Proteoglycans in the nervous system

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Proteoglycans are ubiquitous cell-surface and secreted glycoproteins that are involved in diverse cellular behaviors. The identities of several nervous system proteoglycans, including many of the major species in the mammalian brain, have recently come to light. In addition, recent studies have given new insights into the roles of proteoglycans in nervous system development and function.

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Introduction

Proteoglycans (PGs) are found on the surfaces of all adherent cells, within intracellular vesicles, and in virtually all extracellular matrices (ECMs). They are evolutionarily ancient molecules, and play functional roles in the biology of growth factors, extracellular proteolysis, cell adhesion, lipoprotein metabolism, and virus entry into cells, as well as structural roles in maintaining the physical and mechanical properties of ECMs [1,2•,3,4].

Although many basic characteristics of PGs — their number, their structures, the exact nature of the functions they perform — are still slowly emerging, great progress has been made in recent years. With this recent burst of activity has come increasing recognition of the significance of PGs by neurobiologists, and increasing interest in the postulated roles PGs play in the nervous system. Some investigators have isolated monoclonal antibodies against nervous system molecules that have turned out to be PGs. Other investigators have become intrigued by the fact that many of the molecules that are thought to influence neuronal and glial cell behavior *in vivo*, especially during development, bind PGs. In the last few years, direct assaults on determining the structures of central nervous system (CNS) PGs have been undertaken by several groups. The purpose of this article is to review some of these recent results, and place them into the wider context of what PGs are, and how they are thought to function.

What are PGs?

A protein is called a PG if it contains a covalently attached glycosaminoglycan (GAG). GAGs are linear polysaccharides, typically 20–200 sugars in length, which are usually attached via a characteristic linkage region to serine residues. GAGs are built by the sequential addition of identical disaccharide units onto this linkage region. Only three types of disaccharide may be used, giving rise to three families of GAGs: the heparin/heparan family [D-glucuronic acid $\beta(1\rightarrow4)$ D-*N*-acetyl glucosamine $\alpha(1\rightarrow4)]_n$; the chondroitin/dermatan family [D-glucuronic acid $\beta(1\rightarrow3)$ D-*N*-acetyl galactosamine $\beta(1\rightarrow4)]_n$; and the keratan family [D-galactose $\beta(1\rightarrow4)$ D-*N*-acetyl glucosamine $\beta(1\rightarrow3)]_n$. The sugars of most GAGs are further chemically modified, typically in a sporadic fashion throughout the chain, by *O*-sulfation, *N*-deacetylation followed by *N*-sulfation, and/or epimerization (isomerization) of glucuronic acid to iduronic acid. Subsequently, GAGs are referred to as heparin, heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS) or keratan sulfate (KS). The heparin/HS distinction and the CS/DS distinction only reflect differences in level of modification (i.e. heparin is more highly modified than most HS species; DS contains much more iduronate than CS). As each disaccharide in a GAG chain may be modified to a different degree, the large scale structures of GAGs can be exceedingly complex (e.g. in HS, which can be modified in up to five ways, a hexasaccharide can theoretically have over 30,000 possible chemical structures).

Products of several gene families, including secreted and membrane-inserted polypeptides, act as the core proteins of major PGs (Table 1). Some bear as few as one GAG chain, whereas others have over a hundred. Although the signals that specify whether a serine residue will bear a GAG are partially understood [2•], it is not known what controls the type of GAG synthesized: examples exist of cores that always bear one type of GAG, cores that bear different GAGs at different sites, and cores that bear different GAGs depending on the cell type in which they are expressed.

Abbreviations

CNS—central nervous system; **CS**—chondroitin sulfate; **DS**—dermatan sulfate; **ECM**—extracellular matrix; **FGF**—fibroblast growth factor; **GAG**—glycosaminoglycan; **HS**—heparan sulfate; **KS**—keratan sulfate; **NCAM**—neural cell adhesion molecule; **NgCAM**—neuron-glial cell adhesion molecule; **PG**—proteoglycan.

Table 1. Cloned PG core proteins.[a]

Cell-surface PGs	
	Syndecan family
	Syndecan (Syndecan-1)
	Fibroglycan (Syndecan-2)
	N-Syndecan/Syndecan-3
	Ryudican/Amphiglycan/Syndecan-4
	Glypican family
	Glypican
	Cerebroglycan
	NG-2
	'Part-time PGs'[b]
ECM PGs	
	Aggrecan family
	Aggrecan
	Versican
	Neurocan
	Small, interstitial PG family
	Decorin
	Biglycan
	Fibromodulin
	Lumican
	Perlecan
	Type IX Collagen
Intravesicular PGs	
	Serglycin
	SV2

[a]Only shown are the obligate PG core proteins, i.e. those that invariably bear GAG chains. A small number of other cell-surface proteins bear GAG chains in some cells, but not others. [b]These 'part-time' PGs include CD44 and the type III transforming growth factor (TGF)-β receptor (reviewed in [1,2•,53]).

Cell surface PGs of the CNS

Early progress toward identifying cell surface PGs of the brain was made by Margolis' group, who detected a single major HSPG in adult brain membranes [5]. Later, Herndon and I [6] found evidence for CSPGs and other, less abundant, HSPGs in adult brain membranes, as well as additional major HSPGs that are present only during development. In the past year, the core proteins of several of these have been identified.

