Brevican, a Chondroitin Sulfate Proteoglycan of Rat Brain, Occurs as Secreted and Cell Surface Glycosylphosphatidylinositol-anchored Isoforms*

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cDNA clones encoding proteins related to the aggregan/versican family of proteoglycan core proteins have been isolated with antisera against rat brain synaptic junctions. Two sets of overlapping cDNAs have been characterized that differ in their 3′-terminal regions. Northern analyses with probes derived from unique regions of each set were found to hybridize with two brain-specific transcripts of 3.3 and 3.6 kilobases (kb). The 3.6-kb transcript encodes a polypeptide that exhibits 82% sequence identity with bovine brevican and is thought to be the rat ortholog of brevican. Interestingly, the polypeptide deduced from the open reading frame of the 3.3-kb transcript is truncated just carboxyl-terminal of the central domain of brevican and instead contains a putative glycosylphosphatidylinositol (GPI) anchor sequence. Antibodies raised against a bacterially expressed glutathione S-transferase-breviscan fusion protein have been used to show that both soluble and membrane-bound brevican isoforms exist.

Treatment of the crude membrane fraction and purified synaptic plasma membranes with phosphatidylinositol-specific phospholipase C revealed that isoforms of brevican are indeed glycosylphosphatidylinositol-anchored to the plasma membrane. Moreover, digests with chondroitinase ABC have indicated that rat brevican, like its bovine ortholog, is a conditional chondroitin sulfate proteoglycan. Immunohistochemical studies have shown that brevican is widely distributed in the brain and is localized extracellularly. During postnatal development, amounts of both soluble and phosphatidylinositol-specific phospholipase C-sensitive isoforms increase, suggesting a role for brevican in the terminally differentiating and the adult nervous system.

Morphogenesis and differentiation as well as functional plasticity in the brain involve a variety of different interactions between neurons and their environment. The extracellular matrix (ECM), a complex agglomeration of glycoproteins and proteoglycans located in the extracellular space, is prominently involved in these interactions. More than 20 different proteoglycan core proteins have been reported to occur in the developing and adult rat brain (1), most of them bearing either chondroitin sulfate or heparan sulfate as glycosaminoglycan (GAG) moieties. A variety of different genes encode brain proteoglycan core proteins that can occur as secreted molecules, such as CAT 301 (2) or the T1 antigen (3), as integral membrane proteins, e.g. NG2 (4) or N-syndecan (5), or as membrane-anchored forms, such as glypicans (6, 7) or cerebroglycan (8). The diversity of proteoglycan core proteins is further amplified by alternative processing at the post-transcriptional and post-translational levels (for review, see Ref. 9).

One of the most intensely studied families of proteoglycans is the aggrecan/versican family. Members of this family include the cartilage proteoglycan aggrecan (10–12), versican, a molecule originally identified in fibroblasts (13) but also highly expressed in the central nervous system (14), as well as the brain-specific proteoglycans neurocan (15) and brevican (16). They share a number of structural features. The amino-terminal domains mediate the interaction with hyaluronic acid (HA) as well as with the link protein, a relatively small polypeptide involved in the aggregation of proteoglycans (17). The link protein consists basically of a HA binding domain. BEHAB, a protein deduced from its cDNA sequence, was suggested to constitute a brain-specific link protein (18, 19).

The domain structure of the core proteins of aggrecan/versican family members is reflected at the genomic level. The genes for rat (20) and mouse aggrecan (21) as well as human versican (22) have a very similar exon/intron organization that resembles the arrangement of functional domains of the corresponding proteins.

All known members of the aggrecan/versican family are soluble chondroitin sulfate proteoglycans, whereas most of the membrane-spanning or glycosylphosphatidylinositol (GPI)-anchored proteoglycans carry heparan sulfate or keratan sulfate side chains (9). Here we report the identification and characterization of two sets of cDNAs encoding isoforms of a member of the aggrecan/versican family of proteoglycan core proteins expressed in the rat brain. One isoform constitutes most likely the rat homolog of bovine soluble brevican, whereas another group of isoforms represents the first example of GPI-anchored proteins of the aggrecan/versican family. GPI-anchored brevican isoforms are up-regulated late during postnatal development.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X79881.

