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Heparan Sulfate Proteoglycans

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Heparan sulfate proteoglycans are found at the cell surface and in the extracellular matrix, where they interact with a plethora of ligands. Over the last decade, new insights have emerged regarding the mechanism and biological significance of these interactions. Here, we discuss changing views on the specificity of protein–heparan sulfate binding and the activity of HSPGs as receptors and coreceptors. Although few in number, heparan sulfate proteoglycans have profound effects at the cellular, tissue, and organismal level.

Heparan sulfate proteoglycans (HSPGs) are glycoproteins, with the common characteristic of containing one or more covalently attached heparan sulfate (HS) chains, a type of glycosaminoglycan (GAG) (Esko et al. 2009). Cells elaborate a relatively small set of HSPGs (~17) that fall into three groups according to their location: membrane HSPGs, such as syndecans and glycosylphosphatidylinositol-anchored proteoglycans (glypicans), the secreted extracellular matrix HSPGs (agrin, perlecan, type XVIII collagen), and the secretory vesicle proteoglycan, serglycin (Table 1). Much of the early work in the field concentrated on composition (size, chain number, and structure of the HS chains), biosynthesis, and binding properties of the chains. In 1985, the first somatic cell mutants altered in HSPG expression were identified (Esko et al. 1985), which allowed functional studies in the context of a cell culture model (Zhang et al. 2006). A decade later, the first HSPG mutants in a model organism (*Drosophila melanogaster*) were identified (Rogalski et al. 1993; Nakato et al. 1995; Häcker et al. 1997; Bellaiche et al. 1998; Lin et al. 1999), which was followed by identification of mutants in nematodes, tree frogs, zebrafish, and mice (Tables 2 and 3). HS is evolutionarily ancient and its composition has remained relatively constant from *Hydra* to humans (Yamada et al. 2007; Lawrence et al. 2008).

Figure 1 shows in pictorial form many of the systems in which HSPGs participate.

1. HSPGs are present in basement membranes (perlecan, agrin, and collagen XVIII), where they collaborate with other matrix components to define basement membrane structure and to provide a matrix for cell migration.
2. HSPGs are found in secretory vesicles, most notably serglycin, which plays a role in packaging granular contents, maintaining proteases in an active state, and regulating various biological activities after secretion

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Table 1. Heparan sulfate proteoglycans

Class	Proteoglycan	Core mass (kDa)[a]	Chain type (number)[b]	Tissue	Human disease
Membrane-bound	Syndecan-1–syndecan-4	31–45	HS (2–3) in Sdc2 and Sdc4; HS/CS (3–4 HS/1-2 CS) in Sdc1 and Sdc3	Epithelial cells, fibroblasts	
	Glypican-1–glypican-6	57–69	HS (1–3)	Epithelial cells, fibroblasts	Simpson–Golabi–Behmel syndrome (overgrowth) (GPC3) (Pilia et al. 1996); omodysplasia (skeletal dysplasia) (GPC6) (Campos-Xavier et al. 2009)
	Betaglycan (part-time PG)	110	HS/CS (1–2)	Fibroblasts	
	Neuropilin-1 (part-time PG)	130	HS or CS (1)	Endothelial cells	
	CD44v3	37	HS (1)	Lymphocytes	
Secretory vesicles	Serglycin	10–19	Heparin/CS (10–15)	Mast cells, hematopoietic cells	
Extracellular matrix	Perlecan	400	HS (1–4)	Basement membranes	Schwartz–Jampel syndrome (skeletal dysplasia) (Nicole 2000; Arikawa-Hirasawa et al. 2001)
	Agrin	212	HS (2–3)	Basement membranes	
	Collagen XVIII	150	HS (1–3)	Epithelial cells, basement membranes	Knobloch syndrome type I (Sertie et al. 2000)

HS, heparan sulfate; CS, chondroitin sulfate; PG, proteoglycan.
[a]The variation in core mass is because of species differences.
[b]The number of chains is based on the number of putative attachment sites for chain initiation as well as data from the literature; the actual number of chains varies by method, tissue, and species.

such as coagulation, host defense, and wound repair.

3. HSPGs can bind cytokines, chemokines, growth factors, and morphogens, protecting them against proteolysis. These interactions provide a depot of regulatory factors that can be liberated by selective degradation of the HS chains. They also facilitate the formation of morphogen gradients essential for cell specification during development and chemokine gradients involved in leukocyte recruitment and homing.

4. HSPGs can act as receptors for proteases and protease inhibitors regulating their spatial distribution and activity.

5. Membrane proteoglycans cooperate with integrins and other cell adhesion receptors

to facilitate cell-ECM attachment, cell–cell interactions, and cell motility.

6. Membrane HSPGs act as coreceptors for various tyrosine kinase-type growth factor receptors, lowering their activation threshold or changing the duration of signaling reactions.

7. Membrane HSPGs act as endocytic receptors for clearance of bound ligands, which is especially relevant in lipoprotein metabolism in the liver and perhaps in the formation of morphogen gradients during development.

This article is divided into 10 subsections. The first three are written for investigators outside the field who may need some background information on the diversity of HSPGs and the interactions that occur with protein ligands. The subsequent sections describe seven systems that illustrate general principles or ideas that have undergone a significant shift over the last decade. Because of space limitations not all subjects can be considered or treated in appropriate depth and therefore the reader is referred to excellent recent review articles (Tkachenko et al. 2005; Bulow and Hobert 2006; Bishop et al. 2007; Lamanna et al. 2007; Bix and Iozzo 2008; Filmus et al. 2008; Ori et al. 2008; Rodgers et al. 2008; Sanderson and Yang 2008; Iozzo et al. 2009; Couchman 2010).

Table 2. Mutants altered in HSPG core proteins

Gene	Proteoglycan	Phenotype (references)
Sdc1	Syndecan-1	Null allele: viable; increase in inflammation-mediated corneal angiogenesis (Gotte et al. 2002, 2005); corneal epithelial cells migrate more slowly, show reduced localization of α9 integrin during wound closure and fail to increase in proliferation after wounding (Stepp et al. 2002); enhanced leukocyte-endothelial interaction in the retina (Gotte et al. 2002, 2005); increase in medial and intimal smooth muscle cell replication and neointimal lesion after injury (Fukai et al. 2009); reduced cardiac fibrosis and dysfunction during angiotensin II–induced hypertension (Schellings et al. 2010); not required for follicle initiation and development (Richardson et al. 2009); accumulates plasma triglycerides, and shows prolonged circulation of injected human VLDL and intestinally derived chylomicrons (Stanford et al. 2009); juvenile mice resistant to carcinogen-induced tumorigenesis (McDermott et al. 2007); increased basal protein leakage and more susceptible to protein loss induced by combinations of IFN-γ, TNF-α, and increased venous pressure (Bode et al. 2008); exacerbates anti-GBM nephritis shifting Th1/Th2 balance toward a Th2 response (Rops et al. 2007); no role in hepatocyte infection by *Plasmodium yoelii* sporozoites (Bhanot 2002); normal larval development of *Trichinella spiralis*, but modestly reduced Th2 responses during infection (Beiting et al. 2006); less susceptible to *Pseudomonas aeruginosa* infection (Haynes et al. 2005); reduced *P. aeruginosa* infection rate and virulence (Park et al. 2001); protected from *Staphylococcus aureus* beta-toxin-induced lung injury (Hayashida et al. 2009a); exaggerated airway hyperresponsiveness, eosinophilia, and lung IL-4 responses to allergens (Xu et al. 2005); exaggerated CXC chemokines, neutrophilic inflammation, organ damage, and lethality in LPS endotoxemia (Hayashida et al. 2009b); prolonged recruitment of inflammatory cells in dextran sodium sulfate (DSS)-induced colitis and delayed type hypersensitivity (Masouleh et al. 2009; Floer et al. 2010).

