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Proteoglycans of basement membranes

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Abstract. Proteoglycans carrying either heparan sulfate and/or chondroitin sulfate side chains are typical constituents of basement membranes. The most prominent proteoglycan (perlecan) consists of a 400–500 kDa core protein and three heparan sulfate chains. Electron microscopy and cDNA sequencing show a complex and elongated domain structure for the core protein which in part is homologous to that of the laminin A chain. This structure may be varied by alternative splicing and proteolysis. Integration into basement membranes probably occurs by heparan sulfate binding to laminin and collagen IV, core protein binding to nidogen and by limited self assembly. The proteoglycan is in addition a cell-adhesive protein which is recognized by $\beta 1$ integrins. Several more proteoglycans with smaller core proteins (10–160 kDa) apparently exist in basement membranes but are less well characterized. Biological functions include control of filtration through basement membranes and binding of growth factors and protease inhibitors.

Key words. Heparan and chondroitin sulfate; multidomain structure; protein binding; cell adhesion; filtration; growth factors.

Introduction

Basement membranes are specialized extracellular matrices which are abundant in tissues and are usually located in close vicinity to cells. Major constitutents are various forms of collagen IV and laminin as well as smaller glycoproteins such as nidogen, BM-40 and BM-90[82, 103, 104, 119]. They form the supramolecular architectures of basement membranes by self-assembly processes and distinct heterotypic interactions[2, 119]. Proteoglycans, particularly those substituted with heparan sulfate side chains, have also been recognized as major basement membrane components. This was originally indicated in renal basement membranes by regular arrays of polyanionic binding sites for ruthenium red and cationized ferritin[34, 55, 96] and by demonstrating their sensitivity towards heparitinase and nitrous acid[56]. It was also shown that purified basement membranes contain heparan sulfate and smaller amounts of chondroitin sulfate[34, 57, 81]. The first isolation of such a heparan sulfate proteoglycan was achieved from the basement membrane-producing mouse Engelbreth-Holm-Swarm (EHS) tumor[38]. These observations have led to an extensive characterization of such proteoglycans mainly in cell and organ cultures and by immunohistology, with more than 50 references cited in a review for the period 1980–1986[104]. These studies established the existence of basement membrane forms of proteoglycans but provided only few and in part confusing structural details of their molecular relationships.

The EHS tumor was instrumental in overcoming these problems and allowed the separation by CsCl gradient centrifugation of two classes of heparan sulfate proteoglycans with either high (1.5–1.6 g/ml) or low (1.3–1.4 g/ml) buoyant density[31, 39]. As expected the protein content was low in the high density (10–20%) compared to the low density (70–80%) form and subsequent studies allowed structural models for both proteoglycans to be established[31, 84]. Similar forms which differ in buoyant density apparently exist in other tissues, as shown for example by studies with Reichert's membrane or PYS-2 cells[83, 106]. The low density form was then shown by cDNA cloning and complete sequencing to contain a unique core protein, that very probably comprises a single gene product[53, 75, 76]. For this reason, a considerable part of this review will concentrate on this particular basement membrane proteoglycan, perlecan. Similar data are so far not available for high density heparan sulfate proteoglycans. Further proteoglycans with unique core products substituted by chondroitin/dermatan sulfate may also exist in basement membranes. Such side chains are ubiquitous basement membrane components as initially indicated from glycosaminoglycan analyses and also shown convincingly by monoclonal antibodies specific for chondroitin-6-sulfate[14]. Here again the gene products are not very well characterized.

The overwhelming evidence for basement membrane-specific proteoglycans was primarily established as discussed later in antibody studies. By this means, they could also be distinguished from cell membrane-bound proteoglycans[24, 51, 60, 100] and proteoglycans present in cartilage and other interstitial matrices. The discrimination from cell membrane bound proteoglycans was particularly important since these are frequently substituted with heparan sulfate chains and occur simultaneously with basement membrane forms in adjacent anatomical

compartments of tissues and cultured cells. A unique characteristic of the cell membrane-bound forms seems to be their hydrophobic interaction potential and their solubility in detergents, while extraction of the basement membrane forms requires high salt or guanidine concentrations. Meanwhile, it is clear from sequence studies (see other reviews in this series) that these different proteoglycan classes can also be distinguished at the level of their gene products. This does not necessarily indicate that they are functionally independent.

