Molecular Cloning and Expression of Chondroitin 4-Sulfotransferase*

(Received for publication, August 5, 1999, and in revised form, November 29, 1999)

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Chondroitin 4-sulfotransferase (C4ST) catalyzes the transfer of sulfate from 3′-phosphoadenosine 5′-phosphosulfate to position 4 of N-acetylgalactosamine residue of chondroitin. The enzyme has been previously purified to apparent homogeneity from the serum-free culture medium of rat chondrosarcoma cells (Yamauchi, A., Hirahara, Y., Usui, H., Takeda, Y., Hoshino, M., Fukuta, M., Kimura, J. H., and Habuchi, O. (1999) J. Biol. Chem. 274, 2456–2463). The purified enzyme also catalyzed the sulfation of partially desulfated dermatan sulfate. We have now cloned the cDNA of the mouse C4ST on the basis of the amino acid sequences of peptides obtained from the purified enzyme by protease digestion. This cDNA contains a single open reading frame that predicts a protein composed of 352 amino acid residues. The protein predicts a Type II transmembrane topology. The predicted sequence of the protein contains all of the known amino acid sequence and four potential sites for N-glycosylation, which corresponds to the observation that the purified C4ST is an N-linked glycoprotein. The amino acid sequence of mouse C4ST showed significant sequence homology to HNK-1 sulfotransferase. Comparison of the sequence of mouse C4ST with human HNK-1 sulfotransferase revealed ~29% identity and ~48% similarity at the amino acid level. When the cDNA was introduced in a eukaryotic expression vector and transfected in COS-7 cells, the sulfotransferase activity that catalyzes the transfer of sulfate to position 4 of GalNAc residue of chondroitin and desulfated dermatan sulfate was overexpressed. Northern blot analysis showed that, among various mouse adult tissues, 5.7-kilobase message of C4ST was mainly expressed in the brain and kidney.

Chondroitin sulfate proteoglycans are found in various tissues as molecules having divergent molecular architecture (1, 2). Chondroitin sulfate chains attached to chondroitin sulfate proteoglycans appear to play important roles in the formation and maintenance of cartilage tissue, because undersulfation of chondroitin sulfate resulted from the defective synthesis of PAPS3 (3, 4) or defective sulfate transport (5) was found to cause underdevelopment of skeleton. Various chondroitin sulfate proteoglycans have been reported to be present in the brain (6, 7) and to function in the regulation of neurite outgrowth and neural cell adhesion (8–12), neuronal migration (13), and the survival of neurons (14). Chondroitin sulfate chains are also shown to be involved in the interaction with CD44 (15, 16), phospholipase A2 (17), Plasmodium falciparum-infected erythrocytes (18), and L-selectin (19). Chondroitin sulfates have sulfate group at various positions of the component sugars; position 6 and/or 4 of GalNAc residues and position 2 or 3 of GlcA residues. The pattern of sulfation of chondroitin sulfate chains varies with the source of the proteoglycans, development of animal (20–22), and malignant change (23), suggesting that sulfate moieties attached to the specific position of the component sugars may be related to the specific function of the glycosaminoglycan. Characterization and molecular cloning of sulfotransferases, which participate in the formation of the defined structure of chondroitin sulfate, are important to clear the functional roles of chondroitin sulfate.

We have purified chick chondroitin 6-sulfotransferase (C6ST), which catalyzes transfer of sulfate to position 6 of N-acetylgalactosamine residue of chondroitin from PAPS (24), and cloned its cDNA (25). Uronosyl 2-sulfotransferase, which sulfates iduronyl and glucuronyl residues in dermatan/chondroitin sulfate, has been cloned (26). We have previously purified chondroitin 4-sulfotransferase (C4ST), which catalyzes the transfer of sulfate from PAPS to position 4 of N-acetylgalactosamine residue of chondroitin, to apparent homogeneity (27). This enzyme was also found to catalyze the sulfation of partially desulfated dermatan sulfate. In this paper we report the cloning of the cDNA encoding mouse C4ST and the expression of it in COS-7 cells.

