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Molecular Cloning and Expression of an *N*-Acetylgalactosamine-4-*O*-sulfotransferase That Transfers Sulfate to Terminal and Non-terminal β1,4-Linked *N*-Acetylgalactosamine*

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We have identified and characterized an *N*-acetylgalactosamine-4-*O*-sulfotransferase designated GalNAc-4-ST2 (GenBank™ accession number AF332472) based on its homology to HNK-1 sulfotransferase (HNK-1 ST). The cDNA predicts an open reading frame encoding a type II membrane protein of 443 amino acids with a 12-amino acid cytoplasmic domain, a 23-amino acid transmembrane domain, and a 408-amino acid luminal domain containing four potential *N*-linked glycosylation sites. GalNAc-4-ST2 displays a high degree of amino acid sequence identity with GalNAc-4-ST1 (46%), HNK-1 ST (23%), chondroitin 4-*O*-sulfotransferase-1 (C4ST-1) (27%), and chondroitin 4-*O*-sulfotransferase-2 (C4ST-2) (24%). GalNAc-4-ST2 transfers sulfate to the C-4 hydroxyl of terminal β1,4-linked GalNAc in the sequence GalNAc-β1,4GlcNAcβ-R found on *N*-linked oligosaccharides and nonterminal β1,4-linked GalNAc in chondroitin and dermatan. The translated region of GalNAc-4-ST2 is encoded by five exons located on human chromosome 18q11.2. Northern blot analysis reveals a 2.1-kilobase transcript. GalNAc-4-ST2 message is most highly expressed in trachea and to a lesser extent in heart, liver, pancreas, salivary gland, and testis. The I.M.A.G.E. cDNA clone 49547 contains a putative GalNAc-4-ST2 splice form with an open reading frame encoding a protein of 358 amino acids that lacks the transmembrane domain and the stem region. This form of GalNAc-4-ST2 is not retained by transfected cells and is active against chondroitin but not terminal β1,4-linked GalNAc. Thus, as with GalNAc-4-ST1, sequences N-terminal to the catalytic domain contribute to the specificity of GalNAc-4-ST2 toward terminal β1,4-linked GalNAc.

Sulfated carbohydrate structures have been shown to play important roles in a large number of different molecular interactions including symbiotic interactions between plants and nitrogen-fixing bacteria (1), homing of lymphocytes to lymph nodes (2), control of the circulatory half-life of the glycoprotein hormones LH[1] and TSH (3, 4), binding of growth factors by proteoglycans (5–7), and triggering preferential neurite outgrowth (8). HNK-1, an example of such a sulfated glycan, has the structure SO_4-3-GlcUAβ1,3Galβ1,4GlcNAc-R and is found at the nonreducing termini of glycoprotein and glycolipid oligosaccharides (9, 10). The structure was first identified with monoclonal antibodies as an epitope on human natural killer cells (11). The HNK-1 epitope is a hallmark of many neural recognition molecules and displays phylogenetic conservation (12), highlighting its functional importance. It is expressed in the central and peripheral nervous systems during development and regeneration (13, 14) and has recently been recognized to be a crucial player in synaptic plasticity involving inhibitory interneurons in the hippocampus (15). HNK-1 is also a predominant autoantigen in demyelinating diseases of the peripheral nervous system (16).

Expression cloning of the rat and human sulfotransferases responsible for synthesis of the HNK-1 epitope revealed the presence of two sequence motifs that are associated with 3′-phosphoadenosyl-5′-phosphosulfonate (PAPS) binding (17, 18) and are common among all sulfotransferases cloned to date (19–21). Despite the presence of these motifs, HNK-1 sulfotransferase shows only limited similarity to other sulfotransferases outside of the PAPS-binding regions. Nonetheless, three additional sulfotransferases, chondroitin 4-*O*-sulfotransferase 1 (C4ST-1) (22, 23), chondroitin 4-*O*-sulfotransferase 2 (C4ST-2) (23), and *N*-acetylgalactosamine-4-*O*-sulfotransferase 1 (GalNAc-4-ST1) (24, 25), were identified based on their homology to the catalytic C-terminal domain of HNK-1 ST. C4ST-1 and C4ST-2 (Fig. 2) show 25 and 22% identity to HNK-1 ST, respectively, whereas GalNAc-4-ST1 is 23% identical to HNK-1 ST. In addition to the motifs associated with binding PAPS, HNK-1 ST, C4ST-1, C4ST-2, and GalNAc-4-ST1 have three additional motifs of unknown function that are located C-terminal to the PAPS-binding motifs. While homolog-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF332472 and AF332473.

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[1] The abbreviations used are: LH, lutropin; TSH, thyrotropin; HNK-1 ST, HNK-1 sulfotransferase;, C4ST, chondroitin 4-*O*-sulfotransferase; GalNAc-4-ST, *N*-acetylgalactosamine-4-*O*-sulfotransferase; MCO, $(CH_2)_8$-$COOCH_3$; bp, base pair; PAPS, 3′-phospho-adenosine-5′-phosphosulfonate; GlcNAcβ-R, R is the underlying saccharide structure; ORF, open reading frame; htgs, high throughput genomic sequences; CHO, Chinese hamster ovary; hCG, human chorionic gonadotrophin; PNGase F, peptide:*N*-glycosidase F; ΔDi-4S, D-gluco-4-enepyranosideβ1,3GalNAc-4-SO_4; HPLC, high pressure liquid chromatography; PSB, phosphosulfonate-binding site; PB, phosphate-binding site.

ous, these sulfotransferases have distinct saccharide specificities. HNK-1 ST transfers sulfate to the C-3 hydroxyl of terminal β1,3-linked GlcUA in the sequence GlcUAβ1,3Galβ1,4GlcNAcβ-R (17, 18). C4ST-1 and C4ST-2 transfer sulfate to the C-4 hydroxyl of nonterminal β1,4-linked GalNAc in chondroitin and dermatan (22, 23). GalNAc-4-ST1 transfers sulfate to the C-4 hydroxyl of terminal β1,4-linked GalNAc in the sequence GalNAc-β1,4GlcNAcβ-R that is found on *N*-linked oligosaccharides (24, 25).

We have identified and characterized a fourth sulfotransferase that is homologous to HNK-1 ST with 23% identical amino acids. *N*-Acetylgalactosamine-4-*O*-sulfotransferase 2 (GalNAc-4-ST2) transfers sulfate to the C-4 hydroxyl of terminal β1,4-linked GalNAc in the sequence GalNAc-β1,4GlcNAcβ-R and to the C-4 hydroxyl of internal β1,4-linked GalNAc moieties in chondroitin and dermatan. We describe the properties of this novel sulfotransferase that differ in its specificity and expression from other GalNAc-specific sulfotransferases.

EXPERIMENTAL PROCEDURES

Materials—[α-^{32}P]dCTP and Megaprime labeling kit were purchased from Amersham Pharmacia Biotech and human Multiple Tissue Northern blots (MTN®) and human Multiple Tissue Expression (MTE™) arrays from CLONTECH Laboratories, Inc. Bovine LH was provided by the NIDDK National Hormone and Pituitary Program, Dr. A. F. Parlow.

Molecular Cloning of a Human cDNA Homologous to HNK-1 Sulfotransferase—A human EST derived from human infant brain (GenBank™ accession number H15485) with high sequence similarity to rat HNK-1 ST (17) was identified using BLASTN and TBLASTN algorithms (26) against the data bases of GenBank™, EMBL, and DDBJ EST Divisions at the National Center for Biotechnology Information (National Institutes of Health, Bethesda). The insert of the corresponding I.M.A.G.E. Consortium (LLNL) cDNA clone, ID 49547 (IMAGp951B2016 obtained from RZPD, Berlin, Germany) (27), was sequenced and found to contain a complete open reading frame encoding for a 358-amino acid protein showing significant sequence similarity to rat and human HNK-1 sulfotransferases. This sequence was designated as GalNAc-4-ST2(Met-86) (GenBank™ accession number AF332473).

The forward primer 5′-GGG AGA GTG GAG AAG AGA AGA GAA C-3′ and the reverse primer 5′-AAG CCA ATC CAT TTA GTA CCA TCA GA-3′ were used to amplify a 595-bp fragment (nucleotides at position 211–805 in the GalNAc-4-ST2 cDNA) including the putative start codon from first-strand cDNA produced from 100 ng of total RNA obtained from a human glioma and the human kidney 293 cell line using Omniscript® reverse transcriptase (Qiagen). An additional 38 bps (designated exon 4 in Fig. 3) were present in this product when compared with GalNAc-4-ST2(Met-86) and shifted the stop codons located 5′ out of the reading frame of the putative translation start site. Similarity searches identified a bovine EST (GenBank™ accession number BE724107) with sequence homology to the 5′ end of the amplified fragment, allowing us to predict a chimeric sequence from the human and bovine cDNAs that would contain an open reading frame encoding for a putative 443-amino acid protein. Human genomic clones (GenBank™ accession numbers AC009872, AC023575, and AC010854) were analyzed with FGENESH (28), and the predicted exons were evaluated using the chimeric GalNAc-4-ST2 cDNA. Primer pairs derived from exonic sequences were designed and used to amplify the full-length GalNAc-4 ST2 sequence shown in Fig. 1*A* (GenBank™ accession number AF332472) from both human embryonic kidney 293 cell line and glioma cDNA.

