

Proteoglycans and pattern formation

sugar biochemistry meets developmental genetics

While it has been long appreciated that sugar-modified proteins coat the cell surface, their functions are poorly understood. Here, I describe recent genetic studies that demonstrate that one class of sugar-modified proteins, cell-surface proteoglycans, play crucial roles in morphogenesis, growth regulation and tumor suppression. Mutations that affect individual proteoglycans or the enzymes required for glycosaminoglycan synthesis regulate Wingless and Decapentaplegic signaling in *Drosophila*, and body size in mice and humans. Compromising proteoglycan function is also associated with the development of Wilm's tumors and hereditary multiple exostoses. In this review, these biological findings are placed in the context of proteoglycan biochemistry and molecular function.

Proteoglycan is not a term found in the working lexicon of most developmental biologists. These sugar-modified proteins have long been considered the purview of matrix aficionados, and not necessarily of central importance to understanding differentiation and morphogenesis. Therefore, it might come as a surprise to many developmental geneticists that, in the past few years, proteoglycans have been shown to serve critical roles in patterning. Mutations in *Drosophila* that affect proteoglycan core proteins, or the enzymes that produce their carbohydrate modifications, influence the function of several growth factor pathways that are essential for tissue assembly. Similarly, in the mouse, mutations that disrupt proteoglycans or genes that encode glycosaminoglycan biosynthetic enzymes have profound effects on growth and morphogenesis. Whereas the findings that proteoglycans are critical for patterning were derived primarily from studies in model organisms, it is evident that proteoglycans play a role in human pathogenesis as well. A human overgrowth and tumor-susceptibility syndrome, Simpson–Golabi–Behmel dysmorphia (SGBD), is caused by mutations in a gene that encodes an integral membrane proteoglycan, Glypican 3 (*GPC3*)¹. More recently, the *exostoses* (*EXT*) class of tumor-suppressor genes has been found to encode enzymes that are required for the synthesis of the heparan sulfate sugars attached to proteoglycans^{2,3}. Here, I highlight the recent convergence of the genetic, structural and biochemical data that have brought proteoglycans into the fold of developmental genetics and tumor biology.

Proteoglycans – what are they and where are they found?

Proteoglycans are proteins that bear long, unbranched sugar polymers, glycosaminoglycans, which are attached

to specific serine residues of the protein core (see Box 1)⁴. Glycosaminoglycans are polymers of disaccharide units, and different classes of glycosaminoglycans comprise different disaccharide repeats (Fig. 1). For example, chondroitin is a repeat of glucuronic acid and *N*-acetyl galactosamine, whereas heparan sulfate is synthesized as a polymer of glucuronic acid and *N*-acetyl glucosamine. Contrary to the common notion that glycosaminoglycans are monotonous, with a single repeating structural motif, we now know that these sugar polymers show a great deal of structural diversity. Discrete structural forms, generated by complex patterns of deacetylation, sulfation and epimerization, are found in specific tissues and are influenced by aging and disease^{5,6}. A single glycosaminoglycan chain can also contain distinct structural domains, with regions of highly sulfated residues alternating with segments of modest levels of modification.

Proteoglycans are well-known components of the extracellular matrix, including cartilage, basement membranes and connective tissue. However, they are also abundant molecules of the cell surface⁷. Virtually all epithelia express cell-surface proteoglycans, represented principally by glypicans and syndecans. Glypicans are glycosylphosphatidylinositol (GPI)-linked molecules and bear glycosaminoglycans exclusively of the heparan sulfate type. Syndecans are transmembrane proteins, and are decorated with chondroitin sulfate and with heparan sulfate polymers. A discussion of the many proteoglycans found in the matrix and on the cell surface is beyond the scope of any single article. I will therefore limit this review to recent genetic studies of cell-surface proteoglycans and the genes required for the biosynthesis of their glycosaminoglycan modifications (Table 1).

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BOX 1. Glossary**Epimers**

Two isomers of a monosaccharide that differ only in the configuration around a single chiral carbon, for example, the C5 carboxylic acid group in glucuronate versus iduronate.

Epimerase

An enzyme that catalyzes the conversion of one epimer to another.

Exostoses

Cartilagenous tumors of the growth plate associated with hereditary multiple exostoses, autosomal dominant disorders most frequently affecting either *EXT1* or *EXT2*— genes that encode enzymes involved in heparan sulfate synthesis.

Glycosaminoglycan

A type of sugar polymer, comprised of linear disaccharide repeats, and typically attached to specific serine residues of the protein core of proteoglycans.

Glycosylphosphatidylinositol linkage

A membrane anchor comprised of a sugar bridge linking phosphatidylinositol in the membrane to the C terminus of the protein.

Glycosyltransferase

An enzyme that transfers a sugar from a nucleotide-sugar donor, to a substrate.

Glypicans

A family of Glycosylphosphatidylinositol-linked, heparan sulfate-modified proteoglycans, currently represented by six distinct genes in vertebrates; homologs found in *Drosophila* (*dally*), and *Caenorhabditis elegans*.

Heparin

A form of highly sulfated heparan sulfate made in mast cells.

Proteoglycan

A protein bearing covalently attached glycosaminoglycan chains.

Syndecans

A family of transmembrane, chondroitin and heparan sulfate-modified proteoglycans with four members in vertebrates, and related genes in *Drosophila* and in *C. elegans*.