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§ The abbreviations used are: ECM, extracellular matrix; GAG, glycosaminoglycan; HA, hyaluronic acid; GPI, glycosylphosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; kb, kilobase(s); EGF, epidermal growth factor; PCR, polymerase chain reaction.
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Isolation and Sequencing of cDNA Clones—The cDNA clone 37c2 was isolated from a λgt11 expression library constructed from rat brain poly(A)⁺ RNA screened with polyclonal antiserum against synaptic protein fractions from the rat brain (23, 24). Radiolabeled 37c2 cDNA was used to screen a rat brain λgt10 cDNA library (Clontech) for overlapping clones. Hybridization was carried out for 4 h at 65 °C in Rapid-hyb buffer (Amersham Corp.). The cDNA inserts were subcloned into pBluescript vectors (Stratagene) and sequenced from both strands using the fluorescent dye dideoxy termination method in combination with an automated DNA sequencer (Applied Biosystems). Sequences were handled using the GCG sequence analysis package (Genetics Computer Group, Inc.).

Northern Analysis—Total RNA was prepared from liver, heart, muscle, C6 glioma cell line, cerebellum, cortex, hippocampus, and remaining brain regions (i.e. brain stem, striatum, thalamus, hypothalamus) according to Ref. 25. RNA was electrophoretically separated on agarose gels, then transferred to nylon membranes (Amersham Corp.) following standard protocols (26). Hybridization signals were detected using Kodak X-Omat x-ray films or visualized by a Fujix BAS1000 BioImage.

Cloning and Identification of Brevican cDNAs—From a collection of cDNA clones isolated by expression screening with antisera against a rat brain synaptic protein preparation (23, 24) one cDNA, clone 37c2, displayed distinct sequence similarity to cDNAs encoding proteins of the aggrecan/versican family of proteoglycan core proteins (10, 11, 13, 15, 16). The clone was used to isolate a set of 10 overlapping cDNAs. Two of these covered a long open reading frame and were analyzed in detail (Figs. 1 and 2A). As deduced from the nucleotide sequence the encoded protein has 883 amino acid residues and a calculated molecular mass of 96,056. The translation initiation site was assigned to nucleotides 110–112 encoding the first methionine down-stream of an in-frame stop codon (30). The construct was introduced into the pRC/CMV vector (Invitrogen) and lipofected into 293 human embryonic kidney cells. Stably transfected cell lines were selected under G418, and resistant clones were propagated.

RESULTS

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by sequence comparison with other members of the aggrecan/versican protein family, the first 22 amino acids represent a potentially cleavable signal peptide. Thus, the putative mature protein consists of 861 amino acids and has a calculated Mr of 93,871.

Various functional domains can be identified in the protein encoded by the 37c2 cDNAs based on sequence homology to known proteins (Fig. 1). These include an Ig-like fold (amino acids 12–135) and two proteoglycan tandem repeats (amino acids 136–236 and 237–337). All three elements are supposed to act as HA binding region (for review see Ref. 14). Amino acid residues 338–601 represent a region unique to the 37c2 protein. This central domain is relatively rich in glutamic acid residues, which account for 16.5% of all amino acid residues in this region. The carboxyl-terminal portion harbors a single EGF-like repeat (amino acids 602–635), a lectin-like domain (amino acids 636–764), and a complement regulatory protein-like domain (amino acids 765–829). Two potential N-glycosylation sites are located at amino acid positions Asn-107 and Asn-314 (Fig. 2A). In total, 13 Ser-Gly or Gly-Ser dipeptides, potentially serving as GAG attachment sites, occur in the deduced amino acid sequence. Five of these dipeptides have at least one acidic amino acid in the vicinity of either side, which is thought to be essential for GAG attachment (13). Four of these five sites are located in the Glu-rich central domain where GAG attachment to members of the aggrecan/versican family of proteoglycans is thought to occur.

Among the members of the aggrecan/versican family, bovine brevican (16) is most closely related to the protein encoded by the 37c2 cDNAs. The overall sequence identity of the two proteins is 82% with the highest degree of identity located in the HA binding domain (90%) and the carboxyl-terminal homology region comprising the EGF-repeat, the lectin-like domain, and the complement regulatory protein-like domain (84%). The central domain is least conserved, both in length (274 amino acids for the 37c2 protein, 298 amino acids for bovine brevican) and sequence identity (72%), although in both proteins this region is relatively rich in glutamic acid residues. The positions of nine out of 13 Ser-Gly or Gly-Ser dipeptides, the two potential N-glycosylation sites, and all cysteine residues that determine the structure of functional domains are conserved between the 37c2 protein and brevican (Fig. 2A). Thus, the two proteins are likely to be species homologs, and we refer to the new protein as rat brevican.

