C. elegans Genetic Networks Predict Roles for O-GlcNAc Cycling in Key Signaling Pathways

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Abstract: Caenorhabditis elegans is perhaps the best-understood metazoan in terms of cell fate, neural connectivity, nutrient sensing, and longevity. The study of this genetically amenable model has greatly accelerated progress in understanding human aging-associated diseases, such as diabetes and neurodegeneration. The nutrient-responsive cycling of O-GlcNAc on key intracellular targets may play a key, yet unappreciated, role in human disease. Unlike their mammalian counterparts, loss-of-function mutants of ogt-1 (O-GlcNAc Transferase) and oga-1 (O-GlcNAcase) are viable in C. elegans, allowing the impact of the loss of O-GlcNAc cycling to be monitored in a living organism. C. elegans forward and reverse genetics, coupled with proteomics and chemical genomics, reveal networks of interactions and signaling pathways in which O-GlcNAc cycling may participate. The results point to a key regulatory role for O-GlcNAc cycling in cellular functions as diverse as nutrient uptake and salvage, cellular signaling, and transcription. The impact of altered O-GlcNAc cycling on the organism includes many of the hallmarks of aging-associated diseases: altered metabolism, lifespan, stress resistance, and immunity.

O-GlcNAc: INSULIN RESISTANCE AND BEYOND

Type II diabetes mellitus (T2DM) is a metabolic disorder characterized by insulin resistance, which prevents the cellular uptake and utilization of glucose, leading to high blood glucose levels. These high serum glucose levels, in turn, lead to chronic cellular and tissue damage, a phenomenon termed “glucose toxicity”. As a consequence of glucose toxicity, patients with diabetes (90% of whom have type II) are at elevated risk for heart disease, stroke, blindness, kidney disease, nervous system disease, and hearing loss, which can all contribute to decreased lifespan in diabetic patients. Currently, T2DM affects over 23 million Americans, and the Centers for Disease Control estimates that an additional 57 million Americans have ‘pre-diabetes’ and have an increased risk of developing the disease [1]. In recent years, the prevalence of T2DM has markedly increased and the average age of onset has rapidly decreased, leading the CDC to characterize T2DM as an epidemic.

T2DM is associated with several environmental and genetic factors including obesity and physical inactivity [2], as well as age. Although T2DM affects men and women of all races, several ethnic groups have exceptionally high rates of the disease. Epidemiological studies of these groups have helped to identify additional genetic risk factors. The identification of OGA-1 (MGEA5) as a T2DM susceptibility locus in the Mexican-American population [3] was gratifying to researchers who had been working on this protein and its counterpart, OGT-1, as it had been postulated as early as the 1990s that these enzymes played a role in the disease.

OGA-1 is a glucosaminidase that removes the O-GlcNAc (O-linked N-acetyl glucosamine) modification from proteins. This post-translational modification is present on over 500 different proteins in the cell [4]. The action of OGA-1 counteracts the action of OGT-1, the O-GlcNAc transferase, which uses UDP-GlcNAc as its substrate. Glucose and glucosamine levels influence the amount of UDP-GlcNAc available for OGT-1 transfer [5] and generally correlate with the level of O-GlcNAc protein modification [6, 7], suggesting that this hexosamine signaling pathway might act as a cellular nutrient sensor. ~2-5% of the total intracellular glucose is converted to UDP-GlcNAc that is utilized by OGT-1 [8].

Experimental studies that preceded and followed the identification of OGA-1 as a human susceptibility locus support the human genetic link between the hexosamine signaling pathway and T2DM. OGT-1 transcripts and the O-GlcNAc protein modification are enriched in the insulin-producing pancreatic β cells in the rat [9], and further enriched in pancreatic cells of diabetic rats [10]. In addition, transgenic mice that overexpress OGT-1 in muscle [11] or liver [12] develop insulin resistance, as do mammalian cells or rat muscles in culture treated with an inhibitor of OGA-1 [13-15]. In addition to its roles in glucose sensing and diabetes, O-GlcNAc cycling plays a role in many fundamental cellular processes, including transcription, translation, protein localization, proteasome activity, and stress response [4]. Consequently, O-GlcNAc likely plays a role in some of the most pressing health care issues of the 21st century: neurodegenerative diseases, HIV, cardiovascular disease, and cancer [16-19]. It is not clear which of these processes are directly linked to the insulin signaling functions of O-GlcNAc (Alzheimer’s disease has been recently called ‘type III diabetes’) and which are modulated by O-GlcNAc, but are distinct from its role in insulin signaling and resistance.

OGT-1 is known to compete with kinases for available serine and threonine residues that can be modified by either O-GlcNAc or phosphate; however, it is also clear that block-
ing phosphorylation at particular residues is only one mecha-
nism by which OGT-1 and the O-GlcNAc modification func-
tion [20]. For example, inhibition of the GSK-3 kinase in
mammalian COS7 cells results in increased O-GlcNAcyl-
ation of some proteins and decreased O-GlcNAcylation of others
[21]. Unlike phosphorylation, which is regulated by hun-
dreds of phosphatases and kinases, there is only a single
OGA enzyme and a single OGT enzyme in metazoa. Given
that these enzymes are unique and involved in many diverse
and important cellular processes, it is not surprising that both
OGA-1 and OGT-1 are essential in mammals [22, 23].

The essential nature of OGA-1 and OGT-1 is one of sev-
eral complications limiting the study of this pathway in
mammals. Increased activity of OGT-1 is often observed in
mammalian cell culture lines by increasing the glucose or
glucosamine concentration of the media (which in turn in-
creases the levels of UDP-GlcNAc substrate), by overex-
pressing GFAT (the rate-limiting enzyme of the hexosamine
biosynthetic pathway; [24]), or by treating the cells with the
OGA-1 inhibitors like PUGNAc or NAG-thiazoline. All of
these methods do increase the overall level of cellular protein
O-GlcNAc modification; however, they may also induce other
pathways, which make it difficult to determine which of
the resulting phenotypes can be fully attributed to hexo-
samine activity. Excess glucose likely leads to phenotypes
that are not dependent on OGT-1, as only 5% or less of the
intracellular glucose is converted to UDP-GlcNAc. Although
PUGNAc treatment leads to a global increase in the O-
GlcNAc modification, not all proteins respond identically to
treatment: the O-GlcNAc modification of some proteins is
unchanged or even decreased in the presence of PUGNAc
[25]. Moreover, both PUGNAc and thiazoline inhibit other
hexosaminidases in addition to OGA-1 [26, 27]. In whole-
organism mouse and rat models, OGT-1 can be upregulated
in specific tissues, like muscle or liver. However, OGT-1 is
normally expressed in a larger variety of tissues, including
the heart and the brain [28]. These mammalian studies have
provided important molecular and biochemical information
about the functions of O-GlcNAc, but their interpretation is
limited by a lack of integrated signals between genetic net-
worls, from cell-to-cell, and from tissue-to-tissue. Moreover,
the interpretation of these studies is based on molecular or
biochemical changes, as opposed to a functional phenotype
(e.g., animal behavior or survival).

