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Review

Novel aspects of glypican glycobiology

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Abstract. Mutations in glypican genes cause dysmorphic and overgrowth syndromes in men and mice, abnormal development in flies and worms, and defective gastrulation in zebrafish and ascidians. All glypican core proteins share a characteristic pattern of 14 conserved cysteine residues. Upstream from the C-terminal membrane anchorage are 3–4 heparan sulfate attachment sites. Cysteines in glypican-1 can become nitrosylated by nitric oxide in a copper-dependent reaction. When glypican-1 is exposed to ascorbate, nitric oxide is released and participates in deaminative cleavage of heparan sulfate at sites where the glucosamines have a free amino group. This process takes place while glypican-1 recycles via a non-classical, caveolin-1-associated route. Glypicans are involved in growth factor signalling and transport, e.g. of polyamines. Cargo can be unloaded from heparan sulfate by nitric oxide-dependent degradation. How glypican and its degradation products and the cargo exit from the recycling route is an enigma.

Key words. Caveolae; development; growth factors; heparan sulfate; nitric oxide; polyamines; S-nitrosylation.

Introduction

The first member of the family of glycosylphosphatidylinositol (GPI)-anchored heparan sulfate (HS)-substituted proteoglycans, later named glypicans (Gpc), was identified some 15 years ago [1]. The family comprises to date six mammalian members, two in *Drosophila*, called Dally and Dally-like, and one each in *Caenorhabditis elegans* and the zebrafish (called knypek). Potentially localizing to rafts and caveolae, Gpc can be involved in caveolar uptake of biomolecules and viruses and in selective regulation of growth factor and cytokine signalling. The discoveries that mutations in genes involved in glypican assembly cause dysmorphic syndromes in man and aberrant patterning during *Drosophila* development have brought this family of molecules into focus (for recent reviews, see [2–5]). Most of the growth defects associated with aberrant expression of Gpc genes (table 1) are similar to those caused by mutations in genes involved in HS biosynthesis or precursor UDP-sugar formation (table 2). This underscores that many of the functions of Gpc are exerted by the HS side chains. HS chains are strongly polyanionic and contain specific binding sites for a number of growth factors, cytokines and morphogens with cationic binding domains [4, 5].

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Structure of the Gpc core proteins

All mature Gpc core proteins are ~60–70 kDa in size and share a characteristic pattern of 14 conserved cysteine residues, as shown in figure 1. These residues are mainly located to the central domain, but they are also found near the N-terminus. The N-terminal region of the primary translation product contains the signal for translocation to the endoplasmic reticulum (ER). The C-terminal region contains a signal sequence for temporary membrane insertion and subsequent firm attachment via glypiation.

Table 1. The glypican family.

Name	Expression		Functional features
	Embryo	Adult	
Glypican-1 (Gpc-1)	CNS	ubiquitous	involved in FGF2 and Slit signalling
Glypican-2 (Gpc-2, cerebroglycan)	CNS	none	axonal guidance
Glypican-3 (Gpc-3, OCI-5)	ubiquitous	Reduced in CNS	involved in IGF signalling; mutated in SGB-overgrowth syndrome
Glypican-4 (Gpc-4, K-glypican)	kidney, neurons adrenal	reduced in CNS	not known
Glypican-5 (Gpc-5)	kidney, limb buds, CNS	CNS	not known
Glypican-6 (Glp-6)	heart, liver, kidney	also ovary, intestine	not known
Division abnormally delayed (Dally)	epidermal segmental stripes		involved in Wg and Dpp signalling
Dally-like (Dly)	also CNS		involved in Hedgehog signalling
Knypek (Kny)			involved in Wg signalling

Glypicans-1 through 6 are expressed in mammalian tissues (either human, rat or mouse). The known nonmammalian glypican homologs are the two Dally forms expressed in *Drosophila,* knypek in zebrafish and the *C elegans* ortholog.
CNS, central nervous system; Dpp, decapentaplegic; FGF2, basic fibroblast growth factor; IGF, insulin-like growth factor; SGB, Simpson-Golabi-Behmel syndrome; Wg, wingless.

