Human STX Polysialyltransferase Forms the Embryonic Form of the Neural Cell Adhesion Molecule

TISSUE-SPECIFIC EXPRESSION, NEURITE OUTGROWTH, AND CHROMOSOMAL LOCALIZATION IN COMPARISON WITH ANOTHER POLYSIALYLTRANSFERASE, PST*

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PST and STX are polysialyltransferases that form polysialic acid in the neural cell adhesion molecule (N-CAM), although it is not known why these two polysialyltransferases exist. In the present study, we have first isolated cDNA encoding human STX, which includes 5'-untranslated sequence. Northern blot analysis, using this cDNA and PST cDNA previously isolated by us, demonstrated that PST and STX are expressed in different fetal and adult tissues. STX is primarily expressed in embryonic tissues, but only modestly in adult heart, brain, and thymus. PST, on the other hand, is continuously expressed in adult heart, brain, thymus, spleen, small and large intestines, and peripheral blood leukocytes. In various parts of adult brain, the relative amount of PST and STX appears to be substantially different depending on the regions. The analysis by in situ hybridization of mouse adult brain, however, suggests that polysialic acid in the hippocampal formation is synthesized by both STX and PST. HeLa cells doubly transfected with the isolated STX cDNA and N-CAM cDNA supported neurite outgrowth much better than HeLa cells expressing N-CAM alone. However, polysialic acid synthesized by PST appears to be a better substratum than that synthesized by STX. Moreover, the genes for PST and STX were found to reside at chromosome 5, band p21 and chromosome 15, band q26, respectively. These results, taken together, strongly suggest that PST and STX are expressed distinctly in tissue-specific and cell-specific manners and that they apparently have distinct roles in development and organogenesis.

Polysialic acid is a developmentally regulated glycan composed of a linear homopolymer of α-2,8-linked sialic acid residues. Polysialic acid is mainly attached to the neural cell adhesion molecule (N-CAM) and is more abundant in embryonic brain than adult brain (1–3). Presence of this large negatively charged carbohydrate modulates the adhesive property of N-CAM, and removal of polysialic acid increases binding between N-CAMs (4, 5). During embryonic development, the polysialylated embryonic form of N-CAM is restricted to migrating cells (6, 7), and the removal of polysialic acid from the N-CAM during embryonic development affects motor-axon projection patterns (8). Polysialylated N-CAM was also shown to attenuate cell-cell interactions mediated by other cell adhesion molecules (9–11).

Recently, we and others have cloned cDNAs encoding human, hamster, and mouse polysialyltransferases (PST for human, PST-1 for hamster, and ST8Sia II for mouse, respectively) (12–14). The amino acid sequences of PST and PST-1 are more than 97% identical. Both PST and PST-1 directed the expression of polysialic acid on the cell surface. The same studies also revealed that PST is highly homologous to STX (sialyltransferase X, or ST8Sia II) (12, 13), and STX and PST have 59% identity at the amino acid level. STX was originally cloned as a developmentally regulated sialyltransferase from the rat fetal brain (15). Although the substrate specificity of STX was not known at the time of its discovery, the above results prompted investigation of STX as a polysialyltransferase. In support of this speculation, it was shown that STX also directs the expression of polysialic acid in small cell lung carcinoma cell lines (16) and forms polysialic acid in both wild-type N-CAM as well as soluble chimeric N-CAM proteins (17).

Consistent with the presumed roles of polysialic acid, it has been shown that PST facilitates neurite outgrowth (12). HeLa cells were transfected with human PST and N-CAM cDNAs or N-CAM cDNA alone and used as the substratum for the neurite outgrowth assay. When neurons derived from embryonic chicken were grown on these substrata, neurites were much longer and more branched on the substratum cells expressing polysialic acid and N-CAM than those on the substratum expressing N-CAM alone (12). These results clearly indicate that polysialic acid formed by PST facilitates neurite outgrowth. However, it is not known if expression of STX and N-CAM cDNAs also facilitates neurite outgrowth.

