REVIEW ARTICLE

Structure and function of heparan sulphate proteoglycans

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1. Introduction

Heparan sulphate proteoglycans are a complex and heterogeneous family of macromolecules composed of linear sulphated polysaccharide chains, the heparan sulphate moieties, that are covalently attached to protein [1,2,3]. In animal tissues the near-ubiquitous distribution of these components, both on plasma membranes and in the extracellular matrix (ECM), means that they are strategically positioned to regulate interactions between cells and their microenvironment [4,5,6]. Such interactions are of paramount importance for normal cell growth and development and for the maintenance of differentiated cellular functions [7,8]. Because of their relatively large polyanionic and hydrated domains, heparan sulphates must influence the general chemical and physical properties of the pericellular regions. They can, for example, inhibit the diffusion of other macromolecules across basement membranes [9] and they may control the access to cell surfaces of important regulatory molecules such as growth factors, hormones and neurotransmitters and influence local cation balance, especially of Ca^{2+} [10]. There is also ample evidence to show that heparan sulphate proteoglycans display selective patterns of reactivity with several constituents of the ECM and surface membrane (see section 5), and some of these interactions may be specific to particular cell types. In this review we have endeavoured to describe the recent advances in our understanding of these complex polymers. Despite many shortcomings in the available data, we feel that a picture emerges of a proteoglycan family of considerable diversity of structure that appears to reflect a broad spectrum of biological functions. Important questions arise in relation to the mechanisms of polymer biosynthesis and distribution, and the structure–activity relationships at both macromolecular and fine-structural levels.

2. Molecular structure of heparan sulphate

(a) **Monosaccharide composition.** Heparan sulphate is a member of the glycosaminoglycan group of complex carbohydrates that includes other ECM constituents such as chondroitin sulphate, dermatan sulphate and hyaluronic acid. There are no formal criteria for identifying glycosaminoglycans, though molecules described as such are all negatively charged polymers made up almost entirely from repeating disaccharide units. In most glycosaminoglycans the disaccharide repeat consists of an amino sugar and a uronic acid, the only exception being keratan sulphate, in which galactose replaces the uronose

residue. Sulphate groups are found on all glycosaminoglycans apart from hyaluronic acid, and all of the sulphated glycosaminoglycans are covalently linked to protein (see Fig. 1a), forming different classes of proteoglycans. However, it would be an oversimplification to conceive of the sulphated glycosaminoglycans as simple repeat-unit polysaccharides, since considerable chemical and configurational variability can be superimposed upon the component sugars. This is particularly so with the heparan sulphates, which are probably the most complex of all mammalian carbohydrates. The basic repeating-disaccharide unit in heparan sulphate, and in the chemically related compound heparin, is formed from an $\alpha, \beta 1 \rightarrow 4$ -linked sequence of glucosamine and uronic acid [1,2,3; Fig. 1b]. The glucosamine residues are either *N*-acetylated (GlcNAc) or *N*-sulphated (GlcNSO_3^-), and the latter substituent is found only in heparan sulphate and heparin. The amino sugars can also be ester(*O*)-sulphated at C-6. Uronic acid residues are present as glucuronic acid and its C-5 epimer, iduronic acid, and the iduronate residues can be *O*-sulphated, in this instance at C-2. Other *O*-sulphated derivatives that have been identified in very low concentrations in heparin and heparan sulphate are 2-*O*-sulphated glucuronic acid [11, 12] and 3-*O*-sulphated GlcNSO_3^- [13]. This latter 3-*O*-sulphated unit is an essential component of a pentasaccharide sequence in heparin that binds with high affinity to antithrombin III, and is responsible for the potent anticoagulant properties of the polysaccharide [14]. Relatively strong anticoagulant activity is associated with subfractions of heparan sulphate from the vascular endothelium [15], and $\text{GlcNSO}_3(3\text{S})$ has been detected in the proteoglycan from cultured endothelial cells [16]. It is intriguing that although GlcUA(2S) was only recently identified, it is enriched in a nuclear fraction from cultured liver cells [12]. So, despite their apparent rarity, both GlcUA(2S) and $\text{GlcNSO}_3(3\text{S})$ may confer specific properties upon the carbohydrate chain. Since all the constituent sugars in heparan sulphate can be *O*-sulphated, and since the GlcNSO_3 units may be di-*O*-sulphated [$\text{GlcNSO}_3(3\text{S}, 6\text{S})$; see Fig. 1b, ii], this makes a total of ten different monosaccharides. As larger forms of the polysaccharide chain may be composed of over 100 disaccharides, extensive sequence variability could be generated by different arrangements of the ten component sugars. However, there are some restrictions on the sugar combinations that actually occur in the native polymer, so that the full potential for diversification is not fulfilled. It is still not known whether there is a completely defined

Abbreviations used: GlcNAc, *N*-acetylglucosamine; $\text{GlcNAc}(6\text{S})$, *N*-acetylglucosamine 6-sulphate; GlcNSO_3^- , *N*-sulphated glucosamine; $\text{GlcNSO}_3^-(6\text{S})$, *N*-sulphated glucosamine 6-sulphate; $\text{GlcNSO}_3^-(3\text{S})$, *N*-sulphated glucosamine 3-sulphate; $\text{GlcNSO}_3^-(3,6\text{S})$, *N*-sulphated glucosamine 3,6-sulphate; GlcUA, glucuronic acid; $\text{GlcUA}(2\text{S})$, glucuronic acid 2-sulphate; IdUA, iduronic acid; $\text{IdUA}(2\text{S})$, iduronic acid 2-sulphate; UA, uronic acid; ECM, extracellular matrix; EHS, Engelbreth-Holm-Swarm.

- (a) Sugars that form the protein-polysaccharide linkage sequence:
(Disaccharide repeats)–GlcUA β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 3Gal β 1 $\rightarrow$ 4Xyl–Serine
- (b) Disaccharides:

(i) GlcNAc α 1 $\rightarrow$ 4GlcUA β 1 $\rightarrow$ 4

(ii) GlcNSO₃ α 1 $\rightarrow$ 4GlcUA β 1 $\rightarrow$ 4

(iii) GlcNSO₃ α 1 $\rightarrow$ 4IdUA α 1 $\rightarrow$ 4

Fig. 1. Carbohydrate composition of heparan sulphate

The disaccharides (b) making up the major portion of the polysaccharide chain are shown in three forms; an *N*-acetylated type (i) and two *N*-sulphated types, (ii) and (iii). In each example R may be SO₃[−] or H. GlcNSO₃ may be present as a 3-*O*-sulphated or 6-*O*-sulphated derivative and also as a di-*O*-sulphated component, GlcNSO₃(3S,6S).

glycan sequence in heparan sulphate from a single cell type or proteoglycan species, though the significant degree of structural organization that has been demonstrated (see sections 2c and 2d) argues strongly for some predetermination of molecular fine structure [17].

At this juncture it should be noted that, despite the fact that heparan sulphate and heparin are made up of identical sugar residues, they seem to be separate families of *N*-sulphated glycosaminoglycans [17]. Heparin is a mast-cell product and it is highly sulphated; it contains mostly *N*-sulphated hexosamines (>80% of GlcN units are *N*-sulphated) and there is always a molar excess of *O*-sulphates over *N*-sulphates [18]. In heparan sulphates, which are produced by most mammalian cells, the GlcNSO₃ residues constitute about 50% of the total hexosamine, even though there may be significant

variation in the numbers of *O*-sulphate groups (see section 2d).

(b) Sugar combinations. The major constraints that limit sugar sequence variability may be best appreciated by considering two basic features of the arrangement of sugar residues on the reducing side of GlcNSO₃ [11, 19–22, see also section 3d]. These are as follows.

(i) IdUA units are only found attached to C-1 of GlcNSO₃ (i.e. GlcNSO₃ α 1 $\rightarrow$ 4IdUA). A corresponding restriction does not apply to GlcUA.

(ii) *O*-Sulphate groups are located exclusively on disaccharides attached directly to GlcNSO₃. This point is immediately apparent for *O*-sulphated iduronate residues from the restriction already described. However, *O*-sulphated derivatives of GlcUA and GlcNAc are also found exclusively in close proximity to *N*-sulphate groups in the following arrangements:

and

Combining points (i) and (ii) above, it is clear that iduronate residues and *O*-sulphate groups will be largely confined to regions of high GlcNSO₃ content (with *O*-sulphates never separated from *N*-sulphates by more than one monosaccharide), whereas the distribution of GlcNAc units will be correlated with low sulphation and an enrichment of GlcUA. It is therefore essential to know how the different hexosamine substituents are organized in the intact polymer.

