Am. J. Hum. Genet. 64:372–377, 1999

INVITED EDITORIAL
Overgrowth Syndromes and the Regulation of Signaling Complexes by Proteoglycans

Scott B. Selleck

Department of Molecular and Cellular Biology, Division of Neurobiology, University of Arizona, Tucson

The charm of genetics, along with much of its power, lies in its capacity to reveal connections that could never have been anticipated. Pilia et al. (1996) must have been surprised when they uncovered the gene responsible for the overgrowth and tumor-susceptibility syndrome Simpson-Golabi-Behmel syndrome (SGBS). SGBS is an X-linked disorder characterized by pre- and postnatal overgrowth; numerous morphological abnormalities, including congenital heart defects; dysplastic kidneys; vertebral and rib defects; and postaxial hexadactyly. In addition, patients are at high risk for Wilms tumor and neuroblastoma (Neri et al. 1998). SGBS is caused by disruptions of the glypican 3 gene (*GPC3*), which, remarkably, encodes a proteoglycan of the glypican family. The developmental abnormalities associated with SGBS demonstrate that cell-surface proteoglycans can affect tissue growth and morphogenesis and may serve as a novel class of tumor suppressors.

How a proteoglycan affects growth regulation and tumor susceptibility is not immediately obvious, but several laboratories, including my own at the University of Arizona, have found clues at the intersection of cell biology, biochemistry, and genetics. As is often the case, an understanding of a human disease derives in large measure from studies of different organisms and experimental systems. The story that emerges highlights two fundamental features of cell surfaces. First, the plasma membrane is carpeted with sugars, principally as oligosaccharides attached to lipids and proteins. Second, the sugars found on the cell surface show a great deal of structural diversity, with different forms represented in tissue-specific patterns. The function of glycosylation of cell-surface molecules has long been uncertain, but it now appears that one class of sugar-modified proteins, the proteoglycans, influence interactions between other molecules. Moreover, signaling-complex assembly may be regulated, in part, by the interaction of ligands and receptors with the sugar moieties of proteoglycans. It therefore is likely that the different sugar structures found on the cell surface provide the capacity to control the assembly of many varied signaling complexes.

Received December 1, 1998; accepted for publication December 19, 1998; electronically published January 28, 1999.

Address for correspondence and reprints: Dr. Scott B. Selleck, Department of Molecular and Cellular Biology, Division of Neurobiology, University of Arizona, Tucson, AZ 85721. E-mail: selleck@u.arizona.edu

0002-9297/99/6402-0008$02.00

The Players

Proteoglycans are one class of sugar-modified molecules on the cell surface, bearing glycosaminoglycan (GAG) chains attached to serine residues framed within a short "glycanation" acceptor site (SGXG, typically with acidic amino acids on one or both sides; fig. 1) (Esko and Zhang 1996). GAG chains are long, linear polymers of disaccharide units. Unlike certain other classes of proteoglycans, glypicans bear one type of GAG exclusively, namely, heparan sulfate (HS). HS, one of the most prevalent types of GAG found on the cell surface, is synthesized as a repeating polymer of glucuronic acid and N-acetylglucosamine. Heparin, a highly sulfated form of HS, is a potent anticoagulant and is naturally found in mast-cell secretory granules. HS is ubiquitous, occurring on cell surfaces and in the extracellular matrix. Heparin and HS are both synthesized as components of proteoglycans: heparin is attached to the core protein serglycin, whereas HS is attached to a diverse group of core proteins.

HS is not a unique structure but a collection of related molecules (Salmivirta et al. 1996) with different patterns of sulfation, as well as other modifications that take place after the disaccharide polymer is made (fig. 1). These structural variants are found in tissue-specific distributions and show age- and disease-related changes (Maccarana et al. 1996; Feyzi et al. 1998). Different HS forms can be found attached to the same protein core in different cell types and can influence the proteoglycan's biological properties (Sanderson et al. 1994).

