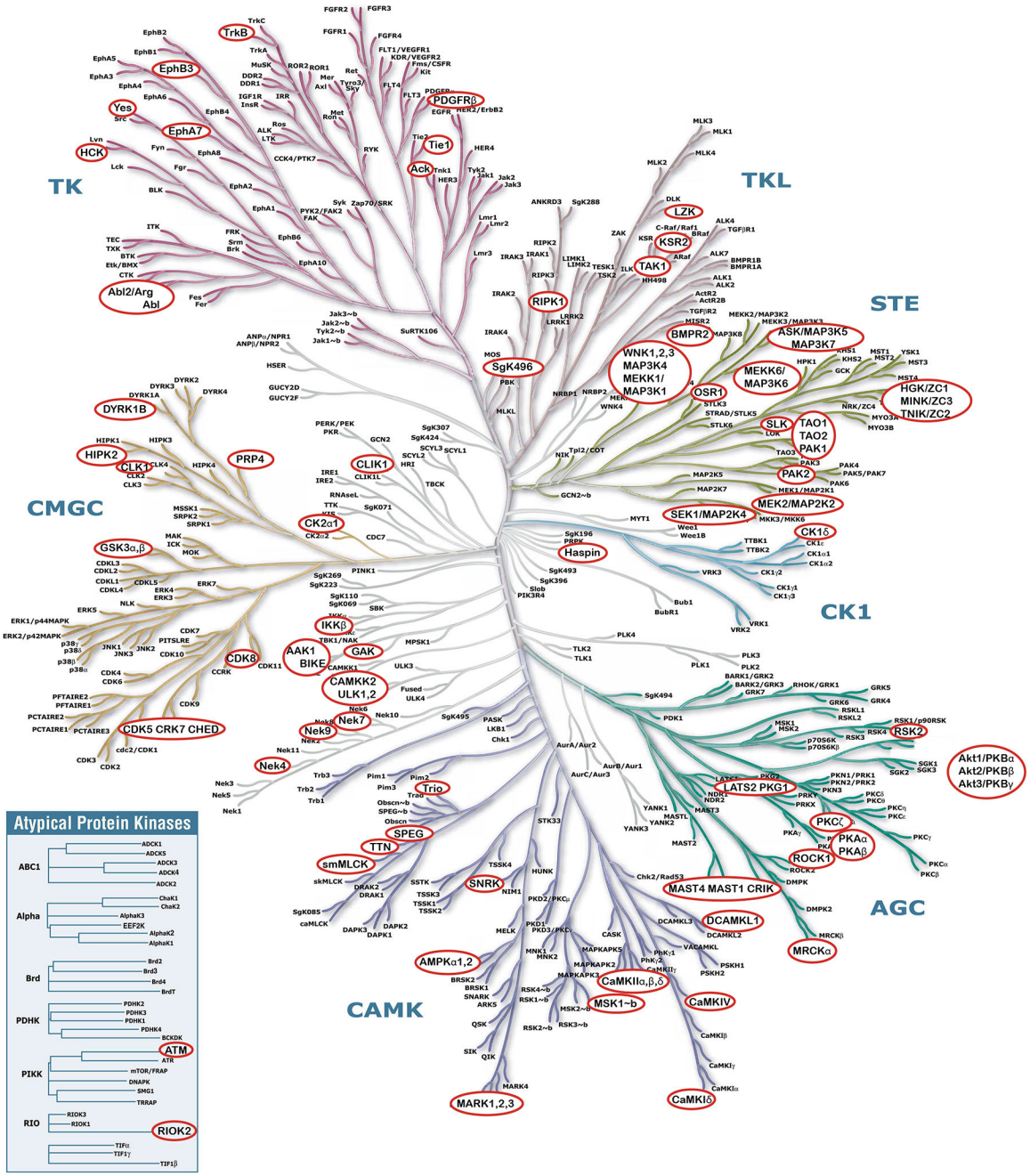

Figure 6.

Human kinome with known O-GlcNAc modified kinases circled in red. Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com).

Table 1.