In Silico Analysis of the Human GalNAc-4-ST2 Locus—A BLASTN search with the GalNAc-4-ST2 and GalNAc-4-ST2(Met-86) sequences against the High Throughput Genomic Sequences (htgs) data base retrieved several entries (GenBank™ accession numbers AC012269, AP001272, AC032032, AC009872, and AC023575) annotated to be derived from chromosome 18q11.2. The genomic clone with the GenBank™ accession number AC010854 was retrieved with a BLASTN search against the nonredundant data base. The organization of the *GalNAc-4-ST2* gene shown in Fig. 3 was deduced by comparing the cDNA of GalNAc-4-ST2 with these genomic sequences.

Construction of pcDNA3.1-GalNAc-4-ST2(Met-86) and pcDNA3.1-GalNAc-4-ST2—The complete open reading frame of GalNAc-4-ST2 was amplified from cDNA prepared from human kidney 293 cells by polymerase chain reaction (PCR) using the following: 1) the 5′-specific primer 5′-ATA TGG ATC CGC CAC CAT GCA GCC ATC TGA AAT G-3′ containing a *Bam*HI site, the consensus Kozak sequence GCCACC (29) and a start codon; and 2) the 3′-specific primer 5′-ATA TTC TAG ACT ACA AAA ATG GAG TTG TAT AAT TAA-3′ containing a stop codon and an *Xba*I site. The open reading frame of GalNAc-4-ST2(Met-86) was amplified by PCR from the human I.M.A.G.E. IMAGE clone ID 49547 clone ID 49547 using the 5′-specific primer 5′-GCG GAT CCG CCA CCA TGC CTG AGG ATG TAC GAG AA-3′ (contains an *Bam*HI site, consensus Kozak sequence GCCACC, and a start codon) and the same 3′ primer that was used to obtain GalNAc-4-ST2, above. PCRs were carried out using Pfu*Turbo*® polymerase (Stratagene) with 35 cycles of a reaction consisting of 45 s denaturation at 95 °C, 60 s annealing at 55 °C, and 60 s of elongation at 72 °C. The PCR fragments had the expected lengths of 1332 and 1077 bp, respectively, and were directionally subcloned into the eukaryotic expression vector pcDNA3.1 (Invitrogen).

Transient Expression of GalNAc-4-ST2 and GalNAc-4-ST2(Met-86)—CHO/Tag cells were transfected with 13 μg of pcDNA3.1-GalNAc-4-ST2, pcDNA3.1-C4ST-1, pcDNA3.1-GalNAc-4-ST2(Met-86), or pcDNA3.1 and 35 μg of LipofectAMINE (Life Technologies, Inc.) in serum-free medium for 6 h per the manufacturer's protocol. Sixty hours after transfection, the cells and medium were collected separately for analysis. Cells were lysed with 200 μl of 20 mM HEPES buffer, pH 7.4, 5 mM $MgCl_2$, 175 mM KCl, 2% Triton X-100, protease inhibitors (23 millitrypsin inhibitor units of aprotinin and 4 μg each of leupeptin, antipain, pepstatin, and chymostatin) per 100-mm diameter culture plate. The homogenate was mixed by rotation for 1 h and sedimented at 12,000 × *g* for 20 min. The supernatant was designated as the cell extract. The culture medium was pooled, sedimented at 12,000 × *g* for 20 min, and the supernatant adjusted to a final concentration of 20 mM HEPES, pH 7.4, and protease inhibitors were added as noted above.

Sulfotransferase Assays—GalNAc-4-sulfotransferase reactions (50 μl) were carried out as described (30) at 28 °C for 15 h. Each reaction contained 15 mM HEPES, pH 7.4, 1% Triton X-100, 40 mM 2-mercaptoethanol, 10 mM NaF, 1 mM ATP, 4 mM magnesium acetate, 13% glycerol, protease inhibitors, 2 μM unlabeled PAPS, 1×10^6 cpm [^{35}S]PAPS, 20 μM GalNAcβ1,4 GlcNAcβ1,2Manα-MCO, and enzyme. [^{35}S]SO_4-GalNAcβ1,4 GlcNAcβ1,2Manα-MCO was separated from [^{35}S]PAPS and labeled endogenous acceptors by passage over a Sep-Pak C_{18} cartridge (Waters) (30). Control reactions were done in the absence of substrate or enzyme. Chondroitin and dermatan GalNAc-4-sulfotransferase activities were determined using a previously described assay (23) and 50 μg of desulfated chondroitin (Seikagaku America, Inc.) or desulfated dermatan (31) as acceptor. HNK-1-sulfotransferase activity was assayed using the acceptor GlcUAβ1,3Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1,$C_2H_4NHCOCF_3$ as described previously (17) except that the reactions were carried out for 15 h.

Product Characterization—[^{35}S]SO_4-GalNAcβ1,4GlcNAcβ1,2Manα-MCO obtained by incubation with GalNAc-4-ST2 or GalNAc-4-ST2(Met-86) for 16 h at 28 °C was isolated on a Sep-Pak C_{18} (Waters) and characterized as described previously with a CarboPak PA1 column (Dionex) (30). Sulfated monosaccharides were released from the [^{35}S]SO_4-GalNAcβ1,4GlcNAcβ1,2Manα-MCO product by mild acid hydrolysis and separated from intact oligosaccharides and other degradation products by gel filtration on Sephadex G-10 in 100 mM NH_4HCO_3 prior to analysis by HPLC on CarboPak PA1 column (Dionex) as described (30).

The [^{35}S]SO_4-labeled chondroitin and dermatan products were digested with 30 milliunits of chondroitinase ABC (Seikagaku America, Inc.) in 100 mM Tris-HCl, pH 8.0, for 16 h at 37 °C. The completeness of digestion was determined by gel filtration on Sephadex G-25 in 100 mM NH_4HCO_3. The released [^{35}S]SO_4-labeled saccharides were characterized by HPLC using a 4.6 × 250 mm MicroPak AX-5 column (Varian Associates). Saccharides were separated using a linear gradient of 10 mM KH_2PO_4 to 450 mM KH_2PO_4 over 40 min at 1.0 ml per min (32). Standards were detected by their absorbance at 210 or 234 nm, and fractions were collected at 0.5-min intervals for determination of radioactivity. In excess of 90% of the [^{35}S]SO_4 label was recovered.

Sulfation of Glycoprotein Acceptors—Glycoproteins were tested as substrates for GalNAc-4-ST2 using the conditions described above for oligosaccharide acceptors with the following modifications. No unlabeled PAPS was added to the [^{35}S]PAPS. Each 50-μl reaction contained 3 μg of purified bovine parotid carbonic anhydrase VI (CA-VI) (33), bovine LH, enzymatically desulfated bovine LH, or asialo human chorionic gonadotrophin (hCG). After 16 h at 28 °C, duplicate reactions were either stopped by addition of an equal volume of sample buffer (10% glycerol, 5% 2-mercaptoethanol, 2% SDS, 0.003% bromphenol blue, and 62.5 mM Tris, pH 6.8) or were digested with 34 microunits of peptide:*N*-glycosidase F (PNGase F) as described (3) prior to addition of

