Biochemical and Biophysical Research Communications **273,** 370–376 (2000)
doi:10.1006/bbrc.2000.2929, available online at http://www.idealibrary.com on IDEAL®

Expression and Transcriptional Regulation of the Human α1,3-Fucosyltransferase 4 (FUT4) Gene in Myeloid and Colon Adenocarcinoma Cell Lines

Akiyoshi Taniguchi,[1] Ryohei Suga, and Kojiro Matsumoto
Department of Clinical Chemistry, School of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan

Received May 22, 2000

In fucosyltransferase genes, mRNA expression is regulated in a cell-type-specific manner. The expression level of human fucosyltransferase 4 (FUT4) mRNA is high in both colon adenocarcinoma and myeloid cell lines. We will demonstrate here cell-specific expression and transcriptional regulation of the FUT4 gene. FUT4 has two different transcription initiation sites that respectively produce long- and short-form mRNAs. To determine the major FUT4 transcript in colon adenocarcinoma and myeloid cell lines, we analyzed the transcriptional starting sites of the FUT4 gene in myeloid and colon adenocarcinoma cell lines, using 5′-RACE, RT-PCR, and luciferase analysis. The results suggested that the expression level of short-form mRNA is higher than the long-form transcript in the colon adenocarcinoma cell lines and that the expression level of long-form mRNA is higher than the short-form transcript in the myeloid cell lines. Using a luciferase assay, we identified a functional DNA portion within FUT4 genomic DNA that confers a colon adenocarcinoma cell line-specific enhancer, located in nucleotide number (nt) −256 to −44, and a myeloid cell line-specific enhancer, located in nt −686 to −582. The present results suggest that these elements play a critical role in the colon adenocarcinoma and leukemia cell-specific transcriptional regulation of the FUT4 gene. © 2000 Academic Press

Key Words: **fucosyltransferase; gene expression; transcriptional regulation; myeloid cell line; colon adenocarcinoma cell line; 5′-RACE; RT-PCR; luciferase assay; enhancer; promoter.**

Fucosylated oligosaccharides expressed on the cell surface are involved in embryogenesis, leukocyte trafficking and inflammation. Expression of fucosylated glycans depends upon fucosyltransferses (FUTs). Recently, six human α1,3-FUTs have been cloned and partially characterized: FUT3, FUT4, FUT5, FUT6, FUT7, and FUT9 (1–9). FUT4 cDNA isolated from the HL-60 cell corresponds to myeloid type fucosyltransferase (6). FUT4 catalyzes the transfer of fucose to type 2 chain-based structures, but is much less active on sialylated substrates (10). FUT4 gene is expressed in several tissues including myeloid and colon cells (11). The expression of the FUT4 gene is regulated in a cell type-specific manner. However, cell type-specific transcriptional regulation of this gene is not well understood.

FUT4 has two closely spaced, in-frame ATGs, and uses two different transcription initiation sites to produce mRNAs that encode either a long or a short form of the protein, originating at either of the two different ATGs (6). However, the first ATG that would be used to produce such a protein does not conform to the consensus sequence for translation initiation. Transcription of the two mRNA forms may be initiated by the promoter regions, pL and pS. Miltiple promotors have been described in sialyltransferase genes (12–20). Cell type specific mRNA isoforms, generated by cell type specific promoters, have been identified in α2,6-sialyltransferase (hST6Gal I) (12–14, 18, 20). It is not known yet which type of FUT4 mRNA is expressed in different cell types.

In the present study, we compared the transcriptional regulation of the FUT4 gene in human colon adenocarcinoma and myeloid cell lines. The 5′-RACE, RT-PCR and luciferase assay results suggested that the short form mRNA is the major transcript in the colon adenocarcinoma cell lines, while long form mRNA is the major transcript in the myeloid cell lines. We identified the cell type specific enhancers using

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases under Accession No. AB041774.

[1] To whom correspondence should be addressed. Fax: +81-47-472-1485. E-mail: taniaki@phar.toho-u.ac.jp.

Copyright © 2000 by Academic Press
All rights of reproduction in any form reserved.

luciferase assay. These elements may play a critical role in colon adenocarcinoma- and leukemia-cell specific transcriptional regulation of the FUT4 gene.

