

Null Mutations in *Drosophila* N-Acetylglucosaminyltransferase I Produce Defects in Locomotion and a Reduced Life Span*

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UDP-GlcNAc:α3-D-mannoside β1,2-N-acetylglucosaminyltransferase I (encoded by *Mgat1*) controls the synthesis of hybrid, complex, and paucimannose N-glycans. Mice make hybrid and complex N-glycans but little or no paucimannose N-glycans. In contrast, *Drosophila melanogaster* and *Caenorhabditis elegans* make paucimannose N-glycans but little or no hybrid or 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 *Mgat1*¹/*Mgat1*¹ null mutants showed no β1,2-N-acetylglucosaminyltransferase 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, *Mgat1*¹/*Mgat1*¹ null mutants are viable but exhibit pronounced defects in adult locomotory activity when compared with *Mgat1*¹/*CyO-GFP* heterozygotes or wild type flies. In addition, in null mutants males are sterile and have a severely reduced mean and maximum life span. Microscopic examination of mutant adult fly brains showed the presence of fused β lobes. The removal of both maternal and zygotic *Mgat1* 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-acetylglucosaminyltransferase I-dependent N-glycans are required for locomotory activity, life span, and brain development in *Drosophila*.

According to recent genome project estimates, the human and fruit fly genomes contain about 24,000 and 14,000 genes, respectively. However, the number of functionally discrete proteins encoded by either of these genomes is probably increased by at least 1 order of magnitude because of post-translational modifications (PTM)³ 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 are composed of several different monomeric sugar components connected by one of six to eight 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 GlcNAc to the amido group of Asn (N-glycans). The first phase of N-glycan synthesis involves the assembly of a lipid-linked precursor Glc₃Man₉GlcNAc₂-pyrophosphate-dolichol and the oligosaccharyltransferase-catalyzed transfer of the Glc₃Man₉GlcNAc₂- 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 Glc₃Man₉GlcNAc₂ to Man₅GlcNAc₂ (5, 6) (Figs. 1 and 2A). The final phase of the pathway (7) occurs in the Golgi apparatus and involves the conversion of Man₅GlcNAc₂-Asn to hybrid, paucimannose, and complex N-glycans (Fig. 2A). UDP-GlcNAc:α3-D-mannoside β1,2-N-acetylglucosaminyltransferase I (GlcNAcTI, encoded by *Mgat1*) converts Man₅GlcNAc₂-Asn to the hybrid N-glycan GlcNAcMan₅GlcNAc₂-Asn. This is followed by the action of α3,6-mannosidase II to form the hybrid N-glycans GlcNAcMan₄GlcNAc₂-Asn and GlcNAcMan₃GlcNAc₂-Asn (Fig. 2A). In vertebrates, GlcNAcMan₃GlcNAc₂-Asn is converted to complex N-glycans by the action of UDP-GlcNAc:α6-D-mannoside β1,2-N-acetylglucosaminyltransferase II (GlcNAcTII) and other branching GlcNAcTs (7). Further action by other glycosyltransferases (galactosyl-, sialyl-, and fucosyltransferases) on the distal nonreducing ends of the glycan creates a large variety of complex N-glycans.

In plants (8), insects (9), and *Caenorhabditis 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 hydrolyze GlcNAcMan₅GlcNAc₂ and acts further downstream on GlcNAcMan₃₋₄GlcNAc₂Fuc₀₋₁ after the action of α3,6-mannosidase II (Fig. 2A) (9). *Drosophila* (11) and *C. elegans* (12) make paucimannose N-glycans (Man₃₋₄GlcNAc₂Asn) but little or no hybrid or complex N-glycans (Fig. 2A). Insect glycoproteins carry relatively large amounts of Man₃₋₄GlcNAc₂ 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 Man₃GlcNAc₂ 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 find-

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³ The abbreviations used are: PTM, post-translational modification; CDG, congenital disorder of glycosylation; FucT, fucosyltransferase; GlcNAcT, N-acetylglucosaminyltransferase; HRP, horseradish peroxidase; MALDI-TOF-MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; MS, mass spectrometry; PNGase, protein N-glycanase; GFP, green fluorescent protein; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonic acid; MES, 4-morpholinethanesulfonic acid; PBS, phosphate-buffered saline; Gnase, β-N-acetylglucosaminidase.

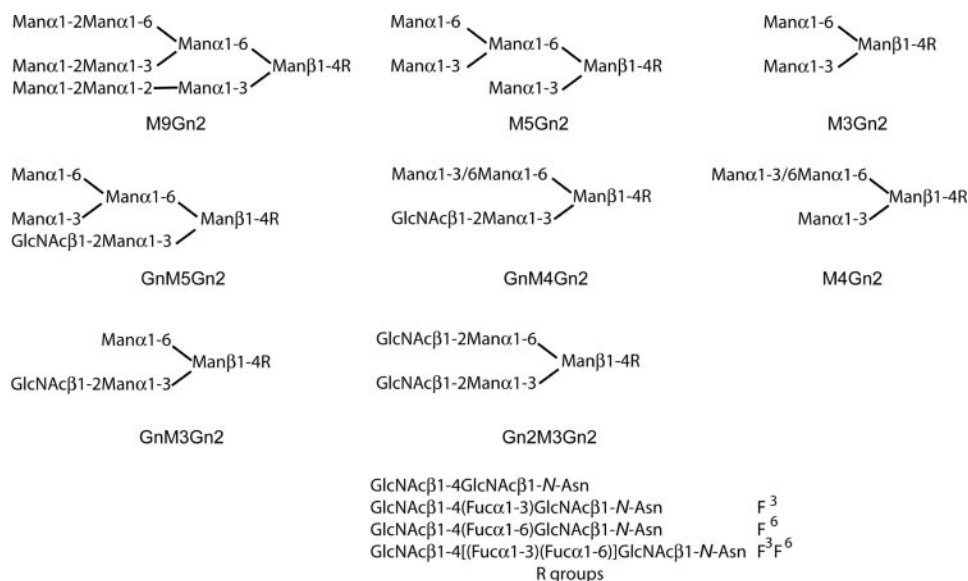


FIGURE 1. **N-Glycan structures in *Drosophila*.**

The names assigned to these structures are used in the synthetic schemes shown in Fig. 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 GlcNAc β 1-2Man α 1-3 moiety and are therefore dependent on prior GlcNAcT1 action. The R group is defined in the figure; there are four R variants depending on the absence or presence of core α 1-3- and α 1-6-linked Fuc residues (designated as F^3 and F^6 , respectively).

