Differences in the apical and basolateral pathways for glycosaminoglycan biosynthesis in Madin–Darby canine kidney cells

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Serylcin with a green fluorescent protein tag (SG-GFP) expressed in epithelial Madin–Darby canine kidney cells is secreted mainly (85%) into the apical medium, but the glycosaminoglycan (GAG) chains on the SG-GFP protein core secreted basolaterally (15%) carry most of the sulfate added during biosynthesis (Tveit et al. (2005) J. Biol. Chem., 280, 29596–29603). Here we report further differences in apical and basolateral GAG synthesis. The less intensely sulfated chondroitin sulfate (CS) chains on apically secreted SG-GFP are longer than CS chains attached to basolateral SG-GFP, whereas the heparan sulfate (HS) chains are of similar lengths. When the supply of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) is limited by chlorate treatment, the synthesis machinery maintains sulfation of HS chains on basolateral SG-GFP until it is inhibited at 50 mM chlorate, whereas basolateral CS chains lose sulfate already at 12.5 mM chlorate and become longer. Apically, incorporation of 35S-sulfate into CS is reduced to a lesser extent at higher chlorate concentrations than basolateral CS, although apical CS is less intensely sulfated than basolateral CS in control cells. Similar to what was found for basolateral HS, sulfation of apical HS was not reduced at chlorate concentrations below 50 mM. Also, protein-free, xyloside-based GAG chains secreted basolaterally are more intensely sulfated than their apical counterpart, supporting the view that separate apical and basolateral GAG synthesis pathways exist for GAG synthesis and sulfation. Introduction of benzyl β-D-xyloside (BX) to the GAG synthesis machinery reduces the apical secretion of SG-GFP dramatically and also the modification of SG-GFP by HS.

Key words: epithelial cell sorting/glycosaminoglycan synthesis/proteoglycan/sulfation/xyloside

Introduction

Polarized cells have evolved sorting mechanisms to supply their specialized membrane domains and the adjacent extracellular milieus with particular lipids, proteins, and glycans. Sorting into the axons and dendrites of neurons and toward the apical and basolateral surfaces of epithelial cells requires molecular interactions of a certain affinity in the Golgi apparatus and/or in recycling endosomes (Rodriguez-Boulan and Musch, 2005). Proteins that traverse the secretory pathway are translocated into the lumen of the endoplasmic reticulum (ER) before most of them acquire glycan modifications that are trimmed off and/or extended throughout the Golgi apparatus. One important class of glycans that attaches to protein cores is glycosaminoglycans (GAGs), which are long linear repeats of characteristic disaccharide units, usually linked to serines with adjacent glycines in the core protein via a xylose-galactose-galactose-glucuronic acid tetrasaccharide (Prydz and Dalen, 2000). The nature of the disaccharide is decisive to whether the proteoglycan (PG) is a heparin/heparan sulfate (HS) or a chondroitin sulfate/dermatan sulfate (CS/DS) PG. PGs with more than one class of GAG chains are called hybrid PGs. Several studies with different cell lines indicate that synthesis of HS chains is completed before the trans-Golgi network (TGN), before the Brefeldin A block, whereas CS chain synthesis is completed in the TGN (Spiro et al., 1991; Sugumaran et al., 1992; Uhlin-Hansen and Yanagishita, 1993; Calabro and Hascall, 1994). The TGN is the exit site of apical and basolateral vesicular carriers from the Golgi apparatus and is also regarded as the Golgi compartment where most sorting processes take place (Griffiths and Simons, 1986). However, indications that more stable membrane microdomains might form earlier in the secretory pathway exist (Bagnat and Simons, 2002; Emery et al., 2003; Paladino et al., 2004; Alfalah et al., 2005), and we have recently promoted the idea that apical and basolateral sorting platforms organize earlier in the Golgi apparatus than the TGN in MDCK cells (Tveit et al., 2005).

