A Single Amino Acid in the Hypervariable Stem Domain of Vertebrate \( \alpha_{1,3/1,4} \)-Fucosyltransferases Determines the Type 1/Type 2 Transfer

CHARACTERIZATION OF ACCEPTOR SUBSTRATE SPECIFICITY OF THE LEWIS ENZYME BY SITE-DIRECTED MUTAGENESIS*

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Alignment of 15 vertebrate \( \alpha_{1,3} \)-fucosyltransferases revealed one arginine conserved in all the enzymes employing exclusively type 2 acceptor substrates. At the equivalent position, a tryptophan was found in \( \text{FUT2} \)-encoded Lewis \( \alpha_{1,3/1,4} \)-fucosyltransferase (Fuc-TIII) and \( \text{FUT5} \)-encoded \( \alpha_{1,3/1,4} \)-fucosyltransferase, the only fucosyltransferases that can also transfer fucose in \( \alpha_{1,4} \)-linkage. The single amino acid substitution Trp\(^{111} \)→Arg in Fuc-TIII was sufficient to change the specificity of fucose transfer from H-type 1 to H-type 2 acceptors. The additional mutation of Asp\(^{112} \)→Glu increased the type 2 activity of the double mutant Fuc-TIII enzyme, but the single substitution of the acidic residue Asp\(^{112} \) in Fuc-TII by Glu decreased the activity of the enzyme and did not interfere with H-type 1/H-type 2 specificity. In contrast, substitution of Arg\(^{115} \) in bovine futb-encoded \( \alpha_{1,3} \)-fucosyltransferase (Fuc-Tb) by Trp generated a protein unable to transfer fucose either on H-type 1 or H-type 2 acceptors. However, the double mutation Arg\(^{115} \)→Trp/Glu\(^{116} \)→Asp of Fuc-Tb slightly increased H-type 1 activity. The acidic residue adjacent to the candidate amino acid Trp/Arg seems to modulate the relative type 1/2 type 2 acceptor specificity, and its presence is necessary for enzyme activity since its substitution by the corresponding amide inactivated both Fuc-TIII and Fuc-Tb enzymes.

Fucosyltransferases are type II transmembrane proteins catalyzing fucose transfer from GDP-fucose to different oligosaccharide acceptors in \( \alpha_{1,2} \), \( \alpha_{1,3} \), \( \alpha_{1,4} \), and \( \alpha_{1,6} \)-linkages. The fucosylated glycoconjugates they produce are blood group and oncodevelopmental antigens (1) and are involved in tumorigenesis (2), embryogenesis (3), normal leukocyte trafficking (4), and leukocyte extravasation in inflammatory reactions (5–7). Since all fucosyltransferases utilize GDP-fucose as donor substrate, their specificity depends on the recognition of the acceptor substrate and the type of linkage formed. Although the primary sequences of six human \( \alpha_{1,3} \)-fucosyltransferases are known, the amino acids involved in the recognition of different acceptor substrates as type 1 (Gal\( \beta_{1,3} \)-GlcNAc) and type 2 (Gal\( \beta_{1,4} \)-GlcNAc) disaccharides or blood group H-type 1 (Fuc1,2Gal\( \beta_{1,3} \)-GlcNAc) and H-type 2 (Fuc1,2Gal\( \beta_{1,4} \)-GlcNAc) trisaccharides are not yet known. These last trisaccharides are better acceptors than the disaccharides because they give higher values of fucose incorporation with a better \( K_m \) and they are unambiguous in the sense that C-2 of Gal is already substituted by Fuc, and therefore, they can accept fucose only on C-3 or C-4 of GlcNAc. This is particularly relevant for Fuc-TIII since up to 4% of \( \alpha_{1,2} \)-fucosyltransferase activity has been found with this enzyme (8, 9).

Two enzymes (Fuc-TIII and Fuc-TV) are able to use both H-type 1 and H-type 2 trisaccharide acceptors, and consequently, they have been called \( \alpha_{1,3/1,4} \)-fucosyltransferases. The Lewis or Fuc-TIII enzyme is \( \sim 100 \) times more efficient on H-type 1 compared with H-type 2 acceptor substrates (10). The Fuc-TV enzyme is more efficient on H-type 2 than on H-type 1 substrates, although the relative type 1/2 activities are of the same order of magnitude (10). Finally, the remaining four \( \alpha_{1,3} \)-fucosyltransferases (Fuc-TIV, Fuc-TVI, Fuc-TVII, and Fuc-TIX) are able to use only type 2 acceptor substrates and are consequently called \( \alpha_{1,3} \)-fucosyltransferases.