A large heparan sulfate proteoglycan (perlecan) is the most prominent component

This major proteoglycan was originally purified from 6 M guanidine-HCl or 7 M urea extracts of the mouse EHS tumor and was shown to have unique properties[39,84]. It has a high protein content (75–80%) contributed by a single polypeptide chain of 400–450 kDa when analyzed by electrophoresis after heparitinase digestion. A precursor protein of about 400 kDa can be detected intracellularly and appears in pulse-chase experiments within 60 min as intact proteoglycan in the culture medium[68]. Chemical analyses indicated the presence of 2–3 heparan sulfate chains of 40–60 kDa and no significant amounts of chondroitin sulfate. Ultracentrifugation demonstrated monomers with a molecular mass of 600–700 kDa together with some aggregates and an elongated shape in neutral buffer[84]. Extensive unfolding was observed in 6 M guanidine-HCl indicating that the purified proteoglycan may have lost some of its native structure. Yet exposure to strongly denaturing solvents was required during extraction and isolation in order to minimize proteolytic degradation and contamination by other basement membrane proteins. Specific antibodies could be raised against the proteoglycan which did not react with laminin, collagen IV and other proteins[24,39]. They recognize mainly disulfide-dependent epitopes on the core protein[84].

Electron microscopy after rotary shadowing or negative staining was an essential step for proposing a model for the proteoglycan[65,84,118]. This revealed an 80 nm-long elongated core protein consisting of 5–7 globular domains of variable size with some small connecting rods (fig. 1). Rotary shadowing also allowed three thin heparan sulfate chains of 100–170 nm length, which were connected to one end of the core protein, to be visualized. This suggested a multidomain structure for the core protein which was supported by showing stable fragments in the range 40–200 kDa after cleavage with neutral proteases[5,67,84]. The asymmetric attachment of heparan sulfate chains was confirmed with a small tryptic fragment (20–30 kDa) possessing all three chains while a 200 kDa fragment lacked the chains but had the shape of a portion of the core protein located at the opposite end[65,84].

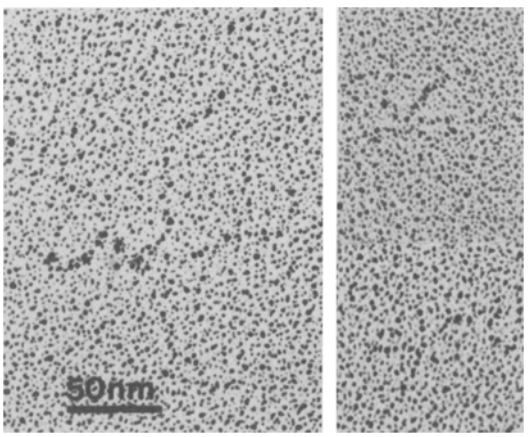

Figure 1. Rotary shadowing images of one low density (left) and two high density (right) proteoglycan molecules purified from the EHS tumor. Note on the left the elongated core protein attached to three faint heparan sulfate chains and on the right the star-like connection of four short heparan sulfate chains to an invisible small core.

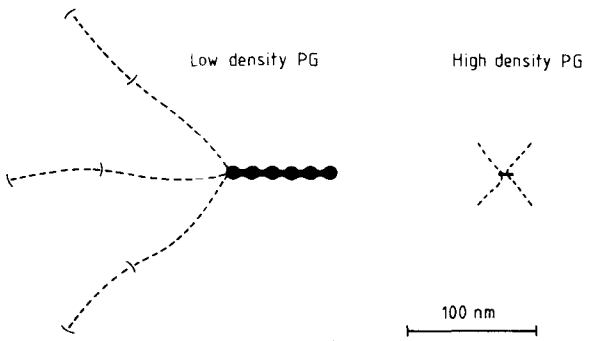

Figure 2. Structural models of low and high density heparan sulfate proteoglycans obtained from the EHS tumor basement membrane. Black contours outline the core protein domains and broken lines the heparan sulfate side chains. Brackets indicate a variability in heparan sulfate length. The models are based on electron microscopy and chemical analyses[31,84]. Molecular masses determined by ultracentrifugation are ~600 kDa (low density form) and 130 kDa (high density form). Reproduced with permission from Paulsson et al.[84].

These data led Paulsson et al.[84] to propose a model for the shape of the large, low density proteoglycan (fig. 2), which indicated that the core protein consists of about six large globular domains connected to each other like pearls on a string. After the multidomain structure was confirmed by sequence analysis, it led to the proposal to name the proteoglycan perlecan[76].

Sequence and domain structure

The first partial amino acid sequences of mouse perlecan were obtained from cDNA clones from EHS tumor and melanoma libraries and shown to correspond to the authentic protein by peptide sequencing and immunological studies[77]. These clones also allowed identification of a 12 kb mRNA encoding the core protein. Subsequently an open reading frame of similar

size was demonstrated by completing the sequence[76]. The sequence predicts a 21-residue signal peptide followed by 3686 amino acid residues for the core protein with a remarkable homology to portions of the laminin A chain. The data also predict a molecular mass of ~400 kDa for the core protein which is in good agreement with chemical observation (see above). Similar sequence analysis of the human core protein[53,75] revealed a 4370 and 4372-residue polypeptide with a calculated mass of ~470 kDa. The extra ~670 residues compared to the mouse sequence have been attributed to seven additional protein modules, as discussed below, and raise the size of the corresponding mRNA to 14 kb. The domains shared by both sequences show 80–87% identity, leaving no doubts that they originate from identical genes. This gene has been localized to human chromosome 1p36.1[22,52]. A nematode gene encoding a perlecan analogue has been recently identified (see below).

