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Genomic Organization and Transcriptional Regulation of the Mouse GD3 Synthase Gene (ST8Sia I): Comparison of Genomic Organization of the Mouse Sialyltransferase Genes[1]

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The genomic organization of the gene encoding the mouse GD3 synthase (ST8Sia I) has been determined. The mouse ST8Sia I gene spans over 100 kilobases of genomic DNA with a unique genomic structure of 5 exons. Analysis of the sequence immediately upstream of the transcription initiation site revealed that the ST8Sia I promoter contained no canonical TATA- or CCAAT-box, but contained a putative Sp1 binding site. Transient transfection experiments demonstrated functional promoter activity of the ST8Sia I promoter in an ST8Sia I–expressing cell line, P19, but not in an ST8Sia I–nonexpressing cell line, NIH3T3. Mobility shift assay and mutation analysis of the promoter region indicated that the Sp1 binding site is involved in the transcriptional regulation of the ST8Sia I gene in P19 cells. Here, the genomic structural analyses of mouse sialyltransferase genes are summarized and the genomic structures of these genes are compared.

Key words: GD3 synthase, genomic organization, molecular cloning, sialyltransferase, transcriptional regulation.

Sialic acids are key determinants of carbohydrate structures that play important roles in a variety of biological functions, like cell–cell communication, cell–substrate interaction, adhesion, and protein targeting. The sialic acid–containing carbohydrates of glycoproteins and glycolipids vary according to the tissue and cell type, and are subject to change during development and oncogenesis (*3*). For the synthesis of sialyl-glycoconjugates, a family of glycosyltransferases called sialyltransferases catalyzes the transfer of a sialic acid from CMP-Sia to an acceptor carbohydrate. The mouse sialyltransferases characterized to date have a type II transmembrane topology and contain highly conserved domains called sialyl motifs L (Long), S (Short), and VS (Very Short) (*4–6*). Based on the high sequence conservativeness of sialylmotifs L and S, PCR-mediated cloning of sialyltransferase cDNAs has been extensively performed (reviewed in Refs. *7* and *8*). Each sialyltransferase exhibits strict specificity for acceptor substrates and linkages. Although three linkages, Siaα2,3Gal, Siaα2,6Gal, and Siaα2,6GalNAc, are commonly found in glycoproteins, and two linkages, Siaα2,3Gal and Siaα2,8Sia, occur frequently in gangliosides, each of these linkages has been found in both gangliosides and glycoproteins.

Gangliosides are expressed predominantly on the outer leaflet of the plasma membrane of most vertebrate cells (*9*), and they are particularly enriched in the central nervous system. The expression patterns of gangliosides during the development of the central nervous system suggest that they play important roles in the development and function of the central nervous system (*10*, *11*). In addition, gangliosides are also considered to be involved in intracellular adhesion (*12*), immune modulation (*13*), growth control, and receptor function (*14*, *15*). The ganglioside GD3

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[9] In addition to the references, the following data also support it: EMBL accession No. AF119390.

Abbreviations: The nomenclature for gangliosides follows the system of Svennerholm (*1*). The abbreviated nomenclature for cloned sialyltransferases follows the system of Tsuji *et al.* (*2*). Sia, sialic acid; NeuAc, *N*-acetylneuraminic acid; CMP-NeuAc, cytidine 5′-monophospho-*N*-acetylneuraminic acid; PCR, polymerase chain reaction; kb, kilobases; bp, base pairs; nt, nucleotides.

(NeuAcα2,8NeuAcα2,3Galβ1,4Glc-Cer) is mainly expressed in the retina and fetal brain (*16, 17*). GD3 is also expressed as one of the major gangliosides on the cell surface of melanomas, gliomas, and neuroblastomas (*18*). It has been suggested that GD3 may play important roles in the neuronal proliferation and differentiation (*19*), the cell adhesion and growth of cultured malignant cells (*20, 21*), and the inductive epithelial–mesenchymal interaction in the kidney development (*22*).

Although the biological importance of GD3 ganglioside has been shown, the mechanisms regulating GD3 ganglioside expression remain unclear. For this purpose, it is important to know the structure and activity of the GD3 synthase gene promoter. For the rat GD3 synthase gene, Zeng *et al.* isolated its promoter region and showed its promoter activity (*23*). But the overall genomic structure of the rat GD3 synthase gene has not been determined. To elucidate the evolutional relation among sialyltransferase genes, it is also important to know the genomic organization of each gene. Recently, we have been analyzing genomic organization and transcriptional regulation of mouse sialyltransferase genes. Here, we describe the genomic organization of the mouse GD3 synthase gene (ST8Sia I) and transcriptional regulation of this gene regulated by Sp1.

So far, 18 mouse sialyltransferase cDNAs have been cloned and their enzymatic properties have been characterized (*19, 24–42*, footnote 9)[9] (Table I). The cloned sialyltransferases can be classified into four families according to the carbohydrate linkages they synthesize, *i.e.*, the β-galactoside α2,3-sialyltransferase family (ST3Gal I–VI), the β-galactoside α2,6-sialyltransferase family (ST6Gal I), the GalNAc α2,6-sialyltransferase family (ST6GalNAc I–VI), and the α2,8-sialyltransferase family (ST8Sia I–V). They can also be classified into subfamilies according to the amino acid sequence similarities and substrate specificity differences (*43*) (Fig. 6). For these sialyltransferases, genomic clones of 14 mouse sialyltransferase genes have been also cloned to date (*28, 36, 44–53*, this work). Our group has been involved in most of these cloning works, so here we compared the genomic structures of mouse sialyltransferase genes and discussed the evolutional relationship among them.

MATERIALS AND METHODS

Isolation of Mouse ST8Sia I Genomic Clones—A C57BL/6 mouse genomic cosmid library was constructed as described previously (*28*), using a pWE15 cosmid vector kit (Stratagene) and *Escherichia coli* XL1-Blue as a host. A C57BL/6 mouse genomic phage library was also constructed by cloning the *Eco*RI–digested genomic DNA fragments into the *Eco*RI site of the λZAP phage vector (Stratagene). The C57BL/6 gemonic libraries were screened with mouse ST8Sia I cDNA as a probe. Genomic DNAs from the positive clones were isolated and subjected to restriction analysis. The locations of the exons of the ST8Sia I gene were determined by PCR with specific oligonucleotide primers or by hybridization.

