RESEARCH COMMUNICATION

Evidence for two distinct α(1,2)-fucosyltransferase genes differentially expressed throughout the rat colon

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Blood-group-ABH antigens are carbohydrate structures widely distributed in numerous tissues. These structures are fucosylated by an α(1,2)-fucosyltransferase. The occurence of at least two α(1,2)-fucosyltransferase genes in the human genome has been strongly suggested by genetic studies, but only one of them has been cloned so far. Specific primers deduced from this human cDNA were used to amplify a fragment of rat genomic DNA (FTA). Screening of a rat colon cDNA library with this probe allowed us to isolate a clearly distinct, but related, cDNA clone (FTB). Both sequences showed considerable sequence similarity to the human α(1,2)-fucosyltransferase cDNA previously cloned. Furthermore, cells transfected with these DNA fragments in antisense orientation displayed a decreased α(1,2)-fucosyltransferase activity, indicating that they both correspond to fragments of α(1,2)-fucosyltransferase genes. Finally, differential expression of these genes was demonstrated in two rat colon-cancer cell lines and throughout the rat colon.

INTRODUCTION

ABH-blood-group antigens are carbohydrate structures shared by glycoproteins and glycolipids, built up in stepwise fashion by specific glycosyltransferases. H-blood-group antigens are made by addition of fucose linked α(1,2) on terminal β-galactosyl residues of precursors. This addition is performed by an α(1,2)-fucosyltransferase (galactoside 2-α-L-fucosyltransferase, EC 2.4.1.69) [1,2]. A- and B-blood-group antigens are constructed by addition of *N*-acetylgalactosamine and galactose linked α(1,3) respectively on H residues. ABH-blood-group antigens are widely distributed in tissues or mucous secretions, and their expression is strictly regulated during embryogenesis [3]. In human, genetic and biochemical observations are consistent with the hypothesis that the formation of the H structures is controlled by two genes (H and Se), coding for two α(1,2)-fucosyltransferases. These enzymes show different tissue-specific expression patterns [4,5]. According to this two-loci model, the human H-blood-group locus determines expression of the H antigen in the erythroid lineage, whereas the Se locus controls H-antigen expression in a variety of secretory epithelia and in secretions [5]. About 20 % of the population is homozygous for the null alleles at the Se locus. These individuals do not present H antigen in their secretions. Moreover, rare individuals lack H determinants in all their tissues. This population, called 'Bombay phenotype', is homozygous for the null alleles at both the H and Se loci [4,5]. Other individuals, called 'para Bombay phenotype', lack this determinant only in their erythrocytes [4]. They are thought to be homozygous for the null alleles only at the H locus. These two enzymes have been purified from human serum, and their substrate specificities have been characterized [6,7]. The H enzyme preferentially fucosylates type 2 precursors, whereas the Se enzyme preferentially reacts with type 1 and 3 acceptors.

ABH antigens are expressed by colonocytes throughout the entire colonic tract during human fetal life [8,9]. Human adult distal-colon epithelium never expresses these antigens, while distal-colon-carcinoma cells often re-express them [3]. Ørntoft et al. [8] have reported that α(1,2)-fucosyltransferase is involved in controlling expression of ABH and Lewis^b structures in both normal and malignant colon.

The expression of histo-blood-group antigens and α(1,2)-fucosyltransferase activity in two rat colon-carcinoma cell clones which differ by their tumorigenic potential has previously been studied in our laboratory [10,11]. Injected subcutaneously into the syngeneic BDIX rats, PROb cells give progressive tumours that spread to lungs, kidneys and lymph nodes, whereas REGb cells give regressive tumours [12]. These two tumoral cell clones also differ by their glycosylation state, especially by the expression of fucosylated molecules. In particular, PROb cells express higher amounts of H antigen than REGb cells, which display high amounts of precursor T antigen [10]. In agreement with these results, PROb cells were found to express higher amounts of α(1,2)-fucosyltransferase than REGb cells [11].

