REVIEW ARTICLE

Syndecans: multifunctional cell-surface co-receptors

David J. CAREY

Henry Hood, M. D., Research Program, Pennsylvania State University College of Medicine, Sigfried and Janet Weis Center for Research, Geisinger Clinic 26-13, 100 Academy Avenue, Danville, PA 17822, U.S.A.

This review will summarize our current state of knowledge of the structure, biochemical properties and functions of syndecans, a family of transmembrane heparan sulphate proteoglycans. Syndecans bind a variety of extracellular ligands via their covalently attached heparan sulphate chains. Syndecans have been proposed to play a role in a variety of cellular functions, including cell proliferation and cell–matrix and cell–cell adhesion. Syndecan expression is highly regulated and is cell-type- and developmental-stage-specific. The main function of syndecans

appears to be to modulate the ligand-dependent activation of primary signalling receptors at the cell surface. Principal functions of the syndecan core proteins are to target the heparan sulphate chains to the appropriate plasma-membrane compartment and to interact with components of the actin-based cytoskeleton. Several functions of the syndecans, including syndecan oligomerization and actin cytoskeleton association, have been localized to specific structural domains of syndecan core proteins.

INTRODUCTION

The presence of heparan sulphate proteoglycans (HSPGs) on the surfaces of most cells and the finding that many extracellular ligands bind to heparan sulphate generated the concept that membrane-associated HSPGs participate in cellular interactions with these ligands. The application of molecular-cloning techniques resulted in the elucidation of the structures of the core proteins of membrane-associated HSPGs. These investigations led to the discovery of the syndecans, a family of sequence-related transmembrane HSPGs that are the principal form of cell-surface HSPG synthesized by many cells. Syndecans bind a variety of extracellular ligands via their covalently attached heparan sulphate chains, and are thought to play important roles in cell–matrix and cell–cell adhesion, migration and proliferation. This review summarizes our current knowledge of the structure, biochemical properties and functions of the syndecans.

STRUCTURES OF SYNDECAN FAMILY HSPGs

Syndecan core proteins

Syndecans are proteoglycans, i.e. they consist of a core protein to which long, unbranched carbohydrate polymers, called glycosaminoglycans, are covalently attached. Most of our knowledge of syndecan structure is based on analysis of the deduced amino acid sequences of syndecan cDNAs. cDNAs coding for four distinct but homologous syndecan core proteins have been cloned from vertebrate cells [1–12]. A proposal for a common nomenclature designates the syndecans numerically, based on the order of cloning of their cDNAs [13]. This convention will be followed in this review. Syndecan cDNAs have been also cloned from *Drosophila melanogaster* [14] and *Caenorhabditis elegans*. A schematic diagram of the predicted structures of these syndecans is shown in Figure 1(a).

All syndecans are type I transmembrane proteins, with an N-terminal signal peptide, an ectodomain that contains several

consensus sequences for glycosaminoglycan attachment, a single hydrophobic transmembrane domain and a short C-terminal cytoplasmic domain. In syndecan-1 and -3 the glycosaminoglycan attachment sites occur in two distinct clusters, one near the N-terminus and the other near the membrane-attachment site, separated by a proline-and-threonine-rich 'spacer'. The latter domain is more prominent in N-syndecan, where it shows significant sequence similarity to mucin-like proteins. Syndecan ectodomain sequences show only limited amino-acid-sequence similarity. This is apparent even when the sequence of a specific syndecan type is compared across species.

The transmembrane and cytoplasmic domains, in contrast, are highly conserved (Figure 1b). Syndecan-1 and -3 and syndecan-2 and -4 can be considered to form subfamilies, based on sequence comparisons within these regions. The most unusual feature of the transmembrane domains is a pattern of regularly spaced small side-chain residues that includes a pair of invariant glycine residues (Figure 1b). The possible functional significance of this structural feature will be discussed below.

The cytoplasmic domains are short but highly conserved (Figure 1b). The sequence of the 13-amino-acid segment immediately following the transmembrane domain is essentially identical in all syndecans, including the invertebrate forms. All syndecan core proteins also contain an identical tetrapeptide sequence at their C-terminal ends. The function of these portions of the cytoplasmic domain are unknown. Interrupting these highly conserved segments is a variable region that shows considerably less sequence similarity among syndecan types (Figure 1b). Interestingly, however, the variable-region sequences are highly conserved for specific syndecans types in different species. The sequence of syndecan-1 in this region is identical in human, mouse, rat and hamster [1,3,4,15]; the corresponding region in syndecan-2 is identical in human, rat and *Xenopus* [2,7,11]. Thus, once the four vertebrate syndecan genes diverged during evolution, there was strong selective pressure to maintain their particular cytoplasmic domain primary structures. This, plus the structural diversity of the ectodomains, suggests that

Abbreviations used: HSPGs, heparan sulphate proteoglycans; GlcNAc, N-acetylglucosamine; bFGF, basic fibroblast growth factor; aFGF, acidic fibroblast growth factor; KGF, keratinocyte growth factor; CHO, Chinese-hamster ovary; HB-GAM, heparin-binding growth-associated molecule; PECAM-1, platelet–endothelial cell adhesion molecule-1; Ig, immunoglobulin; N-CAM, neural-cell adhesion molecule; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; ICAM-1, intracellular adhesion molecule-1.

Figure 1 Structure of syndecans

(a) Diagram illustrating the structures of the four vertebrate and two invertebrate syndecans. The black boxes represent the transmembrane domains. Vertical lines above the boxes indicate positions of attachment of heparan sulphate chains. (b) Amino acid sequences of syndecan transmembrane and cytoplasmic domains. Identical residues are indicated by vertical bars. (c) Structure of heparan sulphate, including linkage region and major modification reactions, denoted by the order of their occurrence during chain modification. For a typical heparan sulphate chain, $n = 50$ or greater. It should be noted that only some of the sugar residues in heparan sulphate are modified by epimerization and sulphation.

different syndecans have evolved to carry out similar, but non-identical, functions.

Genomic organization

In mice and humans, each of the four syndecan genes is located on a different chromosome [16]. Genes coding for syndecans 1,

3 and 4 have been cloned [12,17–19]. They show a strikingly similar exon–intron organization, which supports the idea that the syndecans arose by gene duplication from a single ancestral gene. These genes consist of five exons. Exon 1 encodes the 5'-untranslated region and signal peptide, exon 2 encodes the N-terminal cluster of glycosaminoglycan-attachment sites, exon 3 encodes the ectodomain spacer region, exon 4 encodes the

membrane proximal cluster of glycosaminoglycan-attachment sites and 10 bp of the transmembrane domain sequence, and exon 5 encodes the remainder of the transmembrane domain, cytoplasmic domain and 3'-untranslated region. The position of the intron separating exons 4 and 5, which splits codon 4 of the transmembrane domain, is conserved. The most variable exon, both in terms of sequence and overall length, is exon 3. This exon, which contains the coding information for the spacer domains of syndecans-1 and -3, ranges in size from only 54 bp in syndecan-4 to approx. 700 bp in syndecan-3.

Glycosaminoglycan chains

Syndecans are 'full time' proteoglycans, that is, with the exception of newly synthesized molecules that have not yet undergone the process of glycosaminoglycan chain addition (a post-translational process), the natural occurrence of syndecan molecules without glycosaminoglycan chains has not been described. The addition of glycosaminoglycan chains to syndecans is critical, since these provide all of the known extracellular ligand binding sites on syndecans. While there is no evidence for the existence of alternative splicing of syndecan core proteins as a mechanism to generate structural variants, structurally distinct forms of syndecans can be produced as a result of variations in the number, type, length or fine structure of the attached glycosaminoglycans [20–23].

The majority of glycosaminoglycan chains added to syndecan core proteins are of the heparan sulphate type (Figure 1c), although syndecan-1 [25] and syndecan-4 [26] have been shown to be modified by chondroitin sulphate chains as well. There is evidence for acceptor site preference in syndecan-1, where the membrane proximal sites are modified mostly by chondroitin sulphate and the N-terminal sites are modified predominantly by heparan sulphate [27]. In contrast, addition of chondroitin sulphate or heparan sulphate to syndecan-4 has been reported to be independent of the specific site, although heparan sulphate addition is preferred under most conditions [26].

The choice of glycosaminoglycan type is likely to have important functional consequences. Although chondroitin sulphate chains have a higher net negative charge than heparan sulphate, heparan sulphates exhibit greater binding affinity for most extracellular ligands. The consensus sequence for glycosaminoglycan addition is the amino acid serine (the site of glycosaminoglycan attachment) followed by a glycine. The structures of the linkage tetrasaccharides connecting heparan sulphate and chondroitin sulphate to the core protein are identical. During biosynthesis, the selection of these sites for heparan sulphate chain assembly depends on the activity of α -N-acetylglucosaminyltransferase I, which adds an N-acetylglucosamine (GlcNAc) moiety to the linkage tetrasaccharide substrate and commits it to heparan sulphate synthesis (Figure 1c). Heparan sulphate chain elongation then proceeds through the sequential addition of alternating glucuronic acid and GlcNAc moieties. (Chondroitin sulphate consists of a polymer of alternating glucuronic acid and GalNAc moieties.) Several features of the sequences surrounding the glycosaminoglycan-attachment sites of syndecans have been identified that bias chain synthesis in favour of heparan sulphate [28,29]. Within each mammalian syndecan core protein there is at least one repetitive Ser-Gly sequence. Nearly half (9/19) of the Ser-Gly motifs in the four mammalian syndecans occur within such repeats. The Ser-Gly sites are also surrounded by regions that contain acidic amino acid residues, but are essentially devoid of basic amino acids. Finally, a majority (6/10) of the single Ser-Gly sites and all of the repetitive sites contain at least one aromatic acid within

four amino acids of the primary sequence. The mechanism by which these structural features influence glycosaminoglycan chain addition is not known.

After the glycosaminoglycan polymer chain is assembled, the individual saccharide units are subjected to a number of enzymic-modification reactions. Collectively, these modifications generate what is referred to as the glycosaminoglycan 'fine structure'. Only a handful of the enzymes that catalyse these reactions have been purified or cloned, and details concerning the regulation of their activities are scanty. It is known, however, that, during heparan sulphate biosynthesis, not all sugar residues are modified [24,30]. Moreover, the modification reactions are carried out sequentially, with the products of earlier steps influencing the extent and sites of modification in subsequent steps. N-deacetylation/N-sulphation of GlcNAc occurs first (catalysed by a single polypeptide with both enzyme activities) [31], followed by epimerization of adjacent glucuronic acid residues to iduronic acid. Finally, O-sulphation at various sites is carried out (Figure 1c). This mechanism places some constraints on the total number of structures that can be formed, and also results in the generation of 'domains' within the polymer chain consisting of blocks of highly modified saccharides separated by regions of relatively low modification [24,30]. (Heparin, which is produced by mast cells, contains the same polymeric backbone as heparan sulphate, but is characterized by higher overall levels of the same post-polymerization modifications; thus, the highly modified blocks in heparan sulphate are sometimes referred to as 'heparin-like').

The importance of these modification reactions stems from the fact that high-affinity binding of certain ligands to heparan sulphate has been shown to be dependent on a particular pattern of modification. For example, high-affinity binding of basic fibroblast growth factor (bFGF) to heparan sulphate requires the presence of iduronic acid sulphated at the 2-O position [32–34]. Thus the synthesis of particular heparan sulphate fine structures provides a mechanism for modulating ligand-binding activity of the syndecans.

The mechanisms regulating the activities of these modification enzymes appear to be complex. For example, there is evidence that the extent of modification of heparan sulphate chains on syndecan-4 to a form that binds antithrombin III with high affinity is regulated by the level of available core protein [23]. It has been proposed that high core-protein levels saturate the capacity of a limiting component of the biochemical machinery required for this modification.

