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REVIEW

Glycobiology on the fly: Developmental and mechanistic insights from *Drosophila*

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Drosophila melanogaster **offers many unique advantages for deciphering the complexities of glycan biosynthesis and function. The completion of the *Drosophila* genome sequencing project as well as the comprehensive catalogue of existing mutations and phenotypes have lead to a prolific database where many of the genes involved in glycan synthesis, assembly, modification, and recognition have been identified and characterized. Recent biochemical and molecular studies have elucidated the structure of the glycans present in *Drosophila*. Powerful genetic approaches have uncovered a number of critical biological roles for glycans during development that impact on our understanding of their function during mammalian development. Here, we summarize key recent findings and provide evidence for the usefulness of this model organism in unraveling the complexities of glycobiology across many species.**

Keywords: Development/*Drosophila*/glycosylation

Introduction

The complexities of glycan biosynthesis and structure necessitate the use of a system that affords the advantages of sophisticated genetics as well as reduced genome redundancy. The *Drosophila melanogaster* genome is inherently less redundant (∼14,000 genes in *Drosophila* versus ∼25,000 in humans) (Rubin et al. 2000; Stein 2004; Hahn et al. 2007) and can therefore circumvent the functional redundancy present in many glycosyltransferase families. Additionally, the elegant genetic strategies employed in *Drosophila* have been used historically to uncover and elucidate many complex biological events. Random mutagenesis coupled with sophisticated mapping techniques has yielded the identification of many novel genes responsible for a plethora of evolutionarily conserved regulatory events. Comprehensive databases cataloguing alleles, phenotypes, expression patterns, and interacting partners of the many genes previously characterized further provide an invaluable resource for rapidly deciphering the function of additional newly discovered genes. Continuing efforts to mutagenize every gene in the genome through transposon targeting techniques have provided a wealth of reagents to interrogate the function of a large number of previously uncharacterized genes. Whole genome RNA interference (RNAi) in insect cell culture provides a system for rapidly cataloguing the function of genes in any cellular process for which a screen has been developed (e.g., viability, growth, morphological changes, cell signaling, cell division, cell adhesion) (Kiger et al. 2003; Boutros et al. 2004). Additionally, techniques for tissue- and stage-specific knockdown of gene expression via RNAi in the fly can address the role of specific genes in specific developmental processes. The recent construction of a genome-wide transgenic RNAi library in the fly will enable researchers to rapidly interrogate the developmental consequences of almost any gene of interest (Dietzl et al. 2007).

In recent years, much progress has been made using *Drosophila melanogaster* to study many diverse aspects of glycobiology. In this review, we will summarize recent work elucidating glycan function using the fly as a model system.

Glycan function in *Drosophila*

The role of proteoglycans during Drosophila development

The importance of glycans during *Drosophila* development was first discovered while dissecting the developmental impact of the components of proteoglycans, which consist of a glycosaminoglycan (GAG) chain attached to serine residues of core proteins. While a detailed account of the extensive advances in this field is beyond the scope of this review, we will highlight key points elucidated in the fly. Both secreted and cell-surface proteoglycans exist in the fly. Cell surface proteoglycans consist of two classes: glypicans, which are membrane-bound via a GPI anchor and are modified with heparan sulfate (HS); and syndecans, which are transmembrane proteins modified with heparan sulfate or chondroitin sulfate (CS). Studies in the fly and fly cell culture systems have led to a number of different models describing the role of proteoglycans in hedgehog (Hh), wingless (Wg), fibroblast growth factor (FGF), and decapentaplegic/TGF-β (Dpp) signaling pathways during development (reviewed in Nybakken and Perrimon 2002 and Hacker et al. 2005). These models include: (1) proteoglycans acting as coreceptors to enhance the receptor–ligand interaction; (2) proteoglycans aiding in requisite ligand dimerization for efficient receptor binding; and (3) proteoglycans influencing ligand/morphogen gradient formation by regulating ligand diffusion, transport, stability, secretion, or endocytosis. While initial information defining the role of proteoglycans came from analysis of mutations in genes encoding the core proteins to which the GAG chains are

