Fucosylated oligosaccharides have been proposed to be involved in multiple cell-cell interactions, including mouse blastocyst adhesion and intestine-microbe interactions. To begin to define the regulation and function of terminal α(1,2)fucosylated carbohydrates in these and other tissues, we isolated and characterized a 85-kilobase (kb) genomic region of mouse chromosome 7, 23.2 centimorgans analogous to human chromosome 19q13.3 that encodes three α(1,2)fucosyltransferases. Gene-specific DNA probes from the open reading frames of the mouse fucosyltransferase genes corresponding to human FUT1, FUT2, and SEC1 demonstrate distinct tissue-specific expression patterns by Northern blot analyses. Flow cytometry profiles of cultured cells transfected with DNA segments containing the open reading frames of the mouse genes confirm that each encodes an α(1,2)fucosyltransferase. In uterus and colon, a 3.3-kb FUT2 mRNA represents the major fucosyltransferase gene expressed. Steady-state FUT2 mRNA levels are cyclically regulated during the estrus cycle, increasing 10-fold from early diestrus to a relative maximum in proestrus. In contrast, SEC1 and FUT1 do not show prominently regulated expression in uterus. FUT2 expression localizes to luminal uterine epithelium by in situ hybridization, implying that this gene determines expression of cell surface Fucα1–2Galβ epitopes proposed to mediate blastocyst adhesion.

Implantation competence of uterine epithelium is hormone-dependent (1) and is accompanied by morphological and biochemical changes in the luminal surface of this epithelium (2, 3). Although a variety of molecules have been implicated in the series of adhesive events leading to implantation, including fibronectin and laminin and their integrin counter-receptors (4–11), cell surface heparin sulfate proteoglycans (12–16), and mucins (17), there is also compelling experimental evidence that specific cell surface glycosconjugates mediate the initial adhesive process (18–22). Specifically, the terminal α(1,2)fucosylated oligosaccharide epitope known as the H type 1 moiety undergoes differential expression in the endometrium and early embryo, exhibits regulation during the mouse estrus cycle, and is present at a time appropriate for implantation (23–25). The epitope is also present on uterine epithelium of ovariec-tomized mice only after estrogen injection at a time correlated with endometrial receptivity (24). In addition, exogenous H type 1 oligosaccharide and monoclonal antibodies toward this epitope inhibit blastocyst adhesion in vitro, whereas isomeric oligosaccharides and isotype-controlled antibodies do not inhibit this attachment (25). Furthermore, a potential counter-receptor on hatched mouse blastocysts has been demonstrated by specific binding of fluorescently labeled H type 1 oligosaccharide (26, 27). This binding was specific to the apical surface of mural trophoderm where the initial adhesion to uterine epithelium is seen in the mouse.

The glycosidic linkages of the H type 1 epitope, like other oligosaccharides, are catalyzed by the actions of specific glycosyltransferases (28, 29). The last step in the synthesis of the H type 1 determinant is catalyzed by an α(1,2)fucosyltransferase activity whose expression is restricted to specific tissues. By genetic analysis and cloning studies in humans, two fucosyltransferase genes, FUT1 and FUT2, are known to be capable of synthesizing α(1,2)fucose oligosaccharides such as those found in H type 1 epitopes (30–32). A third DNA segment in humans (called SEC1) appears to be a pseudogene containing gene-inactivating frameshift mutations (31, 32). An α(1,2)fucosyltransferase activity has been demonstrated in preparations of mouse uterine epithelial cells in vitro (33). This enzymatic activity varied 5-fold during the estrus cycle with the highest activity in estrus (33). In addition, in ovariec-tomized mice, the activity was increased in estrogen-treated animals and inhibited in progesterone-treated animals. Furthermore, changes in the biochemical activity were correlated with cyclical changes in mRNA expression in luminal and glandular epithelium by hybridization in situ using a 650-bp probe from a portion of the mouse FUT1 coding exon (34). Northern blot analysis with this probe on uterine RNA identified a single transcript of a size larger than expected for FUT1, based on our own characterization of the mouse FUT1 locus (35). Kinetic analysis using sonicated uterine epithelial samples in vitro was consistent with the presence of one of α(1,2)fucosyltransferase activity (34). FUT1 expression was re-