Glypican

Glypican was first identified as a surface HSPG core protein of human fibroblasts [7]. The mature polypeptide is 53 kDa and is anchored in the plasma membrane by covalently attached glycosylphosphatidylinositol. Both the adult brain HSPG identified by Klinger *et al.* [5], and brain HSPG M12 identified by us [6], are the rat form of glypican ([8]; ED Litwack, CS Stipp, A Kumbasar, AD Lander, unpublished data). *In situ* hybridization studies in the adult brain and spinal cord indicate that glypican mRNA is expressed primarily, if not exclusively, by projection neurons in many, but not all parts of the CNS (ED Litwack, CS Stipp, A Kumbasar, AD Lander, unpublished data) (see Table 2). In the embryo, glypican is also strongly expressed in ventricular zones (regions undergoing neural precursor proliferation) throughout the neuraxis (Fig. 1).

Cerebroglycan

Cerebroglycan, previously called PG M13 [6], is an HSPG with a ~58 kDa core protein, and was first detected in the embryonic and newborn — but not adult — rat brain. Like glypican, it is glycosylphosphatidylinositol-anchored. In fact, glypican and cerebroglycan define a family of lipid-anchored HSPG cores, based on amino acid sequence similarity (CS Stipp, ED Litwack, AD Lander, unpublished data) (see Table 2). *In situ* hybridization studies indicate that cerebroglycan is transiently expressed by postmitotic neurons throughout the CNS (Fig. 1). Evidently, cerebroglycan mRNA appears in neurons shortly after terminal mitosis and disappears after neuronal migration and axon growth have been completed. Interestingly, cerebroglycan is not expressed outside the nervous system.

N-syndecan

N-syndecan (or syndecan-3) is one of four members of the syndecan family of transmembrane core proteins (Table 1). These polypeptides have short (~34 amino acids) cytoplasmic domains that are highly conserved among all family members, and overall sizes varying from 20 kDa (syndecans-2 and -4) to ≥ 42 kDa (syndecan-3). Their extracellular domains are poorly conserved among the different family members, or even for the same syndecan in different mammalian species. *N*-syndecan was cloned by Carey *et al.* [9••], who identified it in rat Schwann cell membranes (see Table 2). High levels of *N*-syndecan mRNA are also found in neonatal rat brain, as well as in many sites outside the nervous system. Expression of this molecule in rat brain peaks at birth, declining to undetectable levels thereafter. Early immunohistochemical studies suggest that this PG is associated with fiber tracts, but it is not yet known whether its source is neuronal or glial.

Syndecan-2

Syndecan-2, also known as fibroglycan, another member of the syndecan family, has not yet been isolated from the brain, but its mRNA has been found there (see Table 2). Based on electrophoretic behavior, syndecan-2 may correspond to brain PG M14 [6].

Table 2. PGs of the mammalian CNS.[a]

Name	Family	GAG	CNS Expression
Syndecan-3	Syndecan	HS	Transiently expressed in perinatal brain; widespread [9••]
Glypican[b]	Glypican	HS	Neuroepithelium; certain adult projection neurons [8][c]
Cerebroglycan[b]	Glypican	HS	Transiently expressed by newly post-mitotic neurons[d]
NG-2	NG-2	CS	O-2A progenitors [10]
Syndecan-2	Syndecan	HS	Unknown [2•]
Neurocan (1D1)	Aggrecan	CS	White matter of developing cerebellum; molecular layer of adult cerebellum. Mostly intracellular in adult [12••,18]
Versican	Aggrecan	CS	White matter [13•]
Aggrecan	Aggrecan	CS	Embryonic chick brain [14•]
Cat-301	Aggrecan?	CS	Subsets of neurons, cerebellum and spinal cord [15•,20]
PG-T1	?	CS	Widespread [16••,17•]
3H1	?	CS/KS	Similar to neurocan [18]
3F8	?	CS	Concentrated in molecular layer of developing and adult cerebellum [18]
6B4	?	CS	Cerebellar and brainstem projection neurons [19•]
Unnamed	?	HS	Transient, in CNS fiber tracts [26••]
SV2 antigen	SV2	KS	Synaptic vesicles [27••]

[a]PGs are referred to by the names of their core proteins, and are grouped according to whether they are cell surface, extracellular matrix/soluble, or intravesicular molecules (see text). In many cases, information on CNS distribution has been based on the examination of only a few brain regions, and is therefore incomplete. [b]Data on distribution of glypican and cerebroglycan are based on *in situ* hybridization; most other data were obtained using antibodies. [c]ED Litwack, CS Stipp, A Kumbasar, AD Lander, unpublished data. [d]CS Stipp, ED Litwack, AD Lander, unpublished data.

NG2

NG2 is a transmembrane CSPG with a 300 kDa core protein [10]. In the brain it is associated with a population of glial precursor cells, the O-2A progenitors (see Table 2), that give rise to oligodendrocytes and a type of astrocyte. The very large core protein of NG2 suggests that it may serve functions other than just bearing CS chains. One such function appears to be the binding of type VI collagen [11].

ECM and 'soluble' PGs of the CNS

Many PGs can be extracted from the brain using physiological buffers without detergent; others require high salt or denaturing conditions. Although it has been argued that some of these molecules may reside in the cytoplasm of cells, most are probably loosely associated with the ECM.