Table 2. Mutations in genes involved in heparan sulfate biosynthesis.

Gene	Species	Enzyme	Phenotype
Sugarless	*Drosophila*	UDP-Glc-dehydrogenase	defective Wg, FGF and Dpp signalling
Fringe connection	*Drosophila*	UDP-sugar antiport	defective Wg, FGF and Dpp signalling
Slalom	*Drosophila*	PAPS-synthase	defective Wg, FGF and Dpp signalling
Tout-velu	*Drosophila*	HS copolymerase	defect in Hedgehog signalling
EXT1	human	HS copolymerase	multiple exostoses syndrome
EXT2	human	HS copolymerase	multiple exostoses syndrome
Sulfateless	*Drosophila*	*N*-deacetylase/*N*-sulfotransferase	defective Wg, FGF and Hedgehog signalling
Pipe	*Drosophila*	2-*O*-sulfotransferase	defective dorso-ventral patterning
HS-2-OST	mouse	2-*O*-sulfotransferase	defective eye, skeletal and renal development
HS-3-OSTs	mouse	3-*O*-sulfotransferases	complex phenotypes; potential defect in blood clotting

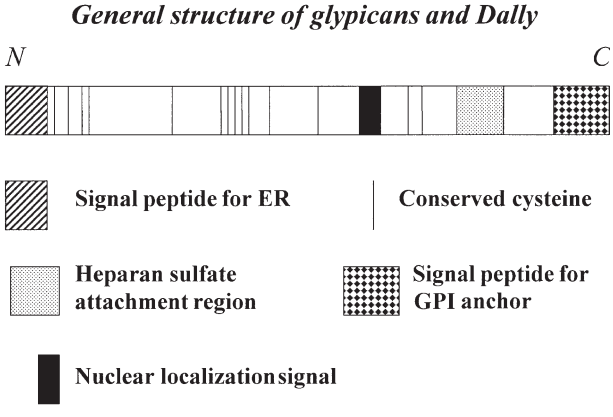

Figure 1. General, schematic structure of glypican core proteins.

The consensus sequence, DSGSGSG, for glycosaminoglycan substitution via serine residues is located between the central domain and the C-terminal GPI anchor. Upstream from the glycosaminoglycan attachment site is a nuclear localization signal [6].

Gpc core protein expression

The *GPC* genes have a very characteristic expression pattern, which is especially evident during development (table 1). Gpc-1 is expressed mainly in the central nervous system (CNS) and skeletal system during development but also in many other tissues in the adult [7].

Gpc-2 is more specifically expressed in the developing brain, especially located to axons and growth cones [8] but does not seem to be present in the adult. Also Gpc-3, -4, -5 and -6 expression is widespread in the embryo but more limited in the adult. This probably reflects important functions for the Gpc variants during tissue morphogenesis. Accordingly, a deletion involving the *GPC1* gene has been detected in brachydactyly type A [9]. Gpc-3 has been implicated as an inhibitor of cell proliferation [10]. Mutations in the human *GPC3* gene are associated with developmental overgrowth, the Simpson-Golabi-Behmel (SGB) syndrome, and Gpc-3 null mice exhibit developmental overgrowth similar to this syndrome [3]. Mutations in Dally and Dally-like result in severe malformations due to impaired gradient formation of the morphogens wingless and decapentaplegic [3, 11, 12]. Mutations in the zebrafish *GPC* gene, called knypek, result in impaired gastrulation due to failure of Wnt11 signalling during induction of appropriate cellular movements [13].

