ACCELERATED PUBLICATION

FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan–protein linkage region

Toshiyasu KOIKE*1, Tomomi IZUMIKAWA*1, Jun-Ichi TAMURA† and Hiroshi KITAGAWA*2

*Department of Biochemistry, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan, and †Department of Regional Environment, Faculty of Regional Sciences, Tottori University, Tottori 680-8551, Japan

INTRODUCTION

Sulfated GAGs (glycosaminoglycans), including HS (heparan sulfate) and CS (chondroitin sulfate), are linear polysaccharides consisting of a repetition of [-4GlcAβ1,3Galβ1,3Galβ1,4Xylβ1-O-Ser], of proteoglycans. Recent mutant analyses in zebrafish suggest that xylosyltransferase I and FAM20B, a protein of unknown function that shows weak similarity to a Golgi kinase encoded by four-jointed, operate in a linear pathway for proteoglycan production. In the present study, we identified FAM20B as a kinase that phosphorylates the xylose residue in the linkage region. Overexpression of FAM20B increased the amount of both chondroitin sulfate and heparan sulfate in HeLa cells, whereas the RNA interference of FAM20B resulted in a reduction of their amount in the cells. Gel-filtration analysis of the glycosaminoglycan chains synthesized in the overexpressing cells revealed that the glycosaminoglycan chains had a similar length to those in mock-transfected cells. These results suggest that FAM20B regulates the number of glycosaminoglycan chains by phosphorylating the xylose residue in the glycosaminoglycan–protein linkage region of proteoglycans.

Key words: chondroitin sulfate, FAM20B, glycosaminoglycan–protein linkage region, heparan sulfate, phosphorylation, xylose kinase.

2-O-phosphorylation of xylose has been detected in the glycosaminoglycan–protein linkage region, GlcAβ1,3Galβ1,3Galβ1,4Xylβ1-O-Ser, of proteoglycans. Recent mutant analyses in zebrafish suggest that xylosyltransferase I and FAM20B, a protein of unknown function that shows weak similarity to a Golgi kinase encoded by four-jointed, operate in a linear pathway for proteoglycan production. In the present study, we identified FAM20B as a kinase that phosphorylates the xylose residue in the linkage region. Overexpression of FAM20B increased the amount of both chondroitin sulfate and heparan sulfate in HeLa cells, whereas the RNA interference of FAM20B resulted in a reduction of their amount in the cells. Gel-filtration analysis of the glycosaminoglycan chains synthesized in the overexpressing cells revealed that the glycosaminoglycan chains had a similar length to those in mock-transfected cells. These results suggest that FAM20B regulates the number of glycosaminoglycan chains by phosphorylating the xylose residue in the glycosaminoglycan–protein linkage region of proteoglycans.

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Key words: chondroitin sulfate, FAM20B, glycosaminoglycan–protein linkage region, heparan sulfate, phosphorylation, xylose kinase.

The nucleotide sequence data reported in this paper for FAM20B have been submitted to the DDBJ, EMBL, GenBank® and GSDB Nucleotide Sequence Databases under accession number AB480690.
EXPERIMENTAL

Materials

[γ-32P]ATP (3000 Ci/mmol) was purchased from PerkinElmer. Unlabelled ATP and bovine liver β-glucuronidase (EC 3.2.1.31) were obtained from Sigma. Shrimp alkaline phosphatase was purchased from Roche Molecular Biochemicals. α-TM (α-thrombomodulin) with a truncated linkage region tetrasaccharide, GlcAβ1-3Galβ1-3Galβ1-4Xyl, was purified and structurally characterized as described previously [16,17]. Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser was chemically synthesized [18]. Galβ1-3Galβ1-4Xyl/β1-O-Ser was prepared by digestion of Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser with alkaline phosphatase.