Most of the PGs in these categories contain CS as their major GAG. Neurocan, a recently cloned CSPG, has a 136 kDa core protein, and contains ~3 CS chains [12••]. Its protein sequence places it in a family with aggrecan — the major ECM PG of cartilage — and versican, an ECM PG first found associated with fibroblasts. Like these other PGs, neurocan binds the ECM polysaccharide hyaluronic acid via a protein domain that is highly conserved in all three family members. Recent evidence suggests that versican and aggrecan are themselves expressed in the human and chicken brain, respectively [13•,14•]. The Cat-301 antigen is yet another large brain CSPG that binds hyaluronic acid, and immunological evidence suggests that it is related to aggrecan [15•]. One additional hyaluronic acid-binding CSPG, the T1 antigen, has been identified in brain, but at least the hyaluronic acid-binding region of this molecule is apparently unrelated to those of the aggrecan family [16••,17•]. Still other brain CSPGs have been identified with monoclonal antibodies, and remain to be fully characterized [14•,18,19•].

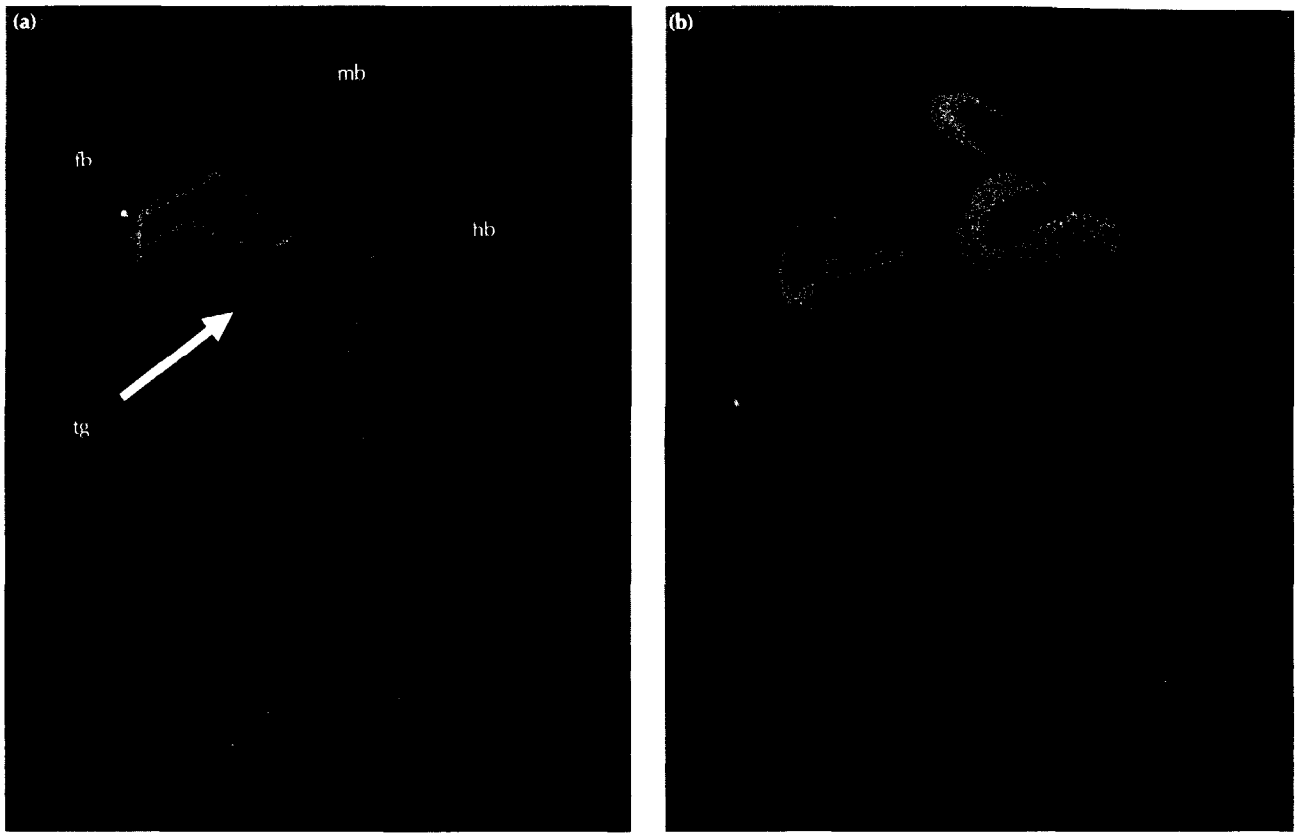

Fig. 1. Expression of glypican and cerebroglycan in the rat embryo. Adjacent sections of embryonic day 14 rats were hybridized with radiolabeled RNA probes specific for **(a)** glypican and **(b)** cerebroglycan mRNA. The images are reverse contrast prints of the resulting autoradiograms. Glypican expression is found throughout the embryo, but is particularly strong in the ventricular zones of the developing CNS. In contrast, cerebroglycan mRNA, which is found only in neural tissue, is not detected in ventricular zones but is found in the layers of immature neurons that form around those zones. In the adult brain, glypican is expressed by subpopulations of neurons, whereas cerebroglycan is absent. fb—forebrain; mb—midbrain; hb—hindbrain; tg—trigeminal ganglion.

The distributions of these CSPGs vary from remarkably uniform throughout the brain (PG T1) to remarkably cell type- and developmental stage-specific. For example, Cat-301 appears around certain subsets of neurons only after activity-dependent critical periods in their development [20]. Another is transiently expressed during axon outgrowth by several types of neurons involved in the cerebellar mossy fiber system [19•].

