



University of Dundee

The role of O-GlcNAcylation in development

Czajewski, Ignacy; van Aalten, Daan M. F.

Published in:
Development

DOI:
[10.1242/dev.201370](https://doi.org/10.1242/dev.201370)

Publication date:
2023

Licence:
CC BY

Document Version
Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

Citation for published version (APA):
Czajewski, I., & van Aalten, D. M. F. (2023). The role of O-GlcNAcylation in development. *Development*, 150(6), Article dev201370. Advance online publication. <https://doi.org/10.1242/dev.201370>

General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

PRIMER

The role of O-GlcNAcylation in development

Ignacy Czajewski¹ and Daan M. F. van Aalten^{1,2,3,*}

ABSTRACT

O-GlcNAcylation is a dynamic post-translational modification performed by two opposing enzymes: O-GlcNAc transferase and O-GlcNAcase. O-GlcNAcylation is generally believed to act as a metabolic integrator in numerous signalling pathways. The stoichiometry of this modification is tightly controlled throughout all stages of development, with both hypo/hyper O-GlcNAcylation resulting in broad defects. In this Primer, we discuss the role of O-GlcNAcylation in developmental processes from stem cell maintenance and differentiation to cell and tissue morphogenesis.

KEY WORDS: O-GlcNAcylation, Glycosylation, Polycomb group proteins, Stem cells, Differentiation

Introduction

Proteins can be post-translationally modified in several different ways, including phosphorylation, ubiquitylation and glycosylation (Khoury et al., 2011; Varki, 2017). O-linked β -N-acetylglucosaminylation (O-GlcNAcylation) is a monoglycosylation that can occur on serine and threonine residues of nucleocytoplasmic proteins (Torres and Hart, 1984; Holt et al., 1987). Like several other forms of glycosylation, O-GlcNAcylation requires uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) as a substrate for the transfer of the sugar moiety onto proteins (Haltiwanger et al., 1990). UDP-GlcNAc is the product of the highly conserved hexosamine biosynthetic pathway (HBP) and its concentrations are generally believed to depend on the levels of various metabolites in the cell, crucially glucose (Fig. 1) (Marshall et al., 2004; Swamy et al., 2016; Chiaradonna et al., 2018), leading to hypotheses about the role of O-GlcNAcylation as a metabolic integrator. However, this may be a tissue-specific effect because recent research has demonstrated that elevated glucose has no effect on UDP-GlcNAc generation in *ex vivo* heart tissue (Olson et al., 2020). Mechanistically, O-GlcNAc transferase (OGT) is the enzyme that installs the sugar moiety on the target protein (Kreppel et al., 1997; Lubas et al., 1997) and the hydrolase O-GlcNAcase (OGA) catalyses its removal (Heckel et al., 1998; Gao et al., 2001). The presence of both an O-GlcNAc ‘writer’ and ‘eraser’ allows the levels of O-GlcNAcylation to rapidly change throughout development (Liu et al., 2012), as well as in response to changing UDP-GlcNAc concentration (Kreppel and Hart, 1999; Mariappa et al., 2011) and stress conditions, such as elevated temperature (Zachara et al., 2004; Radermacher et al., 2014; Mariappa et al., 2018). Modulation of O-GlcNAcylation is, in part,

mediated by post-transcriptional and post-translational regulation of OGT and OGA, although the details of how these mechanisms interplay to control varying O-GlcNAcylation throughout development remain unknown (Whelan et al., 2008; Bullen et al., 2014; Tan et al., 2021). Conversely, an important function of O-GlcNAcylation is the regulation of other post-translational modifications (PTMs), such as ubiquitylation (Ruan et al., 2013; Chen et al., 2021) and phosphorylation (Hart et al., 2011; Bauer et al., 2015), occurring either on or near otherwise modified residues (Kamemura et al., 2002; White et al., 2020), or regulating PTM writers (Dias et al., 2009) and erasers (Liu et al., 2020).

This Primer summarises the current state of knowledge on the role of O-GlcNAcylation in development from loss-of-function studies in various species, highlighting roles in gene regulation and cell signalling. We also discuss emerging themes in this field, including the roles of O-GlcNAcylation in nervous system development and in integrating metabolism with signalling pathways and transcriptional regulation.

O-GlcNAc and loss-of-function studies

O-GlcNAc transferase

In most metazoans, a lack of functional OGT protein is developmentally lethal. This was first observed in *Drosophila*, in which zygotic homozygosity for amorphic alleles of the gene encoding OGT, *super sex combs* (*sxc*), results in lethality at the pupal stage of development (Ingham, 1984; Gambetta et al., 2009; Sinclair et al., 2009). However, in the absence of maternally contributed *sxc* gene product, lethality in *sxc*-null *Drosophila* occurs late in embryonic development or during early larval stages (Ingham, 1984; Gambetta and Müller, 2014). In vertebrates, the importance of OGT is more pronounced. Even in culture, mammalian cells cannot survive in the absence of *Ogt* and the loss of OGT protein is embryonically lethal in mice (Shafi et al., 2000; O’Donnell et al., 2004). Due to this lethality upon global loss of OGT, several groups have attempted to characterise the function of OGT in individual cell types using tissue-specific knockouts. While many conditional knockouts of *Ogt* result in lethality (Cheng et al., 2020; Xiong et al., 2022), this approach has demonstrated the far-ranging roles of the gene in development (Table 1). For example, the excision of *Ogt* in neuronal stem cells results in broad cortical malformations (Cheng et al., 2020), while conditional knockout of the gene in kidney podocytes results in defects in postnatal maturation of these cells (Ono et al., 2017).

It has been recently shown that several individuals with intellectual disability (ID) carry mutations in *OGT*, with family pedigree analysis convincingly indicating the causality of the mutations (Niranjan et al., 2015; Vaidyanathan et al., 2017; Willems et al., 2017; Pravata et al., 2019; Pravata, et al., 2020a). The disorder, termed OGT-linked congenital disorder of glycosylation (OGT-CDG), presents with a range of clinical signs beyond ID that are generally indicative of broad developmental defects, including clinodactyly (curved fingers), short stature and facial dysmorphisms (Pravata et al., 2020b). Several causal *OGT*

¹School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK. ²Institute of Molecular Precision Medicine, Xiangya Hospital, Central South University, Changsha 410000, China. ³Department of Molecular Biology and Genetics, University of Aarhus, Aarhus 8000, Denmark.

*Author for correspondence (daan@mbg.au.dk)

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.

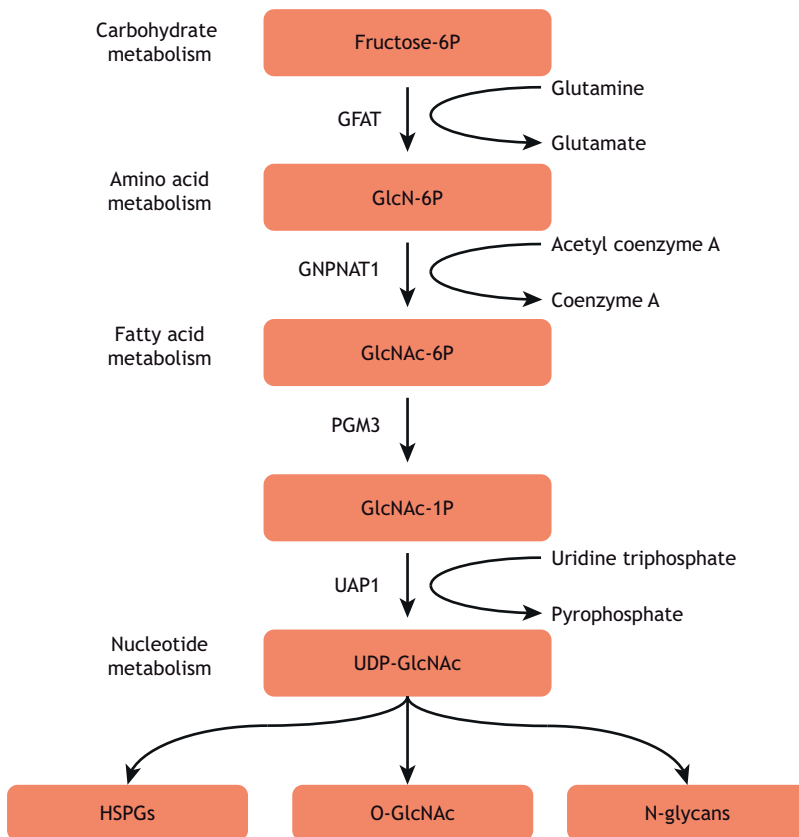


Fig. 1. The hexosamine biosynthetic pathway. The hexosamine biosynthetic pathway is responsible for the generation of UDP-GlcNAc and is highly conserved across metazoans. The first and rate-limiting enzyme of this pathway is glutamine fructose-6-phosphate amidotransferase (GFAT), followed by glucosamine 6-phosphate N-acetyltransferase (GNPNAT1), phosphoglucomutase 3 (PGM3) and UDP-N-acetylhexosamine pyrophosphorylase (UAP1). This pathway uses 2-3% of glucose (Marshall et al., 1991) in the cell and its product UDP-GlcNAc is a crucial substrate for several types of glycosylation, including O-GlcNAcylation, heparan sulphate proteoglycans (HSPGs) and N-glycans.

mutations in ID do not result in measurable decreases in global O-GlcNAcylation in patient-derived fibroblasts (Willems et al., 2017) or when modelled in mouse embryonic stem cells (mESCs) (Pravata et al., 2019), due to compensatory mechanisms, such as OGA downregulation.

O-GlcNAcase

In contrast to OGT deficiency, the lack of OGA appears to be better tolerated by most animals (Table 2). Flies lacking OGA are fully viable and fertile, presenting with only a mild phenotype, such as larger adult size, a semi-penetrant oogenesis defect (Akan et al., 2016, 2021), mild neuronal and behavioural phenotypes (Muha et al., 2020), and increased intestinal stem cell proliferation (Na et al., 2020). Unlike in *Drosophila*, the lack of OGA or its catalytic activity in mice results in broad developmental defects in several organs and pre/perinatal lethality (Table 2) (Yang et al., 2012; Muha et al., 2021). Mice lacking OGA present with reduced body weight as early as embryonic day (E)14.5 (Yang et al., 2012), along with reduced lung alveolar space (Yang et al., 2012), reduced brain size along with a relative increase in ventricle size (Muha et al., 2021), as well as defects in glycogen storage and mobilisation, which are hypothesised to be key contributors to perinatal lethality in the absence of OGA function (Keembiyehetty et al., 2015).

These findings underscore the importance of maintaining appropriate O-GlcNAcylation throughout development, as even slight perturbations to O-GlcNAc homeostasis, as seen in OGT-CDG, can result in pronounced effects. With over 5000 proteins in the human proteome identified as O-GlcNAc modified, some of these modified multiple sites (Wulff-Fuentes et al., 2021); it remains a major challenge to identify functionally important sites and their role in developmental processes.