EXPERIMENTAL PROCEDURES

Purification of Chondroitin 4-Sulfotransferase and Amino Acid Sequencing of the Intact Purified Protein and Peptides Obtained by Enzymatic Digestion of Intact Purified Protein

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1 The abbreviations used are: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; C4ST, chondroitin 4-sulfotransferase; C6ST, chondroitin 6-sulfotransferase; EST, expressed sequence-tagged; PCR, polymerase chain reaction; GlcA, N-glucuronic acid; ΔDi-0S, 2-acetamide-2-deoxy-3-O-(β-D-glucopyranosyluronic acid)-N-galactose; ΔDi-4S, 2-acetamide-2-deoxy-3-O-(β-D-glucopyranosyluronic acid)-4-O-sulfo-D-galactose; ΔDi-6S, 2-acetamide-2-deoxy-3-O-(β-D-glucopyranosyluronic acid)-6-O-sulfod-N-galactose; ΔDi-diSα, 2-acetamide-2-deoxy-3-O-(β-D-glucopyranosyluronic acid)-4-O-sulfo-D-galactose; ΔDi-diSβ, 2-acetamide-2-deoxy-3-O-(β-D-glucopyranosyluronic acid)-4,6-bis-O-sulfo-D-galactose; DMEM, Dulbecco’s modified Eagle’s medium; SSPE, sodium chloride/sodium phosphate/EDTA buffer; HPLC, high performance liquid chromatography.
Cloning of Chondroitin 4-Sulfotransferase
dopainse Digestion—C4ST was purified from the serum-free culture medium of rat chondrosarcoma cells as described previously (27). A portion of the purified C4ST (15 μg as protein) was subjected to SDS-polyacrylamide gel electrophoresis (10% gel) according to the method of Laemmli (28) after reduction and denaturation in loading buffer containing 10% glycerol, 0.1% SDS, and subsequently in 0.1 M sodium phosphate, pH 7.0.

Construction of pCXNC4ST—To construct the plasmid containing the C4ST cDNA named pCXNC4ST, the EcoRI fragment was excised from the pBluescript plasmid and ligated into the EcoRI site of pCX2 expression vector (pCXN2 vector was constructed by Dr. Jun-ichi Miyazaki, Department of Dis-
ease-related Gene Regulation, Faculty of Medicine, University of Tokyo (31) and a gift from Dr. Yasuhiro Hashimoto, Tokyo Metropolitan Institute of Medical Sciences). Escherichia coli XL1-Blue cells were transformed with the ligated mixture and plated on LB ampicillin plates. Recombinant plasmids were cut with Xhol to confirm the correct ori-
ni and introduced into E. coli DH5α. Expression of C4ST was confirmed with Qiagen Plasmid kit (Qiagen). The plasmid, which contained the cDNA fragment in the reversed orientation, was named as pCXNC4ST2 and used for control experiments.

