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Syndecan, a developmentally regulated cell surface proteoglycan that binds extracellular matrix and growth factors

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Cellular behaviour during development is dictated, in part, by the insoluble extracellular matrix and the soluble growth factor peptides, the major molecules responsible for integrating cells into morphologically and functionally defined groups. These extracellular molecules influence cellular behaviour by binding at the cell surface to specific receptors that transduce intracellular signals in various ways not yet fully clear. Syndecan, a cell surface proteoglycan found predominantly on epithelia in mature tissues binds both extracellular matrix components (fibronectin, collagens I, III, V, and thrombospondin) and basic fibroblast growth factor (bFGF). Syndecan consists of chondroitin sulfate and heparan sulphate chains linked to a 31 kilodalton (kDa) integral membrane protein. Syndecan represents a family of integral membrane proteoglycans that differ in extracellular domains, but share cytoplasmic domains. Syndecan behaves as a matrix receptor: it binds selectively to components of the extracellular matrix, associates intracellularly with the actin cytoskeleton when cross-linked at the cell surface, its extracellular domain is shed upon cell rounding and it localizes solely to basolateral surfaces of simple epithelia. Mammary epithelial cells made syndecan-deficient become fibroblastic in morphology and cell behaviour, showing that syndecan maintains epithelial cell morphology. Syndecan changes in quantity, location and structure during development: it appears initially on four-cell embryos (prior to its known matrix ligands), becomes restricted in the pre-implementation embryo to the cells that will form the embryo proper, changes its expression due to epithelial-mesenchymal interactions (for example, induced in kidney mesenchyme by the ureteric bud), and with association of cells with extracellular matrix (for example, during B-cell differentiation), and ultimately, in mature tissues becomes restricted to epithelial tissues. The number and size of its glycosaminoglycan chains vary with changes in cell shape and organization yielding tissue type-specific polymorphic forms of syndecan. Its interactions with the major extracellular effector molecules that influence cell behaviour, its role in maintaining cell shape and its spatial and temporal changes in expression during development indicate that syndecan is involved in morphogenesis.

Introduction

Coordination of the behaviour of individual cells during embryogenesis yields changes in tissue form and function. This coordination is due, largely, to the molecules that integrate the behaviour of the cells into a morphologically and functionally defined group. Two classes of molecules can serve this function, the extracellular matrix components and the peptide growth factors. These molecules act simultaneously on multiple cells and have been implicated in mediating developmentally significant tissue interactions. The extracellular matrix components

are large multidomain molecules that are extensively cross-linked to each other, predominantly by non-covalent bonds, to form an insoluble matrix that extends over multiple cells. The growth factors are small, soluble peptides, that can diffuse locally to affect groups of cells and that may bind with high avidity to components of the extracellular matrix.

Extracellular matrix and growth factors as effector molecules

The extracellular matrix provides a solid substratum that influences the proliferation, differentiation, shape and migration of cells (for review see Bernfield *et al.* (1984)). Although the matrix acts physiologically as an insoluble multicomponent composite, the effects of its individual components have been adduced in cell culture studies. For instance, binding of cells to laminin, a matrix glycoprotein, promotes growth, differentiation and changes in cell morphology (Kleinman *et al.* 1985). Fibronectin, another matrix molecule, also influences cell shape, migration and proliferation. The multiple effects of these molecules on cell behaviour may be attributed to distinct domains that bind to various cell surface receptors and matrix molecules (for example, for laminin, Edgar *et al.* (1984); Liotta (1986); for fibronectin, Hynes (1987); Ruoslahti (1988) and for thrombospondin, Frazier (1987)). Interfering with these interactions can disrupt normal embryogenesis, as has been shown by using peptides containing a sequence (arg–gly–asp) found in several matrix molecules that is recognized by certain integrin matrix receptors (Ruoslahti & Piersbacher 1987; Thiery *et al.* 1985).

Peptide growth factors also control proliferation, differentiation and morphogenesis during development (for review, see Mercola & Stiles (1988)). The actions of the growth factors appear to depend on a cellular 'context'; a peptide growth factor may at one time stimulate proliferation, while later stimulating differentiation. A few examples: TGF-β may either promote or inhibit cell division and differentiation *in vitro*, depending on the cell type, substratum and presence of other growth factors (Roberts *et al.* 1985; for review see Roberts & Sporn (1988)). Basic fibroblast growth factor, bFGF, one of the heparin-binding growth factors, is a potent *in vitro* mitogen of mesoderm-derived cells that can also induce production of various extracellular proteases and cell migration (reviewed in Rifkin & Moscatelli (1989)). *In vivo*, however, its actions are unclear. Exogenous bFGF can induce *Xenopus* ectoderm to acquire mesodermal differentiation (Slack *et al.* 1987), and can induce neovascularization in various tissues (reviewed in Gospodarowicz *et al.* (1987)).

The extracellular matrix substratum is probably the most important factor in generating the cellular context that influences the actions of a growth factor peptide. Cellular adhesion to the matrix is the major determinant of cell shape, which, in turn, is the predominant influence on whether a cell is able to respond, for example, by proliferating, differentiating or migrating (Ingber & Folkman 1989*a*). The precise mechanisms by which cell shape modifies the cellular response are unclear, but it has long been evident that changes in cell shape mediated by the substratum alter the cellular phenotype, (reviewed in Letourneau, *et al.* (1980), and see the classic papers by Emerman & Pitelka 1977; Folkman & Moscona 1978). Growth factors such as members of the TGF-β and PDGF families can also increase the production and accumulation of matrix, or modify cell surface matrix receptors (Rasmussen & Rapraeger 1988), complicating our understanding of their mode of action.