Construction of a soluble form of FAM20B

The cDNA fragment of a truncated form of FAM20B, lacking the first 30 N-terminal amino acids, was amplified by PCR with KIAA 0475 cDNA obtained from the Kazusa DNA Research Institute (Chiba, Japan) as a template using a forward primer (5′-CGGGATCCTCAGCTGCCAACCGGGAGGAC-3′) containing an in-frame BamHI site and a reverse primer (5′-AGAGATCAAACCTGTCGCC-3′), was purified and structurally characterized as described previously [16,17]. Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser was chemically synthesized [18]. Galβ1-3Galβ1-4Xyl/β1-O-Ser was prepared by digestion of Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser with alkaline phosphatase.

Expression of a soluble form of FAM20B and enzyme assays

The expression plasmid (6.0 μg) was transfected into COS-1 cells on 100-mm-diameter plates using FuGENETM 6 (Roche Molecular Biochemicals) according to the manufacturer’s instructions. At 2 days after transfection, 1 ml of the culture medium was collected and incubated with 10 μl of IgG-Sepharose (GE Healthcare) for 1 h at 4°C. The beads recovered by centrifugation at 150 g for 2 min were washed with and then resuspended in the assay buffer, and tested for kinase activity, as described below. Assays for kinase activity were carried out using α-TM, Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser or Galβ1-3Galβ1-4Xyl-O-Ser as an acceptor and ATP as a phosphate donor. Kinase reactions were incubated in reaction mixtures containing the following components in a total volume of 20 μl: 1 nmol of α-TM, Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser or Galβ1-3Galβ1-4Xyl-O-Ser, 10 μM [γ-32P]ATP (1.11 × 106 d.p.m.), 50 mM Tris/HCl (pH 7.0), 10 mM MnCl2, 10 mM CaCl2, 0.1% BSA and 10 μl of the resuspended beads. The mixtures were incubated for 4 h at 37°C.

Characterization of the reaction products

Products of kinase reactions on α-TM were isolated by gel filtration on a Superdex peptide column with 0.2 M ammonium bicarbonate as the eluent. The 32P-labelled tetrasaccharide chains were released from α-TM by mild alkaline treatment using 0.5 M LiOH, derivatized with 2-aminobenzamide and then isolated by gel-filtration HPLC as described in [17]. The isolated tetrasaccharides were digested with β-glucuronidase in 50 mM sodium acetate (pH 5.2) or alkaline phosphatase in 50 mM Tris/HCl (pH 8.0) and 1 mM MgCl2 at 37°C. Periodate oxidation was performed in 0.02 M NaOCl and 0.05 M sodium formate (pH 3.0) as described in [8]. An aliquot of the compound was subjected to gel-filtration chromatography on a column (7.6 mm × 500 mm) of Asahipak GS-320 (Asahi Chemical Industry) using 50 mM ammonium acetate as the eluent at a flow rate of 1 ml/min as described in [17].

Subcellular localization

The cDNA fragment encoding FAM20B was amplified using a forward primer (5′-CGGGATCCACCGGGAGGAC-3′) containing an EcoRI site and a reverse primer (5′-CGGATCCATTCAAGGAGAC-3′) containing a BamHI site. PCR was carried out with KOD-Plus DNA polymerase for 30 cycles at 94°C for 30 s, 53°C for 42 s and 68°C for 180 s in 5% (v/v) DMSO. The PCR fragment was subcloned into the pEGFP-N1 expression vector (Clontech). The Golgi marker vector (pDsRed-Golgi) was constructed using the pECEPF-Golgi vector (Clontech) that harbours a sequence encoding the N-terminal 81 amino acids of human β1-4-galactosyltransferase [20]. The region from pECPF-Golgi was digested with Nhel and BamHI, and subcloned into pDsRed-N1. Combinations of GFP (green fluorescent protein)-tagged and DsRed-Monomer-tagged expression vectors (3.0 μg each) were transfected into HeLa cells on glass-bottom dishes (Matsunami Glass) using FuGENETM 6 according to the manufacturer’s instructions. Fluorescent images were obtained using a laser-scanning confocal microscope, Fluoview (Olympus).