The Fuc-TIII, Fuc-TV, and Fuc-TVI enzymes constituting the primate Lewis subfamily of fucosyltransferases have appeared by two successive duplications of an ancestral Lewis gene, which occurred rather late in evolution (Fig. 1), after the great mammalian radiation and before the separation of higher apes and man from their common evolutionary path (10). These three enzymes share \( \sim 85\% \) sequence identity; the main differences among them are located in the stem amino-terminal region (hypervariable region), whereas their carboxyl-terminal regions are almost identical. Therefore, the differences in type 1/2 specificity among these three enzymes are expected to be determined by amino acid differences in their hypervariable regions. Two different strategies have been developed to define...
the amino acids involved in the relative differences of type 1/type 2 acceptor substrate specificity.

Subdomain swapping of segment 62–110 of Fuc-TIII and the corresponding segment of Fuc-TV increased the type 1 enzyme activity of Fuc-TV (11). Eight amino acids were shown to be Fuc-TV-specific in this area. Site-directed mutagenesis of two of them (Asn28 and Thr27) by the corresponding His and Ile of Fuc-TIII also increased type 1 enzyme activity (12), whereas site-directed mutagenesis of other amino acids in the central area (13) or in the COOH terminus (14) of Fuc-TIII and Fuc-TV did not modify the type 1/type 2 acceptor substrate specificity.

Lowe and co-workers (15) preferred to analyze the more clear-cut difference between Fuc-TIII and Fuc-TV. They divided the hypervariable region into five subdomains and created enzyme chimeras by subdomain swapping. Functional analysis of the chimeras showed that the type 1/type 2 specificity of human Fuc-TIII and Fuc-TV depends on amino acids in the segment corresponding to residues 103–153 of Fuc-TIII. Eleven amino acids in this area were specific to Fuc-TV and were considered as candidates to determine whether or not this α,3-fucosyltransferase can utilize, in addition, type 1 acceptor substrates (15). These 11 amino acids could be reduced to four following the cloning, expression, and peptide sequence analysis of chimpzee Fuc-TIII, Fuc-TV, and Fuc-TVI (10) and the bovine Fuc-Tb enzyme (16). Furthermore, this number could be further reduced to two amino acids specific to Fuc-TV (Arg110 and Glu111) and Fuc-Tb (Arg115 and Glu116) by addition of the hamster Fuc-Tb enzyme to the previous analysis. This study was conducted to define, by site-directed mutagenesis, the contribution to H-type 1/H-type 2 acceptor substrate specificity of the two amino acids Arg115 and Glu116 of Fuc-Tb and the corresponding residues Trp111 and Asp112 of Fuc-TIII.

**FIG. 1.** Phylogenetic tree of the main subfamilies of vertebrate α,3-fucosyltransferases. All these enzymes can use type 2 acceptor substrates; but Fuc-TIII and Fuc-TV can use, in addition, type 1 acceptors, and they are the consequence of the latest gene duplication event within the Lewis subfamily of enzymes. Therefore, it is logical to assume that the capacity to use type 1 acceptors had appeared in the common ancestor of these two enzymes. Values of 100 bootstrap replicates are noted at each divergence point.

EXPERIMENTAL PROCEDURES

**Materials**—Anti-human Fuc-TIII antibody was kindly provided by J. B. Lowe, H-type 1 (Fuc α,2Galβ1,3GlcNAc-biotin) and H-type 2 (Fuc α,2Galβ1,4GlcNAc-biotin) acceptors were purchased from Syntosome (Munich, Germany).

**Site-directed Mutagenesis—**Cloned fos (16) with an additional 3'-untranslated sequence (800 base pairs) that gives better expression was kindly provided by A. Wierinckx, personal communication.

**Fucosyltransferase Assay—**Fucosyltransferase assays were incubated for 1 h at 37°C in a 60-μl volume reaction containing 25 mM sodium cacodylate (pH 6.5), 5 mM ATP, 20 mM MnCl2, 10 mM α-L-fucose, 3 mM GDP-[14C]fucose (310 μCi/mmol; Amersham Pharmacia Biotech), 0.1 mM tri saccharide acceptor, and 50 μg of COS-7 extract proteins. The reaction was stopped by addition of 3 ml of cold water and applied to a Waters Sep-Pak C18 reverse chromatography cartridge. After washing with 15 ml of water, the radiolabeled reaction product was eluted with 2 × 5 ml of ethanol and counted with 2 volumes of biodegradable counting scintillant (Amersham Pharmacia Biotech).

