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Syndecan family of cell surface proteoglycans: developmentally regulated receptors for extracellular effector molecules

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Abstract. Syndecans are a family of integral membrane proteoglycans with conserved membrane-spanning and intracellular domains but with structurally distinct extracellular domains (ectodomains). They are known to function as heparan sulphate co-receptors in fibroblast growth factor signalling as well as to link cells directly to the extracellular matrix. These and other biological activities of syndecans involve specific interactions of the heparan sulphate side chains of syndecans with cytokines and extracellular matrix proteins. Four different vertebrate syndecans, designated as syndecans 1–4 (or syndecan, fibroglycan, N-syndecan and amphiglycan, respectively), are known. During embryonic development, syndecans have specific and highly regulated expression patterns that are distinct from the expression in adult tissue, suggesting an active role in morphogenetic processes. The developmental expression of syndecans is particularly intense in mesenchymal condensates and at epithelium mesenchyme interfaces, where a number of heparan sulphate-binding cytokines and matrix components are also expressed in a regulated manner, often spatially and temporally co-ordinated with the syndecan expression. Recent evidence indicates that the regulation of heparan sulphate fine structure (mainly the number and arrangement of sulphate groups along the polymer) provides a mechanism for the cellular control of syndecan-protein interactions. Furthermore, morphogenetically active cytokines such as fibroblast growth factor-2 and transforming growth factor-β participate in the regulation of syndecan expression and glycosaminoglycan structure. This review discusses the developmental expression and binding functions of syndecans as well as the molecular regulation of specific heparan sulphate-protein interactions.

Key words. Syndecan; heparan sulphate; proteoglycan; growth factor; extracellular matrix; differentiation; tissue interaction.

Introduction

Morphogenetic processes during development as well as in diseases, such as cancer and inflammation, are characterised by profound alterations in the cellular recognition of neighbouring cells and extracellular effector molecules. Cell surface heparan sulphate proteoglycans (HSPGs; for reviews see refs 4, 15, 19, 23, 37, 44) bind several extracellular matrix (ECM) molecules and growth factors. There are at least two types of cell surface PGs. Syndecans, betaglycan and CD44 are integral membrane proteins, while glypican[16] and cerebroglycan[96] are linked to the cell surface by a glycosyl-phosphatidylinositol linkage. Both the glycosaminoglycan (GAG) moieties and the core proteins of cell surface PGs are involved in the recognition of extracellular effector molecules. For example, betaglycan bind transforming growth factor-β (TGF-β) via its core protein and fibroblast growth factor-2 (FGF-2) via its HS chains[1]. The core protein of CD44 recognises a hexasaccharide sequence in hyaluronan[108] whereas its chondroitin suffate (CS) chains bind to type I collagen[115], fibronectin and laminin[39]. Some PGs, like CD44, betaglycan and invariant chain (that acts as a regulator of class II MHC molecule function in T lymphocytes[99]), also occur in a GAG-free form being thus 'part-time' PGs. Some of the 'HSPGs' discussed in this review may also contain CS either exclusively or in combination with HS (hybrid PGs). For example, CD44 from haematopoietic cells is a CSPG[95], whereas its differentially-spliced variant found in epithelial cells is a HSPG[8]. In syndecan-1, the core protein contains mainly HS but, at least in mammary epithelial cells, also about 20% of CS[74]. The precise regulation of the PG core protein glycanation (HS or CS attachment) is not known. A recent report utilising artificial xyloside primers for GAG synthesis suggests that the attachment of CS is the 'default' choice of the synthetic machinery and that certain core protein features such as hydrophobic residues would favour substitution with HS[22]. This and other possibilities are discussed in a recent review by Lindahl and co-workers (see ref. 50 and references therein). The current paper focuses on the developmentally regulated expression and functional characteristics of syndecans,

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a family of cell surface HSPGs. The biological functions of syndecans as receptors for extracellular molecules as well as the underlying molecular recognition mechanisms are also discussed[93].