O-GlcNAcylation in embryonic stem cells

As mentioned above, O-GlcNAcylation is essential from the earliest stages of development and OGT is essential even in mESCs (Shafi et al., 2000). However, beyond being required for general cell viability, O-GlcNAcylation plays an important role in maintaining proliferation and pluripotency (Fig. 2). In mESCs, elevating O-GlcNAcylation by inhibiting OGA delays differentiation, while decreasing O-GlcNAcylation through knockdown of OGT impairs stem cell self-renewal (Jang et al., 2012; Speakman et al., 2014). The effects of O-GlcNAcylation on mESC maintenance appear to occur through the modulation of several pathways, including core pluripotency factors (such as Oct4 and Sox2), auxiliary factors [such as estrogen-related receptor β (ESRRB), phosphokinase PKC ζ and proteasome activator subunit 3 (Psme3)] and epigenetic regulators (Jang et al., 2012; Shi et al., 2013; Miura et al., 2018; Hao et al., 2019; Pecori et al., 2021).

Pluripotency factor targets of O-GlcNAcylation

The effects of O-GlcNAcylation on protein function have largely been revealed by amino acid substitutions of serine/threonine residues that act as O-GlcNAc target sites. For example, alanine mutagenesis of O-GlcNAc sites in the DNA-binding domain of both mouse and human Oct4 (T228A and T235A, respectively) reduces Oct4 transcriptional activity in mouse embryonic fibroblasts and human cancer cells, respectively (Jang et al., 2012; Constable et al., 2017). Evidence that this effect is due to O-GlcNAcylation (and not to other post-translational modifications of Oct4 that occur in the same region) comes from the observation that OGT overexpression increases Oct4 transcriptional activity (Jang et al., 2012; Constable et al., 2017). Similarly, O-GlcNAcylation of serine 25 of ESRRB promotes pluripotency and self-renewal through increasing the stability and transcriptional activity of the protein

Table 1. Summary of O-GlcNAc transferase loss-of-function studies in which O-GlcNAc transferase is either completely absent or its catalytic activity is completely abolished

Species	Genotype (tissue)	Phenotype	Reference
Mouse	α SMA-Cre, <i>Ogt</i> ^{loxP} (smooth muscle)	Postnatal lethality, dilated heart and heart failure	Xiong et al. (2022)
	DAT-Cre, <i>Ogt</i> ^{loxP} (dopaminergic neurons)	Postnatal lethality, reduced weight/food intake and reduced activity	Shao et al. (2022)
	<i>Cyp19</i> -Cre, <i>Ogt</i> ^{loxP} (placenta)	Increased number of β -islets with reduced size after a high-fat diet, reduced weight postnatally, increased corticosterone in males and reduced corticosterone in females	Howerton and Bale (2014); Moore et al. (2021)
	<i>hGFAP</i> -Cre, <i>Ogt</i> ^{loxP} (neural stem cells)	Decreased body and brain weight, post-natal lethality, decreased cortical thickness, and impaired cerebellar development	Cheng et al. (2020); Chen et al. (2022)
	<i>Troponin T</i> -Cre, <i>Ogt</i> ^{loxP} (cardiomyocytes)	Gross morphological heart defects (e.g. small atria and dilated ventricles)	Mu et al. (2020)
	<i>Mlc1f</i> -Cre, <i>Ogt</i> ^{loxP} (skeletal muscle)	Reduced body mass on a high-fat diet	Murata et al. (2018)
	<i>Nphs2</i> -Cre, <i>Ogt</i> ^{loxP} (kidney podocyte)	Impaired post-natal podocyte maturation and proteinuria	Ono et al. (2017)
	<i>Syn1</i> -Cre, <i>Ogt</i> ^{loxP} (neuronal)	Partial embryonic lethality, reduced weight and abnormal locomotor activity	O'Donnell et al. (2004)
	<i>Zp3</i> -Cre, <i>Ogt</i> ^{loxP} (female germ line)	Post-implantation embryonic lethality	Shafi et al. (2000); O'Donnell et al. (2004)
	α MHC-Cre, <i>Ogt</i> ^{loxP} (cardiomyocytes)	Perinatal lethality, increased heart weight and increased ventricular volume	Watson et al. (2014)
<i>Drosophila</i>	<i>sxc</i> ¹ (global knockout)	Lethal homeotic transformations (e.g. ectopic sex combs and Hox gene expression)	Ingham (1984); Gambetta and Müller (2014)
	<i>sxc</i> ^{K872M} (global catalytic inactivity)	Lethality	Mariappa et al. (2015)

(Hao et al., 2019). O-GlcNAcylation also directly modulates protein-protein interactions between Oct4 and ESRRB, as well as other regulators of stemness (Hao et al., 2019). However, in human cells, the effects of O-GlcNAcylation on Oct4 and ESRRB function have primarily been observed in cancer cells. Although additional research in human embryonic stem cells is required to understand the role of O-GlcNAcylation on these two proteins, the effects of O-GlcNAcylation on Oct4 transcriptional activity are not conserved in human embryonic stem cells, casting doubt on the importance of this mechanism in human development (Constable et al., 2017).

Whereas O-GlcNAcylation of Oct4 and ESRRB appears to promote stemness (Jang et al., 2012; Constable et al., 2017; Hao et al., 2019), Sox2 O-GlcNAcylation has mixed effects on maintaining pluripotency and self-renewal, depending on the site modified (Myers et al., 2016; Kim et al., 2021). Sox2 O-GlcNAcylation occurs in the transactivation domain and two neighbouring sites have been the focus of recent studies (Myers et al., 2016; Kim et al., 2021). Alanine mutagenesis of Sox2 threonine 258 increases the expression of early differentiation genes and reduces ectodermal marker genes in mESCs, indicating that threonine 258 O-GlcNAcylation is required for the function of Sox2 in self-renewal and regulation of the expression of ectoderm-lineage genes (Kim et al., 2021). Conversely, O-GlcNAcylation of Sox2 at serine 248 decreases its self-renewal activity and alanine

mutagenesis of this site increases the reprogramming efficiency of somatic cells to induced pluripotent stem cells (Myers et al., 2016). O-GlcNAcylation of Sox2 at serine 248 impairs its interaction with poly ADP-ribose polymerase 1 (Parp1), a protein known to cooperatively regulate stemness, and thus Sox2 genomic binding (Lai et al., 2012). Given the antagonistic effects of O-GlcNAcylation of these two adjacent sites, the regulation of their modification and relative importance during development remains a pertinent question.

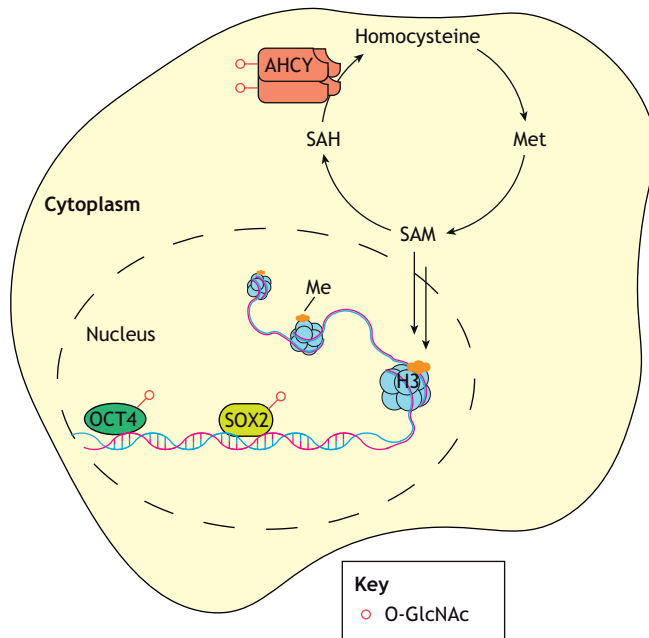
O-GlcNAcylation and epigenetics in pluripotency

In addition to directly controlling transcription factors involved in pluripotency, epigenetic markers required for stem cell maintenance are regulated by O-GlcNAcylation. In particular, DNA and histone methylation are sensitive to OGT activity because methylation modification enzymes and enzymes in the metabolic pathway that generates the most common methyl donor for methylation, S-adenosylmethionine (SAM), are regulated by O-GlcNAcylation (Fig. 2). Depletion of adenosylhomocysteinase (AHCY), a key enzyme in the SAM-generating methionine cycle, reduces the expression of pluripotency markers and increases the expression of differentiation markers through the loss of histone 3 lysine 4 (H3K4) methylation (Zhu et al., 2020). AHCY is O-GlcNAcyated on threonine 136, increasing its catalytic efficiency by almost an order

Table 2. Summary of O-GlcNAc transferase loss-of-function studies in which O-GlcNAc transferase is either completely absent or its catalytic activity is completely abolished

Species	Genotype	Phenotype	Reference
Mouse	<i>Oga</i> KO (global knockout)	Perinatal lethality, reduced embryo size and weight, decreased alveolar space, and decreased placental vascularisation	Keembiyehetty et al. (2015); Yang et al. (2015)
	<i>Oga</i> ^{D285A} (global catalytic inactivity)	Perinatal lethality, reduced body weight and size, and brain ventricle enlargement	Muha et al. (2021)
	<i>Nestin</i> -Cre, <i>Oga</i> ^{loxP} (neural progenitor cells)	Gross brain abnormalities, such as reduced olfactory bulb and pituitary size, as well as enlarged ventricles. Altered neuronal differentiation.	Olivier-Van Stichelen et al. (2017)
<i>Drosophila</i>	<i>Oga</i> ^{KO} (global knockout)	Increased body size, impaired locomotor and habituation function, and overgrowth at the neuromuscular junction	Muha et al. (2020); Akan et al. (2016, 2021)

