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Molecular Cloning and Expression of a Mouse α-1,3 Fucosyltransferase Gene That Shows Homology with the Human α-1,3 Fucosyltransferase IV Gene[1]

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The polymerase chain reaction was used to amplify and clone a fragment of a novel fucosyltransferase cDNA from mouse embryonal carcinoma F9 cells. Southern blot analysis of mouse genomic DNA with the cloned cDNA fragment revealed that there is a single copy of the fucosyltransferase gene per haploid genome. A 5 kb *Eco*RI restriction fragment of the genomic DNA hybridized with the cDNA has been cloned and a 1,697 bp fragment containing the sequence of the cDNA was sequenced. The nucleotide sequence and the deduced amino acid sequence of the clone showed a highest degree of homology with those of human α-1,3 fucosyltransferase IV, *i.e.* 77.1 and 75.5% identity, respectively. Consistent with the sequence similarity, the transfection of a 1,544 bp fragment under the control of the cytomegarovirus enhancer and β actin promoter into COS-1 cells confers α-1,3 fucosyltransferase activity on the cells, resulting in cell surface expression of SSEA-1.

Key words: embryonal carcinoma cells, fucosyltransferase, gene, Lewis x antigen, SSEA-1.

Cell surface carbohydrates undergo marked alterations during the early stages of mammalian embryogenesis (*1*). A class of fucose-labeled high molecular weight poly-*N*-acetyllactosamines called embryoglycans is abundant in preimplantation and early postimplantation embryos, and in embryonal carcinoma (EC) cells, and disappears progressively during differentiation and development (*2, 3*). These glycans carry a number of cell surface markers, of which SSEA-1 (*4*) is the most established one (*5*). This antigen first appears on the surface of cells at the eight-cell stage of mouse embryogenesis and later becomes restricted to specific cell types during development (*6*). Among mouse teratocarcinoma cells, SSEA-1 is exclusively expressed on EC cells and is used as a marker of EC cells. The antigenic determinant of the antigen has been shown to involve the Gal-β1,4-(Fuc-α1,3)-GlcNAc structure, which is also known as Lewis x (*7*). Experiments have suggested that the SSEA-1 antigen may participate in adhesion events leading to compaction (*8-10*). Furthermore, a monoclonal antibody reacting with this epitope structure inhibits the cell-substratum adhesion of F9 EC cells (*11*). Since developmental control of the SSEA-1 epitope is at the level of a fucosyltransferase forming Lewis x structure (*12*), molecular understanding of the antigenic expression requires identification of the fucosyltransferase gene that forms this antigenic epitope.

In this paper, we describe the cloning of a fragment of a novel fucosyltransferase cDNA from mouse F9 EC cells and the cloning of its genomic DNA. Transfection of a 1,544 bp fragment of the genomic DNA under the control of the cytomegarovirus enhancer and β actin promoter into COS-1 cells confers α-1,3 fucosyltransferase activity on the cells, resulting in cell surface expression of SSEA-1.

MATERIALS AND METHODS

cDNA Cloning—The mouse cDNA clone for a fucosyltransferase was isolated by polymerase chain reaction (PCR) from cDNA of the mouse EC cell line, F9. Total RNA was isolated from F9 cells by the acid guanidine isothiocyanate-phenol-chloroform extraction method (*13*). Poly-(A)$^+$ RNA was enriched with oligo(dT)-cellulose (*14*), and used to produce cDNA using MMTV reverse transcriptase (Gibco/BRL) and random-hexamer primers as described previously (*15*). Two oligonucleotides, 5′TTCTACCTGG-CT/CTTCGAGAAC (primer 1) and 5′GAAGTCGTCCAC-GTGGATGAA (primer 2), were synthesized and used as PCR primers. PCR was performed as described previously (*16*). Twenty-five cycles (1 min at 93°C, 2 min at 58°C, and 3 min at 72°C) were performed and the products were isolated from agarose gels for subcloning into Bluescript II KS(+) (Stratagene). The cDNA clones were sequenced using plasmid DNA and T7 DNA polymerase (Pharmacia).