As information on the distribution of these CSPG and CS/KSPG core proteins accumulates, so has information on the distribution of different types of CS and KS chains. Several investigators have observed remarkable cell-type specificity in the binding of anti-CS and anti-KS monoclonal antibodies to brain sections (e.g. [21,22]). During cerebral cortex development, CS is found in the early proliferative neuroepithelium, then later in the marginal zone and subplate regions [23]. With the exception of the subplate, many of the locations of CS expression during development correlate with sites where axons do not grow. For example, CS, as well as KS, are strongly expressed in the roof plate of the spinal cord [24,25].

Recently, a report of an HSPG in brain ECM appeared [26••]. This molecule is found in basal laminae outside of and surrounding the chicken brain, but is also expressed transiently in many developing CNS axon tracts. The core protein size (250 kDa) and basal laminar distribution of this PG are reminiscent of perlecan, a major basement membrane PG, but perlecan itself is not found in CNS axon tracts.

Synaptic vesicle PGs

It has long been known that a PG is a major component of synaptic vesicles isolated from the electric organs of fishes. This PG was recently shown to be a transmembrane KSPG, and appears to be involved in acetylcholine transport into vesicles [27••]. Immunochemical data suggest that this molecule is present in many other types of synaptic vesicles, and might therefore play an important general role in transmitter uptake.

Roles of PGs in the nervous system

Insights into the functions of PGs in the nervous system have come by many routes, direct and indirect, and many of the conclusions are still somewhat preliminary. Highlights of what has been learned are summarized below.

The functions of a family of growth factors are dependent on PGs

All members of the fibroblast growth factor (FGF) family bind GAGs of the heparin/HS class, and apparently must do so to be biologically active [28,29]. Recent studies support a model in which cell-surface HSPGs bind both FGFs and FGF receptors simultaneously, facilitating their interaction [30]. It is known that at least three FGFs — FGF-1, -2 and -5 — are expressed in the nervous system and exert trophic effects on several classes of neurons [31–34,35••]. Recently, Nurcombe *et al.* [35••] have suggested that differences in the type of HS carried by a single core protein can render early neuroepithelial cells selectively responsive either to FGF-1 or to FGF-2. This proposition is supported by evidence in other systems that HS structure can impart specificity to HSPG function (e.g. [36,37•,38,39•]).

The kinetics of action of a family of protease inhibitors are dependent on PGs

The structurally related molecules antithrombin III, heparin cofactor II, and protease nexin I all bind and inactivate certain serine proteases (e.g. thrombin) much more rapidly when appropriate GAGs are present. To a large extent, GAGs act by simultaneously binding both protease and protease inhibitor, confining them to the same locality and thereby facilitating their interaction [38]. Of interest to neurobiologists, protease nexin I is abundantly expressed in the CNS, and is thought to regulate neurite outgrowth and neuronal migration [40].

Cell surface PGs participate in establishing cell–cell and cell–ECM contacts

Although cell surface PGs can apparently be the sole receptors for attachment to certain substrata [37•], PGs usually facilitate interactions mediated through other receptors, such as integrin-dependent cell attachment to ECM molecules [41], and neural cell adhesion molecule (NCAM)-dependent cell–cell adhesion [42,43]. A recent study suggests that cell surface HSPGs are especially important for the interaction of neural cells with fibronectin [44•]. As ECM and cell adhesion molecules are thought to provide important navigational cues to growing axons, the involvement of PGs with such molecules suggests a potential role for PGs in axon guidance. Recent studies in insects support this idea [45••].

ECM PGs regulate cell–cell and cell–matrix interactions

The core protein of at least one PG, perlecan, supports integrin-mediated cell attachment [46]. In contrast, several PGs inhibit the biological activities of ECM and cell adhesion molecules, at least *in vitro*. For example, adsorbed CSPGs or CS/KSPGs can render culture substrata inhospitable for neurite growth [24,25]. Soluble CSPGs from rat brain also inhibit neurite outgrowth by PC12 cells [47]. Neurocan and the 3F8 CSPG of rat brain (but not aggrecan) inhibit homophilic NCAM and neuron-glial cell adhesion molecule (NgCAM)-binding [48•]. A HSPG released by Schwannoma cells specifically blocks the neurite outgrowth-promoting activity of laminin [49]. In some of these cases, the GAG chains of the PGs are required for these actions [24,25,49]; in others they are not [47,48•]. It is not yet known whether these phenomena are direct actions of PGs on neurons, or reflect effects of PGs on the physical characteristics of the culture substratum, so caution must be used in extrapolating these results to *in vivo* settings. Nonetheless, the distributions of some CSPGs are consistent with a 'barrier' function *in vivo* (see above). For example, in the developing retina a receding wave of CS expression marks a front of centripetally directed axons, suggesting that axons are guided by their avoidance of CS. Intriguingly, a CS-degrading enzyme disrupts the timing and direction of retinofugal axons in the developing rat retina [50••].

PGs are involved in the assembly of ECM, and act as binding sites for molecules that associate with the ECM

PGs bind virtually every major ECM component. In addition, molecules such as growth factors (e.g. FGFs) and enzymes (e.g. synaptic acetylcholinesterase) are often immobilized in ECMs through interactions with HSPGs [1,51]. The importance of PGs in ECM structure and function is illustrated by a muscle cell line that is defective in GAG biosynthesis [52•]. This cell line produces an abnormal basal lamina and, probably as a consequence, fails to form acetylcholine receptor clusters. The cells also fail to form such clusters in response to agrin, a GAG-binding ECM molecule that potently induces receptor clusters on normal muscle cells, and is thought to be involved in synaptogenesis *in vivo*.