ing is compatible with extension of glycans with sialic acid. As found previously in vertebrates, insect α 3,6-mannosidase II (20, 21), GlcNAcTII (22), and some α 1,3/ α 1,6-fucosyltransferases (22, 23) require the prior action of GlcNAcT1.

The first committed step toward synthesis of Glc₃Man₉GlcNAc₂-pyrophosphate-dolichol is catalyzed by UDP-GlcNAc:dolichylphosphate GlcNAc-1-phosphate transferase. This step is essential for the synthesis of all N -glycans. Tunicamycin, a GlcNAc analogue that is a competitive inhibitor of GlcNAc-1-phosphate transferase, is toxic to yeast (24) and to mammalian cells in culture (25). Tunicamycin prevents normal mouse embryogenesis (26, 27). GlcNAc-1-phosphate transferase-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 GlcNAcT1 (29). *Mgat1* 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 GlcNAcT1-dependent N -glycans are needed for normal vertebrate development but not for the survival of individual cells. Here we present evidence that GlcNAcT1-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 multienzyme 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 life span, 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 $y^1 w^{67c23}; Mgat1^{KG02444}$ (stock BL-13222; Bloomington Stock Center) contains a P-element insertion at the 5' end of *Mgat1* and was used to generate both the precise excision line *Mgat1*⁺ and the imprecise excision allele *Mgat1*¹. Because *Mgat1*⁺ was generated at the same time as the *Mgat1*¹ allele and is in the same genetic background, this line was used as a control for all of our subsequent analyses (GlcNAcT1 assays, mass spectrometry, immunohistochemistry, locomotion, and life span) and behaved as wild type. *y w; Sp/CyO; SbΔ2-3/TM6, Ubx* was used as a source of transposase. The excision breakpoints were determined by PCR analysis and sequencing using the primers F1 5'-CCGATTGGGG-TAGGTAAAT and R3 5'-CTGAGAGTGGCACACTTTC. The P-element line *l(2)05510⁰⁵⁵¹⁰ cn¹/CyO; ry⁵⁰⁶* contains a lethal mutation in the gene immediately upstream of *Mgat1* (stock BL-12192).

GlcNAcT1 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 mixture. GlcNAcT1 activity was measured using 0.6 mM Man α 1-6[Man α 1-3]Man β 1-*O*-*n*-octyl (Toronto Research Chemicals, Toronto, Canada) as acceptor substrate and 1.2 mM 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 of enzyme in a total volume of 0.04 ml. Time of incubation was 60 min at 37 °C. The assays were carried out as described previously (36). The rate of product formation was proportional to enzyme volume.

Mass Spectrometric Analysis of N-Glycans of Wild Type and Mutant *Drosophila*—Adult flies (~0.5 g of *Mgat1*⁺/*Mgat1*⁺, *Mgat1*¹/*CyO*-GFP, *Mgat1*¹/*Mgat1*¹) were anesthetized with carbon dioxide, suspended in 0.3 ml of water, and boiled for 10 min. The preparation of N -glycans was performed as described previously (11, 37). Proteins were extracted in lysis buffer (35 mM Tris, 8 M urea, 4% CHAPS, 65 mM dithiothreitol, pH 8.0), and centrifuged at 10,000 × *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% trichloro-

