Published in final edited form as:
Glycoconj J. 2013 January ; 30(1): 57–66. doi:10.1007/s10719-012-9442-x.

The N's and O's of *Drosophila* Glycoprotein Glycobiology

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Abstract

The past 25 years have seen significant advances in understanding the diversity and functions of glycoprotein glycans in *Drosophila melanogaster*. Genetic screens have captured mutations that reveal important biological activities modulated by glycans, including protein folding and trafficking, as well as cell signaling, tissue morphogenesis, fertility, and viability. Many of these glycan functions have parallels in vertebrate development and disease, providing increasing opportunities to dissect pathologic mechanisms using *Drosophila* genetics. Advances in the sensitivity of structural analytic techniques have allowed the glycan profiles of wild-type and mutant tissues to be assessed, revealing novel glycan structures that may be functionally analogous to vertebrate glycans. This review describes a selected set of recent advances in understanding the functions of N-linked and O-linked (non-glycosaminoglycan) glycoprotein glycans in *Drosophila* with emphasis on their relatedness to vertebrate organisms.

Keywords

Drosophila; glycosylation; N-linked; O-linked

Introduction and Scope

Given the small size of the organism, it might be expected that *Drosophila melanogaster* would be a less than optimal system in which to pursue structural and functional glycomics. However, the opportunity to address glycan function through genetic manipulation is attracting a growing number of glycobiologists to the field, in addition to mainstream Drosophilists that have come upon glycobiology through unbiased genetic screens or the discovery of unexpected functional connections. Gains in analytic sensitivity realized over the past decade, primarily driven by mass spectrometry, have also enhanced the accessibility of glycan characterization following experimental perturbation or genetic manipulation for many small organisms. Thus, *Drosophila* glycobiology has begun to address major questions of broad relevance for all animals and, importantly, for human diseases. In this review, we focus on a selected handful of such recent advances in understanding the functional impact of N-linked and O-linked glycoprotein glycans in *Drosophila* and point out similarities and differences associated with analogous vertebrate structures. We apologize to our fellow *Drosophila* glycobiologists whose significant results we are unable to discuss due to the constraints imposed by the format of this review. For example, early appreciation for the essential role of glycosaminoglycan-based modulation of morphogen signaling arose from *Drosophila* genetic screens and many fine reviews cover this area very well [1,2]. Likewise, appreciation for the participation of glycosphingolipids in cell signaling and cell-cell

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interactions is growing rapidly and, despite the significant structural differences between *Drosophila* and vertebrate glycosphingolipids, functional and pathophysiological corollaries have emerged [3–12]. *Drosophila* glycosphingolipid glycobiology, *Drosophila* GAG functions, as well as nucleocytoplasmic glycosylation [13,14] are beyond the scope of this review.

The Glycobiology of *Drosophila* N-linked glycans

Mass spectrometry-based techniques have produced total N-linked glycan profiles for glycoproteins harvested from *Drosophila* embryos [15–17]. Unlike vertebrates, *Drosophila* N-linked glycan profiles display an overwhelming abundance of high and paucimannose structures, and a relatively low amount of hybrid and complex glycans [18]. As is also the case in vertebrates, N-linked glycosylation in *Drosophila* is initiated by adding a conserved 14-mer oligosaccharide ($Glc_3Man_9GlcNAc_2$) *en bloc* onto NXS/T/C motifs within newly synthesized polypeptides. Subsequent trimming and processing of this oligosaccharide in the ER/Golgi drives protein maturation and trafficking [19,20]. *Drosophila* has provided a platform to study protein maturation associated with some human diseases. For example, N-linked glycosylation of rhodopsin is critical for its proper trafficking in photoreceptor neurons [21,22]. Mutations that remove the N-linked glycosylation site of human rhodopsin cause autosomal dominant retinitis pigmentosa, a retinal degenerative disorder [23]. The N-linked glycan of *Drosophila* rhodopsin (Rh1) is essential for its exit from the ER [24] but is extensively trimmed or completely removed during its maturation and transport to the light sensing organelle, the rhabdomere [25,26]. Although much of the deglycosylation pathway for Rh1 is unknown, the process has recently been shown to be regulated by a phosphatase (*Drosophila* Metallophosphoesterase, dMPPE) [27]. In *dmppe* mutants Rh1 is incompletely deglycosylated, which renders it more sensitive to endocytic degradation, thereby leading to morphological and functional defects in photoreceptors in aged animal. The relevant target of dMPPE is phosphorylated α-mannosidase II (α-Man-II); dephosphorylated α-Man-II does not act on rhodopsin, thereby blocking full deglycosylation and transport of Rh1 to the rhabdomere. While this extreme processing may be a special case, in which stringent limitations on glycan size are imposed by the constraints of packing protein into a specific subcellular compartment, it remains to be determined how many other glycoproteins are similarly processed and how broadly phosphorylaton/dephosphorylation cycles influence glycoprotein glycan processing.