PCR Amplification of the 5′-cDNA End (RACE)—The 5′-end of mouse ST8Sia I cDNA was amplified by using the 5′-full RACE core set (Takara, Kyoto) and 1 μg of adult mouse kidney poly(A)$^+$ RNA according to the manufacturer's instructions. For cDNA synthesis, the ST8Sia I cDNA specific primer, 5′-CTGGCGAATTATGCTGGGGT-3′ (P2-24–, complementary to the ST8Sia I cDNA coding strand, nt. 875 to 894) was used, and the cDNA was self-ligated. Then two consecutive PCRs were performed, the first with the self-ligated cDNA as a template and the first primer set, 5′-GGAAGACTGCTGTGACCCTG-3′(P2-19+, nt. 558 to 577) and 5′-CCGAGCTAGCATGGCCATGG-3′ (P2-2–, complementary to the ST8Sia I cDNA coding strand, nt. 356 to 375); and the second PCR with 1 μl of the above reaction mixture as a template and the second primer set, 5′-GTGCAACCTGCCTCCCTTGT-3′ (P2-23+, nt. 804 to 823) and 5′-GTGAAGGATGCTCCAGTGGC-3′ (P2-11–, complementary to the ST8Sia I cDNA coding strand, nt. 238 to 257). The amplified products were blunt-ended, cloned into the *Eco*RV site of pBluescript II SK(+), and sequenced.

Analysis of Promoter Activity—A 4.5-kb *Bam*HI fragment containing the ST8Sia I exon 1 was isolated from the obtained cosmid clone and subcloned into the pBluescript SK(+) plasmid. The resultant plasmid, designated as pEX1-B4.5, was used for promoter analysis. An ST8Sia I–

TABLE I. **Mouse sialyltransferases so far cloned.**

Enzyme	Deduced amino acid length	Essential substrates	Accession numbers	References
ST3Gal I	337	Galβ1,3GalNAc-(Protein)*	X73523	(*29*), (*49*)
ST3Gal II	350	Galβ1,3GalNAc-(Lipid)*	X76989	(*30*), (*49*)
ST3Gal III	374	Galβ1,3(4)GlcNAc-	X84234	(*31*), (*49*)
ST3Gal IV	333	Galβ1,4(3)GlcNAc-	X95809	(*31*), (*49*)
ST3Gal V	387	Lac-Cer	Y15003	(*32*), (*33*), (*34*), (*50*), (*51*)
ST3Gal VI	329	Galβ1,4GlcNAc-	AF119390	(*34*), (*42*)
ST6Gal I	403	Galβ1,4GlcNAc-	D16106	(*35*), (*52*), (*53*)
ST6GalNAc I	526	GalNAcα1,*O*-Ser/Thr	Y11274, AJ271036, Y10294-5	(*36*)
ST6GalNAc II	373	GalNAcα1,*O*-Ser/Thr	X93999, X94000	(*28*)
ST6GalNAc III	305	NeuAcα2,3Galβ1,3GalNAc-(Lipid)*	Y11342, Y11343-6	(*29*), (*37*)
ST6GalNAc IV	302	NeuAcα2,3Galβ1,3GalNAc-(Protein)*	Y15779-80, AJ007310, Y19053-7	(*29*), (*37*), (*38*)
ST6GalNAc V	335	GM1b	AB030836, AB028840	(*39*), (*40*)
ST6GalNAc VI	333	GM1b, GT1b, GD1a	B035123	(*41*)
ST8Sia I	355	GM3	X84235, L38677, AJ401102	(*19*), (*24*), ***This study***
ST8Sia II	375	*N*-glycan on NCAM	X83562, X99645-51	(*25*), (*44*)
ST8Sia III	380	α2,3-sialylparagloboside	X80502, X93998	(*26*), (*45*)
ST8Sia IV	359	*N*-glycan on NCAM	X86000, Y09483-8	(*27*), (*46*), (*47*)
ST8Sia V	412	GM1b, GT1b, GD1a, GD3	X98014	(*24*)

Asterisk means preferential but not specific substrate. The studies we took part in are shown in bold. The genomic studies are underlined.

luciferase reporter plasmid, pP2-1246, was constructed by subcloning a 1,246 bp *Bam*HI/*Nco*I fragment from pEX1-B4.5 into the *Bgl*II/*Nco*I sites of pPicaGene-Basic vector II (pPGBII; Toyo-ink, Tokyo). *Sma*I fragments were deleted from pP2-1246, and the remaining plasmid was self-ligated, giving rise to pP2-565. pPGBII-HN was constructed by subcloning a 1,276 bp *Hin*dIII/*Nco*I fragment from pEX1-B4.5 into the *Hin*dIII/*Nco*I sites of pPGBII. An *Xho*I/*Bss*HII fragment was deleted from pPGBII-HN, and the remaining plasmid was blunt-ended and self-ligated, giving rise to pP2-1144. pP2-803 was constructed by deleting an *Nhe*I fragment from pPGBII-HN and self-ligating the remaining plasmid. *Mlu*I/*Pst*I fragments were deleted from pP2-803, and the remaining plasmid was blunt-ended and self-ligated, giving rise to pP2-299.

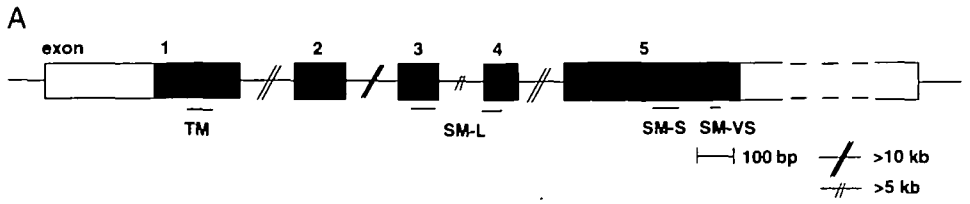
Mouse P19 (embryonal carcinoma) and NIH3T3 (fibroblast) cells were seeded at 1×10^5 cells per 60-mm diameter dish in Dulbecco's Modified Eagle's Medium −10% fetal calf serum 24 h prior to transfection. Each luciferase plasmid (5 μg) and a pSRβ-Gal plasmid (0.5 μg), which served as an internal control of transfection efficiency, were transfected into the cells with LipofectAMINE (GIBCO BRL). After 48 h of transfection, the cells were washed three times with PBS, then lysed with cell lysis buffer (PGC-51, Toyo-ink). Luciferase activity was measured with a PicaGene Luciferase Assay System (Toyo-ink) and a Luminescencer AB-2000 (ATTO, Tokyo). Light activity was measured in triplicate, averaged, and then normalized as to β-galactosidase activity in order to correct for transfection efficiency. β-Galactosidase activity was measured using a Luminescent β-Galactosidase Detection Kit II (Clontech).