Continued

Table 2. *Continued*

Gene	Proteoglycan	Phenotype (references)
Sdc2	Syndecan-2	No mutants reported. Sdc2 antisense impairs angiogenesis in human microvascular endothelial cells (Noguer et al. 2009); morpholinos inhibit cell migration and fibrillogenesis during embryogenesis in zebrafish (Arrington and Yost 2009).
Sdc3	Syndecan-3	Null allele: viable; altered feeding behavior (Strader et al. 2004); no phenotype in synovial endothelial cells (Patterson et al. 2005); enhanced long-term potentiation (LTP) in area CA1 (brain) and impaired performance in tasks assessing hippocampal function (Kaksonen et al. 2002); more sensitive to inhibition of food intake by the melanocortin agonist MTII (Reizes et al. 2003); perturbs laminar structure of the cerebral cortex as a result of impaired radial migration, and neural migration in the rostral migratory stream is impaired (Hienola et al. 2006); novel form of muscular dystrophy characterized by impaired locomotion, fibrosis, and hyperplasia of myonuclei and satellite cells (Cornelison et al. 2004).
Sdc4	Syndecan-4	Null allele: viable; enhanced fibrin deposition in degenerating fetal vessels in the placental labyrinth (Ishiguro et al. 2000); delayed angiogenesis in wound granulation tissue (Echtermeyer et al. 2001); defective subcellular localization of mTOR Complex2 and Akt activation in endothelial cells, affecting endothelial cell size, NOS, and arterial blood pressure (Partovian et al. 2008); decreased macrophage uptake of phospholipase A2-modified LDL (Boyanovsky et al. 2009); mesangial expansion, enhanced matrix collagens I and IV, fibronectin and focal segmental glomerulosclerosis in males, and induction of Sdc2 in glomeruli (Cevikbas et al. 2008); more susceptible to hepatic injury, and thrombin-cleaved form of osteopontin is significantly elevated after concanavalin-A injection (Kon et al. 2008); less damage in osteoarthritic cartilage in a surgically induced model of osteoarthritis (Echtermeyer et al. 2009); explanted satellite cells fail to reconstitute damaged muscle and are deficient in activation, proliferation, MyoD expression, myotube fusion, and differentiation (Cornelison et al. 2004); vibrissae are shorter and have a smaller diameter because of suboptimal response to fibroblast growth factors (Iwabuchi and Goetinck 2006); lower phosphorylation levels of focal adhesion kinase (Wilcox-Adelman et al. 2002); random migration of fibroblasts as a result of high delocalized Rac1 activity (Bass et al. 2007); defective RGD-independent cell attachment to transglutaminase-fibronectin matrices (Telci et al. 2008); impaired suppression of production of IL-1β by TGF-α (Ishiguro et al. 2002); decreased neutrophil recruitment and increased myofibroblast recruitment and interstitial fibrosis after bleomycin-treatment, no inhibition of fibrosis with recombinant CXCL10 protein (Jiang et al. 2010); hypersensitivity to LPS because of decreased TGF-β suppression of IL-1 production in monocytes and neutrophils (Ishiguro et al. 2001).
Gpc1	Glypican-1	Null allele: viable; reduced brain size (Jen et al. 2009). Athymic mutant mice show decreased tumor angiogenesis and metastasis (Aikawa et al. 2008).
Gpc2	Glypican-2	No mutants reported.

Continued

Table 2. *Continued*

Gene	Proteoglycan	Phenotype (references)
Gpc3	Glypican-3	Null allele: viable; resembles Simpson–Golabi–Behmel overgrowth syndrome, including somatic overgrowth, renal dysplasia, accessory spleens, polydactyly, and placentomegaly (Cano-Gauci et al. 1999; Chiao et al. 2002); defects in cardiac and coronary vascular development (Ng et al. 2009); alterations in Wnt signaling, in vivo inhibition of the noncanonical Wnt/JNK signaling, activation of canonical Wnt/β-catenin signaling (Song et al. 2005); increased Hedgehog signaling (Capurro et al. 2008); abnormal rates of proliferation and apoptosis in cortical and medullary collecting duct cells (Grisaru et al. 2001); delay in endochondral ossification, impairment in the development of the myelomonocytic lineage (Viviano et al. 2005).
Gpc4	Glypican-4	Zebrafish *knypek* controls cell polarity during convergent extension (Topczewski et al. 2001); craniofacial skeletal defects in adult fish (LeClair et al. 2009).
Gpc5	Glypican-5	No mutants reported.
Gpc6	Glypican-6	Impaired endochondral ossification and omodysplasia (Campos-Xavier et al. 2009).
Tgfbr3	Betaglycan	Null allele: embryonic lethal; heart and liver defects (Stenvers et al. 2003); defect in seminiferous cord formation in E12.5–13.5 embryos (Sarraj et al. 2010).
Hspg2	Perlecan	Null allele: embryonic lethal (E10–12); developmental angiogenesis altered in zebrafish (Zoeller et al. 2009); high incidence of malformations of the cardiac outflow tract, lack of well-defined spiral endocardial ridges (Costell et al. 2002); lower amounts of collagen IV and laminins in embryonic hearts, reduced function in infarcted hearts from heterozygous mice (Sasse et al. 2008); absence of acetylcholinesterase at the neuromuscular junctions (Arikawa-Hirasawa et al. 2002); cephalic and skeletal abnormalities (Arikawa-Hirasawa et al. 1999); cerebral ectopias, exencephaly (Girós et al. 2007); increased cross-sectional area of myosin heavy chain type IIb fibers in the tibialis anterior muscle (Xu et al. 2010b); diminished osteocyte canalicular pericellular area (Thompson et al. 2011). Exon 3 deletion (*Hspg2*$^{3/3}$) viable: proteinuria after protein loading (Morita et al. 2005); monocyte/macrophage influx impaired in *Hspg2*$^{3/3}$*Col18a1*$^{-/-}$ mice in a model of renal ischemia/reperfusion (Celie et al. 2007). Secreted as CSPG in some tissues (Danielson et al. 1992; Govindraj et al. 2002; Vogl-Willis and Edwards 2004; West et al. 2006), but relationship of CSPG isoform to phenotypes not established.
Prg1	Serglycin	Null allele: viable; secretory granule defects in mast cells (Abrink et al. 2004); dense core formation is defective in mast cell granules (Henningsson et al. 2006); defective secretory granule maturation and granzyme B storage in cytotoxic T cells (Grujic et al. 2005); no effect on macrophages (Zernichow et al. 2006); platelets and megakaryocytes contain unusual scroll-like membranous inclusions (Woulfe et al. 2008); enlargement of multiple lymphoid organs, decrease in the proportion of CD4$^+$ cells, more pronounced airway inflammatory response in older mice (Wernersson et al. 2009); increased virulence of *Klebsiella pneumoniae* (Niemann et al. 2007); defective regulation of antiviral CD8$^+$ T-cell responses (Grujic et al. 2008).

Continued

Table 2. *Continued*

Gene	Proteoglycan	Phenotype (references)
Agrn	Agrin	Null allele: embryonic lethal; reduced number, size, and density of postsynaptic acetylcholine receptor aggregates in muscles; abnormal intramuscular nerve branching and presynaptic differentiation (Gautam et al. 1996,1999); smaller brains (Serpinskaya et al. 1999); abnormal development of interneuronal synapses (Gingras et al. 2007); increased resistance to excitotoxic injury (Hilgenberg et al. 2002); reduced number of cortical presynaptic and postsynaptic specializations (Ksiazek et al. 2007). Floxed allele: Inactivation in podocytes does not affect glomerular charge selectivity or glomerular architecture (Harvey et al. 2007).
Col18a1	Collagen XVIII	Null allele: viable; increased microvascular growth (Li and Olsen 2004); increased angiogenesis associated with atherosclerotic plaques (Moulton et al. 2004); delayed regression of blood vessels in the vitreous along the surface of the retina after birth and lack of or abnormal outgrowth of retinal vessels (Fukai et al. 2002); larger choroidal neovascularization lesions and increased vascular leakage (Marneros et al. 2007); accelerated healing and vascularization rate of excisional dorsal skin wounds (Seppinen et al. 2008); anomalous anastomoses of vasculature; disruption of the posterior iris pigment epithelial cell layer with release of melanin granules, severe thickening of the stromal iris basement membrane zone (Marneros and Olsen 2003); increase in the amount of retinal astrocytes (Hurskainen et al. 2005); more severe glomerular and tubulointerstitial injury in induced anti-GBM glomerulonephritis (Hamano et al. 2010); monocyte/macrophage influx impaired in *Hspg2*$^{3/3}$ *Col18a1*$^{-/-}$ mice in a model of renal ischemia/reperfusion (Celie et al. 2007); mild chylomicronemia (Bishop et al. 2010).

Table 3. Mouse mutants altered in HS biosynthesis

Gene	Enzyme	Phenotype
Xt1	Xylosyltransferase-1	No mutants reported.
Xt2	Xylosyltransferase-2	Null allele: viable; polycystic kidney and livers (Condac et al. 2007).
GalTI (β4GalT7)	Galactosyltransferase I	Human mutants: defective chondroitin substitution of decorin and biglycan in an Ehlers–Danlos patient (Gotte and Kresse 2005; Seidler et al. 2006).
GalTII (β3GalT6)	Galactosyltransferase II	No mutants reported.
Glcat1	Glucuronyltransferase I	Null allele: embryonic lethal (4–8-cell stage) (Izumikawa et al. 2010).
Extl3	*N*-acetylglucosaminyl transferase I	Floxed allele: Inactivation in islets decreases growth and insulin secretion (Takahashi et al. 2009).
Ext1/Ext2	HS Copolymerase (*N*-acetylglucosaminyl-glucuronyltransferase)	Null allele: embryonic lethal (E6-7.5); lack of mesoderm differentiation (Lin et al. 2000; Stickens et al. 2005); heterozygotes develop rib growth plate exostoses (Stickens et al. 2005; Zak et al. 2011); unaltered vascular permeability in heterozygous mice (Xu et al. 2010a).

