NULL MUTATIONS IN DROSOPHILA N-ACETYLGLUCOSAMINYLTRANSFERASE I PRODUCE DEFECTS IN LOCOMOTION AND A REDUCED LIFESPAN

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Running Title: Characterization of Drosophila GlcNAcT1 mutants

Summary

UDP-GlcNAc:α3-D-mannoside β1,2-N-acetylglucosaminyltransferase I (encoded by Mgal1) controls the synthesis of hybrid, complex and paucimannose N-glycans. Mice make hybrid and complex but little or no paucimannose N-glycans. In contrast, Drosophila melanogaster and Caenorhabditis elegans make paucimannose but little or no hybrid nor complex N-glycans. To determine the functional requirement for β1,2-N-acetylglucosaminyltransferase I in Drosophila, we generated null mutations by imprecise excision of a nearby transposable element. Extracts from Mgal1/Mgal1 null mutants showed no β1,2-N-acetyl-glucosaminyltransferase I enzyme activity. Moreover, mass spectrometric analysis of these extracts showed dramatic changes in N-glycans compatible with lack of β1,2-N-acetylglucosaminyltransferase I activity. Interestingly, Mgal1/Mgal1 null mutants are viable but exhibit pronounced defects in adult locomotory activity when compared to Mgal1/Cyo-GFP heterozygotes or wildtype flies. In addition, null mutants are male sterile and have a severely reduced mean and maximum lifespan. Microscopic examination of mutant adult fly brains showed the presence of fused β lobes. The removal of both maternal and zygotic Mgal1 also gave rise to embryos that no longer express the horseradish peroxidase antigen within the central nervous system. Taken together, the data indicate that β1,2-N-acetyl-glucosaminyltransferase I-dependent N-glycans are required for locomotory activity, lifespan and brain development in Drosophila.

Introduction

According to recent genome project estimates, the human and fruit fly genomes contain about 24,000 and 14,000 genes respectively (http://www.ensembl.org). However, the number of functionally discrete proteins encoded by either...
of these genomes is probably increased by at least one order of magnitude due to post-translational modifications (PTM) and processes such as gene splicing. PTM has been implicated in many important processes, e.g., signal transduction cascades, growth, transformation and memory formation (1,2). Glycosylation is one of the most common PTM. Protein-bound glycans are often branched and composed of different monomeric sugar components connected by one of several different linkages. This type of structure confers on glycans the ability to carry a great deal of information in very compact structures and thereby to mediate many different functions (3).

Our laboratory is interested in the synthesis and function of glycans conjugated to proteins by β-linkage of N-acetylglucosamine (GlcNAc) to the amido group of Asn (N-glycans). The first phase of N-glycan synthesis involves the assembly of a lipid-linked precursor Glc\textsubscript{3}Man\textsubscript{3}GlcNAc\textsubscript{2}pyrophosphate-dolichol and the oligosaccharyltransferase-catalyzed transfer of the Glc\textsubscript{3}Man\textsubscript{3}GlcNAc\textsubscript{2}moiety to an Asn residue within an Asn-X-Ser/Thr sequon (4). The second phase involves the processing, within the lumen of the endoplasmic reticulum and Golgi apparatus, of Asn-linked Glc\textsubscript{3}Man\textsubscript{3}GlcNAc\textsubscript{2} to Man\textsubscript{3}GlcNAc\textsubscript{2} (5,6) (Figures 1, 2A). The final phase of the pathway (7) occurs in the Golgi apparatus and involves the conversion of Man\textsubscript{3}GlcNAc\textsubscript{2}-Asn to hybrid, paucimannose and complex N-glycans (Figure 2A). UDP-GlcNAc:α3-D-mannoside β1,2-N-acetylglucosaminyltransferase I (GlcNAcTI, encoded by Mgtat1) converts Man\textsubscript{3}GlcNAc\textsubscript{2}-Asn to the hybrid N-glycan GlcNAcMan\textsubscript{3}GlcNAc\textsubscript{2}-Asn. This is followed by the action of α3,6-mannosidase II to form the hybrid N-glycans GlcNAcMan\textsubscript{3}GlcNAc\textsubscript{2}-Asn and GlcNAcMan\textsubscript{3}GlcNAc\textsubscript{2}-Asn (Figure 2A). In vertebrates, GlcNAcMan\textsubscript{3}GlcNAc\textsubscript{2}-Asn is converted to complex N-glycans by the action of UDP-GlcNAc:α6-D-mannoside β1,2-N-acetylglucosaminyltransferase II (GlcNAcTIII) and other branching GlcNAcTs (7). Further action by other glycosyltransferases (galactosyl-, sialyl- and fucosyltransferases) on the distal non-reducing ends of the glycan creates a large variety of complex N-glycans.

In plants (8), insects (9) and C. elegans (10), an unusual β-N-acetylglucosaminidase removes most of the GlcNAc residues inserted by GlcNAcTI before GlcNAcTII can act. The insect β-N-acetylglucosaminidase cannot hydrolyse GlcNAcMan\textsubscript{3}GlcNAc\textsubscript{2} and acts further downstream on GlcNAcMan\textsubscript{3}GlcNAc\textsubscript{2}Fuc\textsubscript{0,1} after the action of α3,6-mannosidase II (Figure 2A) (9). Drosophila (11) and C. elegans (12) make paucimannose N-glycans (Man\textsubscript{3,4}GlcNAc\textsubscript{2}Asn) but little or no hybrid or complex N-glycans (Figure 2A). Insect glycoproteins carry relatively large amounts of Man\textsubscript{3,4}GlcNAc\textsubscript{2} paucimannose N-glycans with or without α1-6- and/or α1-3-linked fucose residues on the Asn-linked core GlcNAc (13,14). Structures have also been reported with extension of the Man\textsubscript{3}GlcNAc\textsubscript{2} paucimannose N-glycan by addition of GlcNAc to the α1-3-Man terminus with or without further addition of Fuc and Gal residues (15-18). A Drosophila gene encoding a functional sialyltransferase has been reported (19); this finding is compatible with extension of glycans with sialic acid. As previously found in vertebrates, insect α3,6-mannosidase II (20,21), GlcNAcTII (22) and some α1,3/α1,6-fucosyltransferases (22,23) require the prior action of GlcNAcTI.