The nucleotide sequence of one of the isolated cDNAs, clone 37c2/10, differed from that of the other cDNAs starting from nucleotide 1982 and contains 553 additional nucleotides (Figs. 1 and 2B). An nested reverse transcriptase PCR assay confirmed that a corresponding transcript exists in total brain RNA and the cDNA was not artificially recombined during library construction (data not shown). As the nucleotide sequence around the point of sequence divergence (CAG/GTAATT, Fig. 2B) perfectly resembles a splice donor site (31), we wondered whether the clone represents simply an unspliced transcript. To test this hypothesis, Northern blot hybridization was performed using specific PCR amplification products derived from the unique region of each set of cDNAs. As shown in Fig. 3 (lanes 9 and 10), the hybridizing transcript (3.3 kb) is smaller than the major brevican transcript of 3.6 kb, indicating that the RNA represented by the 37c2/10 cDNA must be an alternatively processed mRNA species.

The common protein sequence encoded by both transcripts

FIG. 2. Nucleotide sequence and deduced amino acid sequence of brevican isoforms. A, sequence of the cDNAs encoding the secreted rat brevican isoform. The putative signal peptide is underlined; potential N-glycosylation sites are indicated by an open triangle Ser-Gly and Gly-Ser dipeptide sequences representing potential chondroitin sulfate attachment sites are double underlined with dashed lines; cysteine residues conserved between rat and bovine brevican (16) are indicated by dots. B, sequence of cDNA clone 37c2/10 spanning the unique 3' region that encodes the glycoprotein signal of GPI-anchored brevican. The beginning of the specific sequence at nucleotide 1982 is indicated. The potential splice donor site is dotted underlined. The Ser residue serving as potential site of GPI anchor addition is marked by a filled triangle. The nucleotide sequence of A is available from the GenBank/EMBL/DDJB data banks under the accession number X79881. The sequence of B has been submitted.
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Fig. 3. Northern analysis of brevican transcripts. Nylon filters containing 20 μg lane total RNA from 30-day-old rats were hybridized with radiolabeled 37c2 cDNA (lanes 1−8) or with specific PCR products derived from nucleotides 2326−2618 of the 3.6-kb cDNA (lane 9; probe 1 in Fig. 1) or from nucleotides 1997−2283 of the 3.3-kb cDNA (lane 10; probe 2 in Fig. 1). Tissue distribution was as follows: lane 1, brain stem, striatum, thalamus, and hypothalamus; lane 2, hippocampus; lane 3, cerebellum; lane 4, cerebral cortex; lane 5, liver; lane 6, heart; lane 7, skeletal muscle; lane 8, C6 glioma cells; lanes 9 and 10, cerebral cortex. Note, to detect the 3.3-kb transcript, the filter showing lanes 1−8 had to be overexposed. A shorter exposure visualizes only the 3.6-kb transcript.

Fig. 4. Tissue distribution of brevican immunoreactivity. Immunoblots containing 15 μg of protein/lane of the soluble protein fractions (lanes 1−4) or detergent extracts of crude membrane fractions (lanes 5−7) were developed using rabbit anti-brevican antiserum. Lane 1, 100,000 × g supernatant from brain homogenates; lanes 2−4, chondroitinase ABC-treated 100,000 × g supernatant from brain (2), heart (3), and liver (4). Lanes 5−7, Triton X-100 extracts of untreated brain (5), heart (6), and liver (7) membranes. Apparent molecular weights of major protein bands are indicated.

are consistent with the data for bovine brevican where a 145-kDa core protein and an 80-kDa processing product have been described (16). In addition, two minor protein bands of about 110 and 60 kDa are detected by the antiserum, the identity of which is yet unclear.

Additional brevican immunoreactivity is associated with the crude membrane fraction of brain extracts (Figs. 4 and 5). Detergent extracts of this fraction contain a diffuse high molecular weight smear and immunoreactive bands of 140, 125, and about 80 kDa (Fig. 4, lane 5). Again, the high molecular weight material is chondroitinase-sensitive (Fig. 5A, lanes 2 and 3).

Chondroitinases ABC and AC (not shown) completely eliminate the high molecular weight material, whereas incubation with heparitinase III does not affect these high molecular weight forms, neither in the soluble fraction nor in membranes (data not shown), indicating that brevican bears principally chondroitin sulfate side chains.