Genomic DNA Cloning—Genomic DNA was isolated from the livers of 129 Sv mice according to the method of Herrmann and Frischauf (*17*), restricted with *Eco*RI, and then electrophoresed on low-melting agarose gels. The gel pieces containing a 5 kb fragment that hybridized with the

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Abbreviations: bp, base pair(s); EC, embryonal carcinoma; Fuc-T, fucosyltransferase; kb, kilobase(s); PBS, Dulbecco's phosphate-buffered saline; PCR, polymerase chain reaction.

cDNA were excised. The DNA fragments were isolated from the gel pieces and cloned into the λ ZAP II vector. The λ phage library thus obtained was screened by plaque hybridization using the cDNA as a probe. Four independent positive clones were plaque-purified, and analyzed by agarose gel electrophoresis and hybridization with the cDNA probe after digestion with restriction enzymes. After confirming that each clone showed the same restriction pattern, one representative clone was subjected to further analysis. The DNA was sequenced from both strands as above.

Cells—Mouse F9 EC cells were cultured in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum under a 10% CO_2 atmosphere at 37°C as described previously (*18*). COS-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Mouse myeloid leukemia M1 cells (*19*) were obtained from the Japanese Cancer Research Resources Bank.

Transfection of COS Cells—A 1,544 bp fragment of the genomic DNA clone restricted with *Nae*I and *Stu*I was cloned into a mammalian expression vector, pCAGGS neo, which contains an enhancer derived from cytomegalovirus and the β-actin promoter (*20*), as described previously (*16*). COS-1 cells were transfected with the expression vector by the DEAE-dextran method as described previously (*16*).

Immunofluorescence Staining—COS cells transfected with a fucosyltransferase expression vector (pCMFT-1) or with a control vector (pCAGGS neo) were harvested with Dulbecco's phosphate-buffered saline (PBS) containing 0.5 mM EDTA and 0.02% NaN_3. After washing with a mixture (1:1) of PBS and Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, the cells were incubated with monoclonal antibodies obtained from the following sources; SSEA-1 (Dr. Davor Solter, Max-Planck Institut für Immunbiologie, Freiburg, Germany), anti-Lewis a (clone PR5C5; Cymbus Bioscience, Southampton, UK), anti-sialyl Lewis x (clone KM-93; Seikagaku Kogyo, Tokyo), and anti-sialyl Lewis a (clone 2D3; Seikagaku Kogyo, Tokyo). The cells were then stained with fluorescein isothiocyanate-conjugated anti-mouse IgG (Jackson Laboratories), which reacts with IgG as well as IgM, and photographed under an Olympus microscope using Fujichrome 100 film as described previously (*16*).

Northern Blotting—Poly(A)$^+$ RNA (10 μg) isolated from F9 EC cells and M1 myeloid leukemia cells was fractionated by electrophoresis on a 1% agarose gel and blotted on a nylon membrane (Amersham), and then probed with the genomic DNA fragment radiolabeled by the random priming method (*21*). The hybridization and washing conditions were as described previously (*18*).

RESULTS

Isolation of a cDNA Clone for Mouse α-1,3-Fucosyltransferase—When we started cloning of the α-1,3-fucosyltransferase gene by PCR amplification of F9 cell cDNA, the nucleotide sequences of the cDNA encoding human Lewis blood group fucosyltransferase (Fuc-TIII) (*22*) and α-1,3-fucosyltransferase (Fuc-TIV) (*23*) were available. These sequences showed substantial amino acid identity with each other in their carboxy-terminal portions, *i.e.* within their catalytic domains. Therefore, we synthesized oligonucleotides 1 and 2, and used them as primers for PCR (Fig. 1). After amplification, the expected (162 bp) PCR product was purified by agarose gel electrophoresis and subcloned. Sequencing of the cDNA revealed that the nucleotide sequence (120 bp) was 85.8 and 73.3% identical to the corresponding regions of Fuc-TIV and Fuc-TIII, respectively (Fig. 2). The predicted amino acid sequence of the cDNA also showed a high degree of identity to the corresponding sequences of Fuc-TIV and Fuc-TIII (92.5 and 75.0%, respectively). The high degrees of homology in the nucleotide and amino acid sequences suggested that the cDNA encodes a novel mouse fucosyltransferase. Since the coding regions of all fucosyltransferases so far described are encoded by a single exon (*22-33*), we cloned the genomic DNA encoding the fucosyltransferase using the isolated cDNA fragment as a probe.