Different cell types have been shown to synthesize syndecans with different glycosaminoglycan structures and, in some cases, different functional activities. For example, syndecan-1 molecules produced by simple epithelial cells are modified by more and larger heparan sulphate and chondroitin sulphate chains than syndecan-1 molecules produced by stratified epithelial cells [20]. Heparan sulphate chains on syndecan-1 molecules isolated from different cell lines can also differ in fine structure. These structural differences can result in differences in affinity for type I collagen [22] and in the ability of the syndecan molecules to mediate cell attachment to type I collagen [21]. Similarly, it has been shown that syndecan-1 isolated from NIH-3T3 cells binds laminin [35], in contrast with syndecan-1 isolated from a mammary epithelial cell line, which does not bind laminin [36]. There is evidence that different syndecans can exhibit differences in ligand-binding activity. For example, syndecan-1 (isolated from epithelial cells) binds fibronectin [36], whereas syndecan-3 (isolated from rat brain) does not [37]. It is likely, however, that these differences in ligand-binding activity result from the different sources of these syndecans. A systematic comparison of glycosaminoglycan structures and ligand-binding activities of specific syndecan types

has not been reported. In general, heparan sulphate fine structure appears to reflect the cellular source of the syndecan, and not syndecan type-specific differences.

Syndecan type-specific function might arise as a consequence of differences in the spatial orientations of glycosaminoglycan chains displayed on the cell surface. Although the amino acid sequences of the extracellular domains of specific syndecan types are not highly conserved across species, the number and positions of the glycosaminoglycan chains are conserved. This suggests that the main function of the extracellular domains is to display the glycosaminoglycan chains on the cell surface in a particular spatial orientation, that would be unique for each syndecan type. This could result in subtle, but important, differences in the ability of different syndecans to interact with particular extracellular ligands and/or other membrane receptors. The arrangement of glycosaminoglycan chains as tandem repeats and clusters could also allow for interchain co-operativity. Indirect evidence for such an effect comes from the observation that binding affinities of syndecan-1 for collagens are higher than those measured for the isolated heparan sulphate chains from the proteoglycan [21,22,38].

Expression of syndecans

Consistently with their proposed roles as modulators of cellular activities, syndecans exhibit a complex pattern of cell and development specific expression. An analysis of syndecan expression in a number of tissues and cell lines led to the conclusions that virtually all cells express at least one form of syndecan, most cells express multiple forms, and there is a distinct pattern of syndecan expression that characterizes individual cell types and tissues [39]. Analysis of syndecan mRNA expression in adult mouse tissues, for example, showed that brain contains almost exclusively syndecan-3 mRNA, kidney contains mostly syndecan-4 mRNA, and liver contains high levels of syndecan-1, -2 and -4 mRNAs, but no syndecan-3 mRNA.

Dramatic changes in syndecan expression occur during development and cellular differentiation. Syndecan expression is often associated with morphological transitions or major changes in tissue organization. For example, in rodents there is a period of high-level syndecan-3 expression in the early postnatal central nervous system [12]. Syndecan-3 levels rise rapidly at birth, peak on postnatal day 7, and then decline to low levels in the adult nervous system. This burst of syndecan-3 expression corresponds with the period of oligodendrocyte differentiation and myelin formation in the central nervous system. Syndecan-3 is also expressed transiently during embryonic limb development in chick embryos during the period of mesenchymal condensation [6,10]. In the chick embryo tibia, syndecan-3 expression is restricted to proliferating, immature chondrocytes. Syndecan-3 is not expressed by neighbouring mature, hypertrophic chondrocytes. This has led to the proposal that syndecan is a regulator of proliferation during bone development [40]. In adult-mouse tissues syndecan-1 is expressed almost exclusively by epithelial cells. Within squamous epithelia that undergo regeneration, syndecan-1 expression is lost in the most highly differentiated cells [41]. During embryonic development syndecan-1 is also expressed by various mesenchymal cells, especially during periods of critical epithelium-mesenchyme interactions [42-46]. Syndecan-1 has been shown to be expressed by pre-B-cells in the bone marrow, but expression is lost immediately before the mature B-cells are released into the circulation [47]. This modulation of syndecan-1 expression has been suggested to be important for regulating interactions of these cells with extracellular matrix.

Syndecan expression has also been shown to be induced during wound healing. Syndecan-1 and syndecan-4 expression is induced in the skin after incisional wounding [48,49]. Syndecan-4 expression is induced in carotid artery tissue after balloon-catheter injury of the vessel [50].

The molecular mechanisms responsible for regulation of syndecan expression are beginning to be explored. Syndecan expression can be induced in cultured cells by treatment with growth factors and other agents, but specific responses appear to be unique to individual cell types. For example, bFGF induces syndecan-4 but not syndecan-1 or syndecan-2 expression in aortic vascular smooth-muscle cells [50]. The syndecan-4 response has characteristics of an immediate-early-gene product. In contrast, bFGF induces syndecan-1 expression in 3T3 fibroblasts [51]. Syndecan-1 expression is induced in vascular smooth-muscle cells by treatment with platelet-derived growth factor (PDGF) or angiotensin II [52]. In endothelial cells syndecan-1 expression is subject to negative regulation by tumour necrosis factor- α [53]. Syndecan-1 and syndecan-4 expression by mesenchymal cells at sites of cutaneous wound healing has been shown to be induced by an antimicrobial peptide, PR-39, that is released by inflammatory cells entering the skin [54]. Induction of syndecan expression by PR-39 is selective for mesenchymal cells, and is not detected in epithelial cells or keratinocytes.

In most cells, levels of syndecan synthesis correlate well with syndecan mRNA levels, suggesting that regulation is mainly at the level of gene transcription. Characterization of the promoters of these genes is in progress [17,55], and should yield important information on the molecular mechanisms that regulate syndecan expression. There are some striking exceptions to this general finding, however, where post-transcriptional mechanisms appear to regulate syndecan expression. Thioglycollate-elicited peritoneal mouse macrophages contain significant levels of syndecan-1 mRNA, but this mRNA is not translated into syndecan-1 core protein unless the cells are treated with agents that raise intracellular cAMP levels [56]. Translational control of syndecan-1 expression has also been reported in embryonic kidney mesenchymal tissue [46] and *ras*-transformed mammary epithelial cells [57]. Neonatal and adult-rat heart tissue contain relatively high levels of syndecan-3 mRNA, but synthesis of the proteoglycan is essentially undetectable [58]. When cells from neonatal-rat heart are placed in tissue culture, syndecan-3 synthesis by non-muscle cells (but not cardiomyocytes) can be measured. Thus the culture conditions appear to provide a stimulus that allows for translation of the syndecan-3 mRNA by non-muscle cells.

BIOCHEMICAL PROPERTIES OF SYNDECANS

Core-protein shedding

A common mechanism for turnover of membrane proteins involves their endocytosis and degradation in lysosomes. A significant fraction of syndecans are removed from the cell surface by this mechanism. An additional mechanism for syndecan removal from the cell surface is the spontaneous release of the syndecan ectodomains from the plasma membrane [39]. This release, or membrane shedding, is mediated by a proteolytic activity of unknown identity. The fragments that are released correspond to essentially the entire ectodomain with the attached glycosaminoglycan chains [59,60]. The released fragments retain the ability to bind extracellular ligands, such as bFGF [51]. In cell cultures, these fragments are soluble and accumulate in the culture medium. There is evidence that syndecan shedding also occurs *in vivo*. Extraction of neonatal rat brain tissue with buffer lacking detergent results in the solubilization of a large fraction

of the syndecan-3 that is present, suggesting that these syndecan-3 molecules had lost their membrane attachment [12]. These syndecan-3 molecules are not stained on immunoblots by an antibody directed against the C-terminal cytoplasmic domain, consistently with the loss of the C-terminal domain by proteolysis.

The precise site of cleavage within the ectodomain has not been determined. The dibasic sequence adjacent to the membrane-attachment site in the ectodomain of most syndecans was considered to be a prime candidate for the core-protein cleavage site. This was based on the finding that trypsin releases membrane-associated syndecan-1 in a form that is indistinguishable from that released spontaneously by cells [59,60]. It now appears, however, that this supposition is not correct. The *Drosophila* syndecan core protein lacks basic residues at this site, but is still released from cells by shedding [14]. Moreover, site-directed mutagenesis of these basic residues in mammalian syndecans has no effect on syndecan release by cultured cells (V. K. Asundi and D. J. Carey, unpublished work). With the exception of the dibasic sequence, there is little amino-acid-sequence similarity within the region of the cleavage that would appear to constitute an enzyme-recognition sequence.

Syndecan shedding resembles that which has been observed for other membrane proteins. A large number of transmembrane proteins, including membrane-anchored growth factors, cell-adhesion molecules, cytokine receptors and some enzymes, are proteolytically released from the plasma membrane [61]. This process has been shown to be stimulated by phorbol esters, although the target proteins themselves are not subject to protein kinase C-dependent phosphorylation. The released proteins do not appear to share an identifiable amino-acid-sequence-based recognition site for proteolytic cleavage. In spite of the diversity of the proteins involved, recent genetic evidence suggests there is a common mechanism for their proteolytic release. Mutant cell lines have been isolated that are defective in the shedding of a diverse set of proteins. Independently isolated mutant cell lines have been shown to belong to the same genetic complementation group [61]. Moreover, the shedding of these proteins can be inhibited by treatment of cells with inhibitors of metalloproteinase activity. Together, these results suggest that an extracellular metalloproteinase of unknown identity mediates the proteolytic shedding of transmembrane proteins, including syndecans.

Core-protein-mediated oligomerization

Syndecan core proteins exhibit a propensity to form non-covalently linked dimers and higher-order oligomers. This can be observed readily by SDS/PAGE analysis of syndecan core proteins that have been deglycanated by enzymic digestion. Under these conditions a significant fraction of the core proteins migrate as SDS-resistant dimers. Dimerization is a well-known mechanism for activation of receptor kinases [62]. Intramembrane clustering has also been shown to be important for activation of downstream activities of non-catalytic receptors [63,64] and cell-adhesion molecules [65–68].

The mechanism underlying syndecan-core-protein oligomerization has been investigated using rat syndecan-3 as a model. Recombinant syndecan-3 core proteins form tight, but non-covalent, dimers, tetramers, and higher-order oligomers that can be detected as SDS-resistant complexes after gel electrophoresis, by covalent cross-linking or by gel-permeation chromatography [69]. Analysis of recombinant core-protein domains and site-directed mutants revealed that the transmembrane domain of the core protein is required, but not sufficient, for oligomerization. An adjacent fragment of the

ectodomain expressed in tandem with the transmembrane domain is also required. Interestingly, a segment of the ectodomain as short as four amino acids long is sufficient to confer the ability to form oligomers. The cytoplasmic domain, which is the most highly conserved part of the syndecan core proteins, is not required for oligomerization. Disulphide-bond formation plays no role in syndecan-3 oligomerization, since the smallest active polypeptides do not contain cysteine residues. Syndecan-3 oligomerization can also be observed in membranes of living cells. Chemical cross-linking of syndecan-3 core proteins expressed on the surface of transfected cells reveals that most of the core proteins are present in oligomeric form [69].

The finding that the transmembrane domain is not sufficient for oligomerization suggests that this process is not a result of simple hydrophobic interactions alone, and that a more specific molecular recognition is involved. Inspection of the sequences of the syndecan transmembrane domains reveals the presence of three glycine residues that are conserved from *Drosophila* to human (Figure 1b). Glycine residues are not hydrophobic and are relatively rare in transmembrane sequences. In addition, a regular pattern of spacing of bulky and small side-chain residues can be identified that is also conserved among syndecan transmembrane sequences. This suggests the possibility that syndecan-core-protein oligomerization results from the interdigitation of bulky and small side chains on adjacent core proteins. A similar idea has been proposed as an explanation for the dimerization of some transmembrane receptor kinases [70]. To test this idea, mutant syndecan-3 core proteins were expressed in which two of the conserved glycine residues were changed to leucine residues. Consistently with the model, these syndecan-3 molecules failed to form stable dimers or other oligomers, presumably as a result of steric hindrance by the bulky leucine side chains [69]. There appear to be specific requirements for the structure of the ectodomain segment as well. In syndecan-3 the tetrapeptide adjacent to the transmembrane domain is highly charged (Glu-Arg-Lys-Glu). Mutation of either of the basic amino acids in this segment to alanine decreased the extent of dimer formation that was observed [69]. Together, these results provide strong evidence that syndecan oligomerization results from specific intermolecular interactions.

