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Review

Developmental roles of heparan sulfate proteoglycans: a comparative review in *Drosophila*, mouse and human

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Contents

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Introduction

New biological functions for glycosaminoglycans (GAG) are emerging from the development of some very new and powerful tools to analyze GAG fine structure and pattern of expression. The fantastic combinational possibilities in the biosynthesis of these different chains within the Golgi offers considerable potential for multiple biological activities of distinct GAG chains. Glycobiologists are now able to correlate particular GAG sequences to very specific biological interactions with different proteins, growth factors (GF), ligands or even viruses. Often considered as "decorating" glycoproteins, GAGs are now participating in the fine-tuning of several fundamental processes such as blood coagulation, tissue assembly, adhesion, motility, virus transduction, cell growth, and morphogenesis. Patterning, development processes and morphogenesis are widely studied worldwide, and a lot of effort has been expended to understand these complex processes. In theory, patterning the *Drosophila* embryo should be simple. Morphogen gradients, that are distributed across the unpatterned tissue, activate a combination of transcription factors that interact to define the boundaries of the adult anatomical structures. This concept of morphogens was proposed 50 years ago, however we are still far from understanding how they are regulated. Tabata (2001) recently reviewed the genetics of morphogen gradients, mentioning the function of heparan sulfate proteoglycans (HSPG) in the modulation of morphogens. Moreover, various very interesting studies have highlighted the critical role of proteoglycans (PG) in these processes, in particular through a direct influence on morphogen gradients during development. These different studies demonstrate that this phenomenon is more active and more important than previously thought. Indeed, mutations in *Drosophila* that affect PGs core proteins, or one of their biosynthetic enzymes, influence the function of several GF pathways that are essential for

Abbreviations used in this paper: BM, basement membrane; BMP, bone morphogenetic protein; CS, chondroitin sulfate; Dally, division abnormally delayed; Dfz2, *Drosophila* frizzled 2; Dpp, decapentaplegic; DS, dermatan sulfate; E, embryonic day; ECM, extracellular matrix; EXT, exostose; FGF, fibroblast growth factor; GAG, glycosaminoglycan; GF, growth factor; GlcA, glucuronic acid; GlcNAc, N-acetyl-glucosamine; $GlcNH_2$, glucosamine; Gpc, glypican; GPI, glycosyl-phosphatidylinositol; Hh, hedgehog; HME, hereditary multiple exostose; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; Ndl, nudel; NDST, N-deacetylase/N-sulfotransferase; Per, perlecan; PG, proteoglycan; Sfl, sulfateless; SGBS, Simpson-Golabi-Behmel syndrome; Sgl, sugarless; Syn, syndecan; Ttv, tout-velu; Wg, wingless.

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tissue assembly, differentiation or development. Similarly, in the mouse, mutations that disrupt PGs or genes that encode GAG biosynthetic enzymes can have profound effects on growth and morphogenesis. Whereas the finding that PGs are critical for patterning was derived primarily from studies in model organisms, it is evident that PGs play a role in human pathogenesis as well. In summary, PG was not a term found in the working lexicon of most developmental biologist and they have long not been considered of central importance to understanding differentiation and morphogenesis. The aim of this review is to introduce the knowledge in this new exciting topic which receives actually tremendous interest, but also to help to apprehend and to understand that GAGs are not only inert components of the extracellular matrix (ECM), but that they stand also directly in the heart of very fundamental functions such as morphogenesis. The notion that GAGs play an active role in morphogenesis and cell differenciation stems from an array of converging observations. Basement membrane PGs were shown to be necessary for differenciation of mammary epithelial cells (Parry *et al.,* 1988), while hyaluronan is thought to facilitate the migration of morphogenetically active cells (Toole 2001). Moreover, chondroitin sulfates are important for the maturation of the fetal lung, and the HSPG syndecan-3 is involved in limb bud development (Smith *et al.,* 1990, Kosher 1998). This review compares actual data about biosynthetic genes and core proteins of different HSPGs from *Drosophila* genetics to mice mutations as well as human pathologies. Basically, the surface of most cells and the extracellular matrix are decorated by heparan sulfate (HS) which are the most ubiquitous cell surface GAGs (Bernfield *et al.,* 1992). They are normally present as HSPG and they have been implicated in processes ranging from mechanical support to functions in adhesion, motility, proliferation, differentiation, morphogenesis, control of cell growth, cell adhesion and in the maintenance of a non-thrombogenic surface on the vascular endothelium (Rosenberg 1989; de Agostini and Rosenberg 1991; Carey 1997; Perrimon and Bernfield 2000). Enormous structural heterogeneity is generated through the specific HS chain modifications occurring during their biosynthesis, as well as from the diverse nature of their core proteins (Carey 1997; Perrimon and Bernfield 2000). Tissue-specific isoforms of the biosynthetic enzymes can produce HS chains with distinct sequences and macroscopic organization (Yanagishita and Hascall 1992). Various ligands and their receptors display selectivity in their binding affinities to distinct HS chains structures (Elenius and Jalkanen 1994; Perrimon and Bernfield 2000). These chains can be attached to one or more specific core proteins, each with distinctive tissue-specific expression pattern and cellular localization (Langford *et al.,* 1998). Finally, HSPGs localization can be shifted form the cell surface toward soluble compartments by selective proteolytic cleavage of the extracellular domains bearing the HS chains to yield soluble HSPGs (Kainulainen *et al.,* 1998). Many HSPGs are thus multifunctional molecules that engage in several different specific interactions at the same time (Prydz and Dalen 2000). Studies of these patterns have defined the motifs required for specific interactions with GFs, cytokines, ECM components, enzymes, protease inhibitors and other proteins. The HS biosynthetic enzymes do not modify all the available sugars in the chains, which results in extensive sequence diversity in the final chain. This structural heterogeneity includes a macroscopic organization in which regions of 10-16 highly modified disaccharides alternate with longer regions of relatively unmodified disaccharides. The overall size of the HS chain can vary from 20 to 150 disaccharides, which adds another level of complexity to HS chains (Sanderson *et al.,* 1994; Perrimon and Bernfield 2000). After synthesis, PGs are transported from the Golgi to their destinations: the ECM, the cell surface or intracellular organelles (Carey 1997; Prydz and Dalen 2000). GAG synthesis is initiated by sequential addition of four monosaccharides (Xyl-Gal-Gal-GlcA) (Fig. 1). From this linker tetrasaccharide, the sugar chains are extended by addition of two alternating hexosamine and uronic acid. In heparin and HS, the aminosugar is *N*-acetyl-glucosamine (GlcNAc) and in chondroitin sulfate (CS) and dermatan sulfate (DS) it is *N*-acetyl-galactosamine. The extent of epimerisation of glucuronic acid (GlcA) to iduronic acid and the sulfation pattern of the disaccharide units distinguish heparin from HS, and DS from CS (Rosenberg 1989; de Agostini and Rosenberg 1991; Salmivirta *et al.,* 1996).

Glycosaminoglycan Biosynthetic Enzymes

UDP-Glucose Dehydrogenase (UDP-Glc-DH Genes)

The first *Drosophila* gene involved in HS biosynthesis showing a developmental phenotype is a homologue to vertebrate UDP-glucose dehydrogenase (UDP-Glc-DH), a critical enzyme for the biosynthesis of UDP-GlcA, which is the universal sugar donor for all HS and CS and the first determinant step for GAG biosynthesis (Fig. 1, Table 1). UDP-Glc-DH has been intensively studied. It was described by four groups at the same time, who named it *sugarless*, s*ka*, s*uppenkasper* and *kiwi*, and it is now referred to as *sugarless* (s*gl*) (Binari *et al.,* 1997; Hacker *et al.,* 1997; Haerry *et al.,* 1997). To date, no study implicating mutations of UDP-Glc-DH in mice has been reported and no known syndrome in humans has been related with this very fundamental HS biosynthetic enzyme.

Different studies carried in *Drosophila* implicated *sgl* for normal patterning of the embryonic epidermis. Embryos lacking both the maternal and the zygotic Sgl activity display a cuticle phenotype that is indistinguishable from *wingless (wg)* nulls. Rescue of the s*gl* phenotype with both UDP-GlcA and HSPG demonstrated that Sgl activity in the embryo is crucial for the production of HS in the ECM. Furthermore, injection of the heparin degrading enzyme, heparinase (but not chondroitin, dermatan or hyaluronic acid degrading enzymes) into wild-type embryos leads to the degradation of heparin-like GAGs and a "*wg*-like" cuticular phenotype (Binari *et al.,* 1997). However, if the general synthesis of PGs is perturbed in *sgl* mutants, then it is expected that other signaling pathways, which use PGs as co-receptor(s), may also be disrupted (Hacker *et al.,* 1997). These different pathways are the fibroblast growth factor (FGF) (mediated by Heartless and Breathless) and Hedgehog (Hh) pathways, but the sensitivity and the specificity of these pathways to *sgl* mutants seems to be less, reflecting perhaps another mechanism, or the involvement of other HS biosynthetic enzymes (Selleck 2000). Moreover, different studies demonstrate that PGs facilitate the interaction of Wg with its receptor *Drosophila frizzled2 (Dfz2)* by regulating the diffusion of secreted Wg (Hacker *et al.,* 1997; Cadigan *et al.,* 1998; Strigini and Cohen 2000). One possible model involving HS in a particular signaling pathway came from different studies involving, on the one hand, mutants for *sgl*, and on the other hand, overexpression of Wg protein (Fig. 2). HSPGs could thus be involved in the reduction of the dimensionality of ligand diffusion from three to two dimensions through specific interaction between the effector and the receptor. In this model, the