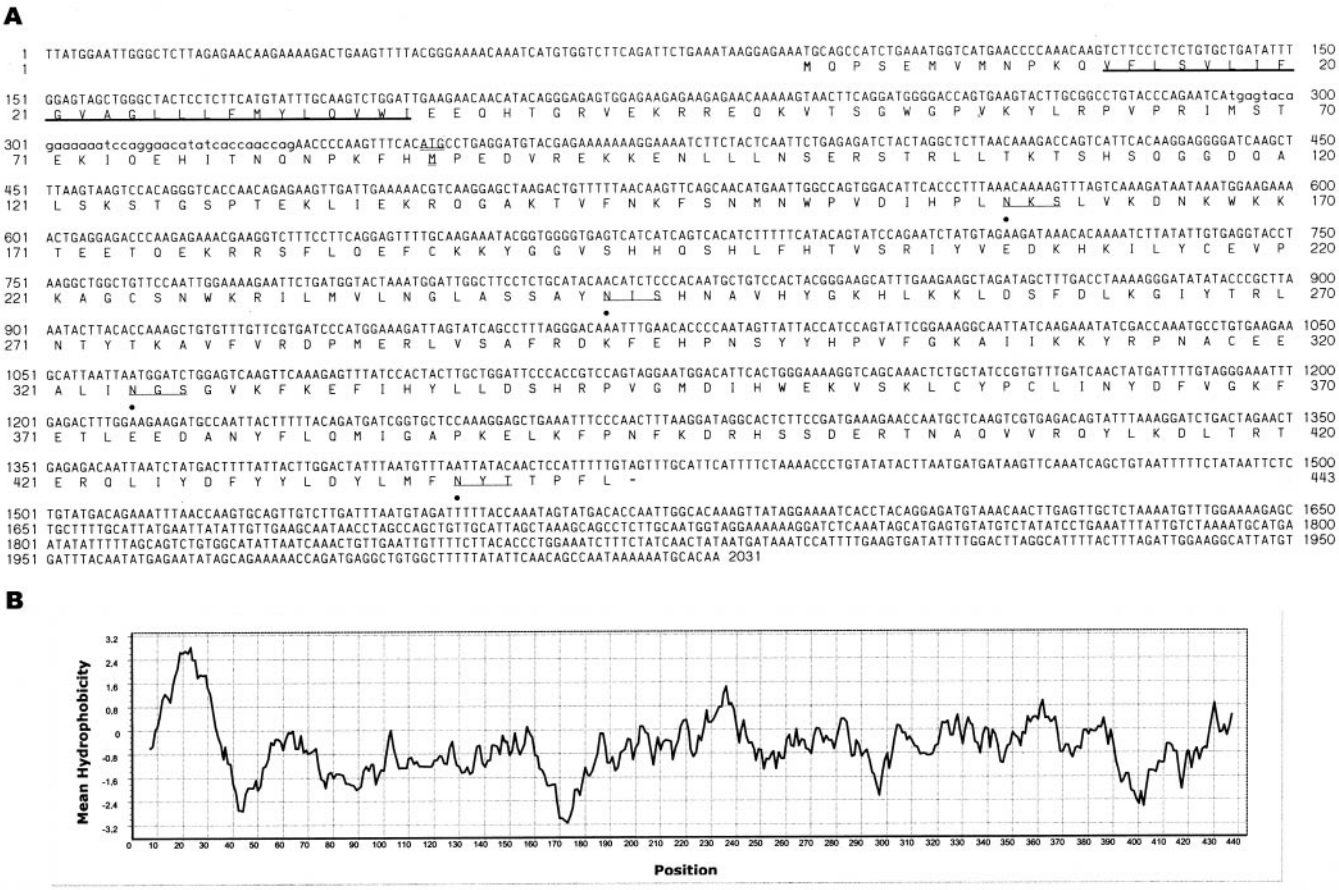


FIG. 1. **Nucleotide and deduced amino acid sequence of human GalNAc-4-ST2 cDNA (GenBank™ accession number AF332472).** *A,* the predicted amino acid sequence of GalNAc-4-ST2 is denoted by *capital letters* below the nucleotide sequence. The single membrane-spanning domain predicted by the hidden Markov model-based algorithm TMHMM (34) and four potential *N*-linked glycosylation sites are indicated by the *bold underline* and by the underline with a ● below the glycosylated Asn, respectively. The truncated form of GalNAc-4-ST2, GalNAc-4-ST2(Met-86), is initiated at the AUG (*double underline*) encoding Met-86 (*double underline*) of GalNAc-4-ST2. The nucleotide sequence derived from exon 4 is shown in *lowercase letters. B*, shows the hydrophobicity plot for GalNAc-4-ST2.

sample buffer. The [^{35}S]SO_4-labeled proteins were separated by SDS-polyacrylamide gel electrophoresis (12% acrylamide) and detected by autoradiography.

Northern Blot and Expression Array Analysis—Human Multiple Tissue Northern (MTN®) blots and Human Multiple Tissue Expression (MTE™) arrays were purchased from CLONTECH Laboratories, Inc. They were hybridized with 5–15 × 10^6 cpm of a GalNAc-4-ST2 specific α-^{32}P-labeled cDNA probe and washed according to the manufacturer's specifications. The membranes were exposed to Biomax MS films (Eastman Kodak Co.) for 2–6 days at −80 °C with intensifying screens. The 503-bp probe used for the labeling reactions corresponds to nucleotide 745–1247 of the GalNac-4-ST2 cDNA (GenBank™ accession number AF332472).

RESULTS

Identification of a Human cDNA Related to HNK-1 ST and GalNAc-4-ST1—We recently identified and characterized a human GalNAc-4-*O*-sulfotransferase, GalNAc-4-ST1 (24), based on its homology to the HNK-1 ST that transfers sulfate to GlcUAβ1,3Galβ1,4GlcNAc-R to produce the HNK-1 epitope SO_4-3-GlcUAβ1,3Galβ1,4GlcNAc-R. In contrast to HNK-1 ST, GalNAc-4-ST1 transfers sulfate to the C-4 hydroxyl of β1,4-linked GalNAc on *N*-linked oligosaccharides such as those found on the glycoprotein hormones LH and TSH (24, 25). Further screening of the EST database at GenBank™, National Center for Biotechnology Information, using the deduced amino acid sequence of the catalytic domain of rat HNK-1 ST (GenBank™ accession number AF022729) identified the human EST H15485. The insert of the corresponding I.M.A.G.E. Consortium (LLNL) cDNA clone ID 49547 contains an open reading frame of 1077 bp with a single in-frame ATG codon at the 5′ end preceded by two in-frame stop codons. The open reading frame predicts a protein of 358 amino acid residues with a molecular mass of 42.1 kDa and four potential *N*-linked glycosylation sites. The predicted protein sequence is closely related to GalNAc-4-ST1 with 44% identical residues. However, no membrane-spanning regions are predicted to be present by hidden Markov model based algorithm TMHMM (34). This suggests the presumptive sulfotransferase, designated as GalNAc-4-ST2(Met-86) (GenBank™ accession number AF332473), is synthesized as a soluble glycoprotein.

Transcripts derived from a human embryonic kidney 293 cell line and glioma cDNA contained an additional 38-bp sequence, later identified as exon 4 (see below), that was not present in GalNAc-4-ST2(Met-86). A virtual cDNA consisting of this amplified sequence and an EST (GenBank™ accession number BE724107) from the 5′ end of the coding region of the bovine orthologue was deduced and used to analyze genomic clones representing the human GalNAc-4-ST2 locus (see below). Primer pairs were designed for the predicted open reading frame and used to amplify the sequence designated GalNAc-4-ST2 in Fig. 1*A* (GenBank™ accession number AF332472) from first strand cDNAs derived from both 293 cells and a glioma. The open reading frame of GalNAc-4-ST2 is preceded at its 5′ end by a stop codon in all three reading frames and predicts a protein of 443 amino acids with four potential glycosylation sites and a molecular mass of 52.1 kDa. GalNAc-4-ST2 is predicted to have a membrane-spanning domain by the TMHMM algorithm (34) and a type II topology. If exon 4 is absent due to

