Glycoconjugate Journal (1997) **14:** 249–258

Cloning of a rat α1,3-fucosyltransferase gene: a member of the fucosyltransferase IV family

Elizabeth M. Sajdel-Sulkowska[1,2,3,5], Frances I. Smith[1,3,4,5], Gherman Wiederschain[1,3,4,5] and Robert H. McCluer[1,3,4,5]*

[1] *Department of Biomedical Sciences, E.K. Shriver Center, Waltham, MA 02254, USA*
[2] *Department of Psychiatry, Massachusetts General Hospital, Boston, MA 02114, USA*
[3] *Department of Psychiatry, Harvard Medical School, Boston, MA 02115, USA*
[4] *Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA*
[5] *Department of Neurology, Harvard Medical School, Boston, MA 02115, USA*

We report the cloning of a rat α1,3-fucosyltransferase gene (*rFuc-T*), isolated from a rat genomic library by a PCR-cross-hybridization based cloning approach using primers derived from the conserved region of human α1,3-Fuc-T sequences. Comparison of the rFuc-T predicted amino acid sequence with those of previously cloned human and murine fucosyltransferases showed highest degree of homology to murine Fuc-TIV (87% identity) and human Fuc-TIV (78% identity), with lower homology (41–49% identity) to Fuc-TIII, V, VI, and VII. COS-1 cells transfected with the *rFuc-T* gene expressed a fucosyltransferase activity with type 2 (Galβ1 → 4GlcNAc)-containing oligosaccharides and the glycolipid acceptor neolactotetraosylceramide but only low activity with sialylated substrates; the SSEA-1/Le^x antigen was detected in transfected cells by immunocytochemistry. Based on these results, we surmise that rFuc-T is a member of the fucosyltransferase IV family. Northern blot analysis with a rFuc-T specific probe indicated a major transcript of 4.2 kb most abundantly expressed in rat spleen; minor transcripts of different sizes were detected in several tissues, including rat brain.

***Keywords*: α1,3-fucosyltransferase, cloning, glycolipid, transfection**

***Abbreviations*: Fuc-T, fucosyltransferase; SSEA-1, stage specific embryonic antigen or Lewis^x (Le^x); ELFT, ELAM-1 ligand fucosyltransferase; ELAM, endothelial-leukocyte adhesion molecule; nLc$_4$Cer, neolactotetraosylceramide (Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glcβ1 → 1Cer); NEM, *N*-ethylmaleimide**

Introduction

Fucosylated glycoconjugates including glycoproteins, glycolipids and proteoglycans expressed on the cell surface, have been suggested to play a key role in cell–cell interactions in many tissues. In the immune system the glycosylated molecules exhibiting an SSEA-1 (Le^x) or sialyl-SSEA-1 epitope, consisting of α-(1,3)-fucosyl-neo-N-acetyllactosamine, expressed on leukocytes are recognized by the selectin adhesion molecules on endothelial cells and platelets [1]. Recognition of the SSEA-1 epitope by B-cells has been shown to lead to the production of IL-10 and PGE2 that down-regulate Th-1 cells and support expansion of Th-2 populations, a process that may be a general phenomenon in schistosomiasis, other parasitic diseases, human immunodeficiency virus infections and a number of metastatic carcinomas [2]. During embryonic development SSEA-1 expression has been implicated in the compaction of the mouse embryo [3], and the aberrant expression of this structure has been observed in association with malignant transformation [4]. Immunohistological data [5, 6] suggests that SSEA-1 may also be involved in the neurodevelopmental process. The observations by Chou *et al.* [7] suggest that in the developing rat cerebellum, the peak of SSEA-1 glycolipid expression correlates with granule cell migration. Because the final step in the biosynthesis of SSEA-1 involves the addition of fucose, fucosyltransferases are likely to play an important regulatory role in SSEA-1 expression and function.

While the possibility of manipulating the immune response and the metastatic process via regulation of the fucosyltransferases triggered the cloning and isolation of several human and a murine genes for Fuc-T, the temporal expression of SSEA-1 glycolipids in developing brain and the development regulation of these enzymes [8] prompted

The nucleotide sequence reported in this paper has been submitted to the Gen bank TM/EMBL Data Bank with accession number U58860.
* To whom correspondence should be addressed.

0282–0080

our efforts to clone the rat gene(s). Several approaches have been used in isolating the human genes, including expression cloning (*Fuc-TIII*, *IV* and *VII*) [9–12] and cross hybridization (*Fuc-TV*, *VI* and *VII*) [13–16]. Sequence analysis of these genes revealed extensive homology between *Fuc-TIII*, *V* and *VI*, all of which are localized on chromosome 19. Human *Fuc-TIV* has less sequence homology to the other human genes, is localized on chromosome 11 [40], and is considered to belong to a separate *Fuc-T* family that includes the mouse *Fuc-TIV* (*mFuc-TIV*) gene [17, 18]. Human *Fuc-TVII* shows little homology to other human *Fuc-T* families and is the only *Fuc-T* that is mapped to chromosome 9 [16]; an alternatively spliced mouse *Fuc-TVII* has been reported recently [19].

Previous enzymatic studies [20] suggested the presence of a Fuc-TIV-like activity in human brain. Based on these studies we used a PCR-cross hybridization based cloning approach with the primers derived from the catalytic region of human α1,3-Fuc-TIV sequence [21, 22]. We report here on the cloning and characterization of a rat *α1,3-Fuc-T* gene that is expressed in rat brain. The rat gene shows a high degree of amino acid identity, 87% and 78%, to the murine and human Fuc-TIV, respectively, and type 2 acceptor specificity, suggesting that it is a member of the Fuc-TIV family. However, the rFuc-T enzyme exhibits different substrate specificities and tissue distribution from those reported for mFuc-TIV. These results suggest that rFuc-T may play a different biological role from previously characterized fucosyltransferases.

Materials and methods

Nomenclature of fucosyltransferases

The nomenclature of fucosyltransferase activities and genes have been discussed [23] and thus *Fuc-TIII*, *Fuc-TIV*, *V*, *VI* and *VII* all represent cloned human α1,3-fucosyltransferase genes. *Fuc-TI* and *II* refer to fucosyltransferases with α1,3 activity expressed in CHO cells. Human *Fuc-TIV* has been cloned more than once, and has also been referred to as *ELFT* and *ELFT-L* [10, 24]. Human *Fuc-TIV* will subsequently be used synonymously with *ELFT-L* in this report. Recently, a murine gene homologous to human *Fuc-TIV* has been cloned and named *mFuc-TIV* [17]. The gene described in this paper represents the first rat α1,3-fucosyltransferase gene that has been cloned; it will be referred to as rat *Fuc-T* or *rFuc-T*.

PCR analysis of rat genomic DNA

Using GeneBank sequence data for human *Fuc-T*s, we designed oligonucleotide primers directed towards a conserved region of human *Fuc-TIV* (accession no. M65030): a forward primer (bp nos 632–657) 5′CTCGGACGTCTTTGTGCCTTATGGCT-3′ and a reverse primer (bp nos 1256–1231) 5′CTACCGCTCGAACCAGCTGGCCAAGT-3′. When human genomic DNA was used as a template, these primers amplified a single PCR product in buffer F containing 5% DMSO (PCR Optimizer, InVitrogen) with an annealing temperature of 52°. When rat genomic DNA was used as template under similar conditions (buffer F without DMSO), three bands were observed. However, a product of the predicted length was most predominant, and was cloned directly into a TA cloning vector (In Vitrogen). One such clone (R-41) was sequenced and found to contain 624 bp that showed 85% identity with the human *Fuc-TIV* gene.

