Modulation of Neural Carbohydrate Epitope Expression in 
Drosophila melanogaster Cells*

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Neural pathways in invertebrates are often tracked using anti-horseradish peroxidase, a cross-reaction due to the presence of core α1,3-fucosylated N-glycans. In order to investigate the molecular basis of this epitope in a cellular context, we compared two Drosophila melanogaster cell lines: the S2 and the neuronal-like BG2-c6 cell lines. As shown by mass spectrometric and chromatographic analyses, only the BG2-c6 cell line expresses α1,3/α1,6-fucosylated N-glycans, a result that correlates with anti-horseradish peroxidase binding. Of all four α1,3-fucosyltransferase homologues previously identified, the core α1,3-fucosyltransferase (FucTA; EC 2.4.1.214) is expressed in the neuronal cell line as well as throughout fly development and in heads and bodies of flies of both sexes. This pattern is distinctive in comparison with the expression of the other three α1,3-fucosyltransferase homologues (FucTB, FucTC, and FucTD). Furthermore, only transfection of FucTA cDNA into S2 cells resulted in expression of the anti-horseradish peroxidase epitope, a result compatible with its substrate specificity in vitro. Finally, silencing of FucTA by RNAi in the neuronal cell line led to a significant reduction of anti-horseradish peroxidase binding. The present study, in conjunction with our previous in vitro data, thereby shows that FucTA is indispensable for expression of the neural carbohydrate epitope in Drosophila cells.

Polyclonal antibodies raised against the plant glycoprotein, horseradish peroxidase (HRP),3 were used for many years in the study of invertebrate neurobiology, although information as to the exact structural basis for this cross-reaction was lacking (1–4). More recently, neural anti-HRP staining has been suggested to be a characteristic of Ecdysozoa (5), one of the two major clades of the animal kingdom. The differences between vertebrates and invertebrates in the expression of antigens recognized by the anti-HRP reagent are well documented (3, 4). As shown in Fig. 1A, the S2 cell line completely lacks the neuronal staining pattern characteristic of invertebrate nervous tissues, whereas the BG2-c6 line shows a neuronal-like staining pattern (Fig. 1A, lower panels). This staining in insects was shown by its sensitivity to reagents that destroy fucose (Fig. 1B). Although this neuronal staining pattern is distinctive in comparison with the expression of the other three α1,3-fucosyltransferase homologues, we have gained further evidence pointing to a key role for FucTA in the biosynthesis of the anti-HRP epitope in Drosophila cells.

1,3-fucosyltransferase (FucTA) (9). Recently, we were also able to demonstrate that anti-HRP staining in C. elegans is due to the activity of FUT-1, a core α1,3-fucosyltransferase (10).

A number of questions, however, remained unanswered, the following in particular. Is FucTA expressed in fly neural tissue, and does it create the anti-HRP epitope in vivo? Are other α1,3-fucosyltransferase homologues involved in neuronal anti-HRP epitope synthesis? Are core α1,3-/α1,6-di-fucosylated N-glycans enriched in fly neuronal tissue? Particularly the last question is problematic, since acquiring enough tissue for glycan analyses is a challenge. To circumvent this, a Drosophila third instar larval neuronal cell line (BG2-c6), previously found to bind anti-HRP (11), was considered as a suitable model for further study of the anti-HRP epitope synthesis; on the other hand, the commonly used Schneider 2 (S2) cell line was also employed in the expectation that these cells, since they are of hemocyte origin, do not bind anti-HRP. By examining n-glycans of these cell lines, performing knock-in and knock-down experiments on α1,3-fucosyltransferase homologues in the respective cell lines, and determining the tissue and stage specificity of the expression of all four α1,3-fucosyltransferase homologues, we have gained further evidence pointing to a key role for FucTA in the biosynthesis of the anti-HRP epitope in Drosophila cells and have demonstrated that the other three fucosyltransferase homologues are not responsible for the formation of anti-HRP epitopes in the neuronal cell line.

MATERIALS AND METHODS

Maintenance and Growth of Insect Cell Lines—The Drosophila neuronal cell line BG2-c6 was kindly provided by Kumiko Ui-Tei, Nippon Medical School, Tokyo, whereas the S2 and S9 cell lines were gifts from Gerald Aichinger (Intercell) and Wolfgang Ernst (Department für Biotechnologie, Universität für Bodenkultur, Wien), respectively. For
expression experiments, S2 cells were grown in Schneider’s *Drosophila* medium (Sigma) supplemented with heat-inactivated 10% fetal bovine serum (FBS), 50 units/ml penicillin G, and 50 µg/ml streptomycin sulfate in 25-cm² cell culture flasks (Sarstedt) at 26 °C. For RNA experiments, neuronal BG2-c6 cells were grown in Shiehls and Sang M3 insect medium (Sigma) supplemented with heat-inactivated 10% FBS, 10 µg/ml bovine insulin (Sigma) in 25-cm² cell culture flasks (Sarstedt) at 26 °C. In order to avoid contamination from bovine serum proteins for the N-glycan analysis, both cell lines were also adapted to serum-free medium (*Drosophila*-SFM; Invitrogen) supplemented with 16.5 mM L-glutamine (Invitrogen) and cultured at 26 °C. We found that both cell lines grew at twice the rate as in the media supplemented with 10% FBS and showed a stronger adherence to the flask surface. For general maintenance, S2 and BG2-c6 cells were split always to a density of 1 × 10⁸ cells/ml after they reached 0.8–2 × 10⁹ cells/ml. Sf9 cells were passaged at 100% confluence using IPL-41 medium (Sigma) supplemented with 3% FBS.

*N-Glycan Preparation from Drosophila Cell Lines*— Typically, 500 mg (wet weight) of S2 or BG2-c6 cell lines grown in serum-free medium (*Drosophila*-SFM; Invitrogen) were resuspended in up to 80 mM HCl, pH 2.0 (HCl was added until pH reached ~2.0) and homogenized with an Ultra Turrax T25 apparatus (high speed). Pepsin (1 mg) was added, and proteolysis was allowed to proceed overnight at 37 °C. The resulting extract was centrifuged, and the supernatant was applied to a Dowex AG50W × 2 column (1.5 × 50 cm); the column was then washed with 50 ml of 2% (v/v) acetic acid. Subsequently, the glycopeptides were eluted with 0.4 M ammonium acetate, pH 6, and orcinol-positive fractions were pooled and concentrated prior to gel filtration (Sephadex G25; elution with 1% (v/v) acetic acid). The resultant glycopeptide fractions were lyophilized and dissolved in 250 µl citrate-phosphate, pH 5, and incubated at 95 °C for 10 min to inactivate any remaining proteases. The N-glycans were then released using 0.375 milliunits of peptide:N-glycosidase A (37 °C, 24 h). The sample was then acidified by adding two volumes of 5% (v/v) acetic acid and applied to a 2-ml Dowex AG50W × 2 column. The column was washed with 2% (v/v) acetic acid, and the unretained orcinol-positive fractions were passed through reverse phase clean-up columns (100 mg; Zorbax; Agilent Technologies). Lyophilized N-glycans were dissolved in water and subject to either MALDI-TOF analysis (see below) or pyridylamination followed by RP-HPLC (12–14).

