Molecular Cloning and Expression of a Third Type of Rabbit
GDP-\(\alpha\)-L-Fucose:β-D-Galactoside 2-α-L-Fucosyltransferase*

Seiji Hitoshi§, Susumu Kusunoki, Ichiro Kanazawa, and Shuichi Tsuji†

From Molecular Glycobiology, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), Saitama 351-01, Japan and the Department of Neurology, Institute for Brain Research, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

Recent molecular investigation revealed that two closely related structural genes encode distinct GDP-α-l-fucose:β-D-galactoside 2-α-L-fucosyltransferases (α,1,2-fucosyltransferases). Some human cancer cells or tissues may express an aberrant α,1,2-fucosyltransferase other than H- and Secretor-type α,1,2-fucosyltransferase. However, definite evidence of the existence of a third type of α,1,2-fucosyltransferase has not been demonstrated. Here we report the molecular cloning of a third type of rabbit α,1,2-fucosyltransferase (RFT-III) from a rabbit genomic DNA library. The DNA sequence included an open reading frame coding for 347 amino acids, and the deduced amino acid sequence of RFT-III showed 59 and 80% identity with those of the previously reported two types of rabbit α,1,2-fucosyltransferase, RFT-I and RFT-II, respectively. COS-7 cells transfected with the RFT-III gene exhibited α,1,2-fucosyltransferase activity toward phenyl-β-Gal as a substrate. Neuro2a (a murine neuroblastoma cell line) cells transfected with the RFT-III gene expressed fucosyl GM1 (type 3 H) but not Ulex europaeus agglutinin-1 lectin reactive antigens (type 2 H). Kinetic studies revealed that RFT-III exhibits higher affinity to types I (Gal/β1,3GlcNAc) than to type 2 (Gal/β1,4GlcNAc) oligosaccharides, which suggests that RFT-III as well as RFT-II is a Secretor-type α,1,2-fucosyltransferase. RFT-III was expressed in the adult gastrointestinal tract. The RFT-I, -II, and -III genes were assigned within 90 kilobases on pulsed field gel electrophoresis analysis. These results constitute direct evidence that, at least in one mammalian species, three active α,1,2-fucosyltransferases exist.

GDP-\(\alpha\)-fucose:β-D-galactoside 2-α-L-fucosyltransferase* catalyzes the fucosylation of terminal β-D-Gal residues and synthesizes H antigens. The activity of α,1,2-fucosyltransferase was detected in various tissues and body fluids of mammals that had several different kinetic characteristics (1–5). In humans, genetic and biochemical studies have indicated that two distinct but closely linked structural genes (H and Se) code α,1,2-fucosyltransferases with tissue-specific patterns (1, 6). The human gene controls the expression of H (Fucα1,2Galβ antigens (along with A or B antigens or both) on erythrocytes, whereas the Se gene determines the soluble A, B, and H antigens in secretory glands, and Lewisb blood group antigens on red cells (for review, see Ref. 7). Homozygosity for null alleles for the H and Se genes yields the rare Bombay blood type and non-Secretor phenotype, respectively. Recent molecular cloning of the H and Se genes provided the molecular basis for the Bombay and para-Bombay blood types and the non-Secretor phenotype, respectively, revealing point mutations within the coding regions that abolish the α,1,2-fucosyltransferase activity (8–10). On the other hand, the Lewis phenotype of erythrocytes and secretory glands revealed the Le(a+b+) and partial Secretor phenotypes in selected Polynesian and Asian individuals (11, 12). These phenotypes, which are virtually absent in Caucasians, are thought to be caused by weak Se phenotype α,1,2-fucosyltransferase activity. The molecular basis of weak Secretor phenotypes, whether weak Se phenotype α,1,2-fucosyltransferase is encoded by an altered Se gene or a gene other than H and Se, has yet to be determined.