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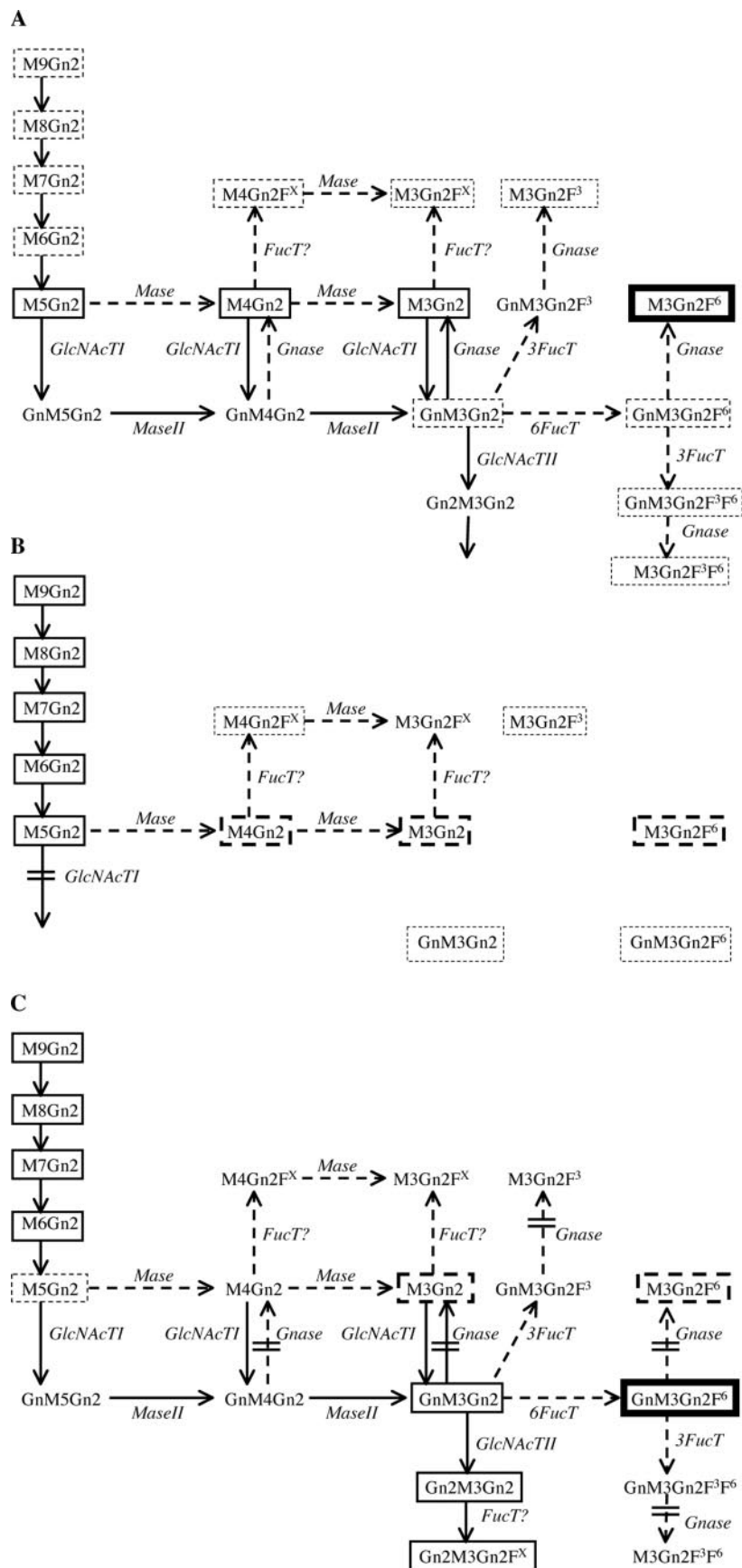


FIGURE 2. *A*, *N*-glycan synthesis in wild type *Drosophila*. This scheme is based on the data in Table 1 and on Paschinger *et al.* (44). The names of the *N*-glycans are defined in Fig. 1. Enzyme names are in *italics*. Reactions shown by *continuous arrows* have been established by *in vitro* assays, and *broken 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

roacetic acid on ice for 1 h. The protein pellet was washed with 1 ml of acetone (three times) and 1 ml of chloroform/methanol/water (10:10:1) (three 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 matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS). The glycans were reduced with NaBH_4 (0.2 ml of 10 mg/ml NaBH_4 in 10 mM NaOH) overnight at room temperature. Borate was removed by adding 2 drops of acetic acid on ice, followed by co-evaporation with 3 ml of ethanol, 3 ml of 1% acetic acid in methanol (five times), and 1 ml of toluene (three times). The reduced glycans were desalted, permethylated (39), and analyzed by MALDI-IT-TOF (where IT indicates ion trap) MS for confirmation. Collision-induced dissociation was performed using MALDI-QIT MS (where QIT indicates quadrupole ion trap) (Kratos-Shimadzu Biotech) with 2,5-dihydroxybenzoic acid as matrix (40). All the structures reported here were confirmed by derivatization and MALDI-IT-MS.

Locomotor 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.

Life Span Determination—The life span of *Mgat1*¹/*Mgat1*¹ adults was compared with *Mgat1*¹/*CyO-GFP* and *Mgat1*⁺⁹/*Mgat1*⁺⁹ flies. Briefly, an overnight egg collection was obtained from *Mgat1*¹/*CyO-GFP* flies and aged for 24–36 h. Upon hatching into first instar larvae, *Mgat1*¹/*Mgat1*¹ homozygotes were sorted from *Mgat1*¹/*CyO-GFP* heterozygotes by the absence (*Mgat1*¹/*Mgat1*¹) or presence (*Mgat1*¹/*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 were 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 life span of each strain was calculated from the time in days when survival reached 50 and 10%, respectively, of the starting population in each of the 10 cohorts of each strain.

Immunocytochemistry—Embryos from homozygous and heterozygous *Mgat1*¹ flies were collected overnight on grape plates, fixed using standard conditions, and double-labeled with antibodies to Elav (a neu-

ron-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, University of Iowa), rabbit anti-GFP, 1:500 (Molecular Probes), and anti-HRP fluorescein isothiocyanate, 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 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; Developmental Studies Hybridoma Bank), 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% DabcoTM (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 version 3.1.7 and Adobe Photoshop version 5.5 software.

RESULTS

Generation of *Mgat1* Mutants—As a first step to identify the *in vivo* function of *Mgat1*, we characterized the genomic structure of the *Mgat1* gene from *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 Fig. 3. As described previously (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 (Fig. 3). In total, we generated three independent deletions that removed various portions of the *Mgat1* gene and failed to complement each other. More 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*