By using an in vitro assay system, both PST and STX added polysialic acid to fetuin and soluble chimeric N-CAM (17, 18). This demonstrates that either PST or STX alone can form polysialic acid by adding the first α-2,8-linked sialic acid to α-2,3-linked sialic acid in a glycoconjugate template, followed by multiple α-2,8-linked sialic acid residues to the acceptor containing NeuNAcα2→8NeuNAcα2→3Gal→R structure. PST and STX thus appear to share common enzymatic properties. On the other hand, it is not known why these two enzymes exist for polysialylation.
In order to address this question, in the present study we have compared the expression profile of PST and STX in various fetal and adult tissues using Northern blot analysis and in situ hybridization. In addition, we have analyzed PST and STX in their capability to promote neurite outgrowth. Moreover, we demonstrate the presence of genes encoding PST and STX on different chromosomes, implying early separation in their evolution. These results strongly suggest that PST and STX play distinct roles in development and organogenesis.

**EXPERIMENTAL PROCEDURES**

**Cloning of Human STX cDNA**—First, cDNA was synthesized from the human fetal brain mRNAs (Clontech) using a 3’-primer and reverse transcriptase. The 3’-primer, ASTX-3-1, is 5’-GGTCTCTCTACGTGGCC-CCCACAT-3’. The last 15 nucleotides in this sequence correspond to nucleotides 1114–1128 of human STX, hSTX sequence (nucleotides 1126–1128 encode the stop codon) (16). The rest of ASTX-3-1 sequence was adapted from the mouse STX (mSTX) sequence (19). By reverse transcriptase-catalyzed reaction, cDNA was produced, and PCR was performed using the formed cDNA as a template to amplify the sequence between nucleotides 96 and 1128 (nucleotides 1–3 and nucleotides 1128–1126 encode the initiation and the stop codon, respectively).

The primer sequences were based on the hSTX sequence (16).

In order to obtain the sequence upstream from nucleotide 96, cDNA was synthesized using a 3’-primer corresponding to nucleotides 188–199, reverse transcriptase, and human fetal brain mRNA as a template. PCR was then carried out using the formed cDNA as a template. The 5’-primer, STX-XhoI, for this PCR is 5’-GGTCTAGACGTGCCCACGGCCGCG-3’. In the last 15 nucleotides encode nucleotides 4–26 of cDNA sequences (19). The rest of STX-XhoI site (denoted by an underline). The 3’-primer sequence corresponds in antisense direction to nucleotides 110–133 of hSTX. The PCR product obtained was cloned into pBluescript II (Strategene) and then digested at HindIII site in the vector and at an internal EcoRI site (nucleotides 98–104). This HindIII-EcoRI fragment was appended to the 5’-end EcoRI and XhoI sites of pcDNAI-STX (Life Technologies, Inc.) using KpnI-RI site of a larger cDNA fragment (encompassing nucleotides 100–1283), producing a full-length cDNA encoding hSTX. The ligated cDNA was cloned into the HindIII and XhoI sites of pcDNAI.

**Northern Blot Analysis of Various Human Tissues**—Northern blots of poly(A)+ RNA from human fetal (19–23 gestational weeks) and adult brains, as well as human multiple tissue Northern blots of poly(A)+ RNA, were purchased from Clontech. The last 15 nucleotides encode nucleotides 4–26 of cDNA sequences (19). The rest of STX-XhoI site (denoted by an underline). The 3’-primer sequence corresponds in antisense direction to nucleotides 110–133 of hSTX. The PCR product obtained was cloned into pBluescript II (Strategene) and then digested at HindIII site in the vector and at an internal EcoRI site (nucleotides 98–104). This HindIII-EcoRI fragment was appended to the 5’-end EcoRI and XhoI sites of pcDNAI-STX (Life Technologies, Inc.) using KpnI-RI site of a larger cDNA fragment (encompassing nucleotides 100–1283), producing a full-length cDNA encoding hSTX. The ligated cDNA was cloned into the HindIII and XhoI sites of pcDNAI.