The glycans on many glycoproteins facilitate protein folding in the ER through interactions with lectinic chaperones and also serve as markers that divert misfolded glycoproteins into the ER-associated degradation (ERAD) pathway. Cytoplasmic peptide:N-glycanase (PNGase) participates in ERAD by catalyzing the cleavage of N-linked glycans from misfolded glycoproteins that have been extruded from the ER. Glycan removal facilitates degradation of the polypeptide by the ubiquitin-proteasome system [28–30]. PNGase is conserved among eukaryotes from yeast to human and it has been demonstrated that its deglycosylation activity is essential for degradation of most target glycoproteins [31,32]. Recently, a *Drosophila* ortholog of cytoplasmic PNGase, PNGase-like (Pngl), was shown to be enzymatically inactive while still retaining its carbohydrate binding activity [33]. The loss of deglycosylation activity of Pngl is due to the lack of the second CXXC motif, which binds a zinc atom essential for enzymatic activity and is also lacking in other dipteran insects. *Drosophila pngl* mutants display severe developmental defects, reduced viability, and adult sterility. These phenotypes were fully rescued by transgenic expression of wild-type Pngl. However, a mutant form of Pngl, in which a key Cys residue of the catalytic triad was converted to Ala, could not rescue the developmental defect. This finding indicates that a biochemical activity other than deglycosylation, which is yet to be determined, is important for at least some of the biological functions of Pngl.

Once successfully folded and transported along the secretory pathway, the most abundant N-linked glycan structures acquired by *Drosophila* glycoproteins are paucimannosidic. The abundance of these highly trimmed glycans is attributable to the activity of a processing β-*N*-acetylglucosaminidase called *fused lobes* (*fdl*). The Fdl enzyme specifically cleaves off the terminal GlcNAc residue added by GlcNAcT1 from the branch point intermediate structure, $GlcNAcMan_3GlcNAc_2$, thereby blocking further elongation of the α3 arm or elaboration of additional branches [34,35]. The "fused lobes" name comes from the observation that *fdl* mutants exhibit morphological changes in the adult brain, where the bilateral mushroom body β-lobes are found aberrantly fused at the midline [36]. The fused lobe phenotype was also observed in *Drosophila mgat1* (GnT1) mutants, which lack the enzyme responsible for transferring GlcNAc onto $Man_5GlcNAc_2$, thereby initiating the formation of hybrid and complex glycans [37]. *Drosophila mgat1* null mutants also exhibited locomotor defects and reduced life span, demonstrating the biological impact of altered N-linked glycan complexity for *Drosophila* central nervous system development and function. More recently, it was reported that tissue-specific knockdown of *Drosophila mgat1* in the nervous system decreased locomotor activity and life span [38]. Furthermore, the reduced life span associated with *Drosophila mgat1* null mutants was not just rescued by neuron-specific expression of Mgat1, even better, their life span was increased significantly compared to control. While these important findings clearly demonstrated that Mgat1-dependent glycan processing in the nervous system contributes to *Drosophila* longevity, it still remains to be understood how one mutation that decreases glycan complexity (Mgat1/GnT1) yields phenotypes so similar to another mutation that increases glycan complexity (Fdl).