(c) Domain structure. There have been many investigations of the arrangement of sugar residues in heparan sulphate. Despite the absence of suitable techniques for determining a complete monosaccharide sequence, effective use has been made of specific procedures of chemical and enzymic degradation of the polysaccharide (Table 1). The most widely used of these methods employs low-pH HNO₂, which gives near-quantitative hydrolysis of all linkages between GlcNSO₃ and uronose units and liberates the *N*-sulphate groups as inorganic sulphate [23]. The bacterial enzyme heparinase may be used to give more selective scission at GlcNSO₃ units, since it recognizes only hexosaminidic linkages between

Table 1. Susceptible glycosidic linkages or sugar residues for reagents that degrade heparan sulphate

Low-pH HNO₂ attacks all hexosaminidic linkages of GlcNSO₃. Heparinase breaks only at highly sulphated disaccharides containing 6-*O*-sulphated GlcNSO₃[−] and 2-*O*-sulphated IdUA, whereas heparitinase shows specificity for *N*-acetylated units. These enzymes may show very weak activity against low-*M_r* substrates [24]. Low-pH periodate/alkali treatments lead to oxidation and scission at GlcUA residues flanked by *N*-acetylated disaccharides [34].

Reagent	Susceptible linkage or sugar
Low-pH HNO ₂	GlcNSO ₃ α 1 $\rightarrow$ 4UA
Heparinase	GlcNSO ₃ α 1 $\rightarrow$ 4IdUA(2S)
Heparitinase	GlcNAc α 1 $\rightarrow$ 4GlcUA
Low-pH periodate/alkali	GlcNAc α 1 $\rightarrow$ 4GlcUA β 1 $\rightarrow$ 4GlcNAc

(d) Sulphation patterns. The nature of the *O*-sulphated sugars in different regions of heparan sulphate is primarily determined by the local density of *N*-sulphate groups. Where these occur in continuous disaccharide sequences the iduronate units are more commonly *O*-sulphated than the adjacent GlcNSO_3^- residues (i.e. $\text{GlcNSO}_3^-\alpha 1 \rightarrow 4\text{IdUA}(2\text{S})\alpha 1 \rightarrow 4\text{GlcNSO}_3^-$) [20,35]. By contrast, in regions composed of alternating *N*-acetylated and *N*-sulphated disaccharides of the general form:

There may be considerable differences in the *O*-sulphate concentration of heparan sulphates from different cell types, despite the relative similarities in their *N*-sulphate contents [17]. These differences seem to be genetically determined, since mammalian cells *in vitro* continue to synthesize their characteristic polysaccharides through many cell doublings. Heparan sulphates may thus be considered to display cellular-level polymorphisms that could have significant implications for the proposed functions of these polymers in cell-cell and cell-matrix recognition (see, for examples, sections 6, 7*b* and 7*d*).

(a) General considerations. The characteristic arrangements of sugar sequences in heparan sulphate result from the substrate specificities of the enzymes which catalyse sulphate transfers and iduronate formation. It should be stated at the outset that there have been very few detailed investigations specifically of heparan sulphate synthesis, and most of our knowledge on the formation of α -sulphated glycosaminoglycans has been derived from studies on heparin synthesis by microsomal preparations

(c) **Assembly of the non-sulphated polysaccharide precursor (glycosyl transfer).** The formation of the repeating disaccharide sequence probably occurs in an endomem-

Scheme 1. Biosynthesis of heparan sulphate

A proposed scheme for the stepwise transformation of a section of the non-sulphated polysaccharide precursor heparan (Structure A) to an *N*-sulphate-rich domain of heparan sulphate (Structure B) is shown. The actual sequence of sugars in structure B is plausible, though unsubstantiated. The hexosamine at position 9 is at the reducing end of the oligosaccharide. If a sugar in the heparan precursor is destined for one permanent modification it is singly underlined, and doubly underlined if two modifications are to take place. The modification pathway begins by the deacetylation and *N*-sulphation of some of the GlcNAc units. This is followed by epimerization of glucuronosyl residues attached to C-1 of GlcNSO₃, *O*-sulphation of IdUA residues and then *O*-sulphation of GlcNAc and GlcNSO₃. Sugars 1–5 make up an alternate sequence of *N*-acetylated and *N*-sulphated derivatives (Structure B), sugars 5–9 form a heparin-like *N*-sulphated repeat sequence. The GlcNSO₃ at position 5 is thus at the junction of two separate domains. It should be emphasized that the precise structures of the intermediates formed during heparan sulphate biosynthesis have not been identified and that the scheme has been arrived at mainly by reference to the biosynthesis of heparin [36,45] and to the established occurrence of particular sugar combinations in heparan sulphate (sections 2*b*–2*d*). Incomplete polymer-level modifications, which are a characteristic feature of heparan sulphate synthesis, are illustrated here by the failure to convert the GlcNAc at position 3 to GlcNSO₃⁻ and by the absence of *O*-sulphate groups from GlcNSO₃⁻ units at positions 1 and 9 and from the IdUA unit at position 8, even though they are potential substrates for the relevant *O*-sulphotransferases. It is possible that polymer *O*-sulphation in the 'block' *N*-sulphated regions is regulated independently from *O*-sulphation of alternate sequences of *N*-acetylated and *N*-sulphated disaccharides.

excluded. It has been established, however, that the non-sulphated polysaccharide precursor is covalently linked to protein before polymer sulphation begins [43].

(d) Polymer-level modifications: sulphation and epimerization. The formation of the non-sulphated polysaccharide precursor, sometimes referred to as 'heparan' or '*N*-acetylheparosan', is the first stage in a complex sequence of events that culminates in the emergence of mature *N*-sulphated macromolecules. These modifications are localized to particular regions of the glycosaminoglycan chains and are brought about by functionally integrated membrane-bound enzymes [44]. The process has been experimentally dissected in biosynthetically active microsomal preparations by restricting the availability of the sulphate precursor 3'-phosphoadenosine 5'-phosphosulphate in the incubation medium. The identification of a relatively discrete series of polysaccharide intermediates with increasing concentrations of sulphate groups and iduronate residues has led to the concept of a *stepwise* mechanism of polymer-level modifications [36,45]. This implies that a particular set of modifications must be completed before the next stage can begin.

A general scheme for the transformation of heparan into heparan sulphate is proposed in Scheme 1. The scheme is based on a combination of limited biosynthetic data [37,38] with extensive information that is available

on the actual sequences of sulphated and non-sulphated sugars that are found in the mature polymers (section 2).

Sulphation begins with the conversion of a proportion (about 50%) of the GlcNAc units in heparan to GlcNSO₃ via a deacetylated intermediate:

Deacetylation is driven by concurrent sulphation of the free amino groups [46], and if we assume that there is no rearrangement of the hexosamine units, then the mainly segregated distribution of the *N*-sulphated and *N*-acetylated disaccharides will be established at this stage.

It can be readily appreciated from the constraints described in section 2(*b*) that further modifications will be confined to the *N*-sulphated domains of the polysaccharide intermediates. Indeed, GlcNSO₃ units are essential for substrate recognition by the subsequent polymer-modifying enzymes (reviewed in ref. [3]). In general terms, the modifications take place in the following sequence: epimerization of GlcUA to IdUA, *O*-sulphation of the IdUA units and finally *O*-sulphation of the hexosamine residues (mainly of GlcNSO₃⁻). A complete polymer modification will therefore produce trisulphated disaccharides of structure:

Such highly sulphated disaccharides are confined to the *N*-sulphated repeat sequences. They are the most

abundant constituent of heparin (and form the heparinase-cleavage site), but they occur with a low frequency in heparan sulphate: instead, disulphated disaccharides, which contain *O*-sulphated iduronate residues, tend to accumulate in the block *N*-sulphated domains [19,35]. However, as we discussed above (section 2*d*), IdUA(2S) is probably absent from the alternating *N*-sulphated and *N*-acetylated regions of heparan sulphate where the *O*-sulphate groups are mainly confined to hexosamine units [21,22]. These distinctions, which are illustrated in Scheme 1, presumably reflect some mechanistic differences in the polymer modification reactions according to the density of *N*-sulphate groups. It is not known how these patterns of modification are controlled and integrated during biosynthesis.

Analyses of HNO₂ hydrolysates of heparan sulphate indicate that, in a small number of instances, *O*-sulphation of GlcNSO₃ occurs without prior conversion of the attached GlcUA to IdUA [20,35]. The GlcUA 5'-epimerase does not recognize as substrates GlcUA units adjacent to *O*-sulphated GlcNSO₃⁻ [45]. An additional important characteristic of heparan sulphate biosynthesis (and to a lesser extent of heparin biosynthesis as well) is that, at each stage of the polymer modification sequence (Scheme 1), only a proportion of the potential substrates are utilized by the modifying enzymes. The only exception is the conversion of GlcNH₃⁺ to GlcNSO₃⁻, which appears to be virtually quantitative. The mode of selection of substrates for modification is unknown.