Extracellular matrix composites and peptide growth factors differ in a fundamental way, yet they share biological effects and often act in concert. Matrix composites are insoluble,

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constitutive, and usually present at a constant concentration, while the growth factors are soluble, inducible and may vary in concentration. However, they both act (i) locally, influencing a well-defined cohort of cells; (ii) via autocrine or paracrine mechanisms, influencing the behaviour of their tissue of origin or of adjacent tissues; (iii) at the cell surface, by binding receptors that transmit signals intracellularly either by activating second messengers or interacting with the actin-containing cytoskeleton. The insoluble substratum provided by the matrix can modulate the effect of the otherwise diffusable growth factors. The matrix binds some growth factor peptides. For example, bFGF is detected in various extracellular matrices (Baird & Ling 1987; Shing *et al.* 1984). Because the matrix can sequester growth factors, it can partition their effects and can serve as a potential reservoir.

The extracellular matrix can contain growth factors in a protected state. The evidence is most impressive for members of the FGF family. Exogenously administered bFGF binds to extracellular matrix and can be released by incubation with either heparan sulphate or heparin as well as by digestion with heparitinase but not chondroitinase (Vlodavsky *et al.* 1987). Heparan sulphate glycosoaminoglycans (GAGs) and proteoglycans synthesized by endothelial cells bind bFGF and protect it from proteolytic degradation (Saksela *et al.* 1988). This interaction does not interfere with the ability of bFGF to bind to its high affinity cell surface receptor (Moscatelli 1988), potentially explaining the ability of heparin to stabilize (Schreiber *et al.* 1985) and even synergize the action of the growth factor (Thornton *et al.* 1983).

A model for the interaction of bFGF with cells and matrix has been proposed in which bFGF released from cells is bound to insoluble matrix heparan sulphate proteoglycan, then solubilized by extracellular enzymes to form a complex containing heparan sulphate (Rifkin & Moscatelli 1989). Haematopoietic growth factors may bind to heparan sulphate proteoglycans (Gordon *et al.* 1987; Roberts *et al.* 1988). Physiologically relevant binding of other growth factors by extracellular matrix is less well documented.

Heparan sulphate proteoglycans are heparin-like molecules at the cell surface

A characteristic of several extracellular matrix molecules and growth factor peptides is their ability to bind to heparin (see reviews in Ofosu *et al.* 1989). This binding can be of high affinity and quite specific. Indeed, several large multidomain extracellular matrix molecules, such as fibronectin, laminin, and thrombospondin, have regions that show this heparin-binding property. Members of the fibroblast growth factor family, including bFGF aFGF, the hst/K-fgf gene product, FGF-5, and the keratinocyte growth factor, bind to heparin, as do other growth factors, such as those in the PDGF family (Rifkin & Moscatelli 1989).

The binding of heparin by extracellular molecules from either the matrix or growth factor groups is curious because heparin is a pharmaceutical product prepared from the degraded heparin proteoglycan that is found intracellularly within the secretory granules of mast cells and basophils. Heparin can exist extracellularly when these cells degranulate, as occurs at sites of specific immune reactions. Thus, heparin binding by these extracellular molecules most likely represents *in vivo* interactions with the heparin-like glycosaminoglycan heparan sulphate.

Heparan sulphate exists in proteoglycans, molecules in which the glycosaminoglycan chain is bound covalently to a protein core. Heparan sulphate proteoglycans are predominantly found at the cell surface or within the matrix immediately adjacent to the cell. Pericellular

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heparan sulphate proteoglycan surrounds fibroblasts and is a major component of the basement membrane that lies subjacent to a variety of parenchymal cells (Hassell *et al.* 1986). Importantly, the same cell type can produce distinct cell surface and basement membrane heparan sulphate proteoglycans, distinguished by their protein cores (Jalkanen *et al.* 1988).

Heparan sulphate proteoglycan is a ubiquitous component of the surfaces of all adherent vertebrate cells. The predominant form contains a membrane-spanning core protein; however, peripheral cell surface components exist as do heparan sulphate proteoglycans that can be released from the cell surface by a phosphatidyl inositol-specific phospholipase C (Carey & Evans 1989). The physiological function of cell surface heparan sulphate proteoglycan is not clear. However, its ubiquitous nature and its affinity for a variety of ligands that affect cell behaviour have motivated us to examine the properties of a highly abundant cell surface proteoglycan that is developmentally regulated.

Syndecan: an integral membrane proteoglycan that binds extracellular matrix and bFGF

Syndecan is a cell surface proteoglycan originally isolated from mouse mammary epithelial cells as a hydrophobic proteoglycan. It is an unusual proteoglycan, containing both heparan sulphate and chondroitin sulphate glycosaminoglycan chains (Rapraeger *et al.* 1985). Subsequently, syndecan has been found on a variety of epithelial cells in mature mouse tissues. Syndecan binds to several extracellular components via its heparan sulphate chains: it binds with high affinity and specificity to the interstitial collagens, types I, III and V (Koda *et al.* 1985), to fibronectin (Saunders & Bernfield 1988), to thrombospondin (Sun *et al.* 1989), and to bFGF (J. Rowland & M. Bernfield, unpublished observations). These binding properties have led to our naming this proteoglycan, syndecan, from the Greek, *syndein*, to bind together (figure 1).