Continued

Table 3. *Continued*

Gene	Enzyme	Phenotype
		Floxed allele of *Ext1*: defective brain morphogenesis and midline axon guidance after *nestin-Cre* inactivation (Inatani et al. 2003); no effect on adaptive immune response in CD15Cre mice (Garner et al. 2008); altered T-cell and dendritic cell homing to lymph nodes in *Tie2Cre* mice (Bao et al. 2010); rib growth plate exostosis formation in Col2Cre mice (Jones et al. 2010; Matsumoto et al. 2010; Zak et al. 2011).
Ndst1	*N*-acetylglucosaminyl N-deacetylase/N-sulfotransferase-1	Null allele: Perinatal lethal; lung hypoplasia, defective forebrain, lens, and skull development (Fan et al. 2000; Ringvall et al. 2000; Grobe et al. 2005; Pan et al. 2006). Floxed allele: decreased chemokine transcytosis and presentation and neutrophil infiltration in *Tie2Cre* mice (Wang et al. 2005); decreased allergen-induced airway hyperresponsiveness and inflammation because of reduction in recruitment of eosinophils, macrophages, neutrophils, and lymphocytes in *Tie2Cre* mice (Zuberi et al. 2009); decreased pathological angiogenesis in *Tie2Cre* mice (Fuster et al. 2007); decreased vascular VEGF-induced hyperpermeability (Xu et al. 2010a); decreased vascular smooth muscle cell proliferation, vessel size, and vascular remodeling after arterial injury in *SM22αCre* mice (Adhikari et al. 2010a); mild effect on T-cell response in *Tie2Cre;Ndst2*$^{-/-}$ mice (Garner et al. 2008); defective lacrimal gland development and Fgf10-Fgfr2b complex formation and signaling in LeCre mice (Pan et al. 2008); defective lobuloalveolar development in mammary gland (Crawford et al. 2010).
Ndst2	*N*-acetylglucosaminyl N-deacetylase/N-sulfotransferase-2	Null allele: viable; mast cell deficiency and defective storage of proteases (Forsberg et al. 1999; Humphries et al. 1999); compounding mutation with Ndst1 reduces L-selectin interactions (Wang et al. 2005).
Ndst3	*N*-acetylglucosaminyl N-deacetylase/N-sulfotransferase-3	Null allele: viable; floxed allele available (Pallerla et al. 2008).
Ndst4	*N*-acetylglucosaminyl N-deacetylase/N-sulfotransferase-4	No mutants reported.
Glce	Uronyl C5 epimerase	Null allele: perinatal lethal; renal agenesis (Li et al. 2003).
H2st	Uronyl 2-O-sulfotransferase	Null allele: perinatal lethal; renal agenesis; skeletal and ocular defects (Bullock et al. 1998; Merry et al. 2001); defective cerebral cortical development (McLaughlin et al. 2003); altered lacrimal gland development (Qu et al. 2011). Floxed allele: altered lipoprotein clearance in *AlbCre* mice (Stanford et al. 2010); altered branching morphogenesis in the mammary gland (Garner et al. 2011).

Continued

Table 3. *Continued*

Gene	Enzyme	Phenotype
H3st1	Glucosaminyl 3-O-sulfotransferase 1	Null allele: partially penetrant lethality; no alteration in coagulation (HajMohammadi et al. 2003); fertility defects because of impaired ovarian function and placenta development (Shworak et al. 2002; HajMohammadi et al. 2003).
H3st2	Glucosaminyl 3-O-sulfotransferase 2	Null allele; viable; no neuronal phenotype (Hasegawa and Wang 2008).
H3st3	Glucosaminyl 3-O-sulfotransferase 3	No mutants reported.
H3st4	Glucosaminyl 3-O-sulfotransferase 4	No mutants reported.
H3st5	Glucosaminyl 3-O-sulfotransferase 5	No mutants reported.
H3st6	Glucosaminyl 3-O-sulfotransferase 6	No mutants reported.
H6st1	Glucosaminyl 6-O-sulfotransferase 1	Null allele: embryonic lethal (Habuchi et al. 2007; Sugaya et al. 2008). Gene trap allele: embryonic lethal; retinal axon guidance defects (Pratt et al. 2006). Floxed allele: systemic inactivation embryonic lethal (Izvolsky et al. 2008); no change in plasma triglycerides in AlbCre mice (Stanford et al. 2010).
H6st2	Glucosaminyl 6-O-sulfotransferase 2	Null allele: viable (Sugaya et al. 2008); HS6ST-2, but not HS6ST-1, morphants in zebrafish show abnormalities in the branching morphogenesis of the caudal vein (Chen et al. 2005).
H6st3	Glucosaminyl 6-O-sulfotransferase 3	No mutants reported.
Hpa	Heparanase, transgene	Accelerated wound angiogenesis, enhanced delayed type hypersensitivity response (Zcharia et al. 2005; Edovitsky et al. 2006; Ilan et al. 2006); accumulation of intracellular crystals of protein Ym1 in macrophages (Waern et al. 2010); resistance to amyloid protein A amyloidosis (Li et al. 2005); age-related enlargement of lymphoid tissue and altered leukocyte composition (Wernersson et al. 2009).
Hpa	Heparanase	Null allele: viable; altered MMP-2 and MMP-14 expression (Zcharia et al. 2009).
Sulf1	Endo-6-sulfatase 1	Null allele: viable; esophageal defect (Ai et al. 2007; Ratzka et al. 2008); enhanced osteoarthritis, MMP-13, ADAMTS-5, and noggin elevated, col2a1 and aggrecan reduced in cartilage and chondrocytes (Otsuki et al. 2010).
Sulf2	Endo-6-sulfatase 2	Null allele: viable; behavioral defects (Lamanna et al. 2006); enhanced osteoarthritis, MMP-13, ADAMTS-5, and noggin elevated, col2a1 and aggrecan reduced in cartilage and chondrocytes (Otsuki et al. 2010). Gene trap allele: *Sulf2*GT(pGT1TMpfs)155Ska, no phenotype (Lum et al. 2007).

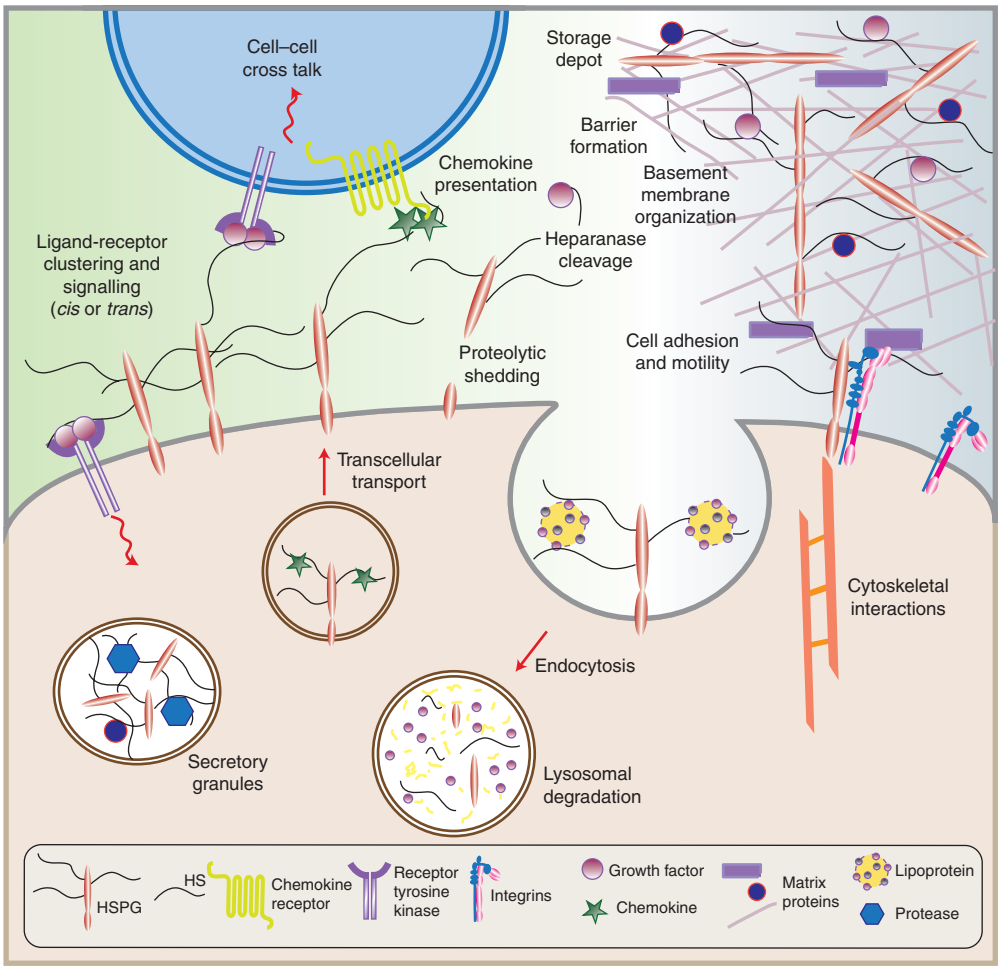

Figure 1. HSPGs have multiple activities in cells and tissues. (Adapted from Bishop et al. 2007; reprinted with permission from Nature Publishing Group © 2007.)