The first committed step towards synthesis of Glc\textsubscript{3}Man\textsubscript{3}GlcNAc\textsubscript{2}-pyrophosphate-dolichol is catalyzed by UDP-GlcNAc:dolichylphosphate GlcNAc-1-phosphate transferase (GPT). This step is essential for the synthesis of all N-glycans. Tunicamycin, a GlcNAc analogue that is a competitive inhibitor of GPT, is toxic to yeast (24) and to mammalian cells in culture (25). Tunicamycin prevents normal mouse embryogenesis (26,27). GPT-null mouse embryos complete preimplantation development but die 4-5 days after fertilization; neither trophoblast nor embryonic endodermal lineages derived from these early embryos survive in culture in vitro indicating that N-glycosylation is needed for the viability of early embryonic cells (28). In contrast, Chinese hamster ovary cells suffer no obvious phenotypic abnormalities in the absence of GlcNAcTI (29). Mgtat1 null mice, however, die at embryonic stage E9.5 days (30,31). The data
indicate that the oligomannose N-glycans made in the first two phases of N-glycan synthesis are essential to the survival of both unicellular and multicellular animals whereas GlcNAcTI-dependent N-glycans are needed for normal vertebrate development but not for the survival of individual cells. We present evidence in this paper that GlcNAcTI-dependent N-glycans are also needed for the normal development of D. melanogaster.

Vertebrate glycan function has been studied by analysis of mice and humans with mutations in genes required for glycosylation (32-34). Such studies are complicated by the fact that synthesis of glycans requires a complex multi-enzyme system acting on a large number of protein targets. In the expectation that the relatively primitive N-glycan synthesis pathway in Drosophila and C. elegans may be more amenable to functional analysis than the vertebrate pathway, we have initiated studies on Mgat1 null mutations in these invertebrates. We have shown that C. elegans Mgat1 deficient mutants are viable and have an apparently normal phenotype when grown under standard laboratory conditions but show decreased survival times when exposed to pathogenic bacteria (35). We have reported the cloning and expression of the Drosophila Mgat1 gene (36). Here we show that null mutations in Drosophila Mgat1 give rise to viable adults with dramatically altered N-glycans that result in pronounced defects in locomotion, a severely reduced lifespan and abnormal brain development.

Experimental Procedures

Fly stocks and generation of mutants

All fly stocks were maintained at room temperature on standard cornmeal agar media. The line y w67c23; Mgat1KGO2444 (Bloomington Stock Center, stock BL-13222) contains a P-element insertion at the 5' end of Mgat1 and was used to generate both the precise excision line Mgat1 +9 and the imprecise excision allele Mgat1-. Since Mgat1 +9 was generated at the same time as the Mgat1- allele and is in the same genetic background, this line was used as a control for all of our subsequent analyses (GlcNAcTI assays, mass spectrometry, immunohistochemistry, locomotion and lifespan) and behaved as wild type. +9; Sp/CyO: ShΔ2-3/UM6, Ubx was used as a source of transposase. The excision breakpoints were determined by PCR analysis and sequencing using the primers F1: 5’ CCGATTGGGTTAGGTAAT and R3: 5’ CTGAGAGTTGCACACTTTC. The P-element line l(2)05510cn1/CyO; ry506 contains a lethal mutation in the gene immediately upstream of Mgat1 (stock BL-12192).

GlcNAcTI enzyme assay

Fourteen adult flies from each group were homogenized in 0.2 ml of 25 mM MES buffer pH 6.5 containing 1% Triton X-100 and protease inhibitor cocktail. GlcNAcTI activity was measured using 0.6 mM of Manac1-6Manac1-3Manβ1-O-n-octyl (Toronto Research Chemicals, Toronto, Canada) as acceptor substrate and 1.2 mM of UDP-[3H]GlcNAc (100,000 dpm/nmol) as donor substrate. The assay mixture also contained 3.0 mM AMP, 60 mM GlcNAc, 20 mM MnCl2, in 0.05 M MES buffer pH 6.5 and 0.01 or 0.02 ml enzyme in 0.04 ml total volume. Time of incubation was 60 min at 37°C. The assays were carried out as previously described (36). The rate of product formation was proportional to enzyme volume.

Mass spectrometric analysis of N-glycans of wildtype and mutant Drosophila.

Adult flies (~ 0.5 g Mgat1+/Mgat1 +/+ , Mgat1+/CyO-GFP, Mgat1+/Mgat1−) were anesthetized with carbon dioxide, suspended in 0.3 ml water and boiled for 10 min. The preparation of N-glycans was performed as previously described (11,37). Proteins were extracted in lysis buffer (35 mM Tris, 8 M urea, 4% CHAPS, 65 mM DTT, pH 8.0), and centrifuged at 10,000 x g for 15 min. The protein content of the supernatant was determined using the Bradford assay (38). Protein in the supernatant was precipitated with 15% trichloroacetic acid on ice for 1 h. The protein pellet was washed with 1 ml acetone (3 times) and 1 ml chloroform-methanol-water (10:10:1) (3 times), dried under nitrogen and stored at -20°C. The protein pellet was lyophilized prior to
treatment with protein N-glycanase F (PNGase F). The released glycans were purified and subjected to MALDI-TOF-MS (matrix-assisted laser desorption/ionisation-time of flight mass spectrometry). The glycans were reduced with NaBH₄ (0.2 ml of 10 mg/ml NaBH₄ in 10 mM NaOH) at room temperature overnight. Borate was removed by adding two drops of acetic acid on ice, followed by co-evaporation with 3 ml ethanol, 3 ml 1% acetic acid in methanol (5 times), and 1 ml toluene (3 times). The reduced glycans were desalted, permethylated (39) and analyzed by MALDI-IT-TOF (IT = ion trap) MS for confirmation. CID (collision induced dissociation) was performed using MALDI-QIT MS (Kratos-Shimadzu Biotech; QIT = quadrupole ion trap) with 2,5-dihydroxybenzoic acid as matrix (40). All the structures reported in this paper were confirmed by derivatization and MALDI-IT-MS.

**Locomotory Activity**

The locomotory activity of adult male and female flies was measured using a slightly modified open field test (41). Briefly, individual flies from each genotype were placed in a covered petri-dish (15 mm) and allowed to adapt to their environment for 5 min. The length of time adult flies were moving over a period of 3 min was then measured. Twenty-five flies of each genotype were used for each experiment.