**Preparation of a Probe for in Situ Hybridization of Mouse PST (mPST)**—A mouse genomic DNA library derived from 129 SVJ mouse genome (Strategene) was screened with a DNA fragment that represents the 5’-portion of human PST cDNA (12). One of the isolated genomic clones, I15, contains the entire sequence of the translation initiation methionine of mPST judging from the comparison of human PST cDNA and mouse genomic sequences. Subsequently, this was verified by comparison of this sequence and the mPST cDNA sequence reported later (14). The details of the isolation of mouse genomic DNA will be described elsewhere.

Using this clone I15 as a template, mPST cDNA sequence surrounding the initiation methionine (nucleotides 47–113; the first nucleotide of the initiation codon is +1) was amplified by PCR. The 5’-primer and 3’-primer are 5’-GGTCTAGACGTGCCCACGGCCGCG-3’ and 5’-CAGGGGTACCGGATTGGTGCTCTCTG-3’, respectively. The XhoI and KpnI sites are denoted by an underline. The sequence of the 3’-primer ends at the 3’-end of the exon, which was deducted from the comparison of the mouse genomic and human PST cDNA sequences. This amplified cDNA sequence was cloned into XhoI and KpnI sites of pBluescript II SK, and the resultant vector was used as a template for the construction of RNA probes. A digoxigenin-labeled antisense RNA probe was produced using the XhoI-cut template and T7 RNA polymerase with the dIII site in the vector and at an internal KpnI site. The obtained cDNA was cloned into XhoI and KpnI sites of pBluescript II SK, and used as a template for in vivo RNA probes.

**In Situ Hybridization for mPST and mSTX Transcript in the Hippocampal Formation**—Hybrid-Ready tissue slides of mouse adult brain (NIH Swiss strain) were purchased from Novagen. In situ hybridization was performed as described previously (21). The sections were subjected to immunohistochemistry for detection of the hybridized probes by using mouse monoclonal antibody 12F8 conjugated anti-digoxigenin antibody with the DIG nucleic acid detection kit (Boehringer Mannheim). The alkaline phosphatase reaction was demonstrated by 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium in the presence of 10% polyvinyl alcohol (22). Control experiments using sense probes for PST or STX produced no specific signals.

**Immunohistochemistry for Polysialic Acid**—The Hybrid-Ready tissue slides were subjected to the immunohistochemical staining for detection of polysialic acid as described previously (12, 23). A monoclonal antibody 12F8 specific for polysialic acid (24) (Pharmingen) was used in this study, and immunohistochemical detection was performed by indirect method using anti-rat immunoglobulins conjugated with horseradish peroxidase (Dako) (25), followed by counterstaining with hematoxylin. A control experiment was done by omitting the antibody from the staining procedure, and no specific staining was found. The results obtained using antibody 12F8 were confirmed by using antibody 735 (26) or 12E3 (27).