An epithelial tissue depends on a proper, basolaterally localized extracellular matrix with HSPGs that carry GAGs sufficiently sulfated to be able to bind growth factors and other signaling molecules. We have previously shown that MDCK cells secrete most of the newly synthesized HSPGs basolaterally, in terms of incorporated 35S-sulfate label (Svennevig et al., 1991; Sugumaran et al., 1995), whereas CSPGs are almost exclusively secreted apically (Kolset et al., 1999). Inhibition of sulfate activation to 3′-phosphoadenosine-5′-phosphosulfate (PAPS) by the competitive inhibitor chlorate has shown that HS sulfation resists higher chlorate concentrations than sulfation of CS and proteins (Humphries and Silbert, 1988; Fjeldstad et al., 2002). However, it has previously been difficult to study protein core-independent regulatory differences in the synthesis of apical and basolateral GAGs in epithelial cells, because a good model PG that could be quantitatively retrieved after metabolic labeling has been unavailable.

In this work, we have taken advantage of the fact that the PG serglycin coupled to green fluorescent protein (SG-GFP),...
when expressed in MDCK II cells, is produced as a hybrid PG and secreted 85% apically and 15% basolaterally (Tveit et al., 2005). This allows us to study both HS and CS chain synthesis along the apical and basolateral secretory pathways. CS-GAG chains attached to SG-GFP secreted apically are longer than CS chains on SG-GFP secreted basolaterally, even if the apical GAGs are less sulfated. Protein-free GAG chains linked to xylosides recovered from the basolateral medium are also more intensely sulfated than their apical counterparts. When the supply of active sulfate (PAPS) is limited by chlorate treatment, priority is given to sulfation of HS on both apically and basolaterally secreted SG-GFP molecules, whereas sulfation of apical CS resists higher chloride concentrations than the basolateral counterpart. This differential response indicates that HS-GAG synthesis and sulfation in the apical and basolateral secretory pathways are regulated differently and is a further support for the idea that these pathways are segregated during GAG synthesis in the Golgi apparatus.

Results

We have previously shown that PGs secreted from the basolateral membrane domain of epithelial MDCK II cells are more intensely sulfated than those secreted into the apical medium. Even when the same PG protein core (serglycin with C-terminal GFP [SG-GFP]) was immune isolated from the two opposite medium reservoirs of transfected MDCK II cells (MDCK II, the covalently attached GAG chains on basolaterally secreted SG-GFP were several times more intensely sulfated than those on apically secreted SG-GFP, and the patterns of CS and HS sulfation were different (Tveit et al., 2005). We now present experiments where we have investigated differences in chain lengths and regulation of sulfation of apical and basolateral GAG chains. To address such differences, the GAG chains have been removed from their SG-GFP protein core after immune precipitation, allowing separate studies of CS and HS chains. Figure 1 shows a Sepharose CL-6B chromatogram with a comparison of the chain lengths of GAGs derived from apically and basolaterally secreted SG-GFP. Clearly, the major peak of basolateral GAGs has a lower average $K_{av}$ than the apical counterpart, although basolateral GAGs are more intensely sulfated (Tveit et al., 2005). The average CS and HS chain lengths were estimated after differential degradation and by use of a standard curve with $K_{av}$ values for dextrans of different sizes (data not shown), giving apical CS-GAG chains with an average size of 67.0 ± 2.1 kDa and basolateral chains of 43.1 ± 2.0 kDa. HS chains derived from the hybrid SG-GFP PG were more similar in size after apical (34.1 ± 3.8 kDa) and basolateral (29.6 ± 2.4 kDa) retrieval (Table I).

We investigated whether the apical and basolateral pathways for GAG synthesis would respond similarly or differently to a regulatory challenge, such as reduction of the cellular PAPS level by the competitive inhibitor of PAPS synthesis, chlorate (Baeuerle and Huttner, 1986; Humphries and Silbert, 1988). Treatment with 50 mM chlorate did not alter the secretion polarity (Figure 2A) or the CS : HS ratio of SG-GFP significantly (Figures 2B and 8B) but confirmed the finding that HSPG sulfation in general is more resistant to chlorate treatment than CSPG in MDCK cells (Fjeldstad et al., 2002). Treatment of MDCK II cells with 50 mM chlorate reduced the incorporation of $^{35}$S-sulfate into SG-GFP by 98% (Figure 3B), and CS-GAGs on SG-GFP secreted into the apical and basolateral media were now similar in size because the basolateral chains became longer (Table I). HS chains did not change significantly in length upon chlorate treatment and resisted dramatic reduction in the sulfation level until 50 mM chlorate was employed. To address the distribution of the remaining sulfate groups on SG-GFP after chlorate treatment of the cells, immune precipitation (IP) from media from one or two pooled filters was performed, followed by HS or CS degradation of individual samples. The SDS–PAGE gel (Figure 3B) shows that treatment of MDCK II cells with 50 mM chlorate essentially blocks sulfation of CS on both apical and basolateral SG-GFP. However, sulfation of CS on basolateral SG-GFP is close to maximally reduced already at 12.5 mM chlorate, whereas this chlorate concentration only reduces the sulfation of apical CS chains by

