ticular regard to the incorporation of the glucosaminyl 3-O-sulphate marker group. A capsular polysaccharide derived from *Escherichia coli* K5, having the same [GlcA-GlcNAc]-, structure as the initial polymerization product in heparin biosynthesis, was chemically N-deacetylated and N-sulphated and used as a substrate for detergent-solubilized mastocytoma microsomal enzymes, in the presence of [35S]PAPS (M. Kusche & U. Lindahl, unpublished work). Analysis of the products showed 35S-labelled components representative of all the major disaccharide units recognized in heparin, including IdoA-containing species formed through the action of the GlcA C-5 epimerase. A small proportion, 1–2% of the total labelled material, had acquired high affinity for AT, thus suggesting that formation of the AT-binding pentasaccharide sequence did not require any as yet unidentified ‘committing’ structural element, in addition to the simple alternating [GlcA-GlcNAc] sequence. Likewise, a heparin-derived pentasaccharide with the structure, GlcNSO₃-GlcA-GlcNSO₃-IdoA-GlcNSO₃, yielded a 35S-labelled AT-binding component during similar incubation, whereas isomeric pentasaccharides with IdoA and GlcA in reversed sequence, or with two IdoA or two GlcA units, did not; nor did they incorporate any glucosaminyl 3-O-sulphate group (M. Kusche, R. Reynertson, L. Rodén & U. Lindahl, unpublished work). These findings emphasize the importance of the appropriate monosaccharide sequence in formation of the binding region. Further experiments, involving an analogous octasaccharide substrate and detailed structural characterization of subfractions obtained by separation of the 35S-labelled products on AT-Sepharose, led to the proposal illustrated in Fig. 2. The incorporation of the 3-O-sulphate group concludes polymer modification, and requires an acceptor structure which contains all other structural components needed for AT-binding [6]. These findings raised the question as to the structural difference between heparin with high and low affinity for AT. Detailed structural analysis between corresponding high-affinity and low-affinity fractions showed that the difference was essentially restricted to the presence or absence of the glucosaminyl 3-O-sulphate group [7]. On the other hand, the latter fractions were found to contain appreciable quantities of the -IdoA-GlcNAc(6-O-SO₃)-GlcA-GlcNSO₃- tetrasaccharide sequence, the three reducing-terminal units of which form part of the postulated acceptor sequence for the 3-O-sulphate group (Fig. 2). The two remaining positions of the acceptor regions were not examined in the low-affinity heparin; however, since these positions are occupied by the most abundant disaccharide unit -[IdoA(2-O-SO₃)-GlcNSO₃(6-O-SO₃)]- in heparin, it would seem reasonable to assume that low-affinity heparin contains the acceptor sequence for 3-O-sulphation. Yet this material did not yield any labelled high-affinity components on incubation with [35S]PAPS, in the presence of the solubilized 3-O-sulfotransferase. While the reason for this failure is unknown, 3-O-sulphation may conceivably be restricted by as yet unidentified structural elements that are preferentially expressed in polysaccharide sequences selected for the generation of low-affinity heparin. The mechanism behind such inhibition and its release appears particularly intriguing in view of the markedly non-random distribution of functional AT-binding regions in the heparin proteoglycan (Fig. 1).


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**Heparan sulphate proteoglycans of human fibroblasts**

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Heparan sulphate proteoglycans occur in close association with cells. They accumulate at cell–cell and cell–matrix interfaces, both as plasma-membrane-associated molecules and as integral components of the pericellular matrix. By binding to cell surface and matrix components, they seem to function as part of the ‘glue’ that sticks cells and tissues together. However, by activating cellular receptors, growth factors and proteinase inhibitors, these proteoglycans apparently also act as cell-surface- and matrix-associated ‘catalysts’ that influence cell growth, modulate the assembly and remodeling of the matrix, and control the activity of coagulation factors and other extracellular proteinases. These functions are primarily based on the binding interactions and allosteric effects of the heparan sulphate chains, but also depend on the interactions and associations of the proteins which carry these glycosaminoglycans. These core proteins come in a variety of forms, seem to specify the accumulation of the proteoglycans at their sites of occurrence, and may be subject to regulatory events that modulate proteoglycan abundance and function. Studies on the heparan sulphate proteoglycans of human lung fibroblasts support these concepts.

