THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 5, pp. 2935–2942, January 27, 2012

Chondroitin Sulfate "Wobble Motifs" Modulate Maintenance and Differentiation of Neural Stem Cells and Their Progeny*

Published, JBC Papers in Press, November 17, 2011, DOI 10.1074/jbc.R111.298430

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Chondroitin sulfate/dermatan sulfate (CS/DS) proteoglycans, major components of the central nervous system, have the potential to interact with a wide range of growth factors and neurotrophic factors that influence neuronal migration, axon guidance pathways, and neurite outgrowth. Recent studies have also revealed the role of CS/DS chains in the orchestration of the neural stem/progenitor cell micromilieu. Individual functional proteins recognize a set of multiple overlapping oligosaccharide sequences decorated to give different sulfation patterns, which are termed here "wobble CS/DS oligosaccharide motifs," and induce signaling pathways essential for the proliferation, self-renewal, and cell lineage commitment of neural stem/progenitor cells.

The discovery of populations of multipotent self-renewing neural stem cells within fetal and adult brains has raised the hope of developing new therapeutic strategies for CNS disorders. Neural stem/progenitor cells (NSPCs)[3] are defined as self-renewing multipotential cells that can generate all types of neural cells, including neurons and glia (astrocytes and oligodendrocytes). In an adult brain, NSPCs are present in two distinct regions: in the subgranular zone of the dentate gyrus of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles (1–4). During brain development, the neuroepithelial cells of the neural tube expand and self-renew by symmetric division. With increasing thickness of the neuroectoderm, radial glial cells emerge and fulfill the role of neural stem cells. In the first wave, these cells self-renew by symmetrical divisions. In parallel, an asymmetric division pattern develops in which each division cycle gives rise to a radial glial cell and a neuronal progenitor. This phase of neurogenesis is followed by a phase of gliogenesis. In many regions of the CNS, oligodendrocytes precede the formation of astrocytes, which constitute the final population that is formed in the developing CNS (5). The radial glial cells can transform into astrocytes, and the subpopulation of astrocytes in the SVZ has been identified as NSPCs in the adult brain (6). Thereafter, the radial glia recede. Adult forms of radial glia are preserved as Bergmann glia and Müller glia solely in the cerebellum and retina, respectively (7, 8). Thus, NSPCs, which are characterized by their high proliferative potential while retaining self-renewal and pluripotency, encompass neuroepithelial cells, radial glial cells, and SVZ astrocytes (9, 10). The self-renewal and differentiation properties of NSPCs are modulated by intrinsic factors such as transcription factors, intercellular interactions, and extrinsic factors present in the extracellular matrix (ECM). Understanding how these factors regulate the differentiation of NSPCs is essential to exploit potential therapeutic applications to treating various neurodegenerative disorders and spinal cord injuries.

Neural Stem Cell Niche

The microenvironment where the NSPCs reside and maintain their self-renewal, proliferation, and differentiation is termed the "stem cell niche." The neural stem cell niche consists of restricted sets of cell types and contains a specialized microenvironment (11–13) composed of glycoproteins, mainly tenascin C (14–16); proteoglycans (PGs) bearing heparan sulfate (HS), chondroitin sulfate (CS), or dermatan sulfate (DS) side chains; and cell adhesion molecules such as polysialic acid (17), SSEA-1 (stage-specific embryonic antigen-1)/Lewis X (18), human natural killer-1 antigen (19), prominin (20), and gp130 (21). Studies using knock-out mice have underscored the importance of ECM components in CNS development (22, 23). Tenascin C-deficient mice display behavioral abnormalities (24) and deficits in the stem cell compartment, including a delayed acquisition of the EGF receptor (15), reduced proliferation (25), and accelerated differentiation of oligodendrocyte precursors (26–28). Studies using animals deficient in the genes involved in HS biosynthesis have provided information concerning the roles of HS in mammalian brain development (29, 30). Thus, the ECM in which the NSPCs reside has a number of critical roles in the development, function, and repair after injury of the CNS, yet minimal investigation in this area has been carried out. The mammalian brain is a rich source for

* This work was supported by grants from the Deutsche Forschungsgemeinschaft, the Bundesministerium für Bildung und Forschung, the Deutscher Akademischer Austausch Dienst, the International Graduate School for Neuroscience, and the Ministerium für Innovation, Wissenschaft, Forschung und Technologie des Landes Nordrhein-Westfalen (to A. F.) and by a grant from the Matching Program for Innovations in Future Drug Discovery and Medical Care from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to K. S.).