Screening of a rat genomic library

A rat genomic library in Lambda Fix II (Stratagene) was screened by hybridization with the 624 bp rat clone R-41. Three strongly hybridizing plaques and 15 weakly hybridizing ones were observed. Two strongly hybridizing phages (EKS-1 and EKS-2) were plaque-purified and the insert DNA was analysed by restriction enzyme digestion followed by Southern blot analysis with the R41 probe. A *Sac*I-digested, approximately 3500 bp fragment (R-59) was identified and isolated from EKS-1, and a *Xho*I-digested, approximately 4500 fragment (R-85) was isolated from EKS-2. Both fragments were subcloned into the vector pIBI31 (IBI) and partially sequenced.

DNA sequencing

The initial sequencing of clones R-59 and R-85 revealed an unusually high GC content and thus an IsoTherm DNA Sequencing Kit (Epicentre Technologies) based on isothermal sequencing at 65° was used according to the manufacturer's instructions. Sequence analysis and alignment were performed with the Wisconsin Sequence Analysis Package.

Northern blot hybridization

A Multiple Tissue Northern (MTN) Blot, containing 2 μg of polyadenylated mRNA per lane, was purchased from Clontech. The blot was prehybridized in 5 Prime → 3 Prime, Inc. Northern hybridization buffer containing 50% formamide at 42° for several hours and then hybridized overnight at 42° in the same solution containing additional 10% Dextran sulfate and the *Bam*HI-*Hinc*II ^{32}P-labelled fragment of the *rFuc-T* gene, that had been radiolabelled with ^{32}P by random priming (PrimeItII, Stratagene). This sequence begins at position −192 in the untranslated 5′ region of *rFuc-T* and ends at +197 position in the coding region. The blot was washed in a low stringency buffer (2 × SSC, 0.1% SDS) at 25 °C for 40 min, followed by a moderate stringency wash (2 × SSC, 0.1% SDS) at 50 °C for 40 min and a high stringency wash (0.1 × SSC, 0.1% SDS) at 50 °C for 15 min. Following hybridization with rFuc-T probe, the blot was stripped and hybridized with a digoxygenin-labelled hamster actin antisense RNA probe [25]. The blot was prehybridized and hybridized under the conditions described above. Following the hybridization the blot was washed

in a low stringency buffer at 25 °C, followed by a high stringency wash at 68 °C. The actin signal was measured using a chemiluminescent detection method (Boehringer and Mannheim Biochemicals) according to the manufacturer's directions.

Preparation of expression constructs

Sequencing of R-59 revealed that the rFuc-TIV coding region was flanked by a 5′-*Bam*HI site and a 3′-*Eco*RI site. Thus, an insert of approximately 1500 bp was isolated from a *Bam*HI/*Eco*RI digestion of R-59, and subcloned into the expression vector pcDNA3 (In Vitrogen). This construct was named pcDNA3-rFuc-T.

Transfection of COS-1 cells

COS-1 cells were propagated in DMEM medium containing 10% fetal calf serum, and plated at about 50% confluence the day before transfection. Cells were transfected using Lipofectamine (GIBCO-BRL), according to manufacturer's instructions, and harvested 48 h later.

Immunocytochemistry of transfected cells

Cells were plated onto 10 mm coverslips coated with polylysine 24 h before transfection. At 48 h following transfection, the cover slips were washed in PBS and the cells fixed with 4% paraformaldehyde for 15 min. Cells first were reacted with the anti-SSEA-1 monoclonal antibody 43B-11 [26], followed by staining with fluorescein-conjugated goat anti-mouse, F(ab′)2 fragment IgM (Boehringer Mannheim Biochemicals).

Fucosyltransferase assay

Transfected COS-1 cells were homogenized in 20 mM MOPS/NaOH buffer (pH 7.4) with Triton X-100 (0.5%). The homogenate was extracted for 1 h with the same buffer on ice, centrifuged at $800 \times \mathbf{g}$, for 15 min at 2 °C, and the supernatant aliquoted (100 µl each), and stored at −80 °C prior to enzyme assays. The reaction mixture (50 µl) contained 2.5 µmol of 3-(*N*-morpholino)propansulfonic acid (MOPS)/NaOH buffer (pH 7.4) 0.25 µmol $MnCl_2$, 5 µmol of NaCl, 0.5 µmol of ATP, 40 µg *N*-acetyllactosamine (LacNAc) (104 nmol), 0.5 nmol of GDP-L-[^{14}C]-fucose (283 mCi mmol^{-1}) (DuPont-New England Nuclear) which was diluted to a specific activity of ∼ 54 000 cpm nmol^{-1} with unlabelled GDP-fucose (Sigma), and 20 µl of cell supernatant (∼ 40–60 µg protein) as enzyme source. After incubation at 37 °C for 1–2 h, the reaction was terminated by the addition of 0.5 ml of cold water and the mixture was applied to a 1 ml column of AG 1-X8 anion resin (BioRad). The column was washed with 1 ml of water, and the combined aqueous effluents containing the fucosylated products were collected in scintillation vials and counted after addition of 4.5 ml of scintillation cocktail (Ready Safe, Beckman). When sialylated oligosaccharides were used as acceptors the column elution included 2 ml of 0.15 M NaCl; under this condition GDP-fucose remained bound to the column [27]. Sensitivity towards the inhibitor, *N*-ethylmaleimide (NEM) was determined with LacNAc by pre-incubation of the cell homogenates containing 10 mM NEM for 1 h at 0 °C. When nLc_4Cer was used as acceptor (8–10 µg unless otherwise noted), the reaction was sonicated for 1 min to allow the formation of glycolipid/detergent micelles. The enzymatic reaction was stopped by the addition of 0.5 ml of TUP-0.1 M KCl mixture [28] ($CHCl_3$:MeOH:H_2O; 60:960:940, 7.35 g KCl) and the mixture was applied to a pre-packed reverse phase C_{18} cartridge (Bond Elut C_{18}. Analytichem International) equilibrated with TUP-KCl mixture. The cartridge was washed with 1 ml TUP mixture and with H_2O (3 ml) and eluted with 2 ml of $CHCl_3$:MeOH (2:1) and 2 ml methanol [29]. The eluate was dried under a stream of nitrogen and the incorporation of fucose was determined by scintillation counting with Beckman cocktail. Values were corrected for fucose incorporation into endogenous acceptors.

Protein was determined in diluted cell extracts by a microassay (96-well microtitre plate) procedure with BCA Protein Assay Reagent (Pierce, Rockford, II, USA); absorbance at 562 nm was determined via a microtitre plate reader (BT 2000) Microkinetics Reader Spectrophotometer, (Fisher Biotech, Pittsburg, PA, USA). The standard curve consisted of a series of known concentrations of bovine serum albumin. The rate of the enzyme reaction was shown to be linear with time and with protein concentration within the parameters defined above.

Fucosyltransferase acceptors *N*-acetyllactosamine, and lacto-*N*-tetraose, were obtained from Accurate Chemical Co. Lacto-*N*-biose, and Lacto-*N*-neotetraose, were purchased from V-LABS. Lactose, 2′-fucosyllactose; GDP-fucose, *N*-ethylmaleimide and taurodeoxycholate (sodium salt) were obtained from Sigma. 3′-Sialyl-*N*-acetyllactosamine, and 6′-Sialyl-*N*-acetyllactosamine, were purchased from Oxford GlycoSystems. Lacto-*N*-fucopentaose I, was obtained from BioCarb. 6′-fucosyllactose, and 6-Fucosyllactose, were synthesized by Dr C.D. Warren (E.K. Shriver Center). Lipid substrates were isolated from bovine red blood cells by Dr O. Koul (E.K. Shriver Center).