MATERIALS AND METHODS

Cell culture. Human colon adenocarcinoma (HT-29, SW48 and COLO201) and leukemia cell lines (HL-60, U-937 and THP-1) were obtained from the American Type Culture Collection (U.S.A.). Cells were maintained in the media containing 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin: RPMI 1640 (Nissui, Japan) for HL-60, U-937 and THP-1 and Dulbecco's modified Eagle's medium (Nissui, Japan) for HT-29, SW-48 and COLO201.

5′-RACE analysis. Amplification of the 5′ end of FUT4 cDNA was performed according to the manufacturer's instructions (5′-RACE System for Rapid Amplification of cDNA ends, Gibco BRL, U.S.A.). First-strand cDNA was synthesized from 3 μg of total RNA using the gene specific primer, 5′-TCGCGACGGGGTTGGCGACGCCCA-3′. After digestion of template mRNA with RNase H at 30°C for 30 mim, cDNA was precipitated with spin cartridge. A homopolymeric tail was then added to the 3′-end of the cDNA using TdT and dCTP. The dC-tailed cDNA was used as the template for the first PCR amplification using a bridged anchor primer as the sense primer and 5′-TAGGTGATCAGCGCCGTACACGTCAA-3′ as the anti-sense primer. Thirty-five cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min were performed. The resulting PCR products were diluted 100-fold with sterile water, and amplified under the same conditions using anchor primer as the sense primer and 5′-CCAGCACACAGACGGTCCATGGCA-3′ as the anti-sense primer. The PCR amplicon was ligated into pCR2.1-TOPO (Invitrogen, U.S.A.), and sequenced using GeneRapid DNA sequencing system (Amersham Pharmacia Biotech, UK).

RT-PCR analysis of FUT4 mRNA. RT-PCR were performed as described previously (18, 19). Four μg of total RNA was heated at 70°C for 10 min and placed on ice for 2 min. Reverse transcription into cDNA was achieved using the Super Script preamplification system (Gibco BRL, U.S.A.) according to the manufacturer's protocol with random hexamers as initiation primers in a final reaction volume of 22 μl. Four μl of the retrotranscription reaction were subjected to PCR amplification. Specific primers were as follows; long form (forward), 5′-TGCGCGGCAGCTGCTTTAGAAGGTCTC-3′; long form (reverse), 5′-GCACCGGCTCTCATGCTGTAGCTGCGG-3′; FUT4 (forward), 5′-CAGCTGGTTCGAGCGGTGAAGCCGCGCT-3′; FUT4 (reverse), 5′-CAGAAAAACGTGAATCGGGAACAGTTGTGT-3′. Twenty-two cycles of PCR amplification consisting of denaturation at 94°C for 30 s, annealing at 54°C for 1 min and extension at 72°C for 1 min were allowed to proceed in a Perkin–Elmer/Cetus thermal cycler.

Cloning of the 5′-flanking region of the FUT4 pL and pS promoters. Cloning and isolation of the 5′-flanking region of pL and pS promoter was performed using GenomeWalker kit (Clonetech, USA) according to the manufacturer's instructions. *Eco*RV digested human genomic DNA was ligated with a double stranded oligonucleotide containing an anchor sequence, which functioned as a primer binding site for subsequent PCR amplification. Primary PCR was performed with the provided adapter primer (AP1) and a gene specific primer, GSP1 (5′-CAGCACACAGACGGTCCATGGCA-3′). Secondary PCR using the provided adapter primer (AP2) and gene specific primer, GSP2 (5′-GTCGGCGAGCCCCACGGTGCCCCCTA-3′) was performed. The PCR amplicon was ligated into pCR2.1-TOPO (Invitrogen, U.S.A.), and sequenced using the GeneRapid DNA sequencing system (Amersham Pharmacia Biotech, UK).

Luciferase assay. Transient transfection was performed using Effectene Transfection Reagent (Qiagen, Germany) for HL-60 and U-937 cells and DMRIE-C reagent (Gibco BRL, U.S.A.) for HT-29 and SW-48 cells. Luciferase assays were performed as described previously (16, 17). Cells were plated at a density of approximately 1–3 $\times$ 10^5 cells per 35-mm dish, and then transfected with 1 μg of pGL constructs and 0.1 μg of pRL-CMV (Promega, U.S.A.), containing the CMV promoter located downstream of the *Renilla* luciferase gene, as an internal control for variations in transfection efficiency. After 24 h, cells were harvested and cell lysates were prepared. *Firefly* and *Renilla* luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega, U.S.A.).

