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Genomic structure and promoter analysis of the human α1,6-fucosyltransferase gene (*FUT8*)

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GDP-L-Fuc:N-acetyl-β-D-glucosaminide α1,6-fucosyltransferase (α1,6FucT) catalyzes the transfer of a fucosyl moiety from GDP-fucose to the asparagine-linked GlcNAc residue of complex N-glycans via α1,6-linkage. We have cloned the genomic DNA which encodes the human α1,6FucT gene (*FUT8*) and analyzed its structure. It was found that the gene consists of at least nine exons spanning more than a 50 kbp genomic region, and the coding sequence is divided into eight exons. The translation initiation codon was located at exon 2, and thus exon 1 encodes only 5′-untranslated sequences. Transcription initiation site of *FUT8* was determined by 5′-rapid amplification of the cDNA end and a primer-extension analysis using the total RNA isolated from SK-OV-3 cells, which have a high level of α1,6FucT activity. We then characterized the *FUT8* promoter region by a reporter gene assay. The luciferase reporter assay indicated that the 5′-flanking region of exon 1, which covered about 1 kbp, conferred the promoter activity in SK-OV-3 cells. This region contains potential binding sites for some transcription factors, such as bHLH, cMyb, GATA-1, as well as a TATA-box, but not a CCAAT motif. 5′-Untranslated sequences found in ESTs and the cDNA for the *FUT8* suggest the presence of an additional exon(s) at the upstream of the first exon identified in this study, and therefore, the transcription of the gene would be regulated by multiple promoters.

Key words: α1,6-fucosyltransferase/gene promoter system/genomic organization/alternative splicing/*N*-glycan

Introduction

α1,6-Fucosyltransferase (α1,6FucT) transfers a fucosyl residue to position 6 of the asparagine-linked (*N*-linked) *N*-acetylglucosamine residue of an *N*-glycan, and is involved in biosyntheses of complex types of *N*-linked oligosaccharides in glycoproteins (Longmore and Schachter, 1982; Kobata, 1992; Miyoshi *et al., 1999a*). This enzyme is widely distributed in nature and its products, α1,6-fucosylated oligosaccharides, are found in numerous glycoproteins in a variety of tissues (Miyoshi *et al., 1999a*).

The α1,6-fucose content of serum glycoproteins, such as ceruloplasmin and transferrin, is typically low, under normal conditions, which is probably due to the very low levels of α1,6FucT activity in the liver where these proteins are synthesized. The activity of α1,6FucT, however, is increased considerably during the development of malignant liver diseases (Huchinson *et al.*, 1991), and, as a result, the α1,6-fucose contents of some serum glycoproteins are elevated under these conditions. In particular, sugar chains of α-fetoprotein, a well-established tumor marker which is produced by hepatocellular carcinomas, are abundantly α1,6-fucosylated, whereas those produced in non-tumorous liver diseases are only slightly fucosylated, if at all (Taketa *et al.*, 1990; Huchinson *et al.*, 1991; Ohno *et al.*, 1992). Thus, the expression of α1,6FucT in the liver is closely associated with changes in oligosaccharide structure during hepatocarcinogenesis. In addition, germ cell tumors such as yolk sac tumors also produce highly fucosylated α-fetoprotein (Aoyagi *et al.*, 1993).

In order to investigate the molecular basis of the increased expression of α1,6FucT, which is associated with the cancer-associated alteration in oligosaccharide structure, we purified and characterized α1,6FucTs from pig brain and a human gastric cancer cell line, and then cloned the cDNAs for these enzymes (Uozumi *et al.*, 1996; Yanagidani *et al.*, 1997). The enzymes were found to be nearly identical, even though they were cloned using different mRNA sources. We also isolated a genomic clone for the human α1,6FucT gene (*FUT8*) and used this to map the gene on human chromosome 14q24.3 by fluorescence *in situ* hybridization (Yamaguchi *et al.*, 1999). Northern blot analyses have shown that a good correlation exists between the increased α1,6FucT activities in hepatocellular carcinomas and the elevated levels of the *FUT8* mRNA (Miyoshi *et al.*, 1997; Noda *et al.*, 1997, 1998). This supports the view that the *FUT8* is certainly responsible for the increased fucosylation which is associated with hepatocellular carcinoma.

