INTRODUCTION

Proteoglycans (PGs) consist of a protein portion and long, unbranched polysaccharides (glycosaminoglycans or GAGs). The latter have a high negative charge, owing to the presence of acidic sugar residues and/or modification by sulphate groups. The acidic sugar alternates with an amino sugar in repeated disaccharide units. The GAGs adopt an extended conformation, attract cations, and bind water. Hydrated GAG gels enable joints and tissues to absorb large pressure changes.

In addition to buffering pressure changes, PGs play important roles in control of growth and differentiation. Particular sulphation patterns in the GAG chains allow interactions, normally of ionic nature, with growth factors, for example. Recent studies have identified ~30 PG protein cores. These cores are not just scaffolds for GAGs: they contain domains that have particular biological activities (Iozzo, 1998). Many PGs are thus multifunctional molecules that engage in several different specific interactions at the same time.

After synthesis PGs are transported from the Golgi to their destinations: the extracellular matrix (ECM), the cell surface or intracellular organelles. Such vectorial transport requires mechanisms for recognition, sorting and delivery, which are especially important in cells such as epithelial cells and neurons, where the cell membrane comprises separate domains. Recognition and sorting must require determinants in the GAG chains and/or in the PG protein cores. Here we discuss how and where chondroitin sulphate (CS)/dermatan sulphate (DS) and heparan sulphate (HS)/heparin GAGs are synthesised, and how these GAGs influence the sorting of PGs to the sites at which they act.

SUMMARY

Proteoglycans are widely expressed in animal cells. Interactions between negatively charged glycosaminoglycan chains and molecules such as growth factors are essential for differentiation of cells during development and maintenance of tissue organisation. We propose that glycosaminoglycan chains play a role in targeting of proteoglycans to their proper cellular or extracellular location. The variability seen in glycosaminoglycan chain structure from cell type to cell type, which is acquired by use of particular Ser-Gly sites in the protein core, might therefore be important for post-synthesis sorting. This links regulation of glycosaminoglycan synthesis to the post-Golgi fate of proteoglycans.

Key words: Proteoglycan, Glycosaminoglycan, Sorting, Polarised cell, Epithelial cell, Glyceran

VARIATION IN PROTEOGLYCAN COMPOSITION

PGs were initially grouped together because of the high negative charges of their GAG chains, which make separation from other molecules by ion-exchange chromatography easy. PGs are, however, not that similar. The core protein size ranges from 10 kDa to >500 kDa, and the number of GAG chains attached varies from one to >100 (for review, see Poole, 1986; Rouslahti, 1988; Kjellén and Lindahl, 1991; Silbert and Sugumaran, 1995). In addition, several PGs carry GAG chains of more than one type (hybrid PGs; Rapraeger et al., 1985; Sugahara et al., 1992a) and/or have additional N-linked or O-linked sugar modifications.

Not all PGs are ‘full-time’ PGs. Some proteins, such as MHC class II invariant chain, thrombomodulin and the transferrin receptor are ‘part-time’ PGs (Fransson, 1987), alternatively spliced variants having GAG-initiation sites. PGs such as versican and CD44 also occur as alternatively spliced forms (Greenfield et al., 1999; Dours-Zimmermann and Zimmermann, 1994; Naso et al., 1994) whose sugar modifications vary. A variant of versican without CS-attachment sites has been discovered, and thus the PG versican could also be regarded as a part-time PG (Iozzo, 1998).

GAG (except for in keratan sulphate (KS); Baker et al., 1975; Stein et al., 1982) synthesis is initiated by sequential addition of four monosaccharides (xylose (Xyl), galactose (Gal), galactose and glucuronic acid (GlcA); see Fig. 1). From this linker tetrasaccharide, the sugar chains are extended by addition of two alternating monosaccharides, an aminosugar and GlcA. In heparin and HS, the aminosugar is N-acetylgalactosamine (GlcNAc) and in CS/DS it is N-acetylgalactosamine (GalNAc; see Fig. 2). The extent of
epimerisation of GlcA to iduronic acid (IdoA) and the sulphation pattern of the disaccharide units distinguish heparin from HS, and DS from CS (see Figs 1 and 2). In KS, the GAGs are initiated as N-linked or O-linked oligosaccharides and extended by addition of GlcNac and Gal.