Of flies, mice and men: patterning defects and proteoglycans

Direct evidence linking proteoglycans to pattern formation and growth-factor signaling *in vivo* came from studies of a gene that affects cell division in the *Drosophila* visual system. *division abnormally delayed* (*dally*) mutants were isolated based on cell-division abnormalities in the larval brain; *dally* mutants show delayed cell-cycle progression for subsets of dividing cells in the visual system⁸. The isolation of a collection of *dally* alleles revealed that *dally* was also required for the morphogenesis of many tissues, including the wing, genitalia, eye and antenna. Sequencing of a *dally* cDNA revealed homology throughout the coding sequence to glypicans. Biochemical analysis of Dally has confirmed that, like the vertebrate glypicans, Dally is linked to GPI and modified with heparan sulfate⁹.

Within a few months of the first report on *dally*, a human overgrowth and tumor-susceptibility syndrome, SGBS, was found to be caused by mutations in *GPC3* (Ref. 1), one of several glypicans related to *dally*. In addition to overgrowth of all somatic tissues, SGBD patients have a predisposition to Wilm's tumor of the kidney, neuroblastomas and display a host of morphological abnormalities, including heart defects, dysplastic kidneys, vertebral and rib defects, and polydactyly¹⁰. Recently, a mouse knockout in *Gpc3* has been reported and, like its human counterpart, it shows significant overgrowth and renal defects¹¹. Together, studies of *dally* and *GPC3* have demonstrated that a family of conserved cell-surface proteoglycans is required for the control of growth, cell division and patterning during development. However, these

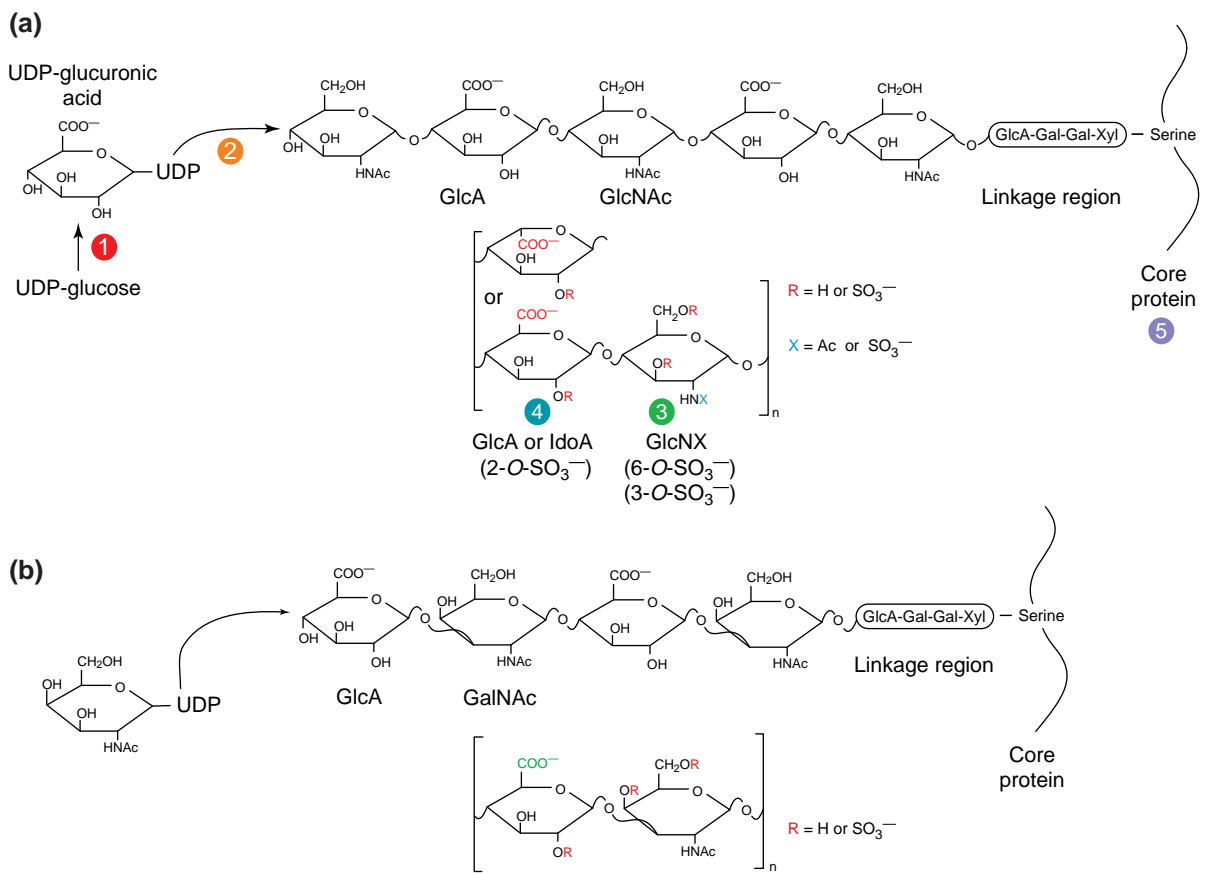
initial reports left unanswered how, at the molecular level, the loss of glypican function could produce such a broad range of developmental and growth abnormalities. Studies directed largely by earlier biochemical analyses soon revealed that cell-surface proteoglycans affect patterning, at least in part, by controlling growth-factor signaling.