The crude membrane fraction was digested with PI-PLC to prove the existence of GPI-anchored isoforms. As shown in Fig. 5A (lanes 4 and 5), most of the immunoreactivity is released from the membranes by this treatment. Digestion of the crude membrane fraction with GPI-specific phospholipase C produces similar results (not shown). Incubation of the membranes under identical conditions but without enzyme does not release brevican immunoreactivity into the supernatant. Thus, we conclude that GPI-anchored isoforms of brevican do occur. These isoforms are a 140-kDa protein that is similar in size as the major soluble form, a 125-kDa protein that does not occur in the soluble protein fraction, and an 80-kDa isoform that co-migrates with the upper half of the soluble 70−80-kDa material.

In order to clarify which GPI-anchored brevican isoforms are derived from the 3.3-kb transcript, human embryonic kidney cells 293 were transfected with the 3.3-kb cDNA. On Western blots of isolated crude membranes from untransfected 293 cells, no brevican immunoreactivity is detectable (Fig. 6, lane 1). Stable transfectants express high amounts of brevican. After chondroitinase treatment, three distinct isoforms with apparent molecular masses of 90, 125, and 140 kDa are observed (Fig. 6, lane 2). Crude membrane fractions of these transfectants were treated with PI-PLC to test whether GPI anchoring occurs. Whereas the 140-kDa isoform is not affected by the enzyme, the 90- and 125-kDa isoforms are at least in part released into the supernatant (Fig. 6, lanes 3 and 4). This demonstrates that correct glypiation of the 90- and the 125-kDa isoform can occur in 293 cells.

The original rat brevican clone was isolated using antibodies against a synaptic protein preparation. Therefore, we exam-
Figure 5. Identification of GPI-anchored brevican isoforms in the rat brain. A, immunoblots contain chondroitinase ABC-treated soluble protein fraction (lane 1); untreated crude membrane fraction (lane 2); chondroitinase ABC-treated crude membrane fraction (lane 3); supernatant of chondroitinase ABC and PI-PLC-treated membranes (lane 4); pellet of chondroitinase ABC and PI-PLC-treated membranes (lane 5). Lanes 1-3 contain 15 μg of protein/lane; the protein contents of lanes 4 and 5 add up to 15 μg. B, immunoblots of brevican isoforms released by PI-PLC from chondroitinase ABC-treated crude membranes (lane 1) and synaptic membranes (lane 2). The synaptic membrane-enriched isoform is indicated by an asterisk. Immunoblots were developed using rabbit anti-brevican antibodies.

Figure 6. Expression of GPI-anchored brevican isoforms in stably transfected HEK 293 cells. 15 μg/lane of crude membrane fractions from untransfected (lane 1) or stably transfected 293 cells (lane 2) were treated with chondroitinase ABC and immunoblotted. Lane 3 contains the chondroitinase ABC treated PI-PLC-released supernatant, and lane 4 contains the corresponding pellet from 15-μg crude membrane proteins of transfected cells.

Expression of Brevican during Postnatal Development—The expression of many proteoglycans is subjected to massive changes during development (1). To study the developmental regulation of brevican isoforms, we have performed an immunoblot analysis of chondroitinase-digested soluble and PI-PLC-released protein extracts from rat brains collected at postnatal days P1 through P64. The soluble 140- and 70–80-kDa isoforms are present at birth, and their amounts steadily increase from days P4 to P64 (Fig. 7A). The complexity of GAG attachment also increases during this developmental period (not shown). The GPI-anchored isoforms are barely detectable around birth. A strong increase of these isoforms occurs after day P8 (Fig. 7B). In conclusion, brevican expression is generally up-regulated during terminal differentiation of the rat brain.

Immunohistochemical Localization of Brevican—We have used three different antisera against the glutathione S-transferase-37c2/12 fusion protein to study the distribution of brevican in the rat brain at postnatal day P30. The antisera cannot discriminate between the various brevican isoforms. Brevican immunoreactivity is found in all parts of the brain. A typical extracellular staining surrounding cell bodies is observed, for instance around hippocampal pyramidal cells (Fig. 8, A and B) or cerebellar granule cells and Purkinje cells (Fig. 8C). Primary dendrites of hippocampal pyramidal cells and Purkinje cells are also stained at their surfaces. In neuropil areas, the distribution of brevican immunoreactivity is diffuse. Staining appears not to be restricted to neurons.

DISCUSSION

This study describes the cloning of the complete coding sequence for two isoforms of a rat member of the aggrecan/versican family of proteoglycan core proteins. One isoform has the features of ECM core proteins that are secreted into the extracellular space, the other one is likely to be attached to the cell membrane via a GPI anchor. Biochemical data demonstrate that both soluble and GPI-anchored isoforms of this proteoglycan exist. Immunohistochemical studies with antisera that recognize both types of isoforms revealed an extracellular localization throughout the brain, suggesting that the protein is indeed a component of the ECM.