Creation of stably transfected cell lines

The cDNA fragment encoding FAM20B was amplified from KIAA 0475 cDNA as a template using a forward primer (5′-CGGAATTCCTGAGAGAAGGAC-3′) containing an EcoRI site and a reverse primer (5′-CGGATCCATTCAAGGAGAC-3′) containing a BamHI site. PCR was carried out with KOD-Plus DNA polymerase for 30 cycles at 94°C for 30 s, 53°C for 42 s and 68°C for 180 s in 5% (v/v) DMSO. The PCR fragments were subcloned into the EcoRI/BamHI site of the pCMV expression vector (Invitrogen). The expression plasmid was transfected into HeLa cells, and colonies were selected as described in [3].

FAM20B silencing in HeLa cells was performed using MISSION shRNA (short hairpin RNA) (Sigma). A hairpin construct identified by The RNAi Consortium clone number TRCN0000138872 was used. The shRNA plasmid (6.7 μg) was transfected into HeLa cells on 100-mm-diameter plates using FuGENETM 6 according to the manufacturer’s instructions. Transfectants were cultured in the presence of 0.4 μg/ml puromycin. Resultant colonies were then picked up and propagated for experiments.

Quantitative real-time RT (reverse transcription)–PCR

The cDNA was synthesized from total RNA extracted from HeLa cells as described in [4]. Primer sequences were as follows: FAM20B, forward primer 5′-AGAGATCAAACCTGTCGCC-3′ and reverse primer 5′-CCAAAGTGTACGACATCCCT-3′; and glyceraldehyde-3-phosphate dehydrogenase, forward primer 5′-ATGGGTTGAAACCATGAGAAGTA-3′ and reverse primer 5′-GGCAGTAGGCATGGCAGAC-3′. Quantitative real-time RT–PCR was performed as described in [4].

GAG analysis

GAGs from HeLa cells were prepared as described in [3]. The purified GAG fraction was digested with chondroitinase ABC or a mixture of heparinase and heparitinase, and then the digests...
were derivatized with 2-aminobenzamide and analysed by HPLC as described in [3].

**Gel-filtration chromatography of GAGs**

To determine the chain length of GAGs, the purified GAG fraction was subjected to reductive -elimination using NaBH₄/NaOH, and then analysed by gel-filtration chromatography on a column (10 mm × 300 mm) of Superdex 200 eluted with 0.2 M ammonium bicarbonate at a flow rate of 0.4 ml/min. Fractions were collected at 3.0 min intervals, freeze-dried and digested with chondroitinase ABC or a mixture of heparinase and heparitinase. The digests were derivatized with 2-aminobenzamide, and then analysed by HPLC on an amine-bound PA-03 column [3].

### RESULTS AND DISCUSSION

FAM20B consists of 409 amino acids with type II transmembrane protein topology and shows weak similarity (21.4 %) to a protein named Four-jointed, which was recently identified as a Golgi kinase that phosphorylates a subset of cadherin domains (see Supplementary Figure S1 at http://www.BiochemJ.org/bj/421/bj4210157add.htm) [15]. To examine whether FAM20B could phosphorylate xylose on the GAG–protein linkage region, a soluble form of FAM20B was generated by replacing the first 30 amino acids of FAM20B with a cleavable insulin signal sequence and a Protein A IgG-binding domain, as described in the Experimental section, and then soluble FAM20B was expressed in COS-1 cells as a recombinant protein fused with the Protein A IgG-binding domain. When the expression plasmid containing the putative kinase–Protein A fusion was expressed in COS-1 cells, an approx. 76 kDa protein was secreted, as shown by Western blotting (see Supplementary Figure S2 at http://www.BiochemJ.org/bj/421/bj4210157add.htm). The fused putative kinase expressed in the medium was adsorbed on IgG–Sepharose beads to eliminate endogenous kinases, and then the protein-bound beads were used as an enzyme source. The bound fusion protein was assayed for xylose kinase activity using various linkage region compounds as acceptor substrates. As shown in Table 1, the trisaccharide and the amount of GAG isolated from each of the representative stable clones were analysed by HPLC, as described in the Experimental section. The results showed a correlation between the expression levels of FAM20B and the total amounts of both CS and HS in these stable clones. In addition, while no significant change was detected in the disaccharide composition and the amount of GAG isolated from each of the representative stable clones, differences in that of CS were observed corresponding to the expression level of FAM20B. Notably, the increase in the expression level of FAM20B was concomitant with the increase in the proportion of CS 6-sulfate to 4-sulfate. Together, results these indicated that the xylose kinase regulates the sulfation profile of CS chains as well as the total amount of GAG synthesized in cells.