Syndecan family of cell surface proteoglycans

At present, four different vertebrate syndecans are known (designated as syndecans 1–4, see fig. 1; for review, see refs 4, 19, 37). The name 'syndecan' is based on two Greek words $\sigma\upsilon\nu\delta\varepsilon\iota\nu + \gamma\lambda\upsilon\chi\upsilon\zeta$. The first word (syndein) has the meaning 'to keep together by binding' and originates from the verb $\sigma\upsilon\nu\delta\varepsilon\omega$, 'to bind together', whereas the second word (glychos) means 'sweet' giving rise to the term glycan. 'Fibroglycan', in turn, refers to the abundant occurrence of this PG in fibroblasts and in the connective tissue. 'N-syndecan' has been named so because it was first cloned from the rat brain and is expressed in the nervous system during development. The name 'amphiglycan' is derived from Greek $\alpha\mu\phi\iota$, meaning 'on both sides', since this PG is expressed by both epithelial and connective tissue cells. An alternate name for the same PG is 'ryuodocan' that is derived from the Japanese 'ryudousei' for fluidity. The investigators cloned this PG from an endothelial cell cDNA library and it is thought to participate in anticoagulative mechanisms acting in the cardiovascular system.

In *Drosophila melanogaster*, only one syndecan gene exists, coding for a 395 amino acid core protein that carries HS chains[93]. Each of the syndecans 1–4 has been cloned and sequenced from more than one species (see fig. 1). Inter- and intraspecies sequence comparisons indicate that the transmembrane and cytoplasmic domains of syndecans are structurally closely related to each other, sharing 51–82% amino acid homology and fully conserved intracellular tyrosine residues. Studies with syndecan-1 carrying truncated cytoplasmic domains indicate that the full-length intracellular part is required for normal inside-out transport and for proper apical-basolateral compartmentalisation[61]. In addition, a role for the cytoplasmic domains in the intracellular signalling has been suggested. Although tyrosine, serine and threonine are found in these domains, there is so far no data suggesting that they would be phosphorylated, e.g., upon binding to extracellular molecules. In immunocytochemical analysis, syndecan-1 colocalises with actin filaments[11,49,73] and in mouse mammary epithelial cells there is a fraction of syndecan-1 that remains cell-associated after combined detergent-salt extraction[73]. However, a careful study utilising cytoplasmically-truncated forms of syndecan-1 showed that the cytoplasmic domain is not required for the association with the detergent-insoluble cytoskeleton[62]. In the same study, the detergent-insolubility was shown to be sensi-

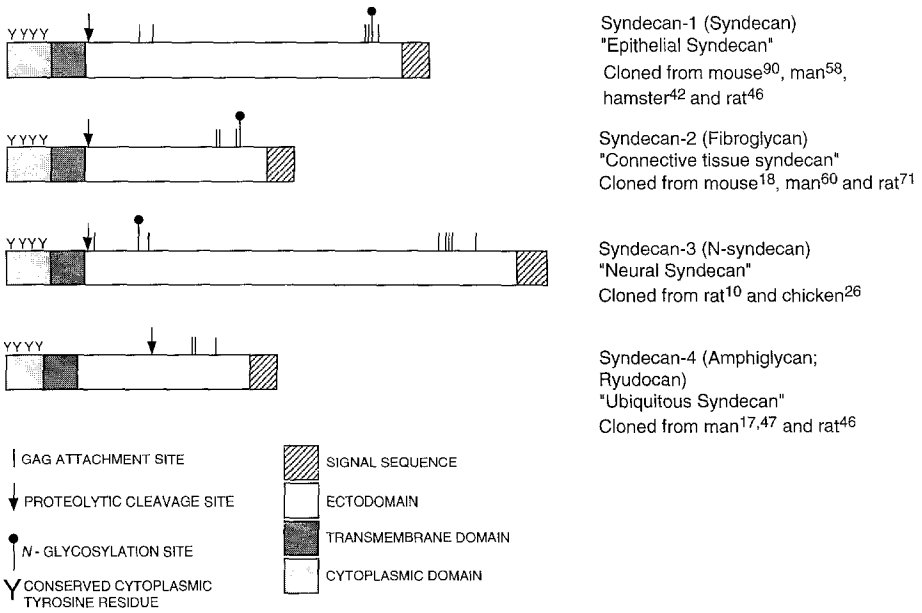

Figure 1. Schematic diagram showing structures of vertebrate syndecans.
In addition to syndecans 1–4, a syndecan cloned from *Drosophila melanagaster* is also known[93]. The *Drosophila* syndecan resembles most closely syndecan-3. Tissue localisation of different syndecans is also given but it should be noted that syndecans are more widely expressed during development. For example, the 'epithelial' syndecan-1 is expressed in mesenchymes and the 'neural' syndecan-3 is expressed in the limb buds. Conserved cytoplasmic tyrosine residues as well as putative glycosaminoglycan attachment, N-linked glycosylation and protease cleavage sites are shown. In syndecan-1 and -3 as well as in the *Drosophila* syndecan, the glycosaminoglycan attachment sites are found at both ends of the extracellular domain, whereas in syndecan-2 and -4 they are found in the amino-terminal, or distal, end of the core protein only. Throughout this review, a systematic nomenclature that designates all of these proteoglycans as syndecans and numbers them from one to four according the order of the elucidation of their core protein structure has been used. Alternate names are given in the figure.