We recently reported that the overexpression of *FUT8* in hepatoma cells suppresses intrahepatic metastasis after splenic injection in athymic mice (Miyoshi *et al.*, 1999b), suggesting that the altered oligosaccharide structure resulting from excessive α1,6-fucosylation affects metastatic potential. Since the elevated expression of α1,6FucT activities has been reported

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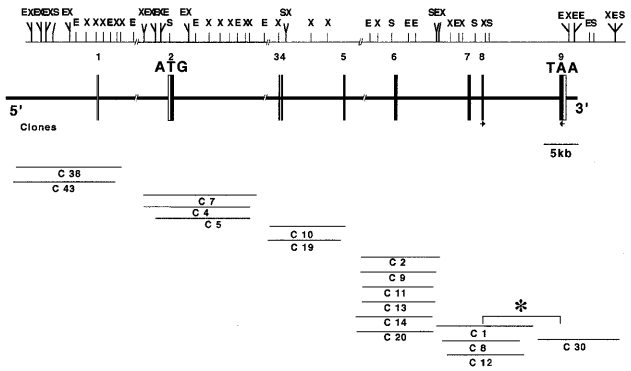

Fig. 1. Genomic structure of the human *FUT8*. Solid boxes correspond to the ORF of the FUT8 gene. Open boxes indicate the noncoding regions at the 5′- and 3′-end. Restriction sites recognized by *Eco*RI (E), *Sac*I (S), and *Xba*I (X) are shown. The positions of genomic phage clones are indicated as solid bars below. An asterisk (*) is the intron 8 estimated by PCR. The arrows show the PCR primers used for gap-filling in intron 8.

in a variety of cancers, in addition to liver cancer (Miyoshi *et al.*, 1997), *FUT8* may also play a role in other malignant diseases. Thus, the expression of this enzyme is likely to contribute to the malignant characteristics of cancer cells, and, hence, an examination of the mechanism for the *FUT8* expression is of importance in comprehending malignant potentials, such as invasive and metastatic capabilities of the cells.

This study was undertaken in order to elucidate the mechanism which controls the regulation of the expression of *FUT8* by means of a structural and functional characterization of the gene. The human *FUT8* was cloned and characterized, and its promoter activity was demonstrated via a reporter assay. The findings herein will provide useful information regarding the investigation of malignant characteristics of cells in terms of alterations in oligosaccharide structure.

The novel nucleotide sequence data reported in this study have been submitted to the DDBJ, EMBL, and GenBank sequence data banks and are available under accession numbers AB032567, AB032568, AB032569, AB032570, AB032571, AB032572, AB032573, AF038280, and AF038281.

Results

Isolation and characterization of the human FUT8

A human genomic DNA library (λFIXII) was screened using the entire cDNA for human *FUT8* (Yanagidani *et al.*, 1997) as a probe. The screening of ~10^6 plaques led to the identification of 17 independent positive clones. DNA fragments from the digestion of the genomic clones by restriction endonucleases were subjected to Southern hybridization to identify the exon-containing fragments. After subcloning the DNA fragments, which were hybridizable to the cDNA probe, into a plasmid vector, they were subjected to sequence analyses. Figure 1 shows the structure of the human *FUT8*, along with the restriction enzyme sites and positions covered by the isolated clones. The isolated genomic clones covered the entire coding region of the *FUT8* cDNA (Yanagidani *et al.*, 1997). The coding sequence was divided into eight exons, and, in addition, the most 5′-upstream exon, designated as exon 1 in this study, was found to encode only 5′-untranslated sequences of the cDNA. The nucleotide sequences of all exons were found to be identical to the cDNA sequences (Yanagidani *et al.*, 1997). The exon–intron boundaries all followed the GT-AG rule that introns begin with GT and end with AG (Mount, 1982), and were in agreement with the consensus sequences for the splicing donor and acceptor sites (Table I). Although all coding exons were covered by the isolated clones, no overlap in the four intronic sequences was observed. Among the four introns, 1, 2, 5, and 8, the length of intron 8 was successfully estimated by genomic PCR, but attempts to determine the sizes of the other three introns by other methods resulted in failure. Thus, the size of the genomic region is predicted to be longer than 50 kb, but the exact size remains unknown.

