Molecular Cloning and Expression of a Human Chondroitin Synthase

We have identified a human chondroitin synthase from the HUGE (human unidentified gene-encoded large proteins) protein data base by screening with two keywords: “one transmembrane domain” and “galactosyltransferase family.” The identified protein consists of 802 amino acids with a type II transmembrane protein topology. The protein showed weak homology to the β1,3-galactosyltransferase family on the amino-terminal side and to the β1,4-galactosyltransferase family on the carboxyl-terminal side. The expression of a soluble recombinant form of the protein in COS-1 cells produced an active enzyme, which transferred not only the glucuronic acid (GlcUA) from UDP-[1-14C]GlcUA but also N-acetylgalactosamine (GalNAc) from UDP-[3H]GalNAc to the polymer chondroitin. Identification of the reaction products demonstrated that the enzyme was chondroitin synthase, with both β1,3-GlcUA transferase and β1,4-GalNAc transferase activities. The coding region of the chondroitin synthase was divided into three discrete exons and localized to chromosome 15. Northern blot analysis revealed that the chondroitin synthase was divided into three discrete exons and localized to chromosome 15. The protein showed weak homology to the β1,3-galactosyltransferase family on the amino-terminal side and to the β1,4-galactosyltransferase family on the carboxyl-terminal side. The expression of a soluble recombinant form of the protein in COS-1 cells produced an active enzyme, which transferred not only the glucuronic acid (GlcUA) from UDP-[1-14C]GlcUA but also N-acetylgalactosamine (GalNAc) from UDP-[3H]GalNAc to the polymer chondroitin. Identification of the reaction products demonstrated that the enzyme was chondroitin synthase, with both β1,3-GlcUA transferase and β1,4-GalNAc transferase activities. The coding region of the chondroitin synthase was divided into three discrete exons and localized to chromosome 15. Northern blot analysis revealed that the chondroitin synthase gene exhibited ubiquitous but markedly differential expression in the human tissues examined. Thus, we demonstrated that analogous to human heparan sulfate polymerases, the single polypeptide chondroitin synthase possesses two glycosyltransferase activities required for chain polymerization.

Chondroitin sulfate belongs to the glycosaminoglycan family and occurs as proteoglycans that are expressed on the surface of most cells and in extracellular matrices. They are covalently linked to a wide range of core protein families. The glycosaminoglycan chains are increasingly implicated as important regulators of many biological processes, such as cell proliferation and recognition, extracellular matrix deposition, and morphogenesis (for reviews see Refs. 1–3). Recently, chondroitin sulfate as well as heparan sulfate have attracted much attention because they play an important role in neural network formation in the developing mammalian brain (for reviews see Refs. 4 and 5). Although a number of sulfotransferases involved in chondroitin sulfate synthesis as well as glycosyl- and sulfotransferases required for heparan sulfate synthesis have been molecularly cloned (for a review see Ref. 6), glycosyltransferases that are responsible for chondroitin sulfate have not been cloned except for a bacterial chondroitin synthase (7). This lack of cloning has hampered vigorous investigation into the biological functions of this essential molecular species in mammalian systems.

Sulfated glycosaminoglycans including chondroitin sulfate and heparin/heparan sulfate have a linear polymer structure that is composed of repeating disaccharide units of glucuronic acid (GlcUA) and N-acetylgalactosamine (GalNAc) or GlcUA and GlcNAc, respectively. Both chains are covalently attached to serine residues in core proteins via a unique tetrasaccharide structure, GlcUA(1–3)Gal(1–4)Xyl(1–3)GalNAc(1–4)Gal(1–3)Gal(1–4)Xyl(1–3)GalNAc (for reviews see Refs. 8 and 9). In glycosaminoglycan biosynthesis, the disaccharide units are formed by alternate monosaccharide addition from corresponding UDP sugars to the nonreducing end of the elongating chain (for reviews, see Refs. 9 and 10). Sulfotransferases involved in the biosynthesis of the repeating disaccharide region of heparan/heparan sulfate have been purified from bovine serum, and cDNA cloning has demonstrated that a single polypeptide catalyzes both GlcNAc and GlcUA transferase reactions, designated heparan sulfate polymerase, and that it is encoded by a member of the hereditary multiple exostoses gene family of tumor suppressors (11). In contrast, although GlcUA transferase II (GlcAT-II) and GalNAc transferase II (GalNAcT-II) that are involved in the biosynthesis of chondroitin sulfate disaccharide units have been extensively purified from chick cartilage (12) and fetal bovine serum (13), cDNA cloning has not been achieved because of the difficulty in purifying the enzymes to homogeneity.