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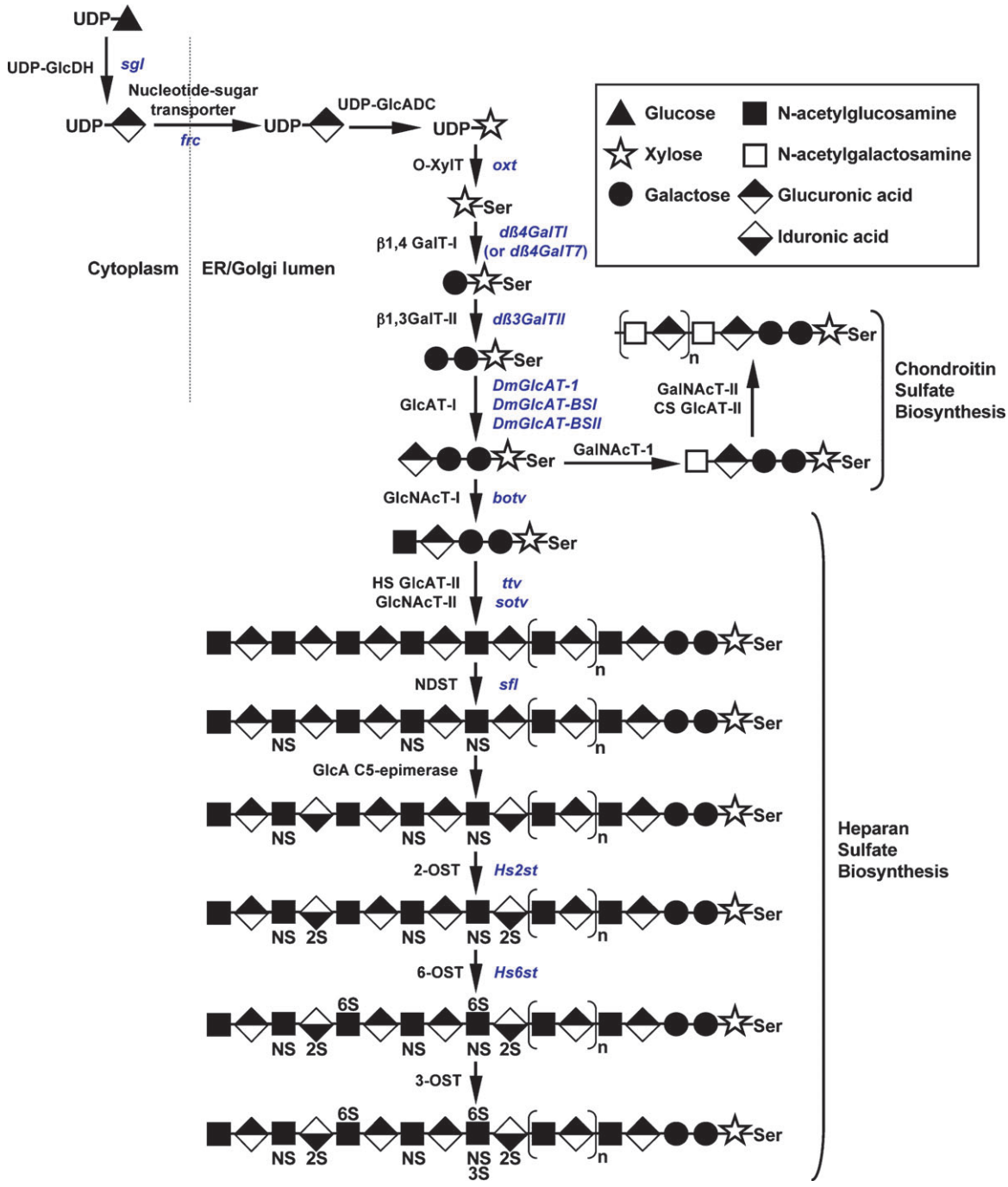

Fig. 1. Biosynthesis of glycosaminoglycans. The initiation of chondroitin sulfate (CS) and the complete synthesis of heparan sulfate (HS) are shown. Enzymes responsible for catalyzing each step are shown in black and the corresponding *Drosophila* genes are shown in blue. Enzyme abbreviations are as follows: UDP-GlcDH, UDP-glucose dehydrogenase; UDP-GlcADC, UDP-glucuronic acid decarboxylase; *O*-XylT, polypeptide *O*-xylosyltransferase; β1,4GalT-I, xylose-β1,4-galactosyltransferase; β1,3GalT-II, galactose-β1,3-galactosyltransferase; GlcAT-I, galactose-β1,3-glucuronyltransferase; GalNAcT-I, glucuronic acid-β1,4-*N*-acetylgalactosaminyltransferase; CS GlcAT-II, chondroitin sulfate GalNAc-β1,3-glucuronyltransferase; GalNAcT-II, glucuronic acid-β1, 4-*N*-acetylgalactosaminyltransferase; GlcNAcT-I, glucuronic acid-α1,4-*N*-acetylglucosaminyltransferase; HS GlcAT-II, heparan sulfate GlcNAc-β1, 4-glucuronyltransferase; GlcNAcT-II, glucuronic acid α1,4-*N*-acetylglucosaminyltransferase; NDST, *N*-deacetylase/*N*-sulfotransferase; 2-OST, 2-*O*-sulfotransferase; 6-OST, 6-*O*-sulfotransferase; 3-OST, 3-*O*-sulfotransferase.

attached, genetic screens have revealed (and continue to reveal) the importance of genes responsible for GAG biosynthesis and modification (Figure 1).

GAG synthesis is initiated by the addition of a xylose to the peptide backbone of proteins destined to become proteoglycans (Figure 1). *Drosophila* has one xylosyltransferase (encoded by the *oxt* gene) responsible for catalyzing this addition (Wilson 2002; Brunner et al. 2006). Defects in this gene have not yet been characterized. The core xylose is then further extended by the addition of galactose through the action of a