Table I. Exon–intron boundaries of the human *FUT8*

Exon number	Position in the cDNA (bp)	Exon size (bp)	Sequences at exon–intron junction
1	–260 ~ –230	31	——TTCAGgttag
2	–229 ~ 203	432	cacagCATGT——CTCCGgtagg
3	204 ~ 319	116	tacagGATAC——AAATGgtagg
4	320 ~ 482	163	aacagGTCTG——GAAAGgtact
5	483 ~ 597	112	tacagGTCTA——TTCAGgtgca
6	598 ~ 830	236	gacagAATCC——CACTGgtaat
7	831 ~ 1082	252	aacagGTCAG——ATTGGgtaag
8	1083 ~ 1259	177	cgcagAGTCC——ACAAAgtaag
9	1260 ~ 2493	1234	actagGTACC——ATTTTaaaag

Identification of the transcription initiation site

To determine the transcriptional initiation site of the *FUT8*, the 5′ end of the mRNA was analyzed by 5′-rapid amplification of the cDNA end (5′RACE) and a primer-extension analysis. Prior to these analyses, we surveyed several ovarian cancer cell lines using the enzyme activity assay and Northern blot analysis in order to identify a cell line that expresses a sufficiently high level of the *FUT8* mRNA. As shown in Figure 2, the SK-OV-3 cells were found to express the highest level of mRNA as well as activity among the five cell lines examined.

Total RNA extracted from the SK-OV-3 cells was reverse-transcribed, and a homopolymeric dA-tail was then attached to the resulting first-strand of cDNA using terminal nucleotidyltransferase with dATP. As shown in Figure 3A, the DNA was subjected to the first PCR using the oligo dT anchor primer and the gene specific primer SP-1, resulting in the amplification of approximately a 0.5 kbp fragment. This PCR product was then used for the second PCR with a pair of the anchor primer and the gene specific primer SP-2. The PCR products were subcloned into the plasmid vector followed by sequence analysis (Figure 3A). It was found that a putative 5′ end of the mRNA for the *FUT8* extended by 257 nucleotides from the translation start codon, as revealed by the results from analyses of several clones (Figure 3B). To further confirm the transcription initiation sites, a primer-extension analysis was performed. This analysis suggests that the transcription of the

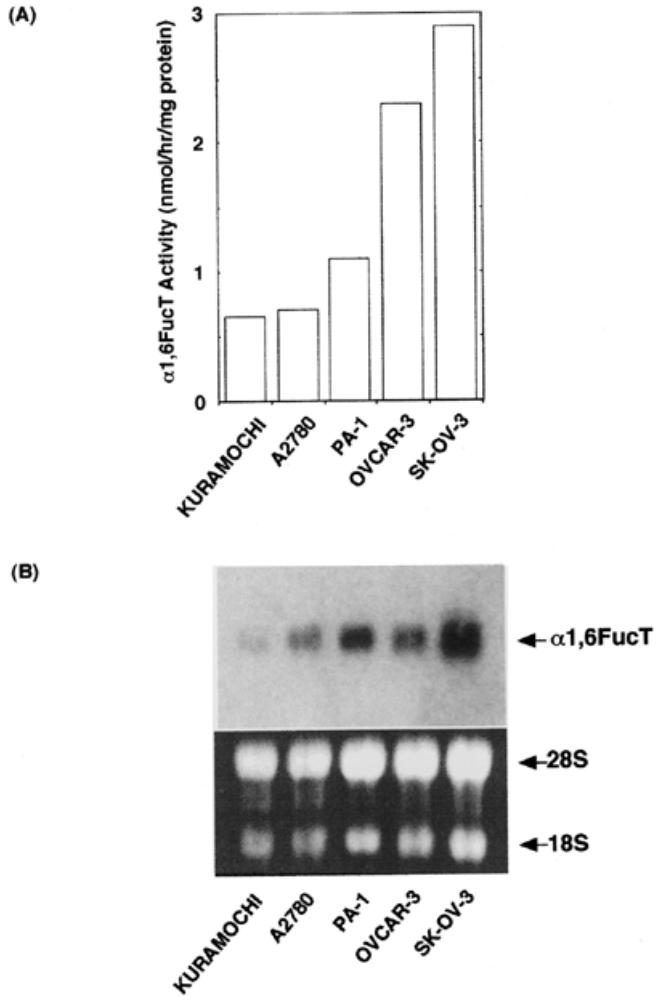

Fig. 2. Expression of *FUT8* in various carcinoma cell lines. (**A**) α1,6FucT activities. (**B**) Northern blot of the *FUT8* mRNA in the cell lines (top). Ethidium bromide staining shows a comparable amount of RNA in each lane (bottom).