Membrane localization of syndecans

There is evidence that syndecans are localized to specific plasma-membrane compartments and, moreover, that different syndecans can have different sites of localization. Immunocytochemical staining of mouse tissues with anti-syndecan-1 antibodies revealed that this proteoglycan is expressed by most epithelial cells. Among epithelial cell types, the distribution of staining on the plasma membrane ranges from staining over the entire cell surface (squamous epithelia, such as cornea and tongue) to staining of only the basolateral surface (cuboidal and columnar epithelia, such as mammary duct and trachea) [40]. An analysis of syndecan-1 distribution on the surface of cultured mammary epithelial cells revealed that, in newly plated cultures, the proteoglycan is present initially over the entire cell surface, but becomes restricted to the basolateral surface as the cells become polarized [71].

The specific mechanisms that are responsible for selective syndecan-1 targeting and/or membrane retention are not known. There is evidence suggesting a role for syndecan binding to the actin cytoskeleton in this process, perhaps triggered by extracellular-matrix-dependent cross-linking of syndecan molecules on the cell surface [71]. Such a mechanism would indicate a role for the cytoplasmic domain of syndecan-1 in targeting of the

proteoglycan in polarized epithelial cells. There is experimental evidence to support this conclusion. Expression of syndecan-1 in transfected Madin–Darby canine kidney cells results in the targeting of the proteoglycan to the basolateral surface. Deletion of the C-terminal 12 amino acids of the cytoplasmic domain results in the appearance of syndecan-1 in both the basolateral and apical compartments of the plasma membrane [72].

Syndecan-4 presents a different situation with respect to subcellular localization. Syndecan-4 is localized to areas of focal adhesion in a number of adherent cell types, including fibroblasts, smooth-muscle cells and endothelial cells [73]. Focal adhesions (or focal contacts) are specialized regions of the plasma membrane that mediate tight adhesion between the cell and the underlying extracellular matrix. These structures contain a number of specialized membrane and cytoskeletal proteins and are sites for membrane attachment of actin filament bundles [74].

There is some disagreement over whether syndecan-4 is an obligatory component of focal adhesions that is required for their assembly, or is recruited to existing focal adhesions as a result of the activation of a cellular signalling mechanism. Evidence for the latter comes from the finding that, in quiescent fibroblasts, syndecan-4 is not detected by immunofluorescent staining in all focal adhesions [75]. The appearance of syndecan-4 in focal adhesions is stimulated by treatment of the cells with serum or with activators of protein kinase C (phorbol esters). Furthermore, calphostin C, a protein kinase C inhibitor, blocks the serum- or phorbol-ester-stimulated change in syndecan-4 staining.

FUNCTIONS OF SYNDECANS

General considerations

It is generally accepted that heparan sulphate-mediated binding of extracellular ligands is central to syndecan functional activity.

Thus any attempt to understand syndecan function must take into account several general features of extracellular ligand–heparan sulphate binding. First, a large number of such ligands have been identified. These encompass an amazing variety of molecules, including growth factors, extracellular-matrix proteins, cell–cell adhesion receptors, enzymes and other proteins (Table 1). Secondly, in most cases the specificity of binding of these ligands to syndecans appears to be relative and not absolute. For example, although bFGF binds to specific sugar sequences within heparan sulphate chains, every syndecan that has been examined appears to contain these sequences and binds bFGF. Variations in binding affinity have been reported, but these generally reflect quantitative and not qualitative differences in binding. These two features appear to rule out the notion that syndecans function as ligand-activated signalling receptors in the usual sense. They fail to exhibit the specificity that would be expected, and is generally observed, in such systems. Related to this is the third general feature of syndecan ligands, which is that nearly all of these ligands also bind to other receptors that do exhibit a higher degree of specificity and, in some cases, have been shown to mediate ligand-dependent activation of cytoplasmic signalling activities (Table 2).

If syndecans are not receptors in the classical sense, and they are not the primary sites for cellular interactions with their extracellular ligands, then what is their function? Our working hypothesis is that syndecans are essential regulators of ligand-dependent activation of primary signalling receptors at the cell surface. Syndecans accomplish this by binding, via their covalently attached glycosaminoglycan chains, extracellular ligands for primary signalling receptors at the cell surface. Syndecan-mediated binding of extracellular ligands at the cell surface can have several important consequences, as indicated below.

(1) By sequestering ligands on the two-dimensional surface of the plasma membrane, syndecan binding increases the effective

Table 1 Heparin sulphate-binding proteins

Abbreviations used: GGF, glial growth factor; ARIA, acetylcholine-receptor-inducing activity; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor; NK1 and NK2, naturally occurring splice variants of HGF; wnt-1, vertebrate homologue of wingless gene product of *Drosophila*.

Polypeptide growth factors	Extracellular-matrix proteins
<i>Heparin-binding growth-factor family</i>	Fibronectin
aFGF (FGF-1)	Laminin
bFGF (FGF-2)	Collagens
KGF (FGF-4)	Thrombospondin
	Tenascin
<i>EGF-related growth factors</i>	
HB-EGF	Cell–cell adhesion molecules
GGF	L-selectin
ARIA	N-CAM
	PECAM
<i>Hepatocyte growth factors</i>	Mac-1
HGF	
NK1	Lipid-binding proteins
NK2	LDL
	Lipoprotein lipase
<i>Pleiotrophin-related</i>	
HB-GAM	Blood-coagulation factors
Midkine	Antithrombin III
	Tissue factor
<i>Others</i>	Platelet factor IV
VEGF	
PDGF (A-chain variant)	Other
wnt-1	Influenza virus
	Diphtheria toxin
Enzymes	Prion proteins
Lipoprotein lipase	
Acetylcholinesterase	

Table 2 Syndecans as cell-surface co-receptors

Ligand	Primary receptors	Function	Role of syndecan
Growth factors	Growth-factor-receptor kinases	Activate intracellular pathways leading to cellular proliferation	Increase binding affinity of growth factor for receptor
Extracellular-matrix adhesive proteins	Integrins	Cell–matrix adhesion; activation of intracellular signalling pathways	Increase strength of adhesion by binding to secondary site on ligand, and cytoskeletal attachment
Ig superfamily cell-adhesion molecules	Ig cell-adhesion molecules (homophilic interaction)	Cell–cell adhesion; cytoskeletal organization	Increase strength of adhesion by binding to secondary site on homophilic receptor, and cytoskeletal attachment
Heterophilic cell-adhesion molecules (e.g. MAC-1, L-selectin)	Selectins, integrins, other cell-adhesion molecules	Cell–cell adhesion; cytoskeletal organization	Increase strength of adhesion by providing counter-receptor for heterophilic receptors, and cytoskeletal attachment

concentration of the ligands at the cell surface, thereby enhancing their ability to bind and activate signalling receptors. This effect would be especially apparent at low solution concentrations of the ligand. In some cases this would have the effect of altering the apparent threshold for receptor activation (Figure 2).

(2) Syndecans increase the range of binding interactions with extracellular ligands at the cell surface. Syndecans provide cell-surface binding sites for extracellular ligands that are composed of extended carbohydrate polymers (heparan sulphate chains) covalently linked to membrane-immobilized proteins. Recent measurements of force–distance interactions between ligands attached to flexible tethers and immobilized receptors reveal several unique properties of such systems. Specifically, a large attractive force can be measured between the receptors and ligands with a range that corresponds to the extended length of the tether [76]. The existence of this ‘tether potential’ has two important consequences. It extends spatially the effective range of binding interactions. Secondly, it increases the apparent affinity of receptor for the ligand. By carrying out the combined roles of extended tethers and receptors, the heparan sulphate chains on syndecans extend the range and increase the apparent affinities of binding interactions at the cell surface. Some simple calculations illustrate how this might apply to syndecans. It has been estimated that integrin receptors, for example, extend from the surface of the plasma membrane for a distance of approx. 20 nm [77]. A typical heparan sulphate chain with a molecular mass of 25000 [30] is predicted to form an extended linear polymer with an estimated length of approx. 60 nm [78] (some heparan sulphate chains are even longer). From the discussion of the ‘tether potential’ summarized above this would extend the effective range of cellular interactions with integrin/heparan sulphate ligands by a factor of 3 over what would occur in the absence of HSPGs (Figure 2a) and increase the apparent affinity of ligands for integrins.

(3) The spatially localized distribution of syndecan molecules on the cell surface (e.g. in focal adhesions) would impose a similar restricted distribution on bound ligands and sequester them within specific plasma-membrane domains. In theory this could either enhance or inhibit ligand-stimulated activation of the primary receptor, depending on its localization within the membrane. Membrane shedding can be considered an extreme example of a change in membrane compartmentation, with the potential to sequester ligands away from the primary signalling receptors on the membrane.

(4) Syndecan binding promotes dimerization/oligomerization of bound ligands, which enhances activation of primary signalling receptors. Oligomerization results from the multivalent nature of

the ligand-binding sites on syndecans (i.e. the multiple glycosaminoglycan chains, each with multiple ligand-binding sites) and from core-protein-mediated oligomerization of the syndecans themselves.

(5) Syndecans provide secondary binding sites for adhesive interactions that complement binding interactions mediated by primary receptors and strengthen the adhesive force.

(6) Syndecan association with the cytoskeleton, in addition to providing a mechanism for specific membrane localization, also helps to stabilize cell adhesion. Stabilization of the cytoskeleton increases adhesive strength generally (a stiffened cell is detached less readily than a deformable cell). Cytoskeleton association of transmembrane adhesion receptors (such as syndecans) might also be dictated by the need to prevent membrane ‘pull-out’ of the receptors. In artificial ligand–receptor interaction systems, binding components have been shown to be physically pulled from the lipid bilayer [76]. This occurs when the force required to break the ligand–receptor bond is greater than the force required to pull the receptor from the membrane. For transmembrane proteins anchored in the membrane by only protein–lipid interactions, the latter force is rather small. In addition to this stabilizing effect, syndecans, via their cytoplasmic domains, could recruit specific cytoplasmic proteins to the membrane at sites of adhesive interactions.

The following sections review the experimental data on functional activities of syndecans. While *in vitro* binding experiments have identified a large number of potential syndecan ligands, it has been difficult in some cases to provide convincing evidence for syndecan-specific functional activities in living cells. This is in part a result of the fact that syndecans are not the principal cell-surface receptors for most of these ligands. Identifying functional activities of syndecans often requires the use of quantitative assays or measurements of specific parameters. In spite of these problems, however, there is now good evidence for roles of syndecans in diverse cell functions, including growth-factor activation, cell–extracellular matrix adhesion and cell–cell adhesion.

Modulation of growth-factor-receptor activation

A large number of polypeptide growth factors are known to bind to heparan sulphate (or heparin) (Table 1). In every case where this has been examined, binding of the growth factors to syndecans is also observed [37,79,80]. Growth factors that demonstrate this characteristic include members of the heparin-binding-growth-factor family, of which bFGF is the best-studied example, but also hepatocyte growth factor (HGF) [81,82], a

Figure 2 Syndecans as co-receptors

(a) Syndecans bind ligands (red ovals) via their heparan sulphate chains. The bound ligands are immobilized on the plasma membrane, which facilitates their binding to primary signalling receptors. Localization of syndecans to specific membrane compartments, such as focal adhesions, can sequester ligands near co-receptors that are localized to the same structures. The distribution of syndecan on the cell surface is dictated in part by association with the cytoskeleton via their cytoplasmic domains. (b) For soluble ligands, such as growth factors, binding to syndecans stabilizes ligand–receptor interactions and results in enhanced activation at low ligand concentrations (c).

splice variant of PDGF [83], heparin-binding epidermal growth factor (EGF) [84,85], vascular endothelial growth factor [86], neuregulins [87] and others [88,89].

The importance of cell-surface heparan sulphate in the activation of signalling receptors by growth factors was first suggested in studies which showed that a loss of cell-surface heparan sulphate, caused either by treatment with a metabolic inhibitor of heparan sulphate synthesis (sodium chlorate) [90] or mutation of a gene coding for an enzyme of heparan sulphate biosynthesis [91], resulted in the failure of bFGF to bind and

activate the FGF-receptor kinase under the experimental conditions used. Many subsequent studies have confirmed these initial observations and have extended them to include other heparan sulphate-binding growth factors [84,85,88]. These findings led to the concept that binding of bFGF (and presumably other heparan sulphate-binding growth factors) to the receptor kinase has an absolute requirement for heparan sulphate.