Proteoglycans and the regulation of growth factor signaling

In the early 90s, studies using tissue-culture systems established that cell-associated heparan sulfate can affect growth-factor signaling in a profound way. Inhibition of glycosaminoglycan sulfation using chlorate, or removal of heparan sulfate from the cell surface with heparin lyases, dramatically reduced the signaling activity of fibroblast growth factors (FGFs)^{12,13}. Based on these observations, integral membrane proteoglycans were proposed to serve as growth-factor coreceptors, affecting the assembly of signaling complexes on the cell surface (Fig. 2a). Recent crystallographic studies suggest that heparin (a highly sulfated form of heparan sulfate) promotes the assembly of a signaling complex through contacts with FGF and with its receptor¹⁴. Since the demonstration that heparan sulfate proteoglycans are critical for FGF signaling, similar studies have shown a role for heparan sulfate in signaling mediated by Wingless¹⁵, heparin-binding epidermal growth factor¹⁶ and hepatocyte growth factor¹⁷.

Evidence that proteoglycans influence growth-factor signaling *in vivo* came initially from the analysis of *dally* mutants¹⁸. After identifying *dally* as a glypican-encoding gene, my laboratory began a series of studies to determine

FIGURE 1. Two common glycosaminoglycans



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|---|--|
| <p>1 UDP-glucose dehydrogenase (<i>sugarless</i>)
Converts UDP-glucose to UDP-glucuronate</p> <p>2 HS co-polymerase (<i>EXT1</i>, <i>EXT2</i>, <i>tout-velu</i>)
Glycosyl transferase that adds alternating GlcNAc and GluA residues to growing heparan sulfate chain. <i>tout-velu</i> is not the only <i>EXT</i>-related gene in <i>Drosophila</i> and is probably not exclusively required for heparan sulfate biosynthesis.</p> | <p>3 <i>N</i>-deacetylase/<i>N</i>-sulfotransferase (<i>Ndst2</i>, <i>sulfateless</i>)
Removes acetyl group of GlcNAc and replaces it with a sulfate group.</p> <p>4 HS-2-<i>O</i>-sulfotransferase (<i>Hs2st</i>)
Adds sulfate group to 2-<i>O</i> position of iduronate or glucuronate.</p> <p>5 Cell surface core proteins, glypicans (<i>GPC3</i>, <i>dally</i>), syndecans</p> |
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Structure of (a) heparan sulfate and (b) chondroitin sulfate. Both polymers are covalently linked to the polypeptide at serine residues and share a common tetrasaccharide linker. Heparan sulfate (HS) is synthesized as an alternating polymer of glucuronic acid (GlcA) and *N*-acetyl glucosamine (GlcNAc), whereas chondroitin consists of alternating GlcA and *N*-acetyl galactosamine (GalNAc) residues. Both polymers are modified, with some of these modifications shown in brackets below the respective chains (modified position highlighted in color). The carboxylic acid group (COO⁻) of GlcA can be inverted by an epimerase to form iduronic acid in both polymers (both forms shown for heparan sulfate, the COO⁻ group highlighted in green for chondroitin sulfate). Circled numbers indicate biosynthetic steps where mutants have been identified either in *Drosophila* or mouse (see Table I for references).

if *Dally* influenced patterning by affecting the activity of two known heparan sulfate-binding growth factors, Decapentaplegic (*Dpp*) and *Wingless* (*Wg*). We found that, in several tissues, *dpp* signaling is extremely sensitive to the levels of *dally* function. Compromising *dally* reduces the expression of genes activated by *Dpp*, without appreciably affecting the levels of *dpp* expression (Fig. 3). In addition, ectopic *dally*⁺ (the plus sign indicating wild type) expression can enhance the patterning activity of *Dpp*. Our findings provided direct evidence that a single

proteoglycan is capable of affecting patterning mediated by a growth factor during development.

Dally's effects are not limited to *Dpp* signaling, however. Recent experiments have shown that *dally* modulates patterning directed by *Wg* in the embryonic epidermis and wing imaginal disc^{9,19}. *dally* mutants show segment-polarity defects in the larval cuticle, and genetic interactions with *wg* and *Dfz2* (Ref. 19), a component of the *Wg* receptor²⁰. Ectopic *Dally* can rescue *wg* partial loss-of-function mutants and can potentiate *Wg* signaling, without changing

the levels of Wg (Ref. 9). These findings support a model in which proteoglycans enhance the activity of growth factors at the cell surface by promoting the assembly of signaling complexes.

The ability of Dally to enhance Dpp as well as Wg signaling raises a question about the specificity of proteoglycans in modulating growth-factor signaling. Interestingly, during genitalia development in *Drosophila*, *dally* affects Dpp and not Wg signaling, whereas in the embryonic epidermis, Dally influences Wg but not Dpp-directed patterning⁹. While the molecular mechanism of these differential activities of Dally is not known, it is apparent that a proteoglycan can serve as a tissue-specific modulator of a growth-factor signaling pathway. It is possible that the nature of the glycosaminoglycan chain attached to Dally regulates its participation in one signaling pathway or another. This hypothesis is supported by the analysis of an *Hs2st* mutant in mouse (see below). *Hs2st* encodes an enzyme (heparan sulfate 2-O-sulfotransferase) that places a sulfate on the 2-O position of iduronate, and a gene-trap insertion in *Hs2st* affects kidney, eye and skeletal development rather selectively³⁷.

Genes required for glycosaminoglycan biosynthesis affect signaling of multiple growth-factor families

Proteoglycan function can be potentially compromised by mutations that affect either the protein core, or the biosynthesis of the covalently attached glycosaminoglycan chains. Glycosaminoglycan synthesis occurs directly on the protein core, it takes place in the golgi, and it requires a host of nucleotide sugar transporters and glycosyltransferases. The basic repeating unit of the disaccharide polymer is further modified by epimerases and sulfotransferases to generate structurally diverse forms that show tissue-specific distributions.