There is also regional variability to the epimerisation and

**Figure 1**: The different steps in the synthesis of CS, DS, HS and heparin glycosaminoglycan chains of the GlcA-Gal Xyl-linker region.
sulphation in each GAG chain. Studies of these patterns have defined the motifs required for specific interactions with growth factors, cytokines, matrix components, enzymes and other proteins (Salmivirta et al., 1996). The minimal requirement for binding to GAGs may differ from protein to protein – for instance fibroblast growth factors FGF-1 and FGF-2 are recognised by different HS structures expressed in discrete domains of the HS polymers (Kreuger et al., 1999). Chlorate treatment (5-20 mM) of MDCK cells reduces 6-O-sulphation; higher concentrations (50 mM) also reduce 2-O-sulfation, but N-sulphation of HSPG is not affected (Safaiyan et al., 1999). In parallel with the dose-dependent loss of 6-O-sulphation there is a reduction in binding to FGF-1, whereas binding to FGF-2 is essentially unchanged (Kreuger et al., 1999).

WHERE ARE PROTEOGLYCANS SYNTHESISED?

The cell takes up the building blocks for GAG synthesis, monosaccharides and sulphate, through specialised transporter complexes in the plasma membrane. Sugars (with a few exceptions) and sulphate are then activated by nucleotide consumption in the cytosol to form UDP-sugars and 3'-phosphoadenosine 5'-phosphosulphate (PAPS), respectively (Fig. 3). Specific transporters then translocate UDP-sugars and PAPS (Mandon et al., 1994) into the endoplasmic reticulum (ER) and Golgi lumens (Hirschberg and Snider, 1987; Hirschberg et al., 1998). Glycoproteins and glycolipids are also often sulphated. PAPS is the universal donor of sulphate to all sulphotransferases, both in the Golgi and the cytosol.

The linker tetrasaccharide

Although the lumen of the Golgi apparatus is the main site for GAG synthesis, the formation of the linker tetrasaccharide might start earlier in the secretory pathway (Fig. 4). In chicken chondrocytes, xylosylation clearly takes place in a pre-Golgi compartment (Kearns et al., 1991, 1993; Vertel et al., 1993). Xylosylation was more efficiently catalysed by detergent-treated Golgi fractions than ER fractions from rat liver (Nuwayhid et al., 1986), and Lohmander et al. (1980, 1989) have also put forward kinetic arguments for Golgi localisation of xylosyl transferase (XT) in rat chondrosarcoma cells. CHO cells lacking either XT (Esko et al., 1985), galactosyl transferase I (GT I; Esko et al., 1987) or glucuronic acid transferase I (GlcAT I; Bai et al., 1999) can synthesise neither HS nor CS, which indicates that the pathways for synthesis of the linker tetrasaccharides of these GAG types share enzymes. Cells lacking GT I still synthesise GAGs on xylosides*, however (Esko et al., 1987), which indicates that an alternative GT activity can synthesise xyloside-based GAGs. Actually,

<table>
<thead>
<tr>
<th>GAG</th>
<th>Hexuronic or Iduronic acid</th>
<th>Galactose</th>
<th>Hexosamine</th>
<th>Disaccharide composition</th>
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<tbody>
<tr>
<td>Heparan sulphate/ Heparin</td>
<td>D-glucuronic acid (GlcA) L-iduronic acid (IdoA)</td>
<td>-</td>
<td>D-glucosamine (GlcNAc)</td>
<td>GlcA β(1→4) GlcNAc α(1→4)</td>
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<tr>
<td>Keratan sulphate</td>
<td>Galactose (Gal)</td>
<td>D-glucosamine (GlcNAc)</td>
<td>Gal β(1→4) GlcNAc β(1→3)</td>
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<tr>
<td>Chondroitin sulphate</td>
<td>D-glucuronic acid (GlcA)</td>
<td>-</td>
<td>D-galactosamine (GalNAc)</td>
<td>GlcA β(1→3) GalNAc β(1→4)</td>
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<tr>
<td>Dermatan sulphate</td>
<td>D-glucuronic acid (GlcA) L-iduronic acid (IdoA)</td>
<td>-</td>
<td>D-galactosamine (GalNAc)</td>
<td>IdoA β(1→3) GalNAc β(1→4)</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>D-glucuronic acid (GlcA)</td>
<td>-</td>
<td>D-glucosamine (GlcNAc)</td>
<td>GlcA β(1→3) GlcNAc β(1→4)</td>
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Figure 2: Structure of the different glycosaminoglycan chains.

The structure of the repeating disaccharides in the different types of glycosaminoglycan chains is drawn without sulphation. The different sulphation positions in each GAG are marked by encircling with a dashed red line ( evidenza).

*Xylosides: compounds in which xylose is coupled to a hydrophobic group. Xylosides cross membranes and initiate GAG synthesis, bypassing the need for xylosylated core proteins. The GAGs initiated on xylosides are in almost all cases of the CS type.
decorin protein cores contain xylose and one or two Gal residues in the presence of xylosides (Moses et al., 1999). The competition between synthesis of xyloside-based GAGs and endogenous CSPG might thus be mainly at the level of chain polymerisation. Recently, a cDNA encoding a novel GT was transfected into GT-I-deficient CHO cells, restoring the PG synthesis (Okajima et al., 1999). Patients with Ehlers-Danlos syndrome have previously been shown to exhibit reduced GT I activity (Quentin et al., 1990), and two missense substitutions have now been identified in the GT I gene (Almeida et al., 1999).