The two homologous sequences were interpreted to indicate the presence of five distinct domains numbered I–V. Each domain except one is composed of different combinations of six protein motifs (fig. 3). The N-terminal cysteine-free domain I (172 residues) has no counterpart in other proteins and was considered to be the major site for heparan sulfate attachment[53,75,76]. Domain II (~330 residues) consists of four cysteine-rich motifs first identified in the LDL receptor[116] and one N-CAM module. Such motifs are thought to mediate LDL-binding to the receptor and may have similar

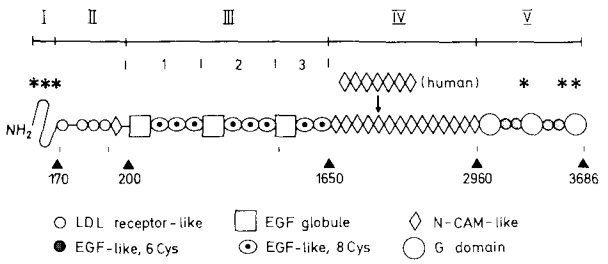

Figure 3. Predicted domain structure and protein modules of the core protein of the large heparan sulfate proteoglycan (perlecan). The model is derived from a mouse cDNA sequence[76]. Seven extra N-CAM modules present in the human sequence[53,75] are denoted by the arrow. Major domains are identified on top by roman numbers and subdomains by arabic numbers. Numbers under arrow heads denote approximate sequence positions (mouse) of domain borders and asterisks approximate positions of putative heparan sulfate attachment sites (see table). The predicted protein modules include two 40-residue motifs each with 6 cysteines that were originally discovered in the LDL receptor[116] and in epidermal growth factor (EGF) and its precursor[1]. EGF-like repeats (~60 residues) with 8 cysteines and EGF globules (~250 residues) are homologous to those of laminin[7]. The latter structure apparently arises by insertion of a cysteine-free globular domain between Cys-3 and Cys-4 of a typical laminin EGF motif[3,7]. N-CAM denotes 90-residue motifs with a single disulfide bridge typical for the immunoglobulin family[113]. G domains are globules (150–180 residues) homologous to those in the laminin A chain[7].

functions in the proteoglycan. It is in this context of interest that LDL modulates the expression of the proteoglycan in endothelial cells[79]. Domain III (~1170 residues) is composed of three subdomains each starting with a globule encased by an EGF-like motif and followed by two or three laminin type EGF motifs[7]. These subdomains show significant internal sequence identity (30–40%) and a comparable identity with similar laminin A chain sequences. Domain IV (1300 residues in the mouse) consists of 14 consecutive motifs characteristic of the modules found in the large immunoglobulin/receptor family of proteins and which have considerable internal homology including two cysteines that are supposed to form a disulfide bridge[113]. The best, although low, homology (20–25% identity) exists with N-CAM. The most C-terminal domain V consists of three globular domains similar to one another and the G domains of laminin A chain (~30% identity) which are interspersed by 4 EGF-like motifs. Such G domains in laminin provide a major cell-binding site recognized by integrins[21]. Together these data demonstrate that the core protein represents one of the largest and most diverse single chain multidomain proteins.

The extra sequences predicted for the human core protein provide seven additional N-CAM motifs to domain IV[53,75]. This additional structure is for the sake of simplicity shown in figure 3 as a single entity inserted somewhere in the middle of domain IV but this positioning is not obvious from computer-assisted sequence alignments. Solutions to this problem may come from sequences of genomic clones and the identification of exon borders. This may also allow studies to determine whether variations of the core protein sequences are due to alternative splicing. Limited analyses of mRNA sizes by Northern hybridization have so far not lent support to this possibility[53,75,77].

Domains III and V of the core protein are homologous to short and long arm structures, respectively, of the laminin A chain, except for some variations in the number of EGF-like repeats separating the globular domains[7]. The heptad repeat sequences of the rod in laminin's long arm, which form a triple-coiled α helix, are replaced by domain IV in the core protein, which very probably has a different conformation. Since the α helical sequences of laminin determine chain assembly and selection[28], it is unlikely that the core protein can associate with these structures and should therefore not be considered as an isoform of laminin chains. Rotary shadowing images of laminin[7] would also predict that the core domain III appears as three globules connected by short rods and domain V as a large globe not being sufficiently resolved into subdomains. A fifth globular domain may be contributed by domains I and/or II. Domain IV, however, may have a rod-like structure approaching in length the entire core protein[53], for which no electronmicroscopical evidence exists. It was

Putative heparan sulfate attachment sites similar to the consensus sequences E/DGSGD/E[120] and SGXG[8] in the large heparan sulfate proteoglycan. The mouse sequences[76] are shown with human substitutions underneath.