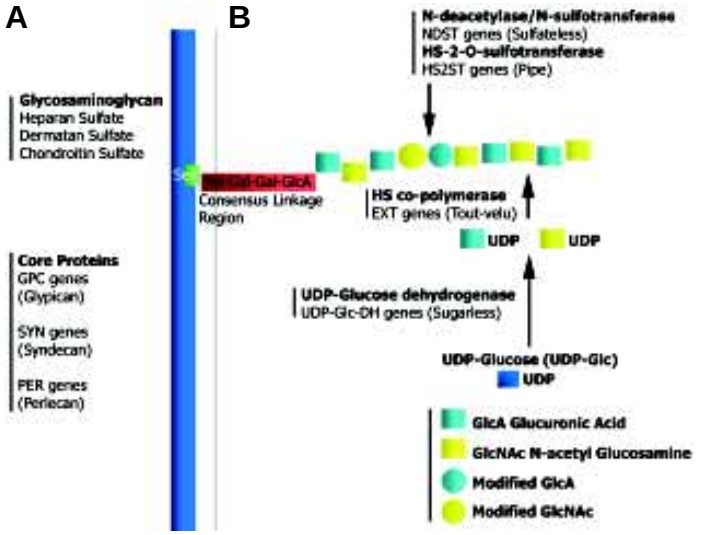

Fig.1. General structure of proteoglycans and biosynthetic enzymes. (A) *Core proteins have been classified into three distinct families. In mammals, the Syndecan family* (SYN genes) *includes four members which are membrane spanning proteins, the Glypican family* (GPC genes) *contains at least six members bound to the cell surface via a Glycosylphosphatidyl-inositol-anchor, basement membrane proteoglycans are secreted, and Perlecan* (PER gene) *is a major component of basement membranes.* **(B)** *Glycosaminoglycans (heparan, dermatan and chondroitin sulfates) are attached to the core protein by a consensus linkage region. Multiple biosynthetic enzymes are involved in the polymerization of these long chains (UDP-Glucose dehydrogenase, HS co-polymerase), which are then highly modified by other sets of highly specialized enzymes (N-deacetylase/N-sulfotransferase, HS-2-O-sulfotransferase). Tissue-specific isoforms of the biosynthetic enzymes and incomplete modification in the Golgi can produce enormous heterogeneity in the glycosaminoglycan chains with distinct sequences and macroscopic organization generating different ligand-binding properties.*

function of PGs is to increase the local concentration of Wg ligand to its receptor (Fig. 2A). In that way, binding of Wg to specific PGs on the cell surface reduces the diffusion of Wg. In the absence of PGs, the concentration of Wg protein presented on the cell surface may be lower than its threshold concentration, and the efficiency of Wg signaling will be reduced (Fig. 2B). Finally, overexpression of ectopic Wg protein can compensate for the loss of PGs on the cell surface and therefore bypass this specific interaction (Fig. 2C) (Binari *et al.,* 1997; Hacker *et al.,* 1997; Cadigan *et al.,* 1998). This *sgl* mutant and the morphogenetic pathway in which this gene is involved (Wg pathway), demonstrated first: the very high specificity of the pathway and second: the important phenotype engaged by the loss of a GAG biosynthetic enzyme.

GlcNAc/GlcA Polymerase (EXT Genes)

The second set of genes discussed which is involved in HS biosynthesis is related to HS co-polymerases (*EXT* gene family), including the glycosyl-transferase that adds alternating GlcNAc and GlcA residues to growing HS chains (Fig. 1, Table 1). These genes have been extensively studied because they were first related to a human pathology known as hereditary multiple exostoses (HME). They have also been cloned in mice, but to date knockout mouse models are not available to study this gene function *in vivo* and to compare mice phenotypes with human pathology (Clines *et al.,* 1997). Nevertheless in *Drosophila*, a related homologue of this gene family has been cloned and named *tout-velu (ttv)* (Lin and Wells 1997; Bellaiche *et al.,* 1998). Mutants for *ttv* showed an interesting developmental phenotype suggesting an unexpected level of specificity of the HSPG involved in a major morphogenetic pathway, the Hh pathway.

The *Drosophila* gene *ttv* has been identified as an enzyme involved in the elongation of HS chains (Fig. 1). Mutations in the *ttv* gene were first isolated based on the inability of Hh molecules to move through a field of cells (Bellaiche *et al.,* 1998). Further studies suggested that the Hh molecule requires HSPGs either to be trapped by receiving cells or to move from cell to cell. Because *ttv* encodes a HS polymerase it is surprising that, in the absence of Ttv activity, only Hh signaling is affected and not Wg and FGF signaling, as observed in *sgl* and *sulfateless* (*sfl*) mutants. This, together with the observation that the overall HS concentration is reduced in *ttv* mutants, suggests that other *Drosophila ext* genes may exist (The *et al.,* 1999). Besides recently, *Dextl3* (*Drosophila Ext*-like3) gene has been characterized with structural determinants of GlcNAc transferase (Kim *et al.,* 2002). A possible explanation of the specificity of Ttv for Hh action is that Hh signaling may be more sensitive to a reduction in HSPG concentration than Wg and FGF signaling. According to the "quantitative" model, Wg and FGF signaling pathways would not be affected in *ttv* embryos because HS synthesized by another *Drosophila* Ext are sufficient to allow these pathways to function. Moreover, Hh-specific HSPGs may exist and *ttv* may be responsible for their production (Stark *et al.,* 1998; Perrimon and Bernfield 2000). Thus, Ext proteins may generate specific GAG chains, perhaps in a complex with certain HS-modifying enzymes. According to this model, the biosynthesis of sufficient amounts of Hh-specific HS would depend upon Ttv and confer specificity of Ttv to Hh signalling (Bellaiche *et al.,* 1998; The *et al.,* 1999). Alternatively, according to a "qualitative" model, the specificity of Ttv to Hh signaling suggests the existence of Hh-

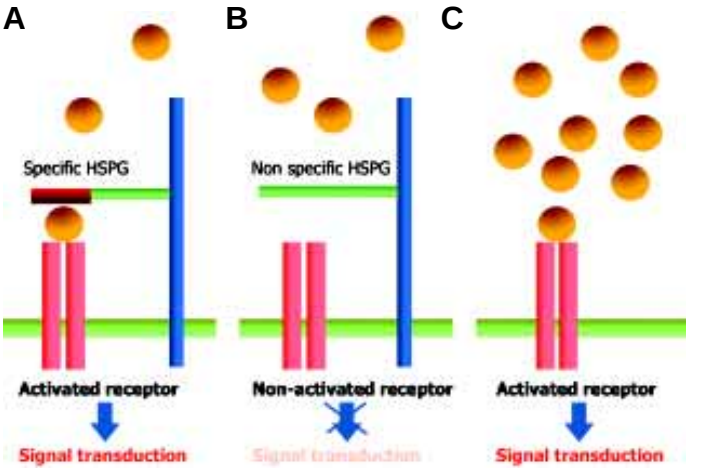

Fig.2. Qualitative and quantitative models of ligand-receptor activation and co-receptor function of specific HSPG. (A) *Qualitative model: specific HS chains act as specific co-receptors during receptor-ligand interaction.* **(B)** *Loss of specific HSPG: the ligand concentration is below the limit required for receptor activation.* **(C)** *Quantitative model: receptor activation by HSPG co-receptor can be bypassed by ectopic overexpression of the ligand.*

specific HS and the core protein sequence of the HSPG would be critical for defining its specificity in terms of HS chains, cell-type localization, pattern of expression and concentration (Toyoda *et al.,* 2000). However, the observation that membrane-targeted Hh requires HSPGs suggests that there is a transport mechanism for Hh allowing it to move from cell to cell. It is possible that HSPGs are required to target Hh to a specific subcellular compartment. The transport of Hh might involve ''lipid rafts,'' which are microdomains in the plasma membrane rich in sphingolipids, cholesterol, and glycosyl-phosphatidylinositol (GPI)-anchored proteins. Interestingly, Hh has been reported to localize in the detergent-insoluble fractions of cell extracts, characteristic for proteins found in "lipid rafts". Perhaps a GPI-anchored HSPG, such as a glypican (Gpc) molecule, is required to localize Hh in these rafts. Indeed, the transfer of GPI-anchored proteins between cells has been observed, and Hh might be transferred from cell to cell in this way (The *et al.,* 1999).

A murine homologue of the human *EXT2* gene has been cloned recently and isolated by screening a mouse brain cDNA library. An open reading frame of ~2.2 Kb was detected bearing ~90% homology in the coding region with the human *EXT*2 gene. The predicted mouse Ext2 protein is 718 amino acids in length and is 94.7% identical with the human *EXT2* gene product over the entire length of both proteins. When comparing only the last one-third of the human and mouse proteins the identity increases to 99.5% (Clines *et al.,* 1997; Stickens and Evans 1997; Lin and Wells 1997; Stickens *et al.,* 2000). However, a knockout mouse for this *ext2* gene is not available and there is no report of a known phenotype in mice close to HME.

Human *EXT* genes were related in humans to the pathology known as HME. HME is an autosomal dominant disorder characterized by the formation of cartilage-capped tumors or exostoses that develop from the growth plate of endochondral bone. This condition can lead to skeletal abnormalities, short stature and malignant transformation of exostoses to chondrosarcomas or osteosarcomas (McCormick *et al.,* 1998). Linkage analyses have identified three different genes for HME: *EXT1, EXT2* and *EXT3*. Recently, several genes have been identified that share significant sequence homology with the *EXT* genes and named *EXTL* (-like) or *EXTL1, EXTL2,* and *EXTL3* (Lind *et al.,* 1998). *EXTL* genes map to diverse chromosome locations and have not been associated with HME. Most HME cases have been attributed to missense or frameshift mutations in either *EXT1* or *EXT2* (Wuyts and Van Hul 2000; McCormick *et al.,* 2000). The majority of these mutations cause loss of function, which is consistent with the presumed tumor-suppressor function of the *EXT* genes (Wuyts and Van Hul 2000). Different studies have demonstrated that EXT1 is a predominantly ER-localized glycoprotein whose expression enhances the synthesis of cell surface HS (McCormick *et al.,* 1998), and EXT1 and EXT2 harbor both GlcA transferase and GlcNAc transferase activities that catalyze the polymerization of HS (McCormick *et al.,* 1998; McCormick *et al.,* 2000). The identification of EXT1 as an ER-resident protein suggests that it exerts its effects on the cell-surface HS expression by modifying PGs traversing the secretory organelles *en route* to the cell surface. Moreover, several ER-resident membrane proteins including glycosyltransferases involved in carbohydrate metabolism share the putative type II configuration proposed for EXT1 (McCormick *et al.,* 1998). The precise defect in HS biosynthesis in HME is unclear. Complete elimination of HS-polymerase activities would result in total absence of GAG. Partial loss of activity might lead to the formation of fewer and/or shorter chains. However, it seems likely that the polymerase interacts with one or more of the enzymes that catalyze the various modification reactions through which the nonsulfated