A mutant MDCK cell line that exhibits reduced import of UDP-Gal into the Golgi lumen, has dramatically reduced synthesis of KS, but essentially normal CS and HS production (Toma et al., 1996). Gal is incorporated into the polymerising GAG chain in KS, whereas in CS/DS and HS/heparin Gal is found only in the linker tetrasaccharide (see Fig. 1). GTs involved in KS chain polymerisation (GT III and IV) and linker tetrasaccharide synthesis (GT I and II) might have different Km for UDP-Gal, but another possibility is that linker tetrasaccharide synthesis is localised to a compartment excluded from the MDCK cell Golgi fraction initially characterised by Brändli et al. (1988). A pre-Golgi UDP-Gal transporter activity has not been demonstrated (Kawakita et al., 1998), but a functional GT I has been localised to the ER (Sprong et al., 1998). UDP-Gal therefore must also be available in the lumen of this compartment.

Enzyme studies and a study using xylosides to prime GAG synthesis indicated that GT I and II localise to different sub-regions of rat liver Golgi (Sugumaran et al., 1992; Etchison et al., 1995). Also, the last enzyme needed for synthesis of the linker tetrasaccharide, GlcAT I, has a dual density distribution after gradient fractionation. The distribution resembles that of GlcAT II, which is involved in CS polymerisation, but is clearly different from those of GT I and II (Sugumaran et al., 1998).

Dual localisation of enzymes involved in GAG synthesis could be interpreted in the context of the recently revived Golgi cisternal maturation model, in which a fraction of the transferases is constantly being transported retrogradely in vesicles from maturing Golgi cisternae (Bonfanti et al., 1998; Mironov et al., 1998; Glick and Malhotra, 1999).

**WHAT DETERMINES WHETHER THE GLYCOSAMINOGLYCAN CHAIN BECOMES CHONDROITIN SULPHATE/DERMATAN SULPHATE OR HEPARAN SULPHATE/HEPARIN?**

After completion of the linker tetrasaccharide, the addition of the fifth saccharide determines whether the GAG chain becomes CS/DS or HS/heparin. This sugar is GlcNAc in the case of HS/heparin and GalNAc in the case of CS/DS. GlcNAcT I (Fritz et al., 1997) and GalNAcT I mediate addition of these sugars (Sugumaran et al., 1998) and are postulated to be distinct from those enzymes used in elongation of the GAG chains of both CS (Rohrmann et al., 1985) and HS (Fritz et al., 1994). Nadanaka et al. (1999), however, provide evidence that the same enzyme catalyses the addition of the fifth sugar (GalNAc) to the linker tetrasaccharide and GalNAc addition during CS polymerisation.

Kitagawa et al. (1995) have shown that an α-GalNAcT is able to add GalNAc to the linker tetrasaccharide to produce structures that have ‘unproductive’ pentasaccharides because a β-linkage is required for further elongation of CS chains. The same group has recently shown that this enzyme also catalyses the addition of α-GlcNAc (Kitagawa et al., 1998, 1999), which is the proper sugar in HS chain synthesis. This indicates that the enzyme is the GlcNAcT I active in HS and heparin synthesis. This enzyme could be important for regulation, capping some linker tetrasaccharides with α-GalNAc and preventing their elongation. Several other factors might also regulate GAG-type synthesis (see below).

**The amino acid sequence flanking the serine residue**

Some differences in the consensus amino acid sequences for attachment of CS and HS have been found. Most HSPGs contain repetitive (Ser-Gly)n segments (n > 1) and a nearby consensus amino acid sequence for acidic residues (Zhang et al., 1995), whose position and composition are quite flexible. Substitution of these acidic residues in betaglycan gave more CS and less HS (Zhang and Esko, 1994). Three Ser-Gly-Asp motifs accepted mainly HS if an acidic motif was present N-terminally. Changing acidic residues to amines increased the proportion of CS as did deletion of one or two GAG sites (Dolan et al., 1997). Acidic clusters also occur in some CSPGs (Bourdon et al., 1987; Brinkmann et al., 1997) and are thus necessary, but not sufficient, for HS modification. Tryptophan residues also influence the choice of GAG for a particular site. In betaglycan, a Trp → Ala mutation near the Ser-Gly pair causes a shift from HS to CS production, whereas introduction of a tryptophan residue near a CS site caused a shift to HS production (Zhang and Esko, 1994). Removal of 110 residues from the basement membrane PG perlec an reduced the HS content slightly (Dolan et al., 1997).