A O-GlcNAc in wild type



B Loss of O-GlcNAc

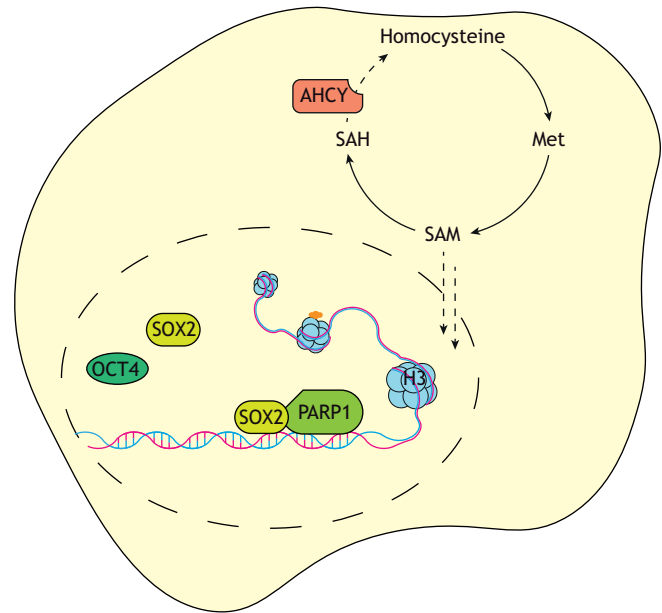


Fig. 2. Role of O-GlcNAcylation in embryonic stem cell maintenance. (A) Mouse embryonic stem cells (mESCs) with normal O-GlcNAcylation (O-GlcNAc) are maintained by a variety of transcriptional regulators. Some of the most important, the Yamanaka factors, can be used to induce pluripotency in terminally differentiated cells. Among these are OCT4 and SOX2, both of which are functionally O-GlcNAc modified. OCT4 transcriptional activity is regulated by O-GlcNAc in both its DNA-binding and transactivation domains, with increased O-GlcNAc promoting transcriptional activity. The effects of O-GlcNAc of SOX2 are more site specific: although both occur in the transactivation domain, SOX2 O-GlcNAcylation can either increase its transcriptional activity or change its DNA occupancy by altering its interactions with other proteins important in maintenance of stemness. Histone (H3) methylation (Me) is also important for perpetuating stemness. A crucial metabolic pathway for maintaining appropriate levels of methylation is the methionine (Met) cycle that produces S-adenosyl methionine (SAM), a donor substrate for methyltransferases. O-GlcNAc plays a crucial role in this cycle by enhancing the activity of adenosylhomocysteinase (AHCY), which converts S-adenosylhomocysteine to homocysteine and adenosine. (B) Loss of O-GlcNAcylation disrupts transcriptional regulation, e.g. by changing SOX2 protein interactions with co-factors such as poly [ADP-ribose] polymerase 1 (PARP1), which results in differentiation.

of magnitude through facilitating AHCY homotetramerization and thereby promoting stem cell maintenance (Zhu et al., 2020). O-GlcNAcylation also regulates the function of histone methyltransferases directly (Chu et al., 2014; Lo et al., 2018; Parween et al., 2022), although this function has been investigated more extensively in the context of differentiation and so is discussed in greater detail later.

In the context of the role of DNA methylation in stem cell maintenance, the interplay between the TET (Ten-eleven translocation) family of proteins and OGT has been a focus of research. TET proteins (TET1-TET3) are key enzymes responsible for demethylating DNA through converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine and in so doing regulate gene expression (Tahiliani et al., 2009). TET1 and TET2 are particularly enriched in mESCs, and a loss of protein function results in earlier differentiation (Ito et al., 2010; Shi et al., 2013). The loss of self-renewal and increased differentiation upon knockdown of TET1 and/or TET2 is likely due to their role in derepression (by hydroxylating 5mC) of core pluripotency factors, primarily Nanog (Ito et al., 2010). One of the first functions ascribed to OGT/TET interactions was the stabilising effect of O-GlcNAcylation on TET1 (Shi et al., 2013); however, more recent research shows a more-complex relationship between OGT and the TET family of proteins. TET1 activity is regulated by its interaction with OGT, with OGT binding-deficient TET1 unable to rescue defects in hematopoietic stem cell production in TET knockout zebrafish (Hrit et al., 2018). The mechanistic details of

how OGT and O-GlcNAcylation affect stability and activity of TET proteins remain to be fully understood, although extensive crosstalk between O-GlcNAcylation and phosphorylation occurs on TET proteins (Bauer et al., 2015). TET proteins have also been found to regulate OGT activity, promoting O-GlcNAcylation of chromatin-associated protein complexes and histones (Chen et al., 2013; Deplus et al., 2013). For example, both TET2 and TET3 promote O-GlcNAcylation of host cell factor 1 (HCF1), which regulates the integrity of the H3K4 methyltransferase complex SET1/COMPASS, as well as its binding to chromatin (Deplus et al., 2013). Although the consequences of this interaction on stem cell maintenance have not been investigated, SET1 is known to promote mESC colony formation, potentially through its interaction with Oct4, enhancing its activity (Fang et al., 2016).

It is important to note that, despite the high degree of conservation of O-GlcNAc sites on the pluripotency factors mentioned here, the phenotypic effects of perturbations in O-GlcNAc addition and removal on pluripotency maintenance in human pluripotent stem cells are milder relative to mouse cells (Maury et al., 2013; Andres et al., 2017). Human embryonic and induced pluripotent stem cells are unperturbed by OGA or OGT manipulation, although increasing or decreasing O-GlcNAcylation does accelerate their rate of differentiation (Maury et al., 2013; Andres et al., 2017). Whether this is a result of broader cellular differences between mouse and human pluripotent stem cells (Schnerch et al., 2010) or of differences in the role of O-GlcNAcylation on target proteins remains to be fully explored.

O-GlcNAcylation in differentiation and cell fate determination

The first function ascribed to OGT (before its molecular characterisation) was its role as a polycomb group (PcG) protein (Ingham, 1984). More recently, the diverse roles of O-GlcNAcylation in differentiation and cell fate determination have become more extensively investigated. The importance of O-GlcNAcylation in neuronal development is particularly pronounced; however, many unanswered questions remain regarding mechanisms regulated by this PTM, across different germ layers.

OGT as a polycomb group gene

Proteins encoded by PcG genes are important transcriptional regulators that prevent ectopic gene expression during development. Notable targets of PcG silencing are the homeotic Hox genes, making PcG proteins central to the maintenance of cell identity in the anterior-posterior axis (Struhl and Akam, 1985; Golbabapour et al., 2013). PcG proteins assemble into histone-modifying complexes that act in concert to maintain gene repression at loci adjacent to genomic regions called polycomb response elements (PREs) (Muller and Bienz, 1991; Horard et al., 2000; Kassis and Brown, 2013). In *Drosophila*, O-GlcNAcylation prevents the aggregation of a stoichiometric component of polycomb repressive complex 1 (PRC1), Polyhomeotic (Ph) (Fig. 3) (Gambetta and Müller, 2014). In the absence of OGT activity, or the serine/threonine-rich domain of Ph that is O-GlcNAc modified, Ph forms aggregates that prevent PRC1-mediated maintenance of Hox gene repression, such as *ultrabithorax* (Ubx) (Gambetta and Müller, 2014). Although it has not been investigated whether this observation translates to vertebrates, human orthologs of Ph (PHC2 and PHC3) also aggregate *in vitro* in the absence of O-GlcNAcylation (Gambetta and Müller, 2014). In human embryonic stem cells, the catalytic subunit of PRC1 (RING1B) is also modified by O-GlcNAc, altering its chromosomal distribution (Maury et al., 2015): O-GlcNAcylated RING1B is enriched at genes implicated in neuronal differentiation and development, relative to

the unmodified protein (Maury et al., 2015). O-GlcNAcylation decreases upon neuronal differentiation of human ESCs (Maury et al., 2013); therefore, the loss of RING1B O-GlcNAcylation may be important for the derepression of genes involved in neuronal differentiation.

In addition to PRC1, early genetic interaction studies proposed a role for OGT, and by extension O-GlcNAcylation, in the function of other PcG complexes. In *Drosophila*, *sxc* amorphic alleles enhance homeotic transformations as a result of mutations in elements of either the polycomb repressive complex 2 (PRC2) or the polycomb repressive-deubiquitylase complex (PR-DUB) (Campbell et al., 1995). Recent research has demonstrated the molecular functions of O-GlcNAcylation in these complexes in mammalian systems. The primary focus has been on the histone methyltransferase subunit enhancer of zeste homologue 2 (EZH2) of PRC2 (Fig. 3). *In vitro*, O-GlcNAcylation increases EZH2 stability, specifically when this protein is not in complex with other PRC2 components, and enhances its histone methyltransferase activity (Chu et al., 2014; Lo et al., 2018). However, *in vivo* research indicates that elevated O-GlcNAcylation can also negatively regulate EZH2 function. In a rat model of maternal hyperglycaemia, a condition leading to elevated embryonic O-GlcNAcylation, EZH2 has increased phosphorylation at threonine 311 (Parween et al., 2022). Phosphorylation at this site hinders the association between EZH2 and SUZ12, another PRC2 component (Wan et al., 2018). The consequent deregulation of PRC2 activity results in misexpression of neurogenic genes, causing elevated neuronal marker expression at earlier stages of development, a phenotype that can be reversed by OGT inhibition (Parween et al., 2022). However, how O-GlcNAcylation positively regulates phosphorylation of EZH2 remains unknown. Threonine 311 of EZH2 is phosphorylated by AMP-activated protein kinase (AMPK) (Wan et al., 2018), which is likely more active when O-GlcNAcylated in specific contexts (Bullen et al., 2014; Xu et al., 2014). However, it remains unknown whether this mechanism is responsible for the phenotypes seen in hyperglycaemic mouse embryos, or whether other mechanisms of O-GlcNAc/phosphorylation crosstalk are involved. The effects of

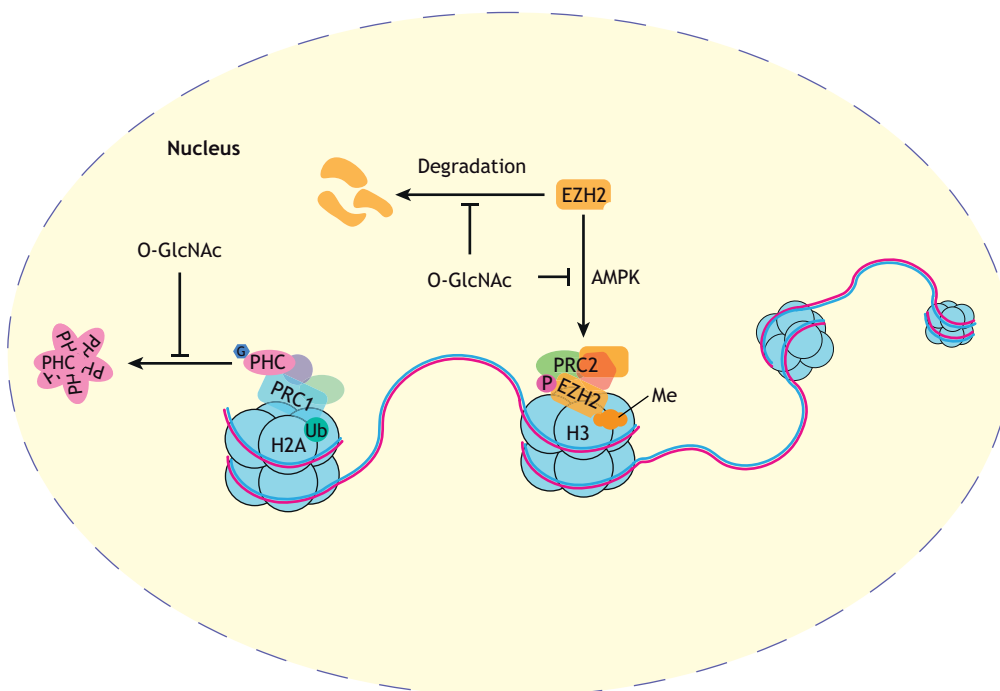


Fig. 3. O-GlcNAcylation in PcG protein function. O-GlcNAcylation (O-GlcNAc) is important for the functioning of several PcG proteins; notably, preventing the aggregation of polyhomeotic (PHC), a component of polycomb repressive complex 1 (PRC1), which catalyses the ubiquitylation (Ub) of histone 2A (H2A). In histone 3 (H3) methylating (Me) polycomb repressive complex 2 (PRC2), O-GlcNAc appears to play an important role in preventing the incorporation of enhancer of zeste homologue 2 (EZH2) into the complex by preventing its phosphorylation by AMP-activated protein kinase (AMPK). However, in addition to this role, when not in complex with other components of PRC2, O-GlcNAc prevents the degradation of EZH2. G, O-GlcNAc; P, phosphorylation.