Mobility Shift Assay—For the ST8Sia I promoter, the DNA fragment comprising nucleotides −205 to 61 was prepared from pP2-565 by digestion with *Mlu*I and *Sse*8387I, then end-labeled with [α-^{32}P]dCTP using Klenow fragment. Binding assays were performed as described previously (*46*). Two synthetic DNA fragments, 5′-GGGCTACCACGTGACTGCTG-3′ and 5′-CCAGCAGTCACGTGGTAGCC-3′ were annealed and after two synthetic DNAs had been annealed used as a non-specific competitor. Sp1-specific competitor DNA and anti-Sp1 antibodies were purchased from Promega and Santa Cruz Biotechnology, respectively.

Site-Directed Mutagenesis of the Sp1 Binding Site—For the ST8Sia I promoter, an Sp1 binding site-replaced mutant, pP2-565(Sp1*), was constructed as follows. A 1,247-bp *Sph*I–*Sac*I fragment of pP2-565 was subcloned into pKF-19k (Takara), then subjected to site-directed mutagenesis using mutagenic primers, 5′-GCGGCGGCGCAGGAAGCGCTGAAAGGGCGGTGC-3′ for the Sp1 binding site (nt. −137 to −105, the synthetic *Aor*51HI site is underlined) with a Mutan-Super Express Km kit (Takara). From this mutagenized plasmid, the *Sma*I–*Nco*I fragment was excised and subcloned into pPGB II, giving rise to the Sp1 binding site-replaced mutant, pP2-565(Sp1*). This plasmid was verified by restriction analysis and sequencing.

RESULTS

Isolation of Mouse ST8Sia I Genomic Clones—Screening of C57BL/6 mouse genomic libraries with mouse ST8Sia I cDNA resulted in the isolation of five independent non-overlapping clones. The location of the ST8Sia I exons were determined by hybridization and PCR. The ST8Sia I gene was divided into 5 exons and seemed to span over 100 kilobases of genomic DNA, judging from the results of hybridization and long accurate PCR, although we did not determine the exact sizes of introns (Fig. 1). Sequencing was also performed to determine the exon–intron splice junctions. The sequences of the splice junctions of the ST8Sia I gene obey the GT-AG rule (*54*). The splice junctions of the ST8Sia I gene in exons 1-2, 3-4, and 4-5 were after the second nucleotide of the amino acid codon, and that in exons



B

	Exon (bp)	Splice donor	intron	Splice acceptor
		77		79
1	548	GCC AGT CTC TTC AGgtacctac	ctctcctagG	AGA CAA ATG GAA
		Ala Ser Leu Phe (Arg)		Arg Gln Met Glu
		126		130
2	145	CTC TTC CCC CAG gtgagtggag	ctgttgctag	GCA ACC CCC TTC
		Leu Phe Pro Gln		Ala Thr Pro Phe
		162		164
3	110	AAT TTT GTC ATG CGgtaagaga	cttttctagG	TGC AAC CTG CCT
		Asn Phe Val Met (Arg)		Cys Asn Leu Pro
		193		195
4	93	ATA ATT CGC CAG AGgtaagact	ttcccatagA	TTT GAA AAC CTG
		Ile Ile Arg Gln (Arg)		Phe Glu Asn Leu

Fig. 1. **Genomic structure of the mouse ST8Sia I gene.** (A) Exon/intron structure of the ST8Sia I gene. Five independent genomic clones were isolated. The protein coding region and the untranslated region are shown by filled rectangles and open rectangles, respectively. It should be noted that at least two transcripts (9.0 kb, 2.4 kb) differing in the length of the 3′-untranslated region have been observed (*19*). TM, transmembrane domain; SM-L, sialyl motif L; SM-S, sialyl motif S; SM-VS, sialyl motif VS. (B) Exon/intron junctions of the ST8Sia I gene. The nucleotide sequences at the intron (lowercase letters) and exon (uppercase letters) junctions are shown. The derived amino acid sequence is displayed below the nucleotide sequence. The conserved amino acid residues among the ST8Sia I, II, and IV genes are underlined. The numbering of amino acid residues starts at the initiator methionine as +1.

2-3 was between codons. It should be noted that the amino acid residues around the splice junction of exons 3-4 (encoding the sialyl motif L) are conserved among ST8Sia I, II, and IV (Figs. 1 and 2). These are located in the middle of the sialyl motif L. In addition, we found that codons for Arg in the sialyl motif L are highly conserved as a splice junction among many sialyltransferase genes (Fig. 2). Exon 1 contained the entire 5′-untranslated region and the beginning of the coding region to amino acid residue 77, containing a cytoplasmic domain, a hydrophobic signal anchor sequence, and a part of the stem region. Exon 2 encoded a part of stem region and the putative active domain of the enzyme. Exon 3-5 encoded the putative active domain including sialyl motifs L, S and VS, and exon 5 contained the 3′-untranslated region.

Mapping of the Transcription Initiation Sites—The major transcription initiation site of the ST8Sia I gene was determined by 5′-RACE PCR with RNA recovered from adult mouse kidney. Sequencing of 5′-RACE PCR products revealed that most of the transcription initiation sites were mapped at the adenosine 315 nucleotides upstream from the translation initiation codon ATG, which is designated as nucleotide position +1 (Fig. 3).

Promoter Analysis of the ST8Sia I Gene and Demonstration of Promoter Activity—Analysis of the sequence immediately upstream of the transcription initiation site revealed that the ST8Sia I promoter contained no canonical TATA or CCAAT box, but contained a putative Sp1 binding site at nucleotide position −125 (Fig. 3). There are also putative elements for binding transcription factors, NF-Y (*55*), Pbx-1 (*56*), MZF1 (*57*), and GATA (*58*). The promoter structure of the mouse ST8Sia I gene is quite similar to that of the rat GD3 synthase gene (*23*).

To characterize the regions regulating the transcription activity of the ST8Sia I gene, chimeric reporter plasmids encoding the luciferase gene and different lengths of the promoter region of the ST8Sia I gene were constructed. The resultant chimeric constructs were transfected into an ST8Sia I–expressing cell line, P19, and an ST8Sia I–nonexpressing cell line, NIH3T3, for analysis of the ST8Sia I promoter activity. As a negative control, the plasmid pPGBII, containing the promoterless luciferase gene, was transfected into parallel cultures. The luciferase activity due to each luciferase reporter plasmid was normalized for β-galactosidase activity by co-transfecting an internal control plasmid, pSRβ-Gal, carrying the β-galactosidase gene under the control of the SRα promoter, and expressed as a percentage of the SV40 promoter activity.