Isolation of Genomic DNA Clones for Mouse α-1,3-Fucosyltransferase—Southern blot analysis of mouse genomic DNA restricted with *Eco*RI revealed a single band at 5 kb (Fig. 3A). The *Eco*RI-restricted fragment of the corresponding region was purified and used to construct a partial genomic DNA library with the λ ZAP II vector. The library

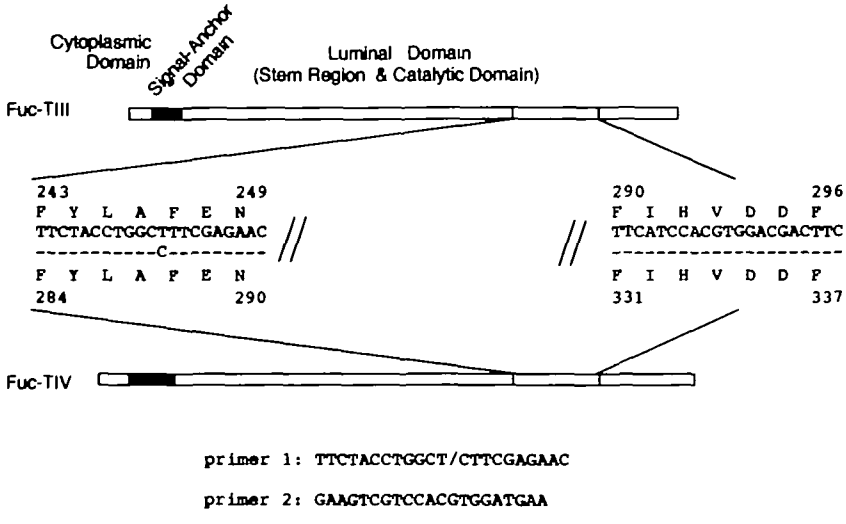

Fig. 1. **Schematic representation of α-fucosyltransferases, and the sequences and locations of the PCR primers.** α-Fucosyltransferases are composed of an amino-terminal cytoplasmic domain followed by a signal-anchor domain, a stem region, and a large carboxy-terminal catalytic domain oriented within the lumen of the Golgi cisternae. Parts of the published nucleotide sequences of human Fuc-TIII (*22*) and Fuc-TIV (*23*), with their predicted amino acid sequences above or below the nucleotide sequences are compared. Dashed lines in the Fuc-TIV sequence denote the DNA sequence identity between Fuc-TIII and Fuc-TIV. The nucleotide sequence difference is indicated within the dashed line representing the Fuc-TIV sequence. Amino acid residues are numbered.

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mouse cDNA  TTCTACCTGGCCTTCGAGAACTCACGGCACGTGGATTACATCACCGAGAAGCTGTGGCGGAATGCCTTTCTGGCTGGGGCC  81
            F  Y  L  A  F  E  N  S  R  H  V  D  Y  I  T  E  K  L  W  R  N  A  F  L  A  G  A
  Fuc-TIV   ----------T----------G-A----C-------T-------------C-----C--C--G--G--C--------G
            -  -  -  -  -  -  -  -  Q  -  -  -  -  -  -  -  -  R  -  -  -  -  L  -  -  -  -
  Fuc-TIII  ----------------------CTT----CCC--C-------------------A----C---C-GGA---CT-----
            -  -  -  -  -  -  -  -  L  -  P  -  -  -  -  -  -  -  -  -  -  -  L  E  -  W  -