Beneath the large shadow cast by the highly abundant paucimanose glycans, in-depth mass spectrometric analysis of the minor glycans in *Drosophila* tissues has revealed the presence of di-, mono-, and non-fucosylated hybrid/complex N-linked glycans with Gal or GalNAc extenstions, forming LacNAc or LacdiNAc termini, respectively [15–17]. Terminal LacNAc groups are found to be capped by acidic sugars, either sialic acid or glucuronic acid, but similarly capped LacdiNAc termini have not yet been detected [39,40]. The identity of the *Drosophila* enzyme responsible for LacNAc formation on N-linked glycans is still unresolved. Three orthologs of mammalian β4GalT-1 have been annotated in the *Drosophila* genome and their enzymatic properties have been investigated [41–44]. Of these three candidates, the enzyme with the least similarity to mammalian β4GalT-1 is more closely related to mammalian β4GalT-7, which transfers Gal to Xyl in the production of the glycosaminoglycan linker tetrasaccharide [41]. The other two genes appear to encode invertebrate β4GalNAcTs (β4GalNAcTA and B) rather than β4GalTs since they exhibit in vitro donor preference for UDP-GalNAc over UDP-Gal [44,45]. Based on the sequence differences between invertebrate β4GalNAcTs and mammalian β4GalTs and the crystal structure of bovine β4GalT-1, it has been proposed that a single amino acid mutation at the nucleotide sugar binding site (isoleucine/leucine in invertebrate β4GalNAcTs to tyrosine in mammalian β4GalTs) occurred over 500 million years ago, shifting the ancestral specificity from UDP-GalNAc to UDP-Gal. Indeed, switching the corresponding isoleucine residue of *Drosophila* β4GalNAcTA to tyrosine dramatically changed the donor preference of the enzyme [46]. Nevertheless, these observations do not exclude the possibility that invertebrate β4GalNAcTs transfer Gal onto GlcNAc in vivo. Such a low efficiency enzyme activity might partially explain why *Drosophila* N-linked glycan profiles contain much less LacNAc-containing glycans than those of mammalian species. The relative paucity of LacdiNAc terminated N-linked glycans may reflect differential compartmentalization of enzyme, acceptor glycoprotein, and UDP-GalNAc/UDP-Gal. Consistent with this proposal, β4GalNAcTB appears to be primarily responsible for generating LacdiNAc groups on *Drosophila* glycosphingolipids, but requires an escort protein (GABPI) to pilot it towards its functionally appropriate Golgi localization [47].

Unlike the putative *Drosophila* galactosyltransferase activities, little ambiguity surrounds the identity of the sialyltransferase that caps hybrid and complex glycans in *Drosophila*. A single sialyltransferase gene (DSiaT) has been identified in *Drosophila* [48]. Consistent with glycomic characterization of the sialylated glycans detected in *Drosophila* embryos, DSiaT is most homologous to the vertebrate ST6Gal1 enzyme [15]. *DSiaT* expression is restricted to a subset of central nervous system neurons where its activity is proposed to modulate neuronal excitability. Therefore, DSiaT mutants display various abnormalities including reduced life span, locomotor defects, temperature-sensitive paralysis, and altered neuromuscular junction morphology [49]. Interestingly, in vitro characterization of DSiaT enzyme activity indicated that LacdiNAc is a better acceptor than LacNAc, but sialylated LacdiNAc N-linked glycans have not yet been detected in *Drosophila* tissues [15,16,48]. LacNAc groups on N-linked glycans are also modified by addition of GlcA [16]. However, the relevant biosynthetic machinery and biological function(s) of glycoprotein glucuronylation are yet to be identified.

