Glycosylation-related Gene Expression in Prion Diseases

PrPSc ACCUMULATION IN SCRAPIE INFECTED GT1 CELLS DEPENDS ON β-1,4-LINKED GalNAc-4-SO4 HYPOSULFATION*

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Several lines of evidence indicate that some glycoconjugates are efficient effectors of the normal cellular prion protein (PrPC) conversion into its pathogenic (PrPSc) isoform. To assess how glycoconjugate glycan moieties participate in the biogenesis of PrPSc, an exhaustive comparative analysis of the expression of about 200 glycosylation-related genes was performed on prion-infected or not, hypothalamus-derived GT1 cells by hybridization of DNA microarrays, semiquantitative RT-PCR, and biochemical assays. A significant up- (30-fold) and down- (17-fold) regulation of the expression of the ChGlN1 and Chst8 genes, respectively, was observed in prion-infected cells. ChGlN1 and Chst8 are involved in the initiation of the synthesis of chondroitin sulfate and in the 4-sulfation of non-reducing N-acetylgalactosamine residues, respectively. A possible role for a hyposulfated chondroitin in PrPSc accumulation was evidenced at the protein level and by determination of chondroitin and heparan sulfate amounts. Treatment of Sc-GT1 cells with a heparan mimetic (HM2602) induced an important reduction of the amount of PrPSc, associated with a total reversal of the transcription pattern of the N-acetylgalactosamine-4-O-sulfotransferase 8. It suggests a link between the genetic control of 4-O-sulfation and PrPSc accumulation.

Prion diseases are characterized by the refolding of a normal cell surface glycoprotein, the cellular prion protein (PrPC), into an abnormal β-sheet-enriched form that is insoluble in detergents and called PrPSc because it is partially resistant to protease K treatment (1–3). The primary structure of PrPC contains two potential N-glycosylation sites on Asn180 and Asn190 in rat and mouse, several potential O-glycosylation sites and a C-terminal glycosylphosphatidylinositol (GPI) anchor (4, 5). It also contains specific glycosaminoglycan binding sites (6), including three that bind heparan sulfate proteoglycans, which are specific components of the extracellular matrix (7). Glycans directly linked to PrP participate in the strain diversity, in cell to cell transfer of GPI-anchored PrP, allowing the transport of both normal and infectious protein and, as a consequence, the propagation of infection (8, 9). Although the putative cofactors involved in the structural trans-conformation of PrPSc into the pathogenic form are not yet identified, glycans and/or glycoconjugates are proper candidates. N-glycosylation of PrP, which was shown to interfere with PrPSc accumulation, participates in the control of the accessibility of PrP determinants involved in its conversion, in relation to prion strain diversity and resistance (10–12). The relative membrane mobility allowed by the GPI anchor, leads the prion protein to confine in “rafts” (spingolipid and cholesterol rich semi-ordered membrane microdomains), a location which is essential for the conformational conversion of PrPSc into PrPSc (13–15). The ability of PrP to bind heparan sulfate proteoglycans has evidenced the importance of these glycoconjugates for the conversion process. Three regions of PrP, residues 23–52, 53–93, and 110–128, were identified as being able to bind heparin and heparan sulfate (7). They can also be involved in the PrP binding to the LRP/LR laminin receptor that leads to PrP endocytosis, thus suggesting that proteoglycans can modulate the subcellular trafficking of PrP (16, 17). Nevertheless the effect of heparan sulfates seems to be paradoxical as they can stimulate or inhibit PrPSc formation. Depending on their level of sulfation, a variety of sulfated exogenous glycans such as dextran sulfate, pentosan polysulfate, and heparan mimic can inhibit PrPSc accumulation in cell cultures (18–23). Inversely, heparan sulfate has been shown to be associated with cerebral prion amyloid plaques and with the more diffuse PrPSc deposits that appear in early stages of prion diseases (24, 25). Moreover, heparinase III sensitive-heparan sulfate proteoglycans, that are probably hyposulfated, can participate in the metabolism of prions and stimulate the cell-free conversion of PrPSc into PrPSc (26–28). While the function of the complex formed by PrP and sulfated glycans has still to be determined, the molecular interactions between these two partners seem to have a pivotal role in prion diseases especially in the structural conformation of PrP.