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1 The abbreviations used are: FUT1, α1,2fucosyltransferase H locus; FUT2, α1,2fucosyltransferase Secretor locus; SEC1, α1,2fucosyltransferase third locus; bp, base pair(s); kb, kilobase pair(s); PCR, polymerase chain reaction; Fucosyl GM1, Fucol2Galβ1,3GalNAcβ1,4 NeuAcβ2,3Galβ1,4Glcβ1,1ceramide; UEA-1, Ulex europaeus agglutinin-I.1
ported by these authors to decrease during early pregnancy by reverse transcriptase-PCR and confirmed by sequencing of the resulting 358-bp band (34). Taken together, these experimental data suggest involvement of both the H type 1 epitope and the enzymes that catalyze the synthesis of this epitope in blastocyst adherence. We now identify and characterize three mouse α(1,2)fucosyltransferase genes capable of expressing the H type 1 oligosaccharide. Using relatively short 200-bp gene-specific probes, we find that steady-state FUT2 mRNA levels are cyclically regulated during the estrus cycle, increasing ~10-fold from early diestrus to a relative maximum in proestrus. FUT1 was also detected in uterus but did not show regulated expression and accumulated at relatively lower steady-state mRNA levels. FUT2 expression localizes to luminal uterine epithelium by in situ hybridization, implying that this gene determines expression of cell surface Fucα1→2Galβ epitopes proposed to mediate blastocyst adherence.

EXPERIMENTAL PROCEDURES

Library Screening and Sequence Analysis—A commercially available P1 genomic library (strain 129P2/OlaHsd, Incyte Genomics, St. Louis, MO) was screened using PCR primers that generated a 432-bp fragment from the 3′-region of the open reading frame of mouse FUT1 (35): forward primer, 5′-TAC TCT GTG GGG TCT GAG TGG-3′ and reverse primer, 5′-GAT GCC CAC CCA CTC GGG CAG G-3′. Positive clones were transferred by transduction to Escherichia coli N53516 to increase plasmid yield. Plasmid preparations were performed according to the manufacturer’s instructions by alkaline lysis of isopropyl-β-D-thiogalactoside-induced cultures. Restriction digests were performed with NotI, SfiI, SalI, and PciI. Restriction fragments of 2–85 kb were mapped by pulsed field gel electrophoresis in a 1% agarose gel on a Bio-Rad CHEF Mapper for 16 h at 14°C, 6 v/cm with switching times of 2.16–6.67 s and a ramping constant of 0.130. Plasmid Southern blotting (36) using a double-stranded DNA probe corresponding to the 432-bp PCR amplicon was performed to identify smaller restriction fragments for subcloning and sequencing in vector pNEB193 (New England BioLabs, Beverly, MA). Resulting plasmids were mapped by partial digestion restriction mapping (37) and DNA sequencing. Automated DNA sequencing was performed on Applied Biosystems DNA sequencers in the University of Michigan DNA Sequencing Core Facility. Sequencing runs with overlapping forward and backward primers were aligned using the program Sequencher (Gene Codes, Ann Arbor, MI).