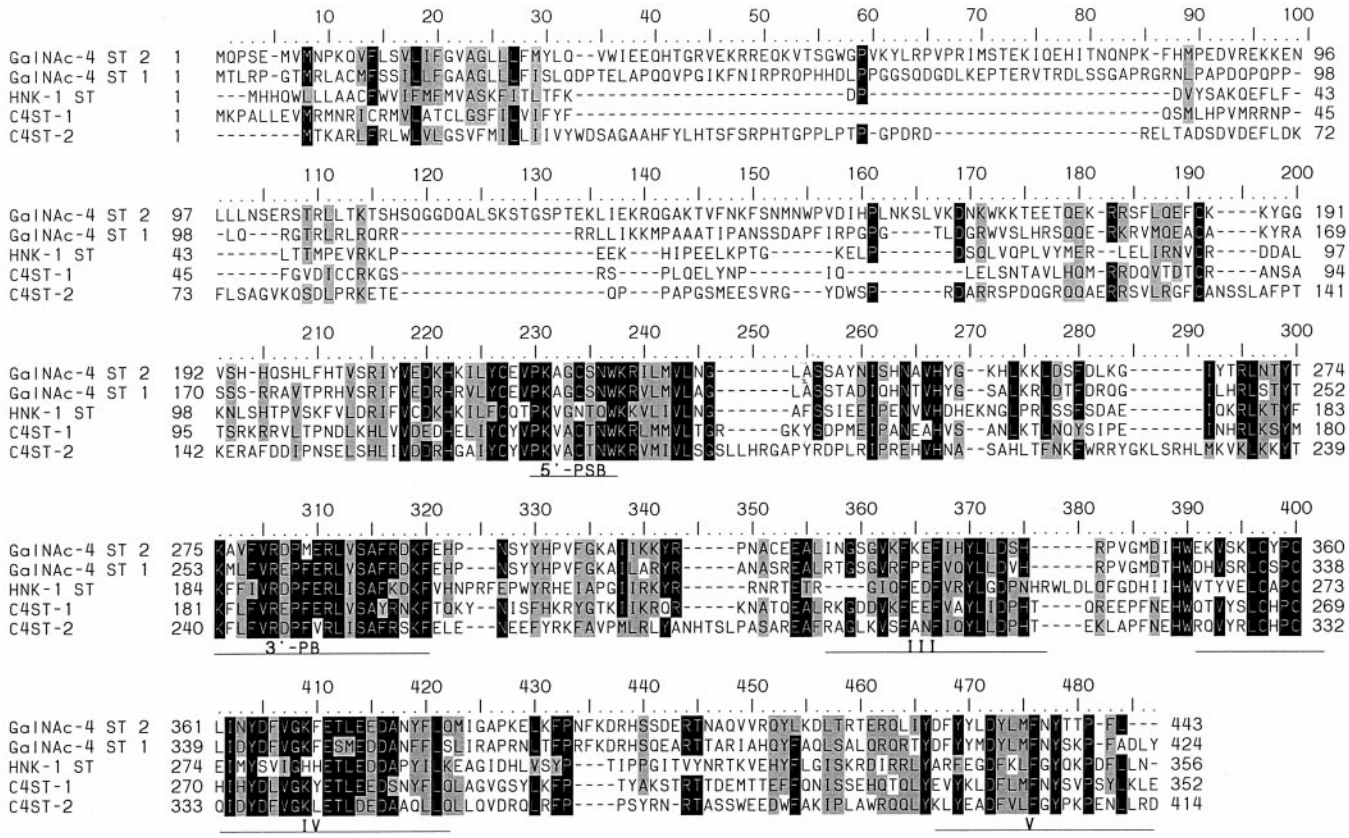


FIG. 2. **Comparison of amino acid sequences of human GalNAc-4-ST2, GalNAc-4-ST1, C4ST-1, C4ST-2, and HNK-1 ST.** Alignment was performed using the ClustalW program implemented in the BioEdit suite (35). Introduced gaps are shown as *hyphens,* and aligned amino acids are *boxed* (*black* for identical residues and *dark gray* for similar residues). Putative binding sites for the 5′-phosphosulfonate group (5′-PSB) and 3′-phosphate group (3′-PB) of PAPS, and three additional highly conserved domains (III–V) are marked.

alternative splicing, GalNAc-4-ST2(Met-86) would be initiated from the alternative ATG start codon located at the position of the Met that is equivalent to amino acid 86 in full-length GalNAc-4-ST2 (Fig. 1).

Multiple alignment of the protein sequence of GalNAc-4-ST2 with other members of the HNK-1 sulfotransferase family using the ClustalW algorithm implemented in the BIOEDIT suite (35) (Fig. 2) revealed that GalNAc-4-ST2 is 46% identical to GalNAc-4-ST1. GalNAc-4-ST2 also has a high percentage of identical amino acids when compared with other HNK-1 ST-related sulfotransferases as follows: 23% to human HNK-1 ST, 27% to human chondroitin 4-*O*-sulfotransferase-1 (C4ST-1), and 24% to human chondroitin 4-*O*-sulfotransferase-2 (C4ST-2) (Fig. 2). Regions with the highest degree of identity include the putative 5′-phosphosulfonate-binding site (5′-PSB), the putative 3′-phosphate-binding site (3′-PB), and three regions designated III, IV, and V (23) that do not have an identified function.

Genomic Organization and Chromosome Localization of GalNAc-4-ST2—BLAST similarity searches of the htgs and the nonredundant data set were performed using the GalNAc-4-ST2 cDNA sequence and retrieved multiple matching sequences (see "Experimental Procedures"). Among the genomic clones retrieved GenBank™ accession numbers AP001272 and AP001087 were both annotated to be mapped on chromosome 18q11.2. Comparisons between the cDNA and the genomic sequences AC009872, AC023575, and AC010854 showed that the coding region of GalNAc-4-ST2 is distributed over five exons (Fig. 3*A*). The sizes of the untranslated regions of exon 1 and exon 5 have not yet been determined. As noted above, GalNAc-4-ST2(Met-86) does not contain exon 4. Synthesis of GalNAc-4-ST2(Met-86) is therefore initiated from the alternative start codon located at amino acid position 86 of GalNAc-4-ST2 producing a protein that does not have a transmembrane domain (Fig. 3*B*). Each of the exons identified, including exon 4, was found to have conserved donor and acceptor splice sites (Fig. 3*C*).

GalNAc-4-ST2 Transfers Sulfate to Terminal β1,4-Linked GalNAc on N-Linked Oligosaccharides—Since GalNAc-4-ST2 is closely related to GalNAc-4-ST1, the specificity of GalNAc-4-ST2 for saccharides terminating with β1,4-linked GalNAc was examined using the substrates shown in Table I. pcDNA3.1-GalNAc-4-ST2 was transfected into CHO/Tag cells, and cell extracts were prepared for analysis. Like GalNAc-4-ST1, GalNAc-4-ST2 transfers sulfate to GalNAcβ1,4 GlcNAcβ1,2Manα-MCO and to GalNAcβ1,4 GlcNAcβ-MCO. GalNAc-4-ST2 does not transfer sulfate to either Galβ1,4GlcNAcβ-MCO or GlcNAcβ1,2Manα-MCO (Table I) indicating that the β1,4-linked GalNAc is required and likely the sugar was modified with sulfate. The oligosaccharide acceptors in Table I were tested at 20 and 100 μM, concentrations that are equal to and 5-fold greater than the K_m of 15 μM that we previously reported for GalNAcβ1,4 GlcNAcβ1,2Manα-MCO with the GalNAc-4-sulfotransferase found in the pituitary gland (36). As we have observed with the GalNAc-4-sulfotransferase from the pituitary gland (30, 36) and with GalNAc-4-ST1 (24), GalNAc-4-ST2 transfers sulfate to GalNAcβ1,4GlcNAcβ1,2Manα-MCO more efficiently than to GalNAcβ1,4GlcNAcβ-MCO.

The sulfated product was characterized as described previously (30) to establish the location of the sulfate. The [^{35}S]SO_4-labeled GalNAcβ1,4 GlcNAcβ1,2Manα-MCO product comigrated with authentic SO_4-4-GalNAcβ1,4 GlcNAcβ1,2Manα-MCO when analyzed by HPLC (Fig. 4*A*). Following mild acid hydrolysis, GalNAc-4-SO_4 was the only product obtained (Fig.

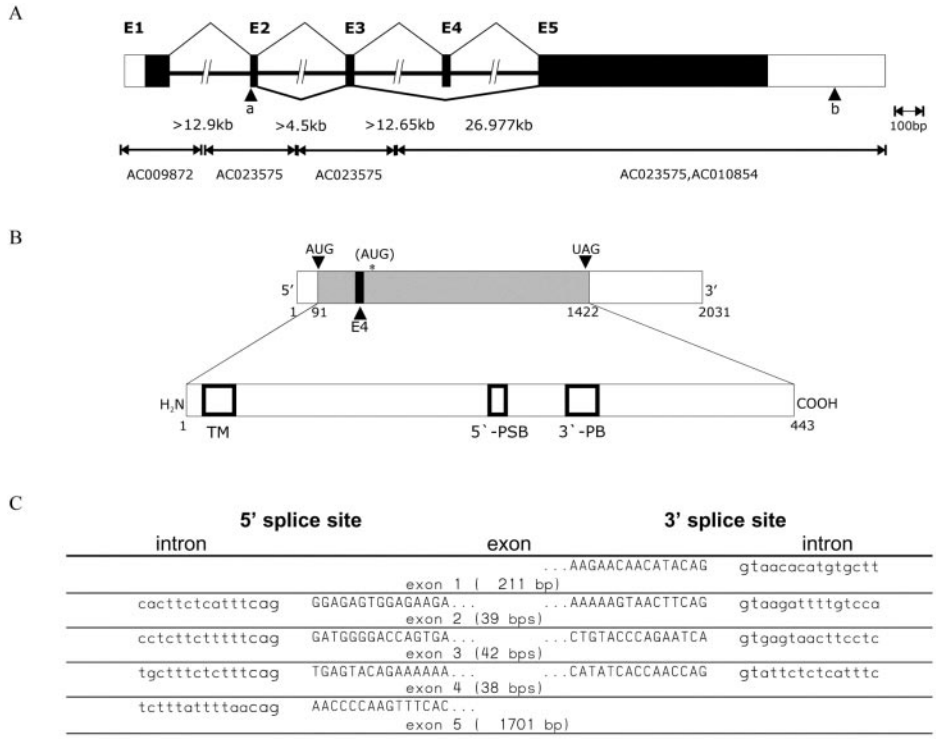


FIG. 3. **Structure of the human *GalNAc-4 ST2* gene.** *A,* exons that contribute to the 5′- and 3′-untranslated region are *boxed*. Exonic sequences that are transcribed into the coding sequence are *boxed* and *shaded* in *black*. Exons are numbered from 1 (*E1*) to 5 (*E5*). Relevant genomic clones are denoted with their GenBank™ accession numbers *below* the schematic. The splicing pattern for GalNAc-4-ST2 is shown *above* and for GalNAc-4-ST2(Met-86) *below* the schematic. Note that I.M.A.G.E. cDNA clone 49547 only contains exonic sequences from the position marked *a* to the position marked *b*. *B,* mRNA and the translated GalNAc-4-ST2 protein are shown. Nucleotide positions are denoted *below* the mRNA scheme. *TM* denotes the transmembrane domain. The, AUG in brackets marks the start codon for GalNAc-4-ST2(Met-86) that would be generated if exon 4 is absent. *C,* the intron-exon boundaries are shown for the human GalNAc-4-ST2 gene. Exon-derived sequences are capitalized. Note that all boundaries have an invariant intron derived GT and AG at the donor and acceptor splice sites.