**Assay for Neurite Outgrowth on HeLa Cells Stably Expressing N-CAM and PSA**—Since HeLa cells were negative for both PSA and N-CAM, they were transfected by a plasmid pBluescriptI-1-neo-N-CAM encoding N-CAM140 (26) using LipofectAMINE (Life Technologies, Inc.). After selection with G418, clonal cell lines stably expressing N-CAM were selected by staining with anti-N-CAM antibody, CD56 (Dako). A clonal HeLa cell line expressing N-CAM was then transfected with pcDNAI-STX and a plasmid pSvSvHyB encoding the hygromycin resistance gene, and HeLa cells expressing both N-CAM and polysialic acid (detected by 12F8 antibody), named HeLa-N-CAM+STX cells, were selected. HeLa cells expressing both N-CAM and PST, termed HeLa-N-CAM+PST, were established as described (12). These cells were cultured as monolayers in Lab-Tek chamber slides (Nunc). Sensitive neurons were obtained from the dorsal root ganglia of 10-day-old chicken embryos. These neurons were dissociated with trypsin (0.5%), counted, seeded at low density over HeLa cell monolayers, and cultured in minimum essential medium containing 10% fetal bovine serum (29, 30). Nerve growth factor was included in the sensory neuron culture. Neuron-HeLa cell co-cultures were grown for 15 h, fixed with 4% paraformaldehyde in phosphate-buffered saline, and stained with anti-neurofilament antibody RM0270 (31), followed by fluorescein isothiocyanate-conjugated anti-mouse IgG antibody. Neurite lengths were measured with the Metamorph Imaging system (Universal Imaging Corp., West Chester, PA) as described previously (32). The mean neurite lengths and the number of branch points per neuron were compared among the different substrate conditions by Student’s t test.

**Western Blot Analysis**—HeLa-N-CAM, HeLa-N-CAM+PST, and HeLa-N-CAM+STX cells were subjected to Western blotting using the ECL Western blot detection system (Amersham) as described (12) with a modification. Briefly, the cell pellet was centrifuged and a portion of the pellet was digested with endo-N (32). The cells were then dissolved in 1% Nonidet P-40 containing a protease inhibitor mixture (Boehringer Mannheim) in phosphate-buffered saline, and the supernatant obtained after centrifugation was incubated with an equal volume of wheat germ agglutinin (WGA)-agarose (EY Laboratories). After incubation with rotation, the suspension was briefly centrifuged and the WGA-agarose beads were recovered. The glycoproteins were dissociated from WGA-agarose by heating at 85°C for 3 min in the sample buffer for electrophoresis (18). The proteins, separated by 5% SDS-polyacrylamide gel electrophoresis, were blotted to PVDF membrane and treated in 0.01 N HCl at 80°C for 20 min, followed by blocking with 10% skim milk. The membrane was then incubated with anti-N-CAM antibody, CD56 (Becton Dickinson). For detection of polysialic acid, a monoclonal antibody 12F8 was incubated with 12E3 antibody (27). The 12E3 antibody was kindly provided by Dr. Yasumasa Arai, Juntendo Medical School, Tokyo, Japan.

**Fluorescence in Situ Hybridization Analysis of PST and STX Genes**—P1 plasmid human genomic DNA library was screened by PCR as described (34). The 5’ and 3’ primers for PCR of PST gene correspond
to the PST sequence of nucleotides 599–608 and that of nucleotides 757–776, respectively. Purified DNA from one of the clones, clone 6029, was labeled with digoxigenin-dUTP by nick translation. Labeled probe was combined with sheared human DNA and hybridized to normal metaphase chromosomes derived from phytohemagglutinin-stimulated peripheral blood leukocytes, and the chromosome banding was carried out as described (35).

Similarly, P1 plasmid human genomic DNA library was screened for STX gene by PCR. The 5'- and 3'-primers for this PCR correspond to the STX sequence of the nucleotides 954–972 and that of the nucleotides 1101–1118, respectively. Purified DNA from one of the isolated clones, clone 6747, was used as a probe as described above.

**RESULTS**

**Isolation of a Human STX cDNA**—There were two reports on human STX (hSTX) sequences that differ in several nucleotides, including the stop codon (16, 33). In order to determine whether or not either sequence was correct and if allelic differences might exist, we cloned hSTX cDNA. First we cloned the cDNA encompassing nucleotides 96–1128 by synthesizing cDNA using human brain mRNA as a template. This was only possible using the nucleotide sequence reported by Scheidegger et al. (16). When primers were synthesized according to the 3'-end sequence reported by another laboratory (33), no PCR product was formed. We then tried to extend this sequence upstream to 5'-untranslated sequence since there is no information on this sequence of human STX even in the data bank. This sequence was also necessary to express STX protein in mammalian cells. The 5'-untranslated sequence was obtained by reverse transcriptase-PCR using the 5'-primer sequence adapted from mouse STX and human fetal brain mRNA as a template. This 5'-upstream sequence was ligated to the above large cDNA fragment at a common EcoRI site to form a cDNA encompassing a single long open reading frame predicted to code the entire hSTX polypeptide.