**Matrix-assisted Laser Desorption Ionization Mass Spectrometry**— Aliquots of 0.8 µl of undervariated or pyridylaminated N-glycans were applied to a flat sample plate and dried immediately under mild vacuo; subsequently, 1 µl of matrix (2% (w/v) 2,5-dihydroxybenzoic acid in 30% (v/v) acetonitrile or 0.03 M 1-hydroxyisouquinoline plus 0.1 M 2,5-dihydroxybenzoic acid in 50% (v/v) acetonitrile) was added, and the samples were dried once more (15, 16). MALDI-TOF mass spectra were acquired on a DYNAMO (Thermo BioAnalysis, Hemel Hempstead, UK) linear time-of-flight mass spectrometer with a dynamic extraction setting of 0.1. External mass calibration was performed with pyridylaminated N-glycans or with a partial dextran hydrolysate. On-plate α-fucosidase digestion was performed using 0.2 milliunits of bovine kidney α-fucosidase (Sigma) in 10 mM ammonium acetate, pH 5, with incubation in a “wet chamber” at 37 °C for 1 h. On-plate α-mannosidase digestion was performed using ~10 milliunits of jack bean α-mannosidase (Sigma; repurified on Sephacryl S200) in 50 mM ammonium acetate, pH 5, with incubation in a “wet chamber” at 37 °C for 5 h.

**Reverse Phase and Normal Phase HPLC of Pyridylaminated N-Glycans**— After crude gel filtration to remove excess 2-aminopyridine, pyridylaminated oligosaccharides were fractionated by reverse phase chromatography on an ODS column (0.4 × 25 cm), following the previously published methods (12, 14), at a flow rate of 1.5 ml/min. The starting buffer was 0.1 M ammonium acetate, pH 4.0, and a gradient increasing at 1% per min using 30% (v/v) methanol was applied. Columns were calibrated daily in terms of glucose units with a pyridylaminated partial dextran hydrolysate (3–11 glucose units). Fractions containing fucosylated structures were further fractionated on a normal phase HPLC (PalPak Type N) column in order to remove accompanying oligomannosidic structures. The buffer A used was made up by mixing a stock solution of 3% acetic acid (pH 7.3, adjusted with triethylamine) and 10% acetonitrile and acetonitrile in a 25:75 ratio, whereas buffer B was made up by mixing the stock solution and acetonitrile in a 75:25 ratio. The following gradient was applied: 0–5 min, 10% B; 5–45 min, 10–100% B; 45–55 min, 100% B; 55–56 min, 100–10% B; 56–65 min, 10% B. Selected fractions or the entire N-glycan pool were subject to exo- or endoglycosidase digestions as follows: *Canavalia ensiformis* (jack bean) α-mannosidase (200 milliunits in 20 µl of 50 mM sodium acetate, 0.1 mM zinc chloride, pH 4.2); C. *ensiformis* ß-hexosaminidase (5 milliunits in 20 µl of 0.1 M sodium citrate, pH 5.0); bovine kidney α-fucosidase (4 milliunits in 20 µl of 0.1 M sodium citrate, pH 5.0). In addition, core α1,3-fucose-containing fractions were specifically defucosylated with hydrofluoric acid (48% w/v) overnight at 4 °C as previously described (17).

**Construction of Insect Expression Vectors**— In order to construct plasmids encoding all four α1,3-fucosyltransferases homologues suitable for expression in insect cells, PCR were performed using the following primers: FusTA (CG6869, GenBank™ accession number AJ302045), forward primer 5′-CGGGGTACCTCGAGCAATAGGATGCTG-3′ and reverse primer 5′-CGGAATTCATCTCGACTGATGTC-3′; FusTB (CG4435, GenBank™ accession number AJ302046), forward primer 5′-CGGGTACCAGGATATCAGCAGTGATG-3′; FusTC (CG40305, GenBank™ accession number AJ302047), forward primer 5′-CGGGTACCAGGATATCAGCAGTGATG-3′ and reverse primer 5′-CGGAATTCATCTCGACTGATGTC-3′.

For FusTA, the Tf24 gene; in the latter case, the sequences were cloned in Pichia expression vectors (where the insert originated from the Tf24 strain cDNA) (9), whereas the 5′-end (first 675 nt) was generated by PCR from Canton S female body cDNA. Complete ORFs of FusTB and FusTC as well as the first 675 nt of FusTC were amplified from cDNA and subcloned into pGEM-T vector (Promega). Subsequently, complete ORFs with or without the natural stop codon of all four FusTc were cloned into the pLZT/VS/His vector (Invitrogen) carrying a gfp-zeo gene; in the latter case, the sequences were cloned in frame with a 3′ sequence encoding the V5 and His, epitopes using the following reverse primers: FusTA, 5′-CGGGATCCATCTCGACTGATGTC-3′; FusTB, 5′-GCTCTAGAATATTGAAACTAGTTCG-3′; FusTC, 5′-CGGGATCCAGGATATCAGCAGTGATG-3′; FusTD, 5′-GGATTCTCGAGGAAGGGTGTGAAGTATGAT-3′. The sequence and orientation of all cloned cDNAs were verified by sequencing.

**Transfection of Drosophila Schneider 2 Cells**— The pLZT/VS/His vectors with native stop codons were introduced to *Drosophila* S2 cells.
using TransFectin Lipid Reagent (Bio-Rad), according to the manufacturer’s protocol for adherent cells (comparable results where also obtained with the standard calcium phosphate transfection method).