Proteoglycans are most often thought of as extracellular-matrix components, but some, like those of the glypican family, are integral membrane proteins attached to the plasma membrane via a glycosyl-phosphatidyli-

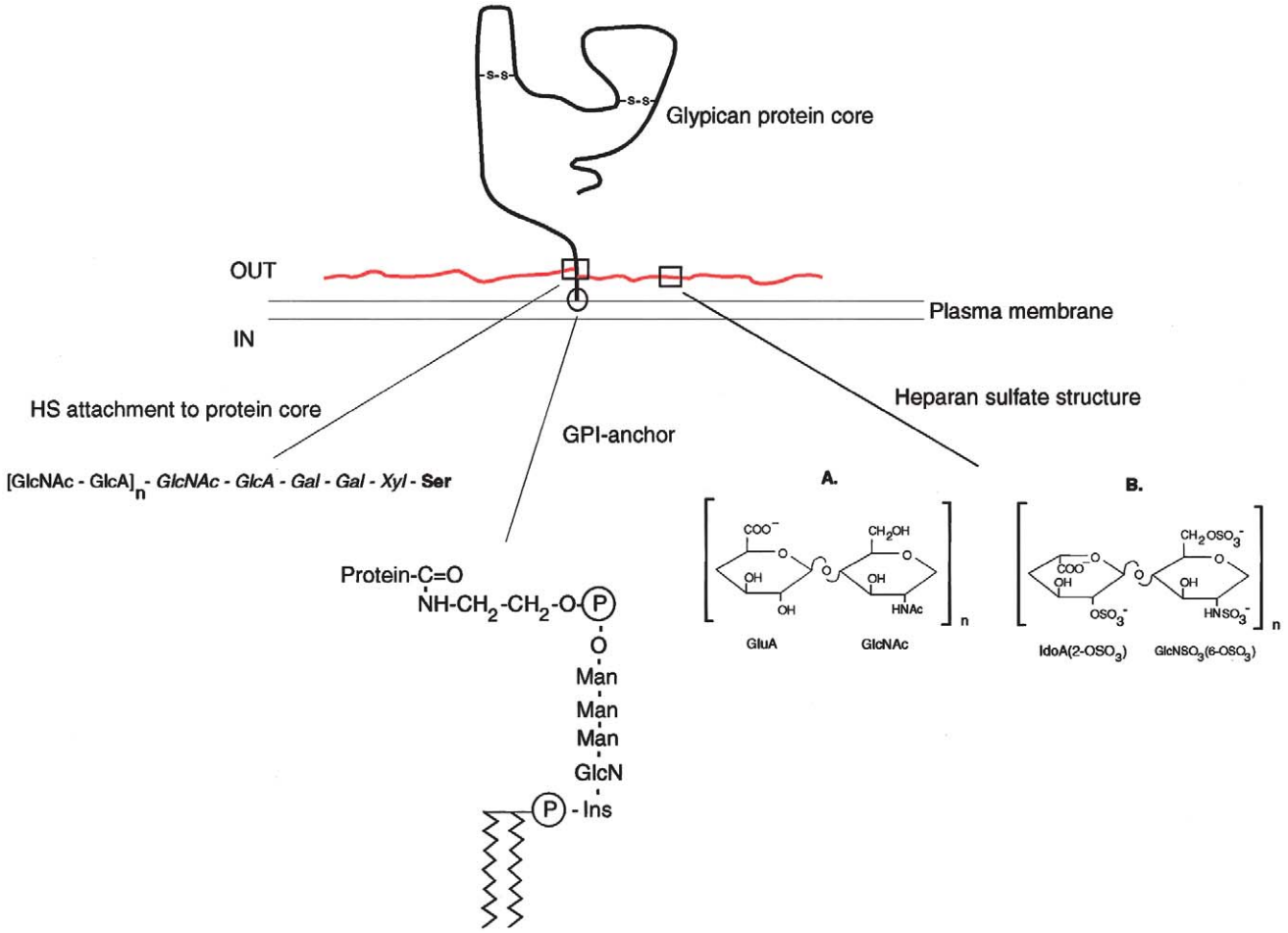

Figure 1 Organization of glypicans. The polypeptide chain of glypicans is completely extracellular, with numerous disulfide bridges producing a highly compact globular domain. GAG chains of the HS type are attached to serine residues near the carboxy terminus, via a carbohydrate linker (in italics). The protein is covalently linked to the plasma membrane at its carboxy terminus, via a GPI attachment. HS is a linear polymer of disaccharide units. *A*, Unmodified unit. *B*, Sulfated form of HS that usually represents <10% of all disaccharide units in a chain. GlcNAc = N acetyl glucosamine; GlcA = glucuronic acid; Gal = galactose; Xyl = xylose; Man = mannose; GlcN = glucosamine; Ins = inositol; IdoA = iduronic acid; P = phosphate.

nositol (GPI) linkage (Lander et al. 1996). At present, five distinct glypican genes have been described in vertebrates and one in *Drosophila*. *GPC3* is responsible for SGBS, and a *Drosophila* glypican, *division abnormally delayed* (*dally*), affects both cell-division patterning and morphogenesis during development (Nakato et al. 1995). All the glypicans share considerable sequence homology, including a set of 14 cysteine residues at conserved positions, and sites of GAG attachment near the carboxy terminus, where the protein bears the GPI linkage (fig. 1).