Results

Cloning of the *rFuc-T* gene

Oligonucleotide primers directed towards the carboxy terminal end of the human *α1,3-Fuc-TIV* gene were used to amplify and clone a crossreactive sequence from rat genomic DNA in a TA cloning vector. This clone was then used to probe a rat genomic lambda phage library, and inserts from two strongly hybridizing clones were subcloned (R-59 and R-85) and sequenced. Initially, both inserts were sequenced in parallel, but when both were found to be identical in the first 500 bp, we proceeded only with clone R-59.

A

```
400 AAATACGGAAGGAAGCTGACTCTGCTCTAAGTTCTGCAGTGGGATCGCACCCCTACCCGCCACCCCTTCGAGGTGCAGGTCTCCAGTGCAAGACTCTCT -301
300 CTGCAAGGCGAGGACTCAGGTCACCTAGTCGCGGGACCAGCGAACGCATCAAGAGGCCACGACCCCCGGTGAGGCGCCATCCGGGCGCAGTTTCGGAGCC -201
200 CAGGATCCTTGGCTGGGCAGGGGAACCAGCGCAGGCGCAGCAGGAAACGCCCCAGGCCGGGCCCGCAGGGCTTGGAGAGCAGTGTCTGGGAAGGCCAGCG -101
100 TGGCGCCGCGCCAGGGCCACCGGCCCGGTTCACCTCGTCCTGGACTTGGGAGGGGCGAGGCACCGCCCGTGGGCCCGCTGATGCCCCGCCCGCCCCTCT -1

  1 ATGGCCCCGGCCGGGCGAAAGCTACAGCATGAGAGCCGGTGCCGCCCCTCACGGCCCGTGGACGCGTGGCGAGCCCGCGGCGACCACTCGTGGGCGCTGCA 100
  1 M A P A G R K L Q H E S R C R P S R P V D A W R A A A T T R G R C M 34

101 TGGGGACCCCAGGGGCCCGGCGAACGGCACGGCGCGGAGGCTGGGGATTACCCCGGACCTCCTCTGGGCTGGCAGCAGCGGGACTACTATGCACCGCGTT 200
 35  G T P G A R R T A R R G G W G L P R T S S G L A A A G L L C T A L  67

201 GACTGCCTGCCTCTGTTGGGGACAGCTGCCGCCGCTGCCCTGGGCGTCCCCAGCCCCGCAACGCCCGGTGAGTGTGCTGCTCTGGTGGGAACCCTTTGGG 300
 68  T A C L C W G Q L P P L P W A S P A P Q R P V S V L L W W E P F G 100

301 GGTCGCGGCGGCCACTCCAAGCCTCCCCCCGATTGCAGCCTGCGCTTCAACATCAGCGGCTGCCGCTTGCTCACCGACCGCGCGGCCTACGGGGAGGCAC 400

101 G R G G H S K P P P D C S L R F N I S G C R L L T D R A A Y G E A Q 134

401 AAGCAGTGCTGTTCCACCACCGCGACCTGGTGAAGGGACCCCCCGACTGGCCCCCGCCCTGGGGCGCCCAAGAACGCACCGATGAGGCTCTGGAGCTACG 500
135  A V L F H H R D L V K G P P D W P P P W G A Q E R T D E A L E L R 167

501 CGTGTTCGACGACCAGGAGGGAGCGGTGATGCTAGCTAGAGAAGCCCTGGAGACCACAGGCTCTCGTCCCCCGGGTCAGCGGTGGGTGTGGATGAACTTC 600
168  V F D D Q E G A V M L A R E A L E T T G S R P P G Q R W V W M N F  200

601 GAATCACCCTCCCACACTCCAGGGCTGCGGGGCTTGGCTAAGGACCTCTTCAATTGGACACTATCCTACCGGACCGATTCGGATATCTTCGTGCCCTATG 700
201 E S P S H T P G L R G L A K D L F N W T L S Y R T D S D I F V P Y G 234

701 GCTTCCTCTATCCCAGGAGCCATCCAGCCGAACAGCCTTCAGGTCTGGGCCCCCCGCTGGCCCGCAAGCGGGGACTGGTGGCCTGGGTTGTGAGCCACTG 800
235  F L Y P R S H P A E Q P S G L G P P L A R K R G L V A W V V S H W 267

801 GAACGAGCGCCAGGCACGGGTCCGTTACTACCATCAGCTGAGGCGGCACGTGTCTGTGGACGTGTTTGGCCGGGCGGGACCCGGACAACCAGTGCCAGCC 900
268  N E R Q A R V R Y Y H Q L R R H V S V D V F G R A G P G Q P V P A  300

901 GTCGGGTTGCTGCACACGGTGGCCCGCTACAAGTTTTACCTGGCGTTTGAGAACTCACAGCACGTGGATTACAACACTGAGAAGCTGTGGCGGAATGCCT 1000
301 V G L L H T V A R Y K F Y L A F E N S Q H V D Y N T E K L W R N A F 334

001 TCCTGGCTGGGGCCGTGCCAGTGTTACTTGGTCCCGATCGTGCCAACTATGAGGGCTTCGTGCCTCGTGGTTCCTTCATCCATGTGGATGATTTCCCTAG 1100
335  L A G A V P V L L G P D R A N Y E G F V P R G S F I H V D D F P S  367

1101 TGCTGCCTCCCTGGCTGCCTACCTGCTCTTTCTCGACCGAAACGTGGCTGTCTATCGCCGCTACTTCCACTGGCGTCGGAGCTATGCGGTGCACATCACT 1200
 368 A A S L A A Y L L F L D R N V A V Y R R Y F H W R R S Y A V H I T  400

1201 TCTTTCTGGGATGAGCCATGGTGCCAGACATGCAGGGCCGTCCAGACCTCTGGGGACCAGCCCAAGAGTATCCACAACTTGGCAGACTGGTTCCAGCGAT 1300
 401 S F W D E P W C Q T C R A V Q T S G D Q P K S I H N L A D W F Q R * 433

1301 GAGGCTATTCTGCTGTGAATTGATGACATCTCCTTGACTGTTTTTCTAAATACCCAATTTTGCTTTATGGGAA                          1373
```

