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Identification of a cDNA encoding a plant Lewis-type α1,4-fucosyltransferase

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Recently, it has been found that plants, including tomato (*Lycopersicon esculentum*), express the Lewis-a epitope, Galβ1,3(Fucα1,4)GlcNAc, on some N-glycans. By searching the EST database, it was possible to identify a tomato cDNA encoding a protein, designated FucTC, of 413 amino acids with homology to plant and mammalian α1,3/4-fucosyltransferases. The cDNA was expressed in *Pichia pastoris* and the recombinant enzyme was found to transfer fucose from GDP-Fuc (K_m 16 μM) to lacto-*N*-tetraose (Galβ1,3GlcNAcβ1,3Galβ1,4Glc; K_m 80 μM) as well as to β1,3- and β1,4-galactosylated N-glycans. It is concluded that FucTC is responsible for the biosynthesis of Lewis-a on N-glycans in tomato.

Keywords: fucosyltransferase, tomato, Lewis-a

Abbreviations: LNFPII, lacto-*N*-fucopentaose II; LNFPIII, lacto-*N*-fucopentaose III; MALDI-TOF MS, matrix-assisted laser desorption/ionisation time-of-flight spectrometry. For example N-linked oligosaccharide structures see Figure 1.

Introduction

The N-glycans of plants display many features not found in mammals, such as core α1,3-fucosylation and β1,2-xylosylation, while lacking certain modifications such as β1,4- or α1,3-galactosylation, sulphation or sialylation [1]. However, it has recently become obvious that a wide variety of plants contain N-glycans, such as $(G^3F^4)(G^3F^4)XF^3$ (see Figure 1 for an explanation of glycan structures), which carry the Lewis-a (Le^a) epitope [2,3]. Indeed in a recent survey, most plant foodstuffs studied contained such N-glycans, with particularly high amounts being found in foods such as apple, banana, celery and kiwifruit, which are often associated with allergy [4]. One of the enzymes responsible for the biosynthesis of Le^a-epitopes, an α1,4-fucosyltransferase, has been detected in some plant sources, such as sprouting mung beans and sycamore or myrtle cells [2,3,5–7]. The purification of this enzyme to homogeneity has, though, not been reported. Other fucosyltransferases found in plants do not catalyse this reaction, e.g., core α1,3-fucosyltransferases [8,9] or xyloglucan α1,2-fucosyltransferases (in Arabidopsis encoded by *AtFUT1-10*) [10,11].

The Le^a epitope is better known as a human histo-blood group antigen, sialylated forms of which are found in high amounts on cancer cells of the digestive tract [12]. In humans, α1,4-fucosylation is primarily mediated by the Lewis fucosyltransferase, Fuc-TIII, a member of the α1,3-fucosyltransferase family, which can catalyse the formation of both α1,3- and α1,4-fucose linkages [13]. Of the other human members of this enzyme family only Fuc-TV appears to have a low α1,4-fucosylation activity, in addition to its predominant α1,3-fucosylation capability [14]. Considering this dual functionality, database searching was performed and a number of plant EST clones displaying homology to mammalian α1,3/4-fucosyltransferases, but which were distinct from plant core α1,3-fucosyltransferases, were identified. In the present report, the full characterisation of a cDNA clone from tomato (*Lycopersicon esculentum*), which is already known to express Le^a-epitopes [4], and the activity of the encoded recombinant enzyme are described.

Experimental procedures

EST identification and cloning of tomato FucTC cDNA

The Genbank database was searched using the tBLASTn program using the sequences of mammalian α1,3-fucosyltransferases. In Arabidopsis, a novel gene (encoding FucTC) was identified [9] and the predicted sequence used to search

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The nucleotide sequence of tomato FucTC cDNA has been deposited in the EMBL database under the accession number AJ313193.

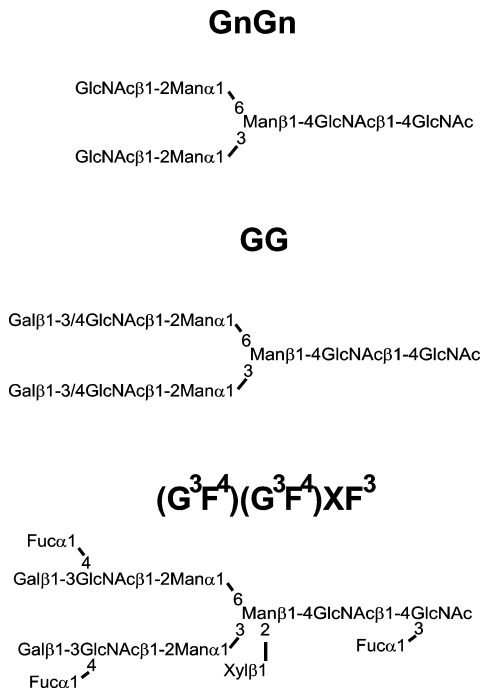

Figure 1. Examples of N-linked oligosaccharide structures referred to in this study. The abbreviations refer to the non-reducing terminal sugars in an anti-clockwise manner (first the substitution of the α1,6-arm, then that of the α1,3-arm): M refers to a terminal mannose, Gn to a terminal N-acetylglucosamine, G to a terminal galactose, F to a fucose and (GF) to a branch carrying both a terminal galactose and a fucose residue. Superscripts refer to defined linkages of the galactose or fucose residues.