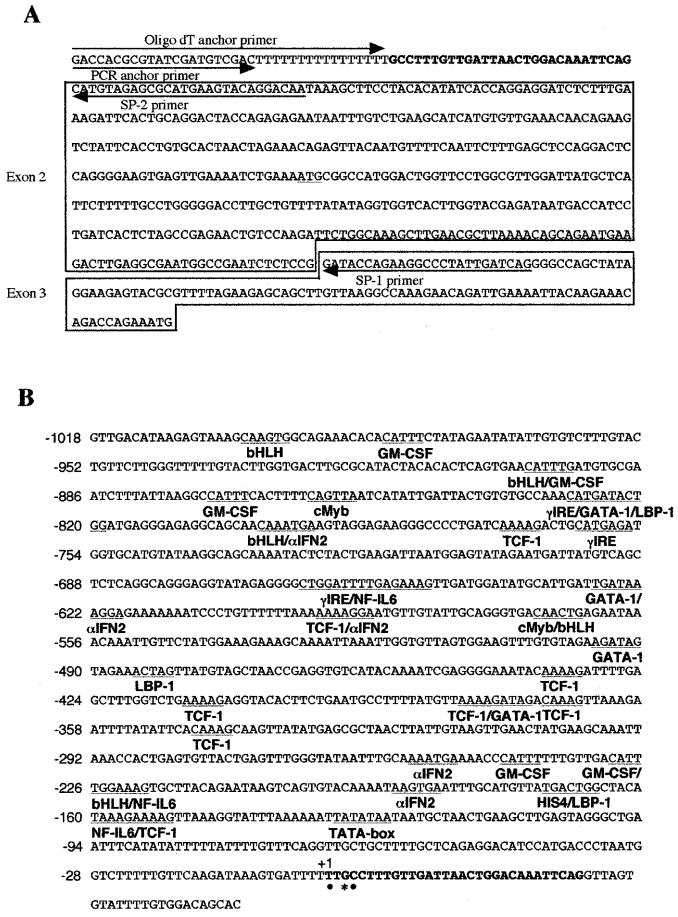

Fig. 3. The 5′-untranslated region of the human *FUT8* mRNA by 5′RACE and the 5′-flanking genomic sequences of the region. (**A**) The bold letters show exon 1 sequence determined by 5′RACE using SK-OV-3 cells. Exons 2 and 3 are boxed. The underlined ATG indicates initiation codon. (**B**) The sequences are the proximal promoter region of the *FUT8* including 31 bases of exon 1 (bold letters). Dotted nucleotides are transcription-initiation sites determined by primer-extension analysis. An asterisk (*) indicates the 5′ end of 5′RACE products. Potential sequences for transcriptional elements are indicated.

FUT8 starts at –259 and –256 bp upstream of the translation initiation codon in the SK-OV-3 cells (Figure 3B). Therefore, we conclude that the sites determined herein represent transcription start sites in the cell line examined.

Nucleotide sequences and promoter activities of the 5′ upstream region

Sequence analysis of ~1 kbp of the 5′-flanking region of the determined transcription initiation site revealed that the region contained a typical TATA-box sequence but not a canonical CCAAT motif (Figure 3B). In order to explore the issue of whether the upstream region functions as a promoter, we constructed a reporter plasmid in which a firefly luciferase gene was fused to the 20 bp downstream of the transcription initiation site. When SK-OV-3 cells were transfected with the plasmid containing the entire 1 kbp fragment, the reporter activity was found to be about 8 times higher than that in the case of a control plasmid with no extra DNA fragment (Figure 4). Therefore, we conclude that the identified region serves as a promoter in the *FUT8*. For a more detailed exploration of the region responsible for the promoter activity, several truncated forms of the promoter sequences were prepared. These were inserted into the reporter plasmids and were also subjected to the reporter assay. As shown in Figure 4, all the truncated constructs exhibited much lower luciferase activity, as compared with that in the whole 1 kbp fragment. It is thus likely that essential sequences for enhancing the *FUT8* transcription are located between –1015 and –910 bp region and the downstream sequences had no significant contribution to the activity in this cell line.