The structure of the syndecan core protein has been deduced from its full-length cDNA (Saunders *et al.* 1989). The derived protein sequence defines three distinct polypeptide domains: (i) a 34 amino acid cytoplasmic domain at its carboxy terminus; (ii) a 25 amino acid hydrophobic transmembrane domain, and (iii) a 235 amino acid cysteine-free extracellular domain (figure 2). The extracellular domain contains five regions bearing ser–gly sequences that represent two distinct chondroitin sulphate (Bourdon *et al.* 1987) and three heparan sulphate putative attachment sites. Importantly, the only dibasic sequence in the mature protein serving as a potential protease-susceptible site is immediately adjacent to the region embedded within the plasma membrane.

Comparison of the syndecan core protein sequence with sequences available in protein databases reveals that the syndecan sequence is unique. However, a recently described protein sequence derived from a partial cDNA corresponding to a cell surface heparan sulphate proteoglycan from human diploid fibroblasts (Marynen *et al.* 1989) shows great similarity (figure 3). The protein core of this proteoglycan is substantially larger than that of syndecan. A partial sequence of its extracellular domain is available; this sequence is 15% identical with the syndecan sequence, but they differ substantially in various characteristics; that from the human fibroblast proteoglycan shows multiple dibasic potential proteolytic cleavage sites, two cysteines, and distinct sequences at different regions of the protein correspond to the glycosaminoglycan putative attachment sites. However, the transmembrane domains are 52%

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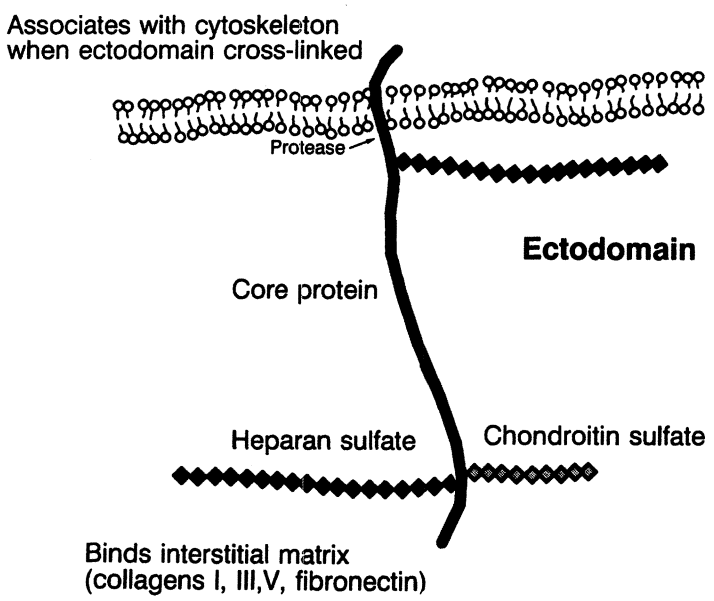

FIGURE 1. Model of syndecan at the cell surface. Note the intracellular, transmembrane and extracellular (denoted as ectodomain) domains of the core protein. The cytoplasmic domain associates with the actin-containing cytoskeleton when the ectodomain is cross-linked at the cell surface. A protease-susceptible site on the ectodomain is adjacent to the plasma membrane; cleavage at this site releases the ectodomain. The ectodomain bears both chondroitin sulphate and heparan sulphate chains, and the latter bind interstitial matrix components and bFGF.

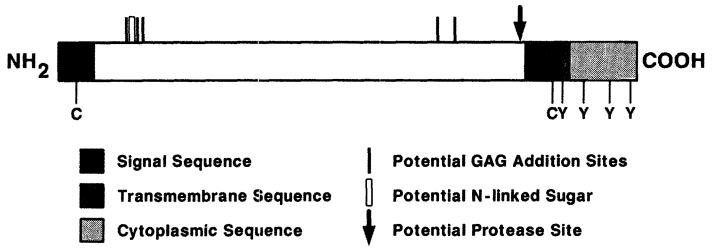

FIGURE 2. Structure of the syndecan core protein deduced from its cDNA sequence. The 33 kDa peptide has a carboxy terminal cytoplasmic domain, a hydrophobic transmembrane domain, a large extracellular domain, and a signal sequence. The extracellular domain contains five glycosaminoglycan putative attachment sites, a potential *N*-linked sugar attachment site and one potential proteolytic cleavage site. The transmembrane domain contains the single cysteine in the mature protein. The cytoplasmic domain contains three tyrosines. (After Saunders *et al.* 1989.)

identical and the cytoplasmic domains are 66% identical in sequence, with three small gaps. These structural similarities suggest that the fibroblast heparan sulphate proteoglycan core protein is related to the syndecan core protein. Syndecan probably represents a family of integral membrane heparan sulphate proteoglycans that differ in extracellular domains, but share cytoplasmic domains. Members of this family would have distinct extracellular interactions, depending on the needs of the cell type of origin, but analogous intracellular functional interactions.