In this study, to assess the genetic basis of the intervention of glycoconjugates in prion disorders, we used the derived hypothalamic neuronal GT1 cell line, which is a proper model to simulate PrPSc accumulation that occurs at the late stages of the disease. Based on the use of a DNA microarray, we examined the expression of genes related to glycosylation and showed that the PrPSc accumulation depends not only on ex-
pression of genes involved in heparan and chondroitin synthesis but probably also on chondroitin sulfation.

**EXPERIMENTAL PROCEDURES**

**Chemicals—**Heparan mimetic (HM) was purchased from Professor D. Barritault (University of Paris XII). It was obtained by controlled chemical substitution of T40 dextran with defined amounts of carboxymethyl (CM), sulfite (S), and hydrophobic groups such as benzylamide (Bn) (29). The molecule used was HM2602 that contains 88% CM, 20% Bn, and 50% S substitutions per dextran unit (23).

**Cell Cultures—**GT1-7 cell line derived from immortalized murine hypothalamic GnRH neurons (30). ScGT1 cells correspond to GT1-7 cells infected with the scrapie Chandler isolate. These cells, kindly provided by S. Lehmann (Montpellier, France), persistently express high amounts of PrPSc (31).

**GT1 and ScGT1 Cells** were treated for 6 days (two passages) with 10 μg of HM2602 per ml of medium. As a control, a set of GT1 and ScGT1 cells were grown without HM for the same time. PrPSc accumulation analysis was carried out as described previously (32).

**DNA Microarray Analysis—**DNA microarrays were prepared by ourselves on polysine slides (CML, Menzel-Glas). They contained 165 hybridization units (each DNA fragment was about 450 bp) specific of the main murine glycosylation-related genes, that belong to the glycosyltransferases (111 units), glycosidases (30 units), glycosyltransferase (1 unit), lectins (3 units), and sulfotransferases (20 units) gene families. The slides also contained 23 controls units corresponding to 10 normalization units (Arabidopsis genes), 7 elongation units, and 1 positive and 5 negative controls. Each unit was present in triplicate.

**Labeled cDNA synthesis and microarray hybridizations** were performed as described in the MICROXAM™ TSA™ labeling and detection kit (PerkinElmer Life Sciences). Equal amounts of cDNA corresponding to total RNA from GT1 cells and fluorescein-labeled cDNA corresponding to total RNA from ScGT1 cells were hybridized on the slide. Various amounts (1–100 pg) of control RNAs (Arabidopsis RNAs, SpotReport-10 Array Validation System from Stratagene) were added to each batch of RNA samples for normalization. Hybridizations were carried out overnight at 65 °C in a hybridization chamber (Corning).

**After washings,** cDNA were revealed by streptavidin-horseradish peroxidase (HRP) and Cy5-tyramide. Fluorescein-labeled cDNAs were revealed using an anti-fluorescein-horseradish antibody and Cy3-tyramide. Cy3 and Cy5 fluorescence signals were measured using a GMS 418 Array Scanner (MWG). The raw data were analyzed using the Array-Pro Analyser software.

**Hybridizations** were repeated three times with biotin-labeled cDNA generated from RNAs from GT1 cells and fluorescein-labeled cDNA generated from RNAs from ScGT1 cells. Three other hybridizations were made with reversed Cy3 and Cy5 labeling.

**Data reported** were analyzed as the mean fold transcript level increase or decrease in the ScGT1 compared with GT1 cells. The fold change in relative transcript level (RTL) between ScGT1 and GT1 cells must be ≥2 or ≤2 for being taken into account.

**Real-time PCR Analysis—**Relative quantitative RT-PCR analyses were carried out in triplicate on the ABI Prism™ 5700 sequence detection system (PE Applied Biosystems). Reactions were performed in 25 μl, cDNA used as template were obtained by reverse transcription of GT1 and ScGT1 cell cultures, Glycosaminoglycans Quantification—GT1 and ScGT1 cell cultures, at three levels of confluence, were washed in phosphate-buffered saline and lysed in 400 μl of K2HPO4 100 mM pH 8.0, Triton X-100 0.5% buffer. The protein content of a 100-μl aliquot was quantified (Micro BC assay, Amersham Biosciences), and the remaining 300 μl of protein sample was digested at 56 °C for 12 h with proteinase K (50 μg/ml final). The enzyme was heat-inactivated at 90 °C for 10 min, and the mixture was centrifuged (10 000 × g, 10 min and 20 °C) through a Ultrafree 0.2-μm filter (Microcon).