Conclusions

Although much still needs to be learned about nervous system PGs, the identities of many of the major species in the brain are now known. Tracking down the functions of these molecules will probably not be easy. Their biological activities are likely to reside in their capacity to regulate, possibly in subtle ways, the functions of the molecules they bind. Moreover, the repertoire of molecules they bind will probably depend in part on the precise structures of their GAG chains, structures which defy easy analysis. Nevertheless, PGs are likely to continue to receive increasing attention in neurobiology, as their *in vivo* distributions and *in vitro* activities suggest that they are widely involved in nervous system development and function.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. RUOSLAHTI E: **Proteoglycans in Cell Regulation.** *J Biol Chem* 1989, **264**:13369–13372.

2. • BERNFIELD M, KOKENYESI R, KATO M, HINKES MT, SPRING J, GALLO RL, LOSE EJ: **Biology of the Syndecans: a Family of Transmembrane Heparan Sulfate Proteoglycans.** *Annu Rev Cell Biol* 1992, **8**:365–393.

A cogent review that brings together historical, structural and functional information about all members of the syndecan family, the major family of transmembrane HSPGs.

3. SHIEH MT, WUDUNN D, MONTGOMERY RI, ESKO JD, SPEAR PG: **Cell Surface Receptors for Herpes Simplex Virus Are Heparan Sulfate Proteoglycans.** *J Cell Biol* 1992, **5**:1272–1281.

4. LANDER AD, CALOF AL: **Extracellular Matrix in the Developing Nervous System.** In *Molecular Genetics of Nervous System Tumors.* Edited by Levine AJ, Schmidek HH. New York: Wiley-Liss; 1993:341–355.

5. KLINGER MM, MARGOLIS RU, MARGOLIS RK: **Isolation and Characterization of the Heparan Sulfate Proteoglycans of Brain.** *J Biol Chem* 1985, **260**:4082–4090.

6. HERNDON ME, LANDER AD: **A Diverse Set of Developmentally Regulated Proteoglycans Is Expressed in the Rat Central Nervous System.** *Neuron* 1990, 4:949–961.

7. DAVID G, LORIES V, DECOCK B, MARYNEN P, CASSIMAN J-J, VAN DEN BERGHE H: **Molecular Cloning of a Phosphatidylinositol-Anchored Membrane Heparan Sulfate Proteoglycan From Human Lung Fibroblasts.** *J Cell Biol* 1990, **111**:3165–3176.

8. KARTHIKEYAN L, MAUREL P, RAUCH U, MARGOLIS RK, MARGOLIS RU: **Cloning of a Major Heparan Sulfate Proteoglycan From Brain and Identification as the Rat Form of Glypican.** *Biochem Biophys Res Commun* 1992, **188**:395–401.

9. •• CAREY DJ, EVANS DM, STAHL RC, ASUNDI VK, CONNER KJ, GARBES P, CIZMECI-SMITH G: **Molecular Cloning and Characterization of N-Syndecan, a Novel Transmembrane Heparan Sulfate Proteoglycan.** *J Cell Biol* 1992, **117**:191–201.

This paper presents the cloning of a partial *N*-syndecan (syndecan-3) cDNA, and identifies this molecule as a major HSPG of Schwann cells and developing brain.

10. NISHIYAMA A, DAHLIN KJ, PRINCE JT, JOHNSTONE SR, STALLCUP WB: **The Primary Structure of NG2, a Novel Membrane-Spanning Proteoglycan.** *J Cell Biol* 1991, **114**:359–371.

11. STALLCUP WB, DAHLIN K, HEALY P: **Interaction of the NG2 Chondroitin Sulfate Proteoglycan with Type VI Collagen.** *J Cell Biol* 1990, **111**:3177–3188.

12. •• RAUCH U, KARTHIKEYAN L, MAUREL P, MARGOLIS RU, MARGOLIS RK: **Cloning and Primary Structure of Neurocan, a Developmentally Regulated, Aggretating Chondroitin Sulfate Proteoglycan of Brain.** *J Biol Chem* 1992, **267**:19536–19547.

The cloning of neurocan is described. The cDNA that was obtained encodes a protein similar to aggrecan and versican, including an amino-terminal hyaluronate binding domain, and carboxy-terminal epidermal growth factor (EGF)-like, lectin-like and complement regulatory-like domains. Evidence is presented that the adult brain contains only a truncated form of neurocan, lacking the amino-terminal region.

13. • PERIDES G, RAHEMTULLA F, LANE WS, ASHER RA, BIGNAMI A: **Isolation of a Large Aggregating Proteoglycan from Human Brain.** *J Biol Chem* 1992, **267**:23883–23887.

Versican is isolated from human brain and shown to bind hyaluronic acid. The relationship of versican to GHAP (glial hyaluronic acid binding protein) is also discussed. GHAP, a previously characterized protein, appears to be an amino-terminal fragment of versican.

14. • KRUEGER R, HENNIG AK, SCHWARTZ NB: **Two Immunologically and Developmentally Distinct Chondroitin Sulfate Proteoglycans in Embryonic Chick Brain.** *J Biol Chem* 1992, **267**:12149–12161.