Recently, aberrant α,1,2-fucosyltransferase activity, which synthesized Leβ from Lea or Leb from the Leα determinant, or both, was found in cancer cells and tissues, suggesting the possibility of a third distinct α,1,2-fucosyltransferase gene (13, 14). In the rabbit, the possibility of a third type of α,1,2-fucosyltransferase was suggested by immunohistochemical studies on DRG neurons. We recently cloned two types of rabbit α,1,2-fucosyltransferase, RFT-I and RFT-II, showing that RFT-I but not RFT-II is expressed in postnatal rabbit brain (15). RFT-I shows comparable affinities to types 1, 2, and 3 acceptors, which suggests that the binding specificity of RFT-I is primarily restricted to the terminal β-D-Gal residues of acceptors. In rabbit DRG neurons, fucosyl GM1 (type 3 H) is readily detected immunohistochemically on embryonic day 25, followed by the appearance of UEA-1 lectin-reactive antigens (type 2 H) postnatally (16, 17). UEA-1 lectin-reactive antigens of DRG neurons in postnatal rabbits could be formed through fucosylation catalyzed by RFT-I. In contrast, fucosyl GM1 observed in DRG neurons from embryonic day 25 rabbits might not be the product of RFT-I because UEA-1 lectin-reactive antigens are not detected at that stage. This observation suggests the existence of another type of α,1,2-fucosyltransferase that catalyzes preferential fucosylation to type 3 rather than type 2 glycochains.

Here we report the molecular cloning of a third type of rabbit α,1,2-fucosyltransferase, which could synthesize fucosyl GM1. This is the first direct evidence that, at least in one mammalian species, three active α,1,2-fucosyltransferases exist.
Two bands on rabbit genomic Southern blotting. Another 2 using PC/Gene (Teijin System Technology, Osaka, Japan). Termination method using an Autocycle DNA sequencing kit and an A.L.F. DNA sequences were determined by the dideoxynucleotide chain-termination enzymes and then subcloned into vector plasmid pUC119. The homogeneity of plaques of the rabbit genomic DNA library were screened and a plaque of the rabbit genomic DNA library was fractionated on a denaturing formaldehyde-agarose gel (1.2%) and then digested with 0.2 mg/ml proteinase K and 1% Sarkosyl. Digestion of DNA suspended in agarose blocks was carried out using 10 units of EcoRI; Cedarlane, Hornby, Canada), suspended in agarose blocks, and then digested with 0.2 mg/ml proteinase K and 1% Sarkosyl. Digestion of DNA suspended in agarose blocks was carried out using 10 units of restriction endonucleases in appropriate restriction buffers at 37 °C for 3 h. Pulsed field gel electrophoresis was performed using pulse times of 0.2–18 s linearly ramped at 200 V for 18 h. After electrophoresis, the gels were stained with ethidium bromide to visualize size markers.

Pulsed Field Gel Electrophoresis and Northern Hybridization—Rabbit lymphocytes were prepared by density gradient (Lymphohyte-Rabbit; Cedarlane, Hornby, Canada), suspended in agarose blocks, and then digested with 0.2 mg/ml proteinase K and 1% Sarkosyl. Digestion of DNA suspended in agarose blocks was carried out using 10 units of restriction endonucleases in appropriate restriction buffers at 37 °C for 3 h. Pulsed field gel electrophoresis was performed using pulse times of 0.2–18 s linearly ramped at 200 V for 18 h. After electrophoresis, the gels were stained with ethidium bromide to visualize size markers followed by alkali transfer onto nylon membranes (Nytran; Schleicher & Schuell). Hybridization was performed in 6× SSC, 5× Denhardt’s, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA at 65 °C.

Total RNA was prepared by the guanidium thiocyanate method and subcloning of polymerase chain reaction fragments (229 base pairs) used as probes were described previously (15). We used the polymerase chain reaction fragments as probes for further cloning of α1,2-fucosyltransferase, because these probes hybridized to more than two bands on rabbit genomic Southern blotting. Another 2×10⁶ plaques of the rabbit genomic DNA library were screened and a positive plaque containing a 16.8-kb (RG193) insert was isolated to homogeneity.

