

Role of heparan sulfate proteoglycans in cell–cell signaling in *Drosophila*

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Abstract

Heparan sulfate proteoglycans (HSPGs) are abundant molecules associated with the cell surface and extracellular matrix, and consist of a protein core to which heparan sulfate (HS) glycosaminoglycan (GAG) chains are attached. Although these molecules have been the focus of intense biochemical studies *in vitro*, their biological functions *in vivo* were unclear until recently. We have undertaken an *in vivo* functional study of HSPGs in *Drosophila*. Our studies, as well as others, demonstrate the critical roles of HSPGs in several major signaling pathways, including fibroblast growth factor (FGF), Wnt, Hedgehog (Hh) and TGF- β . Our results also suggest that specific HS GAG chain modifications, as well as specific HSPG protein cores, are involved in specific signaling pathways. © 2000 Elsevier Science B.V./International Society of Matrix Biology. All rights reserved.

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1. Introduction

Heparan sulfate proteoglycans (HSPGs) are cell surface macromolecules that consist of a protein core to which heparan sulfate (HS) glycosaminoglycan (GAG) chains are attached (Bernfield et al., 1999). Several families of HSPGs can be classified according to the structure of the protein cores (Fig. 1). In addition to the diverse nature of the core proteins, enormous structural heterogeneity can be generated through specific HS chain modifications during their biosynthesis (Fig. 2). Thus, both the nature of the core proteins and modifications of HS-GAG chains can determine the specificity and function of HSPGs. A large body of evidence, mainly from *in vitro* biochemical studies, have implicated that HSPGs are important in a variety of cellular functions such as

cell adhesion, motility, proliferation, differentiation and morphogenesis (Iozzo, 1998; Bernfield et al., 1999). In the context of signal transduction, HSPGs have been proposed to act as co-receptors for a number of growth factors, internalization of receptors and transport of signaling molecules. However, the function of HSPGs *in vivo* has been unclear. Recent genetic studies in both *Drosophila* and mice have begun to uncover the functions of HSPGs *in vivo* in specific signaling pathways involved in cell differentiation and morphogenesis. This review summarizes the work from our laboratory on the *in vivo* functional studies of HSPGs in *Drosophila*. For a more extensive review, see Perrimon and Bernfield (2000).

2. Identification and isolation of mutations involved in the biosynthesis of HS GAGs

The development of the *Drosophila* embryo requires several key-signaling molecules for its pattern-

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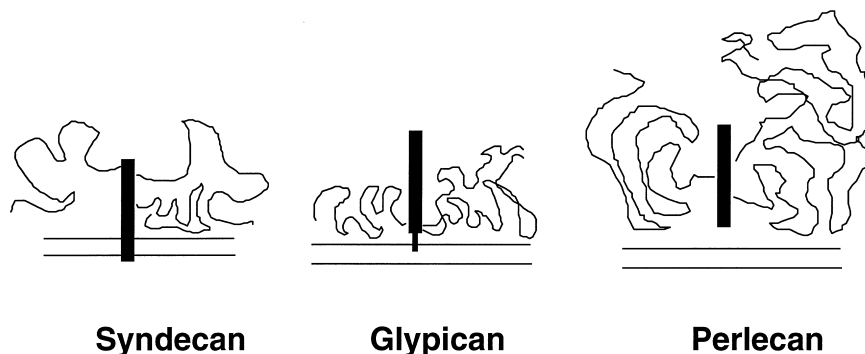


Fig. 1. Structures of HSPGs associated with cell surfaces. The syndecan and glypican family members are two major cell surface HSPGs, both of which are integral membrane proteins. The syndecan core proteins are transmembrane proteins that contain a highly conserved short (34–38 residue) carboxy-terminal cytoplasmic domain. The HS chains on the syndecans are linked to serine residues that are distal from the plasma membrane. The glypican core proteins are disulfide-stabilized globular core proteins linked to the plasma membrane by a GPI linkage. The HS chains on the glypicans are linked to serine residues adjacent to the plasma membrane. Perlecan are secreted HSPG bearing HS chains linked to amino termini of the core protein. In mammalian tissues, perlecan is present in nearly all basement membranes as well as cartilage.