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[3] The abbreviations used are: NSPC, neural stem/progenitor cell; SVZ, subventricular zone; ECM, extracellular matrix; PG, proteoglycan; HS, heparan sulfate; CS, chondroitin sulfate; DS, dermatan sulfate; GAG, glycosaminoglycan; GlcUA, D-glucuronic acid; IdoUA, L-iduronic acid; C/D-ST, chondroitin/dermatan sulfotransferase; C4ST, chondroitin 4-*O*-sulfotransferase; D4ST, dermatan 4-*O*-sulfotransferase; C6ST, chondroitin 6-*O*-sulfotransferase; U2ST, uronosyl 2-*O*-sulfotransferase; GalNAc4S-6ST, GalNAc-4-sulfate 6-*O*-sulfotransferase; PTPζ, protein-tyrosine phosphatase-ζ; RPTPβ, receptor PTPβ; PTN, pleiotrophin; MK, midkine; HGF, hepatocyte growth factor; E, embryonic day; BLBP, brain lipid-binding protein; GLAST, glutamate aspartate transporter; ES, embryonic stem.

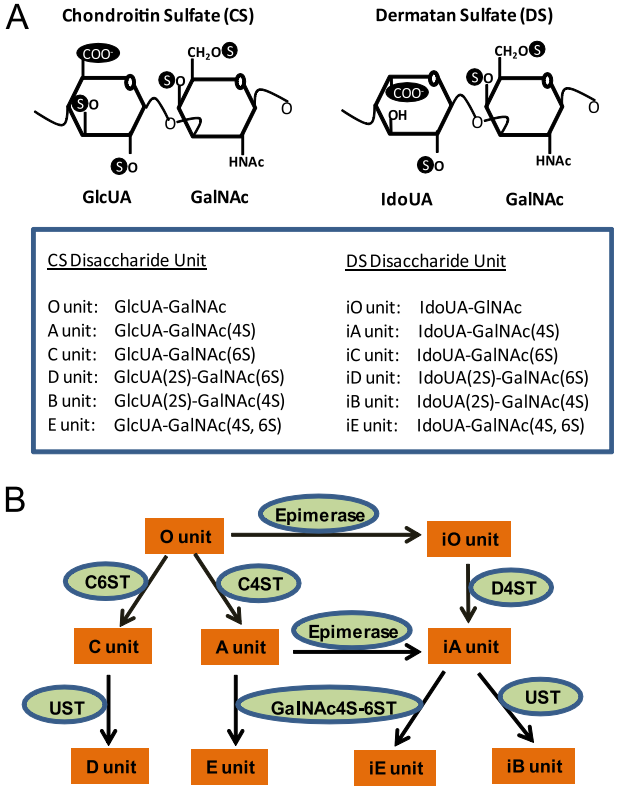

FIGURE 1. **Structure of CS/DS disaccharide unit.** *A*, the repeating disaccharide units of CS are composed of GlcUA and GalNAc residues. GlcUA often undergoes epimerization to form IdoUA and generates the DS disaccharide units along CS chains. CS units are named traditionally, and the corresponding DS units are indicated by "i," which stands for IdoUA. Sulfation of the sugar residues, indicated by "S," occurs at the 2nd, 4th, and 6th carbon positions in the ring (32). Depending on the position of sulfation, there are six major CS/DS disaccharide units that generate numerous polymer sequences. The enormous diversity thus generated by the various disaccharide units can result in the formation of various sets of wobble motifs along the CS/DS hybrid chains. *B*, biosynthetic pathways of CS/DS chains. *epimerase*, glucuronyl C5-epimerase; *UST*, uronyl 2-*O*-sulfotransferase.

carbohydrates, which occur in the form of PGs, glycoproteins, and glycolipids. This minireview will focus on the role played by CS/DS-PGs in CNS development, with particular emphasis on the maintenance and differentiation of NSPCs.