Cell-Surface Proteoglycans and the Assembly of Signaling Complexes

The phenotypes of SGBS patients, as well as those that I and my colleagues have characterized in *dally* mutant flies, raise the question of how a cell-surface proteoglycan affects cellular physiology. A great deal of evidence indicates that GAG chains are critical for proteoglycan function. HS is a negatively charged molecule that binds a variety of extracellular proteins, including growth factors. Landmark studies in the early 1990s showed that cell-surface HS is a required component for fibroblast growth factor (FGF) signaling in tissue-culture cells (Rapraeger et al. 1991; Olwin and Rapraeger 1992). Cells with functional FGF receptors (FGFRs) are incapable of FGF signaling if HS is enzymatically removed or its synthesis blocked by treatment with chlorate.

These findings lead to the proposal that HS-modified proteoglycans (HSPGs) serve as growth-factor coreceptors, which affect the delivery or assembly of ligands

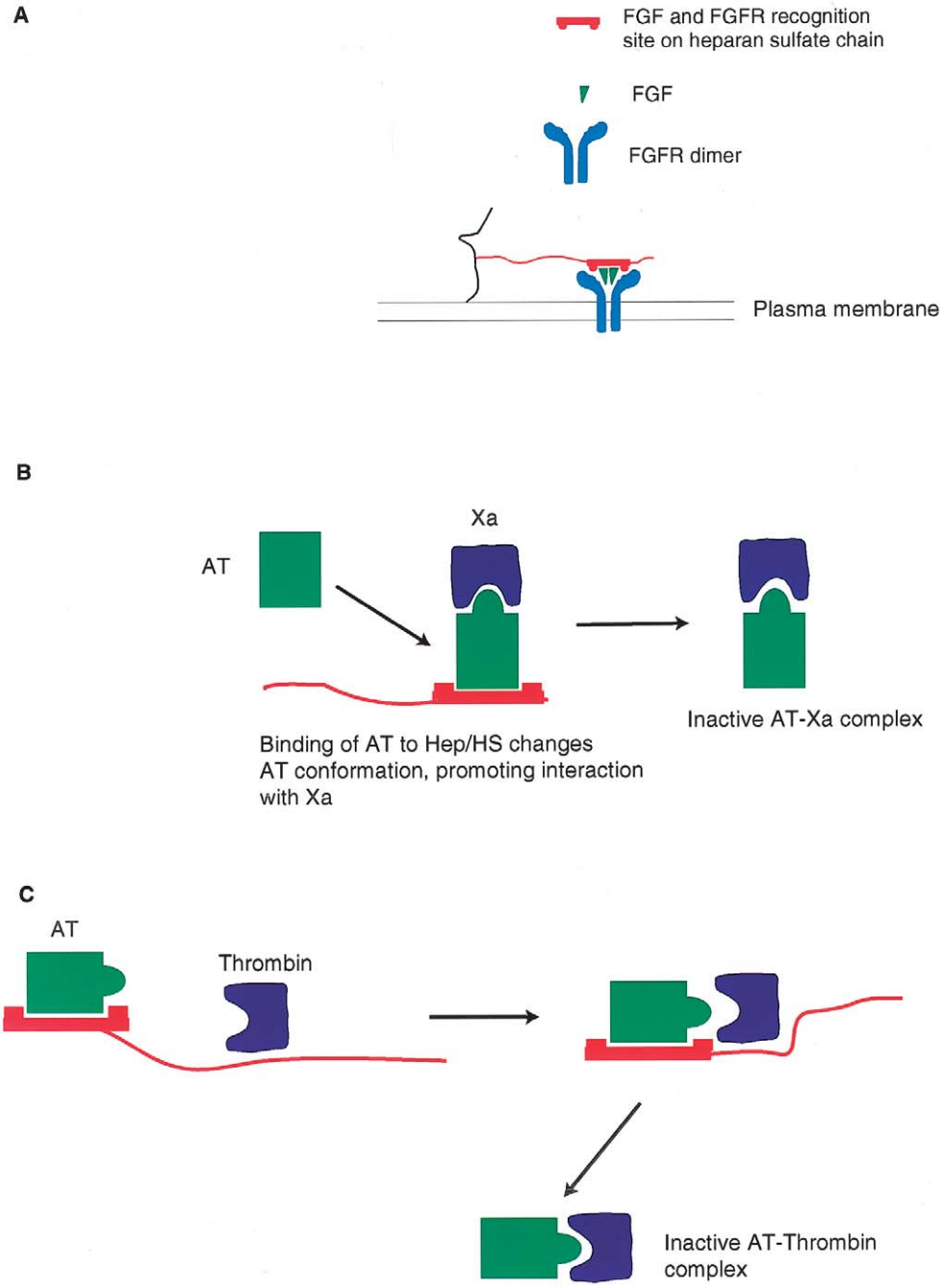

Figure 2 Effects on signaling, by cell-surface proteoglycans. *A*, Proteoglycans as growth-factor coreceptors. FGF binds to specific sequences within an HS polymer, bringing together one or more FGF molecules. In addition, the FGFR recognizes HS, promoting assembly of the proteoglycan-FGF-FGFR complex. *B*, Proteoglycans as allosteric regulators. Heparin ("Hep") or HS can affect the conformation of AT, inducing a conformational change so that it binds the protease factor Xa ("Xa"), producing an inactive AT-Xa complex. *C*, Proteoglycans as bridging molecules. Heparin or HS can promote the association of AT with thrombin when both of these proteins bind the same heparin/HS chain. Their rate of association is accelerated by their proximity on the heparin/HS chain.

into signaling complexes. The prevailing view holds that HSPGs are important in the dimerization of FGF, which in turn is required for receptor dimerization and signaling. By addition of a twist to this model, there is evidence that an HS-binding domain on the FGFR itself is also required for signaling, suggesting that HSPGs promote FGF and FGFR association by bringing them into close proximity (fig. 2; also see Rapraeger 1995).

