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Biochemistry

Expression cloning of a human polysialyltransferase that forms the polysialylated neural cell adhesion molecule present in embryonic brain

(glycosyltransferase/polysialic acid/developmental regulation/neurite outgrowth)

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ABSTRACT **Polysialic acid is a developmentally regulated posttranslational modification of the neural cell adhesion molecule (N-CAM). It has been suggested that this large anionic carbohydrate modulates the adhesive property of N-CAM, but the precise function of polysialic acid is not known. Here we describe the isolation and functional expression of a cDNA encoding a human polysialyltransferase. For this expression cloning, COS-1 cells were cotransfected with a human fetal brain cDNA library and a cDNA encoding human N-CAM. Transfected COS-1 cells were stained with a monoclonal antibody specific for polysialic acid and enriched by fluorescence-activated cell sorting. Sibling selection of recovered plasmids resulted in a cDNA clone that directs the expression of polysialic acid on the cell surface. The deduced amino acid sequence indicates that the polysialyltransferase shares a common sequence motif with other sialyltransferases cloned so far. The polysialyltransferase is, however, distinct by having two clusters of basic amino acids. The amount of the polysialyltransferase transcripts correlates well with the formation of polysialic acid in various human tissues, and is abundant in the fetal brain but not in the adult brain. Moreover, HeLa cells stably expressing polysialic acid and N-CAM promoted neurite outgrowth and sprouting. These results indicate that the cloned polysialyltransferase forms polysialylated, embryonic N-CAM, which is critical for plasticity of neural cells.**

Polysialic acid (PSA) is a developmentally regulated carbohydrate composed of a linear homopolymer of α-2,8-linked sialic acid residues. PSA is mainly attached to the neural cell adhesion molecule (N-CAM). While polysialylated N-CAM is abundant in embryonic tissues, the majority of N-CAM in adult tissues lacks this unique glycan (1–3). Presence of this large negatively charged carbohydrate modulates the adhesive property of N-CAM, and removal of PSA increases binding between N-CAMs (1, 2). During embryonic development, the polysialylated, embryonic N-CAM is restricted to specific cell types where cells are migrating (4, 5), and the removal of PSA from the N-CAM during embryonic development was reported to influence motor-neuron projections (4). Other studies suggest that the polysialylated form of N-CAM also attenuates cell–cell interactions carried by other cell surface receptors (2, 6, 7). Recent studies of N-CAM knockout mice demonstrated defects in spatial learning and memory, due to an anomaly in the olfactory bulb and hippocampus, where PSA is continuously present also in adults (8, 9). These results strongly suggest that polysialylation regulates the function of N-CAM. The polysialylated N-CAM was also found in tumors derived from neuroendocrine and hematopoietic cells (10, 11). It has been suggested that the presence of PSA in tumor cells promotes metastatic migration into specific tissues (11, 12). Due to its presence in tissues undergoing synaptic rearrangement and cell migration, PSA is implicated in reducing N-CAM adhesion and thus perhaps allowing increased neurite outgrowth and cellular mobility.

Despite the fact that PSA apparently plays a critical role in development and oncogenesis, tools available for studying the functions of PSA have been limited to endoneuraminidase (13) and antibodies specific to PSA (14). To overcome this problem, we describe here the isolation of a cDNA encoding the human polysialyltransferase (PST)† by transient expression cloning (15, 16) in COS-1 cells expressing human N-CAM.