TABLE I

The GalNAc-4-ST2 transfers sulfate to terminal β1,4-linked GalNAc

CHO/Tag cells were transfected with pcDNA3.1-GalNAc-4-ST2. After 60 h in culture cells were harvested and solublized in 200 μl of 1% Triton X-100. The cell extract (10 μl) was tested for transfer of [^{35}S]SO_4 (10^6 cpm/nmol [^{35}S]PAPS) to the acceptors shown. Results are expressed as pmol incorporated/h/100-mm plate.

Acceptor	GalNAc-4-ST2	
	20 μM	100 μM
	pmol/h/plate	
GlcNAcβ1,2Manα-MCO	0.11	0.12
GalNAcβ1,4 GlcNAcβ1,2Manα-MCO	10.47	31.47
GalNAcβ1,4 GlcNAcβ-MCO	5.46	24.10
Galβ1,4 GlcNAcβ1,2Manα1,6Manβ-MCO	0.18	0.15

4*B*). Thus, GalNAc-4-ST2 transfers sulfate exclusively to the C-4 hydroxyl of the terminal GalNAc in GalNAcβ1,4 GlcNAcβ1,2Manα-MCO.

We also examined GalNAc-4-ST2 for its ability to transfer sulfate to glycoproteins bearing *N*-linked oligosaccharides terminating with the sequence GalNAcβ1,4 GlcNAcβ1,2Manα such as are found on the glycoprotein hormones LH and TSH (37) and carbonic anhydrase VI (CA-VI) (33). GalNAc-4-ST2 transfers sulfate to bovine CA-VI isolated from parotid gland and to bovine LH (Fig. 5). The *N*-linked oligosaccharides on CA-VI isolated from parotid glands bear terminal β1,4-linked GalNAc due to the absence of sulfotransferase in the parotid gland (33). In contrast, >90% of the GalNAc on the *N*-linked oligosaccharides of LH is substituted with sulfate (38–40). Following removal of terminal sulfate moieties from the *N*-linked oligosaccharides on LH by digestion with GalNAc-4-sulfatase, there is a marked increase in the amount of sulfate transferred to LH. This indicates the sulfate is added to the terminal β1,4-linked GalNAc that has been exposed by the digestion (Fig. 5). The sulfate label is released from CA-VI and LH by digestion with PNGase F further confirming its location on *N*-linked oligosaccharides. Glycoproteins bearing oligosaccharides with terminal β1,4-linked Gal, such as asialo-hCG, were not modified with sulfate (not shown). Thus, GalNAc-4-ST2, like GalNAc-4-ST1, is capable of specifically modifying oligosaccharides on glycoproteins bearing terminal β1,4-linked GalNAc. We did note that GalNAc-4-ST1 shows a preference for LH while GalNAc-4-ST2 prefers CA-VI, suggesting the peptide portion of these glycoproteins may contribute to recognition by one or the other GalNAc-4-ST.

GalNAc-4-ST2 Transfers Sulfate to Internal β1,4-Linked GalNAc Moieties in Chondroitin and Dermatan—Even though GalNAc-4-ST2 displays the highest degree of identity with GalNAc-4-ST1, it is also homologous to C4ST-1, C4ST-2, and HNK-1 ST. We therefore examined extracts and medium from CHO/Tag cells transfected with pcDNA3.1-GalNAc-4-ST2 and pcDNA3.1-GalNAc-4-ST2(Met-86) for transfer of sulfate to chondroitin, dermatan, and the HNK-1 precursor GlcUAβ1,3Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1,$C_2H_4NHCOCF_3$. No evidence of transfer to GlcUAβ1,3Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1,$C_2H_4NHCOCF_3$ was seen (not shown). Following transfection of pcDNA3.1-GalNAc-4-ST2 into CHO/Tag cells, 15% of the sulfotransferase activity directed at GalNAcβ1,4 GlcNAcβ1,2Manα-MCO was found in the cell extract and 85% in the medium (Fig. 6). Release of GalNAc-4-ST2 into the medium most likely results from proteolytic cleavage as has been seen for a number of transferases (41) including GalNAc-4-ST1 (24). Whereas GalNAc-4-ST2 in the cell extract is able to transfer sulfate to chondroitin, there is a 3-fold increase in the relative rate of transfer of sulfate to chondroitin *versus* GalNAcβ1,4 GlcNAcβ1,2Manα-MCO for GalNAc-4-ST2 that has been released into the medium as compared with GalNAc-4-ST2 retained by the cell (Fig. 6).

When pcDNA3.1-GalNAc-4-ST2(Met-86) is expressed in CHO/Tag cells, virtually all of the activity directed at either GalNAcβ1,4 GlcNAcβ1,2Manα-MCO or chondroitin is present in the medium indicating little or no retention in the Golgi in the absence of the N-terminal transmembrane domain and stem region. Notably the GalNAc-4-ST2(Met-86) released into the medium transfers sulfate to chondroitin while showing little transfer to GalNAcβ1,4 GlcNAcβ1,2Manα-MCO (Fig. 6).

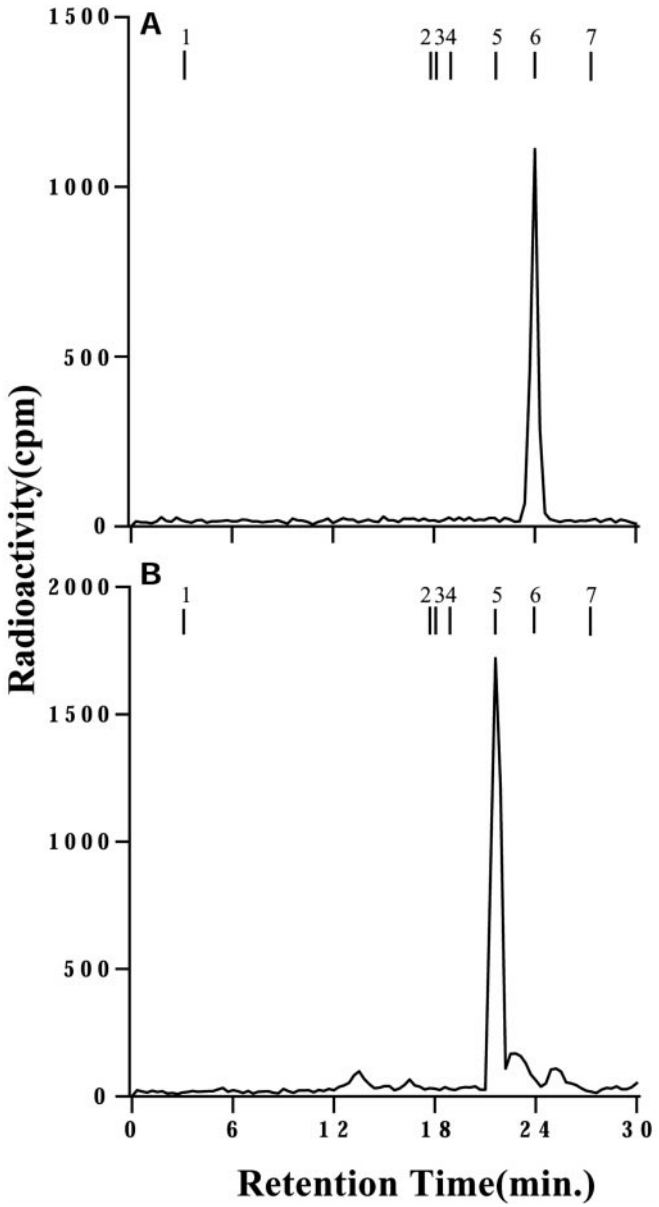


FIG. 4. **GalNAc-4-ST2 transfers sulfate exclusively to C-4 of the β1,4-linked GalNAc.** GalNAcβ1,4 GlcNAcβ1,2Manα-MCO was incubated with [^{35}S]PAPS and GalNAc-4-ST2 and the [^{35}S]SO_4-labeled product isolated by passage over a Sep-Pak C_{18}. *A,* analysis of the [^{35}S]SO_4-GalNAcβ1,4 GlcNAcβ1,2Manα-MCO product on a CarboPak PA1 column. *B,* analysis of the [^{35}S]SO_4-GalNAcβ1,4 GlcNAcβ1,2Manα-MCO product on CarboPak PA1 following mild acid hydrolysis. The elution positions of standards are indicated by the numbers: *1,* GlcNAc-3-SO_4; *2,* SO_4; *3,* GalNAc-3-SO_4; *4,* SO_4-3-GalNAcβ1,4 GlcNAcβ1, 2Manα-MCO; *5,* GalNAc-4-SO_4; *6,* SO_4-4-GalNAcβ1,4 GlcNAcβ1, 2Manα-MCO; and *7,* GalNAc-6-SO_4