Position in mouse[a)]	Domain	Sequence
42–46	I	DASGD SI
48–52	I	LGSGD
53–57	I	VGSGD L
3229–3232	V	SGAG
3487–3491	V	EGSGG
3570–3574	V	LGSGE

[a)]Numbering without signal peptide (21 residues[76])

therefore suggested that two extra cysteines located at each end of domain IV may by disulfide-bridging form a large loop and thus an additional globular domain[76]. This predicted shape would come close to that observed experimentally (see figs 1 and 2) as an average structure[65,84,118].

Both core protein structures contain some 50 Ser-Gly sequences which could serve for heparan sulfate attachment. Evidence or predictions for larger consensus sequences are scarce but include D/EGSGD/E and GSXG[8,120]. Six such sequences are, with small variations, conserved in the mouse and human sequence (table). Interestingly, they are clustered either at the N- or C-terminal end of the core protein (fig. 3) in agreement with predictions from electron microscopy (figs 1 and 2). While the first sequence analyses favored an N-terminal localization[76], recent preliminary fragment data support a C-terminal location[5]. It is obvious that more precise biochemical data are required to settle this question. There is also sequence evidence for 10–12 N-linked oligosaccharide acceptor sites. Studies of glomerular basement membrane[25] and EHS tumor proteoglycans[84] indicate the occupation of several N- and O-linked acceptor sites.

Aspects of structural variability

The identification of very similar core protein sequences by three different laboratories are still in contrast with quite variable core protein sizes reported for large heparan sulfate proteoglycans from various basement membrane or cell sources. Since sequencing was done with cDNA libraries obtained from seven different cell types[53,75,76], this indicates that the domain structure outlined in figure 3 represents an abundant prototype of the core protein. This is also supported by showing a single chromosomal locus and by restriction fragment length polymorphism patterns indicating a single copy gene[22,52]. Variations of the core protein structure may, however, occur by alternative mRNA splicing[53,75] or by proteolytic processing to a ~250 kDa core protein fragment still possessing the heparan sulfate chains[61,84,93]. Whether the latter event represents a physiological process or an artefact of extraction and isolation conditions is, however, not known.

Taken together the data define a core protein size of 250–500 kDa as a possible range, matching the core protein prototype of perlecan. A number of studies have demonstrated such core proteins in heparan sulfate proteoglycans obtained from epithelial, endothelial, tumor and Schwann cells and some tissues[27,40,42,47,61,69,78,93,101,114]. In most instances it was also shown that these proteoglycans reacted with antisera against perlecan, indicating that they are identical or closely related. More definitive proof for this relationship has so far only been reported for proteoglycans from human lung fibroblasts by peptide sequencing[42] and from bovine endothelial cells by electron microscopy and peptide finger-printing[93]. Smaller core protein sizes (110–170 kDa) but a high protein content were observed for heparan sulfate proteoglycans from glomerular and lens capsule basement membranes[25,74,109], although this is in conflict with some biosynthetic data[61,101]. This emphasizes a broad occurrence of perlecan in basement membranes but does not exclude the possibility that genes for other large core proteins may be expressed by cells involved in basement membrane synthesis. The identification of such novel proteoglycans will, however, become crucially dependent on peptide or cDNA sequencing.

Another way of diversifying proteoglycan structures is by posttranslational modifications, in particular variations in the glycosaminoglycan side chains. Such variability is known to exist for the size of the heparan sulfate chains (30–70 kDa)[32,39,84,105] in different preparations of EHS tumor proteoglycan. Heparan sulfates of these proteoglycans also show a considerably low level of O-sulfation[85,102,105] which may be a consequence of cell transformation[17,86]. On the other hand, two novel 3-O sulfated glucosamine structures not related to the antithrombin-binding site were found in bovine glomerular basement membrane but not lens capsule[26]. Hybrid proteoglycans possessing both heparan sulfate and chondroitin/dermatan sulfate chains have also been identified[48,73,105,114]. A hybrid perlecan that was substituted with the usual heparan sulfate chains and a single 17 kDa chondroitin sulfate, has also been obtained from a cultured EHS tumor cell line[16].