EXPERIMENTAL PROCEDURES

Isolation of a Human PST cDNA Clone. CHO cells were found to express PSA by indirect immunofluorescent staining using monoclonal antibody 735 (14). COS-1 cells were negative for PSA even after transfection with a cDNA, pHβAPr-1-neo-NCAM (hereafter pHβA-NCAM), encoding a transmembrane form (140 kDa) of N-CAM (17). COS-1 cells (2.4×10^7 cells) were thus cotransfected with 40 μg of a human fetal brain cDNA library constructed in pcDNAI (Invitrogen) and 40 μg of pHβA-NCAM by Lipofectamine (GIBCO/ BRL). After 48 hr, PSA-positive cells were isolated by fluorescence-activated cell sorting (FACStar, Becton Dickinson) using antibody 735, which reacts with PSA of eight or more residues (18). Plasmid DNA was isolated by the Hirt procedure (19) from the sorted cells and then amplified in the host bacteria MC1061/P3 in the presence of ampicillin and tetracycline. The pcDNAI vector contains *supF* suppressor tRNA, so that MC1061/P3 cells containing pcDNAI are resistant to both ampicillin and tetracycline. In contrast, MC1061/P3 cells having pHβA-NCAM are resistant to ampicillin but not to tetracycline. Because of this difference, only plasmids derived from pcDNAI were rescued and amplified by this procedure, allowing the isolation of plasmids responsible for PSA expression.

These plasmids isolated from the sorted COS-1 cells were subjected to sibling selection and divided into 23 plates. Plasmid DNA prepared from each plate was separately transfected into COS-1 cells together with pHβA-NCAM, and the transfectants were screened by immunofluorescence microscopy using antibody 735, to isolate a single plasmid encoding a human PST cDNA, pcDNAI-PST. The nucleotide sequence was determined by using a Sequenase kit (Amersham) with oligonucleotides synthesized according to the flanking sequence and identified sequences within the insert as described (16).

Northern Blot Analysis of Various Human Tissues. Samples of poly(A)$^+$ RNA from human fetal and adult brains pur-

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Abbreviations: N-CAM, neural cell adhesion molecule; PSA, polysialic acid; PST, polysialyltransferase.
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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L41680).

chased from Clontech were electrophoresed in a 1.2% agarose gel containing 2.2 M formaldehyde and transferred to a nylon filter (Micron Separations, Westboro, MA). Human multiple tissue Northern blots of poly(A)$^+$ RNA were purchased from Clontech and these blots were hybridized with a gel-purified cDNA insert of pcDNAI-PST after labeling with [α-^{32}P]dCTP by random-oligonucleotide priming (Prime-IT II labeling kit; Stratagene) (16).

Detection of PSA in Human Tissues. Surgical specimens from the pathology file of Shinshu University Hospital and Nagano Children's Hospital, Japan, were examined. Fetal tissues were obtained upon therapeutic abortions. All tissue samples were fixed for 24 hr in a cold 4% paraformaldehyde/0.1 M phosphate buffer, pH 7.4, and then embedded in paraffin and sectioned at 3-μm thickness. Deparaffinized and hydrated sections were immersed in absolute methanol containing 0.3% H_2O_2 for 30 min. Immunohistochemical staining with antibody 735 was performed by the avidin–biotin-peroxidase complex method (20) (Vectastain ABC kit; Vector Laboratories) followed by counterstaining with methyl green. A control experiment was performed by omitting antibody 735 from the staining procedure, and no specific staining was found. The samples were examined under a Zeiss Axioplan microscope.

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 Lipofectamine with pcDNAI-PST and pHβA-NCAM or with pHβA-NCAM alone. After selection with G418, clonal cell lines stably expressing both N-CAM and PSA or N-CAM alone were selected by staining with anti-N-CAM antibody (Dako) or with antibody 735. These cells were cultured as monolayers in Lab-Tek chamber slides (Nunc). Sensory neurons were obtained from the dorsal root ganglia of 10-day chicken embryos. Ventral portions of the spinal cords, which contained predominantly motor neurons, were dissected from 6-day chicken embryos. Both groups of neurons, shown to express both N-CAM and PSA (21), were dissociated with trypsin (0.5%), counted, seeded at low density over HeLa cell monolayers, and cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Nerve growth factor (NGF) was included in sensory neuron culture. Neuron–HeLa cell cocultures were grown for 15 hr, then fixed with 4% formaldehyde in phosphate-buffered saline and stained with anti-neurofilament antibody RMO270 (22) followed by fluorescein isothiocyanate-conjugated anti-mouse IgG antibody. Neurite lengths were measured with the JAVA morphometric system (Jandel, Corte Madera, CA) by epifluorescence in a Zeiss Axiovert 405M microscope equipped with a video camera (Optronics International, Chelmsford, MA). The longest neurite of each neuron was measured for 30 neurons within adjacent fields in triplicate experiments. Only neurons whose neurites did not overlap with others were included. The mean neurite lengths and the number of neurite branches per neuron occurring on the substrates were compared by Student t test.