We previously reported that the GlcAT-II and GalNAcT-II responsible for chondroitin sulfate biosynthesis were coeluted at 160 kDa by gel filtration and were not separable through various chromatographies (13). Hence, we hypothesized that analogous to human heparan sulfate polymerases, a single large polypeptide, namely chondroitin synthase with GlcAT-II and GalNAcT-II activities, would be responsible for chondroitin sulfate biosynthesis. To clone this putative human chondroitin synthase, we took advantage of the HUGE (human unidentified gene-encoded large proteins) protein data base at the Ka- gashinada-ku, Kobe 658-8558, Japan. Tel.: 81-78-441-7570; Fax: 81-78-441-7571; E-mail: k-sugar@kobepharma-u.ac.jp.

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EXPERIMENTAL PROCEDURES

Materials—UDP-[U-14C]GlcUA (285.2 mCi/mmol), UDP-[3H]GalNAc (10 Ci/mmol), UDP-[3H]GalNAc (60 Ci/mmol), and UDP-[3H]Gal (15 Ci/mmol). The abbreviations used are: GlcUA, D-glucuronic acid; GalNAc, N-acetyl-D-galactosamine; GlcAT-II, β1,4-N-acetylgalactosaminyltransferase II; GlcNAc, N-acetyl-D-glucosamine; GlcAT, β1,3-glucurononyltransferase; kb, kilobase(s); MES, 4-morpholineethanesulfonic acid.
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Cuv/ml) were purchased from PerkinElmer Life Sciences. Unlabeled UDP-GlcUA, UDP-GalNAc, UDP-GlcNAc, and UDP-Gal were obtained from Sigma. Chondroitin (a chemically desulfated derivative of whale cartilage chondroitin sulfate A), various chondroitin sulfate variants, and Arthrobacter aurouscens chondritolase (EC 4.5.2.5) were purchased from Seikagaku Corp. (Tokyo, Japan). Galβ-3-Galβ-4Xyl was a gift from Dr. Nancy B. Schwartz (University of Chicago). Purified α-thrombomodulin (15) was provided by the Research Institute of Dui-ichi Pharmaceutical Co. (Tokyo, Japan) and purified linkase (triacetylchondroitin sulfate, bovine liver, 1000 units/mg) from Sigma. Chondroitin (a chemically desulfated derivative of whale cartilage chondroitin sulfates A), various chondroitin sulfate variants, and Arthrobacter aurouscens chondritolase (EC 4.5.2.5) were purchased from Seikagaku Corp. (Tokyo, Japan). Galβ-3-Galβ-4Xyl was a gift from Dr. Nancy B. Schwartz (University of Chicago). Purified α-thrombomodulin (15) was provided by the Research Institute of Dui-ichi Pharmaceutical Co. (Tokyo, Japan) and purified linkase (triacetylchondroitin sulfate, bovine liver, 1000 units/mg) from Sigma.