4. Proteoglycan localization

In 1971, Kraemer [47,48] suggested that heparan sulphate is likely to be a ubiquitous component of cultured mammalian cells and that it occurs partly as a membrane-associated component. Subsequent investigations have substantiated this proposition, with most cell types in culture producing cell-surface-associated proteoglycans. The form of association with the plasma membrane has itself been the subject of much investigation, with two suggested models, namely intercalation into the membrane and binding to a cell-surface receptor.

Beginning with Kjllen and co-workers' [49] observations with rat liver cells, a number of studies [50–55] have demonstrated the ability of various cell types (i.e. endothelial, Chinese-hamster ovary and brain cells) to specifically bind exogenous heparin or heparan sulphate, allowing the possibility of a receptor-like association of the proteoglycan with the plasma membrane, mediated by the polysaccharide chains. Hepatocytes apparently possess approx. 4×10^6 receptors per cell, and the dissociation constant for the interaction is of the order of 10^{-7} M [52]. Binding does not appear to be a prelude to internalization and degradation of the proteoglycan [52] and is thus distinct from the receptor-mediated endocytosis involving recognition of the protein core, which has been demonstrated for proteoglycans in fibroblasts [56–57]. Many cells [33,55,58–61], including fibroblasts and hepatoma cells, do not appear to possess significant quantities of receptor-bound proteoglycans (as assessed by the lack of released proteoglycan on addition of heparin). The significance of these cellular differences is not clear. In hepatoma [60] and other transformed cells [55] the lack of receptor-bound proteoglycan has been correlated with an undersulphation of the heparan sulphate chains rather than a deficiency

of the receptor itself. This undersulphation leads to a demonstrably weaker affinity of the polysaccharide for the receptor.

The evidence for a membrane-intercalated lipophilic proteoglycan, although substantial, is indirect, deriving from such observations as the ease of extraction with detergents, interaction with detergent micelles, binding to the hydrophobic gel octyl-Sepharose and insertion into liposomes [51,53,60–64]. Direct evidence, such as the identification of an intramembrane domain with a lipophilic amino acid sequence, or, in the case of a putative transmembrane molecule, the identification of a cytoplasmic domain, is lacking.

In normal rat fibroblasts the plasma-membrane proteoglycan aligns with actin-containing stress fibres within the cells, indicating that it may provide a means of direct communication between structural elements of the stromal and cytoplasmic matrices. However, in transformed rat fibroblasts actin-containing stress fibres cannot be identified and the heparan sulphate proteoglycan is diffusely distributed [65].

The turnover of plasma-membrane proteoglycans has been studied in a number of cell-culture systems. Endocytosis followed by intracellular breakdown seems to be their principal metabolic fate [35], although some shedding into the growth medium has been observed in cultured colon [66] and granulosa cells [67]. In the latter, the endocytosed proteoglycan fraction may follow different degradation pathways, one rapid ($t_{1/2}$ of 25 min), the other proceeding more slowly ($t_{1/2}$ of 4 h) with initial stages of proteolytic and endoglycosidic cleavage occurring within a non-acidic endosome compartment [67,68].

Heparan sulphate proteoglycans also occur in both stromal matrices and basement membranes. They are usually found in association with networks of fibrillar material and at interfibrillar junctions, often in apparent co-distribution with collagens, fibronectin and laminin [65,69–72]. Transformed cells in culture sometimes lack a detectable extracellular matrix [69,73], even though the major matrix macromolecules are synthesized, albeit in decreased amounts. Heparan sulphate produced by transformed cells is undersulphated [33,74–78], and it may be unable to participate in interactions with other structural macromolecules that are essential for matrix biogenesis.

5. Proteoglycan structure

(a) **Technical difficulties.** In common with many other types of proteoglycan, structural analyses have been complicated both by heterogeneity and polydispersity, and by the fact that several cell types may contribute to the proteoglycans isolated from tissue sources. These problems are further compounded by the lack of appropriately calibrated methods of M_r determination, particularly for intact proteoglycan molecules, and to a lesser extent for the glycosaminoglycan chains. Cell cultures can provide more homogeneous cell populations for study, but the low quantities of the proteoglycans that they produce have precluded the application of ultracentrifugal or light-scattering techniques for more reliable size estimations. These factors have made comparisons between proteoglycans from different sources rather problematic.

(b) **Size and structural organization.** The apparent M_r values for the proteoglycans so far described vary be-

Table 2. Composition of some heparan sulphate proteoglycans

Origin	$10^{-3} \times M_r$			No. of heparan sulphate chains	References
	Intact proteoglycan	Protein core	Heparan sulphate chains		
Embryonic-human skin fibroblasts (cell membrane)	350	150 (dimer)	20	8–12 (dimer)	[83,84]
Rat hepatocytes (cell membrane)	73.5	17–30	14	3–4	[51]
Human colon carcinoma (organ culture)	850–950	240	30	30–40	[66]
Rat ovarian granulosa cells (cell culture medium)	400–500	—	28	6–8	[85–86]
Rat and bovine glomerular basement membranes (tissue extract)	130–200	18	14–25	4	[87,88–90]
Mouse EHS sarcoma (tissue extract)*	750–800	350–400	60–70	4–6	[91–93]
	400	—	29	4	[94]
Rat pheochromocytoma cells (conditioned medium)	350	80	15	18	[95]

* Two differing sets of analytical data referring to the large EHS-sarcoma proteoglycan are presented.

tween approx. 70000 for hepatocytes [51] and ascites hepatoma [79] up to an estimated $(1-2) \times 10^6$ for aortic endothelial cells [80], colon/colon carcinoma [66,81] and bovine lung [82]. These wide variations appear to reflect differences both in the structure of the protein cores and in the number and length of the heparan sulphate chains. Comparative data for the more extensively characterized proteoglycan species are summarized in Table 2. In addition to heparan sulphate, both *N*- and *O*-linked oligosaccharides have been identified on a number of protein cores [53,83,85, 90]. Surprisingly, two recent reports have indicated that the mouse mammary epithelial proteoglycan possesses a minority of chondroitin sulphate chains on the same polypeptide as heparan sulphate [96,97].

At present there is still insufficient information to allow construction of detailed models of heparan sulphate proteoglycans; however, a number of recent studies have provided some insights into certain aspects of structural organization. Yanagishita & Hascall [85], working with the secreted ovarian granulosa-cell proteoglycan, have identified three major domains of indeterminate arrangement within the proteoglycan, comprising (a) an interspersed grouping of 6–8 heparan sulphate chains and 10–15 *O*-linked oligosaccharides, (b) an *O*-linked-oligosaccharide-substituted domain, and (c) an *N*-linked-oligosaccharide-substituted domain. A tendency for the heparan sulphate chains to be clustered has also been observed with the skin fibroblast [83] and glomerular-basement-membrane [90,98] proteoglycans and may be a general phenomenon. Parthasarathy & Spiro [98] isolated a Pronase-released glycosaminoglycan-peptide from the glomerular proteoglycan and showed that 64% of the amino acids were accounted for by serine and glycine. In order to accommodate four heparan sulphate chains, every seventh amino acid residue (on average) may be substituted for by polysaccharide. Such a tight clustering with a preponderance of intervening glycine residues ($\sim 50\%$ of remaining amino acids) has similarities to the glycosaminoglycan-linked Ser-Gly repeats found in both the heparin proteoglycan [99] and a chondroitin sulphate proteoglycan from rat yolk-sac tumour [100].

The most thoroughly characterized proteoglycan is probably that derived from the cell surface of confluent embryonic-human skin fibroblasts. This proteoglycan

has an M_r of approx. 350000 and is so far unique in being a disulphide-bonded dimer. It comprises apparently identical monomer units of approx. M_r 140000 with protein cores of M_r less than 80000 [83,84]. This dimer has some structural similarities to the transferrin receptor molecule and, interestingly, Fransson and co-workers [101] demonstrated that the proteoglycan could bind transferrin *in vitro*, as assayed by co-immunoprecipitation with an anti-transferrin antibody. This activity was located on the protein core of each monomer in close proximity to the hydrophobic domain [102]. However, the inability of the cell-surface proteoglycan to bind transferrin *in situ* makes the physiological significance of this phenomenon uncertain.

(c) **Structural homologies.** Despite difficulties in making meaningful comparisons of proteoglycans on the basis of physical criteria, interesting data have been acquired by using antibodies that bind to protein-core determinants. Such studies have revealed cross-reactivity, and therefore some homology, between the proteoglycans derived from the widely studied basement-membrane-producing yolk-sac carcinoma and Engelbreth-Holm-Swarm (EHS) sarcoma, with other basement membranes *in vivo*, e.g. kidney, liver, skin, cornea, Reichert's membrane and amnion [103–105]. Likewise, antibodies against the rat liver membrane proteoglycan cross-react with the cell surfaces of both EHS sarcoma cells and parietal endoderm cells, but not with the basement membranes laid down by these cells [105]. There are thus at least two structurally dissimilar proteoglycan core proteins being synthesized in these tissues, with different distributions. The antibody approach has also proved useful in confirming the indications from pulse-chase experiments that the smaller protein-deficient basement-membrane proteoglycan from EHS sarcoma is structurally related to, and probably a physiological degradation product of, the larger, protein-rich, molecule [92,93].

