Molecular Cloning and Expression of a Bovine α(1,3)-Fucosyltransferase Gene Homologous to a Putative Ancestor Gene of the Human FUT3-FUT5-FUT6 Cluster

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Only one bovine gene, corresponding to the human cluster of genes FUT3-FUT5-FUT6, was found by Southern blot analysis. The cognate bovine α(1,3)-fucosyltransferase shares 67.3%, 69.0%, and 69.3% amino acid sequence identities with human FUC-T3, FUC-T5, and FUC-T6 enzymes, respectively. As revealed by protein sequence alignment, potential sites for asparagine-linked glycosylation and conserved cysteines, the bovine enzyme is an intermediate between FUC-T3, FUC-T5, and FUC-T6 human enzymes. Transfected into COS-7 cells, the bovine gene induced the synthesis of an α(1,3)-fucosyltransferase enzyme with type 2 substrate acceptor pattern specificity and induced expression of fucosyltransferase family (FUC-T3, FUC-T5, and FUC-T6) human enzymes. Transfected into COS-7 cells, the bovine gene induced the synthesis of an α(1,3)-fucosyltransferase enzyme with type 2 substrate acceptor pattern specificity and induced expression of fucosylated type 2 epitopes (Leα and sialyl-Leα), but not of type 1 structures (Leα or sialyl-Leα), suggesting that it has an acceptor specificity similar to the human plasma FUC-T6. However, no enzyme activity was detected in bovine plasma. Gene transcripts are detected on tissues such as bovine liver, kidney, lung, and brain. The type 2 sialyl-Leα epitope was found in renal macula densa and biliary ducts, and Leα and Leα epitopes were detected on the brush border of epithelial cells of small and large intestine, suggesting a tissue distribution closer to human FUC-T5, but fucosylated type 1 structures (Leα, Leβ, or sialyl-Leα) were not detected at all in any bovine tissue.

Cell surface fucosylated oligosaccharides have received a substantial amount of attention because they play a role in inflammation-mediated cell adhesion and are frequently modified in malignant cells (1–5). The biosynthesis of these glycoconjugates requires the ordered action of several glycosyltransferases, of which fucosylation is the last step (6).

Five human α(1,3)-fucosyltransferase genes have been cloned as follows: FUT3 encodes the Lewis α(1,3,1,4)-fucosyltransferase or FUC-T3 enzyme (7–10), FUT4 encodes the myeloid α(1,3)-fucosyltransferase or FUC-T4 enzyme (11, 12), FUT5 encodes an unspecified type of α(1,3)-fucosyltransferase called FUT5 (13), FUT6 encodes the plasma α(1,3)-fucosyltransferase or FUC-T6 (14, 15), and FUT7 encodes the leukocyte α(1,3)-fucosyltransferase or FUC-T7 (16, 17). Three out of these five genes (FUT3, FUT5, and FUT6) constitute a cluster within 1 centimorgan on human chromosome 19p13.3 (18, 19) and share more than 90% sequence identity (14, 20). The individual members of the human α(1,2)-fucosyltransferase family, H1 (21, 22) and Se (23, 24) and the human α(1,3)-fucosyltransferase family (FUC-T3, FUC-T4, FUC-T5, FUC-T6, and FUC-T7), are discriminated by differences in substrate specificities, cation requirements, sensitivity to inhibitors, and tissue distribution (25–28). Besides humans and to a lesser extent mice (29), little is known about the molecular mechanisms that determine the tissue-specific (30) and the developmentally regulated expression patterns of fucosyltransferases (31–34).

Previous histochemical data having revealed the absence of α(1,4)-fucosylated structures and the prevalence of αGal-type 2 epitope, on bovine red cells and tissues (35), we chose to isolate and characterize the bovine genes homologous to the human FUT3-FUT5-FUT6 cluster to identify the enzymes and glycoconjugate epitopes present in this species. Only one gene, named futb, homologous to the three genes of the human cluster was detected on genomic DNA. We also observed the corresponding mRNA transcript on different bovine tissues such as

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) X87810.

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liver, kidney, brain, and lung. The predicted sequence of the cognate enzyme has a transmembrane type II topology and presents a high degree of identity with the three human enzymes. Nevertheless, the bovine enzyme has a type 2 acceptor substrate specificity, like human FUC-T6, as demonstrated by (i) type 2 acceptor specificity of the activity detected in homogenates of COS-7 cells transfected with futb, (ii) immunofluorescence detection of type 2 α(1,3)-fucosylated epitopes (Lea and sialyl-Lea) on COS-7 cells transfected with futb, and (iii) the presence of fucosylated type 2 epitopes (Lea, Le, and sialyl-Lea) on normal bovine tissues.

The position of the bovine gene on the mammalian phylogenetic tree of fucosyltransferase genes suggests that this gene may be the orthologous homologue of an ancestor gene, from which has derived the present human FUT3-FUT5-FUT6 cluster of genes.

**EXPERIMENTAL PROCEDURES**

**Nomenclature**—The gene described represents the first bovine fucosyltransferase gene. It will be designated fut and the cognate α(1,3)-fucosyltransferase enzyme Futb.

**PCR Amplification of the Bovine fut Probe**—PCR was performed as described previously (36) in a mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9 at 25°C), 1.5 mM MgCl2, 150 μM each of dATP, dCTP, dGTP, and dTTP, 50 ng of DNA, 100 pmol of PCR primers (Table 1), and 1.25 units of Taq DNA polymerase (Promega) in a total volume of 25 μl. After heating at 95°C for 4 min, 35 cycles were performed (denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min). The PCR product was analyzed on 1.4% agarose gel electrophoresis. The 459-bp band was eluted from the gel, cloned into the Smal site of pBluescript II KS+ (Stratagene), and sequenced.