ing, particularly in Wingless (Wg), a member of Wnt family, and Hedgehog (Hh). Maintenance of the normal segmentation pattern of the developing embryo is the result of reciprocal interactions between cells producing and cells receiving the Wg protein in the adjacent segments (Siegfried and Perrimon, 1994). Segmentation can be clearly visualized in the cuticle of the hatched larva as an alternating pattern of

‘naked’ cuticle and cuticle covered with small hairs called denticles. Wg signaling is required for specification of the ‘naked’ cuticle. Loss-of-function mutations involved in Wg signaling lead to a cuticle pattern with the absence of naked cuticle. Through a feed back mechanism, Hh is required for maintenance of Wg production. Mutations in Hh signaling result in a loss of *wg* expression and thus, lead to

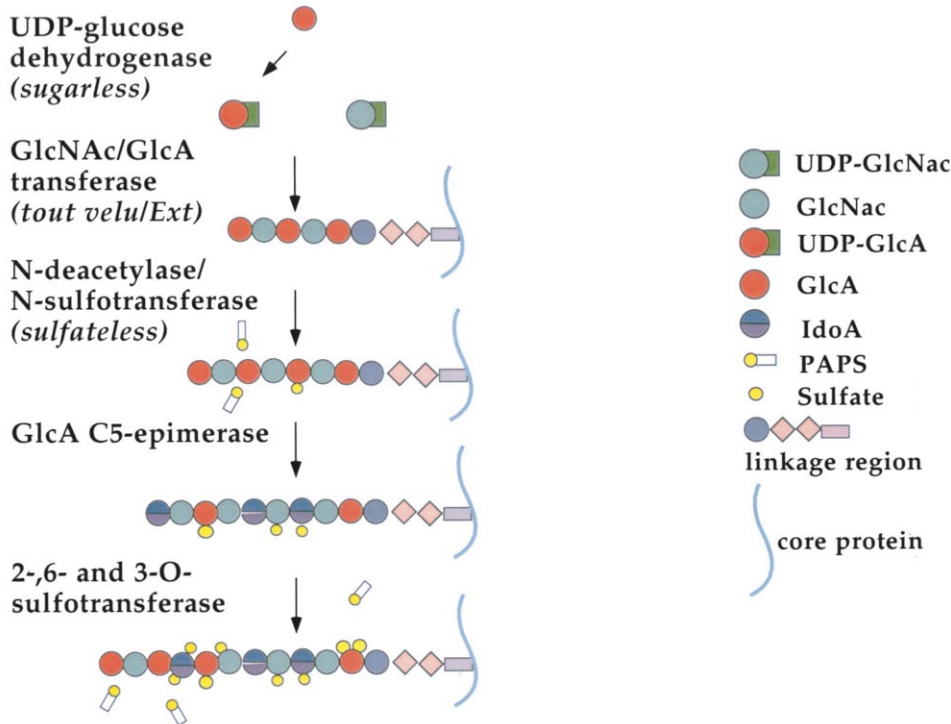


Fig. 2. HS chain biosynthesis (adapted from Perrimon and Bernfield, 2000). The chains are synthesized on the core protein by the sequential action of individual glycosyltransferases. A common tetrasaccharide linkage region is formed, followed by the addition of alternating GlcA and GlcNAc residues, producing in turn the precursor chain. This chain is then enzymatically modified by deacetylation and *N*-sulfation, epimerization and *O*-sulfation, yielding individual chains whose sequence is distinct from all the other chains.

similar cuticle defects as in *wg* mutants. Mutants with *wg*- or *hh*-like cuticle phenotypes are classified as segment polarity genes (Siegfried and Perrimon, 1994).