**Determination of Kinetic Parameters**—The apparent Km values for GDP-fucose of the wild-type enzyme and mutated variants were determined using 10–250 μM GDP-fucose with 5 μM GDP-[14C]fucose in each

Both Fuc-Tb and Fuc-Tb are orthologous homologous to the ancestor of the human Fuc-TIII, Fuc-TV, and Fuc-TVI enzymes.

3 R. Mollicone and R. Oriol, unpublished results.

4 A. Wierinckx, personal communication.

5 FUT3 to FUT7 and FUT9 are the Genome Data Base names of the six human α,3-fucosyltransferase genes.

6 Twenty-five microliters of water, the radiolabeled reaction product was eluted with 2 × 5 ml of ethanol and counted with 2 volumes of biodegradable counting scintillant (Amersham Pharmacia Biotech).
One Amino Acid Defines Type 1/Type 2 Activity of Lewis Enzyme

Table I

Primer bases used in polymerase chain reaction to obtain each of the desired mutations

| Enzyme mutation | Base changes | Primers | Position |
|-----------------|--------------|---------|----------|
| Fuc-TIII Trp111 → Arg | T → A, G → A | 5′-GATGACGCGGTTCGCTGGTG−3′ (antisense) | 321–302 |
| | | 5′-GTGCCAACAAAGTATATCCTG-3′ (sense) | 322–342 |
| Asp112 → Glu | T → A | 5′-GATGACGCGGTTCGCTGGTG−3′ (antisense) | 321–302 |
| | | 5′-GTGCCAACAAAGTATATCCTG-3′ (sense) | 322–342 |
| Fuc-TIII Trp111 → Arg/Asp112 → Glu | T → A, G → A | 5′-GATGACGCGGTTCGCTGGTG−3′ (antisense) | 321–302 |
| | T → A | 5′-GTGCCAACAAAGTATATCCTG-3′ (sense) | 322–342 |
| Asp112 → Asn | G → A | 5′-GATGACGCGGTTCGCTGGTG−3′ (antisense) | 321–302 |
| | 5′-GTGCCAACAAAGTATATCCTG-3′ (sense) | 322–342 |
| Fuc-Tb and Fuc-th | Glu16 → Asp | | |
| | G → C | 5′-GACGACGGCGGCTCCGTTAGGGTGAC−3′ (antisense) | 332–309 |
| | | 5′-GTGCCAACCCGGGAGTCAAGCAC−3′ (sense) | 334–357 |
| Arg15 → Trp | C → T | 5′-GAGGACCCGCTCCGTTAGGGTGAC−3′ (antisense) | 332–309 |
| | | 5′-GTGCCAACCCGGGAGTCAAGCAC−3′ (sense) | 334–357 |
| Glu16 → Gln | G → C | 5′-GACGACGGCGGCTCCGTTAGGGTGAC−3′ (antisense) | 332–309 |
| | | 5′-GTGCCAACCCGGGAGTCAAGCAC−3′ (sense) | 334–357 |

*Positions of primers are given in comparison with adenine residues of the initiation codon assigned as base one.

Acceptor Substrate Specificity of Fuc-TIII Mutants—For each Fuc-TIII mutant, fucose transfer reactions were conducted with H-type 1 and H-type 2 acceptors. Under the experimental conditions used, Fuc-TIII had a very weak activity with the H-type 2 acceptor compared with that obtained with the H-type 1 acceptor (Table III).

The recombinant Fuc-TIII enzyme sharing the single mutation Trp111 → Arg lost its type 1 acceptor activity and acquired type 2 acceptor activity. The double mutation Trp111 → Arg/Asp112 → Glu conferred to the recombinant enzyme higher activity (~2-fold increase with the H-type 2 acceptor), which became equivalent to the activity obtained with the native Fuc-TIII enzyme using the H-type 1 acceptor. The single conservative substitution of Fuc-TIII (Asp112 → Glu) decreased by about half the activity of the enzyme without changing its relative type 1/type 2 acceptor substrate specificity, suggesting that the nature of the acidic residue (Asp or Glu) does not by itself determine the H-type 1/H-type 2 transfer activity, although this acidic residue can help to modulate the relative rates of enzyme activities.