| Table I. Molecular weights of GAG chains on secreted SG-GFP upon treatment with chlorate (50 mM) or BX (1 mM) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Apical (kDa ± SE) | Basolateral (kDa ± SE) |
| CS-GAG                         | 67.0 ± 2.1       | 43.1 ± 2.0       |
| HS-GAG                         | 29.6 ± 2.4       | 34.1 ± 3.8       |
| Chlorate                       | 67.1 ± 5.7       | 66.0 ± 4.0       |
| BX                              | 39.2 ± 2.0       | 37.0 ± 2.0       |

$^3$H-GlcN-labeled SG-GFPs secreted from filter-grown MDCK II cells when expressed in MDCK II cells, is produced as a hybrid PG and secreted 85% apically and 15% basolaterally (Tveit et al., 2005). We now present experiments where we have investigated differences in chain lengths and regulation of sulfation of apical and basolateral GAG chains. To address such differences, the GAG chains have been removed from their SG-GFP protein core after immune precipitation, allowing separate studies of CS and HS chains. Figure 1 shows a Sepharose CL-6B chromatogram with a comparison of the chain lengths of GAGs derived from apically and basolaterally secreted SG-GFP. Clearly, the major peak of basolateral GAGs has a lower average $K_{av}$ than the apical counterpart, although basolateral GAGs are more intensely sulfated (Tveit et al., 2005). The average CS and HS chain lengths were estimated after differential degradation and by use of a standard curve with $K_{av}$ values for dextrans of different sizes (data not shown), giving apical CS-GAG chains with an average size of 67.0 ± 2.1 kDa and basolateral chains of 43.1 ± 2.0 kDa. HS chains derived from the hybrid SG-GFP PG were more similar in size after apical (34.1 ± 3.8 kDa) and basolateral (29.6 ± 2.4 kDa) retrieval (Table I).

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35% (Figure 3B and C). In relative terms, more and more of the remaining sulfate is associated with HS chains with increasing chlorate concentrations, indicating that the cellular machinery prioritizes HS sulfation when the PAPS availability is low.

Another way to challenge the synthesis and transport machinery of PGs is by introduction of xylosides (Schwartz, 1979). The xylose portion allows polymerization of protein-free GAG chains, usually of the CS type (Schwartz et al., 1974; Galligani et al., 1975), whereas the hydrophobic portion facilitates access to the polymerizing enzymes. Exactly at what stage of GAG synthesis the xyloside becomes a competitor for the enzymatic machinery is not clear, but competition is evident at the level of GAG chain polymerization (Moses et al., 1999).

The benzyl-xyloside (BX) itself acquired GAG chains sensitive to c-ABC treatment (data not shown), as is generally the case for xylosides. These CS chains followed the trends observed previously (Figure 4A and B) by being predominantly secreted apically (Kolset et al., 1999), whereas the basolaterally secreted xyloside-based GAGs were more intensely sulfated (Tveit et al., 2005). The xyloside treatment had a dramatic effect on the apical secretion of SG-GFP protein cores. Whereas these are mainly secreted into the apical medium in control cells, the secretion is nonpolarized in the presence of BX (Figure 5). On the basis of confocal
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laser fluorescence microscopy of the SG-GFP, it seems that the protein core to some extent accumulates in the ER in the presence of BX (data not shown), indicating a reduced capacity for entry of SG-GFP into the apical pathway. The incorporation of $^3$H-glucosamine ($^3$H-GlcN) and $^{35}$S-sulfate is reduced for both apical and basolateral SG-GFP (Figure 6A and B), manifested as shorter CS-GAGs (Table I). These shorter GAGs contain lesser $^{35}$S-sulfate per incorporated $^3$H-GlcN, whereas apical SG-GFP still carries less intensely sulfated GAGs than the basolateral counterpart (Figure 6C). The amount of CS attached to xylosides was (20 times that of CS on SG-GFP apically and 16 times that of CS on SG-GFP basolaterally. An additional observation is that treatment of MDCK# II cells with BX reduces the extent of HS modification more than that of the CS modification on basolateral SG-GFP; in other words, the relative fraction of CS chains on SG-GFP is increased, seen as more HNO$_2$-resistant and less chondroitinase-A,B,C (cABC)-resistant material in the basolateral medium of BX-treated cells (Figure 7). This is contrary to the observation that xylosides generally knock down CS synthesis (Klein et al., 1989; Margolis et al., 1991) and is surprising because MDCK cells tend to maintain HS synthesis/sulfation as long as possible when challenged by other inhibitors (Fjeldstad et al., 2002). At the apical side, the CS : HS ratio on SG-GFP was not changed by BX treatment (Figure 8B and C), although the total incorporation of $^{35}$S-sulfate and $^3$H-GlcN is reduced to 20–30% of controls (Figure 8A).