**The sulfated glycosaminoglycan (GAGs) amount** was determined using the Biocolor assay (Tebu) according to Barbosa’s procedure (34). Briefly, 1 ml of dimethylmethylen blue (DMMB) solution was added to
TABLE II
Relative transcript levels (RTL) of the glycosylation-related genes in ScGT1 versus GT1 cells

| Gene name | Encoded protein | Relative transcript level (ScGT1 vs. GT1 cells) |
|-----------|----------------|-----------------------------------------------|
| ChGl1     | Chondroitin β-1,4 N-acetylgalactosaminyltransferase | +5.5 ± 2.2                                   |
| Chat8     | Carbohydrate (N-acetylgalactosamine 4-O) sulfotransferase 8 | +4.9 ± 1.7                                   |
| Mfn2      | β-1,3-4-N-Acetylgalactosaminyltransferase 8 | −3.5 ± 1.7                                   |
| B3gnt1    | β-1,3-Galactosyltransferase 1 | −3.1 ± 1.0                                   |
| Chat1     | Chondroitin-6-sulfotransferase | −2.8 ± 0.6                                   |
| Hexa      | β-Hexosaminidase α chain | −2.8 ± 0.6                                   |
| Gla        | Galactosidase A | −2.7 ± 0.3                                   |
| HsGnt1    | Heparan sulfate 6-sulfotransferase 1 | −2.5 ± 0.5                                   |
| ST6GalNAcIII | α-N-Acetylgalactosaminide-α-2,6-sialyltransferase III | −2.4 ± 0.2                                   |
| Ogt       | UDP-N-acetylgalactosamine-peptide N-acetylgalactosaminyltransferase | −2.4 ± 0.4                                   |
| Neu1      | Neuraminidase 1 | −2.3 ± 0.3                                   |
| B4galt6   | β-1,4-Galactosyltransferase 6 | −2.2 ± 0.2                                   |

Table values are the mean of 3–6 experiments carried out using the 165 glycosylation-related genes DNA microarray tool described under "Experimental Procedures." RTL is defined as the ratio of Cy3/Cy5 normalized fluorescence. Only genes whose RTL values were up or down threshold 2 were considered.

an adjusted 100-μl aliquot of digested sample, shocked for 30 min, and centrifuged at 12,000 × g for 10 min. After discarding the supernatant, 1 ml of a DMBB decomplexation solution (4 M guanidine hydrochloride in 10% propan-1-ol and acetate tri-hydrate buffer 50 mM pH 6.8) was added to the pellet. The mixture was shocked for 30 min and its absorbance was measured at 656 nm. The sulfated GAG amount was determined by comparison with a chondroitin sulfate solution curve.

For chondroitin sulfate quantification, a 100-μl aliquot of proteinase K-digested sample mixed with 100 μl of sodium nitrite (0.5%) and acetic acid (33%). The reaction was stopped by addition of 100 μl of ammonium sulfamate (12.5%). Remaining chondroitin sulfate was quantified in 100 μl of nitric acid reaction mixture as described above.

### Western Blotting Analysis of ChGl1 and Chat8-encoded Enzymes—
Antigenic peptides were designed from the N-terminal regions of carbohydrate (N-acetylgalactosamine 4-O) sulfotransferase 8 (GalNAc-4-STT1, NP_786341) and chondroitin sulfate N-acetylgalactosaminyltransferase I (NP_780349) mouse protein sequences. Rabbit antibodies were generated by Eurogentec (Liege, Belgium). Only the CQAPDQRPRPHKAAGS peptide belonging to GalNAc-4-STT1 sequence proved to be immunogenic.

For Western blotting, GT1 and ScGT1 cells were washed in PBS, lysed in 300 μl of Tris-HCl, 50 mM pH 6.8, glycerol 10% (v/v), SDS 1% (w/v) buffer, and sonicated. The protein amount was determined according to the biocin chonic acid procedure (Sigma). 60 μg of total protein were separated by electrophoresis on a 10% polyacrylamide gel. After transfer (200 mA, 1 h 30 min), the nitrocellulose membrane was incubated for 2 h in 10 ml of 1% blocking reagent (Western blocking reagent, Roche Applied Science). The incubation with the anti-GalNAc-4-STT1 antibodies (1:100 dilution) was carried out overnight at 4 °C in 0.5% blocking solution. As a control, an anti-TFIIId Western blotting was also performed (Anti-TFIIId dilution: 1:750, Santa Cruz Biotechnology). Detections were performed using a goat anti-rabbit IgG HRP-coupled antibody (Dako). The chemiluminescent reaction (Roche Applied Science) was revealed by an Amersham Biosciences Hyperpressor.