B

```
     1                                                  50                                                 100
 V   ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
III  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
 VI  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
 IV  MRRLWGAARK PSGAGWEKEW AEAPQEAPGA WSGRLGPGRS GRKGRAVPGW ASWPAHLALA ARPARHLGGA GQGPRPLHSG TAPFHSR*SG E*QRRLEPQ*
rFuc-T ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ......MAPA GRK......L
mFuc-T ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ......**** RQE......*
 VII  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........  ..........
     101                                                150                                                200
 V   ..........  ..........  ..........  ......MDPL GPAKPQ*LWR *CLAG*LFQL *VAVCFFSY* RVSRDDATGS PRPGLMAVEP VTGAPNGSRC
III  ..........  ..........  ..........  ......MDPL G*AKPQ*PWR *CLAA*LFQL *VAVCFFSY* RVSRDDATGS PR........ ...APSGSSR
 VI  ..........  ..........  ..........  ......MDPL GPAKPQ*SWR CCLTT*LFQL *MAVCFFSY* RVS*DD*TV. .......... ...YPNGSRF
 IV  *******S*T *A*****E*A LPV*A**A*W GSPTAAAGG* RGW*R*R*** W*VCV***** *T****ITVA ********** *.......... ..........
rFuc-T QHESRCRPSR PVDAWRAAAT TRGRCMGTP. ......GARR TARRGGWGLP RTSSGLAAAG LLCTALTACL CWGQLPLPLW A.......... ..........
mFuc-T ********** T********VA ****H*E**. ......*Y** RT*C****** *SV*S***V* *******TFI ********** *.......... ..........
 VII  ..........  ..........  ..........  ....MNN*GH GPT*RLR**.  ....GV**GVA **AALWLLW* ....*GSA*R G.......... ..........
     201                                                250                                                300
 V   QDSMATPAHP TLLI***TW* *NTPVALPRC SEMVP...GA AD*NITA*SS V*PQ*D**IV **W*IMYN*S ANL**..... .......... ..........
III  QD..TTPTRP TLLI***TW* *HIPVALSRC SEMVP...GT AD*HITA**K V*PQ*DT*IV **W*IMSN*K SRL**..... .......... ..........
 VI  PDSTGTPAHS IPLI***TW* *NKPIALPRC SEMVP...GT AD*NITA**K V*PQ*D**IV ***EVMYN*S AQL*R..... .......... ..........
 IV  ....**T*** ********** ****DSAPR* ****R***** ********** S********* ********** *******I*A H*A*EVD*** L*YE*A*A..
rFuc-T ....SPAPQR PVSVLLWWEP FGGRGGHSKP PPDCSLRFNI SGCRLLTDRA AYGEAQAVLF HHRDLVKGPP DWPPPWGAQE RTDEALELRV FDDQEGAVML
mFuc-T ....****** L*G******* *R****YP*S ********** ********** ********** *******ELH ********R* ***K**V*** ********T*
 VII  ....T***** TITI*V*HW* *T..DQPPEL *S*TCT*YG* AR*H*SAN*S LLAS*D**V* ***E*QTRRS HL*..... .......... ..........
     301                                                350                                                400
 V   ........PT **Q****I*F SM****NCRH *EA*D.GY** L*M***S*** **T***W*E* W*...G**AH PPLN*SA*TE ****A**N*K PDS******Q
III  ........SP **Q****I*F *L*P*PNCQH *EA*D.RY** L*M***S*** **T***W*E* W*...G**AH PPLN*SA*TE ****A**N*K PDS******Q
 VI  ........SP *RQ****I*F SM*****CWQ *KAMD.GY** L*M***S*** **T***W*E* W*...G**AH PPLN*SA*TE ****A**N*G PNS******Q
 IV  *A***A*SSP ********** *******g** **S**SN*** ******A*** V*****Y*** ****GDP*** *A***SR*Q* *********D **********
rFuc-T AREALETTGS RPPGQRWVWM NFESPSHTPG LRGLAKDLFN WTLSYRTDSD IFVPYGFLYP RSHPAEQPSG LGPPLARKRG LVAWVVSHWN ERQARVRYYH
mFuc-T TGK****V** ********** ********** ********** ********** V********S **D*T***** ***Q****** *******N** *H********
 VII  .......LAQ **R**P***A SM******H* *SH*.RGI** *V****R*** ******R*E* HW..... AS***PA*SR VA*****NFQ ***L*A*L*R
     401                                                450                                                500
 V   S*QA*LK*** Y**SH..K*L *KGTMME*LS ********** *L*P**I*** *****LE*W* ***V***S*S ***R*L*PDA *******Q*P KD**R**QE*
III  S*QA*LK*** Y**SH..K*L *KGTMME*LS ********** *L*P**I*** *****LE*W* ***V***S*S ***R*L*PDA *******Q*P KD**R**QE*
 VI  S*QA*LK*** Y**SH..K*L *QGTMME*LS ********** *L*P**I*** *****LE*W* ***V***S*S ***R*L*PDA *******Q*P KD**R**QE*
 IV  **SQ**T*** ***G****** *EI******* ********** ***L**I*** *****L**** ***V****** ***R*****A ********** S***S*****
rFuc-T QLRRHVSVDV FGRAGPGQPV PAVGLLHTVA RYKFYLAFEN SQHVDYNTEK LWRNAFLAGA VPVLLGPDRA NYEGFVPRGS FIHVDDFPSA ASLAAYLLFL
mFuc-T **S******* ***T***R** **I******* ********** *R****I*** ********** ***V****** ***R*****A ********N* **********
 VII  **AP*LR*** ****N.*R*L C*SC*VP*** Q*R***S*** ***R**I*** F****LV**T ***V***P** T**A***ADA *V*****G** RE***F*..T
     501                                                550
 V   *KDH*R*LS* *R**ETLRPR SF*.*ALAF* KA*WKL*QES .RYQTVRSI* A**T.
III  *KDH*R*LS* *R**ETLRPR SF*.*ALDF* KA*WKL*QES .RYQTVRSI* A**T.
 VI  *KDH*R*LS* *R**ETLRPR SF*.*ALAF* KA*WKL*EES .RYQT.RGI* A**T.
 IV  ***P****** ********** ********** RV*Q***RA* *R****R*** S**E*
rFuc-T DRNVAVYRRY FHWRRSYAVH ITSFWDEPWC QTCRAVQTSG DQPKSIHNLA DWFQR
mFuc-T ********** *R****F*** *******Q** R**Q****** ********** *****
 VII  GM*ESR*Q*F *A**D*VR*R LFTD*R*RF* AI*DRYPHLP .RSQVYED*E G**QA
```

Nucleotide and amino acid sequence of the *rFuc-T* is presented in Figure 1A. The coding region of *rFuc-T* is 1302 bp long and encodes a peptide 433 amino acids long. A comparison of *rFuc-T*, recently cloned *mFuc-T*, and human *Fuc-TIV* indicates that the rat sequence shares the high nucleotide homology with the murine and human *Fuc-TIV* (89% and 78% identity, respectively). Comparison to the other human *Fuc-T* genes shows a considerably lower homology (60–65% identity). These results suggest that the newly isolated sequence may represent a member of the *Fuc-TIV* family, which we have termed *rFuc-T*. The predicted amino acid sequence encoded by the *rFuc-T* gene shows 87 and 78% identity with *mFuc-TIV* and human *Fuc-TIV*, respectively, 49% identity with human *Fuc-TVII*, and 41–43% identity with *Fuc-TIII*, *V* and *VI* (Figure 1B). Like mFuc-T and human *Fuc-TIV*, *rFuc-T* has two in-frame potential initiation codons, but the longer form of both rat and murine genes contains 99 fewer amino acids in the cytoplasmic tail as compared to the human Fuc-TIV, and threonine in place of serine in the GDP-fucose binding domain.

The *rFuc-T* gene encodes a fucosyltransferase activity and directs SSEA-1 expression in transfected COS-1 cells

To determine if the open reading frame of the *rFuc-T* encodes an enzyme activity involved in the synthesis of SSEA-1 COS-1 cells were transfected with the eukaryotic expression vector containing rFuc-T, pcDNA3-rFuc-T. COS-1 cells were selected for these experiments because they do not express α1,3-Fuc-T activity or SSEA-1 antigen but maintain both the donor GDP-fucose and various precursors [9]. As shown in Figure 2, the transfected population expressed SSEA-1, as assayed by immunocytochemistry. Transfection of COS-1 cells with a plasmid expressing epitope-tagged rFuc-T revealed that the same percentage of cells that stained for the presence of the epitope also stained for the surface expression of SSEA-1 (data not shown). Enzyme activity was measured in the COS-1 cells before and after transfection with the *rFuc-T* gene. Fucosyltransferase activities with LacNAc and nLc_4Cer as potential acceptors are presented in Figure 3. The results obtained for both acceptors demonstrate that transfected cells show a dramatic increase in enzyme activity over untransfected cells. The endogenous activities for transfected cells without LacNAc and nLc_4Cer were 2% and 10%, respectively. It is, however, important to note that the fucosyltransferase activity with the glycolipid acceptor nLc_4Cer was revealed only when both 0.2% Triton X-100 and 1% taurodeoxycholate were added to the reaction mixture. The other detergents, Triton X-100 (0.2%) or combination of Triton X-100 (0.2%) and taurocholate (1%) were ineffective.