the dbEST database. One cDNA clone (Genbank AW621252; EST312050/cLEX11I22 from tomato root during/after root set) with homology to Arabidopsis FucTC at the 5′-end, and so potentially full-length, was obtained from the Clemson University Genome Institute. Sequencing of the clone was completed using the BigDye system (PE Biosystems) using both tomato- and vector-specific primers. A fragment encoding the putative soluble form of tomato FucTC (i.e. lacking the putative transmembrane domain) was amplified from the clone using Expand (Roche Biochemicals), the primers TomC5/*Eco*RI 5′-CCG*GAATTC*ACTTCACACTTCTTC-3′ and TomC2/*Kpn*I 5′-CGG*GGTACC*TCAAGAGGCTTTTG-CATTTC-3′ and an Eppendorf MasterCycler PCR machine (3 min @ 95°C, 40 cycles of 1 min @ 55°C, 3 min @ 72°C and 1 min @ 95°C, followed by a final extension step of 8 min @ 72°C). RT-PCR was also performed using RNA, prepared using Trizol reagent (Life Technologies), from young developing leaves of three-week old tomato plants (cv. Ailsa Craig).

PCR products were purified after gel electrophoresis using the QIAGEN gel extraction kit and cloned using the ZeroBlunt kit (Invitrogen). Clones were selected and the FucTC fragment was excised from plasmid DNA with *Eco*RI and *Kpn*I, gel-purified and ligated into the pICZαC vector (Invitrogen) cut with the same enzymes. Alternatively, digested tomato leaf RT-PCR products were used directly to construct the recombinant pICZαC plasmid. Expression vector DNA (ca. 10 μg) from PCR-positive *E. coli* clones was cut with *Sac*I prior to electroporation into *Pichia pastoris* cells. Positive yeast clones were identified after PCR of genomic DNA using primers specific for the host *AOX1* gene.

Production and assay of recombinant tomato FucTC

For the screening of tomato FucTC clones, expression of recombinant fucosyltransferase was induced by methanol as described previously for Arabidopsis and Drosophila fucosyltransferases [9,15]. Yeast clones were grown in 10 ml MGYC (medium with glycerol, yeast nitrogen base and casamino acids) overnight at 30°C, washed with yeast nitrogen base and resuspended in the same volume of MMYC (medium with methanol, yeast nitrogen base and casamino acids). Methanol (final volume 1%) was added every 24 hours thereafter and the medium was collected after three or four days of induction. The culture medium was concentrated ten-fold using Vivaspin concentrators. For medium-scale expression, a portion of one yeast clone was grown overnight in 10 ml MGYC, washed and then diluted ten-fold in 100 ml MMYC. In this case, optimal expression (ca. 1 U/L) was achieved after 48 hours induction; concentration of the medium was found not to be necessary.

Assay of recombinant tomato FucTC

The standard assay for α1,3- and α1,4-fucosyltransferase activities was performed using pyridylaminated lacto-*N*-tetraose (Gal*β*1,3GlcNAc*β*1,3Gal*β*1,4Glc-PA) and lacto-*N*-neo-tetraose (Gal*β*1,4GlcNAc*β*1,3Gal*β*1,4Glc-PA): 5 μl enzyme solution was added to 2 μl 0.5 mM acceptor, 1 μl 0.4 M MES, pH 6, 0.5 μl 0.2 M $MnCl_2$ and 1 μl 10 mM GDP-Fuc. The incubation was allowed to proceed at 37°C and the assay mixture was analysed by reverse-phase chromatography on a Hypersil ODS column (0.4 × 25 cm) at room temperature. The column was equilibrated with 0.1 M ammonium acetate, pH 4, and a gradient of 1% per minute of 30% methanol was applied. The eluate was monitored using a fluorescence detector (320/400 nm) and the retention times of the products compared with standard LNFPII and LNFPIII, the α1,4- and α1,3-fucosylated forms of lacto-*N*-tetraose and lacto-*N*-neo-tetraose respectively. For the metal ion dependence studies the HPLC-based assay was employed with the difference that, instead of 0.5 μl 0.2 M $MnCl_2$, 0.5 μl of 0.2 M solutions of the relevant cation or EDTA were added to the enzyme incubation.