**Membrane-associated proteoglycans**

Cell-surface-associated heparan sulphate proteoglycans have now been isolated from a large number of different tissues and cells in culture [1, 2]. Several of these heparan sulphate proteoglycans have tentatively been identified as membrane proteins, based on the ability to solubilize these components with detergent, or to extract the proteoglycans as detergent-susceptible aggregates by using strong chaotropes. Furthermore, these proteoglycans are able to interact with hydrophobic gels (e.g. octyl-Sepharose) and can be intercalated into liposomes. In cultured human lung fibroblasts, the membrane heparan sulphate proteoglycans represent 20–30% of the total cellular proteoglycan [3]. Their unique hydrophobic properties and their isolation from plasma membranes that were washed in hypertonic salt solutions suggest that the core proteins of these proteoglycans are intercalated into the lipid bilayers of the membranes. In lung fibroblasts, highly
purified preparations of these hydrophobic heparan sulphate proteoglycans appear to contain multiple distinct molecular species, as heparitinase treatment yields monomeric core proteins of 125, 90, 64, 48, and 35 kDa and a disulphide-bonded dimeric ~64 kDa core protein composed of ~35 kDa subunits [4]. The reactivity of a panel of monoclonal antibodies raised against these proteoglycan and comparative peptide maps indicate that the core proteins belong to at least three different groups of molecules [5]. More recently, these findings and conclusions were corroborated by isolating cDNA clones that encode the primary structures of the 48 kDa and the 64 kDa core proteins.

The 64 kDa core protein cDNA sequence predicts an N-terminal extracellular core protein domain that carries the heparan sulphate chains, a transmembrane domain of 25 amino acid residues, and a short C-terminal domain that is 32 amino acids long [6]. The structure of the extracellular domain of this core protein seems unique, since a polyclonal rabbit antibody against this domain does not cross-react with the other cell surface proteoglycan core proteins [6]. The cytoplasmic domain of this proteoglycan, in contrast, displays extensive structural similarity with the cytoplasmic domain of Syndecan, an integral membrane proteoglycan from murine origin [7]. This similarity includes the presence of three tyrosine residues which do not occur in contexts that have been identified, so far, as consensus sequences for tyrosine phosphorylation [Fig. 1]. The conservation of these structures in different species and in different proteoglycans implies that this domain may be functionally important, possibly for interaction with the cytoskeleton. Such interactions are suggested by the co-localization of heparan sulphate proteoglycan and actin during changes in cell shape in fibroblasts [8], and when cell polarity is established in epithelial cells [9].

The detergent-insoluble residue of the fibroblast cultures, in contrast, contains only a single major heparan sulphate proteoglycan [12]. Several lines of evidence indicate that this proteoglycan is closely related or identical to the large heparan sulphate proteoglycans that occur in basement membranes [13]. It is composed of a large core protein of ~400 kDa that carries a few long heparan sulphate chains and that reacts with a polyclonal antiserum raised against the low-density proteoglycan isolated from the Engelbreth Holm Swarm tumour. Conversely, four monoclonal antibodies that were raised against the fibroblast proteoglycan react with proteoglycans produced by several cultured epithelial cell lines and intensely stain basement membranes. Since each of the four monoclonal antibodies is directed against a separate epitope, since the fibroblast proteoglycan is able to completely block the reactivity of the polyclonal antiserum with human basement membranes, and since the N-terminal amino acid sequences of two cyanogen bromide fragments from the fibroblast core protein closely match sequences predicted from the analysis of cDNA clones for murine basement membrane proteoglycan, the structural similarity appears to be extensive [14].

In culture, the matrix proteoglycan is one of the major heparan sulphate proteoglycans produced by the fibroblasts, accounting for nearly half of the protein-bound 35S-halogenated sulphate that accumulates when confluent cells are labelled with 35SO4 for 48 h. Immunocytochemical studies indicate that this proteoglycan also accumulates around fibroblastic cells in situ, and suggest that this proteoglycan is a nearly ubiquitous component of cell-matrix interfaces [14], basement membranes, however, represent cell-matrix interfaces where these proteoglycans are particularly abundant, ubiquitous, or both. Close examination of the epithelial tissues using ultracytochemistry and immunogold labelling, reveals that some monoclonal antibodies preferentially decorate the stromal aspects of the basement membranes, suggesting that these proteoglycans adopt a specific topological orientation with respect to the interstitial matrix and possibly interact with it [14]. Studies in vitro support this notion. In long-term cultures, the proteoglycan accumulates around the cells in fibrils that form a loose meshwork. Destruction of the heparan sulphate residues in this ‘matrix’ by treatment with heparitinase does not destroy this fibrillar pattern, not does it release the core protein [11]. This indicates that the intact proteoglycan is not required for the physical integrity of the meshwork and suggests that the core protein is directly incorporated in these fibrils.