**Lifespan Determination**

The lifespan of \( \text{Mgat1}^{+/+} / \text{Mgat1}^{+/+} \) adults was compared to \( \text{Mgat1}^{+/+}/\text{CyO-GFP} \) and \( \text{Mgat1}^{+/-}/\text{Mgat1}^{+/-} \) flies. Briefly, an overnight egg collection was obtained from \( \text{Mgat1}^{+/+}/\text{CyO-GFP} \) flies and aged for 24-36 hrs. Upon hatching into first instar larvae, \( \text{Mgat1}^{+/+}/\text{Mgat1}^{+/+} \) homozygotes were sorted from \( \text{Mgat1}^{+/+}/\text{CyO-GFP} \) heterozygotes by the absence (\( \text{Mgat1}^{+/+}/\text{Mgat1}^{+/+} \)) or presence (\( \text{Mgat1}^{+/+}/\text{CyO, GFP} \)) of a GFP marker. The sorted larvae were then transferred to vials containing standard medium and allowed to develop. Adult flies were removed as soon as they eclosed and placed into fresh vials (10 flies/vial). The starting population size for each genotype was 100. The males and females were kept in separate vials. Dead flies were scored and vials were changed every 3 days. For statistical analysis, the mean and maximum lifespan of each strain was calculated from the time in days at which survival reached respectively 50% and 10% of the starting population in each of the 10 cohorts of each strain.

**Immunocytochemistry**

Embryos from homozygous and heterozygous \( \text{Mgat1}^{+/+} \) flies were collected overnight on grape plates, fixed using standard conditions and double labeled with antibodies to Elav (a neuron-specific antigen) and horseradish peroxidase (HRP) or GFP and HRP as described (42). Primary antibodies used were rat anti-Elav, 1:10 (clone 7E8A10, Developmental Studies Hybridoma Bank (DSHB), University of Iowa), rabbit anti-GFP, 1:500 (Molecular Probes), and anti-HRP FITC, 1:500 (ICN Biomedicals). The secondary antibodies used were donkey anti-rat Cy3, 1:500 (Molecular Probes), and donkey anti-rabbit Cy3, 1:500 (Molecular Probes). Whole mount brains from immobilized adults 1-2 days post-eclosion were dissected in cold PBS (pH 7.2), fixed for 15 min at room temperature (RT) in 4% paraformaldehyde in PBS, and washed in PBT (PBS with 0.3% Triton X-100). The brains were then blocked for 30 min at room temperature in 5% normal donkey serum (Chemicon) in PBT. Primary and secondary antibody labeling was performed overnight at 4°C in blocking solution. Antibodies used were mouse anti-Fasciclin II (FasII), 1:5 (clone 1D4, DSHB) and donkey anti-mouse Cy3, 1:500 (Molecular Probes). Washes between steps were performed with PBT at room temperature. Embryos and brains were mounted in antifade (2% DABCO (Sigma), 70% glycerol in 0.12 M Tris-HCl, pH 7.6). Epifluorescent images were acquired with a Leica DMRA-2 microscope equipped with a Hamamatsu Orca-ER digital camera. Images were processed using Improvision OpenLab v3.1.7 and Adobe Photoshop v5.5 software.

**Results**

**Generation of Mgat1 Mutants**

As a first step to identify the \textit{in vivo} function of \( \text{Mgat1} \), we characterized the genomic structure of the \( \text{Mgat1} \) gene from \textit{Drosophila}. Unlike C.
elegans, which contains three Mgat1 genes, the Drosophila genome contains a single Mgat1 gene that is contained within 3.2 kb of genomic DNA and is flanked at the 5' end by the gene l(2)05510 and at the 3' end by the gene CG13424 (43). The predicted intron/exon structure of Drosophila Mgat1 is illustrated in Figure 3. As previously described (36), Mgat1 gives rise to a 2.8 kb cDNA predicted to encode a 458 amino acid protein with 52% amino acid sequence identity to human GlcNAcT1. To determine the functional requirement for Mgat1 in flies, we then generated a series of Mgat1 mutants by imprecise excision of a transposable P-element, KG02444, located within the first exon of Mgat1, 545 bp 5′ of the start-ATG (Figure 3). In total, we generated three independent deletions that removed various portions of the Mgat1 gene and failed to complement each other. Importantly, all of these mutants complemented the mutation in the gene located immediately 5′ to Mgat1, l(2)05510, demonstrating that our excisions are specific to Mgat1. All of the Mgat1 mutants were homozygous viable although the adults appeared sluggish (see below). To identify potential null mutants in Mgat1, we used PCR analysis to map the breakpoints and found one line, Mgat1′ that deleted most of the first and all of the second exon including the translational start site. At the same time, we also identified a precise excision of the P-element, Mgat1′9, which restored the Mgat1 locus and was subsequently utilized as a wild type, genetic control for all of our remaining experiments.

To confirm that Mgat1′ was in fact a null allele, we then measured GlcNAcT1 activity from extracts derived from Mgat1′/Mgat1′ flies and compared these to Mgat1′/CyO-GFP and the wild type precise excision line Mgat1′9/Mgat1′9. We found that homozygous mutant flies had no detectable GlcNAcT1 activity whereas heterozygotes exhibited intermediate levels (105 +/- 29 pmol/hr/mg protein) compared to the wild type controls (259 +/- 40 pmol/hr/mg protein).

Comparison of N-glycans from wild type and Mgat1 mutant flies.

To determine if the synthesis of oligomannose, hybrid, complex and paucimannose N-glycans was affected in the Mgat1′/Mgat1′ mutants we examined the levels of N-glycans released by PNGase F using MALDI-TOF mass spectrometric analysis (40). The N-glycan patterns obtained from wild type (Mgat1′9/Mgat1′9) (Table 1, Figure 4A) and mutant (Mgat1′/Mgat1′) (Table 1, Figure 4B) flies were compared. As previously described (11,13,14), the dominant N-glycan structures found in wild type adult flies were paucimannosidic (M3Gn2F6, M3Gn2, M4Gn2) and oligomannosidic (M5Gn2) N-glycans (Table 1, Figure 2A). In contrast, Mgat1′/Mgat1′ flies showed a dramatic decrease in the amount of M3Gn2F to almost undetectable levels (Table 1, Figure 2B). The amount of M3Gn2F3 in wild type flies is very small (14,44) indicating that almost all the decrease in M3Gn2F in mutant flies is due to M3Gn2F6. Furthermore, since both core α3-FucT (14) and core α1,6-FucT (44) require the prior action of GlcNAcT1, the very small MS peak for M3Gn2F seen in GlcNAcT1-null fly extracts (Table 1) is due to a different as yet uncharacterized FucT (Figure 2B, product M3Gn2F6). M4Gn2F, GmM3Gn2 and GnM3Gn2F were not detected in Mgat1′/Mgat1′ flies, and M3Gn2 and M4Gn2 were moderately decreased (Table 1). The mutant flies also showed a significant accumulation of M5Gn2 and small increases in M6Gn2, M7Gn2, M8Gn2 and M9Gn2 (Table 1); these structures are synthesized upstream of the GlcNAcT1 block. The values for the heterozygous flies were intermediate between the wild type and mutant values (Table 1).

