Site-specific Fucosylation of Sialylated Polylactosamines by α1,3/4-Fucosyltransferases-V and -VI Is Defined by Amino Acids Near the N Terminus of the Catalytic Domain*

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Fucose transfer from GDP-fucose to GlcNAc residues of the sialylated polylactosamine acceptor NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-ceramide leads to two isomeric monofucosyl antigens, VIM2 and sialyl-Leα. Human α1,3/4-fucosyltransferase (FucT)-V catalyzes primarily the synthesis of VIM2, whereas human FucT-VI catalyzes primarily the synthesis of sialyl-Leα. Thus, these two enzymes have distinct “site-specific fucosylation” properties. Amino acid sequence alignment of these enzymes showed that there are 24 amino acid differences in their catalytic domains. Studies were conducted to determine which of the amino acid differences are responsible for the site-specific fucosylation properties of each enzyme. Domain swapping (replacing a portion of the catalytic domain from one enzyme with an analogous portion from the other enzyme) demonstrated that site-specific fucosylation was defined within a 40-amino acid segment containing 8 amino acid differences between the two enzymes. Site-directed mutagenesis studies demonstrated that the site-specific fucosylation properties of these enzymes could be reversed by substituting 4 amino acids from one sequence with the other. These results were observed in both in vitro enzyme assays and flow cytometric analyses of Chinese hamster ovary cells transfected with plasmids containing the various enzyme constructs. Modeling studies of human FucT using a structure of a bacterial fucosyltransferase as a template demonstrated that the amino acids responsible for site-specific fucosylation map near the GDP-fucose-binding site. Additional enzyme studies demonstrated that FucT-VI has ~12-fold higher activity compared with FucT-V and that the Trp124/Arg110 site in these enzymes is responsible primarily for this activity difference.

α1,3/4-Fucosyltransferases (FucTs)3 catalyze transfer of fucose to GlcNAc residues present in lacto or neolacto series structures of cell-surface glycolipids and glycoproteins. Fucosylated structures are known to accumulate in many human cancers (1–4), function as ligands for leukocyte adhesion during inflammation (4, 5), and undergo developmental regulation (6–8). The diversity of naturally occurring α1,3/4-fucosylated structures found in human cells is controlled by a family of six distinct yet related FucTs with individual tissue distribution properties and acceptor substrate preferences. Among these six FucTs, FucT-III, FucT-V, and FucT-VI share >85% amino acid sequence homology and originated from a common ancestral gene via gene duplication (9–11). Genes for these enzymes form a cluster on chromosome 19 in humans (12–15). FucT-IV, FucT-VII, and FucT-IX share a lower amino acid sequence homology both between each other and with FucT-III, FucT-V, and FucT-VI. Additionally, they have distinct chromosomal localization. Genes for FucT-IV, FucT-VII, and FucT-IX have been mapped to chromosomes 11, 9, and 6, respectively (16–18).

Although all of these enzymes catalyze fucose transfer to (LacNAc)Galβ1-4GlcNAc chain acceptors to produce α1,3-fucosylated products, only FucT-III and FucT-V have been shown to catalyze fucose transfer to Galβ1-3GlcNAc chain acceptors in an α1,4-linkage (19–22). This property correlates with the presence of an aromatic amino acid residue in the acceptor-binding domain of the enzymes (22). In addition, more subtle differences in acceptor specificity exist. FucT-VII is specific for sialylated acceptors (23). Conversely, FucT-IX has specificity for neutral acceptors (24), and FucT-IV has a preference for neutral acceptors, but will also catalyze fucose transfer to sialylated acceptors (25).

Accepter specificity differences have also been observed between FucT-V and FucT-VI. FucT-V preferentially transfers fucose to internal GlcNAc residues of polylactosamine structures compared with distal GlcNAc residues (26) and has been shown to catalyze the cell-surface expression of the VIM2 anti-

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3 The abbreviations used are: FucTs, α1,3/4-fucosyltransferases; nLc6Cer, neolactohexosylceramide; type 1, Galβ1-3GlcNAc; type 2, (LacNAc)Galβ1-4GlcNAc; H-type 1, Fuca1-2Gal β1-3GlcNAc; H-type 2, Fuca1-2Gal β1-4GlcNAc; MOPS, 4-morpholinepropanesulfonic acid; FACS, fluorescence-activated cell sorter; MFI, median fluorescence intensity; CHO, Chinese hamster ovary. Glycolipids are abbreviated according to the recommendations of the IUPAC-UAB Joint Commission on Biochemical Nomenclature (44).
gen (CD65s, III<sup>2</sup>FucVI<sup>3</sup>NeuAc-nLc<sub>6</sub>) in cells transfected with a human FucT-V expression vector (14). In contrast, FucT-VI preferentially transfers fucose to distal GlcNAc residues of neutral and sialylated polylactosamine structures, leading to the Le<sup>e</sup> and sialyl-Le<sup>e</sup> determinant structures, including V<sup>3</sup>FucVI<sup>3</sup>NeuAc-nLc<sub>6</sub> Cer by desialylation with 1% acetic acid and of the acceptor oligosaccharide, producing Lex or sialyl-Lex (see Fig. 1). We refer to this transferase specificity as "site-specific fucosylation."

We have conducted a series of structure-function studies utilizing domain swaps and site-directed mutants of FucT-V and FucT-VI to identify the amino acids responsible for site-specific fucosylation of internal versus distal GlcNAc residues of VI<sup>3</sup>NeuAc-nLc<sub>6</sub>Cer. The results demonstrate that site-specific fucosylation is controlled by a 40-amino acid segment near the N terminus of the catalytic domain where 8 amino acid differences are located between FucT-V and FucT-VI. Amino acids at both ends of this segment are involved in defining the site-specific fucosylation properties of each enzyme. Substitution of as few as 2 of the 8 amino acids from one FucT into the sequence of the other is sufficient to alter its site-specific fucosylation properties. Our results also demonstrate that inherent differences in enzyme activity between FucT-V and FucT-VI correlate with a single amino acid change near the C terminus of the 40-amino acid segment.