TABLE 1

HSPG GENES INVOLVED IN DEVELOPMENT

	Drosophila		**Mouse**		**Human**	
	Gene (Abbreviation, number of genes)	Pathway or Pathology	Gene (Abbreviation, number of genes)	Pathway or Pathology	Gene (Abbreviation, number of genes)	Pathway or Pathology
Glycosaminoglycan Biosynthetic Enzymes						
UDP-Glucose dehydrogenase (*Biosynthesis of UDP-Glc*)	*Sugarless* (*Sgl*, 1)	*Wingless* Pathway (Fig. 2)	UDP-Glc-DH (Ugdh, 1)	No Ugdh deficient mice available	UDP-Glc-DH (UGDH, 1)	No human related mutations
GlcNAc/GlcA polymerase (*Addition of alterning GlcNAc and GlcA residues to growing HS chains*)	*Tout-velu* (*ttv*) and *Dextl3* (2)	*Hedgehog* Pathway	Ext-1/-2 (~95-99% homology with human EXT genes, 2)	No Ext deficient mice available	EXT-1/-2/-3 (3) EXTL-1/-2/-3 (3)	HME (Hereditary Multiples Exostoses)
N-deacetylase/ N-sulfotransferase (*Modifications ofthe different GAG chains*)	*Sulfateless* (*sfl,* 1)	*Wingless* and *FGF receptor* pathway	Ndst-1/-2/-3 (3)	Ndst-1 deficient mice: neonatal lethality. Ndst-2 deficient mice: viable, but alterate heparin biosynthesis	NDST-1/-2 (2)	No human related mutations
HS-2-O-sulfotransferase (*Final modifications. Addition of specific sulfate groups*)	*Pipe* (1)	Activation of *Spätzle*. Defects in dorso-ventral polarity	Hs2st, Hs3st, Hs6st (multiples subfamilies members)	Hs2St deficient mice: neonatal lethality	HS2ST, HS3ST, HS6ST (multiples subfamilies members)	No human related pathologies
Proteoglycan Core Proteins						
Syndecan (*Transmembrane core proteins*)	*Dsyn* (1)	No detailed study	Syn-1/-2/-3/-4 (4)	No highly relevant phenotypes	SYN-1/-2/-3/-4 (4)	No human related pathologies
Glypican (*GPI-anchor core proteins*)	*Dally, Dally-like* (*dly*) (2)	*TGF-_* (Dpp) and *Wingless* pathways	Gpc-1/-2/-3/-4/-5/-6 (6)	Gpc-3 deficient mice: SGBS like syndrome	GPC-1/-2/-3/-4/-5/-6 (6)	SGBS (Simpson-Golabi-Behmel syndrome, GPC-3 mutations)
Perlecan (*Secreted in BM*)	*Per* (1)	No detailed study	Per (1)	Per deficient mice: high mortality and cartilage malformations	PER (1)	No human related pathologies

precursor polysaccharide is converted into the mature, sulfated product (Lind *et al.,* 1998). A mutation in the appropriate EXT protein might affect such interaction as well as the initial polymerization reaction itself, with presently unpredictable effects on the structure of the final product. Indeed many different types of tumors are associated with distinct changes in GAG structure. Although the mechanisms behind these changes are generally unknown, the alterations may be expected to affect functional interactions with a variety of proteins that are potentially involved in neoplastic transformation.

N-Deacetylase/N-Sulfotransferase (NDST Genes)

Once HS chains are elongated, modifications of the different GAG chains are catalyzed by various enzymes while specific GlcNAc N-deacetylation/N-sulfation are catalyzed by a single-chain polypeptide GlcNAc N-deacetylase/N-sulfotransferase (NDST) (Fig. 1, Table 1). Three different isozymes with high sequence similarity have been identified in mice and named Ndst-1, Ndst-2, and Ndst-3 (Kusche-Gullberg *et al.,* 1998; Aikawa and Esko 1999), but only *ndst-1* and *ndst-2* deficient mice are currently available (Fan *et al.,* 2000; Ringvall *et al.,* 2000). Further informations about *ndst* gene function came from *Drosophila* studies were a homologue, named *sulfateless (sfl)* (Lin *et al.,* 1999), was recently implicated in HS biosynthesis, showing important phenotypes, affecting both Wg and FGF receptor pathways. However no known human pathology is related to this gene family.

The biological functions of FGFs in *Drosophila* and vertebrates, include the regulation of cell proliferation, differentiation, survival, motility and tissue patterning. Different studies demonstrate that FGFs relay their signals through high affinity transmembrane protein tyrosine kinase receptors (Pellegrini *et al.,* 2000). In addition to its high affinity receptor, biochemical studies indicate that heparin/HSPGs act as low affinity FGF co-receptors that facilitate FGF signal transduction (Ornitz 2000; Pellegrini *et al.,* 2000). While the precise mechanism by which HS participate in FGF receptor activation remains unclear, different studies suggest that HS may stabilize or induce the formation of FGF dimers or a ternary complex composed of ligand plus high and low affinity receptors (Lin *et al.,* 1999; Ornitz 2000). By affecting the FGF receptor signaling pathway, *sfl* mutations provide an *in vivo* model for examining the involvement of these HS in FGF receptor signaling. One model, similar to the one developed about *sgl* function, suggests that the binding of FGF to abundant but low affinity HS on the cell surface limits the free diffusion of the ligand, thereby increasing its local concentration and the probability that it will interact with less abundant high affinity signaling receptors. A variation of this model proposes that FGF monomers are capable of self-associating, a process that is stabilized by HS. This hypothesis predicts that elevated levels of FGF should compensate at least in part for a loss of dimer stabilization mediated by HS.

In the mouse, *ndst* genes have been connected to lung and mast cells function. *Ndst-1* deficient mice developed immaturity of type II pneumocytes, and as a consequence died shortly after birth. The development of other organs, however, showed no pathological defects, suggesting that Ndst-1 played a non-redundant role in the differentiation of type II pneumocytes. Two models may explain this phenotype. Ndst-1 may be required for the modification of subtypes of HS that are necessary for the differentiation of type II pneumocytes. Alternatively, reduced sulfation in *ndst-1* deficient mice may quantitatively reduce the amount of HS that is available for multiple signaling processes during lung development (Fan *et al.,* 2000; Ringvall *et all.* 2000). The compact structure of the lungs from *ndst-1* deficient embryos indicates that they contain an elevated number of cells. Furthermore, differentiation of these cells seems to be delayed since extracellular deposits of surfactant are less frequently observed in *ndst-1* deficient embryos compared to their wild-types littermates. The condition of the *ndst-1* deficient newborns therefore resembles the respiratory distress syndrome seen in premature infants (Fan *et al.,* 2000; Ringvall *et al.,* 2000). The development of the respiratory system requires epithelial-mesenchymal interaction, mediated by specific FGF ligand-receptor signaling, together with additional modulation by other peptide GFs, including epidermal GF, platelet-derived GF-A and transforming GF, as well as by ECM components. HSPGs could function as co-receptors for all these GFs through several mechanisms, including facilitating or stabilizing ligand-ligand dimerization and subsequent ligand-receptor interaction as well as altering their effective concentration (Pellegrini *et al.,* 2000). In summary, Ndst-1 may be required for the modification of a lung-specific HS that is necessary for the activity of a specific signaling molecule (Fan *et al.,* 2000).

Connective-tissue-type mast cells express little or no Ndst-1, but contain large amounts of the transcript encoding Ndst-2. Female and male *ndst-2* deficient mice were viable, fertile and showed no obvious pathological phenotype at 20 months. In addition, histological examination of different organs revealed no apparent defects (Forsberg *et al.,* 1999). However, different experiments showed that mast cells of *ndst-2* deficient mice lack sulfated heparin demonstrating that this enzyme is crucial for heparin biosynthesis. In contrast, no differences is observed in the N-sulfation pattern of HS, isolated from control and *ndst-2*knockout mice, so Ndst-1 and -3 might compensate for the lack of Ndst-2 in the biosynthesis of HS (Forsberg *et al.,* 1999; Humphries *et al.,* 1999). These *ndst-2* deficient mice have fewer connective-tissue-type mast cells. These cells have an altered morphology and contain severely reduced amounts of histamine and mast-cell proteases (Forsberg *et al.,* 1999). These results support the hypothesis that individual members of the Ndst protein family may be required for the modifications of HS subtypes that are required for specific cellular functions during development. Considering Ndst-1 widespread expression, it is interesting that most organs appear to develop normally in the mutant. Although it is possible that Ndst-1 is only required in the developing lung, it is more likely that in other tissues and organs the function of Ndst-1 can be compensated for by other members of the Ndst family. Examination of mice null for both *ndst-1* and other *ndst* family members will allow addressing these issues (Wodarz and Nusse 1998; Ringvall *et al.,* 2000).

In humans, FGF self-association in the absence of HS has been observed by different techniques at physiological ligand concentrations (DiGabriele *et al.,* 1998; Filla *et al.,* 1998). In addition, elevated FGF-2 levels exert biological effects on cultured cells that fail to synthesize HS (Lin *et al.,* 1999). Thus, under normal conditions, the concentration of FGFs seems to be limiting, and necessitates the presence of HS to augment or stabilize ligand dimerization and subsequent FGF receptor activation. To date no human pathology as been related to the NDST gene family.