All chimeric constructs exhibited some degree of functional promoter activity in ST8Sia I–expressing cell line, P19, but very low or no significant promoter activity in ST8Sia I–nonexpressing cell line, NIH3T3, indicating that the ST8Sia I promoter is a cell-type specific promoter (Fig. 4). Deletion of the upstream sequences containing a GT-rich region (pP2-1144) increased the promoter activity slightly in P19 cells, and further deletion of the promoter to nucleotide position −444 (pP2-803) gave a high level of transcription activity comparable to that of the SV40. This suggests that some negatively regulating elements are included in the upstream region from −784 to −444. Further deletion of the promoter to nucleotide position −206 (pP2-565) reduced the promoter activity, although its level was still significant. This suggests that there are important elements for transcription activation in P19 cells in the deleted sequences. Further deletion of the promoter to nucleotide position 61 (pP2-299) greatly reduced the promoter activity, indicating that the upstream region from −205 to 61 contains the minimum promoter for expression in P19 cells.

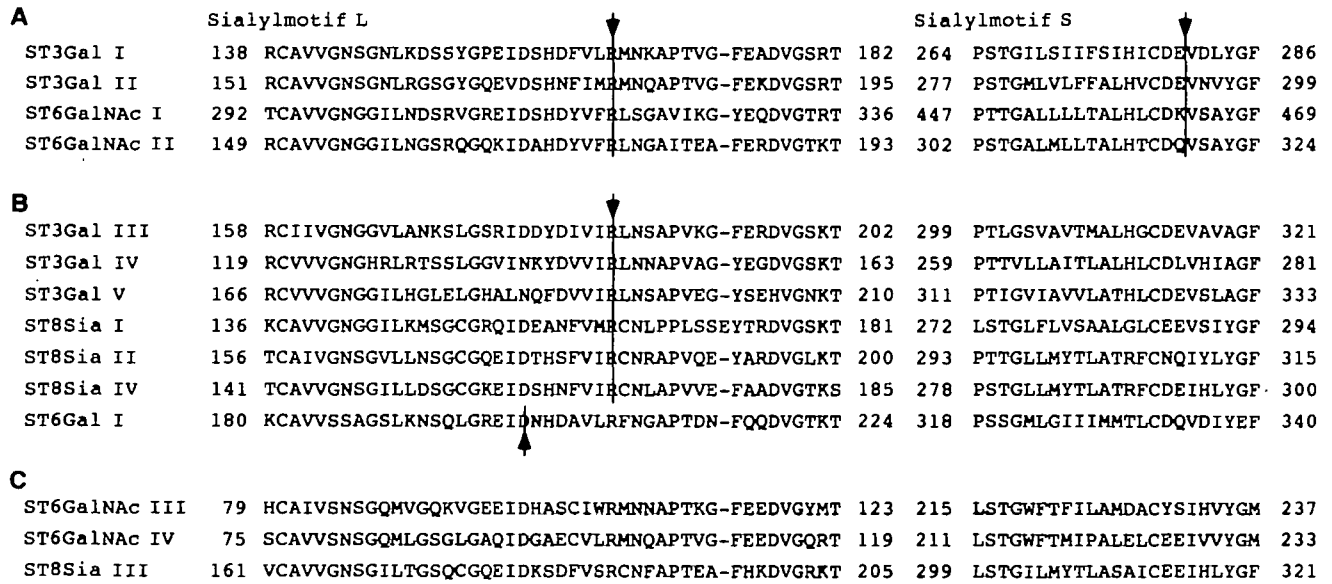


Fig. 2. **Split patterns of sialyl motifs.** Split positions of exons encoding sialyl motifs are indicated by vertical lines. (A) The sialyltransferase genes are shown whose exons encoding sialyl motifs L and S are split by introns. The split positions of sialyl motifs L and S are highly conserved among these members. (B) The sialyltransferase genes are shown whose exons encoding the sialyl motif L are split by an intron but exons encoding the sialyl motif S are not. The split positions of sialyl motif L are conserved among all these members except the ST6Gal I gene. (C) The sialyltransferase genes are shown whose exons encoding sialyl motifs L and S are not split.