mouse cDNA  GTGCCAGTGGTGCTGGGCCCAGATCGTGCCAACTATGAGCGCTTCGTGCCTCGTGGCGCCTTCATCCACGTGGACGACTTC 162
            V  P  V  V  L  G  P  D  R  A  N  Y  E  R  F  V  P  R  G  A  F  I  H  V  D  D  F
  Fuc-TIV   -----G----------------C----------C---------T-----C--C-------------------------
            -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -  -
  Fuc-TIII  -----C--------------CAGCA-AAG------C---A-G---C----A-CC-A-----------------------
            -  -  -  -  -  -  -  S  -  S  -  -  -  -  -  L  -  P  D  -  -  -  -  -  -  -  -
```

Fig. 2. **The nucleotide sequence of mouse α-fucosyltransferase cDNA, the predicted amino acid sequence of the protein, and comparison of them with those of human Fuc-TIII and Fuc-TIV.** Nucleotides are numbered on the right. Dashed lines in the human Fuc-T DNA and amino acid sequences denote identity with the mouse sequences. The nucleotide and amino acid sequence differences are indicated within the dashed line representing each sequence.

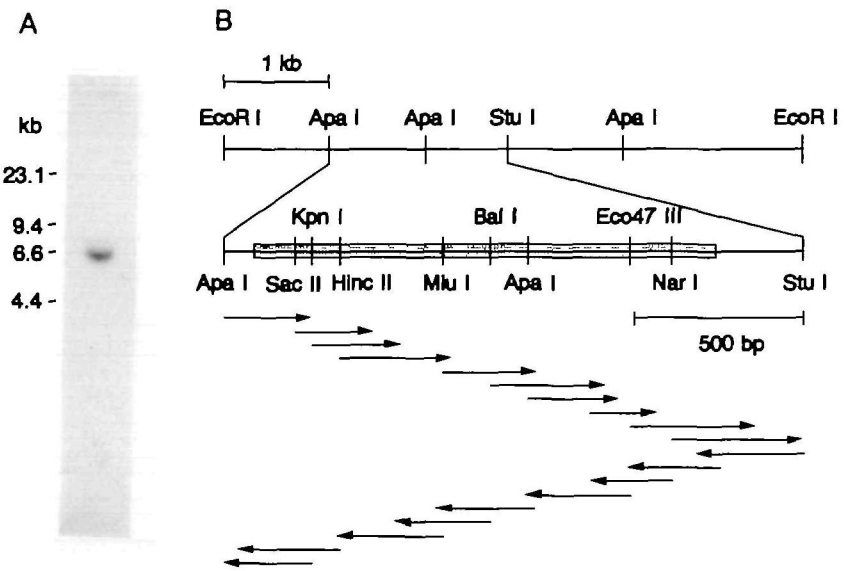

Fig. 3. **Southern blot analysis of, restriction enzyme mapping of, and sequencing strategy for the mouse α-fucosyltransferase gene.** A: Southern blot analysis of the mouse α-fucosyltransferase gene. Genomic DNA was digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, transferred onto a nitrocellulose filter, and then hybridized with the labeled cDNA probe. kb, kilobases. B: Schematic representation of the α-fucosyltransferase gene. The isolated genomic clone and restriction enzymes used to determine the nucleotide sequence are shown. The open reading frame is depicted by a thick bar. bp, base pair(s).

was screened with the 162 bp cDNA fragment and four positive clones were purified. Mapping with several restriction enzymes revealed that the clones were identical. The nucleotide sequence of the entire region surrounding the coding region of the cDNA fragment was determined according to the strategy shown in Fig. 3B.

DNA sequence analysis of this genomic fragment revealed a single long reading frame predicted to yield a protein with striking amino acid sequence similarity to human Fuc-TIV (Fig. 4, and see below). The 5′ part of the sequence is very GC-rich, these two bases accounting for more than 80% of the total. There are two ATG codons at nucleotides 143 and 242 that are preceded by an in-frame stop codon at nucleotide 14. Although these two ATG codons are embedded in the consensus sequence for translation initiation (*34*), the latter gives a higher score (*53*) than the former (*47*). Therefore we assigned the latter ATG codon as the initiation codon. It is predicted that translation from this ATG codon gives a protein of 400 amino acids long with a calculated molecular weight of 45,640. The overall structure of the deduced protein seems to be that of a type II transmembrane protein typical