The RG193 DNA fragments were digested with appropriate restriction enzymes and then subcloned into vector plasmid pUC119. The DNA sequences were determined by the dideoxynucleotide chain-termination method using an Autocycle DNA sequencing kit and an A.L.F. DNA sequencer (Pharmacia Biotech Inc.). The sequences were analyzed using PC/Gene (Teijin System Technology, Osaka, Japan).

Pulsed Field Gel Electrophoresis and Northern Hybridization—Rabbit lymphocytes were prepared by density gradient (Lymphohyte-Rabbit; Cedarlane, Hornby, Canada), suspended in agarose blocks, and then digested with 0.2 mg/ml proteinase K and 1% Sarkosyl. Digestion of DNA suspended in agarose blocks was carried out using 10 units of restriction endonucleases in appropriate restriction buffers at 37 °C for 3 h. Pulsed field gel electrophoresis was performed using pulse times of 0.2–18 s linearly ramped at 200 V for 18 h. After electrophoresis, the gels were stained with ethidium bromide to visualize size markers followed by alkali transfer onto nylon membranes (Nytran; Schleicher & Schuell). Hybridization was performed in 6× SSC, 5× Denhardt’s, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA at 65 °C.

Total RNA was prepared by the guanidium thiocyanate method and purified by ultracentrifugation through 5.7 M CsCl. Poly(A)-rich RNA was purified with Oligotex-dT30 (Takara). The poly(A)-rich RNA (5 μg) was fractionated on a denaturing formaldehyde-agarose gel (1.2%) and then transferred onto a nylon membrane. Northern filters were hybridized in 50% formamide, 5× saline/sodium/sulfate/EDTA, 5× Denhardt’s, 0.5% SDS, 0.25% sodium lauryl Sarkosyl, and 100 μg/ml denatured salmon sperm DNA at 37 °C. To quantify the RNA loading, the Northern filters were rehybridized with a labeled cDNA fragment of rabbit glyceraldehyde-3-phosphate dehydrogenase.

All probes were labeled with [α-³²P]dCTP, using the random priming method. The gene probes used for hybridizations were a 1.8-kb SalI-SacI fragment from 3′-UTR of RFT-I, a 0.7-kb SacI-PstI fragment from 5′-UTR of RFT-II (15), a 0.6-kb SacI-EcoRI fragment from 3′-UTR of RFT-III for pulsed field gel electrophoresis, and a 0.3-kb PstI-NaeI fragment from 5′-UTR and the coding region of RFT-III for Northern blotting.

Expression of α1,2-Fucosyltransferase (RFT-III)—A 1.3-kb PstI fragment of RG193 DNA containing the full open reading frame of RFT-III was ligated into mammalian expression vector pcD-SRα (19), yielding pcD-SRα-RFT-III. The single insertion in the correct orientation was finally analyzed with restriction enzymes.

Neuro2a (a murine neuroblastoma cell line) cells were transiently transfected with pcD-SRα-RFT-III by means of LipofectAMINE according to the manufacturer’s instructions. The cells were trypsinized and divided into several dishes at 24 h post-transfection. The cells from one dish were stained with FITC-labeled-UEA-1 lectin or anti-fucosyl GM1 antibody at 72 h post-transfection. After washing with PBS, the cells were fixed with formaldehyde for 3 min, washed, and then incubated in 1% bovine serum albumin/PBS. After washing briefly with PBS, the cells were incubated in 2 ng/ml FITC-labeled-UEA-1 lectin in 1% bovine serum albumin/PBS for 1.5 h or in anti-fucosyl GM1 monoclonal antibody for 1.5 h and then washed with PBS, followed by incubation with FITC-labeled anti-mouse Ig (G and M) for 1 h. After washing three times with PBS, the cells were observed under a fluorescence microscope. The cells from another dish were trypsinized, fixed with formaldehyde, and stained as above for flow cytometry analysis using FACSCalibur (Becton Dickinson). Transfection of the gene was verified by measuring fucosyltransferase activity using cell extract from another dish.