Expression and tissue distribution

Tissue staining by antibodies has been from the first studies[38] an instrumental tool for the identification of basement membrane proteoglycans. Matrix staining can be clearly demonstrated for large basement membranes (i.e. EHS tumor, Reichert's membrane) and distinguished from the staining patterns for cell membrane-

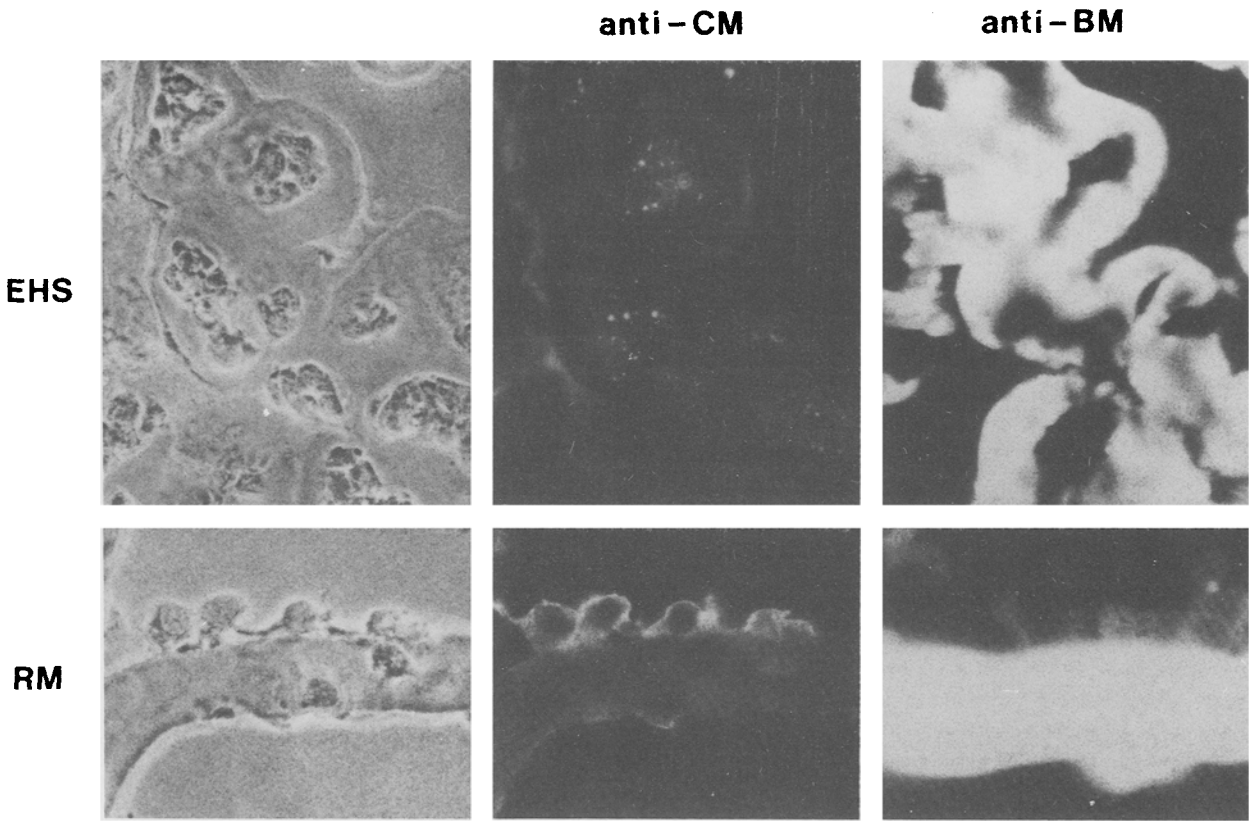

Figure 4. Indirect immunofluorescence localization of cell membrane-bound and basement membrane forms of heparan sulfate proteoglycan in Reichert's membrane (RM) and EHS tumor (EHS). Antisera were against the large proteoglycans from EHS tumor (anti-BM) and from rat liver membranes (anti-CM). The left panel shows phase contrast pictures of the sections stained with anti-CM, demonstrating the cellular localization of the stained regions. Reproduced with permission from Dziadek et al.[24].

bound proteoglycans (fig. 4). Polyclonal antisera against the perlecan core protein[24,93,95] and various monoclonal antibodies specific for either the core protein or heparan sulfate[15,27,40,42,44,48,75,98,108] showed very similar staining of almost all basement membranes in the body. This indicates that perlecan and related proteoglycans are ubiquitous basement membrane components as is also indicated by biochemical studies. The proteoglycan is already expressed at the 2–4 cell stage of mouse embryos and later deposited in all embryonic basement membranes, suggesting an important role during development[24]. In the developing intestine it is produced by epithelial cells and then assembles with mesenchyme-derived collagen IV into a basement membrane[97]. Whether perlecan is also a mesenchymal product is unknown but suggested from observations with lung[42] and skin[75] fibroblasts.

Immunogold and other electronmicroscopical techniques were used for a more precise ultrastructural localization of the core protein in renal, corneal and vessel basement membranes and Reichert's membrane[20,35,42,44,93,94,100]. Even though different staining intensities were observed with different basement membranes, a specific topological orientation such as described for laminin[94] has so far not been recognized. This will require further studies with antibodies of defined domain specificity. In other studies, 4.5 nm-wide double-track structures were stained in glomerular basement membrane and Reichert's membrane and claimed to represent submorphological structures produced by the proteoglycan[36,45].