Catalog of kinases with at least one mapped glycopeptide reported from biochemical or glycoproteomics studies. For proteins with multiple reported glycosites, sites on different peptides are separated by a comma, while sites found from the same peptide are separated by slashes. If one site contained 3 or more peptide spectral matches (PSMs) across large-scale proteomic studies, that site is bolded. Proteins that had more than five reported glycosites from the same peptide sequence or were ambiguously localized within the peptide from large-scale proteomic studies, are designated as "N/A."

Protein names	Gene name(s)	Organism	Reported Glycosite(s)	Uniprot accession(s)	Citation
1-phosphatidylinositol 3-phosphate 5-kinase kinase	PIKFYVE, KIAA0981, PIP5K3	Homo sapiens	T90, S1433/T1436/T1438	Q9Y2I7	84
Activated CDC42 kinase 1	TNK2 ACK1, Tnk2, Ack1	Homo sapiens; Mus musculus	N/A	Q07912; O54967	92, 30, 91, 93
AP2-associated protein kinase 1	AAK1, KIAA1048, Aak1, Kiaa1048	Homo sapiens; Mus musculus	T441/S447/**T448**, **T507**/ S519; N/A	Q2M2I8; Q3UHJ0	92, 30, 84
6-phosphofructokinase type C, platelet type	PFKP, PFKF	Homo sapiens	T538/S546	Q01813	84
BDNF/NT-3 growth factors receptor	NTRK2 TRKB	Homo sapiens	N/A	Q16620	30
BMP-2-inducible protein kinase	BMP2K BIKE HRIHFB2017	Homo sapiens	T381	Q9NSY1	92, 30
Bone morphogenetic protein receptor type-2	BMPR2 PPH1, Bmpr2	Homo sapiens; Mus musculus	T803/**T805**; S547/S548/ S549/ S550	Q13873; O35607	30, 91
Breakpoint cluster region protein	BCR BCR1 D22S11, Bcr Kiaa3017	Homo sapiens; Mus musculus	S400; N/A	P11274; Q6PAJ1	30
Calcium/calmodulin-dependent (CaM) protein kinase I delta, II alpha, II beta, II delta, IV	CAMK1D CAMKID, Camk1d; CAMK2A CAMKA KIAA0968, Camk2a; Camk2b, mCG_122182; Camk2d, Kiaa4163; Camk4	Homo sapiens; Mus musculus; Caenorhabditis elegans	Human CaMKIV: T57/ S58, S137, S189, S344/345, S356	Q8IU85, Q8BW96; Q9UQM7, P11798; P08414, Q5SVJ0; Q6PHZ2	30, 93, 91
Calcium/calmodulin-dependent protein kinase kinase 2	CAMKK2 CAMKKB KIAA0787	Homo sapiens	T56	Q96RR4	30
Casein kinase I isoform delta	Csnk1d Hckid	Mus musculus	T344/T347, S382	Q9DC28	91
Casein kinase II subunit alpha	CSNK2A1 CK2A1, Csnk2a1 Ckiia	Homo sapiens; Mus musculus	S347; N/A	P68400; Q60737	30, 91
cGMP-dependent protein kinase 1	PKG1 PRKG1 PRKG1B PRKGR1A PRKGR1B	Homo sapiens	T151	Q13976	84
Citron Rho-interacting kinase	CIT CRIK KIAA0949 STK21	Homo sapiens	N/A	O14578	30
Creatine kinase S-type, mitochondrial	Ckmt2	Rattus norvegicus	S51	A0A0G2JVQ1	122
Cyclin-dependent kinase 8, 12, 13	CDK8, Cdk8; CDK12 CRK7 CRKRS KIAA0904, Cdk12 Crk7 Crkrs Kiaa0904; CDK13 CDC2L CDC2L5 CHED KIAA1791, Cdk13 Cdc2l5 Kiaa1791	Homo sapiens, Mus musculus	Human CDK12: T592/ S593/S597/ S601 Human CDK13: T1286/ **S1287**/T1292 Mouse CDK13: T1286	P49336, Q8R3L8; Q9NYV4, Q14AX6; Q14004, Q69ZA1	96, 97, 98, 92, 84, 91