**Bovine Genomic Library Screening**—Approximately 2 × 108 bacterial colonies representing more than four bovine genomes (37), with recombinant cosmids bearing bovine DNA fragments of 35-klb base average size (bovine genomic library prepared from semen DNA, Stratagene), were screened. Filters were prehybridized at 4°C for 1.4% agarose gel electrophoresis. The 459-bp band was eluted from the gel, cloned into the Smal site of pBluescript II KS+ (Stratagene), and sequenced.

**Reverse Transcriptase-PCR Detection of futb Transcripts**—All cDNAs were synthesized using 1 μg of poly(A) RNAs (CLONTECH) and the Marathon cDNA Amplification Kit (CLONTECH). First strand synthesis was made with the Moloney murine leukemia virus reverse transcriptase and a modified oligo(dT) primer containing two degenerate nucleotide positions at the 3’ end: 5’-TTCTAGAGTACCCGCGCGCG(T)30N3-3’. These nucleotides position the primer at the start of the poly(A) tail and thus eliminate the 3’ heterogeneity inherent to conventional oligo(dT) priming (39, 40). Second strand synthesis was performed (41) with a mixture of Escherichia coli DNA polymerase I, RNase H, and E. coli DNA ligase (Clontech). One percent of each cDNA preparation was then amplified by a first PCR in the presence of the U1 and L1 primers (Table 1). Aliquots of each reaction were then amplified by a second PCR in the presence of U2 and L2 primers (Table 1). The [32P] PCR reaction mixture contained 2.5 nM each of dATP, dCTP, dGTP, and dTTP, 10 pmol of primers; 25 nmol of MgCl2, and 1.25 units of Taq DNA polymerase (Promega). The conditions for the first PCR reactions were as follows: one cycle of denaturation at 94°C for 2 min, annealing at 60°C for 15 s and extension at 72°C for 2 min, followed by 35 cycles; denaturation at 94°C for 10 s, annealing at 60°C for 15 s and extension at 72°C for 40 s, supplemented by 1 s at each cycle, plus a last extension at 72°C for 7 min. The second PCR reaction was started by denaturation at 94°C for 2 min, annealing at 68°C for 15 s, and extension at 72°C for 2 min, followed by 35 cycles; denaturation at 94°C for 10 s, annealing at 68°C for 15 s, and extension at 72°C for 40 s, supplemented with 1 s at each cycle and a last extension at 72°C for 7 min. Final products were analyzed in 1.5% agarose gel electrophoresis, and PCR segments were sequenced after gel extraction (PCR pure-bind Kit, CLONTECH).

**Transfection and Expression of the Bovine Fucosyltransferase Gene**—The 1213-bp AvrII-HindIII fragment from the pFL44-futb plasmid containing futb, was cloned between SacI and XbaI sites, into the pFL44 plasmid (Fig. 2A). After amplification in bacteria, the 1247-bp insert was isolated by EcoRI-HindIII digestion and cloned between EcoRI-HindIII sites into the mammalian expression plasmid pcDNA3/Amp (Invitrogen) (Fig. 2B). A plasmid containing a well oriented insert was selected and designated pcDNA3/Amp-fut. COS-7 cells were transiently transfected using DEAE-dextran (42) and expression incubation time of 48 h.

**Fucosyltransferase Enzyme Assay**—Transfected cells were homogenized at 4°C in 1% Triton X-100. Each assay contained in a total volume of 60 μl: 50 μg of the protein cell homogenate or 25 μl of plasma, 25 mM cacodylate buffer, pH 6.5, 4 mM ATP, 20 mM MnCl2, 10 mM MgCl2, 1 μM GDP, 1 μM [14C]fucose (Amersham Corp., 300 mCi/mmol), and 5 μl of 1 mg/ml solution of the different synthetic 8-methoxycarbonyl trisaccharide acceptors. The mixture was incubated for 2 h at 37°C, and the reaction was stopped by addition of 3 ml of water, centrifuged, and the supernatant applied to a conditioned Sep-Pak C18 reverse chromatography cartridge (Waters, Milford), attached to a 10-ml syringe. The unreacted [14C]fucose and its hydrolysis products were eluted with 25 ml of H2O, and the radiolabeled reaction products were eluted with two 5-ml portions of methanol collected directly into scintillation vials and counted with 1 volume of Instagel (Packard, IL) in a liquid scintillation beta counter (43).

**Synthetic Oligosaccharide Acceptors**—Trisaccharide acceptor substrates with the 8-methoxycarbonyl group, R = (CH2)5COOCH3, were obtained from Chembiochem (Alberta Research Council, Edmonton, Alberta, Canada).

The αGal-type 2, Galα1–3Galβ1–4GlcNAcβ-R was synthesized by incubating 10 mg of Galβ1–4GlcNAcβ-R with 35 millimolar of α(1,3)galactosyltransferase (44) and 4 mg of UDP-Gal in 30 mM sodium cacodylate buffer, pH 6.5, containing 0.1% Triton X-100 and 20 mM MnCl2 at 37°C for 48 h. After 3, 6, 24, and 28 h of incubation, an additional 4.3 mg of UDP-Gal donor was added to the reaction mixture. The product was isolated on three tandem Sep-Pak C18 cartridges, washed with 150 ml of water, and then eluted with 45 ml of methanol, which was evaporated to dryness and the residue chromatographed on an IATROBEAD column (21 × 180 mm) and washed with 60 ml of 4:1 dichloromethane/methanol to remove Triton X-100. The product was eluted with 65:35:2 dichloromethane/methanol/ethyl acetate to dryness (final yield: 12 mg of trisaccharide). The NMR spectrum of this product showed signals for the new anomeric H-1 proton of the αGal residue at δ 5.146 ppm (J = 4.0 Hz) (45).