One of only a few studies to look at PGs in the avian brain. Aggrecan and its mRNA are found to be transiently expressed in the embryonic chick brain. Unlike cartilage aggrecan, the brain form contains only CS and not KS. Also detected in chick brain was a PG with a similar core size, but that can be distinguished from aggrecan by the presence of both CS and KS chains, reactivity with the HNK-1 monoclonal antibody, and lack of developmental regulation.

15. • FRYER HJL, KELLY GM, MOLINARO L, HOCKFIELD, S: **The High Molecular Weight Cat-301 Chondroitin Sulfate Proteoglycan from Brain is Related to the Large Aggregating Proteoglycan from Cartilage, Aggrecan.** *J Biol Chem* 1992, **267**:9874–9883.

The Cat-301 immunoreactive CSPG of brain is shown to have properties similar to those of aggrecan. Also, authentic aggrecan from cartilage is shown to react with Cat-301, as well as to another monoclonal antibody that recognizes the same brain PG as Cat-301. Nevertheless, the Cat-301 antigen can be distinguished from aggrecan by virtue of containing few, if any, KS chains and having a lower buoyant density (indicative of a lower carbohydrate/protein ratio) than aggrecan. The data suggest that Cat-301 is either a modified form of aggrecan, or a new member of the aggrecan family.

16. •• IWATA M, CARLSON S: **A Large Chondroitin Sulfate Proteoglycan Has the Characteristics of a General Extracellular Matrix Component of Adult Brain.** *J Neurosci* 1993, **13**:195–207.

The T1 antigen is a large CSPG that can only be extracted from rat brain with chaotropic agents (e.g. guanidine). It appears to be expressed in all parts of the CNS, and is therefore proposed to be a general ECM component. It fails to react with an antiserum directed against a 15 amino acid sequence that is present in the hyaluronic acid-binding domain of aggrecan, is 100% conserved in versican, and 80% conserved in neurocan. This suggests that the T1 antigen may not belong to the aggrecan family.

17. • IWATA M, WIGHT TN, CARLSON SS: **A Brain Extracellular Matrix Proteoglycan Forms Aggregates with Hyaluronan.** *J Biol Chem* 1993, **268**:15061–15069.

Even though the T1 antigen is thought to be unrelated to aggrecan and veriscan, it too binds hyaluronic acid. Using affinity co-electrophoresis, a ~1 nM dissociation constant for hyaluronic acid was measured.

18. RAUCH U, GEO P, JANETZKO A, FLACCUA A, HILGENBERG L, TEKOTTE H, MARGOLIS RK, MARGOLIS RU: **Isolation and Characterization of Developmentally Regulated Chondroitin Sulfate and Chondroitin/Keratan Sulfate Proteoglycans of Brain Identified with Monoclonal Antibodies.** *J Biol Chem* 1991, **266**:14785–14801.

19. • MAEDA N, MATSUI F, OOHIRA A: **A Chondroitin Sulfate Proteoglytcan That Is Developmentally Regulated in the Cerebellar Mossy Fiber System.** *Dev Biol* 1992, **151**:564–574.

Monoclonal antibodies were prepared using soluble brain PGs as immunogen. An antibody (6B4) is described that recognizes a CSPG with a 250 kDa core protein. Using immunohistochemistry, the antigen was detected around cerebellar Purkinje cells, Golgi cells and deep cerebellar neurons, as well as in brainstem nuclei that project mossy fibers to the cerebellum. Transient immunoreactivity was also found on ponto-cerebellar fibers during development.

20. ZAREMBA S, NAEGELE J, BARNSTABLE C, HOCKFIELD S: **Neuronal Subsets Express Multiple High-Molecular-Weight Cell-Surface Glyconjugates Defined by Monoclonal Antibodies Cat-301 and VC1.1.** *J Neurosci* 1990, **10**:2985–2995.

21. FUJITA SC, TADA Y, MURAKAMI F, HAYASHI M, MATSUMURA M: **Glycosaminoglycan-Related Epitopes Surrounding Different Subsets of Mammalian Central Neurons.** *Neurosci Res* 1989, 7:117–130.

22. BERTOLOTTO A, ROCCA G, CANAVESE G, MIGHELI A, SCHIFFER D: **Chondroitin Sulfate Proteoglycan Surrounds a Subset of Human and Rat CNS Neurons.** *J Neurosci Res* 1991, **29**:225–234.

23. SHEPPARD AM, HAMILTON SK, PEARLMAN AL: **Changes in the Distribution of Extracellular Matrix Components Accompany Early Morphogenetic Events of Mammalian Cortical Development.** *J Neurosci* 1991, **11**:3928–3942.

24. SNOW DM, LEMMON V, CARRINO DA, CAPLAN AI, SILVER J: **Sulfated Proteoglycans in Astroglial Barriers Inhibit Neurite Outgrowth *in Vitro*.** *Exp Neurol* 1990, 109:111–130.

25. SNOW DM, WATANABE M, LETOURNEAU PC, SILVER J: **A Chondroitin Sulfate Proteoglycan May Influence the Direction of Retinal Ganglion Cell Outgrowth.** *Development* 1991, 113:1473–1485.