The cells were incubated at 26 °C and harvested between the second and the fifth day of expression. A small aliquot of the cells was analyzed by confocal laser-scanning microscopy with a UV light source to confirm the presence of GFP fluorescence within the cells as an indication of a successful transfection procedure. Harvested cells were counted, spun down (1000 × g for 10 min), and washed twice with PBS. Cell pellets were either processed immediately or left frozen at −80 °C. Cell pellets were lyzed in radioimmuno precipitation buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 0.4 mM EDTA, 10% glycerol) supplemented with Complete-mini protease inhibitor mixture without EDTA (Roche Applied Science) or His tag protease inhibitor mixture (Sigma). Different volumes of radiolabeled precipitation buffer were used to normalize the number of cells per microliter (normally 2.5 × 10⁶ cells/µL). After lysis for 10 min at room temperature and 20 min on ice, cells were spun down at 14,100 × g at 4 °C for 30 min. Supernatants (10 µL/lane) were then analyzed by Western blotting as described below.

Transfection of Sf9 Cells—pIZT/V5-His vectors carrying complete ORFs of all four Drosophila α1,3-fucosyltransferase homologues with either the native stop codon or in frame with V5 coding sequence were used to transfect Sf9 cells using Cellfectin reagent (Invitrogen) following the manufacturer’s protocol for insect cells. A small aliquot of the cells was analyzed by confocal laser-scanning microscopy with a UV light source to confirm the presence of GFP fluorescence within the cells as an indication of successful transfection. Cells were collected 48 h post-transfection, washed once with PBS, and stored at −80 °C. For assaying, cells were lysed using 50 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Triton X-100 supplemented with Complete-mini protease inhibitor mixture without EDTA (Roche Applied Science) or His tag protease inhibitor mixture (Sigma) following the lysis protocol for Drosophila S2 cells. Different volumes of the buffer were used to normalize the number of cells per volume (to 1.25 × 10⁶ cells/µL).

Western Blotting—Cell lysates were mixed with 2× Laemmli loading buffer followed by electrophoresis on 12.5% SDS-polyacrylamide gels and transferred to nitrocellulose. The nitrocellulose sheets were reversibly stained with 0.5% Ponceau S (in 1% acetic acid) to verify that equal amounts of proteins were present in every lane. Subsequently, the membrane was blocked for 1 h with 1% bovine serum albumin in 50 mM Tris, pH 7.5, 1% Tween 20, bound antibodies were detected by alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma), followed by color detection using nitro blue tetrazolium/5-bromo-4-chloro-3-indolyolphosphate as substrate. Alternatively, the expression of recombinant proteins in Sf9 cells was verified by probing the extracted proteins on Western blots with anti-V5 antibody (Invitrogen; 1:5,000), followed by anti-mouse IgG (Fc-specific, alkaline phosphatase conjugate, 1:30,000; Sigma).

Assay of Fucosyltransferase Activity—Activity of core α1,3-fucosyltransferase from insect cell cultures was measured as previously described (18) with modifications using 100 mM MES, pH 6.5, 10 mM MnCl₂, 1 mM AMP, 210 µM 6-acetamido-6-deoxyoctanspermin, 0.4 mM GDP-fucose, and 25 µM dapsylated glycopeptide, with the sequence GENR in either the GnGn-, GnGnF₆-, MM-, or GnGNglycoforms (see Fig. 1 for oligosaccharide structures) supplemented with Complete-mini protease inhibitor mixture (Roche Applied Science). Reaction tubes were incubated at 23 °C and analyzed after 18 h. For analysis by MALDI-TOF MS, 0.5 µl of a 1:10 dilution of the reaction mixture was mixed with 0.5 µl of 1% α-cyano-4-hydroxycinnamic acid (Fluka; in 70% acetonitrile) on a MALDI plate. An increase in glycopeptide mi/z by 146.1 Da indicated the transfer of one fucose residue to the reducing terminal N-acetylgalosamine of the glycopeptide. For testing the substrate specificity of the recombinant Drosophila core α1,3-fucosyltransferase, a FLAG-tagged form of FucTA was expressed in Pichia pastoris under control of the AOX1 promoter using a modified form of the pPICZαC vector (Western blotting with anti-FLAG antibody showing a protein with apparent size of Mr 75,000). After 4 days of expression at 16 °C, the enzyme was concentrated 10-fold (using UltraFree centrifugal concentration devices, molecular weight cut-off 30,000) and tested with dapsylated Mgn-glycopeptide, which had been prepared from dapsylated MM-glycopeptide by the activity of FLAG-tagged human GnTI expressed also in P. pastoris at 16 °C, as well as with dapsylated MgnF₆-glycopeptide that was prepared from the dapsylated Mgn-glycopeptide by the activity of P. pastoris expressed C. elegans FUT-8 core α1,6-fucosyltransferase. The assays were performed using 0.1 mM glycopeptide, 2 mM GDP-fucose, 40 mM MES, pH 6.5, 10 mM MnCl₂ at 30 °C for 4 h prior to MALDI-TOF MS analysis as described for the cell line cell assays. The enzyme was also tested using a dansylated Man₉GlcnAc₂-glycopeptide, which was prepared after Pronase digestion of Aspergillus oryzae amylase. The resulting dansyl-Man₉GlcnAc₂-glycopeptide, which was analyzed by MALDI-TOF-MS, 0.5 µl of a 1:10 dilution of the reaction mixture was applied to the plate. An increase in glycopeptide mi/z/ by 146.1 Da indicated the transfer of one fucose residue to the reducing terminal N-acetylgalosamine of the glycopeptide.

Developmental Stages and RT-PCR—Canton S or w−; wild type D. melanogaster flies were maintained at room temperature. For different developmental stages, w−; wild type was used; after a 1-h “precollection” phase, eggs were collected for 1.5 h on standard apple juice Petri dishes, and different developmental stages were collected. For the tissue specificity (i.e. gender-separated heads and bodies), the Canton S strain was used. Total RNAs from various stages were isolated by TRIZol reagent (Invitrogen) followed by a first strand cDNA synthesis using Superscript III reverse transcriptase (Invitrogen) and oligo (dT)₁₆ as primer. Expression of Drosophila fucosyltransferase genes FucTA, FucTB, FucTC, and FucTD was analyzed by performing a 32–35 cycle PCR (55–60 °C for 30 s, 72 °C for 2 h; 94 °C for 30 min) using first strand cDNAs from staged flies and the following primers: FucTA, forward primer 5’-CGCCATGATCCTTCACT-3’ and reverse primer 5’-GTTTCTCTGT-GAATGCCGCTG-3’ (58 °C, 35 cycles); FucTB, forward primer 5’-CGCATCCACAAAGGC-3’ and reverse primer 5’-CGACAGGTTT-GTGGAGTAG-3’ (57 °C, 35 cycles); FucTC, forward primer 5’-CTT-ATCGATTGACTCGGAGT-3’ and reverse primer 5’-CGCCGAA-TTCTTCAACACGTATTCCGGTTTG-3’ (55 °C, 32 cycles); FucTD, forward primer 5’-CAAATGCCGATAGACACTC-3’ and reverse primer 5’-GTCGGAACCGCCGGACG-3’ (60 °C, 35 cycles). The cDNAs were normalized against the rp49 transcript, coding for a ribosomal protein. The primers used, producing a 440-bp fragment, were as follows: rp49-fw, GACCATCACCAGCCAGCATA; rp49-rev, TCCGACCAGGTTAACAAGAAC (60 °C).