Expression Studies in COS-7 Cells—Expression vectors for mouse FUT2 and SEC1 were designed using restriction sites obtained from the DNA sequences around each open reading frame and subcloned into the expression vector pcDNAI (Invitrogen, Carlsbad, CA). The plasmid pcDNAI-SEC1 contains the predicted open reading frame of mouse FUT1 (35): a 347-bp PCR fragment (35), Fuc-TIV, Ncol-SapI segment (39); α(1,3)Gal-T, PacI/PstI segment (40); β-actin, CLONTECH product 7760-7C. The Expand High Fidelity PCR kit (Roche Molecular Biochemicals, Mannheim, Germany) was used for amplification of the probe sequences. The resulting DNA fragments were purified with a Qiagen QIAquick gel extraction kit column (Qiagen, Valencia, CA), restricted with EcoRI and HindIII, re-purified, and cloned into pGEM-4Z (Promega, Madison, WI). DNA sequencing confirmed the correct amplification of the probe sequences. Southern and Northern blot probes for Southern and Northern blot analyses following standard protocols (36, 38). To summarize, total RNA was collected from various flash-frozen mouse organs and run twice over oligo-dT columns to enrich for polyadenylated RNA. Ultraviolet absorbance at 260 nm was determined for each sample, and equal volumes (3–5 μg) of polyadenylated RNA from each tissue were run on formaldehyde agarose gel electrophoresis. The gels were transferred to nylon membranes (Hybond-N, Amersham Pharmacia Biotech) and prehybridized overnight in a formamide-based nucleic acid hybridization buffer. 25 ng of oligonucleotide probes was random-primer–primed with [32P]dCTP using the Rediprime DNA-labeling system (Amersham Pharmacia Biotech) resulting in a specific activity of 1–2 × 108 cpm/μg of DNA probe. Blots were hybridized for 24–36 h, then washed with 2× SSC, 0.2% SDS at room temperature for 10 min three times, then 0.5× SSC, 0.2% SDS at 65°C for 15 min twice. Washed blots were quantified on a PhosphorImager then subjected to autoradiography for the final images. Other mouse gene studies by Northern blot analysis used previously published study of FUT1 expression (35, 42). The 288-bp FUT1 probe corresponds to bp 81–308 of the FUT1 open reading frame (35). The 214-bp FUT2 probe corresponds to bp –20 to 194 of the FUT2 open reading frame. To compare in situ hybridization with the previously published study of FUT1 expression in uterus, a less selective 530-bp FUT1 probe that shares 70% similarity between FUT1 and FUT2 was derived from an EcoRI/PstI fragment corresponding to bp 556–1085 of the FUT1 open reading frame (34). Frozen sections of mouse uterus were processed in duplicate. Each slide contained adjacent sections for hybridization with antisense and sense control probes. Single-stranded RNA probes were synthesized using the RNase script transcriptase (Stratagene, La Jolla, CA) and Sp6 promoters as previously described (35) using linearized plasmids. After hybridization under glass coverslips overnight, slides were washed twice under high stringency (50% formamide, 2× SSC, 20 mM dithiothreitol at 65°C for 30 min), dipped in photographic emulsion, and exposed for 2–4 weeks at 4°C. Slides were developed and examined under darkfield microscopy.

RESULTS

Molecular Cloning of an 85-kb Genomic Segment Containing the Open Reading Frames of Three α(1,2)Fucosyltransferase Genes—Because the three human α(1,2)fucosyltransferase genes are physically linked within an 65-kb region of human chromosome 19, it was reasoned that a similar linkage may be found in the mouse (31). A P1 phage library was chosen for screening because of the relatively large insert size in P1 clones of 75–95 kb (43). Because the three human α(1,2)fucosyltransferase genes showed the highest degree of sequence similarity in their 3′-regions (32), PCR primers for the P1 screening were chosen from the presumed catalytic region in the 3′-end of a previously cloned mouse FUT1 open reading frame (35) to maximize the potential cross-hybridization with several α(1,2)fucosyltransferase genes. Four positive P1 clones with inserts of 70–85 kb were obtained by screening a mouse genomic P1 library (see “Experimental Procedures”). The clones were characterized by pulsed field gel electrophoresis restriction mapping and plasmid Southern blotting using probes derived from mouse FUT1. Upon subcloning and sequencing of the largest clone, three
α(1,2)fucosyltransferase open reading frames were obtained (Fig. 1A). From the T7-end of the P1 vector, 9257 bp were sequenced, including the open reading frame of FUT1 (GenBank accession number AF214655). The DNA coding sequence of 129P2/OlaHsd mouse FUT1 was identical to our previously published sequence of NIH Swiss mouse FUT1 (35). From the Sp6 end of the P1 vector, nine overlapping subclones were serially mapped by EcoRI restriction digestion and plasmid Southern blotting (Fig. 1B). Open reading frames for mouse genes FUT2 (GenBank AF214656) and SEC1 (GenBank AF214657) were determined by sequencing 6762-bp and 4705-bp contigs, and are similar to GenBank accession number sequences AF064792 and Y09889/AF113532, respectively. The order and relative spacing of the three loci (FUT1, FUT2, and SEC1) on mouse chromosome 7, 23.2 centimorgans (35), is similar to the homologous genes on human chromosome 19q13.3 (31) and implies gene duplication, as has been postulated for the human FUT1 and FUT2 loci.