HS-2-O-Sulfotransferase (HS2ST Genes)

The large family of HS-O-sulfotransferases is involved in final modifications of the HS chains by catalyzing the transfer of specific sulfate groups in different positions within long HS chains (Fig. 1, Table 1). Recently, interesting insights came from genetic studies of the *hs2st* homologue in *Drosophila* (*pipe*) that implies it in different developmental patterning. Different members of this important gene family have been intensively studied in mice (*hs2st*, *hs3st*, *hs6st*), but only *hs2st* was found to be involved in developmental processes in mice and no human pathology has been associated to this gene family.

The *Drosophila pipe* gene encodes an Hs2st, and its mutation produces defects in the establishment of dorso-ventral polarity in the embryo (Sen *et al.,* 1998). The phenotype of *Pipe* mutants is less severe than that of *sfl* and *sgl* mutants consistent with the fact that Hs2st exerts limited modifications on HS chains. *Pipe* is expressed in the ventral part of the egg chamber and has been proposed to activate Toll ligand Spätzle. The effect of *pipe* on Toll signaling is probably indirect. The role of the PG involved might be either to activate or to assemble a protease complex which activates Spätzle or assembles Spätzle to the ventral side of the egg chamber (Perrimon and Bernfield 2000). However, mutations that affect follicular polarity conversely alter the spatial pattern of *pipe* expression (Sen *et al.,* 1998; Morata and Sanchez-Herrero 1999). It appears that Pipe acts to mediate the sulfation at the 2-O position of hexuronic acid moieties of HS/heparin or DS GAGs attached to a PG required for dorso-ventral pattern formation. Currently, *nudel (ndl)* and *wind* are the only two identified dorsal group genes, in addition to *pipe*, that are expressed in the somatic follicle cells. *Ndl* encodes a large protein that carries a potential N-terminal signal sequence for secretion, several repeated motifs reminiscent of those found in ECM proteins, and two separate regions that exhibit homology to the catalytic domains of serine proteases. The presence in Ndl of three potential sites for GAG addition suggests the possibility that Ndl may be covalently linked to the GAGs that form substrates for Pipe enzymatic activity (Sen *et al.,* 1998; Nilson and Schupbach 1998). Although PGs and their associated GAGs have been linked to a number of specific functions and mechanisms, a common theme that has emerged from these studies is the modulation of protein/protein or protein/ECM interactions by GAGs, particularly sulfated GAGs. The serine proteolytic cascade that leads to the formation of the Toll ligand has been compared to the proteolytic process required for blood clotting. By analogy, it could be postulated then, that sulfation of a particular GAG by Pipe sets in motion the protease cascade leading to the formation of active Spätzle (Sen *et al.,* 1998).

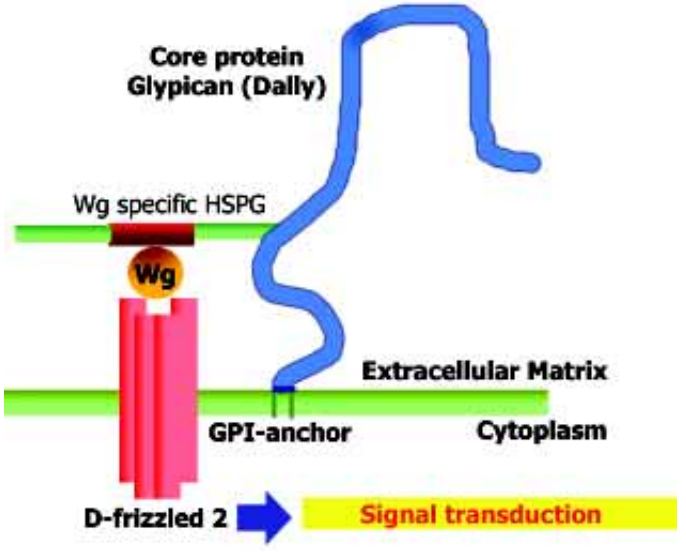

Fig.3. Co-receptor function of Dally for the Wg-transducing receptor D-frizzled-2. *Specific HS chains attached close to the cell surface of a GPI-anchored core protein (GPC) enhance the probability of interaction of the specific ligand (Wg) with its receptor (DFrizzled-2).*

In the mouse, the developmental role of the *hs2st* mouse gene has been characterized by gene trap mutation (Bullock *et al.,* 1998). Mice homozygous for this mutation die in the neonatal period and invariably exhibit bilateral renal agenesis and defects of the eye and skeleton. Cleft palate and polydactyly also occur with incomplete penetrance. The Hs2st enzymatic activity catalyzes 2-O-sulfation of L-iduronic acid in HS but is thought not to transfer sulfate to other GAG substrates, such as DS, and CS (Kobayashi *et al.,* 1996). Deficiency in 2-O-sulfation may be accompanied by a secondary increase in N-sulfated glucosamine residues in HS and altered degradation products and some of these modifications may have additional biological significance. Several biochemical and *in vitro* studies have shown that 2-O-sulfation in particular is essential if HS is to interact with certain GFs such as FGF-2. Therefore, modulating the levels of 2-O-sulfation may confer an additional level of specificity on some, but not all, GF-receptor interactions. HSPG structure varies extensively between tissues during development (Bernfield *et al.,* 1992), and one means of achieving this diversity is illustrated by the regulated expression of *hs2st* (Bullock *et al.,* 1998). The phenotype observed in *hs2st* deficient mice, however, indicates an early requirement for 2-O-sulfation in the initial condensation of the metanephric mesenchyme (Bullock *et al.,* 1998). Therefore, impaired FGF-2 signaling could explain many of the defects observed in *hs2st* mutants. Mice homozygous for a null mutation in *fgf2*, however, show no defects in kidney morphogenesis or skeletogenesis demonstrating that simple failure of FGF-2 signaling is not an adequate explanation for the *hs2st* mutant phenotype. Because other members of the FGF family are expressed in the developing kidney, the phenotype observed in *hs2st* deficient embryos may be attributable to perturbation of more than one FGF-signaling pathway (Bullock *et al.,* 1998). HSPGs can interact with a variety of ECM components via their HS chains (Bernfield *et al.,* 1992). Clearly, metanephric mesenchymal condensation requires alterations in adhesive properties and defective sulfation might prevent such changes (Bullock *et al.,* 1998). However, it is intriguing, considering the gene's widespread early expression, that *hs2st* is not essential for early embryogenesis and that certain tissues that express *hs2st* later in development, such as the lungs and teeth, appear to develop normally in the mutants. Although it is possible that 2-O-sulfation is only required in the developing kidney, skeleton, and eye it is more likely that in other tissues and organs Hs2st function may be compensated for by other sulfotransferases. Therefore, the phenotype of *hs2st* deficient embryos may reflect those sites where Hs2st is the only 2-O-sulfation enzyme expressed (Bullock *et al.,* 1998).

Several documented human disorders involve abnormalities of the kidney, eye, skeleton, and palate, but no human genetic disorders with defects reminiscent of the murine phenotype have been described to date.

The different studies involving HS biosynthetic enzymes demonstrated very interesting clues and redundant mechanisms in which HS act often in the same way. Heparan sulfates are able to concentrate specific morphogens close to their relative receptors or close to their specific target cells. On the other hand, through this tremendously high complexity, defined HS fine sequences are shown to be involved in very specific morphogenetic pathways. This reading suggests that actual studies have revealed only the tip of the iceberg, and that further effort will be needed to correlate directly specific HS sequences with particular ligand interactions. Several different HS biosynthetic enzymes are now directly linked to specific biologically active HS that are involved in specific morphogenetic pathways. For example, Hh has long been considered to simply diffuse along a basic gradient. It is now clear that this phenoma is active and mediated by specific HS glycoforms that are instrumental not only in the enhancement of the ligand concentration close to the receptor but also as active partners in the transport of this morphogen through long distances toward its target cells. What have we learned from these different studies? On the one hand, there is a canonical biosynthetic pathway for HS and other GAGs. Mecanisms of biosynthesis in the Golgi is apparently highly conserved through evolution, generating a tremendous diversity in the sequences of GAGs. On the other hand, particular mutants of HS biosynthetic enzymes have demonstrated a very high specificity for some of the morphogenetic pathways involved, together with wider phenotypes. This may be explained by the presence of compensatory effects of the different members of the same gene family in mice and humans compared to *Drosophila* where a single member of each gene family exist.

Proteoglycan Core Proteins

The involvement of HSPG in developmental pathways has also been examined at the level of possible functions of the different HSPG core proteins present at the cell surface (Yanagishita and Hascall, 1992). Several HSPG core proteins have been cloned and they have been classified in three distinct families. In mammals, the Syndecan (Syn) family counts four members which are membrane spanning proteins, the Glypican (Gpc) family contains at least six members which are bound to the cell surface via a GPI-anchor (Lories *et al.,* 1989; David *et al.,* 1990; Veugelers *et al.,* 1999), and finally basement membrane (BM) PGs are secreted and Perlecan (Per) is a major component of these structures. Although some human pathologies are related to core protein mutations such as the well-defined syndrome called Simpson-Golabi-Behmel (SGBS), much less is known about the phenotypes associated with mutations in these different core proteins. In *Drosophila*, mutations in both *syn (Dsyn)* and one of the *gpc* gene, *division abnormally delayed* (*dally*), have been studied. However, detailed phenotypic analysis are only available for *dally* (Nakato *et al.,* 1995). In mice, targeted disruptions of *gpc* genes yield either no apparent abnormalities or a phenotype similar to the SGBS (Gonzalez *et al.,* 1998). Targeted disruption of the *syn1* gene, the main Syndecan gene expressed by epithelia and plasma cells, yields apparently well developed and normal fertile mice despite the early and intense expression of *syn1* during embryogenesis (Bernfield *et al.,* 1992). *Syn4* deficient mice show also no or little phenotype associated with the impairment of focal adhesion formation, but only under restricted conditions. In contrast, *per* deficient mice show a very severe phenotype with high level of lethality and many abnormalities (Costell *et al.,* 1999; Arikawa-Hirasawa *et al.,* 1999).