**Cell Membrane Fluorescence Staining**—Transfected cells were trypsinized and distributed in 96-well conic-bottom microtiter plates (3 × 103 cells/well). Oligosaccharide epitopes were stained by 30 min incubation with first monoclonal antibodies (50 μl per well), washed
twice in phosphate-buffered saline, pH 7.5, and incubated for 30 min with fluorescein isothiocyanate-labeled sheep anti-mouse Ig's second antibodies (Pasteur Diagnostics, Marnes la Coquette, France). Each reaction was stopped by sucking off the reagent, after 10 min centrifugation of the plates at 2000 rpm, and then cells were resuspended and washed (3 × 3) in phosphate-buffered saline. Stained and washed cells were resuspended in 10 ml of phosphate-buffered saline/paraformaldehyde 4%. Then 5 µl of Mowiol 4:80 (Hoechst, Frankfurt, Germany) were added, and they were mounted under coverslides for observation, on a Leitz SM-Lux epifluorescence microscope. Positive and negative cells were counted with a 25 × oil-immersion NPL-fluotar objective.

Tissue Immunofluorescence Staining—Routine formalin-fixed/paraffin-embedded sections were deparaffinated and stained by indirect (monoclonal antibodies) or direct (lectin) immunofluorescence. They were incubated for 30 min in a wet chamber with the first monoclonal antibody, washed, and stained for 30 min with fluorescein isothiocyanate-labeled sheep anti-mouse Ig's second antibody (Pasteur Diagnostics, Marnes la Coquette, France). Direct staining was performed for 30 min with fluorescein isothiocyanate-labeled *Ulex europaeus* lectin 1 (UEA1) and fluorescein isothiocyanate-labeled *Griffonia simplicifolia* isoelectin 1-B4 (GSI-B4) (Vector Laboratories, Burlington, CA). Stained slides were washed again and mounted under coverslides with 1 drop of

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**FIG. 1.** Nucleotide sequence of the bovine α(1,3)-fucosyltransferase gene (*futb*) and comparison to human *FUT6*, *FUT5*, and *FUT3* sequences. The adenine residues of the putative initiation codons are assigned as residue 1. Dashed lines below the *futb* sequence alignments are indicated by dots and are not taken into account for nucleotide numbering. The catalytic domain *FUT3* (303 bp) and *futb* (301 bp) probes are indicated by open triangles and solid arrowheads on *FUT3* and *futb* sequences. The stem domain *futb* probe (459 bp) was obtained by PCR, with the thick underlined sense primer and the antisense primer, whose complementary sequence is stippled underlined (see Table I and “Experimental Procedures”).
EcoI plasmid pcDNAI/Amp between subcloned into the mammalian expression plasmid, designated pcDNAI/Amp-
isolated by infection experiments in COS-7 cells. Correct orientation, was selected for trans-
frame of entry containing the full open reading

FIG. 2. Subcloning of the coding region of futb into the mammalian expression plasmid pcDNAI/Amp. The 5.8-kilobase pair BamHI restriction fragment encompassing the potential futb structural gene was cloned into the plasmid pFL44 and was designated pFL44-
futb (A). The 1213-bp AvrII-SacI fragment containing the full open reading frame of futb was cloned into the pFL44 between SacI and XbaI sites. Then it was isolated by EcoRI-HindIII digestion and subcloned into the mammalian expression plasmid pcDNAI/Amp between EcoRI and HindIII sites (B). A representative plasmid, designated pcDNAI/Amp-
futb, containing a single insertion in the correct orientation, was selected for transfection experiments in COS-7 cells.

Mowiol 4:80 (34) and observed under the epifluorescence microscope.

Mouse Monoclonal Antibodies—Anti-Lea were obtained from Sigma (CD15), VaBlotech (SSEA1), and ChembioMed (82H15); anti-Leb (069, 070, 071), anti-A (013), and anti-B (026) were obtained from the Second International Workshop on Monoclonal Antibodies Against Human Red Blood Cells (46); anti-sialyl-Lea was from VaBlotech (RM93) and P. I. Terasaki (UCLA) (TT19A6); anti-Leb (75.12) was from P. Avner (Pasteur Institute, Paris, France); anti-Lea (7LE), anti-Leb (2.25LE), and anti-sialyl-Lea (19.9) were from J. Bara (INSERM U55, St. Antoine Hospital, Paris, France).

Molecular Phylogeny—Twelve selected complete coding sequences available from the GenBank/EMBL data base (Table II), were aligned with the Clustalw 1.5 program, and the genetic distances in the matrix were analyzed with the Phylip phylogeny package, using the Fitch-Margoliash (47) least square method with evolutionary clock.2 The phylogenetic tree was drawn from the Phylip dendrogram with the NJ method.2 The accession numbers in Table II correspond to the nucleotide sequences numbering of Fig. 1.