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). GlcNAcT1 adds GlcNAc in β 1–2 linkage to the Man α 1–3 arm of M5Gn2 to form the hybrid *N*-glycan GnM5Gn2 (36, 69). Two Man residues are removed from GnM5Gn2 by the action of α 3,6-mannosidase II (*Masell*) to form the truncated hybrid *N*-glycans GnM4Gn2 and GnM3Gn2 (6). A specific β -*N*-acetylglucosaminidase not found in vertebrates (*Gnase* (9, 49)) removes the GlcNAc added by GlcNAcT1 to form M4Gn2 and M3Gn2 paucimannose *N*-glycans. GlcNAcTII (70) 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 GlcNAcTII. The substrates, products, and reactions of the core α 1,6-FucT (6FucT) and α 1,3-FucT (3FucT) are shown (44). Both core α 1,6-FucT (FucT6) (44) and α 1,3-FucT (FucT4) (45) in *Drosophila* are dependent on prior GlcNAcT1 action. FucT6 cannot act on structures with a core α 1–3-linked Fuc and must therefore act before FucT4 to make the small amounts of M3Gn2F³ in wild type *Drosophila* (14, 44). GlcNAcT1-null flies make small amounts of fucosylated M3Gn2 (M3Gn2F^X) (Table 1) indicating a GlcNAcT1-independent path to this structure; neither of the previously reported core FucTs are responsible because both enzymes require prior GlcNAcT1 action (44). The site and linkage of the Fuc on M3Gn2F^X are unknown. GlcNAcT1-null flies make relatively large amounts of M3Gn2 and M4Gn2 (Table 1) suggesting that a GlcNAcT1-independent α -mannosidase (*Mase*) acts on M5Gn2 upstream of GlcNAcT1; such a mannosidase has been reported in *Spodoptera frugiperda* (71) but not in *Drosophila*. *B*, *N*-glycan synthesis in *Mgat1*-null flies. The figure is based on MS analysis of the *N*-glycan structures in GlcNAcT1-null flies (Table 1) and was obtained by removing all arrows dependent on the action of GlcNAcT1 from the wild type fly scheme (see A). The definitions of names and arrows are as for A. 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 GlcNAcT1-null flies (Table 1); this M3Gn2F is neither M3Gn2F³ nor M3Gn2F⁶ (the respective FucTs require prior GlcNAcT1 action) and has been designated as M3Gn2F^X (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 M4Gn2F^X indicate that the structure may be an intermediate in the pathway to M3Gn2F^X. *C*, *N*-glycan synthesis in *fdl*-null flies. The figure is based on MS analysis of the *N*-glycan structures in the *fdl* and *Df(2R)achi²* fly strains; *fdl* flies have a hypomorphic mutation in the *fdl* gene, and *Df(2R)achi²* 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 GlcNAcT1 (49). The definitions of names and arrows are as for Fig. 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 (M3Gn2F^X, unboxed) in *fdl*-null flies (49) cannot be due to either of the previously described core FucTs because both routes require *Gnase* action; suggested routes to M3Gn2F^X 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).

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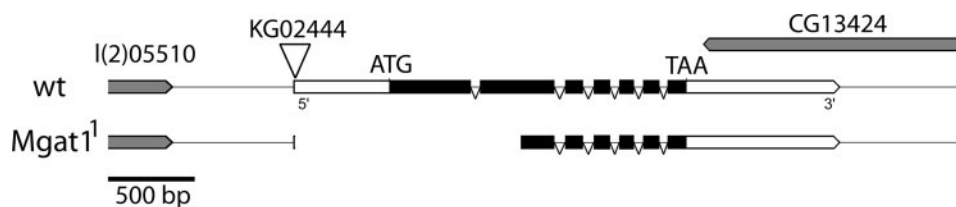


FIGURE 3. **Structure of the *Mgat1* locus.** *Mgat1* consists of 7 exons (boxes) spanning 3.2 kb. Exons 1 and 7 contain both untranslated sequences (open boxes) and parts of the open reading frame (filled boxes). The two genes flanking *Mgat1*, *l(2)05510* and *CG13424*, are shown with their orientation.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

TABLE 1

MALDI-TOF-MS analysis of underivatized *N*-glycans of *Mgat1*⁺⁹/*Mgat1*⁺⁹, *Mgat1*¹/*CyO-GFP*, and *Mgat1*¹/*Mgat1*¹ 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; the data are 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 parentheses (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.

<i>N</i> -Glycan structures	<i>m/z</i> M + Na ⁺	Relative height of peak		
		<i>Mgat1</i> ⁺⁹ / <i>Mgat1</i> ⁺⁹	<i>Mgat1</i> ¹ / <i>CyO-GFP</i>	<i>Mgat1</i> ¹ / <i>Mgat1</i> ¹
Oligomannose				
Hex ₅ HexNAc ₂ (M5Gn2)	1258.4	68	35	100
Hex ₆ HexNAc ₂ (M6Gn2)	1420.5	8	15	12
Hex ₇ HexNAc ₂ (M7Gn2)	1582.7	4	14	10
Hex ₈ HexNAc ₂ (M8Gn2)	1744.7	4	16	10
Hex ₉ 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 ₃ 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) ^a	1337.5	4	4	2

^a This peak has been identified by derivatization and MALDI-IT-TOF as a (Hex)₈ polymer consistent with fly food. The other peaks associated with fly food (marked with an asterisk in Fig. 4B) do not overlap with any of the other *N*-glycan peaks in the table.

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*¹, 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*¹ was in fact a null allele, we then measured GlcNAcT1 activity from extracts derived from *Mgat1*¹/*Mgat1*¹ flies and compared these to *Mgat1*¹/*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/h/mg protein) compared with the wild type controls (259 ± 40 pmol/h/mg protein).

Comparison of *N*-Glycans from Wild Type and *Mgat1* Mutant Flies—To determine whether the synthesis of oligomannose, hybrid, complex, and paucimannose *N*-glycans was affected in the *Mgat1*¹/*Mgat1*¹ 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 and Fig. 4A) and mutant (*Mgat1*¹/*Mgat1*¹) (Table 1 and Fig. 4B) flies were compared. As described previously (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 and Fig. 2A). In contrast, *Mgat1*¹/*Mgat1*¹ flies showed a dra-

matic decrease in the amount of M3Gn2F to almost undetectable levels (Table 1 and Fig. 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 because of M3Gn2F⁶. Furthermore, because both core α1,3-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 because of a different as yet uncharacterized FucT (Fig. 2B, product M3Gn2F^X). M4Gn2F, GnM3Gn2, and GnM3Gn2F were not detected in *Mgat1*¹/*Mgat1*¹ 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). M3Gn2F³F⁶ (with both an α1-3- and α1-6-linked Fuc residue on the same Asn-linked core GlcNAc), Gn2M3Gn2, and Gn2M3Gn2F (Fig. 2A) have been reported in the 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, and 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 Fig. 2, A and B.