Protein names	Gene name(s)	Organism	Reported Glycosite(s)	Uniprot accession(s)	Citation
Cyclin-G-associated kinase	GAK	Homo sapiens	S1130	O14976	84
DDB1- and CUL4-associated factor 1	DCAF1 KIAA0800 RIP VPRBP	Homo sapiens	T891	Q9Y4B6	30
Diacylglycerol kinase	Dgkh, Dgkd	Mus musculus	N/A	D3YXJ0, E9PUQ8	91
Dual serine/threonine and tyrosine protein kinase	DSTYK KIAA0472 RIP5 RIPK5 SGK496 HDCMD38P	Homo sapiens	N/A	Q6XUX3	92
Dual specificity mitogen-activated protein kinase kinase 2, 4	MAP2K2 MEK2 MKK2 PRKMK2; MAP2K4 JNKK1 MEK4 MKK4 PRKMK4 SEK1 SERK1 SKK1	Homo sapiens	**T396**/T398; S7/S36	P36507; P45985	84
Dual specificity protein kinase CLK1	CLK1 CLK	Homo sapiens	T302	P49759	84
Dual specificity tyrosine-phosphorylation-regulated kinase 1B	Dyrk1b	Mus musculus	S624	Q9Z188	91
Ephrin type-A receptor 7	EPHA7 EHK3 HEK11	Homo sapiens	T437/S444	Q15375	84
Ephrin type-B receptor 3	EPHB3 ETK2 HEK2 TYRO6	Homo sapiens	S390	P54753	84
Glycogen synthase kinase-3 alpha, beta	GSK3A; Gsk3B	Homo sapiens; Mus musculus	T106; T7/T8/S9	P49840; Q9WV60	30, 91
Homeodomain-interacting protein kinase 2	HIPK2, Hipk2 Nbak1 Stank	Homo sapiens; Mus musculus	N/A; S1009	Q9H2X6; Q9QZR5	98, 93
Inhibitor of nuclear factor kappa-B kinase subunit beta	IKBKB, IKKB	Homo sapiens; Mus musculus	N/A	O14920; O88351	123
Kinase superfamily with octicosapeptide/Phox/Bem1p domain-containing protein	At2g35050, At1g79570, At5g57610	Arabidopsis thaliana	T13	O64768, A0A1P8ASK1, Q9FKL3	91, 94
Kinase suppressor of Ras 2	Ksr2	Mus musculus	T212	Q3UVC0	91
MAP/microtubule affinity-regulating kinase 3	Mark3 Emk2 Mpk10	Mus musculus	S494/S495	Q03141	91
Microtubule-associated serine/threonine-protein kinase 1, 4	MAST1 KIAA0973 SAST; Mast4	Homo sapiens; Mus musculus	S799; S2165	Q9Y2H9; Q811L6	84, 91, 93
Misshapen-like kinase 1	MINK1 B55 MAP4K6 MINK YSK2 ZC3, Mink1 Map4k6 Mink	Homo sapiens; Mus musculus	T672; T675/S676/S677/S685	Q8N4C8; Q9JM52	30, 91
Mitogen-activated protein kinase 18	MPK18 At1g53510 F22G10.12 T3F20.17	Arabidopsis thaliana	S415/T416	Q9C5C0	91, 94
Mitogen-activated protein kinase kinase kinase 1, 4, 5, 6, 7, 13	MAP3K1 MAPKKK1 MEKK MEKK1; MAP3K4 KIAA0213 MAPKKK4 MEKK4 MTK1; Map3k5 Ask1 Mekk5; MAP3K6 ASK2 MAPKKK6 MEKK6; MAP3K7 TAK1, Map3k7 Tak1; MAP3K13 LZK	Homo sapiens; Mus musculus	S297; S201, T300; T1227/S1228; N/A; T446/T448; T440/S441; S492/S495; T560	Q13233; Q9Y6R4; O35099; O95382; O43318, Q62073, A2AP92; O43283	84, 30, 91, 92