Expression of a Soluble Form of the Novel Glycosyltransferase and Enzyme Assay—The expression plasmid (6.7 μg) was transfected into COS-1 cells on 100-mm plates using FuGENETM I (Roche Molecular Biochemicals) according to the manufacturer's instructions. Two days after transfection, 1 ml of the culture medium was collected and incubated with 10 μl of IgG-Sepharose (Amersham Pharmacia Biotech) for 1 h at 4 °C. The beads recovered by centrifugation were washed with and then resuspended in the assay buffer and tested for GalNAc transferase, GlcUA transferase, Gal transferase, and GlcNAc transferase activities, as described below. Acceptors used for Gal transferase activities were asialo-ovine submaxillary mucin (300 μg) or GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc (1 nmol). The assay mixture contained 10 μl of the resuspended beads, an acceptor substrate, 8.57 μM UDP-[3H]GalNAc (5.60 × 10^5 dpm), 50 mM MES buffer, pH 6.5, 10 mM MnCl2, and 171 μM sodium citrate in a total volume of 30 μl (25). The assay mixture for Gal transferase was similar, except that the acceptor substrate was 40 μM UDP-[3H]GalNAc (3.00 × 10^5 dpm) and 100 μM sodium citrate in a total volume of 30 μl (26). After incubation for 2 h at 37 °C, the assay mixtures were analyzed by gel filtration on a Superdex Peptide column equilibrated with 30 mM sodium citrate buffer, pH 6.0, or with 22 mM of β-glucuronidase in a total volume of 30 μl of 0.05 M sodium citrate buffer, pH 6.0, or with 22 mM of β-glucuronidase in a total volume of 30 μl of 0.05 M sodium citrate buffer, pH 6.0, or with 22 mM of β-glucuronidase in a total volume of 30 μl of 0.05 M sodium citrate buffer, pH 6.0, or with 22 mM of β-glucuronidase in a total volume of 30 μl of 0.05 M sodium citrate buffer, pH 6.0.

RESULTS

In Silico Cloning of a Putative Human Chondroitin Synthase cDNA—Two keywords, “one transmembrane domain” and “galactosyltransferase family,” were used to screen the HUGe protein data base (www.kazusa.or.jp/huge/) at the Kazusa DNA Research Institute (Chiba, Japan) with two keywords, “one transmembrane domain” and “galactosyltransferase family,” revealed a clone (ID KIAA0990; GenBankTM accession number AB023207) that was then obtained from the Kazusa DNA Research Institute. Analysis of this clone revealed a single open reading frame with weak sequence similarity to human core 1 UDP-Gal:GalNAc-R β1,3-Gal transferase (GenBankTM accession number AF155582) on the 5′-untranslated region of 494 base pairs, a single open reading frame with weak sequence similarity to human core 1 UDP-Gal:GalNAc-R β1,3-Gal transferase (GenBankTM accession number AB024434) on the 5′-untranslated region of 1.7 kb with a presumptive polyadenylation signal (accession number NT 012074.3) identical to the cDNA sequence. Comparison between the cDNA and the genome sequence revealed the genomic organization of this novel glycosyltransferase gene.

Northern blot analysis (CLONTECH) membrane was used for the analysis. One μg of polyadenylated RNA was loaded in each lane. The membrane was probed with a gel purified, radiolabeled (~1 × 10^6 cpm/μg), 0.84-kb chondroitin synthase-specific fragment corresponding to nucleotides 631-1469 of the KIAA0990 cDNA (GenBankTM accession number AB023207).

RESULTS

In Silico Cloning of a Putative Human Chondroitin Synthase cDNA—Two keywords, “one transmembrane domain” and “galactosyltransferase family,” were used to screen the HUGe protein data base, because a type II transmembrane protein topology is characteristic of many other glycosyltransferases cloned to date, and GalNACT-II is a β1,4-GalNAc transferase that might belong to β1,4-Gal transferase family. HUGe database screening identified a clone (KIAA0990) containing a 5′-untranslated region of 494 base pairs, a single open reading frame of 631-1469 of the KIAA0990 cDNA (GenBankTM accession number AB023207).