The syndecan cDNA nucleotide sequence shows a remarkable similarity to the corresponding regions of the human insulin receptor cDNA (Ebina *et al.* 1985). Nucleotide sequences at both the 5′ (99 base pairs, 67% identical with four small gaps) and 3′ (35 base pairs, 80% identical) untranslated regions of the syndecan cDNA, show this similarity. The similar location of these

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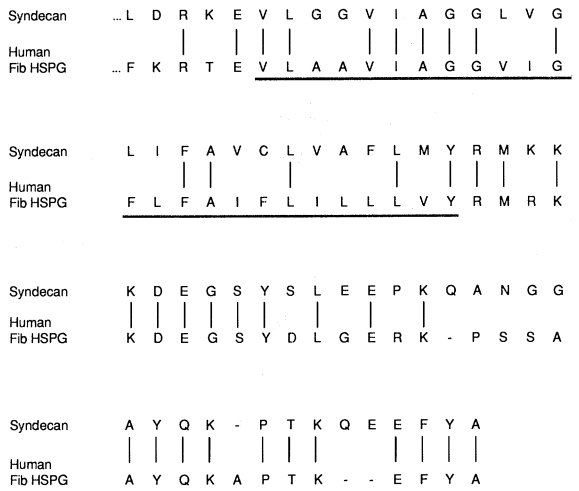

FIGURE 3. The amino acid sequences of the transmembrane and cytoplasmic domains of syndecan are similar to those of a human fibroblast heparan sulphate proteoglycan (Fib HSPG). The transmembrane domains (underlined) are 52% identical and the cytoplasmic domains are 66% identical, with three small gaps. (From Marynen *et al.* 1989; Saunders *et al.* 1989.)

regions in both cDNAs and the large size of the 5′ untranslated regions suggest that these sequences are shared translational control elements. Because insulin is a known growth factor, this sequence similarity is intriguing.

Syndecan is a matrix receptor

Although syndecan binds cells to a variety of components of the interstitial extracellular matrix, such binding, despite its high specificity and affinity, is insufficient evidence to categorize the proteoglycan as a physiologically relevant matrix receptor. However, syndecan shows several biological properties that are consistent with its role as a matrix receptor, (for reviews, see Rapraeger *et al.* (1986*b*, 1987)). Most of this work comes from studies of cultured mammary epithelial (NMuMG) cells. Prior to confluence, syndecan surrounds these cells. Its ectodomain is shed, presumably by cleavage at the site adjacent to the plasma membrane, from their apical cell surfaces into the medium as a non-lipophilic proteoglycan (Jalkanen *et al.* 1987). Upon reaching confluence, shedding of syndecan decreases rapidly and the molecule becomes localized solely at basolateral cell surfaces. Simple epithelial cells *in situ* show an identical localization of syndecan at their basolateral cell surfaces (Hayashi *et al.* 1987). Thus, syndecan localizes at a site adjacent to the matrix on polarized epithelial cells, the appropriate site for a matrix receptor.

The level of cell surface syndecan correlates with cell type. NMuMG mammary epithelial cells have *ca.* 10^6 molecules per cell, and cultured mouse keratinocytes have more (Hinkes *et al.* 1988). However, cultured mesenchymal cells have barely detectable levels of syndecan. Enhancing the staining by the use of immunogold silver, shows that NIH 3T3 and Balb/c 3T3 cells have syndecan over their entire surfaces. The amount of syndecan however, is small: there is *ca.* 100-fold less cell surface syndecan on these mesenchymal cells than on NMuMG mammary epithelial cells. Interestingly, however, these cells contain only four- to sixfold less syndecan mRNA, which suggests substantial post-transcriptional regulation of syndecan expression in these cells (M. Kato & M. Bernfield, unpublished observations).

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Syndecan binds cells to components of the interstitial matrix. The isolated ectodomain binds to fibrillar collagen with high affinity, showing a Kd of *ca.* 1 nM (Koda *et al.* 1984, 1985). This affinity, plus the large number of syndecan molecules per cell translates into a very tight association between cell and matrix. This association might stabilize the morphology of epithelial cell sheets. Upon suspension of the cells in the presence of EDTA, the syndecan extracellular domain is shed from the cell surface and is not replaced while the cells remain in suspension (Jalkanen *et al.* 1987). These results suggest a mechanism by which a tight, syndecan-mediated association of cells with matrix can be loosened to accommodate cell movements or changes in cell shape.

Cross-linking of syndecan in the plane of the membrane causes it to associate intracellularly with the actin-containing cytoskeleton (Rapraeger *et al.* 1986*a*). Indeed, isolated syndecan binds directly or indirectly to F-actin (Rapraeger & Bernfield 1982). Such cross-linking would accompany the binding of syndecan to an insoluble matrix component. Thus, on simple epithelia, syndecan can act as a matrix anchor; it binds cells to matrix, can stabilize epithelial sheets by linking the matrix to the cytoskeleton, and can be cleaved at the plasma membrane when cells are induced to change shape (figure 4).

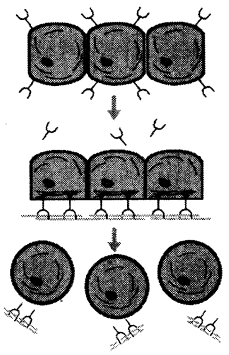

FIGURE 4. Syndecan stabilizes epithelial cell sheets. Epithelial cells are linked together by cell adhesion molecules. Syndecan is mobile in the plane of the membrane and surrounds subconfluent cultured cells (upper section of the diagram). When the cells become confluent, syndecan polarizes exclusively to the basolateral cell surface and binds to the underlying insoluble matrix. The matrix cross-links syndecan in the plane of the membrane, causing it to associate intracellularly with the actin cytoskeleton. This physical linkage stabilizes the morphology of the epithelial cell sheet (middle section). Change in cell shape leads to cleavage of the syndecan ectodomain, disrupting the linkage (lower section).

SYNDECAN HAS MULTIPLE FUNCTIONS

Mammary epithelial cells made syndecan-deficient acquire fibroblastic morphology and cell behaviour

Simple epithelial cells grow in culture as islands of closely adherent polygonal cells. These cell sheets appear to be maintained in part, by E-cadherin, a cell adhesion molecule, because expression of this molecule in fibroblasts causes them to become epithelioid in morphology (Nagafuchi *et al.* 1987) and antibodies directed against this molecule cause epithelia to become fibroblastic (Behrens *et al.* 1989). However, if syndecan is a matrix anchor that stabilizes epithelial morphology, as we have proposed, then making cells deficient in syndecan should alter their morphology.