COS-7 cells (60-mm culture dish) were transiently transfected with 5 μg of pcD-SRα-RFT-III using the DEAE-dextran procedure (20). The cells were washed with PBS, washed with 25 ml 4-morpholineethanesulfonic acid for 10 min, and then collected with a rubber policeman and pelleted by centrifugation. The pellets were resuspended in 100 μl of cold 1% Triton X-100 and then sonicated briefly.

Fucosyltransferase Assay—The fucosyltransferase assays were performed according to previous reports (15, 21) in a mixture of 25 mM sodium phosphate (pH 6.1), 5 mM ATP, 30 μM GDP-fucose, 3 μM GDP-[¹⁴C]fucose (10.5 GBq/mmol), the enzyme solution, and substrates in a final volume of 10 μl. Each reaction mixture for oligosaccharide acceptors was incubated at 37 °C for 2 h and then applied to a Silica Gel 60 high performance thin layer chromatography plate (Merck). The plate was developed with ethanol/pyridine-1-butanol-water/acetic acid (100:10:10:30). When glycolipids were used as substrates, the reaction mixture was applied to a C-18 Sep-Pak cartridge (Waters-Millipore, Milford, MA) after 2 h of incubation at 37 °C, washed with 2 ml of water, and then eluted with 1 ml of methanol. The eluate was then applied to a high performance thin layer chromatography plate, which was developed with chloroform/methanol/0.5% CaCl₂ (55:45:5). For glycoprotein acceptors, the reaction was terminated by the addition of 10 μl of SDS-polyacrylamide gel electrophoresis loading buffer after 2 h of incubation at 37 °C, and the incubation mixtures were directly subjected to SDS-polyacrylamide gel electrophoresis. The radioactivity on each plate and gel was visualized and determined with a BAS2000 radionuclide analyzer (Fuji Film, Tokyo, Japan).

RESULTS

Cloning and Nucleotide Sequence of a Third Type of Rabbit α1,2-Fucosyltransferase—On screening of a rabbit genomic...
DNA library with polymerase chain reaction fragments (229 base pairs) as probes, one positive clone, RG193, was obtained (Fig. 1). Sequence analysis revealed that RG193 contained an entire open reading frame with three closely located in-frame methionine codons in the starting region (Fig. 2A). The last of the three methionine codons was a good candidate for an initiator, accompanied by the Kozak consensus translation initiation sequence. The open reading frame beginning with the last methionine codon encodes 347 amino acids with a predicted molecular mass of 39 kDa (RFT-III). The deduced amino acid sequence contains a putative transmembrane region in the NH2-terminal region, indicating RFT-III is a type 2 protein, as is usual with glycosyltransferases. RFT-III contains three potential N-linked glycosylation sites, two of which are commonly observed among rabbit and human α1,2-fucosyltransferases. Comparison of the primary structure of RFT-III revealed significant (59 and 80%) amino acid identity with those of RFT-I and RFT-II, respectively. The putative extracellular region composed of the glycine residue at position 66 to the alanine residue at position 339 of RFT-III was highly conserved among not only rabbit but also human α1,2-fucosyltransferases (Fig. 2B), suggesting that this region is an active domain. In particular, this region was highly conserved among RFT-II and RFT-III, containing a 515-base-pair stretch of identical bases with two exceptions. The deduced amino acid identities between rabbit and human α1,2-fucosyltransferases are summarized in Table I. In this case, the amino acids were translated from the Sec1 DNA sequence (10), correcting the frameshift that disrupted the open reading frame.
The deduced amino acid identities of the entire sequence, accompanied by the putative cytoplasmic, transmembrane, and stem/active domains in parentheses, are shown. Amino acid length and calculated molecular weight of polypeptide deduced from each gene are also shown. The deduced amino acids for Sec1 were translated, correcting the frameshift that disrupts the reading frame of Sec1 and yielding a long reading frame appropriately corresponding to other α1,2-fucosyltransferases.

|          | Rabbit | Human |
|----------|--------|-------|
|          | M_r  | (FUT1)$^a$ | Se(Sec2)$^b$ | Sec1$^b$ |
| RFT-I(373 aa) | 42,098 | 80 (59/88) | 59 (10/70) | 53 (17/64) |
| RFT-II(354 aa) | 40,082 | 54 (12/68) | 74 (28/86) | 76 (70/79) |
| RFT-III(347 aa) | 39,469 | 59 (12/70) | 83 (54/89) | 67 (22/79) |

$^a$ DNA sequence from Ref. 8.
$^b$ DNA sequence from Ref. 10.
$^c$ DNA sequence from Ref. 15.

aa, amino acid.