6. Interaction of heparan sulphate proteoglycans with matrix macromolecules

Heparan sulphate, either as free chains or as proteoglycan, is capable of interaction with a variety of matrix macromolecules *in vitro*. However, the degree of

Fig. 2. Extracellular matrix and plasma-membrane forms of heparan sulphate proteoglycans

Diagrammatic representation of three differing proteoglycan structures: (a) large basement-membrane proteoglycan from EHS sarcoma [92,94], (b) cell-surface proteoglycan from ovarian granulosa cells [86] and (c) cell-surface proteoglycan from confluent cultures of embryonic-human skin fibroblasts [83,84]. In (a) there is, as yet, no information on possible substitution with *N*- and *O*-linked oligosaccharides. The cell-surface proteoglycans (b,c) are represented as possessing hydrophobic polypeptide domains by which they can be anchored within the lipid bilayer. There is generally strong evidence for clustering of the heparan sulphate chains. Likewise, the various glycan species do tend to occupy separate domains along the protein core, but the actual positions and relative sequence are purely speculative. The fibroblast proteoglycan (c) possesses a transferrin-binding site *in vitro* [101] that appears to be located on the protein core near to both the hydrophobic domain and the interchain disulphide bond [102].

specificity, the structural requirements and the likely stability of the interactions under physiological conditions are often unclear.

(a) Collagens and basement membranes. Some heparan sulphates [106,107], including the mammary-epithelial-cell proteoglycan [108,109], can bind to type I collagen. The latter proteoglycan interacts with a high affinity (K_d 10^{-9} – 10^{-10} M) and an apparent stoichiometry of one proteoglycan molecule per 80–200 collagen monomers (a single fibril?) [108]. Interestingly, free heparan sulphate chains were inactive, even though exogenous heparin chains were able to compete with proteoglycan for binding. Probably the multivalency of the polysaccharides in a proteoglycan is a significant factor here, and the protein core itself may express collagen-binding activity as has been demonstrated for the protein component of a dermatan sulphate proteoglycan [110]. The epithelial proteoglycan would also bind collagen types III and V, but not types II and IV [109].

The affinity of the basement-membrane-derived EHS-tumour proteoglycan for the basement-membrane-specific type IV collagen apparently resides in the polysaccharide chains, although binding appears weak under

physiological conditions [94,111]. Both the triple-helical and the globular NC1 domains of the collagen monomer interact [94].

Heparan sulphate chains [112,113] and basement-membrane proteoglycans [94,111,114] bind relatively strongly to laminin, another characteristic basement-membrane protein. Binding occurs at physiological ionic strength with a reported stoichiometry of 3–10 laminin molecules per proteoglycan molecule [114].

(b) Fibronectin. One interaction which has received much attention has been that with fibronectin, although the situation is still obscure. Various heparan sulphates (and proteoglycans) bind weakly with intact fibronectin [60,94,111,115–117], although myeloma heparan sulphate has been stated not to interact [107]. Comparisons of binding activities are confused by the relative weakness of the interaction *in vitro* and the multiplicity of assay conditions, although a dependence of binding on the degree of sulphation of the heparan sulphate has been established by the demonstration that rat liver proteoglycan has a greater affinity for fibronectin than has the relatively less-sulphated hepatoma proteoglycan under identical assay conditions [60]. The binding sites on the

fibronectin molecule apparently coincide with the three or four heparin-binding domains identified by Gold and colleagues [118]. Although the data obtained *in vitro* indicate that the binding of heparan sulphate is too weak to constitute a stable association *in vivo*, several strands of evidence suggest that such an association actually exists. Evington and colleagues [119] have estimated from fluorescence measurements *in vitro* that the dissociation constant for the interaction at physiological ionic strength may be of the order of only 10^{-5} M. However, coupled with estimates for the likely cell-surface concentrations of both heparan sulphate and fibronectin, they suggest that there may be sufficient scope for stable complexes to form. In addition, cross-linking studies *in vivo* [120] have demonstrated the close proximity of fibronectin and sulphated proteoglycans on the surface of fibroblasts. Immunoprecipitation of fibronectin from fibroblast extracts can also co-precipitate heparan sulphate and chondroitin sulphate proteoglycans, and density-gradient centrifugation of matrix extracts can float fibronectin and heparan sulphate proteoglycan at identical positions, even at relatively high ionic strength (2 M-CsCl)[70]. The possibility of additional, unidentified, components mediating these interactions has not been ruled out, however. Certain proteinase-generated fragments of fibronectin can bind heparan sulphate more strongly than the intact molecule [117], implying that there may be cryptic binding sites that are accessible only after unfolding of the fibronectin. Such unfolding or 'activation' might be induced by binding to other matrix macromolecules, e.g. collagen [121]. Matrix deposition may thus involve multiple co-operative interactions between these macromolecules, as heparan sulphate has also been implicated in fibronectin fibrillogenesis and the binding of fibronectin to type I collagen [122–124].

(c) Cellular adhesion. Cell–matrix interactions as modelled *in vitro* by cell–substratum adhesion studies appear to involve heparan sulphate proteoglycan in some instances [125–127]. For example, the adhesion sites of newly attached fibroblasts are enriched in both fibronectin and cell-surface proteoglycan [65,115,128,129].

Both heparin [130] and heparan sulphate [131] apparently induce conformational changes in fibronectin, and the findings of Johansson and colleagues [132,133] would favour such an activation (possibly in conjunction with collagen) as a prerequisite for maximal activity of fibronectin's cell-binding domain. Recent studies with neural retina cells have shown that attachment occurs via interaction of the cells with discrete supramolecular complexes, comprising various proteins and glycosaminoglycans termed 'adherons'. Exogenous heparan sulphate inhibits cellular adhesion to adheron-coated substrata, suggesting that the cell-surface proteoglycan may be the adheron receptor at the cell surface [134,135]. The corresponding heparan sulphate-binding molecules in the adheron appear to be two proteins of M_r 20000 and 170000 [136,137].

(d) Self-affinity. Fransson and co-workers [138–141] have demonstrated an ability of structurally similar heparan sulphate chains to self-associate. This is dependent on the presence of multiple contact zones, with a highly copolymeric structure, separated by *N*-acetylated repeat sequences. Both the cell-surface- and culture-medium-derived proteoglycans from human skin fibro-

blasts will aggregate with Sepharose-bound heparan sulphate chains *in vitro* [142]. Quantitative binding data are now needed to ascertain whether such self-association can occur under physiological conditions.

(e) Protein core interactions. The above picture of potential proteoglycan interactions has been heavily influenced by the relative ease of assessing the binding properties of heparan sulphate as compared with those of the protein core. However, the protein component may yet prove to be a significant reactant in its own right (see also transferrin binding in section 5b). For example, a hyaluronate-binding activity has been identified in colon [81] and lung [82] proteoglycans which, by analogy with the cartilage chondroitin sulphate proteoglycans [143], may be dependent upon the protein core. The necessity for denaturing conditions in order to extract the large EHS basement-membrane proteoglycan (the precursor of the smaller, protein-deficient, proteoglycan, which only requires saline for extraction) would also point to a considerable interactive role for the protein core [92].

7. Functional aspects

(a) Heparan sulphate and DNA regulation. Heparan sulphate has been identified as a constituent of the nuclear fraction from cultured melanoma [144] and liver [12] cells, observations that raise the possibility of its involvement in nuclear structure or regulation. Heparan sulphates from a number of cell lines are able to inhibit DNA polymerase *in vitro* [145,146], and exogenous heparan sulphate will also inhibit endogenous DNA synthesis in isolated liver nuclei [146]. None of these observations prove a physiological role for the polysaccharide. However, Kinoshita and colleagues have provided strong evidence that a nuclear proteoglycan, probably heparan sulphate, is an essential determinant of genetic regulation in sea-urchin embryos [147–149]. If proteoglycan synthesis is inhibited by addition of β -D-xylosides, embryonic development does not proceed beyond the blastula stage [150,151].

(b) Neuronal cells. Interest in heparan sulphate proteoglycans in neuronal tissues is expanding rapidly and providing new insights into biological functions. They are the major proteoglycans of the mammalian nervous system [152], and investigations into their distribution and activity has been considerably assisted by the fact that they are more potent as antigens than their counterparts from other sources. In general, reactivity with antibodies that recognize neural cell-surface proteoglycans increases markedly during ganglionic growth and periods of rapid dendritic elaboration, reaching maximum levels in the young adult [153,154]. Heparan sulphate proteoglycans are also the major antigenic components of cholinergic synaptic vesicles of many animal species [155–157]. After fusion of these vesicles with the synaptic plasma membrane, the proteoglycans may become firmly embedded in the synaptic basal lamina [155], although some molecules are internalized during retrieval of the synaptic membrane and these may help in maintaining the integrity of vesicle membranes during multiple cycles of secretion and endocytosis [157].