We have also shown that treatment of PROb cells with retinoic acid decreased α(1,2)-fucosyltransferase activity and modified kinetic parameters (K_m and $V_{max.}$), suggesting the existence of several enzymes [11]. To test this hypothesis, we first isolated a fragment of a rat α(1,2)-fucosyltransferase gene and used it to search for cross-hybridizing cDNAs. The present paper describes the development and characterization of DNA fragments corresponding to genes encoding two distinct α(1,2)-fucosyltransferases.

MATERIALS AND METHODS

Cell culture

PROb and REGb rat colon-adenocarcinoma cell clones (kindly provided by Dr. F. Martin, INSERM U158, Dijon, France) were cultured in complete medium (RPMI 1640, 5 % fetal-calf serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin) as previously described [10].

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The nucleotide sequences reported have been submitted to the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers L26009 (FTA) and L26010 (FTB).

Isolation of the FTA fragment

A specific probe for a rat α(1,2)-fucosyltransferase was synthesized by PCR amplification of PROb genomic DNA using oligonucleotides deduced from the human sequence [13] using the GeneJockey software. Primers were:

FT1: 5′-CCCTGCTCTTGGACTTTCTTCC-3′
FT2: 5′-AAGGTGCCAATGGTCATAATGG-3′

A total of 30 cycles of PCR were performed with 250 ng of PROb genomic DNA in 1 × PCR buffer [10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 % (w/v) gelatin] and 40 pmol of each primer as follows: 94 °C for 30 s; 52 °C for 30 s; 72 °C for 30 s. After phenol/chloroform extraction and ethanol precipitation, the amplified fragment was blunt-ligated in the *Sma*I restriction site of the pBluescript plasmid (Stratagene, La Jolla, CA, U.S.A.). The FTA probe used in hybridization experiments was released by digestion of this plasmid with *Xba*I and *Hin*dIII.

Cloning of the FTB cDNA

Construction of the PROb cDNA library has been previously described [14]. The unamplified library was plated at a density of 2×10^4 plaque-forming units/150-mm-diameter dish. Lifts were done with colony-plaque-screening hybridization-transfer membrane discs (New England Nuclear). The nylon filters were prehybridized for 1 h at 65 °C in 2 × SSC (0.3 M NaCl/0.015 M sodium citrate, pH 7.0), containing 5 % SDS and 10 % dextran sulphate. Hybridization was performed for 20 h at 62 °C in the same solution containing $(0.5–1.0) \times 10^6$ c.p.m./ml of probe (FTA) labelled by random priming. Following hybridization, the membranes were washed with 1 × SSC/1 % SDS at room temperature, 1 × SSC/1 % SDS at 62 °C, 0.2 × SSC/0.1 % SDS at 62 °C, then exposed to Amersham Hyperfilm-MP. A secondary screening was performed under the same conditions. The insert of the isolated phage was amplified by PCR using oligonucleotides specific for λgt10 as described by the supplier (Clontech, Palo Alto, CA, U.S.A.). After digestion with *Eco*RI, the fragment was ligated in pBluescript plasmid.

DNA sequencing

Sequence analysis of FTA and FTB was performed by the dideoxy chain-termination method [15] using a T7 sequencing kit (Pharmacia, Uppsala, Sweden), and primers specific for the plasmid (T3 and T7) or designed from partial sequencing. The final sequence was determined from both strands of several plasmids.

Transient transfection assays

FTA or FTB DNA fragments were inserted in antisense orientation in the *Eco*RI cloning site of pSG5 plasmid (Stratagene). Orientation of the inserts was confirmed by sequencing. PROb transient transfectants were produced by transfection with Lipofectin reagent (Gibco BRL, Bethesda, MD, U.S.A.). Plasmids (20 μg) were transfected on subconfluent PROb cells cultured in 75-mm^2 flasks as described by the manufacturer. After 16 h, fresh medium was added and α(1,2)-fucosyltransferase assays were performed 48 h later.

α(1,2)-fucosyltransferase activity

These assays were performed as previously described using the synthetic acceptor phenyl β-D-galactopyranoside, which is specific for α(1,2)-fucosyltransferase activity [16].