**RFT-I, RFT-II, RFT-III**

*Fig. 3. Pulsed field gel electrophoresis analysis.* Sequential hybridization with RFT-I, -II, and -III gene probes on pulsed field gel electrophoresis blots separating DNA fragments. All probes recognized a common Sall (90 kb) restriction fragment, and the RFT-II and -III gene probes recognized common NotI (100 kb) and common Mulu (105 kb) restriction fragments.

**Fig. 4. Northern blot analysis of RFT-III.** Poly(A)-rich rabbit RNAs (5 μg) were electrophoresed on a denaturing formaldehyde-agarose gel, transferred onto a nylon membrane, and hybridized with the 0.3-kb PstI-Nad fragment of RFT-III (upper panel). The lower panel shows the same blot rehybridized with a labeled rabbit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe.

**Fig. 5. Expression of fucosyl GMI on RFT-III transfected Neuro2a cells.** A, Neuro2a cells were transiently transfected with 5 μg of pcD-SRa or pcD-SRa-RFT-I, -II, or -III using LipofectAMINE. The cells were stained with FITC-labeled UEA-1 lectin or anti-fucosyl GMI monoclonal antibody followed by FITC-labeled anti-mouse Ig (G and M) at 72 h post-transfection and observed under fluorescent microscopy. B, transfected Neuro2a cells from another dish were trypsinized, stained as above, and subjected to flow cytometry. Transfection of RFT-I, -II, or -III was verified by measuring fucosyltransferase activity using the cell extract from another dish.
Northern blot analysis revealed that a 1.8-kb mRNA of RFT-III was expressed in the adult gastrointestinal tract (Fig. 4). The expression of minor 5.0- and 6.4-kb mRNAs of RFT-III was also observed in the adult colon. Other secretory glands such as salivary and mammary glands did not express RFT-III.

Expression of RFT-III and Enzyme Assaying—Neuro2a cells transiently transfected with pcDNA-RFT-I, -II, and -III showed a 1,2-fucosyltransferase activity of 1.93, 0.12, and 0.52 pmol/h/μl of enzyme, respectively, whereas parent Neuro2A cells contained no activity. Neuro2A cells transfected with RFT-

### Table II

| Acceptor                  | RFT-III | RFT-I | RFT-II |
|---------------------------|---------|-------|--------|
|                           | K_m (mM) | V_{max}/K_m (×10^{-11} l/min) | K_m (mM) | V_{max}/K_m (×10^{-11} l/min) | K_m (mM) | V_{max}/K_m (×10^{-11} l/min) |
| Phenyl-β-D-Gal            | 5.7     | 0.82  | 4.0    | 2.3    | 17.1  | 0.13  |
| Galβ1,3GlcNAc             | 1.5     | 0.49  | 3.1    | 11.4   | 2.2   | 0.36  |
| Galβ1,4GlcNAc             | 6.7     | 0.65  | 4.2    | 8.9    | 12.5  | 0.21  |
| Galβ1,3GalNAc             | 1.0     | 0.75  | 3.5    | 5.8    | 4.6   | 0.50  |
| Lacto-N-tetraose          | 1.6     | 0.30  | 3.5    | 3.0    | 4.8   | 0.51  |
| Lacto-N-neotetraose       | 4.2     | 0.23  | 5.5    | 1.2    | 12.5  | 0.29  |