The secreted isoform of the rat protein characterized here has 82% sequence identity with bovine brevican (16). Both proteins are of similar size. Therefore, we assume that the two proteins are orthologs. This assumption is supported by protein data; soluble bovine brevican occurs in two isoforms, a 145-kDa mature form and an 80-kDa amino-terminally truncated form (16). Antisera against rat brevican fusion proteins also recognize proteins of similar sizes in the soluble fraction of rat brain homogenates. Moreover, bovine brevican as well as the rat protein are brain-specific. Both proteins act as conditional proteoglycans, i.e., they can occur as proteoglycan and as free proteins.

The central domains of the two proteins have substantially diverged. The sequence identity is only 72% as compared with...
90 and 84% for the HA binding domain and the carboxyl-terminal homology region, respectively. Attachment of chondroitin sulfate is thought to occur to the central domain. Rat brevican contains four typical GAG attachment sites as defined by Ref. 13. These are at positions Ser-391, Ser-524, Ser-528, and Ser-539. Bovine brevican contains three putative sites at positions homologous to Ser-391, Ser-528, and Ser-539 (16).

Another putative member of the aggregan/versican family is BEHAB (18). BEHAB is more than 99% identical with the amino-terminal 363 amino acids of pre-brevican, indicating that both are most likely derived from the same gene and that BEHAB is a carboxyl-terminally truncated form of brevican. However, the existence of BEHAB as an actual protein has not been demonstrated yet. The distribution of BEHAB transcripts in the rat brain as revealed by in situ hybridization (18, 19) is in favorable accord with the distribution of brevican immunoreactivity observed in this study.

Differential RNA processing and the alternate use of exons have been described as mechanisms to increase the molecular diversity of ECM components (e.g. Refs. 33–35). Our data show that this is also the case for brevican, where secreted and GPI-anchored isoforms are synthesized from alternatively processed transcripts of the same gene. A potential splice donor site located on the 3.3-kb brevican transcript at the point of divergence from the 3.6-kb transcript suggests that the glycilation signal may be encoded by a DNA segment, which is removed as an intron from transcripts for the secreted brevican isoform. Indeed, the organization of the mouse brevican gene implies that transcripts for the GPI-anchored isoform are synthesized by “read through” into a 2-kb intron located between the exons for the carboxyl terminus of the central domain and the EGF-like domain.

Besides alternative processing of transcripts, the heterogeneity among brevican isoforms may reflect differential modification of primary translation products. After chondroitinase ABC digestion of the soluble protein fraction, the two major bands of 140 and 70–80 kDa are likely to represent unprocessed and amino-terminally processed brevican, respectively (16). In the crude membrane fraction, three major PI-PLC-sensitive protein bands of 140, 125, and 80 kDa are detectable. Human embryonic kidney 293 cells stably transfected with the cDNA of the 3.3-kb transcript express 140-, 125-, and 90-kDa core proteins with the latter two being sensitive to treatment with PI-PLC. Thus, 293 cells are capable of correctly inserting brevican isoforms into the plasma membrane via a GPI-anchor. The 90-kDa isoform synthesized by transfected cells coincides with the size of the in vitro translation product obtained from in vitro transcripts of the 3.3-kb cDNA, suggesting that it represents the primary glypiated translation product. Glypilation is thought to be an early event in the cascade of post-translational modifications that occurs in the endoplasmic reticulum (32). In the rat brain, only minor amounts of the 90-kDa material are detectable. The 125-kDa isoforms found in brain membranes and in transfected cells are both released from membranes by PI-PLC. They may be identical products. In contrast, the 140-kDa isoforms differ in their PI-PLC sensitivity; the brain membrane isoform is PI-PLC-releasable, and the isoform expressed in 293 cells is not. The reason for this difference is unclear. In the brain, an additional 80-kDa isoform occurs, which is released from membranes by PI-PLC. This may be a proteolytic degradation product of the larger forms, as the relative amount of the 80-kDa material varies from preparation to preparation. Taken together, these data show that multiple isoforms are derived from the 3.3-kb transcript, although the modifications leading to the differences in migration in SDS-polyacrylamide gel electrophoresis are yet unknown.