Construction of plasmids for luciferase assay. The following oligonucleotide primers were designed (pGL-1027pL, 5′-GAGGTACC-ATCATCCAAACTATTTTGCATA-3′; pGL-615pL, 5′-TTGGTACCGT-CTCGGCGGGCCACGAGAT-3′; and pGL-520pL, 5′-AAGGTACCGTCG-CTGGCGGGTGGCTA-3′) and 5′-TTAAGCTTGCCGCGCAGGAGCAGG-CCCTAG-3′ (pGL-1027pS, 5′-GAGGTACCATCATCCAAACTATTTTGC-ATA-3′; pGL-615pS, 5′-TTGGTACCGTCTCGGCGGGCCACGAGAT-3′; pGL-520pS, 5′-AAGGTACCGTCGCTGGCGGGTGGCTA-3′; pGL-256pS, 5′-TTGGTACCGCGCTTGTGGGGCGCGGCC-3′; and pGL-44pS, 5′-TTGGTACCGCCGCGGGCCCTGCCCTGCT-3′) and 5′-CCAAGCTT-GGAGGAGCGGCACCGGCTCT-3′. Restriction sites incorporated into the primers are underlined. Twenty five cycles of PCR amplification consisting of denaturation at 98′C for 20 s, and annealing and extension at 68°C for 1 min were allowed to proceed in a Perkin–Elmer/Cetus thermal cycler. A single band was obtained by agarose gel electrophoretic analysis. The PCR products were digested with *Kpn*I and *Hin*dIII restriction enzymes and cloned into the *Kpn*I and *Hin*dIII sites of the pGL3-Basic vector (Promega, U.S.A.).

RESULTS

Analysis of the Transcriptional Starting Sites of FUT4 mRNA in Colon Adenocarcinoma and Myeloid Cell Lines

FUT 4 has two different transcription initiation sites that respectively produce long and short form mRNA. Figure 1 shows a schematic diagram of the 5′-untranslated region of FUT4 cDNAs. To determine the major FUT4 transcript in colon adenocarcinoma and myeloid cell lines, we analyzed the transcriptional starting sites of the FUT4 gene in myeloid and colon adenocarcinoma cell lines, using 5′-RACE analysis. After subcloning the PCR products and sequencing individual bands, we identified two types of cDNA, namely long form and a short form. 5′-RACE analysis of colon adenocarcinoma cell lines, HT-29 and SW-48 cells resulted in major extension products of short form cDNA (Fig. 2). In contrast, the long form cDNA were barely detectable by 5′-RACE analysis. Thus, HT-29 and SW-48 cells would be predicted to contain predominantly the short form of FUT4.

In myeloid cell lines, HL-60 and U-937, both types of cDNAs were detected by 5′-RACE analysis. The level of long form cDNA was much higher than short form in HL-60 and U-937 cells using 5′-RACE analysis (Fig. 2). Thus, HL-60 and U-937 cells would be predicted to contain predominantly the long form of FUT4.

Comparison of Expression Level of Long-Form mRNA Isoforms in Myeloid and Colon Adenocarcinoma Cell Lines by RT-PCR

The results of 5′-RACE analysis suggest that the expression level of long form mRNA is high in myeloid

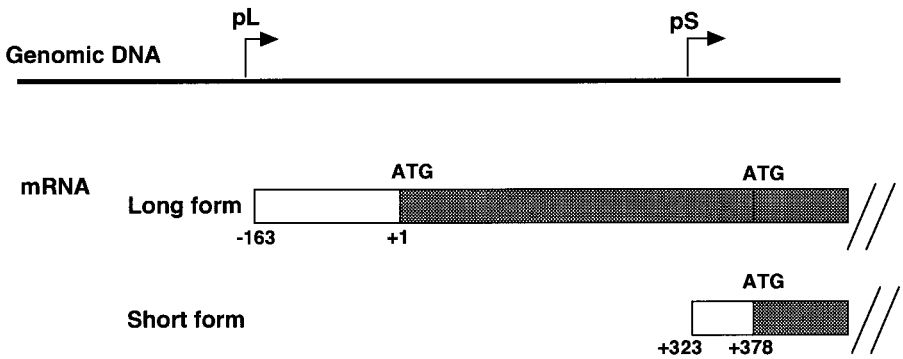

FIG. 1. Comparison of structures of 5′-regions in long and short form mRNA isoforms. Black boxes represent coding sequences and open boxes denote 5′-untranslated sequences.