The acceptor specificity of COS-1 cell homogenates for a panel of oligosaccharides is shown in Table 1. Under the conditions used for screening, the type 1 (Galβ1 → 3GlcNAc) containing oligosaccharide LNT and 3′-SLN were poor acceptor substrates for the recombinant fucosyltransferase. The enzyme also was unable to utilize Lac, 2′-FL, 6′-FL, 6-FL, LNB, LNFI and 6′-SLN as acceptors. The type 2 (Galβ1 → 4GlcNAc) containing tetrasaccharide, LNnT was a better acceptor than the type 2 disaccharide, LacNAc. Results shown in Table 1 are representative of several experiments. In order to further investigate the catalytic properties of the rFuc-T, reactions were carried out in the presence of NEM. The enzyme activity was resistant to inhibition by NEM (approximately 92% of control).

Tissue-specific expression of *rFuc-T*

To test for the expression of the newly cloned rat gene, we hybridized the *rFuc-T* probe to RNA from a panel of adult rat tissues. Northern blot analysis (Fig. 4) indicated that multiple transcripts are expressed in most of the rat tissues examined. The major transcript of 4.2 kb is most abundantly expressed in spleen, and at least two transcripts are expressed in brain and testes.

Discussion

Based on sequence similarity and chromosomal localization, the cloned human α1,3-fucosyltransferase genes are divided into three groups: group 1 includes the closely related *Fuc-TIII*, *V*, and *VI*, group 2 includes human *Fuc-TIV* and *mFuc-TIV*, and group 3 includes *Fuc-TVII*. An alternative classification is based on comparing the specificities of fucosyltransferases towards various acceptors in different tissue and body fluids, and groups the enzymes into at least three types: Lewis, myeloid and plasma type. However, as many as five groups have been suggested using this system [20]. These two classification systems have been reconciled in some cases and thus *Fuc-TIII*, *IV*, and *VI* correspond to the Lewis, myeloid and plasma type,

Figure 1. DNA and predicted amino acid sequence of *rFuc-T*. (Panel A) The complete DNA sequence of *rFuc-T* is shown with the derived translation using the single-letter code given below the nucleotide sequence. Numbers on top and bottom count nucleotide and amino acid residues, respectively. SP1 binding sites are shown in italics and underlined. (Panel B) The amino acid sequence alignment of *rFuc-T*, *mFuc-T* and human *Fuc-TIV* with other human *α1,3 Fuc-T*s was generated using the PileUp program of the University of Wisconsin Genetics Computer Group. The putative transmembrane domains of individual *Fuc-T*s are underlined. The suggested NEM-binding residue (cysteine) and its substitutions to threonine or serine are shown in bold letters, and the potential glycosylation sites are marked in italics and underlined. * – indicates amino acids identical with corresponding *rFuc-T* residues; ⋯ – indicates amino acids that have no counterpart in aligned genes; I–NEM inhibition and putative GDP-fucose binding residue.

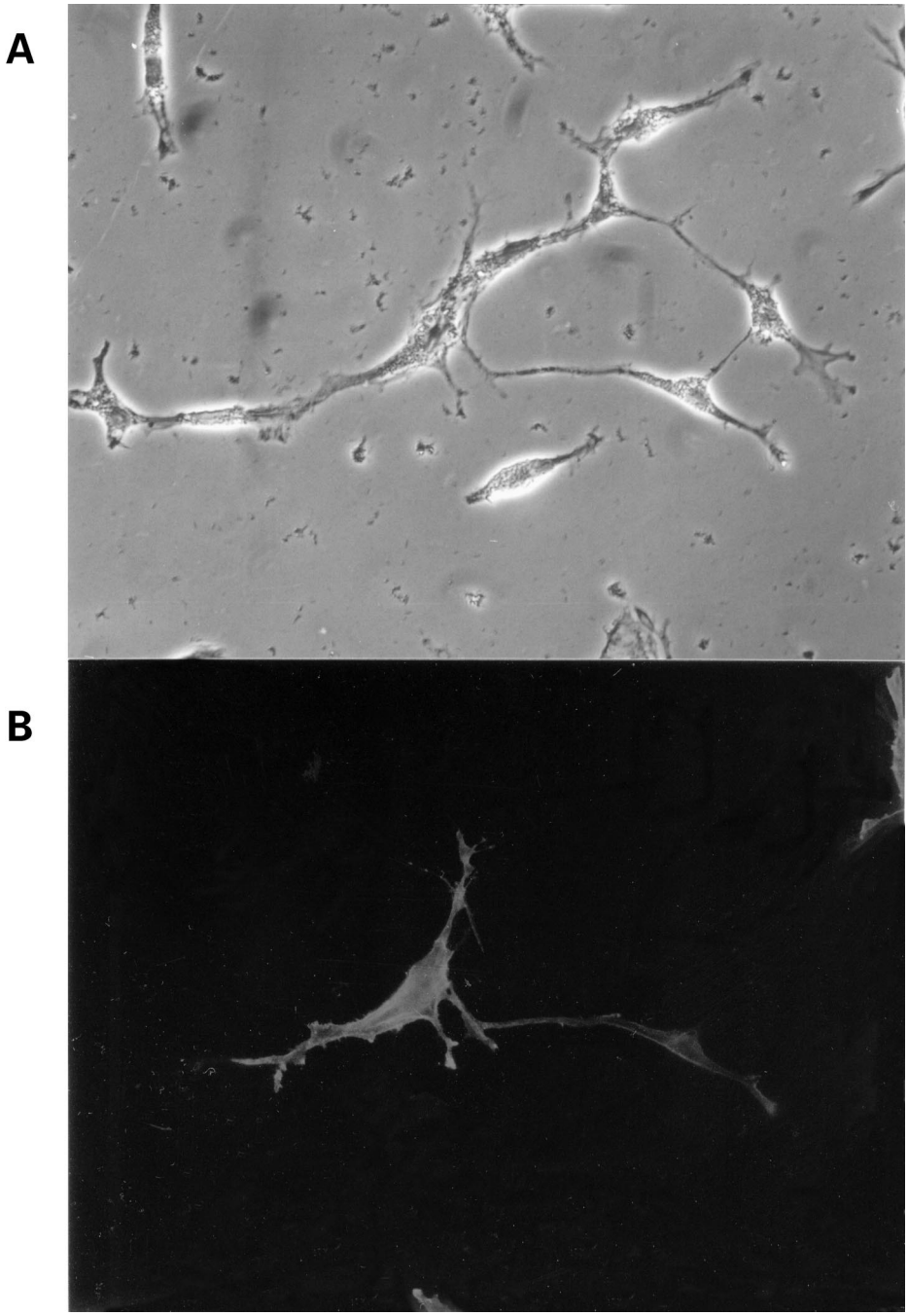

Figure 2. Immunocytochemistry of COS-1 Cells Transfected with the *rFuc-T* gene. (Panel A) shows cells viewed under phase. (Panel B) shows cells viewed using fluorescent light. Cells were transiently transfected with the *rFuc-T* gene and reacted with anti-SSEA-1 antibodies. These results indicate that the rat gene encodes a fucosyltransferase capable of producing the epitope SSEA-1.

respectively. Enzyme activity studies show that myeloid type activity is the exclusive type of fucosyltransferase expressed in the embryo, and that with development, individual tissues acquire tissue specific patterns of fucosyltransferases. The only adult cells or tissues that have been demonstrated to retain the unaltered embryonic type of enzyme are leukocytes and brain. However, embryonic and adult human brain express enzymes that can be distinguished by activity with divalent ions. The enzyme from embryonic brain utilizes manganese more efficiently than cobalt, the adult brain is equally active with both ions [20, 39].