Glypican Mutations (GPC Genes)

All of the structural features of this protein family are shared by the product of the *Dally* gene in *Drosophila* (Table 1). The Dally protein is formed by a signal sequence, followed by a ~50-kDa N-terminal domain with a characteristic pattern of 14 highly conserved cysteine residues, which form a presumably extended region containing 2 or 3 Ser-Gly GAG attachment sequences near the plasma membrane. Moreover, a C-terminal sequence is involved in the formation of a GPI-linkage to the plasma membrane. However, sequence homologies and exons organization of the six Gpc genes suggest that they form three subfamilies, Gpc1 and 2, Gpc3 and 5, and Gpc4 and 6 (Bernfield *et al.,* 1999).

Gpc from *Drosophila* (*dally*) was identified in a genetic screen for mutations affecting cell division patterning during the visual system development. Mutations in *dally* are pleiotropic, affecting antennal, eye, genitalia and wing morphogenesis. The *dally* cDNA shows homology to Gpc in the protein coding sequence and the degree of sequence homology of *dally* to each member of this vertebrate family is similar to their homology to one another, supporting the assignment of *dally* to this gene family (Nakato *et al.,* 1995). Treatment of cells expressing Dally with phosphatidylinositol-phospholipase-C quantitatively releases it from the cell surface, demonstrating its GPI-linkage to the outer leaflet of the plasma membrane (Tsuda *et al.,* 1999). Dally bears HS chains on two criteria: it is sensitive to a chemical cleavage reaction specific for N-sulfated Glc residues found in HS and also to heparin lyase-II, an enzyme that selectively cleaves HS chains. Like the vertebrate glypicans, Dally does not bear CS because it is insensitive to chondroitinase ABC (Nakato *et al.,* 1995; Jackson *et al.,* 1997; Tsuda *et al.,* 1999). *Dally* seems to be involved in many different GF pathways acting as co-receptor implicating, on the one hand TGF-β and on the other hand Wg. However, the loss of Dally activity, in both the embryo and imaginal discs, generates phenotypes reminiscent of loss of Wg activity. Genetic experiments are consistent with a model in which Dally acts as a co-receptor for the Wg-transducing receptor encoded by the seven-transmembrane protein Dfz2 (Fig. 3) (Haerry *et al.,* 1997; Tsuda *et al.,* 1999; Lin and Perrimon 1999). Interestingly, *dally* expression is developmentally regulated and is co-expressed with *dfz2* in the embryo (Cadigan *et al.,* 1998). The level of the HSPG co-receptor has to be tightly regulated for proper Wg signaling. Recently, a *Drosophila* member of the TGF-β/Bone morphogenetic protein (BMP) superfamily, Decapentaplegic (Dpp), has been shown to have morphogen activity. Gradients of Dpp can affect patterns of gene expression and are critical for establishing the anterior-posterior axis of the wing, as well as the proximal-distal axis of the leg (Jackson *et al.,* 1997). One potential site for regulating Dpp signaling is at the cell surface, where Dpp binds to its signaling receptor, a heteromer of both type I and type II serine/threonine kinases encoded by the *punt, saxophone* and *thickvein* genes (Nakato *et al.,* 1995; Jackson *et al.,* 1997). *Dally* mutants show segment-polarity defects in the larval cuticle, and genetic interactions with Wg and Dfz2. Ectopic *dally* can rescue W*g* partial loss-of-function mutants and can potentiate Wg signaling, without changing the levels of Wg. These findings support a model in which PGs enhance the activity of

different GFs at the cell surface by promoting the assembly of signaling complexes (Tsuda *et al.,* 1999). The ability of Dally to enhance Dpp as well as Wg signaling raises a question about the specificity of PGs in modulating GFs or signaling. During genitalia development in *Drosophila*, Dally affects Dpp but not Wg signaling, whereas in the embryonic epidermis, Dally influences Wg but not Dpp-directed patterning. While the molecular mechanism of these differential activities of Dally is not known, it is apparent that a PG can serve as a tissue-specific modulator of a GF signaling pathway (Selleck 2000). Genetic analyses of *dpp-dally* interactions showed that *dally* does not serve the same role in all tissues. *Dally* mutant phenotypes are enhanced by decreasing *dpp* function in the eye, antenna and genitalia, but are suppressed by *dpp* mutations in the wing. Increasing *dpp* function likewise rescues eye, antenna and genitalia defects, while enhancing wing abnormalities. These findings show that the interaction between Dpp and Dally in the wing cannot be accounted for by the trivial explanation that existing *dally* alleles affect the wing to a lesser degree (Jackson *et al.,* 1997). Another *Drosophila* glypican gene, *dally-like (dly)* has been recently characterized and has been shown to be involved in Wg signaling. Dly co-localizes with Wg and inhibition of the *dly* gene activity implicates a function for Dly in Wg reception (Baeg *et al.,* 2001). These results confirm that different glypican-like core proteins are involved in the organization of the extracellular distribution of Wg.

Different core proteins knockout mice have recently been described. They are available for *gpc3*, *syn1* and *syn4* and recently for *per*, but if some shows relatively important phenotypes, such as *gpc3* knockout mice, other core proteins expressed during embryogenesis, such as Syn1 and 4, are not required for normal developmental processes. *Gpc3* deficient mice exhibit several of the clinical features of SGBS patients, including developmental overgrowth, perinatal death, cystic and dysplastic kidneys, and abnormal lung development. Other abnormalities observed in some SGBS patients, such as hernias, heart defects, polydactyly, and vertebral and rib malformations, are not present in the mutant mice. Conversely, *gpc3* deficient mice display mandibular hypoplasia and an imperforate vagina, two clinical features that have not been described in SGBS patients. It is interesting to note that an imperforate vagina could be the result of deficient apoptosis of the vaginal epithelial cells during sexual maturation (Gonzalez *et al.,* 1998; Cano-Gauci *et al.,* 1999). The similarities between SGBS and Beckwith-Wiedemann syndrome have suggested the hypothesis that Gpc3 regulates IGF-2 activity. Furthermore, the IGF-2 receptor-deficient mice display a degree of developmental overgrowth similar to the *gpc3* knockout mice. However, in contrast to the IGF-2 receptor knockout, the *gpc3* deficient mice do not show any increase in circulating or local expression of IGF-2 (Cano-Gauci *et al.,* 1999).

SGBS in humans (*GPC3* gene disruption), is an X-linked disorder characterized by phenotypes closely related to those observed for *gpc3* deficient mice (Cano-Gauci *et al.,* 1999). In addition, patients are at high risk for Wilm's tumor and neuroblastoma. Death during infancy is very frequent, usually as a result of pneumonia. It is also important to note that *GPC3* is highly expressed during development in the tissues that are affected in SGBS patients (Pellegrini *et al.,* 1998; Cano-Gauci *et al.,* 1999; Selleck 1999). The phenotypes of SGBS patients raise the question of how a cell-surface PGs affects cellular physiology. As seen for mutation in HS biosynthetic enzyme pathway, HSPG may serve as GF co-receptors, which affect the delivery or assembly of ligands 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 (Pellegrini *et al.,* 2000). In addition to this model, there is evidence that an HS-binding domain on FGF receptor itself is also required for signaling, suggesting that HSPGs promote FGF and FGF receptor association by bringing them into close localization (Selleck 1999). These models provide a basis for the understanding of how the loss of *GPC3* might cause the overgrowth, patterning defects, and tumor susceptibility that characterize SGBS. The evidence from *Drosophila* and from cell-culture systems suggests that many different GFs are likely to be affected by the loss of *GPC3*. Clearly, Wnt and TGF/BMP signaling could be compromised in SGBS patients. TGF is a well-known inhibitor of cell-cycle progression, and the loss of *GPC3* could compromise TGF-mediated control of cell division. Disruptions in TGF-signaling components, and in TGF- receptors are associated with tumor progression, suggesting that the neoplasia associated with SBGS could reflect a loss of normal TGF activity (Selleck 1999). 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 summary, the many SGBS phenotypes found probably reflect the variety of signaling events that are affected by loss of this cell-surface PG. As a complement, human K-glypican/GPC4 (*GPC4)* also maps to the same chromosome as *GPC3*. The GPC4 protein is encoded by nine exons. These two genes are arranged in a tandem array, the 5' end of *GPC4* flanking the 3'end of *GPC3*. Unlike the *GPC3* mRNA, the *GPC4* mRNA is nearly ubiquitous. Analysis of DNA samples from eight patients with diagnosis of SGBS identified one individual with a deletion that involves the entire *GPC4* gene and the last two exons of *GPC3*. The tight clustering of *GPC3* and *GPC4*, with deletions that occasionally affect both genes, may be relevant for explaining the variability of the SGBS phenotype (Veugelers *et al.,* 1998).

Syndecan Mutations (SYN Genes)

The initial cell surface HSPG to be characterized was termed Syndecan, from the Greek, syndein, to bind together (Saunders *et al.,* 1989; Vihinen *et al.,* 1993; Kim *et al.,* 1994; Carey *et al.,* 1997). Four distinct membranes spanning core proteins were cloned and these molecules constitute a defined family of transmembrane HSPGs that appears to regulate cellular responses to the microenvironment (Lories *et al.,* 1989; Bernfield *et al.,* 1992; Kainulainen *et al.,* 1998; Perrimon and Bernfield 2000) (Table 1). Their chromosomal locations, exons organization, and sequence relationships with the single *Drosophila* Syn (*Dsyn*) suggest that the gene family arose by gene duplication and divergent evolution from a single ancestral gene, and that Syn1 and 3, and Syn2 and 4, represent subfamilies. Each gene product is a single type I membrane-spanning protein with an apparently extended extracellular domain of varying size that contains covalently attached HS chains distal from the plasma membrane (Bernfield *et al.,* 1992; Bernfield *et al.,* 1999). In *Drosophila*, the single *Dsyn* may replace the functions of the entire complement of vertebrate syndecans (Spring *et al.,* 1994). Unfortunately, there is no detailed study involving mutations in *Dsyn*.