Neuronal cells in culture are stimulated to produce neurites in the presence of substrates containing heparan sulphate proteoglycans [158,159]. Morphogenic activity probably requires a combination of the proteoglycan with

other extracellular constituents such as laminin [160,161]. Innervation of embryonic muscle is accompanied by an early accumulation of heparan sulphate proteoglycans in the muscle membranes at the sites of synapse formation [162]; this is followed by localization of acetylcholine receptors. Denervated muscles retain their proteoglycans (but not the acetylcholine receptors) in regions of the original synapse, where they might provide a guidepath for regenerating nerves [163,164]. Furthermore, a specific interaction between surface proteoglycans of neuronal cells and the asymmetric form of acetylcholinesterase seems to be essential for the association of the enzyme with the synaptic basal lamina [165,166]. These findings strongly implicate heparan sulphates in the biogenesis of membrane specialization at synaptic junctions.

(c) Lipid metabolism. Lipoprotein lipases hydrolyse the triacylglycerol component of very-low-density lipoproteins and chylomicrons to free fatty acids and glycerol. Early observations on the antilipaemic properties of intravenous heparin [167] and the related increase in serum lipoprotein lipases suggested that this enzyme was located on the interior lining of blood vessels, perhaps attached to a heparin-like molecule. Heparan sulphate and heparin will bind to lipoprotein lipase under experimental conditions [168]. It is now established that cultured endothelial cells synthesize heparan sulphate [32, 80, 169–171], and the glycosaminoglycan chains probably represent an essential component of the lipoprotein lipase-binding site of endothelial surfaces [172,173]. In the intact endothelium, the binding stoichiometry has been estimated at one enzyme molecule for each proteoglycan [174]. A heparan sulphate fraction from peripheral blood stimulates lipoprotein lipase activity [175], but it remains to be established whether the endothelial cell-bound enzyme is in a similarly active form. The proteoglycan might facilitate lipid metabolism by bringing the enzyme into close contact with cell-surface receptors for lipoproteins.

(d) Cell growth. The predominant pericellular localization of heparan sulphate proteoglycans has prompted many investigators into proposing that they play important roles in controlling cell growth. *In vitro*, the growth of Schwann cells requires direct contact with neuronal cells, and heparan sulphate has been identified as an important constituent of the mitogenic stimulus [176]. However, heparan sulphate more frequently displays growth-inhibitory properties. Glutaraldehyde-fixed monolayers of confluent fibroblasts inhibited the growth of freshly seeded cells, and the inhibition was relieved by heparitinase or HNO_2 treatment [177]. Heparan sulphate from normal rat liver inhibits the growth of confluent hepatoma cells in culture [178]. Perhaps the clearest data on growth inhibition relates to smooth-muscle cells, which are inhibited by heparan sulphate polysaccharides produced by confluent, but not sub-confluent, cultures of the same cell type [179], implying considerable specificity in this effect. Similarly, endothelial cells release heparan sulphate fragments that inhibit the growth of vascular smooth-muscle cells [180,181]. Such fragments, which are enriched in sulphate, could be produced by excision from endothelial-cell heparan sulphate by platelet-derived endoglucuronidase. In this context, it is significant that, under experimental conditions, heparan sulphate is important for maintaining the non-thrombogenic pro-

perties of the endothelial surface [182,183], an effect presumably mediated by its ability to bind antithrombin III [15,16] and also thrombin [184]. It will be important to establish whether the various properties of growth control, anticoagulation and lipoprotein lipase binding (section 7c) are present on the same, or different, endothelial-cell proteoglycans. Attention should also be drawn to an alternative view of heparan sulphate as a promoter of angiogenesis, a process requiring endothelial-cell growth and migration [185]. The argument is based partly on some structural similarities between endothelial-cell heparan sulphate and heparin, which is known to stimulate angiogenesis under certain conditions [186]. The proposal merits experimental scrutiny.

(e) Basement-membrane permeability. We have already referred to the important contribution made by heparan sulphate proteoglycans to the organized molecular framework that exists in basement membranes. These membranes regulate the passage of cells, glycoproteins and small ions between the luminal spaces lined by epithelial and endothelial cells and the surrounding connective-tissue stroma. Malfunction of basement membranes would have a deleterious effect on organ and tissue homeostasis. The importance of the heparan sulphate proteoglycan component in this regard is stressed by the observations that the kidney glomerular basement membrane, which separates the circulating blood from the urinary spaces, becomes permeable to ferritin [9] and albumin [187] after treatment with heparan sulphate-degrading enzymes. Nephrotic kidneys, which also allow the diffusion of serum proteins into the urine, are almost completely deficient in basal-lamina heparan sulphate [188]. There is also experimental evidence to indicate that a decreased content of heparan sulphate could be responsible for some of the pathological findings in diabetes [189].

(f) Cancer metastasis. The dissemination of tumour cells to various organs and tissues is dependent upon a variety of factors, one of which is the ability of these cells to migrate across normal architectural barriers such as epithelial and endothelial basement membranes. The actual mechanism of migration is poorly understood, but often some disruption of the integrity of basement membranes is involved. Melanoma and lymphoma cells release an endoglycosidase that specifically degrades heparan sulphate [190,191] and which will liberate heparan sulphate oligosaccharides from basement membranes produced by endothelial cells *in vitro*. Highly metastatic cells produce larger quantities of this enzyme than do low-metastatic variants [192]. The melanoma enzyme seems to attack the *N*-acetylated regions of heparan sulphate, hydrolysing the glucuronosyl linkages, $\text{GlcUA}\beta 1 \rightarrow 4\text{GlcNAc}$ [193]. Enzymes with similar specificities have been identified from several mammalian cells and tissues (section 2c), and it is not known if the melanoma enzyme has distinctive properties. It may be possible to inhibit endoglycosidases by chemically synthesizing structural analogues of the susceptible sugar linkages, and such compounds could be useful inhibitors of cancer metastasis.

8. Future directions

It is inevitable that modern molecular-biological techniques will strongly influence research into heparan

Fig. 3. Interactions of heparan sulphate proteoglycan in the pericellular domain

Some interactions of heparan sulphate proteoglycans are schematically represented in vascular tissues by showing an endothelial-cell layer, with basement membrane, overlying a stromal matrix. Heparan sulphate proteoglycans are distributed at various possible cellular and matrix sites, where specific proteoglycan structures may display differing patterns of reactivity with other macromolecules (as listed), thus contributing to the specific functions indicated.

sulphate in the immediate future. We can anticipate the molecular cloning and sequencing of the genes coding for the proteoglycan core proteins (this has already been achieved for a chondroitin sulphate proteoglycan [100]). Homologies between different protein cores can then be sought: the tendency for polysaccharide chains to be clustered in the proteoglycan suggests that similar amino acid sequences may be present in these regions. Amino-acid-sequence data should confirm the existence of a hydrophobic section in plasma-membrane proteoglycans. It will also be possible to examine the influence of the protein core upon the structure of the attached heparan sulphate.

Heparan sulphate biosynthesis is still poorly understood, and more refined methods are needed to isolate and characterize the metabolic intermediates. Glycosaminoglycan sequence analysis still lags behind that achieved for protein and nucleic acids, although polyacrylamide-gel electrophoresis might make a useful contribution to these studies in the near future [194]. If the sugar sequence is highly ordered in heparan sulphate, there must be some form of template or blueprint that predetermines these sequences.

As we have discussed at length and have summarized in Fig. 3, there is now compelling evidence for believing that heparan sulphate proteoglycans are important for the regulation of cell-cell and cell-matrix interactions. Such properties almost certainly depend upon the ability of the proteoglycans to bind with different components of the extracellular matrix and thereby promote the formation of an organized network of extracellular macromolecules. We need to pursue the problem of the molecular structure of the plasma-membrane receptors and extracellular-matrix binding sites for heparan sulphate proteoglycans and the ability of these interacting molecules to recognize specific sugar or amino acid sequences. What is the form and purpose of the information that is relayed across the plasma membrane when surface proteoglycans interact with complementary

structures in the pericellular regions? Is this 'informational flow' compromised if the polysaccharide chain is undersulphated, as in transformed cells, and does this then limit the ability of the micro-environment to regulate tumour-cell growth and migration?