We have isolated a full length rat gene, *rFuc-T*, from a rat genomic library. We sequenced 1773 bp, which corresponds

to an open-reading frame of 1302 bp, flanked by 400 bp upstream and 71 bp downstream (Figure 1A). The site(s) of transcription initiation have not yet been mapped, and so it is not clear whether we have sequenced the promoter region. However, the 5′ region of this gene is extremely GC-rich, and potential binding sites for the transcriptional activator SP1 (CCCGCC) are found at positions −12, −16, and −27 (numbered according to the first in-frame methionine as +1). A cluster of SP1 binding sites is frequently found in the 5′ region of housekeeping genes [30]. No other *α1,3-Fuc-T* gene promoters have yet been characterized. It should be noted that the term housekeeping generally is used to describe enzymes that are widely distributed and perform essential metabolic functions, but does not imply 'unregulated' [30].

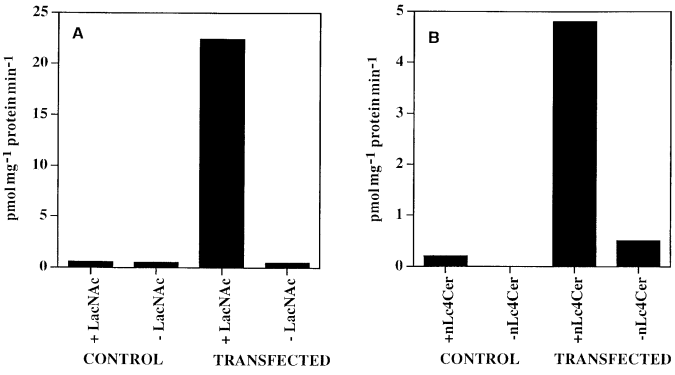

Figure 3. Fucosyltransferase Activity in COS-1 Cells. (Panel A) LacNAc acceptor. (Panel B) nLc_4Cer acceptor. Incubation mixture in A contained 0.2% Triton X-100; incubation mixture in (B) contained 0.2% Triton X-100 plus 1% taurodeoxycholate. Each reaction mixture contained 1 nmol of GDP-fucose.

The nucleotide sequence analysis of *rFuc-T* indicates that the coding region of the rat gene is highly similar to both murine *mFuc-TIV* and human *Fuc-TIV*, and comparison of the predicted amino acid sequence indicates 87 and 78% identity with murine and human *Fuc-TIV*, respectively (Figure 1B). Similar comparison with other *Fuc-T*s showed only 49% identity with *Fuc-TVII* and 41–43% with *Fuc-TIII*, *V* and *VI* (Figure 1B). The *rFuc-T* gene encodes a peptide of approximate molecular weight 48 779 Da. Like murine and human *Fuc-TIV*, it encodes two closely spaced, in-frame 5′-methionine residues, that could initiate 433 bp and 399 bp polypeptides, respectively (Figure 1A). This observation is of interest, as another glycosyltransferase, β1,4 galactosyltransferase (GalTase), also has two closely spaced, in-frame 5′-methionine residues, and it has been shown that this gene has two major transcription initiation sites, producing a long mRNA that initiates 5′ to the first ATG, and a short mRNA that initiates between the two ATGs [31]. The longer form of the GalTase protein is preferentially expressed on the cell surface. Interestingly, the presence of fucosyltransferase activity on the cell surface has been reported in mouse germ cells [32] and rat Sertoli cells [33]. It will be important to characterize more fully the mRNA produced by *rFuc-T* gene, to determine whether the mechanism used by the *GalTase* gene could also be used by certain members of the Fuc-TIV family of glycosyltransferases. The longer forms of both rat and murine predicted proteins differ from the human in the length of the cytoplasmic domain by 99 amino acids and there is a deletion of seven amino acids following the second ATG codon in both rat and murine genes (Figure 1).

Most previously characterized glycosyltransferases share common structural features: a cytoplasmic tail of 6–27 amino acids, a single transmembrane domain of 16–33 residues, a stem region of 35–37 residues and a long

Table 1. Acceptor specificity of the rat fucosyltransferase expressed in transfected COS-1 cells.

Chemical name and abbreviation	*Acceptors*	
	Structure	*Relative activity (%)*
N-Acetyllactosamine (LacNAc)	Galβ1 → 4GlcNAc	100
Lacto-*N*-Biose (LNB)	Galβ1 → 3GlcNAc	–
Lactose (Lac)	Galβ1 → 4Glc	–
2′-Fucosyllactose (2′-FL)	Fucα1 → 2Galβ1 → 4Glc	–
6′-Fucosyllactose (6′-FL)	Fucα1 → 6Galβ1 → 4Glc	–
6-Fucosyllactose (6-FL)	Galβ1 → 4(Fucα1 → 6)Glc	–
3′-Sialyl-*N*-acetyllactosamine (3′-SLN)	NeuAcα2 → 3Galβ1 → 4GlcNAc	2.5
6′-Sialyl-*N*-acetyllactosamine (6′-SLN)	NeuAcα2 → 6Galβ1 → 4GlcNAc	–
Lacto-*N*-tetraose (LNT)	Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glc	1.8
Lacto-*N*-neotetraose (LNnT)	Galβ1 → 4GlcNAcβ1 → 3Galβ1 → 4Glc	113
Lacto-*N*-fucopentaose I (LNF I)	Fucα1 → 2Galβ1 → 3GlcNAcβ1 → 3Galβ1 → 4Glc	–

Relative rates for each oligosaccharide acceptor are expressed as a percentage of the fucosylation of *N*-acetyllactosamine (LacNAc). Acceptor concentration was 1 mM. The fucosyl residue donor GDP-Fucose was used in a concentration of 10 μM. Relative activity of 100% corresponds to 8.4 pmol min^{-1} mg $prot^{-1}$ of fucosylated LacNAc. A dash indicates less than 1% of acceptor was fucosylated.