RESULTS

Isolation of a cDNA Clone That Determines the Expression of PSA in COS-1 Cells. COS-1 cells were cotransfected with a human fetal brain cDNA library in pcDNAI and pHβA-NCAM. Plasmid DNA, recovered from anti-PSA antibody 735-positive COS-1 cells, was subjected to sibling selection with sequentially smaller, active pools, identifying a single plasmid, pcDNAI-PST, that directed the expression of PSA at the cell surface as detected by antibodies 735 (Fig. 1*D*) and 12E3 (data not shown), which react with PSA (14, 23). The staining with antibody 735 was abolished by pretreatment with endoneuraminidase, which hydrolyzes PSA (Fig. 1*F*). Untransfected COS-1 cells were not stained by monoclonal antibody M6703, which is specific for trisialosyl residues (24). M6703 weakly but definitely reacted with COS-1 cells transfected with PST and N-CAM cDNAs (data not shown), indicating that PST forms at least trisialosyl or longer polysialosyl residues and that the majority of the synthesized product was likely to be highly polymerized sialic acid.

To confirm that N-CAM contains PSA, HeLa cells stably transfected with N-CAM and PST were subjected to Western blot analysis. N-CAM displayed a smeared band above 140 kDa but was converted to a sharp band at 140 kDa after endoneuraminidase digestion (Fig. 1*G*, compare lane 3 with lane 2), indicating that PSA was attached to N-CAM.

Predicted Amino Acid Sequence of PST. The cDNA insert encoding PST contains an open reading frame predicting a protein of 359 amino acid residues with a molecular mass of 41,279 Da (Fig. 2*A*). A hydropathy plot predicts that this protein has a type II transmembrane topology, having a short cytoplasmic sequence at the amino terminus, followed by the transmembrane domain, and then by the so-called stem region and a large catalytic domain, which presumably reside in the Golgi lumen. This topology has been found in all mammalian glycosyltransferases so far cloned (25). The deduced amino acid sequence revealed that PST has a strong homology with another recently cloned α-2,8-sialyltransferase, GD3 synthase (27.0% identity), which forms a disialosyl structure in glyco-