Restriction Map and Sequence Comparison of Mouse FUT2 and SEC1 Open Reading Frames—Mouse FUT2 and SEC1 are encoded by single open reading frames contained within relatively small continuous genomic DNA segments (Fig. 2A). The human FUT2 locus also maintains a single coding exon, although there are one or possibly more 5’-exons derived from this human gene. We do not yet know if the mouse FUT2 is similar in this regard, because we have not yet characterized the structures of mouse FUT2 cDNAs. When compared with the orthologous human fucosyltransferases, mouse FUT2 and SEC1 display 82% and 77%, respectively, nucleotide sequence identity (Fig. 2B). The corresponding mouse polypeptides predicted by these open reading frames show conservation of type-2 transmembrane topology, because each is predicted to maintain short intracellular amino termini, transmembrane regions of 14–20 amino acid residues, and conservation of predicted N-glycosylation sites.

In contrast to the human gene, the coding region of mouse SEC1 extends beyond the region in human SEC1 where a 2-base pair deletion results in a frameshift and consequent inactivation of fucosyltransferase activity. Mouse SEC1 maintains an in-frame translation similar to FUT2, including a high degree of sequence similarity to FUT2, and conservation of three predicted N-glycosylation sites. The open reading frames of mouse FUT2 and SEC1 share 75% sequence identity overall, with 50-bp regions that vary from 25% to 99% identity (Fig. 2C).

Expression of Mouse FUT2 and SEC1 in Cultured Cells—Our previous studies have documented that mouse FUT1 encodes an α(1,2)fucosyltransferase (35). To determine if the genomic DNA sequences for FUT2 and SEC1 encode functional α(1,2)fucosyltransferases, 1.3-kb restriction fragments containing the open reading frames of each gene were cloned into mammalian expression vectors, sequenced to assure correct orientation, and transiently transfected into COS-7 cells as described under “Experimental Procedures.” In our previous studies, we found that blood group A and B glycosyltransferases are expressed in COS-7 cells, whereas α(1,2)fucosyltransferase, and H blood group molecules, are absent from these cells (35). Because H blood group molecules are used as substrates by the A and B transferases, the A and B transferases in COS-7 cells thus cannot form A or B antigens. However, when transfected with an α(1,2)fucosyltransferase expression vector, the cells can then form H blood group molecules, and these can in turn be used as substrates by the A and B transferases to form A and B blood antigens (35). In the present analysis, anti-H and anti-A antibodies were used in similar flow-cytometry analyses on COS-7 cells transiently transfected with expression constructs for FUT2 and SEC1. Mouse FUT2 demonstrates accumulation of A and H antigens (Fig. 3). Although significantly lower in apparent activity compared with human and mouse FUT2, cells transfected with the mouse SEC1 vector also accumulate A and H antigens.