In the last few years, screens for mutations that affect patterning in *Drosophila* have uncovered genes involved in glycosaminoglycan biosynthesis. Analysis of these mutants has demonstrated that glycosaminoglycans affect signaling that is mediated by multiple growth factors, including but not necessarily limited to, Wg, Dpp, FGFR and Hedgehog (Hh). The first of these genes was described by three groups and named variously as *sugarless*, *suppenkasper* and *kiwi*, and now referred to as *sugarless* (*sgl*)^{21,22}. This gene encodes a protein with homology to vertebrate UDP glucose dehydrogenase, an enzyme critical for the biosynthesis of UDP-glucuronic acid. UDP-glucuronic acid is the sugar donor for all glucuronate-containing glycosaminoglycans, including heparan and chondroitin sulfate. *sgl* is required for normal patterning of the embryonic epidermis, and embryos lacking both the maternal and the zygotic *sgl* activity display a cuticle phenotype that is indistinguishable from *wg* nulls. Patterning events in the embryo that are known to be directed by Wg and not Hh are compromised in *sgl* mutants, indicating that, at the very least, this gene affects Wg signaling²¹.

sgl mutations were also recovered in a genetic screen for suppressors of ectopic Dpp signaling²³, indicating that glycosaminoglycan biosynthesis can influence Dpp-directed patterning, as earlier studies of *dally* had suggested. Further analysis of *sgl* revealed that it is required for FGFR signaling mediated by *heartless* and *breathless*²⁴.

More recently, a second *Drosophila* gene with sequence homology to a known glycosaminoglycan biosynthetic

TABLE 1. Genetics of proteoglycan and glycosaminoglycan biosynthesis

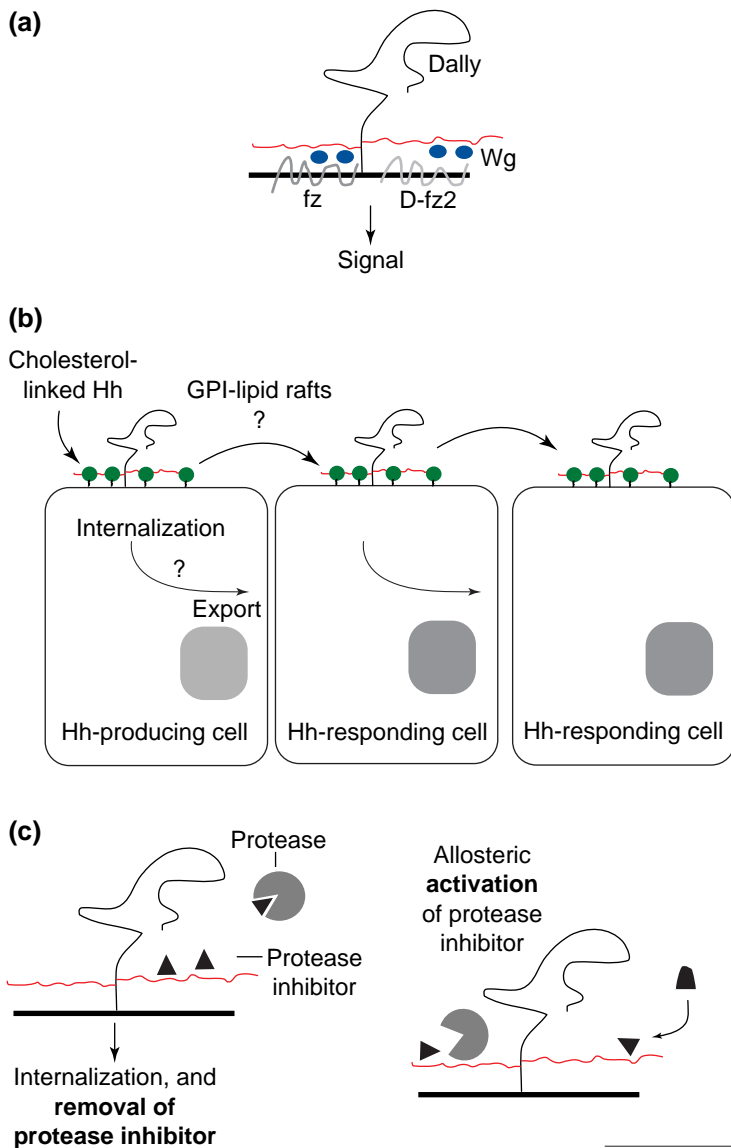
Gene and associated protein, activity	Associated phenotype	Refs
<i>division abnormally delayed</i> (<i>dally</i>), a <i>Drosophila</i> GPI-linked, heparan sulfate-modified proteoglycan	Defects in Wg and Dpp signaling	8, 9, 18, 19, 29
glypican 3 (GPC3)(human): GPI-linked, heparan sulfate-modified proteoglycan	Overgrowth, skeletal abnormalities, Wilm's tumor	1
<i>Gpc3</i> (<i>mouse</i>): GPI-linked, heparan sulfate-modified proteoglycan	Overgrowth, dysplastic kidneys	11, 41
<i>sugarless</i> : a <i>Drosophila</i> gene related to vertebrate UDP-glucose dehydrogenase; required for synthesis of glucuronate-containing polymers	Defects in Wg, FGFR, and Dpp signaling	21–24
<i>sulfateless</i> : a <i>Drosophila</i> gene related to vertebrate <i>N</i> -deacetylase <i>N</i> -sulfotransferase, a heparan sulfate-modifying enzyme	Defects in Wg, FGFR and Hh signaling	19, 24, 30
<i>NDST-2</i> (<i>mouse</i>): <i>N</i> -deacetylase <i>N</i> -sulfotransferase	Loss of mast cell heparin, defects in secretory granules	25, 26
<i>EXT1</i> (<i>human</i>): encodes a heparan sulfate co-polymerase	Multiple exostoses tumors	2, 3, 28
<i>EXT2</i> (<i>human</i>): encodes a heparan sulfate co-polymerase	Multiple exostoses	3
<i>tout-velu</i> : <i>Drosophila</i> gene related to <i>EXT1</i>	Selective defect in Hh signaling and distribution	27, 29, 30
heparan 2-O-sulfotransferase (<i>mouse</i>): a heparan sulfate-modifying enzyme	Renal agenesis, eye and skeletal abnormalities	37
<i>pipe</i> , a <i>Drosophila</i> gene related to glycosaminoglycan 2-O-sulfotransferases	Defects in dorsal-ventral patterning, controls generation of ventralizing signal	38