tive to the removal of HS chains. The intracellular colocalisation patterns of syndecans may vary, since, in fibroblasts, syndecan-4 but not syndecan-2 is present at the focal adhesion sites, where it colocalises with vinculin as well as with $\beta 1$ and $\beta 3$ integrin subunits[117]. This (and other studies[30, 34] discussed below) may imply that syndecans have a cooperative role with integrins in cell adhesion, but the results do not provide direct evidence of interaction of syndecan with intracellular molecules.

In contrast to the cytoplasmic and the membrane-spanning domains, the extracellular or ectodomains are structurally distinct from each other (there is both a large interspecies variation in a particular syndecan gene and a large intraspecies variation between the genes/cDNAs coding for syndecans 1–4) although they share some homology at the putative GAG attachment regions. Unlike the ectodomains of the syndecan family, the extracellular domains of two phosphatidyl inositol-linked cell surface HSPGs, glypican and cerebroglycan, are more homologous, sharing, e.g., conserved cysteine residues[96]. The structural diversity of the syndecan ectodomains, and the fact that all known extracellular functions of syndecans occur via their GAG chains, may indicate that the most important function of the core proteins of syndecan ectodomains is to provide positional distribution of the GAG chains at the cell surfaces (see fig. 2). The common features of syndecan ectodomains are the occurrence of 1) serine-glycine residue pairs surrounded by sequences typical for GAG attachment and 2) basic residue pairs near the plasma membrane thought to be target sites for proteases such as plasmin and trypsin[38]. Ectodomains of syndecan-1, -2 and -3 also contain potential asparagine residues for oligosaccharide attachment. However, biochemical studies performed using epithelial cell-derived syndecan-1 did not reveal the presence of N- or O-linked oligosaccharides[116]. The syndecan family can further be divided into two subclasses, since syndecan-1 and -3, and correspondingly syndecan-2 and -4, are structurally more related to each other than to the two other syndecans. The most important distinction is that syndecan-1 and -3 contain a cluster of GAG-attachment sites at both ends of the ectodomain whereas syndecan-2 and -4 have their GAG attachment sites at the distal (amino-terminal) end of the ectodomain. Also the developmental expression patterns of syndecan-1 and -3, and similarly, of syndecan-2 and -4, resemble each other[43], thus further indicating the existence of two syndecan 'subfamilies'. It is possible that the presence of separate GAG clusters is important for the ability of syndecan-1 to bind fibronectin and FGF-2 simultaneously (cf. ref. 81; see below). Interestingly, a recent report by Kokenyesi and Bernfield[48] demonstrated that in NMuMG mammary epithelial and in Chinese hamster ovary cells, the carboxy-terminal GAG attachment sites of syndecan-1 are substituted with CS and the amino-terminal sites with HS. This may indicate that syndecan-3 could also exist as a hybrid HS/CS PG in some cells. However, comparative studies of binding properties of syndecans are needed to establish possible differences in their ability to interact with other molecules. Syndecan-3 also contains a threonine-serine-proline-rich domain located between the proximal and distal GAG attachment site clusters[10, 26]. Although in mucin-like proteins these types of domains are often heavily O-glycosylated, studies done with Schwann cell-derived syndecan-3 showed that its core protein is glycanated by HS only[10]. Taken together, there is no evidence so far that syndecan core proteins would carry N- or O-linked oligosaccharides.

Developmentally regulated expression of syndecans

An active role for a cell surface-associated acid mucopolysaccharide protein in the branching morphogenesis of the salivary gland was suggested over twenty years ago[3]. Today, it is generally accepted that cell surface PGs have an important role in the regulation of morphogenetic processes during embryonic development. The developmental importance of PGs is exemplified by the fact that the frequency of congenital GAG/PG synthesis deficiency is low, indicating that most of such mutations are likely to be lethal in utero. In addition GAGs other than HS are involved in the regulation of cell growth and differentiation. CS, for example, is necessary for normal retinal development[7]. In a developing rat retina model, enzymatic removal of CS resulted in premature retinal ganglion cell differentiation and aberrant axonal outgrowth[7].