RESULTS

Molecular Cloning of a Bovine Fucosyltransferase Gene—Using the bovine futb probe (459 bp), obtained as described under “Experimental Procedures” and corresponding to the stem domain of FUT3, FUT5, and FUT6 human genes, the hybridization screening of a bovine genomic library for an

TABLE II

| Accession number | Species | Locus | DNA cd | Protein amino acids | Ref. | bp |
|------------------|---------|-------|--------|--------------------|------|----|
| M53531           | Human   | FUT1  | 1098   | 365                | 64   |    |
| U17894           | Human   | FUT2  | 999    | 332                | 24   |    |
| X53578           | Human   | FUT3  | 1086   | 361                | 7    |    |
| M8596            | Human   | FUT4  | 1218   | 405                | 11   |    |
| M81485           | Human   | FUT5  | 1125   | 374                | 13   |    |
| L01698           | Human   | FUT6  | 1080   | 359                | 14   |    |
| X75031           | Human   | FUT7  | 1029   | 342                | 16   |    |
| X900256          | Rabbit  | rufut2| 1122   | 373                | 65   |    |
| X900255          | Rabbit  | rufut2| 1065   | 354                | 65   |    |
| U33457           | Mouse   | mufut4| 1302   | 433                | 29   |    |
| U45990           | Mouse   | mufut7| 1170   | 389                | 66   |    |
| X87810           | Bovine  | futb  | 1098   | 365                | 64   |    |

2 All programs are available at CIS INFOBIOGEN, 7 rue Guy Moncquet, BP8, 94801 Villejuif Cedex, France; E-mail: bioinfo@infobiogen.fr; WEB: http://www.infobiogen.fr.
revealed that they share 67.3, 69, and 69.3% amino acid sequence identity, respectively (Fig. 3).

Seven cysteine residues are common to human FUC-T3, FUC-T5, and FUC-T6 (16); the bovine enzyme has these same 7 cysteines (Fig. 3), including the bovine Cys-147, which determines N-ethylmaleimide sensitivity (50). As expected, 90% of the Futb activity determined on type 2 acceptors was inhibited by pretreatment with 8 mM N-ethylmaleimide for 1 h at 37°C. Consequently, Futb behaves as FUC-T3, FUC-T5, and FUC-T6 and is different from the human myeloid (FUC-T4) and leukocyte (FUC-T7) enzymes, which are resistant to N-ethylmaleimide (26).

Two potential consensus sites for asparagine-linked glycosylation (bovine amino acids 158 and 189) are common to the bovine Futb and the human FUC-T3, FUC-T5, and FUC-T6 enzymes. Another bovine glycosylation site (Asn-100) is close to a similar glycosylation site in human FUC-T5 (Asn-105) and FUC-T6 (Asn-91). Finally, a fourth glycosylation site is only present in human FUC-T5 (Asn-46) and FUC-T6 (Asn-60) (14), and it is absent from human FUC-T3 and bovine enzymes (Fig. 3).

Protein sequence alignment of bovine Futb and human FUC-T3, FUC-T5, and FUC-T6 enzymes. Amino acid sequence identities are indicated with dashed lines and gaps with dots. The double underlined amino acids in the bovine α(1,3)-fucosyltransferase enzyme correspond to the putative transmembrane domain. Solid circles represent common cysteine residues. Solid triangles represent potential asparagine-linked glycosylation sites. The underlined sequence in the hyper-variable domain, between amino acids 115 and 155, corresponds to the sequence of subdomains 4 and 5 (51).

Southern Blot Analyses of Bovine Genomic DNA—FUT3 and futb catalytic domain probes were used to sample both bovine and human genomes for cross-hybridizing DNA sequences. Regardless of the restriction enzyme used, both probes identify the same three bands corresponding to human FUT3, FUT5, and FUT6 genes in human genomic DNA (14). Alternatively, but also regardless of the restriction enzyme used, the same two probes recognize only one band corresponding to the bovine gene identified above in bovine genomic DNA (Fig. 4A). As a control, the low stringency hybridization of bovine DNA with futb probe revealed a similar pattern (Fig. 4B and C). At these low stringencies two other bands were detected resulting from unspecific hybridization due to the high amount of repeated satellite DNA. The 1.4-kilobase pair band (Fig. 4B and C) corresponded to an already described bovine satellite (52), and the other around 1.8-kilobase pair (Fig. 4B) is not well characterized. Altogether, these results suggest that in the bovine genome, there is a single gene, futb, related to the human enzyme family of α(1,3)-fucosyltransferases.

Identification of mRNA Transcripts of futb—cDNAs obtained by reverse transcriptase-PCR on mRNA transcripts from bovine liver, kidney, lung, and brain were probed by nested PCR using specific primer pairs corresponding to the futb stem domain (Table I and Fig. 1). In all cases, the clear-cut amplified band of 333 bp (Fig. 5) was eluted and sequenced. A complete identity between sequences of the amplified DNAs and futb was found, certifying that the bovine gene is effectively transcribed in the probed bovine tissues.
enzymes. The same experiment was performed as described in 0.2
3 to those obtained with the human FUT6 not type 1 (Lea or sialyl-Lea) epitopes. These results are similar

termine transient expression of type 2 (Le x and sialyl-Lex) but
comparatively to COS-7 cells transfected with the human

type 1 and type 2 epitopes on transfected cells (Table IV),

in vitro were analyzed to assess enzyme activity (Table III) and the appearance of fucosylated

hybridized at high stringency (see "Experimental Procedures") with a futb probe constituted of 301 bp (I) and a human FUT3 probe of 303 bp (II). B, low stringency hybridization of bovine DNA was obtained using the same futb (I) and human FUT3 (II) probes. Conditions were as follows: hybridization at 65 °C followed by three washings at 42 °C with 2 × SSC. Lane 1 contains EcoRV/EcoRI digests and lane 2 contains BamHI/EcoRI digests. futb denotes the bovine gene fragments. The main bovine satellite (52) is indicated by an arrow at 1.4 kilobase pairs. C, low stringency hybridization. The same experiment was performed as described in B, but hybridization temperature was 42 °C and only one washing at 42 °C with 0.2 × SSC was done.