Although FGF is the prototype among growth factors that are affected by HSPGs, it is not unique. The activity of other growth factors—including wingless (Wg), a *Drosophila* Wnt (Reichsman et al. 1996), transforming growth factor–β (TGF-β) (Lopez-Casillas et al. 1993), hepatocyte growth factor (Zioncheck et al. 1995), and heparin-binding epidermal growth factor (Aviezer and Yayon 1994)—have all been shown to be affected by the presence of cell-surface HS. In addition, vascular endothelial growth factor, interleukin-3, granulocyte macrophage colony–stimulating factor, and interferon-γ all bind HSPGs, raising the possibility that a very large number of protein factors are influenced by the expression and structure of proteoglycans (Nelson et al. 1995). However, evidence that proteoglycans act in growth-factor–signaling pathways during development comes largely from studies of the fruit fly.

In Vivo Studies of Dally, a Fly Glypican

Many biochemical and tissue-culture studies support the hypothesis that cell-surface proteoglycans, like GPC3 in humans, affect growth-factor signaling during development. It is not yet known whether defects in growth-factor–signaling pathways account for the abnormalities in SGBS patients, but tools to address this question have recently become available for the mouse. *Gpc3* knockout mice have been described, and these mice show many of the defects found in SGBS patients, including generalized overgrowth (J. Filmus, personal communication). We therefore can look forward to more-detailed studies of the molecular and cellular abnormalities that follow from *GPC3* dysfunction.

At present, the best evidence of a role for glypicans in growth-factor signaling during development derives from work with two secreted, heparin/HS-binding growth factors that play key roles in *Drosophila* developmental patterning: decapentaplegic (Dpp), a TGF-β superfamily member most closely related to bone morphogenetic protein (BMP)–2 and BMP-4, and Wg, a Wnt family member. To test the hypothesis that mutations in glypicans affect growth-factor signaling, my colleagues and I began by examining whether phenotypes observed in *dally* mutants were a consequence of compromised Dpp function. A variety of experiments showed this to be the case (Jackson et al. 1997). For example, *dally* mutants show defects in the activation of known Dpp target genes, without affecting the expression of Dpp, and reduction in Dpp function greatly increases the severity of *dally* phenotypes. Similarly, overgrowth defects produced by misexpression of Dpp can be rescued by reducing *dally* function, which supports the argument that *dally* serves to influence cellular responses to Dpp. Our studies showed that *dally* works, at least in part, by affecting the function of Dpp.