O-GlcNAcylation on EZH2 may also, in part, explain why human iPSCs acquire neuronal markers earlier in differentiation in the presence of OGA inhibitors (Parween et al., 2017).

The component of the PR-DUB that genetically interacts with *sxc* is Additional sex combs (*Asx*) (Milne et al., 1999). *Asx* is important for the functioning of both PcG and the opposing trithorax group (TrxG) proteins, and, therefore, plays a role in both restricting and promoting the expression of Hox genes (Milne et al., 1999; Li et al., 2017). All the human orthologs of *Asx* are O-GlcNAcylated (Wulff-Fuentes et al., 2021), with O-GlcNAcylation stabilising the ortholog *Asx*-like 1 (ASXL1) (Inoue et al., 2018). The functional significance of the relationship between OGT and ASXL1 remains understudied; however, some evidence indicates that reduced O-GlcNAcylation of ASXL1 results in deficient myeloid differentiation by inhibiting H3K4 methylation (Inoue et al., 2018).

O-GlcNAc in cell fate determination

Several studies have found that the precise regulation of O-GlcNAcylation is required for cells to differentiate into a specific fate. Several cell types, such as cardiomyocytes (Kim et al., 2009), myocytes (Ogawa et al., 2012), keratinocytes (Sohn et al., 2014), neutrophils and erythroid cells (Zhang et al., 2019), require reduced levels of O-GlcNAcylation to acquire their cell fate, while several others, such as osteoblasts (Koyama and Kamemura, 2015), adipocytes (Ishihara et al., 2010) and chondrocytes (Andrés-Bergós et al., 2012), require increased levels of O-GlcNAcylation to differentiate. In some cases, the inability of cells to acquire a specific identity upon O-GlcNAc deregulation may simply reflect the role of O-GlcNAcylation in maintaining stemness. However, important roles for O-GlcNAcylation in the function of cell fate-determining transcription factors have also been identified, such as Sp1 (Yang et al., 2001; Sohn et al., 2014). In keratinocytes, upon differentiation, global levels of O-GlcNAcylation decrease, including O-GlcNAcylation of Sp1. This results in a de-repression of Sp1 transcriptional activity, which increases the expression of genes encoding structural proteins important for the formation of the protective outer layer of the epidermis: the stratum corneum (Sohn et al., 2014).

O-GlcNAc and neural development

The role of O-GlcNAcylation in neuronal differentiation has been of particular interest in recent years due to its relevance to OGT-CDG. An increase in mESC O-GlcNAcylation impairs differentiation towards a neuronal cell fate and mice lacking *Oga* in neuronal precursor cells present with increased numbers of immature neurons (Olivier-Van Stichelen et al., 2017). Conversely, the loss of O-GlcNAcylation in neural stem cells (NSCs) through genetic ablation of *OGT* using Cre-Lox recombination or knockdown with short hairpin RNA (shRNA) results in a depletion of these cells and impairs neurogenesis (Cheng et al., 2020; White et al., 2020; Chen et al., 2021; Shen et al., 2021), although reports of mechanisms causing this phenotype are conflicting. One explanation for impaired neurogenesis in the absence of OGT is via STAT3, a key transcription factor that promotes a glial cell fate (Cao et al., 2010; Hong and Song, 2014). O-GlcNAcylation of STAT3 prevents its phosphorylation on a nearby residue, thereby inhibiting its transcriptional activity (White et al., 2020). Although this effect has primarily been investigated in the context of ageing, it also extends to NSCs isolated from postnatal day (P) 1 mice. More recent research challenges the notion that O-GlcNAcylation impairs differentiation towards a neural fate and suggests that impaired

neurogenesis occurs primarily because of NSC depletion. Specifically, shRNA knockdown of OGT in mouse embryos results in the preferential acquisition of a neuronal cell fate by transfected cells, an effect that can also be observed in human forebrain organoids (Shen et al., 2021). This shift towards a neuronal cell fate is accompanied by a reduction in the number of proliferating NSCs, explaining the reduced total number of differentiated neurons upon loss of OGT. Further supporting this mechanism of impaired neurogenesis, loss of OGT in mouse adult NSCs reduces proliferation and results in increased differentiation towards a neuronal cell fate, at the expense of glial differentiation (Chen et al., 2021). Mechanistically, this shift towards a neuronal cell fate can be explained by reduced levels of either β -catenin or reduced Notch signalling (Chen et al., 2021; Shen et al., 2021). Disentangling how such mechanisms may interact in the developing brain is crucial for the understanding conditions such as OGT-CDG or hyperglycaemia-induced neural tube defects (Kim et al., 2017).

O-GlcNAcylation in cell and tissue morphogenesis

Beyond controlling stem cell proliferation, differentiation and cell identity, OGT activity can also modulate morphogenesis. For example, in mouse embryos from hyperglycaemic dams, defects in neural tube closure can be reversed by OGT inhibition (Kim et al., 2017). In zebrafish, *ogt* overexpression disrupts epiboly and microtubule formation, resulting in thicker shorter microtubules (Webster et al., 2009). However, the mechanistic roles of O-GlcNAcylation in these phenotypes has not been further investigated.

Signalling pathways

More-detailed insights into the role of O-GlcNAcylation in morphogenesis have been gained from research in *Drosophila*, from genetic disruption of components of the HBP. For example, loss of *nesthocker* (*nst*), the *Drosophila* ortholog of phosphoglucomutase 3 (*PGM3*), results in defective cell migration of mesodermal cells during gastrulation and later defective tracheal formation (Mariappa et al., 2011). These defects are a result of altered fibroblast growth factor (FGF) signalling, wherein a crucial adaptor protein, Downstream of FGFR (*Dof*), requires O-GlcNAcylation for appropriate function. This is illustrated by phenotypic rescue of *nst* mutants by overexpression of a chimeric receptor retaining the FGF receptor extracellular domain while the intracellular domain is substituted for a tyrosine kinase that is not dependent on *Dof* for signal transduction. Although mechanistic details of the role of O-GlcNAcylation on *Dof* are not understood, the lack of additional research in this area is likely due to *Dof* not being conserved in mammals (Mariappa et al., 2011).

In addition to FGF signalling, O-GlcNAc is also linked to bone morphogenetic protein (BMP) signalling. In *Drosophila*, excessive activity of the BMP ligand decapentaplegic (*Dpp*) manifests most visibly as a tissue morphogenesis phenotype, with dorsal puckering from excess dorsal movement of epithelial cells. This phenotype is also observed in *sxc* mutant embryos and when the HBP is disrupted, e.g. in *Drosophila* that are homozygous for a hypomorphic allele of the gene *Mummy* (*mmy*) (Moulton et al., 2020). *mmy* is the ortholog of the human gene UDP-N-acetylglucosamine pyrophosphorylase (*UAP1*) (Humphreys et al., 2013), which encodes an enzyme that catalyses the formation of UDP-GlcNAc from N-acetylglucosamine-1-phosphate and UTP – the final step in the HBP (Mio et al., 1998) (Fig. 1). Although proteomics approaches have identified that some elements of the *Dpp* transcriptional activator complex (AP-1) are O-GlcNAc

modified (Selvan et al., 2017), the phenotypes seen in *my* mutants are more likely conveyed through defective O-GlcNAcylation of the type I BMP receptor saxophone (Sax) (Moulton et al., 2020). In the absence of functional OGT, the *Drosophila* SMAD ortholog, Mothers against dpp (Mad), is ectopically phosphorylated by Sax, causing the dorsalising phenotypes seen in *my* and *sxc* mutant embryos. Interestingly, embryos with wild-type *sxc* from mothers fed a sugar-free diet present with a similar phenotype to embryos homozygous for an amorphic allele of *sxc*, demonstrating that the role of O-GlcNAcylation in conveying metabolic signals in development is conserved from *Drosophila* to mammals (Moulton et al., 2020). Whether this particular mechanism is conserved in vertebrates remains to be investigated, although the putatively modified region of Sax is conserved in its human orthologs (Moulton et al., 2020).

Neurite morphology

Another understudied function of O-GlcNAcylation is its role in neurite outgrowth and axonal branching. OGA overexpression in chicken embryonic forebrain neurons is associated with increased primary axon length and the number of neurons entering a branching program (Francisco et al., 2009), whereas knockout of OGT in mouse embryonic cortical neurons reduces their total axonal length (Cheng et al., 2020). The mechanism by which loss of O-GlcNAcylation promotes axon branching is hypothesised to be downstream of protein kinase A (PKA) because PKA activation increases axon branching and this is prevented when OGA is inhibited (Francisco et al., 2009); however, specific conveyors have not been further investigated. O-GlcNAcylation also affects dendrite outgrowth in cultured hippocampal and cortical neurons (Cheng et al., 2020; Shen et al., 2021), with neurite outgrowth of the former rescued by overexpression of β -catenin (Shen et al., 2021), which is reduced in cells lacking OGT. β -Catenin is stabilised by O-GlcNAcylation (Olivier-Van Stichelen et al., 2014); therefore, it is likely that the effects of reduced O-GlcNAcylation on neurite outgrowth are directly mediated by this role of OGT. Similar defects have been observed in models of OGT-CDG. In *Drosophila*, both loss of O-GlcNAcase and mutations in *sxc* that are analogous to those seen in individuals with ID result in disrupted neuromuscular junction formation (Muha et al., 2020; Fenckova et al., 2022), while neurons differentiated from mESCs carrying a patient mutation are characterised by reduced neurite outgrowth (Pravata et al., 2019).