cell lines, compared with colon adenocarcinoma cell lines. In order to compare the expression level of long form transcript in colon adenocarcinoma and myeloid cell lines, we performed RT-PCR analysis using long form specific primer. As shown in Fig. 3, long form specific primer gave fragments with the expected size after amplification of cDNA from myeloid cell lines, but in colon adenocarcinoma cell lines, long form mRNA was barely detected. The long form mRNA was detectable in colon adenocarcinoma cell lines, when sensitive assay condition was used (deta not shown). The results suggest that the expression level of long form isoform mRNA is high in the myeloid cell line, when compared with colon adenocarcinoma cell lines.

Cloning of the 5′-Flanking Region of the FUT4 pL and pS Promoters

To clarify the transcriptional regulation of the FUT4 gene in myeloid and colon adenocarcinoma cell lines, we cloned and identified the 5′-flanking region of FUT4 pL and pS promoter from a human genome library. The sequence of this region is shown in Fig. 4. The transcription start sites of 5′-RACE are indicated by arrows. The 5′-flanking region lacks canonical TATA or CCAAT boxes, but contains several putative transcriptional factor binding sites such as MZF-1, AML-1a, AP-1, Sp1, and Lyf-1.

Deletion Analysis of pL and pS Promoters

To identify the cell type-specific enhancer elements of pL and pS promoters, we prepared luciferase constructs carrying 5′-deleted FUT4 pL and pS promoters (Fig. 5A) and transfected them into HT-29, SW-48, HL-60 and U-937 cell lines. Plasmids pGL-1027pS, pGL-615pS, pGL-520pS and pGL-256pS containing pS promoter had very high luciferase activities in HT-29 and SW-48 cell lines, whereas plasmids pGL-1027pL, pGL-615pL and pGL-520pL containing pL promoter did not (Fig. 5B). These results suggest that the activity of pL promoter is much higher than pS promoter activity in HT-29 and SW-48 cell lines. In HT-29 and SW-48 cells, extension of the 5′-deletion of the pS promoter to nt −44 (pGL-44pL) reduced expression to approximately 10–20% of that of the promoter that was deleted to nt −256 (pGL-256pS) (Fig. 5B). In contrast to colon adenocarcinoma cell lines, truncation at −44 had no effect on pS promoter activity in HL-60 and

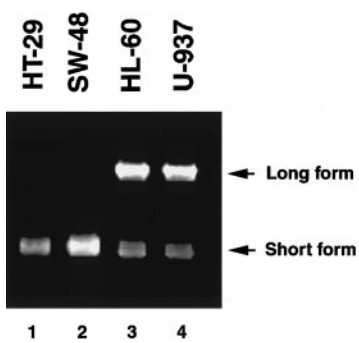

FIG. 2. 5′-RACE analysis of FUT4 mRNA. mRNAs from HT-29 (lane 1), SW-48 (lane 2), HL-60 (lane 3), and U-937 (lane 4) were subjected to 5′-RACE analysis. The PCR products were run on 2% agarose gels and stained with ethidium bromide.

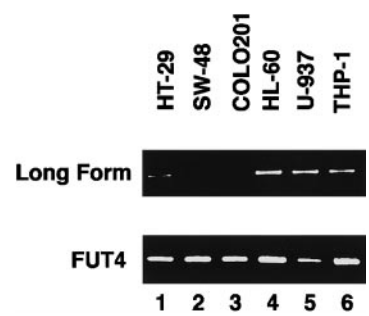

FIG. 3. RT-PCR analysis of long form mRNA isoform. Complementary DNAs from HT-29 (lane 1), SW-48 (lane 2), COLO201 (lane 3), HL-60 (lane 4), U-937 (lane 5) and THP-1 (lane 6) were used as target DNA in the PCR analysis. The primers used were as follows: upper panel, long form; bottom panel, FUT4. The PCR products were run on 2% agarose gels and stained with ethidium bromide.