CS/DS in CNS

Cloning of various sulfotransferases and glycosyltransferases involved in the synthesis of sulfated glycosaminoglycan (GAG) chains of PGs has revealed crucial functions of GAGs in development and pathophysiology. CS-GAGs are detectable in the ECM and at cell surfaces in the CNS from an early stage of development (31). Immunostaining of brain sections using antibodies CS-56 (specific for CS-A and CS-C), MO-225, and 473HD (both of which recognize octasaccharide sequences containing an A–D tetrasaccharide sequence composed of A and D disaccharide units; for abbreviations of disaccharide units, see Fig. 1 and Ref. 32) and mAb 2H6 (which recognizes a C–C tetrasaccharide sequence) revealed the existence of CS chains in the neurogenic regions of embryonic and adult brains (14, 16). CS-PGs have also been found deposited between Purkinje cell surfaces and the processes of Bergmann glia (not a "classic" neurogenic region, however) (33). In addition, mAb 2A12, which is specific for iD-containing DS decasaccharide(s), showed the distribution of DS chains in the hippocampus and cerebellum of postnatal day 7 mice (34).

CS chains are heterogeneous molecules with repeating disaccharide units (-4GlcUAβ1–3GalNAcβ1-) (Fig. 1). The structural complexity of CS chains is generated biosynthetically under the control of multiple sulfotransferases and DS C5-epimerases, which generate a DS domain along CS chains by converting GlcUA into L-iduronic acid (IdoUA). Depending on the number and positions of sulfate groups, a rich variety of CS or DS disaccharide units can be generated (for detailed information about CS biosynthesis, refer to reviews in Refs. 32 and 35–37). Sulfate groups are transferred from 3′-phosphoadenosine 5′-phosphosulfate to the specific acceptor sites in CS/DS chains by chondroitin/dermatan sulfotransferases (C/D-STs) that are located in the Golgi apparatus (32, 35). These enzymes are classified into the following four groups: chondroitin/dermatan 4-*O*-sulfotransferases (C4ST/D4ST), chondroitin 6-*O*-sulfotransferase (C6ST), uronosyl 2-*O*-sulfotransferase (U2ST), and GalNAc-4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST). Three C4ST isoforms (38–40), two C6ST isoforms (41, 42), two DS epimerase isoforms (DS-epi1 and DS-epi2) (43, 44), D4ST1 (45), U2ST (46), and GalNAc4S-6ST (47) have been identified in mammals. Gene expression levels of these enzymes correlated with the amount of sulfated products that corresponded to each enzymatic activity (48), which holds the promise that studies of gene expression of C/D-STs will yield more detailed insights into the sulfation profiles of CS, DS, and their hybrid chains.

The expression of CS biosynthetic enzymes in the postnatal brain is dynamically regulated during development (49). It has been demonstrated that the ratio of C4ST and C6ST activities forming the specific sulfation profile changes markedly with development in the embryonic chick brain (50). *In situ* hybridization of mouse brain revealed that the sulfotransferase genes, including *C4ST1* and *C4ST2*, which are involved in the synthesis of A units, a precursor for B/iB units, in addition to the GalNAc4S-6ST gene, which synthesizes E/iE units, are ubiquitously expressed in the developing brain, whereas expression of the *D4ST1* and *U2ST* genes (which synthesize iA and D/iD/B/iB units, respectively) is restricted to the cerebellum (32, 44, 49, 51, 52). Recently, Akatsu *et al.* (44) also found that the *DS-epi2* rather than *DS-epi1* is the predominant isoform that is ubiquitously expressed in the developing brain after birth, and its expression correlated with the presence of high levels of IdoUA-containing iD units and iB units at every developmental stage. On the basis of these observations, we speculate that, like HS, CS in the brain also has structural motifs composed of oversulfated and/or IdoUA-containing disaccharide units that change markedly with embryonic development.