single β1,4-galactosyltransferase (β1,4 GalT-I) encoded by the gene *dβ4GalT7* (also known as *dβ4GalTI*) (Nakamura et al. 2002; Takemae et al. 2003). RNAi to *dβ4GalT7* in *Drosophila* impaired HS and CS biosynthesis and resulted in abnormal wing and leg morphology, phenocopying defects in Hh and Dpp signaling (Nakamura et al. 2002; Takemae et al. 2003). The next step in the pathway is catalyzed by a proteoglycan β1,3-galactosyltransferase (encoded by the *dβ3GalTII* gene; Figure 1), which transfers galactose to the Galβ1-4Xyl disaccharide core. RNAi to *dβ3GalTII* resulted in decreased levels of heparan sulfate proteoglycans (HSPGs) and decreased levels of extracellular Wg (Ueyama et al. 2008). The fly also expresses three glucuronyltransferases, encoded by the genes *DmGlcAT-I*, *DmGlcAT-BSI*, and *DmGlcAT-BSII* (BS stands for "broad specificity"). DmGlcAT-I demonstrated specificity for transferring GlcA to the linkage region trisaccharide (Galß1-3Galß1-4Xyl) of proteoglycans (Kim et al. 2003). In contrast, DmGlcAT-BSI and -BSII transferred GlcA to a wide range of substrates, including proteoglycans, glycolipids, and glycoproteins, suggesting their potential involvement in the synthesis and extension of a variety of glycans in the fly (Kim et al. 2003). Interestingly, *DmGlcAT-BSI* and *DmGlcAT-BSII* are widely expressed during development, suggesting that GlcA may serve as a major negatively charged sugar in the fly, as sialic acid addition appears to be much more restricted (see section on *N*-Linked glycosylation).

The next steps of GAG biosynthesis are catalyzed by members of the EXT (hereditary multiple exostosin) gene family. *Brother of tout velu (botv)* encodes an *N*-acetylglucosoaminyltransferase that adds GlcNAc to the tetrasaccharide core of HSPGs and may also contribute to chain elongation (Han et al. 2004; Izumikawa et al. 2006). *Tout velu (ttv)* and *sister of tout velu (sotv)* encode enzymes which form a complex responsible for the sequential, repeating addition of GlcA and GlcNAc to elongate the HS chains (Han et al. 2004; Izumikawa et al. 2006). Mutations in *ttv*, *sotv*, or *botv* result in impared Wg, Hh and Dpp signaling as well as reduced or abrogated HS synthesis, indicating the crucial role of GAGs in morphogen gradient formation (Toyoda et al. 2000; Takei et al. 2003; Han et al. 2004; Dasgupta et al. 2007). Additionally, mutations in the *sugarless (sgl)* or *sulfateless (sfl)* genes (encoding the enzymes UDP-glucose dehydrogenase and *N*-deacetylase/*N*-sulfotransferase, respectively) (Figure 1), displayed aberrant tracheal morphogenesis, similar to defects in FGFR signaling (Lin et al. 1999; Selleck 2000; Toyoda et al. 2000;). Recent studies have further demonstrated strict temporal control of GAG synthesis during *Drosophila* embryonic development. The mechanism, which involves developmentally regulated translational control of *ttv* and *sgl*, is thought to be an evolutionarily conserved means of modulating growth factor activity and morphogen gradient formation at specific times during development by regulating GAG biosynthesis (Bornemann et al. 2008).

Additional genes involved in GAG biosynthesis and modification include two encoding sulfotransferases (*Hs2st* and *Hs6st*). While mutation of either gene alone did not result in significant defects due to compensatory sulfation, mutation of both genes resulted in severe defects in FGF signaling, indicating the importance of overall GAG sulfation levels as opposed to specific sulfation patterns for signaling in certain developmental contexts (Kamimura et al. 2006; Xu et al. 2007). Also, the nucleotide sugar transporter, Fringe connection (encoded by the *frc* gene) transports UDP-GlcA (and other nucleotide sugars) into the ER/Golgi, further influencing proteoglycan biosynthesis, as well as the synthesis of other glycans (Goto et al. 2001; Selva et al. 2001). From these studies, it has become widely appreciated that the GAG component of proteoglycans and the enzymes regulating their biosynthesis function in many diverse aspects of development.

N-Linked glycosylation in Drosophila

Recent mass spectrometry studies by North et al. (2006) and Aoki et al. (2007) have defined the structure of *N*-linked glycans present on protein substrates during *Drosophila* embryonic development (Figure 2). The predominant *N*-glycan structures found consist of high mannose and paucimannose and may be fucosylated at the chitobiose core. Only minor amounts of hybrid, bi- and tri-antennary complex glycans were observed, with some containing α2,6-linked sialic acid (North et al. 2006; Aoki et al. 2007). This composition differs considerably from that seen in mammals, where *N*-glycans are predominantly hybrid and complex, with abundant sialylation (Gagneux and Varki 1999). However, *Drosophila N*-glycans appear to be more similar to mammalian *N*-glycans than those from another model organism, *Caenorhabditis elegans*, which contain unique high fucose, phosphorylcholine, and methylated structures (Cipollo et al. 2005; Hanneman et al. 2006; Paschinger et al. 2008). Interestingly, the *N*-glycan profile of the fly changes as development proceeds, suggesting specific regulation of the glycosylation machinery and roles for certain glycan structures during different stages of development (Aoki et al. 2007).

A number of genes involved in *N*-glycan biosynthesis in *Drosophila* have recently been characterized. *Wollknauel* (*wol*) encodes a UDP-glucose:dolichyl-phosphate glucosyltransferase responsible for dolichol-sugar synthesis. Mutations in this gene result in an unfolded protein response and disruption of embryonic patterning (Haecker et al. 2008). Mutations in other genes involved in later stages of *N*-glycan synthesis appear to primarily affect nervous system development and function. The abundant paucimannose *N*-linked glycans in the fly are attributed to the gene *fused lobes* (*fdl*), which encodes an *N*-acetylglucosaminidase that is responsible for cleaving the *N*-acetylglucosamine (GlcNAc) of hybrid *N*-glycans to form paucimannose *N*-glycans (Leonard et al. 2006). Mutations in this gene cause altered central nervous system (CNS) development resulting in fusion of the mushroom body β lobes of the brain (Boquet et al. 2000). Paradoxically, mutations in the *Drosophila* Mgat1 orthologue, which transfers GlcNAc to the paucimannose *N*-glycans to produce hybrid *N*-glycans, result in a similar "fused lobes" phenotype (Sarkar et al. 2006), suggesting key roles for both hybrid and paucimannose *N*-glycans in CNS development. In addition to the fused lobe phenotype, *Drosophila Mgat1* mutants display reduced locomotory activity, reduced lifespan, and sterility in males, indicating roles for β1,2-*N*-acetylglucosaminyltransferase I-dependent *N*-glycans in reproduction and homeostasis in the adult fly (Sarkar et al. 2006).