This is also consistent with the finding that both the intact proteoglycan and its heparitinase-resistant core bind specifically and with high affinity to fibronectin, another major constituent of the pericellular matrix of fibroblasts and many

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**Fig. 1. Alignment of the sequences of the putative transmembrane and cytoplasmic domains of Syndecan [7] and of the fibroblast proteoglycan [6]**

The ectodomains share only 7% sequence similarity.

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**SYNDECAN, 253-311**

FIBROGLYCAN, 145-201

Alignment 60, Matches 29, Mismatches 23, Score 114, Homology 58%
other cell types [15]. This binding interaction, which occurs at physiological salt concentrations, is also displayed by glycosaminoglycan-free core protein fragments obtained by thermolysin fragmentation of the proteoglycan, and by thermolysin and chymotryptic fibronectin fragments that fail to bind heparin under the conditions of the assay, ruling out the possible contribution of residual glycosaminoglycan. Moreover, isolated core protein prevents the binding of intact proteoglycan to intact fibronectin, implying that the interaction is operative in the physiologically relevant ligands. Similar fibronectin-binding behaviour is also displayed by the core protein of the low-density proteoglycan of the Engelbreth Holm Swarm tumour, but not, e.g., by the core proteins of the membrane proteoglycans from fibroblasts, which is further evidence for the relatedness of the murine and the fibroblast matrix proteoglycans. These data indicate that fibronectin may function as a ‘docking’ protein for the matrix anchor structures that reside in the core protein of these pericellular proteoglycans. Together with the evidence that the core protein of the matrix proteoglycan interacts with cell surface components [16], that it assumes a vectorial orientation in the pericellular matrix [14], and that it codistributes with focal contacts during early adhesion cell responses [17], this functional association of the proteoglycan core and fibronectin suggests that the matrix proteoglycan plays a role in the anchorage of individual cells and cell sheets to the interstitial matrix, possibly in concert with the heparan sulphate proteoglycans that are directly embedded in the plasma membrane.

Prospects

Future investigations will hopefully further clarify the structure and function of the heparan sulphate proteoglycans and establish their role in the formation of stable cell-matrix contacts. Undoubtedly, the availability of genetic probes will be helpful in clarifying the significance of the structural heterogeneity of these components. This structural heterogeneity may be related to the modulation of cell-matrix attachment, but may also reflect the involvement of heparan sulphate proteoglycans in other cell surface processes, in which the cells take advantage of the catalytic and versatile binding properties of heparan sulphate residues. These include the binding growth factors, the control of pericellular proteolysis, and cell-cell adhesion, which seems to involve interactions between members of the integrin and of the immunoglobulin supergene families, and heparan sulphate proteoglycans. Nature may have successfully experimented with variations on a basic theme: heparan sulphate-mediated facilitation of specific molecular interactions.

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Secretory heparin in murine mastocytoma cell lines

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Proteoglycan synthesis begins in the endoplasmic reticulum by translation of the core protein and ends in the Golgi, where the glycosaminoglycan chains polymerize and undergo sulphation. Core protein containing a membrane-spanning domain move through the constitutive pathway of secretion and appear as membrane-bound components of the cell surface. If the core lacks a membrane-spanning segment, the proteoglycan may be secreted into the extracellular matrix or it may be sorted to secretory granules for storage. The different cellular locations suggest diverse biological roles for proteoglycans.

To study proteoglycan function, we have isolated a large collection of Chinese hamster ovary (CHO) cell mutants defective in proteoglycan assembly. Based on cell hybridization studies, the mutants belong to different complementation groups. Each group is designated by a three-letter acronym, pgs, which stands for proteoglycan synthesis, and an upper-case letter which refers to a genetic locus. Each complementation group corresponds to genetic lesions in a specific gene which encodes an enzyme involved in proteoglycan assembly. For example, pgsA mutants lack xylosyltransferase and pgsB mutants lack galactosyltransferase I.