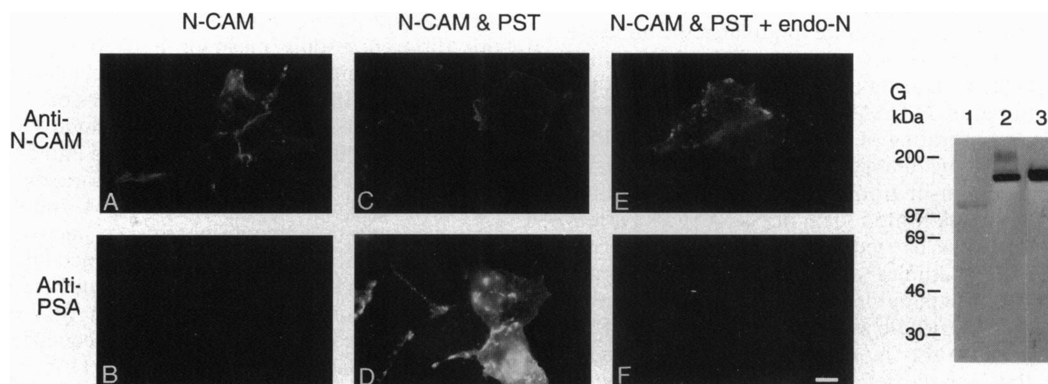

FIG. 1. Expression of PSA by pcDNAI-PST. (*A–F*) COS-1 cells were cotransfected with pcDNAI-PST (*C–F*) or pcDNAI (*A* and *B*) together with pHβA-NCAM. Sixty-four hours after transfection, the cells were fixed and examined by incubation with anti-PSA antibody 735 (*B, D,* and *F*) or anti-N-CAM antibody (Dako) (*A, C,* and *E*), followed by fluorescein isothiocyanate-conjugated anti-mouse IgG. In *E* and *F*, the cells were digested with endoneuraminidase (endo-N) before staining. (Bar = 20 μm.) (*G*) Cell lysate from 2.0×10^6 of the parent HeLa cells (lane 1) or HeLa cells stably expressing both N-CAM and PSA was subjected to SDS/PAGE before (lane 2) or after (lane 3) endoneuraminidase treatment and analyzed by Western blotting with anti-N-CAM antibody (Becton Dickinson) as described (7). The immunoreaction was visualized by the ECL Western blot detection system (Amersham).

A

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-212                                CGCAAACAGGGCGAGAGGTCGCTGGGCAGCGTTCGAGGACCAGAGGGAGCTCGGCCACAGAAGACCCCAGTGATCTGATCCCGGGATCCCGG -121
-120 CTCCAAGCTCTCCTCGCATTTTACAGATTTCACCCCCGCGACTATCTCCCCAAAACGGAGCCTTTATATCAAGAGAAGGTGCGGGAGCTGGGGCAACCAGGACTTTCTCGGGCACCCAAG   -1

   1 ATGCGCTCCATTAGGAAGAGGTGGACGATCTGCACAATAAGTCTGCTCCTGATCTTTTATAAGACAAAAGAAATAGCAAGAACTGAGGAGCACCAGGAGACGCAACTCATCGGAGATGGT  120
   1 M  R  S  I  R  K  R  W  T  I  C  T  I  S  L  L  L  I  F  Y  K  T  K  E  I  A  R  T  E  E  H  Q  E  T  Q  L  I  G  D  G    40

 121 GAATTGTCTTTGAGTCGGTCACTTGTCAATAGCTCTGATAAAATCATTCGAAAGGCTGGCTCTTCAATCTTCCAGCACAATGTAGAAGGTTGGAAAATCAATTCCTCTTTGGTCCTAGAG  240
  41 E  L  S  L  S  R  S  L  V  N  S  S  D  K  I  I  R  K  A  G  S  S  I  F  Q  H  N  V  E  G  W  K  I  N  S  S  L  V  L  E    80
                             *                                                                       *
 241 ATAAGGAAGAACATACTTCGTTTCTTAGATGCAGAACGAGATGTGTCAGTGGTCAAGAGCAGTTTTAAGCCTGGTGATGTCATACACTATGTGCTTGACAGGCGCCGGACACTAAACATT  360
  81 I  R  K  N  I  L  R  F  L  D  A  E  R  D  V  S  V  V  K  S  S  F  K  P  G  D  V  I  H  Y  V  L  D  R  R  R  T  L  N  I   120
                                                                                                                      *
 361 TCTCATGATCTACATAGCCTCCTACCTGAAGTTTCACCAATGAAGAATCGCAGGTTTAAGACCTGTGCAGTTGTTGGAAATTCTGGCATTCTGTTAGACAGTGAATGTGGAAAGGAGATT  480
 121 S  H  D  L  H  S  L  L  P  E  V  S  P  M  K  N  R  R  F  K  T  C  A  V  V  G  N  S  G  I  L  L  D  S  E  C  G  K  E  I   160

 481 GACAGTCACAATTTTGTAATAAGGTGTAATCTAGCTCCTGTGGTGGAGTTTGCTGCAGATGTGGGAACTAAATCAGATTTTATTACCATGAATCCATCAGTTGTACAAAGAGCATTTGGA  600
 161 D  S  H  N  F  V  I  R  C  N  L  A  P  V  V  E  F  A  A  D  V  G  T  K  S  D  F  I  T  M  N  P  S  V  V  Q  R  A  F  G   200