Monoclonal antibodies are likely to be of immense value in identifying functional domains of the proteoglycan core protein, and perhaps also of the glycosaminoglycan chain. Immunological and molecular-biological approaches will permit analysis of changing patterns of proteoglycan-gene expression and the ultrastructural localization of the proteoglycan antigens during cell differentiation in embryonic and adult tissue. It is conceivable that, in common with certain other glycoproteins, embryonic forms of proteoglycan genes will be re-expressed in adult malignancies. In view of the extensive variation in structure and location of heparan sulphate proteoglycans, it is unlikely that a single function will be ascribed to these macromolecules. As their biological roles become defined we are likely to gain new insights into the molecular basis of cell development, and this could have profound implications for our understanding and treatment of a wide range of diseases, including cancer, blood-vessel disorders, diabetes, nephritis and inherited and acquired dysfunctions of the nervous system. We predict with confidence that the next few years will not be dull in heparan sulphate proteoglycan research.

References

1. Lindahl, U. & Höök, M. (1978) *Annu. Rev. Biochem.* **47**, 385-417
2. Rodén, L. (1980) in *The Biochemistry of Glycoproteins and Proteoglycans* (Lennarz, W. J., ed.), pp. 267-371, Plenum Publishing Corp., New York
3. Comper, W. D. (1981) *Heparin (and Related Polysaccharides)*, Gordon and Breach, New York
4. Kraemer, P. M. (1979) in *Surfaces of Normal and Malignant Cells* (Hynes, R. O., ed.), pp. 149-198, John Wiley and Sons, New York

5. Gallagher, J. T. & Hampson, I. N. (1984) *Biochem. Soc. Trans.* **12**, 541–543
6. Höök, M., Kjellén, L., Johansson, S. & Robinson, J. (1984) *Annu. Rev. Biochem.* **53**, 847–869
7. Bissell, M. J., Hall, H. G. & Parry, G. (1982) *J. Theor. Biol.* **99**, 31–68
8. Hay, E. D. (1981) *J. Cell Biol.* **91**, 205s–223s
9. Kanwar, Y. S., Linker, A. & Farquhar, M. G. (1980) *J. Cell Biol.* **86**, 688–693
10. Öbrink, B., Pertoft, H., Iverius, P.-H. & Laurent, T. C. (1975) *Connect. Tissue Res.* **3**, 187–193
11. Bienkowski, M. J. & Conrad, H. E. (1985) *J. Biol. Chem.* **260**, 356–365
12. Fedarko, N. S. & Conrad, H. E. (1986) *J. Cell Biol.* **102**, 587–599
13. Lindahl, U., Bäckström, G., Thunberg, L. & Leder, I. G. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6551–6555
14. Thunberg, L., Bäckström, G. & Lindahl, U. (1982) *Carbohydr. Res.* **100**, 393–410
15. Marcum, J. A. & Rosenberg, R. D. (1984) *Biochemistry* **23**, 1730–1737
16. Marcum, J. A. & Rosenberg, R. D. (1985) *Biochem. Biophys. Res. Commun.* **126**, 365–372
17. Gallagher, J. T. & Walker, A. (1985) *Biochem. J.* **230**, 665–674
18. Taylor, R. L., Shively, J. E., Conrad, H. E. & Cifonelli, J. A. (1973) *Biochemistry* **12**, 3633–3637
19. Cifonelli, J. A. & King, J. (1977) *Biochemistry* **16**, 2137–2141
20. Jacobsson, I., Höök, M., Pettersson, I., Lindahl, U., Larm, O., Wirén, E. & Von Figura, K. (1979) *Biochem. J.* **179**, 77–87
21. Sanderson, P. N., Huckerby, T. N. & Nieduszynski, I. A. (1984) *Biochem. J.* **223**, 495–505
22. Linker, A. (1979) *Biochem. J.* **183**, 711–720
23. Shively, J. E. & Conrad, H. E. (1976) *Biochemistry* **15**, 3943–3950
24. Merchant, Z. M., Kim, Y. S., Rice, K. G. & Linhardt, R. J. (1985) *Biochem. J.* **229**, 147–156
25. Linker, A. & Hovingh, P. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 43–46
26. Oosta, G. M., Favreau, M. L., Beeler, D. L. & Rosenberg, R. D. (1982) *J. Biol. Chem.* **257**, 11249–11255
27. Höök, M., Wasteson, Å. & Oldberg, Å. (1975) *Biochem. Biophys. Res. Commun.* **67**, 1422–1428
28. Silva, M. E., Dietrich, C. P. & Nader, H. B. (1976) *Biochim. Biophys. Acta* **437**, 129–141
29. Cifonelli, J. A. & King, J. (1975) *Connect. Tissue Res.* **3**, 97–104
30. Linker, A. & Hovingh, P. (1973) *Carbohydr. Res.* **29**, 41–62
31. Hampson, I. N., Kumar, S. & Gallagher, J. T. (1984) *Biochim. Biophys. Acta* **801**, 306–313
32. Winterbourne, D. J., Schor, A. M. & Gallagher, J. T. (1983) *Eur. J. Biochem.* **135**, 271–277
33. Winterbourne, D. J. & Mora, P. T. (1981) *J. Biol. Chem.* **256**, 4310–4320
34. Fransson, L.-Å., Sjöberg, I. & Havsmark, B. (1980) *Eur. J. Biochem.* **106**, 59–69
35. Bienkowski, M. J. & Conrad, H. E. (1984) *J. Biol. Chem.* **259**, 12989–12996
36. Riesenfeld, J., Höök, M. & Lindahl, U. (1980) *J. Biol. Chem.* **255**, 922–928
37. Riesenfeld, J., Höök, M. & Lindahl, U. (1982) *J. Biol. Chem.* **257**, 7050–7055
38. Silbert, J. E. & Baldwin, C. T. (1984) *Glycoconjugate J.* **1**, 63–71
39. Walter, P., Gilmore, R. & Blobel, G. (1984) *Cell* **38**, 5–8
40. Lohmander, L. S. & Kimura, J. H. (1986) in *Articular Cartilage Biochemistry* (Schlyerbach, R., Hascall, V. & Keuttner, K., eds.), Raven Press, New York, in the press
41. Helting, T. & Lindahl, U. (1972) *Acta. Chem. Scand.* **26**, 3515–3523
42. Forsee, W. T. & Rodén, L. (1981) *J. Biol. Chem.* **256**, 7240–7247
43. Lidholt, K., Jacobsson, K. G., Riesenfeld, J. & Lindahl, U. (1985) Personal communication
44. Jacobsson, I. & Lindahl, U. (1980) *J. Biol. Chem.* **255**, 5094–5100
45. Jacobsson, I., Lindahl, U., Jensen, J. W., Rodén, L., Prihar, H. & Feingold, D. S. (1984) *J. Biol. Chem.* **259**, 1056–1063
46. Riesenfeld, J., Höök, M. & Lindahl, U. (1982) *J. Biol. Chem.* **257**, 421–425
47. Kraemer, P. M. (1971) *Biochemistry* **10**, 1437–1445
48. Kraemer, P. M. (1971) *Biochemistry* **10**, 1445–1451
49. Kjellén, L., Oldberg, Å., Rubin, K. & Höök, M. (1977) *Biochem. Biophys. Res. Commun.* **74**, 126–133
50. Kraemer, P. M. (1977) *Biochem. Biophys. Res. Commun.* **78**, 1334–1340
51. Oldberg, Å., Kjellén, L. & Höök, M. (1979) *J. Biol. Chem.* **254**, 8505–8510
52. Kjellén, L., Oldberg, Å. & Höök, M. (1980) *J. Biol. Chem.* **255**, 10407–10413
53. Klinger, M. M., Margolis, R. U. & Margolis, R. K. (1985) *J. Biol. Chem.* **260**, 4082–4090
54. Glimelius, B., Busch, C. & Höök, M. (1978) *Thromb. Res.* **12**, 773–782
55. Winterbourne, D. J. (1982) *Biosci. Rep.* **2**, 1009–1015
56. Prinz, R., Schwermann, J., Buddecke, E. & Von Figura, K. (1978) *Biochem. J.* **176**, 671–676
57. Glossl, J., Schubert-Prinz, R., Gregory, J. D., Damle, S. P., Von Figura, K. & Kresse, H. (1983) *Biochem. J.* **215**, 295–301
58. Vogel, K. G. & Dolde, J. (1979) *Biochim. Biophys. Acta* **552**, 194–200
59. Hurst, R. E., Parmley, R. T., Nakamura, N., West, S. S. & Denys, F. R. (1981) *J. Histochem. Cytochem.* **29**, 731–737
60. Robinson, J., Viti, M. & Höök, M. (1984) *J. Cell Biol.* **98**, 946–953
61. Rapraeger, A. C. & Bernfield, M. (1985) *J. Biol. Chem.* **260**, 4103–4109
62. Kjellén, L., Pettersson, I. & Höök, M. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5371–5375
63. Norling, B., Glimelius, B. & Wasteson, Å. (1981) *Biochem. Biophys. Res. Commun.* **103**, 1265–1272
64. Rapraeger, A. C. & Bernfield, M. (1983) *J. Biol. Chem.* **258**, 3632–3636
65. Woods, A., Höök, M., Kjellén, L., Smith, C. G. & Rees, D. A. (1984) *J. Cell Biol.* **99**, 1743–1753
66. Iozzo, R. V. (1984) *J. Cell Biol.* **99**, 403–417
67. Yanagishita, M. & Hascall, V. C. (1985) *J. Biol. Chem.* **260**, 5445–5455
68. Yanagishita, M. & Hascall, V. C. (1984) *J. Biol. Chem.* **259**, 10270–10283
69. Hayman, E. G., Oldberg, Å., Martin, G. R. & Ruoslahti, E. (1982) *J. Cell Biol.* **94**, 28–35
70. Hedman, K., Johansson, S., Vartio, T., Kjellén, L., Vaheri, A. & Höök, M. (1982) *Cell* **28**, 663–671
71. Laurie, G. W., Leblond, C. P. & Martin, G. R. (1982) *J. Cell Biol.* **95**, 340–344
72. Leivo, I. (1983) *J. Histochem. Cytochem.* **31**, 35–45
73. Alitalo, K., Keski-Oja, J., Hedman, K. & Vaheri, A. (1982) *Virology* **119**, 347–357
74. Underhill, C. B. & Keller, J. M. (1975) *Biochem. Biophys. Res. Commun.* **63**, 448–454
75. Underhill, C. B. & Keller, J. M. (1977) *J. Cell Physiol.* **90**, 53–59
76. Winterbourne, D. J. & Mora, P. T. (1978) *J. Biol. Chem.* **253**, 5109–5120
77. Keller, K. L., Keller, J. M. & Moy, J. N. (1980) *Biochemistry* **19**, 2529–2536