of mammalian glycosyltransferases (*35*) with a short amino-terminal cytoplasmic tail of 23 amino acids, a signal-anchor domain of 26 amino acids, and a large carboxy-terminal domain. Sequence analysis also predicts two potential *N*-linked glycosylation sites, at amino acid residues 84 and 185. No polyadenylation signal was found in the 3′-noncoding region of the gene sequenced in the present study, but one ATTTA motif, implicated in mRNA instability (*36*), was found in the region.

The Cloned DNA Fragment Determines the Expression of an α-1,3 Fucosyltransferase—To determine if the cloned DNA fragment encodes a functional α-1,3 fucosyltransferase, the putative coding region segment was cloned into a mammalian expression vector and then subjected to transfection experiments. Expression plasmid pCMFT-1 (see "MATERIALS AND METHODS") was transfected into the COS-1 cell line, a mammalian host that does not normally express α-1,3 fucosyltransferase activity (*22*). Cells transfected with pCMF-1 or the control vector (pCAGGS neo) were analyzed by immunofluorescence staining. As shown in Fig. 5A, COS-1 cells transfected with pCMF-1 exhibited surface expression of the SSEA-1 (Lewis x) determinant but did not express either the sialyl SSEA-1 (sialyl Lewis x) (Fig. 5B), Lewis a or sialyl Lewis a antigen (not shown). The expression of the SSEA-1 determinant is dependent on the cloned DNA fragment, since the control vector or the vector with the DNA fragment in the reverse orientation did not direct the expression of the determinant (data not shown).

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                                                                    GGGCCCGCAGGGCTAGGAGAACAGTGTCTGGAAGGGTCAGGGTGGCGCTGGGCCAGGGCTG   61
CCGGCCCGGCTCACCTTGTCCTGGACTTGGGAGGGGCGAGGCACCGCCCGTGGGTCCGCCGATGCCCCGCCCGCCCCCTCCATGGCTCCG  151

GCCCGGCAAGAGCTACAGCATGAGAGCCGGTGCCGCCCCTCACGGACGGTGGACGCGTGGCGAGCCGCGGTGGCCACTCGTGGACGCCAC  241

ATGGAGACGCCGGGGTACCGGCGTCGCACACGGTGTGGAGGCTGGGGACTACCCCGGAGCGTCTCTTCGCTGGCGGCAGTGGGACTACTG  331
M  E  T  P  G  Y  R  R  R  T  R  C  G  G  W  G  L  P  R  S  V  S  S  L  A  A  V  G  L  L

TGCACCGCGTTGACCACCTTCATCTGCTGGGGACAGTTGCCGCCACTGCCCTGGGCGTCCCCAGCTCCGCAACGCCTGGTGGGTGTGCTG  421
C  T  A  L  T  T  F  I  C  W  G  Q  L  P  P  L  P  W  A  S  P  A  P  Q  R  L  V  G  V  L

CTCTGGTGGGAACCCTTTAGGGGGCGCGGCGGCTACCCCAAGTCTCCCCCCGATTGCAGCCTGCGCTTCAACATCAGCGGCTGCCGCTTG  511
L  W  W  E  P  F  R  G  R  G  G  Y  P  K  S  P  P  D  C  S  L  R  F  N  I  S  G  C  R  L

CTCACCGACCGCGCGGCCTACGGGGAGGCTCAGGCAGTGCTGTTCCACCACCGCGACCTGGTGAAGGAACTCCACGACTGGCCCCCGCCC  601
L  T  D  R  A  A  Y  G  E  A  Q  A  V  L  F  H  H  R  D  L  V  K  E  L  H  D  W  P  P  P

TGGGGCGCCCGAGAACGCACGGATAAGGCGCTGGTACTACGCGTGTTCGACGACCAGGAGGGAGCAGTGACGCTAACTGGCAAAGCCCTG  691
W  G  A  R  E  R  T  D  K  A  L  V  L  R  V  F  D  D  Q  E  G  A  V  T  L  T  G  K  A  L