Discussion

Fucosyltransferases catalyze the transfer of a fucosyl moiety from GDP-fucose in α-linkage to an appropriate acceptor substrate. In mammals, fucosyltransferases involved in the formation of α1,2-(FucTH and FucTSe), α1,3-(α1,3/4-) (FucTIII–FucTVII and FucTIX), and α1,6-fucose residues (α1,6FucT) are also known (Breton *et al.*, 1998; Costache *et al.*, 1997). Among the nine human fucosyltransferase genes

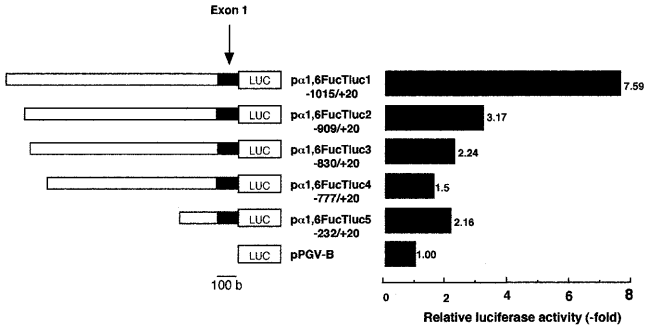

Fig. 4. Promoter activities of the 5′-flanking sequences of the human FUT8. Activities of the human *FUT8* promoter were examined in SK-OV-3 cells. Promoter/luciferase gene constructs are shown on the left. Results are expressed as the relative luciferase activity compared to the activity obtained by transfecting with the promoterless plasmid pPGV-B.

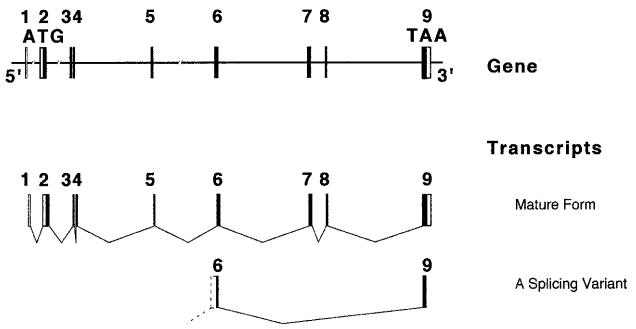

Fig. 5. Alternative splicing of the human *FUT8* in retina.

(*FUT1–FUT9*) reported to date (Larsen *et al.*, 1990; Kelly *et al.*, 1995; Kukowska-Latallo *et al.*, 1990; Goelz *et al.*, 1990; Weston *et al.*, 1992a,b; Sasaki *et al.*, 1994; Yanagidani *et al.*, 1997; Kaneko *et al.*, 1999), the ORFs of *FUT1* to *FUT6* are encoded by single exons, and the ORF of the *FUT7* is assembled from two exons. On the other hand, our findings indicate that the ORF of the *FUT8* unusually encompasses 8 exons. The human *FUT8* product consists of 575 amino acid residues without a consensus *N*-glycosylation site, whereas the other fucosyltransferases have much fewer amino acid residues, about 400 or less. In addition, the *FUT8* has been mapped to the chromosomal region 14q24.3 (Yamaguchi *et al.*, 1999) and this localization is distinct from those of some α1,2- and α1,3-fucosyltransferases, in which the genes are clustered or located close together. Thus, it is shown that *FUT8* is unique and distinct from other fucosyltransferases in terms of both its structural and genetical features.