To compare the mouse enzymes to human FUT2, the apparent Michaelis-Menten constant (K_m) for the artificial substrate phenyl-β-D-galactoside was assayed in extracts from tran-
siently transfected COS-7 cells (see “Experimental Procedures”). While authentic α(1,2)fucosyltransferase acceptors include O- and N-linked type I and II glycans as well as glycolipids, phenyl-β-D-galactoside is typically chosen as a representative acceptor because of its ease of purification. Extracts from cells transfected with FUT2 fucosylate phenyl-β-D-galactoside with an apparent Michaelis-Menten constant (Kₘ) of 10.2 mM. This Kₘ is similar to the Kₘ determination for this substrate using human FUT2 (11.5 mM (32)) (Fig. 4). Unexpectedly, the expression vector containing the open reading frame of FUT2 and SEC1 were compared in 50-bp increments. The 5' ends contained the region of least similarity. Gene-specific probes were designed from the first 200 bases of the 5' end of each gene. Potential asparagine-linked glycosylation sites are indicated with the Y-shaped symbol. C, comparison of percent nucleotide identity between FUT2 and SEC1. The sequences of the open reading frames of FUT2 and SEC1 had no detectable fucosyltransferase activity in this assay under circumstances where we identify cell surface H and A epitopes in SEC1-transfected cells. These observations suggest that the physiological acceptors for SEC1, some of which must be made by COS cells, are not highly similar to the in vitro substrate phenyl-β-D-galactoside.

Tissue-specific Expression Patterns of Mouse FUT2 and SEC1—To study mRNA expression of the FUT2 and SEC1 loci, we sought to generate relatively short gene-specific DNA probes with less than 35% sequence identity, because the open reading frames of FUT2 and SEC1 contain long regions of 75% to 99% similarity. From the 5' coding regions, ~200 bp of each gene were subcloned by PCR (see “Experimental Procedures”; Fig. 5A). To test the specificity of the probes, Southern blot analyses were performed (Fig. 5B). The FUT2 214-bp and SEC1 208-bp probes identify single bands (Fig. 5B).

Using these gene-specific probes, Northern blot analysis discloses that FUT2 and SEC1 show distinct patterns of mRNA expression in adult tissues (Fig. 6). The FUT2 probe identifies a single transcript of ~3.3 kb expressed most abundantly in uterus, stomach, and colon, and at a lower level on ovary. In contrast, the SEC1 probe hybridizes to multiple transcripts in testes/epididymis and thymus. The major transcript is 2.6 kb in size, and additional transcripts of ~2.3, 1.6, and 1.3 kb in size are also identified. The SEC1 probe also cross-hybridizes to multiple faint bands in other tissues. Although these may represent partially processed or alternatively spliced forms of authentic SEC1 transcripts, or transcripts emanating from other genes, their precise nature is not yet known. As a comparison to these expression patterns, the hybridization pattern of the major FUT1 transcript we have previously described in the same tissues is also shown (35).

To directly determine if the changes in uterine α(1,2)fucosylated glycans and α(1,2)fucosyltransferase activity observed during the rodent estrus cycle (33) correspond to alteration in fucosyltransferase mRNA accumulation and, if so, to determine how each of these three loci may contribute to this process, Northern blot analyses were completed using mRNA prepared from uteri taken during each stage of the estrus cycle. Northern blot analysis of uterine polyadenylated RNA from early diestrus, late diestrus, proestrus, estrus, and metestrus 2 demonstrates that FUT2 represents the major α(1,2)fucosyltransferase gene expressed in mouse uterus (Fig. 7). By PhosphorImager quantification, levels of FUT2 transcripts are greatest in proestrus and estrus, with 10- to 12-fold increases over early diestrus. Steady-state FUT1 transcript levels represent ~5% of that of FUT2 when compared with Northern blot probes of similar specific activity, but also show a 2- to 3-fold increase in...
steady-state levels from early diestrus to proestrus (second row), similar to the variation in steady-state actin mRNA during the estrus cycle. The apparent variation in FUT1 transcript accumulation may thus reflect slight differences in the amounts of mRNA loaded in each lane of the Northern blot or may reflect some subtle regulation of the FUT1 locus during the cycle. Two unrelated glycosyltransferase genes whose corresponding carbohydrate epitopes are not known to be regulated in the estrus cycle, α(1,3)fucosyltransferase IV (Fuc-TIV) and α(1,3)galactosyltransferase (α(1,3)Gal-T), show constitutive expression in uterus by Northern analysis.