Expression of Gpc is often correlated with neoplastic transformation. Glypican-1 is overexpressed in human breast cancer cells and thereby supports and maintains the mitogenic effect of several HS-binding growth factors [14]. Gpc-3 is overexpressed in human hepatocellular carcinoma [15] but silenced in human breast cancer [16]. Generally, Gpc-3 expression seems be upregulated in tumours originating from tissues that normally express Gpc-1 but not Gpc-3. In contrast, Gpc-3 expression tends to be downregulated in tumours derived from tissues normally expressing Gpc-3 [17]. When expression of the relevant Gpc is silenced in tumour cells using antisense RNA technology, both growth-factor response and tumour formation are depressed [14, 18].

Posttranslational modification and secretion

After transport into the ER and attachment to the membrane via the GPI anchor, folding of the Gpc core protein should take place. There are sites for N-linked glycosylation near the N-terminus, and these oligosaccharides are likely to participate in the general quality-control process [19]. It has always been assumed, but never proven, that the 14 conserved Cys residues engage in disulfide bond formation, forming a tight, central globular domain in the Gpc core proteins.

The glycosaminoglycan attachment region present in the Gpc protein is similar, sometimes almost identical, to glycosaminoglycan attchment sites in other proteoglycan core protein families. These proteins are either substituted with the galactosaminoglycans chondroitin/dermatan sulfate or with the glucosaminoglycans HS/heparin, sometimes alternatively, sometimes with both [20, 21]. The glycosaminoglycan attachment sites in all of these proteins are first substituted with a common tetrasaccharide spacer, called the protein-to-carbohydrate linkage region, with the structure GlcAβ1-3Galβ1-3 Galβ1-4Xylβ1-O-Ser (see also fig. 2).

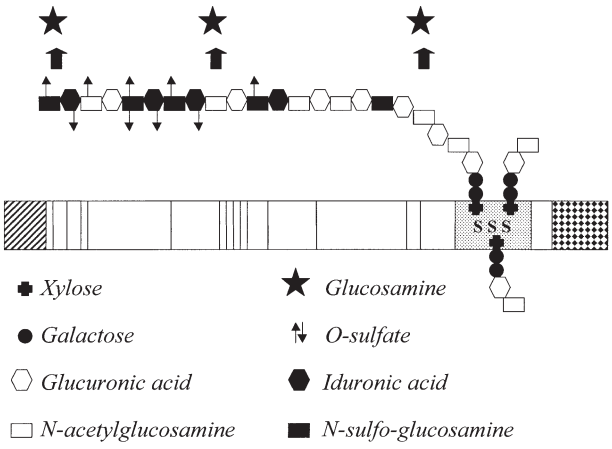

Figure 2. Posttranslational heparan sulfate synthesis on Gpc-1. Build-up of only one chain is shown for simplicity. Arrows pointing upwards are 6-*O*-sulfates; arrows pointing downwards are either 3-*O*-sulfates in GlcN or 2-*O*-sulfates in IdoA.

Initiation of HS synthesis then depends on whether an αGlcNAc residue (open rectangle in fig. 2) is added to C-4 of the GlcA of the linkage tetrasaccharide, instead of a β1GalNAc3 residue or other capping residues [4, 5]. The former reaction is catalyzed by unique αGlcNAc transferases that can recognize sequences in the core protein [4, 22]. Three enzymes (EXTL1, EXTL2 and EXTL3) that are able to catalyze this step have been identified/cloned and shown to participate in HS biosynthesis [23].

Polymerization of HS could involve as much as three different copolymerase genes generating a backbone of more than 200 glucuronic acid-glucosamine repeats $(\text{-4GlcA}\beta\text{1-4GlcNAc}\alpha\text{1-})_n$ (fig. 2). EXTL3 can also participate in elongation, and two other specific copolymerases, EXT1 and EXT2, have been cloned and localized to the Golgi [24]. Both EXT1 and EXT2 need to be expressed in order to acquire efficient HS polymerization in vivo [25]. In vitro polymerization studies have confirmed these results [26, 27]. Mice deficient in EXT1 are incapable of synthesizing HS and die in utero [28]. The EXT terminology is derived from the finding that mutations in the *EXT* genes cause hereditary multiple exostosis syndrome (table 2), which is a bone disorder with potential for malignant transformation [29].