Understanding communication between tissues is funda-
mentally important to understanding the role of O-GlcNAc
in human disease, since, for example, it is known that cellu-
lar signals between the pancreas, muscle, and fat are neces-
sary for the development of insulin resistance. The homeo-
static mechanisms in metazoa, including the endocrine sys-
tem and anabolic and catabolic metabolism, are robust and
interconnected. Understanding how signals are integrated
from different genetic networks is especially important if we
are to understand how O-GlcNAc regulates diverse cellular
processes, and which of its phenotypes are connected to, and
which are independent of, insulin signaling. In order to take
a genetic, whole-organism approach to studying hexosamine
signaling and its role in human disease, a model experimen-
tal organism is necessary.

THE USE OF C. elegans AS A MODEL SYSTEM TO
STUDY O-GlcNAc

The nematode Caenorhabditis elegans is an excellent
genetic model system to study the mechanism of the O-
GlcNAc modification, its effect on nutrient sensing and insu-
lin resistance, and its genetic connections to other pathways.
Both the OGA-1 and OGT-1 proteins are unique and highly
conserved in C. elegans (68% and 88% similarity to the hu-
man orthologs, respectively; [28, 29]). Unlike the mammal-
ian mutants, which are embryonic lethal, catalytically null
mutants of C. elegans oga-1 and ogt-1 are viable and fertile
[28-30], allowing easier observation of their phenotypes.
Multiple null alleles of ogt-1 are available, allowing verifica-
tion of the observed phenotypes and potentially interesting
allele-specific phenotypes. Viable knockouts allow the con-
sequences of increased O-GlcNAc protein modification to be
observed without non-specific inhibitors and allow the sepa-
ration of glucose-induced phenotypes that are dependent on
OGT-1 and those that are not.

The simplicity of the nematode system (small size, short
generation time, and large number of progeny) allows rapid
and large-scale analysis of genetic questions. Studying hexo-
samine signaling in C. elegans could identify connections
between the hexosamine pathway and other genetic networks
via genetic screens for unknown factors that interact with
OGT-1 and OGA-1 to modify insulin or nutrient signaling.
In addition, the fate of each of the C. elegans 959 somatic
cells is known from development through adulthood [31, 32],
and the connections between the 302 neurons in the
adult have been mapped [33, 34], providing a unique fram-
work for integrating genetic and developmental information.

C. elegans homologs have been identified for ~60-80%
of human genes [35]. The insulin-like signaling pathway is
well studied in C. elegans and components of the pathway
are conserved between C. elegans and humans [36]. As in
humans, insulin signaling in C. elegans has been linked to
many biological processes, including life span, fat storage,
reproduction, and the stress response. Because the genetic
requirements for insulin signaling are known in C. elegans,
genetic connections between the insulin and other signaling
pathways can be identified and studied. In mammals, signal-
ing from the insulin receptor negatively regulates the down-
stream FOXO1 transcription factor, which is required for the
development of insulin resistance in insulin receptor knock-
out mice [37]. Likewise, activation of the C. elegans insulin-
like receptor DAF-2 negatively regulates the downstream
FOXO homolog DAF-16, which is required for many of the
phenotypes observed in daf-2 mutants (Fig. 1). The recent
discovery that mammalian FOXO1 is activated by O-GlcNAc
modification in response to glucose or glucosamine [38-40]
provides yet another potential mechanism for O-GlcNAc’s
modulation of insulin signaling. Because it is known which
daf-2 phenotypes are DAF-16-dependent or independent, the
analysis of this well-characterized system makes it straight-
forward to distinguish genetically which effects of OGA-1
and OGT-1 are FOXO-dependent and which function in
separate pathways. This is key for identifying other functions
of hexosamine signaling and determining if any of these ac-
tivities are independent of insulin signaling.
Several experiments have established genetic and protein interaction networks in *C. elegans* [41-43], and this data, in addition to traditional genetic assays, can be mined for potential proteins and pathways that interact with OGA-1 and OGT-1. To date, genetic or synthetic interactions have been identified between OGT-1 and four other *C. elegans* genes: DAF-2, BAR-1, PMK-1, and LET-756. These interactions define a role for hexosamine signaling in four genetic networks: insulin signaling, Wnt/β-catenin signaling, p38 MAP kinase signaling, and FGF signaling, respectively. In this review, we will discuss the implications of each of these interactions and the potential of the *C. elegans* system to contribute to our understanding of the role of hexosamine signaling in diverse cellular processes and human diseases.

**C. elegans AS A MODEL FOR THE ROLE OF HEXOSAMINE SIGNALING IN INSULIN SIGNALING AND INSULIN RESISTANCE: OGT-1 AND OGA-1 INTERACT GENETICALLY WITH DAF-2**

In humans, signaling through the insulin receptor triggers a cascade that results in the repression of FOXO transcription factor activity. Similarly, signaling through the *C. elegans* insulin-like receptor DAF-2 initiates a cascade that ends in the down-regulation of the FOXO transcription factor DAF-16 by its retention in the cytoplasm (Fig. 1). Loss-of-function mutations in *daf-2* result in a variety of phenotypes, including altered metabolism, an alternative larval stage termed dauer, extended lifespan, and increased stress and pathogen resistance [44]. Many of the phenotypes of *daf-2* mutants are dependent upon DAF-16/FOXO. DAF-16 directly or indirectly regulates over 700 target genes in *C. elegans* [45-47] that in turn regulate these varied cellular processes.

![Diagram](image)

**Fig. (1). Role of O-GlcNAc in Insulin Signaling.** The DAF-2 insulin-like signaling cascade leads to the cytoplasmic retention of the DAF-16/FOXO transcription factor. In the absence of insulin signaling, the active transcription factor translocates to the nucleus (gray arrow), modulating expression of target genes. OGT-1 attenuates the signal at multiple points in the pathway. Known O-GlcNacylated targets are shown as filled symbols. In this simplified schematic, factors described in the text are shown with both the mammalian and *C. elegans* protein names.