Fig. 1A shows the 5'-region of the nucleotide and amino acid sequences of hSTX obtained by the above experiments. The nucleotide sequence from codon 1 to the stop codon was found to be identical to that of Scheidegger et al. (16). The amino acid sequences of hSTX and hPST have 58.8% identity. As shown in Fig. 1B, the similarity is more significant in the catalytic domains of STX and PST.

Expression of STX and PST Transcripts in Various Human Fetal and Adult Tissues—In order to understand how STX and PST differ, the expression of STX and PST transcripts were examined by Northern blot hybridization using the same blot. Although the expression of hSTX and hPST transcripts were reported individually (12, 33), there is no report on the direct comparison of two enzymes side by side, nor is there any report on STX expression in various parts of the brain. The results shown in Fig. 2 demonstrate that STX transcripts are much more abundant in fetal brain than adult brain (for adult brain, see the whole brain in multiple brain tissues). Among adult tissues, the STX transcript is relatively abundant in thymus and heart, and moderately present in the small intestine, colon, and skeletal muscle. Among adult brain tissues, the STX transcript is present prominently in hippocampus, medulla oblongata, and putamen and moderately in amygdala, cerebral cortex, subthalamic nucleus, and cerebellum. In contrast, the PST transcript is more prominent in amygdala, subthalamic nucleus, cerebral cortex, and occipital pole (Fig. 2). PST is more widely present in non-neural cells. In particular, a substantial amount of the PST transcript is present in spleen and peripheral blood leukocytes, where STX is hardly expressed. PST was also expressed in placenta where STX was not detected. These results suggest that STX is more dramatically
reduced during embryonic development and its expression is restricted to only a few tissues in adult. PST is also substantially reduced during development of brain but persists in some non-neural tissues.

The specific hybridization by PST or STX probe was confirmed by the fact that only PST or STX transcript could be detected in certain tissues. For example, only PST transcript was detected in placenta while only STX transcript was detected in cerebellum. Moreover, the relative intensity of each transcript in different tissues did not change even after more stringent washings.

Expression of mPST and mSTX Determined by in Situ Hybridization in the Hippocampus—The above results indicate that STX is more dominant than PST in the hippocampus. In order to determine which cells express these enzymes, in situ hybridization was carried out using mouse brain. In the hippocampus formation, a significant amount of the signal for the mPST transcript was detected in the granule cells of the dentate gyrus as well as the pyramidal cells medially located in the CA1 and CA3 fields of the Ammon’s horn (Fig. 3A). In CA3, the mPST transcript was barely detectable, and the CA2 field lacked the mPST transcript. In contrast, a strong signal for the mSTX transcript was detected not only in CA1 and the granule cells of the dentate gyrus, but also in CA2 and CA3 (Fig. 3B). Polysialic acid, on the other hand, was expressed in the granule cells of the dentate gyrus and the mossy fibers arising from these granule cells (Fig. 3D), and this staining pattern is consistent with results shown in the previous report (36). These results combined together indicate that the polysialic acid in the granule cells are coordinately synthesized by mPST and

Fig. 2. Northern blot analysis of STX and PST in various human tissues. Each lane contained 2 μg of poly(A)+ RNA. The blots were first probed with 32P-labeled hSTX cDNA (STX). After hSTX probe was removed, the same blots were hybridized with hPST cDNA (PST).