Southern-blot analysis

Rat genomic DNA was extracted from liver using a standard method [17]. A 15 μg sample of DNA was digested with *Bam*HI or *Hin*dIII, electrophoresed on a 1 % agarose gel, denatured and transferred to Amersham Hybond-N. The nylon filter was prehybridized for 1 h at 65 °C in 2 × SSC/5 % SDS/10 % dextran sulphate. Hybridization was performed for 16–20 h at 65 °C in the same solution containing $(0.5–1.0) \times 10^6$ c.p.m./ml of probe (FTA or FTB) labelled by random priming. Following hybridization, the membranes were washed with 2 × SSC/1 % SDS at room temperature, 1 × SSC/1 % SDS at 65 °C, 0.2 × SSC/0.1 % SDS at 65 °C, then exposed to Amersham Hyperfilm-MP. A 1.2 kb DNA fragment obtained by digestion with *Hin*dIII of the human α(1,2)-fucosyltransferase gene (kindly provided by Dr. John B. Lowe, Howard Hughes Medical Institute, Ann Arbor, MI, U.S.A.), was hybridized at 58 °C, and washed with 2 × SSC/1 % SDS at room temperature, 1 × SSC/1 % SDS at 58 °C, 0.5 × SSC/0.1 % SDS at 58 °C.

Northern-blot analysis

Adult rat colon was cut in three parts corresponding to proximal, median and distal sections. Epithelial cells were removed with a glass slide. Total cytoplasmic RNA was extracted from colon tissue or cultured cells with RNAzol (Bioprobe Systems, Montreuil, France). A 15 μg portion of total RNA was denatured, electrophoresed on a 1 % agarose gel, and transferred to Amersham Hybond-N membranes. Hybridization was performed as described above. A human β-actin probe was used as a control for equal loading of RNA in each well.

RESULTS AND DISCUSSION

Oligonucleotides derived from the human α(1,2)-fucosyltransferase sequence [13] were used to amplify rat genomic DNA. The 422-bp fragment isolated, which will be referred to as 'FTA', showed considerable sequence identity (79 %) with the human sequence as determined by DNA sequencing (Figure 1a). This probe was then used to screen a PROb cDNA library. A unique positive clone was isolated and subcloned in pBluescript plasmid. Sequencing of this clone (FTB) revealed a significant sequence identity (67 %) with FTA (Figure 1a). Translation of these nucleotide sequences after alignment with the reading frame of the human gene revealed that the similarities between the amino acid sequences of FTA and FTB to the human α(1,2)-fucosyltransferase were 80 and 74 % respectively (Figure 1b). The protein encoded by the FTB gene has a longer C-terminus than the human protein (Figures 1b and 1c). These results suggest that FTA and FTB are members of a α(1,2)-fucosyltransferase family. This hypothesis is strengthened by several observations. On the one hand, there is no significant sequence similarity between the human α(1,2)-fucosyltransferase (H enzyme) and any of the known human α(1,3)-fucosyltransferases, although they share the same donor substrate, GDP-fucose [18]. It has also been shown that there is no sequence similarity between glycosyltransferases of different families [18]. On the other hand, members of the α(1,3)-fucosyltransferase gene family show considerable sequence similarities [19].

To confirm that FTA and FTB both correspond to fragments of α(1,2)-fucosyltransferase genes, we transfected PROb cells with plasmids containing FTA or FTB inserts in antisense

(a)

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FT A ATCACCTCCG GGAACAGATT CGCCGGGAAT TCACCCTGCA TGACCACCTA
FT A CGGGAAGACG CACAGCGCCT GTTGAGTGGG CTCCGGATAG GCCCTGCAGG
FT A CATCCGTCCT CGTACCTATG TGGGTGTTCA CGTGCGTCGT GGAGACTATT

FT A TGGAGGTGAT GCCTAACCGC TGGAAGGGAG TGGTGGGTGA CCGAGCTTAC
FT B                ----TGTG --------C- ------C--- ---G-G----