N-linked glycan processing occurs in a stepwise fashion, dictated by the topographic distribution of processing enzymes across the Golgi apparatus. Many longstanding questions remain regarding the trafficking and regulation of glycan processing enzymes in this organelle. Unlike the classic depictions of stacked cisternae that are characteristic of the Golgi found in most vertebrate cells, the Golgi of *Drosophila* cells consists of discrete puncta distributed throughout the cytoplasm, implying the existence of robust intraorganellar targeting mechanisms [50,51]. Cellular and genetic analysis has demonstrated the existence of discrete trafficking pathways that independently support glycoprotein or glycosaminoglycan processing through the *Drosophila* Golgi apparatus [52]. Recently, RNAi and classic mutagenesis screens have taken advantage of the tissue specific expression of a unique difucosylated N-linked glycan to characterize genes that regulate the glycosylation machinery. These approaches have revealed the participation of specific RNA binding proteins in regulating the expression of glycosyltransferases [53] and identified a role for protein phosphorylation in Golgi trafficking [54]. Similarly, RNAi screens targeting a broad range of protein kinases in vertebrate cells have also implicated phosphorylation as a central regulator of Golgi trafficking during interphase, in addition to the previously identified importance of cell cycle kinases for redistribution of the Golgi during mitosis [55,56]. Further characterization of Golgi-directed kinases and their substrates will likely provide new mechanistic links between cell signaling and glycomic plasticity for all classes of glycans.

The Glycobiology of *Drosophila* O-linked glycans

While the first characterizations of *Drosophila* N-linked glycans began to appear in the literature during the mid-1980s[57,40], it was another decade before lectin histochemistry and biochemical analysis documented the distribution and biosynthesis of O-linked glycoprotein glycosylation in the organism [39,58,59]. The passing of yet another decade, the first of this century, witnessed evidence for the inducible biosynthesis of core 1 disaccharide, the spatial and temporal regulation of polypeptide GalNAcT expression, and novel influences of specific O-glycan structures on cell signaling [60–63]. In particular, the demonstration that modification of the *Drosophila* Notch receptor protein with O-linked Fuc affects its signaling, and that extension of O-Fuc with GlcNAc modulates Notch's ability to discriminate between ligands, opened fertile new ground for coupling genetic analysis with functional glycomics [64]. Analogous modifications of vertebrate Notch proteins were identified contemporaneously with the *Drosophila* work, raising awareness of how little we really understood about the functions of O-linked glycosylation in any organism [65,66]. In the opening years of the second decade of this century, *Drosophila* O-linked glycoprotein

glycobiology continues to draw parallels and contrasts with vertebrate O-glycan functions in developmental and disease contexts.

As in other animals, the *Drosophila* genome contains multiple polypeptide GalNAcT genes, called PGANTs in *Drosophila* and ppGalNAcTs in vertebrates, which encode candidate enzymes for the initiation of mucin-type O-linked glycan formation. Currently, 9 PGANT gene products have been shown to have enzymatic activity, while another 4 candidates remain to be validated. As in other organisms, the *Drosophila* PGANTs fall into two broad families, those that transfer GalNAc to naked peptide and those that prefer to transfer GalNAc to peptides that are already modified by glycan. Systematic analysis has demonstrated that individual PGANTs are expressed in characteristic, but dynamic and frequently overlapping patterns during development [62]. In some *Drosophila* tissues only one PGANT is expressed or is highly dominant over others, providing unique opportunities to assess the function of mucin-type glycosylation through RNAi or mutagenesis strategies. These approaches have demonstrated that in various tissue contexts specific PGANTs regulate cell adhesion, modulate extracellular matrix composition, participate in epithelial morphogenesis, or are otherwise essential for viability [67–69]. These phenotypes resonate well with the pathophysiology of human diseases, such as autoimmunity, cancer progression, and congenital heart disease, in which altered mucin type O-glycosylation has been implicated [70–75]. Therefore, the accessibility of the *Drosophila* system for further mechanistic characterization of phenotypes may yield new insight and new targets for understanding several disease processes.