open reading frame was cloned into the mammalian expression vector pcDNAI/Amp (Fig. 2B), and the resulting plasmid (pcDNAI/Amp-futb) was introduced into COS-7 cells. The cells were analyzed to assess in vitro substrate acceptor pattern of enzyme activity (Table III) and the appearance of fucosylated type 1 and type 2 epitopes on transfected cells (Table IV), comparatively to COS-7 cells transfected with the human FUT3, FUT5, and FUT6 genes.

Both analyses demonstrate that pcDNAI/Amp-futb can determine transient expression of type 2 (Leα and sialyl-Leα) but not type 1 (Leα and sialyl-Leα) epitopes. These results are similar to those obtained with the human FUT6 construct (Tables III and IV). However, in quantitative terms the bovine enzyme is about 10-fold less efficient than the human FUC-T6 for the three type 2 acceptors tested. The amount of fucose incorporated by the bovine enzyme on H-type 2 is 7-fold lower than that of FUC-T5 and similar to that obtained with the FUC-T3 human enzyme. The amounts of fucose incorporated onto αGal-type 2 and sialyl-type 2, by the bovine enzyme, are intermediate between those observed with human FUC-T3 and FUC-T5 enzymes (Table III).

Molecular Phylogeny of Fucosyltransferase Genes—We have added to the human paralogous fucosyltransferase tree (20) the sequences of orthologous animal fucosyltransferase genes to make a combined phylogenetic tree. This new tree suggests that separation of mouse, rabbit, and bovine species from the main evolutionary pathway, during the great mammalian radiation, about 80 millions years ago (Fig. 6), occurred after the duplication events which originated the ancestral H and Se (1,2)-fucosyltransferase genes and the divergence of the ancestors of the myeloid, leukocyte, and Lewis α(1,3)-fucosyltransferase loci but before the duplication events that originated the present FUT3, FUT5, and FUT6 human genes. Consequently, the bovine fucosyltransferase gene, described in this paper, may be the orthologous homologue of an ancestor gene, which originated the present human FUT3-FUT5-FUT6 cluster.

Tissue Enzyme Distribution—Human FUC-T6 activity is mainly found in plasma, whereas human FUC-T3 is absent from plasma. Using the same conditions established for detection of human α(1,3)-fucosyltransferase activity, no enzyme activity was detected in two different samples of bovine plasma. Alternatively, α(1,3)-fucosyltransferase activity has been reported in mesenteric lymph nodes, suggesting the presence of another calf enzyme functionally homologous to the human FUC-T4 (55).

Immunofluorescent Detection of Oligosaccharide Epitopes on Normal Bovine Tissues—Bovine tissues present an immunofluorescent pattern of carbohydrate epitopes similar to other lower mammals and new world monkey tissues but quite different from human and old world monkey tissues (35). Vascular endothelium, leukocytes, and red cells are strongly positive with the anti-αGal isoelectin GSI-B4 and are completely nega-

Fig. 4. Southern blots of human and bovine genomic DNA. A, human (H) and bovine (B) genomic DNA were digested with the restriction enzymes EcoRV/EcoRI (lanes 1 and 3), and BamHI/EcoRI (lanes 2 and 4) and electrophoresed on agarose gels. Results are derived from a master gel and blot, containing reiterated sets of the two digests (10 μg of digested DNA/lane). Each strip, containing two digests, was separately hybridized at high stringency (see "Experimental Procedures") with a futb probe constituted of 301 bp (I) and a human FUT3 probe of 303 bp (II). B, low stringency hybridization of bovine DNA was obtained using the same futb (I) and human FUT3 (II) probes. Conditions were as follows: hybridization at 65 °C followed by three washings at 42 °C with 2 × SSC. Lane 1 contains EcoRV/EcoRI digests and lane 2 contains BamHI/EcoRI digests. futb denotes the bovine gene fragments. The main bovine satellite (52) is indicated by an arrow at 1.4 kilobase pairs. C, low stringency hybridization. The same experiment was performed as described in B, but hybridization temperature was 42 °C and only one washing at 42 °C with 0.2 × SSC was done.

Fig. 5. futb transcripts in some bovine tissues. cDNAs were prepared as described under "Experimental Procedures." Nested PCR reactions were performed with primers U1 and L1 for the first PCR and U2 and L2 for the second PCR (Table I). The amplification product (333 bp) was observed in all the tissues tested as follows: lane 2 (brain), lane 3 (liver), lane 4 (lung), and lane 5 (kidney); lane 6 was a control without DNA.

FIG. 4.

FIG. 5.

Bovine α(1,3)-Fucosyltransferase Gene 8769

Human FUT6 activity is present mainly in plasma, whereas human FUC-T3 is absent from plasma. Using the same conditions established for detection of human α(1,3)-fucosyltransferase activity, no enzyme activity was detected in two different samples of bovine plasma. Alternatively, α(1,3)-fucosyltransferase activity has been reported in mesenteric lymph nodes, suggesting the presence of another calf enzyme functionally homologous to the human FUC-T4 (55).