It appears that fucosyltransferase genes have evolved from a single ancestral gene by successive duplications, followed by subsequent, divergent evolution, as suggested by Costache *et al.* (1997). The phylogenetic tree in their report also suggested that the *FUT8* is the orthologous homologue of the common ancestor of the α1,2- and α1,3-fucosyltransferase genes. Our result concerning the genomic organization is consistent with these proposals, which have been made on the basis of the sequence comparison (Costache *et al.*, 1997; Breton *et al.*, 1998), that the *FUT8* is the oldest gene of the fucosyltransferase family and that gene duplication of the *FUT8* or its closely related ancestral gene is the origin of the diversity in this enzyme family, since it is possible that the other fucosyltransferase genes initially arose by RNA-mediated gene duplication which may yield an intronless gene from an ancestral intron-containing gene (McCarrey, 1987; McCarrey, 1990).

A FASTA search of the GenBank database identified 20 ESTs encoding human *FUT8*. Of these ESTs, five, all of which were derived from the retina, encode sequences containing exons 6 and 9 but lack exons 7 and 8, suggesting that the latter two exons are alternatively spliced out in these transcripts (Figure 5). The lack of exons 7 and 8 causes a frame-shift in exon 9, resulting in the premature termination of translation. As a result, this splicing variant of the transcript encodes a much shorter protein, which contains only 308 amino acid residues. The variant protein would be unable to catalyze α1,6-fucosylation because it lacks the protein domain which has been predicted to play an essential role in enzyme activity through the binding of the donor substrate (Breton *et al.*, 1998; Perrin *et al.*, 1999). Although many ESTs for the *FUT8* have been reported in several tissues, the ESTs for the splicing variant were found only in retina. Thus, it seems likely that the variant is produced via retina-specific alternative splicing and may have a role which is independent of the fucose-transferring activity. Although we attempted to screen human adult and fetal retina cDNA libraries, the cDNA for the splicing variant has not been isolated.

The reporter assay indicated that approximately a 1.0 kb 5′-flanking region of the transcription initiation site actually serves as a promoter in SK-OV-3 cells. However, the identified transcription initiation site appears to be located more downstream than would have been expected by the position of typical TATA-box (Figure 3B), because the motif is generally localized within a 30 nucleotide sequence upstream of the transcription initiation site of the genes. The luciferase reporter assays using successively truncated forms of the 5′-flanking region demonstrated that the region between –1015 to –910 is responsible for enhancing the expression of the *FUT8* in SK-OV-3 cells, an ovarian cancer cell line. This region contains consensus sequences for bHLH and GM-CSF-responsive elements. In many ovarian cancer cells, it is known that GM-CSF often forms an autocrine loop as well as other cytokines such as IL-1, IGF-1, M-CSF, GM-CSF, and TNF-α (Burke *et al.*, 1996).

A search of the database also identified four human *FUT8* ESTs which have sequences of 200–450 bp upstream from the transcription initiation site determined here. Hence, it is entirely possible that an additional upstream exon(s) and other promoter(s) in the human *FUT8* exist, although we identified only one promoter region localized upstream of the noncoding exon next to the exon containing the translational initiation codon. An increasing number of vertebrate genes have been found to be regulated by multiple promoters, and, in the case of glycosyltransferases, multiple promoters have also been reported for many genes such as the α2,3-sialyltransferase ST3Gal IV (Kitagawa *et al.*, 1996), the *N*-acetylglucosaminyltransferase III (Koyama *et al.*, 1996), the *N*-acetylglucosaminyltransferase V (Saito *et al.*, 1995), a β1,4-galactosyltransferase (Shaper *et al.*, 1995; Rajput *et al.*, 1996) and α1,3-fucosyltransferases (Cameron *et al.*, 1995). The *FUT8* could also be possibly transcribed from multiple promoters to regulate its expression in a cell-type- or tissue-specific manner.

Identification of the promoter region(s) responsible for the cancer-associated elevation of α1,6FucT level would be one of the most important issues. A comprehensive study involving the possible multiple promoter system would be required to understand the more detailed mechanism for the regulation of α1,6FucT gene expression.

Materials and methods

Screening of a genomic library

A human genomic DNA library constructed in λFIXII (Stratagene, La Jolla, CA) was screened with the full-length cDNA clone which encodes for the human *FUT8* (Yanagidani *et al.*, 1997) as a probe. Hybridization was carried out with the ^{32}P-labeled cDNA probe overnight at 42°C in a hybridization solution which contained 50% formamide. The filters were then washed three times in 2× SSC containing 0.1% SDS at 55°C, and were exposed to x-ray films at –80°C overnight. λPhages were purified by glycerol density-gradient ultracentrifugation and subjected to proteinase K digestion. After phenol/chloroform extraction, the λphage DNAs were precipitated in 80% ethanol and subjected to further analyses.