We hypothesized that cells might be made syndecan-deficient by reducing their mRNA level with a syndecan antisense cDNA. Plasmid vectors containing the powerful β-actin promoter

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with and without the full length coding region of syndecan cDNA in the antisense configuration were transfected into NMuMG mammary epithelial cells and 40 stable clones were isolated (Saunders *et al.* 1989). The vector-only transfectants show the same morphology and levels of cell surface syndecan as the parental epithelial cells. The antisense transfectants show reduced cell surface syndecan, but the levels correlate with cellular morphology: clones showing greater than 40% of control levels grow as discrete islands of epithelial cells, while those showing less than 5% of control levels are fibroblastic, growing as individual fusiform cells with extensive filopolia that under- and overlap adjacent cells. This result corresponds with the finding that fibroblasts contain markedly lower levels of cell surface syndecan than epithelial cells.

Syndecan is apparently required for the NMuMG cells to maintain epithelial morphology, presumably by binding the cells to the fibronectin-rich matrix on the dish. There appears to be a threshold of cell surface syndecan below which the cells change their morphology, cellular organization and behaviour. The syndecan-deficient cells are no longer anchored to their substratum in an epithelial cell sheet, but now migrate as individual cells. Thus, syndecan-deficiency also causes the cells to lose their organization as an epithelial sheet, implying that either syndecan or epithelial cell shape itself is needed to maintain this cellular arrangement *in vitro*.

Syndecan shows polymorphic forms specific to various cell types

The molecular mass and cell surface localization of syndecan vary according to cell type (Hayashi *et al.* 1987; Sanderson & Bernfield 1988). Syndecan from simple epithelia has a modal molecular mass of 160 kDa and localizes to the basolateral cell surface, both *in vivo* and *in vitro*. However, syndecan from stratified epithelia has a modal molecular mass of 92 kDa, both *in vivo* and *in vitro*, and localizes over the entire cell surface. Although present at *ca.* 1% the level of that on epithelial cells, syndecan from mesenchymal cells has a modal mass of 300 kDa and localizes over the entire cell surface (M. Kato & M. Bernfield, unpublished observations). The difference in syndecan mass between these various polymorphic forms is because of differences in the number and size of the glycosaminoglycan chains on these molecules (table 1).

Whether these different polymorphic forms of syndecan have distinct functions is unclear. Each has a different localization on the cell surface and the major variation is in the number

TABLE 1. SYNDECAN SHOWS TISSUE-SPECIFIC POLYMORPHIC FORMS.

(The modal size of syndecan and the size of its glycosaminoglycan-free core protein were determined by SDS-PAGE. The actual sizes are smaller because the core protein is known to migrate spuriously (Saunders *et al.* 1989). The size of the glycosaminoglycan chains was determined by Sepharose CL-6B chromatography. The number of chains was calculated, based on these sizes and the proportion of each glycosaminoglycan type found after radiosulphate labelling of the tissues or cells. (See Sanderson & Bernfield 1988.)

	modal mass		core protein	glycosaminoglycan chains: chondroitin sulphate		glycosaminoglycan chains: heparan sulphate	
syndecan form	(kDa)	cell or tissue	(kDa)	no.	mass (kDa)	no.	mass (kDa)
stratified epithelial	92	stratified keratinocytes	69	1	7	1	21
		vagina	69	1	11	1	16
simple epithelial	160	NMuMG cells	69	2	17	2	39
		uterus	69	2	21	2	21
mesenchymal	300	3T3 cells	69	2	21	2	93

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and size of heparan sulphate chains. For example, the smallest polymorphic form is on stratified cells, such as in the epidermis and vagina. Its small size, localization surrounding the cells, and its absence from the least adhesive, most superficial cells (Hayashi *et al.* 1987) have led to the hypothesis that this heparan sulphate-poor form of syndecan may mediate cell–cell adhesions on stratified epithelia. On the other hand, the form on fibroblasts could accumulate in adhesion plaques, as previously suggested (reviewed in Lark & Culp (1987)) and mediate tight local adhesions because of its large complement of heparan sulphate (Izzard *et al.* 1986; Woods *et al.* 1986).

Syndecan binds bFGF

Low- and high-affinity binding sites for bFGF have been demonstrated on a variety of cell types (Neufeld & Gospodarowicz 1986; Olwin & Hauschka 1986; Moscatelli 1987). The high-affinity binding sites (*ca.* 10^{-10} M binding affinity) represent the functional receptor for bFGF, whereas the lower-affinity binding sites apparently involve a heparan sulphate molecule (Moscatelli 1987). These heparan sulphate molecules protect bFGF from proteolytic degradation, can displace bFGF from extracellular matrix binding sites and do not interfere with interaction of bFGF with its high-affinity receptor (Saksela *et al.* 1988; Moscatelli 1988). Syndecan also binds bFGF via its heparan sulphate chains (J. Rowland & M. Bernfield, unpublished observations). Thus, the binding of matrix components to the heparan sulphate chains of syndecan would compete with or otherwise modify its binding of bFGF.

Syndecan expression is morphogenetically regulated

Syndecan is expressed before organogenesis

In pre-implantation mouse embryos, syndecan is first detected at the four-cell stage (Sutherland *et al.* 1988). These cells show intracellular staining, but at the eight-cell stage, syndecan becomes localized on the external surface of the blastomeres adjacent to the zona pellucida, but not at the cell surface between the blastomeres. However, at the morula stage, syndecan is found on the entire blastomere surface, often prominent at the interface between the outer and inner cell populations. Thus, syndecan is initially expressed and becomes localized to the cell surface some time before expression of its matrix ligands.