**EXPERIMENTAL PROCEDURES**

**Materials**

COS-7 cells were obtained from American Type Culture Collection (Manassas, VA). VI<sup>3</sup>NeuAc-neolactohexosylceramide (VI<sup>3</sup>NeuAc-nLc<sub>6</sub>Cer) was isolated from bovine erythrocytes as described previously (29). nLc<sub>6</sub>Cer was prepared from VI<sup>3</sup>NeuAc-nLc<sub>6</sub>Cer by desialylation with 1% acetic acid and boiling for 1 h. nLc<sub>6</sub>Cer was obtained after dialysis with water in Spectrapor 3 dialysis tubing. The detergent G-3634-A was obtained from Dr. S. Basu (University of Notre Dame, Notre Dame, IN). Galβ1-4GlcNAc-8-methoxycarbonyloctyl glycoside was obtained from Dr. Ole Hindsgaul (Carlsberg Laboratory). GDP-<sup>14</sup>C]fucose and GDP-[<sup>3</sup>H]fucose were obtained from PerkinElmer Life Sciences. All restriction enzymes were purchased from New England Biolabs (Beverly, MA). Oligonucleotide primers were synthesized by Operon Technologies (Alameda, CA). The QuikChange site-directed mutagenesis kit was purchased from Stratagene (La Jolla, CA).

**Preparation of FucT Domain Swap and Point Mutant Constructs**

Full-length wild-type FucT-V in pcDNA3.0 and full-length wild-type FucT-VI in pcDNA3.1 were the starting materials for construction of all domain swap mutants (Table 1) and point mutants (Table 2). To create swap set 1, existing BamHI sites (two in FucT-V, one in FucT-VI, and one in the pcDNA vectors) had to be removed and one created in the desired location in both FucT-V and FucT-VI. A naturally occurring restriction site in both FucTs, Msel, was utilized to create swap set 2.
Expression of Recombinant FucTs

Wild-type and mutant constructs were transfected into COS-7 cells using Lipofectamine 2000. Thirty μg of plasmid DNA was used per 10-cm dish. Four days post-transfection, cells were washed with cold phosphate-buffered saline and scraped into microcentrifuge tubes. After two washes with 50 mM cacodylate (pH 6.7), cells were lysed with 0.2% Triton X-100 in 50 mM cacodylate (pH 6.7). The FucT activity present in the cell lysate was analyzed directly, or the lysate was stored at 4 °C for up to 4 weeks prior to assay. (Control reactions were incubated for 2–8 h at room temperature and stopped by the addition of 100 μl of CHCl3/CH3OH/H2O (10:5:1) with incubation at 37 °C for 30 min in the solvent. The glycolipid remaining at the origin of the dried chromatography paper was extracted twice with 5-ml washes of CHCl3/CH3OH/H2O (10:5:1) with incubation at 37 °C for 30 min in the solvent. The desialylated product was removed, spotted onto a high-performance TLC plate (Silica Gel 60; EM Science, Gibbstown, NJ), and developed in a solvent system consisting of n-propyl alcohol/water/concen-

Enzyme Assays

Galβ1-3GlcNAc (Type 1), (LacNAc)Galβ1-4GlcNAc (Type 2), Fucα1-2Galβ1-3GlcNAc (H-type 1), and H-type 2 (Fucα1-2Galβ1-4GlcNAc) 8-Methoxycarbonylglucosyl glycosides acceptor (type 1, 2, H-type 1, and H-type 2; see Table 1 for structures), and 5 μl of enzyme fraction in a total volume of 20 μl. The reaction mixtures were incubated at 37 °C. Each enzyme preparation was assayed at four time points ranging from 30 min to 6 h. The reactions were stopped by the addition of 400 μl of water, and the reaction products were separated from substrate by reverse-phase chromatography (Sep-Pak C18) and quantified as described previously (30).

Glycolipid Acceptors—Reaction mixtures contained 2.5 μmol of HEPES (pH 7.2), 30 μmol of either VI3NeuAc-nLc6Cer or nLc6Cer as the glycolipid acceptor, 100 μg of G-3634-A detergent, 1 μmol of MnCl2, 15 nmol of GDP-[14C]fucose (30,000 cpm/nmol), and 200–500 μg of protein from 0.2% Triton X-100-solubilized membrane fractions of transfected COS-7 cells in a total reaction volume of 0.1 ml. The reaction mixtures were incubated for 2–8 h at room temperature and stopped by the addition of 100 μl of CHCl3/CH3OH (2:1). The entire reaction mixture was streaked onto a 4-cm wide strip of Whatman No. 3 paper and chromatographed overnight with water. The glycolipid remaining at the origin of the dried chromatography paper was extracted twice with 5-ml washes of CHCl3/CH3OH/H2O (10:5:1) with incubation at 37 °C for 30 min in the solvent. The solvent was removed with a nitrogen stream. To analyze the products formed from reaction mixtures containing VI3NeuAc-nLc6Cer as the acceptor, the isolated product was desialylated by boiling in 1 ml of 1% acetic acid for 1 h. Five ml of absolute EtOH was added to the cooled tubes, and the mixture was dried with a nitrogen stream. The desialylated product was transferred to a 0.5-ml Eppendorf tube, dried, and dissolved in 20 μl of CHCl3/CH3OH (2:1). For reaction mixtures utilizing nLc6Cer as the acceptor, the desialylation step was omitted. A 5-μl aliquot from the processed reaction mixture was removed, spotted onto a high-performance TLC plate (Silica Gel 60; EM Science, Gibbstown, NJ), and developed in a solvent system consisting of n-propyl alcohol/water/concen-

Table 2: PCR primers and templates for FucT mutations

The primers listed (5′ to 3′) are the upper primers only; the lower primers are of the same length and complementary to the upper primers. Primers listed for EcoNI and BamHI knock-outs (ko) were used to remove naturally occurring restriction sites in the templates indicated. Primers listed for BamHI and SacII swaps were used to create unique restriction sites in FucT-V or FucT-VI. WT, wild-type.