***Mgat1*¹/*Mgat1*¹ Mutant Males Are Sterile**—Although *Mgat1*¹/*Mgat1*¹ homozygotes were viable, we found that homozygous mutant males, but not females, were sterile. To determine the nature of the

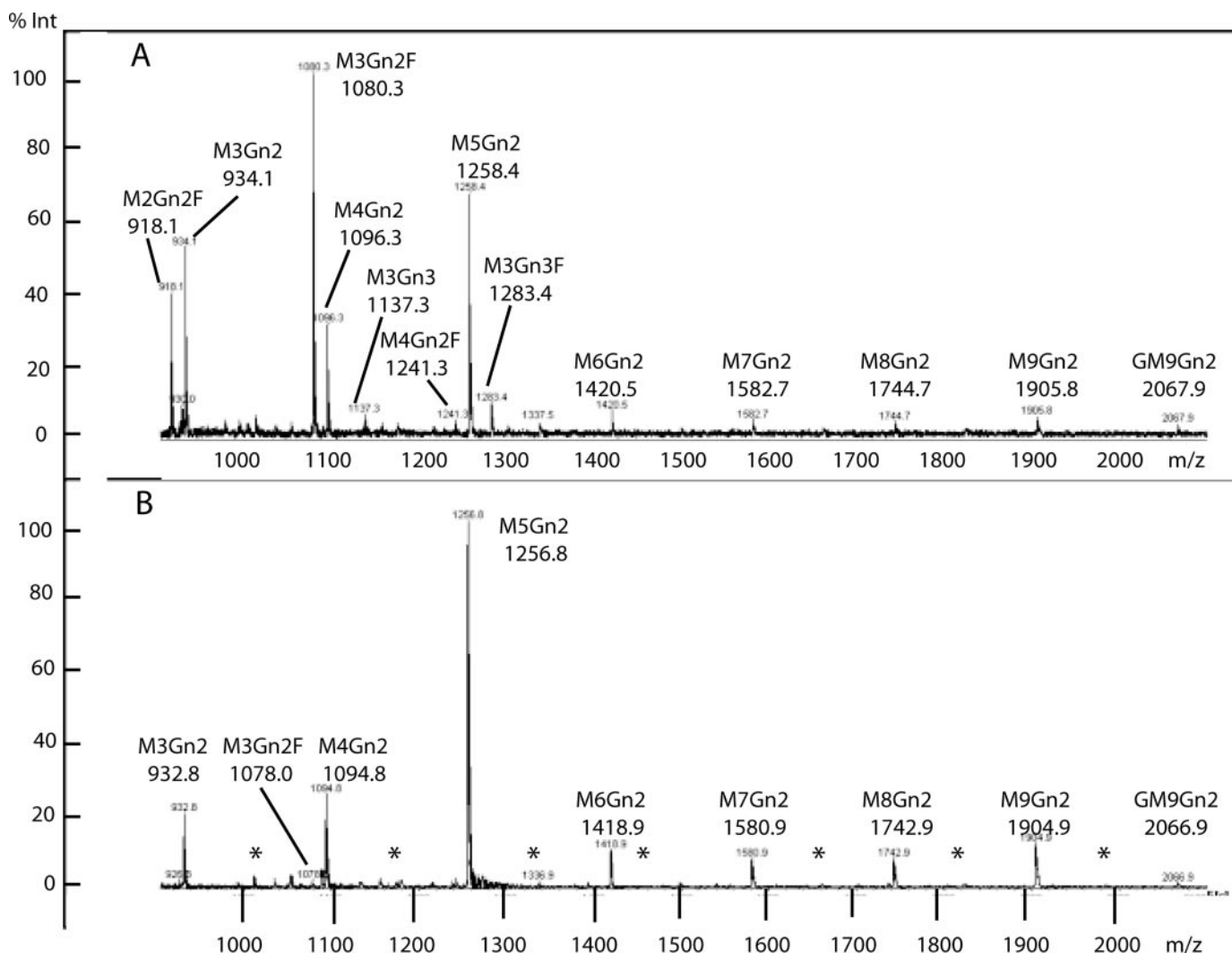


FIGURE 4. *Mgat1*¹ homozygotes exhibit altered *N*-glycan profiles. *A*, MALDI-TOF MS analysis of neutral *N*-glycans of *Mgat1*^{+/+}/*Mgat1*^{+/+} homozygotes. The structure names (*M* = Man; *Gn* = GlcNAc; *F* = Fuc; *G* = Glc; see Fig. 1 for structures) and the *m/z* values for [*M* + Na]⁺ ions are shown above the peaks. *B*, MALDI-TOF MS of neutral *N*-glycans of *Mgat1*¹/*Mgat1*¹ homozygotes. The locations marked with an asterisk correspond to peaks obtained after MS analysis of fly food and are because of 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)₆ polymer consistent with fly food.

sterility, we examined the testes from *Mgat1*¹/*Mgat1*¹ males and found that they produced normal levels of mobile sperm, indicating that the sterility was not because of defects in spermatogenesis (data not shown). We then determined whether *Mgat1*¹/*Mgat1*¹ males were able to mate with and fertilize females. In wild type flies, females store sperm within structures called spermathecae after mating. Although we were able to detect mobile sperm within the spermathecae of females mated to *Mgat1*^{+/+}/*Mgat1*^{+/+} or *Mgat1*¹/*CyO-GFP* males, we could not detect any sperm in females mated to *Mgat1*¹/*Mgat1*¹ males (data not shown). This suggests that the sterility defect is associated with a failure of *Mgat1*¹/*Mgat1*¹ males to mate.