In contrast to mammals, which have dozens of genes encoding sialyltransferases, the *Drosophila* genome has only one sialyltansferase gene (*SiaT*) (Koles et al. 2004). The *Drosophila* SiaT encodes an α2,6 sialyltransferase that is evolutionarily related to the vertebrate ST6 sialyltransferase (Koles et al. 2004). This enzyme acts on oligosaccharides and glycoproteins but not

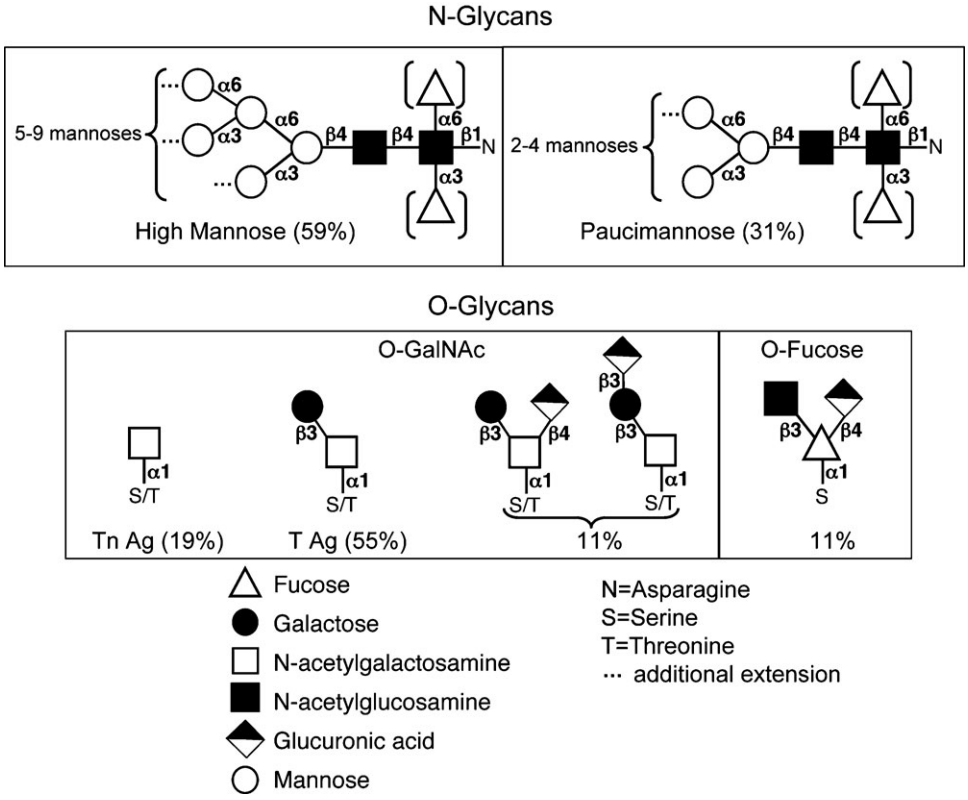

Fig. 2. *N*- and *O*-linked glycan structures present in *Drosophila melanogaster*. Shown are the major types of *N*-linked and *O*-linked glycans found in *Drosophila* and their relative abundance. *N*-Glycans consist primarily of high mannose (59%) and paucimannose (31%); hybrid (7%) and complex (3%) structures are much less abundant. Brackets indicate that an α1,3 fucose or α1,6 fucose may also be present in these structures. Mucin-type *O*-linked glycans are predominantly comprised of the Tn antigen (Tn Ag) and T antigen (T Ag or Core 1) structures. The only detectable protein *O*-fucose glycan in *Drosophila* is a glucuronyl trisaccharide (Aoki et al. 2008).

on glycolipids in vitro. *SiaT* is expressed in a very tissue- and stage-specific fashion, being found only in a subset of cells in the developing CNS (Koles et al. 2004). Indeed, mutations in *SiaT* result in adults with reduced lifespan, reduced tolerance to heat, and reduced locomotory activity, supporting a role for sialic acid modification of glycans in nervous system function (Koles et al. 2004). To date, additional genes involved in *N*-glycan biosynthesis have not yet been characterized in the fly but ongoing work by many groups centers around investigating the role of these glycans in protein structure, function, processing, and stability influencing fly development and homeostasis.

O-Linked glycosylation in Drosophila

O-GlcNAc. *O*-Linked GlcNAc was the first *O*-glycan to be detected in *Drosophila*. Early studies using lectin staining as well as radiolabeling, identified *O*-GlcNAc in distinct banding patterns along polytene chromosomes (Kelly and Hart 1989), providing evidence for the presence of this glycan on nuclear and chromatin-associated proteins in *Drosophila*. However, most of our current understanding of the *O*-GlcNAc modification has come from studies in mammalian systems, although a number of groups are now analyzing *O*-GlcNAc function in the fly.