Fig. 3. Expression of mPST and mSTX transcripts, and polysialic acid in the hippocampal formation. In situ hybridization of the mouse hippocampus with an antisense probe for mPST (A) and mSTX (B), and a sense probe for mSTX (C). The sense probe for mPST also provided negative staining. Immunohistochemical staining for polysialic acid using a mAb 12F8 is shown in D. CA1–3, fields of the Ammon’s horn; DG, dentate gyrus; MF, mossy fibers. Bar = 500 μm.
mSTX, and most likely transported to the mossy fibers through axoplasmic flow.

Expression of Polysialylated N-CAM by STX—We have shown previously that the expression of PST and N-CAM cDNAs in HeLa cells resulted in the expression of polysialic acid on N-CAM (12). In the present study, HeLa cells were stably transfected with the cloned STX and N-CAM cDNAs. As shown in Fig. 4, HeLa cells express both N-CAM and polysialic acid detected by immunofluorescent staining using anti-N-CAM and anti-polysialic acid antibodies (Fig. 4, G and H). The immunostaining of polysialic acid was abolished after endo-N treatment of the cells (Fig. 4 J). In parallel, HeLa cells transfected with N-CAM and PST cDNAs were examined. Those HeLa cells showed a positive staining for N-CAM before and after endo-N treatment (Fig. 4, C and E), while polysialic acid staining disappeared after endo-N treatment (Fig. 4, D and F).

In order to determine whether or not STX actually formed polysialic acid on N-CAM, the cell lysates from the above stably transfected HeLa cells were subjected to Western blot analysis. A diffused high molecular band detected in cell lysates was converted to a sharp low band after endo-N treatment (Fig. 5, left panel, lanes 5 and 6). Almost identical results were obtained on HeLa cells expressing both N-CAM and PST (Fig. 5, left panel, lanes 3 and 4). Such a broad smear band was not obtained in parental HeLa cells which were transfected only with N-CAM cDNA (Fig. 5, left panel, lanes 1 and 2). These results indicate that both STX and PST can form polysialic acid on N-CAM.

In order to specifically detect the polysialylated form of N-CAM, the blot was incubated with anti-polysialic acid antibody,
12E3, without acid pretreatment. As shown in Fig. 5 (right panel), a highly polysialylated form of N-CAM with high molecular weight was detected in both PST and STX-transfected cells (lanes 3 and 5) and those broad bands with high molecular weight were not detected after endo-N treatment (lanes 4 and 6). These highly sialylated N-CAM are much larger than those detected by anti-N-CAM antibody (compare right and left panels), consistent with the results reported previously (37).

Neurite Outgrowth on HeLa Cells Transfected with STX and N-CAM—Neural cell migration and axon outgrowth are influenced by polysialic acid expression on neural cells (29, 30). To determine the effect of polysialic acid synthesized by STX on N-CAM, HeLa cells expressing N-CAM alone was used as a parental cell line in establishing HeLa-N-CAM+STX. Neuronal cells derived from dorsal root ganglion were grown on HeLa substrate cells and found to exhibit modest outgrowth on confluent monolayers of HeLa cells expressing N-CAM alone (Fig. 6A). In contrast, neurons cultured on HeLa cells, which were transfected with both STX and N-CAM to express polysialylated N-CAM, grew neurites significantly longer and exhibited more branching (Fig. 6C) than those grown on N-CAM alone (Fig. 6A). This enhanced effect by polysialic acid was abolished once the substrate cells were treated with endo-N before the assay (data not shown). Very similar results were obtained on HeLa cells that were transfected with PST and N-CAM to express polysialylated N-CAM (Fig. 6B), as shown previously (12).