In mammalian cells, the O-GlcNAc protein modification interacts molecularly with insulin receptor substrates as well as the downstream FOXO transcription factor. Exposure of adipocytes to the OGA-1 inhibitor PUGNac enhances phosphorylation of the insulin receptor substrate IRS1 (Fig. 1). In contrast, high glucose or PUGNac exposure inhibits the phosphorylation of Akt in response to insulin, which in turn inhibits its kinase activity [12]. Both IRS1 and Akt are modified by O-GlcNAc [12], suggesting that this response is direct. Although the O-GlcNAC modification has an opposite effect on the phosphorylation of these two proteins, the end result is the same: the attenuation of insulin signaling.

In diabetic patients, changes in carbohydrate and fat metabolism accompany insulin resistance. Likewise, insulin signaling also regulates fat storage in *C. elegans*, as *daf-2* insulin receptor mutants have increased fat stores [48]. *ogt-1* mutants also exhibit changes in fat metabolism; however, the effect is opposite that of *daf-2* mutations. Levels of triacylglycerol and sterol esters are reduced ~70% in *ogt-1* mutants compared to wild type [29, 30], and a reduction in fat accumulation has also been observed in *ogt-1* RNAi knockdown experiment by Nile Red staining [41]. Interestingly, *oga-1* mutants also have decreased fat stores, as triacylglycerol and sterol esters are reduced ~40% in *oga-1* mutants [29, 30]. This decrease in fat storage is accompanied by an increase in carbohydrate storage. Disaccharide trehalose and glycogen levels are increased in both *ogt-1* (~2.3 fold increased compared to wild type) and *oga-1* mutants (~1.6 fold increased) [29, 30]. These data indicate that OGA-1 and OGT-1 are both necessary for proper carbohydrate and fat metabolism, and may also suggest that the dynamic cycling of the O-GlcNAC modification is important in this process.

Proper energy metabolism is important for many processes, including fertility. Obese and diabetic women have reduced fertility rates [49]. Several lines of evidence suggest that proper energy metabolism is also necessary for fertility in *C. elegans*. Mutations in the *daf-2* insulin-like receptor [50], high media concentrations of glucose [51], or mutations that reduce desaturation of fatty acids (*fat-6; fat-7; [52]) all inhibit *C. elegans* fertility. Measuring fertility rates in *C. elegans* oga-1 and ogt-1 mutants (either alone or in combination with *daf-2* mutations or nutrient stress) is simple and illustrates the utility of genetic model systems elucidate the role of O-GlcNAC in complex phenotypes.

The altered fat metabolism observed in *C. elegans daf-2* mutants is dependent on DAF-16 [53]. It is not known how DAF-16 integrates varied environmental and genetic signals to differentially regulate its target genes. If, like its mammalian homolog FOXO1, DAF-16 is modified by O-GlcNAC, this modification might provide target gene specificity. For example, the fatty acid desaturases FAT-5, FAT-6, and FAT-7 are required for proper fat storage and are targets of DAF-16 [48]. Since O-GlcNAC is involved in responding to nutrient levels, insulin signaling, and fat storage, it could be the connection between FOXO1 and these phenotypes. Simple genetic assays in *C. elegans* will allow the determination of which phenotypes/target genes require the O-GlcNAC modification of FOXO1 (DAF-16 and OGT-1 dependent) and which do not (DAF-16 dependent; OGT-1 independent).
In *C. elegans*, environmental cues (e.g., nutrient or temperature stress) or mutations in daf-2 can lead to an alternative larval stage known as dauer. The dauer stage provides a convenient assay for assessing insulin signaling. A daf-2 temperature-sensitive mutant will produce dauer larvae at temperatures above 16°C, with the percentage of dauer increasing as temperature increases, even in the absence of nutrient stress (Fig. 2). Dauers form constitutively in temperature-sensitive daf-2 mutants at 25°C. ogt-1 and oga-1 single mutants do not form dauer larvae in the absence of nutrient stress [29, 30]. In daf-2 ogt-1 double mutants, the number of dauers produced is reduced over three fold compared to the daf-2 mutant alone, indicating that these worms are insulin hypersensitive [29, 30]. Experiments at low temperature (21-22°C) suggest that daf-2;oga-1 double mutants produce increased dauers compared to daf-2 single mutants, indicating insulin resistance [29]. However, this is not observed at higher temperatures (23-25°C; MA Mondoux, JA Hanover, and MW Krause, unpublished data). Because dauer formation in daf-2 mutants is known to be DAF-16 dependent [54], it is important to determine whether dauer formation in daf-2 ogt-1 and daf-2;oga-1 double mutants is DAF-16 dependent. Dependence of the dauer phenotype on DAF-16 would establish that this modulation of dauer formation is specific to the insulin-signaling pathway.

Fig. (2). Common *C. elegans* Genetic Assays. (A) Dauer Assay. In a daf-2 temperature-sensitive mutant, more dauer larvae are formed as temperature increases. Mutations that partially suppress dauer formation, like ogt-1, produce fewer dauer larvae in the daf-2 background at semi-permissive temperatures. (Adapted from [29]; photos from CA Wolkow). (B) Lifespan Assay. Adults are monitored for survival, often in the presence of 5’fluorodeoxyuridine (FUDR) to prevent progeny growth from complicating the analysis. Mean lifespan is defined as the day at which 50% of the population is alive. Some mutations shorten lifespan; others, like daf-2, extend lifespan. (Adapted from [139]; photos from CT Murphy). (C) Pathogen Killing Assay. Similar to the lifespan assay, L4 larvae or adults are exposed to a pathogen and then assayed for survival, with mean survival defined as the time at which 50% of the population is alive. Some mutations increase pathogen resistance, and others, like bar-1, are more susceptible to killing by the pathogen. (Adapted from [82]; photo JJ Ewbank and C Couillault).
In addition to the insulin-signaling pathway, the TGF-β and guanylyl cyclase pathways can also contribute to dauer formation in *C. elegans* [55]. Just as O-GlcNAc modulates insulin signaling, it likely plays a role in the TGF-β signaling pathway, which is also responsive to glucose and plays a role in the development of diabetes [56]. Increasing O-GlcNAc levels via high glucose concentrations, overexpression of GFAT, or use of the OGA-1 inhibitor streptozotocin all lead to increased transcription and DNA binding of the upstream stimulatory factors (USF) at the TGF-β promoter in mammalian cells [57]. The USF proteins themselves are not modified by O-GlcNAc [57], suggesting that O-GlcNAc controls this pathway via transcriptional or post-translational regulation of some other factor(s). Investigating the genetic interactions between OGT/OGA and the TGF-β pathway in *C. elegans* could provide new insights into the role of O-GlcNAc in this pathway and will help to distinguish how O-GlcNAc contributes to T2DM via insulin signaling, TGF-β signaling, and the interactions between these networks.