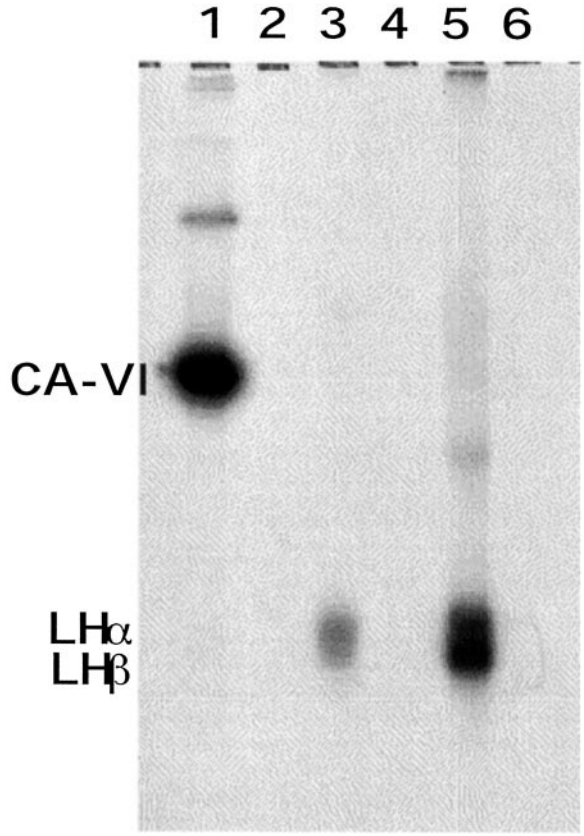


FIG. 5. **GalNAc-4-ST2 transfers sulfate to *N*-linked oligosaccharides terminating with the sequence GalNAcβ 1,4GlcNAcβ 1,2Manα on glycoproteins.** GalNAc-4-ST2 was incubated with 3 μg each of bovine CA-VI (from parotid gland), LH, or desulfated LH (treated with GalNAc-4-sulfatase) and [^{35}S]PAPS. An equal amount of each reaction was digested with PNGase F to release *N*-linked oligosaccharides. No sulfated products were seen in control lanes containing hCG, asialo-hCG, or media from cells transfected with pcDNA3.1 with no cDNA (not shown). *Lane 1,* CA-VI; *lane 2,* CA-VI + PNGase F; *lane 3,* LH; *lane 4,* LH + PNGase F; *lane 5,* desulfated LH; and *lane 6,* desulfated LH + PNGase F.

Furthermore, GalNAc-4-ST2 and GalNAc-4- ST2(Met-86) that have been released into the medium transfer sulfate to dermatan as well as to chondroitin. Thus the relative rates of transfer to GalNAcβ1,4 GlcNAcβ1,2Manα-MCO, chondroitin, and dermatan differ significantly for GalNAc-4-ST2 in the cell (0.4:0.1:0), GalNAc-4-ST2 released into the medium (2.3:1.7:1.0), and GalNAc-4-ST2(Met-86) released into the medium (0.2:4.5:1.0). This suggests that the transmembrane domain and stem region dictates the specificity for terminal GalNAc and also has an impact on recognition of chondroitin *versus* dermatan.

The location of the sulfate in the [^{35}S]SO_4-chondroitin and in [^{35}S]SO_4-dermatan products was determined by digestion with chondroitinase ABC and HPLC analysis (Fig. 7). Both chondroitin and dermatan yielded predominantly the sulfated disaccharide ΔDi-4S (D-gluco-4-enepyranosideβ1,3GalNAc-4-SO_4). The same result was obtained with the product produced by human C4ST-1 expressed in CHO/Tag cells (not shown). Thus GalNAc-4-ST2 transfers sulfate predominantly to the C-4 hydroxyl of internal GalNAc moieties in both chondroitin and dermatan.

Expression Pattern of GalNAc-4-ST2—Array and Northern blot analyses were used to determine the expression pattern for GalNAc-4-ST2 in human tissues (Fig. 8 and Fig. 9, respectively). A strong signal was obtained for the trachea with the human Multiple Tissue Expression (MTE^{TM}) array system when probing with radiolabeled GalNAc-4-ST2 cDNA (Fig. 8, *7H*). Significantly weaker signals were detected in the following tissues listed in order of decreasing intensity of hybridization signal: fetal lung (*G11*), adult pancreas (*B9*), testis (*F8*), and salivary gland (*E9*) stronger than pituitary gland (*D3*), apex of the heart (H4), lung (*A8*), prostate (*E8*), and mammary gland (*F9*) stronger than heart (*A4*), liver (*A9*), and the spinal cord (*E3*). Even though the signals did not reproduce well and could not be quantitated by densitometry, they are considered specific since the negative controls showed no visual signal (Fig. 8, *12A–H*). In addition, a specific species of mRNA could be detected by Northern blot analysis in a number of the tissues that provided a weak signal by array analysis (Fig. 9). A transcript of ~2.1 kilobase pairs was detected by Northern

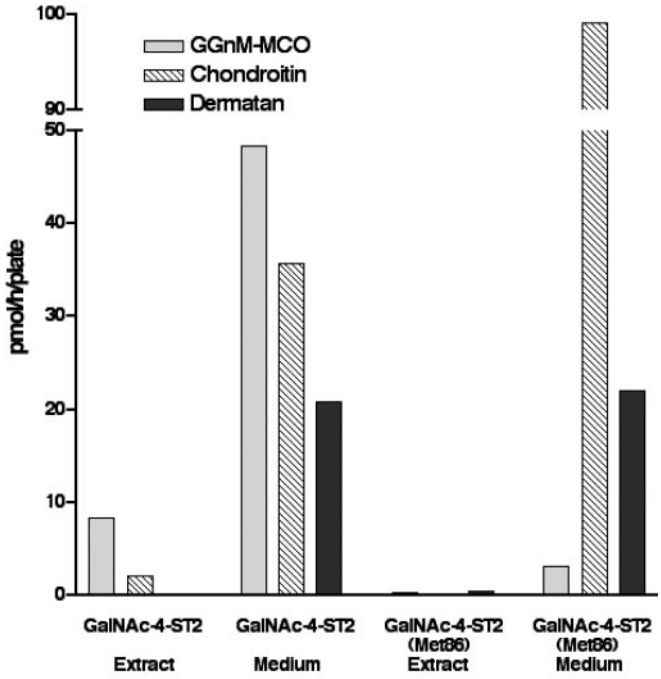


FIG. 6. **GalNAc-4-ST2 transfers sulfate to GalNAcβ 1,4GlcNAcβ 1,2Manα, chondroitin, and dermatan.** CHO/Tag cells were transfected with pcDNA3.1-GalNAc-4-ST2, pcDNA3.1-GalNAc-4-ST2(Met-86), or pcDNA3.1. After 60 h in culture cells were harvested and solubilized in 200 μl of 1% Triton X-100. The medium was collected and concentrated 10-fold to 2.0 ml by ultrafiltration. Each assay utilized 10 μl of either the cell extract or concentrated medium. Incorporation is reported as pmol/h/100-mm plate. Basal levels of incorporation have been subtracted. The transferase reactions were carried out with 20 μM GalNAcβ1,4GlcNAcβ 1,2Manα-MCO (*gray bar*), 50 μg of chondroitin (*striped bar*), or 50 μg of dermatan (*dark bar*) in a 50-μl reaction containing [^{35}S]PAPS (1×10^6 cpm/nmol) for 16 h. the products were separated as described under "Experimental Procedures."