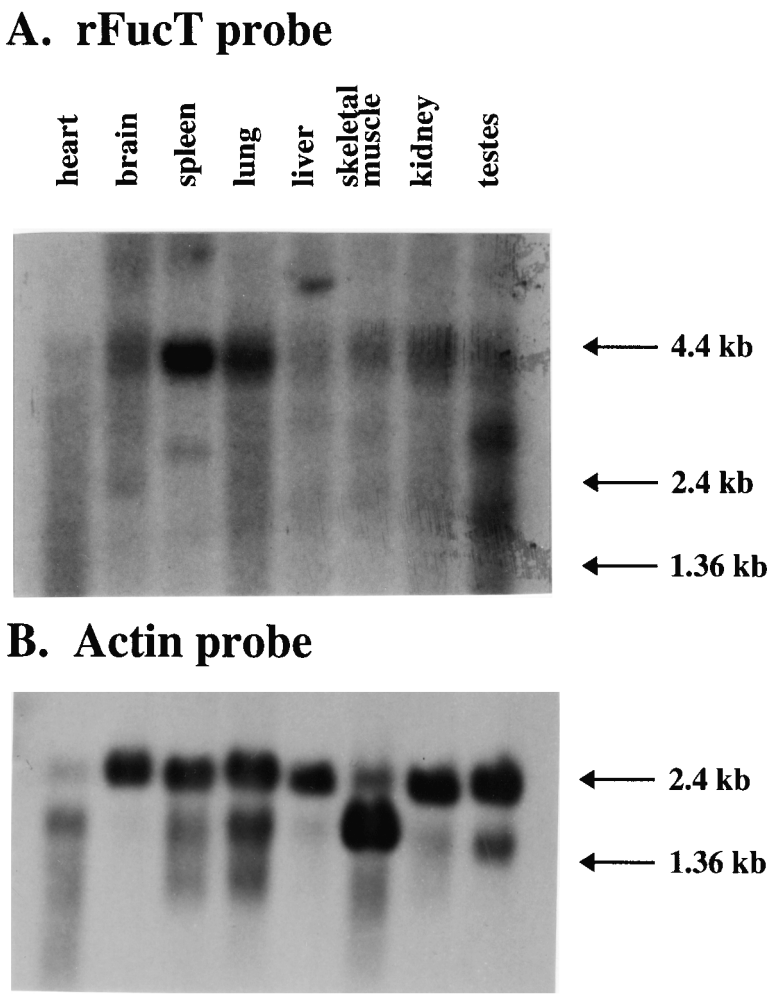

Figure 4. Expression of the *rFuc-T* gene in adult rat tissue. (Panel A) shows a Northern blot, containing 2 μg of polyadenylated mRNA per lane, hybridized with a *rFuc-T* probe. (Panel B) shows Northern blot hybridization with actin probe.

catalytic domain. The rFuc-T sequence, while conforming to the overall structural arrangement, shows some differences. As mentioned above, use of the most 5′ ATG encodes unusually long cytoplasmic tails for the rFuc-T, mFuc-TIV and human Fuc-TIV, 48, 48, and 147 residues, respectively. The putative transmembrane domain in rFuc-T, mFuc-TIV and human Fuc-TIV is longer (33 residues) than those in other cloned glycosyltransferases (generally about 19 residues). The significance of this longer domain is unclear, but its amino acid sequence is highly conserved between the *rFuc-T* and the *mFuc-TIV* and human *Fuc-TIV* genes, with the exception of a different cysteine residue distribution in this domain. There are multiple amino acid substitutions between rat and human peptides, including 62 different residues in the catalytic domain that are likely to contribute to the unique acceptor specificity of the rFuc-T [34, 35].

The GDP fucose binding domain contains threonine at position 328 (Figure 1B) of both rFuc-T and mFuc-TIV, while the human Fuc-TIV contains serine; neither form of the enzyme is NEM sensitive. This difference may affect the catalytic properties of the enzymes [38]. Fuc-Ts III, V and VI all contain a cysteine residue in the GDP-fucose binding domain and are inhibited by NEM (Figure 1B, position 328). The *rFuc-T* gene encodes two possible glycosylation sites. The first glycosylation site (NIS, positions 239–241 in Figure 1B) is unique to rFuc-T, mFuc-TIV and human Fuc-TIV whilst the second site (NWT at positions 339–341, Figure 1B) is present in all *α1,3-Fuc-T* genes so far characterzed.

COS-1 cells, which do not express α1,3-Fuc-T activity [9], were transfected by the *rFuc-T* gene and the acceptor specificity of the recombinant *rFuc-T* was analysed. Our data (Table 1) shows that rFuc-T prefers type 2 (Galβ1 → 4GlcNAc) over type 1 (Galβ1 → 3GlcNAc) structures. rFuc-T has a very narrow acceptor specificity pattern with neutral type 2 chain substrates. LNnT is a slightly better acceptor than LacNAc, suggesting that a β anomeric configuration of GlcNAc and a longer oligosaccharide chain with penultimate Gal- and reducing Glc-residues are

preferred by rFuc-T. Under the conditions used for screening, sialylated LacNAc derivative 3′-SLN was a poor acceptor and 6′-SLN did not serve as an acceptor for rFuc-T. These results are consistent with those obtained by deVries *et al.* [34], who reported that both 3′-SLN and 6′-SLN were very poor acceptors for the recombinant full length human Fuc-TIV. However, a different group of investigators [17] reported that the both human and murine Fuc-TIV recombinant enzymes utilized 3′-SLN, although the human enzyme exhibited a lower affinity than did the murine enzyme. The reasons for this discrepancy are not yet clear. The other previously cloned human α1,3-Fuc-Ts show very different characteristics: 3′-SLN is a good acceptor for recombinant Fuc-TVII [16] and Lac and 2′-FL are good acceptors for recombinant Fuc-TIII and Fuc-TV. Both type 2 chain-based substrates as well as type 1 chain-based substrates could serve as acceptors for Fuc-TV [34].

The rFuc-T, unlike human Fuc-TIV [34], was able to fucosylate a glycolipid acceptor. Fucosylation of nLc_4Cer was observed only when the incubation mixture contained both 0.2% Triton X-100 and 1% taurodeoxycholate. It is important to note that in these experiments the concentration of nLc_4Cer in the incubation mixtures was much less than the concentration of LacNAc, and that under similar assay conditions human Fuc-TIV was not able to fucosylate nLc_4Cer [34]. However, it would be important to confirm differences in acceptor specificity with cloned rFuc-T, mFuc-T and human Fuc-TIV within the same experiment to validate these conclusions.

It is clear that the *rFuc-T* gene encodes a fucosyltransferase activity since the SSEA-1 antigen is expressed on transfection of COS-1 cells with this gene. However it is not yet clear whether the rFuc-T is involved in fucose addition to glycolipids and/or glycoproteins *in vivo*, because the SSEA-1 epitope occurs on both glycoconjugates and the antibody used in the immunocytochemical experiments does not distinguish between these two types of antigens. The possibility that different enzymes control the addition of fucose to glycolipids and glycoproteins has been raised by previous studies [36]. Studies by deVries *et al.* [34] on human Fuc-TIV suggest that truncation of the transmembrane and stem domains confers preference towards glycoprotein acceptors. This suggestion is consistent with the preference for glycolipid acceptors exhibited by human Fuc-TV, that contains an expanded stem region on comparison with other Fuc-Ts (Figure 1B). Our *in vitro* studies measuring fucosyltransferase activity in homogenates from transfected COS-1 cells suggest that rFuc-T has higher activity with oligosaccharide acceptors than with glycolipid acceptors, but makes no predictions about glycoprotein substrates. It should be noted, however, as pointed out by Goelz *et al.* [27], that the acceptor specificity as established by *in vitro* studies of enzymes does not necessarily reflect products formed in intact cells. Thus, different cell lines transfected with Fuc-T may express different products perhaps as a result of different complements of cellular glycosyltransferases and thus different repertoires of substrates. Future studies will be directed to comparing lipid and protein substrates *in vitro* as well as characterizing the nature of SSEA-1 and other fucosylated products in a variety of transfected cells to address this question.

The pattern of tissue expression of *rFuc-T* and *mFuc-TIV* genes appears to be different. The relative expression of *rFuc-T* is higher in spleen than in lung or kidney; a similar comparison of relative tissue expression of *mFuc-TIV* shows high expression in lung, lesser expression in spleen and only a trace in kidney [17]. Furthermore, *rFuc-T* probes detected several transcripts of different size in testes and brain, whereas *mFuc-TIV* probes detected only a single transcript in mouse testes and little hybridization in mouse brain [17]. These differences suggest either that the rat and mouse genes are closely related but different members of the same family, or could be interpreted as indicating different tissue regulation of the same gene in different species. The tissue distribution of the human *Fuc-TIV* gene has not yet been reported. The observation of multiple transcripts detected by the *rFuc-T* probe suggests that, as is the case for some other glycosyltransferase [31] and fucosyltransferase [15, 19, 37] genes, the use of alternative transcription initiation or polyadenylation sites, or alternative splicing, may be involved in regulating the tissue specific expression of the *rFuc-T* gene. The expression of *rFuc-T* in brain indicates that this gene will provide an important tool in the quest for understanding the role of SSEA-1 antigens during neurological development.