Two key issues related to the role of heparan sulphate in growth-factor activation have been the subject of much investigation and speculation. These are the identity of the active cell-surface heparan sulphate species that exert these effects, and the mechanism by which heparan sulphate exerts this effect.

Immediately after the discovery of a role of heparan sulphate in growth-factor-receptor activation, syndecans were proposed to be likely participants in this process [92]. This was based on the logical, but circumstantial, argument that syndecans are a major form of membrane-associated HSPGs on many cells, and that syndecans do indeed bind bFGF and other growth factors. Direct evidence that syndecans functioned as growth-factor activators was not forthcoming, however. In fact, several lines of evidence to the contrary were reported. First, it was observed that overexpression of syndecan-1 in cultured cells strongly inhibited bFGF-induced cell proliferation [93]. Subsequently it was reported that purified preparations of soluble syndecan-1 or syndecan-2 inhibited bFGF-receptor binding in cell-free assays [94]. Finally, it was reported that perlecan, the large basement-membrane HSPG, was active in promoting bFGF-receptor binding [95]. Together, these findings led to the hypothesis that perlecan was the HSPG species that promoted bFGF-receptor activation, and that syndecans were natural inhibitors of this process.

A recent study, however, provides the basis for an alternative interpretation of these findings. When K562 cells, which normally produce only low levels of cell-surface HSPGs, are transfected with FGF-receptor cDNA, binding of bFGF to the receptor can be measured, but bFGF binding to the receptor is stimulated by addition of heparin [96]. When these cells were stably transfected with syndecan-1, syndecan-2 or syndecan-4 cDNAs, syndecan expression was shown to support increased bFGF binding to the receptor to a level that was equivalent to what was observed in heparin-treated control cells. Moreover, in syndecan-expressing cells there was no additional binding in the presence of heparin. Thus syndecan expression stimulated bFGF-receptor binding over levels that were observed in control cells with low levels of endogenous HSPG synthesis. The effects of syndecan expression on bFGF binding were shown to be dependent on the heparan sulphate moieties of the proteoglycans. An effect of syndecan expression was also observed using a bioassay for FGF-receptor activation, namely the down-regulation of glycophorin A expression. Syndecan-expressing cells responded at significantly lower bFGF concentrations than control cells, although the maximal responses at high bFGF concentrations were essentially identical. Interestingly, in these experiments there was no evidence for dependence on a particular species of cell-surface HSPG. Similar effects were seen in cells expressing syndecan-1, syndecan-2 or syndecan-4.

The apparent discrepancy between these findings and previously obtained results that seemed to support the opposite conclusion was potentially resolved by results of cell-free receptor-binding assays. Syndecan-4 was observed to increase the affinity of bFGF for the receptor over what was observed in the absence of the HSPG, but only when the syndecan and receptor were co-immobilized on the surface of a bead [96]. The presence of soluble syndecan-4 had no effect on the binding of bFGF to the receptor.

Several important conclusions can be drawn from these findings. First, the ability of syndecans to promote bFGF–receptor interactions appears to be limited to proteoglycans that are in what the authors termed the *cis* mode [96], i.e. HSPGs that are associated with the membrane. This provides a potential explanation for the reported inhibitory effect of soluble syndecans [94], which would bind bFGF and sequester it in a soluble form away from the receptor. Secondly, the effect of syndecans on bFGF activity is most apparent at submaximal concentrations of bFGF, and essentially disappears at high concentrations. The net effect of the presence of HSPGs, therefore, is to shift the growth-factor dose–response curve to the left (Figure 2c).

These findings raise the issue of whether heparan sulphate is required for growth-factor binding to the receptor kinase. Results of earlier experiments carried out with soluble ectodomain constructs of the FGF receptor led to the conclusion that heparan sulphate was essential for bFGF–receptor binding [97]. More recent studies have disputed this claim, however, and support the alternative notion that heparan sulphate binding increases the affinity of bFGF for the receptor, but is not required for binding [19,98,99]. Additional evidence that HSPGs are not required comes from measurements of bFGF binding and activity in heparan sulphate-deficient cells. Receptor binding, internalization and mitogenic activity of bFGF were demonstrated to occur in the absence of heparan sulphate and to the same maximal levels as observed in heparan sulphate-containing cells [100]. The lack of heparan sulphate simply reduced the binding affinity and apparent potency of bFGF in these assays.

This finding also provides a potential explanation for the earlier conclusion that heparan sulphate is required for receptor binding. Measurements of binding or other responses dependent on receptor activation carried out at bFGF concentrations that just produce a maximal or near-maximal response in the presence of a normal complement of cell-surface HSPGs would produce low (or even undetectable) responses in the absence of heparan sulphate. Such data might be interpreted as suggesting an obligatory role for heparan sulphate.

Several models have been proposed to explain the effect of heparan sulphate on bFGF binding to the receptor kinase. The simplest of these is that binding to heparan sulphate induces a structural change in the growth factor to a form that binds the receptor with higher affinity. There is little evidence to support this idea, however, and some evidence to the contrary. Specifically, binding to heparin or heparan sulphate does not induce a detectable change in the structure of bFGF as determined by analysis of the crystal structure [101]. It is possible that heparan sulphate binding induces conformational changes in other ligands, although this remains to be established. Another model that has been proposed is that heparan sulphate binding induces dimerization of growth factors which, in turn, promotes dimerization of receptor kinases and facilitates activation by autophosphorylation [97,102,103]. Heparan sulphate binding does promote bFGF and acidic fibroblast growth factor (aFGF) dimerization [97,103]. *In vitro*, this requires relatively high concentrations of the glycosaminoglycan, however. The importance of this property to biological activity remains to be established. A third model proposes that heparan sulphate molecules bind to both the growth factor and the receptor [78,104,105]. This provides a potential explanation for the finding that stimulation of bFGF binding to receptor requires a minimum heparan sulphate length of 10–12 monomer units [34]. This is longer than the size required for bFGF binding of heparan sulphate (4–7 monomer units) [32,101]. It has been suggested that the extra length is required to generate a bifunctional agent that binds growth factor and receptor simultaneously. Binding of

some FGF receptor isoforms to heparan sulphate has been demonstrated [104], although this has been reported to be dependent on high concentrations of magnesium (25 mM) [105]. Additional experimental evidence will be required before this model can be generally accepted. It has also been suggested that binding of bFGF to cell-surface heparan sulphate stabilizes the growth factor–receptor complex [98,100]. This is consistent with results of a kinetic analysis of bFGF–receptor binding, which indicated that heparan sulphate increases the affinity of bFGF–receptor binding mainly by decreasing the off-rate constant [98]. Finally, as discussed above, the functional consequences of growth-factor sequestration on the plasma membrane by binding to syndecans can explain most or all of the empirical data, without the need for invoking more elaborate molecular mechanisms.

An observation that appears to be at odds with this model is the finding that bFGF–receptor interactions can be activated by addition of soluble heparin. Typically, this effect occurs at low concentrations of heparin (typically less than about 1 µg/ml). Higher concentrations of heparin, in fact, inhibit bFGF activity. A potential explanation for this phenomenon is that the range within which exogenous heparin potentiates bFGF binding to receptor corresponds to the concentration at which most heparin is adsorbed to the plasma membrane through non-covalent binding to heparin-binding proteins. At higher heparin concentrations these binding sites will be saturated and the amount of soluble heparin will exceed the amount of membrane-associated heparin. Since both bound and soluble forms of heparin bind bFGF, the latter situation would result in sequestration of bFGF in an inactive form in the medium. This explanation is consistent with the findings that syndecans potentiate bFGF binding to receptor when they are associated with the membrane, but inhibit bFGF–receptor binding when added in soluble form [96]. This also provides an explanation for the observation that syndecan overexpression in a cell line in which most of the expressed syndecan is shed from the membrane leads to decreased responsiveness to bFGF [106].

This discussion of the role of HSPGs in growth factor–receptor activation has been based almost entirely on data derived from analysis of bFGF and its receptor. While there are indications that other heparan sulphate-binding growth factors respond in qualitatively similar manners to changes in levels of cell-surface heparan sulphate [84,85,87,88], there is limited evidence that this is not universally the case. Keratinocyte growth factor (KGF) is a member of the heparin-binding-growth-factor family, whose receptor is a splice variant of the FGF-receptor 2. This receptor also binds aFGF. It has been shown that heparan sulphate has opposite effects on aFGF and KGF binding to the receptor. Binding of aFGF is enhanced, whereas binding of KGF is inhibited [107]. The mechanism underlying these findings is not known.

The potential to regulate growth-factor activity by means of heparan sulphate-dependent interactions provides an attractive system for fine tuning cellular responses to growth factors in the extracellular environment. This could be especially important during development, where rapid changes in cellular responses are required. Adjustments in the thresholds for cellular response to growth factors could result from changes in the levels of syndecans expressed, the subcellular compartmentation of syndecans or changes in the structures of glycosaminoglycans attached to syndecans. That such a mechanism is used is consistent with the available data. Rapid changes in levels of syndecan expression have been observed at critical periods of development (see above). In the developing neuroepithelium there is evidence for a developmentally regulated change in the

relative binding affinities of heparan sulphate chains for bFGF and aFGF that matches the temporal pattern of expression of these growth factors [108].

Cell–extracellular matrix adhesion

The primary receptors that mediate cell adhesion to extracellular-matrix proteins are receptors of the integrin family [109]. An additional property of many extracellular-matrix proteins is their ability to bind glycosaminoglycans, especially heparan sulphate (Table 2). These proteins also bind to syndecans, via the heparan sulphate chains of the proteoglycans [35,36,38,110–112]. Typically, heparan sulphate-binding activity of extracellular-matrix proteins is mediated by structural domains that are distinct from those associated with integrin-binding activity. The ability of syndecans to bind extracellular-matrix proteins makes it logical to suggest that syndecans participate in cell–extracellular matrix adhesion. Investigations of the role of syndecans in cell–extracellular matrix adhesion are plagued, however, by several complications. Integrins, as the primary matrix receptors expressed by most cells, can mediate cell–matrix adhesion in the absence of heparan sulphate. Also, the method that is most often used to prepare cells for measurements of cell adhesion, namely suspension by treatment with trypsin, results in a loss of syndecans from the cell surface by proteolysis [60].

These problems were addressed in a study investigating the role of syndecan-1 in the adhesion of mammary epithelial cells to fibronectin-coated surfaces. Syndecan-1 is the major cell-surface HSPG expressed by these cells. Trypsin-treated mammary epithelial cells attach to fibronectin-coated surfaces, but the attachment is entirely dependent on integrin-mediated adhesion [113]. This is based on the finding that adhesion of trypsin-treated cells to fibronectin is inhibited by Arg-Gly-Asp-containing peptides that block the activity of fibronectin-binding integrins. In contrast, when the cells are prepared by EDTA-mediated release (bivalent cations are required for integrin activity) integrin-blocking peptides no longer inhibit binding to fibronectin. Inhibition of adhesion in this case requires a combination of integrin-blocking peptide plus soluble heparin or purified syndecan-1 ectodomain. Heparin and the soluble syndecan-1 ectodomain act as competitive inhibitors of binding interactions mediated by heparan sulphate chains on cell-surface HSPGs. This interpretation is supported by the additional finding that treatment of EDTA-released cells with heparitinase (to remove cell-surface heparan sulphate chains), renders cell adhesion to fibronectin inhibitable by integrin blocking peptides alone. Syndecan-dependent adhesion of mammary epithelial cells is mediated by the heparin-binding domain of fibronectin. The purified heparin-binding domain supports cell attachment, and this is inhibited by soluble heparin but not integrin-blocking peptides.