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predicting that the protein has a type II transmembrane topology characteristic of many Golgi-localized glycosyltransferases cloned to date (Fig. 1). Data base searches indicated that the amino acid sequence displayed weak sequence similarity to human core 1 UDP-Gal:GalNAc 1,3-Gal transferase (GenBank™ accession number AF155582) on the amino-terminal side and to human UDP-Gal:GlcNAc 1,4-Gal transferase II (GenBank™ accession number AB024434) on the carboxyl-terminal side. A characteristic feature of homologous glycosyltransferase genes is that different members may encode enzymes that have different donor or acceptor sugar specificities, but the nature of the sugar linkage formed is often retained (31). Thus, the features of the identified protein sequence suggest that the identified gene product might possess both 1,3-GlcUA transferase (GlcAT-II) and 1,4-GalNAc transferase (GalNAcT-II) activities. In addition, a homologue of the identified human gene was found in the Caenorhabditis elegans or Drosophila genome. The sequences and alignment of the hypothetical proteins from human, C. elegans, and Drosophila are shown in Fig. 1. The human sequence shared 36 and 42% identity with that of C. elegans and Drosophila, respectively. Each of the three proteins contained DDD on the amino-terminal side and DXXD on the carboxyl-terminal side (Fig. 1), both of which appear to correspond to the conserved DXXD motif found in most glycosyltransferases (32).

Genomic Organization and Chromosomal Localization—Comparison of the identified cDNA sequence with the genome sequence deposited in the Human Genome Project Data Base revealed the genomic structure and chromosomal localization of the gene. The gene spans over 40 kb, and the coding region of the gene was divided into three discrete exons as shown in Fig. 2. The intron/exon junctions followed the GT/AG rule (33) and were flanked by conserved sequences (data not shown). This gene is located on human chromosome 15.

Expression of a Soluble Form of the Putative Glycosyltransferase and Characterization as Chondroitin Synthase—To facilitate the functional analysis of the putative glycosyltransferase, a soluble form of the protein was generated by replacing the first 46 amino acids of the putative glycosyltransferase with a cleavable insulin signal sequence and a protein A IgG-binding domain as described under “Experimental Procedures,” and then the soluble putative glycosyltransferase was expressed in COS-1 cells as a recombinant enzyme fused with the protein A IgG-binding domain. The fused enzyme expressed in the medium was adsorbed onto IgG-Sepharose beads to eliminate endogenous glycosyltransferases, and then the en-
zyme-bound beads were used as an enzyme source. The bound fusion protein was assayed for glycosyltransferase activity using a variety of acceptor and donor substrates. As shown in Table I, the activity was detected using polymer chondroitin with either UDP-GlcUA or UDP-GalNAc and using chondroitin sulfate C with UDP-GalNAc. In addition, a weak activity was detected using chondroitin sulfate A with UDP-GlcUA and using chondroitin sulfate C with UDP-GalNAc, respectively (Table II). In contrast, no other activities were detected using other acceptor substrates with either UDP-GlcUA, UDP-GalNAc, UDP-GlcNAc, or UDP-Gal as a donor substrate. These included GlcAT-I, GalNAc transferase I involved in the initiation of chondroitin sulfate biosynthesis, hyaluronic synthase, core 1 UDP-Gal:GalNAc-R β1,3,4-Gal transferase, and UDP-GlcNAcβ-R β1,4-Gal transferase activities. No detectable glycosyltransferase activity was recovered from a Superdex Peptide column subjected to digestion with chondroitinase AC-II as described under “Experimental Procedures.” The undigested sample (filled squares), the chondroitinase AC-II digest (filled circles), or the β-gluconuridase digest (filled triangles) was applied to a column of Superdex Peptide, and the respective effluent fractions (0.4 ml each) were analyzed for radioactivity. Arrows indicate the elution positions of the authentic saturated disaccharide (arrow 1, UDP-GlcAβ1–3Galβ1–4Xy1; arrow 2, [3H]GalNAc). B, the GalNAc transferase reaction products recovered from a Superdex Peptide column were subjected to digestion with chondroitinase AC-II as described under “Experimental Procedures.” The undigested sample (filled squares) or the chondroitinase AC-II digest (filled circles) was applied to a column of Superdex Peptide, and the respective effluent fractions (0.4 ml each) were analyzed for radioactivity. Arrows indicate the elution positions of the authentic saturated disaccharide (arrow 1, GlcUAβ1–3Galβ1–4Xy1; arrow 2, [3H]GalNAc).