#### RESULTS
Expression Analysis of Glycosylation-related Genes in ScGT1 versus GT1 Cells—ScGT1 cells are a proper model to study PrPSc accumulation. They were established on the basis of a derived hypothalamic neuronal cell line that expresses high amount of PrPSc (eight times more than in the widely used neuroblastoma N2a cell line) and they persistently accumulate high levels of PrPSc (31, 35). The origin of these cells makes them the proper cellular model for studying the process of brain PrPSc deposition that occurs in prion disorders (36).

Upon the 165 glycosylation-related genes whose relative transcript levels were estimated by hybridization on a DNA microarray, twelve presented a significant variation in ScGT1 cells as compared with GT1 cells (Table II). These genes belong to the glycosyltransferase (6 genes), glycosidase (3 genes) and carbohydrate sulfotransferase (3 genes) families. Among them, ChGl1 and Chat8 genes showed the most important variations in their relative transcript levels (RTL). ChGl1, which encodes the chondroitin sulfate N-acetylgalactosaminyl-transferase I (CsGalNAcT-I), was 5.55-fold overexpressed in ScGT1 cells.

The Chat8 gene, which encodes the N-acetylgalactosamine 4-O sulfotransferase 8 (GalNAc-4-STT1), was 4.87-fold underexpressed in ScGT1 cells (Table II). The CsGalNAcT-I enzyme is directly involved in glycosaminoglycan synthesis, particularly chondroitin sulfate (37, 38). The GalNAc-4-STT1 enzyme transfers a SO4 radical from 3'-phosphoadenosine-5'-phosphosulfate (PAPS) donor substrate to the hydroxyl at C4 position of a β1,4-linked N-acetylgalactosamine containing acceptor sulfate. When the enzyme is free from the Golgi membrane or experimentally truncated, the SO4 radical can be transferred on a β1,4-linked GalNAc residue belonging to a chondroitin glycan (39).

Results from the microarray were confirmed by semiquantitative RT-PCR (Fig. 1A). ChGl1 was found to be 30.2-fold overexpressed and Chat8 17.13-fold underexpressed in ScGT1 cells. Compared with the microarray data, the semiquantitative RT-PCR analysis shows for both genes even higher relative transcript level differences, indicating that DNA microarray technology underestimates changes in mRNA amounts, particularly for the most highly expressed genes.

To correlate these transcripts levels with protein amounts, specific antibodies toward both polypeptides were designed. An important and reproducible decrease in the amount of GalNAc-4-STT1 was found in ScGT1 cells as compared with GT1 cells (Fig. 1B).

Immunoochemistry of N-acetylgalactosamine 4-O sulfotransferase 8 in Mouse Brain—To assess enzyme localization in mouse brain, search for GalNAc-4-STT1 was performed (Fig. 2) using the purified antibody directed against the N-terminal epitope of the enzyme located in its stem region (see "Experimental Procedures"). Both occipital cortex and lateral hypothalamus brain regions stained positively. In few cortical neurons, the presence of the enzyme was evidenced in pericellular space and cytoplasmic membrane (Fig. 2A). GalNAc-4-STT1 was also detected as vesicular string in some neurites from other cells (Fig. 2B). A higher number of neurons from lateral hypothalamus showed a positive staining inside their cytoplasm and dendritic cell extensions (Fig. 2C). Altogether, this staining was in good agreement with the presence in neuronal cells of a significant non-Golgi enzyme fraction.

Measurement of Glycosaminoglycan and Chondroitin Sulfate Amounts—The amount of chondroitin sulfate, which depends on the balance between CsGalNAcT-I and chondroitinase activities, was quantified both in GT1 and ScGT1 cells. To this aim, total GAG and chondroitin sulfate amounts were measured at different levels of cell confluence (low confluence, proliferation phase, high confluence). The amount of total GAG did
Table III, which correlates well with the lower amount of Ext2 respecti -

three independent cell cultures. The relative transcript level represents the fold change of mRNA levels in ScGT1 versus GT1 cells. Data correspond to the mean value of three independent cell cultures. B, Western blotting analysis. 50 μg of total cell proteins were separated on SDS-PAGE. GalNAc-4-ST1 enzyme was immunodetected (see “Experimental Procedures”). As a control of the amount of protein, an anti-TfIId Western blot was also performed.