In other experiments syndecan-1 has also been shown to mediate adhesion of cells to collagen type I [114]. These results demonstrate that cells can utilize both integrins and syndecans to mediate cell–extracellular matrix adhesion. In this dual receptor system the extracellular matrix ligand binds simultaneously to the two receptors by means of distinct structural domains. An obvious question is: what is the function of this dual receptor system? Intuitively, the simultaneous binding of a ligand to two receptors would lead to a stronger interaction (Figure 3a). Similarly to what has been observed with growth factors, syndecans, acting through their heparan sulphate chains, could also influence integrin–ligand affinity, although this has not been examined directly. The strength of adhesive interactions would

Figure 3 Syndecans as co-receptors in cell adhesion

(a) Cells bind to extracellular-matrix ligands, such as fibronectin, by means of integrins (left) and syndecans. Syndecans bind to discrete structural domains (red oval) on adhesive proteins. (b) The strength of cell–matrix adhesion is further enhanced by clustering of integrins and syndecans and concomitant association with the cytoskeleton. (c) Heparan sulphate-mediated binding of syndecans to specific structural domains of adhesion receptors (red ovals), such as N-CAM, reinforces and stabilizes homophilic binding mediated by other structural domains. Adhesive stability is also provided by association of syndecans with the cytoskeleton. (d) By binding to heterophilic counter-receptors, such as L-selectin or Mac-1 (red), syndecans provide one component of paired receptor systems. The syndecan–counter-receptor binding reinforces and stabilizes interactions mediated by other pairs of adhesion receptors, such as E-selectin binding to carbohydrate ligands on neutrophils. Adhesive stability is also provided by association of syndecans with the cytoskeleton.

also be enhanced by clustering of receptors in the membrane and stabilized by syndecan–cytoskeleton association (Figure 3b).

Integrin binding and clustering are also linked to transduction of extracellular-matrix-dependent signalling events through activation of focal adhesion kinase and other pathways, and recruitment of cytoskeletal proteins to the membrane [68,115,116]. It is likely that syndecans contribute unique functional activities to the process of cell–matrix adhesion. Recent evidence indicates that syndecan-4 can bind protein kinase C, and that this regulates protein kinase C distribution and activity [117]. Protein kinase C binding is dependent on a peptide sequence in the variable region of the syndecan-4 cytoplasmic domain. This suggests that other syndecans might couple to different signalling pathways.

Some of the best evidence for a specific role of syndecans in cell adhesion comes from studies of focal-adhesion formation in response to attachment to fibronectin. Fibroblast adhesion to surfaces coated with intact fibronectin, which contains both integrin-binding and heparin-binding domains, results in cell spreading and formation of focal adhesions. In contrast, when fibroblasts adhere to a large 105 kDa fragment of fibronectin that contains the integrin-binding but not the heparin-binding domain, they fail to form focal adhesions [118]. Focal-adhesion formation by cells attached to the 105 kDa fragment is restored, however, by addition of a specific heparin-binding peptide from

the fibronectin heparin-binding domain. Evidence that cell-surface heparan sulphate molecules are required for the activity of the peptide was provided by the finding that treatment of the cells with heparinase significantly inhibits peptide-stimulated focal-adhesion formation. Results similar to these were obtained when Chinese-hamster ovary (CHO) cell lines that are genetically deficient in their ability to synthesize heparan sulphate were examined [119]. Wild-type CHO cells adhere to intact fibronectin, to the 105 kDa integrin-binding fragment and to a 31 kDa fragment that contains the heparin-binding domain but lacks the integrin-binding domain. Heparan sulphate-deficient CHO cells attach to intact fibronectin and to the 105 kDa fragment, but not to the 31 kDa heparin-binding fragment. Although they adhere to intact fibronectin, heparan sulphate-deficient CHO cells fail to produce focal adhesions.

In these experiments the identity of the cell-surface heparan sulphate molecules responsible for focal-adhesion formation was not determined. The finding that syndecan-4 is localized to focal adhesions in a variety of cell types [73,75] led to the proposal that syndecan-4 fulfils this role. Direct evidence for this is still lacking, however. As described above, there is disagreement over whether syndecan-4 is required for focal-adhesion formation or is recruited to focal adhesions only under certain conditions.

There is also evidence for a role of syndecan-3 in cell-matrix adhesion in the developing central nervous system. There is a burst of high-level syndecan-3 expression in the central nervous system during the early postnatal period [12]. Heparin-binding growth-associated molecule (HB-GAM, also called pleiotrophin) is an 18 kDa secreted protein that is expressed in the central nervous system with a developmental time course that is essentially identical with that of syndecan-3 [120]. Purified HB-GAM promotes the attachment of a variety of cells and is a potent inducer of neurite outgrowth from embryonic or early-postnatal cortical neurons [121]. Neurite-outgrowth activity of HB-GAM is inhibited by soluble heparan sulphate, suggesting the involvement of cell-surface HSPGs in this interaction. Several lines of evidence support the conclusion that syndecan-3 functions as an HB-GAM receptor in the central nervous system [122]. HB-GAM affinity chromatography of extracts of newborn-rat brain results in the purification of syndecan-3. Purified syndecan-3 binds with high affinity to HB-GAM ($K_d \approx 0.4$ nM) in *in vitro* assays; the binding is mediated by the heparan sulphate chains of syndecan-3. Syndecan-3 is present on the surface of cortical neurons spreading on surfaces coated with HB-GAM. Soluble syndecan-3 or anti-syndecan-3 antibodies block HB-GAM-dependent neurite outgrowth. Inhibitor studies suggest that neurite-outgrowth activity of HB-GAM is dependent on interactions with heparan sulphate molecules that contain 2-O-sulphated iduronic acid; structural analysis of heparan sulphate chains on neonatal-rat brain syndecan-3 revealed the presence of contiguous blocks of disaccharide units with an unusually high content of this modification [123]. An important question that remains to be answered is whether there are additional HB-GAM-binding proteins that co-operate with syndecan-3 to mediate biological activity, analogously to the integrin-syndecan dual-receptor system that mediates adhesion to fibronectin or collagen.

Cell-cell adhesion

Syndecans also have been suggested to play a role in cell-cell adhesion, based on the finding that several well-characterized cell-adhesion molecules bind heparan sulphate (Table 2). In at least one case there is direct evidence demonstrating a role for syndecans in cell-cell adhesion. Some lymphoid cells express

syndecan-1 [47]. It has been shown that certain human myeloma cell lines have lost their ability to synthesize syndecan-1. The loss of syndecan-1 expression correlates with the inability of the cells to adhere to one another in a rotation-mediated aggregation assay [114]. Stable transfection of these cells with syndecan-1 cDNA restores cell-cell adhesion activity [124]. Evidence for a direct role of syndecan-1 in the adhesion process comes from the finding that cell adhesion is inhibited by soluble heparan sulphate or purified syndecan-1, or by treatment of the cells with heparitinase. Analysis of the composition of aggregates formed by syndecan-1 expressing and non-expressing cells demonstrated that syndecan-1-mediated adhesion is heterophilic in nature, i.e. it results from the binding of syndecan-1 on one cell to a different counter-receptor on the adjacent cell. Interestingly, transfection of the cells with syndecan-4 cDNA also restores cell-cell adhesion activity, whereas transfection with cDNA coding for another membrane proteoglycan, betaglycan, has no effect on adhesion [124].

In these experiments the identity of the syndecan-1 counter-receptor was not determined. There are several candidates for syndecan counter-receptors among the known cell-cell adhesion receptors. The immunoglobulin (Ig) superfamily of adhesion molecules are transmembrane proteins that contain a variable number of Ig homology repeats in their extracellular domains and have been shown to mediate homophilic cell-cell adhesion. One such receptor is platelet-endothelial cell adhesion molecule-1 (PECAM-1), which is expressed by platelets, endothelial cells and other cells. PECAM-1 binds heparan sulphate. Heparan sulphate-binding activity is localized to a specific PECAM-1 structural domain [125]. A role for cell-surface HSPGs in PECAM-1-mediated adhesion is demonstrated by the finding that adhesion is inhibited by soluble heparan sulphate, but not other glycosaminoglycans, or by treatment of the cells with heparinase.

Neural-cell adhesion molecule (N-CAM) is another member of the Ig superfamily of adhesion receptors. N-CAM also binds heparan sulphate. This activity has been localized to the second Ig loop in the extracellular domain. Although N-CAM mediates cell-cell adhesion predominantly by a homophilic binding mechanism, a role for heparan sulphate binding in N-CAM-mediated adhesion is suggested by several findings. N-CAM-mediated adhesion is inhibited by soluble heparan sulphate or by peptides derived from the heparin-binding domain of N-CAM [126]. Deletion of the heparin-binding domain or generation of point mutations that alter basic residues within this domain, significantly reduces N-CAM adhesion activity.

Another group of potential syndecan counter-receptors are the selectins. Selectins are a family of membrane proteins that contains variable numbers of EGF-like repeats in their extracellular domains and mediate cell adhesion by binding to specific carbohydrate ligands. Selectins are widely expressed on leucocytes and endothelial cells and have been shown to be important in leucocyte trafficking [127]. Recent data have shown that certain carbohydrate ligands that bind L-selectin on the surface of endothelial cells are in fact heparan sulphate [128,129]. The L-selectin-binding heparan sulphate molecules have not been identified, but syndecans are a major form of cell-surface HSPGs expressed by endothelial cells [9].

Mac-1 is a leucocyte integrin that binds a variety of ligands, including intercellular adhesion molecule-1 ('ICAM-1') and fibrinogen. Mac-1 has also been shown to bind directly to heparan sulphate [130]. Assays carried out under conditions of physiological flow showed that Mac-1-heparan sulphate binding is not sufficient to mediate neutrophil adhesion. Neutrophils also express carbohydrate ligands that bind to E-selectin. Neutrophil

binding to E-selectin-coated surfaces causes reversible tethering (rolling), but is not able to support firm adhesion (arrest) at physiological shear stresses. When E-selectin and heparan sulphate are co-adsorbed on to a substratum, both receptors are utilized, so that E-selectin-mediated rolling allows the binding of Mac-1 to heparan sulphate to occur, resulting in firm adhesion of neutrophils at physiological shear stresses [130].

Each of these cases is an example of the use of a dual-receptor system for cell adhesion in which one of the components of the system is a membrane-associated HSPG, and the participation of the HSPG appears to increase the overall strength of the binding interaction (Figure 3).

Cell migration

Cell migration is a complex activity that is dependent on interaction of cells with an adhesive surface (to provide traction) and mediated by directed membrane and cytoskeleton assembly. Studies with cultured cells have shown a close correlation between cell–substratum adhesive strength and migration rates [131]. Evidence for a role of syndecans in modulating cell-migration activity comes from studies of lymphocyte migration. As described above, some lymphocyte cell lines lose syndecan expression. These cells exhibit markedly enhanced rates of migration through collagen gels. Transfection of these cells to restore syndecan expression produces a significant reduction in migration rates [132]. This is dependent on heparan sulphate-mediated interactions, based on the finding that addition of soluble heparan sulphate or treatment of the cells with sodium chlorate causes an increase in migration rates of syndecan-expressing cells to levels comparable with those of cells that lack syndecan.

Syndecan–cytoskeleton association

The activities of transmembrane receptors that function in cell adhesion have been shown to be dependent, at least in part, on interactions of the cytoplasmic domains of these proteins with the cytoskeleton. Integrin receptors bind talin [133] and α -actinin [134] and other cytoskeleton-associated proteins [65,68,117], selectins bind α -actinin [135], and cadherins bind catenins [136]. In the case of integrin receptors it has been shown that one of the elements that regulates cytoskeleton association is clustering of the receptors within the plane of the membrane [65]. This is postulated to occur in response to binding of extracellular ligands, which generally consist of immobilized polymers (e.g. fibronectin fibrils) in the extracellular matrix. The high degree of amino-acid-sequence similarity of the cytoplasmic domains of syndecans strongly suggests that these domains carry out an essential function. There is evidence that at least one function of syndecan cytoplasmic domains is to mediate association with the cytoskeleton.