Concluding remarks

In summary, O-GlcNAcylation exerts broad effects on development by modulating a variety of cellular processes, including transcriptional regulation, epigenetics and metabolic pathways. The role of O-GlcNAcylation in these processes is diverse, although key among them is the crosstalk of O-GlcNAcylation with other PTMs. An emerging theme in the role of O-GlcNAc in development is its connection to metabolic states, in part mediated through the HBP (Mariappa et al., 2011; Humphreys et al., 2013; Moulton et al., 2020; Wong et al., 2020). In humans, defective maternal metabolic homeostasis, such as poorly managed pregestational diabetes mellitus, can also affect development (Ornoy et al., 2015). Interestingly, much like mutations in *OGT*, some of the most pronounced clinical manifestations of maternal pregestational diabetes mellitus during development affect the central nervous system, including neural tube defects and autism (Ornoy et al., 2015). Initial studies have shown that maternal hyperglycaemia can negatively impact embryonic development, both through altering embryonic (Kim et al., 2017) and placental O-

GlcNAcylation (Dela Justina et al., 2017, 2018). The study of O-GlcNAcylation in development may lead to crucial discoveries related to the effects of metabolic states on development, particularly relevant in the light of an increase in individuals affected by metabolic disorders (Saklayen, 2018).

Acknowledgements

We thank members of our laboratory for their critical feedback, including Laurin McDowall, Veronica Pravata and Conor Mitchell.

Funding

The authors' research is funded by a Wellcome Trust Investigator Award (110061) and by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (T001682). Open access funding provided by the University of Dundee. Deposited in PMC for immediate release.

References

- Akan, I., Love, D. C., Harwood, K. R., Bond, M. R. and Hanover, J. A. (2016). *Drosophila* O-GlcNAcase deletion globally perturbs chromatin O-GlcNAcylation. *J. Biol. Chem.* **291**, 9906-9919. doi:10.1074/jbc.M115.704783
- Akan, I., Halim, A., Vakhrushev, S. Y., Clausen, H. and Hanover, J. A. (2021). *Drosophila* O-GlcNAcase mutants reveal an expanded glycoproteome and novel growth and longevity phenotypes. *Cells* **10**, 1026. doi:10.3390/cells10051026
- Andrés-Bergós, J., Tardío, L., Larranaga-Vera, A., Gómez, R., Herrero-Beaumont, G. and Largo, R. (2012). The increase in O-linked N-acetylglucosamine protein modification stimulates chondrogenic differentiation both in vitro and in vivo. *J. Biol. Chem.* **287**, 33615-33628. doi:10.1074/jbc.M112.354241
- Andres, L. M., Blong, I. W., Evans, A. C., Rumachik, N. G., Yamaguchi, T., Pham, N. D., Thompson, P., Kohler, J. J. and Bertozzi, C. R. (2017). Chemical modulation of protein O-GlcNAcylation via OGT inhibition promotes human neural cell differentiation. *ACS Chem. Biol.* **12**, 2030-2039. doi:10.1021/acscchembio.7b00232
- Bauer, C., Göbel, K., Nagaraj, N., Colantuoni, C., Wang, M., Müller, U., Kremmer, E., Rottach, A. and Leonhardt, H. (2015). Phosphorylation of TET proteins is regulated via O-GlcNAcylation by the O-Linked N-Acetylglucosamine transferase (OGT). *J. Biol. Chem.* **290**, 4801-4812. doi:10.1074/jbc.M114.605881
- Bullen, J. W., Balsbaugh, J. L., Chanda, D., Shabanowitz, J., Hunt, D. F., Neumann, D. and Hart, G. W. (2014). Cross-talk between two essential nutrient-sensitive enzymes O-GlcNAc Transferase (OGT) and Amp-Activated Protein Kinase (AMPK). *J. Biol. Chem.* **289**, 10592-10606. doi:10.1074/jbc.M113.523068
- Campbell, R. B., Sinclair, D. A. R., Couling, M. and Brock, H. W. (1995). Genetic interactions and dosage effects of Polycomb group genes of *Drosophila*. *Mol. Gen. Genet.* **246**, 291-300. doi:10.1007/BF00288601
- Cao, F., Hata, R., Zhu, P., Nakashiro, K. I. and Sakanaka, M. (2010). Conditional deletion of Stat3 promotes neurogenesis and inhibits astrogliogenesis in neural stem cells. *Biochem. Biophys. Res. Commun.* **394**, 843-847. doi:10.1016/j.bbrc.2010.03.092
- Chen, Q., Chen, Y., Bian, C., Fujiki, R. and Yu, X. (2013). TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* **493**, 561-564. doi:10.1038/nature11742
- Chen, J., Dong, X., Cheng, X., Zhu, Q., Zhang, J., Li, Q., Huang, X., Wang, M., Li, L., Guo, W. et al. (2021). Ogt controls neural stem/progenitor cell pool and adult neurogenesis through modulating Notch signaling. *Cell Rep.* **34**, 108905. doi:10.1016/j.celrep.2021.108905
- Chen, L., Li, Y., Song, Z., Xue, S., Liu, F., Chang, X., Wu, Y., Duan, X. and Wu, H. (2022). O-GlcNAcylation promotes cerebellum development and medulloblastoma oncogenesis via SHH signaling. *Proc. Natl. Acad. Sci. USA* **119**, e2202821119. doi:10.1073/pnas.2202821119
- Cheng, J., Wu, Y., Chen, L., Li, Y., Liu, F., Shao, J., Huang, M., Fan, M. and Wu, H. (2020). Loss of O-GlcNAc transferase in neural stem cells impairs corticogenesis. *Biochem. Biophys. Res. Commun.* **532**, 541-547. doi:10.1016/j.bbrc.2020.08.084
- Chiaradonna, F., Ricciardiello, F. and Palorini, R. (2018). The nutrient-sensing hexosamine biosynthetic pathway as the hub of cancer metabolic rewiring. *Cells* **7**, 53. doi:10.3390/cells7060053
- Chu, C. S., Lo, P. W., Yeh, Y. H., Hsu, P. H., Peng, S. H., Teng, Y. C., Kang, M. L., Wong, C. H. and Juan, L. J. (2014). O-GlcNAcylation regulates EZH2 protein stability and function. *Proc. Natl. Acad. Sci. USA* **111**, 1355-1360. doi:10.1073/pnas.1323226111
- Constable, S., Lim, J. M., Vaidyanathan, K. and Wells, L. (2017). O-GlcNAc transferase regulates transcriptional activity of human Oct4. *Glycobiology* **27**, 927-937. doi:10.1093/glycob/cwx055
- Dela Justina, V., Gonçalves, J. S., De Freitas, R. A., Fonseca, A. D., Volpato, G. T., Tostes, R. C., Carneiro, F. S., Lima, V. V. and Giachini, F. R. (2017). Increased O-linked N-acetylglucosamine modification of NF- κ B and augmented cytokine production in the placentas from hyperglycemic rats. *Inflammation* **40**, 1773-1781. doi:10.1007/s10753-017-0620-7