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TABLE 1
N-Glycans of Drosophila BG2-c6 and S2 cell lines

| Structure               | m/z (OS) [M + Na]+ | Retention time (reverse phase) | Fraction no. (BG2-c6 cells) | Fraction no. (S2 cells) | Occurrence in BG2-c6 cells (MS) | Occurrence in S2 cells (MS) |
|-------------------------|--------------------|--------------------------------|-----------------------------|-------------------------|-------------------------------|------------------------------|
|                         | glucose units      |                                 |                             |                         |                               |                               |
| UMF6<sup>+</sup>         | 917.8              | 12.5                            | 10                          | ND<sup>a</sup>          | 1.4                           | 2.8                           |
| MM                      | 933.8              | 4.6/7/5.7/8                     | 3.7, 8                      | 10                      | 9.3                           | 4.6                           |
| MUFP<sup>+</sup>/UMF<sup>+</sup> | 1084.0             | 7.5/7.8/12.5                   | ND                          | 13                      | 19.7<sup>d</sup>             | 16.0                          |
| MMF<sup>+</sup>          | 1080.0             | 5.0                             | 4                           | ND                      | 1.2<sup>e</sup>              | ND                            |
| Man4                    | 1096.0             | 7.8                             | 8                           | ND                      | 1.8                           | ND                            |
| MMFP<sup>+</sup>         | 1226.1             | 6.1/7.8                         | 6.8                         | ND                      | 19.0                          | ND                            |
| Man5                    | 1258.1             | 4.6/7.5                         | 3.7                         | 10                      | 9.0                           | 5.1                           |
| GnMF<sup>+</sup> or MGn<sup>+</sup> | 1283.2            | ND                             | ND                          | ND                      | ND                            | 2.2                           |
| GnGn                    | 1340.2             | ND                             | ND                          | ND                      | 0.7                           |                               |
| Man6                    | 1420.3             | 4.0/6.1                         | 2.6                         | ND                      | 7.4                           | 5.6                           |
| GnMF<sup>+</sup>         | 1429.3             | 10.0                            | 9                           | ND                      | 0.3                           | ND                            |
| GnGnF<sup>+</sup>        | 1486.4             | ND                             | ND                          | ND                      | 0.4                           |                               |
| Man7                    | 1582.8             | 3.2/4/0.0/5.0/5.8               | 1.2, 4, 5                   | 2, 3, 4                 | 8.9                           | 11.4                          |
| Man8                    | 1744.5             | 3.2/4.6/6.6                    | 1.3                         | 1.3, 8                  | 10.3                          | 19.7                          |
| Man9                    | 1906.7             | 3.2/5.0                         | 1.4                         | 2, 3, 4                 | 9.0<sup>e</sup>             | 22.7                          |
| GlcMan8                 | 1906.7             | 5.8                             | 5                           | ND                      | 0.2<sup>e</sup>             | ND                            |
| GlcMan9                 | 2068.8             | 4.0/6.1                         | 2.6                         | 5                      | 0.9                           | 5.3                           |
| Glc2Man9                | 2290.2             | 6.5                             | ND                          | 7                      | ND                            | 0.5                           |

<sup>a</sup> Based on the known reverse phase HPLC retention time of MUF6<sup>+</sup>.  
<sup>b</sup> ND, not detected.  
<sup>c</sup> Structures expected to have core N-acetylglucosamine isomerised to N-acetylmannosamine.  
<sup>d</sup> MALDI-TOF MS ratio was estimated after analysis of structures on reverse phase HPLC.  
<sup>e</sup> Presence of the structure confirmed after normal phase HPLC.

**RESULTS**

A *Drosophila Neuronal Cell Line Is Enriched in Difucosylated N-Glycans.—**Due to the challenges in collecting a sufficient amount of *Drosophila* heads to show by direct HPLC or MS analyses that core α1,3-linked fucose is enriched in the neural tissue, we decided to look for suitable *Drosophila* cell lines to examine the expression of this epitope. The BG2-c6 cell line isolated from third instar larval central nervous system cells has been previously shown to bind anti-HRP (11); on the other hand, readily available and commonly used *Drosophila* S2 cells (20) of hemocyte origin were expected not to bind anti-HRP. The complete N-glycosylation profile of neither cell line has previously been described; thus, we prepared N-glycans from these cells after culturing them in serum-free medium. The MALDI-TOF MS profiles (Fig. 2) indicate that the BG2-c6 cells have difucosylated N-glycans similar to those we found previously in the glycan profile of whole adult flies (see Fig. 1 for oligosaccharide structures). Indicative of an enrichment of these structures in neuronal cells, these glycans accounted for 20% of the total N-glycans in the BG2-c6 cells (Fig. 2A and Table 1), as opposed to 0.8% in whole adult flies (9). On the other hand, these structures were apparently absent from S2 cells (Fig. 2B and Table 1).

In order to prove that the difucosylated N-glycans from BG2-c6 cells are indeed carrying core α1,3-fucose, the complete N-glycan pool was
pyridylaminated and fractionated by RP-HPLC (Fig. 3A); fraction 8 contained two dominant species with m/z values of 1012.0 and 1304.2, suggestive of the presence of both MM and MMF3F6. Removal of the α1,6-fucose residue from the latter by bovine kidney α-fucosidase should result in an earlier retention time on reverse phase columns, whereas removal of the α1,3-fucose residue by acid hydrolysis with hydrofluoric acid should result in a later retention time (12, 14, 21). Indeed (Fig. 3B), treatment of the difucosylated species (isolated and separated from MM structure by normal phase HPLC) using these two methods resulted in the predicted changes in chromatographic behavior, which in both cases correlated with the loss of m/z 146, equivalent to the removal of one fucose residue, as shown by MALDI-TOF MS analysis.