O-Mannose. The role of *O*-linked mannose on protein substrates is of great interest as mutations in the glycosyltransferases responsible for this modification in humans result in muscular dystrophies (Muntoni et al. 2004). *Drosophila* has two genes, *rotated abdomen (rt)* and *twisted (tw)*, encoding protein *O*-mannosyltransferases that are orthologues of the vertebrate POMT1 and POMT2 *O*-mannosyltransferases (Martin-Blanco and Garcia-Bellido 1996; Willer et al. 2003; Ichimiya et al. 2004; Lyalin et al. 2006). Similar to vertebrates, RT and TW appear to be required simultaneously, perhaps acting as a heterocomplex responsible for the transfer of mannose to protein substrates. Mutations in either gene result in defects in muscle development leading to a rotated abdomen phenotype in adults (Martin-Blanco and Garcia-Bellido 1996; Lyalin et al. 2006). In mammals, the major substrate of the *O*-mannosyltransferases is α-dystroglycan. *Drosophila* also has a dystroglycan (Dg) that, when mutated, results in muscle phenotypes similar, but not identical to those seen in *tw* and *rt* mutants (Haines et al. 2007). These enzymatic and phenotypic similarities between the fly and mammals suggest that *Drosophila* will be a valuable model system for deciphering the mechanistic role of *O*-linked mannose in muscle development and function. Additionally, the orthologue of the mammalian POMGnT-I (which extends the core mannose through the addition of GlcNAc) has not been found in *Drosophila*, suggesting that *O*-mannose glycans in the fly may be simpler in structure and thus more amenable to experimental analysis.

O-Linked GalNAc (Mucin-type O-glycosylation). Mucin-type *O*-linked glycosylation and the family of UDP-*N*-acetylgalactosamine:polypeptide *N*-acetylgalactosaminyltransferases

Table I. Summary of *Drosophila pgants*

Name	CG no.	Activity	Expressed in[a]
pgant1	CG8182	Peptide/glycopeptide transferase	Embryonic gut, salivary glands, antennomaxillary complex, and posterior spiracles; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female
pgant2	CG3254	Peptide/glycopeptide transferase	Embryonic brain and trachea; third instar larval wing and eye-antennal imaginal disks; adult male and female heads
pgant3	CG4445	Peptide/glycopeptide transferase	Embryonic gut, posterior spiracles, pharynx, esophagus, and epidermis; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female
pgant4	CG31956	Glycopeptide transferase	Embryonic gut and proventriculus; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female bodies
pgant5	CG31651	Peptide/glycopeptide transferase	Embryonic gut, salivary glands, antennomaxillary complex, posterior spiracles, and epidermis; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female
pgant6	CG2103	Glycopeptide transferase	Embryonic gut, salivary glands, antennomaxillary complex, and epidermis; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female
pgant7	CG6394	Glycopeptide transferase	Embryonic gut, salivary glands, antennomaxillary complex, and epidermis; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female
pgant8	CG7297	Peptide/glycopeptide transferase	Embryonic gut; adult male and female
pgant35A	CG7480	Peptide/glycopeptide transferase	Embryonic gut, salivary glands, trachea, and posterior spiracles; third instar larval wing, eye-antennal, leg and haltere imaginal disks; adult male and female
NA	CG30463[b]	ND	Embryonic amnioserosa, gut, and salivary glands; third instar larval wing, eye-antennal, leg and haltere imaginal disks
NA	CG10000[b]	ND	Embryonic gut
NA	CG31776[b]	ND	Embryonic gut and antennomaxillary complex; third instar larval wing, eye-antennal, leg and haltere imaginal disks

[a]based on previous studies by PCR (Ten Hagen et al. 2003) and whole-mount in situ hybridization (Tian and Ten Hagen 2006).
[b]putative isoforms.
NA, not applicable; ND, not detected.

(PGANTs) that initiates it are conserved in *Drosophila*. However, mucin-type *O*-glycans in flies are less extended relative to their mammalian counterparts, consisting primarily of the core 1 structure (T Ag; Galβ1-3GalNAcα1-O-S/T) (North et al. 2006), the Tn antigen (Tn Ag; GalNAcα1-O-S/T), and the core 1 structure modified with GlcA attached to either the Gal or GalNAc (Aoki et al. 2008; Breloy et al. 2008; M. Tiemeyer, personal communication) (Figure 2). No evidence for sialylated *O*-glycans has been found.

The fly has 12 putative genes encoding PGANTs, 9 of which have demonstrated biochemical activity in vitro (Schwientek et al. 2002; Ten Hagen and Tran 2002; Ten Hagen et al. 2003) (Table I). These genes display dynamic spatial and temporal regulation, suggesting that their coordinated expression determines what proteins (and what regions within those proteins) acquire *O*-glycans at various stages during development (Table I) (Tian and Ten Hagen 2006). Indeed, tissue staining using lectins and antibodies has illustrated the diversity of cells and organs expressing *O*-glycans at specific stages of development (Fredieu and Mahowald 1994; D'Amico and Jacobs 1995; Tian and Ten Hagen 2007a; Figure 3). Of note, is the unique spatial expression of mucin-type *O*-glycans along the presumptive apical and luminal regions of developing tubular tissues (Figure 3), which may play key roles in proper tube formation (see below).