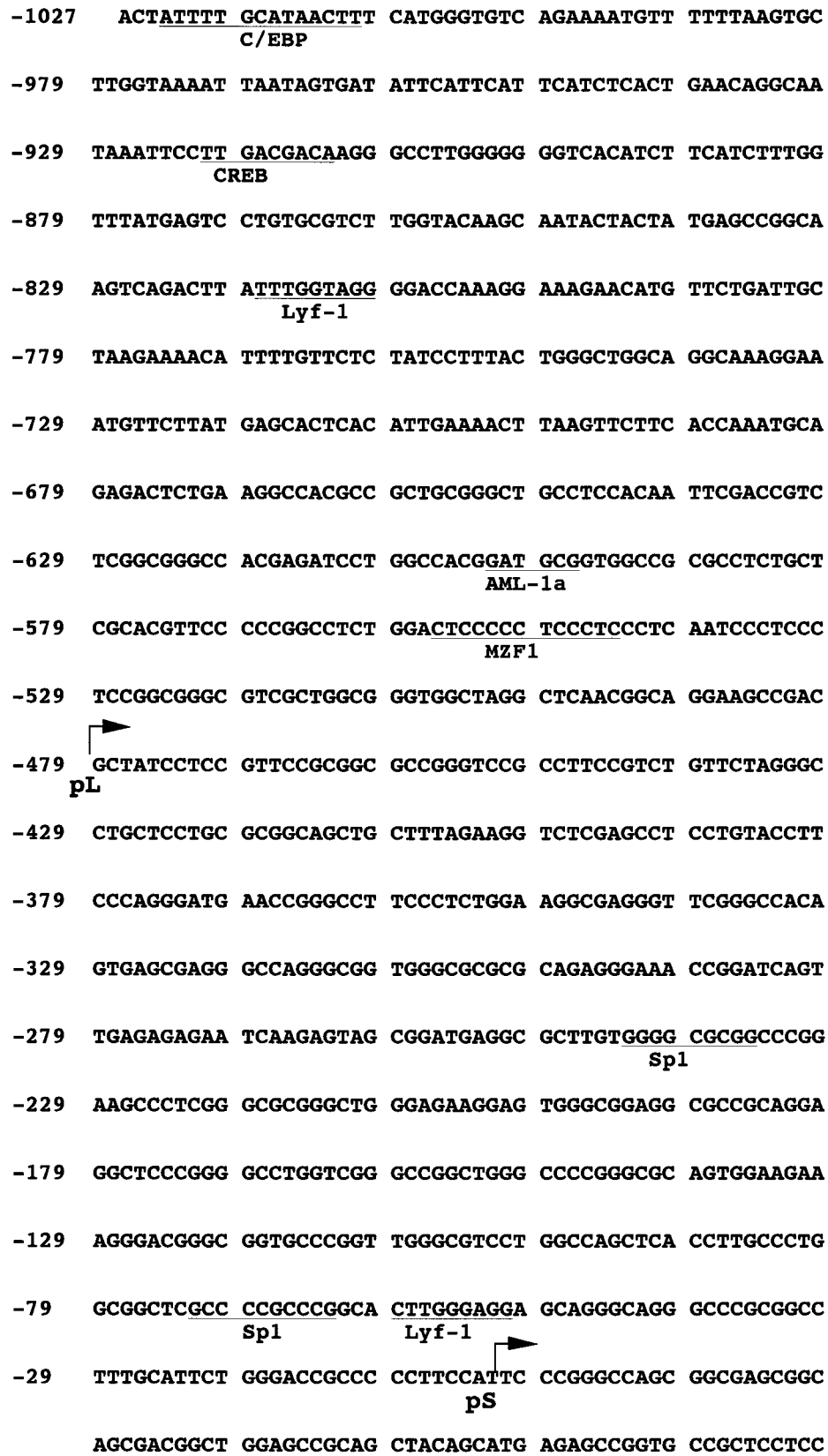

FIG. 4. Nucleotide sequence of pL and pS promoters. Potential regulatory elements are underlined and indicated below the sequence. The transcription start sites are marked with arrows. Nucleotides are numbered with the transcription-initiation site of the short form, designated as +1. The nucleotide sequence of the pL and pS promoters has been submitted to the GeneBankTM/EMBL Data Bank with Accession No. AB041774.