Furthermore, reverse phase fraction 9 contained one species with an m/z value of 1507.4, corresponding to the mass of either GnMF or MGnFF. A partial jack bean hexosaminidase digest of a GnMF standard was analyzed by RP-HPLC and compared with an α-fucosidase digest of fraction 9; as judged by co-elution, it was concluded that fraction 9 contained the difucosylated structure GnMF3F6 with nonreducing N-acetylgalactosamine linked to the α1,6-arm and not to the α1,3-arm (data not shown).

Expression of α1,3-Fucosyltransferase Homologues in Drosophila—In order to investigate the genetic basis for the observed core α1,3-fucosylated N-glycans, we initiated studies of the expression of all four α1,3-fucosyltransferase homologues from the fly. Although previous data suggest that FucTA functions as a core α1,3-fucosyltransferase, it could be theoretically possible that FucTB, FucTC, and FucTD also contribute to anti-HRP epitope expression (9). Thus, in order to examine whether we could infer function from the expression pattern, we determined the transcript levels of all four α1,3-fucosyltransferase homologues in different stages of the fly, in male and female heads and bodies and in the BG2-c6 and S2 cell lines. If FucTA is responsible for the expression in vivo of core α1,3-fucosylated N-glycans in flies, then this gene should be transcribed throughout the life cycle (with the possible exception of the first 8 h before development of the neural system), in heads and bodies of both sexes (with an enrichment of neural tissue in heads) and in BG2-c6 cells; on the other hand, FucTA should be absent from S2 cells. The absence of FucTB, FucTC, and FucTD from any stage of the life cycle and their presence in S2 cells would be compatible with a potential inability to form the anti-HRP epitope.

Total RNA was prepared from heads and bodies of both male and female flies, from various developmental stages and from the two cell lines; the quantities of RNA used in each PCR were prenormalized on the basis of the levels of the transcript encoding RP49, a constitutively expressed ribosomal protein. Primers were designed across introns, so as to ensure that genomic DNA contamination could not result in a PCR product of the same size as an RT-PCR product. Consistent with our hypothesis, the results (Fig. 4) indicated that FucTA is expressed in heads and bodies of male and female flies, in all developmental stages, and in BG2-c6 cells, whereas it is apparently absent from S2 cells under the given conditions (under no conditions were we able to amplify the complete FucTA open reading frame from S2 cells). On the other hand, FucTB transcripts are present only in 16-h-old embryos, heads of both sexes, and male bodies as well as in BG2-c6 cells and S2 cells; FucTC is expressed in all stages but only in adult bodies and male heads and seemingly is not present in female heads and BG2-c6 and S2 cells; FucTD is strongly expressed in male bodies and, weakly, in the pupal stages and S2 cells.
Anti-HRP Epitope of Drosophila

Gain of Anti-HRP Staining in Schneider 2 and Sf9 Cells—Our next experiments were directed at examining which of the four α1,3-fucosyltransferase homologues in Drosophila (FucTA, FucTB, FucTC, and FucTD) could confer anti-HRP staining to Drosophila S2 cells. Previously, we have only demonstrated that FucTA is enzymatically active in vitro (9); using the finding that S2 cells lacked difucosylated glycans, we decided to express the complete reading frames of all four homologues in these cells. When designing the expression constructs, we considered the differences between the various sequences of each fucosyltransferase present in the data bases; these polymorphisms (whether natural or PCR-generated) could theoretically lead to acquisition or loss of enzymatic activity of the encoded proteins. For instance, our previously published FucTB sequence (AJ302947) encodes a protein of 425 residues; however, the genomic sequence lacks nucleotides 530 and 531 and so would encode a protein of only 185 amino acids (see chromosome X shotgun sequence AABU01002757.2). On the other hand, the unpublished FucTD sequence of Petit et al. (AF441265) contains an Ala-Thr sequence, whereas the Drosophila Gene Collection clone encodes a Thr-Pro at this position (AY075216). Using portions of the reading frames of either Canton S-derived clones, our previous clones, or Drosophila Gene Collection clones, we generated four forms of FucTA, two forms of each FucTB and FucTC, and three forms of FucTD in order to cover most of the polymorphisms.

Two days after transfection, the acquisition of anti-HRP binding was examined by Western blotting. An extract of BG2-c6 cells was used as a positive control (Fig. 5A, lane 2). Only S2 cells transfected with FucTA gained the epitope (Fig. 5A, lane 5), whereas untransfected or mock-transfected S2 cells and S2 cells transfected with FucTB, FucTC, FucTD or empty vector showed only background staining with anti-HRP. The
extracts of transfected cells were analyzed for fucosyltransferase activity using dabsylated GnGnF6-glycopeptides (see Fig. 1 for relevant structures). All extracts transferred a fucose residue to dabsylated GnGn-glycopeptide to a similar extent, an expected result considering the lower affinity of FucTA for that particular substrate (9) and possibly due to relatively high endogenous activity of core α1,6-fucosyltransferase. On the other hand, only extracts of FucTA-transfected cells transferred a fucose residue to the dabsylated GnGnF6-glycopeptide (Fig. 5B). The extracts of cells expressing Drosophila fucosyltransferase homologues were further tested with dabsylated MM- and GGN-glycopeptides as substrates, but no transfer of fucose was detected.

Subsequently, constructs encoding all four fucosyltransferases with either the native stop codon or encoding C-terminally V5/His6-tagged forms were used to transfect Sf9 cells, which in our hands display a higher transfection efficiency than S2 cells. After 2 days of transient expression, extracts of the cells were analyzed for fucosyltransferase activity using dabsylated GnGn-, MM-, GGN-, and GnGnF6-glycopeptides. All extracts transferred a single fucose residue to a dabsylated GnGn-glycopeptide to a similar extent, except that FucTA-transfected cells could transfer a second fucose to this substrate. Also, the FucTA-transfected cells fucosylated GnGnF6 completely in 23 h, indicating a much higher activity of recombinant FucTA expressed in Sf9 cells than in S2 cells. None of the extracts of cells transfected with FucTB, FucTC, and FucTD ORFs displayed any transfer above background to this substrate (data not shown). The dabsylated MM-glycopeptide was quickly degraded under the given conditions, presumably due to an endogenous endoglycosidase H activity present in extracts of Sf9 cells, and therefore could not be used to estimate any fucosyltransferase activity. Furthermore, Western blotting confirmed that only with FucTA was there an obvious increase in anti-HRP staining upon transfection of fucosyltransferase cDNAs into Sf9 cells (Fig. 5C, lane 7). On the other hand, all four Drosophila α1,3-fucosyltransferase homologues were successfully expressed in Sf9 cells as judged by use of the anti-V5 antibody (Fig. 5C, lanes 1–5).