![Image](368x306 to 495x530)

FIG. 3. Identification of the putative human glycosyltransferase reaction products. A, the GlcUA transferase reaction products recovered from a Superdex Peptide column were subjected to digestion with chondroitinase AC-II or β-glucuronidase as described under “Experimental Procedures.” The undigested sample (filled squares), the chondroitinase AC-II digest (filled circles), or the β-glucuronidase digest (filled triangles) was applied to a column of Superdex Peptide, and the respective effluent fractions (0.4 ml each) were analyzed for radioactivity. Arrows indicate the elution positions of the authentic saturated disaccharide (arrow 1, UDP-GlcAβ1–3Galβ1–4Xy1; arrow 2, [3H]GalNAc). B, the GalNAc transferase reaction products recovered from a Superdex Peptide column were subjected to digestion with chondroitinase AC-II as described under “Experimental Procedures.” The undigested sample (filled squares) or the chondroitinase AC-II digest (filled circles) was applied to a column of Superdex Peptide, and the respective effluent fractions (0.4 ml each) were analyzed for radioactivity. Arrows indicate the elution positions of the authentic saturated disaccharide (arrow 1, GlcUAβ1–3Galβ1–4Xy1; arrow 2, [3H]GalNAc).

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**TABLE I**

Acceptor specificity of the glycosyltransferase secreted into the culture medium by transfected COS-I cells towards polymer chondroitin and chondroitin sulfate

| Acceptor          | Donor        | Activity[^a^] pmol/ml medium/h |
|-------------------|--------------|-------------------------------|
| Chondroitin       | UDP-GlcUA    | 5.2[^f^]                      |
| Galβ1–3Galβ1–4Xy1 | UDP-GlcUA    | ND[^d^]                       |
| GlcUAβ1–3GalNAc   | UDP-GalNAc   | 1.4[^e^]                      |
| GlcUAβ1–3GalNAc   | UDP-GalNAc   | 0.4                           |
| GlcUAβ1–3Galβ1–4Xy1–O-Ser | UDP-GalNAc | ND                            |
| α-Thrombomodulin  | UDP-GalNAc   | ND                            |
| (GlcUAβ1–3GalNAc) | UDP-GalNAc   | ND                            |
| Asialo-ovine submaxillary mucin[^c^] | UDP-Gal | ND                            |
| GlcNAcβ1–3Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAc | UDP-Gal | ND                            |

[^a^]: The values represent the averages of two independent experiments.
[^b^]: α-Thrombomodulin contains a tetrasaccharide linkage GlcUAβ1–3Galβ1–3Galβ1–4Xy1 (16).
[^c^]: Asialo-ovine submaxillary mucin possesses a large number of GalNAc1-O-Ser/Thr residues.
[^d^]: Under the incubation conditions used, GlcUA or GalNAc incorporation into polymer chondroitin was proportional to the incubation time up to 8 h (data not shown).
[^e^]: ND, not detected (<0.1 pmol/ml medium/h).

[^f^]: The values represent the averages of the two independent experiments.

Under the incubation conditions used, GlcUA or GalNAc incorporation into polymer chondroitin was proportional to the incubation time up to 8 h (data not shown).

[^g^]: ND, not detected (<0.1 pmol/ml medium/h).
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III, quantitatively yielding a 3H-labeled peak at the position of free [3H]GalNAc, which was separable from GlcUA

lane 4

skeletal muscle; under

were hybridized with a probe for the chondroitin synthase, as described

human tissues.