In addition to their effects on Dpp (and, by extension, other TGF-β family members), proteoglycans are also implicated in Wnt signaling. Genetic screens for mutations affecting Wg signaling in *Drosophila* suggest that this growth factor also requires GAG biosynthesis for its normal function during embryonic development. Three laboratories identified mutations in a *Drosophila* gene encoding a protein with homology to UDP-glucose dehydrogenase (dUDPG-DH, known as *sugarless, suppenkasper,* or *kiwi*) (Binari et al. 1997; Häcker et al. 1997; Haerry et al. 1997). UDPG-DH is required for the synthesis of UDP-glucuronate. Glucuronate is one of the two backbone sugars of HS, and UDP-glucuronate serves as the nucleotide sugar donor. Mutations in *Drosophila* UDPG-DH disrupt Wg signaling in the early embryo and are lethal. Interestingly, overexpression of Wg can compensate for the loss of UDPG-DH, suggesting that HSPGs serve to enhance the signaling activity of Wg but are not sine qua non components of the receptor (Häcker et al. 1997). Our experiments, in collaboration with Hiroshi Nakato's group, indicate that *dally* influences Wg signaling during embryonic development (M. Tsuda and S. B. Selleck, unpublished data). These findings suggest that *dally* serves as a component of the Wg receptor, influencing the assembly of an active signaling complex. Our studies also indicate that *dally* is not simply a general enhancer of growth-factor signaling but participates selectively in either Dpp or Wg signaling, in a tissue-specific manner.

Polysaccharide Structure and Tissue Patterning

We have hypothesized that different sugar modifications of Dally might govern its participation in different growth-factor–signaling assemblies. Two recent genetic studies lend credence to this idea and suggest that sulfation of HS is one such biologically relevant sugar modification required for discrete patterning events during development. First, a gene trap–induced mutation in a gene encoding an HS-modifying enzyme, HS-2-sulfotransferase (HS-2st), produces renal agenesis as well as eye and skeletal abnormalities (Kobayashi et al. 1997; Bullock et al. 1998). *HS-2st* shows tissue-specific patterns of expression, and, because 2-O-sulfation can affect the interaction with HS of growth-factor ligands, it seems likely that tissue-specific modifications of HS regulate HSPG growth-factor assemblies in the mouse.

More direct evidence of a role for 2-O-sulfotransferase activity in patterning comes from studies of genes controlling axis formation in *Drosophila*. The dorsal-ventral axis of the embryo begins to be established before fertilization and requires the follicle cells that surround the oocyte (reviewed in Anderson 1998). In the absence of normal follicle-cell function, embryos are "dorsalized" and completely lack ventral structures. A critical role

served by these follicle cells is the activation of the ventralizing signal, that is, the secreted protein Spätzle. This activation is restricted to the ventral region of the developing embryo and depends on the expression in the follicle cells of the gene *pipe,* which encodes a protein homologous to vertebrate 2-O-sulfotransferase (Sen et al. 1998). Ectopic *pipe* expression is sufficient to ventralize dorsal cells, suggesting that localized 2-O-sulfation of an HSPG is critical to the activation of the ventralizing signal provided by Spätzle.

Several other genes required, in follicle cells, for Spätzle activation encode secreted proteases, and protease activity has been shown to be critical for the generation of ventral structures. The homology of *pipe* to a vertebrate 2-O-sulfotransferase suggests that a proteoglycan may be required for the proteolytic processing of Spätzle. Perhaps, for example, a 2-O-sulfated HSPG forms a docking site for the proteolytic activation of Spätzle. Although one must acknowledge the caveat that *pipe* has not yet been shown to act as a 2-O-sulfotransferase, these findings emphasize that HS-modifying enzymes and their proteoglycan substrates are likely to play critical roles in spatially regulated signaling events during development.