Transfer of fucose to N-glycans was also monitored by MALDI-TOF MS of incubations in which dansylated fibrin glycopeptides were used as substrates. Bovine fibrinogen has been previously shown to contain primarily sialylated biantennary N-glycans with two terminal *β*1,4-galactose residues [16], but NMR also demonstrates the presence of *β*1,3-linked galactose [17]. In this laboratory, putative G^3G^4 and G^3G^3 structures were calculated to account for 11.2% and 2.2%, respectively, of total asialofibrin glycans as judged

by their insensitivity to *Aspergillus oryzae* β-galactosidase, an enzyme previously shown to remove β1,4-galactose residues [18]. The remaining galactose residues on the fibrin glycans are, however, completely removed with bovine testes β-galactosidase, an enzyme which prefers β1,3-galactose linkages (data not shown).

For assay of fucosyltransferase, either complete asialoglycopeptides or a preparation enriched in terminal β1,3-galactose were purified. In the latter case, complete desialylated fibrin glycopeptides were treated with *A. oryzae* β-galactosidase, dansylated and (in order to remove the majority of the resulting predominant GnGn structure) partially purified by HPLC yielding a mixture of GnGn, GGn/GnG and GG. The conditions for assay of fucosyltransferase were similar to those described above with the pyridylaminated tetrasaccharides: 250–500 pmol of dansylated glycopeptide were dried in microcentrifuge tubes, then 5 µl of enzyme, 1 µl 0.4 M MES, pH 6, 0.5 µl 0.2 M $MnCl_2$, 2.5 µl water and 1 µl 10 mM GDP-Fuc were added. After the timepoints stated in the figure legends or in the text, 1 µl of the reaction mixture was withdrawn and diluted tenfold with water. 0.8 µl diluted sample was then mixed with 0.8 µl 1% (w/v) α-cyanohydroxycinnaminic acid in 70% (v/v) acetonitrile and allowed to dry on a sample platen prior to MALDI-TOF MS analysis.

Results

Identification and analysis of tomato FucTC cDNA

Searching of the Arabidopsis genome indicates the presence of three genes encoding α1,3-fucosyltransferase-like proteins; the first two (designated FucTA and FucTB)[1] show high homology to the mung bean core α1,3-fucosyltransferase, the third (preliminarily designated FucTC) shows some sequence characteristics more akin to mammalian Lewis-type fucosyltransferases [9]. Since the Le^a antigen and requisite α1,4-fucosyltransferase activities have been detected in some plants, FucTC was considered a candidate for the plant α1,4-fucosyltransferase [2,3,5–7]. However, brassicas, including the model organism Arabidopsis, apparently lack any glycans larger than GnGnXF[3] [4,19,20]. Also attempts to detect enzyme activity in yeast transformed with Arabidopsis FucTC cDNA were unsuccessful [9]; therefore, it was decided to clone FucTC from a plant in which these epitopes have been found.

BLAST searching indicated the presence of ESTs with homology to parts of Arabidopsis FucTC from barley, cotton, *Lotus japonicum*, maize, *Medicago truncatula*, potato, soybean and tomato, whereas in rice two homologues (designated FucTC and FucTD) and in *Brassica oleracea* one homologue were identified from genomic sequences. Amongst these species, studies in this laboratory using MALDI-TOF MS indicated that around 3% of the N-glycans of tomato and potato carry one or two Le^a determinants [4]. Fortuitously, a publicly-available EST from tomato displayed homology to the very 5′-end of the Arabidopsis FucTC cDNA and sequencing of this tomato cDNA indicated that it encodes a protein consisting of 413 amino acids (M_r 46269) with one potential N-glycosylation site and a typical Golgi glycosyltransferase type II N-terminal putative transmembrane structure (Figure 2). Except for eight nucleotide changes or deletions, the determined sequence is also consistent with four further tomato ESTs, BE449780 (from plants pre-anthesis), BG628342 (from flowers), BF051851 (from developing/immature green fruit) and BI421553 (tomato callus) encoding the middle and C-terminal regions of tomato FucTC. A fragment corresponding to the soluble form of FucTC cDNA was also isolated by RT-PCR of tomato leaf RNA and expressed in *E. coli* using the pCR® T7/NT® TOPO system: the resultant MALDI peptide map was compatible with the determined nucleotide sequence (data not shown). The sources of the ESTs and of the RT-PCR product suggest that the FucTC gene is transcribed in various parts of the tomato plant.