M3Gn2F6 (with both an α1-3- and α1-6-linked Fuc residue on the same Asn-linked core GlcNAc), Gm2M3Gn2 and Gm2M3Gn2F (Figure 2A) have been reported in extracts of flies (14) and in cultured Drosophila cells (45); however, these structures were not observed in the analysis shown in Table 1 probably because we used PNGase F to release N-glycans whereas Fabini et al. (14) and Rendic et al. (45) used PNGase A. Biosynthetic pathways based on the structural
analyses in Table 1 and the work of others (14,45) are shown in Figures 2A and 2B.

**Mgat1**/**Mgat1** mutants are male sterile

Although *Mgat1*/*Mgat1* homozygotes were viable, we found that homozygous mutant males, but not females, were sterile. To determine the nature of the sterility we examined the testes from *Mgat1*/*Mgat1* males and found that they produced normal levels of mobile sperm indicating that the sterility was not due to defects in spermatogenesis (data not shown). We then determined whether *Mgat1*/*Mgat1* males were able to mate with, and fertilize females. In wild type flies, females store sperm within structures called spermathecae after mating. While we were able to detect mobile sperm within the spermathecae of females mated to *Mgat1*/* or *Mgat1*/CyO-GFP males, we could not detect any sperm in females mated to *Mgat1*/*Mgat1* males (data not shown). This suggests that the sterility defect is associated with a failure of *Mgat1*/*Mgat1* males to mate.

**Mgat1**/**Mgat1** mutants exhibit defects in locomotory activity and a reduced lifespan

Consistent with the observation that *Mgat1*/*Mgat1* males did not mate with wild type females, we also found that *Mgat1*/*Mgat1* adults appeared sluggish and slower in their movements. To quantify any potential locomotory defects, we measured the amount of time mutant and control flies spent moving during a 3 min period using an open field test. We found that *Mgat1*/*Mgat1* adults showed >95% reduction in movement compared to heterozygous and wild type controls (Table 2). Specifically, we found that *Mgat1*/*Mgat1* males moved for 7.1 ± 16.3 seconds while *Mgat1*/CyO-GFP males moved for 153 ± 15.4 seconds and *Mgat1*/*Mgat1* males moved for 156 ± 20.0 seconds. Similar differences were observed in mutant females compared to controls. Overall, *Mgat1*/*Mgat1* males and females differed significantly from all other groups (p<0.001).

In addition to the locomotory defects, *Mgat1*/*Mgat1* mutants also appeared to die earlier than wild type flies. Of note, mutant adults were recovered only when mutants were isolated as first instar larvae and allowed to develop at low density in the absence of wildtype larvae. Under these conditions, approximately two thirds of the mutants eclosed as pupae. The remaining third appear to die throughout larval development. Mutant adults that emerged showed a normal external morphology but appeared to die earlier than their wildtype or heterozygous counterparts.

To determine if there was a significant difference in lifespan between homozygous mutants and controls, we determined the mean and maximum lifespan for both males and females (Table 3). We found that *Mgat1*/*Mgat1* males and females had a severely reduced lifespan compared to both *Mgat1*/CyO-GFP and *Mgat1*/CyO-GFP controls. Specifically, *Mgat1*/*Mgat1* males had a mean lifespan of 12.8 days (50% survival) and a maximum lifespan of 16.7 days (10% survival) whereas *Mgat1*/*Mgat1* females had a mean lifespan of 13.9 days and a maximum lifespan of 21.3 days. This is significantly different than either *Mgat1*/CyO-GFP flies (mean lifespan for males = 67.9 days, maximum lifespan = 79.1 days; mean lifespan for females = 86.1 days, maximum lifespan = 93.6 days) or *Mgat1*/CyO-GFP flies (mean lifespan for males = 75.1 days, maximum lifespan = 80.1 days; mean lifespan for females = 76.7 days, maximum lifespan = 84.1 days). Mutant flies grown under sterile conditions showed the same marked reduction in lifespan indicating that infection by pathogenic microorganisms was not responsible for the reduced lifespan (data not shown). Taken together, these data clearly demonstrate that *Mgat1* is required for locomotory activity and survivorship in flies.

The Central Nervous System (CNS) of maternal-null *Mgat1*/*Mgat1* embryos does not bind anti-HRP

Antibodies raised against the plant glycoprotein HRP have been used to specifically label *Drosophila* and *C. elegans* neurons. A specific α1,3-fucosyltransferase (α1,3-FucT) that adds an α1-3-linked fucose to the proximal *N-*glycan core is essential for synthesis of the HRP epitope in both *C. elegans* (46) and *Drosophila*...
In vitro studies of the α1,3-FucT (FUT-1) required for HRP epitope synthesis by *C. elegans* have shown that the enzyme does not require the prior action of GlcNAcTI (46). Furthermore, a *C. elegans* strain with null mutations in all three GlcNAcTI genes (37) displayed normal staining with anti-HRP in the complete absence of GlcNAcTI enzyme activity (46). In contrast, the *Drosophila* α1,3-FucT (FucTA) required for synthesis of the HRP epitope acts only on substrates that require the prior action of GlcNAcTI (14,45). However, we initially observed that *Mgat1*/*Mgat1* mutant and *Mgat1*/CyO-GFP control embryos exhibit similar levels of staining with anti-HRP (data not shown). A possible explanation for this discrepancy is the presence of maternally derived *Mgat1* mRNA in the *Mgat1*/*Mgat1* mutant embryos. Indeed, retention of maternally derived *Mgat1* mRNA has been demonstrated in pre-implantation *Mgat1* mutant mouse embryos (47). Consistent with this possibility, we found that unlike *Mgat1*/CyO-GFP, *Mgat1*+/*Mgat1* or other wild type brains, *Mgat1*/Mgat1* adult fly brains do not show anti-HRP staining (data not shown). To prove that the anti-HRP immunoreactivity observed in *Mgat1*/Mgat1* embryos was due to maternal contribution, we examined embryos obtained from *Mgat1*/Mgat1* females mated to *Mgat1*/CyO-GFP males. The resulting *Mgat1*/Mgat1* embryos (which lack any maternal contribution) were negative for anti-HRP staining, whereas sibling *Mgat1*/CyO-GFP embryos (which also lack any maternal contribution) exhibited normal anti-HRP staining (Figure 5). This finding suggests that maternally derived *Mgat1* mRNA is responsible for the presence of the HRP epitope in null mutant embryos consistent with the observation that *Drosophila* FucTA requires prior GlcNAcTI action (14,45).