Functions of CS/DS Chains in Developing Brain

Genetically engineered mice deficient in protein-tyrosine phosphatase-ζ (PTPζ)/receptor PTPβ (RPTPβ) CS-PG, which is a receptor protein-tyrosine phosphatase with one transmembrane domain and two intracellular tyrosine phosphatase modules (an isoform of this gene that comprises the complete ectodomain is released as CS-PG and known as phosphacan/DSD-1-PG in rat and mouse, respectively), exhibit an age-de-

pendent impairment of spatial learning and enhancement of long-term potentiation in the hippocampus (53). This is suggested to be due to impairment in the signaling of pleiotrophin (PTN)/midkine (MK) because PTPζ/RPTPβ is a receptor for these cytokines. Similarly, knockdown of other CS-PGs such as neurocan and brevican in mice also showed no obvious abnormalities in the brain, but the maintenance of long-term potentiation was disrupted, supporting the function of CS-PG in memory and learning (35). The reported functions of brain CS/DS chains in neuritogenesis are controversial because they can act as promoters as well as inhibitors (32, 35, 54–59). Such apparently contradictory functions are probably attributable to the structural diversity of CS/DS chains. CS/DS chains bind and present neurotrophic factors such as PTN, MK, and hepatocyte growth factor (HGF) to neuronal cells to promote neurite outgrowth (32, 56, 60, 61). PTN and MK serve as ligands for PTPζ/RPTPβ, and the affinity binding of these cytokines is dependent upon the D and E disaccharide units of the CS chains in PTPζ/RPTPβ (62–64). The preferred HGF-binding sites on neuronal cell surface CS are composed of oversulfated iB and E disaccharide units. However, *in vitro* experiments for studying the neurite outgrowth-promoting activity of CS/DS chains are dependent on the cell types used. For example, CS-E, which promotes neurite outgrowth of embryonic mouse hippocampal neurons *in vitro* (60), is a potent inhibitor of dorsal root ganglion explants from chick embryos (65). Similarly, phosphacan/DSD-1-PG also has opposing effects on neuritogenesis depending upon the neuronal lineages (66). In view of these findings, further investigations are needed for a better understanding of the neurite outgrowth-promoting activity of CS/DS chains *in vitro*.

CS in the brain also functions as an axon guidance molecule. Injection of chondroitinase ABC, a bacterial enzyme that degrades CS and hyaluronic acid, into developing nervous system structures leads to deviations in axon guidance pathways (67, 68). In addition, oversulfated CS has been shown to influence the migration of cortical neurons, which is mediated by a PTPζ/RPTPβ-PTN/MK signaling complex (69). Knockdown of U2ST (the enzyme involved in the synthesis of B and D units, both of which contain GlcUA 2-*O*-sulfate) and GalNAc4S-6ST in embryonic cortex neural progenitor cells resulted in severe defects in the radial migration of cortical neurons, suggesting that the expression of oversulfated CS structures changes the behavior of neurons, possibly by modifying their modes of interaction with ECM components (70). In addition, oversulfated CS has been shown to reinforce integrin signaling, leading to the selective growth of CS-positive nascent axons (71). Removal of CS by chondroitinase ABC induces the formation of unstable axons in hippocampal neurons that undergo multiple extensions and growth retardation (71). More recently, Nakanishi *et al.* (72) have shown that, in addition to its interaction with CS chains, PTN can also interact with the core protein of the brain-specific CS-PG neuroglycan. Mikami *et al.* (73) have shown that CS can function via the cell surface receptor contactin-1. Considering these findings, it is tempting to suggest that CS/DS-PGs are master regulators in CNS development.