Sequence alignments (Figure 3) showed that the tomato enzyme (entire sequence) is 69% identical to Arabidopsis FucTC and 60% identical to a predicted reading frame of a rice homologue, as well as 72% identical to *Beta vulgaris* (sugar beet) α1,4-fucosyltransferase [21]. In comparison to mammalian enzymes, tomato FucTC is 22% identical to human Fuc-TVII between residues 115 and 395 and 25% identical to human Fuc-TIX between residues 122 and 376. Like the FucTC homologues from Arabidopsis, barley, maize, Medicago, soybean and rice, tomato FucTC has an SYVTEK motif, rather than the DY(I/V)TEK motif found in other α1,3-fucosyltransferases from plant, Drosophila, nematode, fish or mammals [22,23] or the GY(I/V)TEK motif found in *Helicobacter pylori* and *Vibrio cholerae* α1,3-fucosyltransferases [24,25]. All the aforementioned plant FucTC homologues contain a CRLC motif, which is also present in all plant core α1,3-fucosyltransferases: this motif corresponds to a cysteine pair also conserved in mammals [26]. On the other hand, plant FucTC homologues lack the SNC(A/G)ARN sequence found in plant and *Drosophila* core α1,3-fucosyltransferases [8,9,15], while FucTC sequences also have a WXW motif (marked with × in Figure 3) found in most mammalian Lewis-type fucosyltransferases.

Identification of FucTC as an α1,3/4-fucosyltransferase

Tomato FucTC was expressed under control of the *AOX1* alcohol oxidase promoter in *Pichia pastoris* as a fusion protein with the α-mating factor secretion signal (M_r 43067 after signal cleavage). A number of yeast clones were screened by assay of

[1]Considering the recent numbering of *Arabidopsis thaliana* α1,2-fucosyltransferase homologues as *AtFUT1-10* (Ref. 11), it is hereby proposed to designate the genes encoding *Arabidopsis thaliana* FucTA, FucTB and FucTC as *AtFUT11*, *AtFUT12* and *AtFUT13*. The tomato (*Lycopersicon esculentum*) FucTC gene is therefore *LeFUT13*.

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ggcttgtagagatctcaaacccttacaaattaatactacataaattttggtctaa
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Figure 2. Predicted amino acid sequence of tomato Lewis-type α1,3/4-fucosyltransferase (tomato FucTC). The amino acid sequence is shown below the cDNA sequence. The putative transmembrane domain and potential N-glycosylation site are underlined.

the medium for secreted fucosyltransferase activity. All were found to express a fucosyltransferase activity as determined by an HPLC-based assay, similar to one previously used with zebrafish fucosyltransferases [27], using fluorescently-tagged tetrasaccharide substrates allowing simultaneous product quantitation and identification. Acceptors for both α1,3- and α1,4-fucosyltransferases were employed: respectively pyridylaminated lacto-*N*-neo-tetraose (Galβ1,4GlcNAcβ1,3Galβ1,4Glc; type II acceptor) and pyridylaminated lacto-*N*-tetraose (Galβ1,3GlcNAcβ1,3Galβ1,4Glc; type I acceptor). For negative controls, supernatants of Pichia transformed with constructs encoding soluble forms of, and verified by enzyme activity assays to express, either Arabidopsis core α1,3-fucosyltransferase (FucTA) or bovine β1,4-galactosyltransferase I were used (cf. Ref 9.).

Most previous reports on plant Lewis fucosyltransferases suggested that they probably act primarily as α1,4-fucosyltransferases [2,3,5–7], although some α1,3-fucosylation activity towards α1,2-fucosylated type II acceptors has also been found [5,7]. Indeed, the recombinant tomato enzyme was active towards both pyridylaminated lacto-*N*-tetraose and lacto-*N*-neo-tetraose, albeit with an approximately 200-fold lower rate of conversion for the latter as compared to the former (Figure 4): i.e., conversion of the former was 70% within 30 minutes, whereas only 14% of the latter was converted in 20 hours. With both substrates, the products co-eluted with the relevant pyridylaminated fucosylated standards LNFPII and LNFPIII; no such products were seen when supernatants of *Pichia* expressing soluble forms of bovine β1,4-galactosyltransferase I (see chromatograms C and F, Figure 4) or Arabidopsis FucTA were employed as negative controls.

The K_m values for pyridylaminated lacto-*N*-tetraose and GDP-Fuc were determined using this HPLC method. The K_m for the acceptor was determined to be 80 μM and for