In both mammals and *C. elegans*, insulin signaling plays a role in varied processes including fat metabolism and fertility. The insulin genetic network is connected to many different networks (including the TGF-β signaling pathway and others to be discussed in this review). Studying O-GlcNAc’s role in the insulin-signaling network in *C. elegans* allows the observation of its effects on multiple pathways and on multiple phenotypes. Because O-GlcNAc modifies so many proteins, understanding the integrated effect of O-GlcNAc modification on multiple genetic networks will be crucial for therapeutic development.

**A ROLE FOR HEXOSAMINE SIGNALING IN THE Wnt/β-CATENIN SIGNALING PATHWAY: OGT-1 INTERACTS SYNTHETICALLY WITH BAR-1 IN C. elegans**

Just as signaling through the mammalian insulin receptor regulates the nuclear localization of the downstream transcription factor FOXO, Wnt signaling regulates the nuclear localization of β-catenin (BAR-1 in *C. elegans*; Fig. 3). In the nucleus, β-catenin complexes with TCF family transcription factors to regulate the expression of at least 100 target genes that regulate cellular proliferation, differentiation, transformation, migration, and adhesion [58]. Given its role in these processes, it is not surprising that mutated β-catenin has been found in many human cancers, including colorectal cancer and melanoma [59].

β-catenin is modified by O-GlcNAc in mammalian cells [13, 60], and this modification seems to play a role in its nuclear localization. Modification by O-GlcNAc regulates the binding of β-catenin to its nuclear binding partner TCF-4, but not to the APC tumor suppressor protein [61], which functions in the nuclear export of β-catenin [62, 63].

![Fig. (3). Role of O-GlcNAc in β-catenin Signaling.](image-url)

The Wnt signaling cascade leads to β-catenin’s translocation to the nucleus (gray arrow), where it can bind the transcription factors TCF-4 or FOXO to regulate gene expression. In the absence of Wnt ligand, GSK-3 phosphorylation of β-catenin results in its retention in the cytoplasm and its eventual degradation by the proteasome. The insulin-signaling pathway (blue) converges on the Wnt/β-catenin network (yellow). OGT-1 modifies multiple factors in the pathway (filled symbols) and negatively regulates β-catenin’s nuclear localization.
ver, treatment of human prostate and breast cancer cell lines with the OGA-1 inhibitor PUGNAC results in decreased nucleic localization of β-catenin [61]. This decreased nuclear localization is accompanied by decreased transcription of β-catenin targets [61]. Defects in the O-GlcNAc modification of β-catenin may be another path to oncogenesis: prostate cancer cells lines have low levels of O-GlcNAcylated β-catenin [61], suggesting that the increased nuclear localization of unmodified β-catenin and subsequent transcriptional changes could contribute to transformation in these cells. TGF-β1 can also induce nuclear localization of β-catenin in human mesenchymal stem cells [64], although the role of the O-GlcNAc modification in this process has not been examined.

In addition to its connections to the hexosamine and TGF-β networks, the Wnt/β-catenin signaling pathway is connected to the insulin-signaling pathway. β-catenin and FOXO physically interact in mammalian cells, as do heterologously expressed BAR-1 and DAF-16 [65, 66]. β-catenin enhances FOXO transcriptional activity [65, 66]. In addition to this positive role for β-catenin in FOXO transcriptional activation, FOXO negatively regulates β-catenin/TCF transcriptional activity and reduces binding of β-catenin to TCF-4 [66, 67]. FOXO and TCF bind to the same region of the β-catenin protein [65]. Furthermore, the interactions between β-catenin/FOXO and BAR-1/DAF-16 are increased in response to oxidative stress [65, 66], and the interaction between β-catenin and TCF-4 is decreased in response to oxidative stress [67]. Together, these data suggest that FOXO and TCF compete for β-catenin binding to control the transcriptional response to Wnt signaling, insulin signaling, and stress.

Since β-catenin and FOXO are both modified by O-GlcNAc, the modification of one or both of these proteins could affect their interaction, activity, and/or nuclear localization, and may influence whether FOXO or TCF ‘wins’ the competition for β-catenin binding. The β-catenin/TCF complex is known to interact with c-Jun [68], which is also modified by O-GlcNAc [69], raising the possibility that O-GlcNAc modification could be a general mechanism controlling the selection of β-catenin’s binding partners and therefore its transcriptional activation program. The c-Jun N-terminal kinases (JNKs) are known to be involved in the progression of type II diabetes [70], and it is possible that c-Jun’s interaction with β-catenin and/or its O-GlcNAc modification influences its availability for phosphorylation by JNK. In C. elegans, JNK can directly phosphorylate DAF-16 [71], suggesting another connection point in the OGT-1, insulin signaling, and β-catenin genetic networks.

In addition to its regulation by O-GlcNAc, β-catenin is regulated via phosphorylation by the cytoplasmic kinase GSK-3 [72, 73], which leads to β-catenin’s subsequent ubiquitination and degradation by the proteasome [74] (Fig. 3). GSK-3 modifies a wide range of substrates, including many targets of OGT-1. Also, like OGT-1, GSK-3 is thought to play a crucial role in diabetes, Alzheimer’s disease, and cancer [75-77]. Inhibition of GSK-3 by lithium treatment increases the levels of O-GlcNAcylated β-catenin [78], and changes the O-GlcNAc modification state of at least 45 additional proteins, with increases in O-GlcNAcylation observed for some proteins and decreases observed for others [21].

GSK-3 itself is modified by O-GlcNAc [79], suggesting that O-GlcNAc could potentially negatively regulate β-catenin activity through at least two mechanisms: first, by maintaining β-catenin in the cytoplasm via O-GlcNAc modification, and second, by promoting the degradation of β-catenin via O-GlcNAc modification of GSK-3 or a GSK-3 regulator (for example, Akt). Phosphorylation of GSK-3 Ser-9 contributes to its inactivation [80], and is used as a marker for GSK-3 activity. In C. elegans, both GSK-3 and GSK-3 phospho-Ser-9 protein levels are increased in ogt-1 (2.2 fold increased compared to wild type) and oga-1 (1.3 fold increased) mutants [29]. In contrast, treating adipocytes with insulin and the OGA-1 inhibitor PUGNAC has no discernable effect on GSK-3 levels but decreases GSK-3-phospho-Ser-9 levels [13]. These results may indicate a difference in the O-GlcNAc regulation of GSK-3 between mammals and C. elegans, or they may highlight the caveats of extrapolating function and phenotype from cell culture experiments that rely on chemical inhibitors and lack integration of cell-to-cell and tissue-to-tissue signals.