Restriction mapping of the FUT8

A restriction map of cloned *FUT8* was established using the FLASH nonradioactive gene mapping kit (Stratagene) according to the manufacturer's instructions. Briefly, λphage DNAs were digested to completion with *Not*I and then partially digested with *Eco*RI, *Xba*I, and *Sac*I. These partially digested DNAs were separated on agarose gels and their blots were performed in duplicate. The membranes were subjected to hybridization using a alkaline phosphatase-conjugated probes, which were specific to either of the T3 and T7 promoters. The blots were reacted with a chemiluminescent substrate for alkaline phosphatase, and then exposed to x-ray films.

Determination of exon/intron boundaries

The λphage DNAs were digested with various restriction enzymes and characterized by Southern hybridization. The restriction enzyme-digested DNA fragments, which were hybridizable to the cDNA probe, were subcloned into pBluescript II KS$^+$. The entire exons and exon/intron boundaries were directly sequenced for both strands in an ABI PRISM 310 DNA Genetic Analyzer using a DNA sequencing kit (PE Biosystems Inc., USA). Intron sizes were determined by Southern blotting, restriction mapping, and long accurate polymerase chain reaction (LA-PCR) analyses of the genomic DNAs using an LA-PCR kit (Takara, Shiga, Japan).

Cell culture

All cells were maintained at 37°C in 5% CO_2. SK-OV-3 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, and 200 μg/ml kanamycin. OVCAR-3, A2780 and Kuramochi cells were maintained in RPMI1640 supplemented with 5% FCS and 200 μg/ml kanamycin. PA-1 cells were maintained in Eagle's minimal essential medium supplemented with 5% fetal calf serum and 200 μg/ml kanamycin.

Protein determination

The protein concentration was determined with a Bio-Rad protein assay kit using bovine serum albumin as the standard.

Assay for α1,6FucT activity

α1,6FucT activity was analyzed using a 4-(2-pyridylamino)-butylamine-labeled oligosaccharide as previously described (Uozumi *et al.*, 1996). The reaction was performed in 100 mM MES-NaOH buffer (pH 7.0) containing 1% Triton X-100, 500 μM GDP-fucose as the donor substrate, and 5 μM of the 4-(2-pyridylamino)-butylamine-labeled sugar chain as the acceptor substrate. After the sample addition, the mixture was incubated at 37°C for 2 h and then boiled for 1 min to terminate the reaction. The reacted mixture was centrifuged for 10 min at 10,000 × *g* and the resulting supernatant was applied to a high-performance liquid chromatography apparatus equipped with a TSK-gel and ODS-80TM column (4.6 × 150 mm) (Tosoh, Tokyo, Japan) in order to separate and quantify the product. The elution was isocratically performed at 55°C with 20 mM sodium acetate buffer containing 0.15% butanol (pH 4.0). Fluorescence of the column elute was detected with a fluorescence detector (Shimadzu, Kyoto, Japan, model RF-10AXL) at excitation and emission wavelengths of 320 and 400 nm, respectively. The amount of the product was estimated from the fluorescence intensity.

RNA isolation and Northern blotting

Total RNAs were isolated from several lines of ovarian cancer cells with TRIzol Reagent (Life Technologies Inc.). A total of 10^6 cells were homogenized in 1 ml of TRIzol Reagent and were incubated for 5 min at room temperature. After chloroform extraction, the total RNAs were precipitated in 50% isopropyl alcohol. Twenty micrograms of the total RNAs were electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde and then transferred onto a Zeta-probe membrane (Bio-Rad, Hercules, CA) by capillary action. The membrane was hybridized with the ^{32}P-labeled 2.7 kbp fragment of the *FUT8* cDNA as a probe. Hybridization was carried out overnight at 42°C in the hybridization solution, which contained 50% formamide. The membrane was then washed three times with 2× SSC containing 0.1% SDS at 55°C, and exposed overnight to x-ray films at –80°C.