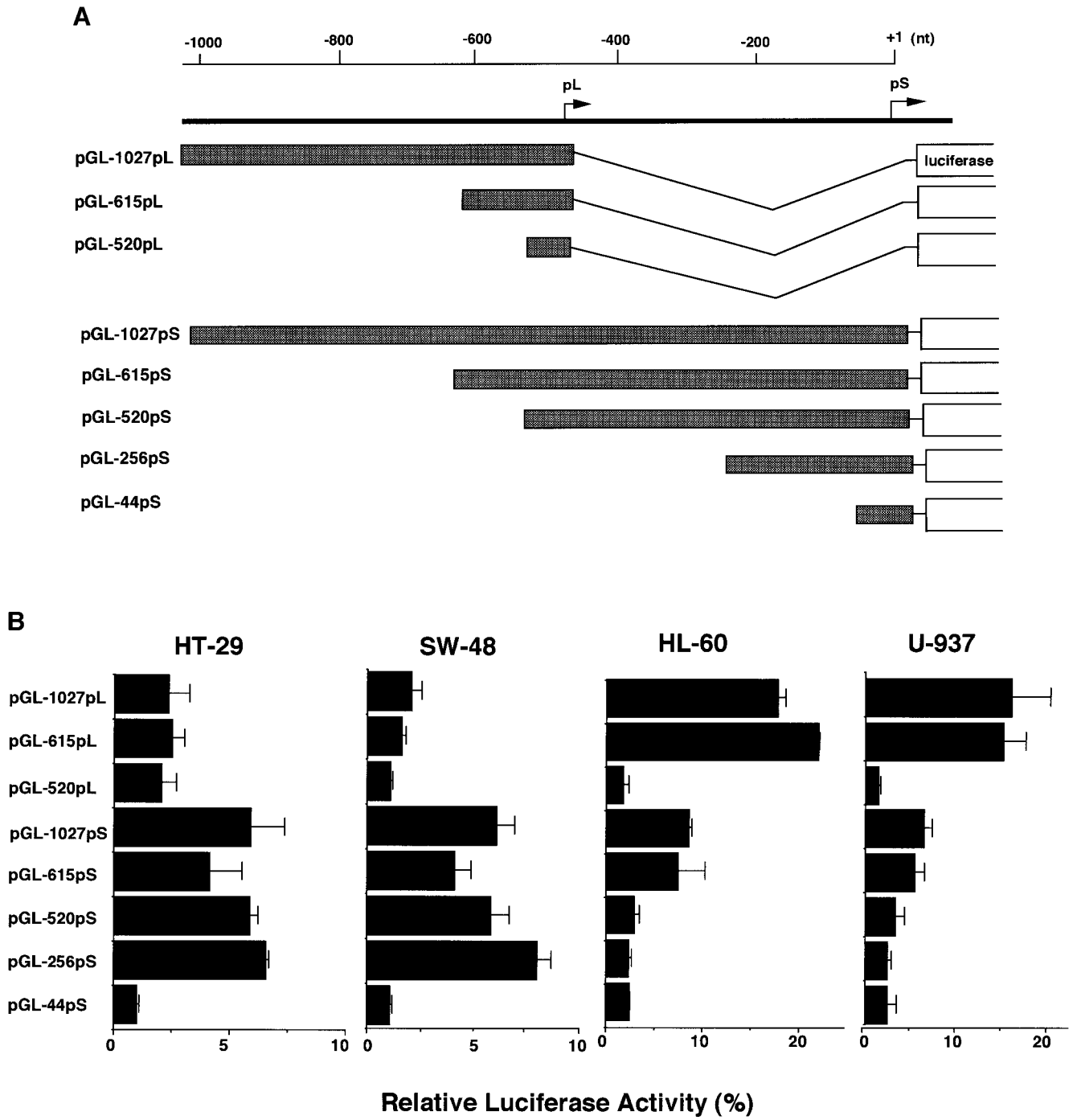

FIG. 5. Deletion of analysis of pL and pS promoters in FUT4 gene. Structure of the 5′-deletion mutants of L and S promoters (A). Each *firefly* luciferase construct was co-transfected into HT-29, SW-48, HL-60 and U-937 (B) with the *Renilla* luciferase expression vector (pRL-SV40) as the internal control. Relative luciferase activities were normalized to luciferase activity of the pGL3-Control that contained the SV40 promoter-enhancer sequences upstream of the luciferase gene. Each value represents the mean activity of luciferase detected in three independent experiments. Bars indicate standard deviation of the mean activities.

U-937 cells (Fig. 5B). These results suggest that the nt −256 to −44 region acts as an enhancer in HT-29 and SW-48 cell lines.