CS/DS-PGs Expressing Functional "Wobble Oligosaccharide Motifs" Are Localized in NSPC Niche

CS-PGs are major components of the neural stem cell niche, and using mAb CS-56, brain-specific CS-PGs consisting of neurocan, phosphocan, and neuroglycan have been detected in the ventricular zone of embryonic day (E) 14 fetal rat telencephalon, in which NSPCs are abundant (74, 75). NSPCs by themselves participate in the construction of their own milieu by synthesizing CS-PGs, including lectican PG family members (aggrecan, versican, neurocan, and brevican) (76), and depositing them in their surroundings. Consistent with these observations, several CS-PGs were detected in neurospheres, which are cellular aggregates that grow in suspension and are composed of NSPCs and differentiating progeny. Using mAb 473HD, specific CS-structural motifs could be clearly attributed to cells positive for NSPC and radial glial markers, including nestin, brain lipid-binding protein (BLBP), and glutamate aspartate transporter (GLAST) (77–79). Phosphacan/DSD-1-PG is another component present in the postnatal and adult NSPC niche and in neurospheres (55, 66, 80). This CS-PG is selectively recognized by mAb 473HD, and its particular CS epitope, enriched with the D, A, and B disaccharide units (32), is functionally active and promotes neurite outgrowth of several types of CNS neurons (55).

In situ hybridization of E13 mouse brain showed a prominent expression of various sulfotransferase genes such as *C4ST1*, *C6ST1*, *D4ST*, and *U2ST* in the ventricular zones of the dorsal and ventral telencephalon (51). It has to be remembered that this is the same region where the 473HD epitope, consisting of A, B, and D CS disaccharides, resides and is presumably synthesized in a pathway involving the sulfotransferases for these disaccharide units. NSPCs cultured as neurospheres also maintain the expression of these enzymes (51). However, the expression of these C/D-STs changes during the lineage-specific differentiation of NSPCs. Yamauchi *et al.* (81) have recently reported that the expression of *C4ST1*, *C4ST2*, and *C6ST1* mRNAs decreases and that of *U2ST* and DS C5-epimerase mRNAs increases during the differentiation of NSPCs to neurons and astrocytes. They also showed that the expression of *GalNAc4S-6ST* is lower only in astrocytes, whereas the expression of *D4ST1* is lower in neurons and higher in astrocytes. This provides insights into the role of CS/DS hybrid chains (characterized by disulfated disaccharide units such as B/iB, D/iD, and E/iE) in critically modulating cytokine signaling involved in the lineage-specific differentiation of NSPCs. Further inhibition of sulfation of CS/DS chains using sodium chlorate in cells from secondary neurospheres of E13 mouse cerebral cortex resulted in a significant dose-dependent decrease in the number of neurospheres. This decrease in neurosphere population could not be rescued by the addition of individual purified GAG chains, including heparin, CS-B, CS-D, or CS-E (51). The possible explanation for this is the difference in the sulfation pattern of GAGs from CNS and non-CNS sources. Thus, neural stem cell maintenance might require the information of a "sulfation code," as has been proposed for neurite branching influenced by HS chains in the nematode (82). Such a hypothetical code would differ for neural stem cell self-renewal *versus* growth and

TABLE 1

PTN-binding "wobble GAG motifs" expressed by CS/DS hybrid chains during CNS development

CS/DS hybrid chains isolated from embryonic pig brain were fractionated using a PTN affinity column, and the PTN-bound oligosaccharide fractions were sequenced by HPLC after digestion with chondroitinase B, which cleaves GalNAc-IdoUA but not GalNAc-GlcUA linkages, followed by fluorescent labeling (84). Shown are the PTN-binding motifs in the CS/DS chains containing IdoUA, which therefore are not rigid but rather flexible. PTN thus binds not just one set of a particular sequence but an overall functional domain structure in the GAG chain. On the basis of these findings, we propose a "wobble hypothesis," which states that the growth factor-binding moieties on GAGs are a set of multiple overlapping oligosaccharide sequences with similar conformation and distributed electrostatic potential but not just one specific sequence. "i" represents IdoUA, and "*X*" represents any disaccharide unit, including A, C, D, E, B, or T (trisulfated) units.