In a genetic screen to characterize the maternal effects of zygotic lethal mutations (Perrimon et al., 1996), we have identified several segment polarity genes including *sugarless* (*sgl*) (Binari et al., 1997; Haecker et al., 1997; Haerry et al., 1997), *sulfateless* (*sfl*) (Lin and Perrimon, 1999) and *tout velu* (*ttv*) (Bellaiche et al., 1998; The et al., 1999). In the absence of both maternal and zygotic expression of these genes, embryos die with a segment polarity phenotype. The cuticle phenotypes of these mutants are reminiscent of the phenotypes exhibited by mutations in either *wg* or *hh*, suggesting that these genes are involved in either or both of these signaling pathways. Molecular characterization of these genes revealed that they encode enzymes involved in the biosynthesis of HS GAGs. As illustrated in Fig. 2, *Sgl* encodes a homolog of UDP-D-glucose dehydrogenase (Hempel et al., 1994), which produces UDP-D-glucuronic acid that is a substrate for the biosynthesis of HS GAGs. *Sfl* is a homolog of heparan sulfate *N*-deacetylase/*N*-sulfotransferase (Hashimoto et al., 1992) that is required for the modification of HS GAG. Molecular characterization of *ttv* revealed that it encodes a member of the *Ext* gene family implicated in the human multiple exostoses (*Ext*) syndrome (Stickens et al., 1996). Both tissue culture experiments (McCormick et al., 1998) and biochemical studies (Lind et al., 1998) suggest that *Ext* proteins function as HS polymerases. The cuticle phenotypes associated with *sgl*, *sfl* and *ttv* as segment polarity genes suggest that HSPGs play key roles in Wg and/or Hh signaling.

3. HSPGs are required for Wg signaling

The *wg*-like cuticle phenotypes associated with mutations involved in the biosynthesis of HS GAGs implicate that HSPGs are required for Wg signaling. To further demonstrate a role of HSPGs in Wg signaling, we examined the development of several other embryonic tissues that require Wg activity. These include the development of the stomatogastric nervous system (SNS) and the second mid-gut constriction. Examinations of both *sgl* and *sfl* null embryos revealed that both the development of the SNS and the mid-gut constriction are abnormal, and resemble those found in *wg* mutants (Haecker et al., 1997). Altogether, our results implicate a requirement for HSPGs activity in Wg signaling during embryonic development.

HSPGs are also required for Wg signaling in the development of *Drosophila* imaginal discs. Wg is re-

quired for dorso/ventral (D/V) patterning and acts as a short-range inducer to activate the expression of several genes such as *neuralized* (*neu*) at the wing margin. Wg also functions as a morphogen to directly activate the transcription of several target genes, including *distalless* (*dll*), in a concentration-dependent manner. In *sfl* mutant wing discs, the expression of *neu* is abolished and *Dll* expression is also markedly reduced. These results indicate a role for HSPGs in both short-range and long-range Wg effects during wing-disc development (Lin and Perrimon, 1999).

To explore the mechanism(s) of how HSPGs participate in Wg signaling, we have ectopically expressed Wg protein in *sgl* or *sfl* null embryos using the UAS-Gal4 technique. Over expression of Wg protein can partially rescue Wg signaling in a dose-dependent manner in *sgl* and *sfl* null embryos (Haecker et al., 1997). These results suggest that HSPGs function in Wg signaling by limiting its diffusion and thereby facilitating the binding of Wg to its receptor (Haecker et al., 1997).

Consistent with our genetic evidence for a role of HSPG in Wg signaling, experiments in tissue culture demonstrated that Wg protein can directly bind to heparin. Wg signaling can be inhibited by removal of the heparan sulfate with heparinase or by treatment of cells with sodium perchlorate, a competitive inhibitor that blocks the sulfation of proteoglycans (Reichsman et al., 1996). Altogether, the results from both in vivo and in vitro experiments strongly argue that HSPGs play a key role in Wg signaling.

4. In vivo evidence for HSPGs function in the FGF signaling pathways

A large body of biochemical and cellular evidence shows that HSPGs are an essential component for signaling mediated by the FGF protein family (Ornitz, 2000). However, there has been no direct demonstration that HSPGs are required for the biological activity of FGFs in a developmental system in vivo. The availability of HS-GAG biosynthesis mutants allowed us to examine the in vivo role of HSPGs in FGF signaling. Two FGF receptors, Heartless (*Htl*) and Breathless (*Btl*) have been identified in *Drosophila*. *Htl* and *Btl* are required for the migration of mesodermal and tracheal cells respectively. In both *sgl* and *sfl* null mutants, the migration of mesodermal and tracheal cells is defective, phenotypes reminiscent of *htl* and *btl* null mutants (Lin et al., 1999). We have further demonstrated that both *Htl*- and *Btl*-dependent MAPK activation is significantly reduced in the embryos lacking *sfl* and *sgl* activities. Furthermore, a constitutively activated form of *Htl* partially rescues the mesodermal cell migration defects in *sfl* and *sgl*

mutants. These results provide the first in vivo evidence that HSPGs are essential for FGF signaling.