Because the Wnt/β-catenin signaling pathway has complex interactions with a variety of genetic networks, C. elegans is an excellent system to study the role of O-GlcNAc in β-catenin signaling. β-catenin/bar-1 was used as a ‘query’ gene in a systematic genetic interaction analysis (SGI) in C. elegans, and OGT-1 was identified as a BAR-1 genetic interactor based on a slow growth phenotype in bar-1 mutant ogt-1 RNAi lines [41]. As suggested by the interactions between β-catenin/FOXO and BAR-1/DAF-16 [65, 66], the β-catenin pathway interacts with the DAF-2 insulin-like signaling pathway in C. elegans. Similar to daf-2 ogt-1 double mutants [29, 30], daf-2;bar-1 double mutants form fewer dauer larvae compared to daf-2 single mutants [65]. Furthermore, a bar-1 RNAi knockdown results in an ~50% reduction of fat stores [41], which is similar to the defect in fat metabolism observed in ogt-1 mutants [29, 30] and opposite to the increase in fat stores observed in daf-2 mutants [81]. bar-1 mutants also have decreased lifespan and increased sensitivity to oxidative stress, similar to the observed lifespan and stress resistance defects in daf-16 mutants [65]. BAR-1 is required for full expression of the DAF-16 target gene sod-3 in response to oxidative stress in C. elegans [65, 66], although there is no change in basal sod-3 levels in bar-1 mutants [82]. These data suggest that β-catenin could modulate dauer formation, lifespan, and stress response through its interaction with DAF-16/FOXO.

In addition to its roles in fat metabolism, insulin signaling, and the oxidative stress response, BAR-1 also plays a role in the C. elegans response to the opportunistic human pathogen Staphylococcus aureus. bar-1 mutants fail to induce expression of several genes up-regulated in the wild-type response to S. aureus and are more sensitive to killing by the pathogen [82]. BAR-1’s role in the response to pathogen stress could be related to its role in the insulin-signaling network. daf-2 mutants are resistant to several pathogens, and this response is DAF-16 dependent [83]. bar-1 RNAi suppresses the daf-2 resistance to S. aureus [82]. This low
level of resistance is similar to the sensitivity observed in bar-1 RNAi alone and is more severe than daf-16;daf-2 mutants, which have a wild-type level of pathogen sensitivity [82, 83], suggesting that BAR-1’s role in the pathogenesis response is at least partially distinct from DAF-16/FOXO. It is important to determine how and if ogt-1 mutations affect pathogenesis, as we know that β-catenin and FOXO, key factors in the pathogen response network, are modified by O-GlcNAc.

The discovery of a genetic interaction between ogt-1 and bar-1 in C. elegans complements the biochemical and molecular data implicating O-GlcNAc in the regulation of β-catenin in mammalian cells. In addition, the known functions of bar-1 in C. elegans lifespan regulation, stress response, and pathogen resistance suggest new roles for OGT-1 and O-GlcNAc. In the next section, we will discuss how this proposed role for OGT-1 in the C. elegans innate immune response is reinforced by a physical interaction with a p38 MAP kinase.

**HEXOSAMINE SIGNALING AND THE MAP KINASE NETWORK: C. elegans OGT-1 INTERACTS PHYSICALLY WITH PMK-1**

Like the insulin, TGF-β, and β-catenin signaling networks, the p38 mitogen-activatived protein (MAP) kinase pathway responds to extracellular signals and transduces the signal to regulate gene expression. The p38 MAP kinase pathway regulates genes that play roles in apoptosis, cell cycle, differentiation, development, and inflammation [84]. The p38 MAP kinase network is also connected to the hexosamine-signaling network. In mammalian cells, p38 MAP kinase physically interacts with the C-terminus of OGT-1 [85]. Phosphorylation and activation of p38 by the upstream MAP kinase kinase MKK3 are required for its binding to OGT-1 (Fig. 4; [85]). p38 and OGT-1 interact in cells transfected with wild-type or constitutively active MKK3, but not in cells transfected with a dominant negative MKK3 [85]. Reciprocally, increasing O-GlcNAc levels (via high glucose or overexpressing GFAT) increases the level of phosphorylated p38 in mammalian cells [86]. In addition to their physical interaction, OGT-1 and the p38 MAP kinase share several target substrates, including the neurofilament peptide NF-H [87, 88]. Under glucose deprivation conditions, p38 inhibition does not affect the interaction between OGT-1 and NF-H; however, p38 inhibition abolishes the O-GlcNAcylation of NF-H, suggesting that p38 modulates OGT-1’s catalytic activity, but not its recruitment to substrates [85].

Another shared substrate is the transcriptional co-activator PGC-1α (PPAR-γ co-activator 1α), which is modified by O-GlcNAc [39], p38 MAP kinase [89], and GSK-3 [90]. PGC-1α binds to OGT-1 and targets OGT-1 to FOXO, increasing its O-GlcNAcylation and activity [39]. PGC-1α acts as a co-activator for many different proteins, including the PPARs (α, β, and γ), which are members of the nuclear hormone receptor (NHR) family. The NHR family regulates fat and glucose metabolism in mammals. PPARα is modified by both p38 MAP kinase and GSK-3 [91]. PPAR γ has been implicated in diabetes: it is expressed in adipocytes and in the liver of obese patients, and several anti-diabetic drugs act as PPARγ ligands [92]. Mammalian FOXO1 interacts with PPARγ in a two-hybrid assay, and the two proteins antagonize each other in reporter gene assays [93]. Together, these data demonstrate that PGC-1α is a possible connection point between multiple signaling networks. Increased O-GlcNAc levels improve cellular stress response in mammalian cardiac tissue, and this seems to be mediated by p38 MAP kinase [94]. Furthermore, the induced transcription of the TGF-β1 gene observed in the presence of increased O-GlcNAc via high glucose or overexpression of GFAT is dependent on p38 MAP kinase [86], suggesting a link between the OGT-1, TGF-β, and p38 genetic networks (Fig. 5).
The physical interaction between p38 and OGT-1 is evolutionarily conserved, as the C. elegans p38 homolog PMK-1 was identified as a physical interactor of OGT-1 in a high-throughput yeast two-hybrid screen [43]. The PPAR nuclear hormone receptors, which are direct targets of p38 and GSK-3 and indirect targets of OGT-1 in mammals, are also evolutionarily conserved. The C. elegans genome encodes a large number of NHRs, and although it is not clear by sequence which are the orthologs of the PPARs, functional conservation suggests that C. elegans NHR-49 is similar to PPARα [48]. NHR-49 is important for expression of the fatty acid desaturases FAT-5 and FAT-7, and mutations in these genes lead to altered fat storage and fertility defects [95, 96]. NHR-49 and FAT-7 are also necessary for wild-type lifespan [96]. O-GlcNAc may regulate this arm of the fat metabolism pathway, as it is known to directly target some mammalian NHRs [97, 98]. For example, increased O-GlcNAc levels inhibit the estrogen and progesterone NHRs directly by decreasing their transcription and increasing transcription of co-repressors [99]. Since OGA-1 and OGT-1 are both required for maintaining proper fat metabolism in C. elegans [29, 30], it will be interesting to see whether this phenotype is dependent on NHRs, and/or whether nhr-49 fat metabolism and lifespan phenotypes are dependent on hexosamine signaling.