Wobble GAG motif
iC-C-D-C-i*X*
iA-C-D-C-i*X*
iC-A-D-C-i*X*
iD-C-D-C-i*X*
iC-D-D-C-i*X*
iC-D-iD-C-i*X*
iE-D-A-D-i*X*
iE-D-iA-D-i*X*

proliferation behavior because the latter could be rescued by defined CS and heparin (82). Thus, the patterned level of sulfation in CS/DS of the neural stem cell niche may allow or even instruct NSPC behavior by modulating the activities of growth factors and cytokines.

It should be noted, however, that the term sulfation code (73, 82) may not be appropriate. The structural information contained by specific sulfation patterns of GAG oligosaccharide motifs is not rigid but flexible, and functional proteins such as growth factors recognize the overall organization of functional GAG domains or motifs (83), but not just one set of a combination of peculiar sulfate groups at specific positions (83, 84). Rather, the common features of the conformation of the overlapping multiple oligosaccharide molecules and of the electrostatic potential distribution over the surface of the sugar molecules are crucial for recognition, as has been revealed by structural investigation for the CS and DS oligosaccharides that bind mAbs (85) or PTN/HGF using biochemical and computational approaches (86, 87). Hence, for such structural entities, we propose the term "wobble GAG oligosaccharide motifs." Table 1 shows the wobble CS motifs for PTN binding.

CS/DS Chains Promote Proliferation and Self-renewal of NSPCs

Stem cell maintenance and differentiation are governed by local cues found in the microenvironment (11, 88). The proliferation capacities and motility of NSPCs are retained even in the adult brain, and therefore, understanding the mechanism of NSPC proliferation and differentiation is of great therapeutic significance for a wide variety of clinical disorders, including cell replacement strategies. NSPCs display multipotentiality and adopt a wide range of phenotypes in response to various stimulating factors from the microenvironment. These responses are partially mediated by the CS/DS chains that are found on the surface of NSPCs and in the niche. Degradation of CS chains by chondroitinase ABC both *in vivo* and *in vitro* reduces NSPC proliferation and the differentiation of radial glia to neurons, favoring the maturation of the gliogenic subtype of radial glia and the formation of astrocytes in the telencephalon (77, 78). Deglycanation of CS reduced the number of neurospheres and proliferating NSPCs. Utilizing the clonal density assay, it was demonstrated that CS can also promote self-renewal of early telencephalic NSPCs grown in a neurosphere culture. Twice as many secondary neurospheres originated from cell suspensions derived from untreated primary cortical and striatal neurospheres compared with neurospheres that had been exposed to chondroitinase ABC. The findings described provide the first experimental evidence for CS/DS chains in regulating the self-renewal and proliferation of NSPCs. This is reminiscent of the functions of HS, which contributes to the self-renewal and proliferation of embryonic stem (ES) cells (89, 90). Recently, Tham *et al.* (91) reported that soluble phosphacan/DSD-1-PG can stimulate the survival of neural stem cells by preferential signaling through the EGF receptor, JAK, and PI3K pathways. The same study also revealed that CS-PGs can enhance the survival of neural stem cells derived from ES cells and thus can be used as a tool to generate ES cell-derived neural stem cells (91).