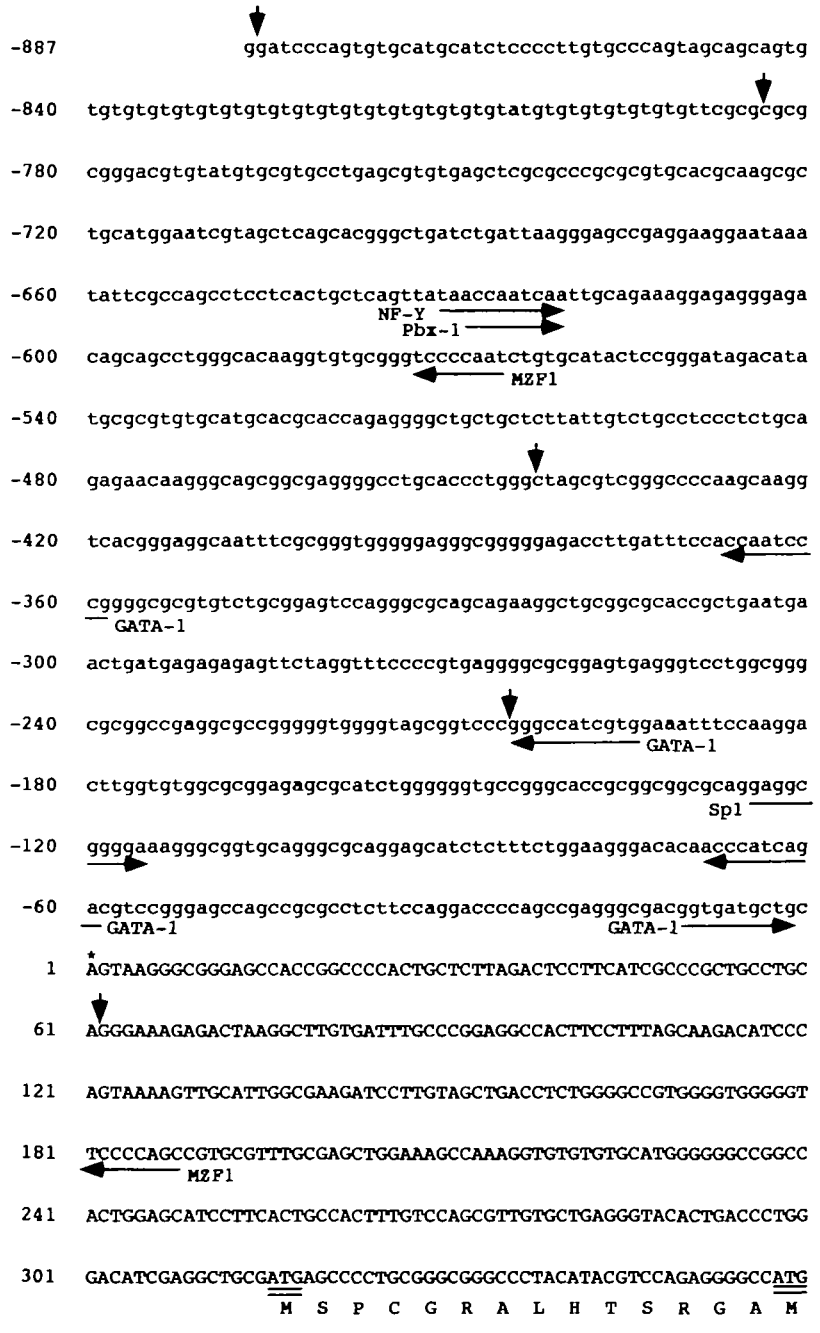


Fig. 3. **Nucleotide sequence of the 5′-flanking region of the ST8Sia I gene.** The exon sequence is shown in uppercase letters, while that of the untranscribed region is given in lowercase letters. The major transcription initiation site in kidney determined by 5′-RACE PCR is indicated by an asterisk. The numbering of the nucleotides begins with the major transcription initiation site as +1. The translation initiation codon is double underlined. The consensus binding sequences of some transcription factors are shown by arrows. For the detection of promoter activity, the start point of each construction is indicated by an arrowhead.

Involvement of Sp1 in the Transcription of the ST8Sia I Gene—Although the ST8Sia I promoter contained no canonical TATA- or CCAAT-box in its minimum promoter region, it contained a putative Sp1 binding site at nucleotide position −125. The Sp1 binding sequence is frequently found in the housekeeping gene promoters. In addition, it has been shown that Sp1 is involved in the transcription of the mouse ST8Sia II, ST8Sia IV, ST6GalNAc III, and ST6GalNAc IV genes (*44*, *46*, *48*). To determine whether or not this putative Sp1 binding site is recognized by Sp1 and involved in the transcriptional regulation of the ST8Sia I gene, we performed a mobility shift assay experiment.

The DNA fragment comprising nucleotides −205 to 61, which contains the putative Sp1 binding site, was prepared from pP2-565 by digestion with *Mlu*I and *Sse*8387I and used in the mobility shift experiment. The recombinant Sp1 bound to this fragment (Fig. 5, lane 6). A corresponding band to that observed in the presence of recombinant Sp1 appeared in the presence of the nuclear protein extract of P19 cells (lane 2). This shifted band was abolished by the addition of a non-labeled specific competitor for Sp1 binding (lane 4), but not by the addition of a non-labeled non-specific competitor (lane 3). Moreover, the shifted band was abolished by the addition of anti-Sp1 polyclonal antibodies (lane 5), indicating that Sp1 in the nuclear extract of P19 cells recognizes the putative Sp1 binding site in this fragment.

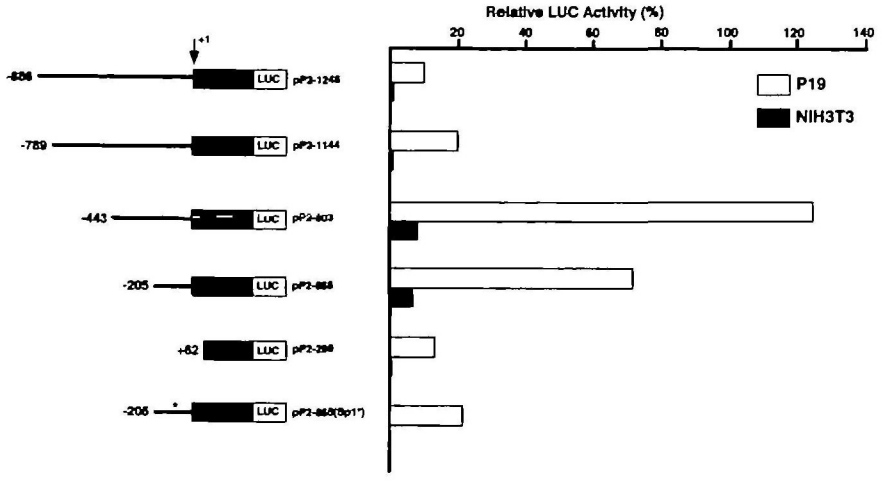


Fig. 4. **Promoter activity of the ST8Sia I gene and identification of the regulatory regions.** Schematic representation of DNA constructs containing various lengths of the promoter linked to the luciferase gene (pPGBII). Each DNA fragment subcloned into the luciferase reporter plasmid is defined by its position in the promoter relative to the transcription initiation site (+1). Luciferase activity was measured in cells transfected with the reporter plasmids, and normalized to the β-galactosidase activity of a co-transfected internal control plasmid, pSRβ-Gal, and expressed as a percentage of the SV40 promoter activity. The mutated Sp1 binding site is shown by an asterisk. Relative luciferase activities in P19 cells and NIH3T3 cells are shown by open rectangles and filled rectangles, respectively.

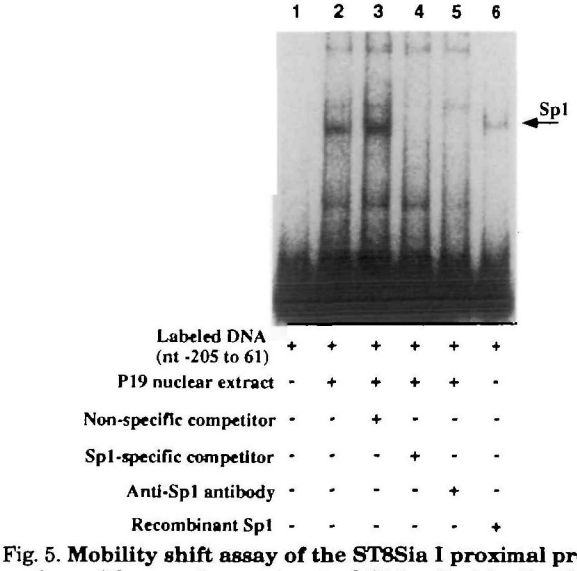


Fig. 5. **Mobility shift assay of the ST8Sia I proximal promoter region with a nuclear extract of P19 cells.** The 5′-end labeled DNA fragment of the ST8Sia I proximal promoter region comprising nucleotides −205 to 61 (lane 1) was incubated with a nuclear extracts of P19 cells either alone (lane 2) or with 25 times the amount of the non-specific competitor (lane 3), and with 25 times the amount of the Sp1-specific competitor (lane 4), and the anti-Sp1 antibodies (lane 5), and then subjected to the mobility shift assay. Lane 6 shows the results of a mobility shift assay involving 0.4 footprinting units of recombinant Sp1 instead of the nuclear extract of P19 cells.