Production of core protein mRNA can also be shown in various tissues by in situ and Northern hybridization[65,75]. Transforming growth factor-β upregulates core protein production in cultured colon carcinoma cell by stabilizing the mRNA[23]. Such studies are at an early stage and may provide much more useful information in the near future.

Binding of matrix proteins

The integration of large proteoglycans such as perlecan into the basement membrane matrix is very likely to depend on both core protein and glycosaminoglycan structures. Laminin and collagen IV were initially identified as binding partners both by ligand assays[115] and by electronmicroscopy of preformed complexes[64]. Since the proteoglycan used was exposed to reducing agents, it showed a collapsed structure and no distinction could be made between core protein and heparan sulfate chains serving as a binding structure.

A crucial role of the core protein for binding was strongly indicated from the need to use denaturing solvents for proteoglycan extraction. Nidogen was shown to interact with the core protein but not with the heparan sulfate chains[6]. The binding site of nidogen was mapped to its central globular domain G2 which also has collagen IV-binding activity. Nidogen, via its laminin-binding domain G3, could also mediate the formation of a complex between proteoglycan and laminin in a heparan sulfate independent fashion[6]. Such ternary complexes can also form between laminin, nidogen and collagen IV and are considered to play an important role in basement membrane architecture[2]. The core protein is also involved in a self assembly process. Incubation of the proteoglycan at 37 °C results in the formation of dimers and larger, stellate proteoglycan clusters[118]. Dimers are formed by binding between the ends of the core protein opposite to the heparan sulfate attachment region, indicating that the side chains play no role in this process. Other studies indicated, in addition, self aggregation of the proteoglycan into even larger morphological structures, which could be independent from the laminin and collagen IV networks[36,45]. The core protein was also essential for binding the proteoglycan to a matrix produced by cultured fibroblasts[40].

Additional interactions of the proteoglycan are mainly or exclusively mediated by the heparan sulfate chains, as shown for laminin, collagen IV and fibronectin in ligand assays[6]. Binding could be efficiently inhibited by heparin and to a lower extent by EHS tumor heparan sulfate and chondroitin sulfate. The binding site of laminin was localized to a fragment from the end of its long arm[6] and that of collagen IV probably to a central region of its triple helix[64]. Conflicting results about core protein and heparan sulfate involvement in proteoglycan binding to fibronectin were, however, reported in other studies[40,41]. A similar but hybrid proteoglycan from human placenta was isolated by fibronectin affinity chromatography and showed binding to fibronectin and laminin but not to collagen IV[48]. A strongly undersulfated heparan sulfate proteoglycan can be obtained by chlorate inhibition of cultured cells[9]. This resulted in impaired matrix assembly and laminin-proteoglycan binding, indicating that polyanionic interactions contribute to an intact basement membrane architecture. Several more biological functions of heparan sulfate are discussed at the end of the review.

Cell-binding properties

Initial indications that perlecan, like laminin and collagen IV, belongs to the cell-adhesive proteins came from studies with hepatocytes[11,12]. Yet, the cell surface proteins binding to the core protein were all smaller than typical integrin chains which are the main cellular receptors for mediating cell adhesion. A subsequent study, however, showed that several tumor cells attach and spread on the proteoglycan substrate and that this is mediated by $\beta 1$ integrins but not by the typical laminin receptor ($\alpha 6\beta 1$)[5]. Cell-binding could not be attributed to either the core protein or the heparan sulfate chains and apparently requires cooperation of both structures. The single RGD sequence of mouse perlecan, which is not conserved in the human sequence[53,75], seems not to be involved in these interactions.

A heparan sulfate proteoglycan from corneal endothelial cells has been implicated in stimulating neurite outgrowth of peripheral neurones[62]. Later studies indicated that a laminin-proteoglycan complex was responsible for this activity and the role of the proteoglycan in this process has remained undecided[18,63]. Studies with purified perlecan showed, however, that it potentiates neurite length[36].

An exciting observation was recently made for a core protein gene *unc*-52 of the nematode *C. elegans*[32]. Certain mutations of this gene destabilize the body wall muscle structure presumably by interfering with cell-matrix interactions. Sequence analysis of the *unc*-52 gene demonstrated a truncated form of the perlecan core protein ($\sim$2450 amino acid residues) consisting mainly of homologous domains II, III and IV but missing domains I and V (D. G. Moerman, personal communication). The data also indicated alternative splicing of domain IV. Further studies on such mutants will be of considerable benefit for the understanding of the biological functions of this proteoglycan type.