*Mgat1*¹/*Mgat1*¹ Mutants Exhibit Defects in Locomotory Activity and a Reduced Life Span—Consistent with the observation that *Mgat1*¹/*Mgat1*¹ males did not mate with wild type females, we also found that *Mgat1*¹/*Mgat1*¹ 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*¹/*Mgat1*¹ adults showed >95% reduction in movement compared with heterozygous and wild type controls (Table 2). Specifically, we found that *Mgat1*¹/*Mgat1*¹

*Mgat1*¹ males moved for 7.1 ± 16.3 s, *Mgat1*¹/*CyO-GFP* males moved for 153 ± 15.4 s, and *Mgat1*^{+/+}/*Mgat1*^{+/+} males moved for 156 ± 20.0 s. Similar differences were observed in mutant females compared with controls. Overall, *Mgat1*¹/*Mgat1*¹ males and females differed significantly from all other groups (*p* < 0.001).

In addition to the locomotory defects, *Mgat1*¹/*Mgat1*¹ 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 wild type larvae. Under these conditions, approximately two-thirds of the mutants eclosed as pupae. The remaining third appeared to die throughout larval development. Mutant adults that emerged showed a normal external morphology but appeared to die earlier than their wild type or heterozygous counterparts.

To determine whether there was a significant difference in life span between homozygous mutants and controls, we determined the mean and maximum life span for both males and females (Table 3). We found that *Mgat1*¹/*Mgat1*¹ males and females had a severely reduced life span compared with both *Mgat1*¹/*CyO-GFP* and *Mgat1*^{+/+}/*Mgat1*^{+/+} controls. Specifically, *Mgat1*¹/*Mgat1*¹ males had a mean life span of 12.8

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days (50% survival) and a maximum life span of 16.7 days (10% survival), whereas *Mgat1*¹/*Mgat1*¹ females had a mean life span of 13.9 days and a maximum life span of 21.3 days. This is significantly different from either *Mgat1*¹/*CyO-GFP* flies (mean life span for males = 67.9 days and maximum life span = 79.1 days; mean life span for females = 86.1 days and maximum life span = 93.6 days) or *Mgat1*⁺⁹/*Mgat1*⁺⁹ flies (mean life span for males = 75.1 days and maximum life span = 80.1 days; mean life span for females = 76.7 days and maximum life span = 84.1 days). 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). Taken together, these data clearly demonstrate that *Mgat1* is required for locomotory activity and survivorship in flies.

The Central Nervous System of Maternal-null *Mgat1*¹/*Mgat1*¹ 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 α 1,3-fucosyltransferase (α 1,3-FucT) that adds an α 1–3-linked fucose to the proximal *N*-glycan core is essential for synthesis of the HRP epitope in both *C. elegans* (46) and *Drosophila* (14, 45). *In vitro* studies of the α 1,3-FucT (FUT-1) required for HRP epitope synthesis by *C. elegans* have shown that the enzyme does not require the prior action of GlcNAcT1 (46). Furthermore, a *C. elegans* strain with null mutations in all three GlcNAcT1 genes (37) displayed normal staining with anti-HRP in the complete absence of GlcNAcT1 enzyme activity (46). In contrast, the *Drosophila* α 1,3-FucT (FucTA) required for synthesis of the HRP epitope acts only on substrates that require the prior action of GlcNAcT1 (14, 45). However, we initially observed that *Mgat1*¹/*Mgat1*¹ mutant and *Mgat1*¹/*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*¹/*Mgat1*¹ mutant embryos. Indeed, retention of maternally derived *Mgat1* mRNA has been demonstrated in pre-implantation *Mgat1*^{-/-} mouse embryos (47). Consistent with this possibility, we found that unlike *Mgat1*¹/*CyO-GFP*, *Mgat1*⁺⁹/*Mgat1*⁺⁹, or other wild type brains, *Mgat1*¹/*Mgat1*¹ adult fly brains do not show anti-HRP staining (data not shown). To prove that the anti-HRP immunoreactivity observed in *Mgat1*¹/*Mgat1*¹ embryos was because of maternal contribution, we examined embryos obtained from *Mgat1*¹/*Mgat1*¹ females mated to *Mgat1*¹/*CyO-GFP* males. The resulting *Mgat1*¹/*Mgat1*¹ embryos (which lack any maternal contribution) were negative for anti-HRP staining, whereas sibling *Mgat1*¹/*CyO-GFP* embryos (which also lack any maternal contribution) exhibited normal anti-HRP staining (Fig. 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 GlcNAcT1 action (14, 45).

The major *Drosophila* *N*-glycan structure with two Fuc residues on the Asn-linked GlcNAc of the core is M3Gn2F³F⁶ (Fig. 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 M3Gn2F³F⁶ (45). M3Gn2F³F⁶ is at least partly responsible for the staining of fly neurons with anti-HRP antibody. Mass spectrometric analysis shows a small amount of M3Gn2F in the adult *Mgat1*¹/*Mgat1*¹ mutants (Figs. 2B and 4B), indicating the presence of GlcNAcT1-independent FucTs not involved in synthesis of the HRP epitope.