Acceptor Substrate Specificity of Fuc-Tb Mutants—The native bovine α1,3-fucosyltransferase Fuc-Tb utilizes exclusively type 2 acceptors (16). Any single substitution of the Fuc-Tb enzyme at each of the candidate amino acids (Arg115 → Trp or Glu116 → Asp) generated an inactive enzyme with both acceptor substrates (Table III). Only a very small increase in activity on the H-type 1 acceptor was observed from 7 ± 0.5 to 11 ± 0.5 pmol/h/mg of protein with the double mutation Arg115 → Trp/ Glu116 → Asp, which also conserved a weak activity (58 ± 3 pmol/h/mg of protein) on the H-type 2 acceptor.

Involvement of Acidic Residue Asp112 of Fuc-TIII and Glu116 of Fuc-Tb in Enzyme Activity—In all α1,3-fucosyltransferases listed in Table II, the candidate amino acid involved in H-type 1/H-type 2 specificity is followed by an acidic residue (Asp or Glu). Aspartic acid is found in enzymes able to transfer fucose either on the H-type 1 or H-type 2 acceptor (Fuc-Tb or Fuc-TIV, Fuc-TV), but also in enzymes acting only on the H-type 2 acceptor (Fuc-TIV, and Fuc-TIX), whereas Glu is present exclusively in the α1,3-fucosyltransferases such as Fuc-TVI, Fuc-T VII, Fuc-Th, and Fuc-Tb.

The presence of Asp or Glu in wild-type Fuc-TIII or its variants seems to modulate enzyme efficiency (Table III). However, in Fuc-Tb, only the presence of Glu, as in the native enzyme, was able to confer activity to the protein. Indeed, the Glu116 → Asp change was associated with enzyme activity loss, whatever the substrate acceptor.

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*These programs are available on the server from Cis Infobiogen (E-mail: bioinfo@infobiogen.fr; WEB: http://www.infobiogen.fr).
One Amino Acid Defines Type 1/Type 2 Activity of Lewis Enzyme

Asterisks identify the four previously defined potential candidate amino acids based on the multiple alignment of the Lewis subfamily of enzymes (10). Hyphens indicate amino acids identical to the human Fuc-TIII enzyme.

| Acceptor specificity | a1,3-PFT* subfamily | Enzyme | GenBank accession | Amino acid sequence | Ref. |
|----------------------|----------------------|--------|-------------------|---------------------|------|
| Type 1 and 2         | Lewis                | Fuc-TIII | X53578             | 101PQADTVVHWDMSNPKRSI121 | 18   |
|                      | Fuc-TIII (chimera)   | Y14033 |       | 112---A---WD---Y---Y---A---132 | 10   |
|                      | Fuc-TV               | M81485 |       | 114---A---WD---Y---SAN---134 | 38   |
|                      | Fuc-TV (chimera)     | Y14034 |       | 114---A---WD---Y---SAN---134 | 10   |
| Type 2 only          | Fuc-TV-1             | L01698 |       | 100---A---WD---Y---SAN---120 | 39   |
|                      | Fuc-TV-2             | Y14035 |       | 100---A---WD---Y---SAN---120 | 10   |
|                      | Fuc-T (bovine)       | X87510 |       | 105---A---WD---REV---SHR---QG---125 | 16   |
|                      | Fuc-T (hamster)      | U78737 |       | 102---G---A---F---RE---SP---R---L---122 | GenBank/EBI |
| Leukocyte            | Fuc-TVH              | X78031 |       | 86---A---V---F---REL---TRR---SHL---106 | 4    |
|                      | Fuc-TV (mouse)       | U45980 |       | 86---A---V---F---REL---TRQ---SSL---106 | 4    |
| Myeloid              | Fuc-TIV              | M58596 |       | 105-G-GE-QA-LF---RDLVYKPGPDPW-125 | 41   |
|                      | Fuc-TIV (mouse)      | U33457 |       | 131-GE-QA-LF---RDLVYKPGPDPW-151 | 42   |
|                      | Fuc-TIV (rat)        | U58860 |       | 95-GE-QA-LF---RDLVYKPGPDPW-154 | 43   |
|                      | Fuc-TIV (chicken)    | U73678 |       | 93-GE-QA-LF---RDLVYKPGPDPW-123 | 44   |
|                      | Fuc-TIV (mouse)      | AB015426 | | 101NKS---A-L---I-RD-SWDLT---L121 | 45   |

* FT, fucosyltransferase.