Further Substrate Specificity Studies with FucTA—Previously, we had only tested the transfer of fucose by FucTA with MM, GnGn, and Gal-Gal substrates. However, while considering both the putative action of a processing hexosaminidase in insect cells (22) and the fact that MMF3F6, rather than GnMF3F6, is the major difucosylated species in flies, we wished to confirm that Mgf, in addition to GnGn, is a substrate for the fucosyltransferase. To this end, FucTA was tested with Mgf and MgfF6 substrates. When recombinantly expressed in P. pastoris, FucTA transfers a fucose residue to dabsylated Mgf and MgfF6-glycopeptides (Fig. 6). It also transfers to a much lower extent to dabsylated GGN-glycopeptide, but not to dabsylated Mm- or MGF-glycopeptides (data not shown). Furthermore, in assays with the dansylated Man5-glycopeptide in the presence or absence of GnTI and core α1,6-fucosyltransferase, GnTI-dependent transfer was observed, particularly when the core α1,6-fucosyltransferase was also present, as shown by a shift to lower retention times, thereby generating either Man5GnF5 or Man5GnF5 in vitro (Fig. 6E). On the basis of these data, we presume that the prior action of GnTI (23) is required for creating substrates for FucTA, whereas subsequent modification by GnTI (24) and the core α1,6-fucosyltransferase (25) are not required for the activity of FucTA, although prior core α1,6-fucosylation appears to improve the efficiency of the action of the FucTA. Whereas the action of mannosidase II may well result in more efficient transfer by core fucosyltransferases, our findings in both this and our previous studies (25) support the hypothesis that GnTI is the entry point for the generation of fucosylated paucimannosidic N-glycans in Drosophila. Furthermore, we can account for the synthesis of the major difucosylated structure in Drosophila, MMF3F6.

Reduction of FucTA Expression in a Neuronal Cell Line—The detection of core α1,3-fucose on its N-glycans (see above) and the anti-HRP binding characteristics of BG2-c6 cells (11) indicated that this line is a suitable model for examination of core fucosylation in vivo. Therefore, since FucTA is a proven core α1,3-fucosyltransferase (9), we assumed that specific targeting of FucTA transcripts by RNAi could result in a reduction of binding to anti-HRP.

Double-stranded RNAs based on portions of the FucTA, FucTB, FucTC, FucTD, and Nervana genes were synthesized (Nervana being an anti-HRP epitope of Drosophila
**Anti-HRP Epitope of Drosophila**

These were then incubated with BG2-c6 cells, which after 4 days of culture were then subject to Western blotting analysis using anti-HRP; as a control, a monoclonal antibody against Nervana was also employed. As shown in Fig. 7A, anti-HRP staining was reduced in BG2-c6 cells incubated with double-stranded RNA encoding part of the FucTA sequence (a similar reduction was attained when the cells were incubated with mixtures including this RNA; data not shown). However, no such diminution was observed if double-stranded RNA corresponding to any other fucosyltransferase or Nervana had been present. The same result was acquired by incubating dsRNA-treated BG2-c6 cells with anti-HRP antibodies, followed by anti-rabbit-FITC and flow cytometry; only cells treated with dsRNA encoding a part of FucTA reduced the binding of anti-HRP to the surface of intact cells (Fig. 7B). In order to verify whether the RNAi knock-down was specific for the targeted genes, the relative amounts of transcripts of the α1,3-fucosyltransferase homologues shown to be present in BG2-c6 cells (cf. Fig. 4; i.e. FucTA and FucTB) were estimated by performing RT-PCR from rp49-normalized cDNA. RNA was prepared from cells treated without dsRNA and from cells treated with dsRNA corresponding to ORFs of FucTA, FucTB, and nrv2.2. The cells treated with dsRNA corresponding to either FucTA or FucTB ORF contained significantly reduced amounts of the respective transcripts while leaving amounts of other transcripts unaltered, demonstrating that the RNAi knock-down was specifically affecting the targeted genes (Fig. 7C). In the case of anti-Nervana Western blots (data not shown), only the M, 37,000 band was no longer seen when Nervana double-stranded RNA was present. Thus, we believe that, since this method was successful with both a carbohydrate and a protein epitope, RNA-mediated interference is a valid method for investigation of the BG2-c6 cell line.

**DISCUSSION**

Twenty years after the first description of the use of anti-HRP for the staining of neural tissue in *Drosophila* (1), we determined the structural basis for this cross-reaction by demonstrating the presence of core α1,3-fucosylated N-glycans and of a relevant fucosyltransferase gene (FucTA) (9). However, as indicated by antibody-binding, mass spectrometric, and HPLC data, we only had in vitro evidence that FucTA was capable of synthesizing the anti-HRP epitope. Identifying α1,3-fucosyltransferase FUT-1 as the enzyme responsible for anti-HRP staining in *C. elegans* (10) also supported the role of core α1,3-fucosyltransferases in the neuronal anti-HRP epitope synthesis in invertebrates in general. In the present study, we made use of two different *Drosophila* cell lines in order to investigate the biological role of FucTA in vivo and to show that, of the four α1,3-fucosyltransferase homologues, only FucTA is indispensable in synthesis of the anti-HRP neuronal epitope in these cells.