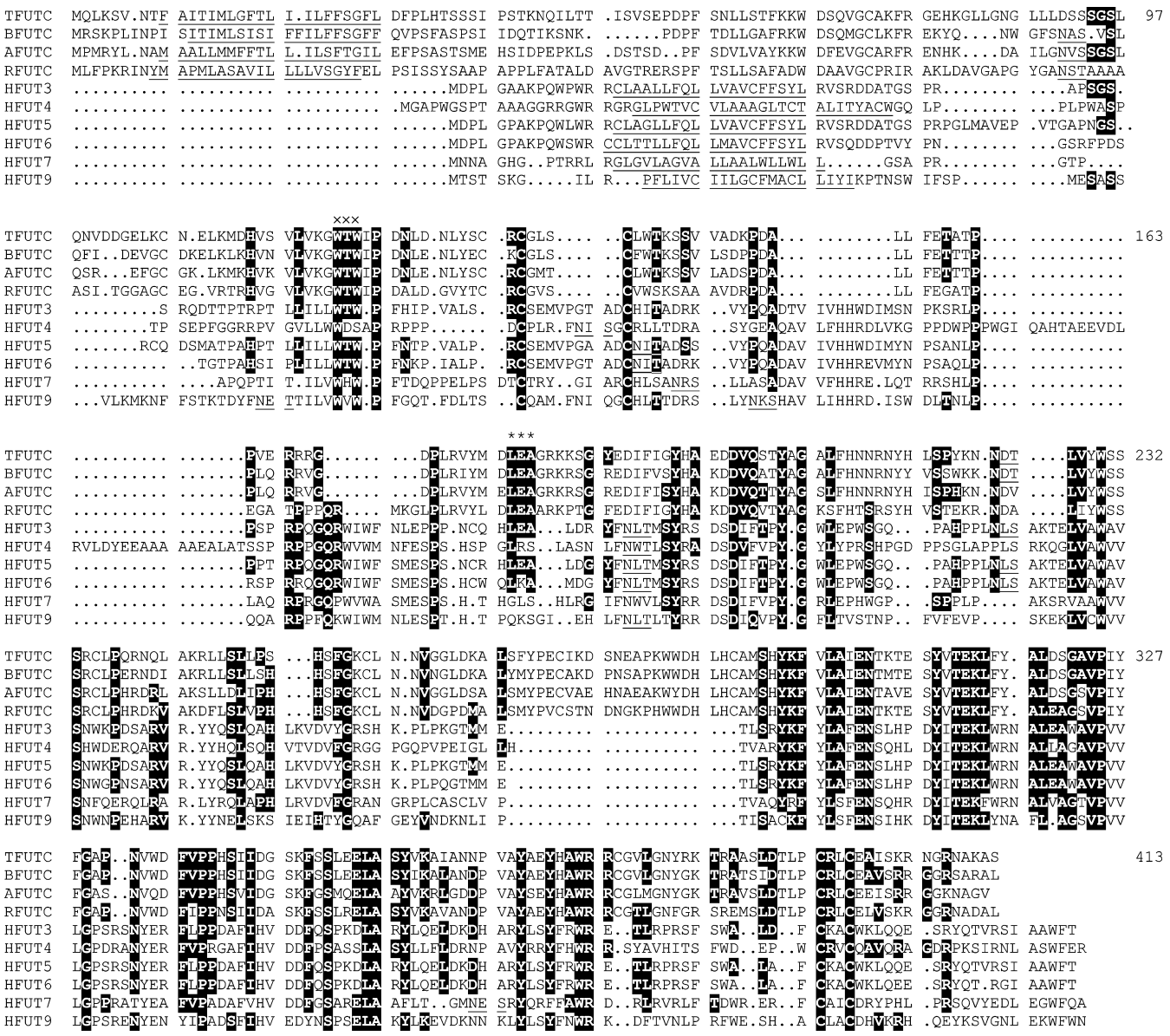

Figure 3. Alignment of plant and mammalian Lewis-type α1,3/4-fucosyltransferases. Sequences shown are: tomato (TFUTC; from this study), sugar beet (BFUTC; see Ref. 21), Arabidopsis (AFUTC; see Ref. 9), rice (RFUTC; as predicted from genomic DNA), HFUT3, HFUT4, HFUT5, HFUT6, HFUT7 and HFUT9 (human α1,3/4-fucosyltransferases III, IV, V, VI, VII and IX). Highlighted are regions where at least one sequence from one group (plant or human) shares identity with at least two sequences from another group (mammal or plant), whereas glycosylation sites and transmembrane domains are underlined. The numbering is according to that of tomato FucTC.

GDP-Fuc 16 μM, as compared to values determined for the mung bean α1,4-fucosyltransferase of 170 μM and 7.8 μM for 8-methoxycarbonyllacto-*N*-tetraose and GDP-Fuc [5]. The metal ion dependence of the enzyme present in the medium was also tested using the same HPLC-based methodology: whereas, as compared to samples in which 10 mM EDTA or no added cation were present, 10 mM Mn^{2+}, Fe^{2+} or Mg^{2+} stimulated activity (respectively, 110%, 100% and 40% stimulation), while 10 mM Cu^{2+} or Zn^{2+} inhibited the enzyme (respectively, 90% and 40% inhibition). As with the mung bean enzyme [5], but in contrast to the results with the myrtle enzyme [7], cations acted as non-essential activating co-factors.