Biochemical studies have revealed a hierarchy of action within the PGANT family, with certain members acting on previously unmodified peptides as initiating transferases (peptide transferases) and others acting to further modify previously glycosylated substrates (glycopeptide transferases), further supporting the coordinated action of PGANTs in dictating *O*-glycosyaltion patterns. Biochemical analyses also demonstrated that fly and mammalian orthologues have similar substrate preferences and preferred sites of GalNAc addition within those substrates (Ten Hagen et al. 2003; Gerken et al. 2008). This functional conservation may indicate conserved biological roles for members of this glycosyltransferase family that have been maintained over the course of evolution.

While other extending transferases have not been characterized in the fly, genes encoding core 1 β3-galactosyltransferases (core 1 β3-GalTs) have been identified (Muller et al. 2005). In contrast to mammals, which have one core 1 β3-GalT whose activity requires a chaperone (Ju et al. 2002; Ju and Cummings 2002), in vitro studies in the fly suggest that there may be as many as four functional core 1 β3-GalTs that do not appear to require a chaperone for activity (Muller et al. 2005). It is of note that the majority of mucin-type *O*-linked glycans in the fly consist of the unmodified core 1 structure (North et al. 2006; Aoki et al. 2008), suggesting that evolutionary pressures may have favored expansion of core 1 β3-GalTs in the fly. Regulation of core 1 β3-galactosyltransferase activity appears to be under different genetic controls in flies versus mammals; the multiple fly core 1 β3-GalT genes exhibit unique spatial expression patterns (Muller et al. 2005) whereas the single mammalian core 1 β3-GalT gene is ubiquitously expressed (Ju et al. 2002; Ju and Cummings 2002). Additionally, the activity of the mammalian transferase is influenced by expression of its chaperone, and possibly other competing transferases.

Crucial roles for mucin-type *O*-glycosylation were first demonstrated in *Drosophila*, where one member of the family (*pgant35A*) was found to be recessive lethal (Schwientek et al. 2002; Ten Hagen and Tran 2002). This was the first demonstration that mucin-type *O*-glycosylation was required for viability in any organism. Additional work on this enzyme has recently revealed a role during tracheal tube formation (Tian and Ten Hagen 2007b), consistent with the abundant presence of *O*-glycans in this organ (Figure 3). Mucin-type *O*-glycans

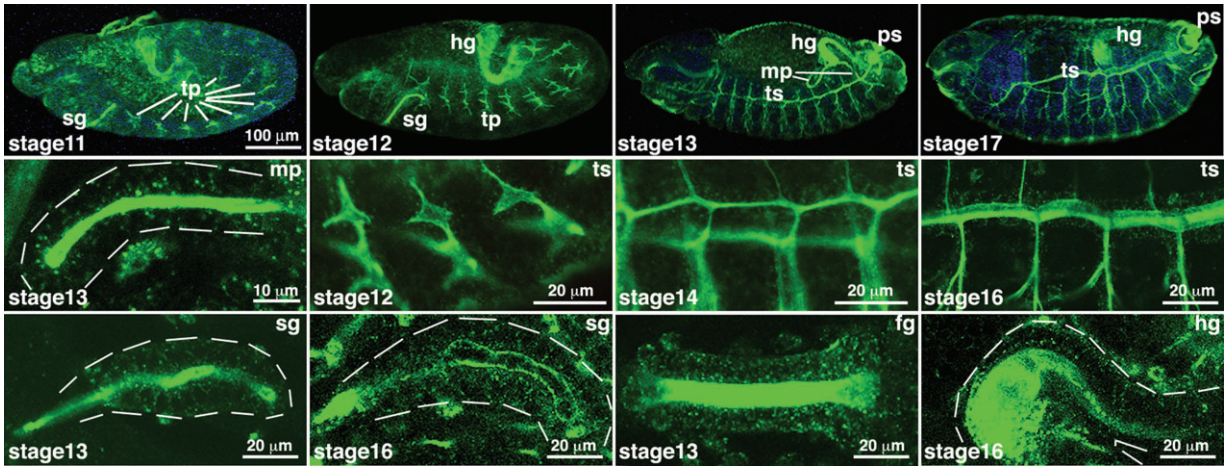

Fig. 3. Mucin-type *O*-linked glycan expression is found throughout *Drosophila* embryogenesis. Tn Ag (GalNAcα1-S/T) was detected by immunofluorescence and confocal imaging using antibodies directed against this glycan (described in Tian and Ten Hagen 2007a). Embryos at various stages of development (shown in the bottom-left corner of each image) are shown across the top row. The bottom and middle rows show enlarged images of developing tubular structures (denoted in the top-right corner of each image). *O*-Glycans are abundant along the apical and luminal regions of the developing organs shown. Dashed white lines are included to illustrate the outer boundaries of certain organs. fg, foregut; hg, hindgut; mp, malpighian tubules; ps, posterior spiracles; sg, salivary gland; tp, tracheal placodes; ts, tracheal system. Adapted from *Glycobiology*, **17,** 820-827 (2007) by copyright permission of Oxford University Press.

normally present along the apical and luminal surfaces of the developing respiratory system in the fly were absent in *pgant35A* maternal/zygotic mutants. Proteins normally seen along the apical and luminal regions of the tracheal system were found in cytoplasmic vesicles, and septate junction (tight junction) proteins normally found along the lateral regions of cells comprising the tracheal tubes were mislocalized to more apical positions, indicating defects in apicobasal polarity. The resultant tracheal tubes were irregular in shape and diameter and lacked an intact diffusion barrier. These results suggest a role for mucin-type *O*-glycans in proper formation of the apical and luminal surfaces of the tracheal system, possibly by influencing trafficking/maintenance of proteins destined for those surfaces. Roles for mucin-type *O*-glycans in mammalian organ formation and tubulogenesis were also seen in mice deficient for the core 1 β3-GalT, where mice displayed defective vasculature formation and died embryonically from fatal brain hemorrhages (Xia et al. 2004). Additionally, hypomorphic mutations in core1 β3-GalT resulted in defective glomeruli and proximal tubules in the kidney, adding additional support for the role of mucin-type *O*-glycans in tubulogenesis across diverse species (Alexander et al. 2006).