 601 GGCTTTCGAAATGAGAGTGACAGAGAAAAATTTGTGCATAGACTTTCCATGCTGAATGACAGTGTCCTTTGGATTCCTGCTTTCATGGTCAAAGGAGGAGAGAAGCACGTGGAGTGGGTT  720
 201 G  F  R  N  E  S  D  R  E  K  F  V  H  R  L  S  M  L  N  D  S  V  L  W  I  P  A  F  M  V  K  G  G  E  K  H  V  E  W  V   240
              *                                            *
 721 AATGCATTAATCCTTAAGAATAAACTGAAAGTGCGAACTGCCTATCCGTCATTGAGACTTATTCATGCTGTCAGAGGTTACTGGCTGACCAACAAAGTTCCTATCAAAAGACCCAGCACA  840
 241 N  A  L  I  L  K  N  K  L  K  V  R  T  A  Y  P  S  L  R  L  I  H  A  V  R  G  Y  W  L  T  N  K  V  P  I  K  R  P  S  T   280

 841 GGTCTTCTCATGTATACACTTGCCACAAGATTCTGTGATGAAATTCACCTGTATGGATTCTGGCCCTTCCCTAAGGATTTAAATGGAAAAGCGGTCAAATATCATTATTATGATGACTTA  960
 281 G  L  L  M  Y  T  L  A  T  R  F  C  D  E  I  H  L  Y  G  F  W  P  F  P  K  D  L  N  G  K  A  V  K  Y  H  Y  Y  D  D  L   320

 961 AAATATAGGTACTTTTCCAATGCAAGCCCTCACAGAATGCCATTAGAATTCAAAACATTAAATGTGCTACATAATAGAGGAGCTCTAAAACTGACAACAGGAAAGTGTGTAAAGCAATAA 1080
 321 K  Y  R  Y  F  S  N  A  S  P  H  R  M  P  L  E  F  K  T  L  N  V  L  H  N  R  G  A  L  K  L  T  T  G  K  C  V  K  Q end  359
                       *
1081 AGCACATTTTGAAACAAACAATATGCACTTCTTTTCTGAGATGCTTCCGAAGATTTGAAAATAGGATCCAAAACACGGCTGGGTTTCAGCATCCACCAATGAACTGAAAGGTGAATAAAG 1200
1201 GACGTTCATGAGAAATCGACTACCAGCTGATGAAATACCTGCAAAGTGCTCTAAAAATTAAATATTTTGACTTTAAGGGTCCTAGTAAGTGCCACTTCCACTAAGAATACAGTTTGAATG 1320
1321 TATAATCAGTAGTGTTTACAAGATCCAACAGTGCACTCATCATTAGTTAACAAAGCAAATATGTTCATCACTGTCAGGCTGCCCACAGCAACACCAAGCATATTAGAAGAGGAACCCCAG 1440
1441 GAACGCAACTCAGACCTTGGGAAATTAAACCATCCTTGTCAGCAGAAGCCAAGATGGAAGCAGTTTGAGCAATGAAATCCGTAAGATTAAACAACTCAAGTAAATGCTTCAGTCAGGACT 1560
1561 CTGAGTCTGATCATGAATTTTATGTTTTAATTTATGTTTTTTTTTTTGTCTTCTGGAATCTCTTTTGGTTTGGATATTGGGATGCTTAGAAATCCTTTCTGAGATGCATATGAGTGAGGA 1680
1681 AA                                                                                                                       1682
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B

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PST     101 SFKPGDVIHYVLDRRRTLNISHDLHSLLPEVSPMKNRRFKTCAVVGNSGILLDSECGKEIDSHNFVIRCNLAPV.VEFAADVGTKSDFITM 190
STX1    116 TL----I---IF--DS-M-V-QN-YE---RT--L--KH-Q---I-----V--N-G--Q---T-S----------.Q-Y-R---L-T-LV-- 205
GD3ST    83 NSPM-KSMW-DGEFLYSFT-DNSTY--F-QAT-FQ.LPL-K------G---KK-G--RQ--EA---M----P-LSS-YTK------Q-V-A 172
α2,3ST  104 ER--NNLSDT-KE.LFR-.VPGNVDPM-NK..RL..VGCRR--------N-K--SY-P-----D--L-M-K--T.-G-E-----RTTHHLV 187
                                                   <--              Sialylmotif L             -->
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FIG. 2. (*A*) Full-length nucleotide and amino acid sequences of PST. The signal/membrane-anchoring domain is underlined, and sialyl motif L (residues 141–185) and sialyl motif S (residues 278–300) are shown by double underlining. Potential N-glycosylation sites are marked by asterisks. (*B*) Amino acid sequences surrounding sialylmotif L (residues 101–190 in PST) are shown for PST, STX1, GD3 sialyltransferase, and α2,3-sialyltransferase. Amino acid residues identical to PST are shown by hyphens. Two clusters of basic amino acid residues are underlined.