gene was shown to affect both Wg and FGFR signaling. *sulfateless* (*sfl*) bears striking homology to *N*-deacetylase *N*-sulfotransferases (NDST), enzymes that are essential for the modification of heparan sulfate polymers^{19,24}. Indeed, *N*-deacetylation *N*-sulfation is an early step in heparan sulfate modification, and would therefore probably affect the structural diversity of the polymer considerably.

In vertebrates, there are currently four distinct known genes that encode NDST enzymes. The data from *Drosophila* suggests that these genes will be essential for normal patterning. Recently, *Ndst2* knockouts have been reported, and although they are viable and fertile, these animals are defective in mast-cell heparin biosynthesis and secretory-granule assembly^{25,26}; the phenotype of an *Ndst1* knockout mouse indicates that this gene is required for viability and the normal morphogenesis of multiple organ systems (L. Kjellen, pers. commun.).

The third gene that has been identified in *Drosophila* that affects glycosaminoglycan biosynthesis is *tout-velu* (*ttv*) (which translates to 'all hairy', a reference to the cuticle phenotype)²⁷. The cloning and sequencing of *ttv* showed that it shared a high degree of homology to *EXT1*, a gene that is associated with cartilaginous tumors of the growth plate called exostoses. Exostoses have been linked to three distinct genetic loci, and two of these, *EXT1* and *EXT2*, encode related proteins²⁸. Several lines of evidence show that *EXT1* and *EXT2* are involved in heparan sulfate biosynthesis. First, a mouse cell line that is resistant to herpes simplex virus (HSV) infection shows a selective loss of heparan sulfate that can be rescued by transfection with an *EXT1* cDNA (Ref. 2). Second, a protein purified on the basis of its ability to catalyze the sequential addition of glucuronic acid and *N*-acetyl glucosamine to the growing heparan sulfate chain, a heparan sulfate copolymerase, is

FIGURE 2. Models for proteoglycan function



Somewhat surprisingly, it appears that *ttv*⁺ is selective for Hh signaling, with FGF and Wg-directed patterning unaffected by loss of *ttv* gene function³⁰. The Hh-signaling defects associated with loss of *ttv* contrasts with the widespread effects on Wg and FGFR signaling in *sgl* and *sfl* mutants. There are other *EXT*-related genes in *Drosophila* and it is possible that some proteoglycans require genes other than *ttv* for their heparan sulfate modification. A comparison of the glycosaminoglycans that remain in animals bearing these different mutations also has the potential to reveal which polymer forms are required for which signaling pathways.

Proteoglycans can affect the tissue distributions of signaling molecules

The detailed analysis of *ttv* function in imaginal discs using genetic mosaics has revealed a fascinating twist to this story²⁷. Loss of *ttv* function does not abrogate the ability of cells to respond to Hh (although it is reduced), but rather alters the distribution of Hh, preventing its dispersal from Hh-synthesizing cells across a domain of 8–10 cells that respond to Hh. Clearly, a heparan sulfate-modified proteoglycan is affecting the transport of Hh across the wing epithelium. How could a proteoglycan be involved in this process? Several mechanisms are possible. GPI-linked proteins have been shown to transfer from the plasma membrane of one cell to another when these cells are in contact³¹. Presumably, because GPI-linked proteins are inserted in only the outer leaflet of the plasma membrane, they can 'flip' between adjacent outer leaflets under the appropriate conditions. Regardless of the mechanism, the demonstrated transfer of GPI-linked proteins between cells suggests that a GPI-linked proteoglycan that binds Hh permits contact-mediated diffusion of Hh from cell to cell (Fig. 2b). Proteoglycans have also been implicated in the regulation of endocytosis of cell surface ligands³², and a heparan sulfate proteoglycan could potentially distribute Hh by endocytosis and transcellular transport.

Hh is not the only growth factor that has discrete distributions across tissues that are critical for its patterning activity. Wg shows an asymmetric distribution with respect to the Wg-producing cells in the embryonic epidermis, and there is good evidence to suggest that endocytosis is critical for this regulated distribution of Wg (Refs 33, 34). For example, the expression of a gene encoding a dominant-negative form of *Drosophila* dynamin that blocks endocytosis affects Wg-mediated patterning, even when it is expressed in cells that do not make Wg. These findings suggest that endocytic transport of Wg is important for controlling its patterning activity. Two recent studies show that *hb* and *naked* influence the range of Wg transport, demonstrating that the control of Wg distribution is tightly regulated^{34,35}.