Most of the studies dealing with the developmentally-regulated expression of syndecans are restricted only to syndecan-1, and it is therefore impossible to compare the expression pattern of different syndecans in morphogenetic processes in detail. In adult tissues, syndecan-4 has the widest expression pattern[17], whereas syndecan-3 appears to be almost solely expressed in embryonic and neonatal tissues[10]. Syndecan-1 is expressed in epithelia[29] and also in leukocytes such as B-lymphocytes[86] and myeloma cells[87]. Syndecan-2, originally cloned from fibroblasts[60] and hepatocytes[71], is particularly abundant at tissue level in parenchymal organs like liver and kidney[43].

In the developing mouse, syndecan-1 is first detected throughout the embryo at the four-cell stage[98]. After gastrulation, highest syndecan-1 expression is found in the ectoderm and in the endoderm[98], that differentiate into syndecan-1-positive epithelial structures in adults[29]. During secondary inductions occurring via epithelium-mesenchyme interactions, the inductive potential first resides in the presumptive epithelium which then induces mesenchymal condensation and, e.g., in develop-

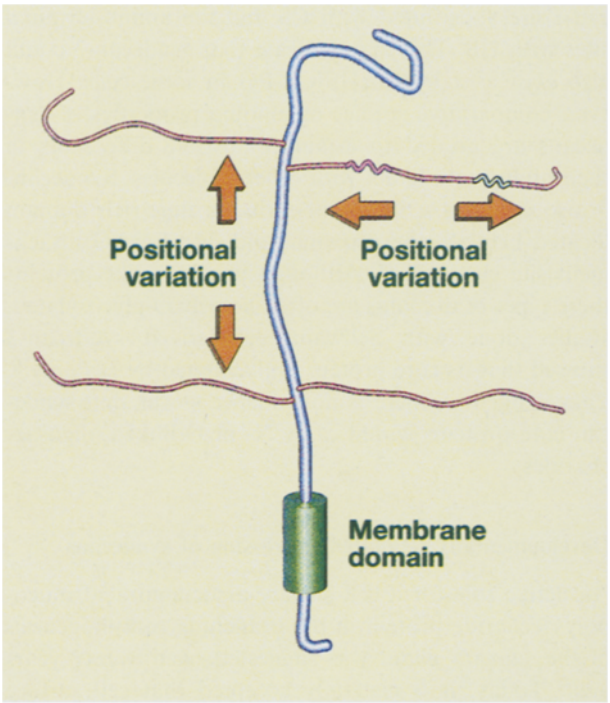

Figure 2. Side-chain dependent variation in syndecan-1 structure and function.
The putative attachment sites (serine residues on the core protein) will determine the location of side chains (thinner pink rods) with respect to the membrane domain (green cylinder). Different cores (large blue rod) can differ in the location of serine attachment sites, and in the same core protein these sites can be differentially substituted providing a possibility for a positional variation of side chains along the core. Side chain modifications (twisted dark and green regions) can further influence the mode and frequency of interactions of the side chains with extracellular effector molecules.