To examine further the physiological relevance of the xylose kinase, we investigated whether overexpression or knockdown of FAM20B changes the amount of GAG in HeLa cells. As shown in Table 2, the disaccharide composition and the amount of GAG isolated from each of the representative stable clones was similar to that in mock-transfected cells. In addition, while no significant change was detected in the disaccharide composition of HS among these stable clones, differences in that of CS were observed corresponding to the expression level of FAM20B. Notably, the increase in the expression level of FAM20B was observed in the proportion of CS 6-sulfate to 4-sulfate. Together, results these indicated that the xylose kinase regulates the sulfation profile of CS chains as well as the total amount of GAG synthesized in cells.

For this analysis, the length of CS and HS chains obtained from FAM20B-overexpressing and mock-transfected cells were compared. Gel-filtration analysis using a Superdex 200 column revealed that the length of CS and HS chains in FAM20B-overexpressing cells was similar to that in mock-transfected cells (Figure 3), although the short CS chains in the overexpressing cells were particularly augmented. These findings indicated that the increase in the amount of CS and HS in FAM20B-overexpressing cells was mainly caused by gel-filtration HPLC, resulting in two fluorescent components: 32P-labelled (peak I) and unlabelled tetrasaccharides (peak II) (Figure 1A). Peak I was shifted to a position corresponding to 2-aminobenzamide-derivatized GlcAβ1-3Galβ1-3Galβ1-4Xyl by alkaline phosphatase digestion. Peak II was co-eluted with authentic 2-aminobenzamide-derivatized GlcAβ1-3Galβ1-3Galβ1-4Xyl when they were co-injected, indicating that it was derived from the unused acceptor substrate. Thus peak I was analysed below. The 32P-labelled tetrasaccharide (peak I) was converted into 32P-labelled trisaccharide after treatment with -glucuronidase (Figure 1B). This 32P-labelled trisaccharide was resistant to periodate oxidation (Figure 1C). In contrast, after phosphate removal by alkaline phosphatase (Figure 1D), the trisaccharide became sensitive to periodate oxidation (Figure 1E). As expected, the 2-aminobenzamide-derivatized peaks in Figures 1(D) and 1(E) were eluted at the positions of the 2-aminobenzamide-derivatized Galβ1-3Galβ1-4Xyl derived from peak II (Figure 1A) treated with -glucuronidase (Figure 1F) and the products obtained after periodate oxidation of the 2-aminobenzamide-derivatized Galβ1-3Galβ1-4Xyl (Figure 1G) respectively. As the 2-aminobenzamide was attached to C-1 of xylose, the phosphate must have been attached to C-2, making the C-1/C-2 and the C-2/C-3 glycols resistant to periodate oxidation. These findings clearly showed that FAM20B is a xylose kinase that phosphorylates C-2 of the xylose residue in the GAG–protein linkage region of the tetrasaccharide sequence of α-TM.

To examine the intracellular localization of xylose kinase, a full-length form of FAM20B fused with EGFP (enhanced GFP) at the C-terminus (FAM20B–EGFP) was generated as described in the Experimental section. FAM20B–EGFP was then co-expressed with a Golgi marker (Golgi–DsRed) or an ER (endoplasmic reticulum) marker (ER–DsRed) in HeLa cells and analysed by confocal microscopy. FAM20B–EGFP (Figure 2A) was co-localized with the Golgi–DsRed marker (Figure 2C), whereas FAM20B–EGFP (Figure 2D) was not completely co-localized with the ER–DsRed marker (Figure 2F). These results suggested that FAM20B acts as a xylose kinase in the Golgi apparatus.