A BIRD'S-EYE VIEW OF STRUCTURE AND ASSEMBLY

An idealized picture of a HSPG is shown in Figure 2. Each proteoglycan consists of a protein and one or more covalently attached HS chains. Comprehensive reviews have appeared on the assembly process and structural characterization of the chains, and therefore these subjects will not be discussed further here (Esko and Selleck 2002; Sugahara and Kitagawa 2002; Sasisekharan et al. 2006; Ori et al. 2008; Laremore et al. 2009). However, several features are important to consider in the context of their biological activities. (1) HSPGs are polyanionic and have unusual hydrodynamic volume because of the presence of long HS chains (40–300 sugar residues, ~20–150 nm), sulfate groups, and uronic acids. Thus, different HSPGs often copurify by techniques that rely simply on the anionic characteristics of the chains or gel filtration. HS and other sulfated GAGs are amongst the most highly negatively charged biopolymers in nature and variation in the number and length of the chains gives rise to enormous polydispersity. (2) Some proteoglycans contain only one GAG chain (e.g., CD44v3 and betaglycan), whereas others have

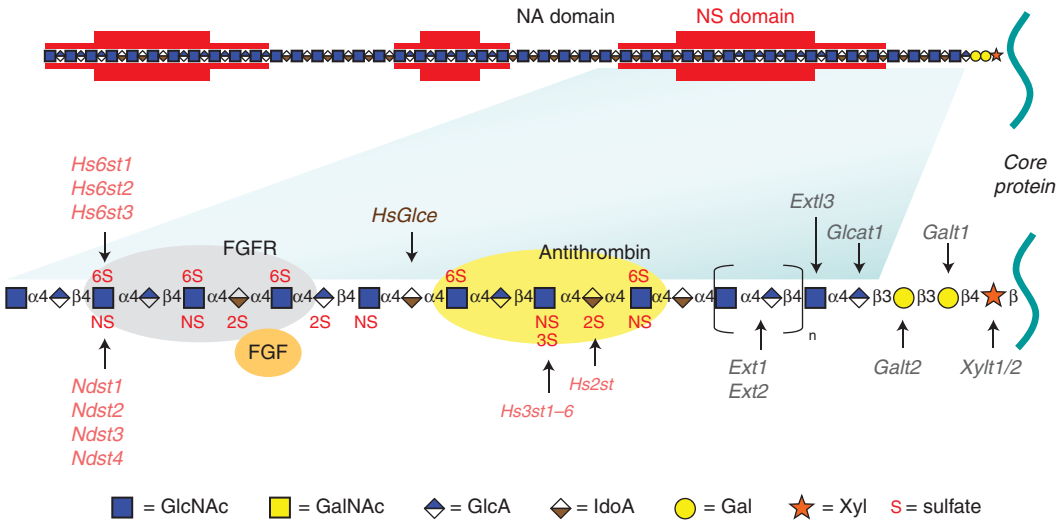

Figure 2. Heparan sulfate (HS) structure. HS biosynthesis initiates by the attachment of xylose to specific serine residues in HSPG core proteins followed by the formation of a linkage tetrasaccharide, glucuronic acid-galactose-galactose-xylose (GlcA-Gal-Gal-Xyl). Extl3 attaches the first *N*-acetyl-D-glucosamine (GlcNAc) residue and an enzyme complex composed of Ext1 and Ext2 alternately adds GlcA and GlcNAc to the nascent chain. The chains simultaneously undergo a series of processing reactions that begins by the removal of the acetyl groups from clusters of GlcNAc residues and substitution of the free amino groups with sulfate, catalyzed by one or more *N*-deacetylase-*N*-sulfotransferases (Ndst). The C5 epimerase (HsGlce) epimerizes D-glucuronic acids immediately adjacent to *N*-sulfoglucosamine units to L-iduronic acid (IdoA). A series of *O*-sulfotransferases can then add sulfate: uronyl 2-*O*-sulfotransferase (Hs2st) adds sulfate at C2 of the iduronic acids (and less frequently to glucuronic acids), 6-*O*-sulfotransferases (Hs6st1-3) add sulfate at C6 of the N-sulfoglucosamine units and less frequently to *N*-acetylglucosamine, and 3-*O*-sulfotransferases (Hs3st1, 2, 3a, 3b, 4, 5, 6) add sulfate at C3 of glucosamine units (N-sulfated or N-unsubstituted). As shown in the top of the figure by red shading, the modifications occur in clusters of variable length (N-sulfated or NS domains), which are interspersed by unmodified domains (N-acetylated or NA domains). The regions at the junction of these domains are sometimes called NA/NS domains (not shown) because the extent of processing is less. The modified domains make up binding sites for protein ligands as depicted for antithrombin, FGF and FGF receptor. The HS chains can be further modified once they arrive at the cell surface or in the ECM by two endosulfatases (Sulf1 and Sulf2), which remove specific sulfate groups located at C6 of glucosamine units, or by the action of extracellular heparanase or extracellular proteases (not shown). (Figure adapted from Bishop et al. 2007; reprinted with permission from Nature Publishing Group © 2007.)

three to five chains (e.g., syndecan). Furthermore, the stoichiometry of GAG chain substitution can vary depending on source and growth conditions. "Part-time" proteoglycans can exist with or without a GAG chain (Table 1). (3) Some proteoglycans contain other types of glycans (e.g., asparagine-linked [*N*-linked] and serine/threonine-linked [*O*-linked] mucin-type chains). Some proteoglycans, such as syndecan-1, contain both HS and chondroitin/dermatan sulfate, another type of GAG. Other types of posttranslational modifications can occur (e.g., phosphorylation on cytoplasmic domains of *trans*-membrane proteoglycans). (4) The number and sulfation state of the chains can vary according to growth conditions and in response to growth factors. (5) The arrangement of negatively charged sulfate groups and the orientation of the carboxyl groups specify the location of ligand-binding sites. Furthermore, the sulfated residues are clustered in regions along the chain containing mixtures of iduronic acid and glucuronic acid (NS domains, Fig. 2) and are separated by nonsulfated domains rich in glucuronic acid (NA domains). (6) The pattern of sulfation, extent of uronic

acid epimerization, and organization of the modified residues is generally thought to depend on the cell type in which HS is expressed rather than on the nature of the core protein (Kato et al. 1994). Thus, the overall composition of HS on different core proteins expressed by the same cell appears to be similar, but great variation occurs between cell types. This concept might be an oversimplification, as some variation has been suggested to occur in ligand-binding properties and composition dependent on the core protein (Shworak et al. 1993; Tveit et al. 2005).

GENERALIZATIONS ABOUT THE INTERACTION OF PROTEIN LIGANDS WITH HS PROTEOGLYCANS

HSPGs bind to many ligands, usually via the HS chains. In fact heparin, a highly sulfated form of HS, is often used as an "affinity" matrix for purifying proteins, and many of the growth factors in use today were purified by heparin affinity chromatography. Heparin-binding ligands include growth factors, cytokines, chemokines, enzymes, enzyme inhibitors, and extracellular matrix proteins. In other areas of glycobiology, glycan-binding proteins are referred to as "lectins," a designation based on the presence of a carbohydrate recognition domain defined by characteristic protein folds or sequence motifs indicating their membership in an evolutionarily conserved gene family (Varki et al. 2009). In contrast, proteins that bind to HS appear to have evolved by convergent evolution; that is, they do not possess a specific fold or recognizable amino acid sequence pattern (Esko and Linhardt 2009). Heparin-binding sites often occur on the external surface of proteins or in shallow grooves lined with positively charged amino acids. Attempts have been made to define "consensus" sequences in heparin-binding proteins based on content and spacing of positively charged amino acid residues within linear sequences (Cardin and Weintraub 1989; Hileman et al. 1998; Capila and Linhardt 2002). However, the binding site for HS is often defined by positive residues contributed by noncontiguous segments of the protein. Although electrostatic interactions contribute much of the binding energy, hydrogen-bonding, van der Waal interactions, and hydrophobic effects also participate (Conrad 1998; Capila and Linhardt 2002).

The binding of a ligand to HS follows the same principles that underlie the interaction of other macromolecules, but the following considerations are important.

- Dissociation constants for HS-dependent ligands range from millimolar to nanomolar values. Many growth factors bind with high affinity (e.g., fibroblast growth factors, FGFs), whereas many matrix proteins bind with low affinity (e.g., fibronectin). However, affinity does not dictate selectivity; some low affinity ligands achieve high avidity through dimerization (e.g., chemokines) or by clustering (e.g., fibronectin fibrils).
- The HS chains can facilitate diffusion of ligands by allowing them to bind and slide or dissociate/reassociate through adjacent binding sites (mass action).
- Binding can lead to a conformational change in the protein. The best-studied example is the allosteric effect of heparin on antithrombin.
- HS can act as a template to approximate two proteins next to each other. Antithrombin inactivation of thrombin serves as the paradigm for this type of interaction, but other examples include the association of some growth factors with their receptor tyrosine kinases (e.g., FGF with FGF receptors).

In addition to the HS chains, the protein core of HSPGs can also bind ligands. For example, the *Drosophila* glypican ortholog, Dally, can directly interact with a number of morphogens, such as decapentaplegic (Dpp) and bone morphogenetic factor 4, in the absence of HS chains (Kirkpatrick et al. 2006). Expression of HS-deficient Dally can rescue several mutant phenotypes in Dally-deficient flies, indicating that a number of biologically relevant functions are mediated by the glypican protein core independently of HS. In mammals, the glypican-3

core protein interacts with hedgehog (Hh) independently of HS, and *Gpc3*-null embryos display increased Hh signaling, which might explain the overgrowth phenotype observed in patients lacking glypican-3 (Simpson–Golabi–Behmel syndrome) (Capurro et al. 2008). As discussed below, a peptide sequence in the core protein of syndecan-1 interacts with αVβ3 and αVβ5 integrins and modulates cell adhesion (Beauvais et al. 2004; McQuade et al. 2006). Finally, perlecan, collagen XVIII, and agrin are large proteins composed of multiple, functionally independent domains that can bind to other matrix components and growth factors (Iozzo et al. 2009).