To clarify the involvement of this Sp1 binding site in the transcription of ST8Sia I mRNA, we constructed an Sp1 binding site-replaced mutant of pP2-565, designated pP2-565(Sp1*), and analyzed its promoter activity in P19 cells (Fig. 4). The Sp1 binding site-replaced mutant pP2-565-(Sp1*) caused a significant reduction in the promoter activity to 21.2% as compared with the wild-type construct (71.1%). This result corresponded to those of the mobility shift experiments, and indicated that the Sp1 binding site is involved in the basic transcription of ST8Sia I mRNA. The residual promoter activity of pP2-565(Sp1*) suggests that there are other transcription factors involved in the basic transcription of ST8Sia I mRNA besides Sp1.

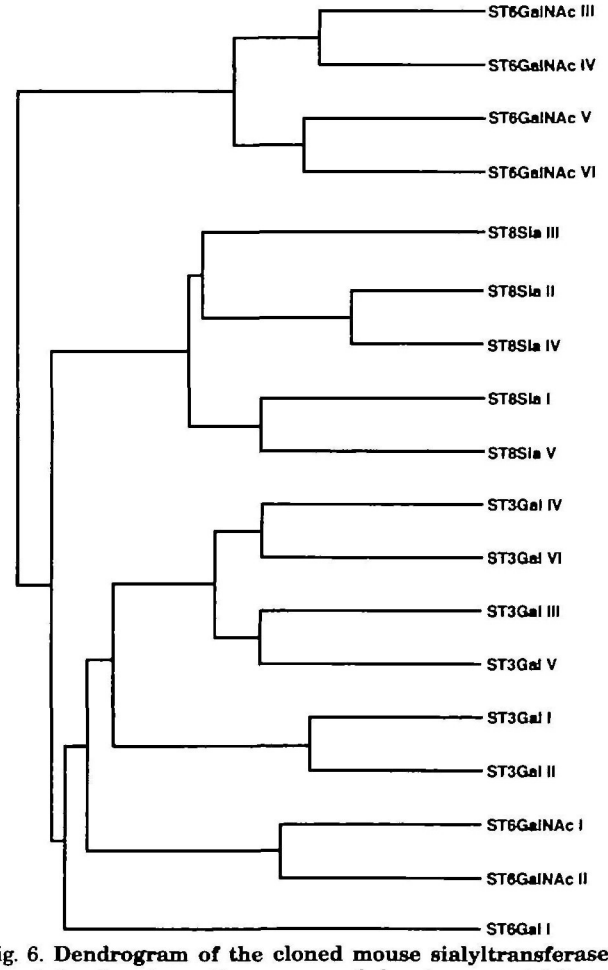


Fig. 6. **Dendrogram of the cloned mouse sialyltransferases.** The deduced amino acid sequences of cloned mouse sialyltransferases were analyzed by the method of Higgins and Sharp (*43*) with some modifications, and a dendrogram was constructed.

DISCUSSION

In this study, we determined the genomic organization of the mouse ST8Sia I gene and characterized its promoter region by means of transient transfection assays with a

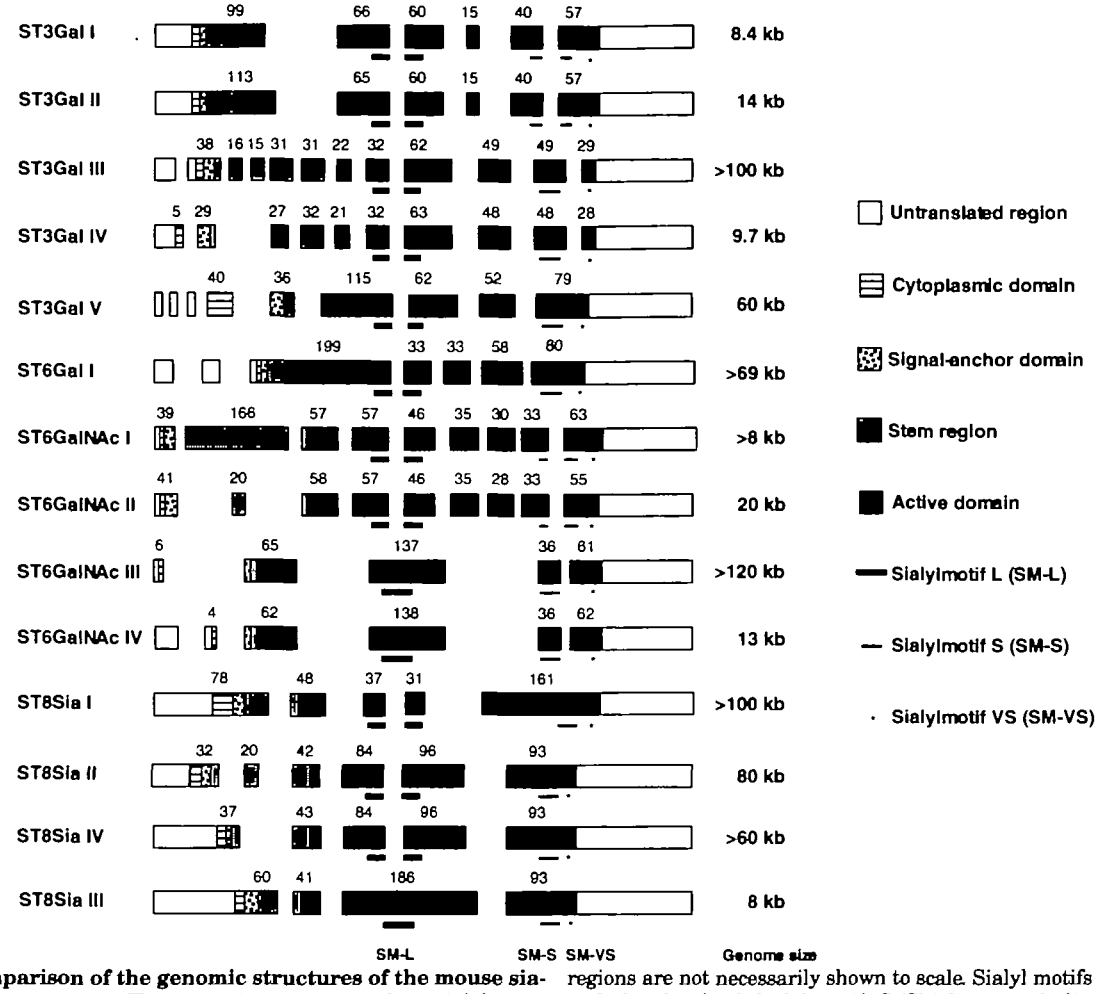


Fig. 7. **Comparison of the genomic structures of the mouse sialyltransferase genes.** The genomic structures of 14 sialyltransferase genes are presented. The putative protein domain structure is represented schematically by a rectangle, which is subdivided to show the major structural elements of the protein. Note that untranslated regions are not necessarily shown to scale. Sialyl motifs L (SM-L) are underlined in bold. Sialyl motifs S (SM-S) are underlined. Sialyl motifs VS (SM-VS) are shown as asterisks. Sialyl motifs L and S of some genes are split by introns (Fig. 2).