***Mgat1*¹/*Mgat1*¹ Flies Exhibit a Fused Lobe Phenotype**—Because *Drosophila* *Mgat1*¹/*Mgat1*¹ mutants did not stain with anti-HRP, we could not use this marker to determine whether the locomotory defects

TABLE 2

*Mgat1*¹/*Mgat1*¹ adult flies have reduced locomotory activity

The locomotory activity of *Mgat1*¹/*Mgat1*¹ 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.
♂♂ <i>Mgat1</i> ⁺⁹ / <i>Mgat1</i> ⁺⁹	156	20.0
♀♀ <i>Mgat1</i> ⁺⁹ / <i>Mgat1</i> ⁺⁹	160	10.2
♂♂ <i>Mgat1</i> ¹ / <i>CyO-GFP</i>	153	15.4
♀♀ <i>Mgat1</i> ¹ / <i>CyO-GFP</i>	155	17.9
♂♂ <i>Mgat1</i> ¹ / <i>Mgat1</i> ¹	7.1	16.3
♀♀ <i>Mgat1</i> ¹ / <i>Mgat1</i> ¹	5.4	3.7

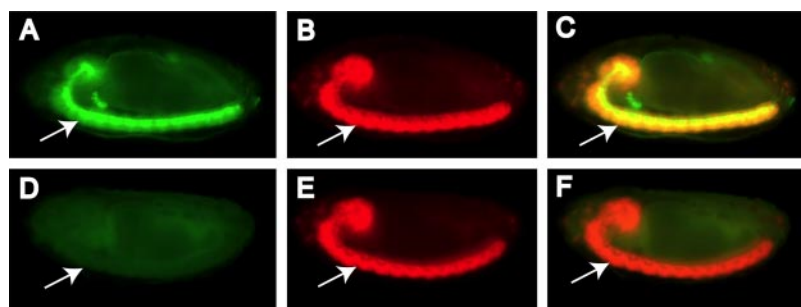
TABLE 3

*Mgat1*¹/*Mgat1*¹ mutants exhibit a severely reduced life span

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) life span for each genotype is shown in days.

Genotype	Mean	Max.	Std. Dev.
♂♂ <i>Mgat1</i> ⁺⁹ / <i>Mgat1</i> ⁺⁹	75.1	80.1	2.5
♀♀ <i>Mgat1</i> ⁺⁹ / <i>Mgat1</i> ⁺⁹	76.7	84.1	6.2
♂♂ <i>Mgat1</i> ¹ / <i>CyO-GFP</i>	67.9	79.1	6.2
♀♀ <i>Mgat1</i> ¹ / <i>CyO-GFP</i>	86.1	93.6	5.2
♂♂ <i>Mgat1</i> ¹ / <i>Mgat1</i> ¹	12.8	16.7	2.6
♀♀ <i>Mgat1</i> ¹ / <i>Mgat1</i> ¹	13.9	21.3	3.9

FIGURE 5. Maternal-null *Mgat1*¹/*Mgat1*¹ embryos exhibit no anti-HRP immunoreactivity. *Mgat1*¹/*CyO-GFP* (A–C) and *Mgat1*¹/*Mgat1*¹ (D–F) embryos were collected from *Mgat1*¹/*Mgat1*¹ females mated to *Mgat1*¹/*CyO-GFP* males and double-labeled with anti-HRP (green; A and D) and anti-Elav (red; B and E) antibodies. C and F are merges of the left two panels. The time of exposure in D was more than double that of A. The central nervous system is marked by the arrow.



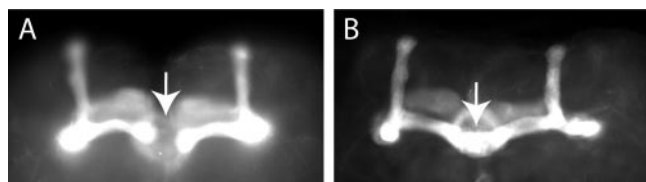


FIGURE 6. *Mgat1¹/Mgat1¹* mutants exhibit a fused lobe phenotype. Whole mount brains from *Mgat1⁺⁹/Mgat1⁺⁹* (A) and *Mgat1¹/Mgat1¹* (B) adults were immunolabeled with anti-FasII antibodies to detect mushroom body lobes. Only the *Mgat1¹/Mgat1¹* brains exhibited a fused β lobe phenotype. The arrow points to a gap between the mushroom body β lobes that is present in wild type and *Mgat1¹/CyO-GFP* brains but reduced by >50% in all *Mgat1¹/Mgat1¹* brains and is completely absent in 40% of mutant brains.

observed in our mutants were because of defects in adult brain structures. To circumvent this problem, we therefore immunostained whole mount brains from control *Mgat1⁺⁹/Mgat1⁺⁹* flies and *Mgat1¹/Mgat1¹* mutants with anti-FasII, which labels a subset of axons (including the mushroom bodies) within the central nervous system. Although we did not observe any gross morphological defects using either light microscopy or FasII staining, we did find that *Mgat1¹/Mgat1¹* mutants exhibit a fused lobe phenotype (Fig. 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*-acetylglucosaminidase (Fig. 2A, *Gnase*) that removes the GlcNAcT1-dependent GlcNAc residue to form paucimannose *N*-glycans in *Drosophila*. Indeed it has been reported recently (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. *Df(2R)achi²* flies have a null mutation in the *fdl* gene, but there are five other mutant genes in this fly strain; however, none of these genes appear to be involved in glycan metabolism (49).