The HS backbone is more or less extensively modified in various ways (fig. 2). First another family of HS-specific enzymes, *N*-deacetylase/*N*-sulfotransferase (NDST) replace *N*-acetyl in GlcNAc with *N*-sulfate (filled rectangle in fig. 2). NDST-1 modifies the chain in a regional fashion, whereas type 2 causes more extensive, consecutive replacements. NDST-3, which has a 10-fold higher deacetylase than sulfotransferase activity, could be re-

sponsible for the formation of glucosamines with free amino groups ($GlcNH_3^+$, star symbol in fig. 2). Free amino groups could also be generated by *N*-desulfation. *N*-sulfation is followed by C-5 epimerization that converts neighbouring GlcA to IdoA (open to filled hexagonal in fig. 2).

Finally, various *O*-sulfotransferases (OSTs) generate extensive and variable sulfation of $GlcNSO_3$ (3-OST and 6-OST) and IdoA (2-OST), but also occasionally of GlcNAc and GlcA [4]. Enzymes involved in HS biosynthesis can occur in functional complexes in the Golgi [30]. All of this results in efficient synthesis of HS chains consisting of sections, variable in length and sulfation, separated by completely unmodified GlcA-GlcNAc sections, also variable in length (fig. 2). As many of the modifications are incomplete and as the specificities of e.g. various isoforms of 6-OSTs and 3-OSTs are dependent on structures generated by preceding enzymes (NDST and 2-OST), regions with unique binding sequences are generated [31–34].

It should be added that mutations in genes involved in HS assembly should be more severe than mutations in Gpc genes only. Defective HS formation will result in inadequate glycanation not only of Gpc but also of several other proteoglycans, including agrin, perlecan, serglycin, betaglycan and the syndecans (see table 2).

The GPI anchor of the mature Gpc proteoglycans should favour apical over basolateral sorting in polarized cells. However, the major part of cell-surface Gpc-1 is unexpectedly expressed at the basolateral surfaces. Removal of the HS-attachment region resulted in extensive apical targeting, suggesting that HS substitution antagonized the GPI-sorting signal [35]. Alternatively, basolateral forms may be more stationary if their HS chains interact with extracellular matrix molecules, e.g. in basement membranes. Another functional consequence of the GPI anchor in Gpc could be the sorting into specialized membrane domains rich in sphingolipids and cholesterol, so-called rafts, or into the raft-derived, caveolin-coated invaginations called caveolae (for reviews, see [36, 37]).

Recycling of Gpc and degradation of Gpc HS

Internalization

Cross-linking of caveolar constituents induces uptake via endosomes that are budding off from caveolae [36, 37]. HS chains can self-associate [38], and therefore Gpc HS chains could mediate cross-linking and induce caveolar internalization.

Brefeldin A (BFA) is a fungal metabolite that has been used extensively to manipulate secretion as well as recycling via endosomal compartments [39]. Depending on the cell type, treatment with BFA can inhibit anterograde transport in the secretory pathway as well as arrest recycling vesicles trafficking the route plasma membrane-endosomes-Golgi [36, 37, 40]. For example, BFA inhibits caveolar uptake and delivery of cholera toxin B and SV40 virus.

We showed that when metabolic radiolabelling of secretory proteoglycans was inhibited by BFA, Gpc-type HS proteoglycan was still radiolabelled. Furthermore, cell-surface biotinylated Gpc proteoglycan reappeared radiolabelled after incubation of cells with radiosulfate in the presence of BFA [41]. We could later show that the HS chains of Gpc underwent both degradation and resynthesis during recycling [42]. Degradation of HS was partly enzymatic, as treatment with the heparanase-inhibitor suramin partly prevented generation of HS oligosaccharides and resulted in accumulation of Gpc proteoglycan with truncated HS chains.