FT A CTCCAGAAAG CCATGGACTG GTTCCGGGCC CGGCACAAAG ACCCCATCTT
FT B --GG-A--G- --C----TAT ---------A --CT-TTC-T CT--AG----

FT A TGTGGTCACC AGCAATGGCA TGAGATGGTG TTTGGAGAAC ATTGACACAT
FT B C-----T--A -----C--T- --GCC----- CCG------- ---A-TG-T-

FT A CCCACGGTGA TGTGGTCTTC GCTGGCAATG GACAGGAGGG TACGCCGGGG
FT B ---GA--A-- C-GT--G--- --G------- -TATT----- GT----A-CC

FT A AAGGACTTCG CACTGCTCAC CCAGTGTAAT CACA
FT B ---------- -G-------- ------C--C ----CCATCA TGACTATTGG

FT B GACCTTTGGG ATTTGGGCTG CCTACCTGGC AGGTGGTGAT ACCATCTACT
FT B TAGCCAACTA CACCCTTCCG GATTCTCCGT TCCTCAAAGT CTTTAAGCCA
FT B GAGGCAGCCT TCCTACCCGA ATGGGTGGGC ATCCCTGCCG ATCTGTCCCC
FT B ACTCCTTAAG GCATTAACAC CAGCCTGTCC TCGGTCCCAC TTCCACCTCA
FT B AGGCAAAAGG AGTCACTTGT TACGTCGCAG GAAGAGCCTT CTGATGGGAA
FT B GAAGTGTGCC TTGGGCTGCC AACGAGAAGT ACTAACATGT TCAGAAAGCA
FT B TATTTACATG TTAGATGCGG CCAGCTCAGC TCCAACATTT GTTAACAGCT
FT B CAGACATCAT CAGTGGCCCC TGTTCCTGAA GACTCCTAGC AGTGCCATCA
FT B CCGTTTTTCA GCACAGCTGA CCCATGGCGC CAGACGGGCT GCTACCCGCA
FT B GACAGCCACT TCAGAGGCAG GCAAGAAAAG GCACGCTATG GATCGTTTTC
FT B TTTAGGTTCC TTTTGAAGGG TGAGACAAAC TTGAGTCTGC CTACTTTTTT
FT B TTTTTTTTCC CATCTCAGAA GTAGAAAGAA AATTGCAAAA ATCTAGGCTC
FT B AACAGTGGTG GCATACACTT AAGGAGACAG AGGCCAAATA GATCTCTGAG
FT B TTTGAGGCTA GCCTGGGCTA CACAGAAATC TTATTTTTAA AAAATATATA
FT B TTTTTTCAAA AATCCAAAAC AAATAAAAAT GTGCCTTTC
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(b)

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FT A        ----- ---------- ---D--RL-S G--I-PA-I- ---Y------
FT HUM WTFFHHLREQ IRREFTLHDH LREEAQSVLG QLRLGRTGDR PRTFVGVHVR
       180
FT A   -----E---N --------R- --QK------ ---KD-I--- -----R--L-
FT B           N V-----A-RG --EK-L-M-- --YSS----- -----A--R-
FT HUM RGDYLQVMPQ RWKGVVGDSA YLRQAMDWFR ARHEAPVFVV TSNGMEWCKE

FT A   -----H---V ---N---G-- G--------- --
FT B   --NA-R--GV ---N-I-GS- A--------- ---------- -I--------
FT HUM NIDTSQGDVT FAGDGQEATP WKDFALLTQC NHTIMTIGTF GFWAAYLAGG

FT B   --I----Y-- ---P---V-- ---------- --P------L KALT-ACPRS
FT HUM DTVYLANFTL PDSEFLKIFK PEAAFLPEWV GINADLSPLW TLAKP•

FT B   HFHLKAKGVT CYVAGRAF•
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(c)

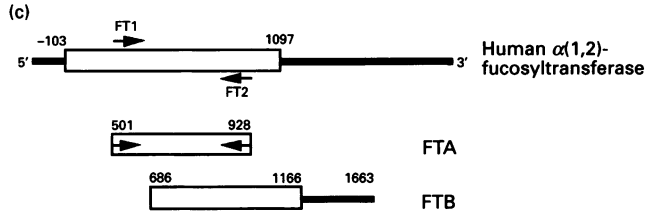

Figure 1 Sequence analysis of clones FTA and FTB

(**a**) Nucleic acid sequence of FTA (FT A) and FTB (FT B). Broken lines indicate nucleic acid sequence identity. (**b**) Deduced amino acid sequences and comparison with the human (HUM) α(1,2)-fucosyltransferase gene. (**c**) Schematic representation of FTA and FTB inserts aligned on the human gene. PCR primers (FT1 and FT2) are represented by arrows. Open boxes correspond to the reading frame and thick black lines to untranslated sequences.