An early study provided indirect evidence that syndecan-1 on the basal surface of epithelial cells could associate with the actin cytoskeleton [71]. It was proposed that cytoskeleton association was triggered in response to binding of syndecans in the membrane to fibronectin molecules in the basal extracellular matrix. Part of the evidence for cytoskeleton association came from the finding that syndecan-1 was insoluble in non-ionic detergents. A later study, however, questioned the ability of syndecan-1 to associate with the cytoskeleton. Incubation of cells in low-pH medium (pH 5) results in the conversion of syndecan-1 into a form that is not extractable by non-ionic detergent. Detergent-insolubility of the syndecan-1 molecules in these cells is not dependent on the cytoplasmic domain, but, instead, on the

heparan sulphate chains [137]. Thus detergent-insolubility of syndecan-1 appears to result from an extracellular interaction, and not from an intracellular (i.e. cytoskeletal) interaction.

This apparent dilemma was resolved in experiments utilizing rat Schwann cells that were stably transfected with syndecan-1. Schwann cells do not normally synthesize syndecan-1, but syndecan-1 is very similar in its cytoplasmic domain to syndecan-3, the form normally expressed by these cells. Stable expression of syndecan-1 in Schwann cells results in enhanced spreading of the cells on fibronectin and laminin-coated substrata and a striking reorganization of microfilaments [138]. There is a transient co-localization of cell-surface syndecan-1 and actin filaments during cell spreading, suggesting association of syndecan-1 with the cytoskeleton, but this is lost when spreading is completed.

These results suggested the possibility that syndecan–cytoskeleton association is subject to dynamic regulation. Ligand-mediated clustering of integrins has been shown to activate integrin–cytoskeleton interactions [65]. This process can be mimicked by binding of anti-integrin antibodies, which induces clustering of the receptors on the cell surface. There is evidence that a similar mechanism promotes syndecan association with the cytoskeleton. Incubation of syndecan-1-transfected Schwann cells in medium containing cross-linking anti-syndecan-1 antibodies results in clustering of the syndecan-1 molecules on the plasma membrane and co-localization with the underlying actin filaments [139]. Antibody-clustered syndecan-1 molecules are present on the external side of the plasma membrane, as revealed by immunogold electron-microscopic staining. The distribution of antibody-clustered syndecan-1 molecules on the cell surface is altered by disruption of actin filaments caused by cytochalasin D treatment of the cells. Actin filament co-localization results from the association of syndecan-1 molecules on the cell surface with the actin cytoskeleton by a mechanism that is dependent on a specific region of the cytoplasmic domain [140]. In cells expressing a membrane-associated form of syndecan-1 that lacks the cytoplasmic tail, antibody-mediated clustering is observed, but the clusters do not co-localize with actin filaments. Deletion of a 12-amino-acid segment from the middle of the cytoplasmic domain, or point mutation of a conserved tyrosine residue within this region, abolishes actin-filament co-localization of antibody-clustered syndecan-1 [140]. Surprisingly, although syndecan-1 molecules that lack the cytoplasmic domain do not associate with actin filaments, they are not extractable by Triton X-100 after antibody-mediated clustering. These results, together with a previous report on low-pH-induced syndecan aggregation [137], demonstrate that detergent-insolubility of syndecan-1 results from a process that is not related to cytoskeleton association.

Preliminary experiments have shown that similar results are obtained with syndecan-3 in Schwann cells (V. K. Asundi and D. J. Carey, unpublished work). As described above, the sequences of the cytoplasmic domains of syndecan-1 and syndecan-3 are very similar, including the region that is critical for syndecan–actin-filament association. Interestingly, the sequence of syndecan-4 in this region, which overlaps the variable portion of the cytoplasmic domain, differs significantly from syndecan-1 and syndecan-3. Unlike these syndecans, syndecan-4 is found in focal adhesions [73,75]. These findings suggest that one function of the variable region of the cytoplasmic domains might be to promote distinct modes of association of different syndecans with the cytoskeleton.

The fact that cytoskeleton association of syndecan-1 is triggered by antibody-mediated clustering suggests that cytoskeleton association is regulated by ligand-dependent clustering.

Preliminary experiments have shown that incubation of syndecan-1-expressing Schwann cells with beads coated with bFGF or type I collagen results in syndecan-1 clustering at the cell/bead interface and recruitment of actin filaments (K. A. Bendt and D. J. Carey, unpublished work). Regulation of cytoskeleton association by syndecan clustering also provides a potential function for syndecan-core-protein self-association. Consistently with this, preliminary results have shown that dimerization-deficient forms of syndecan-3 do not co-localize with actin filaments after antibody-mediated clustering in transfected Schwann cells (V. K. Asundi and D. J. Carey, unpublished work).

The mechanisms responsible for syndecan-cytoskeleton association are not known. By analogy to other transmembrane receptors, cytoskeleton association is likely to be mediated by intermediary proteins that bind both syndecans and actin filaments. The identification of such proteins is an active area of current research. The finding that mutation of a tyrosine residue prevents actin-filament association of syndecan-1 [140] suggests a possible role for tyrosine phosphorylation in this process. This is an attractive possibility, but one for which there is no direct evidence. Tyrosine phosphorylation of syndecans can be demonstrated *in vitro* and in transfected cells (V. K. Asundi, K. A. Bendt and D. J. Carey, unpublished work), but the functional consequences of this modification are not known.

Several functional consequences of syndecan-cytoskeleton association can be considered. Syndecans could recruit cytoskeletal proteins to specific sites on the plasma membrane and so actively participate in adhesion-dependent cytoskeletal organization. As described above, association with the cytoskeleton would also stabilize adhesive interactions. Cytoskeleton association could be used as a mechanism to impose a particular subcellular distribution on syndecans (syndecan-4 in focal adhesions, for example). Cytoskeleton association could also result in internalization of syndecan molecules and their bound ligands. This might be especially relevant for extracellular ligands that are not immobilized by association with the extracellular matrix or the plasma membrane of an adjacent cell. Examples of such ligands include enzymes such as lipoprotein lipase [141,142]. Binding and internalization of ligands by heparan sulphate-dependent pathways will be discussed below.

Maintenance of differentiated phenotype and suppression of tumour growth

As described in the preceding subsections, there is evidence that a large number of cellular processes are modulated by syndecans. Consistently with this, there are several reports describing dramatic changes in cellular phenotype that occur as a result of changes in syndecan expression. For example, it has been shown that suppression of endogenous syndecan-1 expression in epithelial cells by transfection with antisense cDNA causes a striking change in cell morphology, from a flattened cuboidal shape characteristic of epithelial cells to an elongated fusiform morphology [143]. The fusiform transfectants lose expression of E-cadherin, gain the ability to migrate in collagen gels and acquire anchorage-independent growth. Thus syndecan-1 expression appears to be required for maintenance of a differentiated epithelial phenotype.

A similar conclusion was reached from an investigation of the effects of syndecan-1 expression on tumour-cell phenotype. The cells of an epithelial-derived tumour-cell line, S115, have lost the ability to synthesize syndecan-1. These cells exhibit a transformed phenotype, characterized by a poorly organized actin cytoskeleton and the ability to form colonies in soft agar. Transfection

of the cells with syndecan-1 cDNA results in a dramatic reversal of the transformed phenotype [144]. Syndecan-1 expression causes a reorganization of actin filaments as well as inhibition of soft-agar colony formation. Somewhat surprisingly, these effects on the transformed phenotype were observed with both wild-type syndecan-1 and a mutant form of syndecan-1 that lacked the transmembrane and cytoplasmic domains and was not anchored to the membrane. This result suggests that the effects of syndecan-1 expression are caused by the ectodomain of the HSPGs. Additional evidence for this comes from the finding that purified syndecan-1 ectodomain inhibits the growth of S115 tumour cells, but not normal epithelial cells that express endogenous syndecan-1 [145]. The inhibition is dependent on the syndecan-1 heparan sulphate chains, since the inhibitory activity is abolished by digestion of the proteoglycan with heparitinase. Attachment of the glycosaminoglycans to the syndecan core protein is essential, however, on the basis of the finding that addition of soluble heparan sulphate had no effect on tumour-cell growth. The molecular mechanisms responsible for this effect of syndecan-1 are not known.

The apparent relationship between syndecan-1 expression and normal epithelial cell phenotype led Jalkanen and co-workers to investigate the use of syndecan-1 expression as a prognostic marker for patients with cancer. These investigators reported a striking correlation between loss of syndecan-1 expression and mortality in a series of patients with a variety of tumours of the head and neck [146]. They suggested that syndecan-1 expression could be a valuable criterion for assessing outcome and planning appropriate treatments. Whether this finding extends to other kinds of tumours, and whether expression of other syndecans could be of similar diagnostic value, have not been investigated.

Other ligands

Cell-surface HSPGs, including syndecans, also bind extracellular ligands that are not growth factors and do not mediate cell adhesion (Table 2). These include enzymes (e.g. lipoprotein lipase, acetylcholinesterase), components of the blood-coagulation cascade (e.g. antithrombin III) and lipid carrier proteins (e.g. low-density lipoprotein) [141,147–149]. Like all other known syndecan-binding activities for extracellular ligands, these interactions are mediated by the glycosaminoglycan chains of the proteoglycans. In some cases, syndecan binding appears to play an obvious role in sequestering these proteins to the appropriate physiological compartment. For example, binding of antithrombin III to syndecan molecules on the luminal surfaces of endothelial cells would contribute to the establishment of a non-thrombogenic lining for blood vessels [150]. In other cases, the main function of syndecan binding might be to mediate internalization and possible degradation of the bound proteins. Heparan sulphate-dependent internalization of several extracellular ligands has been reported, including bFGF, lipoprotein lipase and thrombospondin [142,151–153]. There is evidence that the kinetics of internalization and final destinations of ligands that utilize this pathway are distinct from those mediated by more conventional internalization pathways. Details of the syndecan-dependent internalization mechanism are not known. Preliminary data from our laboratory have shown that syndecan-1 can facilitate the internalization of bound ligands, and that this is stimulated by cross-linking of syndecan molecules on the cell surface and requires the cytoplasmic domain of the core protein (D. J. Carey, unpublished work).

Cell-surface HSPGs have also been implicated in the binding and internalization of some pathological agents, including certain

toxins [154,155] and viruses [148]. Direct evidence for syndecans mediating these processes is still lacking, however.

SUMMARY

The current state of knowledge of the structure and function of syndecans has been reviewed. A working model for syndecan function was proposed that states that the main function of syndecans is to modulate ligand-dependent activation of primary signalling receptors at the cell surface. This model is consistent with the large number and variety of syndecan ligands, the lack of absolute binding specificity, and the fact that most of these ligands also bind to other primary receptors at the cell surface. In this dual-receptor model, syndecan-dependent binding of ligands increases the apparent affinity of cell–ligand interactions. Syndecan binding interactions also increase the apparent strength or quality of cell–matrix or cell–cell adhesive interactions. Syndecan activity is postulated to be dependent on sites of membrane localization and, in some cases, limited to syndecans immobilized on the cell surface. An essential feature of the functional activity of syndecans is their ability to associate with actin filaments. There is evidence that this is triggered by ligand-induced clustering of syndecan molecules on the cell surface and is mediated by a specific region of the cytoplasmic domain. Syndecans can oligomerize through non-covalent self-association of their core proteins. It is postulated that this regulates syndecan functional activities, such as cytoskeleton association.

A great deal remains to be learned with respect to syndecan function. A few important questions for the future are: (1) do different syndecans carry out distinct functions; if so, what are the unique functions of each type? (2) how is the fine structure of syndecan heparan sulphate chains regulated and what are the functional consequences of these modifications? (3) what are the functions of the syndecan cytoplasmic domains? Specifically, what are the identity and functions of cytoplasmic proteins that interact with syndecans? Do syndecans bind cytoplasmic signalling proteins? If so, what is the functional consequence of their association with syndecans? Answers to these questions will enable us to begin to understand the functional differences between syndecans and the other major class of membrane-associated HSPGs, the glypicans. These proteoglycans bind the same extracellular ligands, but are anchored to the membrane by a lipid anchor and lack transmembrane and cytoplasmic domain structures. (4) What are the molecular mechanisms that regulate development and cell-specific syndecan expression? Finding answers to these questions should keep scientists investigating these unique and fascinating proteins busy for many years.