In the early blastocyst, syndecan is found at the surfaces of cells in the inner cell mass and is prominent on the external surface of the trophectoderm. Upon hatching of the blastocyst from the zona pellucida, syndecan disappears completely from the external trophectoderm surface and is predominant at the interface of the primitive ectoderm and primitive endoderm, the site of earliest extracellular matrix accumulation. Here syndecan co-localizes with its matrix ligands, collagens type I and III.

In post-implantation embryos, syndecan expression correlates with differentiation in both extraembryonic and embryonic lineages, but in opposite ways (figure 5). With differentiation, extraembryonic structures, such as the extraembryonic ectoderm and the ectoplacental cone, lose syndecan expression, except for the giant cells. In contrast, cells of the embryonic lineage that will form the embryo itself retain syndecan during their development. Syndecan is lost from the primitive endoderm but is expressed by the primitive ectoderm and little change occurs during primitive streak formation and mesoderm migration. However, with further differentiation, expression changes; for example, the neural plate, notochord and pre-cardiac mesoderm lose syndecan expression (figure 5). Hence, syndecan is expressed at an early stage

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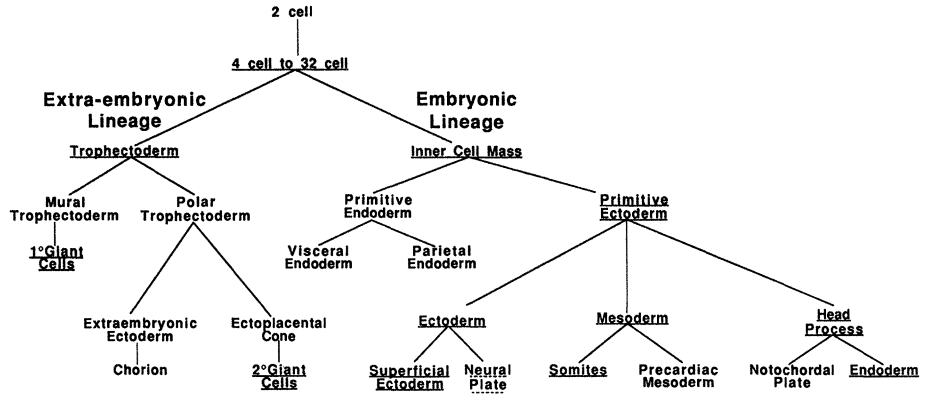

FIGURE 5. Syndecan expression during early mouse development. The cells or tissues that stain for syndecan with a serum antibody directed against the syndecan ectodomain are underlined. The dashed line indicates slight staining.

in development. It appears before its known matrix ligands, accumulates where these ligands deposit and is expressed most prominently in the cells that will form the definitive embryo.

Syndecan expression is dictated by embryonic tissue interactions

The formation of organs is often guided by epithelial–mesenchymal interactions during which the tissues undergo morphogenetic movements involving rapid changes in the adhesive properties of their constituent cells. These interactions are reciprocal; each tissue influences the morphogenesis of the other. Syndecan expression changes as a consequence of epithelial–mesenchymal interactions in a fashion consistent with its role as a matrix anchor that maintains the morphology of epithelial sheets. However, its expression during these interactions also implies additional functions.

Syndecan expression correlates with the tissue interactions responsible for development of the tooth (Thesleff *et al.* 1988). Syndecan is present on the early oral epithelium, which induces condensation of the neural crest-derived jaw mesenchyme. These latter cells now express syndecan and induce the epithelium to become the enamel organ, which then loses its stain. Thus, the target tissue in each of these interactions undergoes both a morphogenetic change and a change in syndecan expression. Subsequently, there is a loss of syndecan when the cells undergo further differentiation, analogous to that seen in various mature epithelia.

The pattern of syndecan staining in the developing tooth is mimicked during the morphogenetic changes that accompany formation of the optic, vibrissal, nasal and otic placodes and the apical ectodermal ridge (Trautman *et al.* 1987). In these tissues, the mesenchyme influences the overlying epithelium to change shape. The placode-forming cells lose their syndecan while the closely associated condensing mesenchymal cells become transiently positive. Once epithelial cell shape becomes stabilized, syndecan expression often reappears.

These changes in syndecan during reciprocal tissue interactions are prominent in the developing kidney where the ureteric bud epithelium induces the nephrogenic mesenchymal cells to become the secretory tubular epithelium (Vainio *et al.* 1989). Uninduced metanephric mesenchyme shows no stain, but induced mesenchyme expresses syndecan even when induced by a heterotypic tissue. With initial branching of the ureteric bud the entire mesenchyme expresses syndecan, most intensely on the cells that will condense into pretubular aggregates.

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These continue to express syndecan while the adjacent mesenchyme loses stain. Syndecan is expressed at the same time as epithelial tubules form, consistent with its role in stabilizing the shape of epithelial cells. However, it is lost completely after tubule formation, when these cells differentiate into the secretory epithelium. Syndecan expression in the nephrogenic mesenchyme is the first indication of renal tubule formation, preceding the changes in the extracellular matrix that are known to occur. It correlates with formation of the epithelium, but as in other developing systems, it is lost with mesenchymal cell differentiation.