Protein names	Gene name(s)	Organism	Reported Glycosite(s)	Uniprot accession(s)	Citation
Mitogen-activated protein kinase kinase kinase kinase 4	MAP4K4 HGK ZC1 KIAA0687 NIK	Homo sapiens	T840	O95819	84
Myosin light chain kinase, smooth muscle	MYLK MLCK MLCK1 MYLK1	Homo sapiens	N/A	Q15746	92
Non-specific serine/threonine protein kinase	Akt3	Mus musculus	N/A	Q6NXW0	91
Pantothenate kinase 2, mitochondrial	PANK2 C20orf48	Homo sapiens	T182	Q9BZ23	84
Phosphatidylinositol 4-phosphate 3-kinase C2 domain-containing subunit alpha, beta, delta	PIK3C2A; PIK3C2B; PIK3CD	Homo sapiens	T334, T1327/S1335; N/A; S950	O00443; O00750; O00329	30, 78, 84
Phosphoenolpyruvate carboxykinase (ATP)-like protein	At4g37870	Arabidopsis thaliana	N/A	Q0WWL8	91, 94
Phosphoglycerate kinase 1	PGK1 PGKA MIG10 OK/SW-cl.110	Homo sapiens	N/A	P00558	78
Phosphotransferases/inositol or phosphatidylinositol kinase	At4g36080	Arabidopsis thaliana	S3248	F4JPL0	91, 94
Platelet-derived growth factor receptor beta	Pdgfrb Pdgfr Pdgfr1	Mus musculus	S308	P05622	91
Probable gluconokinase	IDNK C9orf103	Homo sapiens	S125	Q5T6J7	84
Protein kinase superfamily protein	At1g73460 T9L24.35 T9L24_35	Arabidopsis thaliana	S191/S193	F4HQ88	91, 94
Putative 3-phosphoinositide-dependent protein kinase 2	PDPK2P PDPK2	Homo sapiens	T182	Q6A1A2	84
Pyruvate kinase PKM	PKM OIP3 PK2 PK3, Pkm Pk3 Pkm2 Pykm	Homo sapiens; Mus musculus	T50; S37	P14618; P52480	30, 91
Receptor-interacting serine/threonine-protein kinase 1	RIPK1 RIP RIP1	Homo sapiens	S330/S331	Q13546	30
Rho-associated protein kinase 1	ROCK1	Homo sapiens	S1336	Q13464	84
Ribosomal protein S6 kinase alpha-3	RPS6KA3 ISPK1 MAPKAPK1B RSK2	Homo sapiens	S556	P51812	84
Ribosomal protein S6 kinase alpha-5	RPS6KA5 MSK1	Homo sapiens	S187	O75582	84
Serine-protein kinase ATM	ATM	Homo sapiens	S601, S941, T2608	Q13315	84
Serine/threonine-protein kinase 35	STK35 CLIK1 PDIK1 STK35L1	Homo sapiens	N/A	Q8TDR2	92
Serine/threonine-protein kinase DCLK1	Dclk1 Dcamkl1 Dclk	Mus musculus	T156	Q9JLM8	91
Serine/threonine-protein kinase haspin	HASPIN GSG2	Homo sapiens	S299	Q8TF76	84
Serine/threonine-protein kinase LATS2	LATS2 KPM	Homo sapiens	S277	Q9NRM7	84
Serine/threonine-protein kinase LMTK3	LMTK3 KIAA1883 TYKLM3, Lmtk3 Aatyk3	Homo sapiens; Mus musculus	S1316; S535, S1280	Q96Q04; Q5XJV6	30, 91
Serine/threonine-protein kinase MARK1	Mark1 Emk3 Kiaa1477	Mus musculus	N/A	Q8VHJ5	91