Molecular Models for HSPG Functions in SGBS

The genetic studies of mice and flies show that proteoglycans and HS biosynthetic and modifying enzymes affect patterning during development. The activities of these genes are expressed at least in part by their influence on the signaling of two key secreted growth-factor families, the Wnts and the TGF-β/BMPs. However, what precisely are HSPGs doing at the cell surface, and how might they affect signaling there? One model, referred to above, maintains that HSPGs serve as growth-factor coreceptors by helping to deliver ligands to the signaling receptor. However, HSPGs might also affect molecular assemblies at the cell surface by other means, and there is experimental support for two other mechanisms: (1) HS as an allosteric regulator changing the conformation of proteins that bind to specific HS sequences and (2) HSPGs as "molecules of encounter" facilitating the interaction between two other components. As shown in figure 2, both these mechanisms play a role in the regulation, by HS, of blood coagulation on the luminal surface of endothelial cells (Rosenberg et al. 1997). The serine protease inhibitor antithrombin (AT) inactivates the proteases thrombin and factor Xa as a means of regulating the coagulation cascade. Heparin—a highly sulfated form of HS—accelerates AT-protease interactions by two different mechanisms. First, heparin binding to AT induces conformational changes that accelerate AT-Xa–complex generation. Second, both AT and thrombin bind to one long-chain heparin molecule, promoting AT-thrombin association. The regulation of proteases by HSPGs might provide a paradigm for understanding how *pipe* controls dorsal-ventral patterning during *Drosophila* development.

These models provide a basis for the understanding of how loss of GPC3 might cause the overgrowth, patterning defects, and tumor susceptibility that characterize SGBS. It has been suggested that GPC3 binds to insulin-like growth factor–2 (IGF2) and that loss of GPC3 might increase the levels of IGF2, hence promoting tissue growth. However, rat Gpc3 does not bind to IGF2, which casts some doubt on this model (Song et al. 1997), although it is possible that GPC3 affects IGF2 activity indirectly, perhaps by regulating IGF2-binding proteins. It seems very unlikely, however, that all the phenotypes associated with SBGS will be accounted for by defects in IFG2 activity alone. The evidence from *Drosophila* and from cell-culture systems suggests that many different growth factors are likely to be affected by loss of GPC3. Clearly, Wnt and TGF-β/BMP signaling could be compromised in SGBS patients. TGF-β is a well-known inhibitor of cell-cycle progression, and the loss of GPC3 could compromise TGF-β–mediated control of cell division. Disruptions in TGF-β–signaling components, including the Smads, and in TGF-β receptors are associated with tumor progression (Moskaluk and Kern 1996), suggesting that the neoplasia associated with SBGS could reflect a loss of normal TGF-β activity.

Recently, mouse Gpc3 has been shown to affect apoptosis in different tissue-culture cell lines (Gonzalez et al. 1998). Perhaps some of the phenotypes of SGBS patients, such as hexadactyly, are the consequence of defects in programmed cell death during development. In this regard, it is interesting to note that TGF-β/BMPs have been implicated in the induction of programmed cell death during limb morphogenesis (Merino et al. 1998). In sum, the many SGBS phenotypes found probably reflect the variety of signaling events that are affected by loss of this cell-surface proteoglycan. The analysis of proteoglycans will yield a great deal of insight into the molecular topography of the cell surface and the orchestration of signaling events that take place there.

References

Anderson KV (1998) Pinning down positional information: dorsal-ventral polarity in the *Drosophila* embryo. Cell 95: 439–442

Aviezer D, Yayon A (1994) Heparin-dependent binding and autophosphorylation of epidermal growth factor (EGF) receptor by heparin-binding EGF-like growth factor but not EGF. Proc Natl Acad Sci USA 91:12173–12177

Binari RC, Staveley BE, Johnson WA, Godavarti R, Sasisekharan R, Manoukian AS (1997) Genetic evidence that hep-

arin-like glycosaminoglycans are involved in wingless signaling. Development 124:2623–2632

Bullock SL, Fletcher JM, Beddington RS, Wilson VA (1998) Renal agenesis in mice homozygous for a gene trap mutation in the gene encoding heparan sulfate 2-sulfotransferase. Genes Dev 12:1894–1906

Esko JD, Zhang L (1996) Influence of core protein sequence on glycosaminoglycan assembly. Curr Opin Struct Biol 6: 663–670

Feyzi E, Saldeen T, Larsson E, Lindahl U, Salmivirta M (1998) Age-dependent modulation of heparan sulfate structure and function. J Biol Chem 273:13395–13398

Gonzalez AD, Kaya M, Shi W, Song H, Testa JR, Penn LZ, Filmus J (1998) OCI-5/GPC3, a glypican encoded by a gene that is mutated in the Simpson-Golabi-Behmel overgrowth syndrome, induces apoptosis in a cell line–specific manner. J Cell Biol 141:1407–1414