TABLE II. **Chromosomal localization of mouse and human sialyltransferase genes.**

Gene	Mouse chromosome	Locus information	Human chromosome	Locus information
ST3Gal I	UN		8	8q24.3, D8S529-D8S1710 (145.9–149.8 cM)
ST3Gal II	UN		16	D16S3031-D16S3139 (83.7–86.6 cM)
ST3Gal III	UN		1	1p34-p33
ST3Gal IV	UN		11	11q23-q24, D11S934-D11S1351 (131.7–136.9 cM)
ST3Gal V	UN		2	2q11.2, D2S289-D2S2371 (107.1–107.7 cM), D2S388-D2S113 (111.5–115.3 cM)
ST3Gal VI	UN		3	D3S1552-D3S1603 (113.0–115.7 cM)
ST6Gal I	16	15.5 cM	3	3q27-q28, D3S1553-D3S1580 (178.1–213.7 cM)
ST6GalNAc I	11	11E2	17	17q25
ST6GalNAc II	UN		17	17q25, D17S785-D17S836 (104.7–114.0 cM)
ST6GalNAc III	UN		UN	
ST6GalNAc IV	UN		9	D9S1821-D9S159 (137.6–142.7 cM)
ST6GalNAc V	UN		1	1p31.1
ST6GalNAc V	UN		9	D9S1821-D9S159 (137.6–142.7 cM)
ST8Sia I	6	60.0 cM	12	12p12.1-p11.2, D12S358-D12S1596 (27.2–48.1 cM)
ST8Sia II	UN		15 (8)	15q26 (Chr8, D8S560-D8S1820 (42.2–54.2 cM))
ST8Sia III	18		18	18q21
ST8Sia IV	UN		5	5q21, D5S494-D5S492 (107.8–116.3 cM)
ST8Sia V	UN		18	D18S460-D18S1118 (67.1–68.9 cM)

There is a discrepancy between FISH and ePCR results for the chromosomal localization of the human ST8Sia II gene. UN, unknown.

luciferase-reporter system and a gel mobility shift assay using a nuclear extract of P19 cells.

The ST8Sia-family contains five known members. They can be classified into three subfamilies according to the amino acid sequence similarities (*43*) (Fig. 6). ST8Sia I and V show 44.0% amino acid sequence identity to each other. ST8Sia II, III, and IV can be classified into a large subfamily, which can be further divided into two (Fig. 6). ST8Sia III shows 38.0 and 39.3% amino acid sequence identity to ST8Sia II and IV, respectively, and ST8Sia II and IV show 66.9% amino acid sequence identity to each other. It should be also noted that ST8Sia II and IV show similar substrate specificity toward *N*-glycan on NCAM (*25*, *27*) (Table I). So far, the genomic structures of the ST8Sia I, II, III, and IV genes have been also determined (*44–47*, this study). Although the split patterns of coding sequences for sialyl motifs L are conserved among some sialyltransferase genes including the ST8Sia I, II, and IV genes (Fig. 2), the genomic structure of the ST8Sia I gene seems unique when compared to those of so far determined α2,8-sialyltransferase genes and other sialyltransferase genes (Fig. 7). For the ST8Sia I gene, the lengths of coding sequence of exons encoding sialyl motif L are shorter and that of the exon encoding sialyl motif S is longer than those of corresponding exons of other α2,8-sialyltransferase genes. This suggests that the ST8Sia I gene may not share a common ancestral gene with the ST8Sia II, III, and IV genes. We do not know the genomic structure of the ST8Sia V gene at present, but it may have similar properties to that of the ST8Sia I gene judging from their amino acid sequence identity (Fig. 6). On the other hand, the ST8Sia II and IV genes are composed of six and five exons, respectively, and the genomic structures of these genes are quite similar to each other (Fig. 7). The exons encoding sialyl motifs L are divided by introns but not for exons encoding sialyl motifs S for the ST8Sia I, II, and IV genes. The ST8Sia III gene has the simplest genomic structure among sialyltransferase genes so far cloned, being composed of only four exons (*45*). The exons encoding sialyl motifs L and S are not divided by introns for the ST8Sia III gene. It should be also noted that the genomic structure of the ST8Sia III gene would show more similarity to those of the ST8Sia II and IV genes if the third exon of the ST8Sia III gene were split at an appropriate position (Fig. 7). This suggests that the ST8Sia II, III, and IV genes share a common ancestral gene.

The similarities among genomic structures, amino acid sequences and linkage- and substrate-specificities are also observed in other sialyltransferase subfamilies. ST3Gal I and II show 47.1% amino acid sequence identity to each other and similar substrate specificity (*29–31*) (Fig. 6 and Table I). The genomic structures of these genes are quite similar to each other (*49*) (Fig. 7). On the other hand, ST3Gal III and IV show 37.1% amino acid sequence identity to each other and similar substrate specificity toward Galβ1,(3 or 4)GlcNAc-residues (*29–31*) (Fig. 6 and Table I). Although these genes contain different numbers of exons, they show significant similarity in some exon–intron boundaries (Fig. 7). It should be also noted that the genomic structure of the ST3Gal V gene would show more similarity to those of the ST3Gal III and IV genes if the exons 6 and 9 of the ST3Gal V gene were split at appropriate positions. This suggests that the ST3Gal III, IV, and V genes share a common ancestral gene.