| Construct | Primer | Template |
|-----------|--------|----------|
| I126V FucT-V | CACCCACTGAGAGATCTGACATCTAACCC | WT FucT-V |
| V112I FucT-VI | CACCCACTGAGAGATCTGACATCTAACCC | WT FucT-VI |
| T87/K FucT-V | TGGGCTTTTAAAAACCCGTCATCG | WT FucT-V |
| K74T FucT-VI | TGGGCTTTTAAAAACCCGTCATCG | WT FucT-VI |
| D125E FucT-V | CACCCACTGAGAGATCTGACATCTAACCC | WT FucT-V |
| E111D FucT-VI | CACCCACTGAGAGATCTGACATCTAACCC | WT FucT-VI |
| TKDE FucT-V | TGGGCTTTTAAAAACCCGTCATCG | WT FucT-V |
| KTED FucT-VI | TGGGCTTTTAAAAACCCGTCATCG | WT FucT-VI |
| S110R FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| S111K FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| K97S FucT-VI | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| RK FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| SS FucT-VI | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| W124R FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| R110W FucT-VI | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| TKWR FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| KTRW FucT-VI | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| REV FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| WDI FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| KREV FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| WDI FucT-VI | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| KREV FucT-V | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| EcoNI ko | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| 5BamHI ko1 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| 5BamHI ko2 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| 6BamHI ko3 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| 6BamHI ko4 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| 6BamHI ko5 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| BamHI swap5 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
| BamHI swap6 | ATCCACTGCCACACATGATGTCATCG | WT FucT-V |
Glycolipid Acceptors and Products

| Name                        | Abbreviation | Structure                        |
|-----------------------------|--------------|----------------------------------|
| IB FucTI/NeuAcLc6Cer        | VIM2         | NeuAα2→3Galβ1→4GlcNAcβ1→3Galβ1→4[Fucα1→3]GlcNAcβ1→3Galβ1→4Glcβ1→Cer |
| V FucTI/NeuAcLc6Cer         | sialy Lex    | NeuAα2→3Galβ1→4[Fucα1→3]GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→Cer |
| Sialyl nLc6Cer              | nLc6Cer      | NeuAα2→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→Cer |
| nLc6Cer                     | nLc6Cer      | Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→Cer |

**Di and Tri saccharide 8 methoxyacycloxyoctyl acceptors**

| Abbreviation | Structure       |
|--------------|-----------------|
| Type 1 chain  | Galβ1→3GlcNAc O (CH2)α COOCH3 |
| Type 2 chain  | Galβ1→4GlcNAc O (CH2)α COOCH3 |
| H type 1      | Fucα1→2Galβ1→3GlcNAc O (CH2)α COOCH3 |
| H type 2      | Fucα1→2Galβ1→4GlcNAc O (CH2)α COOCH3 |

**FIGURE 1. Structures of the glycolipid acceptors and products and di- and trisaccharide acceptors referred to in this study.** The glycolipid acceptor VI NeuAc-nLc6Cer was isolated from bovine erythrocytes as described previously (29). nLc6Cer was prepared from VI NeuAc-nLc6Cer by acid treatment. The di- and trisaccharide acceptors used contained an 8-methoxyacycloxyoctyl aglycone.

**Flow Cytometry**

Twenty-four to 48 h after transfection, CHO cell monolayers were washed twice with calcium- and magnesium-free phosphate-buffered saline (Invitrogen) and then incubated with cell dissociation buffer (enzyme-free, phosphate-buffered saline-based; Invitrogen) for 5 min at 37 °C. The cells in suspension were washed once with fluorescence-activated cell sorter (FACS) staining buffer (calcium- and magnesium-free phosphate-buffered saline (Invitrogen) and then incubated with cell staining buffer. The transfected cells were then run such that an identical number (5–10,000 depending on the experiment) of CD65s-positive cells were collected for all constructs. To determine the median fluorescence intensity (MFI) of the transfected cells, the profiles of the mock-transfected and transfected cells were overlaid, and the percentage of transfected cells that stained positively and the MFI of the positively stained population were assessed. The cells were analyzed on a FACSscan (BD Biosciences), and data were further analyzed using FlowJo (TreeStar Inc.). Positive antibody staining was determined by setting the staining of the mock-transfected cells as the negative population and then determining the percentage of the transfected cells that stained positively, and the MFI of that population was determined.

**RESULTS**

Previous studies have demonstrated that cells transfected with full-length FucT-V and FucT-VI express a different complement of cell-surface fucosylated antigens (14, 26). Cells transfected with FucT-V preferentially produce VIM2, whereas cells transfected with FucT-VI preferentially produce sialyl-LeX (Fig. 1). These results indicate that FucT-V and FucT-VI preferentially fucosylate different GlcNAc residues in substrates that contain repeating Galβ1-4GlcNAc (LacNAc) disaccharides, with FucT-V preferentially transferring fucose from GDP-fucose to the internal GlcNAc residue and FucT-VI to the GlcNAc residue near the nonreducing terminus. Domain swapping and site-directed mutagenesis of full-length FucT-V and FucT-VI were used to identify the amino acids within the FucT-V and FucT-VI sequences responsible for this substrate specificity (i.e., site-specific fucosylation).

**Site-specific Fucosylation of Extended Chain Glycolipid Acceptors by Wild-type FucT-V and FucT-VI**—Table 3 shows the distribution of the fucose transfer to the III- and V-GlcNAc residues of VI NeuAc-nLc6Cer catalyzed by wild-type FucT-V and FucT-VI. The results demonstrate that FucT-V transfers >80% of the fucose to the internal III-GlcNAc residue, producing the VIM2 product, whereas the reverse occurs with FucT-VI: >80% preferential fucosylation of the V-GlcNAc residue, producing the sialyl-LeX product. A TLC analysis of these reaction products is shown in Fig. 2 (lanes 1 and 2, respectively). The preferential fucosylation of the III-GlcNAc residue is retained by FucT-V when the non-sialylated acceptor nLc6Cer is used (Fig. 2, lane 3). Interestingly, FucT-VI does not show site-specific fucosylation with the neutral glycolipid (Fig. 2, lane 4).

Table 3 also shows the relative activity of the recombinant wild-type FucT-V and FucT-VI enzymes using LacNAc-O-(CH2)α-COOCH3 as the acceptor. The results from these assays demonstrate that the relative activity of FucT-VI is 12-fold higher than that observed for FucT-V with this acceptor.

**FucT-V and FucT-VI Domain Swaps**—There are 39 amino acid differences (including a 14-amino acid insertion in FucT-V) in the N-terminal non-catalytic domain between the FucT-V and FucT-VI sequences. Much higher sequence homology occurs in the catalytic domain of these enzymes, with 24 amino acid differences occurring in the C-terminal catalytic domains, which contain ~300 residues (see alignment in Fig. 3). To determine which of the amino acid residue(s) are responsible for the site-specific fucosylation properties of these enzymes, a series of domain swap protein constructs were prepared, and their enzymatic properties were analyzed. Domain swap constructs were produced as shown in Table 1 and were generally composed of varying amounts of the N-terminal portion of one enzyme spliced onto the corresponding C-terminal portion of the other enzyme. In this way, constructs containing varying numbers of the amino acids from the N- and C-terminal portions of the enzyme
that differ can be analyzed to successively refine the search for the amino acid(s) that impart the site-specific fucosylation property observed in the FucTs.