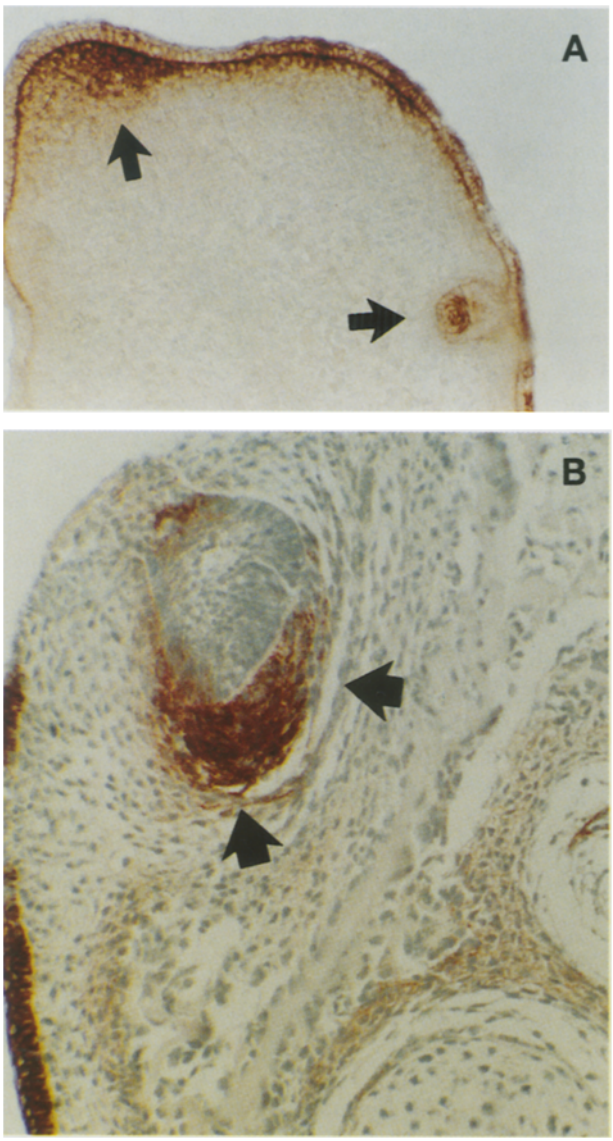

Figure 3. Developmental expression of syndecan-1 in 13-day-old mouse embryo.
Syndecan-1 was localised in mouse tissues using a monoclonal antibody 281–2. In addition to the appearance of syndecan-1 in stratified epithelia, a strong induction of syndecan-1 expression is seen in the mesenchyme of facial processes and a forming hair papilla (arrows, panel *A*). In panel *B*, a developing tooth shows strong induction of syndecan-1 in condensing mesenchyme (arrows). (Courtesy of Prof. Irma Thesleff, Institute of Dentistry, University of Helsinki.)

ing tooth, a shift of the inductive potential to the mesenchyme[63]. During organogenesis, syndecan-1 is intensely but transiently expressed in condensing mesenchymes of tooth (ref. 100; see fig. 3), kidney[110], lung[6], limb[92] and hair follicle[104], as well as in mesenchymes associated with the placode-forming ectoderm of eye and ear[104]. All studies discussed above imply that syndecan-1 is expressed in organogenic mesenchymes in a spatiotemporally regulated manner[100,111]. In tooth, syndecan-1 expression correlates positively with tenascin-C expression[101] and with mesenchymal cell proliferation[112], suggesting that syndecan-1 may participate in the matrix recognition and in the growth control of mesenchymal cells. Experiments utilising mouse-specific syndecan-1 antibody and epithelium-mesenchyme and mesenchyme-mesenchyme rat-mouse combination cultures have indicated that mesenchyme grown with epithelium can further induce syndecan-1 expression in uninduced mesenchyme[113]. The authors concluded that diffusible signals may mediate the inductive capacity, and hence also the syndecan-1 expression, during epithelium-mesenchyme interactions. The precise nature of these signals, i.e., the factors regulating the expression of syndecan-1 during development, is not known. In vitro, however, a combination treatment with FGF-2 and TGF-β results in increased syndecan-1 expression in mesenchyme-derived NIH-3T3 cells[20]. Interestingly, both FGFs (e.g. FGF-3, a product of the *int-2* gene; (cf. ref. 109) and TGF-β (cf. refs 32, 109) are expressed in the dental mesenchyme or in the adjacent budding epithelium, thus suggesting that molecules belonging to FGF- and TGF-families may be the biological regulators of syndecan-1 expression. Since TGF-β

alters the GAG composition of syndecan-1 (ref. 72), it could be possible that it also alters the binding properties, e.g., for growth factors. HSPGs bind FGFs[20,42] and are involved in cellular response to members of the FGF family[75,118]. This has been described also at the level of specific HS structures, needed for FGF-1, FGF-2 and FGF-4 interactions[68]. These observations suggest that growth factor signalling could be delicately controlled by altered syndecan-1 expression level and HS fine structure. An additional feedback mechanism could be that high syndecan-1 expression level may also down-regulate the mitogenic effect of FGF-2, as suggested by a study by Mali and co-workers[57] using syndecan-1 transfected NIH-3T3 cells that express over 10-fold more syndecan-1 than wild-type cells. Interestingly, a further study shows that purified syndecan-1 ectodomain suppresses the growth of several tumour cell lines while it does not affect the growth of non-transformed cell lines[56]. The growth inhibition was not seen if the cells were treated with free HS chains or with the GAG-free core protein alone. It is therefore possible that syndecan-1 restrictively controls mitogenic stimuli in tumour cells. Additional factors, which may be involved in the developmental regulation of syndecan-1 expression, include interleukin-6, a cytokine affecting B-cell differentiation[91]. For a general review of cytokine effects on PG metabolism, see ref. 65.