To determine which cell types within the uterus express FUT2, frozen sections of mouse uterus during the estrus cycle were analyzed by in situ hybridization. Specific binding of the FUT2 antisense probe is detected in uterine luminal epithelial cells, whereas the FUT1 antisense probe shows no specific signal (Fig. 8). In contrast, a less selective FUT1 probe (see “Experimental Procedures”) that shares 70% similarity between FUT1 and FUT2 shows a signal in epithelial cells most likely due to cross-hybridization with FUT2 transcripts.

**DISCUSSION**

A wide variety of α(1,2)fucosylated glycans have been described in humans, mice, and other mammals (44). Expression of these terminal glycan structures is precisely regulated during development, in a tissue-specific manner in the postnatal period, and is characterized by dynamic expression in several physiological and pathological contexts. However, definitive functional correlates for the careful regulation of these structures remain elusive. To identify such functions in the context of an intact mammalian organism, we have sought to develop strains of mice with induced mutations in the α(1,2)fucosyltransferase loci that control the expression of α(1,2)fucosylated glycoconjugates. A first step in this process involves the isolation and characterization of the cognate α(1,2)fucosyltransferase genes. We report here the characterization of three closely linked mouse α(1,2)fucosyltransferase loci that correspond to the human H and Se blood group α(1,2)fucosyltransferase genes, and a human α(1,2)fucosyltransferase pseudogene.

A 100-kb genomic region of human chromosome 19q13.3 contains the three human α(1,2)fucosyltransferase loci (31). In rabbits, three α(1,2)fucosyltransferases with sequence similarity to the human loci each demonstrate enzymatic activity in expression constructs, including rabbit SEC1 (45, 46). Although the chromosomal locations of the rabbit α(1,2)fucosyltransferase loci have not been mapped, all three α(1,2)fuco-
The SEC1 locus in mice predicts a protein of similar size to the other members of the (1,2)fucosyltransferase family and is clearly transcribed in a tissue-specific manner. However, the recombinant product of this locus in mice did not give clear activity when tested with one prototypical substrate in vitro, suggesting the possibility that this polypeptide does not exhibit (1,2)fucosyltransferase activity. However, dozens of known mammalian glycans have been described with potential for utilization as an acceptor substrate for this SEC1 locus-encoded protein (i.e. type 1–6 glycans, fucosyl GM1, and other glycolipids). Such substrates have yet to be tested in vitro with this protein and remain candidates for SEC1-dependent fucosylation. In addition, the attendant possibility exists that mouse SEC1 may act on novel acceptors. For example, a schistosome (1,2)fucosyltransferase has been documented to add an α(1,2)fucose to an α(1,3)fucose (48), although such activity is as yet unprecedented in mammalian species. There is also the possibility that the SEC1 protein is not an α(1,2)fucosyltransferase but may have other functions, enzymatic or otherwise. These issues remain to be explored experimentally.

Multiple lines of evidence support the hypothesis that adhesive interactions between the mouse blastocyst and the uterine epithelium during implantation involves a specific α(1,2)fucosylated carbohydrate epitope. The H type 1 moiety undergoes differential expression in luminal epithelium of the endometrium and exhibits hormonal regulation during the mouse estrus cycle and in ovariectomized mice at a time correlated with endometrial receptivity (23–25). In vitro experiments demonstrate that fluorescently labeled H type 1 oligosaccharide binds specifically to hatched mouse blastocysts and imply the existence of a counter-receptor for H type 1 glycans on the blastocyst (26, 27). The hypothesis is supported by in vitro blastocyst adhesion assays, where exogenous H type 1 oligosaccharide and monoclonal antibodies toward this epitope specifically inhibit blastocyst adhesion in vitro (25). Because very little is known about the expression of fucosylated glycans and their corresponding fucosyltransferases in the uterus of other mammals, it remains to be determined if these observations will apply generally to other species. In rat uterus, carbohydrate antigens based on the Gal-β-1-GlcNAc backbone structure are expressed but include some carbohydrate antigens not expressed in mouse (A and B antigen) or under different steroidal regulation (H type 1) (49).