The results shown in Table 3 demonstrate that all domain swap mutants retained FucT activity. Analysis of the site-specific fucosylation properties of swap sets 1 and 2 demonstrated that it was possible to swap at least the first 85 amino acids from FucT-V for the analogous N-terminal 71 amino acids from FucT-VI without impacting the site-specific fucosylation property of FucT-V. This swapped region contains a total of 39 amino acid differences, including a 14-amino acid insertion in FucT-V that precedes the enzyme's catalytic domain. Similarly, swapping the first 71 amino acids from FucT-VI for the N-terminal 85 residues from FucT-V resulted in an enzyme with FucT-VI-like specificity. In each case, site-specific fucosylation was retained and was defined by the enzyme form on the C-terminal side of the swap. Thus, the N-terminal portion of these enzymes, containing most of the sequence heterogeneity, is not involved in defining their site-specific fucosylation properties. Analysis of the relative activity of FucT-V and FucT-VI demonstrated that wild-type FucT-VI had a 12-fold higher activity compared with wild-type FucT-V. The level of FucT activity observed for each domain swap protein reflected the activity level of the wild-type enzyme domain on the C-terminal side of each swap.

Additional swaps were created that focused on exchanging the more C-terminal portions of the catalytic domain between FucT-V and FucT-VI (swap sets 3 and 4). This resulted in the exchange of up to 16 of the 24 catalytic domain amino acid differences occurring at the most C-terminal portion of each enzyme. These enzyme swaps also retained the site-specific fucosylation and relative activity properties of the parental enzymes. However, in contrast to the results with swap sets 1 and 2, the enzyme characteristics observed (i.e. activity and site-specific fucosylation) were defined by the enzyme form at the N-terminal side of the swap. Taken together, the results obtained with swap sets 1–4 demonstrate that both the site-specific fucosylation and relative enzyme activity properties of FucT-V and FucT-VI are defined by a region of each protein containing 8 amino acid differences spanning a 40-amino acid segment composed of amino acids 87–126 of FucT-V and amino acids 73–112 of FucT-VI.

To confirm that this region of the FucT sequence is responsible for the site-specific fucosylation and activity differences observed, recombinant FucTs were prepared in which only the regions containing the 8 amino acid differences were swapped.

### Table 3

Relative enzyme activity of various FucT swap constructs

| Enzyme Construct | % VIM2 | % sialyl-Le<sup>a</sup> | Relative Activity |
|------------------|--------|------------------------|------------------|
| FucT-V           | 81     | 19                     | 100              |
| FucT-VI          | 19     | 81                     | 1200             |
| 1a               | 16     | 84                     | 1030             |
| 1b               | 79     | 21                     | 93               |
| 2a               | 19     | 81                     | 1068             |
| 2b               | 82     | 18                     | 130              |
| 3a               | 80     | 20                     | 77               |
| 3b               | 24     | 79                     | 1329             |
| 4a               | 74     | 26                     | 112              |
| 4b               | 30     | 70                     | 980              |
| 5a               | 14     | 86                     | 1248             |
| 5b               | 84     | 16                     | 79               |

<sup>a</sup> Domain swap constructs are shown graphically and defined in Table 1.
The same enzymatic properties were found for these swaps as for swap sets 3 and 4. Thus, the site-specific fucosylation and relative activity differences between FucT-V and FucT-VI are controlled by 1 or more of the 8 amino acid differences found in the 40-amino acid segment composed of amino acids 87–126 of FucT-V and amino acids 73–112 of FucT-VI (see alignment in Fig. 3).

**Contribution of Various Amino Acids to Site-specific Fucosylation**—The contribution of 1 or more of these critical 8 amino acids to the site-specific fucosylation properties of FucT-V and FucT-VI was evaluated by carrying out a set of site-directed mutagenesis studies. The results obtained with a large number of mutants are provided in Fig. 4, which shows the percent of fucose transfer to the III-GlcNAc residue (VIM2 product) for the wild-type and mutant FucTs. The results obtained for mutants S110R, S111K, D125E, and I126V of FucT-V and the corresponding mutants of FucT-VI (R96S, K97S, E111D, and V112I) show that these single amino acid substitutions do not substantially impact the preferential fucosylation properties of the FucT construct compared with the respective wild-type enzyme (Fig. 4A). Thus, each FucT-V mutant shows a significant preference for transfer of fucose from GDP-fucose to the internal GlcNAc residue, creating the VIM2 antigen. The FucT-VI mutants preferentially catalyzed the synthesis of sialyl-Lex.

Unlike the previous set of single amino acid mutants, the T87K FucT-V mutant and the K73T FucT-VI mutant transferred fucose nearly equally to the III- and V-GlcNAc residues.

**FIGURE 2.** TLC of products from [14C]fucose transfer to VI3NeuAc-nLc6Cer or nLc6Cer catalyzed by the indicated enzymes. The results for transfer to VI3NeuAc-nLc6Cer are shown after initial desialylation with 1% acetic acid and boiling for 1 h. The positions of the III- and V-GlcNAc monofucosyl products are indicated. The slower migrating band corresponds to III- and V-GlcNAc difucosyl products. Lane 1, transfer to VI3NeuAc-nLc6Cer catalyzed by wild-type FucT-V; lane 2, transfer to VI3NeuAc-nLc6Cer catalyzed by wild-type FucT-VI; lane 3, transfer to nLc6Cer catalyzed by wild-type FucT-V; lane 4, transfer to nLc6Cer catalyzed by wild-type FucT-VI. In lanes 1 and 2, the products produced correspond to VIM2 (III-GlcNAc product) and sialyl-Le" (V-GlcNAc product). In lanes 3 and 4, the products produced correspond to non-sialylated VIM2 (III-GlcNAc product) and Le" (V-GlcNAc product). The plate was developed in a solvent composed of n-propyl alcohol/water/concentrated NH4OH (7:3:1) and autoradiographed. The amount of radioactivity present in each band was determined by scraping the silica corresponding to each individual band from the plate and determining the amount of radioactivity present via liquid scintillation counting. Radioactivity incorporated into the monofucosyl product bands (counts/min for the VIM2 product (III-GlcNAc) followed by counts/min for the sialyl-Le" product (V-GlcNAc)) was as follows: 405 and 95 cpm (lane 1), 60 and 255 cpm (lane 2), 645 and 165 cpm (lane 3), and 410 and 425 cpm (lane 4).