Heparan sulfation affects patterning during development

The polymer of glucuronic acid and *N*-acetyl glucosamine that is synthesized to produce heparan sulfate is modified extensively to generate discrete structural forms in different tissues. One of the enzymes involved in this process is heparan sulfate 2-*O*-sulfotransferase (Hs2st), which places a 2-*O* sulfate on glucuronic acid, or its epimer, iduronate³⁶. A gene-trap insertion in the mouse *Hs2st* gene was recently shown to cause renal agenesis, as well as defects in the eye and skeleton³⁷. These findings suggest

(a) As an example of how a cell-surface proteoglycan might serve as a growth factor co-receptor, Dally, with its heparan sulphate chains (red), is shown bound to Wingless (Wg; blue), and affecting the assembly of a receptor complex that includes Fz and D-fz2. (b) *tout-velu* mutants disrupt heparan sulfate biosynthesis and they have been shown to be required for normal Hedgehog (Hh; green) distributions across the imaginal wing disc. Proteoglycans might affect endocytosis and the subsequent export of complexes, or 'contact-mediated' diffusion of growth factors bound to glycosylphosphatidylinositol- (GPI-) linked proteoglycans. (c) Heparan sulfate proteoglycans have been shown to activate protease inhibitors (antithrombin III; thimble shape → triangle) and promote association of inhibitor with protease³⁹, as well as to remove protease inhibitors from the cell surface⁴⁰, providing a mechanism for the activation of a protease.

EXT2. Indeed, *in vitro*, EXT1 and EXT2 both have heparan sulfate co-polymerase activity³. Finally, structural analysis of glycosaminoglycans from *ttv* mutants has shown that these animals have greatly reduced levels of heparan sulfate but not chondroitin sulfate, as would be predicted if *ttv* encodes a heparan sulfate-specific co-polymerase²⁹. Characterization of *ttv* mutant embryos with an antibody that recognizes a structural feature of heparan sulfate chains exposed after scission with a heparin lyase, also supports the conclusion that normal heparan sulfate biosynthesis requires *ttv*⁺ (Ref. 30).

that discrete heparan sulfate forms are essential for regulating specific growth-factor pathways, and that regulated synthesis of proteoglycans is critical for normal development.

The proposal that specific glycosaminoglycan modifications are critical in regulating discrete patterning processes was furthered by the characterization of a *Drosophila* gene that affects dorsal–ventral patterning in the embryo. *pipe* is required for specifying the ventral fate of cells in the early embryo, and has been shown to play a role in the proteolytic activation of Spätzle, the presumed ligand for the Toll receptor. Activation of Toll in the embryo is required for cells to adopt a ventral fate and *pipe* is central to restricting these events to the future ventral side of the embryo³⁸. So what is Pipe? It shows homology to glycosaminoglycan 2-O sulfotransferases, once again suggesting that modification of the glycosaminoglycan moieties of proteoglycans is critical for the control of signaling events during development.

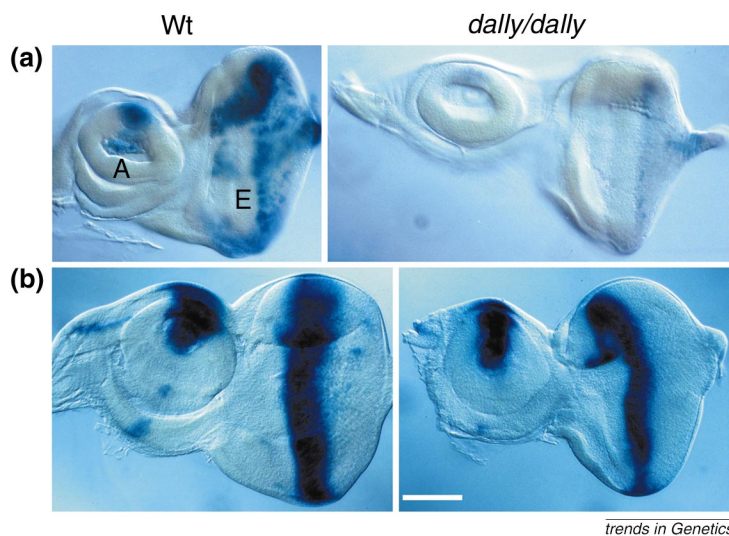
The importance of glycosaminoglycans and proteoglycans in the control of proteases is not a new theme (Fig. 2c). Heparin, a highly sulfated form of heparan sulfate used clinically as an anticoagulant, binds to antithrombin III (ATIII), inducing a conformational change that is critical for the ATIII-mediated inhibition of the Factor X protease³⁹. This provides an example of a glycosaminoglycan activating a protease inhibitor. Proteoglycans are also implicated in removing protease inhibitors from the cell surface. Internalization of tissue factor pathway inhibitor (TFPI), a protease inhibitor also involved in the regulation of coagulation, is mediated in part by cell-surface heparan sulfate proteoglycans⁴⁰. This latter observation suggests a mechanism for proteoglycan-mediated regulation of Spätzle activation. If a heparan sulfate proteoglycan with 2-O-sulfated chains were required to internalize and remove a protease inhibitor, this would provide the means of locally activating the protease that is responsible for processing Spätzle.