Relative activity

| Acceptor                  | RFT-III | RFT-I | RFT-II |
|---------------------------|---------|-------|--------|
|                           | %       | %     | %      |
| Phenyl-β-D-Gal (25 mM)    | 100 (1.70)a | 100 (3.38)b | 100 (0.21)b |
| Asialofetuin (1 mg/ml)    | 2.7     | 2.4   | ND     |
| Asialo-α1-acid glycoprotein (1 mg/ml) | 0.6 | 0.9 | ND |
| G_{α1} (2 mM)             | 31.0    | 19.7  | 15.7   |
| Paragloboside (2 mM)      | 5.6     | 23.0  | ND     |
| Lacto-N-fucopentaose II (2 mM) | 3.0 | 2.3 | 13.8 |
| Lacto-N-fucopentaose III (2 mM) | 2.0 | 1.1 | ND |

a From Ref. 15.
b The actual activities (pmol/h/μl of enzyme) of fucosyltransferases are shown in parentheses. ND, not detected.

**Fig. 6. Lineweaver-Burk plots.** Lineweaver-Burk plots used to calculate the K_m and V_{max}/K_m values are shown. Experiments were performed in triplicate and typical plots are shown.

**Phenyl-β-D-Gal**

**Biose**

**Tetraose**

Expression of RFT-III and Enzyme Assaying—Neuro2A cells transiently transfected with pcDNA-RFT-I, -II, and -III showed α1,2-fucosyltransferase activity of 1.93, 0.12, and 0.52 pmol/h/μl of enzyme, respectively, whereas parent Neuro2A cells contained no activity. Neuro2A cells transfected with RFT-
a1,2-fucosyltransferase.

III expressed fucosyl GM1 but not UEA-1 lectin-reactive antigens (Fig. 5, A and B), which suggested that the binding specificity of RFT-III differed between type 2 and type 3 glycochains or between glycoproteins and glycolipids. Neuro2a cells transfected with RFT-I expressed both fucosyl GM1 and UEA-1 lectin-reactive antigens, and Neuro2a cells transfected with RFT-II expressed neither of them (Fig. 5, A and B). Cell extracts of COS-7 cells transfected with pcD-SRα-RFT-III contained a1,2-fucosyltransferase activity that transferred radio-labeled fucose to phenyl-β-D-Gal as a substrate. As shown in Table II and Fig. 6, the acceptor specificity of RFT-III was comparable with that of RFT-II. RFT-III showed higher affinity for types 1 and 3 acceptors than for type 2 acceptors and phenyl-β-D-Gal, like RFT-II and human Se-type a1,2-fucosyltransferase. RFT-III could transfer fucose to asialofetuin and asialo-α1-acid glycoproteins as well as GM1 ganglioside. The relative activity of RFT-III toward GM1, as to glycoproteins was higher than that of RFT-I. RFT-III could also fucosylate lacto-N-fucopentaoaxes II and III, α1,3- and α1,4-fucosylated oligosaccharides, respectively.

DISCUSSION

In this and previous work (15) we reported the molecular cloning of three types of rabbit α1,2-fucosyltransferase, RFT-I, -II, and -III. These results constitute direct evidence that, at least in one mammalian species, three active α1,2-fucosyltransferases exist, one H type and two Se types, based on kinetic analysis.

RFT-I exhibits comparable kinetic properties and significant structural homology with human H-type α1,2-fucosyltransferase, indicating that RFT-I is a counterpart of human H. RFT-II and -III show higher affinity to types 1 and 3 acceptors than to type 2 acceptors and phenyl-β-D-Gal. The kinetic parameters of RFT-II and -III are comparable with those of human Se-type α1,2-fucosyltransferase (2, 3, 5). RFT-II and -III genes share remarkably conserved base pair sequence in the putative active domain (95%) as compared with the RFT-I gene. RFT-II and -III are thought to constitute Se-type α1,2-fucosyltransferase family. A recent report (10) of the molecular cloning of human Se gene enables us to compare it with RFT-II and -III genes, as it was revealed that RFT-III exhibits higher amino acid identity with human Se than RFT-II. These findings led us to conclude that RFT-III is a counterpart of the human Se gene.