The studies we report here identify FUT2 as the mouse α(1,2)fucosyltransferase gene responsible for directing expression of α(1,2)fucosyltransferase activity, and presumably H type 1 structures, in the mouse uterus. FUT2 mRNA levels are regulated in the uterus, either by transcription or mRNA stability, during the estrus cycle. The expression pattern of FUT2 during the estrus cycle parallels the known α(1,2)fucosyltransferase activity detected in uterine epithelial preparations in vitro such that the highest level of its mRNA occurred in proestrus, 12–24 h preceding the maximal enzymatic activity seen in estrus. By in situ hybridization, accumulation of FUT2 transcripts is maximal in the luminal epithelium of the uterus during proestrus. This location, and timing, are coincident with the elaboration of the luminal, cell surface, α(1,2)fucosylated H type 1 epitopes also observed in this physiological context. By contrast, FUT1 mRNA accumulates to levels representing <5% of that of levels achieved by FUT2, as assessed by Northern blotting, using FUT1 and FUT2 probes of similar specific activity. We observe a 2- to 3-fold increase in steady-state levels of FUT1 transcripts between early diestrus and proestrus; this modest increase is minimally greater than the variation in steady-state levels of actin mRNA during the estrus cycle.
Regulated Uterine Expression of α(1,2)Fucosyltransferase

**Fig. 6.** Northern blot analysis of three mouse α(1,2)fucosyltransferase transcripts in adult mouse tissues. Multiple Northern blots were prepared and hybridized as described under “Experimental Procedures.” Each lane contains 3 µg of polyadenylated mRNA isolated from the mouse tissues indicated above each lane. Each blot was probed using gene-specific probes corresponding to FUT1, FUT2, or SEC1. Transcript sizes, in kilobases, were estimated from [35S]RNA markers.

FUT2 transcripts were more abundant than steady-state FUT1 transcripts, and varied more dramatically during the estrus cycle, from 10- to 12-fold.

FUT1 was the first mouse α(1,2)fucosyltransferase molecularly cloned (35) and has been proposed to be responsible for regulation of α(1,2)fucosylated H type 1 epitopes in uterine epithelium. An estrogen-dependent α(1,2)fucosyltransferase activity is observed to vary 5-fold during the estrus cycle, when assessed using mouse uterine epithelial cells isolated from mice during the cycle (33). Using a 650-bp DNA probe encoding ~50% of the FUT1 open reading, and in situ hybridization procedures, Sidu and Kimber (34) detected cyclical changes in the accumulation of a cognate mRNA in uterine luminal and glandular epithelium. Northern blot analysis with this probe on uterine RNA identified a single transcript of a size larger than expected for FUT1, although DNA sequence analysis of a 358-bp reverse transcriptase-PCR product from uterine epithelium aligned with a portion of FUT1. Our observations indicate that FUT2 transcripts accumulate to a substantially greater level than FUT1 transcripts in the mouse uterine epithelium. We estimate that the in situ hybridization probe used by Sidu and Kimber (34) to assign FUT1 expression to mouse uterine epithelia shares 70% sequence similarity with the corresponding segment of FUT2. Thus, in the in situ hybridization experiments reported by these authors (34), cross-hybridization of this probe with FUT2 transcripts that are abundant in uterine epithelia could have led to misassignment of these as FUT1-derived. Indeed, our own in situ hybridization analyses indicate that a FUT2-specific probe identifies transcripts in uterine epithelial cells, whereas a FUT1-specific probe does not react with transcripts in these cells. In our hands, a non-selective probe corresponding to the one used by Sidu and Kimber (34) does indeed hybridize to uterine epithelia; but, when considered with our in situ data using FUT1- or FUT2-specific probes and with the fact that FUT2 mRNA accumulates in the uterus to a much higher steady-state level compared with FUT1, these results indicate that, in uterine epithelial cells, this non-selective probe identifies FUT2 transcripts and not FUT1 transcripts. Kinetic analysis of α(1,2)fucosyltransferase activity in vitro is also consistent with the presence of one type of α(1,2)fucosyltransferase (34). Considered together, these data indicate that FUT2 represents the major α(1,2)fucosyltransferase gene expressed in mouse uterus. Further analysis of FUT1 and FUT2 functions in blastocyst-uterine epithelium interactions will be aided by molecular characterization of these genes and can be further tested in systems such as genetically manipulated mice.