Loss of syndecan expression also correlates with mesenchymal cell differentiation during limb development (M. Solursh, M. Kato & M. Bernfield, unpublished observations). In early limb buds, syndecan is on the surface ectoderm and is distributed throughout the mesenchyme. Here, as in the kidney and tooth, the epithelium transiently induces syndecan expression in the mesenchyme. However, with chondrogenic differentiation in the central limb core, syndecan is lost from these cells but remains in the peripheral and distal mesenchyme until these cells differentiate into the components of the dermis.

Thus, during organogenesis, syndecan expression follows morphogenetic rather than histologic boundaries. Its expression tracks epithelial–mesenchymal interactions in a variety of systems, becoming expressed in condensed mesenchyme as a consequence of the interaction. Based on the cell culture studies, this mesenchymal expression was unexpected, and is transient, appearing only when and where the tissue interaction occurs. Its expression also correlates in time and space with the stability of epithelial sheets. It is consistently lost when and where epithelia change shape, and appears when and where epithelia form.

Syndecan polymorphic forms correlate with changes in cell shape and organization

Syndecan has distinct polymorphic forms on simple and stratified epithelia. Changes in these forms acompany induced changes in cell shape and organization, both *in vitro* and *in vivo*. For example, primary murine keratinocytes grow as round monolayer cells in media containing low calcium ion concentration (0.05 mM) but differentiate to flat cells and stratify when the calcium concentration is increased (1.2 mM) (Hennings *et al.* 1980). With stratification, syndecan size decreases relative to its size in monolayer cultures because of a change in size and number of its glycosaminoglycan chains (Hinkes *et al.* 1988), duplicating the polymorphic forms found in simple and stratified epithelia *in vivo* (Sanderson & Bernfield 1988).

These induced changes in cell shape and organization and in syndecan structure can also be seen *in vivo* (Boutin *et al.* 1988). Newborn vaginal epithelium can be induced by uterine stroma to develop as a simple, uterine epithelium, while uterine epithelium can be induced by vaginal stroma to form a statified vaginal epithelium. When syndecan is detected by a mouse-specific monoclonal antibody in experimental rat–mouse tissue recombinations, changes in syndecan localization and structure accompany the morphologic changes: if the induced epithelium is simple, syndecan localizes basolaterally and is large in size, if the induced epithelium is stratified, syndecan localizes over the entire cell surface and is small. Thus, the structural form of syndecan is a characteristic of the cell shape or organization.

B lymphocytes express and lose syndecan at specific stages of differentiation

Considerable evidence shows that interactions between haematopoietic cells and bone marrow stroma play a regulatory role in blood-cell development (Dexter *et al.* 1982). Fibrillar collagens and fibronectin are major components of the marrow stroma and syndecan binds to

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these matrix molecules. Therefore, we assessed whether hematopoietic cells express syndecan (Sanderson *et al.* 1989). Of the haematopoietic cells in the bone marrow, syndecan is found only on precursor B cells. Furthermore, syndecan expression changes with pre-B cell maturation in the marrow and at distinct stages of lymphocyte development. Syndecan is present on pre-B and immature B cells, but is lost when B cells release from the marrow. Syndecan is absent from circulating and peripheral B lymphocytes but is re-expressed upon their differentiation into plasma cells within interstitial matrices (figure 6). Therefore, during B cell differentiation, syndecan is expressed only when and where the cells associate with extracellular matrix.

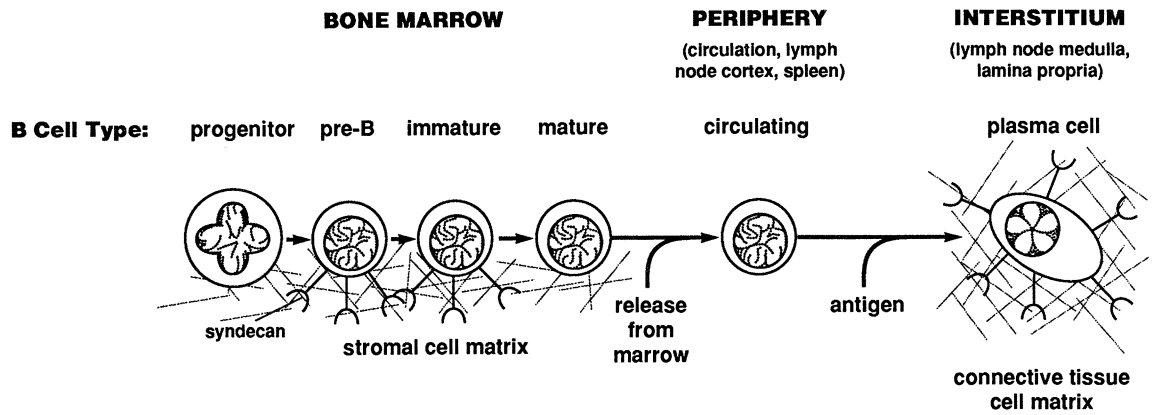

FIGURE 6. Syndecan is expressed on B lymphocytes when they associate with interstitial matrix. The expression of syndecan as a cell surface receptor is diagrammed at distinct stages and sites of B lymphocyte differentiation. Syndecan is initially expressed on pre-B and immature B cells in the bone marow but is lost as cells mature and exit the marrow. Circulating B cells lack syndecan, but re-express it upon their terminal differentiation into plasma cells within interstitial matrices.