The RFT-I, -II, and -III genes are assigned within approximately 90 kb, both RFT-II and -III genes being located in 3' region of RFT-I, based on the results of pulsed field gel electrophoresis. The physical relationship of the RFT-I, -II, and -III genes is consistent with that of the human H, Se, and Sec1, α1,2-fucosyltransferase-related pseudogenes (22). These results suggest that RFT-II corresponds to the human Sec1 gene. This idea is further supported by the structural analysis showing that the putative cytoplasmic, transmembrane, and stem domains of RFT-II and human Sec1 are well conserved as compared with other α1,2-fucosyltransferases (Table I). In this case, an ancestral Se gene is thought to have been duplicated into two related genes, one of which was subsequently inactivated by the frameshift mutations in humans. Site-directed point mutation analysis of the Sec1 gene, which corrects the frameshift, and kinetic studies on α1,2-fucosyltransferase activity of the mutants will provide further information on the relationship between RFT-II and Sec1. It is difficult to know the exact correspondence of recently cloned fragment of rat or porcine α1,2-fucosyltransferases (23, 24) to RFT-I, -II, or -III, because all α1,2-fucosyltransferases, including the human and rabbit H type and Se types, exhibit high homology.

It is interesting to consider that RFT-II represents a weak Se-type α1,2-fucosyltransferase. The weak Se-type α1,2-fucosyltransferase was postulated based on the results of Lewis phenotype analysis in Polynesian people. The Lewis antigens on erythrocytes are regulated by two fucosyltransferases, Se-type α1,2- and Lewis α1,3/4-fucosyltransferases. With the conventional analysis method, three Lewis phenotypes of erythrocytes were found in Caucasian adults, Le(a−b−), Le(a+b−), and Le(a−b+). When Se-type α1,2-fucosyltransferase is active (or in Secreto), most of the type 1 precursor is converted into type 1 H, which can be transformed into Leb by Lewis α1,3/4-fucosyltransferase. On the other hand, a fourth Lewis phenotype, Le(a+b−), was found on erythrocytes from selected Polynesian individuals. In addition, low levels of salivary ABH antigens, that is partial secretion, were found in saliva from Le(a+b−) and Le(a−b+) individuals, suggesting the presence of a weak Se-type α1,2-fucosyltransferase (11, 12). Molecular analysis of the Se and Sec1 genes of Polynesian people, especially of partial Secreto individuals, will facilitate determination of whether or not RFT-II corresponds to the human Sec1 gene.

The fucosyltransferase assay showed that the relative activity of RFT-III toward GM1, as to glycoproteins was higher than that of RFT-I. RFT-III could also synthesize fucosyl GM1 from GM1 but not UEA-1 reactive antigens when expressed in Neuro2a cells, where RFT-I could form both and RFT-II could synthesize neither under the same transfection conditions. RFT-III is a good candidate for the enzyme that synthesizes fucosyl GM1 expressed in a subpopulation of neurons of rabbit embryonic DRG (16, 17), although we could not detect the expression of RFT-III in embryonic brain. It is possible that the expression of RFT-III is restricted to specific regions or specific types of neurons. In situ hybridization analysis will provide further information.

Recently, aberrant α1,2-fucosyltransferase activity that synthesized Leb from Lea or Leb from the Leα determinant, or both, was found in cancer cells or tissues (13, 14). The classical models assume that Leb and Leα determinants are synthesized through the sequential actions of α1,2- and α1,3/4-fucosyltransferases through H determinants. In this case, α1,2-fucosyltransferase is not postulated to catalyze the fucosylation of Lea or Leα determinants. Accordingly, the α1,2-fucosyltransferase activity that formed Leb from Leα or Leb from the Leα determinant, or both, was supposed to represent an aberrant or new enzyme. In this study, however, we demonstrated that enzyme preparations from COS-7 cells transfected with rabbit α1,2-fucosyltransferases contained activity that fucosylated Leb or Leα or both determinants. Cancer cells or tissues of gastrointestinal origin might, we think, express an unusually large amount of H or Se α1,2-fucosyltransferase but not express an unusual α1,2-fucosyltransferase.