The C. elegans NHR DAF-12 is a candidate for the functional ortholog of PPARγ, as it interacts with DAF-16 and with mammalian FOXO1 [93]. The role of O-GlcNAc in DAF-12 regulation is not known; however, both the insulin signaling and TGF-β signaling pathways converge upon DAF-12 in dauer formation [100]. Therefore, O-GlcNAc could potentially modulate this NHR signaling network via the modification of FOXO or other insulin signaling factors, and/or via modulation of TGF-β transcription.

Like the β-catenin and insulin signaling pathways, the p38 MAP kinase pathway responds to several different kinds of stress in C. elegans, including heat, osmotic, and pathogen...
stress. PMK-1 was identified in a screen for *C. elegans* mutants with enhanced susceptibility to the nematode and human pathogen *Pseudomonas aeruginosa* [101]. Other components of the p38 MAP kinase signaling pathway were also identified in this screen, including SEK-1 (*C. elegans* ortholog of MKK3) and NSY-1 (*C. elegans* ortholog of ASK1) [101]. The role of the p38 MAP kinase pathway in pathogen response is conserved from *C. elegans* to mammals, as ASK1 is also required for the innate immune response in mice [102].

A connection between the p38 MAP kinase pathway and the insulin signaling pathway in the pathogen response is suggested by the biochemical interactions between OGT-1, PGC1-α, and FOXO. In *C. elegans*, genetic evidence connects the p38 MAP kinase-signaling pathway to the DAF-2/DAF-16 insulin-signaling network. Loss of PMK-1 or SEK-1 eliminates the daf-2 mutant resistance to *P. aeruginosa* [103], just as loss of BAR-1 eliminates the daf-2 mutant resistance to *S. aureus* [82]. PMK-1 seems to act in parallel to BAR-1 in the *C. elegans* immune response. *pmk-1, bar-1* double mutants are more susceptible to killing by *S. aureus* than either single mutant alone [82]. Furthermore, loss of *bar-1* does not affect the induction of PMK-1-dependent genes in response to *P. aeruginosa*; nor does loss of *pmk-1* affect the induction of BAR-1-dependent genes in response to *S. aureus*. Interestingly, *bar-1* mutants seem to be more susceptible to *S. aureus*, and *pmk-1* mutants are more susceptible to *P. aeruginosa* [82], suggesting that *C. elegans* has pathogen-specific response programs [104].

How do the insulin, β-catenin, and p38 MAP kinase pathways contribute to the innate immune response? DAF-16, BAR-1, and PMK-1 are all necessary for the pathogen resistance observed in daf-2 insulin-like receptor mutants [82, 83, 103]. However, daf-2;bar-1 and daf-2;pmk-double mutants are more sensitive to killing by *P. aeruginosa* than daf-16, daf-2 double mutants [82, 103]. These data suggest that both BAR-1 and PMK-1 are necessary for the increased pathogen resistance observed in daf-2 mutants, but that both pathways also function in parallel to insulin signaling.

As expected for parallel pathways, daf-16, pmk-1 double mutants are more sensitive to *P. aeruginosa* than either single mutant alone [103]. Furthermore, there is very little overlap between genes up-regulated by PMK-1 and genes up-regulated by DAF-16 [45, 46, 103]. PMK-1 and DAF-16 do share some common targets, however: several genes that are upregulated by PMK-1 are downregulated by DAF-16, including C-type lectins [103]. C-type lectins can act as pathogen recognition receptors in a variety of organisms, including mammals. The C-type lectin domain was originally described as a carbohydrate-recognition motif, though not all C-type lectins bind carbohydrates [105]. The *C. elegans* genome contains nearly 300 genes thought to encode for C-type lectins, and different subsets of these genes are induced in the wild-type response to the *C. elegans* pathogens *P. aeruginosa* [103], *Serratia marcescens* [106], and *Microbacterium nematophilum* [107].

The C-type lectins are just one example of a large class of genes that are a common part of the immune response but show pathogen-specific expression patterns. In addition to pathogen specificity, there is also pathway specificity, as the p38 MAP kinase pathway and insulin signaling pathway (and presumably the β-catenin pathway) regulate overlapping but distinct sets of target genes in response to pathogen stress [108]. The TGF-β signaling pathway [106] and the JNK signaling pathway [109] are also involved in the *C. elegans* innate immune response, which could implicate O-GlcNAc in at least five different genetic networks that regulate the pathogen response. Since O-GlcNAc and OGT-1 modulate the insulin, β-catenin, p38 MAP kinase, and TGF-β signaling networks (Fig. 5), it is important to analyze the pathogen response in *ogt-1* mutants (alone or in combination with *bar-1, pmk-1, daf-2*, etc.). We can then begin to understand how the O-GlcNAc modification influences innate immunity in both general and pathogen-specific pathways.

**A NOVEL CONNECTION TO THE FGF NETWORK:**

**LET-756**

The same *C. elegans* SGI analysis that identified BAR-1 as a synthetic interactor of OGT-1 also identified LET-756 [41]. LET-756 is an FGF–like ligand. Unlike β-catenin and p38, there is no biochemical or molecular evidence in mammalian cells that links OGT-1 or O-GlcNAc with the closest LET-756 mammalian ortholog, the fibroblast growth factor FGF-20. This highlights the usefulness of the *C. elegans* model system to identify novel interactions and connections to the hexosamine-signaling network.