Demonstration of fucosylation of N-glycans by FucTC

The activity of the enzyme towards N-glycan acceptors was tested by a MALDI-based assay. As described under EXPERIMENTAL PROCEDURES, use was made of the fact that bovine fibrin oligosaccharides contain both non-reducing terminal β1,3- and β1,4-linked galactose residues [17]. Thus, a preparation of dansylated fibrin glycopeptides enriched in

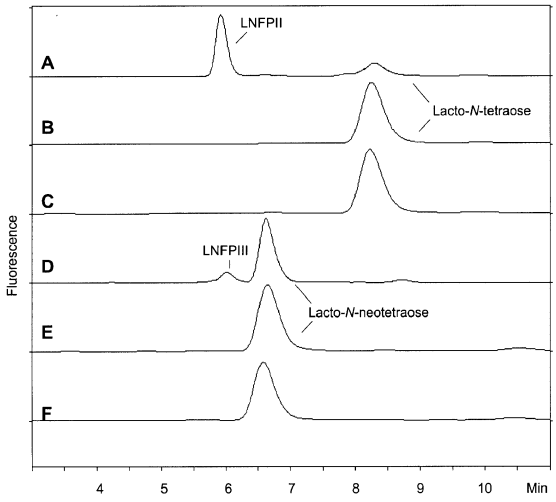

Figure 4. HPLC-based assay of fucosyltransferase activity. Supernatant of Pichia expressing tomato FucTC was incubated with pyridylaminated lacto-*N*-tetraose for 30 minutes in the absence (A) or presence (B) of GDP-Fuc or incubated with pyridylaminated lacto-*N*-neo-tetraose for 20 hours in the absence (D) or presence (E) of GDP-Fuc. As an additional control, pyridylaminated lacto-*N*-tetraose (C) or pyridylaminated lacto-*N*-neo-tetraose (F) were incubated for 20 hours with GDP-Fuc and supernatant of Pichia expressing bovine β1,4-galactosyltransferase I. The annotations of LNFPII and LNFPIII peaks are based on the retention times of standards, which under these HPLC conditions co-elute.

β1,3-galactosylated oligosaccharides (to be exact, a mixture of GnGn, GnG^3 and G^3G^3) was incubated with medium from Pichia expressing either tomato FucTC, Arabidopsis FucTA or bovine β1,4-galactosyltransferase, in the absence or presence of GDP-Fuc. Conversion of the GnG^3/G^3Gn and G^3G^3 peaks (m/z 2166 and 2327) to $Gn(G^3F^4)$ and $(G^3F^4)(G^3F^4)$ (m/z 2311 and 2618; consistent with the respective addition of one or two fucose residues) was observed only with the sample where FucTC and GDP-Fuc were present (Figure 5B). The GnGn peak was not converted by tomato FucTC, but was (as expected) a substrate for Arabidopsis FucTA. While the transfer of fucose to galactose-containing substrates was observed with supernatants of Pichia transformed with constructs encoding tomato FucTC derived from either the purchased cDNA clone or RT-PCR of tomato leaf mRNA, it was absent from supernatants of Pichia expressing bovine β1,4-galactosyltransferase I (see Figure 5A).

In addition, when a preparation of complete asialofibrin dansylated glycopeptides, which had not been subject to any prior galactosidase treatment, was used, it was found that after 30 and 60 minutes there was a ratio of GG to G(GF)/(GF)G of around 100 : 8. This is suggestive of a rapid conversion of much of the putatively β1,3-galactosylated fraction. After overnight incubation (Figure 5D), however, consistent with the assays with lacto-*N*-neo-tetraose, the main GG peak (mainly G^4G^4 with some G^4G^3/G^3G^4 and G^3G^3) was over 50% converted to G(GF)/(GF)G or (GF)(GF), again indicating a significant, but slower, α1,3-fucosylation activity. Again, the fucosylation of the N-glycans was not mediated by supernatants of Pichia expressing bovine β1,4-galactosyltransferase I (see Figure 5C).

Discussion

The data in this paper indicate that tomato FucTC is a Lewis-type α1,3/4-fucosyltransferase which transfers fucose to the antennae of N-glycans. It belongs to a sub-family of α1,3/4-fucosyltransferase homologues previously identified on the basis of protein homologies [9], which are present in plants, yet are distinct from those α1,3-fucosyltransferases, such as Arabidopsis FucTA and mung bean FucT-C3, which fucosylate the core Asn-linked *N*-acetylglucosamine residue. The plant Lewis-type fucosyltransferase sub-family is also of interest since tomato FucTC has significantly higher α1,4- than α1,3-fucosyltransferase activity. This property is shared by relatively few fucosyltransferases for which data is available, specifically human Fuc-TIII [13] and the Lewis enzyme from one of three *Helicobacter pylori* isolates [24].