The major *Drosophila* N-glycan structure with two Fuc residues on the Asn-linked GlcNAc of the core is M3Gn2Fβα (Figure 2A), although it represents only about 0.4-1.0% of the total N-glycans in wild type flies (14); however, a cultured *Drosophila* cell line has been reported with a 19% content of M3Gn2Fβα (45). M3Gn2Fβα is at least partly responsible for the staining of fly neurons with anti-HRP antibody (44). Mass spectrometric analysis shows a small amount of M3Gn2F in the adult *Mgat1*/*Mgat1* mutants (Figures 2B, 4B) indicating the presence of GlcNAcTI-independent FucTs not involved in synthesis of the HRP epitope.

*Mgat1*/Mgat1* flies exhibit a fused-lobe phenotype

Since *Drosophila* *Mgat1*/Mgat1* mutants did not stain with anti-HRP we could not use this marker to determine if the locomotory defects observed in our mutants were due to defects in adult brain structures. To circumvent this problem, we therefore immunostained whole mount brains from control *Mgat1*+/Mgat1* flies and *Mgat1*/Mgat1* mutants with anti-FasII, which labels a subset of axons (including the mushroom bodies) within the CNS. Although we did not observe any gross morphological defects using either light microscopy or FasII staining, we did find that *Mgat1*/Mgat1* mutants exhibit a fused lobe phenotype (Figure 6). Specifically, we observed >50% reduction in the separation of the β lobes in all of the brains that we examined, with full fusion observed in 40% of the samples. This phenotype is similar to that observed in fused lobe (fdl) mutant flies (48). *fdl* mutants were first identified in an enhancer trap screen for genes that are expressed during late larval development in structures that will give rise to the central complex of the adult brain. In the case of *fdl*, insertion of the enhancer trap element also results in mildly penetrant defects in the adult brain consisting of fused β lobes in the mushroom body (48).

Interestingly, the *fdl* gene is highly homologous to hexosaminidase genes in other species suggesting that it may encode the β-N-acetylgalactosaminidase (Gnase, Figure 2A) that removes the GlcNAcTI-dependent GlcNAc residue to form paucimannose N-glycans in *Drosophila*. Indeed it has recently been reported (49) that the *fdl* gene encodes a hexosaminidase with the same substrate specificity as Gnase. Léonard *et al.* (49) studied two Fdl-deficient *Drosophila* lines. The *fdl* fly has a hypomorphic mutation in the *fdl* gene and shows the fused lobe phenotype; no other genes are mutated in this fly.
Function participates in oligosaccharide production and function (52). Protein-bound glycans have many functions (32,33,52), e.g., cell adhesion, control of the immune system, embryonic development and differentiation, and have been implicated in diseases such as metastatic cancer.

N-glycans occur primarily on secreted and membrane-bound proteins. Oligomannose N-glycans (Man3,4GlcNAc2, Figure 1), found in both unicellular and multicellular eukaryotes, are ancient structures essential for the viability of all cells. GlcNAcTII-dependent N-glycans (Figure 2) appeared in evolution at about the same time as multicellular organisms and are essential for normal mouse (30,31) and human (53,54) embryonic development consistent with a major function for these N-glycans in cell-cell and cell-environment interactions. Over twenty genes encoding enzymes involved in N-glycosylation have been implicated as causes of congenital disorders of glycosylation (CDG) in humans (34,55); the CDGs are a family of genetic multisystemic disorders with severe nervous system involvement. Both mice (56) and humans (53,54) have been reported with null mutations in Mgat2, the gene encoding GlcNAcTII downstream of GlcNAcTI (Figure 2A). Human Mgat2 deficiency is named CDG-IIa and is characterized by severe psychomotor retardation.

Whereas the GlcNAcTI-dependent structures in vertebrates are complex N-glycans with antennary extensions of the Man1-6(Mano1-3)Man1-4GlcNAc1-4GlcNAc core (Figure 2), plants, insects and C. elegans synthesize predominantly GlcNAcTII-dependent Man3,4GlcNAc2, paucimannose N-glycans (Figure 2) instead of complex N-glycans. Although the paucimannose N-glycans in Drosophila are modified by fucosylation (11,14,44), these structures lack the antennary branches typical of vertebrate complex N-glycans. We have determined the phenotypic effects of a null mutation in Mgat1 in flies in the expectation that the functions of GlcNAcTII-dependent N-glycans can be more readily analyzed in this organism than in vertebrates. The Mgat1'/Mgat1' adult flies are null mutants since extracts showed no GlcNAcTI
enzyme activity and mass spectrometric analysis showed dramatic changes in N-glycans compatible with lack of GlcNAcTI enzyme activity. Mutant adults were recovered only when animals were removed from the vial at the larval stage and allowed to develop at low density. Under these conditions, mutant pupae eclosed normally and adults showed a normal external morphology but had a significantly reduced life span. Mutant flies grown under sterile conditions showed the same marked reduction in life span indicating that infection by pathogenic microorganisms was not responsible for the reduced life span (data not shown). Mgat1<sup>−/−</sup>/Mgat1<sup>−/−</sup> mutants were also significantly more sluggish than wild type flies. Moreover, this defect in locomotion is likely responsible for the observed male sterile phenotype since mutant males produced motile sperm but were unable to mate with either mutant or wildtype females. Although we did not observe any gross morphological defects in the brains of adult mutants that could account for the locomotory phenotype, we did find that the brains of adult mutant flies did not react with an antibody to HRP. Furthermore, microscopic examination of the mutant brains showed the presence of fused β lobes within structures called mushroom bodies. Interestingly, many studies have shown that mushroom bodies are required for learning and memory in <i>Drosophila</i>. At present, the severe locomotory defects observed in Mgat1<sup>−/−</sup>/Mgat1<sup>−/−</sup> mutants preclude us from determining if the fused lobe phenotype would result in defects in learning and memory. Nonetheless, our data clearly indicate that GlcNAcTI-dependent N-glycans are required for normal development of the nervous system of the fly. Future studies, involving the identification of additional alleles or the ability to rescue the locomotory defects may allow us to determine if N-glycans are also required for higher processes such as those involved in learning and memory.