Small heparan sulfate proteoglycans

Several more heparan sulfate proteoglycans, which are substantially smaller than perlecan, have been purified from different basement membrane sources but have so far not been characterized up to the sequence level. Extraction of the EHS tumor with 0.5 M NaCl released such a 130 kDa proteoglycan, which consisted of four 29 kDa heparan sulfate chains connected to a small core protein ($\sim$10 kDa) and appeared homogeneous by ultracentrifugation[31]. Rotary shadowing revealed four thin strands connected at one end (fig. 1) and led to the proposal of a star-like model (fig. 2). This proteoglycan was a weaker immunogen than perlecan but shared with it some epitopes of different affinities[24,39]. Binding studies revealed weak interactions ($K_d \sim 10^{-6}$ M) with laminin, collagen IV and fibronectin that could be abolished by 0.1–0.2 M NaCl and heparin[31]. Another study based on pulse-chase experiments with the EHS tumor questioned the existence of such an individual proteoglycan and interpreted the data as indicating a breakdown product of perlecan[68]. Still another pulse-chase study, together with peptide map data, disagreed with this interpretation[59]. This study, however, apparently identified several proteoglycans with 60 kDa heparan

sulfate and 17 kDa chondroitin sulfate chains and core proteins in the range 21–34 kDa. The fragmentation hypothesis is also not supported by electronmicroscopical evidence for different sizes and arrangements of heparan sulfate chains (fig. 1). Nevertheless, the question whether such small proteoglycans are products of unique genes still needs to be settled by peptide sequencing and cDNA cloning.

A 130 kDa heparan sulfate proteoglycan with 26 kDa side chains and a 18 kDa core protein has been purified from glomerular basement membranes[58]. Despite the remarkable similarity to the EHS tumor variant[31], their identity remains to be determined. The same needs to be established for a 150 kDa heparan sulfate proteoglycan from teratocarcinoma cells with 25 kDa side chains but a 50 kDa core protein[60]. Antibodies against this proteoglycan specifically stain basement membranes. A small heparan sulfate proteoglycan with a 40 kDa core protein has been isolated from rat liver microsomes and shown by antibody staining to be localized in the space of Disse and basement membranes of blood vessels and bile ducts[99]. Since there was no cross-reaction with antisera to typical basement membrane proteoglycans this indicates a novel component. This may also be the case for an endothelial cell heparan sulfate proteoglycan with a 22 kDa core protein characterized by a unique N-terminal sequence[10]. It matches an unpublished endothelial core protein cDNA sequence (EMBL data base Acc. No. M81786) which shows a distinct homology to the cell membrane proteoglycan syndecan.

Chondroitin sulfate proteoglycans

The presence of chondroitin sulfate in a large variety of basement membranes was initially demonstrated by chemical analysis and staining with a specific monoclonal antibody[14, 104]. This led to a search for proteoglycans with novel core proteins that are mainly substituted with chondroitin sulfate. A component with 3-fold more chondroitin than heparan sulfate but a core protein identical or similar to that of perlecan was identified in a rat yolk sac tumor[114]. A more unique proteoglycan was, however, found in biosynthetic studies with yolk sacs from 14.5 day rat embryos[46]. It was of large size with a 130 kDa core protein, of high buoyant density (> 1.45 g/ml) and possessed mainly chondroitin-6-sulfate chains. A presumably identical or very similar component was then isolated from rat Reichert's membrane and kidneys[71]. It consisted of a 150 kDa core protein and about twenty 17 kDa chondroitin sulfate chains. Monoclonal antibodies raised against the core protein stained almost all basement membranes in adult rat tissues[71, 72], indicating the ubiquitous distribution of an apparently novel proteoglycan. In this context, it is, however, noteworthy that biosynthetic and chemical studies with mouse Reichert's membrane showed mainly high and low density heparan sulfate proteoglycans similar to those of the EHS tumor and only little amounts of small chondroitin sulfate proteoglycan[83, 85]. Whether this reflects species differences or different synthetic rates[45] remains to be established. High and low density proteoglycans with different chondroitin sulfate substitutions on a 47 kDa core protein have been observed in human endothelial cell culture[69]. A giant chondroitin sulfate proteoglycan seems to be associated with Schwann cells and electric organ basement membranes[50]. Electron microscopy indicated a bottle-brush structure perhaps similar to cartilage proteoglycan and chemical analysis revealed core protein subunits. This suggests that basement membranes possess several more core proteins that serve as chondroitin sulfate carriers besides those in hybrid proteoglycans as mentioned before.

Functions and biological aspects

Perlecan and several other proteoglycans are mandatory constituents of basement membranes and therefore, as discussed, essential for major features of their architecture. They may also contribute to basement membrane diversity. Some of the proteoglycans can be cell-adhesive and are thus involved in the anchorage of cells to basement membranes. With their polyanionic sites, they add unique structural and functional properties to basement membranes. It was especially this feature which has attracted many studies that led to an understanding of the role of basement membranes in a broader biological context. Important aspects of these studies will be briefly discussed in the following three sections.