- Dela Justina, V., Dos Passos Junior, R. R., Bressan, A. F., Tostes, R. C., Carneiro, F. S., Soares, T. S., Volpato, G. T., Lima, V. V., Martin, S. S. and Giachini, F. R. (2018). O-linked N-acetyl-glucosamine deposition in placental proteins varies according to maternal glycemic levels. *Life Sci.* **205**, 18-25. doi:10.1016/j.lfs.2018.05.013
- Deplus, R., Delatte, B., Schwinn, M. K., Defrance, M., Méndez, J., Murphy, N., Dawson, M. A., Volkmar, M., Putmans, P., Calonne, E., et al. (2013). TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. *EMBO J.* **32**, 645-655. doi:10.1038/emboj.2012.357
- Dias, W. B., Cheung, W. D., Wang, Z. and Hart, G. W. (2009). Regulation of calcium/calmodulin-dependent kinase IV by O-GlcNAc modification. *J. Biol. Chem.* **284**, 21327-21337. doi:10.1074/jbc.M109.007310
- Fang, L., Zhang, J., Zhang, H., Yang, X., Jin, X., Zhang, L., Skalnik, D. G., Jin, Y., Zhang, Y., Huang, X., et al. (2016). H3K4 methyltransferase Set1a is a key Oct4 coactivator essential for generation of Oct4 positive inner cell mass. *Stem Cells* **34**, 565-580. doi:10.1002/stem.2250
- Fenckova, M., Muha, V., Mariappa, D., Catinozzi, M., Czajewski, I., Blok, L. E. R., Ferenbach, A. T., Storkebaum, E., Schenck, A. and Van Aalten, D. M. F. (2022). Intellectual disability-associated disruption of O-GlcNAc cycling impairs habituation learning in *Drosophila*. *PLOS Genet.* **18**, e1010159. doi:10.1371/journal.pgen.1010159
- Francisco, H., Kollins, K., Varghis, N., Voadlo, D., Vosseller, K. and Gallo, G. (2009). O-GlcNAc post-translational modifications regulate the entry of neurons into an axon branching program. *Dev. Neurobiol.* **69**, 162-173. doi:10.1002/dneu.20695
- Gambetta, M. C. and Müller, J. (2014). O-GlcNAcylation prevents aggregation of the polycomb group repressor polyhomeotic. *Dev. Cell* **31**, 629-639. doi:10.1016/j.devcel.2014.10.020
- Gambetta, M. C., Oktaba, K. and Müller, J. (2009). Essential role of the glycosyltransferase Sxc/Ogt in polycomb repression. *Science* **325**, 93-96. doi:10.1126/science.1169727
- Gao, Y., Wells, L., Comer, F. I., Parker, G. J. and Hart, G. W. (2001). Dynamic O-glycosylation of nuclear and cytosolic proteins: cloning and characterization of a neutral, cytosolic beta-N-acetylglucosaminidase from human brain. *J. Biol. Chem.* **276**, 9838-9845. doi:10.1074/jbc.M010420200
- Golbabapour, S., Majid, N. A., Hassandarvish, P., Hajrezaie, M., Abdulla, M. A. and Hadi, A. H. A. (2013). Gene silencing and polycomb group proteins: An overview of their structure, mechanisms and phylogenetics. *OMICs* **17**, 283-296. doi:10.1089/omi.2012.0105
- Haltiwanger, R. S., Holt, G. D. and Hart, G. W. (1990). Enzymatic addition of O-GlcNAc to nuclear and cytoplasmic proteins. Identification of a uridine diphospho-N-acetylglucosamine:peptide beta-N-acetylglucosaminyltransferase. *J. Biol. Chem.* **265**, 2563-2568. doi:10.1016/S0021-9258(19)39838-2
- Hao, Y., Fan, X., Shi, Y., Zhang, C., Sun, D. e, Qin, K., Qin, W., Zhou, W. and Chen, X. (2019). Next-generation unnatural monosaccharides reveal that ESRRB O-GlcNAcylation regulates pluripotency of mouse embryonic stem cells. *Nat. Commun.* **10**, 4065. doi:10.1038/s41467-018-07882-8
- Hart, G. W., Slawson, C., Ramirez-Correa, G. and Lagerlof, O. (2011). Cross Talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease. *Annu. Rev. Biochem.* **80**, 825-858. doi:10.1146/annurev-biochem-060608-102511
- Heckel, D., Comtesse, N., Brass, N., Blin, N., Zang, K. D. and Meese, E. (1998). Novel immunogenic antigen homologous to hyaluronidase in meningioma. *Hum. Mol. Genet.* **7**, 1859-1872. doi:10.1093/hmg/7.12.1859
- Holt, G. D., Snow, C. M., Senior, A., Haltiwanger, R. S., Gerace, L. and Hart, G. W. (1987). Nuclear pore complex glycoproteins contain cytoplasmically disposed O-linked N-acetylglucosamine. *J. Cell Biol.* **104**, 1157-1164. doi:10.1083/jcb.104.5.1157
- Hong, S. and Song, M. R. (2014). STAT3 but not STAT1 is required for astrocyte differentiation. *PLoS One* **9**, e86851. doi:10.1371/journal.pone.0086851
- Horard, B., Tatout, C., Poux, S. and Pirrotta, V. (2000). Structure of a Polycomb Response Element and in vitro binding of polycomb group complexes containing GAGA factor. *Mol. Cell. Biol.* **20**, 3187-3197. doi:10.1128/mcb.20.9.3187-3197.2000
- Howerton, C. L. and Bale, T. L. (2014). Targeted placental deletion of OGT recapitulates the prenatal stress phenotype including hypothalamic mitochondrial dysfunction. *Proc. Natl. Acad. Sci. USA* **111**, 9639-9644. doi:10.1073/pnas.1401203111
- Hrit, J., Goodrich, L., Li, C., Wang, B. A., Nie, J., Cui, X., Martin, E. A., Simental, E., Fernandez, J., Liu, M. Y., et al. (2018). OGT binds a conserved C-terminal domain of TET1 to regulate TET1 activity and function in development. *eLife* **7**, e34870. doi:10.7554/eLife.34870
- Humphreys, G. B., Jud, M. C., Monroe, K. M., Kimball, S. S., Higley, M., Shipley, D., Vrablik, M. C., Bates, K. L. and Letsou, A. (2013). Mummy, A UDP-N-acetylglucosamine pyrophosphorylase, modulates DPP signaling in the embryonic epidermis of *Drosophila*. *Dev. Biol.* **381**, 434-445. doi:10.1016/j.ydbio.2013.06.006
- Ingham, P. W. (1984). A gene that regulates the bithorax complex differentially in larval and adult cells of *Drosophila*. *Cell* **37**, 815-823. doi:10.1016/0092-8674(84)90416-1
- Inoue, D., Fujino, T., Sheridan, P., Zhang, Y. Z., Nagase, R., Horikawa, S., Li, Z., Matsui, H., Kanai, A., Saika, M., et al. (2018). A novel ASXL1-OGT axis plays roles in H3K4 methylation and tumor suppression in myeloid malignancies. *Leukemia* **32**, 1327-1337. doi:10.1038/s41375-018-0083-3
- Ishihara, K., Takahashi, I., Tsuchiya, Y., Hasegawa, M. and Kamemura, K. (2010). Characteristic increase in nucleocytoplasmic protein glycosylation by O-GlcNAc in 3T3-L1 adipocyte differentiation. *Biochem. Biophys. Res. Commun.* **398**, 489-494. doi:10.1016/j.bbrc.2010.06.105
- Ito, S., D'Alessio, A. C., Taranova, O. V., Hong, K., Sowers, L. C. and Zhang, Y. (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* **466**, 1129-1133. doi:10.1038/nature09303
- Jang, H., Kim, T. W., Yoon, S., Choi, S.-Y., Kang, T.-W., Kim, S.-Y., Kwon, Y.-W., Cho, E.-J. and Youn, H.-D. (2012). O-GlcNAc regulates pluripotency and reprogramming by directly acting on core components of the pluripotency network. *Cell Stem Cell* **11**, 62-74. doi:10.1016/j.stem.2012.03.001
- Kamemura, K., Hayes, B. K., Comer, F. I. and Hart, G. W. (2002). Dynamic interplay between O-glycosylation and O-phosphorylation of nucleocytoplasmic proteins: alternative glycosylation/phosphorylation of Thr-58, a known mutational hot spot of c-Myc in lymphomas, is regulated by mitogens. *J. Biol. Chem.* **277**, 19229-19235. doi:10.1074/jbc.M201729200
- Kassis, J. A. and Brown, J. L. (2013). Polycomb Group Response Elements in *Drosophila* and vertebrates. *Adv. Genet.* **81**, 83-118. doi:10.1016/B978-0-12-407677-8.00003-8
- Keembiyehetty, C., Love, D. C., Harwood, K. R., Gavrilova, O., Comly, M. E. and Hanover, J. A. (2015). Conditional knock-out reveals a requirement for O-Linked N-Acetylglucosaminase (O-GlcNAcase) in metabolic homeostasis. *J. Biol. Chem.* **290**, 7097-7113. doi:10.1074/jbc.M114.617779
- Khoury, G. A., Baliban, R. C. and Floudas, C. A. (2011). Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. *Sci. Rep.* **1**, 90. doi:10.1038/srep00090
- Kim, H. S., Park, S. Y., Choi, Y. R., Kang, J. G., Joo, H. J., Moon, W. K. and Cho, J. W. (2009). Excessive O-GlcNAcylation of proteins suppresses spontaneous cardiogenesis in ES cells. *FEBS Lett.* **583**, 2474-2478. doi:10.1016/j.febslet.2009.06.052
- Kim, G., Cao, L., Reece, E. A. and Zhao, Z. (2017). Impact of protein O-GlcNAcylation on neural tube malformation in diabetic embryopathy. *Sci. Rep.* **7**, 11107. doi:10.1038/s41598-017-11655-6
- Kim, D. K., Lee, J. S., Lee, E. Y., Jang, H., Han, S., Kim, H. Y., Hwang, I. Y., Choi, J. W., Shin, H. M., You, H. J., et al. (2021). O-GlcNAcylation of Sox2 at threonine 258 regulates the self-renewal and early cell fate of embryonic stem cells. *Exp. Mol. Med.* **53**, 1759-1768. doi:10.1038/s12276-021-00707-7
- Koyama, T. and Kamemura, K. (2015). Global increase in O-linked N-acetylglucosamine modification promotes osteoblast differentiation. *Exp. Cell Res.* **338**, 194-202. doi:10.1016/j.yexcr.2015.08.009
- Kreppel, L. K. and Hart, G. W. (1999). Regulation of a cytosolic and nuclear O-GlcNAc transferase. Role of the tetratricopeptide repeats. *J. Biol. Chem.* **274**, 32015-32022. doi:10.1074/jbc.274.45.32015
- Kreppel, L. K., Blomberg, M. A. and Hart, G. W. (1997). Dynamic glycosylation of nuclear and cytosolic proteins: cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. *J. Biol. Chem.* **272**, 9308-9315. doi:10.1074/jbc.272.14.9308
- Lai, Y. S., Chang, C. W., Pawlik, K. M., Zhou, D., Renfrow, M. B. and Townes, T. M. (2012). SRY (sex determining region Y)-box2 (Sox2)/poly ADP-ribose polymerase 1 (Parp1) complexes regulate pluripotency. *Proc. Natl. Acad. Sci. USA* **109**, 3772-3777. doi:10.1073/pnas.1108595109
- Li, T., Hodgson, J. W., Petruk, S., Mazo, A. and Brock, H. W. (2017). Additional sex combs interacts with enhancer of zeste and trithorax and modulates levels of trimethylation on histone H3K4 and H3K27 during transcription of hsp70. *Epigenetics & Chromatin* **10**, 43. doi:10.1186/s13072-017-0151-3
- Liu, Y., Li, X., Yu, Y., Shi, J., Liang, Z., Run, X., Li, Y., Dai, C., Grundke-Iqbal, I., Iqbal, K., et al. (2012). Developmental regulation of protein O-GlcNAcylation, O-GlcNAc transferase, and O-GlcNAcase in mammalian brain. *PLoS One* **7**, e43724. doi:10.1371/journal.pone.0043724
- Liu, C., Shi, Y., Li, J., Liu, X., Xiahou, Z., Tan, Z., Chen, X. and Li, J. (2020). O-GlcNAcylation of myosin phosphatase targeting subunit 1 (MYPT1) dictates timely disjunction of centrosomes. *J. Biol. Chem.* **295**, 7341-7349. doi:10.1074/jbc.RA119.012401
- Lo, P. W., Shie, J. J., Chen, C. H., Wu, C. Y., Hsu, T. L. and Wong, C. H. (2018). O-GlcNAcylation regulates the stability and enzymatic activity of the histone methyltransferase EZH2. *Proc. Natl. Acad. Sci. USA* **115**, 7302-7307. doi:10.1073/pnas.1801850115
- Lubas, W. A., Frank, D. W., Krause, M. and Hanover, J. A. (1997). O-linked GlcNAc transferase is a conserved nucleocytoplasmic protein containing tetratricopeptide repeats. *J. Biol. Chem.* **272**, 9316-9324. doi:10.1074/jbc.272.14.9316
- Mariappa, D., Sauer, K., Mariño, K., Turnock, D., Webster, R., Van Aalten, D. M. F., Ferguson, M. A. J. J., Müller, H. A. J., Marino, K., Turnock, D., et al. (2011). Protein O-GlcNAcylation is required for fibroblast growth factor signaling in *Drosophila*. *Sci. Signal.* **4**, ra89. doi:10.1126/scisignal.2002335