Role of nitric oxide

Results obtained in studies on proteoglycan turnover in vascular endothelial cells suggested a nonenzymatic, autodegradation of HS in cell extracts [43]. When it became apparent that endogenously formed nitric oxide (NO) could degrade HS, we could show that the nonenzymatic HS degradation in Gpc-1 is catalyzed by NO [44]. HS is deaminatively cleaved by NO at sites where the glucosamines are N-unsubstituted ($GlcNH_3^+$). These residues are preferentially located to sites near the core protein (fig. 2) in Gpc-1 HS [45] as well as in HS derived from other proteoglycans [46]. Inhibition of endogenous polyamine synthesis generates, via an unknown mechanism, an increased number of $GlcNH_3^+$ residues scattered throughout the HS chains in Gpc-1 [47]. The NO-sensitive sites tend to be more common to the reducing side of the sites cleaved by heparanase (an endo-β-glucuronidase acting on unsubstituted GlcA; fig. 2).

More recently, we showed that the NO-catalyzed degradation of HS in Gpc-1 is dependent on a Cu(II)/Cu(I) redox cycle and that it is triggered by ascorbate [48]. The copper redox cycle is involved in S-nitrosylation (SNO) of free cysteine thiols in the Gpc-1 core protein. When SNO-containing Gpc-1 is exposed to ascorbate, NO is released. The nitroxyl anions generated from NO in the copper redox cycle cleave the HS chains at the $GlcNH_3^+$ residues, releasing HS fragments terminating with anhydromannose (anMan) at the reducing end (fig. 3). This self-pruning process can also be demonstrated in vivo [48].

By using various specific antisera and monoclonal antibodies, recycling Gpc-1 can be detected by confocal microscopy in intracellular compartments that are associated with uptake of cholera toxin B, and that contain caveolin-1 as well as the sorting endosome marker Rab9 [49]. Gpc-1 is partly S-nitrosylated and contains HS chains with $GlcNH_3^+$ residues. When recycling is arrested by

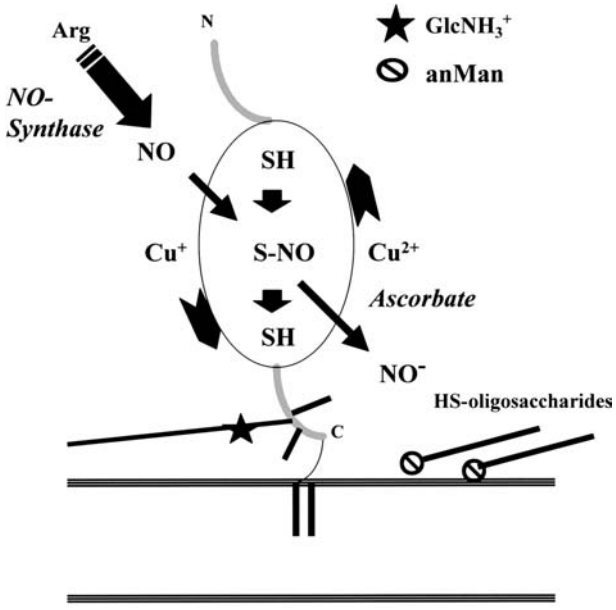

Figure 3. Model of Gpc-1 with intrinsic SNO groups generating NO for the deaminative cleavage of its own HS chains.

BFA, Gpc-1 colocalizes strongly with caveolin-1 but not with Rab9. BFA-arrested Gpc-1 contains HS chains with many $GlcNH_3^+$ residues, but there are very few SNO groups in the core protein. Inhibition of heparanase with suramin induces accumulation of SNO-containing Gpc-1 in caveolin-1-rich compartments. In NO-deprived cells, SNO-containing Gpc-1 is undetectable, but heparanase can generate HS oligosaccharides that colocalize strongly with caveolin-1. In cells where NO release from SNO-containing Gpc-1 is stimulated, either by ascorbate or by the growth state of the cell, N-unsubstituted glucosamines in Gpc-1 HS are almost undetectable, whereas anMan-containing HS fragments are generated, some of which are present in acidic vesicles [48, 49].