The O-linked modifications of Notch continue to engage the interest of glycobiologists for their novelty, diversity, and functional significance. In both vertebrates and *Drosophila*, O-linked Fuc on EGF repeats was the first functional glycan modification of Notch to be described. The loss of O-Fuc was demonstrated genetically and biochemically to decrease Notch signaling, leading to the hypothesis that the O-Fuc moiety contributed to receptor activation. Subsequent work revealed that enzymatically inactive O-fucosyltransferase (OFUT-1 in *Drosophila*) rescued Notch signaling and reversed intracellular Notch accumulation associated with an enzyme null mutant. These findings led to the proposal that OFUT-1 acts as a chaperone, ensuring the proper folding and/or trafficking of the Notch receptor and that this activity did not require transfer of O-Fuc to Notch [76,77]. Rather, the addition of O-Fuc to Notch primarily serves to provide a substrate for glycan elongation by Fringe, producing GlcNAcβ3Fuc, which establishes the ligand binding specificity of Notch [64]. In vertebrates, the disaccharide generated by Fringe is rapidly elongated by the addition of a Gal and sialic acid (SA) residue, generating a tetrasaccharide that has not been detected in *Drosophila*. In fact, the major O-Fuc glycan in *Drosophila* is a branched trisaccharide, GlcNAcβ3(GlcAβ4)Fuc, carrying GlcA rather than SA as its acidic residue [78]. The GlcAT responsible for branching the Fringe product is yet to be identified.

O-Fucosylation of Notch requires GDP-Fuc and is thought to occur in the endoplasmic reticulum, although direct evidence for ER transport of this nucleotide sugar has been lacking. Mutations in two *Drosophila* genes, now known as *efr* and *gfr*, have identified transporters with overlapping influence on Notch signaling. Efr transports GDP-Fuc, as well as UDP-GlcNAc and UDP-Xyl, into the ER, providing what should be an essential pool of GDP-Fuc for O-fucosylation of Notch. Gfr transports GDP-Fuc into the Golgi, where it can serve as donor substrate for fucosylation of N-linked glycans [79]. Loss of Gfr results in significant underfucosylation of N-linked glycoprotein glycans, but also produces mild, temperature sensitive Notch phenotypes [80]. Thus, it is proposed that GDP-Fuc taken up into the Golgi by Gfr may be delivered to the ER by retrograde transport where it makes an important contribution to the total pool of nucleotide sugar required for O-fucosylation of Notch [79,81]. The *Drosophila* Gfr gene is homologous to a human GDP-Fuc transporter

(solute carrier family member 35C1, SLC35C1), which is mutated in a congenital disorder of glycosylation (CDG type IIc, also known as Leukocyte Adhesion Deficiency Type II or LADII) that is characterized by growth retardation, intellectual disability, and immune deficiency [82,83]. While the immune deficiency of this disorder arises primarily from loss of fucosylated glycans that mediate leukocyte trafficking, the developmental phenotypes may reflect altered Notch signaling [81]. In contrast to Gfr, Efr transports GDP-Fuc directly into the ER pool expected to contribute to O-fucosylation of Notch. However, Efr mutants do not display Notch phenotypes, indicating that retrograde transport of GDP-Fuc from the Golgi is sufficient to maintain Notch signaling. In the Efr/Gfr double mutant, Notch phenotypes more severe than those seen in the Gfr single mutant are detected. The clear demonstration of genetic interactions between Gfr and Efr may reveal the existence of feedback mechanisms that regulate the distribution of nucleotide sugars across the secretory pathway or may suggest that a significant portion of Notch O-fucosylation actually occurs in the Golgi. The development of better subcellular markers and sensitive methods to determine the distribution of GDP-Fuc (and other nucleotide sugars) would help to clarify the mechanisms that regulate Notch O-fucosylation.