First of all, it was necessary to show that the two cell lines, BG2-c6 and
S2 cells were relevant models (positive and negative, respectively). This necessitated the analysis of the N-glycans of both cell lines; indeed, this is the first time that the complete N-glycan profile of any Drosophila cell line has been examined. The results are generally in agreement with the data obtained with other insect cell lines in that oligomannose and core α1,6-fucosylated N-glycans were found. Furthermore, in the neuronal cell line, previously found to express the anti-HRP epitope (11), difuco-
sylated N-glycans binding both α1,3- and α1,6-linked fucose residues were detected at a level enriched as compared with whole adult flies, these structures being apparently absent from S2 cells, which do not express this epitope as judged by our Western blot data. This is also in agreement with the glycan structures found on two recombinant glyco-
proteins expressed in S2 cells (26). Thus, there appears to be a corre-
lation between expression of the anti-HRP epitope and the presence of core α1,3-linked fucose. This is, perhaps, not surprising, since horserad-
ish peroxidase is a plant glycoprotein, on which around 80% of the glycans carry core α1,3-fucose (7) and since core α1,3-fucosylated glyco-
conjugates inhibit the binding of anti-HRP to embryonic neural tissue (9). Various insect lines were previously shown to express glycoproteins containing core α1,3-linked fucose; some have a low degree of α1,3-
fucosylation (Sf-21 and Bm-N cells), whereas up to 30% of the glycans of the IZD-Mb-0503 cell line are α1,3-fucosylated glycans, also predomi-
nantly in difucosylated form (27). However, our data suggest that S2

Another line of evidence to suggest that FucTA is a mediator of this
epitope’s expression comes from our RT-PCR data. The particular pat-
tern of presence and absence of transcripts under given conditions
would strongly suggest that expression of FucTA correlates with the
appearance of the anti-HRP epitope. The data from the present study
are comparable with the rather limited data available from other
sources. The data from the EST database (NCBI) indicates the presence
of Drosophila FucTA in 0–24 h old embryos. In situ RNA hybridization
analysis of Drosophila FucTA expression in embryos shows that expres-
sion of this gene takes place in the central nervous system throughout

The presence of MMF\textsuperscript{3F6} structures in the neuronal cell line, tak-
ing into account that recombinantly expressed FucTA was previ-
uously shown to act on GnGn and GaGal structures (9), raised a
question as to how this structure is actually created in the cell line.
Our new finding that FucTA acts on MgN (and Mg\textsuperscript{4F6}) but not on
GnM structures is in agreement with the evidence of an N-glycan
processing hexosaminidase in insects (22) and with the previously
proposed glycosylation order in invertebrates (25), where it is expected
that MgN is modified by the action of α1,6-fucosyltrans-
ferase and FucTA, yielding Mg\textsuperscript{4F6}, which is, in turn, processed by
the hexosaminidase, finally yielding the MMF\textsuperscript{3F6} structure. It is also
noteworthy that all substrates demonstrably utilized by FucTa
(MgN, Mg\textsuperscript{4F6}, Man\textsuperscript{5Gn}, Man\textsuperscript{5GnF6}, GnGn, GnGn\textsuperscript{4F6}, GaGal,
and GNGN) contain the residue transferred by GnTI. On the other hand,
MM, GnM, and Man\textsuperscript{5} (the former two requiring the prior action
of GnTI and hexosaminidase during their biosynthesis) were not
detectably substrates, although, of course, an extremely low transfer
in vivo by FucTA or other FucTs to such glycans cannot be ruled out.
Interestingly, this substrate specificity is completely the opposite of
that of the other proven invertebrate core α1,3-fucosyltransferase, C.
elegans FUT-1 (10).

Another line of evidence to suggest that FucTA is a mediator of this
epitope’s expression comes from our RT-PCR data. The particular pat-
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sion of this gene takes place in the central nervous system throughout

differently expressed ribosomal protein. Relative amounts of Rp49, FucTA, and FucTB tran-
scripts in cells treated with no dsRNA (lane 1), with FucTA dsRNA (lane 2), with FucTB
dsRNA (lane 3), and with nrv2.2 dsRNA (lane 4) were estimated by RT-PCR.

FIGURE 7. Reduction of anti-HRP binding to Drosophila BG2-c6 neuronal cell line
 treated with dsRNA from Drosophila FucTA. A, BG2-c6 cells were incubated for 4 days
 in the presence of dsRNA corresponding to parts of the ORFs of either one of the four
 Drosophila fucosyltransferase homologues and the Nervana nrv2.2 fragment. Extracts of
cells treated with FucTA, FucTB, FucTC, and FucTD dsRNA probed with anti-HRP antibody
are shown in lanes 2, 3, 4, and 5, respectively. Extracts of cells incubated without dsRNA
(lane 1) and with Nervana nrv2.2 dsRNA (lane 6) were used as negative controls. These
data were reproduced with two other different dsRNAs corresponding to different parts
of FucTA ORF. Reversible Ponceau S staining was used to confirm the presence of equal
amounts of protein in each lane. B, BG2-c6 cells were incubated for 4 days in the presence
of dsRNA corresponding to parts of the ORFs of either one of the four Drosophila fuco-
syltransferase homologues and the Nervana nrv2.2 fragment. Treated cells were incu-
bated with anti-HRP, washed with PBS, and further incubated with anti-rabbit FITC anti-
body and subjected to flow cytometry. The thick line shows the profile with BG2-c6 cells
 treated with dsRNA corresponding to FucTA. The negative control (S2 cells) is shown using
a dotted line, whereas the positive control (untreated BG2-c6 cells) is shown using a thin line.
Cells treated with dsRNA corresponding to FucTB, FucTC, FucTD, and Nervana
nrv2.2 fragments showed the same binding pattern as untreated BG2-c6 cells (data not
shown). C, BG2-c6 cells were incubated for 4 days in the presence of dsRNA correspond-
ing to parts of the ORFs of the two Drosophila fucosyltransferase homologues shown to
be present in BG2-c6 cells (i.e., FucTA and FucTB; cf. Fig. 4) and the Nervana nrv2.2 frag-
ment. Total RNA was prepared from harvested cells and used to synthesize cDNA, which
was subsequently normalized by RT-PCR of rp49 transcripts, which encode a constitu-

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the last few stages of embryonic development (stages 13–16 equivalent to 10–15-h-old embryos) (28). These data are in agreement with the expression pattern acquired in this study; furthermore, they also show that the expression of *Drosophila* FucTA correlates spatially and temporally with expression of the anti-HRP epitope in flies (1).

In contrast, FucTB is, according to the microarray data, only expressed in the first 12 stages (although we also see low levels of transcript in the later developmental stages); the seemingly stronger expression of FucTB in male bodies is compatible with the presence of many corresponding adult testes ESTs in the data base. Interestingly, FucTB ESTs containing one intron have been isolated from adult head, but the FucTB fragments we detect in heads by RT-PCR are of the correctly spliced size. It also appears that FucTB is expressed more in male flies and predominantly in the testis, since other recent data indicate 14.5 times stronger expression in testis over ovaries and 8.9 times stronger expression in testis over samples of male flies without gonads (29). Other data from the EST data base are compatible with the expression panel, indicating expression of FucTB in testis, adult heads, and 0–24-h-old embryos.