Cell surface expression of α(1,2)fucosylated oligosaccharides has also been explored in the gastrointestinal tract; the nature of the fucosyltransferases responsible for regulating these structures in that tissue is clarified to some extent by our studies. Lectin binding studies demonstrate complex developmental programs for expression of fucosylated oligosaccharides in the gastric-colonic and crypt-villus axes (50) and imply an interesting dependence on bacterial colonization of the intestine (51). Whereas the epithelium of ileum of conventionally housed mice is characterized by a complex cell-type-specific expression pattern of α(1,2)fucosylated glycoconjugates, the intestine of a germ-free mouse is virtually deficient in such fucosylated glycans. Colonization of germ-free mice with *B. thetaiotaomicron* stimulates expression of α(1,2)fucose epitopes by the ileal enterocyte, in coincidence with accumulation of a previously unidentified α(1,2)fucosyltransferase mRNA as assessed by a reverse transcriptase-PCR approach. By contrast, colonization with an isogenic strain of *B. thetaiotaomicron* defective in l-fucose metabolism is not accompanied by induction of α(1,2)fucosyltransferase gene expression (51). A comparison of the sequences of the PCR primers used by Bry and colleagues (51) with the three mouse α(1,2)fucosyltrans-
ferase genes we characterize here indicates that the primers were nearly identical and allows us to conclude that either SEC1 or FUT2 could have been amplified in their studies. Dynamic and tissue-specific expression of α(1,2)fucosylated oligosaccharides has been observed in other, anatomically related physiological and pathological contexts, although little is yet known about the α(1,2)fucosyltransferase genes responsible for such control. For example, the α(1,2)fucosyltransferase activity in the rat small intestine varies developmentally, in response to hydrocortisone during suckling, and by nutritional state (52–54). Conventionalization of ex-germ free mice stimulates fucosylation of the glycolipid GM1, in concert with in increases in expression of major histo compatibility complex class II molecules and Thy-1 and induction of cytolytic activity of intraepithelial lymphocytes (55). These observations suggest a role for α(1,2)fucosylated oligosaccharides in the development of the mucosal architecture and immune system of the small intestine (55). This general notion is supported by the observation that mouse Peyer’s patch follicle-associated epithelial cells endocytose and transcytose the α(1,2)fucose-specific lectin Ulex europaeus agglutinin-I (UEA-I) in a gut-loop in vivo model that may recapitulate microorganism adhesion and uptake (56), and by the observation that UEA-I lectin binding to dome epithelium of gut-associated lymphoid tissues exhibits developmental regulation (57). In thymus, medullary epithelial cells are labeled by [3H]fucose (58), and α(1,2)fucose epitopes have been demonstrated in the thymic medulla of mice by binding of the lectin UEA-I (59). Nonetheless, a critical role for α(1,2)fucosylated epitopes in the thymus is not yet known, even though carbohydrate-lectin interactions have been implicated in several immune-related activities (60). The identity of the α(1,2)fucosyltransferase loci responsible for control of these processes will be facilitated by the sequences we report here. Importantly, insight into the functional relevance of these observations should be facilitated by generation and analysis of mice with induced null mutations at the FUT1, FUT2, or SEC1 loci.

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