The capacity of CS/DS chains to bind to various growth factors might impinge on the proliferation rate of NSPCs. The ability of CS to induce stem cell proliferation has recently been documented in the nematode, and the involvement of chondroitin and CS in controlling embryonic cell division is mediated by regulating proper embryonic cytokinesis (35, 92, 93). Studies have also shown that CS serves as a docking site for growth factors and thereby modulates responsiveness to FGF-2 in embryonic NSPCs (94). In experiments carried out by Sirko *et al.* (78), NSPCs were grown as freely floating neurospheres in defined media containing the growth factors EGF and FGF-2, and therefore, the secreted or cell surface CS-PGs can intervene in the FGF-2- or EGF-dependent signaling pathways and thereby foster NSPC proliferation and self-renewal. This could be effected either through the manner by which CS-GAGs bind and store factors in the pericellular environment (48) or by their serving as *cis*-acting cofactors for growth factor receptors, analogous to the role played by HS-PGs with respect to the FGF receptor (54). Indeed, the growth factors PTN and MK, which have been associated with the proliferation of NSPCs, were also secreted into the neurosphere-conditioned media and strongly interacted with the CS chains of phosphacan/DSD-1-PG (94, 95). The profound effect of elimination of CS/DS chains in inhibiting the proliferation and self-renewal of NSPCs is likely due to an impact on a multitude of signaling pathways. This corroborates with studies of tenascin C, a glycoprotein that interacts with phosphacan/DSD-1-PG (96, 97). Tenascin C facilitates NSPC development by altering the response of cells to mitogenic growth factors, and one possibility is that this could be due to the interaction of tenascin C with CS-PGs. This assumption is further supported by the finding that tenascin C stimulates contactin-dependent neurite outgrowth (98), and recently, it has been shown that contactin also serves as a receptor for CS-E (73).

CS/DS Chains Participate in Decision of NSPC Fate

The subject of cell fate, also referred to as the ultimate differentiated state to which a cell has become committed, is governed by a set of intrinsic transcriptional regulators but also by

the unique local microenvironment (99). Toward the end of embryogenesis and during early postnatal life, astrocytes and oligodendrocytes are generated mainly from NSPCs, whereas neurogenesis has largely ceased (1, 5). This timely differentiation of NSPCs is dependent on the presence of CS, and the elimination of CS/DS from NSPCs inhibits neurogenesis and increases gliogenesis (78). In these studies, telencephalic neurospheres were used as a model for NSPCs because these cellular aggregates self-renew in response to FGF-2 and EGF and give rise to neurons, astrocytes, and oligodendrocytes upon differentiation. Elimination of CS chains from neurospheres reduced the number of BLBP-positive neurogenic neural progenitor cells and increased the number of GLAST-positive radial glial cells that preferentially generate astroglia (78). *In vivo* removal of CS/DS chains from the cerebral ventricles of E13–14 embryos reduced the number of phosphohistone H3-positive and BrdU-positive proliferating cells (phosphohistone H3 and BrdU are markers for mitosis and DNA synthesis, respectively) in the area close to the ventricular surface, indicating that CS/DS regulates the proliferation of precursor cells residing in the ventricular zone of the developing brain. Further removal of CS chains has been shown to decrease the expression of the neural precursor markers nestin and BLBP and to increase the number of GLAST-positive precursor cells in the cortical regions (78). These studies show the essentiality of CS/DS chains for the timely differentiation of neurogenic-positive radial glia to neurons. It was interpreted that CS/DS might be part of the control machinery that delays the onset of gliogenesis and promotes self-renewal of stem cells and neurogenic precursors (78).

The timely differentiation of NSPCs into neurons and glia is differentially controlled by CS via regulation of the responsiveness to FGF-2 and EGF (79). Using the neurosphere culture model, it was observed that the selective removal of CS/DS chains preferentially affected neurosphere formation of NSPCs in response to FGF-2 rather than in response to EGF. Quantification of BrdU-positive cells on chondroitinase ABC-treated neurospheres revealed that FGF-2-sensitive NSPCs require intact CS chains for proliferation. Furthermore, the enzymatic removal of CS-GAGs suppressed the expansion of FGF-2-responsive, BLBP-positive, and preferentially neurogenic NSPCs. Removing CS chains favored the increase in GLAST-positive and EGF-responsive progenitors that preferentially generate astroglia. The preferential expansion of GLAST-positive radial glial cells can reflect an enhanced propensity of dividing cortical progenitors to generate glia-restricted progenitors, with a concomitant decrease in the generation of BLBP-expressing neurogenic progenitors. This switch in phenotype of neural progenitor cells most likely corresponds to radial glial cell subtypes that appear at later developmental stages (100). In this way, a selective expression of CS-PGs in subsets of cells may account for the generation of NSPC diversity. The gradually declining or changing disaccharide composition of CS-GAGs during forebrain development results in CS/DS chains with functional "wobble motifs" that can direct the NSPCs to neurogenic and/or gliogenic lineage commitment. Still, there is no extensive *in vivo* evidence for the roles of CS-PGs in the control of neural stem cells. Further studies based on gene knock-out for CS-PGs will help to fully understand the precise role of this sugar molecule in CNS development.