Many isoforms of CD44 are modified by addition of GAGs. Most sites accept CS only, but a Ser-Gly-Ser-Gly motif in exon V3 also accepts HS. Substituting an acidic eight residue sequence downstream of this motif with eight residues from downstream of a CS site in exon E5 changes the modification correspondingly (Greenfield et al., 1999). Incubation of different cell types with xylosides has indicated that generation of CS-GAGs is a default modification: HS-GAGs on xylosides are rare. However, there are indications that xylosides are segregated from CSPG core proteins early during biosynthesis (Moses et al., 1999) and that different CSPG core proteins might even be segregated from each other (Vertel et al., 1989; Wong-Palms and Plaas, 1995). It is thus not clear whether some protein cores have more stringent requirements for modification by CS-GAG than others.

**Access of UDP-sugars and the presence of glycosaminoglycan-synthesising enzymes in the Golgi**

Formation of CS/DS and HS/heparin requires UDP-Xyl, UDP-Gal, UDP-GlcA, UDP-GlcNAc and UDP-GalNAc in the Golgi and/or ER lumens. Uptake of UDP-sugars, ATP and PAPS into Golgi vesicles in vitro increases the concentration 50- to 100-fold in comparison with the incubation medium (Hirschberg and Snider, 1987). The UDP-Gal transporter (Ishida et al., 1996; Miura et al., 1996) and the UDP-GlcNAc transporter (Guillen et al., 1998) from mammalian species have
been characterised and cloned, whereas the UDP-GalNAc transporter from rat liver has been characterised but not yet cloned (Puglielli et al., 1999a).

Reduced levels of UDP-Gal in the Golgi lumen in intact MDCK cells give reduced levels of KS (Toma et al., 1996). In an in vitro system, the chain length of heparin GAGs produced is determined by the ratio of UDP-GlcNAc to UDP-GlcA (Lidholt et al., 1988). In a similar way, the ratio of UDP-GalNAc and UDP-GlcNAc could influence the extent of CS/DS versus HS/heparin synthesis. Normally, UDP-GalNAc is formed by epimerisation of UDP-GlcNAc, which is catalysed in the cytosol by UDP-GlcNAc-4-epimerase, an enzyme that also catalyses epimerisation of UDP-glucose to UDP-Gal (Piller et al., 1983). A kidney cell line deficient in this enzyme has defects in the synthesis of glycolipids and the N-linked O-linked carbohydrate chains of glycoproteins. The defect can be corrected by exogenous Gal and GalNAc (Kingsley et al., 1986). Interestingly, a UDP-GlcNAc pyrophosphorylase isolated from kidney can use GalNAc-1-P and UTP to catalyse the formation of UDP-GalNAc (Szumilo et al., 1996). Thus, alternative pathways for formation of UDP-Gal and UDP-GlcNAc seem to exist. The enzymatic systems producing the different UDP-sugars, and the translocators, are thus likely to influence the concentration of UDP-sugars in the Golgi lumen and therefore also GAG-chain synthesis.

Modifications of the linker region
Several modifications of the linker region have been discovered: phosphorylation of C-2 of xylose, sulphation at various positions and epimerisation of the last sugar of the linker tetrasaccharide. The C-2 of xylose is a major phosphorylation site in both CSPG (Oegma et al., 1984; Sugahara et al., 1992a,b,c) and HSPG (Fransson et al., 1985) in certain tissues (Fig. 5). However, Xyl is generally not phosphorylated in PGs derived from tissues that have abundant extracellular matrix (Sugahara et al., 1995a,b; Cheng et al., 1996). Phosphorylation of C-2 in Xyl is most prominent after addition of the two Gal residues (Moses et al., 1999), whereas addition of the first GlcA residue is followed by rapid dephosphorylation (Moses et al., 1997). In PGs produced by melanomas, this dephosphorylation is complete (Spiro et al., 1991), whereas chondrosarcoma aggrecan contains phosphate even in secreted PGs (Oegma et al., 1984). This is also the case when high concentrations of xylosides are added to cells (Greve and Kresse, 1988; Moses et al., 1999). Dephosphorylation might be inefficient in some tumour cells and at high production rates and, in some way, cause the altered PG synthesis seen in these cells. The role of C-2 phosphorylation is thus not clear, but it might provide a signal for secretory transport of PGs or for further modifications of the growing glycan chain (Moses et al., 1997, 1999). The phosphorylation of the linker residues might occur in the ER, in the Golgi or in both locations, since functional ATP transporters are present in both membrane systems (Guillen and Hirschberg, 1995; Puglielli et al., 1999b).