Regulation of recycling

Self-pruning of recycling Gpc-1 is controlled by the availability of copper ions, NO and a reducing agent, which could be ascorbate as this is actively taken up by e.g. brain cells [50]. Copper ions are usually not free, but bound to various cuproproteins. One such GPI-linked cuproprotein is the cellular prion protein PrP^C. This protein is enriched in caveolae and taken up via nonclassical, caveolin-containing early endocytic structures and delivered to the pericentriolar region [51]. We have very recently shown that PrP^C colocalizes with Gpc-1 in paranuclear, caveolin-1-positive sites and that it can deliver Cu(II) ions to Gpc-1 and support autodegradation of HS [52]. The interaction is mediated by the HS chains. In prion null cells, SNO-containing Gpc-1 is undetectable, but S-nitrosylation of Gpc-1 can be restored by ectopic expression of PrP^C. Obviously, stimulation of NO formation, which increases SNO formation in general, would also regulate Gpc-1 turnover [53].

Autocatalyzed NO- and ascorbate-dependent cleavage of HS in Gpc-1 can also be supported by Zn(II), which simultaneously inhibits Cu-dependent autocleavage [52]. As Zn(II) is redox inert, the mechanism must be different, probably a transnitrosation reaction involving transient formation of SNO groups. This finding is particularly relevant for Alzheimer's research, because free Zn(II) ion is released from the presynaptic neuron during transmission and has been implicated as a causative agent [54].

Functions of Gpc

Signalling

Cell surface HS is a substituent of Gpc as well as of members in the syndecan family and betaglycan [2, 4]. Syndecan core proteins are type I transmembrane proteins with the HS chains located near the N-terminus. In addition, the protein core should have an extended shape. Therefore, HS chains of syndecans are mainly at a distance from the cell surface and primarily interact with the extracellular matrix proteins. Moreover, the cytoplasmic C-terminal end of syndecans can transmit signals to the cytoskeleton [55]. In contrast, the HS chains in Gpc are situated close to the membrane surface (fig. 3). As HS fine structure appears to be more cell- than proteoglycan-specific, syndecans and Gpc can contain similar HS chains and can bind the same ligands [56]. Both Gpc and syndecan ectodomains are shed from cells in a regulated manner with retained binding properties for ligands [35, 57]. Gpc shedding may be catalyzed either by phosphatidylinositol-specific phospholipases C and D, which cleave in the GPI anchor, or by proteases [1]. Gpc cleaved off by phospholipase C retains the phosphoinositol moiety, which can be recognized by a receptor for internalization and degradation [58].

The HS binding sites for cationic ligands are usually of pentasaccharide size. The prototype for a unique sequence is the antithrombin III-binding pentasaccharide in heparin, -GlcNAc(6-OSO_3)-GlcA-$GlcNSO_3$(3,6-OSO_3)-IdoA(2-OSO_3)-$GlcNSO_3$(6-OSO_3)-, which has also been identified in glypican HS [59]. The minimal binding sequence for fibroblast growth factor-2 differs from the antithrombin-binding sequence only in two places, the position of the sulfate in the nonreducing terminal sugar, and in the absence of a 3-O-sulfate in the central sugar residue. HS sequences may also be exploited as binding sites for intruders, like viruses and microbes. For example, the herpes simplex virus gains entry via interaction between a viral glycoprotein gD and a HS sequence that is also similar to the antithrombin-binding sequence, except that it has opposite polarity, a different sulfate posi-

tion in the reducing terminal sugar and a free amino group in the central glucosamine ($GlcNH_3^+$; see [4, 5, 56]).