**FIGURE 3.** Amino acid alignments of human FucT-V and FucT-VI. A ClustalW alignment of the FucT-V and FucT-VI amino acid sequences is shown, and the shaded letters show amino acid differences between the two sequences. The restriction enzyme sites used to generate the domain swaps are shown (arrows). The amino acid segment within the outlined box is the 40-amino acid segment containing amino acids capable of directing site-specific fucosylation of sialylated polylactosamines.
FIGURE 4. Comparison of [14C]fucose transfer to the III- and V-GlcNAc residues of the VI3NeuAc-t-Lc6Cer acceptor catalyzed by wild-type FucT-V and FucT-VI and the indicated derived site-directed mutant enzymes. The conditions of the assay and analysis were as described under “Experimental Procedures.” Each bar represents the percent of VIM2 product formed relative to the total amount of the fucosylated glycolipid products formed (i.e., (VIM2 product/VIM2 + sialyl-Le^a product) × 100) as shown at the bottom of each panel (i.e., x axis). The wild-type and mutant proteins evaluated are indicated to the left of each panel. A, amino acid substitutions that do not substantially impact the preferential fucosylation properties of the FucT (FT) construct compared with the respective wild-type enzyme. B, amino acid substitutions that reduce the preferential fucosylation properties of the FucT construct compared with the respective wild-type enzyme. C, amino acid substitutions that reverse the preferential fucosylation properties of the FucT construct compared with the respective wild-type enzyme.
of VI3NeuAc-nLcαCer, generating approximately equal amounts of the VIM2 and sialyl-Leα products (Fig. 4B). These residues are located near the N terminus of the region identified in the domain swap studies as being critical for the site-specific fucosylation property of the FucTs. A similar loss of transfer selectivity occurred for the W124R FucT-V mutant and R110W FucT-VI mutant (Fig. 4B). Interestingly, these residues are located 37 amino acid residues away from Thr87 and Lys73 of FucT-V and FucT-VI near the C terminus of the region identified in the domain swap studies as being critical for the site-specific fucosylation property of the FucTs.

The T87K/D125E FucT-V and K73T/E111D FucT-VI double mutants were prepared and found to have site-specific fucosylation properties similar to those of the T87K and K73T mutants, respectively. Additionally, the W124R/D125E/I126V FucT-V and R110W/E111D/V112I FucT-VI triple mutants behaved similarly to the W124R and R110W single mutants in terms of site-specific fucosylation properties. The T87K/V89I/A101T FucT-V and K73T/I75V/T87A FucT-VI triple mutants gave results (data not shown) similar to those of the T87K FucT-V and K73T FucT-VI single mutants, respectively.

In contrast to the mutants described above, substituting 4 of the amino acids from FucT-VI (T87K/W124R/D125E/I126V) into the FucT-V sequence produced a fucosylated product pattern (72% sialyl-Leα) similar to that of wild-type FucT-VI (Fig. 4C). Thus, incorporating only 4 amino acids from the FucT-VI sequence into FucT-V is sufficient to essentially reverse the site-specific fucosylation pattern of FucT-V. The complementary mutant of FucT-VI (K73T/R110W/E111D/V112I) produced predominantly VIM2 (75% VIM2), a pattern similar to that of wild-type FucT-V. Therefore, 4 amino acids from the FucT-V sequence reverse the site-specific fucosylation pattern of FucT-VI. FucT-V mutants containing two of these four substitutions (i.e. T87K/W124R) had site-specific fucosylation properties resembling those of wild-type FucT-VI, whereas the complementary mutant of FucT-VI (K73T/R110W) had the site-specific fucosylation properties of FucT-V. However, the change observed for these two amino acid mutants was not as dramatic as that observed for the proteins containing all four mutations.

**Flow Cytometric Analysis of VIM2 (CD65s) Expression in Transfected Cells**—To determine whether the results obtained from the *in vitro* enzyme assays described above could be replicated in an intact cell system, flow cytometric analyses of Chinese hamster ovary (CHO) cells transfected with plasmids encoding either wild-type or mutant FucTs were performed for VIM2 expression using the anti-CD65s antibody. Transfection efficiencies in several experiments ranged from 10 to 30%. In one experiment, ∼7.2 and 9.4% of the CHO cells transfected with constructs of wild-type FucT-V and the T87K/W124R/D125E/I126V FucT-V mutant, respectively, were positive for cell-surface VIM2 (CD65s) expression. Only a small percentage of CD65s-positive cells were detected in cells transfected with either wild-type FucT-VI or the K73T/R110W/E111D/V112I FucT-VI mutant.

The CD65s MFI for wild-type FucT-V was nearly 9-fold higher than that for wild-type FucT-VI (Table 4). The domain swap mutant of FucT-VI containing amino acids 87–126 of FucT-V (swap set 5a in Table 1) had an MFI that was even greater than that of wild-type FucT-V and >6-fold higher than the domain swap mutant of FucT-V containing amino acids 73–112 of FucT-VI (swap set 5b in Table 1). Finally, the FucT-V mutant with 4 amino acids (T87K/W124R/D125E/I126V) from FucT-VI had the highest MFI for VIM2 expression, whereas its counterpart (FucT-VI with K73T/R110W/E111D/V112I from the FucT-V sequence) had the lowest MFI for VIM2 expression. A comparison of the results obtained from the *in vitro* enzyme assays using the sialylated glycolipid acceptor (VI3NeuAc-nLcαCer) and those from the FACS analyses demonstrated that both approaches gave similar results, with some variation in relative values. Thus, those constructs that produced more VIM2 glycolipid product also expressed a higher level of VIM2 antigen on the cell surface as detected by the anti-CD65s antibody.

**FucT Activity of Site-directed Mutants**—As described above, FucT enzyme activity measurements of the domain swaps with VI3NeuAc-nLcαCer as the acceptor substrate demonstrated that 1 or more of the 8 amino acid differences occurring between amino acids 87 and 126 of FucT-V and amino acids 73 and 112 of FucT-VI are responsible for the approximate 12-fold difference in enzyme activity observed when comparing wild-type FucT-V and FucT-VI (Table 2). Enzyme activity measurements were made for each of the site-directed mutants created and compared with those for the wild-type enzymes.