Transient Expression of Chondroitin 4-Sulfotransferase cDNA in COS-7 Cells—COS-7 cells (obtained from Riken Cell Bank, Tsukuba, Japan) were plated in 35-mm culture dishes at a density of 8 × 10^4 cells/dish. Volume of the medium was 10 ml. The medium used was DMEM containing penicillin (100 units/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum (Life Technologies, Inc.), and cells were grown at 37 °C in 5% CO_2, 95% air. When the cell density reached 3 × 10^6 cells/dish (48 h after plating), COS-7 cells were transfected with pCXNC4ST, pCXNC4ST2, or vector alone. The transfection was performed using the DEAE-dextran method (32). 5 ml of the prewarmed DMEM containing 10% NuSerum (Collaborative Biomedical Products) was mixed with 0.2 ml of phosphate-buffered saline containing 10 mg/ml DEAE-dextran plus 2.5 mg/ml chloroquine solution. 15 μg of the recombinant plasmid was mixed with the solution, and the mixture was added to the cells. The cells were incubated for 4 h in a CO_2 incubator. The medium was replaced with 5 ml 10% fetal bovine serum phosphate-buffered saline. After the cells were left at room temperature for 2 min, the dimethyl sulfoxide solution was aspirated and 25 ml of DMEM containing penicillin (100 units/ml), streptomycin (50 μg/ml), and 10% fetal bovine serum was added. The cells were incubated for 67 h, washed with DMEM alone, scraped, and extracted with 10 ml Tris-HCl, pH 7.2, 10 mM MgCl_2, 2 mM CaCl_2, 0.5% Triton X-100, and 20% glycerol for 30 min on a rotatory shaker. The extracts were centrifuged at 10,000 × g for 20 min, and the sulfotransferase activities in the supernatant fractions were measured using chondroitin or desulfated dermatan sulfate as acceptors. The sulfotransferase activities were assayed by the method described previously (27). The standard reaction mixture contained 2.5 μmol of imidazole HCl, pH 6.8, 1.25 μg of protein and 0.1 μmol of dithiothreitol, 25 nmol (as glucuronic acid) chondroitin, 50 μmol [35S]PAPS (about 5.0 × 10^6 cpm) (33), and enzyme in a final volume of 50 μl. When 25 nmol (as GalNAc) partially desulfated dermatan sulfate was used as acceptor, the amount of protamine chloride was increased to 10 μg. Partially desulfated dermatan sulfate was prepared from pig skin dermatan sulfate as described (27). The degree of the desulfation was calculated as 83% from the proportion of total unsaturated disaccharides formed after chondroitinase ABC digestion. When the desulfated dermatan sulfate was digested with chondroitinase ACII, the yield of DA-0S was only 5% of the total unsaturated disaccharides formed after chondroitinase ABC digestion (27). The reaction mixtures were incubated at 37 °C for 20 min, and the reaction was stopped by immersing the reaction tubes in a boiling water bath for 1 min. After the reaction was stopped, 35S-labeled incubation products were separated by preparative thin-layer chromatography followed by gel chromatography with a Fast Desalting Column as described previously and radioactivity was determined. For determining the incorporation into position 4 and position 6 of GalNAc residues, 35S-labeled chondroitin and 38S-labeled desulfated dermatan sulfate were digested with chondroitinase ACII and chondroitinase ABC, respectively. The resulting unsaturated disaccharides (DA-0S and DA-6S) were separated with paper chromatography (24), and their radioactivities were measured.

Preparation of a FLAG-C4ST Fusion Protein—Recombinant C4ST was also expressed as a fusion protein with FLAG peptide. A DNA fragment, which codes for full open reading frame, was amplified by PCR using mouse C4ST cDNA as a template. The 5' and 3' primers were CGCAACGTTATGAAACCCGGCGCTGCTGGAAGT and GAAATTTCTCAATTCACACTGAGATTGTAGT, respectively. At 5' end of the oligonucleotide primers, restriction enzyme recognition sites were introduced: HindIII site for the sense primer and EcoRI site for the antisense primer. The PCR product was digested with EcoRI and HindIII and subcloned into these sites of pFLAG-CMV-2 plasmid (Eastman Kodak Co.). The resulting plasmid was transfected in COS-7 cells, and the recombinant protein was obtained from the cells supernatant. The extracts were applied to an anti-FLAG monoclonal antibody-conjugated affinity column (Kodak) equilibrated with the buffer used for the elution. The absorbed materials were eluted with FLAG peptide under the conditions recommended by the manufacturer.

HPLC Separation of 35S-Labeled Products after Digestion with Chondroitinase ACII or Chondroitinase ABC—Separation of the degradation
products formed from 35S-labeled chondroitin and 35S-labeled desulfated dermatan sulfate after digestion with chondroitinase ACII or chondroitinase ABC was carried out by HPLC using a Whatman Partisil 10-SAX column (4.5 mm × 25 cm) equilibrated with 10 mM KH2PO4. The column was developed with gradient elution as shown in Fig. 4. The flow rate was 1 ml/min, and the column temperature was 40 °C. 0.5-ml fractions were collected.