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Protein names	Gene name(s)	Organism	Reported Glycosite(s)	Uniprot accession(s)	Citation
Serine/threonine-protein kinase MARK2	Mark2 Emk	Mus musculus	N/A	Q05512	91
Serine/threonine-protein kinase MRCK alpha	CDC42BPA KIAA0451, Cdc42bpa Kiaa0451	Homo sapiens; Mus musculus	S1609; S1596	Q5VT25; Q3UU96	30, 91
Serine/threonine-protein kinase N1	PKN1 PAK1 PKN PRK1 PRKCL1	Homo sapiens	S559/S561	Q16512	84
Serine/threonine-protein kinase Nek4, Nek7, Nek9	NEK4 STK2; NEK7; NEK9 KIAA1995 NEK8 NERCC	Homo sapiens	Nek7: S260/S264 Nek9: T877	P51957; Q8TDX7; Q8TD19	92, 84, 30
Serine/threonine-protein kinase OSR1	OXSR1 KIAA1101 OSR1	Homo sapiens	N/A	O95747	92
Serine/threonine-protein kinase PAK 2	PAK2	Homo sapiens	T169/T178	Q13177	84
Serine/threonine-protein kinase PRP4	PRPF4B KIAA0536 PRP4 PRP4H PRP4K	Homo sapiens	S20, S356	Q13523	84
Serine/threonine-protein kinase RIO2	RIOK2 RIO2	Homo sapiens	S239	Q9BVS4	84
Serine/threonine-protein kinase TAO1, TAO2	TAOK1 KIAA1361 MAP3K16 MARKK; TAOK2 KIAA0881 MAP3K17 PSK PSK1 UNQ2971/ PRO7431	Homo sapiens	N/A	Q7L7X3; Q9UL54	92
Serine/threonine-protein kinase ULK2	ULK2 KIAA0623, Ulk2 Kiaa0623	Homo sapiens; Mus musculus	T613; T613, T727	Q8IYT8; Q9QY01	30, 91, 93
Serine/threonine-protein kinase WNK1, WNK2, WNK3	WNK1 HSN2 KDP KIAA0344 PRKWNK1, Wnk1 Hsn2 Prkwnk1; WNK2 KIAA1760 PRKWNK2 SDCCAG43 P/ OKcl.13, Wnk2 Kiaa1760; WNK3 KIAA1566 PRKWNK3, Wnk3	Homo sapiens, Mus musculus	WNK1 Human: T1848/ S1849/S1850 WNK1 Mouse: S1230, S1844, T1945, T2291, S2301, T2376 WNK2 Human: T1604/ S1606 WNK2 Mouse: T1698 WNK3 Human: T709, T974, T1312 WNK3 Mouse: S1161	Q9H4A3, P83741; Q9Y3S1, Q3UH66; Q9BYP7, Q80XP9	92, 30, 84; 91, 93
SNF-related serine/threonine-protein kinase	Snrk	Mus musculus	N/A	Q8VDU5	91
STE20-like serine/threonine-protein kinase	Slk Kiaa0204 Stk2	Mus musculus	S1230	O54988	91
Striated muscle preferentially expressed protein kinase	SPEG APEG1 KIAA1297	Homo sapiens	S1880	Q15772	84
Titin	TTN	Homo sapiens	S4651, T4659, S10385, S33976	Q8WZ42	84
TRAF2 and NCK-interacting protein kinase	TNIK KIAA0551, Tnik Kiaa0551	Homo sapiens; Mus musculus	S568; S539, T577	Q9UKE5; P83510	92, 30; 93
Triple functional domain protein	Trio	Mus musculus	N/A	Q0KL02	91
Tyrosine-protein kinase	Abl2	Mus musculus	T822	B2RQ57	91

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Protein names	Gene name(s)	Organism	Reported Glycosite(s)	Uniprot accession(s)	Citation
Tyrosine-protein kinase ABL1, ABL2	ABL1 ABL JTK7; Abl2 Arg	Homo sapiens; Mus musculus	S855; T872	P00519; Q4JIM5	30, 93
Tyrosine-protein kinase HCK	HCK	Homo sapiens	S347/S351	P08631	84
Tyrosine-protein kinase receptor Tie-1	TIE1 TIE	Homo sapiens	N/A	P35590	92
Tyrosine-protein kinase Yes	Yes1 Yes	Mus musculus	N/A	Q04736	91
Uridine-cytidine kinase 2	UCK2 UMPK	Homo sapiens	T106	Q9BZX2	84
Bifunctional UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase	GNE	Homo sapiens	N/A	Q9Y223	114
5’-AMP-activated protein kinase	PRKAA2, PRKACA; PRKACB; AMPK	Homo sapiens	N/A	P54646, P17612, P22694	111 99
RAC-alpha serine/threonine-protein kinase 1; 2	AKT1; AKT2	Homo sapiens; Rattus norvegicus	T305/T312; N/A	P31749; P47197	100, 104
Protein kinase C zeta type	Prkcz	Mus musculus	T410 (also a phosphosite)	Q02956	124
Cyclin-dependent-like kinase 5	CDK5	Homo sapiens	S46, T245/T246/S247	Q00535	125
Serine/threonine-protein kinase ULK1	ULK1 KIAA0722	Homo sapiens	T754	O75385	110

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