In sum, we have challenged the biosynthesis of one PG (SG-GFP) in different ways, and the apical and basolateral pathways have responded differently. This adds to the accumulating evidence that these two pathways are separated before the TGN and thus might be regulated differently.

Discussion

In this work, we have studied differences that occur in post-translational processing of a model PG in the apical and
basolateral secretory pathways of epithelial MDCK cells. MDCK II cells transfected to express the human SG variant in frame with the GFP (SG-GFP) were metabolically labeled with $\text{{}^{35}S}$-sulfate, $\text{{}^{3}H}$-GlcN, or $\text{{}^{35}S}$-cysteine/methionine ($\text{{}^{35}S}$-Cys/Met) before the secreted PG was immune precipitated from the apical and basolateral media. SG-GFP is secreted both as a hybrid PG, with CS and HS chains, and as a pure CSPG. We have previously found that the fraction of SG-GFP (85%) secreted apically acquires GAG chains with less sulfate than the basolaterally secreted SG-GFP (15%). We have now demonstrated another difference between apical and basolateral CS chains on SG-GFP—the apical chains are significantly longer (Table I). Also, xylose-based CS chains followed the general pattern—those secreted basolaterally contained more sulfate than those secreted to the apical medium. A reduction in the supply of active sulfate, by chlorate treatment, had different effects on the apical and basolateral pathways. Sulfation of HS chains on the basolaterally secreted SG-GFP was maintained, whereas CS chains rapidly lost sulfate. SG-GFP secreted at the apical membrane carried CS chains that resisted higher chlorate concentrations than the basolateral counterpart. Apical HS sulfation was similar to that of basolateral HS not dramatically changed before 50 mM chlorate was applied. This indicates that apical and basolateral CS chains on SG-GFP are substrates for different sets of enzymes that respond differently to reduced PAPS levels. We have previously found that CS on basolateral SG-GFP is mainly 6-O-sulfated, whereas the apical counterpart is mainly 4-O-sulfated (Tveit et al., 2005). These enzymes might have different Kms for PAPS in MDCK cells, because a higher PAPS Km has been reported for 6-O-sulfation of CS (40 μM) than for 4-O-sulfation (0.25 μM) in other biological contexts (Sugumaran et al., 1995; Yamauchi et al., 1999). Chlorate treatment also increased the length of basolateral CS-GAG chains. This would imply that a reduced sulfation level postpones the termination of the CS chains. The sulfation patterns of CS chains from apical and basolateral SG-GFP were obtained from cells grown in medium with a high glucose level, whereas the $\text{{}^{3}H}$-GlcN metabolic labeling was performed with a reduced level of glucose in the medium. Still in the context of this work (results not shown) and of previous work (Safaiyan et al., 1999; Fjeldstad et al., 2002), variable glucose concentration has not influenced GAG synthesis, measured as chain length.

While chlorate treatment did not alter the polarity of SG-GFP transport, BX treatment did reduce apical SG-GFP transport to levels corresponding to basolateral transport. The underlying mechanism cannot be deduced in detail from this observation, but it is possible that the xyloside, which is preferentially transported apically, displaces SG-GFP from apical carriers. Alternatively, the hydrophobic moiety of the xyloside might interfere with the formation of the apical transport platform by disturbing the formation of a proper lipid domain. The basolateral pathway is less sensitive to xyloside treatment, but GAG chains on both apical and basolateral SG-GFP are shorter in the presence of BX; again, the apical pathway is affected the most (Table I).