**Northern Blot Hybridization—Mouse Multiple Tissue Northern blot** Filters (CLONTECH) (on which 2 μg of poly(A+) RNAs from various adult mouse tissues were blotted) were prehybridized in ExpressHyb solution (CLONTECH) at 68 °C. Hybridization was carried out in the same solution containing 32P-labeled probe for 1 h at 68 °C. The radio-active probe was prepared from the cDNA fragment excised from the pBluescript II plasmid by the random oligonucleotide-prime labeled method (30) using [α-32P]dCTP. The filters were washed at room temperature in 2 × SSC, 0.05% SDS, and subsequently in 0.1 × SSC, 0.1% SDS at 50 °C. The membrane was exposed to x-ray film at −80 °C with an intensifying screen.

**FIG. 1.** Analysis of PCR products formed from the first strand cDNA of poly(A+) RNA from rat chondrosarcoma cells. Sequences of primer F and primer R and conditions for PCR were described under “Experimental Procedures.” Lanes 1 and 4, both primer F and primer R were used; lane 2, only primer R was used; and lane 3, only primer F was used. The template cDNA was present in lanes 1–3 and absent in lane 4. The band indicated by an arrowhead was used for the screening of mouse brain cDNA library. bp, base pairs.

**FIG. 2.** Nucleotide sequence of the C4ST cDNA and the predicted amino acid sequence and hydropathy plot of the protein. A, the predicted amino acid sequence is shown below the nucleotide sequence. Peptides from which amino acid sequence data were obtained are underlined. Four potential N-linked glycosylation sites are indicated by dots. The putative transmembrane hydrophobic domain is boxed. The site at which C4ST might be cleaved during the secretion is indicated with a triangle. B, the hydropathy plot was calculated by the method of Kyte and Doolittle (49) with a window of 11 amino acids.

**TABLE I**

| Sample     | Endoproteinase | Amino acid sequence |
|------------|----------------|---------------------|
| 60-kDa protein | Trypsin | XPLQXLNYNP          |
| Peptide 1  | Trypsin      | LVASYR              |
| Peptide 2  | Trypsin      | EPFFER              |
| Peptide 3  | Trypsin      | LAGYSGLK            |
| Peptide 4  | Trypsin      | TNLQXYP             |
| Peptide 5  | Asp-N        | DSNVYLR             |
| Peptide 6  | Asp-N        | DLVQK               |

**Cloning of Chondroitin 4-Sulfotransferase**

8977

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RESULTS

Generation of PCR Probe to Screen cDNA Library—We obtained amino acid sequences of six peptides from the purified C4ST (Table I). We also determined amino-terminal amino acid sequence of the intact protein. When the sequence of peptide 3 was used for the homology search, we found that the peptide matched a mouse EST cDNA containing 505 nucleotides (accession number AA218236). Examination of the sequence of the cDNA indicated the presence of the nucleotide sequences corresponding to peptide 1, 2, 4, 5, and 6, although the reading frame was not the same. We thought that the clone must be a cDNA fragment encoding a partial sequence of C4ST. From the sequence of the EST cDNA, oligonucleotide primers were designed to amplify a DNA fragment with 374 nucleotides. The sequence of primer R was found in both the mouse EST cDNA and another rat EST cDNA (accession number AI044878), but the rat sequence corresponding to primer F was not available; therefore we used the sequence of the mouse EST cDNA to design primer F. PCR was carried out using the first strand cDNA (indicated by an arrowhead in Fig. 1). A DNA fragment with about 350-base pair was amplified in the presence of both forward and reverse primers and the first strand cDNA (indicated by an arrowhead in Fig. 1). The sequence of this DNA fragment was almost identical to that of the mouse EST cDNA (data not shown); therefore, we used the PCR product for the preparation of a probe for the screening.