Mammalian cells in culture suffer no obvious phenotypic abnormalities when deprived of GlcNAcTI (29) consistent with the hypothesis that these cells do not require the kind of cell-cell interactions provided by vertebrate N-glycans. Mgat1<sup>−/−</sup> <i>C. elegans</i> are viable and have an apparently normal phenotype when grown under standard laboratory conditions but show altered survival times when exposed to pathogenic bacteria (35) suggesting that worms developed GlcNAcTI-dependent paucimannose N-glycans to cope with a hostile bacterial environment. This report shows that a null mutation of Mgat1 in <i>Drosophila</i> resulted in severe developmental abnormalities and supports the hypothesis that the fly uses GlcNAcTI-dependent N-glycans to mediate some cell-cell interactions during development. Mgat1<sup>−/−</sup> mice die <i>in utero</i> at embryonic day 9.5 (30,31) indicating a need for GlcNAcTI-dependent complex N-glycans during development. This graded change in importance and functions of GlcNAcTI during evolution is probably related to the differences in the types of N-glycans synthesized downstream of GlcNAcTI by the various organisms.

The dystrophin glycoprotein complex (DGC) is an assembly of proteins spanning the sarcolemma of vertebrate skeletal muscle cells. An O-mannosyl glycan (sialyα2-3Galβ1-4GlcNAcβ1-2Manα1-O-Ser/Thr) (57,58) on α-dystroglycan, one of the components of the DGC, has been identified as a receptor for laminin and other extra-cellular ligands. Defects in this O-mannosyl glycan have been associated with a distinct group of autosomal recessive congenital muscular dystrophies (59,60). Protein O-mannosyl β1,2-N-acetylglucosaminyltransferase 1 (POMGlcNAcT1), a homolog of GlcNAcTI, catalyzes the synthesis of the GlcNAcβ1-2Manα1-O-Ser/Thr moiety on α-dystroglycan. POMGlcNAcT1 is deficient in patients with Muscle-Eye-Brain disease, a congenital muscular dystrophy (61,62). <i>Drosophila</i> has functional genes encoding homologs of α-dystroglycan and the two protein β-O-mannosyltransferases (POMT1 and POMT2) that incorporate O-Man residues into α-dystroglycan (63). However, BLAST analysis of the <i>Drosophila</i> genome with either the vertebrate Mgat1 or POMGlcNAcT1 nucleotide sequence identifies Mgat1 as the only homologous gene (36,63). The enzyme encoded by <i>Drosophila</i> Mgat1 is unable to catalyze the transfer of GlcNAc to the Manα1-O-Ser/Thr moiety on α-dystroglycan (63). It can therefore be
concluded that the phenotype observed in the \( Mgat1^{+/+}/Mgat1^{+/+} \) null flies is due to abnormal \( N \)-glycan structures and is not related to \( \alpha \)-dystroglycan function.

Null mutations in the glycosylation pathways of \( Fuc\alpha 1-O\text{-}Ser/\text{Thr} \) (Notch), \( Xyl\beta 1-O\text{-}Ser \) (proteoglycans) and \( Man\alpha 1-O\text{-}Ser/\text{Thr} \) (\( \alpha \)-dystroglycan) glycoproteins in \textit{Drosophila} result in defective development (63-65). However, the role of \( N \)-glycans in fly development has received relatively little attention.

It has been estimated that as many as 50% of all proteins in humans and mice may be \( N \)-glycosylated (66). It is probable that many proteins are also \( N \)-glycosylated in \textit{Drosophila}. If a mutant animal with a defect in the \( N \)-glycosylation pathway shows an abnormality, it is essential to identify the protein or proteins targeted by the mutation. Although this is usually a difficult task, it has been achieved in some cases (67). The phenotype of \textit{Drosophila} \( Mgat1^{+/+}/Mgat1^{+/+} \) mutants is probably due to several different GlcNAcTI targets. However, the absence of the HRP epitope in mutant neurons indicates that the protein or proteins that carry the \( \alpha 1-3 \)-fucosylated \( N \)-glycan associated with the HRP epitope are among these targets. Polyclonal anti-HRP antibodies have been used to purify \textit{Drosophila} proteins carrying the HRP epitope (68). Neurotactin, fasciclin I and II, neuroglian and three receptor protein tyrosine phosphatases were identified and many other bands were seen on Western blotting with anti-HRP antibody (68). These proteins can be used to study the roles of \( N \)-glycans in protein function in \textit{Drosophila}.

**Acknowledgements**

We thank Dr. K. Iliadi for assistance and helpful discussions regarding the behavioral and lifespan experiments and Dr. J. Brill for helpful discussions about male fertility. We also thank Haddas Grosbein for her assistance with fly genetics. The anti-FasII monoclonal antibody 1D4 developed by Dr. C.S. Goodman and the anti-Elav monoclonal antibody 7E8A10 developed by Dr. G.M. Rubin were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242. This work was supported by funds from the Canadian Institutes of Health Research (CIHR) to H.S. and G.L.B., NSERC funds to G.L.B., and a CIHR Doctoral Research Award to P.A.L. G.L.B. is the recipient of a Canada Research Chair in Molecular and Developmental Neurobiology.
Figure Legends

Figure 1. N-glycan structures in Drosophila. The names assigned to these structures are used in the synthetic schemes shown in Figure 2 (M = Man; Gn = GlcNAc; F = Fuc). Oligomannose N-glycans have from 5 to 9 Man residues; only M9Gn2 and M5Gn2 are shown. M3Gn2 and M4Gn2 are paucimannose N-glycans. The remaining structures have a GlcNAcβ1-2Manα1-3 moiety and are therefore dependent on prior GlcNAcTI action. The R group is defined in the figure; there are four R variants depending on the absence or presence of core a1-3- and a1-6-linked Fuc residues (designated as F3 and F6 respectively).