The FucT-V mutant containing the FucT-VI amino acid substitutions T87K/W124R/D125E/I126V had FucT enzyme activity 12-fold greater than that of wild-type FucT-V (Table 5). Similarly, the mutant containing only three of the FucT-VI substitutions (W124R/D125E/I126V) had a nearly equivalent increase in FucT activity. The FucT-V mutant containing the W124R single substitution had a slightly lower activity increase (9-fold). Other FucT-V single, double, and triple mutants had FucT activity that differed only modestly compared with wild-type FucT-V.
The corresponding FucT-VI mutants produced a pattern similar to that observed with the FucT-V mutants. Thus, the R110W FucT-VI mutant had activity slightly less than but similar to that of wild-type FucT-V, as did all of the mutants containing the Arg-to-Trp substitution at amino acid 110 of the FucT-VI sequence. Most of the other FucT-VI mutants had FucT activities similar to that of wild-type FucT-VI. Exceptions were K97S, which had ~50% higher activity compared with wild-type FucT-VI, and K73T/I75V/T87A, which had ~2-fold lower activity compared with wild-type FucT-VI.

Fucosyltransferase Activity for H-type 1 and H-type 2 Acceptors—Wild-type FucT-V is known to transfer fucose to both type 1 and type 2 acceptors and their corresponding α1,2-fucosylated derivatives, H-type 1 and H-type 2. Previously, Dupuy et al. (22) reported that, unlike wild-type FucT-V, the W124R FucT-V mutant cannot transfer fucose to an H-type 1 acceptor, but has a 4-fold higher FucT activity with an H-type 2 acceptor compared with wild-type FucT-V. They also reported that the R110W FucT-VI mutant is inactive with both H-type 1 and H-type 2 acceptor substrates.

We also found that wild-type FucT-V could catalyze the transfer of fucose to both H-type 1 and H-type 2 substrates, whereas the W124R FucT-V mutant had no activity with the H-type 1 acceptor (Table 6). However, we found that the activity of the W124R FucT-V mutant with an H-type 2 acceptor was dramatically increased (14-fold) compared with wild-type FucT-V and was similar to that of wild-type FucT-VI. The W124R/D125E/I126V FucT-V triple mutant had an activity profile mirroring that of the W124R FucT-V mutant.

In contrast to wild-type FucT-V, wild-type FucT-VI did not transfer fucose to the H-type 1 acceptor. When Arg110 of the FucT-VI sequence was changed to Trp, the resulting mutant had dramatically lower activity compared with wild-type FucT-VI with the H-type 2 acceptor and approximately half the activity found with wild-type FucT-V. Interestingly, this mutant was only slightly less active with an H-type 1 acceptor compared with wild-type FucT-V. The R110W/E111D/V112I FucT-VI triple mutant gave an activity pattern like that of the R110W mutant.

### DISCUSSION

The six cloned human FucTs (FucT-III, FucT-IV, FucT-V, FucT-VI, FucT-VII, and FucT-IX) comprise a family of type II transmembrane glycoproteins composed of the same domain structures that share substantial amino acid sequence homology, particularly in their C-terminal catalytic domains (31). The existence of this series of related enzymes has provided a basis to study structure-function relationships of differing protein segments and specific amino acids occurring therein. Each of these enzymes has a common property of binding the donor substrate GDP-fucose, and multiple studies have defined amino acids involved in GDP-fucose binding and/or catalysis (32–37). However, both significant and subtler differences in acceptor oligosaccharide specificity exist. One such subtle difference is the site-specific fucosylation properties of FucT-V and FucT-VI with fucose transfer to sialylated polylactosamine structures. FucT-V has been shown to favor fucosylation of internal GlcNAc residues, yielding the VIM2 determinant structure, whereas FucT-VI preferentially fucosylates distal GlcNAc residues, leading to the sialyl-Leα determinant structure (14, 26). The difference in the fucosylation specificity of the two FucTs leads to the production of products that have different biological functions and antigenicity (38, 39).

We have conducted a series of studies utilizing recombinant enzymes prepared by reciprocally swapping portions of the catalytic domain between FucT-V and FucT-VI and by site-directed mutagenesis of 1 or more amino acid residues to define the amino acids that are responsible for the site-specific fucosylation properties. There exist a total of 24 amino acid differences between FucT-V and FucT-VI in the catalytic domain. Domain swapping isolated a 40-amino acid segment containing 8 of these differences that was found to be responsible for the VIM2 versus sialyl-Leα product specificity.

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**TABLE 5**

Relative activity of wild-type FucT-V and FucT-VI and derived site-directed mutants

| Protein | Relative activity |
|---------|------------------|
| FucT-V  |                  |
| WT      | 100              |
| T87K/K1235E/I126V | 1205          |
| W124R/D125E/I126V | 1107          |
| W124R   | 895              |
| T87K/K124R | 433            |
| S110R   | 295              |
| S111K   | 286              |
| H126N   | 222              |
| T87K/D125E | 146            |
| D125E   | 96               |
| T87K/V89A/A101T | 87            |
| T87K   | 66               |
| FucT-VI |                  |
| WT      | 1200             |
| K73T/R110W/E111D/V112I | 26            |
| R110W/E111D/V112I | 38            |
| R110W   | 69               |
| K73T/R110W | 26            |
| R96S    | 1354             |
| K97S    | 1994             |
| V112I   | 1504             |
| K73T/E111D | 1078        |
| E111D   | 1030             |
| K73T/I75V/T87A | 653           |
| K73T    | 516              |

* Relative activity compared with wild-type FucT-V with the type 2 acceptor.

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**TABLE 6**

Fucose transfer to type 1 and type 2 chain acceptors catalyzed by FucT-V and FucT-VI constructs

| Protein | Relative activity |
|---------|------------------|
| FucT-V  |                  |
| WT      | 100 ± 10%        |
| W124R   | 895 ± 9%         |
| W124R/D125E/I126V | 1107 ± 60%     |
| FucT-VI |                  |
| WT      | 1273 ± 90%       |
| R110W   | 68 ± 8%          |
| R110W/E111D/V112I | 38 ± 4%        |

* WT, wild-type; ND, none detected.

* Relative enzyme activity compared with wild-type FucT-V with the type 2 acceptor is shown.
Product analysis of fucose transfer by site-directed mutants containing single and multiple amino acid changes among these 8 differences demonstrated that most of these amino acid differences are unrelated to the site-specific fucosylation properties of the FucTs. In contrast, the T87K mutant of FucT-V and the corresponding K73T mutant of FucT-VI showed little site-specific fucosylation preference. Similarly, significant reductions in site-specific fucosylation preferences were observed with the W124R mutant of FucT-V and the R110W mutant of FucT-VI. Mutants of FucT-V containing the W124R change along with changes in the adjacent amino acids (D125E and I126V) lost virtually all selectivity between internal versus distal GlcNAc residues. The same result was obtained with the R110W/E111D/V112I triple mutant of FucT-VI. Although the individual D125E and I126V mutants of FucT-V (and the individual E111D and V112I mutants of FucT-VI) did not, on their own, impact site-specific fucosylation results, the REV versus WDI sequence had a larger impact than the R110W and W124R point mutations alone.