orientation. Fucosyltransferase activity was determined in such transfected cells and compared with PROb cells transfected with the pSG5 plasmid. In both FTA- and FTB-antisense-transfected cells, α(1,2)-fucosyltransferase activity was significantly decreased [1.26±0.09 nmol/h per mg ($n = 3$) and 0.40±0.01 nmol/h per mg ($n = 3$) respectively] as compared with control cells (1.90±0.30 nmol/h per mg; $n = 4$). The activity in cells transfected with the plasmid pSG5 was not significatively different from that of parental PROb cells (2.15±0.30 nmol/h per mg; $n = 5$). As phenyl β-D-galactopyranoside, the synthetic substrate used in the assay, is a specific acceptor for α(1,2)-

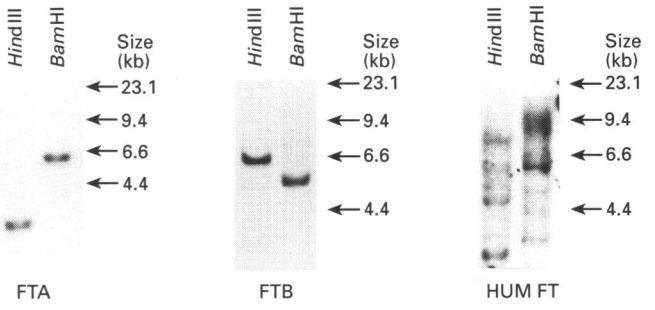

Figure 2 Southern-blot analysis of rat genomic DNA with FTA, FTB and human α(1,2)-fucosyltransferase (HUM FT) probes

DNA was digested with *Bam*HI or *Hin*dIII, electophoresed on agarose gels and transferred to nylon membranes. Hybridization and washes of FTA and FTB probes were performed at high stringency. Hybridization and washes of human α(1,2)-fucosyltransferase probe were performed at a lower stringency (see the Materials and methods section).

fucosyltransferase [16], we conclude that both fragments correspond to α(1,2)-fucosyltransferase genes. However, the decrease in enzyme activity observed with the two antisense DNA fragments cannot be compared, since the inserts differ in length. Furthermore, the two different enzymes might have different specificities, as has been shown for human α(1,2)-fucosyltransferases, the H enzyme being more reactive with phenyl β-D-galactopyranoside than the Se enzyme [6,7]. Since FTA shows the greatest sequence identity with that of the human enzyme, it is likely that FTA is a fragment of the rat gene corresponding to the human α(1,2)-fucosyltransferase cloned by Larsen et al. [13]. To test this hypothesis, rat genomic DNA was hybridized with a human α(1,2)-fucosyltransferase probe at low stringency (Figure 2). The human probe hybridized to several DNA fragments. The strongest signals corresponded to the pattern of hybridization obtained with the FTA probe (~5.5 kb for *Bam*HI-digested DNA, ~2.5 kb for *Hin*dIII-digested DNA). The conclusion, therefore, is that FTA probably represents the rat gene corresponding to the human α(1,2)-fucosyltransferase gene. The number of hybridization bands also suggests the presence in the rat genome of other genes or pseudogenes similar in sequence to the α(1,2)-fucosyltransferase gene, as described for α(1,3)-fucosyltransferases [19].

Expression of FTA and FTB genes in PROb and REGb rat cancer-cell lines was determined by Northern-blot analysis (Figure 3). The FTA gene is transcribed in both PROb and REGb cells. Although hybridization with a β-actin probe demonstrated a difference in RNA content in the wells (~2-fold difference as determined by densitometric scanning analysis), it is clear that transcription of the FTA gene is much higher in PROb cells than in REGb cells. In contrast, the FTB gene was found to be transcribed only in PROb cells. Hybridization signals were never detected with REGb RNA, even after prolonged exposure (results not shown). Altogether, both genes are much more expressed in PROb cells than in REGb cells. This is in good agreement with α(1,2)-fucosyltransferase activities, which are 5-fold higher in PROb than in REGb cells [10,11]. It has been shown that increased expression of H-blood-group antigen and, conversely, decreased expression of β-galactoside precursors of this antigen correlated with increased tumorigenicity in this experimental model of colon cancer [20]. As FTB is expressed only in the progressive PROb cells, it is tempting to speculate that FTB plays a role in the tumorigenic process. Hybridization of genomic DNA prepared from PROb and REGb cells with FTA or FTB probes did not reveal any evidence of gene rearrangement, and