26. •• HALFTER W: **A Heparan Sulfate Proteoglycan in Developing Avian Axonal Tracts.** *J Neurosci* 1993, 13:2863–2873.
A search for monoclonal antibodies against retinal basal lamina and optic nerve turns up four antibodies that recognize a single, large HSPG. Although this molecule exhibits biochemical properties similar to those of perlecan, its expression in CNS fiber tracts indicates that is not identical to perlecan. Until this report, it was not clear that any HSPGs were present in the ECM of the brain. Interestingly, like several other ECM molecules (e.g. laminin and fibronectin), expression of this PG in the parenchyma of the brain appears to be transient.

27. •• SCRANTON TW, IWATA M, CARLSON SS: **The SV2 Protein of Synaptic Vesicles Is a Keratan Sulfate Proteoglycan.** *J Neurochem* 1993, 61:29–44.
An important clarification of a complicated story. 'Two' synaptic vesicle molecules, a large PG and a major vesicle glycoprotein called SV2 are shown to be forms of the same integral membrane protein, bearing either long or short KS chains. This helps to bring together findings in other labs that the synaptic vesicle PG is involved in acetylcholine transport, and that the SV2 protein is evolutionarily related to bacterial transport proteins.

28. YAYON A, KLAGSBRUN M, ESKO JD, LEDER P, ORNITZ, DM: **Cell Surface, Heparin-like Molecules are Required for Binding of Basic Fibroblast Growth Factor to its High Affinity Receptor.** *Cell* 1991, 64:841–848.

29. RAPRAEGER AC, KRUFKA A, OLWIN BB: **Requirement of Heparan Sulfate for bFGF-Mediated Fibroblast Growth and Myoblast Differentiation.** *Science* 1991, 252:1705–1708.

30. KAN M, WANG F, XU J, CRABB JW, HOU J, MCKEEHAN WL: **An Essential Heparin-Binding Domain in the Fibroblast Growth Factor Receptor Kinase.** *Science* 1993, 259:1918–1921.

31. WALICKE P: **Basic and Acidic Fibroblast Growth Factors Have Trophic Effects on Neurons from Multiple CNS Regions.** *J Neurosci* 1988, 8:2618–2627.

32. ELDE R, CAO Y, CINTRA A, BRELJE TC, PELTO-HUIKKO M, JUNTTILA T, FUXE K, PETTERSSON RF, HOKFELT T: **Prominent Expression of Acidic Fibroblast Growth Factor in Motor and Sensory Neurons.** *Neuron* 1991, 7:349–364.

33. GÓMEZ-PINILLA F, LEE JW-K, COTMAN CW: **Basic FGF in Adult Rat Brain: Cellular Distribution and Response to Entorhinal Lesion and Fimbria-Fornix Transection.** *J Neurosci* 1992, 12:345–355.

34. HUGHES RA, SENDTNER M, GOLDFARB M, LINDHOLM D, THOENEN H: **Evidence that Fibroblast Growth Factor 5 is a Major Muscle-Derived Survival Factor for Cultured Spinal Motoneurons.** *Neuron* 1993, 10:369–377.

35. •• NURCOMBE V, FORD MD, WILDSCHUT JA, BARLETT PF: **Developmental Regulation of Neural Response to FGF-1 and FGF-2 by Heparan Sulfate Proteoglycan.** *Science* 1993, 260:103–106.
In this provocative study, an HSPG was isolated from mouse neuroepithelial cells at two stages of development, and tested for binding to FGF-1 and FGF-2. HSPG from embryonic day 9 cells preferentially bound FGF-2, whereas HSPG from day 11 cells preferentially bound FGF-1. Similar specificity was seen in the ability of the HSPG samples to potentiate the actions of FGF-1 and FGF-2. The developmental switch in binding coincides with a switch in expression of FGFs in the neuroepithelium, from FGF-2 to FGF-1, over the same period.

36. SALMIVIRTA M, ELENIUS K, VAINIO S, HOFER U, CHIQUET-EHRISMANN R, THESLEFF I, JALKANEN M: **Syndecan from Embryonic Tooth Mesenchyme Binds Tenascin.** *J Biol Chem* 1991, 266:7733–7739.

37. • SANDERSON RD, SNEED TB, YOUNG LA, SULLIVAN GL, LANDER AD: **Adhesion of B Lymphoid (MPC-11) Cells to Type I Collagen is Mediated by the Integral Membrane Proteoglycan, Syndecan.** *J Immunol* 1992, 148:3902–3911.
This study demonstrates that a cell surface HSPG can directly mediate cell adhesion, and that a single HSPG can have different affinities for an ECM ligand, depending, presumably, on the structure of its GAG chains.

38. OLSON ST, BJÖRK I: **Role of Protein Conformational Changes, Surface Approximation and Protein Cofactors in Heparin-Accelerated Antithrombin-Proteinase Reactions.** In *Heparin and Related Polysaccharides.* Edited by Lane DA. New York: Plenum Press; 1992:155–165.

39. • SAN ANTONIO JD, SLOVER J, LAWLER J, KARNOVSKY MJ, LANDER AD: **Specificity in the Interactions of Extracellular Matrix Proteins with Subpopulations of the Glycosaminoglycan Heparin.** *Biochemistry* 1993, 32:4746–4755.
A direct demonstration that some ECM molecules (e.g. laminin, fibronectin and type I collagen) can bind preferentially to subpopulations of the GAG heparin. The implication is that features in the structure of heparin/HS determine binding specificity. Thus, carbohydrate structure is likely to regulate the functions of HSPGs.