REFERENCES

- Saunders, S., Jalkanen, M., O'Farrell, S. and Bernfield, M. (1989) *J. Cell Biol.* **108**, 1547–1556
- Marynen, P., Zhang, J., Cassiman, J. J., Van den Berghe, H. and David, G. (1989) *J. Biol. Chem.* **264**, 7017–7024
- Kiefer, M. C., Stephans, J. C., Crawford, K., Okino, K. and Barr, P. J. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6985–6989
- Mali, M., Jaakola, P., Arvilommi, A.-M. and Jalkanen, M. (1990) *J. Biol. Chem.* **265**, 6884–6889
- Carey, D. J., Evans, D. M., Stahl, R. C., Asundi, V. K., Conner, K. J., Garbes, P. and Cizmeci-Smith, G. (1992) *J. Cell Biol.* **117**, 191–201
- Gould, S. E., Upholt, W. B. and Koshier, R. A. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3271–3275
- Pierce, A., Lyon, M., Hampson, I. N., Cowling, G. J. and Gallagher, J. T. (1995) *J. Biol. Chem.* **267**, 3894–3900
- David, G., Van der Schueren, B., Marynen, P., Cassiman, J. J. and Van den Berghe, H. (1992) *J. Cell Biol.* **118**, 961–969
- Kojima, T., Shworak, N. W. and Rosenberg, R. D. (1992) *J. Biol. Chem.* **267**, 4870–4877
- Gould, S. E., Upholt, W. B. and Koshier, R. A. (1995) *Dev. Biol.* **168**, 438–451
- Rosenblum, N. D., Botelho, B. B. and Bernfield, M. (1995) *Biochem. J.* **309**, 69–76
- Carey, D. J., Conner, K., Asundi, V. K., O'Mahony, D. J., Stahl, R. C., Showalter, L. J., Cizmeci-Smith, G., Hartman, J. and Rothblum, L. I. (1997) *J. Biol. Chem.* **272**, 2873–2879
- Bernfield, M., Kokenyesi, R., Kato, M., Hinkes, M. T., Spring, J., Gallo, R. L. and Lose, E. J. (1992) *Annu. Rev. Cell Biol.* **8**, 365–393
- Spring, J., Paine-Saunders, S., Hynes, R. and Bernfield, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 3334–3338
- Cizmeci-Smith, G., Asundi, V. K., Stahl, R. C., Teichman, L. J., Chernousov, M. A., Cowan, K. and Carey, D. J. (1992) *J. Biol. Chem.* **267**, 15729–15736
- Spring, J., Goldberger, O. A., Jenkins, N. A., Gilbert, D. J., Copeland, N. G. and Bernfield, M. (1994) *Genomics* **21**, 597–601
- Hinkes, M. T., Goldberger, O. A., Neumann, P. E., Kokenyesi, R. and Bernfield, M. (1993) *J. Biol. Chem.* **268**, 11440–11448
- Vihinen, T., Auvinen, P., Alanen-Kurki, L. and Jalkanen, M. (1993) *J. Biol. Chem.* **268**, 17261–17269
- Baciu, P. C. and Goetinck, P. F. (1995) *Mol. Biol. Cell* **6**, 1503–1513
- Sanderson, R. D. and Bernfield, M. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9562–9566
- Sanderson, R., Turnbull, J., Gallagher, J. and Lander, A. (1994) *J. Biol. Chem.* **269**, 13100–13106
- Kato, M., Wang, H. and Bernfield, M. (1994) *J. Biol. Chem.* **269**, 18881–18890
- Shworak, N. W., Shirakawa, M., Collic-Joualt, S., Liu, J., Mulligan, R. C., Birinyi, L. K. and Rosenberg, R. D. (1994) *J. Biol. Chem.* **269**, 24941–24952
- Turnbull, J. E. and Gallagher, T. (1991) *Biochem. J.* **273**, 553–559
- Rapraeger, A., Jalkanen, M., Endo, E., Koda, J. and Bernfield, M. (1985) *J. Biol. Chem.* **260**, 11046–11052
- Shworak, N. W., Shirakawa, M., Mulligan, R. C. and Rosenberg, R. D. (1994) *J. Biol. Chem.* **269**, 21204–21214
- Kokenyesi, R. and Bernfield, M. (1994) *J. Biol. Chem.* **269**, 12304–12309
- Zhang, L. and Esko, J. (1994) *J. Biol. Chem.* **269**, 19295–19299
- Zhang, L., David, G. and Esko, J. D. (1995) *J. Biol. Chem.* **270**, 27127–27136
- Lyon, M., Deakin, J. A. and Gallagher, J. T. (1994) *J. Biol. Chem.* **269**, 11208–11215
- Wei, Z., Swiedler, S. J., Ishihara, M., Orellana, A. and Hirschberg, C. B. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3885–3888
- Turnbull, J. E., Fernig, D. G., Ke, Y., Wilkinson, M. C. and Gallagher, J. T. (1992) *J. Biol. Chem.* **267**, 10337–10341
- Maccarana, M., Casu, B. and Lindahl, U. (1993) *J. Biol. Chem.* **268**, 23898–23905
- Walker, A., Turnbull, J. E. and Gallagher, J. T. (1994) *J. Biol. Chem.* **269**, 931–935
- Salmivirta, M., Mali, M., Heino, J., Hermonen, J. and Jalkanen, M. (1994) *Exp. Cell Res.* **215**, 180–188
- Elenius, K., Salmivirta, M., Inki, P., Mali, M. and Jalkanen, M. (1990) *J. Biol. Chem.* **265**, 17837–17843
- Chernousov, M. A. and Carey, D. J. (1993) *J. Biol. Chem.* **268**, 16810–16814
- San Antonio, J., Karnovsky, M., Gay, S., Sanderson, R. and Lander, A. (1994) *Glycobiology* **4**, 327–332
- Kim, C., Goldberger, O., Gallo, R. and Bernfield, M. (1994) *Mol. Cell Biol.* **5**, 797–805
- Shimazu, A., Nah, H.-D., Kirsch, T., Koyama, E., Leatherman, J. L., Golden, E. B., Koshier, R. A. and Pacifici, M. (1996) *Exp. Cell Res.* **229**, 126–136
- Hayashi, K., Hayashi, M., Jalkanen, M., Firestone, J. H., Trelstad, R. L. and Bernfield, M. (1997) *J. Histochem. Cytochem.* **35**, 1079–1088
- Vainio, S., Jalkanen, M. and Thesleff, I. (1989) *J. Cell Biol.* **108**, 1945–1954
- Solursh, M., Reiter, R. S., Jensen, K. L., Kato, M. and Bernfield, M. (1990) *Dev. Biol.* **140**, 83–92
- Vainio, S., Jalkanen, M., Vaahtokari, A., Sahlberg, C., Mali, M., Bernfield, M. and Thesleff, I. (1991) *Dev. Biol.* **147**, 322–333
- Boutin, E. L., Sanderson, R. D., Bernfield, M. and Cunha, G. R. (1991) *Dev. Biol.* **148**, 63–74
- Vainio, S., Jalkanen, M., Bernfield, M. and Saxen, L. (1992) *Dev. Biol.* **152**, 221–232
- Sanderson, R. D., Lalor, P. and Bernfield, M. (1989) *Cell Regul.* **1**, 27–35
- Elenius, K., Vainio, S., Laato, M., Salmivirta, M., Thesleff, I. and Jalkanen, M. (1991) *J. Cell Biol.* **114**, 585–595
- Gallo, R., Kim, C., Kokenyesi, R., Adzick, N. S. and Bernfield, M. (1996) *J. Invest. Dermatol.* **107**, 676–683
- Cizmeci-Smith, G., Langan, E., Youkey, J., Showalter, L. J. and Carey, D. J. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17**, 172–180
- Elenius, K., Maatta, A., Salmivirta, M. and Jalkanen, M. (1992) *J. Biol. Chem.* **267**, 6435–6441
- Cizmeci-Smith, G., Stahl, R. C., Showalter, L. J. and Carey, D. J. (1993) *J. Biol. Chem.* **268**, 18740–18747
- Kainulainen, V., Nelimarkka, L., Jarvelainen, H., Laato, M., Jalkanen, M. and Elenius, K. (1996) *J. Biol. Chem.* **271**, 18759–18766