Ongoing studies of *Drosophila* and vertebrate Notch signaling continue to reveal the importance of novel O-linked glycan modifications [84]. Molecular characterization of a temperature sensitive mutation, called *rumi*, that produces Notch phenotypes at the restrictive temperature identified a novel protein with a candidate glycosyltransferase domain [85]. Biochemical characterization of recombinant Rumi revealed that the protein possesses an O-glucosyltransferase activity specific for EGF domains, such as those in the Notch extracellular domain. Addition of O-Glc to Ser/Thr residues by Rumi occurs at a consensus that is distinct from O-Fuc addition and individual Notch EGF repeats can carry both O-Fuc and O-Glc, or either alone [86]. Three *rumi* candidate homologues were identified in vertebrates, but only one retains enzymatic activity [87]. Knockout of the enzymatically active mouse *rumi*, called a POGLUT (protein O-glucosyltransferase), results in lethality at embryonic day 9.5 with accompanying defects in neural tube development, somitogenesis, and cardiovascular elaboration [88]. While some aspects of the mouse knockout phenotypes can be attributed to altered Notch signaling, the full range of defects are broader than can be explained by loss of Notch, suggesting additional POGLUT targets in vertebrates. Additional targets for Rumi have not yet been identified in *Drosophila*. Interestingly, unlike O-Fuc addition, ligand binding to Notch is not affected in *rumi* mutants but proteolytic cleavage of Notch is impaired, blocking propagation of the intracellular signal normally transmitted by liberation of the Notch cytoplasmic domain [85,88]. Further characterization of Drosphila Rumi and vertebrate POGLUT has revealed that these enzymes possess two activities. In addition to transferring Glc, they also transfer Xyl to Ser/Thr residues [87]. In theory, the addition of O-Xyl provides a substrate for glycosaminoglycan elongation, but it is currently unclear whether O-Xyl addition results in elaboration of a GAG chain on Notch or any other protein substrate, or whether O-Xyl by itself can modulate Notch function. O-Glc residues on Notch EGF repeats in *Drosophila* are extended by the addition of a Xyl to form the Xylα3Glc disaccharide [89]. In vertebrates, an additional Xyl is added to form the Xylα3Xylα3Glc trisaccharide, which has not been detected in *Drosophila* [78,90]. While the vertebrate XylT enzymes have been identified, *Drosophila* homologues have not been pursued and phenotypes associated with loss of the *Drosophila* or vertebrate enzymes remain to be explored [89].

Further expanding the diversity of O-linked glycans on Notch, recent reports have placed O-GlcNAc on EGF repeats of *Drosophila* (and mouse) Notch protein [91,92]. While O-linked GlcNAc has been extensively studied as a modification of nuclear and cytoplasmic proteins, these recent findings are the first to place O-GlcNAc on the extracellular domain of a transmembrane protein [93]. Extracellular O-GlcNAc modification is achieved by an

enzyme (EOGT) that is distinct from the enzyme responsible for nucleocytoplasmic additon of O-GlcNAc (OGT) and loss of EOGT results in phenotypes that indicate a role for extracellular O-GlcNAc in cell-matrix interactions [94,95].

Are all of the O-linked glycan modifications of Notch now identified? The answer to this question is not clear and its ultimate resolution will require a substantial advance in current glycoproteomic analytic techniques. As mentioned previously, whole embryo glycomic analysis detects the presence of a branched O-Fuc trisaccharide carrying a GlcA residue. This O-Fuc trisaccharide is a major O-glycan of the *Drosophila* embryo and is, therefore, likely to be found on proteins other than just Notch [78]. Does this glycan even exist in linkage to Notch? An answer to this question will only come from analysis of the glycosylation of Notch protein isolated from developing tissues. Constructs expressed in cell culture are unlikely to reproduce the genetic, epigenetic, and cell-cell interactions that drive endogenous glycosylation of vitally important receptor proteins.

Similar considerations pertain for understanding the full diversity of glycoprotein acceptors that carry other O-glycan modifications. Protein O-mannosylation in *Drosophila* is carried out by the coordinated activity of two proteins, POMT-1 and POMT-2 (also known as *rotated* and *twisted abdomen*, respectively), which modify the *Drosophila* dystroglycan protein [96–98]. Loss of O-mannosylation in *Drosophila* results in muscle degeneration which phenocopies the pathophysiology of human congenital muscular dystrophies [99,96]. But unlike O-mannosylation of vertebrate alpha-dystroglycan, *Drosophila* O-Man has not yet been shown to be elongated in a POMGNT-1 or Large dependent manner [100]. The existence of other O-Man modified proteins in *Drosophila* and vertebrates is predicted by several lines of evidence but comprehensive glycoproteomic identification of these targets remains to be achieved in either organism [101,102]. Additionally, a second protein O-fucosyltransferase (POFUT-2 in vertebrates) links Fuc to Ser/Thr residues of thrombospondin repeats (TSRs) but not to EGF repeats; a *Drosophila* homolog (OFUT-2) has been annotated but not characterized [103,104]. In vertebrates, this O-Fuc on TSR repeats can be extended by addition of a Glc residue, generating a Glcβ3Fuc disaccharide [103]. Loss of this glucosyltransferase activity, which has not yet been identified in *Drosophila*, results in Peters Plus syndrome, characterized by eye defects, short stature, developmental delay, and cleft lip/palate [105]. An essential goal for future glycomic and glycoproteomic analysis of O-linked glycosylation in *Drosophila* is to establish the endogenous diversity of glycoproteins carrying specific glycan structures. In so doing, glycoproteomic analysis can identify new targets for genetic and glycobiological analysis that may be relevant for understanding the function of analogous glycans in vertebrate systems.