Immunofluorescent Detection of Oligosaccharide Epitopes on Normal Bovine Tissues—Bovine tissues present an immunofluorescent pattern of carbohydrate epitopes similar to other lower mammals and new world monkey tissues but quite different from human and old world monkey tissues (35). Vascular endothelium, leukocytes, and red cells are strongly positive with the anti-αGal isoelectin GSI-B4 and are completely nega-
tive with ABH and either of type 1 or type 2 Lewis-related reagents.

Bovine pancreas present strong staining with GSI-B4 of vascular endothelium, canaliculi, and ducts and weak staining of acinar cells and ducts, as illustrated on Fig. 7A. For comparison strong staining of acinar cells and ducts with anti-A and no staining at all of vascular endothelium are illustrated in Fig. 7B. None of the reagents tested stained the endocrine cells of the islets of Langerhans (white star, Fig. 7A).

Kidney cortex shows strong staining, with GSI-B4, of vascular endothelium of glomeruli, intertubular capillaries, and larger vessels and weak staining of the epithelial cells of proxi-

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**Table III**

| Acceptor Oligosaccharides         | Transfected constructions |
|-----------------------------------|---------------------------|
|                                   | pcDNAI/Amp | Human FUT3 | Human FUT5 | Human FUT6 | Bovine futb |
| Type-1                            |            |            |            |            |              |
| Fucα1-2Galβ1-3GlcNAcβ-R*          | 0          | 50,000     | 11,960     | 0          | 0           |
| Type-2                            |            |            |            |            |              |
| Fucα1-2Galβ1-4GlcNAcβ-R           | 0          | 2,690      | 19,100     | 24,900     | 3,000       |
| Galα1-3Galβ1-4GlcNAcβ-R           | 0          | 1,350      | 3,920      | 19,100     | 2,390       |
| Neuααα2-3Galβ1-4GlcNAcβ-R         | 0          | 650        | 4,450      | 22,900     | 1,880       |

* R, (CH₂)₈COOCH₃.

**Table IV**

Percentage of fluorescent positive cells detected with monoclonal antibodies

Anti-type 1 (Le⁺ and sialyl-Le⁺), anti-type 2 (Le⁺ and sialyl-Le⁺), and anti-human blood group B, on COS-7 cells are transfected with the pcDNAI/Amp vector alone or containing either human FUT3, FUT5, or FUT6 or the bovine futb constructs.

| Monoclonal antibodies | Transfected constructions |
|-----------------------|---------------------------|
|                       | pcDNAI/Amp | Human FUT3 | Human FUT5 | Human FUT6 | Bovine futb |
| Type 1                |            |            |            |            |              |
| Le⁺                   | 7LE        | 0          | 15         | 4          | 0          | 0           |
| Le⁺                   | 069        | 0          | 32         | 4          | 0          | 0           |
| Le⁺                   | 070        | 0          | 40         | 6          | 0          | 1           |
| Le⁺                   | 071        | 0          | 40         | 4          | 0          | 1           |
| Sialyl-Le⁺            | 19.9       | 0          | 53         | 22         | 0          | 0           |
| Type 2                |            |            |            |            |              |
| Le⁺                   | 82H5       | 0          | 29         | 36         | 35         | 22          |
| Le⁺                   | SSEA1      | 0          | 42         | 54         | 40         | 28          |
| Le⁺                   | CD15       | 0          | 18         | 22         | 25         | 14          |
| Sialyl-Le⁺            | TT19A6     | 0          | 12         | 30         | 25         | 20          |
| Sialyl-Le⁺            | KM93       | 0          | 31         | 28         | 23         | 18          |
| Human blood group B   | B          | 026        | 0          | 0          | 0          | 0           |

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**Fig. 6. Phylogenetic tree of cloned human and animal fucosyltransferase genes.** Loci names of paralogous human fucosyltransferase genes (plain text) and the corresponding orthologous animal genes (shaded) are indicated on the right. The points of divergency between human and mammalian fucosyltransferase genes, represented by gray circles, are located at a similar genetic distance (assumed to correspond to about 80 million years).
imal convoluted tubules (Fig. 7C). The only stain observed in kidney, with other Lewis-related reagents, was seen with anti-sialyl-Lea, and it was specifically located on cells of the macula densa of the juxtaglomerular apparatus (Fig. 7D), but the glomeruli and the rest of the renal parenchyma were negative.

Liver vascular endothelium and hepatocytes were strongly stained with GSI-B4, whereas biliary ducts were weakly stained with GSI-B4 (Fig. 7E). No other staining was detected with other Lewis-related reagents, with the exception of a weak staining of biliary ducts with anti-sialyl-Lea (Table V).

All vascular endothelium and bronchial epithelium of lung were strongly stained with GSI-B4 (Table V).

Type 2-fucosylated (Lea and Leb) epitopes were detected only on the brush border of epithelial cells of small (Fig. 7G) and large intestine (Fig. 7H), whereas fucosylated type 1 (Lea, Leb, sialyl-Lea) was not detected at all in any of the cow intestinal sections studied (Table V). Presence of Lea epitope has also been reported on bovine pituitary hormones (54).

Human blood group A and/or H epitopes were not present on bovine red cells nor on vascular endothelium but were found in exocrine epithelial cells of pancreas (Fig. 7B), renal distal convoluted tubules, biliary ducts, lung, small intestine, and colon (Fig. 7F and Table V).

**DISCUSSION**

The bovine futh gene is similar in its structure to FUT3, FUT5, and FUT6 human genes, and the corresponding cognate enzymes are nearly identical in their COOH-terminal regions (catalytic domain).