In conclusion, the results demonstrating the expression of SSEA-1 antigen by COS-1 cells transfected with the *rFuc-T* gene, and the detection of enzyme activity with type 2 oligosaccharide acceptors in these cells, indicate that the *rFuc-T* gene encodes an α1,3-fucosyltransferase. The high sequence homology between *rFuc-T*, *mFuc-TIV* and human *Fuc-TIV* suggests that the *rFuc-T* gene is a member of the *Fuc-TIV* family. However, it is not yet clear whether *rFuc-T* is a true homologue exhibiting some differences in acceptor specificity and tissue expression from mouse and human genes, or whether it is a new, closely related member of this family. The issue may be resolved upon the cloning and characterization of additional α1,3-fucosyltransferase genes.

Acknowledgements

We thank Dr Christopher D. Warren (E.K. Shriver Center) for a synthesis of 6′-Fucosyllactose, and 6-Fucosyllactose, Dr Omanand Koul (E.K. Shriver Center) and Raegan McDonald for preparation of lipid substrates, Rhonda Doll for assistance in the transfection procedures, Denise Chou for help in the fluorescence immunostaining, Peter McCaffery and Ursula Dräger for providing the 43B11 SSEA-1 antibody, and Denise Brescia for excellent editing

and word processing assistance. We also thank Drs Rosella Mollicone and Rafael Oriol for helpful discussions and suggestions. Supported by Grant HD05515 (to R.H.M.) from the National Institute of Child Health and Human Development, National Institutes of Health.

References

1 Fukuda M (1992) In *Cell Surface Carbohydrates in Hematopoietic Cell Differentiation and Malignancy* (Fukuda EM, ed.) pp. 127–59. Boca Raton, FL: CRC Press.
2 Velupillai P, Harn DA (1994) *Pro Nat Acad Sci* **91**: 18–22.
3. Fenderson BA, Eddy EM, Hakomori S (1990) *Bioassays* **12**: 173–9.
4 Kim YS, Itzkowitz SH, Yuan M, Chung YS, Satake K, Umeyama K, Hakomori S (1988) *Cancer Res* **48**: 475–82.
5 Marani E, Mai K (1992) *Histochem J* **24**: 852–68.
6 Mai J, Marani E, Hakomori S (1992) Special Issue: CD15. *Histochem J* **24**: 852–68.
7 Chou DKH, Suzuki Y, Jungalwala FB (1996) *Glycoconj J* **13**: 1–11.
8 Wiederschain G, Koul O, McCluer RH (1995) *Glycoconj J* **12**: S9.
9 Kukowska-Latallo JF, Larsen RD, Nair RP, Lowe JB (1990) *Genes Develop* **4**: 1288–303.
10 Lowe JB, Kukowska-Latallo JF, Nair RP, Larsen RD, Marks RM, Macher BA, Kelly RJ, Ernst LK (1991) *J Bio Chem* **266**: 17467–77.
11 Kumar R, Potwin B, Muller WA, Stanley P (1991) *J Biol Chem* **266**: 21777–83.
12 Sasaki K, Kurata K, Funayama K, Nagata M, Watanabe E, Ohta S, Hanai N, Nishi T (1994) *J Biol Chem* **269**: 14730–7.
13 Weston BW, Nair RP, Larsen RD, Lowe JB (1992a) *J Biol Chem* **267**: 4152–60.
14 Weston BW, Smith PL, Kelly RJ, Lowe JB (1992b) *J Biol Chem* **267**: 24575–84.
15 Natsuka S, Gersten KM, Zenita K, Kannagi R, Lowe JB (1994) *J Biol Chem* **269**: 16789–94.
16 Natsuka S, Lowe JB (1994) *Current Opinion in Structural Biology* **4**: 683–91.
17 Gersten KM, Natsuka S, Trinchera M, Petryniak B, Kelly RJ, Hiraiwa N, Jenkins NA, Gilbert DJ, Copeland NG, Lowe JB (1995) *J Biol Chem* **270**: 25047–56.
18 Ozawa M, Muramatsu T (1996) *J Biochem* **119**: 302–8.
19 Smith PL, Gersten KM, Petryniak B, Kelly RJ, Rogers C, Natsuka Y, Alford III JA, Scheidegger EP, Natsuka S, Lowe JB (1996) *J Biol Chem* **271**: 8250–9.
20 Mollicone R, Candelier JJ, Mennesson B, Couillin P, Venot AP, Oriol R (1992) *Carbohydr Res* **228**: 265–76.
21 Sajdel-Sulkowska EM, Smith FI, McCluer RH (1994) *Glycobiology* **4**: 741.
22 Sajdel-Sulkowska EM, Smith FI, McCluer RH (1995) *Glycoconjugate J*. **12**: 476–7.
23 Mollicone R, Candelier JJ, Reguigne I, Couillin P, Fletcher A, Oriol R (1994) *Transfus Clin Biol* **1**: 91–7.
24 Goelz SE, Hession C, Goff D, Griffiths B, Tizard R, Newman B, Chi-Rosso G, Lobb R (1990) *Cell* **63**: 1349–56.
25 Dodemont HJ, Soriano P, Quax WJ, Ramaekers F, Lensha JA, Groenen MA, Bernardi G, Blemendal H (1982) *EMBO J* **1**: 167–71.
26 Ko AI, Drager UC, Harn DA (1990) *Proc Nat Acad Sci* **87**: 4159–63.
27 Goelz S, Kumar R, Potvin B, Sundaram S, Brickelmaier M, Stanley P (1994) *J Biol Chem* **269**: 1033–40.
28 Folch J, Lees M, Sloane-Stanley GH (1957) *J Biol Chem* **226**: 497–509.
29 Williams MA, McCluer RH (1980) *J Neurochem* **35**: 1351–61.
30 Dynan WS (1986) *Trends in Genet* **2**: 196–7.
31 Shur BD (1991) *Glycobiology* **1**: 563–75.
32 Cardullo RA, Armant DR, Millette CF (1989) *Biochem* **28**: 1611–17.
33 Raychoudhury SS, Millette CF (1993) *Molecular Reproduction and Development* **36**: 195–202.
34 DeVries T, Srnka CA, Palcic MM, Swiedler SJ, Vandeneijnden DH, Macher BA (1995) *J Biol Chem* **270**: 8712–22.
35 Xu Z, Vo L, Macher BA (1996) *J Biol Chem* **271**: 8818–21.
36 Howard DR, Fukuda M, Fukuda MN, Stanley P (1987) *J Biol Chem* **262**: 16830–7.
37 Cameron HS, Szczepaniak D, Weston BW (1995) *J Biol Chem* **270**: 20112–22.
38 Holmes EH, Xu Z, Sherwood AL, Macher BA (1995) *J Biol Chem* **270**: 8145–51.
39 Mollicone R, Gibaud A, Francois A, Ratcliffe M, Oriol R (1990) *Eur J Biochem* **191**: 169–76.
40 Reguigne I, James MR, Richard CW 3rd, Mollicone R, Seawright A, Lowe JB, Oriol R, Couillin P (1994) *Cytogenet Cell Genet* **66**: 104–6.

Received 10 June 1996, revised 1 August 1996, accepted 9 August 1996