Syndecan-2 has a developmentally-regulated expression pattern distinct from that of syndecan-1 (ref. 18). Syndecan-2 is expressed throughout the mesenchyme-derived tissues in developing mouse but its expression is particularly intense in areas where active morphogenesis takes place, such as in mesenchymal condensates and at epithelial-mesenchymal interfaces. In these sites the expression is partially overlapping with the expression of syndecan-1 (ref. 18). Syndecan-3, originally cloned and sequenced from neonatal rat Schwann cells, is strongly expressed in neonatal brain and cardiac muscle while its expression levels in adult tissues are low[10]. During avian development, syndecan-3 is transiently expressed in condensing chondrogenic mesenchyme of the limb[26] while syndecan-4 is expressed in the nervous system and in muscle[2]. In situ hybridisation analyses of whole mouse embryos suggest that syndecan-1 and -3 are expressed earlier in development than syndecan-3 and -4 and that, in developing tissues, syndecan-1 and -3 are expressed in adjacent regions[25,43]. However, the data available at present are insufficient for detailed conclusions of the developmental expression of different members of the syndecan family.

Syndecans as receptors for extracellular effector molecules

Most of the proposed functions of syndecans are related to their ability to recognise extracellular effector molecules. Syndecan-1 interacts with several ECM components and with various factors associated with the cellular growth control (table). It is so far unclear whether other syndecans show binding properties similar to or distinct from syndecan-1. Chernousov and Carey[13] have reported that syndecan-3 binds FGF-2 but not FGF-1 or collagens, fibronectin and laminin, thus suggesting that its function would be related only to the FGF receptor system during development[13,26]. Syndecan-3 binds also to another molecule involved in neuronal growth control, namely heparin-binding growth-associated molecule[76] (known also as pleiotrophin or HB-GAM (refs 77, 78), that was identified as a detergent-soluble receptor for HB-GAM in ligand-affinity assays using neuronal culture extracts[76]. Different binding assays produce controversial data concerning syndecan binding properties and affinities. For example, while solid phase analyses suggest that syndecan-1 binds to collagen types I, III and V but not to types II and IV (ref. 45), gel retardation assays[84] (for heparin-ECM protein interactions see also ref. 85) indicate that syndecan-1 binds to type IV collagen with a higher affinity than to type I collagen. In solid phase assays, not only syndecan-3 but also syndecan-1 appear to have much stronger affinity to FGF-2 than to FGF-1 or FGF-7 (M. Salmivirta, T. Hiltunen and M. Jalkanen, unpublished data). Nevertheless, more functional cell binding studies suggest that syndecan-1 functions as a matrix anchoring molecule in epithelial[89] and lymphoid[87] cells.

All interactions of syndecan ectodomains known so far occur via the GAG chains rather than via the core

Table. Ligands of syndecan-1. The table summarises the extracellular effector molecules known to bind syndecan-1. It should be noted, however, that syndecans from different biological sources are functionally different in their binding to, for example, tenascin-C (ref. 80) and type I collagen (refs 87, 88). Syndecan-3 is known to bind HB-GAM (ref. 76) as well as FGF-2 (ref. 13) but not type I collagen or fibronectin (ref. 13), suggesting that marked functional differences may exist between syndecans.

Ligand	Reference
ECM molecules	
Type I Collagen	21, 45, 80, 84
Type II Collagen	84
Type III Collagen	84
Type IV Collagen	84
Type V Collagen	84
Fibronectin	21, 80, 89
Thrombospondin	97
Tenascin-C	80
Amphoterin (p30)	83
Laminin	82
Growth-associated molecules	
FGF-2	20, 42
Midkine	64
HB-GAM	64