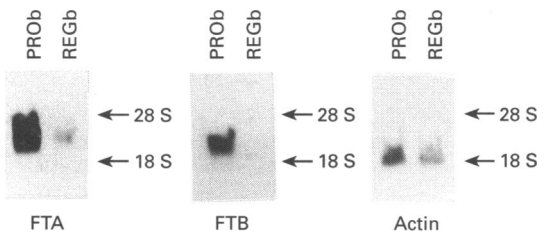

Figure 3 Northern blot of total RNA from PROb and REGb cells using FTA and FTB probes

A 15 μg portion of total RNA was loaded per lane. Hybridization was performed at high stringency. A human β-actin probe was used as control.

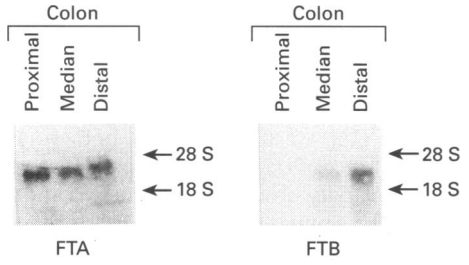

Figure 4 Expression of FTA and FTB genes in proximal, median and distal rat colon

A 15 μg portion of total RNA was loaded per lane. Hybridization was performed at high stringency (see the Materials and methods section). No difference in β-actin hybridization was seen, indicating that equal amounts of RNA were present in each lane (results not shown).

no amplification that would account for the difference in mRNA content (results not shown). Therefore, transcription of these genes is most likely increased in PROb cells, though we cannot exclude a difference in mRNA stability between the two cell lines. A, B, H, Lewis^a and Lewis^b antigens are among the major carbohydrate antigens expressed by human colonic epithelial cells. The ABH antigens are expressed throughout the human fetal colon. However, at birth and during adulthood, only the Lewis^a antigen is expressed along the colonic tract [21,22]. The ABH and Lewis^b immunodeterminants become restricted to colonocytes of the proximal, but not distal, colon [21]. In rat, lectin-binding experiments performed with *Ulex europaeus* (gorse) agglutinin, a lectin which recognizes H type 2 or Lewis^Y structures [23], was found to label more goblet cells of the adult proximal colon than of the distal colon [24,25]. Freeman et al. [26] found regional differences in the distribution of glycoconjugates of goblet cells in the rat colonic tract and suggested that there are significant differences in the biological characteristics of colonic mucosa throughout the rat colon. In order to analyse the expression of FTA and FTB genes in the colonic tract, RNA extracted from proximal, median and distal colon were hybridized with both probes (Figure 4). The FTA gene is transcribed throughout the colonic tract. The expression of the FTB gene seems to follow a gradient from distal to proximal colonic tract: FTB is strongly expressed in the distal part of the colon, weakly in the median portion, and no expression was found in the proximal region. It is conceivable that these two enzymes have different substrate specificities, as demonstrated for human α(1,2)-fucosyltransferases and for the members of the α(1,3)-fucosyltransferase gene family [27]. The differential expression of the FTA and FTB genes would thus be involved in α(1,2)-fucosylation of different precursor structures throughout the rat colon.

In conclusion, our results represent the first direct molecular evidence for the existence of two related, but distinct, α(1,2)-fucosyltransferase genes. They are co-expressed in tumorigenic PROb cells, and one of them is differentially regulated throughout the rat colonic tract. We previously described a correlation between α(1,2)-fucosylation of colon carcinoma cells and cytolysis mediated by lymphokine-activated killer cells [11,12]. We are therefore establishing stable transfectant cell lines which should permit an insight into the role of these residues in tumorigenesis. The isolation and characterization of the full-length cDNAs encoding FTA and FTB are also in progress.

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