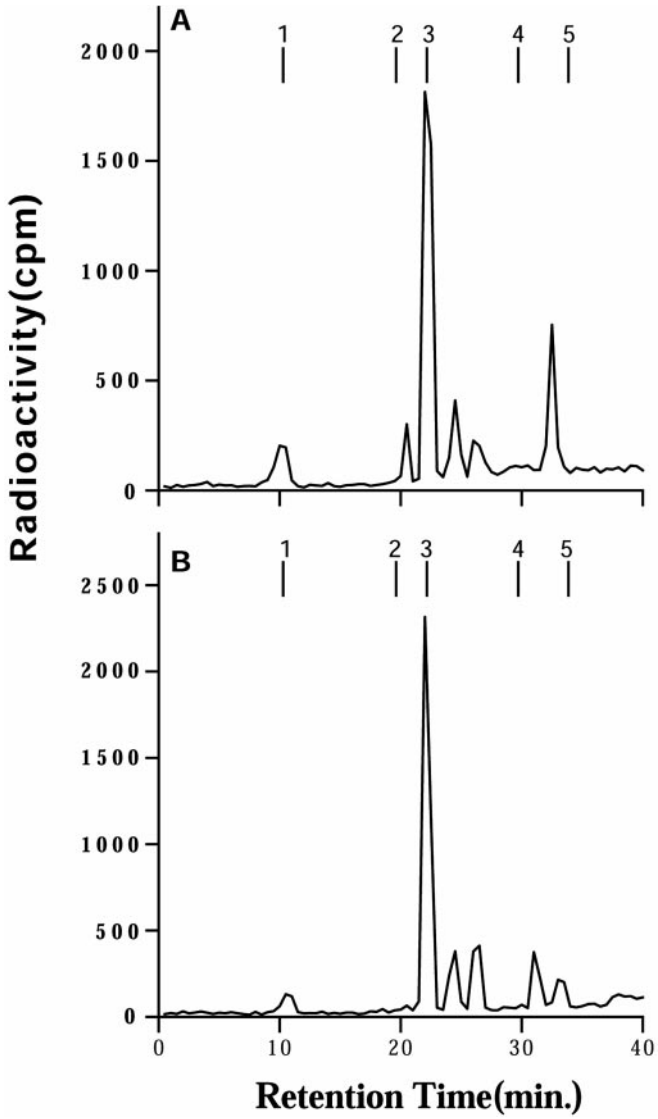


FIG. 7. **GalNAc-4-ST2 transfers sulfate to the C-4 hydroxyl of internal β1,4-linked GalNAc residues in chondroitin.** Chondroitin (50 μg) (*A*) and dermatan (50 μg) (*B*) were incubated with GalNAc-4-ST2 and [^{35}S]PAPS. The labeled product was separated from free label by gel filtration and digested with 30 milliunits of chondroitinase ABC in 100 mM Tris acetate buffer, pH 8.0, for 6 h at 37 °C. The product was analyzed on a Micropak AX-5 column (Varian) developed with a gradient of 10–450 mM KH_2PO_4 over 40 min at a flow rate of 1.0 ml/min. The elution times for for authentic standards are indicated by the following numbers: *1,* GalNAc-4-SO_4; *2,* ΔDi6 (D-gluco-4-enepyranosideβ1,3 GalNAc-6-SO_4); *3,* ΔDi4 (D-gluco-4-enepyranosideβ1,3GalNAc-4-SO_4); *4*, ΔDi-diS_E (D-gluco-4-enepyranosideβ1,3GalNAc-4,6-diSO_4); and *5,* free SO_4.

blot analysis in heart, liver, and pancreas, with a significantly lower signal in lung.

DISCUSSION

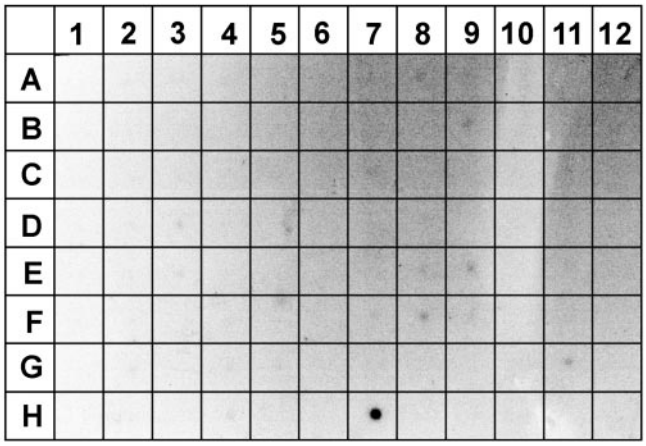
We and others recently reported the cloning and characterization of a GalNAc-4-*O*-sulfotransferase, GalNAc-4-ST1, based on its homology to HNK-1 ST (24) and C4ST (25), respectively. GalNAc-4-ST1 is highly expressed in the pituitary and other regions of the brain. GalNAc-4-ST1 accounts for the addition of sulfate to terminal β1,4-linked GalNAc on the *N*-linked oligosaccharides of LH and other pituitary glycoproteins (24). We have now cloned a second, novel GalNAc-4-*O*-sulfotransferase, GalNAc-4-ST2, utilizing the same strategy. Differences in the specificity and expression GalNAc-4-ST2 as compared with GalNAc-4-ST1 indicate it has a distinct biologic role *in vivo*.

GalNAc-4-ST2 is the fifth member of a family of structurally related sulfotransferases that thus far include HNK-1 ST, C4ST-1, C4ST-2, GalNAc-4-ST1, and GalNAc-4-ST2. Since HNK-1 ST was the first member to be cloned, we and others (24, 25) have used the term HNK-1 ST family to describe this family. Like all other sulfotransferases the members of HNK-1 family of sulfotransferases have two motifs that are hypothesized to mediate binding of the 5′-phosphosulfonate (5′-PSB in Fig. 2) and 3′-phosphate (3′-PB in Fig. 2) group of the high energy donor PAPS (3′-phospho-adenosine-5′-phosphosulfonate). Three additional regions (III, IV, and V in Fig. 2) located C-terminal to the 5′-phosphosulfonate and 3′-phosphate-binding regions also have a high percentage of identical amino acids, whereas the cytosolic, transmembrane, and stem regions have few identical amino acids. GalNAc-4-ST2 and GalNAc-4-ST1 have the highest percentage of identical amino acids with 46%. In contrast, C4ST-1 and C4ST-2 have 29% identical amino acid residues. The multiple sequence alignment in Fig. 2 indicates that GalNAc-4-ST2 is 23% identical to HNK-1 ST, 27% to C4ST-1, and 24% to C4ST-2.

The specificities of the members of the HNK-1 sulfotransferase family are summarized in Table II. HNK-1 ST is the only family member that transfers sulfate to the C-3 hydroxyl of terminal β1,3-linked glucuronic acid. Each of the other family members transfers sulfate to the C-4 hydroxyl of β1,4-linked GalNAc. In the case of C4ST-1 and C4ST-2 the GalNAc is found within the repeating disaccharide sequences of chondroitin and dermatan, *i.e.* it is not located at the nonreducing terminus. In contrast, GalNAc-4-ST1 and GalNAc-4-ST2 transfer sulfate to GalNAc moieties located at the nonreducing termini of oligo-

saccharide acceptors. Thus, like GalNAc-4-ST1 and GalNAc-4-ST2, HNK-1 ST transfers sulfate to a terminal β-linked sugar. The specificities of GalNAc-4-ST1 and GalNAc-4-ST2 are indicative of a structural relationship with C4ST-1 and C4ST-2. Whereas the native, membrane-associated form of GalNAc-4-ST1 does not transfer sulfate to chondroitin, the native, membrane-associated form of GalNAc-4-ST2 transfers sulfate to chondroitin as well as to GalNAcβ1,4 GlcNAcβ1,2Manα-MCO but at a lower rate. The truncated forms of GalNAc-4-ST1 and GalNAc-4-ST2, *i.e.* GalNAc-4-ST1(Met119) and GalNAc-4-ST2(Met-86), both transfer sulfate to nonterminal β1,4-linked GalNAc residues in chondroitin. GalNAc-4-ST1(Met-119) retains its ability to transfer sulfate to the terminal GalNAc of GalNAcβ1,4 GlcNAcβ1,2Manα-MCO, whereas GalNAc-4-ST2(Met-86) no longer transfers sulfate to GalNAcβ1,4 GlcNAcβ1,2Manα-MCO.

Thus, for both GalNAc-4-ST1 and GalNAc-4-ST2 the transmembrane and stem region have a major impact on the specificity for terminal *versus* internal β1,4-linked GalNAc. The presence of the stem and transmembrane domains markedly reduce but do not abolish transfer of sulfate to chondroitin and dermatan for GalNAc-4-ST2, whereas these regions completely abolish sulfate transfer to chondroitin by GalNAc-4-ST1. The different rates of sulfate transfer to chondroitin and dermatan seen with GalNAc-4-ST2 proteolytically released into the medium and truncated GalNAc-4-ST2(Met-86) suggest that the stem region may also have an impact on the specificity for the β1,4-linked GalNAc and the adjacent uronic acid. However, more detailed analyses will be required to address these issues fully.