II, quantitatively yielding a 3H-labeled peak at the position of

chondroitin or chondroitin 4-sulfate is found in

C. elegans

but present in higher eukaryotic species such as

human chondroitin synthase is absent in prokaryotes or yeast

droitin synthase described here. In fact, a homologue of the

Notably, however, the bacterial enzyme, composed of 965

chondroitin or chondroitin-like capsular polysaccharides (7).

activities was molecularly cloned from bacteria, which produce

GlcNAc transferase activities (11, 34). Recently, a chondroitin

sulfate polymerases that have both GlcUA transferase and

GalNAc transferase activities but no GalNAc transferase I activity,

that was highly purified from bovine serum had GalNAcT-II

contrast, the chondroitin sulfate-synthesizing enzyme system

has been observed for other Golgi glycosyltransferases (38, 39). In this context, it is noteworthy that the GlcAT-II purified from

chick cartilage microsomes has been reported to have a molecular mass of 80 kDa as determined by SDS-polyacrylamide gel electrophoresis (12). It will be interesting to evaluate whether it has GalNAcT-II activity as well. The cDNA probe for the human chondroitin synthase has now become available and will be a useful tool for investigating the biological functions of chondroitin sulfate, which is ubiquitously expressed and plays an indispensable role in many tissues, particularly the brain. It will also help investigating the possible association of the gene with any disease pathology.

It is presently unknown why GlcAT-II and GalNAcT-II activities detected in the assays were low with polymer chondroitin and chondro-oligosaccharides as acceptor substrates (Table I). Incubation of polymer chondroitin with the recombinant chondroitin synthase in the presence of both UDP-GlcUA and UDP-GalNAc did not augment the chondroitin synthase activity (data not shown). Rather, it is possible that the recombinant chondroitin synthase might not be secreted well into the culture medium because the transfecant homogenates showed higher chondroitin synthase activity than the medium. More efforts to address this issue will be required. Besides, because the GlcUA or GalNAc incorporation into polymer chondroitin was proportional to the incubation time at least for 8 h (data not shown), the difference between the GlcUA and GalNAc transfer to polymer chondroitin (Table I) may reflect the abundance of nonreducing terminal GlcUA and GalNAc on polymer chondroitin.

Characterization of the acceptor substrate specificity of the human recombinant chondroitin synthase revealed that the enzyme showed marked specificity for polymer chondroitin and chondro-oligosaccharides (Table I and II). Neither a trisaccharide linkage, Galβ1–3Galβ1–4Xylβ1, nor α-thrombomodulin containing a tetrasaccharide linkage, GlcUAβ1–3Galβ1–3Galβ1–4Xylβ1, on the native core protein (16) was used as an acceptor substrate. These findings demonstrate that the transfer of GlcUA to polymer chondroitin is mediated by GlcAT-II, distinct from the GlcAT-I that was previously cloned and was involved in the formation of the tetrasaccharide linkage GlcUAβ1–3Galβ1–3Galβ1–4Xyl (24). Additionally, the transfer of GalNAc to polymer chondroitin is mediated by GalNAcT-II, distinct from GalNAc transferase I involved in the initiation of chondroitin sulfate biosynthesis (40). Hence, it is now clear that at least one other gene encoding GalNAc transferase I likely exists in the human genome. Thus, it should be emphasized that a single GalNAc transferase has been proposed to catalyze transferase reactions not only for chain elongation but also for chain initiation of chondroitin sulfate using the soluble enzyme system derived from serum-free culture medium of the human melanoma cell line (41). Both reactions are greatly stimulated by the α-thrombomodulin core protein, which appears to be recognized by the putative chondroitin synthase. In contrast, the chondroitin sulfate-synthesizing enzyme system that was highly purified from bovine serum had GalNAcT-II and GlcAT-II activities but no GalNAc transferase I activity, even when α-thrombomodulin was used as an acceptor (13). Hence, the chondroitin synthase identified here seems to be similar to or identical to the serum enzyme rather than the melanoma enzyme, which possibly possesses three enzyme activities: GalNAcT-II, GlcAT-II, and GalNAc transferase I. Indeed, data base searches yielded two other human genes that are homologous to the chondroitin synthase gene and had weak but significant sequence similarities and a conserved DXD