However, *syn1* deficient mice, the sole cell surface HSPG known to be expressed during early mouse development, show no defects in morphogenesis. These mice are viable, develop normally, are fertile, and phenotypically indistinguishable on either BALB/c or C57BL/6 backgrounds from wild-type littermates by histology of tissues and cytology and chemistry of blood. This normal development, presumably enabled by compensation by other HSPG structural genes, suggests that mutations of a single of these genes in mice may not elucidate their developmental roles (Bernfield *et al.,* 1999). To confirm this hypothesis, *syn4* knockout mice showed no macroscopic abnormalities and reproduced normally (Ishiguro *et al.,* 2000), although Syn4 is expressed, at low levels, in mice ovaries (Ishiguro *et al.,* 1999). Syn1 is the earliest cell surface HSPG to appear during mouse embryogenesis (Bernfield *et al.,* 1993). Its subsequent expression coincides with the cells fated to become the embryo proper, and it is tightly regulated during the epithelial-mesenchymal interactions of organogenesis, and predominates in adult epithelia and plasma cells. Thus far, the sole abnormality detected in the *syn1* deficient mouse is defective repair of skin and corneal wounds. At both sites, lack of Syn1 prevents keratinocytes migrating into the wound from restoring their stable cell-cell and cell-matrix contacts at a normal rate. The result is a marked delay in reconstitution of the normal epithelium. Thus, the essential function of Syn1 appears to be for normal epithelial behavior during wound repair (Bernfield *et al.,* 1993; Langford *et al.,* 1998; *Bernfield et al.,* 1999).

Perlecan Mutations (PER Gene)

Per was first isolated from the murine Engelbreth-Holm-Swarm tumor cell line (Hassell *et al.,* 1980). The three GAG side chains are located at the N-terminal end of the molecule, which also contains numerous globular regions interlinked by rod-like segments (Noonan *et al.,* 1991). This multi-domain PG is one of the most complex gene products because of its enormous dimensions and number of post-translational modifications (Iozzo 1998) (Table 1).

During mouse development, Per is first expressed in two-cell embryos. Then, its expression increases on the external surface of trophectodermal cells of blastocysts. During postimplantation development, Per is detected in blood vessel walls and the developing heart and skeletal muscle. A significant increase in *per* expression occurs during organogenesis of the kidney, lung, liver, spleen, gastrointestinal tract, and cartilage. In contrast to the well-characterized expression pattern, only a few functional properties of Per are known. But apart to bind to ECM component, one property that Per shares with several other PGs is its ability to bind and store GFs (Costell *et al.,* 1999). To test directly the function of Per, the use of mice lacking *per* expression demonstrate that Per is essential for maintaining the integrity of cartilage BM, of contracting cardiac muscle cells and of expanding brain tissue. Despite the ability of Per to interact with several BM components, such as adhesion molecules involved in BM assembly, and with GFs, all BMs form in the absence of Per and appear morphologically normal. Between embryonic day E10.5 and E12.5, about 70 to 80% of *per* deficient embryos died. When live homozygotes were dissected, their hearts were of normal size and shaped with a well-developed myocardium. However, the ventricles were suffused with blood leakage into the pericardial cavity. Different findings suggest that while the loss of *per* is not required for the assembly of BMs on early contracting cardiomyocytes, it is crucial for the maintenance of their structural and functional integrity when subjected to mechanical stress (Costell *et al.,* 1999; Arikawa-Hirasawa *et al.,* 1999). All *per* deficient embryos that survive the first crisis develop brain anomalies. The brain defects were first visible at E10. At this stage, two well-developed BMs separate the ectoderm and the brain tissue from the mesenchyme. The disruption of both BMs caused aberrant fusion of brain tissue with the overlying ectoderm. This type of defect exposed brain tissue to amniotic fluid, which led to the destruction of the tissue and the development of exencephaly. In addition, all *per* deficient embryos surviving the first critical period between E10 and E12.5 develop chondrodysplasia characterized by disproportionate dwarfism, disorganization of the growth plate, cleft palate, and perinatal lethality most probably because of respiratory failure. The cartilage of *per* deficient mice is very soft and structurally disorganized. The long bones are shortened and thickened. Surprisingly, the ultrastructural analysis of *per*-null cartilage revealed a lack of the typical collagen network. The reduced amount and the shortening of collagen fibrils suggests that perlecan plays a pivotal role in maintaining the collagen network. These experimental findings revealed a new function of Per during endochondral bone formation. A mechanistical explanation could be that Per protects the cartilage ECM possibly by binding and inactivating tissue proteases or by masking the ECM proteins and, hence, protecting them from proteolytic attack. A likely explanation for the finding that BM originally form in Per-deficient mice is that other HSPG substitute for the loss of Per and the GAG chains attached to it. A possible candidate is agrin, which is present in most if not all BMs and can also bind GFs, dystroglycan and BM components. Mice lacking the neuronal splice variant of agrin have defects in neuromuscular synaptogenesis but are otherwise apparently normal. The lack of a general phenotype in these mutants could be due to the very low expression of nonneuronal agrin or the functional substitution by Per (Costell *et al.,* 1999; Arikawa-Hirasawa *et al.,* 1999). However, further information will come soon from study in *Drosophila* were a *per* homologue was recently cloned and characterized (Friedrich *et al.,* 2000).

Conclusions

What have we learned during the past few years about the *in vivo* functions of PGs and their associated GAGs? First, these molecules play a critical role in modulating the signaling mediated by secreted GFs that are central to patterning tissues, described also as morphogens, including Wnt, TGF/BMP, FGF and Hh. Second, specific structural forms of GAGs are critical in governing the biological activities of their associated PGs. Often, the biological functions of PGs lie within GAG chains, and sequences within these long chains are fundamental for the whole PGs function. Third, PGs 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 GF signaling pathways could affect morphogenesis, the links between PG function, growth regulation, and even tumor suppression are less obvious. The ability of PGs to control the distribution of signaling molecules across many cell dimensions is a particularly exciting area of investigation. For instance, it has been widely assumed that morphogens distribution was a function of simple diffusion. But now, this is clearly not always so simple, as studies of Hh and Wg in *Drosophila* have documented. It seems that what

we have learned so far is just the beginning of the story, which will be continued by many ongoing studies. For example, a preliminary study of *Dsyn* mutations, suggests a role for this molecule in Wnt signaling and in other different pathways. Clearly, there is also a great deal of analyses needed to understand the varied functions of the different PGs core proteins. As another important genetical tool, a quick look in the *C. elegans* genome provides some idea as to the magnitude of the problem: there are upwards of 25 genes with homology to known CS or HS PGs. Of these, only a few genes have been studied genetically, such as *unc-52*, a gene encoding a protein related to Per that affect myofilament assembly, and several of the *sqv* genes that are involved in GAG biosynthesis (Bulik *et al.,* 2000, Rogalski *et al.,* 2001). Furthermore, the degree of structural diversity that is possible for GAGs is remarkable, with as many as 16 steps involved in their biosynthesis. Multiple genes encoding a single type of GAG-modifying enzyme are common, suggesting that polymeric GAG structures are tightly controlled. The different models currently available are incomplete, because a critical reading of these different papers reveals that they often miss precise biochemical analyses of HS sequences or *in vitro* binding experiments of the different GFs on purified GAG chains. More precise analyses of the specificity of these different ligands and of the core proteins expression could permit to apprehend more easily these different models. One of the striking features of GAGs is their structural diversity, with discrete structural variants found in different tissues. It is evident that the different core proteins are not expressed everywhere at the same time. It is also evident that each of these core proteins bears different HS chains with subtle structure differences. These particular structures are due to combined, sequential and incomplete activities of enzymatic complexes in the Golgi. But this does not explain why some pathways may or may not be influenced by mutations in different HS biosynthetic enzymes. One possible explanation comes from the fact that all these characterizations are done by phenotype analysis without any enzymatic activity studies and HS sequence analysis (Merry *et al.,* 1999). All mutants in HS biosynthetic enzymes have pleiotropic effects on HS biosynthesis, but these different genes impair specifically different morphogenetic pathways. So, probably the activity of these HS in these different pathways depends of specific sequences that happen to be particularly affected by a partial pleiotropic effect. It seems that it is due to a rescue action of isoforms of the different enzymes, which have yet to be characterized.

Summary

In recent years, progress in the fields of development and proteoglycan biology have produced converging evidence of the role of proteoglycans in morphogenesis. Numerous studies have demonstrated that proteoglycans are involved in several distinct morphogenetic pathways upon which they act at different levels. In particular, proteoglycans can determine the generation of morphogen gradients and be required for their signal transduction. The surface of most cells and the extracellular matrix are decorated by heparan sulfates which are the most common glycosaminoglycans, normally present as heparan sulfate proteoglycans. Considerable structural heterogeneity is generated in proteoglycans by the biosynthetic modification of their heparan sulfate chains as well as by the diverse nature of their different core proteins. This heterogeneity provides an impressive potential for protein-protein and protein-carbohydrate interactions, and can partly explain the diversity of proteoglycan involvement in different morphogenetic pathways. In this review, we summarize the current knowledge about mutations affecting heparan sulfate proteoglycans that influence the function of growth factor pathways essential for tissue assembly, differentiation and development. The comparison of data obtained in *Drosophila*, rodents and humans reveals that mutations affecting the proteoglycan core proteins or one of the biosynthetic enzymes of their heparan sulfate chains have profound effects on growth and morphogenesis. Further research will complete the picture, but current evidence shows that at the very least, heparan sulfate proteoglycans need to be counted as legitimate elements of morphogenetic pathways that have been maintained throughout evolution as determinant mechanisms of pattern formation.

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References

AIKAWA, J. and ESKO, J.D. (1999). Molecular cloning and expression of a third member of the heparan sulfate/heparin GlcNAc N-deacetylase/ N-sulfotransferase family. *J.Biol.Chem.* 274: 2690-2695.

ARIKAWA-HIRASAWA, E., WATANABE, H., TAKAMI, H., HASSELL, J.R. and YAMADA, Y. (1999). Perlecan is essential for cartilage and cephalic development. *Nat.Genet.* 23: 354-358.

BAEG, G.-H., LIN, X., KHARE, N., BAUMGARTNER, S. and PERRIMON, N. (2001). Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development* 128: 87-94.