As described earlier, FucTC also shares some features present in mammalian Lewis-type fucosyltransferases, which are absent from the plant and Drosophila core α1,3-fucosyltransferases, suggesting an early evolutionary divergence of the ancestral eukaryotic Lewis enzymes from the core enzymes (see also Figure 6). However, the regions of identity between the tomato sequence and those of the two human and chimpanzee enzymes that have α1,4-fucosyltransferase activity (Fuc-TIII and, to a lesser extent, Fuc-TV) offer relatively few clues as to why they are different from the enzymes that form exclusively α1,3-linkages (Fuc-TIV, Fuc-TVI, Fuc-TVII and Fuc-TIX), other than the possibly coincidental occurrence of an L-E-A motif (marked with asterisks in Figure 2). In particular, there is no residue in the tomato sequence corresponding to the Trp^{111} in human Fuc-TIII (Trp in Fuc-TV, but Arg in other human sequences) claimed to be important for the α1,4-fucosyltransferase activity of Fuc-TIII and Fuc-TV [28]. Also, the bovine genome contains only one example of an *FUT3-FUT5-FUT6* relative encoding an enzyme of solely α1,3-fucosyltransferase activity, indicative that *FUT3* and *FUT5* (being the basis of primate α1,4-fucosylation) diverged at a relatively late stage from *FUT6* (which encodes an enzyme that is an α1,3-fucosyltransferase) [29–31]. Such pieces of evidence would suggest that plant and primate α1,4-fucosyltransferases arose independently during the evolution of the α1,3-fucosyltransferase gene family: this conclusion being supported by the phylogenetic tree analysis of plant and vertebrate sequences (Figure 6), which suggests a distinct separation of plant core-type, plant Lewis-type and vertebrate Lewis-type fucosyltransferases with no direct linkage between plant and primate α1,4-fucosyltransferases.

Amongst plant fucosyltransferases, the bias of tomato FucTC towards α1,4-fucosyltransferase activity is shared with

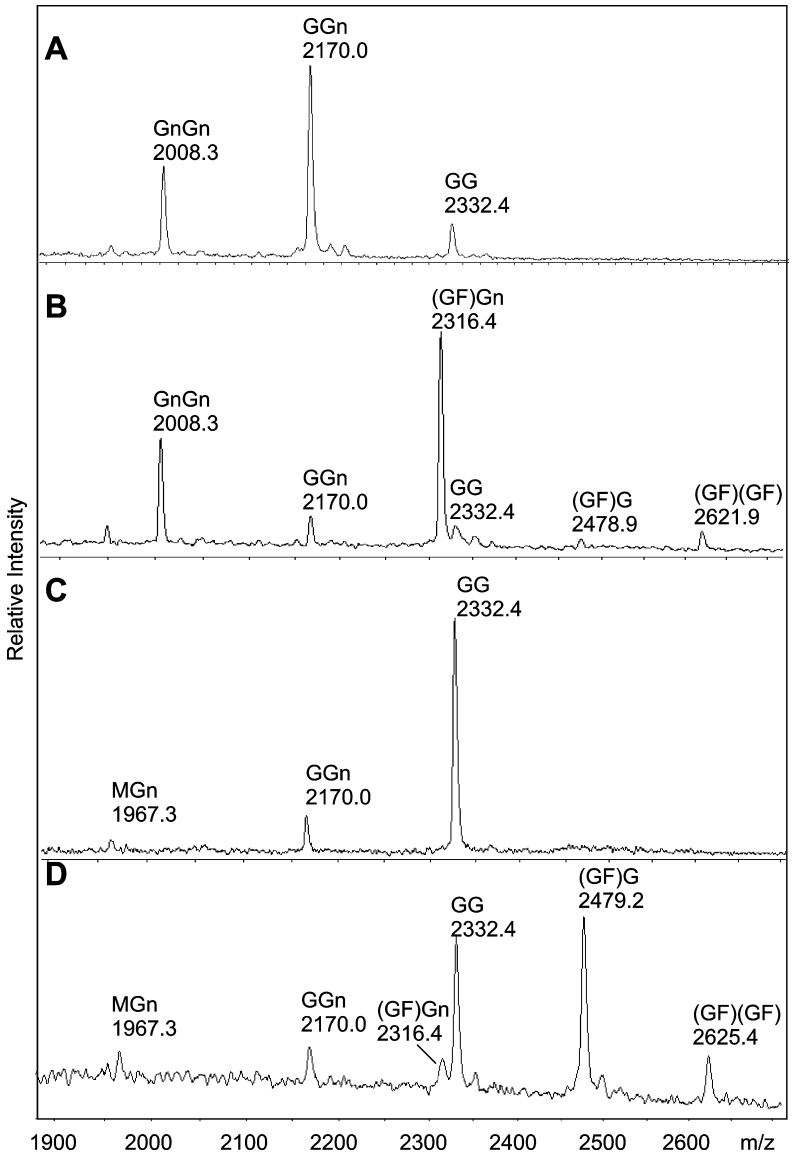

Figure 5. MALDI-based assay of fucosyltransferase activity. For panels A and B, 250 pmol "β3-galactose enriched" dansylated fibrin glycopeptide preparation was incubated for four hours in the presence of GDP-Fuc with supernatant of Pichia expressing either (A) bovine galactosyltransferase or (B) tomato FucTC. For panels C and D, 500 pmol dansylated asialofibrin glycopeptide preparation was incubated overnight in the presence of GDP-Fuc with supernatant of Pichia expressing either (C) bovine galactosyltransferase or (D) tomato FucTC. The designations of the oligosaccharide structures are not intended to indicate any particular structural isomer, merely an overall composition (i.e., GGn may be a mixture of G^3Gn, G^4Gn, GnG^3 and GnG^4). The glycopeptides were predominantly in the $[M+H]^+$ form.