Because syndecan can be rapidly shed from the cell surface, presumably via an endogenous protease, syndecan shedding could be a mechanism used to release B cells from the marrow. We hypothesize that this regulation controls B-lymphocyte stage-specific adhesion: (i) in the marrow, syndecan anchors maturing B cells to stromal matrix; (ii) in lymphoid organs, syndecan anchors plasma cells to the interstitial matrix, and (iii) during the interval between these two stages, syndecan is lost, allowing the cells to be released from the marrow, to circulate within the peripheral compartments, and to migrate to their sites of terminal differentiation.

Syndecan expression varies with tissue type

Immunostaining and Northern analysis of mature tissues reveal that syndecan expression is tissue-specific. In these tissues both serum and monoclonal antibodies directed against the syndecan core protein stain only the surfaces of epithelial cells, Leydig cells and plasma cells (Hayashi *et al.* 1987). Simple cuboidal and columnar epithelial cells, for example, bronchial, pancreatic, mammary ductal and intestinal epithelia, stain at their basolateral surfaces. However, the most differentiated epithelia in these organs fail to stain, for example, alveolar respiratory epithelia and the cells of the pancreatic or mammary acini. Hepatocytes stain at their sinusoidal surfaces but not at their lateral borders. Stratified squamous and transitional epithelial cells stain intensely over their entire surfaces. Again, however, the most superficial and differentiated cells fail to stain, for example the most superficial layers of the corneal, vaginal or oesophogeal epithelia or the umbrella cells of the urinary bladder. Staining

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TABLE 2. SYNDECAN EXPRESSION IS TISSUE SPECIFIC

(Compilation of the results of staining mature mouse tissues with monoclonal antibody 281–2. (After Hayashi *et al.* 1987.))

MnAb 281 staining of adult tissues	
tissue	cell surface stain
epithelium	
stratified: squamous and transitional e.g. epidermis, vagina oesophagus, cornea, bladder.	intense entire cell surface absent from most superficial cells
simple: cuboidal and columnar e.g. mammary, pancreatic ducts, trachea, bronchiole, intestine, uterus.	moderate basolateral surfaces only absent from terminally differentiated cells
mesenchyme	
e.g. endothelia, mesothelia, muscle, fibroblasts, adipocytes, renal tubules, glomeruli	none
plasma cells	intense
nerve	none

is absent on fibroblasts, endothelia, mesothelia and other mesenchymal cells as well as on neural cells and cells derived from neural crest (table 2).

Northern analysis confirms that syndecan is largely restricted to epithelial cells in mature tissues (Saunders *et al.* 1989). Northern blots of poly (A) RNA from newborn skin, adult liver and midpregnant mammary gland reveal two syndecan mRNA species of 2.6 and 3.4 kilobases (kb), whereas no syndecan mRNA was detected in skeletal and cardiac muscle. Interestingly, although no staining is evident in the cerebrum, it yielded a discrete 4.5 kb mRNA. The significance of this observation awaits further study.

SYNDECAN; A POTENTIAL MEDIATOR OF MORPHOGENESIS

During morphogenesis the extracellular matrix and the peptide growth factors act to integrate cells into discrete tissues. Syndecan expression is regulated at specific sites and at precise times during this process. The precise mechanism(s) by which syndecan is involved in morphogenesis is not clear, but importantly, it is integral to the plasma membrane and can interact with both classes of extracellular effector molecules that influence cell behaviour.

The spatiotemporal changes in syndecan expression seen during developmental processes correlate well with the function of syndecan as a matrix anchor, as deduced from its structure, its known binding interactions, its behaviour on cultured cells and the changes in epithelial cell behaviour induced by its deficiency. The strongest evidence is that syndecan maintains cell shape, thus stabilizing the morphology of simple epithelial sheets and potentially promoting the condensation of undifferentiated mesenchymal cells. To a simple approximation, cell shape results from the balance between the tension generated intracellularly by contractile actin microfilaments and the resistance produced by attachment to the solid extracellular matrix substratum (Ingber & Folkman 1989*a*). Because syndecan can physically link the matrix and the actin cytoskeleton, it is involved in controlling cell shape. It can impose stable constraints because its multiple heparan sulphate chains and high abundance produce relatively strong associations. Moreover, cleavage of its extracellular domain from cells provides a unique mechanism to reduce the stability of this linkage.

Syndecan expression in some situations is not understood in light of its matrix anchoring

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function, namely, its appearance during early mouse development before that of its known matrix ligands, its loss from differentiating mesenchymal cells, its high level of expression on stratified epithelia, its polymorphic forms and its absence from terminally differentiated epithelial cells. Moreover, a cell type can contain both syndecan and integrin matrix receptors; both these receptors can bind the cells to the same matrix ligands and can link the matrix to the cytoskeleton (Saunders & Bernfield 1988). These data are most consistent with additional functions for syndecan. One role would be to sequester growth factors at the cell surface. Syndecan will bind bFGF. Matrix heparan sulphate proteoglycans can concentrate bFGF and protect it from degradation (reviewed in Rifkin & Moscatelli (1989)). Growth factors sequestered by syndecan could potentially be released by the binding of matrix ligands.

Recent evidence is consistent with our speculation that relations between matrix and growth factor binding are involved in morphogenesis. bFGF has been shown to either stimulate endothelial proliferation or promote capillary morphogenesis *in vitro* depending upon whether the cells adhere to an insoluble matrix substratum (Ingber & Folkman 1989*b*). bFGF is found in abundance in the embryonic (chick) limb bud and its concentration is highest during the period of active cell proliferation and decreases with cell differentiation (Munaim *et al.* 1988), mimicking the changes in syndecan concentration seen in developing mouse limbs. Whatever the mechanism, however, its extracellular and intracellular interactions, and its changes in expression suggest a key role for syndecan in morphogenesis.

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