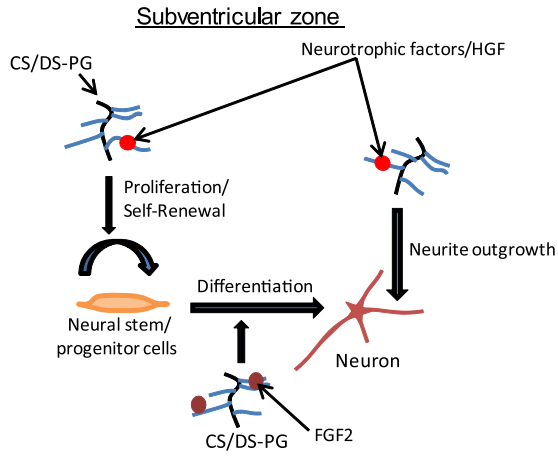

FIGURE 2. **Proposed mechanism of CS-PGs in modulating neural stem cells and their progeny.** CS-PGs, the major components of the neural stem cell niche in the SVZ, interact with various growth factors and neurotrophic factors and promote the proliferation/self-renewal and differentiation of NSPCs and neurite outgrowth of terminally differentiated neurons. CS/DS-PGs interact with growth factors such as FGF and EGF and promote the proliferation and self-renewal of NSPCs. The timely differentiation of NSPCs to neurons is controlled by CS/DS chains, which specifically bind and favor the responsiveness to FGF. Thus, CS-PGs could instruct the NSPCs and direct their lineage commitment. CS/DS-PGs also bind and present neurotrophic factors such as PTN, MK, and HGF to neuronal cells to promote neurite outgrowth. The high affinity binding sites for these factors appear to contain D, E, and/or B disaccharide units and are also enriched in IdoUA. Hence, the ligand-binding sites on the CS/DS hybrid chains are not rigid but flexible, and individual functional proteins recognize a set of multiple oligosaccharide sequences decorated by wobble sulfate groups to give different sulfation patterns. They are termed here "wobble CS/DS motifs" and influence various cellular events, including proliferation, self-renewal, and differentiation of NSPCs and neuritogenesis of terminally differentiated neurons.

Conclusions

The discovery of NSPCs and their ability to differentiate in the adult brain can be viewed as one of the major breakthroughs in the field of neuroscience, yet the application of stem cells for cell replacement therapies is still in its infancy. In an optimistic perspective, much work remains to be done to attain a safe and secure state of empirical research and application. The studies discussed herein for the first time demonstrated that CS/DS carbohydrates play a pivotal role in the orchestration of the NSPC micromilieu. CS-PGs are the major components of the neural stem cell niche, and fine structures of CS/DS chains are dynamically and spatiotemporally regulated during CNS development. CS-PGs fine-tune the neural stem cell microenvironment and mediate various biological processes, including proliferation, self-renewal, cell lineage commitment, and cytokinesis (Fig. 2). Encouraged by these findings, we should exploit and manipulate the powerful influence that the microenvironment holds for stem cell fate decision. The capacity of CS/DS chains containing functional wobble GAG oligosaccharide motifs to possibly instruct NSPCs and direct their lineage commitment opens a new avenue for the use of stem cells in regenerative medicine. The ease of isolation of CS fragments from biological sources, including marine organisms, may yield slow-acting yet safer sugar-based drugs (36) that can harness the processes of neural stem cell-based therapies. Chemical synthe-

sis of active CS/DS fragments is also a powerful approach once various wobble GAG oligosaccharide motifs are elucidated.

Acknowledgments—We thank the many members of the Sugahara and Faissner laboratories who have contributed over the years to much of the knowledge in this minireview.

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