Plasmids pGL-1027pL and pGL-615pL containing pL promoter had very high luciferase activities in HL-60 and U-937 cell lines, whereas plasmids pGL-1027pS and pGL-615pS containing pS promoter did not (Fig. 5B). These results suggest that the activity of pL promoter is much higher than pS promoter activity in HL-60 and U-937 cell lines. In HL-60 and U-937

cells, extension of the 5′-deletion of the pL promoter to nt −520 (pGL-520pL) reduced expression to approximately 10–20% of that of the promoter that was deleted to nt −615 (pGL-615pL) (Fig. 5B). In contrast to myeloid cell lines, truncation at −520 had little or no effect on pL promoter activity in HT-29 and SW-48 cells (Fig. 5B). These results suggest that the nt −615 to −520 region acts as an enhancer in HL-60 and U-937 cell lines. Cell line specific expression of the FUT4 gene may be regulated by these elements, the locations of which are in different regions.

DISCUSSION

The long and short forms of the protein of galactosyltransferase have been reported to be differentially distributed within the cell (21) and that the long form which preferentially goes to the surface plays a direct role in cell-cell interactions (22, 23). However, in the case of rat FUT4, no difference in the intracellular localization between long and short form proteins has been detected by immunocytochemistry, as both show the same pattern of Golgi staining (24). In human FUT4, the first ATG that would be used to produce such a protein does not conform to the consensus sequence for translation initiation, suggesting that both types of mRNA encode the same protein. Why are two promoters used for the production of two mRNA coding the same protein? Multiple promoters have been described in sialyltransferase genes (12–20). Cell type specific mRNA isoforms, which were generated by cell type specific promoters, have been found in the α2,6-sialyltransferase (hST6Gal I) gene (12–14, 18, 20). In the FUT4 gene, we did not find evidence of a clear cell type specificity of each type of mRNA isoform. However, we show here that the expression level of short form mRNA is higher than the long form transcript in the colon adenocarcinoma cell lines and the expression level of long form mRNA is higher than the short form transcript in the myeloid cell lines. The activity of pL promoter is higher than pS promoter activity in colon adenocarcinoma cell lines and the activity of pL promoter is much stronger than pS promoter activity in myeloid cell lines (Fig. 5), suggesting multiple promoters may contribute to the cell type specific transcriptional regulation of the FUT4 gene.

We investigated the expression and transcriptional regulation of the FUT4 gene. The present results suggest that the −615 to −520 region of the pL promoter plays a critical role in the myeloid cell transcriptional regulation of the FUT4 gene and the −256 to −44 region of the pS promoter acts as an enhancer in the colon adenocarcinoma cell line. Several of the transcription factors that are involved in myeloid cells-specific gene regulation have been characterized, such as AML-1a (25), MZF-1 (26, 27), Sp1 (28, 29), and Oct-1 (30–33). We have identified AML-1a and MZF-1 binding sequence motifs in this element (Fig. 4). A mutation of the AML-1a and MZF-1 binding sequence sites did not influence luciferase activity (data not shown), suggesting that an unknown factor binds to this element.