5. Specific function of Ttv in the regulation of Hh movement and signaling

Ttv was initially identified as a segment polarity gene (Perrimon et al., 1996). Molecular characterization of *ttv* revealed that it encodes a member of the *Ext* gene family that functions as HS polymerases. Further analysis of *ttv* reveals that it plays a key role in the movement of Hh through a field of cells (Bellaïche et al., 1998). These studies suggested that the membrane-targeted cholesterol-modified Hh molecule requires HSPGs to be either trapped by receiving cells or move from cells to cells (The et al., 1999). Interestingly, when other signal transduction pathways were examined, it was found that in the absence of *ttv* activity only Hh signaling, but not Wg or FGF signaling, is affected (The et al., 1999). These results argue that biosynthesis of HS GAG can contribute to the specificity of HS GAG in signaling. One model to explain the specificity of Ttv in Hh action is that Hh signaling is more sensitive to a reduction in HSPGs concentration than Wg and FGF signaling. Alternatively, an Hh-specific HSPG may exist and Ttv may be responsible for its synthesis (The et al., 1999). Ttv is not the only example suggesting that specific modification of HS GAG can determine the specificity of proteoglycans in signaling. Other evidence comes from the analysis of *Drosophila pipe* mutant that encodes a putative HS 2-OST (Sen et al., 1998). *Pipe* is expressed in the ventral part of the egg chamber and has been proposed to activate the serine protease cascade that leads to production of the active Toll ligand Spätzle. Another example of specific developmental defects associated with a mutation in a HS 2-OST has been found in mice (Bullock et al., 1998).

6. Role of the HSPG core proteins in signal transduction

In *Drosophila*, a single *syndecan* gene, two *glypican* genes, *dally* and *dally-like* (Baeg et al., unpublished), and a *perlecan* homolog have been identified. However, detailed phenotypic analyses are only available for *dally*. Dally was initially identified as a mutant affecting cell division patterning in the developing central nervous system (Nakato et al., 1995). A detailed analysis of *dally* has implicated Dally in Wg signaling (Lin and Perrimon, 1999; Tsuda et al., 1999). Loss of *dally* activity, both in the embryo and imaginal discs, generates phenotypes reminiscent of loss of

Wg activity. Genetic interaction 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 Frizzled 2 (Lin and Perrimon, 1999; Tsuda et al., 1999). Interestingly, *dally* expression is developmentally regulated and is co-expressed with Frizzled 2 in the embryo (Lin and Perrimon, 1999), suggesting that, as previously observed for Frizzled 2 (Cadigan et al., 1998), the level of the HSPG co-receptor is tightly regulated for proper Wg signaling.

Dally has also been found to act in Decapentaplegic (Dpp, a member of the TGFbeta family) signaling (Jackson et al., 1997). A reduction in *dpp* levels enhances the defects associated with *dally* mutations in the eye, antenna, and genitalia. Furthermore, additional copies of *dpp* rescue the defects in these tissues. These genetic interactions have led to the hypothesis that Dally regulates Dpp activity (Jackson et al., 1997). However, the activity of Dally in Dpp signaling is limited only to imaginal discs since no defects associated with Dpp signaling during embryonic development have been found in *dally* mutant. Taken together, these results suggest that Dally is developmentally regulated and interacts with only a subset of signaling pathways.

7. Perspectives

Genetic analyses of enzymes involved in HS chain biosynthesis and the core proteins have demonstrated critical roles of HSPGs in developmental processes. It is now apparent that HSPGs are implicated in several major signal transduction pathways, and that both the modification of HS biosynthesis and their specific protein cores can contribute to the regulation of specific pathways. It is now important to address how specific modifications and protein cores regulate cell–cell signaling. Furthermore, several signaling molecules including Wg, Hh and Dpp can function as morphogens and we need to better understand the contribution of HSPGs in establishing the protein gradients of these signaling molecules. Further analyses of the mutant phenotypes associated with the remaining enzymes and core proteins will lead to a better understanding of these issues.

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