One effect of BX that had a predominant basolateral manifestation was an inhibitory effect on HS-GAG synthesis on basolateral SG-GFP. In sum, our data support the view that the apical and basolateral secretory pathways are segregated during GAG synthesis in the Golgi apparatus. The regulatory challenges introduced in the experimental system did affect the two pathways differently. It has recently been shown that units of the Golgi complex of imaginal disk cells in Drosophila containing the UDP-sugar transporter Fringe connection are distinct from those containing the sulfotransferase Sulfateless (Yano et al., 2005). A separation of the Golgi apparatus into several, separate functional units could explain much of the diversity observed for glycan modification and would allow a more precise regulatory response. It has been proposed that sorting into the apical and basolateral pathways might occur already at exit from the ER (Rodriguez-Boulan and Musch, 2005). A novel detergent extraction technique, employing Tween 20, makes separation of apical and basolateral proteins with mannose-rich N-glycans possible (Alfalah et al., 2005), indicating that these pathways are segregated at an early stage of the secretory pathway. A concern regarding the differences in modification of apical and basolateral SG-GFP observed in this work and in previous work (Tveit et al., 2005) could be changes occurring to the PGs after their secretion into the medium, during the remainder of the metabolic labeling period (24 h in total). There is, however, a very limited endocytic uptake and transport to a degrading compartment at the apical pole of MDCK cells (Bomsel et al., 1989; Parton et al., 1989), making a chain shortening or sulfatase activity acting on re-endocytosed SG-GFP unlikely to contribute to the observed differences. Still, further experiments are needed to address questions...
concerning the physical segregation of the apical and basolateral pathways in epithelial cells.

Materials and Methods

Construction of plasmid

The plasmid sergly-pEGFP was made as described earlier (Tveit et al., 2005). Expand Long Template PCR System (Roche, Mannheim, Germany) was used together with 5'-ATCGGAAATTCTAGTGCAGAAGCTAAGCAAA-3' and 5'-TTGCAACGTACGATGGATCCTAACAATAGCTCTT-3' to amplify the coding sequence of SG. The PCR product was ligated into the EcoRI and BamHI restriction sites in the expression vector pEGFP-N3 (Clontech, Palo Alto, CA), making sergly-pEGFP.

Cell culture and transfections

MDCK II cells were grown in Dulbecco’s Modified Eagle’s medium (DME) with 5% FBS (PAA, Pasching, Austria), 1% antibiotics and L-glutamine (Biowhitaker, Verviers, Belgium) at 37°C, and 5% CO2. The cells were stably transfected (MDCK II), as previously described (Tveit et al., 2005), with 4 μg sergly-pEGFP and 12 μL Fugene 6 (Roche) for 72 h. For selection of transfected colonies, 1 mg/mL of G-418 (Duchefa, Harlem, The Netherlands) was supplemented to the medium. Several colonies were selected and screened by IP with polyclonal anti-GFP (Abcam, Cambridge, UK). One resulting clone was used for the experiments presented here.

Metabolic labeling and treatment of MDCK II cells

MDCK II cells (10^6) were seeded on 4.7 cm² filters (Costar) in 1.6 mL growth medium added apically, with 2 mL in the basolateral well. The cells were grown to confluence (4 days), before metabolic labeling was carried out with 1 mL medium apically and 2 mL basolaterally, with 0.3 μCi/mL 35S-Cys/Met (PerkinElmer, Boston, MA) using DME without Cys and Met (Sigma), or with 0.3 μCi/mL 35S-SO₄²⁻ (PerkinElmer) using RPMI 1640 without sulfate (Gibco, Paisley,Scotland, UK), or with 0.2 μCi/mL 3H-GlcN (PerkinElmer) using DME without glucose (Gibco). All labeling experiments were of 24-h duration. Apical and basolateral media were harvested, and cell fractions were lysed using 1 mL IP-lysis solution (1% NP-40, 50 mM Tris pH 7.5, 2 mM EDTA, 150 mM NaCl, 35 μg/mL PMSF) added protease inhibitor tablets (Complete, Mini, EDTA free Protease Inhibitor Cocktail Tablets, Roche).

Chlorate treatment was carried out by adding 12.5–50 mM sodium chlorate (final concentration) to both the apical and the basolateral medium before performing metabolic labeling for 24 h. The medium and cell fractions were then harvested, as described in earlier (see Metabolic labeling and treatment of MDCK II cells). BX (a gift from Dr.Clem Robinson, Monash University, Australia) was added at a concentration of 1 mM (final) to both the apical and the basolateral medium of filter-grown MDCK II cells for each of the three different labeling procedures.