78. David, G. & Van Den Berghe, H. (1983) *J. Biol. Chem.* **258**, 7338–7344
79. Mutoh, S., Funakoshi, I., Ui, N. & Yamashina, I. (1980) *Arch. Biochem. Biophys.* **202**, 137–143
80. Oohira, A., Wight, T. N. & Bornstein, P. (1983) *J. Biol. Chem.* **258**, 2014–2021
81. Iozzo, R. V. & Wight, T. N. (1982) *J. Biol. Chem.* **257**, 11135–11144
82. Radhakrishnamurthy, B., Smart, F., Dalferes, E. R., Jr. & Berenson, G. S. (1980) *J. Biol. Chem.* **255**, 7575–7582
83. Carlstedt, I., Cöster, L., Malmström, A. & Fransson, L.-Å. (1983) *J. Biol. Chem.* **258**, 11629–11635
84. Cöster, L., Malmström, A., Carlstedt, I. & Fransson, L.-Å. (1983) *Biochem. J.* **215**, 417–419
85. Yanagishita, M. & Hascall, V. C. (1983) *J. Biol. Chem.* **258**, 12857–12864
86. Yanagishita, M. & Hascall, V. C. (1984) *J. Biol. Chem.* **259**, 10260–10269
87. Kanwar, Y. S., Veis, A., Kimura, J. H. & Jakubowski, M. L. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 762–766
88. Kanwar, Y. S., Hascall, V. C. & Farquhar, M. G. (1981) *J. Cell Biol.* **90**, 527–532
89. Kobayashi, S., Oguri, K., Kobayashi, K. & Okayama, M. (1983) *J. Biol. Chem.* **258**, 12051–12057
90. Parthasarathy, N. & Spiro, R. G. (1984) *J. Biol. Chem.* **259**, 12749–12755
91. Hassell, J. R., Robey, P. G., Barrach, H.-J., Wilczek, J., Rennard, S. I. & Martin, G. R. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4494–4498
92. Hassell, J. R., Leyshon, W. C., Ledbetter, S. R., Tyree, B., Suzuki, S., Kato, M., Kimata, K. & Kleinman, H. K. (1985) *J. Biol. Chem.* **260**, 8098–8105
93. Ledbetter, S. R., Tyree, B., Hassell, J. R. & Horigan, E. A. (1985) *J. Biol. Chem.* **260**, 8106–8113
94. Fujiwara, S., Wiedemann, H., Timpl, R., Lustig, A. & Engel, J. (1984) *Eur. J. Biochem.* **143**, 145–157
95. Matthew, W. D., Greenspan, R. J., Lander, A. D., Reichardt, L. F. (1985) *J. Neurosci.* **5**, 1842–1850
96. Rapraeger, A., Jalkanen, M., Endo, E., Koda, J. & Bernfield, M. (1985) *J. Biol. Chem.* **260**, 11046–11052
97. David, G. & Van Den Berghe, H. (1985) *J. Biol. Chem.* **260**, 11067–11074
98. Parthasarathy, N. & Spiro, R. G. (1981) *J. Biol. Chem.* **256**, 507–513
99. Robinson, H. C., Horner, A. A., Höök, M., Ögren, S. & Lindahl, U. (1978) *J. Biol. Chem.* **253**, 6687–6693
100. Bourdon, M. A., Oldberg, Å., Pierschbacher, M. & Ruoslahti, E. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1321–1325
101. Fransson, L.-Å., Carlstedt, I., Cöster, L. & Malmström, A. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5657–5661
102. Fransson, L.-Å., Cöster, L., Carlstedt, I. & Malmström, A. (1985) *Biochem. J.* **231**, 683–687
103. Oohira, A., Wight, T. N., McPherson, J. & Bornstein, P. (1982) *J. Cell Biol.* **92**, 357–367
104. Fenger, M., Wewer, U. & Albrechtsen, R. (1984) *FEBS Lett.* **173**, 75–79
105. Dziadek, M., Fujiwara, S., Paulsson, M. & Timpl, R. (1985) *EMBO J.* **4**, 905–912
106. Stamatoglou, S. C. & Keller, J. M. (1982) *Biochim. Biophys. Acta* **719**, 90–97
107. Stamatoglou, S. C. & Keller, J. M. (1983) *J. Cell Biol.* **96**, 1820–1823
108. Koda, J. E. & Bernfield, M. (1984) *J. Biol. Chem.* **259**, 11763–11770
109. Koda, J. E., Rapraeger, A. & Bernfield, M. (1985) *J. Biol. Chem.* **260**, 8157–8162
110. Vogel, K. G., Paulsson, M. & Heinegård, D. (1984) *Biochem. J.* **223**, 587–597
111. Woodley, D. T., Rao, C. N., Hassell, J. R., Liotta, L. A., Martin, G. R. & Kleinman, H. K. (1983) *Biochim. Biophys. Acta* **761**, 278–283
112. Sakashita, S., Engvall, E. & Ruoslahti, E. (1980) *FEBS Lett.* **116**, 243–246
113. Del Rosso, M., Cappelletti, R., Viti, M., Vannucchi, S. & Chiarugi, V. (1981) *Biochem. J.* **199**, 699–704
114. Kleinman, H. K., McGarvey, M. L., Hassell, J. R. & Martin, G. R. (1983) *Biochemistry* **22**, 4969–4974
115. Laterra, J., Ansbacher, R. & Culp, L. A. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6662–6666
116. Yamada, K. M., Kennedy, D. W., Kimata, K. & Pratt, R. M. (1980) *J. Biol. Chem.* **255**, 6055–6063
117. Sekiguchi, K., Hakomori, S., Funahashi, M., Matsumoto, I. & Seno, M. (1983) *J. Biol. Chem.* **258**, 14359–14365
118. Gold, L. I., Frangione, B. & Pearlstein, E. (1983) *Biochemistry* **22**, 4113–4119
119. Evington, J. R. N., Luscombe, M. & Holbrook, J. J. (1985) *Biochem. Soc. Trans.* **13**, 243–244
120. Perkins, M. E., Ji, T. H. & Hynes, R. O. (1979) *Cell* **16**, 941–952
121. Williams, E. C., Janmey, P. A., Ferry, J. D. & Mosher, D. F. (1982) *J. Biol. Chem.* **257**, 14973–14978
122. Johansson, S. & Höök, M. (1980) *Biochem. J.* **187**, 521–524
123. Ruoslahti, E. & Engvall, E. (1980) *Biochim. Biophys. Acta* **631**, 350–358
124. Jilek, F. & Hörmann, H. (1979) *Hoppe Seyler's Z. Physiol. Chem.* **360**, 597–603
125. Culp, L. A., Murray, B. A. & Rollins, B. J. (1979) *J. Supramol. Struct.* **11**, 401–427
126. Schubert, D. & LaCorbiere, M. (1980) *J. Biol. Chem.* **255**, 11564–11569
127. Schubert, D. & LaCorbiere, M. (1982) *J. Neurosci.* **2**, 82–89
128. Culp, L. A., Rollins, B. J., Buniel, J. & Hitri, S. (1978) *J. Cell Biol.* **79**, 788–801
129. Rollins, B. J. & Culp, L. A. (1979) *Biochemistry* **18**, 5621–5629
130. Frangou, S. A., Morris, E. R., Rees, D. A., Welsh, E. J. & Chavin, S. I. (1983) *Biopolymers* **22**, 821–831
131. Österlund, E., Eronen, I., Österlund, K. & Vuento, M. (1985) *Biochemistry* **24**, 2661–2667
132. Johansson, S. & Höök, M. (1984) *J. Cell Biol.* **98**, 810–817
133. Johansson, S. (1985) *J. Biol. Chem.* **260**, 1557–1561
134. Schubert, D., LaCorbiere, M., Klier, F. G. & Birdwell, C. (1983) *J. Cell Biol.* **96**, 990–998
135. Schubert, D. & LaCorbiere, M. (1985) *J. Cell Biol.* **100**, 56–63
136. Cole, G. J., Schubert, D. & Glaser, L. (1985) *J. Cell Biol.* **100**, 1192–1199
137. Schubert, D. & LaCorbiere, M. (1985) *J. Cell Biol.* **101**, 1071–1077
138. Fransson, L.-Å., Nieduszynski, I. A. & Sheehan, J. K. (1980) *Biochim. Biophys. Acta* **630**, 287–300
139. Fransson, L.-Å., Havsmark, B., Nieduszynski, I. A. & Huckerby, T. N. (1980) *Biochim. Biophys. Acta* **633**, 95–104
140. Fransson, L.-Å. (1981) *Eur. J. Biochem.* **120**, 251–255
141. Fransson, L.-Å., Havsmark, B. & Sheehan, J. K. (1981) *J. Biol. Chem.* **256**, 13039–13043
142. Fransson, L.-Å., Carlstedt, I., Cöster, L. & Malmström, A. (1983) *J. Biol. Chem.* **258**, 14342–14345
143. Hardingham, T. E., Ewins, R. J. F. & Muir, H. (1976) *Biochem. J.* **157**, 127–143
144. Bhavanandan, V. P. & Davidson, E. A. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2032–2036
145. Winterbourne, D. J. & Salisbury, J. G. (1981) *Biochem. Biophys. Res. Commun.* **101**, 30–37
146. Furukawa, K. & Bhavanandan, V. P. (1983) *Biochim. Biophys. Acta* **740**, 466–475
147. Kinoshita, S. (1971) *Exp. Cell Res.* **64**, 403–411
148. Kinoshita, S. (1974) *Exp. Cell Res.* **85**, 31–40
149. Kinoshita, S. (1976) *Exp. Cell Res.* **102**, 153–161
150. Kinoshita, S. & Saiga, H. (1979) *Exp. Cell Res.* **123**, 229–236