The results summarized in Fig. 7 show clearly that polysialylated N-CAM produced by STX and PST show very similar enhancement in neurite outgrowth, although PST-transfected HeLa cells appear to be better substrates than STX-transfected cells. This difference in the efficiency can be observed in both the length of the neurites and the number of branch points. In experiment 2, for example, the neurite lengths of PST- and STX-expressing HeLa cells appear to be better substrates than STX-transfected cells. Differences in length efficiency can be observed in both the number of branch points. In experiment 2, for example, the neurites of PST- and STX-expressing HeLa cells appear to be better substrates than STX-transfected cells. Differences in length and branch points of neurite outgrowth directed by N-CAM and PST or STX. The length of neurites and branch points formed are compared. Experiments 1 and 2 are shown by open and shaded boxes, respectively, and standard error bars are shown.

PST and STX are very similar. However, the size of minor transcripts differ, confirming the above conclusion that these detected bands are specific to the transcripts of PST and STX. Among different sialyltransferases, the similarity in the amino acid sequences between PST and STX is the highest and no other similarity between different members of sialyltransferase reaches 59% (12, 13). These results suggest that PST and STX are highly related to each other.

In order to determine how closely the PST and STX are related to each other in gene localization, we utilized fluorescence in situ hybridization procedure to localize PST and STX genes. First, P1 plasmids harboring PST and STX genes, named clone 6029 and 6747, respectively, were isolated. Genomic DNA was prepared from these P1 clones and used as a probe. The first clone, 6029, was found to hybridize to the middle of the long arm of a group B chromosome. Two additional experiments using two known probes, which hybridized to 5q34 or 5p15, showed that 6029 is located on chromosome 5. Measurements of 10 specifically hybridized probes to chromosome 5 demonstrated that 6029 containing PST gene is located on chromosome 5 arm q at a position which is 43% of the distance from the centromere to the telomere, and at the area that corresponds to band q21 (Fig. 8, A and C).

Similarly, genomic DNA clone 6747, which contains STX gene, was localized to chromosome 15, band q26 (Fig. 8, B and D). These results clearly indicate that PST and STX are localized on different human chromosomes.

**DISCUSSION**

In the present study, we have isolated human STX cDNA and obtained the 5’-untranslated sequence. It is likely that the adenosine at nucleotide 1 represents the first nucleotide of the translation initiation methionine for the following reasons.
First, the nucleotide sequence surrounding the initiation methionine, CCCACCATG, conforms with Kozak’s sequence, GC-CACCATG, reported for the optimum context for initiation of translation (38). The sequence surrounding the second methionine codon (nucleotides 25–27) is entirely different from this context (see Fig. 1A). Assuming that mSTX and hSTX share the common 5′-untranslated sequence, no methionine codon is found in the upstream sequence (19) as well as in the determined sequence upstream from the presumed initiation methionine (Fig. 1A). Finally, the expression of the obtained cDNA directed the expression of polysialic acid. We have also shown that hSTX and hPST are highly homologues to each other. The similarity is more extensive in the whole catalytic domain, which includes sialyl motif L, the binding site of the donor CMP-NeuNAc (39), and sialyl motif S than in the transmembrane domain and stem region. This similarity in the catalytic domain must reflect that both enzymes have polysialylation activity, whereas dissimilar regions may contribute to the difference in their functions (Fig. 1B).

In the present study, we have shown that hSTX forms polysialic acid on N-CAM, and thus has the same function as mSTX (17). In the previous studies, we and others have shown that PST also forms polysialic acid on N-CAM (12, 13, 17). It is then important to know why these two polysialyltransferases are present and how they work differently. The first clue to understanding this question was derived from Northern blot analysis in the present study. The results obtained with Northern blot analysis were striking (Fig. 2). In human fetal tissues, hSTX is much more abundant in brain than lung, liver, and kidney. Such a tremendous difference was not observed in hPST, although it is still expressed much more in the fetal brain than other fetal tissues. In comparison, both hSTX and hPST are moderately expressed in human adult brain. However, there is a substantial difference in PST and STX expression in other tissues. While hSTX is expressed moderately in thymus and heart, hPST is also expressed in spleen, peripheral blood leu-
ocytes, and small and large intestines. Although hSTX is dominantly expressed in some regions of the adult brain than hPST, no tissue was found where hSTX is expressed but hPST is absent as far as we examined. These combined results indicate that each PST and STX function in specific tissues, in which either enzyme likely plays a dominant role in a particular tissue. The results also suggest that the expression of these two enzymes are differently regulated.