lipids (26, 27). It has also homology with STX1 (58.2%), whose specificity is unknown (28). Much less homology (9.7%) was found with Galβ1→3GalNAc:α-2,3-sialyltransferase (29) (Fig. 2*B*). Among these four glycosyltransferases the highest homology is found in a portion of the catalytic domain, named sialylmotif L, which is shared by all sialyltransferases cloned to date (27, 30, 31). This motif was identified as the binding site to the donor substrate, CMP-NeuNAc (31).

Expression of PST mRNA in Human Tissues. Northern blots of poly(A)$^+$ RNA derived from various human tissues were examined (Fig. 3). A band of 6.5 kb was detected in the poly(A)$^+$ RNA isolated from fetal brain but was barely detectable in the same amount of poly(A)$^+$ RNA from adult brain. The signal from the fetal lung and fetal kidney was strong but that from the fetal liver was not. In adult tissues, the PST transcript was detected in the heart, spleen, and thymus,

FIG. 3. Northern blot analysis of PST in various human tissues. Each lane contained 2 μg of poly(A)$^+$ RNA. The blot for the first two lanes at the far left was made separately and apparently contained less poly(A)$^+$ RNA than the human multiple tissue Northern blots. The same blots were probed by ^{32}P-labeled PST cDNA (PST) or β-actin cDNA (β-actin).

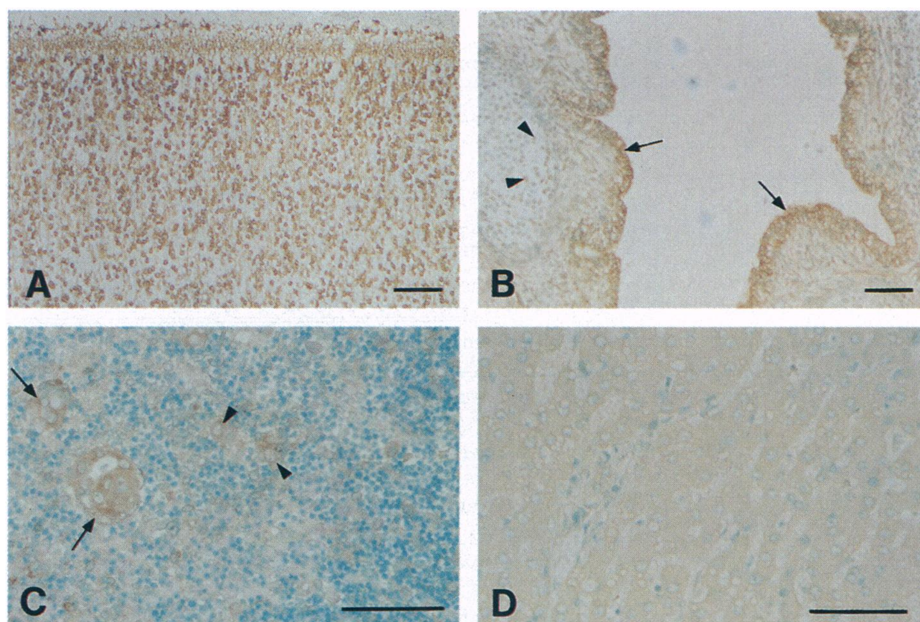

FIG. 4. Expression of PSA in various human tissues detected by monoclonal antibody 735. Paraffin-embedded sections of human fetal, newborn, and adult tissues were stained with antibody 735 by the avidin–biotin-peroxidase complex method. The antibody reacted with neurons in the fetal cerebral cortex (*A*), bronchial epithelia (arrows) and some mesenchymal cells (arrowheads) in cartilage of the fetal lung (*B*), and Hassall's corpuscles (arrows) and epithelial cells (arrowheads) of the newborn thymus (*C*). The hepatocytes in the adult liver did not show any staining (*D*). (Bars = 100 μm.)