Bombay individuals who lack active H and Se genes but who show no apparent abnormal phenotype cast doubt on the physiological role of α1,2-fucosylation of glycoconjugates. However, it remains possible that another α1,2-fucosyltransferase may operate at specific developmental stages or in restricted tissues or regions. This possibility was increased by the present study, in which we showed that at least in one mammalian species three active α1,2-fucosyltransferases exist.

REFERENCES

1. Beyer, T. A., and Hill, R. L. (1980) J. Biol. Chem. 255, 5373-5379
2. Kuriyama, T., and Yoshida, A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4193-4197
3. Le Pendu, J., Cartron, J. P., Lemieux, R. U., and Oriol, R. (1985) Am. J. Hum. Genet. 37, 749-760
4. Sarnesto, A., Köhlin, T., Thunin, J., and Blaszczyk-Thurin, M. (1990) J. Biol. Chem. 265, 15067-15075
5. Sarnesto, A., Köhlin, T., Hindsgaul, O., Thunin, J., and Blaszczyk-Thurin, M. (1992) J. Biol. Chem. 267, 2737-2744
6. Le Pendu, J., Lemieux, R. U., Lambert, F., Dalix, A.-M., and Oriol, R. (1982)
Third Type of Rabbit $\alpha_{1,2}$-Fucosyltransferase

16981

Am. J. Hum. Genet. 34, 402–415
7. Watkins, W. M. (1980) Adv. Hum. Genet. 10, 1–136
8. Larsen, R. D., Ernst, L. K., Nair, R. P., and Lowe, J. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6674–6678
9. Kelly, R. J., Ernst, L. K., Larsen, R. D., Bryant, J. G., Robinson, J. S., and Lowe, J. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5843–5847
10. Kelly, R. J., Rouquier, S., Giorgi, D., Lennon, G. G., and Lowe, J. B. (1995) J. Biol. Chem. 270, 4640–4649
11. Henry, S. M., Berry, A. G., and Woodfield, D. G. (1990) Vox Sang. 58, 61–66
12. Henry, S. M., Oriol, R., and Samuelsson, B. E. (1994) Glycoconj. J. 11, 593–599
13. Blaszczyk-Thurin, M., Sarnesto, A., Thurin, Y., Hindsgaul, O., and Koprowski, H. (1988) Biochem. Biophys. Res. Commun. 151, 100–108
14. Yazawa, S., Nakamura, J., Asao, T., Nagamachi, Y., Sagi, M., Matta, K. L., Tachikawa, T., and Akamatsu, M. (1993) Jpn. J. Cancer Res. 84, 989–995
15. Hitoshi, S., Kusunoki, S., Kanazawa, I., and Tsuji, S. (1995) J. Biol. Chem. 270, 8844–8850
16. Kusunoki, S., Inoue, K., Iwamori, M., Nagai, Y., Mannen, T., and Kanazawa, I. (1992) Neurosci. Res. 15, 74–80
17. Kusunoki, S., Chiba, A., Shimizu, T., and Kanazawa, I. (1994) Biochim. Biophys. Acta 1234, 27–31
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Takebe, Y., Seki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988) Mol. Cell. Biol. 8, 466–472
20. McCutchan, J. H., and Pagano, J. S. (1968) J. Natl. Cancer Inst. 41, 351–357
21. Rajan, V. P., Larsen, R. D., Ajmara, S., Ernst, L. K., and Lowe, J. B. (1989) J. Biol. Chem. 264, 11158–11167
22. Rouquier, S., Lowe, J. B., Kelly, R. J., Fertitta, A. L., Lennon, G. G., and Giorgi, D. (1995) J. Biol. Chem. 270, 4632–4639
23. Piau, J.-P., Labarriere, N., Dabouis, G., and Denis, M. G. (1994) Biochem. J. 300, 623–626
24. Thurin, J., and Blaszczyk-Thurin, M. (1995) J. Biol. Chem. 270, 26577–26580
25. Svennerholm, L. (1980) Adv. Exp. Biol. Med. 125, 533–544