GAGACCGTAGGTTCTCGTCCCCCTGGGCAGAGGTGGGTGTGGATGAACTTCGAATCGCCCTCCCATACTCCAGGGCTGCGGGGCTTGGCC  781
E  T  V  G  S  R  P  P  G  Q  R  W  V  W  M  N  F  E  S  P  S  H  T  P  G  L  R  G  L  A

AAGGACCTCTTCAATTGGACACTGTCCTACCGGACCGACTCGGATGTCTTCGTGCCCTATGGCTTCCTCTATTCCAGGAGCGATCCAACT  871
K  D  L  F  N  W  T  L  S  Y  R  T  D  S  D  V  F  V  P  Y  G  F  L  Y  S  R  S  D  P  T

GAACAGCCTTCAGGCTTGGGCCCCCCCCCTGGCCCGCAAGCAAGGACTGGAAGCCTGGGTTGTGAGCAACTGGAACGAGCACCAGGCACAG  961
E  Q  P  S  G  L  G  P  P  L  A  R  K  Q  G  L  E  A  W  V  V  S  N  W  N  E  H  Q  A  Q

GTCCGTTACTACCACCAGCTGAGTCGGCACGTGTCTGTGGACGTGTTTGGTCGGACGGGACCCGGACGGCCAGTGCCAGCCATCGGGCTG 1051
V  R  Y  Y  H  Q  L  S  R  H  V  S  V  D  V  F  G  R  T  G  P  G  R  P  V  P  A  I  G  L

CTGCACACTGTGGCCCGCTACAAGTTTTATCTAGCCTTTGAGAACTCACGGCACGTGGATTACATCACCGAGAAGCTGTGGCGGAATGCC 1141
L  H  T  V  A  R  Y  K  F  Y  L  A  F  E  N  S  R  H  V  D  Y  I  T  E  K  L  W  R  N  A

TTTCTGGCTGGGGCTGTGCCAGTGGTGCTCGGCCCAGATCGTGCCAACTATGAGCGCTTCGTGCCTCGTGGCGCCTTCATCCACGTGGAC 1231
F  L  A  G  A  V  P  V  V  L  G  P  D  R  A  N  Y  E  R  F  V  P  R  G  A  F  I  H  V  D

GATTTCCCTAATGCTGCTTCCCTGGCTGCCTACCTGCTCTTTCTCGACCGAAACGTGGCTGTCTATCGCCGCTACTTCCGCTGGCGCCGG 1321
D  F  P  N  A  A  S  L  A  A  Y  L  L  F  L  D  R  N  V  A  V  Y  R  R  Y  F  R  W  R  R

AGCTTTGCAGTGCACATCACCTCTTTCTGGGATGAGCAATGGTGCCGGACATGCCAAGCTGTGCAGACCTCTGGGGACCAGCCCAAGAGC 1411
S  F  A  V  H  I  T  S  F  W  D  E  Q  W  C  R  T  C  Q  A  V  Q  T  S  G  D  Q  P  K  S

ATCCACAACTTGGCAGACTGGTTCCAGCGATGAGGCCATTCTGCTGTGAACTGACTACATGGCCTTGGCTGCGTTTTCTAAATACCCAAT 1501
I  H  N  L  A  D  W  F  Q  R

TTTTCTTTATGTGAAGATGAGTTACCAATTAATATTATTTAGCACAAGGGTGGGAAAATTCCCAGGCCCTCGTGGCTTTTTTACAGAGAG 1591

GTGGTCTGGCTTTCTGGTAAGGGGCTGCGGTGTCCTGGGTCAAGGAGGCTGGGCCTTCCTTACCTGTCACCCATGTGAAGAAGTGGGCTA 1681
TCCTGGCTACAGGCCT                                                                            1697
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Fig. 4. **The nucleotide sequence of the mouse α-1,3-fucosyltransferase gene and the predicted amino acid sequence of the protein.** Nucleotides are numbered on the right The predicted signal-anchor domain is denoted by the underline. Two consensus *N*-glycosylation sites are indicated by double-underlines Four potential SP1 binding sites and an ATTTA motif implicated in mRNA instability are underlined

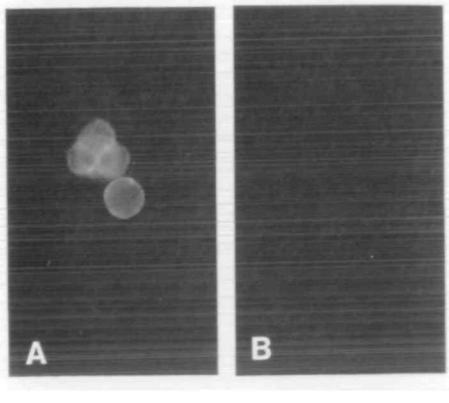

Fig. 5 **Immunofluorescence staining of COS cells transfected with the mouse α-1,3-fucosyltransferase expression vector.** COS-1 cells transfected with the expression vector were stained with anti-SSEA-1 (A) or anti-sialyl Lewis x (B), as described under "MATERIALS AND METHODS."