and moderately in the brain, placenta, lung, large and small intestines, and peripheral blood leukocytes (Fig. 3). In some tissues, a weak band of 3.7 kb was also detected.

Among various parts of the adult brain, a substantial amount of PST mRNA was detected in thalamus, subthalamic nucleus, substantia nigra, and cerebral cortex (Fig. 3). In general, PST is expressed more in forebrain derivatives than in midbrain, hindbrain, and caudal neural tube derivatives.

Immunohistochemical Detection of PSA in Human Tissues. To determine whether or not the expression of PST mRNA is solely responsible for the expression of PSA, paraffin sections of various human tissues were examined by immunostaining with antibody 735. PSA was strongly expressed in neurons of the fetal cerebral cortex, bronchial epithelia of the fetal lung, and Hassall's corpuscles and epithelial cells of the thymus (Fig. 4 *A–C*). Other tissues, including the heart and the small and large intestines, that expressed PST mRNA also expressed PSA. In contrast, those tissues lacking PST mRNA—for example, hepatocytes—were negative also for PSA staining (Fig. 4*D*). The presence of PSA in the fetal kidney represents an oncodevelopmental antigen, since it is also expressed in Wilms tumor but not in the adult kidney (32).

For Northern blots almost identical to those described above, it was reported that the human STX transcript was not detected in the fetal lung and adult intestines (28), where PSA was detected in the present and previous studies (33). Transfection of COS-1 cells with GD3 synthase and N-CAM cDNAs resulted in no staining of PSA (data not shown). These results strongly suggest that PST is solely responsible for the biosynthesis of PSA (see *Discussion*).

Neurite Outgrowth on HeLa Cells Expressing PSA and N-CAM. Neural cell migration and axon outgrowth are influenced by PSA expression on neural cells (4, 21, 34). Neurite outgrowth can be observed on substrate cultures of 3T3 cells transfected to express N-CAM (21). To test how PSA expression on living substrates influences neurite outgrowth, we expressed N-CAM alone or N-CAM and PSA on substrate HeLa cells and measured neurite outgrowth on these cells. Neurons derived from dorsal root ganglion exhibited modest neurite outgrowth on confluent monolayers of either untrans-

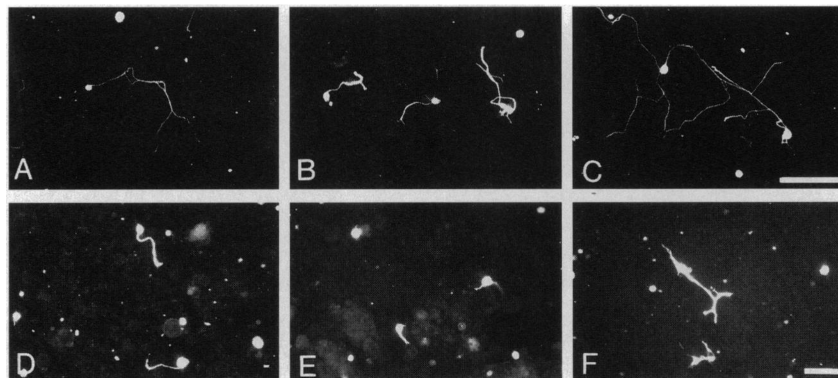

FIG. 5. Neurite outgrowth on confluent untransfected HeLa cells (*A* and *D*), HeLa cells stably expressing N-CAM alone (*B* and *E*), and HeLa cells expressing N-CAM and PSA (*C* and *F*). Neurons from chicken embryonic day 10 dorsal root ganglia (*A–C*) or embryonic day 6 ventral spinal cord (*D–F*) were seeded on the HeLa cells and cultured for 15 hr. The neurites were visualized by immunofluorescent staining for neurofilament. (Bars = 100 μm.)