ON THE SPECIFICITY OF BINDING

Although some ligands bind directly to the HSPG core proteins, the vast majority interact with sulfated domains within HS chains. Early studies of heparin and its interaction with antithrombin guided much of our thinking about the specificity of protein–HS interactions, but recent studies have broadened our view considerably. Heparin has high anticoagulant activity and higher-than-average overall sulfation (heparin contains aproximately 2.3 sulfate groups per disaccharide, whereas typical HS contains approximately 0.8 sulfate groups per disaccharide). The anticoagulant properties of heparin, however, do not depend on overall charge, but instead depend on a unique pentasaccharide with a specific arrangement of sulfate groups and uronic acid epimers, and requires a sulfate group positioned at C3 of the central glucosamine residue as shown in Figure 2 (Lindahl et al. 1980; Atha et al. 1985). This discovery suggested that the interaction of HS with other protein ligands might show similar selectivity.

Interestingly, 3-*O*-sulfated glucosamine residues are quite rare in HS, occurring about 1/20 disaccharides in heparin and less than 1/100 disaccharides in HS or not at all. Nevertheless, the 3-*O*-sulfotransferases comprise the largest family of HS sulfotransferases, with seven members (Fig. 2). Two of the enzymes can produce the antithrombin-binding sequence, whereas the others generate 3-*O*-sulfated sequences distinct in structure from the antithrombin-binding site, suggesting their purpose lies in the formation of binding sites for other ligands. One class of these sites interacts with glycoprotein gD of Herpes simplex virus-1 and appears to be required for infection (Shukla et al. 1999). Endogenous ligands include cyclophilin B (Vanpouille et al. 2007), FGF7 (Ye et al. 2001), and possibly the ectodomain of FGFR1 (McKeehan et al. 1999), but based on the large size of the 3-*O*-sulfotransferase family, other ligands undoubtedly exist.

Most ligands do not require 3-*O*-sulfation, but the study of heparin-antithrombin interaction set the stage conceptually for searching for specific arrangements of sulfated sugars to achieve selective binding. This technically challenging problem was initially approached by fractionating HS into pools that bound to the ligand or that did not, followed by compositional analysis or partial sequencing. Unfortunately, little variation in composition was noted, most likely because binding sites for most proteins represent only five to 12 sugars. To circumvent this problem, partially cleaved preparations were employed, eventually leading to the identification of minimally sized oligosaccharides that bound with reasonable affinity and that in some cases would initiate a biological response. Examples include, but are not limited to, FGF2 (Guimond et al. 1993; Maccarana et al. 1993), platelet-derived growth factor (Feyzi et al. 1997), platelet factor 4 (Maccarana and Lindahl 1993; Stringer and Gallagher 1997), MIP1α (Stringer et al. 2002, 2003), hepatocyte growth factor (scatter factor) (Lyon et al. 1994; Ashikari et al. 1995), vascular endothelial growth factor (Soker et al. 1994; Ono et al. 1999; Ashikari-Hada et al. 2005; Robinson et al. 2006), lipoprotein lipase (Parthasarathy et al. 1994; Spillmann et al. 2006), amyloid (Lindahl and Lindahl 1997), and L-selectin (Norgard-Sumnicht and Varki 1995; Wang et al. 2002). When FGF1 was studied in detail, it was noted that a range of HS octasaccharides that varied in the number as well as the positions of individual sulfate groups could bind with varying affinity (Kreuger et al. 2001). Furthermore, the formation of complexes between

FGF1 and FGF receptors was promoted by a variety of saccharides of differing overall sulfate content. This apparent lack of specificity has a corollary in vivo; in *Drosophila* deletion of 2-*O*- or 6-*O*-sulfotransferases has no effect on FGF signaling and tracheal development, which depends on FGF (Kamimura et al. 2006). Inactivation of either enzyme results in elevated sulfation at other positions, suggesting a form of molecular compensation. Lack of both enzymes impairs FGF signaling and causes multiple patterning deficiencies, indicating that HS is essential but that the receptor ligand complex can accommodate differently sulfated oligosaccharides. It would appear that other ligand-receptor pairs might behave similarly, because many organs and tissues in mice bearing mutations in these sulfotransferases (or the epimerase that interconverts glucuronic acid to iduronic acid) develop normally (Table 3).

Nevertheless, specificity does exist. For example, mice lacking the 2-*O*-sulfotransferase suffer renal agenesis (Bullock et al. 1998), but other tissues develop normally. In the lacrimal gland, a decrease in overall sulfation of HS affects Fgf10-Fgfr2b signaling required for branching morphogenesis (Pan et al. 2008). Similar reduction in overall sulfation alters vasculogenesis (Jakobsson et al. 2006), tumor angiogenesis (Fuster et al. 2007), and vascular hyperpermeability (Xu et al. 2010a). Wnt signaling is specifically affected by removal of 6-*O*-sulfate groups on HS by a pair of cell surface endolytic-6-*O*-sulfatases (the Sulfs) that act at very restricted sites in the chain (Ai et al. 2003; Lamanna et al. 2008). Although the range of biological processes regulated in vivo by the Sulfs remains to be elucidated, growth factors including FGF, Wnt, and GDNF are clearly affected by their loss, resulting in developmental defects and early postnatal lethality (Ai et al. 2007; Holst et al. 2007). Thus, determining whether specific sequences mediate binding and biological action remains an active area of investigation.

Chemokines are a subset of cytokines that interact with HS (Lortat-Jacob 2009). These small (8–10-kDa) secreted proteins signal through G protein–coupled receptors on cell surfaces to control cell migration in development, lymphocyte homing, inflammation, and wound repair. Binding of chemokines to HSPGs allows their local retention, protection from degradation (Sadir et al. 2004), activation by oligomerization (Proudfoot et al. 2003), and presentation to leukocytes (Handel et al. 2005). Interestingly, chemokines usually occur as dimers with positively charged domains oriented in different configurations (Fig. 3). Although no cocrystal structures are currently available, molecular docking suggests that an HS chain might fit along the dimer interface or can span the heparin-binding domains oriented on opposite sides of the dimer. In the latter case, the distance between the two binding sites in the dimer exceeds the length of a typical sulfated domain in HS (around four to eight sugars). Thus, the preferred organization of the bioactive segment of a chain might consist of two short sulfated domains (NS domains, Fig. 2) separated by a nonsulfated linker of a specific length (NA domain) (Lortat-Jacob et al. 2002). Examples of other ligands in which binding sites require extended or discontinuous domains include IFN-γ (Lortat-Jacob et al. 1995) and platelet factor 4 (Stringer and Gallagher 1997), and may include other paired systems, such as FGF/FGFR (Mulloy and Linhardt 2001) or VEGF/VEGFR/Nrp1 (Grunewald et al. 2010). Generating this arrangement would presumably require spatial control over the assembly process, but how this is achieved in vivo is not known (Lindahl and Li 2009).

PROTEOGLYCANS AS CORECEPTORS

In 1991, Yayon et al. (1991) and Rapraeger et al. (1991) reported that cell surface HSPGs facilitate the formation and signaling of FGF2-FGF receptor complexes (Fig. 4A). As described above, exogenous heparin or HS can also potentiate the formation of complexes of FGF with FGF receptors. In this context, the HSPG or heparin is considered a "coreceptor," because its function is to aid the formation of ligand-receptor complexes either through conformational change of ligand and/or receptor or by acting as a template to approximate ligand

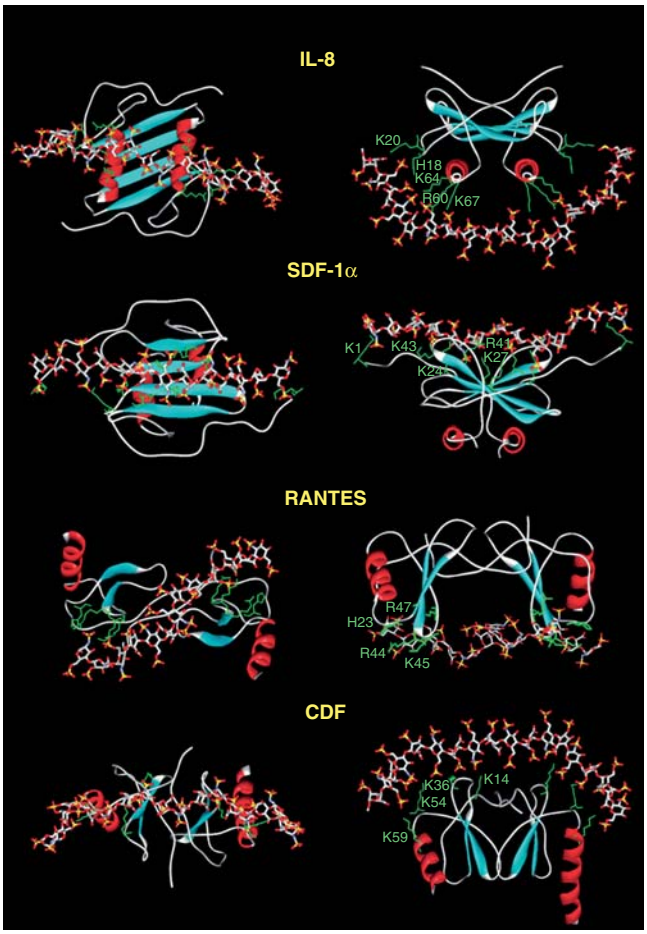

Figure 3. Docking of HS to chemokines. Molecular modeling was used to dock a fully sulfated heparin-like chain to several chemokines. The proteins are represented by ribbons except for the side chains of the basic amino acids directly involved in polysaccharide binding (green). The heparin molecule is represented by sticks. (Data from Lortat-Jacob et al. 2002.)