FIG. 4. Northern blot analysis of the chondroitin synthase in human tissues. Northern blots with RNA from various human tissues were hybridized with a probe for the chondroitin synthase, as described under “Experimental Procedures.” Lane 1, brain; lane 2, heart; lane 3, skeletal muscle; lane 4, colon; lane 5, thymus; lane 6, spleen; lane 7, kidney; lane 8, liver; lane 9, small intestine; lane 10, placenta; lane 11, lung; lane 12, peripheral blood leukocytes.

Expression Pattern of Chondroitin Synthase—Northern blot analysis of mRNA demonstrated a single band of ~5.0 kb for all human tissues examined (Fig. 4). The chondroitin synthase gene exhibited ubiquitous but differential expression in the human tissues examined. Notably, expression was particularly abundant in placenta, followed by spleen, lung, and peripheral blood leukocytes. These findings are in accordance with the observations that chondroitin sulfate proteoglycans are distributed on the surfaces of most cells and in the extracellular matrices in virtually every tissue.

DISCUSSION

We have identified a human chondroitin synthase and demonstrated that the single polypeptide possessed both GlcAT-II and GalNAcT-II activities. This feature is similar to heparan sulfate polymerases that have both GlcUA transferase and GlcNAc transferase activities (11, 34). Recently, a chondroitin synthase with both GlcUA transferase and GalNAc transferase activities was molecularly cloned from bacteria, which produce chondroitin or chondroitin-like capsular polysaccharides (7). Notably, however, the bacterial enzyme, composed of 965 amino acid residues, shows no homology with the human chondroitin synthase described here. In fact, a homologue of the human chondroitin synthase is absent in prokaryotes or yeast but present in higher eukaryotic species such as C. elegans or Drosophila (Fig. 1), being consistent with the findings that chondroitin or chondroitin 4-sulfate is found in C. elegans and Drosophila, respectively (35–37). In addition, although the bacterial enzyme is 87% identical to the bacterial hyaluronan synthase at the nucleotide and the amino acid sequence levels, the human chondroitin synthase shows no homology with the three cloned human hyaluronan synthases. This seeming discrepancy is not unexpected, considering that the bacterial chondroitin synthase appears to be associated with plasma membranes in view of the production of capsular polysaccharides lacking core proteins, whereas vertebrate enzyme(s) are Golgi resident(s) and vertebrate chondroitin sulfate chains are covalently attached to core proteins. In view of the fact that the chondroitin synthesizing enzyme derived from bovine serum appears to have a molecular mass of 160 kDa as estimated by gel filtration (13), the cloned enzyme may form a dimer, as has been observed for other Golgi glycosyltransferases (38, 39). In this context, it is noteworthy that the GlcAT-II purified from chick cartilage microsomes has been reported to have a molecular mass of 80 kDa as determined by SDS-polyacrylamide gel electrophoresis (12). It will be interesting to evaluate whether it has GalNAcT-II activity as well. The cDNA probe for the human chondroitin synthase has now become available and will be a useful tool for investigating the biological functions of chondroitin sulfate, which is ubiquitously expressed and plays an indispensable role in many tissues, particularly the brain. It will also help investigating the possible association of the gene with any disease pathology.

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motif, suggesting that a few more enzymes may be involved in chondroitin sulfate biosynthesis. Characterization of the two gene products is now in progress.

Acknowledgments—We thank Drs. T. Ogawa and K. Yoshida for the enzyme substrates. We also thank M. Tange for technical assistance and the Kazusa DNA Research Institute for providing the KIAA0990 cDNA.

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