FIG. 1 Expression analysis of the ChGn1 and Chst8 genes. A, changes in mRNA levels measured with microarray (open bars) and RT-PCR (closed bars). Total RNA was extracted from ScGT1 and GT1 cells. The relative transcript level represents the fold change of mRNA levels in ScGT1 versus GT1 cells. Data correspond to the mean value of three independent cell cultures. B, Western blotting analysis. 50 μg of total cell proteins were separated on SDS-PAGE. GalNAc-4-ST1 enzyme was immunodetected (see “Experimental Procedures”). As a control of the amount of protein, an anti-TfIId Western blot was also performed.

FIG. 2 GalNAc-4-ST1 enzyme immunolocalization in mouse brain. A and B, sagittal sections of brain cortex showing GalNAc-4-ST1 staining in cytoplasmic membrane, immediate pericellular space (A, magnification ×100) and neurites (B, magnification ×40). C, lateral hypothalamus sections showing the labeling of cytoplasm and dendritic extensions (magnification ×40). Bars: A, 100 μm; B and C, 40 μm.
DISCUSSION

Glycosaminoglycans, specifically heparan sulfate, have been described as being important actors of prion diseases (6, 7, 16, 28, 40). Their function is still under investigation and the role of chondroitin sulfate in these disorders has to be more deeply examined. Heparan sulfates have controversial effects as they can stimulate or inhibit these disorders. By binding on specific PrPC sites, they are important partners in the interaction between PrP and the laminin receptor LRPLRP (7, 16). Heparan sulfates have also been identified as critical factors in Alzheimer amyloidogenesis and they were found to be associated with PrPSc insoluble aggregates and tissue amyloid prion deposits in the brain of infected animals (24, 25, 41, 42). Furthermore, heparinase III-sensitive, possibly hyposulfated, heparans seem to be involved in PrPres metabolism (26). Pentosan polysulfate and usual heparan sulfate have a stimulating effect on the conversion of PrPc into PrPres. Inversely, other glycosaminoglycans, such as chondroitin and keratan sulfate, have no effect on this process (27, 28, 43, 44). Interestingly, in scrapie-infected cell culture and in animal models, exogenous heparan and pentosan polysulfate, contrary to their stimulatory effect, are strong inhibitors of PrPres accumulation, probably through steric competition, while other glycosaminoglycans were neutral (21, 45–48).

Correlation between PrPres and Glycosylation-related Genes—Using our DNA microarray technology, the correlation between the pathologic prion protein accumulation and the expression of numerous glycosylation-related genes was investigated in GT1 cells, cells that derived from the central nervous system. It confirmed the relevance of such a methodology to screen potential changes in glycosylation and demonstrated a profound modification of the expression of some glycosylation-related genes. Modification of the RNA transcript levels of genes involved in glycosaminoglycans synthesis, such as heparan and chondroitin sulfates, or in the 4-O-sulfation of a non-reducing N-acetylgalactosamine residue was evidenced. Most of the genes involved in heparan sulfate synthesis were down-regulated (Table II), whereas GAG and chondroitin sulfate levels varied accordingly (Fig. 3). Only the ChGn1 gene was overexpressed. It encodes the chondroitin sulfate N-acetylgalactosaminyltransferase-I (CsGalNAcT-I), an enzyme involved in the initiation of chondroitin sulfate synthesis (37). Other genes encoding enzymes involved in chondroitin sulfate polymerization (CsGalNAcT-2, CSS, CHPF and CsGlcAT) were found to be stably or underexpressed.