BELLAICHE, Y., THE, I. and PERRIMON, N. (1998). Tout-velu is a Drosophila homologue of the putative tumour suppressor EXT-1 and is needed for Hh diffusion. *Nature* 394: 85-88.

BERNFIELD, M., GÖTTE, M., WOO PARK, P., REIZES, O., FITZGERALD, M.L., LINCECUM, J. and ZAKO, M. (1999). Functions of cell surface heparan sulfate proteoglycans. *Annu.Rev.Biochem.* 68: 729-777.

BERNFIELD, M., HINKES, M.T. and GALLO, R.L. (1993). Developmental expression of the syndecans: possible function and regulation. *Dev.Suppl*, 205-212.

BERNFIELD, M., KOKENYESI, R., KATO, M., HINKES, M.T., SPRING, J., GALLO, R. L. and LOSE, E. J. (1992). Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. *Annu.Rev.Cell Biol.* 8: 365-393.

BINARI, R.C., STAVELEY, B.E., JOHNSON, W.A., GODAVARTI, R., SASISEKHARAN, R. and MANOUKIAN, A.S. (1997). Genetic evidence that heparin-like glycosaminoglycans are involved in wingless signaling. *Development* 124: 2623-2632.

BULIK, A.D., WEI, G., TOYODA, H., KINOSHITA-TOYODA, A., WALDRIP, W.R., ESKO, J.D., ROBBINS, P.W. and SELLECK, S.B. (2000). *sqv-3, -7*, and *-8*, a set of genes affecting morphogenesis in Caenorhabditis elegans, encode enzymes required for glycsoaminoglycan biosynthesis. *Proc.Natl.Acad.Sci.* U.S.A. 97: 10838-10843.

BULLOCK, S.L., FLETCHER, J.M., BEDDINGTON, R.S. and WILSON, V.A. (1998). Renal agenesis in mice homozygous for a gene trap mutation in the gene encoding heparan sulfate 2-sulfotransferase. *Genes Dev.* 12: 1894-1906.

CADIGAN, K.M., FISH, M.P., RULIFSON, E.J. and NUSSE, R. (1998). Wingless repression of Drosophila frizzled 2 expression shapes the Wingless morphogen gradient in the wing. *Cell* 93: 767-777.

CANO-GAUCI, D.F., SONG, H.H., YANG, H., MCKERLIE, C., CHOO, B., SHI, W., PULLANO, R., PISCIONE, T.D., GRISARU, S., SOON, S., SEDLACKOVA, L., TANSWELL, A.K., MAK, T.W., YEGER, H., LOCKWOOD, G.A., ROSENBLUM, N. D. and FILMUS, J. (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.

CAREY, D.J. (1997). Review Article: Syndecans: multifunctional cell-surface co-receptors. *Biochem.J.* 327: 1-16.

CAREY, D.J., CONNER, K., ASUNDI, V.K., O'MAHONY, D.J., STAHL, R.C., SHOWALTER, L., CIZMECI-SMITH, G., HARTMAN, J. and ROTHBLUM, L.I. (1997). cDNA cloning, genomic organization, and in vivo expression of rat N-syndecan. *J.Biol.Chem.* 272: 2873-2879.

CLINES, G.A., ASHLEY, J.A., SHAH, S. and LOVETT, M. (1997). The structure of the human multiple exostoses 2 gene and characterization of homologs in mouse and Caenorhabditis elegans. *Genome Res.* 7: 359-367.

COSTELL, M., GUSTAFSSON, E., ASZODI, A., MORGELIN, M., BLOCH, W., HUNZIKER, E., ADDICKS, K., TIMPL, R. and FASSLER, R. (1999). Perlecan maintains the integrity of cartilage and some basement membranes. *J. Cell Biol.* 147: 1109-1122.

DAVID, G., LORIES, V., DECOCK, B., MARYNEN, P., CASSIMAN, J.-J. and VAN DEN BERGHE, H. (1990). Molecular cloning of a phosphatidylinositol-anchored membrane heparan sulfate proteoglycan from human lung fibroblasts. *J. Cell Biol.* 111: 3165-3176.

DE AGOSTINI, A.I. and ROSENBERG, R.D. (1991). New approaches for defining the molecular basis of anticoagulantly active heparan sulfate production. *Ann.N.Y.Acad.Sci.* 614: 279-288.

DIGABRIELE, A.D., LAX, I., CHEN, D.I., SVAHN, C.M., JAYE, M., SCHLESSINGER, J. and HENDRICKSON, W.A. (1998). Structure of a heparin-linked biologically active dimer of fibroblast growth factor. *Nature* 393: 812-817.

ELENIUS, K. and JALKANEN, M. (1994). Function of the syndecans - a family of cell surface proteoglycans. *J.Cell Biol.* 107: 2975-2982.

FAN, G., XIAO, L., CHENG, L., WANG, X., SUN, B. and HU, G. (2000). Targeted disruption of NDST-1 gene leads to pulmonary hypoplasia and neonatal respiratory distress in mice. *FEBS Lett.* 467: 7-11.

FILLA, M.S., DAM, P. and RAPRAEGER, A.C. (1998). The cell surface proteoglycan syndecan-1 mediates fibroblast growth factor-2 binding and activity. *J.Cell Physiol* 174: 310-321.

FORSBERG, E., PEJLER, G., RINGVALL, M., LUNDERIUS, C., TOMASINI-JOHANSSON, B., KUSCHE-GULLBERG, M., ERIKSSON, I., LEDIN, J., HELLMAN, L. and KJELLEN, L. (1999). Abnormal mast cells in mice deficient in a heparin-synthesizing enzyme. *Nature* 400: 773-776.

FRIEDRICH, M.V., SCHNEIDER, M., TIMPL, R. and BAUMGARTNER, S. (2000). Perlecan domain V of Drosophila melanogaster Sequence, recombinant analysis and tissue expression. *Eur.J.Biochem.* 267: 3149-3159.

GONZALEZ, A.D., KAYA, M., SHI, W., SONG, H., TESTA, J.R., PENN, L.Z. and 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.

HACKER, U., LIN, X. and PERRIMON, N. (1997). The Drosophila sugarless gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis. *Development* 124: 3565-3573.

HAERRY, T.E., HESLIP, T.R., MARSH, J.L. and O'CONNOR, M.B. (1997). Defects in glucuronate biosynthesis disrupt Wingless signaling in Drosophila. *Development* 124: 3055-3064.

HASSELL, J.R., ROBEY, P.G., BARRACH, H.J., WILCZEK, J., RENNARD, S.I. and MARTIN, G.R. (1980). Isolation of a heparan sulfate-containing proteoglycan from basement membrane. *Proc.Natl.Acad.Sci.U.S.A* 77: 4494-4498.

HUMPHRIES, D.E., WONG, G.W., FRIEND, D.S., GURISH, M.F., QIU, W.T., HUANG, C., SHARPE, A.H. and STEVENS, R.L. (1999). Heparin is essential for the storage of specific granule proteases in mast cells. *Nature* 400: 769-772.

IOZZO, R.V. (1998). Matrix proteoglycans: from molecular design to cellular function. *Annu.Rev.Biochem.* 67: 609-652.

ISHIGURO, K., KADOMATSU, K., KOJIMA, T., MURAMATSU, H., TSUZUKI, S., NAKAMURA, E., KUSUGAMI, K., SAITO, H. and MURAMATSU, T. (2000). Syndecan-4 deficiency impairs focal adhesion formation only under restricted conditions. *J.Biol.Chem.* 275: 5249-5252.

ISHIGURO, K., KOJIMA, T., TAGUCHI, O., SAITO, H., MURAMATSU, T. and KADOMATSU, K. (1999). Syndecan-4 expression is associated with follicular atresia in mouse ovary. *Histochem.Cell Biol.* 112: 25-33.

JACKSON, S.M., NAKATO, H., SUGIURA, M., JANNUZI, A., OAKES, R., KALUZA, V., GOLDEN, C. and SELLECK, S.B. (1997). Dally, a Drosophila glypican, controls cellular responses to the TGF- beta-related morphogen, Dpp. *Development* 124: 4113-4120.

KAINULAINEN, V., WANG, H., SCHICK, C. and BERNFIELD, M. (1998). Syndecans, heparan sulfate proteoglycans, maintain the proteolytic balance of acute wound fluids. *J.Biol.Chem.* 273: 11563-11569.

KIM, C.W., GOLDBERGER, O.A., GALLO, R.L., and BERNFIELD, M. (1994). Members of the syndecan family of heparan sulfate proteoglycans are expressed in distinct cell-, tissue-, and development-specific patterns. *Mol.Biol.Cell.* 5: 797-805.

KIM, B.T., KITAGAWA, H., TAMURA, J., KUSCHE-GULLBERG, M., LINDAHL, U. and SUGAHARA, K. (2002). Demonstration of a novel DEXT3 of Drosophila melanogaster as the essential N-acetylglucosamine transferase in the heparan sulfate biosynthesis: chain initiation and elongation. *J.Biol.Chem.* 277: 13659-13665.

KOBAYASHI, M., HABUCHI, H., HABUCHI, O., SAITO, M. and KIMATA, K. (1996). Purification and characterization of heparan sulfate 2-sulfotransferase from cultured Chinese hamster ovary cells. *J.Biol.Chem.* 271: 7645-7653.

KOSHER R.A. (1998). Syndecan-3 in limb skeletal development. *Micr.Res.Tech* 43: 123-130.

KUSCHE-GULLBERG, M., ERIKSSON, I., PIKAS, D.S. and KJELLEN, L. (1998). Identification and expression in mouse of two heparan sulfate glucosaminyl N-deacetylase/N-sulfotransferase genes. *J.Biol.Chem.* 273: 11902-11907.

LANGFORD, J.K., STANLEY, M.J., CAO, D. and SANDERSON, R.D. (1998). Multiple heparan sulfate chains are required for optimal syndecan-1 function. *J.Biol.Chem.* 273: 29965-29971.

LIN, X., BUFF, E.M., PERRIMON, N. and MICHELSON, A.M. (1999). Heparan sulfate proteoglycans are essential for FGF receptor signaling during Drosophila embryonic development. *Development* 126: 3715-3723.