Comparison of MS analyses of wild type flies (Table 1 and Fig. 2A), *Mgat1¹/Mgat1¹* flies (Table 1 and Fig. 2B), and *Df(2R)achi²* flies (Fig. 2C) (49) shows that M3Gn2F⁶, the major *N*-glycan in wild type flies, is decreased by 83% in *Df(2R)achi²* flies and by over 98% in *Mgat1¹/Mgat1¹* flies relative to wild type flies. The only other structure that is significantly decreased in both mutant flies relative to wild type (73 and 59%, respectively) is M3Gn2, the precursor of M3Gn2F⁶. Analysis of *Mgat1¹/Mgat1¹* flies shows that M4Gn2 is decreased by 19%, and M4Gn2F (a very minor component of wild type flies) is not detected in the *Mgat1* null flies; no data were reported for these two structures in the *fdl*-deficient lines (49). M6Gn2, M7Gn2, M8Gn2, and M9Gn2 are increased by 50–250% in both *Mgat1¹/Mgat1¹* and *Df(2R)achi²* flies relative to wild type flies (Fig. 2C). M5Gn2 is increased by 47% in *Mgat1* null and decreased by 54% in *fdl*-null flies. GnM3Gn2F⁶ is present in low amounts in wild type flies and absent in *Mgat1¹*-null flies but is the major *N*-glycan in *fdl*-null flies (Fig. 2C). Wild type, *Mgat1* null, and *fdl*-null flies make little or no GnM3Gn2, Gn2M3Gn2, and Gn2M3Gn2F. It is concluded that the absence of M3Gn2 and M3Gn2F⁶ plays a major role in generating the fused lobe phenotype because these glycans are significantly decreased

in both *Mgat1* null and *fdl*-null flies; a role for other glycans, however, cannot be ruled out on the basis of the above analysis.

DISCUSSION

Formation of the sugar-amino acid linkage occurs throughout the entire phylogenetic spectrum (archaea, eubacteria, and eukaryotes) and involves 13 monosaccharides, 8 amino acids, and at least 41 linkages (50). Three *N*-glycan and 20 *O*-glycan linkages have been reported in eukaryotes (50). It has been estimated that about 0.5–1.0% of the translated mammalian genome participates in oligosaccharide production and function (51). 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 (Fig. 1, Man_{5–9}GlcNAc₂), found in both unicellular and multicellular eukaryotes, are ancient structures essential for the viability of all cells. GlcNAcT1-dependent *N*-glycans (Fig. 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 20 genes encoding enzymes involved in *N*-glycosylation have been inactivated by null mutations in mice (32–34). Eighteen 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 GlcNAcT1 (Fig. 2A). Human *Mgat2* deficiency is named CDG-IIIa and is characterized by severe psychomotor retardation.

Although the GlcNAcT1-dependent structures in vertebrates are complex *N*-glycans with antennary extensions of the Man α 1–6(Man α 1–3)Man β 1–4GlcNAc β 1–4GlcNAc core (Fig. 2), plants, insects, and *C. elegans* synthesize predominantly GlcNAcT1-dependent Man_{3–4}GlcNAc₂ paucimannose *N*-glycans (Fig. 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 GlcNAcT1-dependent *N*-glycans can be more readily analyzed in this organism than in vertebrates. The *Mgat1¹/Mgat1¹* adult flies are null mutants because extracts showed no GlcNAcT1 enzyme activity, and mass spectrometric analysis showed dramatic changes in *N*-glycans compatible with lack of GlcNAcT1 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). *Mgat1¹/Mgat1¹* mutants were also significantly more sluggish than wild type flies. Moreover, this defect in locomotion is likely responsible for the observed male sterile phenotype because mutant males produced motile sperm but were unable to mate with either mutant or wild type 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

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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 *Drosophila*. At present, the severe locomotory defects observed in *Mgat1*¹/*Mgat1*¹ mutants preclude us from determining whether 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 whether *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), which is consistent with the hypothesis that these cells do not require the kind of cell-cell interactions provided by vertebrate *N*-glycans. *Mgat1*^{-/-} *C. elegans* 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 study shows that a null mutation of *Mgat1* in *Drosophila* 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*^{-/-} mice die *in utero* 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 GlcNAcT I during evolution is probably related to the differences in the types of *N*-glycans synthesized downstream of GlcNAcT I by the various organisms.

The dystrophin glycoprotein complex is an assembly of proteins spanning the sarcolemma of vertebrate skeletal muscle cells. An *O*-mannosyl glycan (sialyl α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-*O*-Ser/Thr) (57, 58) on α -dystroglycan, one of the components of the dystrophin glycoprotein complex, has been identified as a receptor for laminin and other extracellular 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 β 1,2-*N*-acetylglucosaminyltransferase 1 (POMGlcNAcT1), a homologue of GlcNAcTI, catalyzes the synthesis of the GlcNAc β 1-2Man α 1-*O*-Ser/Thr moiety on α -dystroglycan. POMGlcNAcT1 is deficient in patients with muscle-eye-brain disease, a congenital muscular dystrophy (61, 62). *Drosophila* has functional genes encoding homologues 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 *Drosophila* genome with either the vertebrate *Mgat1* or *POMGlcNAcT1* nucleotide sequence identifies *Mgat1* as the only homologous gene (36, 63). The enzyme encoded by *Drosophila* *Mgat1* is unable to catalyze the transfer of GlcNAc to the Man α -*O*-Ser/Thr moiety on α -dystroglycan (63). It can therefore be concluded that the phenotype observed in the *Mgat1*¹/*Mgat1*¹ null flies is because of abnormal *N*-glycan structures and is not related to α -dystroglycan function.

Null mutations in the glycosylation pathways of Fuca α 1-*O*-Ser/Thr (Notch), Xyl β 1-*O*-Ser (proteoglycans), and Man α 1-*O*-Ser/Thr (α -dystroglycan) glycoproteins in *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 *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 *Drosophila* *Mgat1*¹/*Mgat1*¹ 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 α 1-3-fucosylated *N*-glycan associated with the HRP epitope are among these targets. Polyclonal anti-HRP antibodies have been used to purify *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 *Drosophila*.

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