Chondroitin Sulfate Proteoglycans and Prion Diseases—The relative increase in the amount of chondroitin sulfate in ScGT1 cells (Fig. 3) suggested a balanced regulation of heparan and chondroitin sulfate synthesis (49) and also a chondroitin sulfate growth-promoting effect in these cells. The changes in the amounts of heparan and chondroitin sulfate in association with PrPres accumulation well correlate with several observations of such modulations after injury of the cerebral nervous system, in Alzheimer’s and prion diseases (50–52). Chondroitin sulfate proteoglycans added to cultures of rat hippocampal or cortical neurons have been described to rescue the cells from excitotoxic damage and to attenuate β-amyloid-induced neurodegeneration (53, 54). An up-regulation of chondroitin sulfate proteoglycans after an injury of central nervous system, specifically around the region of the
lesion, generally leads to an inhibitory effect toward axonal and neurite outgrowth (55–57). However, a correlation between a large amount of chondroitin sulfate proteoglycan in perineuronal nets surrounding neurons (extracellular matrix materials deposited around synaptic endings and in the space between neurons) and the protection toward the formation of materials deposited around synaptic endings and in the space and in the cytoplasmic membrane of neurites and dendritic extension and in the whole cytoplasm (Fig. 2). In contrast to the Golgi-linked enzyme, this non-Golgan isoform is able to transfer a SO_4 group on nonreducing GalNAc moieties of chondroitin and dermatan, suggesting a role for this enzyme in the regulation of chondroitin sulfation (39, 67). The presence of GalNAc-4-ST1 in both fetal brain and in various regions of the central nervous system have suggested the presence on some glycoproteins of N-linked structures ending with GalNAc-4-ST1. The post-translational modifications of TN-R by three distinct sulfated oligosaccharides, contribute to diverse specific functions for TN-R. For example, the addition of O-linked chondroitin sulfate to TN-R contributes to the inhibition of cell adhesion and neurite outgrowth in vitro (80–85). The GalNAc-4-SO_4 modified TN-R has been described to be predominantly synthesized by neurons and to be selectively localized in various brain regions, including the cortex, the hippocampus, the cerebellum and especially in perineuronal nets (79). Therefore, the expression of GalNAc-4-ST1 in a number of specific tissues and brain regions supports the idea that the tightly regulated synthesis of structures ending with β-1,4-linked GalNAc-4-SO_4 in a number of settings, are used in processes requiring specific recognition.

P. F. Gallet, personal communication.
**Possibilities for the Involvement of 4-O-Hypothesized Chondroitin in the Conversion of PrPSc into PrPSc**—The formation of PrPSc seems to involve a direct molecular interaction between PrPC and an unknown PrP isoform acting as template (86). GAG could also participate in this interaction by helping to bring PrPSc and template PrP close enough for interaction to occur (27). Interestingly, efficient sulfated GAG binding sites have been identified in the N-terminal region of PrPSc. More generally, it was shown that polymerized GAGs increase PrPSc, whereas small analogs such as heparan mimetic decrease it (26). Exogenous large chondroitin sulfates, but not chondroitin 6-sulfate, serve as a stop signal that precludes the chondroitin chain extension in ScN2a cells (26). Ben-Zaken et al. (26) suggest that both heparan and chondroitin sulfate can serve as pro-prions cofactors with equal efficiency, but because chondroitin sulfate are poorly present in ScN2a cells, their enzymatic removal by chondroitinase ABC is undetectable in terms of PrPSc production. Inversely, the chondroitin amount is higher than that of heparan sulfate in GT1 cells (Fig. 3) and consequently, in these cells, the efficacy of the large chondroitin sulfate GAG to bind PrPSc isoform could be at least similar to that of heparan sulfate GAG.

The finding that a high ChGn1 transcript level was found in parallel with a down-regulation of the Chst8 gene expression, underlines the possible connection between chondroitin synthesis and its sulfation level. Indeed, Kitagawa et al. (87) evidenced that specific sulfation, particularly 4-O-sulfation, can serve as a stop signal that precludes the chondroitin chain elongation. In this way, our data showing a possible β1-4-linked GalNAc-4-SO4 hypo sulfation, suggest the activation of an endogenous enzyme leading to the synthesis of large hypo sulfated chondroitin. Hyposulfated GAG, mainly present in ScGT1 cells, would be highly effective in promoting PrPSc accumulation by helping to bring PrPSc and template PrP close enough for interaction to occur.

**Why Does Heparan Mimetic Reverse Simultaneously Both PrPSc Accumulation and Chst8 Transcript Levels in ScGT1 Cells**—One possibility is that endogenous hyposulfated chondroitin derivatives could be components of transcription machinery that controls expression of the genes involved in chondroitin synthesis pathway. In addition to their high efficacy for PrPSc clearance, free or PrP-bound heparan mimetic would thus compete with hyposulfated chondroitin derivatives, as regulators of the Chst8 gene transcription.

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