Screening of λgt11 Library—The above described 350-base pair PCR product was labeled with [α-32P]dCTP by the random oligonucleotide-primed labeling method and used as a probe to screen a λgt11 mouse brain cDNA library. About 50 positive clones were observed from 4 × 105 plaques. From the nucleotide sequence, a cDNA clone with 1581 base pairs was found to contain whole open reading frame.

cDNA and Predicted Protein Sequence of the Chondroitin 4-Sulfotransferase—The nucleotide sequence of the C4ST cDNA and the predicted amino acid sequence are shown in Fig. 2A. The amino-terminal sequence upstream from the putative transmembrane domain contains three in-frame ATG codons. A single open reading frame beginning at the first ATG codon predicts a protein of 352 amino acid residues with a molecular mass of 41,629 Da with four potential N-linked glycosylation sites. To determine the location of any transmembrane domain, a hydropathy plot was generated from the translated sequence. Analysis of the plot revealed one prominent hydrophobic segment in the amino-terminal region, 16 residues in length, that extends from amino acid residues 18–33 (Fig. 2B). The NH2-terminal amino acid sequence of the purified C4ST was found in amino acid residues 58–66. The molecular mass of the predicted protein truncated at the amino acid residue 58 was 34,962 Da, which agreed well with the molecular mass of the protein formed after N-glycanase digestion. All the amino acid sequences that have been obtained from the purified protein were found in the predicted protein sequences, although two amino acid residues among these peptides (Arg in peptide 5 and Gln in peptide 6) did not agree with the predicted sequence (Gln291 and Gly277, respectively), confirming that the cDNA clone encodes the purified C4ST protein.

The amino acid sequence of C4ST showed no significant homology with other sulfotransferase so far cloned, except for HNK-1 sulfotransferase (34, 35). Comparison of the sequence of C4ST with human HNK-1 sulfotransferase revealed ~29% identity and ~48% similarity at the amino acid level (Fig. 3).

Expression of Chondroitin 4-Sulfotransferase cDNA in COS-7 Cells—Direct evidence demonstrating that the isolated cDNA encodes the chondroitin 4-sulfotransferase protein was obtained by expressing it in COS-7 cells. COS-7 cells were transfected with the pCXN4CAST, a recombinant plasmid containing the isolated cDNA in the mammalian expression vector pCXN2. The transfected cells were scraped at 67 h after transfection, extracted with gentle shaking in a buffer containing 0.5% Triton X-100, and centrifuged. Sulfotransferase activities in the supernatant fractions were determined using chondroitin or partially desulfated dermatan sulfate (27) as acceptors. Control experiments without vector, and with vector containing the cDNA in the reversed orientation (pCXN4CAST2), were also done. As shown in Table II, when the vector containing the isolated cDNA was used, the sulfotransferase activity, which transfers sulfate to position 4 of GalNAc residue of chondroitin, and the sulfotransferase activity, which transfers sulfate to position 4 of GalNAc residue of partially desulfated dermatan sulfate, were increased 25- and 6-fold, respectively, above those of controls. In contrast, sulfotransferase activity, which transfers sulfate to position 6 of GalNAc residue, was not increased at all. These results demonstrate that the isolated cDNA encodes a protein with sulfotransferase activity identical to the purified C4ST. In Table II, C6ST activity was detected when chondroitin or partially desulfated dermatan sulfate was added to the reaction mixtures. C6ST activity observed in the presence of these acceptors appears to be due to the endogenous enzyme derived from COS-7 cells, since C6ST activity was not overexpressed. To confirm this, we prepared FLAG-C4ST fusion protein and purified with an anti-FLAG monoclonal antibody-conjugated affinity column. The affinity-purified FLAG-C4ST fusion protein was incubated with chondroitin or partially desulfated dermatan sulfate and [35S]PAPS. When the [35S]-labeled chondroitin and the [35S]-labeled partially desulfated dermatan sulfate were digested with chondroitinase B, the digestion at position 4 was detected.
ACII and chondroitinase ABC, respectively, radioactivity was detected only at the position of ΔDi-4S (Fig. 4A and B). When ³⁵S-labeled partially desulfated dermatan sulfate was digested with chondroitinase ACII, about 50% of the total radioactivity was recovered in ΔDi-4S and the remainder appeared in minor peaks with higher retention time (Fig. 4C). These results were identical to those observed previously in the purified C4ST (27). The minor peaks with higher retention time observed when digested with chondroitinase ACII appear to be oligosaccharides as shown previously (27). These results clearly indicate that the expressed FLAG-C4ST fusion protein is able to transfer sulfate only to position 4 of GalNAc residue of chondroitin or partially desulfated dermatan sulfate.