Sulphation of the linker region has been observed only in DS/CS and not in heparin/HS. Both CS and DS may have C-4 sulphation of the second Gal (Sugahara et al., 1988) and C-4 or C-6 sulfation in the first GalNAc after the linker region (Sugahara et al., 1991). Modification by sulphate might influence the degree of synthesis of CS/DS versus HS/heparin.

Distinguishing a CS site from a DS site, and an HS site from a heparin site, probably involves regulation of enzymes involved in GAG elongation in the Golgi complex. The regulation of HS synthesis versus heparin synthesis is complex and probably determined by the proportions of the different sulphotransferases and epimerases (Aikawa and Esko, 1999). CS and DS differ in the critical C5 epimerisation of GlcA to IdoA in DS. Once the first IdoA is formed, the C5 epimerase seems to make more IdoA (Malmstroem et al., 1993). In some PGs isolated from bovine aorta, this epimerisation starts with the GlcA of the linker region (Sugahara et al., 1995a,b). Thus, the first epimerisation reaction might be a trigger for DS synthesis and at the same time prevent the synthesis of CS and HS.

Synthesis of heparan sulphate/heparin
Lidholt et al. suggested that addition of the sixth sugar and of all the following sugars, to HS and heparin precursors is catalysed by a bifunctional 70-kDa enzyme (i.e. it catalyses addition of alternating GlcA and GlcNAc units; Lidholt et al., 1992; Lind et al., 1993). More recently, it has become evident that two different proteins are involved in the catalysis of HS and heparin polymerisation (McCormick et al., 1998; Lind et al., 1998). Both of these are products of tumor suppressor genes (EXT1 and EXT2) of the hereditary multiple exostoses gene family – as is GlcNAc transferase I (EXTL2). The EXT1 and EXT2 gene products are both needed for normal HSPG synthesis in CHO cells, but the exact role of each protein is not clear. EXT1 and EXT2 must form larger hetero-oligomeric complexes to acquire proper Golgi localisation. Such complexes have previously been described for other Golgi enzymes (Nilsson et al., 1993, 1994). Overexpression of only one of EXT1 or EXT2 resulted in ER localisation of the monomeric protein (McCormick et al., 2000). Overexpressed EXTL2 also localised to the ER and might also require a ‘partner’ protein to localise to the Golgi. If the transferases involved in linker tetrasaccharide synthesis also exist in ER and Golgi forms, this would explain some of the difficulties investigators have had in unequivocally localising these enzymes to a particular region of the Golgi.

The growing GAG chains are modified at various positions. The modifications include the following: (1) deacetylation/N-sulphation of GlcNAc units in HS and heparin; (2) epimerisation of GlcA to IdoA in HS and heparin (and also DS); (3) O-sulphation in various positions of the disaccharides of HS, heparin (and CS/DS).

As already mentioned, heparin is modified more extensively than HS but, whereas HS is expressed in virtually all cell types of the body, heparin is synthesised mainly by connective tissue mast cells. However, heparin-like structures have been found in glial cells (Stringer et al., 1999), and highly sulphated HS regions with heparin-like segments have also been found in thymic epithelial cells (Werneck et al., 1999). Three bifunctional N-deacetylase/N-sulphotransferases (DASTs or NDSTs; Wei et al., 1993) have been cloned (Hashimoto et al., 1992; Eriksson et al., 1993; Orellana et al., 1994; Humphries et al., 1997, 1998; Aikawa and Esko, 1999), but the specificity and function of these enzymes are not established. A putative heparin-specific NDST is also expressed in cells that do not produce heparin (Toma et al., 1998).

The bifunctional nature of the enzymes operating in
HS/heparin synthesis is likely to support a high speed of synthesis. The HS/heparin polymerisation rate in the Golgi apparatus has been estimated to be at least 80 monosaccharides per minute (Lidholt et al., 1988). The sulfotransferase activity resides in the C-terminal half of NDSTs (Berninsone and Hirschberg, 1998), with a critical lysine at position 614 (Sueyoshi et al., 1998). The crystal structure of the sulphotransferase (ST) domain of NDST 1 was recently determined (Kakuta et al., 1999).

Whereas only one epimerase that converts GlcA into IdoA (Li et al., 1997) and one each of the STs that catalyse 2-O- (Bai and Esko, 1996; Kobayashi et al., 1999) and 6-O-sulfation (Habuchi et al., 1998) have been cloned, multiple isoforms that have 3-O-ST activity have been cloned and demonstrated to have quite different substrate specificities and to be expressed at different levels in different human tissues (Shworak et al., 1999).

As soon as modification reactions are initiated by an NDST, the other enzymes are able to act. Most of the disaccharides in heparin undergo ‘default’ modification by addition of three sulphate groups per disaccharide, whereas only a minor fraction of HS disaccharides acquire this structure (Salmivirta et al., 1996).