Concluding remarks

More than a century of random mutagenesis screens in *Drosophila* have pursued the identification of developmentally important genes in an unbiased manner. These approaches have repeatedly opened new frontiers for understanding biological processes that are broadly conserved across animal species. Among these important paradigms, several functional consequences of protein glycosylation have been revealed through both forward and reverse genetic strategies. The contribution of N-linked glycans to protein folding and stability, the impact of altered glycosylation on cell signaling, neural activity, adult longevity and fertility, as well as the connection between well-defined glycans and specific disease pathologies have emerged as glycan functions in *Drosophila* with clear parallels in vertebrates. Continued application of genetic strategies to identify new genes, new interactions, and new functions related to *Drosophila* glycans promises to enhance all of glycobiology. But, unlike proteins, glycans are not the translated products of a predictive

linear code. Therefore, rapid genetic advances in *Drosophila* glycobiology will mandate the development of tools for glycan structural analysis that can keep pace with the need to assign biochemical context to new mutants.

Acknowledgments

The authors acknowledge the support of grant R01-GM072839 (to MT) from the National Institutes of Health/ National Institute of General Medicine.

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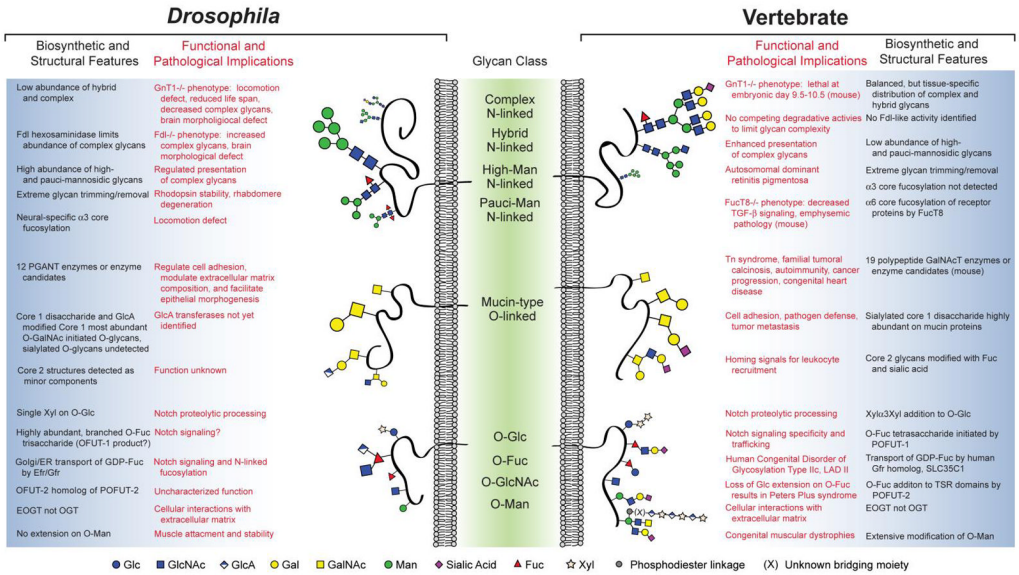

Figure 1. Comparative glycomics of *Drosophila* and vertebrate glycoprotein glycosylation

Representations of N-linked and O-linked glycans are scaled proportionally to their relative abundance in *Drosophila* and vertebrate tissues. Monosaccharide designations are in accordance with the guidelines proposed by the Consortium for Functional Glycomics.