**Northern Blot Analysis—**Among various mouse tissues, C4ST mRNA with 5.7 kilobases was expressed mainly in the brain and kidney (Fig. 5). A weak expression was also observed in the heart, spleen, and lung. A small fragment observed in the testis may not be an intact mRNA, but a degradation product.

**DISCUSSION**

We have cloned a cDNA that encodes the C4ST. The predicted sequence of the protein contains all of the known amino acid sequence and four potential N-linked glycosylation sites. When the cDNA was introduced into a eukaryotic expression vector and transfected in COS-7 cells, the C4ST activity was overexpressed. The predicted protein showed no significant sequence homology with other sulfotransferases except for HNK-1 sulfotransferase. C4ST transfers sulfate to position 4 of GalNAc residue, whereas HNK-1 sulfotransferase transfers sulfate to position 3 of nonreducing terminal GlcA residue. Although acceptor substrate specificity is quite different from each other, C4ST and HNK-1 sulfotransferase seem to belong to a common sulfotransferase family.

Two putative PAPS binding sites were found by x-ray crystallography of estrogen sulfotransferase performed in the presence of adenosine 3′, 5′-bisphosphate (36): one is RSGT for 5′-phosphosulfate binding site and another is RXS for 3′-phosphate binding site. These motifs were observed in every glycosaminoglycan sulfotransferases so far cloned except for heparan sulfate N-deacetylsulfate/N-sulfotransferase, in which the 3′-phosphate binding site is not RXS but IX-S (37). In the predicted mouse C4ST, these motifs were observed; R¹⁵⁵ACT for 5′-phosphosulfate binding site and R¹⁴⁸PPERLVS for 3′-phosphate binding site. It is of note that Cys is present in 5′-phosphosulfate binding site. C4ST was found to require sulfhydryl compounds for the activity. The presence of Cys in the 5′-phosphosulfate binding motif may relevant to the requirement for sulfhydryl compounds.

Some inconsistency in amino acid sequence was observed between peptides obtained from the purified rat C4ST and the predicted mouse C4ST; Arg in peptide 5 and Gln in peptide 6 were changed to Gln and Gly, respectively, in the predicted mouse C4ST. Since peptide 3 adjacent to the carboxyl side of peptide 5 was obtained after trypsin digestion, Arg in peptide 5 must be correct, and the change of Arg to Gln in peptide 5 may reflect the difference between rat C4ST and mouse C4ST. In contrast, it is unlikely that change of Gln to Gly in peptide 6 is due to the species variation, because this change should not be attained after single point mutation. It is most probable that we made an error in the assignment of amino acid residue of peptide 6.
that multiple forms of C4ST may be present in the tissues. Unexpectedly, C4ST showed significant homology in the amino acid sequence with HK-1 sulfotransferase. Oligosaccharides containing 3-O-sulfated GlcA, which was structurally related to the proteoglycan linkage region, have been found in the culture medium of human fibroblast (47) and human urinary thrombomodulin (48). It remains to be determined whether C4ST may share not only amino acid sequence but also substrate specificity with HK-1 sulfotransferase.