A1 brain, whole	B1 cerebral cortex	C1 frontal lobe	D1 parietal lobe
A2 cerebellum, left	B2 cerebellum, right	C2 corpus callosum	D2 amygdala
A3 substantia nigra	B3 accumbens nucleus	C3 thalamus	D3 pituitary gland
A4 heart	B4 aorta	C4 atrium, left	D4 atrium, right
A5 esophagus	B5 stomach	C5 duodenum	D5 jejunum
A6 colon, transverse	B6 colon, descending	C6 rectum	D6 empty
A7 kidney	B7 skeletal muscle	C7 spleen	D7 thymus
A8 lung	B8 placenta	C8 bladder	D8 uterus
A9 liver	B9 pancreas	C9 adrenal gland	D9 thyroid gland
A10 leukemia, HL-60	B10 HELA S3	C10 leukemia, K-562	D10 leukemia, MOLT-4
A11 brain, fetal	B11 heart, fetal	C11 kidney, fetal	D11 liver, fetal
A12 yeast total RNA	B12 yeast tRNA	C12 E.coli rRNA	D12 E. coli DNA
E1 occipital lobe	F1 temporal lobe	G1 p.g. of cerebral cortex*	H1 pons
E2 caudate nucleus	F2 hippocampus	G2 medulla oblongata	H2 putamen
E3 spinal cord	F3 empty	G3 empty	H3 empty
E4 ventricle, left	F4 ventricle, right	G4 interventricular space	H4 apex of the heart
E5 ileum	F5 ilocecum	G5 appendix	H5 colon, ascending
E6 empty	F6 empty	G6 empty	H6 empty
E7 periph. blood leuk.**	F7 lymph node	G7 bone marrow	H7 trachea
E8 prostate	F8 testis	G8 ovary	H8 empty
E9 salivary gland	F9 mammary gland	G9 empty	H9 empty
E10 Burkitt`s ***	F10 Burkitt`s ****	G10 SW480 *****	H10 lung carcinoma, A549
E11 spleen, fetal	F11 thymus, fetal	G11 lung, fetal	H11 empty
E12 Poly r(A)	F12 human Cot-1 DNA	G12 human DNA, 100 ng	H12 human DNA, 500 ng

FIG. 8. **RNA dot blot analysis of GalNAc-4-ST2 transcripts.** The human Multiple Tissue Expression (MTE™) array shown was hybridized with a ^{32}P-labeled human GalNAc-4-ST2-specific cDNA probe (see "Experimental Procedures"). Tissue sources for the RNA are indicated below the *blot*. *Asterisks* indicate the following: *, paracentral gyrus of cerebral cortex; **, peripheral blood leukocytes; ***, Burkitt's lymphoma Raji; ****, Burkitt's lymphoma Daudi; *****, colorectal adenocarcinoma, SW280.

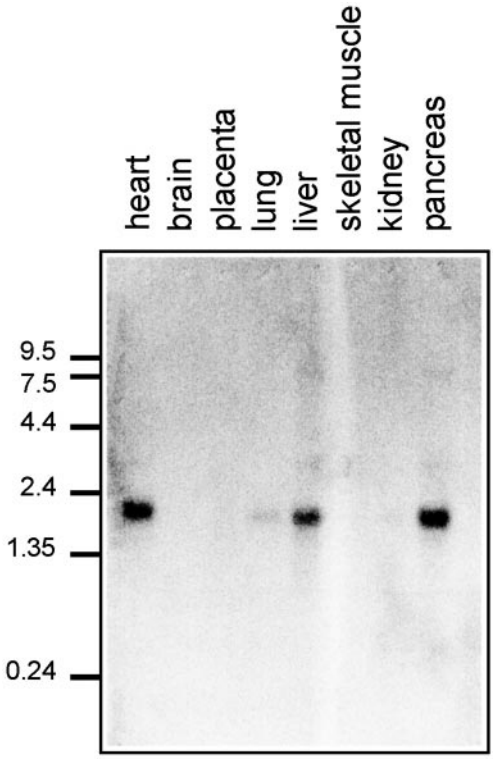


FIG. 9. **Northern blot analysis of GalNAc-4-ST2 transcripts.** Each lane of the MTN® Northern blot contains 2 μg of poly(A)$^+$ RNA and was hybridized with a ^{32}P-labeled cDNA probe specific for human GalNAc-4-ST2 (see "Experimental Procedures"). Tissues used to prepare the RNA are indicated above each lane. Migration positions of standards are indicated at the *left*.

TABLE II
Specificities of the HNK-1 ST family members

The ability of the various members of the HNK-1 family of sulfotransferases to modify the acceptors shown is indicated by a +, −, or +/− for active, inactive, or weakly active, respectively. The saccharide that is modified with sulfate is in each case shown in bold letters. GalNAc-4-ST1 and GalNAc-4-ST2 indicate the native, membrane-associated, intracellular forms.

Sulfotransferases	Acceptors		
	$(GlcA\beta1,3\mathbf{GalNAc}\beta1,4)_n$ $(IdUA\alpha1,3\mathbf{GalNAc}\beta1,4)_n$	**GalNAc**β1,4GlcNAβ	**GlcA**β1,3Galβ1,4
C4ST-1	+	−	−
C4ST-2	+	−	−
GalNAc-4-ST1	−	+	−
GalNAc-4-ST1(Met-119)	+	+	−
GalNAc-4-ST2	+/−	+	−
GalNAc-4-ST2(Met-86)	+	−	−
HNK-1 ST	−	−	+

The five members of the HNK-1 family of sulfotransferases thus far defined are localized to five different chromosomes as follows: HNK-1 ST to chromosome 2 (GenBank™ accession number AC012493), C4ST-1 to chromosome 12q23, C4ST-2 to chromosome 7p22 (23), GalNAc-4-ST1 to chromosome 19q13.1 (24), and GalNAc-4-ST2 to chromosome 18q11.2. The coding sequence for C4ST-2 (GenBank™ accession number AC004840) is found within a single exon. In contrast the coding sequence of GalNAc-4-ST1 is found in 3 exons whereas that for GalNAc-4-ST2 is found in 5 exons. For both GalNAc-4-ST1 and GalNAc-4-ST2 the entire catalytic domain including the 5′-phosphosulfonate-binding site, the 3′-phosphate-binding site, and regions III–V are encoded by a single exon. The additional exons encode the cytosolic domain, the transmembrane domain, and the majority of the stem region. It is these regions that contribute to shifting the specificity away from chondroitin and dermatan to terminal GalNAcβ1,4GlcNAcβ. Thus, GalNAc-4-ST1 and GalNAc-4-ST2 may have evolved from C4ST-1 and C4ST-2 by the introduction of these additional exons that may have markedly altered the properties of the stem region. The presence of these five homologous sulfotransferases on five different chromosomes suggests that they diverged quite some time ago.

Even though GalNAc-4-ST1 and GalNAc-4-ST2 are the most closely related HNK-1 ST family members in terms of their genomic organization and protein sequence identity, the differences in their enzymatic properties and their patterns of expression indicate they have distinct functions *in vivo*. The high level of expression of GalNAc-4-ST1 in pituitary is consistent with its role in adding sulfate to the GalNAcβ1,4GlcNAcβ termini found on *N*-linked oligosaccharides of LH, TSH, proopiomelanocortin, and other hormones (24). Expression of GalNAc-4-ST1 in other regions of the brain indicates that the same structures are present on other glycoproteins produced in these regions. We have, for example, found that GalNAc-4-ST1 and glycoproteins bearing sulfated *N*-linked oligosaccharides are abundant in the cerebellum.[2] In contrast, Northern blots indicate that GalNAc-4-ST2 is not highly expressed in brain but is highly expressed in trachea and to lesser extent in heart, liver, pancreas, salivary gland, testis, and lung. Since GalNAc-4-ST2 can add sulfate to chondroitin as well as to GalNAcβ1,4GlcNAcβ, the actual products produced in these tissues remain to be established. In addition, it is possible that the truncated form of GalNAc-4-ST2, GalNAc-4-ST2(Met-86), is expressed in specific cells and/or tissues. GalNAc-4-ST2(Met-86) is missing exon 4 (see Fig. 3) and does not contain a transmembrane domain or the stem region due to initiation from the alternative start codon that encodes Met-86 in GalNAc-4-ST2. As a result GalNAc-4-ST2(Met-86) is synthesized and released as a soluble chondroitin- and dermatan-specific GalNAc-4-sulfotransferase. Whether the truncated form of GalNAc-4-ST2 is synthesized *in vivo* remains to be established; however, GalNAc-4-ST2(Met-86) and GalNAc-4-ST2 would clearly have different functions.

GalNAc-4-ST1 and GalNAc-4-ST2 appear to have different expression patterns *in vivo*; however, we have found that two cell lines known to produce *N*-linked oligosaccharides terminating with β1,4-linked GalNAc-4-SO_4, human kidney 293 cells, and human SH-SY5Y neuroblastoma cells[3] contain messages for both sulfotransferases. Clearly understanding the relationship of these two closely related sulfotransferases will be important for assessing the role of the sulfated saccharides they produce *in vivo*. The cloning and characterization of a second GalNAc-4-sulfotransferase, GalNAc-4-ST2, that is closely related to GalNAc-4-ST1 but clearly has distinct properties adds further strength to the view that the sulfated saccharides produced have critical biologic roles.

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