The T87K/W124R/D125E/I126V FucT-V mutant preferentially synthesized sialyl-Le^a, similar to wild-type FucT-VI, whereas the K73T/R110W/E111D/V112I FucT-VI mutant closely resembled FucT-V with respect to preferential VIM2 antigen synthesis. Thus, the site-specific fucosylation properties of wild-type FucT-V and FucT-VI were reversed when 4 amino acids were substituted into their wild-type sequence. The T87K/W124R FucT-V and K73T/R110W FucT-VI double mutants retained most of the sialyl-Le^a versus VIM2 specificity of their 4-amino-acid mutant counterparts. Thus, the amino acid positions corresponding to Thr^87 and Trp^124 of FucT-V and Lys^73 and Arg^110 of FucT-V are primarily responsible for site-specific fucosylation properties that lead to the synthesis of either VIM2 or sialyl-Le^a antigens.

The enzyme assay results were confirmed via flow cytometric analysis. The MFI obtained using an antibody specific for the VIM2 structure was increased from 9- to 15-fold in CHO cells expressing recombinant enzymes with a preference for fucosylation of internal GlcNAc residues (e.g. FucT-V and K73T/R110W/E111D/V112I FucT-VI) compared with CHO cells transfected with wild-type FucT-VI.

Previous studies have focused on structure-function properties of amino acids in the region identified in this study as being critical for site-specific fucosylation (19–22). The results collectively demonstrate that the N-terminal portion of the catalytic domain is involved in acceptor oligosaccharide binding. In particular, expression of the aromatic Trp^111 residue of FucT-III (corresponding to Trp^124 of FucT-V) has been shown to be primarily responsible for the type 1 chain acceptor specificity found in FucT-III and FucT-V (22). Additional evidence has demonstrated that higher type 1 chain specificity can be conferred upon FucT-V by substitution of Asn^86 and Thr^87 with His and Ile, respectively, amino acids found in the corresponding positions in FucT-III (20). Taken together, the results independently indicate that, in addition to having a role in defining type 1 versus type 2 chain specificity, amino acids in positions corresponding to Thr^87 and Trp^124 of FucT-V also function in defining a preference for the fucosylation of an internal versus distal GlcNAc residue of sialylated polylactosamines, leading to VIM2 or sialyl-Le^a structures.

The relative activity of wild-type FucT-VI was ~12-fold higher than that found for FucT-V. Most amino acid changes incorporated into the site-directed mutants evaluated in this study had either little or no impact on the FucT activity of the mutant proteins compared with the respective parental wild-type enzymes. In contrast, mutants containing the W124R change in FucT-V and the R111W change in FucT-VI reversed the relative activity relationship compared with their parental wild-type enzymes. In the series of mutants evaluated here, the presence of a Trp residue in this position not only affected the protein’s ability to transfer fucose to type 1 chain acceptors, but also produced proteins with lower FucT activity. In contrast, mutant proteins with an Arg residue in place of the Trp residue had substantially increased FucT activity and lacked type 1 chain acceptor specificity. Therefore, this site also appears to be important for the inherent enzyme activity differences between FucT-V and FucT-VI. Thus, our results confirm and extend the results reported by Dupuy et al. (22).

Although no structural data for mammalian FucTs are presently available, the x-ray crystal structure of the Helicobacter pylori α1,3-FucT was recently solved (40). Using the sequence alignment proposed by Ge et al. (41), we constructed homology models of human FucT-V and FucT-VI with the program MODELLER (42) using the H. pylori crystal structure as the template. The cytoplasmic and transmembrane regions of FucT-V and FucT-VI were not included in the model, and the sequence of the human enzymes was extended by 4 residues beyond the C terminus visible in the H. pylori structure to include C-terminal cysteines that are involved in disulfide bridges. Although sequence identity to H. pylori is low in the N terminus and the C terminus is partially missing from the crystal structure, the cysteines shown to be involved in disulfide bridges in the human enzymes (43) align close enough in space in the resulting homology models to be covalently connected through their sulfur atoms prior to energy minimization of the models. Fig. 5 shows the resulting model of FucT-VI in a ribbon representation as visualized with PyMOL (45). In the final model, the residues identified to determine substrate specificity all align on the same side of the enzyme. Arg^110, Glu^111, and Val^112 (in yellow) are located in a loop directly underneath the acceptor-binding site as indicated by the GDP-fucose in green taken from the H. pylori crystal structure. Lys^73 is ~30 Å away and located near the N terminus. The position of Lys^73 is, however, not accurately defined in the model because of the limited sequence identity between the human and H. pylori N termini and may be closer to the active site in the human enzyme than suggested by the homology models.

Based on the results presented in this study and consistent with the model, it seems likely that Thr^87 and Trp^124 of FucT-V, which are separated by 37 amino acids in the linear sequence, are located in close proximity in three-dimensional space because of protein folding and form a portion of the acceptor-binding site. Protein structural analyses with these enzymes will

Site-specific Fucosylation of Sialylated Polylactosamines
Site-specific Fucosylation of Sialylated Polylactosamines

FIGURE 5. Homology model of the catalytic domain of human FucT-VI derived from the H. pylori crystal structure using the sequence alignment published by Ge et al. (41). Disulfide bridges are indicated in purple and involve cysteines from the N terminus, where sequence identity between the two enzymes and reliability of the model are low. Residues identified to determine site-specific fucosylation substrate specificity are shown in yellow. Of these, Arg110, Glu112, and Val113 are located in a loop directly underneath the GDP-fucose-binding site (in green). Lys23 is located ≤30 Å away from the active site and closer to the less well defined N terminus of the model. Because of the uncertainty of the model in this region, its actual location may be closer to the active site.

be necessary to verify this speculation and to provide information on how various FucTs bind the same substrate but produce different fucosylated products.