Previously we have shown by immunostaining using 735 antibody that thymus and small and large intestines express polysialic acid (12). It has been also shown that NK cells and NK-derived leukemic cells express polysialic acid as well as N-CAM (40, 41). The polysialic acid in these cells are most likely synthesized by PST. In contrast, hSTX was shown to direct the polysialic acid synthesis in small cell lung carcinoma cells (16), and is more dominant in certain parts of adult brain, as shown in the present study. For example, the signal for the mPST and mSTX were detected in those cells, these combined results strongly suggest that both PST and STX are responsible for polysialic acid formation in the dentate granule cells and mossy fibers in the hippocampus. It is noteworthy that N-CAM knockout mice have deficiency in the development of the CA3 region (44), where the STX transcripts are highly expressed. These results are consistent with the conclusion that the defect in N-CAM knockout mice is due to the absence of polysialylated N-CAM (44, 45). The results also suggest that human and mouse probably show common feature in the expression profiles of PST and STX in the hippocampus, since Northern blot analysis of human hippocampus and in situ hybridization analysis of mouse brain showed similar results as a whole.

We have shown previously that neurons extend longer and have more branches on HeLa cells that were transfected with PST and N-CAM cDNAs than those transfected with N-CAM cDNA alone (12). In the present study, we have demonstrated that hSTX can form polysialylated N-CAM, and also introduces the capability of N-CAM to facilitate neurite outgrowth. These results strongly suggest that PST and STX play critical roles in neural development and axon growth. We also noted that HeLa-N-CAM cells transfected with STX appear to be slightly less efficient as substrate in neurite outgrowth than those with PST (Fig. 7). This is rather surprising, since HeLa-N-CAM+STX cells apparently contain more polysialylated N-CAM than HeLa-N-CAM+PST when these particular cell lines utilized are compared (Fig. 5B). It is possible that only a small portion of N-CAM, which is heavily polysialylated, may be critical for neurite outgrowth or that different polysialic acid structures may be synthesized by these two enzymes. Alternatively, too high a concentration of polysialic acid may be less efficient in neurite outgrowth. Further studies are necessary to determine which is the case.

Despite the fact that hPST and hSTX have 59% identity in amino acid sequences, these two enzymes are located on entirely different chromosomes. Moreover, GD3/GT3 synthase, another a-2,3-sialyltransferase related to PST or STX, is located on chromosome 12, band p12 (35). These results strongly suggest that these three sialyltransferases diverged from an ancestral gene early in evolution. These results are similar to those recently reported demonstrating that two different a-2,3-sialyltransferases are located on entirely different chromosomes (46). It will be of significance to analyze the difference in the regulatory sequences between hPST and hSTX genes, since the separation into different chromosomes might have resulted in the divergence of their regulatory elements as well. It is, on the other hand, noteworthy that hPST is located on chromosome 5, band q21. An isoform of a-mannosidase II, a-manIIa, was also located to chromosome 5, band q21, while a-mannosidase II resides on chromosome 15, band q25 (47). Moreover, a tumor suppressor gene, the adenomatous polyposis coli (APC) gene, is located on 5q21 (48). It will be interesting to determine if PST and APC genes are present in close proximity and if the expression of polysialic acid in certain tumors might be caused by gene alteration, which also causes inactivation of APC gene.

In summary, both PST and STX can polysialylate N-CAM, but these two enzymes apparently play distinct roles in different tissues. Such specific expression can be extended to cell-type specific expression as shown in hippocampus. It will be of significance to determine which of these two polysialyltransferases play a dominant role in various tissues and cells.

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