and receptor. In cells, activation usually occurs in *cis* by HSPGs expressed on the same cell as the signaling receptor (Fig. 4A). For example, altering HS sulfation selectively in endothelial cells using the Cre-lox system decreases pathological angiogenesis in vivo (Fuster et al. 2007). Similarly, altering HS in mammary epithelial cells has a striking impact on lobuloalveolar development, in spite of expression of HS in surrounding stromal cells (Crawford et al. 2010). Studies of lens development (Pan et al. 2006), branching morphogenesis in the lacrimal gland (Pan et al. 2008; Qu et al. 2011), axon guidance, and development of the central nervous system (Inatani et al. 2003; Yamaguchi et al. 2010) also suggest that HSPGs act as coreceptors in a cell-autonomous manner.

Nevertheless, the observation that exogenous heparin, HS or HSPGs can activate FGF signaling raised the possibility that HSPGs on one cell type might activate signaling in *trans* on adjacent cells. Kraemer and Yost provided the first in vivo evidence for *trans*-activation in their studies of left–right development in *Xenopus*. In this system ectodermal syndecan-2 transmits in a non-cell-autonomous fashion

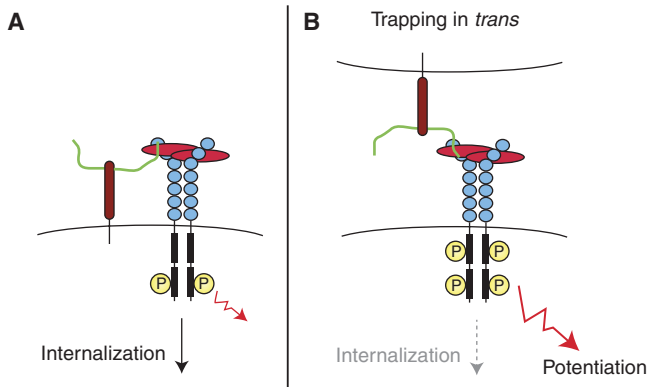

Figure 4. Model for *trans*-activation of VEGF receptor by HSPGs. (*A*) Resident plasma membrane HSPGs can mediate VEGF interactions with its receptor in *cis*, inducing cell signaling and subsequent internalization of the complex. (*B*) HSPGs from an adjacent cell can also mediate VEGF interactions with its receptor in *trans*, delaying internalization of the signaling complex and enhancing VEGF response. (From Jakobsson et al. 2006; reprinted with permission from Elsevier © 2006.)

left–right information to migrating mesoderm via a growth factor signaling pathway (Kramer and Yost 2002). More recently, Jacobsson et al. analyzed VEGF signaling in embryoid bodies derived from mutant stem cell populations that were either deficient in HS biosynthesis or in VEGF receptor expression (Jakobsson et al. 2006). Although both mutant stem cell lines were unable to support VEGF signaling, generation of chimeras restored VEGF signaling and response. A model for how HSPGs might activate VEGF receptors in *trans* is shown in Figure 4B. As discussed below, germline stem cells are maintained in a stem cell niche by short-range *trans*-signaling mediated by glypicans expressed in niche cells acting on the stem cells (Hayashi et al. 2009). *Trans*-activation of receptors by HSPGs could potentially elicit stronger signaling responses by trapping the receptor at the cell surface in an activated state. Overall, the ability of HSPGs to *trans*-activate adjacent cells represents a novel type of cellular cross talk and may play an important role in regulating cellular differentiation and response during development.

Secreted HSPGs also can act in a non-cell-autonomous manner (e.g., by directly eliciting signaling responses in nearby cells). The HSPG agrin acts in this way to induce postsynaptic differentiation at the neuromuscular junction (Bezakova and Ruegg 2003). To carry out this function, agrin is secreted by motor neurons where it activates the receptor muscle-specific receptor tyrosine kinase (MuSK) on adjacent muscle cells. Signaling cascades induced by MuSK signaling result in cytoskeletal reorganization and subsequent aggregation of acetylcholine receptors on muscle cells, priming the neuromuscular junction for activation. Recent studies have shown that proteolytic processing of agrin at the neurological synapses by neurotrypsin releases an active carboxy-terminal fragment that subsequently induces the formation of dendritic filopodia on hippocampal neurons (Matsumoto-Miyai et al. 2009). Other proteoglycans can also undergo proteolytic processing to release bioactive domains that can act in an endocrine fashion, including collagen XVIII (Marneros and Olsen 2001) and perlecan (Bix and Iozzo 2008).

The coreceptor function of some proteoglycans, such as the *trans*-membrane syndecans, can be dynamically regulated by modulating their association with the cell surface through a process known as shedding (Bernfield et al. 1999; Manon-Jensen et al. 2010). Syndecan shedding is mediated by matrix metalloproteinases (MMP1, MMP7, MMP9, ADAM17) (Fitzgerald et al. 2000; Li et al. 2002; Endo et al. 2003; Ding et al. 2005; Brule et al. 2006;

Pruessmeyer et al. 2010). Mechanistically, induced shedding of syndecan-1 appears to involve its cytoplasmic tail, which binds to Rab5, a small GTPase that regulates intracellular trafficking and signaling events (Hayashida et al. 2008). Rab5 may regulate shedding by inducing dissociation of syndecan-1 from β1 integrin (see below), thus exposing a normally cryptic cleavage site. Shedding has a major effect on the localization and signaling capacity of HS-bound ligands (Kato et al. 1998; Park et al. 2000b). For example, syndecan-1 shedding modulates chemokine-dependent inflammatory processes in models of tissue damage caused by noninfectious agents (Li et al. 2002; Xu et al. 2005b; Hayashida et al. 2009b). Syndecan-1 shed by implanted tumor cells can exert biologic effects distal to the primary tumor, driving formation of osteoclasts and bone destruction via bound heat-labile factors (Kelly et al. 2010). Some microorganisms can enhance host cell proteolytic shedding of syndecan-1 resulting in enhanced bacterial colonization (Park et al. 2000a, 2001). In all of these models, the activity of shed syndecan ectodomains depends on the HS chains, suggesting their activity depends on ligands bound to the chains.

PROTEOGLYCANS AS ENDOCYTIC RECEPTORS

Although often overlooked, membrane HSPGs also act as endocytic receptors, and undergo constitutive as well as ligand-induced endocytosis (Williams and Fuki 1997; Belting 2003). Although the precise mechanism of endocytosis is unclear, it appears to occur independently of clathrin, caveolin, and dynamin, but in a lipid-raft-dependent manner involving vesicles of unusual composition (Zimmermann et al. 2005; Wittrup et al. 2010). Ligands bound to the HS chains "piggy back" into the cell through this route. The endocytic activity of HSPGs plays a significant physiological role in lipid metabolism. Recent genetic evidence showed that mice lacking syndecan-1 accumulate both liver-derived and dietary triglycerides in the form of remnant lipoprotein particles (Stanford et al. 2009). Altering the structure of HS by selective inactivation of sulfotransferases in hepatocytes led to the same phenotype and showed that the HS chains on syndecan-1 represent the binding site for the lipoproteins (MacArthur et al. 2007; Stanford et al. 2010).

Other membrane HSPGs, such as syndecan-2 and syndecan-4 and glypicans, also can mediate uptake of ligands in cultured cells (Fuki et al. 2000). Furthermore, uptake can be induced by FGF2 or by antibody-induced clustering (Fuki et al. 1997; Tkachenko et al. 2004; Zimmermann et al. 2005). Although recycling of internalized HSPGs has been observed (Fransson et al. 1995; Zimmermann et al. 2005), most HSPGs end up in lysosomes, where they undergo degradation by lysosomal proteases and exolytic glycosidases and sulfatases. Bound ligands also are degraded, which provides a mechanism for delivering nutrients to cells or for removal of bioactive factors from the environment. The formation of tissue gradients of morphogens (discussed below) may depend in part on continuous clearance of the ligands through an endocytic mechanism (Lander et al. 2002; Marois et al. 2006; Ren et al. 2009).