HS and heparin chains also bind to growth factor receptors [56]. The crystal structure of a dimeric fibroblast growth factor/receptor/heparin ternary complex has been determined [60]. HS/heparin chains make numerous contacts with both ligand and receptor, thereby promoting receptor dimerization and subsequent signalling. The 6-O-sulfate groups are essential for both interactions.

Both Gpc-1, Gpc-3 (see above) and Gpc-4 have been indirectly or directly implicated in growth factor signalling mediated by their HS chains. Gpc-1 expression sensitizes brain endothelial cells to fibroblast growth factor 2-induced mitogenesis, whereas overexpression of syndecan-1 has no such effect [61]. Gpc-1 also acts as an extracellular chaperone for vascular endothelial growth factor and potentiates signalling [62]. In the developing brain, expression of Gpc-4 is correlated with proliferating neural precursor cells [63]. Other potential signalling molecules that interact with Gpc-HS are the amyloid precursor protein of Alzheimer's disease [64] and endostatin [65].

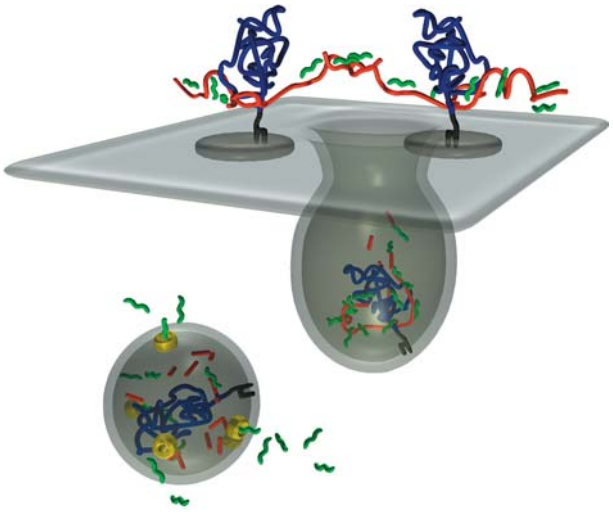

Figure 4. Speculative image of lipid raft-associated Gpc-1 as it binds, internalizes and releases its cargo. Color code: grey, membrane; green, cargo; blue, Gpc-1 core protein; red, HS chains or chain fragments; yellow ring structure, transmembrane channel protein.

Uptake

There are several observations that indicate a role for cell-surface HS proteoglycans in general and Gpc in particular as carriers in a novel cellular uptake mechanism for compounds and complexes with a net positive charge (for review, see [66]). Polyamines constitute a family of small basic molecules that are essential for growth, survival and differentiation of all cells [67]. When intracellular polyamine levels are low, there is upregulation of import from the environment. Involvement of HS proteoglycans in polyamine uptake is indirectly supported by the following findings: HS binds the polyamine spermine very strongly, HS competitively inhibits spermine uptake, and HS-deficient cells have a low spermine uptake [68–70]. Involvement of Gpc is suggested by the observations that polyamine-deprivation increases the $GlcNH_3^+$ content in Gpc-1 HS chains [47], that HS chains in Gpc-1 are cleaved at these $GlcNH_3^+$ units by NO in conjunction with spermine uptake [49] and that NO depletion inhibits spermine uptake in polyamine-deprived cells [47]. Furthermore, RNA interference (RNAi) silencing of Gpc-1 expression abrogates spermine uptake and intracellular delivery [71].

Spermine that is taken up by polyamine synthesis-inhibited cells colocalize with recycling Gpc-1 that is substituted with HS chains rich in N-unsubstituted glucosamines. Unloading of spermine requires degradation of the carrier, i.e. the HS chains. Enzymatic degradation by heparanase appears unlikely, as HS coated with spermine cannot be degraded by bacterial HS lyases [68]. Instead, treatment of cells with ascorbate, which induces release of NO from SNO groups in Gpc-1, results in HS degradation and unloading of sequestered polyamines as well as appearance of deglycanated Gpc-1 protein in the nucleus. We propose that Gpc-1 is a transient resident at the cell surface and binds polyamines to its HS side chains by electrostatic attraction (fig. 4). After transport to endosomes, HS is degraded by NO derived from the SNO groups in Gpc-1. NO should be able to cleave at sites that simultaneously bind polyamines. This is expected to weaken the interaction between HS and polyamines and result in liberation of polyamines that may exit the endosomes via carrier proteins. Gpc is probably also involved in the uptake and nuclear targeting of polyanion-basic peptide complexes such as DNA-HIV-Tat [72].