Figure 2. (A) N-glycan synthesis in wildtype Drosophila. This scheme is based on the data in Table 1 and on Paschinger et al. (44). The names of the N-glycans are defined in Figure 1. Enzyme names are in italics. Reactions shown by continuous arrows have been established by in vitro assays whereas discontinuous arrows are based on other evidence (44). Arrows crossed with double lines indicate reactions that do not occur. The figure shows the conversion of oligomannose N-glycans (M9-5Gn2) to hybrid (GnM5-3Gn2), paucimannose (M4-3Gn2), and complex (Gn2M3Gn2) N-glycans. The major structure in wild type flies is M3Gn2F6 (box with a thick continuous line) (14,44); other structures present in large amounts (32-68% of M3Gn2F6) are boxed with thin continuous lines. The remaining boxed structures (discontinuous lines) are present in amounts less than 10% of M3Gn2F6. Unboxed structures have not been detected by MS but are included in the figure on the basis of other evidence (44). GlcNAcTI adds GlcNAc in β1-2 linkage to the Manα1-3 arm of M5Gn2 to form the hybrid N-glycan GnM5Gn2 (36,69). Two Man residues are removed from GnM5Gn2 by the action of α3,6-mannosidase II (MaseII) to form the truncated hybrid N-glycans GnM4Gn2 and GnM3Gn2 (6). A specific β-N-acetylgalactosaminidase not found in vertebrates (Gnase (9,49)) removes the GlcNAc added by GlcNAcTI to form M4Gn2 and M3Gn2 paucimannose N-glycans. GlcNAcTI acts on GnM3Gn2 to initiate the synthesis of complex N-glycans; this is a minor pathway in plants, insects and C.elegans because Gnase competes more effectively for substrate than GlcNAcTI. The substrates, products and reactions of the core α1,6-FucT (6FucT) and α1,3-FucT (3FucT) are shown (44). Both core α1,6-FucT (FucT6) (44) and α1,3-FucT (FucTA) (45) in Drosophila are dependent on prior GlcNAcTI action. FucT6 cannot act on structures with a core α1-3-linked Fuc and must therefore act before FucTA to make the small amounts of M3Gn2F6 in wild type Drosophila (14,44). GlcNAcTI-null flies make small amounts of fucosylated M3Gn2 (M3Gn2F6) (Table 1) indicating a GlcNAcTI-independent pathway to this structure; neither of the previously reported core FucTs are responsible because both enzymes require prior GlcNAcTI action (44). The site and linkage of the Fuc on M3Gn2F6 is unknown. GlcNAcTI-null flies make relatively large amounts of M3Gn2 and M4Gn2 (Table 1) suggesting that a GlcNAcTI-independent α-mannosidase (Mase) acts on M5Gn2 upstream of GlcNAcTI; such a mannosidase has been reported in Spodoptera frugiperda (71) but not in Drosophila. (B) N-Glycan synthesis in MgaTI null flies. The figure is based on MS analysis of the N-glycan structures in GlcNAcTI-null flies (Table 1) and was obtained by removing all arrows dependent on the action of GlcNAcTI from the wild type fly scheme (Figure 2A). The definitions of names and arrows are as for Figure 2A. The amounts of M5-M9Gn2 (boxes with continuous lines) are increased by 50-250% in the null flies. M3Gn2F6, M3Gn2 and M4Gn2 (boxes with bold discontinuous lines) are reduced by 100, 59 and 19% respectively. A small amount of M3Gn2F was observed in GlcNAcTI-null flies (Table 1); this M3Gn2F is neither M3Gn2F3 nor M3Gn2F6 (the respective FucTs require prior GlcNAcTI action) and has been designated as M3Gn2F5 (not boxed). Structures boxed with thin discontinuous lines are present in low amounts in wild type flies but are absent in the mutant flies. The arrows attached to M4Gn2F5 indicate that the structure may be an intermediate in the pathway to M3Gn2F5. (C) N-Glycan synthesis in fdl-null flies. The figure is based on MS analysis of the N-glycan structures in the fdl and Dfd(2R)achi2 fly strains; fdl flies have a hypomorphic mutation in
the *fdl* gene and *Df(2R)achi*² flies have a null mutation in *fdl* but also have mutations in five other genes (49). The *fdl* gene was recently cloned and shown to encode the β-N-acetylglucosaminidase (Gnase) that removes GlcNAc incorporated by GlcNAcTI (49). The definitions of names and arrows are as for Figure 2A. GnM3Gn2F⁶ (box with bold continuous lines), present in small amounts in wild type flies, is the major N-glycan in *fdl*-null flies. The structures in boxes with thin continuous lines show moderate increases in *fdl*-null flies. M5Gn2 (box with thin discontinuous lines) is decreased by 54%, and M3Gn2 and M3Gn2F⁶ (boxes with bold discontinuous lines) are decreased by 73 and 83% respectively in *fdl*-null flies. The small amount of fucosylated M3Gn2 (M3Gn2F², unboxed) in *fdl*-null flies (49) cannot be due to either of the previously described core FucTs since both routes require Gnase action; suggested routes to M3Gn2F² are shown. The other unboxed structures are either products of Gnase (absent in *fdl*-null flies), or structures not detected by MS, or not reported by Léonard et al. (49).

Figure 3. Structure of the *Mgat1* locus. *Mgat1* consists of 7 exons (boxes) spanning 3.2 kb. Exons 1 and 7 contain both untranslated sequence (open boxes) and parts of the ORF (filled boxes). The two genes flanking *Mgat1*, *l(2)05510* and *CG13424*, are shown with their orientation. *Mgat1* mutants were generated by imprecise excision of the P-element KG02444 (inverted triangle). The breakpoints for one of these deletions, *Mgat1¹⁴*, is illustrated and consists of a 1301 bp deletion that removes most of the first and all of the second exon, including the ATG, but does not affect the flanking genes. Scale bar=500 bp.

Figure 4. *Mgat1¹⁴* homozygotes exhibit altered N-glycan profiles. (A) MALDI-TOF MS analysis of neutral N-glycans of *Mgat1¹⁴/Mgat1¹⁴* homozygotes. The structure names (M = Man; Gn = GlcNAc; F = Fuc; G = Glc; see Figure 1 for structures) and the m/z values for [M + Na]+ ions are shown above the peaks. (B) MALDI-TOF MS of neutral N-glycans of *Mgat1¹⁴/Mgat1¹⁴* homozygotes. The locations marked with an asterisk correspond to peaks obtained after MS analysis of fly food and are due to a polyhexose. Only one peak at m/z 1336.9 corresponds both to a fly food peak and to a potential N-glycan (Hex₃HexNAc₄, see Table 1). Derivatization and MALDI-IT-MS (40) showed this ion to be a (Hex)₈ polymer consistent with fly food.

Figure 5. Maternal-null *Mgat1¹⁴/Mgat1¹⁴* embryos exhibit no anti-HRP immunoreactivity. (A-C) *Mgat1¹⁴/CyO-GFP* and (D-F) *Mgat1¹⁴/Mgat1¹⁴* embryos collected from *Mgat1¹⁴/Mgat1¹⁴* females mated to *Mgat1¹⁴/CyO-GFP* males and double-labeled with anti-HRP (green; A, D) and anti-Elav (red; B, E) antibodies. Panels C and F are merges of the left two panels. The time of exposure in (D) was more than double that of (A). The CNS is marked by the arrow.