Table 1. Kinase activities of fusion proteins secreted into the culture medium by the transfected COS-1 cells

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>32P-labelled</th>
<th>32P-unlabelled</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-TM</td>
<td>102.7 ± 5.4</td>
<td>128.2 ± 24.2</td>
</tr>
<tr>
<td>Galβ1-3Galβ1-4Xylβ1-O-Ser</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Galβ1-3Galβ1-4Xyl(2-O-phosphate)β1-O-Ser</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

The digests were derivatized with 2-aminobenzamide, and then analysed by HPLC on an amine-bound PA-03 column [3].
tetrasaccharide (peak I) and the 32P-labelled compound treated with 2-aminobenzamide (2-AB). The resultant 32P-labelled tetrasaccharide fractions were subjected to gel-filtration HPLC on a column of GS-320 (7.6 mm × 500 mm) using 50 mM ammonium acetate at a flow rate of 1 ml/min, as described in the Experimental section. (A) 2-AB-labelled tetrasaccharide fractions derived from FAM20B reaction products, which were released from α-TM by mild alkaline treatment. Peak I, 32P-labelled tetrasaccharides; peak II, unlabelled tetrasaccharides. (B) A 32P-labelled compound (peak I) treated with β-glucuronidase. (C) Products obtained in (B) after periodate oxidation. (D) The same product after treatment with alkaline phosphatase. (E) The same product after digestion with alkaline phosphatase followed by periodate oxidation. (F) The unlabelled tetrasaccharide (peak II) treated with β-glucuronidase. (G) The products obtained in (F) after periodate oxidation. The eluates were monitored by fluorescence (solid line) at an excitation wavelength of 330 nm and an emission wavelength of 420 nm, and by 32P radioactivity (broken line). Note that the 32P-labelled tetrasaccharide (peak I) and the 32P-labelled compound treated with β-glucuronidase were eluted at fractions 14 and 15 respectively. The arrows denote the elution positions of the following compounds: 1, GlcAβ1-3Galβ1-3Galβ1-4Xyl(2-O-phosphate)-2AB or free 32P phosphate; 2, Galβ1-3Galβ1-4Xyl(2-O-phosphate)-2AB; 3, GlcAβ1-3Galβ1-3Galβ1-4Xyl-2AB; 4, Galβ1-3Galβ1-4Xyl-2AB; 5, 2-AB-C1-fragments.

Table 2 Disaccharide composition of GAGs in control and transfected HeLa cells

<table>
<thead>
<tr>
<th>Disaccharide</th>
<th>Composition (pmol/mg (mol%))</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>FAM20B</td>
</tr>
<tr>
<td>ΔHexAα1-3GalNAC</td>
<td>2.4 ± 0.3 (3)</td>
</tr>
<tr>
<td>ΔHexAα1-3GalNAC(6S)</td>
<td>16.0 ± 1.5 (21)</td>
</tr>
<tr>
<td>ΔHexAα1-3GalNAC(2S)</td>
<td>53.4 ± 6.7 (69)</td>
</tr>
<tr>
<td>ΔHexAβ2(S)α1-3GalNAC(6S)</td>
<td>2.3 ± 0.3 (3)</td>
</tr>
<tr>
<td>ΔHexAα1-3GalNAC(4S,6S)</td>
<td>2.8 ± 0.2 (4)</td>
</tr>
<tr>
<td>Total CS</td>
<td>76.9 ± 7.9</td>
</tr>
<tr>
<td>ΔHexAα1-4GlcNAC</td>
<td>105.8 ± 12.9 (45)</td>
</tr>
<tr>
<td>ΔHexAα1-4GlcNAC(6S)</td>
<td>8.1 ± 1.2 (3)</td>
</tr>
<tr>
<td>ΔHexAα1-4GlcNAC(5S)</td>
<td>76.8 ± 10.0 (33)</td>
</tr>
<tr>
<td>ΔHexAα1-4GlcNAC(5S,6S)</td>
<td>9.5 ± 1.4 (4)</td>
</tr>
<tr>
<td>ΔHexAβ2(S)α1-4GlcNAC(6S)</td>
<td>18.6 ± 1.7 (8)</td>
</tr>
<tr>
<td>ΔHexAβ2(S)α1-4GlcNAC(5S,6S)</td>
<td>17.3 ± 1.8 (7)</td>
</tr>
<tr>
<td>Total HS</td>
<td>236.1 ± 16.7</td>
</tr>
<tr>
<td>Relative expression</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Figure 1 Identification of FAM20B reaction products as 2-0-phosphorylated xylose