- Mariappa, D., Zheng, X., Schimpl, M., Raimi, O., Ferenbach, A. T., Müller, H. A. J. and Van Aalten, D. M. F. (2015). Dual functionality of O-GlcNAc transferase is required for *Drosophila* development. *Open Biol.* **5**, 150234. doi:10.1098/rsob.150234
- Mariappa, D., Ferenbach, A. T. and Van Aalten, D. M. F. (2018). Effects of hypo-O-GlcNAcylation on *Drosophila* development. *J. Biol. Chem.* **293**, 7209-7221. doi:10.1074/jbc.RA118.002580
- Marshall, S., Bacote, V. and Traxinger, R. R. (1991). Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system: role of hexosamine in the induction of insulin resistance. *J. Biol. Chem.* **266**, 4706-4712. doi:10.1016/S0021-9258(19)67706-9
- Marshall, S., Nadeau, O. and Yamasaki, K. (2004). Dynamic actions of glucose and hexosamine on hexosamine biosynthesis in isolated adipocytes: differential effects on glucosamine 6-phosphate, UDP-N-acetylglucosamine, and ATP levels. *J. Biol. Chem.* **279**, 35313-35319. doi:10.1074/jbc.M404133.200
- Maury, J. J. P., Chan, K. K. K., Zheng, L., Bardor, M. and Choo, A. B. H. (2013). Excess of O-linked N-acetylglucosamine modifies human pluripotent stem cell differentiation. *Stem Cell Res.* **11**, 926-937. doi:10.1016/j.scr.2013.06.004
- Maury, J. J. P., El Farran, C. A., Ng, D., Loh, Y. H., Bi, X., Bardor, M. and Choo, A. B. H. (2015). RING1B O-GlcNAcylation regulates gene targeting of polycomb repressive complex 1 in human embryonic stem cells. *Stem Cell Res.* **15**, 182-189. doi:10.1016/j.scr.2015.06.007
- Milne, T. A., Sinclair, D. A. R. and Brock, H. W. (1999). The Additional sex combs gene of *Drosophila* is required for activation and repression of homeotic loci, and interacts specifically with Polycomb and super sex combs. *Mol. Gen. Genet.* **261**, 753-761. doi:10.1007/s004380050018
- Mio, T., Yabe, T., Arisawa, M. and Yamada-Okabe, H. (1998). The eukaryotic UDP-N-acetylglucosamine pyrophosphorylases: gene cloning, protein expression, and catalytic mechanism. *J. Biol. Chem.* **273**, 14392-14397. doi:10.1074/jbc.273.23.14392
- Miura, T., Kume, M., Kawamura, T., Yamamoto, K., Hamakubo, T. and Nishihara, S. (2018). O-GlcNAc on PKC ζ Inhibits the FGF4-PKC ζ -MEK-ERK1/2 pathway via inhibition of PKC ζ phosphorylation in mouse embryonic stem cells. *Stem Cell Rep.* **10**, 272-286. doi:10.1016/j.stemcr.2017.11.007
- Moore, M., Avula, N., Jo, S., Beetch, M. and Alejandro, E. U. (2021). Disruption of O-linked N-acetylglucosamine signaling in placenta induces insulin sensitivity in female offspring. *Int. J. Mol. Sci.* **22**, 6918. doi:10.3390/ijms22136918
- Moulton, M. J., Humphreys, G. B., Kim, A. and Letsou, A. (2020). O-GlcNAcylation dampens Dpp/BMP signaling to ensure proper *Drosophila* embryonic development. *Dev. Cell* **53**, 330-343.e3. doi:10.1016/j.devcel.2020.04.001
- Mu, Y., Yu, H., Wu, T., Zhang, J., Evans, S. M. and Chen, J. (2020). O-linked β -N-acetylglucosamine transferase plays an essential role in heart development through regulating angiopoietin-1. *PLoS Genet.* **16**, e1008730. doi:10.1371/journal.pgen.1008730
- Muha, V., Fenckova, M., Ferenbach, A. T., Catinozzi, M., Eidhof, I., Storkebaum, E., Schenck, A. and Van Aalten, D. M. F. (2020). O-GlcNAcase contributes to cognitive function in *Drosophila*. *J. Biol. Chem.* **295**, 8636-8646. doi:10.1074/jbc.RA119.010312
- Muha, V., Authier, F., Szoke-Kovacs, Z., Johnson, S., Gallagher, J., Mcneilly, A., Mccrimmon, R. J., Teboul, L. and Van Aalten, D. M. F. (2021). Loss of O-GlcNAcase catalytic activity leads to defects in mouse embryogenesis. *J. Biol. Chem.* **296**, 100439. doi:10.1016/j.jbc.2021.100439
- Muller, J. and Bienz, M. (1991). Long range repression conferring boundaries of Ultrabithorax expression in the *Drosophila* embryo. *EMBO J.* **10**, 3147-3155. doi:10.1002/j.1460-2075.1991.tb04876.x
- Murata, K., Morino, K., Ida, S., Ohashi, N., Lemecha, M., Park, S. Y., Ishikado, A., Kume, S., Choi, C. S., Sekine, O., et al. (2018). Lack of O-GlcNAcylation enhances exercise-dependent glucose utilization potentially through AMP-activated protein kinase activation in skeletal muscle. *Biochem. Biophys. Res. Commun.* **495**, 2098-2104. doi:10.1016/j.bbrc.2017.12.081
- Myers, S. A., Peddada, S., Chatterjee, N., Friedrich, T., Tomoda, K., Krings, G., Thomas, S., Maynard, J., Broecker, M., Thomson, M., et al. (2016). SOX2 O-GlcNAcylation alters its protein-protein interactions and genomic occupancy to modulate gene expression in pluripotent cells. *eLife* **5**, e10647. doi:10.7554/eLife.10647
- Na, H. J., Akan, I., Abramowitz, L. K. and Hanover, J. A. (2020). Nutrient-driven O-GlcNAcylation controls DNA damage repair signaling and stem/progenitor cell homeostasis. *Cell Rep.* **31**, 107632. doi:10.1016/j.celrep.2020.107632
- Niranjan, T. S., Skinner, C., May, M., Turner, T., Rose, R., Stevenson, R., Schwartz, C. E. and Wang, T. (2015). Affected kindred analysis of human X chromosome exomes to identify novel X-linked intellectual disability genes. *PLoS One* **10**, e0116454. doi:10.1371/journal.pone.0116454
- O'Donnell, N., Zachara, N. E., Hart, G. W. and Marth, J. D. (2004). Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. *Mol. Cell. Biol.* **24**, 1680-1690. doi:10.1128/MCB.24.4.1680-1690.2004
- Ogawa, M., Mizofuchi, H., Kobayashi, Y., Tsuzuki, G., Yamamoto, M., Wada, S. and Kamemura, K. (2012). Terminal differentiation program of skeletal myogenesis is negatively regulated by O-GlcNAc glycosylation. *Biochim. Biophys. Acta Gen. Subj.* **1820**, 24-32. doi:10.1016/j.bbagen.2011.10.011
- Olivier-Van Stichelen, S., Dehennaut, V., Buzy, A., Zachayus, J.-L., Guinez, C., Mir, A.-M., El Yazidi-Belkoura, I., Copin, M.-C., Boureme, D., Loyalux, D., et al. (2014). O-GlcNAcylation stabilizes β -catenin through direct competition with phosphorylation at threonine 41. *FASEB J.* **28**, 3325-3338. doi:10.1096/fj.13-243535
- Olivier-Van Stichelen, S., Wang, P., Comly, M., Love, D. C. and Hanover, J. A. (2017). Nutrient-driven O-linked N-acetylglucosamine (O-GlcNAc) cycling impacts neurodevelopmental timing and metabolism. *J. Biol. Chem.* **292**, 6076-6085. doi:10.1074/jbc.M116.774042
- Olson, A. K., Bouchard, B., Zhu, W. Z., Chatham, J. C. and Des Rosiers, C. (2020). First characterization of glucose flux through the hexosamine biosynthesis pathway (HBP) in ex vivo mouse heart. *J. Biol. Chem.* **295**, 2018-2033. doi:10.1074/jbc.RA119.010565
- Ono, S., Kume, S., Yasuda-Yamahara, M., Yamahara, K., Takeda, N., Chin-Kanasaki, M., Araki, H., Sekine, O., Yokoi, H., Mukoyama, M., et al. (2017). O-linked β -N-acetylglucosamine modification of proteins is essential for foot process maturation and survival in podocytes. *Nephrol. Dial. Transplant.* **32**, 1477-1487. doi:10.1093/ndt/gfw463
- Ornoy, A., Reece, E. A., Pavlinkova, G., Kappen, C. and Miller, R. K. (2015). Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. *Birth Defects Res.* **105**, 53-72. doi:10.1002/bdrc.21090
- Parween, S., Varghese, D. S., Ardah, M. T., Prabakaran, A. D., Mensah-Brown, E., Emerald, B. S. and Ansari, S. A. (2017). Higher O-GlcNAc levels are associated with defects in progenitor proliferation and premature neuronal differentiation during *in-vitro* human embryonic cortical neurogenesis. *Front. Cell. Neurosci.* **11**, 415. doi:10.3389/fncel.2017.00415
- Parween, S., Alawathugoda, T. T., Prabakaran, A. D., Dheen, S. T., Morse, R. H., Emerald, B. S. and Ansari, S. A. (2022). Nutrient sensitive protein O-GlcNAcylation modulates the transcriptome through epigenetic mechanisms during embryonic neurogenesis. *Life Sci. Alliance* **5**, e202201385. doi:10.26508/lsa.202201385
- Pecori, F., Kondo, N., Ogura, C., Miura, T., Kume, M., Minamijima, Y., Yamamoto, K. and Nishihara, S. (2021). Site-specific O-GlcNAcylation of Psme3 maintains mouse stem cell pluripotency by impairing P-body homeostasis. *Cell Rep.* **36**, 109361. doi:10.1016/j.celrep.2021.109361
- Pravata, V. M., Muha, V., Gundogdu, M., Ferenbach, A. T., Kakade, P. S., Vandadi, V., Wilmes, A. C., Borodkin, V. S., Joss, S., Stavridis, M. P., et al. (2019). Catalytic deficiency of O-GlcNAc transferase leads to X-linked intellectual disability. *Proc. Natl. Acad. Sci. USA* **116**, 14961-14970. doi:10.1073/pnas.1900065116
- Pravata, V. M., Gundogdu, M., Bartual, S. G., Ferenbach, A. T., Stavridis, M., Ünnap, K., Pajusalu, S., Žordania, R., Wojcik, M. H. and Van Aalten, D. M. F. (2020a). A missense mutation in the catalytic domain of O-GlcNAc transferase links perturbations in protein O-GlcNAcylation to X-linked intellectual disability. *FEBS Lett.* **594**, 717-727. doi:10.1002/1873-3468.13640
- Pravata, V. M., Omelková, M., Stavridis, M. P., Desbiers, C. M., Stephen, H. M., Lefebvre, D. J., Gez, J., Gundogdu, M., Ünnap, K., Joss, S., et al. (2020b). An intellectual disability syndrome with single-nucleotide variants in O-GlcNAc transferase. *Eur. J. Hum. Genet.* **28**, 706-714. doi:10.1038/s41431-020-0589-9
- Radermacher, P. T., Myachina, F., Bosshardt, F., Pandey, R., Mariappa, D., Müller, H.-A. J. and Lehner, C. F. (2014). O-GlcNAc reports ambient temperature and confers heat resistance on ectotherm development. *Proc. Natl. Acad. Sci. USA* **111**, 5592-5597. doi:10.1073/pnas.1322396111
- Ruan, H. B., Nie, Y. and Yang, X. (2013). Regulation of protein degradation by O-GlcNAcylation: crosstalk with ubiquitination. *Mol. Cell. Proteomics* **12**, 3489-3497. doi:10.1074/mcp.R113.029751
- Saklayen, M. G. (2018). The global epidemic of the metabolic syndrome. *Curr. Hypertens. Rep.* **20**, 12. doi:10.1007/s11906-018-0812-z
- Schnerch, A., Cerdan, C. and Bhatia, M. (2010). Distinguishing between mouse and human pluripotent stem cell regulation: the best laid plans of mice and men. *Stem Cells* **28**, 419-430. doi:10.1002/stem.298
- Selvan, N., Williamson, R., Mariappa, D., Campbell, D. G., Gourlay, R., Ferenbach, A. T., Aristotelous, T., Hopkins-Navratilova, I., Trost, M. and Van Aalten, D. M. F. (2017). A mutant O-GlcNAcase enriches *Drosophila* developmental regulators. *Nat. Chem. Biol.* **13**, 882-887. doi:10.1038/nchembio.2404
- Shafi, R., Iyer, S. P. N., Ellies, L. G., O'Donnell, N., Marek, K. W., Chui, D., Hart, G. W. and Marth, J. D. (2000). The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. *Proc. Natl. Acad. Sci. USA* **97**, 5735-5739. doi:10.1073/pnas.100471497
- Shao, M. S., Yang, X., Zhang, C. C., Jiang, C. Y., Mao, Y., Xu, W. D., Ma, L. and Wang, F. F. (2022). O-GlcNAcylation in ventral tegmental area dopaminergic neurons regulates motor learning and the response to natural reward. *Neurosci. Bull.* **38**, 263-274. doi:10.1007/s12264-021-00776-8
- Shen, H., Zhao, X., Chen, J., Qu, W., Huang, X., Wang, M., Shao, Z., Shu, Q. and Li, X. (2021). O-GlcNAc transferase Ogt regulates embryonic neuronal