Successful GAG elongation has been postulated to depend on efficient sulphation of the polymer (Lidholt et al., 1989; Lidholt and Lindahl, 1992). In this context, sulphation is a measure of completion of all the modification steps, but the modification generating the possible message to the bifunctional polymerase could also be the epimerase, which introduces a more flexible conformation to the GAG chain. The concept of a coupling between the polymerisation and modification steps has arisen from experiments with microsomal fractions, in which the organisation of the synthesis machinery may be suboptimal. Alternative studies of PG synthesis have also been carried out in intact cells in the presence of inhibitors of sulphation. One such inhibitor, selenate, blocks synthesis of GAG chains, both short and long (Dietrich et al., 1988). Incubations in the presence of other inhibitors, such as chlorate and brefeldin A, produces undersulphated GAG chains of similar length to or longer than those in control cells (Wong-Palms and Plaas, 1995; Kreuger et al., 1999; Safaiyan et al., 1999). A low level of sulphation can still be sufficient to promote chain elongation, as is seen in patients that have achondrogenesis type 1B: sulphation of PGs may be reduced to about 25% of the control level, without any effect on GAG-chain length (Rossi et al., 1996). Still, it is unclear how closely coupled the polymerisation and modification reactions are, and what factors determine how long GAG chains are allowed to grow before termination of synthesis.

Synthesis of chondroitin sulfate/dermatan sulfate

In the case of the CSPG decorin, which has a single GAG chain, N-terminal deletions in the propeptide result in synthesis

![Figure 3: Synthesis pathways for the formation of UDP-sugars and PAPS.](image-url)

**Figure 3:** Synthesis pathways for the formation of UDP-sugars and PAPS.

**Abbreviations:**
- APS: Adenosine-5’-phosphosulphate
- GlcA: Glucuronic acid
- ERGIC: ER-Golgi Intermediate Compartment
- Glc: Glucose
- GlcA: Glucuronic acid
- GlcN: N-Glucosamine
- GlcNAc: N-acetyl-Glucosamine
- Gal: Galactose
- GalNAc: N-acetyl-Galactosamine
- IdoA: Iduronic acid
- PAPS: 3’-Phosphoadenosine-5’-phosphosulphate
- Xyl: Xylose
- UDP-Glc: UDP-Glucose
- UDP-Gal: UDP-Galactose
- UDP-GlcA: UDP-Glucuronic acid
- UDP-GlcNAc: UDP-N-acetylgalactosamine
- UDP-GalNAc: UDP-N-acetylgalactosamine
- UDP-Xyl: UDP-Xylose
- ATP: Adenosine triphosphate
- ADP: Adenosine diphosphate
- UTP: Uridine triphosphate
- PPi: Inorganic pyrophosphate
- NAD+: Nicotinamide adenine dinucleotide phosphate
- NADH: Nicotinamide adenine dinucleotide
- FAD: Flavin adenine dinucleotide
- CoA-SH: Coenzyme A
- ATP + SO42-: Adenosine triphosphate + sulphate
- Endoplasmic reticulum
- Golgi apparatus
- ERGIC

*Synthesis pathways for the formation of UDP-sugars and PAPS needed for synthesis of proteoglycans. Each UDP-sugar and PAPS is actively transported from the cytosol into the Golgi lumen, and into the lumen of ER (in the case of UDP-Xyl), by a corresponding transporter.*
Synthesis and sorting of proteoglycans

of a form of decorin that has a shorter GAG chain (Oldberg et al., 1996). The reason for this early termination is not known. Elongation of CS chains is mediated by two distinct transferases that operate by alternate transfer of GalNAc and GlcA (Richmond et al., 1973). The enzymatic activity that transfers GlcA to the growing GAG chain, glucuronosyl transferase II (Sugumaran et al., 1997), is different from the activity that transfers GlcA to Gal to complete the linker region (Sugumaran et al., 1998). Two CS/DS STs have been cloned, the 6-O-ST (Uchimura et al., 1998) and the 2-O-ST (Kobayashi et al., 1999), whereas a 4-O-ST has been purified from the culture medium of a rat chondrosarcoma cell line (Yamauchi et al., 1999).

Although linker tetrasaccharide synthesis seems to be a common pathway for both HS synthesis and CS synthesis, not only are elongation and modification of CS/DS and HS/heparin GAG chains catalysed by different sets of enzymes, but these events also take place in different subdomains of the Golgi apparatus.

Localisation of enzymatic activities to different regions of the Golgi apparatus has been facilitated by use of the fungal isoprenoid metabolite brefeldin A (BFA, see Fig. 4), because treatment with this drug induces retrograde transport of components of the cis-, medial- and trans-Golgi cisternae to the ER (Lippincott-Schwartz et al., 1990; Sandvig et al., 1991). Golgi enzymes relocated to the ER retain their activity and can still modify their natural substrates, which can no longer exit the ER.