protein. Since the discovery of the first ligands, fibrillar collagens[45] and fibronectin[89], for syndecan-1, an intriguing question about the binding specificity of syndecans has been raised. Current knowledge suggests that syndecan-1 from adherent cells and from some haematopoietic cells binds to some common ligands like type I collagen[21,45,80,87]. In the case of other ligands, however, there appear to be differences in binding between syndecan-1 preparations obtained from different biological sources. For example syndecan-1 from embryonic tooth mesenchyme, but not from mammary epithelial cells, binds to tenascin-C via its HS chains[80], suggesting a developmentally-controlled regulation of syndecan-1 HS composition. During embryonic tooth development, syndecan-1 and tenascin-C are coordinately expressed in the condensing dental mesenchyme[111] that subsequently differentiates into dental papilla giving rise to odontoblasts secreting the organic matrix of dentine. A study of the FGF binding of HSPGs from embryonic neural cell cultures reveals another example of regulated binding specificity. HSPG from cells representing day 9 of development binds to FGF-2 but not to FGF-1, whereas HSPG from day 11 binds FGF-1 but not FGF-2 (ref. 67). In both day 9 and day 11 HSPG preparations, only a single 45-kD core protein was detected that was released from the cell surfaces by trypsin treatment[67]. In myeloid cells, further differences are seen in the binding of syndecan-1 to fibronectin and to type I collagen. In solid phase assays, syndecan-1 from NMuMG cells but not from MPC-11 myeloma cells binds to fibronectin[87]. Syndecan-1 from another myeloma cell line (P3) that has similar syndecan-1 expression level of MPC-11, does not bind type I collagen due to its differential HS fine structure[87,88]. Finally, syndecan-1 from 3T3 cells that overexpress syndecan-1 due to transfection binds laminin, unlike all other forms of syndecan-1 studied so far[82]. Different ECM-protein binding has been described for syndecan-3 and for syndecan-1 (ref. 13). In addition, further differences could possibly be due to the differential number and spacing of GAG chains in syndecans. Structural motifs can also vary along the HS chains, resulting in the variation of recognition structures for extracellular molecules. In syndecan-1 and -3 there are two GAG attachment site clusters located at the opposite ends of the ectodomain (fig. 1). Such a structure may be essential for the simultaneous FGF-2 and fibronectin binding as described for syndecan-1. Syndecan-1 as well as its free chains bind to fibronectin-coated substrata, but only syndecan-1 fibronectin but not HS-fibronectin complexes show FGF-2 binding[81]. Therefore, it is possible that ECM composition can regulate the localisation of growth factor action by immobilising syndecan-1 to sites of, e.g., fibronectin or tenascin-C expression in the cellular microenvironment.

Syndecan-1 evidently functions as a cell-matrix anchor[87,89]. However, the integrins appear to be chiefly responsible for binding cells to ECM protein-coated substrata in most cells (for reviews, see refs 32, 33). It is therefore possible that functions other than matrix-binding per se should be considered for syndecans. Firstly, the presence of GAGs may modulate integrin-mediated cell adhesion both positively and negatively. In melanoma cells, a cell surface CSPG and $\alpha 4\beta 1$ integrin coordinately mediate adhesion to fibronectin[34]. The investigators studied human melanoma cells that bind a fibronectin-derived peptide (termed CS1) in an $\alpha 4\beta 1$-dependent manner. This binding requires the presence of CS although the peptide itself does not bind to CS, suggesting that cell surface CSPGs act as modulators of the integrin function in these cells. Aortic endothelial cells bind via integrins to the core protein of perlecan, a large basement-membrane HSPG[30,66]. In these cells, the binding is inhibited in the presence of either the endogenous perlecan HS or soluble HS, suggesting that HS negatively regulates integrin-mediated adhesion[30]. Syndecan-1 also binds thrombospondin[97] and tenascin-C[80], both of which are considered as 'anti-adhesive' ECM components[14,79]. These proteins, along with osteonectin (BM-40; SPARC), inhibit the spreading and adhesion of many cells in vitro and are therefore thought to be important in dynamic attachment-detachment processes as well as in the generation of cellular compartments in a developing embryo[14,41,79,94]. Syndecan-1 also binds to two developmentally-regulated neurite outgrowth-promoting proteins, amphoterin[69,83] and HB-GAM[78]. In neuroblastoma cells, amphoterin colocalises with tissue plasminogen activator (t-PA)[70]. Interestingly, heparin may act as a stimulator of t-PA induced plasminogen activation, making it therefore possible that syndecan-1 and amphoterin could serve in the formation of rapidly-reversible cell-matrix contacts in, e.g., advancing neurites.