The brevican fusion construct used for antibody production contains, in addition to the relatively unique central domain, a

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Fig. 8. Localization of brevican immunoreactivity in the hippocampus (A and B) and the cerebellum (C) of 30-day-old rats. A distinctive extracellular staining is observed around pyramidal cells (Py) of the hippocampal CA3 region (B). In the radial layer (r) and the mossy fiber layer (stratum lucidum, sl) immunoreactivity coats the dendrites of the pyramidal neurons (B). The cerebellar Purkinje cells (Pu) and their primary dendrites spreading out into the molecular layer (m) are intensely stained at their surfaces (arrowheads in C). The extracellular space in the granule cell layer (g) is filled with reaction product (C). The box in A indicates the hippocampal region detailed in B.

90 and 84% for the HA binding domain and the carboxyl-terminal homology region, respectively. Attachment of chondroitin sulfate is thought to occur to the central domain. Rat brevican contains four typical GAG attachment sites as defined by Ref. 13. These are at positions Ser-391, Ser-524, Ser-528, and Ser-539. Bovine brevican contains three putative sites at positions homologous to Ser-391, Ser-528, and Ser-539 (16).

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The brevican fusion construct used for antibody production contains, in addition to the relatively unique central domain, a
part of the HA binding region, and the EGF-like repeat, which are reasonably conserved among a variety of ECM proteins and cell surface receptors interacting with HA. Therefore, we cannot completely rule out that some of the immunoreactive bands, e.g., the weakly immunoreactive bands observed in both soluble and membrane fractions, represent other HA-binding proteins cross-reacting with the brevican antisera. Although, based on the sizes of recognized proteins, a cross-reaction with known members of the aggrecan/versican family (the identity of the HA-binding region ranges between 57 and 64% (16)) can be excluded.

Perhaps the most important finding of this study is the identification of isoforms of brevican that are anchored to the membrane via glypiation. This is the first example of GPI anchoring in the aggrecan/versican family. All members of this family of proteoglycan core proteins thus far reported to be expressed in the central nervous system, i.e., neurocan, versican, CAT-301, the S103L PG, and brevican, are secreted into the extracellular space and appear as soluble proteins upon cell fractionation (9). They are all chondroitin sulfate-bearing proteins. On the other hand, the two brain proteoglycan core proteins known to be GPI-anchored, glypican and cerebrolysin, (6, 7) and cerebrolysin, (8), also termed M12 and M13 (1), are heparan sulfate proteoglycans.

What could be the biological significance of a glypiated member of the aggrecan/versican family of brain proteoglycans? This mode of anchoring proteins to the cell membrane results in an increased lateral diffusibility along the membrane (36). It enables the protein to be released from the membrane by transcellular phospholipases in a controlled manner. Glypiated proteins are discussed as markers for physiologically important membrane microdomains (37). In polarized cells, such as neurons, a precise targeting to specific plasma membrane domains caused by the GPI signal has been observed (38). In addition, a possible interaction with signal transducing tyrosine kinases was proposed based on immunoprecipitation assays (for review, see Ref. 35). In essence, GPI anchorage makes proteins good candidates to mediate dynamic remodeling of neuronal membranes (39) receiving signals from the ECM and transducing them along and/or through the membrane. This postulated gain of function for the GPI-anchored as compared with secreted brevican isoforms is accompanied by a loss of possible other functions realized by the specific carboxyl-terminal domains of the extracellular brevican isoforms. This portion of the protein comprising the EGF-repeat, the lectin-like domain, and the complement regulatory protein-like domain is conserved among the other members of the aggrecan/versican family. Possible biological functions of this region may include the recognition of carbohydrate structures and the interaction with other proteins. As a working hypothesis, we can assume that the extracellular brevican isoform is an integral ECM component interacting both with HA via its amino terminus and with other ECM components via its carboxyl terminus, whereas the membrane-linked isoform could function as a chondroitin sulfate-bearing cell surface receptor targeting interaction with HA to specific membrane subdomains.

One such subdomain could be the synapse. Interestingly, the developmental appearance of the glypiated brevican isoforms parallels synaptogenesis in the rat central nervous system. Depending on the brain area, synapse formation starts around birth, culminates between postnatal days 15 and 25, and is basically completed after day P30 (40–43). A possible role of particular brevican isoforms in synaptic development and function is also implied by the fact that (i) the original cDNA clone was isolated using antisera against a rat brain synaptic protein preparation and (ii) a glypiated brevican isoform is enriched in the synaptic membrane fraction. A definitive proof for a synaptic localization of glypiated brevican will require specific antibodies for this particular isoform of brevican.

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