Figure 4: The localisation of different proteoglycan-synthesising enzymes in the ER and Golgi apparatus with BFA treatment.

The enzymes responsible for the synthesis of the linker region and GAG polymerisation are found at different locations in the endoplasmic reticulum (ER) and the Golgi apparatus. With Brefeldin A (BFA) treatment, the anterograde transport through the Golgi apparatus is blocked, whereas retrograde transport is intact, which results in transport of cis-, medial- and trans-Golgi cisternae components to the ER. Golgi enzymes relocated to the ER retain their activity and can still modify their natural substrates, which can no longer exit the ER.

Brefeldin A treatment

Enzymes necessary for HS/heparin elongation are transported to the ER, where they catalyse the elongation of HS polymers.

Some enzymes are retained in TGN, which in some cells results in reduced synthesis of HS polymers.

Figure 5: Modification of the linker region.

The extent of phosphorylation increases with the attachment of each Gal, whereas addition of the first GlcA is followed by rapid dephosphorylation.

Sulphate is not yet identified in HS or heparin linker regions. Phosphate has never been found together with sulphate groups in the linker region of CS/DS.

During synthesis of proteoglycans, the linker region might be modified at various positions. Xyl may be phosphorylated in 2-O-position, whereas each Gal may be sulphated at 4-O or 6-O-position, as shown in the figure. The effects of these modifications are not known.
substrates, which consequently do not undergo TGN modifications. In several cell types, synthesis of HSPG is still detectable in the presence of BFA, whereas CSPG synthesis is blocked, which indicates that separate enzymes in different subcompartments of the Golgi complex are involved in HSPG and CSPG synthesis (Spiro et al., 1991; Sugumaran et al., 1992; Fransson et al., 1992; Uhlin-Hansen and Yanagishita, 1993; Calabro and Hascall, 1994). The enzymes necessary for the completion of the synthesis of HSPGs are located in the cis-, medial- and trans-Golgi cisternae, whereas those needed for CSPG synthesis are localised to the trans-Golgi network.

**SORTING OF PROTEOGLYCANS**

PGs are expressed by virtually all vertebrate cells and are also expressed in *Drosophila melanogaster* (Campbell et al., 1987; Cambiazo and Inestrosa, 1990; Spring et al., 1994; Graner et al., 1994) and *C. elegans* (Rogalski et al., 1993; Schimpf et al., 1999). How PGs are sorted and transported to their proper destinations can therefore be studied in many different organisms. After reaching the plasma membrane, PGs can take part in binding and uptake of signalling molecules, such as growth factors and γ-interferon. Cell surface GAGs are internalised and have been detected in endocytic organelles and in the nucleus (Fedarko and Conrad, 1986; Ishihara et al., 1986; Tumova et al., 1999), co-localised with growth factors, but the pathway taken from the cell surface to the nucleus is largely unknown. In rat hepatocytes, HSPG is transported from the TGN to the cell surface in a population of vesicles different from that which transports serum albumin, apolipoprotein E and fibrinogen (Nickel et al., 1994; Barthel et al., 1995). Hepatocytes are polarised cells in vivo, but it is now evident that in non-polarized cells there are also at least two alternative carriers from the TGN to the cell surface (Keller and Simons, 1997). Many neural and endothelial cells possess two secretory pathways from the trans-side of the Golgi apparatus: a regulated pathway and a constitutive pathway. Frequently PGs are sorted to the regulated pathway, being released together with the other contents of the storage granules. The negatively charged PGs are engaged in binding of small positively charged molecules, such as histamine (Grimes and Kelly, 1992; Brion et al., 1992; Castle and Castle, 1998) and proteases (Huang et al., 1998; Lützelschwab et al., 1997). Mast cells from mice that lack the enzyme NDST 2, which is involved in heparin synthesis, fail to store several proteins that are normally bound to heparin in secretory granules. Mast cells from NDST 2 knockout mice contain smaller granules and large empty vacuoles, which indicates that heparin GAGs are needed for normal granule formation (Humphries et al., 1995; Forsberg et al., 1999).

**The role of glycans in apical sorting in epithelial cells**

Similarly to hepatocytes and neurons, epithelial cells have their plasma membranes divided into two domains, the apical and basolateral surfaces. To maintain the polarised organisation, efficient sorting mechanisms target newly synthesised and recycling molecules to the proper surface domain or intracellular location. Epithelial cells must ensure that PG components of the extracellular matrix (ECM) and those that attach the basolateral side of the epithelium to the ECM are transported to the correct side of the monolayer. Basolateral distribution of syndecan-I in MDCK cells requires the terminal 12 amino acids of the cytoplasmic tail (Miettinen et al., 1994), while basolateral secretion of the major basement membrane PG occurs by a pH-dependent mechanism (Caplan et al., 1987). The fact that different sets of PGs are secreted apically and basolaterally indicates that these molecules are actively sorted. The recent discovery that endogenously synthesised CSPG, and hexyl-β-D-xyloside that has CS chains, are mainly secreted apically in MDCK cells suggests that CS chains might contain apical-sorting information (Kolset et al., 1999).