Molecular basis for specific GAG-protein recognition

GAGs, most importantly heparin and HS, bind a plethora of ECM components, cytokines, enzymes and enzyme inhibitors, transcription factors and molecules found in infectious agents[4,36]. Binding of proteins to GAG has generally been considered to be electrostatic in nature. However, recent data convincingly demonstrate that the nature of the interactions may be more complicated and also, more specific. Firstly, analyses of the binding of mutated FGF-2 to low molecular weight heparin show that only about one-third of the binding free energy is derived from purely electrostatic interactions, the rest being derived from non-ionic interactions, hydrogen bonding, van der Waal's packing and hydrophobic interactions[102,103]. Secondly, HS preparations

having the same net charges show differential binding to e.g. type I collagen[85]. Nevertheless, the presence of basic amino acids appears to be important for HS/heparin-protein-interactions, and heparin binding 'consensus sequences' have been identified in proteins composed of clusters of arginine and lysine[9,59]. In some proteins such as antithrombin (also known as antithrombin III), the helical arrangement of basic residues rather than the primary structure itself confers the heparin-binding ability[12] (for review, see ref. 5, and references therein). The 'minimal' (i.e. the shortest and the least-sulphated) specific GAG structures required for binding to antithrombin (see ref. 5) and, in less detailed manner, to FGFs-1 and -2 and to platelet factor-4 (refs 35, 53–55, 107), have been identified. A sulphated pentasaccharide found in heparin containing an essential 3-O-sulphate group[5] binds antithrombin (the occurrence of that particular 3-O-sulphate group explains the existence of high and low anticoagulant activity heparins). The structural requirements for FGF-2-heparin/HS interaction seem to include at least the presence of 2-O-sulphate groups[28,53,105,114]. Using FGF and heparin/HS fragments interacting in solution Maccarana et al.[53] convincingly demonstrated that a pentasaccharide with a single essential 2-O-sulphate in iduronic acid binds FGF, while pentasaccharides lacking this feature but having similar or higher charge density do not bind. Studies made with selectively desulphated oligosaccharides imply that 2-O-sulphates are required for FGF-2 binding while 6-O-desulphated oligosaccharides inhibit the FGF-2 response in cells devoid of endogenous sulphated GAGs. Furthermore, a dodekasaccharide containing both 2-O and 6-O-sulphates restores the mitogenic FGF response[37]. Therefore, the authors suggest that 6-O-sulphates may be involved in binding of FGF-2 to the tyrosine kinase receptor. Other studies show that heparin/HS fragments of similar length but containing predominantly 2-O-sulphate groups fully restore the FGF response[114]. Since heparin/HS binds to FGF receptors[40,106], it has been proposed that FGF-2, its tyrosine kinase receptor (FGFR-4) and HS form a ternary complex resulting in FGF signalling[27]. Alternative possibilities are that HS causes dimerisation of FGFRs without formation of a ternary complex or that HS activates FGF by inducing a conformational change and that the activated FGF subsequently binds to FGFR (for a recent review, see ref. 106). Binding of HS to hepatocyte growth factor appears to be mediated by different structure, in which the presence of non-sulphated iduronic acid residues is important for the interaction while 2-O-sulphated iduronic acid or N-sulphates seem to have only a small contribution to the binding[52].

A single HS species may contain sequences with different composition. In liver HS, three types of stretches are seen, ranging from nonsulphated N-acetylglucosamine-glucuronic acid repeats to sequences that are indistinguishable from heparin, characterised by N-sulphated glucosamine and 2-O-sulphated iduronic acid[51]. Therefore, it is probable that HS contains functional domains that differentially recognise other molecules. Such an ability could be essential for the simultaneous binding of a cytokine and its signalling receptor.

Concluding remarks

The rapid expansion of knowledge of both the number of cell surface PGs and their biological activities raises several important questions. The highly specific developmental expression patterns suggest important regulatory functions for PGs during morphogenesis. However, the presence and the apparently overlapping expression of multiple PGs remains to be explained. One possible explanation could be that different core proteins carry different GAGs with specific structural features. Another level of GAG structure regulation is provided by growth factors that also play a key role in the control of development. Interestingly, many of these growth factors interact with HS or related structures. At present, the enzymatic machinery required for GAG synthesis and modification is under intense investigation. Molecular cloning of the genes coding for GAG synthesising enzymes will reveal their primary structure as well as make it possible to produce large amounts of enzymes for further biochemical studies. Cells or animals carrying mutated or inactivated genes coding for GAG synthesising enzymes or PG core proteins will be the tools used in the near future to elucidate the biological functions of proteoglycans.

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