the sugar beet enzyme, whose properties were published while this manuscript was undergoing review [21].[2] In the latter case, the authors described transfer to short oligosaccharide substrates (with NMR analysis of a relevant product), whereas I have also demonstrated transfer to N-glycans. It will be of interest to examine whether, in combination with a suitable β1,3-galactosyltransferase, plant α1,4-fucosyltransferases can be verified to have a role in the synthesis of Le^a-modified N-glycans *in vivo* and whether the apparent lack of Le^a in Arabidopsis and other brassicas is indeed due to inactivity of this enzyme.

[2]Data on both the tomato and sugar beet α1,4-fucosyltransferases were independently presented at the GLYCO XVI International Symposium on Glycoconjugates, The Hague, 19th–24th August 2001. See: *Glycoconjugate J.* **18**, 64 (Abstract 10.7) and 119 (Abstract 22.38).

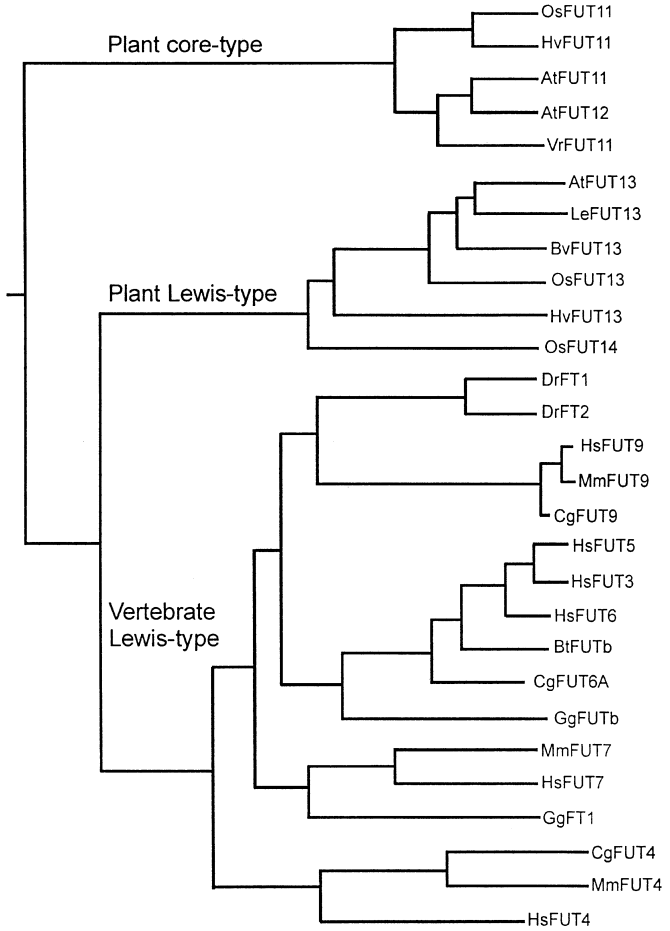

Figure 6. Phylogenetic tree analysis of plant and vertebrate α1,3/4-fucosyltransferases. The following sequences were analysed using the Clustalw and Phylip programs (www.infobiogen.fr/services/menuserv.html): AtFUT11-13 (*Arabidopsis thaliana* FucTA, FucTB and FucTC), BtFUTb (*Bos taurus*, bovine futb), CgFUT4, CgFUT6A and CgFUT9 (*Cricetulus griseus*, hamster Lewis-type fucosyltransferases), DrFT1-2 (*Danio rerio*, zebrafish Lewis-type FT1 and FT2), GgFT1 and GgFUTb (*Gallus gallus*, chicken CFT1 and futb-like sequence), HsFUT4-9 (*Homo sapiens* Fuc-TIII-IX), HvFUT11 and HvFUT13 (*Hordeum vulgare*, barley FucTA and FucTC), LeFUT13 (tomato FucTC), MmFUT4 and MmFUT7 (*Mus musculus* Fuc-TIV and Fuc-TVII), OsFUT11, OsFUT13 and OsFUT14 (*Oryza sativa*, rice FucTA, FucTC and FucTD). Invertebrate fucosyltransferases were not included in the analysis, since only five sequences, highly divergent from other members of the family, have been reported.

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Note added in proof:

Recent antibody-binding and enzyme assay data indicate that the Arabidopsis FucTC (AtFUT13; AtFT4) is indeed active at a low level. See: Léonard R, Costa G, Darrambide E, Lhernould S, Fleurat-Lessard P, Carlué M, Gomord V, Faye L, Maftah A. The presence of Lewis a epitopes in *Arabidopsis thaliana* glycoconjugates depends on an active α4-fucosyltransferase gene. *Glycobiology* in press (2002).

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