Northern Blot Analysis of the Mouse α-1,3 Fucosyltransferase mRNA—Since human Fuc-TIV is expressed in human myeloid cells, including the HL-60 promyelocytic cell line (*23-25*), we were interested to determine whether or not the mouse α-1,3 fucosyltransferase is expressed in mouse myeloid cells. For this, poly(A)$^+$ RNA isolated from M1 myeloid leukemia cells and F9 EC cells was subjected to Northern blot analysis with the genomic DNA probe. We did not detect fucosyltransferase transcripts in either M1 cells or F9 cells (data not shown).

DISCUSSION

The data reported here indicate that we have cloned a mouse gene encoding an α-1,3-fucosyltransferase that is most similar in its catalytic activity and structure to human Fuc-TIV. Since COS-1 cells maintain substrate levels of GDP-fucose and glycosylated acceptors, and can construct surface-localized α(1,3)- and α(1,4)-fucosylated oligosaccharides when transfected with fucosyltransferase expression vectors, they have been used to examine the acceptor substrate specificities of the cloned fucosyltransferase genes *in vivo* (*22, 24, 26-28, 31*). For example, COS-1 cells transfected with human Fuc-TIII expression vectors exhibit surface expression of Lewis x, sialyl Lewis x, Lewis a, and sialyl Lewis a (*22*). By contrast, COS-1 cells transfected with human Fuc-TIV are stained with antibodies specific for Lewis x but not with ones for sialyl Lewis x, Lewis a, or sialyl Lewis a (*24*). Like human Fuc-TIV, COS-1 cells transfected with the mouse fucosyltransferase exhibit surface expression of the Lewis x antigen but do not express the other antigens. The results obtained with the mouse fucosyltransferase also differ from those obtained when COS-1 cells are transfected with human Fuc-TV, Fuc-TVI, or Fuc-TVII, because transfection of all of these fucosyltransferases results in surface expression of the sialyl Lewis x antigen (*26, 28, 31*). Therefore the cloned mouse fucosyltransferase exhibits functional similarity to human Fuc-TIV.

A direct comparison of the amino acid sequence of the mouse fucosyltransferase with that of human Fuc-TIV is shown Fig. 6. The overall identity between these proteins is 75.5%. Although the cytoplasmic domain did not show significant homology, the predicted transmembrane (signal anchor) and luminal domains do. There were marked sequence discontinuities in the amino acid sequence of a region corresponding to residues 123 through 155. This region may correspond to the carboxy-terminal part of the "stem" region of Fuc-TIV and the amino-terminal portion of its catalytic domain. There is a 2-amino acid insertion at residue 145, relative to Fuc-TIV. By contrast, 82.5% of the residues are identical in the carboxy-terminal halves of the two proteins. The two potential *N*-glycosylation sites found in the mouse protein are located at the corresponding positions in human Fuc-TIV.