![Fig. 2. Western blot analysis of wild-type and mutated Fuc-TIII enzymes expressed in COS-7 cells.](image)

To define more precisely the requirement of the acidic residue for enzyme activity, Asp112 of Fuc-TIII was substituted by the corresponding amide Asn. The Fuc-TIII mutant (Asp112→Asn) became inactive with both acceptors (Table III). The same kind of result was obtained with modified Fuc-Tb (Glu116→Gln), which was also unable to transfer fucose on either of the two acceptor substrates (Table III).

Kinetic Parameters of Native and Mutated Enzymes—For Fuc-TIII variants, the apparent Km values for GDP-fucose (~30 μM) were similar to that of the parental enzyme (Table IV). Therefore, amino acid changes in enzyme variants did not modify the affinity of the Fuc-TIII variants for the donor substrate. However, significant changes in the Km values for the H-type 1 and H-type 2 acceptor substrates were observed for native Fuc-TIII compared with the Fuc-TIII mutants. As expected, native Fuc-TIII and Fuc-TIII with the Asp112→Glu mutation had better Km values for H-type 1 than for H-type 2 acceptors, whereas the two Fuc-TIII mutants with the Trp111→Arg change had a good affinity for the H-type 2 acceptor and a poor affinity for the H-type 1 acceptor. It also appeared that the nature of the acidic residue (Asp or Glu) adjacent to the candidate amino acid (Trp or Arg) had a significant effect on the kinetics of the recombinant enzyme. The Trp-Asp association found in wild-type Fuc-TIII is favorable for H-type 1 activity, whereas the Arg-Glu association found in double mutant Fuc-TIII is convenient for H-type 2 activity (Table IV).

DISCUSSION

Fucosyltransferases have a common domain structure including a short NH2-terminal cytoplasmic tail, a signal anchor domain, a stem region, and a globular COOH-terminal catalytic domain. Truncation at the COOH terminus of one or more amino acids induces a dramatic loss of enzyme activity, whereas truncation at the NH2 terminus of as much as 61 amino acids for Fuc-TIII or 75 amino acids for Fuc-TV does not alter the enzyme activity (11, 23). First one (24, 25) and then two (26) peptide conserved motifs were described in the COOH-terminal catalytic domain of all the a1,3-fucosyltransferases, and they are presumed to be involved in GDP-fucose binding (26, 27), whereas the acceptor substrate-binding domain has been tentatively ascribed to a portion of the hypervariable region comprised between either positions 62 and 110 (11) or positions 103 and 153 (15) of Fuc-TIII.

Based on our previous results (10), a short peptide segment corresponding to the sequence Pro101–Leu121 of Fuc-TIII was used for peptide sequence alignment of all known vertebrate a1,3-fucosyltransferases. A single amino acid, Arg in a1,3-fucosyltransferases and Trp in a1,3/1,4-fucosyltransferases, was expected to contribute to the type 1/type 2 acceptor specificity. By site-directed mutagenesis, we have now demonstrated that the single substitution of Trp111 by Arg conferred to the recombinant Lewis enzyme the ability to use efficiently H-type 2 instead of H-type 1 acceptor substrate. This newly acquired activity increased when, in addition, Asp112 was replaced by Glu. The involvement of Arg in fucose transfer was confirmed by the loss of Fuc-Tb activity when Trp substituted for Arg115.

Several invertebrate and bacterial a1,3-fucosyltransferases have been recently found (reviewed in Ref. 28). Two Helicobacter pylori enzymes have been cloned and expressed, and...
they both use type 2 acceptors (24, 25) and have the similarly charged amino acid Lys instead of Arg in the candidate position. A Caenorhabditis elegans α1,3-fucosyltransferase has also been cloned and expressed (29), and it has Glu in the candidate position, but it can transfer Fuc onto acceptors as GalNAcβ1,4GlcNAcβ1-R to generate GalNAcβ1,4(Fucolc6)GlcNAcβ1-R. These nematode oligosaccharides are different from all known type 1 or type 2 oligosaccharides of the lacto or neolacto series. The remaining invertebrate and bacterial putative α1,3-fucosyltransferases have only been defined by sequence homology to vertebrate enzymes, and nothing is known about their enzyme activity or about the structure of the acceptor substrates used. Nevertheless, some of them did not have either Trp or Arg in the corresponding candidate position of the putative acceptor-binding domain, suggesting that as in the case of C. elegans, other acceptors might be used by these enzymes. However, the overall structure of the acceptor substrate-binding domain is, in general, preserved with a conserved His residue before and a carboxylic group (Asp or Glu) after the candidate position.