REFERENCES

1. Alhadeff, J. A. (1989) Crit. Rev. Oncol. Hematol. 9, 37–107
2. Hakomori, S. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10231–10233
3. Hakomori, S. (1989) Adv. Cancer Res. 52, 257–331
4. Toppila, S., Renkonen, R., Penttila, L., Natunen, J., Salminen, H., Helin, J., Maaheimo, H., and Renkonen, O. (1999) Eur. J. Biochem. 261, 208–215
5. Rosen, S. D. (1999) Ann. J. Pathol. 155, 1013–1020
6. Watkins, W. M., and Clarke, J. L. (2001) Adv. Exp. Med. Biol. 491, 231–265
7. Chou, D. K., Suzuki, Y., and Jungalwala, F. B. (1996) Glycoconj. J. 13, 295–305
8. Fukushi, Y., Hakomori, S., and Shepard, T. (1984) J. Exp. Med. 160, 506–520
9. Le Pendu, J. (1989) J. Immunogenet. 16, 53–61
10. Javaud, C., Dupuy, F., Maffah, A., Julien, R., and Petit, J.-M. (2003) Genetica 118, 157–170
11. Couillin, P., Crooijmans, R. P., Fillon, V., Mollicone, R., Groenen, M. A., Adrien-Dehais, C., Bernheim, A., Zoorob, R., Oriol, R., and Candelier, J. J. (2003) Cytogenet. Genome Res. 103, 111–121
12. Kukowska-Latallo, J. F., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990) Genes Dev. 4, 1288–1303
13. Weston, B. W., Nair, R. P., Larsen, R. D., and Lowe, J. B. (1992) J. Biol. Chem. 267, 4145–4160
14. Weston, B. W., Smith, P. L., Kelly, R. J., and Lowe, J. B. (1992) J. Biol. Chem. 267, 24575–24584
15. Nishihara, S., Nakazato, M., Kudo, T., Kimura, H., Ando, T., and Nari- matsu, H. (1993) Biochem. Biophys. Res. Commun. 190, 42–46
16. Kaneko, M., Kudo, T., Iwasaki, H., Shinya, T., Inoko, H., Kozaki, T., Saitou, N., and Narimatsu, H. (1999) Cytogenet. Cell Genet. 86, 329–330
17. Reguigne, I., James, M. R., Richard, C. W., III, Mollicone, R., Seawright, A., Lowe, J. B., Oriol, R., and Couillin, P. (1994) Cytogenet. Cell Genet. 66, 104–106
18. Reguigne-Arnould, I., Wolfe, J., Hornigold, N., Faure, S., Mollicone, R., Oriol, R., and Couillin, P. (1996) C R. Acad. Sci. (Paris) 319, 783–788
19. Legault, D. J., Kelly, R. J., Natsuka, Y., and Lowe, J. B. (1995) J. Biol. Chem. 270, 20987–20996
20. Nguyen, A. T., Holmes, E. H., Whitaker, J. M., Ho, S., Shetterly, S., and Macher, B. A. (1998) J. Biol. Chem. 273, 25244–25249
21. Dupuy, F., Petit, J.-M., Mollicone, R., Oriol, R., Julien, R., and Maftah, A. (1999) J. Biol. Chem. 274, 12257–12262
22. Dupuy, F., Germot, A., Julien, R., and Maftah, A. (2004) Glycobiology 14, 347–356
23. Natsuka, S., Gersten, K. M., Zenita, K., Kannagi, R., and Lowe, J. B. (1994) J. Biol. Chem. 269, 16789–16794
24. Kaneko, M., Kudo, T., Iwashedo, H., Ikehara, Y., Nishihara, S., Nakagawa, S., Sasaki, K., Shinya, T., Inoko, H., Saitou, N., and Narimatsu, H. (1999) FEBS Lett. 452, 237–242
25. Holmes, E. H., and Machar, B. A. (1993) Arch. Biochem. Biophys. 301, 190–199
26. Pykari, M., Toivonen, S., Natsuka, J., Niemela, R., Salminen, H., Aittio, O., Eskstrom, M., Parmanne, P., Valimaki, M., Alais, J., Auge, C., Lowe, J. B., Renkonen, O., and Renkonen, R. (2000) J. Biol. Chem. 275, 40057–40063
27. Sherwood, A. L., and Holmes, E. H. (1999) Glycobiology 9, 637–643
28. Holmes, E. H., Ostrander, G. K., and Hakomori, S. (1986) J. Biol. Chem. 261, 3737–3743
29. Chien, J. L., Li, S. C., Laine, R. A., and Li, Y. T. (1978) J. Biol. Chem. 253, 4031–4035
30. Xu, Z., Vo, L., and Macher, B. A. (1996) J. Biol. Chem. 271, 8818–8823
31. Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L., and Breton, C. (1999) Glycobiology 9, 323–334
32. Holmes, E. H., Xu, Z., Sherwood, A. L., and Macher, B. A. (1995) J. Biol. Chem. 270, 8145–8151
33. Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H., and Bird, M. I. (1997) J. Biol. Chem. 272, 21349–21356
34. Vo, L., Lee, S., Marcinko, M. C., Holmes, E. H., and Macher, B. A. (1998) J. Biol. Chem. 273, 25250–25255
35. Sherwood, A. L., Nguyen, A. T., Whitaker, J. M., Macher, B. A., Stroud, M. R., and Holmes, E. H. (1998) J. Biol. Chem. 273, 25256–25260
36. Sherwood, A. L., Davis, W. C., Ho, S., Macher, B. A., Stroud, M. R., Upchurch, D. A., and Holmes, E. H. (2000) Biochem. Biophys. Res. Commun. 273, 870–876
37. Jost, F., de Vries, T., Knegtel, R. M., and Macher, B. A. (2005) Glycobiology 15, 165–175
38. Macher, B. A., Buehler, J., Scudder, P., Knapp, W., and Feizi, T. (1988) J. Biol. Chem. 263, 10186–10191
39. Becker, D. J., and Lowe, J. B. (2003) Glycobiology 13, 41R–53R
40. Sun, H.-Y., Lin, S.-W., Ko, T.-P., Pan, J.-F., Liu, C.-L., Lin, C.-N., Wang, A. H.-J., and Lin, C.-H. (2007) J. Biol. Chem. 282, 9973–9982
41. Ge, Z., Chan, N. W. C., Palic, M. M., and Taylor, D. E. (1997) J. Biol. Chem. 272, 21357–21363
42. Sali, A., and Blundell, T. L. (1993) J. Mol. Biol. 234, 779–815
43. Holmes, E. H., Yen, T.-Y., Thomas, S., Joshi, R., Nguyen, A., Long, T., Gallet, F., Maffah, A., Julien, R., and Macher, B. A. (2000) J. Biol. Chem. 275, 24237–24245
44. IUPAC-UAB Joint Commission on Biochemical Nomenclature (1998) Eur. J. Biochem. 257, 293–298
45. DeLano, W. L. (2002) The PyMOL Molecular Graphics System, DeLano Scientific LLC, Palo Alto, CA

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