Figure 6. *Mgat1¹⁴/Mgat1¹⁴* mutants exhibit a fused lobe phenotype. Whole mount brains from (A) *Mgat1¹⁴/Mgat1¹⁴* and (B) *Mgat1¹⁴/Mgat1¹⁴* adults were immunolabeled with anti-FasII antibodies to detect mushroom body lobes. Only the *Mgat1¹⁴/Mgat1¹⁴* brains exhibited a fused β lobe phenotype. The arrow points to a gap between the mushroom body β lobes that is present in wild type and *Mgat1¹⁴/CyO-GFP* brains but reduced by >50% in all *Mgat1¹⁴/Mgat1¹⁴* brains and is completely absent in 40% of mutant brains.
Table 1. MALDI-TOF-MS analysis of underivatized N-glycans of \textit{Mgat1}^{+/9} / \textit{Mgat1}^{+/9}, \textit{Mgat1}^{+/}/\textit{CyO-GFP} and \textit{Mgat1}/\textit{Mgat1} flies. All N-glycans reported in this table were reduced and permethylated, and the derivatized glycans were analyzed by MALDI-IT-TOF to confirm the structural assignments; this data is not presented. The height of the MS peak for a particular compound relative to the strongest signal at 100 is shown. The hypothetical N-glycan structures attributed to the MS peaks are shown in brackets (Figure 1; M = Man, Gn = GlcNAc, G = Glc, F = Fuc). The hexose (Hex) and N-acetylhexosamine (HexNAc) structures detected by MS have been identified as Man and GlcNAc on the basis of other analytical techniques carried out on similar compounds in various other species; however, other isomers cannot be ruled out. ND, not detected.

| N-glycan structures | \(m/z\) \([M + Na]^+\) | Relative height of peak \(\text{Mgat1}^{+/9}/\text{Mgat1}^{+/9}\) | Relative height of peak \(\text{Mgat1}^{+/}/\text{CyO-GFP}\) | Relative height of peak \(\text{Mgat1}^{+/}/\text{Mgat1}^{+/}\) |
|---------------------|-----------------------|---------------------------------|-----------------|-------------------|
| Oligomannose        |                       |                                 |                 |                   |
| Hex\(_3\)HexNAc\(_2\) (M5Gn2) | 1258.4               | 68                              | 35              | 100               |
| Hex\(_4\)HexNAc\(_2\) (M6Gn2) | 1420.5               | 8                               | 15              | 12                |
| Hex\(_5\)HexNAc\(_2\) (M7Gn2) | 1582.7               | 4                               | 14              | 10                |
| Hex\(_6\)HexNAc\(_2\) (M8Gn2) | 1744.7               | 4                               | 16              | 10                |
| Hex\(_7\)HexNAc\(_2\) (M9Gn2) | 1905.8               | 4                               | 38              | 14                |
| Hex\(_10\)HexNAc\(_2\) (GM9Gn2) | 2067.9              | 4                               | 5               | 2                 |
| Paucimannose        |                       |                                 |                 |                   |
| Hex\(_3\)HexNAc\(_2\)Fuc (M2Gn2F) | 918.1                | 42                              | 30              | ND                |
| Hex\(_3\)HexNAc\(_2\) (M3Gn2) | 934.1                | 54                              | 35              | 22                |
| Hex\(_4\)HexNAc\(_2\)Fuc (M3Gn2F) | 1080.3              | 100                             | 100             | 2                 |
| Hex\(_4\)HexNAc\(_2\) (M4Gn2) | 1096.3               | 32                              | 15              | 26                |
| Hex\(_4\)HexNAc\(_2\)Fuc (M4Gn2F) | 1241.3              | 4                               | ND              | ND                |
| Hybrid and Complex  |                       |                                 |                 |                   |
| Hex\(_3\)HexNAc\(_3\) (GnM3Gn2) | 1137.3               | 6                               | 2               | ND                |
| Hex\(_3\)HexNAc\(_3\)Fuc (GnM3Gn2F) | 1283.4              | 10                              | 18              | ND                |
| Hex\(_3\)HexNAc\(_4\) (Gn2M3Gn2)\(*\) | 1337.5              | 4                               | 4               | 2                 |

* This peak has been identified by derivatization and MALDI-IT-TOF as a \((\text{Hex})_8\) polymer consistent with fly food. The other peaks associated with fly food (marked with an asterisk in Figure 4B) do not overlap with any of the other N-glycan peaks in the above table.
Table 2. *Mgatl*/Mgatl* adults flies have reduced locomotory activity*. The locomotory activity of *Mgatl*/Mgatl* mutants and controls was measured as the length of time adult flies were moving using an open-field assay over a period of 3 min. The number of animals/group = 25.

| Genotype                  | Movement Time (Seconds) |
|---------------------------|-------------------------|
|                           | Mean  | Std. Dev. |
| *Mgatl*+/+               | 156   | 20.0      |
| *Mgatl*+/+               | 160   | 10.2      |
| *Mgatl*++/CyO-GFP       | 153   | 15.4      |
| *Mgatl*++/CyO-GFP       | 155   | 17.9      |
| *Mgatl*++/*Mgatl*+      | 7.1    | 16.3      |
| *Mgatl*++/*Mgatl*+      | 5.4    | 3.7       |
Table 3. *Mgat1*/ *Mgat1* mutants exhibit a severely reduced lifespan. Adult flies of each genotype were maintained at 25°C in shell vials (10 flies per vial) containing standard cornmeal agar medium. The starting population size for each genotype was 100. Flies were transferred to fresh medium and scored for survivorship every 3 days. The mean (50% survival) and maximum (10% survival) lifespan for each genotype is shown in days.

| Genotype                  | Mean | Max. | Std. Dev. |
|---------------------------|------|------|-----------|
| *Mgat1*+/+ *Mgat1*+/+  | 75.1 | 80.1 | 2.5       |
| *Mgat1*+/+ *Mgat1*/+    | 76.7 | 84.1 | 6.2       |
| *Mgat1*/CyO-GFP          | 67.9 | 79.1 | 6.2       |
| *Mgat1*/CyO-GFP          | 86.1 | 93.6 | 5.2       |
| *Mgat1*/+ *Mgat1*/+     | 12.8 | 16.7 | 2.6       |
| *Mgat1*/+ *Mgat1*/+     | 13.9 | 21.3 | 3.9       |
**Abbreviations**

CDG, congenital disorder of glycosylation; FucT, fucosyltransferase; GlcNAcT, N-acetylglucosaminyltransferase; HRP, horseradish peroxidase; MALDI-TOF-MS, matrix-assisted laser desorption/ionisation-time of flight mass spectrometry; MS, mass spectrometry; PNGase, protein N-glycanase; PTM, post-translational modifications.
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Null mutations in Drosophila N-acetylglucosaminyltransferase I produce defects in locomotion and a reduced lifespan
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J. Biol. Chem. published online March 7, 2006

Access the most updated version of this article at doi: 10.1074/jbc.M512769200

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