ST6GalNAc I and II show 42.0% amino acid sequence identity to each other and similar substrate specificity (*28*, *36*, *59*) (Fig. 6 and Table I). The genomic structures of these genes are also quite similar to each other (*28*, *36*) (Fig. 7). Like the ST3Gal I and II genes, the coding sequences for sialyl motifs L and S are divided by introns for these genes. It is interesting that mouse ST3Gal-family and ST6GalNAc I and II contain conserved four cystein residues known as a Kurosawa motif, Cys-Xaa$^{75-82}$-Cys-Xaa$^{1-2}$-Cys-Ala-Xaa-Val-Xaa$^{150-160}$-Cys (Xaa denotes any amino acid residue) (*37*). Although the ST6GalNAc I and II genes have more exons than the ST3Gal I and II genes, the existence of the Kurosawa motif and the split patterns of coding sequences for sialyl motifs L and S (Fig. 2 and Fig. 7) suggest that the ST6GalNAc I and II genes and ST3Gal I and II genes may share a common ancestral gene. On the other hand, ST6GalNAc III and IV show 40.8% amino acid sequence identity to each other and similar substrate specificity (*37*) (Fig. 6 and Table I). The genomic structures of ST6GalNAc III and IV genes are also quite similar to each other (*48*) (Fig. 7).

ST6Gal I is a unique β-galactoside α2,6-sialyltransferase, and the split pattern of the coding sequence for sialyl motif L of its gene is different from other sialyltransferase genes (Fig. 2), suggesting that the ST6Gal I gene may have evolved independently from an ancestral sialyltransferase gene.

In summary, there is significant correlation between the classification of sialyltransferases according to the amino acid sequence similarities (Fig. 6) and genomic structural resemblance of the so-classified sialyltransferases (Fig. 7). In addition, most sialyltransferase pairs sharing similar genomic structures show similar substrate specificities. Based on these observations, we hypothesize that the sialyltransferases with similar substrate specificities arose from a common ancestral gene through gene duplication. All mouse sialyltransferases characterized to date have sialyl motifs L, S, and VS. Therefore, we think that the most ancient sialyltransferase gene encoding an enzyme having sialyl motifs L, S, and VS was common, and as it evolved it dividerd into several subfamilies of sialyltransferase genes. A viral α2,3-sialyltransferase (v-ST3Gal I) having sialyl motifs L, S, and VS and showing significant similarity with mouse ST3Gal IV has been reported (*60*, *61*). Identification and characterization of such sialyltransferases in viruses and microorganisms may help to elucidate the molecular evolution of sialyltransferase genes.

The chromosomal mapping of mouse sialyltransferase genes has been done for the ST6Gal I and the ST8Sia I genes (*62*, *63*). The ST6Gal I gene is located on mouse chromosome 16 and the ST8Sia I gene is located on mouse chromosome 6. In our database search for the chromosomal localization of mouse and human sialyltransferase genes, we found that the mouse ST6GalNAc I and ST8Sia III genes are located on chromosomes 11 and 18, respectively (Table II). As the chromosomal localization of other mouse sialyltransferase genes has not been determined, we cannot discuss this issue further at present. However, in human cases, chromosomal localization of 17 sialyltransferase genes has been determined or estimated (Table II). Among them, the human ST6GalNAc I and II genes and ST6GalNAc IV and VI genes are located close to each other on

chromosomes 17 and 9, respectively, suggesting that each gene pair is closely related from an evolutional standpoint. However, the human α2,3-sialyltransferase genes are located on different chromosomes (*51*, *64*, *65*) (Table II). This suggests that similar sialyltransferase genes are not necessarily located on the same chromosome. With some exceptions, there appear to be no rules for chromosomal distribution of most of sialyltransferase genes. However, further studies on the chromosomal mapping of sialyltransferase genes may provide useful knowledge on genetic and evolutional relation among sialyltransferase genes.

Although some sialyltransfease genes show genomic structural resemblance, their expression patterns are usually different. As far as we know, the promoter structures of these sialyltransferase genes are not necessarily similar to each other except in regions involved in basic transcription (*44*, *46*, *48*). Tissue- and stage-specific expression of each sialyltransferase gene may be explainable by characteristic promoter structure of each sialyltransferase gene and transcription factors that bind to specific elements on each promoter. However, such transcription factors have not yet been identified for sialyltransferase genes. On the other hand, the mechanisms of basic transcription of some sialyltransferase genes have been analyzed. As we have shown in this study, Sp1 has been shown to be involved in basic transcription of some sialyltransferase genes, such as the ST6GalNAc III and IV genes and the ST8Sia I, II, and IV genes (*44*, *46*, *48*, this study). Sp1 is thought to be a ubiquitous transcription factor associated with the transcriptional regulation of housekeeping genes. It is likely that Sp1 is also involved in basic transcription of other sialyltransferase genes whose transcriptional mechanisms have not been analyzed.

The mutation analysis of the ST8Sia I promoter region suggested that there are other transcription factors involved in the basic transcription besides Sp1. In the case of the mouse ST8Sia IV gene, both Sp1 and NF-Y are synergetically involved in the basic transcriptional regulation of this gene (*46*). Although there is no NF-Y binding site in the proximal promoter region of the ST8Sia I gene, transcription factors which synergetically act with Sp1 may be involved in the basic transcriptional regulation of the ST8Sia I gene. Identification of such transcription factors will be needed for detailed analysis of the transcriptional mechanism of the ST8Sia I gene.

In this study, we identified only one promoter for the ST8Sia I gene. However, transcription of some sialyltransferase genes, such as the ST6Gal I and ST6GalNAc I genes, is regulated by tissue-specific promoters (*36*, *52*, *53*). The expression of the mouse ST6Gal I gene is regulated by at least four physically distinct and independently operating promoters, and each promoter generates an mRNA with identical ST6Gal I open reading frame and differs only in the 5′-untranslated region (*52*, *53*). Transcripts of different sizes are also often observed in the transcription of some sialyltransferase genes (*19*, *31*, *36*, *37*, *45*), and they are tissue-specific in some cases. The regulation mechanisms of these transcriptional diversities remain unclear. The mechanisms of tissue- and stage-specific expression of sialyltransferase genes may be complicated. However, identification of tissue- or stage-specific promoters and transcription factors which regulate tissue- or stage-specific expression of sialyltransferase genes as well as those regulating basic transcription will facilitate understanding of the different expression patterns of each sialyltransferase gene.

The total number of the mouse sialyltransferase genes is unknown at present. But the results of the mouse genome project will answer this question. As we have shown here, some of these sialyltransferase genes should encode enzymes showing similar substrate specificities. The biological significance of these multiple gene families is also unclear at present. One interpretation is that they may be important for fine control of the expression of sialylglycoconjugates, resulting in stage- and tissue-specific variety of glycosylation patterns. Analyses of transcriptional regulation and transgenic animals of sialyltransferase genes, including gene targeting (*66–68*) and overexpression of sialyltransferase genes, will help elucidate of the biological significance of each sialyltransferase and sialylglycoconjugates produced by them.

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