Conclusions and perspectives

So, what have we learned in the past few years about the *in vivo* functions of proteoglycans and their associated glycosaminoglycans? First, these molecules play a critical role in modulating the signaling mediated by secreted growth factors that are central to patterning tissues, including Wnts, transforming growth factor- β /bone morphogenetic proteins (TGF- β /BMPs), FGF and Hh. Second, specific structural forms of glycosaminoglycans are critical in governing the biological activities of their associated proteoglycans. Third, proteoglycans can affect the distribution of signaling molecules across epithelia and, hence, might govern the establishment of morphogen gradients.

Although it is easy to appreciate how defects in multiple growth-factor signaling pathways could affect morphogenesis, the links between proteoglycan function, growth regulation, and tumor suppression are less obvious. The initial proposal, that *GPC3* mutations affected growth in SGBS patients via changes in serum levels of insulin-like growth factor-2 (Ref. 1), has not been borne out by work in the mouse knockout model, which also displays overgrowth^{11,41}. How proteoglycans could influence growth factor activity to restrict tumor development is also a mystery. Perhaps proteoglycans enhance signaling mediated by growth factors that promote cell-cycle arrest or differentiation. TGF- β is one example of a secreted factor

FIGURE 3. Expression of a *dpp* target gene, *optomotor blind* (*omb*), is reduced in *dally* mutants



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(a) Eye (labeled E)—antenna (labeled A) disc complexes from third instar larvae bearing an *omb::lacZ* reporter, and either wild-type or homozygous for *dally*. (b) A *dpp-lacZ* reporter shows mild reduction in expression in *dally* mutants. Wt, wild type. The scale bar represents 100 μ m.

that inhibits cell-cycle progression and plays an important role in tumor suppression⁴². This model is supported by the observation that Dally, a fly glypican, potentiates signaling mediated by the TGF- β /BMP-related protein Dpp (Ref. 18).

The ability of proteoglycans to control the distributions of signaling molecules across many cell dimensions is a particularly exciting area of investigation. It has been widely assumed that morphogen distributions were a function of simple diffusion. This is clearly not always the case, as studies of Hh and Wg in *Drosophila* have documented^{27,33,35}. The analysis of Ttv and its identification as a heparan sulfate biosynthetic enzyme begs the question as to which proteoglycan is controlling Hh movement. Recently, a fine cellular process that extends many cell dimensions, called a cytoneme, has been identified that could mediate long-range signaling⁴³. Perhaps a heparan sulfate proteoglycan governs growth-factor transport by influencing the formation of these processes.

What we have learned thus far is just the beginning of the story. *In vivo* analysis of syndecans, the other major family of integral membrane proteoglycans, is in progress. Preliminary data on these proteins suggest a role for these molecules in Wnt signaling, wound healing, responses to pathogens, and even the control of satiety! Clearly there is a great deal of analysis needed to understand the varied functions of different proteoglycan core proteins. A quick perusal of the *Caenorhabditis elegans* genome provides some idea as to the magnitude of the problem: there are upwards of 25 genes with homology to known chondroitin- or heparan sulfate-modified proteoglycans. Of these, only *unc-52*, a gene that encodes a protein related to perlecan, a heparan sulfate proteoglycan of the basement membrane, has been studied genetically. *unc-52* affects myofilament assembly and the attachment of this complex to the muscle-cell membrane⁴⁴. The interplay between glycosaminoglycan and protein core in dictating function is also largely unexplored. Furthermore, the degree of structural

diversity that is possible for glycosaminoglycans is remarkable, with as many as 16 steps involved in their biosynthesis. Multiple genes encoding a single type of glycosaminoglycan-modifying enzyme are common, suggesting that polymer structure is tightly controlled. Although

the molecular details of proteoglycan function remain largely unknown, it is clear that understanding morphogenesis will require an appreciation of how proteoglycans control events at the cell surface and in the extracellular matrix.