O-Linked Fucose and Glucose in Conserved Signaling Pathways. The identification of the role of glycans in the evolutionarily conserved Notch signaling pathway has generated tremendous interest (reviewed in Stanley 2007). Genetic screens in *Drosophila* initially identified the gene *fringe* (*fng*) as a key regulator of Notch signaling during development (Cohen et al. 1997; Fleming et al. 1997; Panin et al. 1997; Klein and Arias 1998). *fng* expression was shown to enhance the activation of Notch signaling by the ligand Delta while inhibiting activation by the ligand, Serrate (Fleming et al. 1997; Panin et al. 1997). Biochemical and genetic studies revealed that *fng* encodes a glycosyltransferase responsible for the addition of GlcNAc in a β1,3-linkage to *O*-linked fucose present on protein substrates, such as the epidermal growth factor (EGF) repeats of Notch and its ligands, Delta and Serrate (Bruckner et al. 2000; Moloney et al. 2000; Panin et al. 2002). These studies provided the first evidence for a regulatory role of this type of glycan in a highly conserved signaling pathway. Cell culture studies demonstrated that the presence of this glycan affects receptor/ligand binding and may also affect subsequent signaling events (Lei et al. 2003; Okajima et al. 2003). Subsequent studies in mammalian systems have verified the role of these glycans in Notch signaling (reviewed in Stanley 2007), illustrating the utility of *Drosophila* to decipher glycan function across species.

The identification of the GlcNAc-Fuc disaccharide on Notch and its receptors lead to a search for the glycosyltransferase that is responsible for the addition of the core fucose. Two protein *O*-fucosyltransferases (OFUT1 and OFUT2) exist in the fly; OFUT1 is primarily responsible for adding fucose to the EGF repeats of Notch, Delta, and Serrate (Okajima and Irvine 2002; Panin et al. 2002; Okajima et al. 2003) while OFUT2 adds fucose to thrombospondin type I repeats (TSRs), but not EGF repeats (Luo, Koles, et al. 2006; Luo, Nita-Lazar, et al. 2006;). Genetic studies in the fly indicate that the role of the OFUT1 fucosyltransferase in Notch signaling is quite complex and not solely a function of the addition of *O*-linked fucose. In support of this, it has been shown that certain Notch signaling defects in *ofut1* mutants can be rescued by a catalytically inactive form of OFUT1 (Okajima et al. 2005). This is the case during *Drosophila* embryonic neurogenesis, where catalytically inactive OFUT1 can restore Notch signaling during nervous system development (Okajima et al. 2008). This fucosyltransferase-independent function of OFUT1 is further supported by the observation that mutants in the GDP-fucose transporter (*Gmd*) (which fail to transport GDP-fucose into the ER/Golgi and are defective in fucosylation) do not display Notch-related nervous system defects, indicating that fucose addition to Notch per se is not required for nervous system development even though the OFUT1 protein is required (Okajima et al. 2008). Based on Notch localization studies, the authors propose that the OFUT1 protein has a chaperone activity responsible for proper folding,

secretion, and/or cell-surface expression of Notch that is independent of its enzymatic activity (Okajima et al. 2005; reviewed in Stanley 2007).

A number of Notch signaling defects do, however, appear to depend on the fucosyltransferase activity of OFUT1 and are mimicked in *Gmd* mutants (Ishikawa et al. 2005; Okajima et al. 2005). These defects phenocopy *fng* defects, suggesting that the addition of *O*-fucose by OFUT1 in this instance is necessary to form the substrate for the Fng glycosyltransferase. Additional work also indicates that the removal of an *O*-fucose site from Notch affects ligand binding in the absence of Fng, suggesting that the *O*-fucose may function in receptor–ligand interactions independent of serving as a substrate for GlcNAc addition by Fng (Lei et al. 2003). Thus, it appears that OFUT1 performs different functions in different developmental contexts, some of which are dependent upon fucosyltransferase activity and others that involve a chaperone function independent of enzymatic activity. While the mammalian orthologue of OFUT1 (Pofut1) does not appear to have a similar chaperone activity, it is clear that genetic studies in the fly have led to significant insights into the roles of *O*-fucose glycans in a major, conserved signaling pathway in higher eukaryotes.

Most recently, genetic studies in the fly identified yet another glycan involved in Notch signaling (Acar et al. 2008). The protein *O*-glucosyltransferase, Rumi, is responsible for the addition of *O*-linked glucose to serine residues in certain EGF repeats of Notch, serving to regulate Notch folding and/or trafficking. Unlike OFUT1, the influence of Rumi on Notch signaling is dependent on its glycosyltransferase activity. Continued work in the fly will no doubt shed more light on the mechanistic role of these glycans and others in the regulation of Notch signaling during eukaryotic development.