Some amino acids in the subdomains 4 and 5 play an important role in determining the efficiency with which human FUC-T3, FUC-T5, or FUC-T6 use type 1 and type 2 acceptor substrates (51, 55). The bovine enzyme presents, in this region (positions 115–155), a greater homology to the human FUC-T6 enzyme, in good agreement with the exclusive type 2 acceptor substrate specificity of both enzymes. Since the main acceptor chain present in bovine tissue is the Ga1-3Galβ1–4GlcNAc epitope, which is absent from human tissues, but is in vitro an acceptor for human (56), mouse (29), and a bovine lymph node (53) α(1,3)-fucosyltransferases, a special effort was made to add

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**FIG. 7.** Immunofluorescent staining of normal bovine tissues. Strong staining, with the isoelectin I-B4 of *G. simplicifolia*, on vascular endothelium, red cells, and leukocytes of pancreas (A, the white star identifies an islet of Langerhans), kidney (C), and liver (E); anti-A staining on pancreas of an A− cow (B); anti-sialyl-Lea staining, with antibody KM93, on epithelial cells of the macula densa of kidney (D, arrows are located around the glomerulus on the left of the macula densa); anti-H-type 2, *U. europaeus* lectin 1, staining of goblet cells of colonic mucosa of an A− cow (F, arrows point to the surface epithelium). Anti-Lea staining, with 82H5 antibody, of the brush border of surface epithelial cells of small intestine from an A− cow (G); anti-Leb staining, with antibody 75.12, of brush border of surface epithelium of colon from an A− cow (H). Magnification × 250 A–D and G and H, and × 100 E and F.
genes (58). The Le\(x\) epitope is mainly found on neutrophiles, tissues, suggesting that the low amounts of Le\(x\) detected on now is to know if the come this difficulty, we looked for analysis (Fig. 5) showed that extracts from some bovine tissues. Reverse transcriptase-PCR coding sequence plus the 5\(\text{UTR}\) of futb cannot be ascribed to an incorrectly folded mRNA transcript lacking its 5\(\text{UTR}\) (57). Furthermore, the \(\alpha\)-Gal epitope is a good acceptor for the bovine lymph node (1,3)-fucosyltransferase (53). However, preliminary histochemical results suggest that the bean \(\alpha\)-galactosidase digestion removes the \(\alpha\)Gal epitope from tissue sections but does not increase the staining of anti-Le\(a\) on calf tissue. The question now is to know if the (1,3)-fucosyltransferase is effectively expressed in the bovine tissues that we have probed. To overcome this difficulty, we looked for futb transcripts in mRNA extracts from some bovine tissues. Reverse transcriptase-PCR analysis (Fig. 5) showed that futb is transcribed in liver, kidney, brain, and lung.

To control that the lower expression of enzyme activity of futb cannot be ascribed to an incorrectly folded mRNA transcript lacking its 5\(\text{UTR}\), we also transfected COS-7 cells with a pcDNAI/Amp construct containing the futb coding sequence plus the 5\(\text{UTR}\) of a bovine kidney transcript. A high increase of enzyme activity (about 10-fold) was observed with this DNA construct suggesting that the expression of futb is under a tight control of 5\(\text{UTR}\) regions.

Fucosylated glycoconjugate epitopes are synthesized in two compartments in man. Mesodermal cells produce mainly type 2 ABH and Lewis structures (Le\(a\), Le\(b\), sialyl-Le\(a\)) under control of FUT1 (H), FUT4, FUT6, and FUT7 genes, whereas exocrine cells produce mainly type 1 ABH and Lewis structures (Le\(a\), Le\(b\), sialyl-Le\(b\)) under control of FUT2 (Se) and FUT3 (Le) genes (58). The Le\(a\) epitope is mainly found on neutrophiles, brain (26), and epithelial cells of kidney proximal convoluted tubules (34, 59). Sialyl-Le\(a\) is also found on renal proximal convoluted tubules and on monocytes and on hepatocytes (60). Le\(a\) and Le\(b\) are found on biliary and pancreatic ducts (61), on bronchial epithelium, and on surface digestive epithelium, whereas Le\(a\) and Le\(b\) are mainly found on deep glands of digestive mucosa (62). This distribution of glycoconjugate epitopes on human tissues is different from the immunofluorescent pattern found in bovine tissues (Table V and Fig. 7). It suggests that different glycoconjugate epitopes have been selected by different species, in different tissues, and it illustrates that type 2 Le\(a\)-related structures are poorly represented in bovine tissues.

Despite using the same GDP-fucose donor substrate, having the same type II transmembrane topology, a similar location of the catalytic domain in the COOH terminus, a similar size (Table II), and a common three-dimensional folding (63), less than 20% of sequence identity was found between the two main families of \(\alpha\)-2- and \(\alpha\)-3-fucosyltransferase enzymes (63). The present phylogenetic analysis is compatible with a low degree of homology, since the largest genetic distance detected in the Phylip matrix corresponds to these two main families of fucosyltransferases. Consequently, the root of the tree is located between these two families of fucosyltransferases (Fig. 6) (20).

The position of futb on the phylogenetic tree suggests that the duplication event, at the origin of this gene, occurred before the duplication events that originated FUT3, FUT5, and FUT6 human genes. Therefore, it is not surprising that, by Southern blot, whatever the stringency conditions, only one hybridization signal is obtained on bovine genomic DNA (Fig. 4, A–C), regardless of restriction enzymes and human or bovine origin of the probes used. Alternatively, the presence of three hybridization bands on human genomic DNA with the bovine probe confirms that the three human genes have a high degree of sequence homology and cross-hybridize with the bovine probe.