151. Kinoshita, S. & Yoshii, K. (1979) *Exp. Cell Res.* **124**, 361–369
152. Margolis, R. U., Margolis, R. K., Chang, L. B. & Preti, C. (1975) *Biochemistry* **14**, 85–88
153. Grief, K. F. & Reichardt, L. F. (1982) *J. Neurosci.* **2**, 843–852
154. Grief, K. F. & Trenchard, H. I. (1984) *J. Cell. Biochem.* **26**, 127–133
155. Kelly, R. B., Buckley, K. M., Burgess, T. L., Carlson, S. S., Caroni, P., Hooper, J. E., Katzen, A., Moore, H. P., Pfeffer, S. R. & Schroer, T. A. (1983) *Cold Spring Harbor Symp.* **48**, 697–705
156. Stadler, H. & Dowe, G. H. C. (1982) *EMBO J.* **1**, 1381–1384
157. Jones, R. T., Walker, J. H., Stadler, H. & Whittaker, V. P. (1982) *Cell Tissue Res.* **224**, 685–688
158. Greenburg, G. & Gospodarowicz, D. (1982) *Exp. Cell Res.* **140**, 1–14
159. Lander, A. D., Tomaselli, K., Calof, A. L. & Reichardt, L. F. (1983) *Cold Spring Harbor Symp.* **48**, 611–623
160. Edgar, D., Timpl, R. & Thoenen, H. (1984) *EMBO J.* **3**, 1463–1468
161. Matthew, W. D. & Patterson, P. H. (1983) *Cold Spring Harbor Symp.* **48**, 625–631
162. Anderson, M. J. & Fambrough, D. M. (1983) *J. Cell Biol.* **97**, 1396–1411
163. Sanes, J. R., Marshall, L. M. & McMahan, U. J. (1978) *J. Cell Biol.* **78**, 176–198
164. Anderson, M. J., Klier, F. G. & Tanguay, K. E. (1984) *J. Cell Biol.* **99**, 1769–1784
165. Brandan, E., Maldonado, M., Garrido, J. & Inestrosa, N. C. (1985) *J. Cell Biol.* **101**, 985–992
166. Inestrosa, N. C., Matthew, W. D., Rieness, C. G., Hall, Z. W. & Reichardt, L. F. (1985) *J. Neurochem.* **45**, 86–94
167. Hahn, P. F. (1943) *Science* **98**, 19–20
168. Bengtsson, G., Olivecrona, T., Höök, M., Riesenfeld, J. & Lindahl, U. (1980) *Biochem. J.* **189**, 625–633
169. Gamse, G., Fromme, H. G. & Kresse, H. (1978) *Biochim. Biophys. Acta* **544**, 514–528
170. Buonassisi, V. & Colborn, P. (1983) *Biochim. Biophys. Acta* **760**, 1–12
171. Robinson, J. & Gospodarowicz, D. (1984) *J. Biol. Chem.* **259**, 3818–3824
172. Shimada, K., Gill, P. J., Silbert, J. E., Douglas, W. H. & Fanburg, B. L. (1981) *J. Clin. Invest.* **68**, 995–1002
173. Cheng, C.-F., Oosta, G. M., Bensadoun, A. & Rosenberg, R. D. (1981) *J. Biol. Chem.* **256**, 12893–12898
174. Williams, M. P., Streeter, H. B., Wasteman, F. S. & Cryer, A. (1983) *Biochim. Biophys. Acta* **756**, 83–91
175. Felts, J. M., Staprans, I. & Gorman, R. A. (1983) *Life Sci.* **32**, 1659–1664
176. Ratner, N., Bunge, R. P. & Glaser, L. (1985) *J. Cell Biol.* **101**, 744–754
177. Culp, L. A., Rollins, B. J., Buniel, J. & Hitri, S. (1978) *J. Cell Biol.* **79**, 788–796
178. Kawakami, H. & Terayama, H. (1981) *Biochim. Biophys. Acta* **646**, 161–166
179. Fritze, L. M. S., Reilly, C. F. & Rosenberg, R. D. (1985) *J. Cell Biol.* **100**, 1041–1049
180. Castellot, J. J., Jr., Addonizio, M. L., Rosenberg, R. D. & Karnovsky, M. J. (1981) *J. Cell Biol.* **90**, 372–379
181. Castellot, J. J., Jr., Beeler, D. L., Rosenberg, R. D. & Karnovsky, M. J. (1984) *J. Cell Physiol.* **120**, 315–320
182. Colborn, P. & Buonassisi, V. (1982) *Biochem. Biophys. Res. Commun.* **104**, 220–227
183. Marcum, J. A., McKenney, J. B. & Rosenberg, R. D. (1984) *J. Clin. Invest.* **74**, 341–350
184. Shimada, K. & Ozawa, T. (1985) *J. Clin. Invest.* **75**, 1308–1316
185. Long, W. F. & Williamson, F. B. (1984) *Med. Hypotheses* **13**, 385–394
186. Folkman, J. (1985) *Biochem. Pharmacol.* **34**, 905–909
187. Rosenzweig, L. J. & Kanwar, Y. S. (1982) *Lab. Invest.* **47**, 177–184
188. Mynderse, L. A., Hassell, J. R., Kleinman, H. K., Martin, G. R. & Martinez-Hernandez, A. (1983) *Lab. Invest.* **48**, 292–302
189. Rohrbach, D. W., Wagner, C. W., Star, V. L., Martin, G. R., Brown, K. S. & Woon, J.-W. (1983) *J. Biol. Chem.* **258**, 11672–11677
190. Kramer, R. H., Vogel, K. G. & Nicolson, G. L. (1982) *J. Biol. Chem.* **257**, 2678–2686
191. Vlodavsky, I., Fuks, Z., Bar-Ner, M., Ariav, Y. & Schirrmacher, V. (1983) *Cancer Res.* **43**, 2704–2711
192. Nakajima, M., Imamura, T., Di Ferrante, D., Di Ferrante, N. & Nicolson, G. L. (1983) *Science* **220**, 611–613
193. Nakajima, M., Imamura, T., Di Ferrante, N. & Nicolson, G. L. (1984) *J. Biol. Chem.* **259**, 2283–2290
194. Hampson, I. N. & Gallagher, J. T. (1984) *Biochem. J.* **221**, 697–705