Glycosphingolipid (GSL) Function in Drosophila. Glycosphingolipids (GSLs) in *Drosophila* consist primarily of the Manβ1-4Glcβ1-ceramide core (arthro-series), which can be elongated by additional sugars (such as GalNAc, GlcNAc, and Gal) or phosphoethanolamine (reviewed in Seppo and Tiemeyer 2000). The GSL core structure (Glc-ceramide) is synthesized by glucosyl ceramide synthase (DGlcT-1) in flies, which transfers glucose from UDP-glucose to ceramide (Cer) (Kohyama-Koganeya et al. 2004). RNAi to *DGlcT-1* resulted in increased apoptosis, possibly due to increased ceramide levels, which are known to be pro-apoptotic (Kohyama-Koganeya et al. 2004). The next step in GSLs synthesis is controlled by the *egghead (egh)* gene, which encodes a GDP-mannose:βGlc β1,4-mannosyltransferase responsible for forming Manβ1-4Glcβ1-Cer (Wandall et al. 2003, 2005). The *brainiac (brn)* gene, encoding a UDP-GlcNAc:βMan β1,3-GlcNAc transferase, then adds GlcNAc to form GlcNAcβ1-3Manβ1-4Glcβ1-Cer (Muller et al. 2002; Wandall et al. 2005). Mutations in *egh* or *brn* cause loss of apicobasal polarity in the follicular epithelium, indicating a role for these genes in epithelial maintenance and cell adhesion (Goode, Melnick, et al. 1996; Goode, Morgan, et al. 1996). Additionally, these mutants displayed certain neurogenic phenotypes, such as loss of ventral and cephalic epidermal cells and hypertrophy in certain regions of the nervous system. These phenotypes mimicked those seen for certain other signaling molecules (Notch and EGF), suggesting that GSLs synthesized by *egh* and *brn* may be involved in regulating receptor–ligand interactions by altering occupancy in lipid rafts.

GSL chains are further modified by the addition of neutral sugars. To that end, two members of the β1,4-*N*-acetylgalactosyltransferase enzyme family (β1,4 GalNAcTs) have been identified in *Drosophila* (β4GalNAcTA and β4GalNAcTB) (Haines and Irvine 2005). Recent work indicates that β4GalNAcTA and β4GalNAcTB modify GSLs by the addition of GalNAc to the GlcNAcβ1-3Manβ1-4Glcβ1-Cer structure (Chen et al. 2007; Stolz et al. 2008). Analysis of GSL structures in β4GalNAcTA and β4GalNAcTB single mutants indicates that β4GalNAcTB is the major enzyme responsible for GSL modification (Stolz et al. 2008). While mutations in β4GalNAcTB produced epithelial defects in ovarian follicle cells in a small proportion of animals (Chen et al. 2007), β4GalNAcTA mutants displayed altered behavioral (Haines and Irvine 2005) nerve and muscle phenotypes (Haines and Stewart 2007), suggesting that these enzymes are not functionally redundant, but rather have unique roles in vivo. Collectively, these studies demonstrate that two genes with presumably the same enzymatic activity and expression patterns have unique roles in GSL biosynthesis. This raises the possibility that one or both may also be involved in modifying other glycans (Sasaki et al. 2007) or that one may require a cofactor, adaptor, or chaperone that modulates its activity (Chen et al. 2007). However, no effects on viability or fertility were noted, even in animals doubly mutant for both genes, indicating that extension of the GSLs by these enzymes is not required for survival.

Conclusions

The studies summarized herein highlight the recent progress that has been made in defining the genes responsible for glycan biosynthesis in *Drosophila* as well as their unique biological functions through the use of powerful genetic and molecular techniques unique to this organism. Taking advantage of genome-wide RNAi screens both in cell culture as well as in the fly itself will further aid our fundamental understanding of the biological role of glycans and the enzymes that form them. Future studies defining the repertoire of proteins that are glycosylated as well as the glycan binding molecules with which they interact will shed light on the mechanistic role of glycans in many conserved aspects of biology. Recently 205 glycoproteins carrying *N*-linked glycans were identified in the *Drosophila* brain (Koles et al. 2007). The repertoire of proteins carrying this modification is very diverse, including extracellular matrix proteins, cell adhesion proteins, transporters, cell surface receptors, proteases, ion channel components, and enzymes involved in a wide variety of metabolism and cellular functions. Other recent studies have begun to define proteins modified by mucin-type *O*-linked glycosylation in *Drosophila* cells, including those comprising the extracellular matrix, as well as pathogen recognition proteins, stress response proteins, secreted proteases, and protease inhibitors (Schwientek et al. 2007). While a great deal has been discovered in the fly, it only serves to highlight that we are still firmly on the tip of the iceberg in terms of understanding the complex roles of glycans during all stages of eukaryotic development. Ongoing efforts by many talented groups will help to unravel the complexities of glycobiology in many diverse developmental systems and organisms.

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Abbreviations

Cer, ceramide; CNS, central nervous system; EGF, epidermal growth factor; GAG, glycosaminoglycan; Gal, galactose; GalNAc, *N*-acetylgalactosamine; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; GSL, glycosphingolipid; Fuc, fucose; ppGaNTase or ppGalNAcT or *pgant* or PGANT, UDP-GalNAc:polypeptide *N*-acetylgalactosaminyltransferase; Man, mannose.

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