Viruses and other pathogens can exploit HSPGs to transit from the extracellular environment to the inside of cells. The membrane-penetrating peptide, HIV-tat, is released from HIV-infected cells and then enters surrounding cells using HSPGs (Green and Loewenstein 1988; Frankel and Pabo 1988). Based in part on HIV-tat, a number of cell-penetrating-peptides have been generated, typically rich in arginine or lysine residues that will facilitate interaction with HSPGs (Poon and Gariepy 2007). In addition, synthetic positively charged transporters, such as guanidinylated aminoglycosides have been prepared. Conjugation of these carriers to drugs, toxins, enzymes, oligonucleotides, as well as quantum dots can mediate delivery of cargo into the cells via HSPGs (Elson-Schwab et al. 2007; Sarrazin et al. 2010).

HSPGs can also mediate transcellular transport. Wang et al. showed HSPG-mediated chemokine transport across endothelial cells and its dependence on the sulfation state of the HS chains (Wang et al. 2005). As discussed below,

cell surface HSPGs are also engaged in cell adhesion, which is dependent on the interaction of HS chains with ECM proteins such as fibronectin. Whether cell attachment and endocytosis are mutually exclusive or perhaps mediated through different membrane proteoglycans is unclear.

PROTEOGLYCANS AS ADHESION RECEPTORS

HSPGs play several roles in cell adhesion and in the determination of cell shape. These processes depend on binding of cell surface HS to "heparin-binding" domains present in matrix proteins, such as fibronectin, laminins, vitronectin, thrombospondin, and some fibrillar collagens (Bernfield et al. 1999). Syndecan-4 provides an interesting example of a mechanical and functional link between the ECM and the actin cytoskeleton. Syndecan-4 is widely expressed during development and in most adult tissues and is a central component of focal adhesions (Oh and Couchman 2004). Fibroblasts lacking syndecan-4 have an altered actin cytoskeleton and multiple HS chains are required to cluster syndecan-4 on the plasma membrane (Gopal et al. 2010). Consistent with this idea, overexpression of syndecan-4 in CHO cells results in increased focal adhesion formation, organization of cytoskeletal stress fibers, and decreased cell motility (Longley et al. 1999). The activity of syndecan-4 depends on multimerization, which occurs via recruitment of PIP_2, activation of PKC-α, and downstream signaling through the RhoA pathway (Oh et al. 1997a,b,c). Interestingly, multimerization of syndecan-4 is prevented by phosphorylation of its cytoplasmic tail by PKC-δ in response to FGF2 signaling (Murakami et al. 2002). FGF2 is a mitogen and during proliferation, cells need to detach to undergo cytokinesis. Thus, syndecan-4 has a central role in coordinating cytoskeletal changes that take place during adhesion and cell proliferation.

As described in other articles (e.g., Schwartz 2010; Campbell and Humphries 2011; Geiger and Yamada 2011; Watt and Fujiwara 2011), integrins mediate various interactions between cells and ECM components. Integrins can recognize short peptide sequences (e.g., RGD) present in many ECM proteins, and binding leads to activation, intracellular signaling via kinases and other enzymes, and focal adhesion formation (Cox et al. 2006). When fibroblasts attach and spread in response to fibronectin fragments containing the RGD site, the formation of focal adhesions and stress fibers can often take place only if the heparin-binding domain of the fibronectin is also present (Saoncella et al. 1999; Woods et al. 2000; Morgan et al. 2007). The HS chains of syndecan-4 bind to fibronectin, which together with integrin, induce formation of focal adhesions and stress fibers (Fig. 5).

Syndecan-1 (and syndecan-4) also can regulate activation of αvβ3 and αvβ5 integrin by way of interaction of the extracellular domain of the proteoglycan with the β-integrin subunit (Beauvais et al. 2004, 2009; McQuade et al. 2006). The engagement of these receptors occurs outside the cell via a defined peptide segment in syndecan-1, but the activation mechanism is cytoplasmic and occurs via a talin-dependent, inside-out signaling pathway that requires syndecan-1 clustering. The HS chains of syndecan-1 are required, presumably by facilitating syndecan-1 clustering along aggregated ECM components.

HSPGs also can facilitate cell–cell adhesion. During the inflammatory response, endothelial HS interacts with L-selectin on passing leukocytes to aid in the initial tethering of leukocytes to the lumenal surface of the endothelium (Wang et al. 2005; Celie et al. 2009). This interaction may depend on the presence of an unusual N-unsubstituted glucosamine unit in HS (Norgard-Sumnicht and Varki 1995) perhaps in combination with fully sulfated domains (Smits et al. 2010). Interestingly, heparinoids administered intravenously to mice dramatically reduce leukocyte infiltration in response to inflammation (Wang et al. 2002) by disruption of endogenous HS-selectin (Wang et al. 2005) or sialyl $Lewis^{a/x}$-selectin interactions (Koenig et al. 1998; Stevenson et al. 2007). After passing the endothelial cell layer, leucocytes encounter the vascular

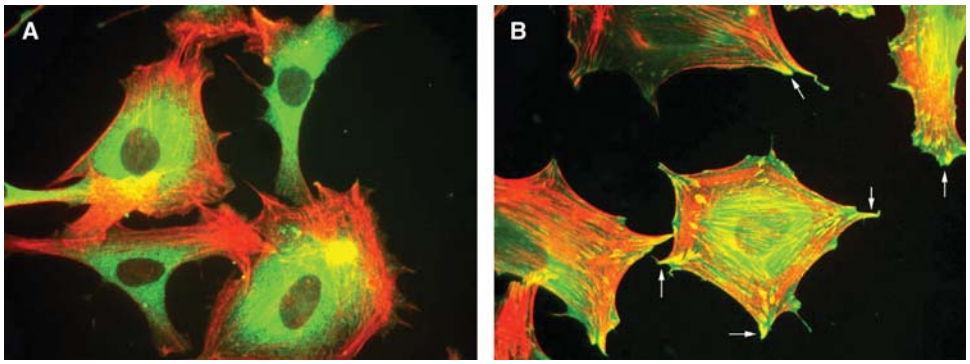

Figure 5. The role of syndecan-4 in focal adhesion. (*A*) Fibroblasts attach and spread through α5β1 integrin on coverslips coated with the integrin-binding domain of fibronectin but they do not form focal adhesions. (*B*) Focal adhesions (arrows) form only after engagement of syndecan-4 HS chains after the addition of the heparin-binding domain (HepII) from fibronectin. (Data for image from Okina et al. 2009.)

basement membrane. Leukocyte migration through this barrier is considered to involve local degradation by matrix metalloproteinases and secreted heparanase, which may be necessary for dissolution of HSPG in the basement membrane (Vreys and David 2007; Li and Vlodavsky 2009).

PROTEOGLYCANS REGULATE GROWTH FACTOR BINDING TO ECM AND CELL MIGRATION

The ECM provides a structural network for mediating and regulating cellular movement (e.g., during development and wound repair). One of the ways the ECM regulates cell migration is to directly bind growth factors, such as platelet-derived growth factor (PDGF), providing directional and stimulatory cues for moving cells (Smith et al. 2009). Interestingly, the association of PDGF with ECM appears to be dependent on HS, but does not involve direct binding to HS (Symes et al. 2010). Similarly, HS-dependent interactions between fibronectin and VEGF have been reported (Mitsi et al. 2006). The mechanism by which HS regulates the binding of growth factors to fibronectin appears to stem from its ability to induce the transition of fibronectin from a globular form to a more stable extended form, revealing growth factor–binding sites (Mitsi et al. 2008). This activity depends on the size and composition of the chains, as shown by studies in which only heparin chains longer than 22 saccharides and with sulfation at the 6-O- and N-positions of glucosamine units retained the ability to modify fibronectin structure and allow VEGF binding (Mitsi et al. 2006).

Tissue-specific expression of different proteoglycans during zebrafish embryogenesis also has been shown to play a role in determining the structure and function of the extracellular matrix. Syndecan-2 expression in the extraembryonic yolk syncytial layer induces fibronectin and laminin matrix assembly throughout the embryo and directs primordial cell migration (Arrington and Yost 2009). Interestingly, overexpression of syndecan-2 in the embryo does not rescue embryonic defects resulting from yolk syncytial layer deficiency, suggesting that proteoglycans in specific cell types can act in a unique manner because of either positional or structural differences. Other studies have also shown that the loss of specific proteoglycans such as syndecan-4 in *Xenopus laevis* can have adverse effects on neural crest cell migration (Matthews et al. 2008) and convergent extension movements (Munoz et al. 2006). These model systems provide a powerful empirical approach for determining the participation of HSPGs in matrix deposition and cell migration.