5′-Rapid amplification of the cDNA end (5′RACE)

Five μg of the total RNA from SK-OV-3 cells were reverse-transcribed with SuperScript II (Life Technologies Inc.) using random primers under the reaction conditions recommended by the supplier. The 5′-end of the human *FUT8* cDNA was cloned by a 5′RACE experiment (Ohara *et al.*, 1989) using the 5′/3′ RACE Kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions. Briefly, a homopolymeric dA-tail was appended to the 3′end of the first-strand cDNA using a terminal nucleotidyl transferase and dATP. The poly dA-tailed cDNA was amplified by PCR using the oligo dT-anchor primer (5′-GACCACGCGTATC-GATGTCGACTTTTTTTTTTTTTTTV-3′) and the gene specific primer SP-1 (5′-CTGATCAATAGGGCCTTCTGG-TATC-3′; corresponds to nucleotides 204 to 228). The reaction conditions were: initial denaturation for 2 min at 94°C, followed by 10 cycles of 15 s at 94°C, 30 s at 55°C, and 40 s at

72°C; and 25 cycles of 15 s at 94°C, 30 sec at 55°C, 40 s + cycle elongation of 20 s for each cycle at 72°C; and then 7 min at 72°C. Ten microliters of the PCR product was further amplified using the PCR anchor primer (5′-GACCACGCGTATCGATGTCGAC-3′) and the gene specific primer SP-2 (5′-TTGTCCTGTACTTCATGCGCTCTACATG-3′; which corresponds to nucleotides –228 to –201). The second PCR product was purified from the agarose gel using a DNA Cell (Daiichi Pure Chemicals, Tokyo, Japan), subcloned into T-vector pT7Blue (R) (Novagen, Darmstadt, Germany), and sequenced.

Primer extension

The 30-mer oligonucleotide primer, 5′-TATTGTCCTGTACTTCATGCGCTCTACATG-3′, of which the sequence corresponds to the position of –199 to –228 from the translation initiation codon and locates in exon 2, was labeled with ^{32}P at the 5′ end by T4 polynucleotide kinase. The labeled oligonucleotide (5×10^5 c.p.m.) was mixed with 50 μg of total RNA from SK-OV-3 cells in 20 μl of 10 mM Tris/HCl, pH8.3, 1 mM EDTA, 250 mM KCl and their hybridization was carried out by heating at 60°C for 1 h followed by incubation at room temperature for 1.5 h. To this mixture, 60 μl of reverse-transcription buffer (16.7 mM KCl, 13.3 mM $MgCl_2$, 23.3 mM Tris/HCl, pH 8.3, 13.3 mM dithiothreitol, 0.33 mM dNTPs, and 0.133 mg/ml actinomycin D), 20 U of Molony mouse leukemia virus reverse transcriptase (RNase H^-), and 20 U of RNase inhibitor were added, and the extension reaction was carried out at 37°C for 1 h. The reaction mixture was subjected to the ethanol-precipitation and then analyzed by urea-denatured polyacrylamide gel electrophoresis.

Construction of reporter plasmid with the firefly luciferase gene

The full-length promoter (–1015 to +20) and 4 deletion constructs (–909, –830, –777, –232 to +20) of the *FUT8* were subcloned into a firefly luciferase reporter plasmid, the PicaGene basic plasmid vector, pPGV-B (Toyo Ink Inc., Tokyo, Japan) which contains neither a eukaryotic promoter nor an enhancer element.

Transfections and reporter assays

Twenty micrograms of the pPGV-B DNA carrying various lengths of the 5′-upstream sequences to be examined and 0.8 μg of the pRL-TK DNA containing sea pansy luciferase, the transcription of which is driven by thymidine kinase promoter, were introduced into approximately 2×10^5 SK-OV-3 cells by electroporation (Chu *et al.*, 1987). The activities of the firefly luciferase as a reporter were determined 48 h after the transfection. Sea Pansy luciferase activities were assayed as an internal control for normalization of the results. Differential activity determination of these luciferases was carried out according to manufacturer's instructions (Kitamura *et al.*, 1999).

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Abbreviations

FucT, fucosyltransferase; *FUT*, FucT gene; LA-PCR, long accurate polymerase chain reaction; 5′RACE, 5′-rapid amplification of the cDNA end.

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