fected HeLa cells (length, 196.3 ± 16.0 μm; Fig. 5*A*) or HeLa cells expressing N-CAM (171.3 ± 14.0 μm; Fig. 5*B*). In contrast, neurons cultured on HeLa cells expressing polysialylated N-CAM grew neurites that were significantly longer (253.6 ± 21.0 μm; $P < 0.05$; Fig. 5*C*) and exhibited more branching (mean, 4.0 branches per neuron) than those grown on N-CAM alone (2.3 branches per neuron). Similar results were obtained with neurons isolated from ventral portions of the spinal cord (compare Fig. 5*F* with Fig. 5 *D* and *E*). The results clearly show that PSA on living cell substrates is a critical regulator of neurite outgrowth.

DISCUSSION

This study describes the isolation of a cDNA clone encoding PST, the enzyme responsible for the formation of α-2,8-linked sialic acid repeats, $(\rightarrow 8\text{NeuNAc}\alpha 2\rightarrow)_n$. For this cloning, COS-1 cells were cotransfected with a cDNA encoding human N-CAM, which is a substrate for PST. Upon introduction of a mixture of plasmids obtained after the first cell sorting, we could detect PSA expression only when N-CAM cDNA was also introduced into COS-1 cells. It was thus critical to express N-CAM in the recipient cells. In this procedure we managed to isolate plasmid DNA only from the cDNA library, by utilizing the difference in antibiotic resistance between two plasmids derived from the cDNA library and plasmids containing N-CAM cDNA. This is a modified and improved method over our previous cloning protocol using CHO cells stably expressing leukosialin, which is a preferred substrate for core 2 β-1,6-*N*-acetylglucosaminyltransferase (15).

The predicted amino acid sequence of PST has several unique characteristics. In particular, PST has two basic amino acid clusters upstream from the amino terminus of sialylmotif L—namely, RRR in residues 114–116 and RRFK in residues 137–140. These clusters are either absent or incomplete in the corresponding sequences of GD3 synthase and STX (Fig. 2*B*). PSA can be composed of as many as 55 sialic acid residues in a chain (12). These basic amino acid clusters in PST may be critical for PST's binding to the acceptor containing multiple, negatively charged sialic acid residues. In fact, the recently cloned hamster PST has almost identical amino acid sequence with human PST (97.8%), including these basic amino acid clusters (35). The cloned PST was found to catalyze the polymerization of sialic acids. The present study, however, does not exclude the possibility that the first disialosyl linkage is formed by another, hitherto unknown enzyme, and PST adds sialic acid residues to this disialosyl group (see also ref. 36). Further studies on the acceptor specificity of PST will be needed to address this matter.

Unexpectedly, PST and PSA are also present in nonneural tissues such as thymus, lung, and peripheral blood leukocytes (Figs. 3 and 4). These results suggest that PSA is synthesized also in nonneural cells and might be attached to glycoproteins other than N-CAM, such as the α subunit of sodium channels (37). In fact, our preliminary experiments using the same Northern blots demonstrated that N-CAM transcripts were barely detected in the fetal lung and kidney, where PST and PSA are present in great quantity. Neural cell development and directional axon growth are guided by the substratum, often neuroepithelial cells. The present study strongly suggests a role for PSA in neurite outgrowth on neuroepithelial cells. This view is supported by previous reports that neuroepithelial cells express both N-CAM and PSA (1, 2). Moreover, the present study suggests that PSA may play an additional role in neuronal regeneration by facilitating neurite outgrowth and branching.

Cell–cell interaction in neural development is mediated not only by N-CAM but also by other adhesive molecules. The addition of PSA to N-CAM and other adhesive molecules might attenuate those cell–cell interactions during development. The PST cDNA obtained in the present study will be a powerful molecular tool to manipulate the expression level of PSA in specific cell types, allowing us to dissect the intricate and complex processes of cell–cell interactions during development.

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