The FGFs respond to extracellular cues in the regulation of varied developmental processes, including cell proliferation and migration, organ and limb formation, and neural development [110]. LET-756 is necessary for early development in *C. elegans*: complete loss of function results in a larval lethal arrest [111]. There are 22 FGFs in mammalian cells and only two in *C. elegans* (LET-756 and EGL-17), simplifying the analysis of FGF function. Phylogenetic and functional analyses suggest that LET-756 is most similar to the FGF-9 superfamily, which also includes FGF-16 and FGF-20 [112].

How and why are the LET-756 and OGT-1 genetic networks connected? In mammals, FGF-9 is expressed in the proliferative cells of the pancreas [113], and OGT-1 is enriched in pancreatic β-cells [9]. One possibility for the interaction between LET-756 and OGT-1 is that this interaction is indirect via interaction of LET-756 with O-GlcNAc-modified protein(s). A *C. elegans* yeast two-hybrid screen for physical interactors of LET-756 identified DAF-21 and an uncharacterized gene, AD-F35A5.4 [114]. DAF-21 is a member of the Hsp90 family of molecular chaperones, which are O-GlcNAc modified in mammalian cells [115]. Similarly, AD-F35A5.4 encodes a protein most similar to human keratin-associated protein 10-4, and the intermediate keratins 1, 8, and 18 are O-GlcNAc modified [116, 117]. A human cDNA library yeast two-hybrid screen for physical interactors of *C. elegans* LET-756 identified human 14-3-3β, and this interaction was confirmed by co-immuno-precipitation [114]. 14-3-3 proteins are modified by O-GlcNAc in mammalian cells, suggesting another potential link between LET-756 and OGT-1. Moreover, the insulin
signaling components PDK1 and IRS1 are substrates for both 14-3-3 [118, 119] and OGT-1 [122].

In *C. elegans*, a genetic interaction has been identified between the fibroblast growth factor LET-756 and OGT-1 and a physical interaction has been identified between LET-756 and 14-3-3. In mammalian cells, biochemical and molecular interactions have been identified between 14-3-3 and OGT-1. However, the mechanism and function of OGT-1’s interaction with FGFs remains elusive. *C. elegans* is an excellent model system to address this question. The FGFs and 14-3-3 proteins are well conserved from *C. elegans* to mammals, but there are fewer FGF and 14-3-3 isoforms in the worm (two FGFs and two 14-3-3s in *C. elegans* vs. 22 and seven, respectively, in mammals) and potentially less redundancy to complicate the analysis. In addition, RNAi can be used to knockdown these genes in adult animals, which allows a whole-organism approach to be used even when the genes are essential for embryonic development. With this simple system and proper genetic tools, *C. elegans* may provide great insight into the novel role of O-GlcNAc in the FGF network.

**TARGETED AND DISCOVERY-DRIVEN APPROACHES TO ELUCIDATE THE ROLES OF O-GlcNAc: FUTURE DIRECTIONS**

The interactions between OGT-1 and the insulin signaling, β-catenin, p38 MAP kinase, and FGF genetic networks in *C. elegans* were identified by both targeted and discovery-driven approaches. These approaches were combined in the SGI screen that identified OGT-1 interactions with BAR-1 and LET-756. RNAi knockdown of 486 genes from linkage group III (discovery-driven) and 372 genes implicated in signal transduction (targeted) was carried out in the context of 11 different *C. elegans* ‘query gene’ mutants chosen to represent different pathways [41]. The *daf-2* query, unlike the *bar-1* and *let-756* queries, did not identify *ogt-1* as a genetic interactor [41], as there was no slow growth phenotype observed in *ogt-1* RNAi in a *daf-2* mutant. However, a targeted test had previously demonstrated that *ogt-1* and *daf-2* genetically interact in the dauer assay [29, 30]. These data suggest that *ogt-1* affects dauer formation, but not fertility, in *daf-2* mutants. The data also underscore that both discovery-driven and targeted approaches, and varied phenotypic outputs, will be necessary to elucidate the diverse functions of O-GlcNAc. In addition to dauer, lifespan, and fertility, there are *C. elegans* assays to evaluate a variety of behaviors, including mating, feeding, learning and memory, locomotion, and chemo- and mechanosensation [120]. Given O-GlcNAc’s modification of many proteins in diverse genetic networks, it seems likely that *ogt-1* and *oga-1* mutations will affect several of these processes. The ease of doing these experiments in *C. elegans* makes a variety of approaches feasible.

Quantitative proteomics is one discovery-driven approach that will be useful for understanding the O-GlcNAc post-translational modification and its role in post-transcriptional regulation. This approach has been used to identify insulin-signaling targets in *C. elegans*. Using 15N-labeled (heavy isotope) wild-type protein as a reference, quantitative mass spectrometry identified 86 proteins that were differentially expressed in *daf-2* mutant worms compared to wild type [121]. This study identified some previously identified DAF-16 targets, like SOD-3, but 51 of the differentially expressed proteins are novel targets of the insulin signaling pathway [121]. In mammalian cells, the quantitative isotope technique has been used in combination with a chemoenzymatic tagging strategy that specifically modifies proteins containing a terminal GlcNAc (QUIC-Tag; [25]). The QUIC-Tag method provides two important sets of data: the identity of O-GlcNAc-modified proteins and their relative abundance in the light vs. heavy samples. Applying the QUIC-Tag method to *C. elegans* would allow the comparison of the O-GlcNAc modification in a variety of genetic backgrounds and environmental conditions (e.g., nutrient stress), and could provide important insights into how this post-translational modification modulates multiple signaling networks.

Chemical genomics using *C. elegans* as a target has also proven to be a useful pathway of discovery for complex signaling cascades. Chemical genetic screens can identify mutants that are hypersensitive or resistant to different compounds [35]. For example, a recent *C. elegans* chemical genetics screen assayed for RNAi clones with increased resistance to the drug parapatraz, which generates reactive oxygen species (ROS). Positive RNAi clones were then tested for effects on lifespan [122]. This screen identified genes and genetic networks previously implicated in the response to ROS (e.g., *daf-2* and other insulin signaling components) as well as novel genes in the TOR (target of rapamycin) nutrient sensing pathway [122], which had not been previously implicated in lifespan regulation. *C. elegans* high-throughput chemical genetic screens are especially useful in determining the mechanism of drug action for drugs with multiple targets. This will be a useful approach for the hexosamine signaling pathway as therapeutics that target OGA-1, OGT-1, or O-GlcNAc-modified proteins must be carefully evaluated to define both the drug target(s) and potential off-target effects.