23P-labelled kinase reaction products were isolated by gel filtration, and 32P-labelled tetrasaccharide chains were released by mild alkaline treatment, and then derivatized with 2-amino benzamide (2-AB). The resultant 32P-labelled tetrasaccharide fractions were subjected to gel-filtration HPLC on a column of GS-320 (7.6 mm × 500 mm) using 50 mM ammonium acetate at the eluent at a flow rate of 1 ml/min, as described in the Experimental section. (A) 2-AB-labelled tetrasaccharide fractions derived from FAM20B reaction products, which were released from α-TM by mild alkaline treatment. Peak I, 32P-labelled tetrasaccharides; peak II, unlabelled tetrasaccharides. (B) A 32P-labelled compound (peak I) treated with β-glucuronidase. (C) Products obtained in (B) after periodate oxidation. (D) The same product after treatment with alkaline phosphatase. (E) The same product after digestion with alkaline phosphatase followed by periodate oxidation. (F) The unlabelled tetrasaccharide (peak II) treated with β-glucuronidase. (G) The products obtained in (F) after periodate oxidation. The eluates were monitored by fluorescence (solid line) at an excitation wavelength of 330 nm and an emission wavelength of 420 nm, and by 32P radioactivity (broken line). Note that the 32P-labelled tetrasaccharide (peak I) and the 32P-labelled compound treated with β-glucuronidase were eluted at fractions 14 and 15 respectively. The arrows denote the elution positions of the following compounds: 1, GlcAβ1-3Galβ1-3Galβ1-4Xyl(2-O-phosphate)-2AB or free [32P]phosphate; 2, Galβ1-3Galβ1-4Xyl(2-O-phosphate)-2AB; 3, GlcAβ1-3Galβ1-3Galβ1-4Xyl-2AB; 4, Galβ1-3Galβ1-4Xyl-2AB; 5, 2-AB-C1-fragments.

Figure 2 Subcellular localization of FAM20B

HeLa cells co-expressing FAM20B–EGFP (A) and Golgi–DsRed (Golgi marker) (B), co-expressing FAM20B–EGFP (D) and ER–DsRed (ER marker) (E) were analysed by confocal microscopy, as described in the Experimental section. The merged images show the precise co-localization of FAM20B with a Golgi–DsRed marker (C), whereas FAM20B–EGFP was not completely co-localized with an ER–DsRed marker (F).

The increased number of CS and HS chains. Although we cannot rule out the possibility that FAM20B may act on other substrates that could contribute to the enhanced effect on GAG biosynthesis, since a direct effect at the cellular level on xylose phosphorylation remains to be formally established, these results suggest that FAM20B regulates the amount of GAG chains by controlling the number of GAG chains and plays an important role in the biosynthesis of GAG.