The homology between the two proteins suggests that the mouse fucosyltransferase can be assigned as the mouse homologue of human Fuc-TIV. In the case of β-1,4-galactosyltransferase (*37, 38*), the overall identity between the mouse and human proteins is 86.0%. The overall identity between the mouse and human α-2,6-sialyltransferases (*39, 40*) is 78.6%, a value close to that between the mouse fucosyltransferase and human Fuc-TIV. The DNA sequences of the coding region and the luminal domain of the mouse fucosyltransferase are 76.8 and 78.0% identical, respectively, to the corresponding regions of the human Fuc-TIV gene. On Southern blot analysis of mouse genomic DNA with human Fuc-TIV cDNA as a probe under several low-stringency conditions, however, we failed to detect the mouse fucosyltransferase gene.

Although we cloned the cDNA encoding the fucosyltransferase from F9 EC cells, the transcript was undetectable on Northern blot analysis under the conditions used. The presence of one ATTTA motif, implicated in mRNA insta-

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mouse    METPGYRRRTRCGGWGLPRSVSSLAAVGLLCTALTTFICWGQLPPLPWASPAP   53
human  MGAPWGSPTAAAGGRRGWRRGRGLPWTVCVLAAAGLTCTALITYACWGQLPPLPWASPTP   60

mouse  QRLVGVLLWWEPFRGRGGYPKSPPDCSLRFNISGCRLLTDRAAYGEAQAVLFHHRDLVKE  113
human  SRPVGVLLWWEPFGGRDSAPRPPPDCPLRFNISGCRLLTDRASYGEAQAVLFHHRDLVKG  120

mouse  LHDWPPPWGARERTDKALVLRVFDDQEGAVTLTGKALETVGSRPPGQRWVWMNFESPSHT  173
human  PPDWPPPWGIQAHTAEEVDLRVLDYEEAAAA--AEALATSSPRPPGQRWVWMNFESPSHS  178

mouse  PGLRGLAKDLFNVTLSYRTDSDVFVPYGFLYSRSDPTEQPSGLGPPLARKQGLEAWVVSN  233
human  PGLRSLASNLFNVTLSYRADSDVFVPYGYLYPRSHPGDPPSGLAPPLSRKQGLVAWVVSH  238

mouse  WNEHQAQVRYYHQLSRHVSVDVFGRTGPGRPVPAIGLLHTVARYKFYLAFENSRHVDYIT  293
human  WDERQARVRYYHQLSQHVTVDVFGRGGPGQPVPEIGLLHTVARYKFYLAFENSQHLDYIT  298

mouse  EKLWRNAFLAGAVPVVLGPDRANYERFVPRGAFIHVDDFPNAASLAAYLLFLDRNVAVYR  353
human  EKLWRNALLAGAVPVVLGPDRANYERFVPRGAFIHVDDFPSASSLASYLLFLDRNPAVYR  358

mouse  RYFRWRRSFAVHITSFWDEQWCRTCQAVQTSGDQPKSIHNLADWFQR              400
human  RYFHWRRSYAVHITSFWDEPWCRVCQAVQRAGDRPKSIRNLASWFER              405
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Fig. 6. **Sequence alignment of the mouse and human α-1,3-fucosyltransferases.** The human α-1,3-fucosyltransferase sequence (*23-25*) is cited from the literature. The amino acid residues of the mouse α-1,3-fucosyltransferase (mouse) conserved in the human α-1,3-fucosyltransferase (human) are denoted by asterisks. Residues *corresponding to the predicted signal*-anchor domain and the two consensus *N*-glycosylation sites are boxed.

bility, may be responsible for the low content of the transcript in F9 cells. It remains possible, however, that F9 cells have another α-1,3-fucosyltransferase, that is encoded by an as yet uncharacterized α-1,3-fucosyltransferase gene, and the latter one is principally involved in the synthesis of SSEA-1 in these cells.

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