REFERENCES

1. Kukowska-Latallo, J. F., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990) *Genes Dev.* **4,** 1288–1303.
2. Weston, B. W., Nair, R. P., Larsen, R. D., and Lowe, J. B. (1992) *J. Biol. Chem.* **267,** 4152–4160.
3. Lowe, J. B., Kukowska-Latallo, J. F., Nair, R. P., Larsen, R. D., Marks, R. M., Macher, B. A., Kelly, R. J., and Ernst, L. K. (1991) *J. Biol. Chem.* **266,** 17467–17477.
4. Sasaki, K., Kurata, K., Funayama, K., Nagata, M., Watanabe, E., Ohta, S., Hanai, N., and Nishi, T. (1994) *J. Biol. Chem.* **269,** 14730–14737.
5. Natsuka, S., Gersten, K. M., Zenita, K., Kannagi, R., and Lowe, J. B. (1994) *J. Biol. Chem.* **269,** 16789–16794.
6. Goelz, S. E., Hession, C., Goff, D., Griffiths, B., Tizard, R., Newman, B., Chi-Rosso, G., and Lobb, R. (1990) *Cell* **21,** 1349–1356.
7. Weston, B. W., Smith, P. L., Kelly, R. J., and Lowe, J. B. (1992) *J. Biol. Chem.* **267,** 24575–24584.
8. Koszdin, K. L., and Bowen, B. R. (1992) *Biochem. Biophys. Res. Commun.* **187,** 152–157.
9. Kaneko, M., Kudo, T., Iwasaki, H., Ikehara, Y., Nishihara, S., Nakagawa, S., Sasaki, K., Shiina, T., Inoko, H., Saitou, N., and Narimatsu, H. (1999) *FEBS Lett.* **452,** 237–242.
10. Mollicone, R., Gibaud, A., Francois, A., Ratcliffe, M., and Oriol, R. (1990) *Eur. J. Biol.* **191,** 169–176.
11. Yago, K., Zenita, K., Ginya, H., Sawada, M., Ohmori, K., Okuma, M., Kannagi, R., and Lowe, J. B. (1999) *Cancer Res.* **15,** 5559–5565.
12. Svensson, E. C., Conley, P. B., and Paulson, J. C. (1992) *J. Biol. Chem.* **267,** 3466–3472.
13. Aasheim, H-C., Aas-Eng, D. A., Deggerdal, A., Blomhoff, H. K., Funderd, S., and Smeland, E. B. (1993) *Eur. J. Biochem.* **213,** 467–475.
14. Lo, N-W., and Lau, J. T. (1996) *Glycobiology* **6,** 271–279.
15. Kitagawa, H., Mattei, M. G., and Paulson, J. C. (1996) *J. Biol. Chem.* **271,** 931–938.
16. Taniguchi, A., and Matsumoto, K. (1998) *Biochem. Biophys. Res. Commun.* **243,** 177–183.
17. Taniguchi, A., and Matsumoto, K. (1999) *Biochem. Biophys. Res. Commun.* **257,** 516–522.
18. Taniguchi, A., Higai, K., Hasegawa, Y., Utsumi, K., and Matsumoto, K. (1998) *FEBS Lett.* **441,** 191–194.
19. Taniguchi, A., Hasegawa, Y., Higai, K., and Matsumoto, K. (1999) *Mol. Cell. Biol. Res. Commun.* **2,** 197–201.
20. Taniguchi, A., Hasegawa, Y., Higai, K., and Matsumoto, K. (2000) *Glycobiology,* in press.
21. Russo, R. N., Shaper, N. L., and Shaper, J. H. (1990) *J. Biol. Chem.* **265,** 3324–3331.
22. Youakim, A., Dubois, D. H., and Shur, B. D. (1994) *Proc. Natl. Acad. Sci. USA* **191,** 10913–10917.
23. Youakim, A., Hathaway, H. J., Miller, D. J., Gong, X., and Shur, B. D. (1994) *J. Cell Biol.* **126,** 1573–1583.
24. Aucoin, J. M., Koul, O., Sajdel-Sulkowska, E. M., Baboval, T., and Smith, F. I. (1998) *Glycoconj. J.* **15,** 671–681.
25. Meyers, S., Downing, J. R., and Hiebert, S. W. (1993) *Mol. Cell. Biol.* **13,** 6336–6345.

26. Morris, J. F., Rauscher, F. J., III, Davis, B., Klemsz, M., Xu, D., Tenen, D., and Hromas, R. (1995) *Blood* **86,** 3640–3647.
27. Robertson, K. A., Hill, D. P., Kelley, M. R., Tritt, R., Crum, B., Van Epps, S., Srour, E., Rice, S., and Hromas, R. (1998) *Leukemia* **5,** 690–698.
28. Rao, J., Zhang, F., Donnelly, R. J., Spector, N. L., and Studzinski, G. P. (1998) *J. Cell. Physiol.* **175,** 121–128.
29. Hauses, M., Tonjes, R. R., and Grez, M. (1998) *J. Biol. Chem.* **273,** 31844–31852.
30. Kim, K. Y., Kweon, K. R., Lee, M. S., Kwak, S. T., Kim, K. E., Hwang, B. D., and Lim, K. (1995) *Biochem. Biophys. Res. Commun.* **213,** 616–624.
31. Lopez-Rodriguez, C., Zubiaur, M., Sancho, J., Concha, A., and Corbi, L. A. (1997) *J. Immunol.* **158,** 5833–5840.
32. Corbi, A. L., and Lopez-Rodriguez, C. (1997) *Leuk. Lymphoma* **25,** 415–425.
33. Tenen, D. G., Hromas, R., Licht, J. D., and Zhang, D. E. (1997) *Blood* **90,** 489–519.