Immune precipitations

Before IP, the apical and basolateral media and the cell fractions were precleared with 60 μL (50–50 slurry) of protein-A-Sepharose (Amersham Biosciences, Buckinghamshire, England, UK) for 1 h at 4°C. IP was performed as described previously (Tveit et al., 2005). All samples were added 1 μL anti-GFP/mL at 4°C overnight, before addition of 60 μL (50–50 slurry) protein-A-Sepharose beads before rotating 3 h at 4°C. The beads were then washed six times with IP-wash solution (50 mM Tris pH 7.4, 150 mM NaCl, 0.05% Triton X-100) with 1% BSA and four times without BSA.

SDS-PAGE and quantification of SG-GFP

After labeling and harvesting, the apical and basolateral media and cell fractions were run on Sephadex G-50 Fine (Amersham) chromatography columns to remove unincorporated radioactive molecules (Spiro et al., 1991). Aliquots (usually 50 μL) of each fraction were then added Insta-Gel II plus (Packard, Groningen, CA) counted in a scintillation counter, and one-tenth of each fraction (1.5 mL total) was analyzed by SDS-PAGE (BioRad, Hercules, CA). IP beads were either added SDS sample buffer (XT, BioRad) directly and run on a 4–12% XT SDS-PAGE with MOPS buffer (BioRad) or first treated for selective GAG degradation before running the gels. The gels were fixed, treated with Amplify (Amersham), and dried. Gels with 3H-GlcN-labeled samples were subjected to autoradiography with Hyperfilm ECL (Amersham). 35S-Cys/Met and 35S-SO₄²⁻--labeled samples in gels were exposed to PhosphorImager screens, scanned (Typhoon 9410 PhosphorImager, Amersham), and quantified by ImageQuant (Amersham).

Selective GAG degradation

After IP of apical and basolateral medium aliquots, each sample was divided into three equal volumes: one for control, one for cABC (Seikagaku, Tokyo, Japan) (75 μL/u/mL) treatment for CS degradation at 37°C overnight, and one for HNO2 treatment for HS degradation (10 min at room temperature), as described (Shively and Conrad, 1976).

Alkaline β-elimination treatment

Beads with IP SG-GFP were resolved in 0.15 M NaCl in 0.05 M Tris–HCl buffer, pH 8.0, and alkaline treated by addition of one-tenth of the sample volume of 5 M NaOH before incubation at room temperature for 20 h to release GAGs from the core proteins. The reaction was stopped by neutralizing with HCl to pH 7–8 and buffered with 1 M Tris–HCl.

Gel filtration chromatography

The released GAGs were treated for degradation with cABC or HNO2 as described. Treated and untreated samples were applied to columns (1 cm diameter × 40 cm) of Sepharose Cl-6B (Amersham Biosciences), along with blue dextran and K2CrO4 as internal standards. The column was eluted with 0.15 M NaCl in 0.05 M Tris–HCl buffer pH 8.0 and 0.1% Triton X-100, at a rate of 6 mL/h. Fractions of 1 mL were collected, and aliquots of each fraction were analyzed for radioactivity in a scintillation counter. The length
of CS and HS-GAG chains was determined from the remaining fraction volumes after HNO₂ or cABC treatment. Standard dextran series of 6, 70, 410, 270, 50, and 12 kDa (Fluka, Buchs, Switzerland) were used for determination of the molecular masses of the GAGs. The fractions with dextran were identified by the phenol sulfuric acid method (Dubois et al., 1951).

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Abbreviations

BX, benzyl-xyloside; cABC, chondroitinase-A,B,C; CS, chondroitin sulfate; Cys/Met, cysteine/methionine; DME, Dulbecco’s Modified Eagle’s medium; ER, endoplasmic reticulum; GAG, glycosaminoglycan; GFP, green fluorescent protein; GlcN, glucosamine; HS, heparan sulfate; IP, immune precipitation; MDCK, Madin–Darby canine kidney; MDCK#, transfected Madin–Darby canine kidney; PAPS, 3’-phosphoadenosine-5’-phosphosulfate; PG, proteoglycan; SG, serylgluc; TGN, trans-Golgi network.

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