In addition to discovery-driven approaches, several genetic networks have tantalizing potential roles for O-GlcNAc regulation and are worth pursuing in a targeted approach. Like the hexosamine signaling pathway, the TOR pathway senses nutrients and regulates the insulin-signaling pathway. There are two distinct TOR complexes, TORC1 and TORC2. Akt phosphorylation and activation, which is countered by O-GlcNAc modification [12], activates TORC1 [123]. TORC2, in turn, phosphorylates Akt [124], creating a feedback loop between the insulin signaling and TOR signaling pathways. It will be interesting to see whether O-GlcNAc modification of insulin signaling pathway components affects their ability to activate TORC1 and/or be modified by TORC2.

In *C. elegans*, TORC1 and TORC2, like the hexosamine signaling pathway, are implicated in insulin signaling and metabolism. Mutation of the *C. elegans* TOR homolog *let-363* leads to a dauer-like arrest and an increase in fat stores [125]. Similarly, mutations in the TORC2 component *rict-1/Rictor* lead to increased fat storage and extended lifespan [126, 127]. These phenotypes are similar to those observed in *daf-2* mutants and oppose to the effects of *ogt-1* mutants. In addition, DAF-16 regulates the transcription of the
TORC1 component daf-15/Raptor [125], further connecting insulin and TOR signaling. With the availability of genetic tools and phenotypic assays that integrate TOR’s effects on multiple pathways, C. elegans will be a useful model system for dissecting the role of O-GlcNAc and OGT in TOR’s regulation of metabolism.

In addition to its connection to insulin signaling and role in metabolism, TOR is also connected to the 14-3-3 network. The 14-3-3 proteins act as molecular chaperones by inducing conformational changes, sequestering proteins in the cytoplasm, or acting as a scaffold to bring protein complexes together [128]. One of the proteins that mammalian 14-3-3 sequesters in the cytoplasm in mammalian cells is CRTC2/TORC2, a central factor in the glucagon expression pathway. CRTC2 is O-GlcNAcylated upon glucose stimulation, which results in a disruption of its binding to 14-3-3 [129]. Once unbound from 14-3-3, CRTC2 translocates to the nucleus and binds to promoters of gluconeogenic genes [129]. 14-3-3 also binds β-catenin and FOXO to sequester these proteins in the cytoplasm [130, 131], and the C. elegans 14-3-3 homolog FTT-2 can bind DAF-16 [132]. It is tempting to speculate that, as is the case for CRTC2, O-GlcNAc modification of β-catenin and/or FOXO could influence their binding to 14-3-3 and therefore their nuclear localization and transcriptional programs.

C. elegans will be a useful model system for studying the role of O-GlcNAc modification and 14-3-3 proteins. There are seven 14-3-3 isoforms in mammals but only two (FTT-1/PAR-5 and FTT-2) in C. elegans, simplifying the analysis of this pathway. 14-3-3 proteins are known to be involved in fat metabolism, dauer formation, and the stress response in C. elegans. Like bar-1 and ogt-1 mutants, fit-2 RNAi reduces fat storage in a Nile Red staining assay [133]. fit-2 RNAi enhances dauer formation in a daf-2 mutant [132, 133], which is the opposite of the ogt-1 and daf-2; bar-1 phenotypes. fit-2 RNAi induces the expression of stress response genes, including the DAF-16 dependent sod-3 gene, even in the absence of stress [133]. Similar to a C. elegans daf-2 mutant [134], fit-2 RNAi knockdown [132, 135] causes DAF-16 to preferentially localize to the nucleus, and the expression of DAF-16 targets are upregulated. These data suggest that FTT-2 sequesters DAF-16 in the cytoplasm but that this interaction is weakened or eliminated when insulin signaling is disrupted. If O-GlcNAc generally modulates transcription factor binding to 14-3-3, we would expect ogt-1 mutants, like fit-2 RNAi, to have severe stress response phenotypes.

In addition to binding FOXO, β-catenin, and the TORG regulator TSC2, 14-3-3 also binds to the deacetylase SIR-2.1 in C. elegans [135]. sir-2.1 is a deacetylase that contributes to lifespan regulation in worms, yeast, flies, and mammals, and it is connected to insulin signaling via its interaction with DAF-16/FOXO [136]. The mammalian homolog of SIR-2.1, SirT1, also interacts with FOXO [137, 138]. In C. elegans, 14-3-3 proteins are required for SIR-2.1’s binding to DAF-16 and the lifespan extension and DAF-16 target gene expression observed when sir-2.1 is overexpressed [135, 136]. It is also not yet known whether the O-GlcNAc modification of 14-3-3 or FOXO influences the SIR-FOXO interaction. Because the OGA-1 protein contains an acetyltransferase domain, one possible model is that acetyltransferase activity of OGA-1 counteracts the deacetylase activity of SIR-2.1, resulting in a dynamic cycling of acetylation and O-GlcNAc in the FOXO complex. A targeted approach to elucidate the role (if any) of O-GlcNAc in SIR-2.1 regulation could provide important insight into the mechanism of aging.

**SUMMARY AND CONCLUSION**

O-GlcNAc modifies hundreds of proteins and is implicated in key cellular processes and human diseases. OGT-1 and OGA-1, the enzymes that modulate O-GlcNAcylaton, are essential in mammals and well conserved, but non-essential, in the nematode C. elegans, making the worm a good model system for studying the modification and its functions. Synthetic, physical, and genetic interactions between OGT-1 and the insulin, β-catenin, and p38 MAP kinase networks in C. elegans confirm the connections suggested by biochemical and molecular interactions between the networks in mammalian cells. Furthermore, the known functions of DAF-2, BAR-1, and PMK-1 in C. elegans suggest new roles for OGT-1 and O-GlcNAc in carbohydrate and fat metabolism, fertility, lifespan, the stress response, and pathogen resistance. The genetic interaction between C. elegans OGT-1 and LET-756 suggests a novel role for O-GlcNAc in the FGF pathway. Importantly, studying the modification in the context of a whole organism allows the observation of the consequences of altered O-GlcNAcylation on multiple, connected genetic networks. This integrated view of signaling networks is essential if we want to understand the role of OGT-1 and O-GlcNAc in human disease.

**REFERENCES**


C. elegans Genetic Networks Predict Roles