In addition, the futb gene that we propose to be the orthologous homologue of the ancestor of the human fucosyltransferase cluster of genes FUT3-FUT5-FUT6 is located on the bovine chromosome 7 (36), which is, by comparative mapping, homologous of the human chromosome 19, bearing the FUT3-FUT5-FUT6 cluster.

In good agreement with the present phylogenetic evolutionary model, we have recently cloned three different chimpanzee genes, each with about 98% sequence identity with the corre-

| Reagents | Vascular endothelium | Pancreas | Kidney tubules | Liver | Biliary ducts | Bronchial epithelium | Brush border | Goblet cells | Brush border | Goblet cells |
|----------|---------------------|---------|---------------|------|--------------|---------------------|-------------|-------------|-------------|-------------|
| Type 1   |                     |         |               |      |              |                     |             |             |             |             |
| Le\(a\)  | 9LE                 |         |               |      |              |                     |             |             |             |             |
| Le\(b\)  | 2.25LE              |         |               |      |              |                     |             |             |             |             |
| Sialyl-Le\(a\) | 19.9 |         |               |      |              |                     |             |             |             |             |
| Type 2   |                     |         |               |      |              |                     |             |             |             |             |
| Le\(a\)  | 82H5                |         |               |      |              |                     |             |             |             |             |
| Le\(b\)  | 75.12               |         |               |      |              |                     |             |             |             |             |
| Sialyl-Le\(a\) | KM93 |         |               |      |              |                     |             |             |             |             |
| H        | UEAI\(^b\)          |         |               |      |              |                     |             |             |             |             |
| \(\alpha\)Gal | GSI-B4\(^c\) |         |               |      |              |                     |             |             |             |             |
| Human blood group \(\alpha\) | A |         |               |      |              |                     |             |             |             |             |
| \(\alpha\)013 | - |         |               |      |              |                     |             |             |             |             |

\(^a\) Mainly negative, but epithelial cells of the macula densa in the juxtaplomerulus apparatus were positive \(\pm\) (Fig. 7D).

\(^b\) UEAI, isolectin 1 of \(U. europaeus\), reacting mainly with the H-type 2 epitope (Fuc1–2 Gal β1–4GlcNAc).

\(^c\) GSI-B4, isolectin I-B4 of \(G. simplicifolia\) reacting mainly with the \(\alpha\)Gal epitope (Galα1–3Gal).

\(^{5}\) A. Oulmouden, A. Wierinckx, J.-M. Petit, M. Costache, M. M. Palcic, R. Mollucelle, R. Oriol, and R. Julien, unpublished results.

\(^{6}\) A. Wierinckx, Ph.D. thesis, in preparation.

\(^{7}\) Mainly negative, but epithelial cells of the macula densa in the juxtaplomerulus apparatus were positive \(\pm\) (Fig. 7D).

\(^{8}\) UEAI, isolectin 1 of \(U. europaeus\), reacting mainly with the H-type 2 epitope (Fuc1–2 Gal β1–4GlcNAc).

\(^{9}\) GSI-B4, isolectin I-B4 of \(G. simplicifolia\) reacting mainly with the \(\alpha\)Gal epitope (Galα1–3Gal).

\(^{10}\) Mainly negative, but epithelial cells of the macula densa in the juxtaplomerulus apparatus were positive \(\pm\) (Fig. 7D).

\(^{11}\) UEAI, isolectin 1 of \(U. europaeus\), reacting mainly with the H-type 2 epitope (Fuc1–2 Gal β1–4GlcNAc).

\(^{12}\) GSI-B4, isolectin I-B4 of \(G. simplicifolia\) reacting mainly with the \(\alpha\)Gal epitope (Galα1–3Gal).

\(^{13}\) Mainly negative, but epithelial cells of the macula densa in the juxtaplomerulus apparatus were positive \(\pm\) (Fig. 7D).

\(^{14}\) UEAI, isolectin 1 of \(U. europaeus\), reacting mainly with the H-type 2 epitope (Fuc1–2 Gal β1–4GlcNAc).

\(^{15}\) GSI-B4, isolectin I-B4 of \(G. simplicifolia\) reacting mainly with the \(\alpha\)Gal epitope (Galα1–3Gal).

\(^{16}\) Mainly negative, but epithelial cells of the macula densa in the juxtaplomerulus apparatus were positive \(\pm\) (Fig. 7D).

\(^{17}\) UEAI, isolectin 1 of \(U. europaeus\), reacting mainly with the H-type 2 epitope (Fuc1–2 Gal β1–4GlcNAc).

\(^{18}\) GSI-B4, isolectin I-B4 of \(G. simplicifolia\) reacting mainly with the \(\alpha\)Gal epitope (Galα1–3Gal).

\(^{19}\) Mainly negative, but epithelial cells of the macula densa in the juxtaplomerulus apparatus were positive \(\pm\) (Fig. 7D).

\(^{20}\) UEAI, isolectin 1 of \(U. europaeus\), reacting mainly with the H-type 2 epitope (Fuc1–2 Gal β1–4GlcNAc).

\(^{21}\) GSI-B4, isolectin I-B4 of \(G. simplicifolia\) reacting mainly with the \(\alpha\)Gal epitope (Galα1–3Gal).
sponding human FUT3, FUT5, and FUT6 genes, suggesting that the divergence of chimpanzee and human species occurred after the duplication events which originated the present FUT3-FUT5-FUT6 cluster of genes.5

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