Häcker U, Lin X, Perrimon N (1997) The *Drosophila sugarless* gene modulates wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis. Development 124: 3565–3573

Haerry TE, Heslip TR, Marsh JL, O'Connor MB (1997) Defects in glucuronate biosynthesis disrupt wingless signaling in *Drosophila*. Development 124:3055–3064

Jackson SM, Nakato H, Sugiura M, Jannuzi A, Oakes R, Kaluza V, Golden C, et al (1997) *Dally,* a *Drosophila* glypican, controls cellular responses to the TGF-β–related morphogen, Dpp. Development 124:4113–4120

Kobayashi M, Habuchi H, Yoneda M, Habuchi O, Kimata K (1997) Molecular cloning and expression of Chinese hamster ovary cell heparan-sulfate 2-sulfotransferase. J Biol Chem 272:13980–13985

Lander AD, Stipp CS, Ivins JK (1996) The glypican family of heparan sulfate proteoglycans: major cell-surface proteoglycans of the developing nervous system. Perspect Dev Neurobiol 3:347–358

Lopez-Casillas F, Wrana JL, Massague J (1993) Betaglycan presents ligand to the TGF beta signaling receptor. Cell 73: 1435–1444

Maccarana M, Sakura Y, Tawada A, Yoshida K, Lindahl U (1996) Domain structure of heparan sulfates from bovine organs. J Biol Chem 271:17804–17810

Merino R, Ganan Y, Macias D, Economides AN, Sampath KT, Hurle JM (1998) Morphogenesis of digits in the avian limb is controlled by FGFs, TGF betas, and noggin through BMP signaling. Dev Biol 200:35–45

Moskaluk CA, Kern SE (1996) Cancer gets *Mad: DPC4* and other *TGF-b* pathway genes in human cancer. Biochim Biophys Acta 1288:M31–M33

Nakato H, Futch TA, Selleck SB (1995) The *division abnormally delayed* (*dally*) gene: a putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in *Drosophila*. Development 121:3687–3702

Nelson RM, Venot A, Bevilacqua MP, Linhardt RJ, Stamenkovic I (1995) Carbohydrate-protein interactions in vascular biology. Ann Rev Cell Dev Biol 11:601–631

Neri G, Gurrieri F, Zanni G, Lin A (1998) Clinical and molecular aspects of the Simpson-Golabi-Behmel syndrome. Am J Med Genet 79:279–283

Olwin BB, Rapraeger A (1992) Repression of myogenic differentiation by aFGF, bFGF, and K-FGF is dependent on cellular heparan sulfate. J Cell Biol 118:631–639

Pilia G, Hughes-Benzie RM, MacKenzie A, Baybayan P, Chen EY, Huber R, Neri G, et al (1996) Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. Nat Genet 12:241–247

Rapraeger AC (1995) In the clutches of proteoglycans: how does heparan sulfate regulate FGF binding? Chem Biol 2: 645–649

Rapraeger AC, Krufka A, Olwin BB (1991) Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. Science 252:1705–1708

Reichsman F, Smith L, Cumberledge S (1996) Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction. J Cell Biol 135: 819–827

Rosenberg RD, Shworak NW, Liu J, Schwartz JJ, Zhang L (1997) Heparan sulfate proteoglycans of the cardiovascular system: specific structures emerge but how is synthesis regulated? J Clin Invest Suppl 100:S67–S75

Salmivirta M, Lidholt K, Lindahl U (1996) Heparan sulfate: a piece of information. FASEB J 10:1270–1279

Sanderson RD, Turnbull JE, Gallagher JT, Lander AD (1994) Fine structure of heparan sulfate regulates syndecan-1 function and cell behavior. J Biol Chem 269:13100–13106

Sen J, Goltz JS, Stevens L, Stein D (1998) Spatially restricted expression of pipe in the *Drosophila* egg chamber defines embryonic dorsal-ventral polarity. Cell 95:471–481

Song HH, Shi W, Filmus J (1997) OCI-5/rat glypican-3 binds to fibroblast growth factor-2 but not to insulin-like growth factor-2. J Biol Chem 272:7574–7577

Zioncheck TF, Richardson L, Liu J, Chang L, King KL, Bennett GL, Fugedi P, et al (1995) Sulfated oligosaccharides promote hepatocyte growth factor association and govern its mitogenic activity. J Biol Chem 270:16871–16878