HS chains could have an opposite effect. The GPI-linked PG glypican was detected predominantly at the basolateral surface of CaCo-2 and MDCK cells (Mertens et al., 1996). A form of glypican that lacks sites for HS attachment was transported strictly to the apical surface in MDCK cells, presumably because of its glycosylphosphatidylinositol (GPI) membrane anchor or N-linked sugars, although the latter question was not addressed. HS chains thus either promote basolateral sorting of glypican or interfere with the recognition of the apical sorting information in the molecule.

Since many GPI-linked proteins are delivered to the apical surface in epithelial MDCK cells (Lisanti et al., 1988), it has been suggested that GPI anchors function as apical sorting signals. However, both secreted and GPI-linked rat growth hormone required N-glycans to be transported apically (Scheiffele et al., 1995; Benting et al., 1999). O-linked sugars might also direct apical sorting in MDCK cells (Yeaman et al., 1997), and both N-linked and O-linked sugars play a role in apical targeting of bovine enteropeptidase (Zheng et al., 1999). For transmembrane proteins, both N-glycans (Gut et al., 1998) and transmembrane domains (Scheiffele et al., 1997) have been proposed to play a role.

The mechanisms underlying carbohydrate-mediated transport and sorting are not known. Simons and co-workers have proposed that sorting is mediated by lectin molecules that accumulate in sphingolipid-cholesterol rafts within the exoplasmic leaflet of the TGN membrane (Simons and Ikonen, 1997; Keller and Simons, 1997). VIP36 has been proposed as a candidate raft-associated lectin (Fiedler et al., 1994), but more recently this protein has been localised to the cis region of the Golgi (Füllekrug et al., 1999) and, thus, to date no sorting lectin has been identified.

Proteins may also be transported apically in a glycan-independent manner (Rodriguez-Boulan and Gonzalez, 1999), and proteins both with (Zheng et al., 1999) and without (Alonso et al., 1997) N-linked sugars are transported to the apical surface without being integrated into rafts, according to the detergent extraction criteria applied for raft association. Thus, different kinds of determinants might mediate apical sorting. A particular protein may contain more than one sorting signal, and glycan signals are often recessive to other sorting signals contained in the protein portion of a molecule (Mellman et al., 1993). This could explain why attachment of a CS chain to a variant of the amyloid-precursor-like protein 2 influences the basolateral sorting of neither the transmembrane-anchored form nor the secretory form of this protein (Lo et al., 1995).

In an alternative model, the sorting lectin is not a transmembrane protein that recycles but a secreted PG.
Versican (Zimmermann and Ruoslahti, 1989) might be the CSPG secreted apically by MDCK cells (Svennevig et al., 1995). This PG is a member of a group called lecintics that, in addition to CS chains, contain several interesting protein core domains (Iozzo, 1998). Among these are two hyaluronic acid (HA)-binding modules near the N terminus and a C-lectin domain at the C terminus (Halberg et al., 1988). Versican has been proposed to bind tenasin-R (Aspberg et al., 1995). L-selectin (Kawashima et al., 1999) and sulphated glycolipids through the C-type lectin domain (Miura et al., 1999). L-selectin has been shown to bind to, in addition to versican, many of the same kinds of ligands as versican and some additional ones (Needham and Schaara, 1993; Kimura et al., 1999; Li et al., 1999; Watanabe et al., 1999). In the Golgi apparatus, the C-lectin domain of versican could bind to sulphated glycolipids. Since HA has been proposed to be synthesised at the cell surface, the two HA-binding domains may be free to bind other sugars in the Golgi apparatus. The HA-binding motifs are structurally similar to C-type lectins, apart from a possible calcium-binding loop (Kohda et al., 1996). Lectin domains at both ends of the versican molecule could thus allow binding of one end to the membrane and binding of glycansated molecules, such as glycoproteins, to the other end. Cross-linking into larger complexes may be necessary for apical sorting of soluble molecules.

More studies are needed if we are to determine the extent to which glycans contain sorting information and to identify the structural determinants involved. The indications that glycosaminoglycans influence the subcellular localisation of proteoglycans raises the possibility that the tissue-specific variability seen in GAG modification directs PGs to different destinations in different tissues. This possibility will link future studies of the regulation of PG synthesis to studies of PG sorting.

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