Previous work by Hindsgaul et al. (30) suggested that oligosaccharide-reactive acceptor hydroxyl groups are involved in a critical hydrogen bond donor interaction with the glycosyltransferases. The main key polar groups on the oligosaccharide acceptors have been identified as the reactive hydroxyls at C-3 or C-4 of GlcNAc and C-6 of Gal (31, 32). On the enzyme side, different amino acids can be involved in this hydrogen bond, but typical hydrogen bond acceptors are His and carboxylates (26), as those found in the conserved amino acid positions flanking the candidate Arg or Trp residue for the definition of the type 1/type 2 enzyme activity. Therefore, these His, Glu, and Asp residues can be considered as candidates for the formation of hydrogen bonds with the acceptor oligosaccharide. Based on recent studies, it seems reasonable to suggest that the active-site base in the protein may be a carboxylate anion (33, 34). In another study, Britten and Bird (35) investigated the amino acids essential for the activity of Fuc-TV1 through chemical modification, and they concluded that the substrate-binding site of the enzyme possesses His residue(s) that are essential for enzyme activity.

It has been suggested, in the case of the oligosaccharyltransferase, that the divalent cation cofactor might be in close proximity to the acceptor oligosaccharide substrate (36). A similar mechanism could occur in fucosyltransferases, and it was recently suggested that Mn2⁺ could interact with a carboxylate anion (Glu or Asp) in the fucosyltransferase on one side and with the oligosaccharide acceptor substrate on the other side (37). The complete loss of enzyme activity by the substitution Asp112 → Asn in Fuc-TIII or Glu416 → Gln in Fuc-Tb suggests that this carboxylate group is necessary for enzyme activity, and therefore, it constitutes a possible candidate for the active-site carboxylate anion.

The Arg115 → Trp substitution in Fuc-Tb was not sufficient to change the specificity of the enzyme toward type 1 acceptors even with the additional mutation Glu116 → Asp. Therefore, Trp seems to contribute to a type 1 activity, but it is not sufficient by itself; and even more than the two tested candidate, Trp and Asp, are probably necessary to change the Fuc-Tb specificity. This is in accordance with the fact that only a very small increase in the number of cells producing Leα and sialyl-Leα antigens was observed in cells transfected by a FucTV1 chimera containing Fuc-TIII subdomains 4 and 5 (positions 103–153) (15). In another work (12), it was shown that two other amino acid changes in Fuc-TV (Asn86 → His and Thr97 → Ile) increased the type 1 activity of the recombinant enzyme.

The molecular phylogeny of fucosyltransferase genes suggested that the duplication events at the origin of the bovine futb (16) and primate FUT6 genes occurred before the duplication that produced the primate FUT3 and FUT5 genes (Fig. 1). Hence, it seems that the common ancestor of FUT3 and FUT5 genes has acquired the capacity to use type 1 acceptors without loss of the ability to use type 2 acceptors. This would help to explain the change in enzyme specificity by a single amino acid substitution in Fuc-TIII, whereas more complex changes are expected in Fuc-Tb or Fuc-TV1 to generate a strong type 1 acceptor activity because other independent mutations might have occurred in Fuc-TV1 and/or Fuc-Tb since their earlier divergence from the common evolutionary path (Fig. 1).

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See “Experimental Procedures” for details.

| Enzyme     | GDP-Fuc | H-type 1 | H-type 2 |
|------------|---------|----------|----------|
|            | K<sub>m</sub> (μM) | V<sub>max</sub> (nmol/h/mg protein) | K<sub>m</sub> (μM) | V<sub>max</sub> (nmol/h/mg protein) | K<sub>m</sub> (μM) | V<sub>max</sub> (nmol/h/mg protein) |
| Fuc-TIII   | 35      | 21       | 0.2      | >3 | 52 | 15 |
| Fuc-TIII Asp<sup>112</sup> → Glu | 30 | 17 | 0.4 | 52 | >3 | 6 |
| Fuc-TIII Trp<sup>111</sup> → Arg | 32 | 19 | >3 | 8 | 0.5 | 58 |

| Fuc-TIII Trp<sup>111</sup> → Arg/Asp<sup>112</sup> → Glu | 31 | 5 | 0.5 | 58 |

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