On the other hand, the Berkeley data do not include information on FucTC (since this was not a predicted reading frame, so no primers were designed); in any case, if in the isogenic *cn bw sp* strain (that used for sequencing the genome (30)) and in the Canton S strain the reading frame is interrupted and if, as we find, transcripts of this gene are not present in female heads and neuronal cell line, then one may conclude that FucTC cannot be relevant to anti-HRP epitope biosynthesis in these fly strains. We also detected FucTC transcripts by *in situ* hybridization in primordial gut (4). Indicative, however, of a conserved pattern of expression among insects for this gene is the isolation of ESTs of a FucTC homologue from *Glossina* gut (GenBank™ accession numbers BX552226, BX552225, BX558576, and BX563797). The expression of the *Drosophila* FucTC in all developmental stages cannot be confirmed by data from the EST data base; to date, FucTC ESTs were only found in adult heads.

Our data, indicating the absence of FucTD transcripts from early stages of fly development, agree with the Berkeley microarray data; also, the large number of FucTD ESTs originate from adult testes and only one from adult heads, which partially concurs with our finding that FucTD is expressed in male bodies but not in male heads or females. In theory, FucTD could therefore be responsible for the previously found anti-HRP epitope expression in male reproductive tissue (1, 4); indeed, *Drosophila* FucTD is found to be predominantly expressed in testis (14.3 times more than in ovaries and 13.8 times more than in samples of male flies without gonads) (29). However, the finding that FucTD does not confer anti-HRP binding to S2 cells (neither at the level we find by RT-PCR to be naturally expressed in these cells nor after overexpression) would argue against such a biochemical function. The expression pattern acquired during the present study for this gene is, however, compatible with expression in testis, since we found transcripts in male bodies (as the nearest “equivalent” to testis). The expression is also seemingly taking place in later developmental stages and in S2 cells. Further indirect evidence that FucTD is not required for anti-HRP epitope expression is that homologues of this gene have as yet not been found in species that do not belong to the *Drosophila* genus (with 20–40% identity on protein level), whereas both FucTA and FucTC homologues are also found in other insects (with 40–80% identity on the protein level). On the other hand, FucTB orthologues from *Drosophila* species and *Bombyx mori* appear to be most related to mammalian FucT-X and FucT-XI. Furthermore, it should be noted that FucTD is missing part of a motif conserved seemingly in all known animal α1,3-fucosyltransferases: instead of the D(Y/I)VTEK motif (31, 32), FucTD has D(Y)PPQ at positions 328–333. Additionally, the hydrophobicity plot of FucTD indicates that, for a type II membrane protein, it has an unusually long cytosolic tail (the predicted transmembrane domain covers amino acids 89–119), showing another difference between FucTD and other core α1,3-fucosyltransferase homologues/enzymes.

Interestingly, the *tollo* gene shown to rescue the epitope in *Drosophila* mutants that lack the staining (33), was also shown to be expressed throughout the *Drosophila* life cycle, even in the S2 cell line lacking the staining (data not shown). The limited data from the EST data base show that Tollo is expressed in 0–24-h-old embryos and in the mbn2 cell line (of hemocyte origin consisting of tumorous blood cells) and possibly in imaginal disks and adult heads. Although suggestive of Tollo not directly taking part in the expression of the anti-HRP epitope, the presence of the Tollo transcripts in the cell line lacking the staining might be misleading; the regulation of the gene product synthesis and activity might be on the translational level or be on the level of the gene product itself. Due to the demonstration that incubation of asialoagglutinin with FucTA results in creation of an anti-HRP epitope *in vitro* (9) and that this enzyme has a role in the synthesis of this epitope in *Drosophila* neuronal cells *in vivo*, the exact role of Tollo in anti-HRP epitope expression remains unclear; one of the possibilities is that the Tollo functions as an upstream regulator of FucTA or that it is involved in biochemical processes that precede the actual transfer of fucose to glycoproteins by FucTA. The role of Tollo in anti-HRP epitope expression and its relation to FucTA are subjects of ongoing research.

Subsequently, we tested all four *Drosophila* α1,3-fucosyltransferase homologues for the ability to confer anti-HRP staining to the otherwise nonstaining S2 cells and for their ability to increase the anti-HRP binding to normally low staining S9 cells. Considering that in S9 cells we could show that recombinant forms of all four fucosyltransferase homologues were indeed expressed, it was demonstrated that, of these four, only FucTA is involved in synthesis of the anti-HRP epitope *in vivo*; this is consistent with our previous *in vitro* and expression panel data. Furthermore, encouraged by the N-glycan analyses and the expression panel results, we used RNA-mediated interference of FucTA expression in the neuronal BG2-c6 cell line using double-stranded RNA with no homology to any other gene; the specificity of the RNAi experiments for the targeted α1,3-fucosyltransferase genes transcribed in the BG2-c6 neuronal cell line was verified by measuring relative amounts of respective transcripts in dsRNA-treated cells. As judged by Western blots, the expression of the epitope was significantly reduced in the neuronal cell line when using the FucTA double-stranded RNA, whereas other double-stranded RNAs encoding other α1,3-fucosyltransferase homologues (FucTB, FucTC, and FucTD) and Nervana had no discernable effect. Furthermore, analysis of RNAi-treated cells by flow cytometry shows a reduction of anti-HRP binding to the surface of cells treated with FucTA dsRNA, suggesting that the cell surface epitopes are also created by the addition of a fucose residue by FucTA. Therefore, both knock-down of *Drosophila* core α1,3-fucosyltransferase homologues (FucTB, FucTC, and FucTD) and Nervana had no discernable effect. Furthermore, analysis of RNAi-treated cells by flow cytometry shows a reduction of anti-HRP binding to the surface of cells treated with FucTA dsRNA, suggesting that the cell surface epitopes are also created by the addition of a fucose residue by FucTA. Therefore, both knock-down of *Drosophila* core α1,3-fucosyltransferase homologues (FucTB, FucTC, and FucTD) and Nervana had no discernable effect.

In summary, the present study is the first to indicate a specific role for...
the core α1,3-fucosyltransferase FucTA in the synthesis of the neural anti-HRP epitope in insects. It is certainly of interest to follow up these studies with larger scale screens to uncover how its expression is controlled as well as to examine the effect of targeted reduction of FucTA transcription in whole animal models. Understanding the biosynthesis of this tissue-specific glycosylation event as well as of a recently identified relevant endogenous C-type lectin (34) opens up the possibility of pursuing new ways toward revealing the function of carbohydrate recognition networks in the fly.

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