- development through modulating Wnt/ β -catenin signaling. *Hum. Mol. Genet.* **31**, 57-68. doi:10.1093/hmg/ddab223
- Shi, F. T., Kim, H., Lu, W., He, Q., Liu, D., Goodell, M. A., Wan, M. and Songyang, Z. (2013). Ten-eleven translocation 1 (Tet1) is regulated by o-linked n-acetylglucosamine transferase (ogt) for target gene repression in mouse embryonic stem cells. *J. Biol. Chem.* **288**, 20776-20784. doi:10.1074/jbc.M113.460386
- Sinclair, D. A. R., Syrzycka, M., Macauley, M. S., Rastgardani, T., Komljenovic, I., Vocadlo, D. J., Brock, H. W. and Honda, B. M. (2009). Drosophila O-GlcNAc transferase (OGT) is encoded by the Polycomb group (PcG) gene, super sex combs (sxc). *Proc. Natl. Acad. Sci. USA* **106**, 13427-13432. doi:10.1073/pnas.0904638106
- Sohn, K. C., Lee, E. J., Shin, J. M., Lim, E. H., No, Y., Lee, J. Y., Yoon, T. Y., Lee, Y. H., Im, M., Lee, Y., et al. (2014). Regulation of keratinocyte differentiation by O-GlcNAcylation. *J. Dermatol. Sci.* **75**, 10-15. doi:10.1016/j.jdermsci.2014.04.010
- Speakman, C. M., Domke, T. C. E., Wongpaiboonwattana, W., Sanders, K., Mudaliar, M., Van Aalten, D. M. F., Barton, G. J. and Stavridis, M. P. (2014). Elevated O-GlcNAc levels activate epigenetically repressed genes and delay mouse ESC differentiation without affecting naïve to primed cell transition. *Stem Cells* **32**, 2605-2615. doi:10.1002/stem.1761
- Struhl, G. and Akam, M. (1985). Altered distributions of Ultrabithorax transcripts in extra sex combs mutant embryos of Drosophila. *EMBO J.* **4**, 3259-3264. doi:10.1002/j.1460-2075.1985.tb04075.x
- Swamy, M., Pathak, S., Grzes, K. M., Damerow, S., Sinclair, L. V., Van Aalten, D. M. F. and Cantrell, D. A. (2016). Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. *Nat. Immunol.* **17**, 712-720. doi:10.1038/ni.3439
- Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L. M., Liu, D. R., Aravind, L., et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner Tet1. *Science* **324**, 930-935. doi:10.1126/science.1170116
- Tan, Z. W., Fei, G., Paulo, J. A., Bellaousov, S., Martin, S. E. S., Duveau, D. Y., Thomas, C. J., Gygi, S. P., Boutz, P. L. and Walker, S. (2021). O-GlcNAc regulates gene expression by controlling detained intron splicing. *Nucleic Acids Res.* **48**, 5656-5669. doi:10.1093/nar/gkaa263
- Torres, C. R. and Hart, G. W. (1984). Topography and polypeptide distribution of terminal N-acetylglucosamine residues on the surfaces of intact lymphocytes. Evidence for O-linked GlcNAc. *J. Biol. Chem.* **259**, 3308-3317. doi:10.1016/S0021-9258(17)43295-9
- Vaidyanathan, K., Niranjana, T., Selvan, N., Teo, C. F., May, M., Patel, S., Weatherly, B., Skinner, C., Opitz, J., Carey, J., et al. (2017). Identification and characterization of a missense mutation in the O-linked β -N-acetylglucosamine (O-GlcNAc) transferase gene that segregates with X-linked intellectual disability. *J. Biol. Chem.* **292**, 8948-8963. doi:10.1074/jbc.M116.771030
- Varki, A. (2017). Biological roles of glycans. *Glycobiology* **27**, 3-49. doi:10.1093/glycob/cww086
- Wan, L., Xu, K., Wei, Y., Zhang, J., Han, T., Fry, C., Zhang, Z., Wang, Y. V., Huang, L., Yuan, M., et al. (2018). Phosphorylation of EZH2 by AMPK suppresses PRC2 methyltransferase activity and oncogenic function. *Mol. Cell* **69**, 279-291.e5. doi:10.1016/j.molcel.2017.12.024
- Watson, L. J., Long, B. W., Demartino, A. M., Brittian, K. R., Readnower, R. D., Brainard, R. E., Cummins, T. D., Annamalai, L., Hill, B. G. and Jones, S. P. (2014). Cardiomyocyte Ogt is essential for postnatal viability. *Am. J. Physiol. Heart Circ. Physiol.* **306**, H142. doi:10.1152/ajpheart.00438.2013
- Webster, D. M., Teo, C., Sun, Y., Wloga, D., Gay, S., Klonowski, K. D., Wells, L. and Dougan, S. T. (2009). O-GlcNAc modifications regulate cell survival and epiboly during zebrafish development. *BMC Dev. Biol.* **9**, 28. doi:10.1186/1471-213X-9-28
- Whelan, S. A., Lane, M. D. and Hart, G. W. (2008). Regulation of the O-linked beta-N-acetylglucosamine transferase by insulin signaling. *J. Biol. Chem.* **283**, 21411-21417. doi:10.1074/jbc.M800677200
- White, C. W., Fan, X., Maynard, J. C., Wheatley, E. G., Bieri, G., Couthouis, J., Burlingame, A. L. and Villeda, S. A. (2020). Age-related loss of neural stem cell O-GlcNAc promotes a glial fate switch through STAT3 activation. *Proc. Natl. Acad. Sci. USA* **117**, 22214-22224. doi:10.1073/pnas.2007439117
- Willems, A. P., Gundogdu, M., Kempers, M. J. E., Giltay, J. C., Pfundt, R., Elferink, M., Loza, B. F., Fuijkschot, J., Ferenbach, A. T., Van Gassen, K. L. I., et al. (2017). Mutations in N-acetylglucosamine (O-GlcNAc) transferase in patients with X-linked intellectual disability. *J. Biol. Chem.* **292**, 12621-12631. doi:10.1074/jbc.M117.790097
- Wong, K. K. L., Liu, T. W., Parker, J. M., Sinclair, D. A. R., Chen, Y. Y., Khoo, K. H., Vocadlo, D. J. and Verheyen, E. M. (2020). The nutrient sensor OGT regulates Hipk stability and tumorigenic-like activities in Drosophila. *Proc. Natl. Acad. Sci. USA* **117**, 2004-2013. doi:10.1073/pnas.1912894117
- Wulff-Fuentes, E., Berendt, R. R., Massman, L., Danner, L., Malard, F., Vora, J., Kahsay, R. and Olivier-Van Stichelen, S. (2021). The human O-GlcNAc database and meta-analysis. *Sci. Data* **8**, 25. doi:10.1038/s41597-021-00810-4
- Xiong, X., Ma, H., Ma, J., Wang, X., Li, D. and Xu, L. (2022). α SMA-Cre-mediated Ogt deletion leads to heart failure and vascular smooth muscle cell dysfunction in mice. *Biochem. Biophys. Res. Commun.* **625**, 31-37. doi:10.1016/j.bbrc.2022.07.106
- Xu, Q., Yang, C., Du, Y., Chen, Y., Liu, H., Deng, M., Zhang, H., Zhang, L., Liu, T., Liu, Q., et al. (2014). AMPK regulates histone H2B O-GlcNAcylation. *Nucleic Acids Res.* **42**, 5594-5604. doi:10.1093/nar/gku236
- Yang, X., Su, K., Roos, M. D., Chang, Q., Paterson, A. J. and Kudlow, J. E. (2001). O-linkage of N-acetylglucosamine to Sp1 activation domain inhibits its transcriptional capability. *Proc. Natl. Acad. Sci. USA* **98**, 6611-6616. doi:10.1073/pnas.111099998
- Yang, Y. R., Song, M., Lee, H., Jeon, Y., Choi, E.-J. J., Jang, H.-J. J., Moon, H. Y., Byun, H.-Y. Y., Kim, E.-K. K., Kim, D. H., et al. (2012) O-GlcNAcase is essential for embryonic development and maintenance of genomic stability. *Aging Cell* **11**, 439-448. doi:10.1111/j.1474-9726.2012.00801.x
- Yang, Y. R., Jang, H. J., Lee, Y. H., Kim, I. S., Lee, H., Ryu, S. H. and Suh, P. G. (2015). O-GlcNAc cycling enzymes control vascular development of the placenta by modulating the levels of HIF-1 α . *Placenta* **36**, 1063-1068. doi:10.1016/j.placenta.2015.08.001
- Zachara, N. E., O'Donnell, N., Cheung, W. D., Mercer, J. J., Marth, J. D. and Hart, G. W. (2004). Dynamic O-GlcNAc modification of nucleocytoplasmic proteins in response to stress: a survival response of mammalian cells. *J. Biol. Chem.* **279**, 30133-30142. doi:10.1074/jbc.M403773200
- Zhang, Z., Parker, M. P., Graw, S., Novikova, L. V., Fedosyuk, H., Fontes, J. D., Koestler, D. C., Peterson, K. R. and Slawson, C. (2019). O-GlcNAc homeostasis contributes to cell fate decisions during hematopoiesis. *J. Biol. Chem.* **294**, 1363-1379. doi:10.1074/jbc.RA118.005993
- Zhu, Q., Cheng, X., Cheng, Y., Chen, J., Xu, H., Gao, Y., Duan, X., Ji, J., Li, X. and Yi, W. (2020). O-GlcNAcylation regulates the methionine cycle to promote pluripotency of stem cells. *Proc. Natl. Acad. Sci. USA* **117**, 7755-7763. doi:10.1073/pnas.1915582117