40. LINDNER J, GUENTHER J, NICK H, ZINSER G, ANTONICEK H, SHACHNER M, MONARD D: **Modulation of Granule Cell Migration by a Glia-Derived Protein.** *Proc Natl Acad Sci USA* 1986, 83:4568–4571.

41. WOODS A, COUCHMAN JR: **Heparan Sulphate Proteoglycans and Signalling in Cell Adhesion.** In *Heparin and Related Polysaccharides.* Edited by Lane DA. New York: Plenum Press; 1992:87–96.

42. COLE GJ, LOEWY A, GLASER L: **Neuronal Cell-Cell Adhesion Depends on Interactions of N-CAM with Heparin-Like Molecules.** *Nature* 1986, 320:445–447.

43. REYES AA, AKESON R, BREZINA L, COLE GJ: **Structural Requirements for Neural Cell Adhesion Molecule-Heparin Interaction.** *Cell Regul* 1990, 1:567–576.

44. • HAUGEN PK, LETOURNEAU PC, DRAKE SL, FURCHT LT, MCCARTHY JB: **A Cell-Surface Heparan Sulfate Proteoglycan Mediates Neural Cell Adhesion and Spreading on a Defined Sequence from the C-Terminal Cell and Heparin Binding Domain of Fibronectin, FN-C/H II.** *J Neurosci* 1992, 12:2597–2608.
Enzymatic removal of HS from rat neuroblastoma cells inhibited their attachment and spreading on fibronectin fragments and peptides, as did antibodies directed against a mouse melanoma HSPG.

45. •• WANG L, DENBURG J: **A Role for Proteoglycans in the Guidance of a Subset of Pioneer Axons in Cultured Embryos of the Cockroach.** *Neuron* 1992, 8:701–714.
Axons in the developing insect nervous system follow highly stereotyped pathways. Using a semi-*in vitro* system, evidence was found that heparin and HS specifically perturb certain axon guidance decisions. Similar perturbations were produced by two HS-degrading enzymes.

46. HAYASHI K, MADRI JA, YURCHENCO PD: **Endothelial Cells Interact with the Core Protein of Basement Membrane Perlecan through β1 and β3 Integrins: an Adhesion Modulated by Glycosaminoglycan.** *J Cell Biol* 1992, 119:945–959.

47. OOHIRA A, MATSUI F, KATOH-SEMBA R: **Inhibitory Effects of Brain Chondroitin Sulfate Proteoglycans on Neurite Outgrowth from PC12D Cells.** *J Neurosci* 1991, 11:822–827.

48. • GRUMET M, FLAGGUS A, MARGOLIS RU: **Functional Characterization of Chondroitin Sulfate Proteoglytcans of Brain: Interactions with Neurons and Neural Cell Adhesion Molecules.** *J Cell Biol* 1993, 120:815–824.
Evidence is presented that the brain CSPGs neurocan (1D1) and 3F8 inhibit homophilic NCAM–NCAM and NgCAM–NgCAM binding (whereas aggrecan does so poorly), and that neurons exhibit some ability to attach to the core proteins of these PGs.

49. MUIR D, ENGVALL E, VARON S, MANTHORPE M: **Schwannoma Cell-Derived Inhibitor of the Neurite-Promoting Activity of Laminin.** *J Cell Biol* 1989, 109:2353–2362.

50. •• BRITTIS PA, CANNING DR, SILVER J: **Chondroitin Sulfate as a Regulator of Neural Patterning in the Retina.** *Science* 1992, **255**:733–736.

This paper raises the provocative hypothesis that the disappearance of CS from the developing mammalian retina sets up a spatial gradient that guides ganglion cell axons toward the optic nerve head. Previous *in vitro* studies had suggested that CS repels retinal axons. In this study, evidence is presented that newly differentiating ganglion cells find themselves within a steep CS gradient oriented away from the optic disk. Evidence is also presented that exposure of developing retinas to a CS-degrading enzyme causes large perturbations in ganglion cell axon guidance, as well as causing ectopic differentiation of ganglion cells.

51. BRANDAN E, MALDONADO M, GARRIDO J, INESTROSA N: **Anchorage of Collagen-Tailed Acetylcholinesterase to the Extracellular Matrix Is Mediated by Heparan Sulfate Proteoglycans.** *J Cell Biol* 1985, **101**:985–992.

52. • GORDON H, LUPA M, BOWEN D, HALL Z: **A Muscle Cell Variant Defective in Glycosaminoglycan Biosynthesis Forms Nerve-Induced but Not Spontaneous Clusters of the Acetylcholine Receptor and the 43 kDa Protein.** *J Neurosci* 1993, **13**:586–595.

Acetylcholine receptor clustering by muscle cells is an important model for early steps in synaptogenesis. In this paper, S27, a variant of C2 mouse myoblasts that is deficient in GAG biosynthesis, is described. Unlike the parent line, S27 cells fail to form spontaneous or agrin-induced acetylcholine receptor clusters. In addition, basal lamina components (e.g. laminin) are also reduced on the surface of these cells. Interestingly, S27 cells are capable of forming acetylcholine receptor clusters at sites of contact by neuronal processes. Possible explanations for the different responses of these cells to clustering signals are discussed.

53. GALLAGHER JT: **The Extended Family of Proteoglycans: Social Residents of the Pericellular Zone.** *Curr Opin Cell Biol* 1989, **1**:1201–1218.

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