References

- Pilia, G. *et al.* (1996) Mutations in *GPC3*, a glypican gene, cause the Simpson–Golabi–Behmel overgrowth syndrome. *Nat. Genet.* 12, 241–247
- McCormick, C. *et al.* (1998) The putative tumour suppressor *EXT1* alters the expression of cell-surface heparan sulfate. *Nat. Genet.* 19, 158–161
- Lind, T. *et al.* (1998) The putative tumor suppressors *EXT1* and *EXT2* are glycosyltransferases required for the biosynthesis of heparan sulfate. *J. Biol. Chem.* 273, 26265–26268
- Lindahl, U. *et al.* (1998) Regulated diversity of heparan sulfate. *J. Biol. Chem.* 273, 24979–24982
- Maccarana, M. *et al.* (1996) Domain structure of heparan sulfates from bovine organs. *J. Biol. Chem.* 271, 17804–17810
- Feyzi, E. *et al.* (1998) Age-dependent modulation of heparan sulfate structure and function. *J. Biol. Chem.* 273, 13395–13398
- Bernfield, M. *et al.* (1999) Functions of cell surface heparan sulfate proteoglycans. *Annu. Rev. Biochem.* 68, 729–777
- Nakato, H. *et al.* (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
- Tsuda, M. *et al.* (1999) A cell surface proteoglycan, Dally, regulates Wingless signalling in *Drosophila*. *Nature* 400, 276–280
- Neri, G. *et al.* (1998) Clinical and molecular aspects of the Simpson–Golabi–Behmel syndrome. *Am. J. Med. Genet.* 79, 279–2839
- Cano-Gauci, D.F. *et al.* (1999) Glypican-3-deficient mice exhibit developmental overgrowth and some of the abnormalities typical of Simpson–Golabi–Behmel syndrome. *J. Cell Biol.* 146, 255–264
- Olwin, B.B. and 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
- Rapraeger, A.C. *et al.* (1991) Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* 252, 1705–1708
- Plotnikov, A.N. *et al.* (1999) Structural basis for FGF receptor dimerization and activation. *Cell* 98, 641–650
- Reichsman, F. *et al.* (1996) Glycosaminoglycans can modulate extracellular localization of the Wingless protein and promote signal transduction. *J. Cell Biol.* 135, 819–827
- Aviezer, D. and 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. U. S. A.* 91, 12173–12177
- Zioncheck, T.F. *et al.* (1995) Sulfated oligosaccharides promote hepatocyte growth factor association and govern its mitogenic activity. *J. Biol. Chem.* 270, 16871–16878
- Jackson, S.M. *et al.* (1997) *dally*, a *Drosophila* glypican, controls cellular responses to the TGF- β -related morphogen, Dpp. *Development* 124, 4113–4120
- Lin, X. and Perrimon, N. (1999) Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signalling. *Nature* 400, 281–284
- Bhanot, P. *et al.* (1996) A new member of the *frizzled* family from *Drosophila* functions as a Wingless receptor. *Nature* 382, 225–230
- Häcker, U. *et al.* (1997) The *Drosophila* *sugarless* gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis. *Development* 124, 3565–3573
- Binari, R.C. *et al.* (1997) Genetic evidence that heparin-like glycosaminoglycans are involved in *wingless* signaling. *Development* 124, 2623–2632
- Haery, T.E. *et al.* (1997) Defects in glucuronate biosynthesis disrupt Wingless signaling in *Drosophila*. *Development* 124, 3055–3064
- Lin, X. *et al.* (1999) Heparan sulfate proteoglycans are essential for FGF receptor signaling during *Drosophila* embryonic development. *Development* 126, 3715–3723
- Forsberg, E. *et al.* (1999) Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. *Nature* 400, 773–776
- Humphries, D.E. *et al.* (1999) Heparin is essential for the storage of specific granule proteases in mast cells. *Nature* 400, 769–772
- Bellaïche, Y. *et al.* (1998) Tout-velu is a *Drosophila* homologue of the putative tumour suppressor *EXT-1* and is needed for Hh diffusion. *Nature* 394, 85–88
- McCormick, C. *et al.* (1999) New perspectives on the molecular basis of hereditary bone tumours. *Mol. Med. Today* 5, 481–486
- Toyoda, H. *et al.* (1999) Structural analysis of glycosaminoglycans in *Drosophila* and *C. elegans* and demonstration that *tout-velu*, a *Drosophila* gene related to *EXT* tumor suppressors, affects heparan sulfate *in vivo*. *J. Biol. Chem.* 275, 2269–2275
- The, I. *et al.* (1999) Hedgehog movement is regulated through tout-velu-dependent synthesis of a heparan sulfate proteoglycan. *Mol. Cell* 4, 633–639
- Kooyman, D.L. *et al.* (1995) *In vivo* transfer of GPI-linked complement restriction factors from erythrocytes to the endothelium. *Science* 269, 89–92
- Mahley, R.W. and Ji, Z.S. (1999) Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* 40, 1–16
- Bejsovec, A. and Wieschaus, E. (1995) Signaling activities of the *Drosophila* *wingless* gene are separately mutable and appear to be transduced at the cell surface. *Genetics* 139, 309–320
- Moline, M.M. *et al.* (1999) Directionality of wingless protein transport influences epidermal patterning in the *Drosophila* embryo. *Development* 126, 4375–4384
- Sanson, B. *et al.* (1999) Engrailed and hedgehog make the range of Wingless asymmetric in *Drosophila* embryos. *Cell* 98, 207–216
- Kobayashi, M. *et al.* (1997) Molecular cloning and expression of Chinese hamster ovary cell heparan-sulfate 2-sulfotransferase. *J. Biol. Chem.* 272, 13980–13985
- Bullock, S.L. *et al.* (1998) Renal agenesis in mice homozygous for a gene trap mutation in the gene encoding heparan sulfate 2-sulfotransferase. *Genes Dev.* 12, 1894–1906
- Sen, J. *et al.* (1998) Spatially restricted expression of pipe in the *Drosophila* egg chamber defines embryonic dorsal–ventral polarity. *Cell* 95, 471–481
- Rosenberg, R.D. *et al.* (1997) Heparan sulfate proteoglycans of the cardiovascular system. Specific structures emerge but how is synthesis regulated? *J. Clin. Invest.* 100, 67–75
- Ho, G. *et al.* (1997) Role of heparan sulfate proteoglycans in the uptake and degradation of tissue factor pathway inhibitor-coagulation factor Xa complexes. *J. Biol. Chem.* 272, 16838–16844
- Song, H.H. *et al.* (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
- Padgett, R.W. *et al.* (1998) TGF-beta signaling, Smads, and tumor suppressors. *BioEssays* 20, 382–390
- Ramirez-Weber, F.A. and Kornberg, T.B. (1999) Cytonemes: cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell* 97, 599–607
- Rogalski, T.M. *et al.* (1993) Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparan sulfate proteoglycan. *Genes Dev.* 7, 1471–1484

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