Acknowledgments—We thank Dr. Jun-Ichi Miyazaki, Department of Disease-related Gene Regulation, Faculty of Medicine, University of Tokyo and Dr. Yasuhiro Hashimoto, Tokyo Metropolitan Institute of Medical Sciences for donating pCJNX2 expression vector.

REFERENCES

1. Kjellén, L., and Lindahl, U. (1991) Annu. Rev. Biochem. 60, 433–475
2. Iozzo, R. V. (1998) Annu. Rev. Biochem. 67, 609–652
3. Pennybacker, J. P., Kimata, K., and Brown, K. S. (1981) Dev. Biol. 81, 280–287
4. Sugahara, K., and Schwartz, N. B. (1982) Arch. Biochem. Biophys. 214, 589–601
5. Rossi, A., Bonventre, J. V., Delezioso, A.-L., Cetta, G., and Superti-Furga, A. (1991) J. Biol. Chem. 267, 18456–18464
6. Ruoslahti, E. (1996) Glycobiology 6, 489–492
7. Oohira, A., Aono, S., Matsui, F., Yasuda, Y., and Tokita, Y. (1996) Connective Tissue Res. 30, 49–56
8. Faisser, A., Clement, A., Locher, A., Streit, A., Mandl, C., and Schachner, M. (1994) J. Cell Biol. 126, 783–799
9. Friedlander, D. R., Miler, P., Karihikian, L., Margolis, R. K., Margolis, R. U., and Grunet, M. (1994) J. Biol. Chem. 269, 669–680
10. Maeda, N., and Noda, M. (1996) Development (Camb.) 122, 647–658
11. Yamada, H., Fredette, B., Shiota, K., Hagihasu, K., Miura, R., Ransh, B., Stickney, W. B., and Yashiro, Y. (1997) J. Neurosci. 17, 7774–7785
12. Dou, C.-L., and Levine, J. M. (1993) J. Neurochem. 68, 1021–1030
13. Maeda, N., and Noda, M. (1998) J. Cell Biol. 142, 203–216
14. Kappler, L. J., Stachowiak, U., Stöcker, C., Hausser, H.-J., Kresse, H., and Müller, H. W. (1993) J. Biol. Chem. 268, 306–318
15. Toyama-Sorimachi, N., Sorimachi, H., Tobita, Y., Kitamura, F., Yagita, H., Suzuki, K., and Miyasaka, M. (1995) J. Biol. Chem. 270, 7434–7444
16. Naujokas, M. F., Morin, M., Anderson, M. S., Peterson, M., and Miller, J. (1993) Cell 74, 257–268
17. Sartipy, P., Johansen, B., Caseme, G., Rosengren, B., Bondjers, G., and Hurtig-Wennander, K. (1995) J. Biol. Chem. 270, 26297–26314
18. Michal, F., and Duffy, P. E. (1995) Science 272, 1502–1504
19. Kawashima, H., Li, Y. F., Watanabe, N., Hirose, J., Hirose, M., and Miyasaka, M. (1999) Int. Immunol. 11, 383–405
20. Kimata, K., Okayama, M., Oohira, A., and Suzuki, S. (1973) Mol. Cell. Biochem. 1, 211–228
21. Habuchi, H., Kimata, K., and Suzuki, S. (1986) J. Biol. Chem. 261, 1031–1040
22. Kita, H., Tsuchiya, K., Tone, Y., and Sugahara, K. (1997) J. Biol. Chem. 272, 31377–31381
23. Adany, R., Heimer, R., Catezors, B., Sorrell, J. M., and Iozzo, R. V. (1990) J. Biol. Chem. 265, 11389–11396
24. Habuchi, O., Matsu, Y., Kato, Y., Oyama, Y., Yasuda, Y., and Noda, M. (1993) J. Biol. Chem. 268, 21968–21974
25. Fukuta, M., Uchimura, K., Nakashima, K., Kato, M., Kimata, K., Shimomura, M., and Habuchi, O. (1993) J. Biol. Chem. 268, 18575–18585
26. Kobayashi, M., Sugumaran, G., Liu, J., Shworak, N. W., Silbert, J. E., and Rosenberg, R. D. (1999) J. Biol. Chem. 274, 10474–10480
27. Yamauchi, S., Hirahara, U., Usui, T., Takeda, Y., Hoshino, M., Fukuta, M., Kimura, H., and Habuchi, O. (1999) J. Biol. Chem. 274, 2456–2463
28. Laemmli, U. K. (1970) Nature 227, 680–685
29. Iwamatsu, A. (1992) Electrophoresis 13, 142–147
30. Kikut, M., and Oka, S. (1999) J. Biochem. 125, 21968–21974
31. Delfert, D. M., and Conrad, H. E. (1985) J. Biol. Chem. 260, 725–735
32. Nishiyama, A., Dahlin, K. J., Prince, J. T., Johnstone, S. R., and Stallcup, W. B. (1997) J. Biol. Chem. 272, 19536–19574
33. Bakker, H., Friedmann, I., Oka, S., Kawasaki, T., Nifant’ev, N., Schaner, M., and Mantei, N. (1997) J. Biol. Chem. 272, 7434–7444
34. Bakker, H., Friedmann, I., Oka, S., Kawasaki, T., Nifant’ev, N., Schaner, M., and Mantei, N. (1997) J. Biol. Chem. 272, 7434–7444
35. Fiig, P. A., and Oka, S. (1998) Anal. Chem. 70, 6–13
36. Noda, M., and Kimata, K. (1991) J. Cell Biol. 114, 211–228
37. Habuchi, H., Kimata, K., and Suzuki, S. (1986) J. Biol. Chem. 261, 1031–1040
38. Kozak, M. (1989) J. Biol. Chem. 264, 108, 260, 270, 272, 273, 276, 294, 296, 297, 316, 318, 320
39. Faissner, A., Clement, A., Lochter, A., Streit, A., Mandl, C., and Shachner, M. (1994) J. Cell Biol. 126, 783–799
40. Rouch, P., Rauch, U., Flax, M., Margolis, R. K., and Margolis, R. U. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2512–2516
41. Nishiyama, A., Dahlin, K. J., Prince, J. T., Johnstone, S. R., and Stallcup, W. B. (1991) J. Cell Biol. 114, 359–371
42. Watanabe, A., Maeda, N., Matsu, F., Kushima, Y., Noda, M., and Oohira, A.
Cloning of Chondroitin 4-Sulfotransferase