- 54 Gallo, R. L., Ono, M., Povsic, T., Page, C., Eriksson, E., Klagsbrun, M. and Bernfield, M. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91**, 11035–11039
- 55 Vihinen, T., Maatta, A., Jaakkola, P., Auvinen, P. and Jalkanen, M. (1995) *J. Biol. Chem.* **271**, 12532–12541
- 56 Yeaman, C. and Rapraeger, A. C. (1993) *J. Cell Biol.* **122**, 941–950
- 57 Kirjavainen, J., Leppa, S., Hynes, N. E. and Jalkanen, M. (1993) *Mol. Biol. Cell* **4**, 849–858
- 58 Asundi, V. K., Keister, B. F., Stahl, R. C. and Carey, D. J. (1997) *Exp. Cell Res.* **230**, 145–153
- 59 Weitzhandler, M., Streeter, H. B., Henzel, W. and Bernfield, M. (1988) *J. Biol. Chem.* **263**, 6949–6952
- 60 Jalkanen, M., Rapraeger, A., Saunders, S. and Bernfield, M. (1987) *J. Cell Biol.* **105**, 3087–3096
- 61 Arribas, J., Coodly, L., Vollmer, P., Kishimoto, T. K., Rose-John, S. and Massague, J. (1996) *J. Biol. Chem.* **271**, 11376–11382
- 62 Heldin, C. (1995) *Cell* **80**, 213–223
- 63 Letourneur, F. and Klausner, R. D. (1992) *Science* **255**, 79–83
- 64 Chacko, G. W., Duchemin, A., Coggeshall, K. M., Osborne, J. M., Brandt, J. T. and Anderson, C. L. (1994) *J. Biol. Chem.* **269**, 32435–32440
- 65 Miyamoto, S., Akiyama, S. K. and Yamada, K. M. (1995) *Science* **267**, 883–885
- 66 Sleeman, J., Rudy, W., Hofmann, M., Moll, J., Herrlich, P. and Ponta, H. (1996) *J. Cell Biol.* **135**, 1139–1150
- 67 Brierley, W. M., Yap, A. S. and Gumbiner, B. M. (1996) *J. Cell Biol.* **135**, 487–496
- 68 Miyamoto, S., Teramoto, H., Coso, O. A., Gutkind, J. S., Burbelo, P. D., Akiyama, S. K. and Yamada, K. M. (1995) *J. Cell Biol.* **131**, 791–805
- 69 Asundi, V. K. and Carey, D. J. (1995) *J. Biol. Chem.* **270**, 26404–26410
- 70 Sternberg, M. J. E. and Gullick, W. J. (1990) *Protein Eng.* **3**, 245–248
- 71 Rapraeger, A., Jalkanen, M. and Bernfield, M. (1986) *J. Cell Biol.* **103**, 2683–2696
- 72 Miettinen, H. M., Edwards, S. N. and Jalkanen, M. (1994) *Mol. Biol. Cell* **5**, 1325–1339
- 73 Woods, A. and Couchman, J. (1994) *Mol. Biol. Cell* **5**, 183–192
- 74 Burrage, K., Fath, K., Kelly, T., Nuckolls, G. and Turner, C. (1988) *Annu. Rev. Cell Biol.* **4**, 487–525
- 75 Baciu, P. C. and Goetinck, P. F. (1995) *Mol. Biol. Cell* **6**, 1503–1513
- 76 Wong, J. Y., Kuhl, T. L., Israelachvili, J. N., Mullah, N. and Zalipsky, S. (1997) *Science* **275**, 820–822
- 77 Hynes, R. O. (1987) *Cell* **48**, 549–554
- 78 Brickman, Y. G., Ford, M. D., Small, D. H., Bartlett, P. F. and Nurcombe, V. (1995) *J. Biol. Chem.* **270**, 24941–24948
- 79 Salmivirta, M., Heino, J. and Jalkanen, M. (1992) *J. Biol. Chem.* **267**, 17606–17610
- 80 Kojima, T., Katsumi, A., Yamazaki, T., Muramatsu, T., Nagasaka, T., Ohsumi, K. and Saito, H. (1996) *J. Biol. Chem.* **271**, 5914–5920
- 81 Lyon, M., Deakin, J. A., Mizuno, K., Nakamura, T. and Gallagher, J. T. (1994) *J. Biol. Chem.* **269**, 11216–11223
- 82 Ashikari, S., Habuchi, H. and Kimata, K. (1995) *J. Biol. Chem.* **270**, 29586–29593
- 83 Feyzi, E., Lustig, F., Fager, G., Spillmann, D., Lindahl, U. and Salmivirta, M. (1997) *J. Biol. Chem.* **272**, 5518–5524
- 84 Johnson, G. R. and Wong, L. (1994) *J. Biol. Chem.* **269**, 27149–27154
- 85 Higashiyama, S., Abraham, J. A. and Klagsbrun, M. (1993) *J. Cell Biol.* **122**, 933–940
- 86 Gitay-Goren, H., Soker, S., Vlodavsky, I. and Neufeld, G. (1992) *J. Biol. Chem.* **267**, 6093–6098
- 87 Sudhalter, J., Whitehouse, L., Rusche, J. R., Marchionni, M. A. and Mahanthappa, N. K. (1996) *Glia* **17**, 28–38
- 88 Reishman, F., Smith, L. and Cumberledge, S. (1996) *J. Cell Biol.* **135**, 819–827
- 89 Mitsiadis, T. A., Muramatsu, T., Muramatsu, H. and Thesleff, I. (1995) *J. Cell Biol.* **129**, 267–281
- 90 Rapraeger, A. C., Krufka, A. and Olwin, B. B. (1991) *Science* **252**, 1705–1708
- 91 Yayon, A., Klagsbrun, M., Esko, J. D., Leder, P. and Ornitz, D. M. (1991) *Cell* **64**, 841–846
- 92 Bernfield, M. and Hooper, K. C. (1991) *Ann. N.Y. Acad. Sci.* **638**, 182–194
- 93 Mali, M., Elenius, K., Miettinen, H. and Jalkanen, M. (1993) *J. Biol. Chem.* **268**, 24215–24222
- 94 Aviezer, D., Levy, E., Safran, M., Svahn, C., Buddecke, E., Schmidt, A., David, G., Vlodavsky, I. and Yayon, A. (1994) *J. Biol. Chem.* **269**, 114–121
- 95 Aviezer, D., Hecht, D., Safran, M., Eisinger, M., David, G. and Yayon, A. (1994) *Cell* **79**, 1005–1013
- 96 Steinfeld, R., Van den Berghe, H. and David, G. (1996) *J. Cell Biol.* **133**, 405–416
- 97 Ornitz, D. M., Yayon, A., Flanagan, J. G., Svahn, C. M., Levi, E. and Leder, P. (1992) *Mol. Cell Biol.* **12**, 240–247
- 98 Nugent, M. A. and Edelman, E. R. (1992) *Biochemistry* **31**, 8876–8883
- 99 Roghani, M., Mansukhani, A., Dell'Era, P., Bellota, P., Basilico, C., Rifkin, D. and Moscatelli, D. (1994) *J. Biol. Chem.* **269**, 3976–3984
- 100 Fannon, M. and Nugent, M. A. (1996) *J. Biol. Chem.* **271**, 17949–17956
- 101 Faham, S., Hileman, R. E., Fromm, J. R., Linhardt, R. J. and Rees, D. C. (1996) *Science* **271**, 1116–1120
- 102 Schwall, R. H., Chang, L. Y., Godowski, P. J., Kahn, D. W., Hillan, K. J., Bauer, K. D. and Zioncheck, T. F. (1996) *J. Cell Biol.* **133**, 709–718
- 103 Spivak-Kroizman, T., Lemmon, M. A., Dikic, I., Ladbury, J. E., Pinchasi, D., Huang, J., Jaye, M., Crumley, G., Schlessinger, J. and Lax, I. (1994) *Cell* **79**, 1015–1024
- 104 Kan, M., Wang, F., Xu, J., Crabb, J. W., Hou, J. and McKeenhan, W. L. (1993) *Science* **259**, 1918–1921
- 105 Kan, M., Wang, F., To, B., Gabriel, J. L. and McKeenhan, W. L. (1996) *J. Biol. Chem.* **271**, 26143–26148
- 106 Mali, M., Elenius, K., Miettinen, H. and Jalkanen, M. (1993) *J. Biol. Chem.* **268**, 24215–24222
- 107 Reich-Slotky, R., Bonneh-Barkay, D., Shaoul, E., Bluma, B., Svahn, C. M. and Ron, D. (1994) *J. Biol. Chem.* **269**, 32279–32285
- 108 Nurcombe, V., Ford, M. D., Wildshut, J. A. and Partlett, P. F. (1993) *Science* **260**, 103–106
- 109 Hynes, R. O. and Lander, A. D. (1992) *Cell* **68**, 303–322
- 110 Koda, J. E., Rapraeger, A. and Bernfield, M. (1985) *J. Biol. Chem.* **260**, 8157–8162
- 111 Sun, X., Mosher, D. F. and Rapraeger, A. (1989) *J. Biol. Chem.* **264**, 2885–2889
- 112 Salmivirta, M., Elenius, K., Vainio, S., Hofer, U., Chiquet-Ehrismann, R., Thesleff, I. and Jalkanen, M. (1991) *J. Biol. Chem.* **266**, 7733–7739
- 113 Saunders, S. and Bernfield, M. (1988) *J. Cell Biol.* **106**, 423–430
- 114 Sanderson, R. D., Sneed, T. B., Young, L. A., Sullivan, G. L. and Lander, A. D. (1992) *J. Immunol.* **148**, 3902–3911
- 115 Juliano, R. L. and Haskill, S. (1993) *J. Cell Biol.* **120**, 577–585
- 116 Yamada, K. M. and Geiger, B. (1997) *Curr. Opin. Cell Biol.* **9**, 76–85
- 117 Oh, E.-S., Woods, A. and Couchman, J. R. (1997) *J. Biol. Chem.* **272**, 8133–8136
- 118 Woods, A., McCarthy, J. B., Furcht, L. T. and Couchman, J. R. (1993) *Mol. Biol. Cell* **4**, 605–613
- 119 LeBaron, R. G., Esko, J. D., Woods, A., Johansson, S. and Hook, M. (1988) *J. Cell Biol.* **106**, 945–952
- 120 Rauvala, H., Vanhala, A., Castren, E., Nolo, R., Raulo, E., Merenmies, J. and Panula, P. (1994) *Dev. Brain Res.* **79**, 157–175
- 121 Raulo, E., Julkunen, I., Merenmies, J., Pihlaskari, R. and Rauvala, H. (1992) *J. Biol. Chem.* **267**, 11408–11416
- 122 Raulo, E., Chernousov, M. A., Carey, D. J., Nolo, R. and Rauvala, H. (1994) *J. Biol. Chem.* **269**, 12999–13004
- 123 Kinnunen, T., Raulo, E., Nolo, R., Maccarana, M., Lindahl, U. and Rauvala, H. (1996) *J. Biol. Chem.* **271**, 2243–2248
- 124 Stanley, M. J., Liebersbach, B. F., Liu, W., Anhalt, D. J. and Sanderson, R. D. (1995) *J. Biol. Chem.* **270**, 5077–5083
- 125 DeLisser, H. M., Yan, H. C., Newman, P. J., Muller, W. A., Buck, C. A. and Albelda, S. M. (1993) *J. Biol. Chem.* **268**, 16037–16046
- 126 Reyes, A. A., Akeson, R., Brezina, L. and Cole, G. J. (1990) *Cell Regul.* **1**, 567–576
- 127 Springer, T. A. (1995) *Annu. Rev. Physiol.* **57**, 827–872
- 128 Norgard-Sumnicht, K. E., Varki, N. M. and Varki, A. (1993) *Science* **261**, 480–483
- 129 Giuffre, L., Cordey, A., Monai, N., Tardy, Y., Shapira, M. and Spertini, O. (1997) *J. Cell Biol.* **136**, 945–956
- 130 Diamond, M. S., Alon, R., Parkos, C. A., Quinn, M. T. and Springer, T. A. (1995) *J. Cell Biol.* **130**, 1473–1482
- 131 Palacek, S. P., Loftus, J. C., Ginsberg, M. H., Lauffenburger, D. A. and Horwitz, A. F. (1997) *Nature (London)* **385**, 537–540
- 132 Liebersbach, B. and Sanderson, R. (1994) *J. Biol. Chem.* **269**, 20013–20019
- 133 Horwitz, A., Duggan, K., Buck, C., Beckerle, M. C. and Burrage, K. (1986) *Nature (London)* **320**, 531–533
- 134 Otey, C. A., Oavalko, F. M. and Burrage, K. (1990) *J. Cell Biol.* **111**, 721–729
- 135 Pavalko, F. M., Walker, D. M., Graham, L., Goheen, M., Doerschuk, C. M. and Kansas, G. S. (1995) *J. Cell Biol.* **129**, 1155–1164
- 136 Hirano, S., Kimoto, N., Shimoyama, Y., Hirohashi, S. and Takeichi, M. (1992) *Cell* **70**, 293–301
- 137 Miettinen, H. and Jalkanen, M. (1994) *J. Cell Sci.* **107**, 1571–1581
- 138 Carey, D. J., Stahl, R. C., Cizmeci-Smith, G. and Asundi, V. K. (1994) *J. Cell Biol.* **124**, 161–170
- 139 Carey, D. J., Stahl, R. C., Tucker, B., Bendt, K. A. and Cizmeci-Smith, G. (1994) *Exp. Cell Res.* **214**, 12–21
- 140 Carey, D. J., Bendt, K. M. and Stahl, R. C. (1996) *J. Biol. Chem.* **271**, 15253–15260
- 141 Misra, K. B., Kim, K. C., Cho, S., Low, M. G. and Bensadoun, A. (1994) *J. Biol. Chem.* **269**, 23838–23844
- 142 Berryman, D. E. and Bensadoun, A. (1995) *J. Biol. Chem.* **270**, 24525–24531
- 143 Kato, M., Saunders, S., Nguyen, H. and Bernfield, M. (1995) *Mol. Biol. Cell* **6**, 559–576
- 144 Leppa, S., Mali, M., Miettinen, H. M. and Jalkanen, M. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92**, 932–936

-
- 145 Mali, M., Andtfolk, H., Miettinen, H. M. and Jalkanen, M. (1994) *J. Biol. Chem.* **269**, 27795–27798
- 146 Inki, P., Hoensuu, H., Grenman, R., Klemi, P. and Jalkanen, M. (1994) *Br. J. Cancer* **70**, 319–323
- 147 Parthasarathy, N., Goldberg, I. J., Sivaram, P., Mulloy, B., Flory, D. M. and Wagner, W. D. (1994) *J. Biol. Chem.* **269**, 22391–22396
- 148 Shieh, M. T., WuDunn, D., Montgomery, R. I., Esko, J. D. and Spear, P. G. (1992) *J. Cell Biol.* **116**, 1273–1281
- 149 Kojima, T., Leone, C., Marchildon, G., Marcum, J. and Rosenberg, R. (1992) *J. Biol. Chem.* **267**, 4859–4869
- 150 deAgostini, A. I., Watkins, S. C., Slayter, H. S., Youssoufian, H. and Rosenberg, R. D. (1995) *J. Cell Biol.* **111**, 1293–1304
- 151 Reiland, J. and Rapraeger, A. C. (1994) *J. Cell Sci.* **105**, 1085–1093
- 152 Chen, H., Strickland, D. K. and Mosher, D. F. (1996) *J. Biol. Chem.* **271**, 15993–15999
- 153 Roghani, M. and Moscatelli, D. (1992) *J. Biol. Chem.* **267**, 22156–22162
- 154 Shishido, Y., Sharma, K. D., Higashiyama, S., Klagsbrun, M. and Mekada, E. (1995) *J. Biol. Chem.* **270**, 29578–29585
- 155 Shyng, S., Lehman, S., Moulder, K. L. and Harris, D. A. (1995) *J. Biol. Chem.* **270**, 30221–30229