Filtration control

Basement membranes serve as a selective filtration barrier for macromolecules, a property which has been especially studied with glomerular basement membranes (GBM). As discussed in recent reviews[29, 54], GBM serve as a size- and charge-selective filter, indicating a functional pore diameter of ~ 9 nm. This was shown with cationic proteins that penetrated GBM very well, while anionic or neutral proteins did not penetrate beyond the lamina rara interna border. Such cationic probes were then used to demonstrate arrays of polyanionic binding sites in GBM, an observation which started research on basement membrane proteoglycans. These polyanionic binding sites are mainly contributed by heparan sulfate as shown by enzymatic degradation, which also causes excessive urinary secretion of anionic proteins. Similar polyanionic networks are also found in many more basement membranes and thought to serve similar functions there.

Massive proteinuria is associated with several human and experimental kidney diseases (diabetic nephropathy, immune complex or autoimmune glomerulo-

nephritis), indicating an impaired filtration control. As shown for diabetic mice, this is correlated with a selectively reduced renal proteoglycan synthesis[29,104]. More recent data showed a similar reduction in perlecan mRNA[65] and that high glucose levels decrease proteoglycan synthesis of mesangial cells[80]. Proteinuria can in addition be caused by anti-proteoglycan antibodies[108] but also by antibodies to laminin or collagen IV[104]. These pathological data therefore support the essential role of proteoglycans in filtration. The molecular mechanism of this process needs, however, still to be elucidated.

Growth control

Growth factors and other cytokines have been recently shown to be stored in the extracellular matrix, emphasizing a particular role of basement membranes and their heparan sulfate proteoglycans[89,90,110,112]. This storage is considered to provide a continuous source of these factors under a tight spatial control. Most of the studies so far have been concentrated on the heparin-binding basic fibroblast growth factor (bFGF). Matrix bound forms were initially isolated from cultured aortic and cornea endothelial cells[111] and their abundance in many cells and matrices was shown by immunohistology of adult and embryonic tissues[13,33]. bFGF could also bind to basement membranes in the chick eye[30], indicating that plenty of binding sites are still available in tissues. The binding of bFGF to corneal basement membranes was shown to be of moderate affinity and was inhibited by heparitinase treatment or by heparin and heparan sulfate[4]. Furthermore, bound bFGF, after release by heparin or heparitinase, was still biologically active. A large heparan sulfate proteoglycan from capillary endothelial cells was shown to bind bFGF by its side chains and to stabilize it against plasmin degradation[91,92]. Plasmin, as well as plaminogen, could release matrix-bound bFGF in active form[92]. This suggests that plasminogen activator and also tissue heparitinases[49] may be responsible for the liberation of tissue bFGF in situ. Two recent studies have in addition demonstrated that heparin or heparan sulfate binding to bFGF is essential for its association to high affinity cellular receptors[87,117]. This cooperation may be realized by simultaneous binding to high and low affinity receptors, the latter being cell-membrane-bound heparan sulfate proteoglycans, or by the action of a bFGF-heparan sulfate complex released from the matrix.

Similar mechanisms of storage, release, stabilization and activation may also exist for other cytokines. Several more heparin-binding growth factors (colony stimulating factor, interleukin 3, platelet factor 4) are known, while others such as transforming growth factor β, bind the core protein of specific proteoglycans (decorin)[90]. They may also include interferon-γ, which binds to the EHS tumor basement membrane with high affinity ($K_d \sim 1$ nM) in a heparan sulfate dependent manner[70]. Another novel 19 kDa heparin-binding factor was shown to be localized in embryonic basement membranes and to stimulate cell proliferation and neurite outgrowth[88,107].

Protease control

Heparin-binding is also known for several proteases and protease inhibitors. A classical example is antithrombin, which inhibits thrombin more efficiently in the presence of heparin. Affinity for antithrombin was also observed for heparan sulfate from basement membrane proteoglycans from Reichert's membrane but not from the EHS tumor[85]. High affinity correlated with a high 3-O-sulfate content, a modification known to be essential for heparin binding to antithrombin. A similar correlation was also found for heparan sulfate obtained from normal and transformed mammary epithelial cells[86] and may exist in many more tissues[43]. It was also observed that anthithrombin binds avidly to the subendothelial matrix and may provide vessels with an anticoagulant reservoir[19].

Such basement membrane localizations are also likely for thrombin, plasminogen, plasminogen activators and their inhibitors, nexin-1 and other proteases and inhibitors involved in homeostatic control[110]. Together with growth factors, they will be important for the control of cell phenotypes, remodelling, angiogenesis, tumor invasion and other biological processes. Those basement membrane components involved in storage and protection need, in most cases, to be identified.

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