LIN, X. and PERRIMON, N. (1999). Dally cooperates with Drosophila Frizzled 2 to transduce Wingless signalling. *Nature* 400: 281-284.

LIN, X. and WELLS, D. (1997). Isolation of the mouse cDNA homologous to the human EXT1 gene responsible for Hereditary Multiple Exostoses. *DNA Seq.* 7: 199-202.

LIND, T., TUFARO, F., MCCORMICK, C., LINDAHL, U. and LIDHOLT, K. (1998). The putative tumor suppressors EXT1 and EXT2 are glycosyltransferases required for the biosynthesis of heparan sulfate. *J.Biol.Chem.* 273: 26265-26268.

LORIES, V., CASSIMAN, J.J., VAN DEN, B.H. and DAVID, G. (1989). Multiple distinct membrane heparan sulfate proteoglycans in human lung fibroblasts. *J.Biol.Chem.* 264: 7009-7016.

MCCORMICK, C., DUNCAN, G., GOUTSOS, K.T. and TUFARO, F. (2000). The putative tumor suppressors EXT1 and EXT2 form a stable complex that accumulates in the Golgi apparatus and catalyzes the synthesis of heparan sulfate. *Proc.Natl.Acad.Sci.U.S.A* 97: 668-673.

MCCORMICK, C., LEDUC, Y., MARTINDALE, D., MATTISON, K., ESFORD, L.E., DYER, A.P. and TUFARO, F. (1998). The putative tumour suppressor EXT1 alters the expression of cell- surface heparan sulfate. *Nat.Genet.* 19: 158-161.

MERRY, C.L., LYON, M., DEAKIN, J.A., HOPWOOD, J.J. and GALLAGHER, J.T. (1999). Highly sensitive sequencing of the sulfated domains of heparan sulfate. *J.Biol.Chem.* 274: 18455-18462.

MORATA, G. and E. SANCHEZ-HERRERO. (1999). Patterning mechanisms in the body trunk and the appendages of Drosophila. *Development* 126: 2823-2828.

NAKATO, H., FUTCH, T.A. and SELLECK, S.B. (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.

NILSON, L.A. and SCHUPBACH, T. (1998). Localized requirements for windbeutel and pipe reveal a dorsoventral prepattern within the follicular epithelium of the Drosophila ovary. *Cell* 93: 253-262.

NOONAN, D.M., FULLE, A., VALENTE, P., CAI, S., HORIGAN, E., SASAKI, M., YAMADA, Y. and HASSELL, J.R. (1991). The complete sequence of Perlecan, a basement membrane heparan sulfate proteoglycan, reveals extensive similarity with laminin A chain, low density lipoprotein-receptor, and the neural cell adhesion molecule. *J.Biol.Chem.* 266: 22939-22947.

ORNITZ, D. M. (2000). FGFs, heparan sulfate and FGFRs: complex interactions essential for development. *Bioessays* 22: 108-112.

PARRY G., FARSON D., CULLEN B. and BISSELL M.J. (1988). p-nitrophenyl-beta-D-xyloside modulates proteoglycan synthesis and secretory differentiation in mouse mammary epithelial cell cultures. *In Vitro Cell Dev.Biol.* 24: 1217-1222.

PELLEGRINI, L., BURKE, D.F., VON DELFT, F., MULLOY, B. and BLUNDELL, T.L. (2000). Crystal structure of fibroblast growth factor receptor ectodomain bound to ligand and heparin. *Nature* 407: 1029-1034.

PELLEGRINI, M., PILIA, G., PANTANO, S., LUCCHINI, F., UDA, M., FUMI, M., CAO, A., SCHLESSINGER, D. and FORABOSCO, A. (1998). Gpc3 expression correlates with the phenotype of the Simpson-Golabi- Behmel syndrome. *Dev.Dyn.* 213: 431-439.

PERRIMON, N. and BERNFIELD, M. (2000). Specificities of heparan sulfate proteoglycans in developmental processes. *Nature* 404: 725-728.

PRYDZ, K. and K. T. DALEN. (2000). Synthesis and sorting of proteoglycans. *J.Cell Sci.* 113 Pt 2: 193-205.

RINGVALL, M., LEDIN, J., HOLMBORN, K., VAN KUPPEVELT, T., ELLIN, F., ERIKSSON, I., OLOFSSON, A.M., KJELLEN, L. and FORSBERG, E. (2000). Defective heparan sulfate biosynthesis and neonatal lethality in mice lacking N-deacetylase/N-sulfotransferase-1. *J.Biol.Chem.* 275: 25926-25930.

ROGALSKI, T.M., MULLEN, G.P., BUSH, J.A., CILCHIRST, E.J. and MOERMAN, D.J. (2001). UNC-52/perlecan isoform diversity and function in Caenorhabditis elegans. *Biochem.Soc. Trans.* 2: 171-176.

ROSENBERG, R.D. (1989). Biochemistry of heparin antithrombin interactions, and the physiologic role of this natural anticoagulant mechanism. *Am.J.Med.* 87: 2S-9S.

SALMIVIRTA, M., LIDHOLT, K. and LINDAHL, U. (1996). Heparan sulfate: a piece of information. *FASEB J.* 10: 1270-1279.

SANDERSON, R.D., TURNBULL, J.E., GALLAGHER, J.T. and LANDER, A.D. (1994). Fine structure of heparan sulfate regulates syndecan-1 function and cell behavior. *J.Biol.Chem.* 269: 13100-13106.

SAUNDERS, S., JALKANEN, M., O'FARRELL, S. and BERNFIELD, M. (1989). Molecular cloning of Syndecan, an integral membrane proteoglycan. *J. Cell Biol.* 108: 1547-1556.

SELLECK, S.B. (1999). Overgrowth syndromes and the regulation of signaling complexes by proteoglycans. *Am.J.Hum.Genet.* 64: 372-377.

SELLECK, S.B. (2000). Proteoglycans and pattern formation: sugar biochemistry meets developmental genetics. *Trends Genet.* 16: 206-212.

SEN, J., GOLTZ, J.S., STEVENS, L. and STEIN, D. (1998). Spatially restricted expression of pipe in the Drosophila egg chamber defines embryonic dorsal-ventral polarity. *Cell* 95: 471-481.

SMITH, C.I., WEBSTER, E.H, NATHANSON, M.A., SEARLS, R.L. and HILFER, S.R. (1990). Altered patterns of proteoglycan deposition during maturation of the fetal mouse lung. *Cell Differ.Dev.* 32: 83-96.

SPRING, J., PAINE-SAUNDERS, S.E., HYNES, R.O. and BERNFIELD, M. (1994). Drosophila syndecan: conservation of a cell-surface heparan sulfate proteoglycan. *Proc.Natl.Acad.Sci.U.S.A* 91: 3334-3338.

STARK, D.R., GATES, P.B., BROCKES, J.P. and FERRETTI, P. (1998). Hedgehog family member is expressed throughout regenerating and developing limbs. *Dev.Dyn.* 212: 352-363.

STICKENS, D., BROWN, D. and EVANS, G.A. (2000). EXT genes are differentially expressed in bone and cartilage during mouse embryogenesis. *Dev.Dyn.* 218: 452-464.

STICKENS, D. and EVANS, G.A. (1997). Isolation and characterization of the murine homolog of the human EXT2 multiple exostoses gene. *Biochem.Mol.Med.* 61: 16-21.

STRIGINI, M. and COHEN, S.M. (2000). Wingless gradient formation in the Drosophila wing. *Curr.Biol.* 10: 293-300.

TABATA, T. (2001). Genetics of Morphogen Gradients. *Nat.Genet.* 2: 620-630.

THE, I., BELLAICHE, Y. and PERRIMON, N. (1999). Hedgehog movement is regulated through tout velu-dependent synthesis of a heparan sulfate proteoglycan. *Mol.Cell* 4: 633-639.

TOOLE, B.P. (2001). Hyaluronan in morphogenesis. *Cell Dev.Biol.* 12: 79-87.

TOYODA, H., KINOSHITA-TOYODA, A. and SELLECK, S.B. (2000). Structural analysis of glycosaminoglycans in Drosophila and Caenorhabditis 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.

TSUDA, M., KAMIMURA, K., NAKATO, H., ARCHER, M., STAATZ, W., FOX, B., HUMPHREY, M., OLSON, S., FUTCH, T., KALUZA, V., SIEGFRIED, E., STAM, L. and SELLECK, S.B. (1999). The cell-surface proteoglycan Dally regulates Wingless signalling in Drosophila. *Nature* 400: 276-280.

VEUGELERS, M., DE CAT, B., CEULEMANS, H., BRUYSTENS, A.M., COOMANS, C.J., VERMEESCH, J., MARYNEN, P. and DAVID, G. (1999). Glypican-6, a New Member of the Glypican Family of Cell Surface Heparan Sulfate Proteoglycans. *J.Biol.Chem.* 274: 26968-26977.

VEUGELERS, M., VERMEESCH, J., WATANABE, K., YAMAGUCHI, Y., MARYNEN, P. and DAVID, G. (1998). GPC4, the gene for human K-glypican, flanks GPC3 on xq26: deletion of the GPC3-GPC4 gene cluster in one family with Simpson-Golabi-Behmel syndrome. *Genomics* 53: 1-11.

VIHINEN, T., AUVINEN, P., ALANEN-KURKI, L. and JALKANEN, M. (1993). Structural organization and genomic sequence of mouse syndecan-1 gene. *J.Biol.Chem.* 268: 17261-17269.

WODARZ, A. and R. NUSSE. (1998). Mechanisms of Wnt signaling in development. *Annu.Rev.Cell Dev.Biol.* 14: 59-88.

WUYTS, W. and VAN HUL, W. (2000). Molecular basis of multiple exostoses: mutations in the EXT1 and EXT2 genes. *Hum.Mutat.* 15: 220-227.

YANAGISHITA, M. and HASCALL, V.C. (1992). Cell surface heparan sulfate proteoglycans. *J.Biol.Chem.* 267: 9451-9454.

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