We demonstrated recently that GlcAT-I could efficiently transfer a D-glucuronic acid residue to the phosphorylated...
trisaccharide–serine Galβ1-3Galβ1-4Xyl(2-O-phosphate)/β1-O-Ser rather than to the non-phosphorylated counterpart Galβ1-3Galβ1-4Xyl/β1-O-Ser [10]. In addition, in rat articular cartilage explants, the introduction of GlcAT-I enhanced GAG synthesis was attributable to an increase in the abundance rather than the length of GAG chains, whereas antisense inhibition of GlcAT-I expression impaired GAG synthesis [21]. Moreover, Bai et al. [22] showed that the transfection of Chinese-hamster ovary cell mutants defective in GlcAT-I with GlcAT-I cDNA augmented GAG synthesis to levels approximately double that in wild-type cells, suggesting that GlcAT-I regulates the expression of GAGs. Hence, phosphorylation of the xylose residue may be required for biosynthetic maturation of the linkage region tetrasaccharide sequence, which may be a prerequisite for the initiation and efficient elongation of the repeating disaccharide region of GAG chains. Xyl-2-O-phosphate has been found in both HS and CS from Drosophila to mammals [1,6,23]. In fact, a homologue of human FAM20B is present in Drosophila, suggesting that the possible involvement of phosphorylation of the xylose residue by FAM20B in the processing and maturation of the growing linkage region might be conserved during evolution.

To date, in addition to FAM20B, two FAM members (FAM20A and FAM20C) have been reported in mammals [14]. Database searches suggested that the amino acid sequence of FAM20B displays 35.7 and 39.1% identity with FAM20A and FAM20C respectively. It was also reported that the FAM20A gene displays the most restricted expression pattern, whereas FAM20B and FAM20C are expressed in a wider variety of tissues and their expression patterns are very similar [14]. It is therefore possible that FAM20A and FAM20C are also involved in phosphorylation of the xylose residue in the linkage region, but exhibit distinct or overlapping acceptor substrate specificities. Characterization of FAM20A and FAM20C is now in progress.

Figure 3  Analysis of the length of CS (A) and HS (B) chains

The purified GAG fraction was subjected to reductive β-elimination using NaBH4/NaOH, and then analysed by gel-filtration chromatography on a column (10 mm × 300 mm) of Superdex 200. The digests of individual fractions obtained with chondroitinase ABC (A) or a mixture of heparinase and heparitinase (B) were derivatized with 2-aminobenzamide (2-AB), and then analysed by HPLC. The amounts of the 2-aminobenzamide derivatives of unsaturated disaccharides were calculated on the basis of fluorescence intensity. The samples from FAM20B-overexpressing cells (C) and the mock-transfected cells (D) are shown. Arrowheads indicate the size of molecular-mass standards (sizes are given in kDa). Results are representative of two series of independent experiments, where the two series of experiments gave essentially identical results.

REFERENCES


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SUPPLEMENTARY ONLINE DATA

FAM20B is a kinase that phosphorylates xylose in the glycosaminoglycan–protein linkage region

Toshiyasu KOIKE*†, Tomomi IZUMIKAWA*†, Jun-Ichi TAMURA† and Hiroshi KITAGAWA*‡

*Department of Biochemistry, Kobe Pharmaceutical University, Higashinada-ku, Kobe 658-8558, Japan, and †Department of Regional Environment, Faculty of Regional Sciences, Tottori University, Tottori 680-8551, Japan

Figure S1 Comparison of the predicted amino acid sequence between human FAM20B and Drosophila Four-jointed

The predicted amino acid sequences were aligned using the program ClustalW (version 1.83). Closed boxes indicate that the predicted amino acid in the alignment is identical between the two sequences. Gaps introduced for maximal alignment are indicated by dashes. The putative membrane-spanning domains are boxed.

Figure S2 Western blot analysis of FAM20B

Culture medium from cells transfected with FAM20B or vector alone was purified with IgG-Sepharose and subjected to SDS/PAGE, and the expression of each Protein A-tagged protein was examined using anti-(mouse IgG) antibody. Lane 1, FAM20B–Protein A; lane 2, vector alone. Molecular masses are indicated in kDa.

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† These authors contributed equally to this work.
‡ To whom correspondence should be addressed (email kitagawa@kobepharma-u.ac.jp).

The nucleotide sequence data reported in this paper have been submitted to the DDBJ, EMBL, GenBank® and GSDB Nucleotide Sequence Databases under accession number AB480690.