PROTEOGLYCANS AND BARRIER ACTIVITY

HSPGs were long thought to be a filtration barrier for charged macromolecules in the kidney, but recent studies cast doubt on this idea. The basement membrane of the kidney filtration structure, the glomerulus, contains HSPGs such as agrin, perlecan, and collagen XVIII, and HS accounts for much of the negative charge in the glomerular basement membrane (GBM) (Miner 1999; Raats et al. 2000). Early studies in which HS was removed by perfusion of rat kidneys with heparinase suggested that HS was essential for filtration of large charged proteins such as ferritin and albumin (Kanwar et al. 1980). Correlations of reduced HS levels in the GBM, increased heparanase expression and proteinuria were also made in patients with various kidney diseases such as diabetic nephropathy, further suggesting a putative barrier function for GBM HS (Makino et al. 1992; Tamsma et al. 1994; van den Hoven et al. 2006; Wijnhoven et al. 2006). However, confusion arose when subsequent in vivo studies failed to substantiate that removal of GBM HS with heparinase can result in acute proteinuria, and in fact enzyme digestion actually prevented proteinuria induced by removal of sialic acids (Wijnhoven et al. 2007a,b). Furthermore, tissue-specific deletion of the major GBM HSPGs in mice does not cause proteinuria (Rossi et al. 2003; Harvey et al. 2007; Goldberg et al. 2009), nor does complete ablation of HS biosynthesis in mouse podocytes until 8 months of age when proximal tubule abnormalities become prevalent (Chen et al. 2008). Taken together, it appears that the actual function of HS in the glomerulus is associated with its control of podocyte behavior and not as an ultrafiltration barrier.

Although HSPGs do not appear to play a substantial role in permselectivity in the kidney, cell surface HSPGs of the syndecan family have been shown to play a major role in maintaining the barrier integrity of the intestinal epithelium (Bode et al. 2006, 2008). The loss of syndecan-1 or its GAG chains from the intestinal epithelium has been shown to correlate with an efflux of plasma proteins into the intestinal lumen, causing a potentially lethal condition known as protein-losing enteropathy (PLE) (Murch et al. 1993, 1996; Westphal et al. 2000). The relationship between loss of syndecan-1 and PLE appears to be because of the ability of syndecan-1 to down-regulate inflammatory cytokines, such as IFN-γ and TNF-α, which work together to disrupt interepithelial integrity. Thus, it is thought that loss of syndecan-1 or its HS chains in the intestine exposes the epithelium to cytokine insult, resulting in the disruption of cell–cell interactions and PLE (Bode et al. 2008). Syndecan-1 may play a direct role in sealing the gaps between intestinal epithelial cells, acting as a physical barrier to prevent protein leakage (Bode et al. 2008). Importantly, the administration of nonanticoagulant heparin to syndecan-1-deficient mice as well as to one patient with PLE has proven effective at correcting protein leakage (Bode et al. 2008; Liem et al. 2008), suggesting that patients suffering from barrier dysfunction diseases associated with HSPG deficiency could be treated similarly.

MORPHOGEN AND CHEMOKINE GRADIENTS

Morphogens are signaling molecules that are expressed in restricted regions of tissue and can form gradients that specify cellular differentiation and patterning during development. Studies of morphogen diffusion in the *Drosophila* wing disk have shown that some morphogens, such as wingless (Wg), hedgehog (Hh) and Dpp, require HS for effective diffusion and will not cross cellular regions deficient in HS (Jackson et al. 1997; The et al. 1999; Tsuda et al. 1999; Baeg et al. 2001; Bornemann et al. 2004). Similar studies in the *Drosophila* wing disc revealed that glypicans are essential for morphogen diffusion (Belenkaya et al. 2004; Han et al. 2005; Yan and Lin 2009). These findings suggest a model of morphogen mobility known as restricted diffusion, where morphogens are transferred from one HSPG to the next at the cell surface, moving from regions of high concentration to regions of low concentration along a path that is defined by the

interacting ligand (Yan and Lin 2009). Although this model might describe the mechanism by which HSPGs regulate short-range morphogen gradients, other modes of morphogen transmission are thought to exist. For example, in studies of morphogen diffusion in the *Drosophila* wing disc, Eaton and colleagues have described exocellular vesicles (argosomes) and lipoprotein particles (Lipophorin) that can mediate the transmission of morphogens over long distances (Greco et al. 2001; Eugster et al. 2007). Interestingly, the packaging of morphogens, such as Wg into argosomes, is HS-dependent and membrane-associated glypicans can recruit Lipophorin containing lipid-modified forms of Hh and Wg to disc tissue. Lander has discussed in detail the complexity of factors that can affect the shape of morphogen gradients and other ways that HSPGs participate in this process (Lander et al. 2002; Lander 2007).

Glypicans may play an important role in morphogen gradient formation because of their mode of attachment to the cell surface. Unlike other proteoglycans, glypicans are bound to the cell membrane via a GPI anchor, allowing diffusion to occur in the outer leaflet of the plasma membrane and affiliation with specific membrane structures such as lipid rafts (Taylor et al. 2009; Gutierrez and Brandan 2010). The ability of glypicans to localize into lipid rafts may allow them to associate more directly with a number of morphogens, such as Hh and Wg, which are themselves lipidated (Rietveld et al. 1999; Zhai et al. 2004). It is also interesting to note that the GPI anchor of glypicans can be cleaved from the cell surface by the hydrolase Notum (Kirkpatrick et al. 2004; Kreuger et al. 2004), a process that may impact the ability of these proteoglycans to regulate morphogen gradients. In support of this idea, overexpression of a secreted form of glypican that lacks a GPI anchor dramatically expands the range of the Hh activity in the *Drosophila* wing disc (Takeo et al. 2005). The reason for this expansion is unclear, but may be caused by a stabilizing effect of this secreted proteoglycan on Hh as it diffuses from its source. Alternatively, secreted glypican may interfere with Hh posttranslational processing events, such as cholesterol modification. HSPGs can modulate morphogen mobility by promoting their association with modifier enzymes such as ADAMs (a disintegrin and metalloproteinases) and transglutaminases (Dierker et al. 2009a,b). Whether the ability of HSPGs to mediate restricted diffusion and morphogen modification represents distinct mechanisms for the control of gradients is currently unclear.

One should also keep in mind that many other factors diffuse through the ECM en route to their final destinations and therefore would encounter HSPGs on the surfaces of cells, in the interstitial matrix or in a basement membrane. For example, lipoprotein lipase is expressed by adipocytes and skeletal and cardiac myocytes, but its site of action is on the lumenal side of the capillary endothelium in resident blood vessels. A recent study has shown that deletion of collagen XVIII results in chylomicronemia caused by decreased presentation of the lipase in the vasculature (Bishop et al. 2010). Because deficiency of collagen XVIII causes thickening of basement membranes (Utriainen et al. 2004), the decreased presentation of the lipase might be caused by delayed diffusion through the basement membrane underlying capillaries in tissues involved in lipolysis. Plasma lipids are normal in perlecan mutants lacking the HS attachment sites (Tran-Lundmark et al. 2008; Bishop et al. 2010), indicating specificity might exist in the interaction of the lipase with HSPGs in the matrix.

STEM CELL NICHE

The generation, maintenance and repair of different tissues during development is regulated by stem cell populations that reside in defined cellular microenvironments known as stem cell niches. These niches are essential for determining the ability of stem cells to retain a self-perpetuating pluripotent state or to differentiate into committed tissue specific progenitors (Nurcombe and Cool 2007). Interestingly, many of the signaling molecules involved in stem cell maintenance, such as Wnts and FGFs, are regulated by HSPGs (Sato et al. 2004; Xu et al. 2005a,c). Furthermore, embryonic

stem cells change the structure of their HS as they differentiate into specific lineages (Johnson et al. 2007; Baldwin et al. 2008).

To directly address the role of HSPGs in stem cell differentiation, mouse embryonic stem cells with mutations in HS biosynthesis have been studied. Embryonic stem cells that lack HS because of *Ext1* gene deficiency are incapable of differentiation on removal of leukemia inhibitory factor, apparently caused by a defective response to FGF (Kraushaar et al. 2010). These findings were corroborated by studies in embryonic stem cells lacking *Ndst1/2*, which also cannot differentiate in response to FGF because of reduced sulfation (Lanner et al. 2010). In addition, mouse embryonic stem cells deficient in *Ndst1/2* were found to be unable to respond to VEGF, preventing their differentiation into blood capillary structures (Jakobsson et al. 2006). Taken together, these studies substantiate the importance of HS in stem cell differentiation at least ex vivo.

To address how HSPGs might regulate stem cells in their native cellular environments, Nakato and colleagues examined whether glypican participated in the maintenance of stem cells in the *Drosophila* germline stem cell niche. Interestingly, this function appears to be related to the ability of glypicans to restrict the localization and activity of the morphogen Dpp to the outer boundary of the niche (Hayashi et al. 2009). Stem cells directly adjacent to this Dpp-rich pocket were shown to be activated in *trans* by this morphogen and remained pluripotent. Daughter cells that were not able to physically associate with this region remained resistant to Dpp signaling and subsequently underwent differentiation. These findings will likely have important implications for stem-cell-based treatments of disease and for the design of synthetic matrices for stem-cell-based tissue engineering.

CONCLUDING REMARKS

The purpose of this article was to provide an overview of HSPGs and their biological roles in the ECM. As described above, HSPGs bind many ligands, modulate numerous cellular activities, and aid in tissue architecture and physiology. The examples selected for presentation represent only a subset of activities associated with HSPGs. However, it is striking that so many essential activities appear to be regulated by such a small family of macromolecules. Understanding how cells regulate the expression and composition of HSPGs to achieve these diverse activities in a coordinated fashion is a major biological problem to solve. The problem may be as complex as unraveling the genetic code, given the enormous complexity of heparan sulfate.

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