45. Thomas, G. J., Shewring, L., MacCarthy, K. J., Couchman, J. R., Mason, R. M., and Davies, M. (1995) *Kidney Int.* 48, 1278–1289

46. Uchimura, K., Kadomatsu, K., Fan, Q.-W., Muramatsu, H., Kurowsawa, N., Kaname, T., Yamamura, K., Fukuta, M., Habuchi, O., and Muramatsu, T. (1998) *Glycobiology* 8, 489–496

47. Shibata, S., Takagaki, K., Nakamura, T., Izumi, J., Kojima, K., Kato, I., and Endo, M. (1995) *J. Biol. Chem.* 270, 13794–13798

48. Wakabayashi, H., Natsuka, S., Mega, T., Otsuki, N., Isaji, M., Naotsuka, M., Koyama, S., Kanamori, T., Sakai, K., and Hase, S. (1999) *J. Biol. Chem.* 274, 5436–5442

49. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* 157, 105–132
Molecular Cloning and Expression of Chondroitin 4-Sulfotransferase
Shinobu Yamauchi, Satoka Mita, Taeko Matsubara, Masakazu Fukuta, Hiroko Habuchi,
Koji Kimata and Osami Habuchi

J. Biol. Chem. 2000, 275:8975-8981.
doi: 10.1074/jbc.275.12.8975

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