Remaining issues

To understand the structure-function relationship for Gpc core proteins, e.g. the location of metal-ion binding sites and S-nitrosylated cysteines, we need to determine the crystal structure of Gpc proteins.

As Gpc core proteins do not penetrate membranes, the signalling mechanism must be different from the classical one. Presumably, interactions between proteins within lipid rafts mediate signalling. Nonreceptor tyrosine kinases such as *c-fyn* are likely candidates [73].

The roles of Gpc in brain development, nerve regeneration, neurodegenerative diseases and cancer are likely to become major issues. Gpc-1, -3- and -4 have been implicated in patterning, trophic activity, amyloid formation, as well as cancer cell invasion and metastasis [74–78].

A follow-up of earlier observations on the involvement of glypicans in haematopoiesis and blood coagulation would also be of interest, e. g. the role of Gpc-3 in the regulation of tissue factor inhibitor [79] and Gpc-4 in the differentiation of haemotopoietic-progenitor cells [80].

With regard to uptake and recycling, it remains unclear how the Gpc-1 core protein, the HS degradation products and the cargo exit from the endosomal pathway (fig. 4). Generation of HS oligosaccharides should increase the osmotic pressure, possibly leading to vesicle rupture. Moreover, recycling Gpc-1 can carry secretory forms of phospholipase A_2 from caveolae to the perinuclear area, where the enzyme releases fatty acids from membrane lipids [81]. This would simultaneously result in the formation of lysophospholipids with potential membrane-permeabilizing ability.

The heparanase and NO cleavage sites are close together in the Gpc-1 HS sequence [47]. Moreover, the heparanase cleavage sites are in the sulfated region of HS but yet inhibited by 3-O-sulfation [82]. However, 3-O-sulfation can take place on $GlcNH_3^+$, the NO target [31]. Hence, combined enzymatic and non enzymatic HS cleavage, especially in polyamine-deprived cells, may generate small highly sulfated HS oligosaccharides. It is possible that spermine and such HS oligosaccharides form electroneutral complexes that can penetrate membranes.

When cargo and carrier have been transferred to the cytosol, the nuclear localization signal in the Gpc-1 core protein would provide guidance into the nucleus [6]. Previous studies have demonstrated that Gpc-1 core protein can be detected in the nuclei of neurons, glia cells and chinese hamster ovary cells. The nuclear appearance of Gpc-1 in C6 glioma cells varied with different phases of the cell cycle, suggesting that Gpc-1 could be involved in regulation of cell division [6]. In chinese hamster ovary cells, the nuclear appearance of Gpc-1 was induced by ascorbate [71]. Interestingly, ascorbate has been shown to promote differentiation of neural stem cells into neurons and astrocytes and to induce expression of differentiation-specific genes [83]. Whether this is due to release of NO or to the nuclear targeting of Gpc-1 core protein remains to be seen.

There are also possibilities for mutual regulation of NO and polyamine levels through the involvement of Gpc-1. Increased NO formation inhibits ornithine decarboxylase, the key enzyme in polyamine biosynthesis [84]. This would result in a demand for increased uptake from the environment. As increased NO formation also increases S-nitrosylation of Gpc-1, the latter acquires increased capacity for HS degradation and release of cargo. Conversely, when polyamine levels are high, NO synthesis is inhibited [85, 86], S-nitrosylation decreases and so should uptake from the environment.

Processing and modulation of Gpc are not confined to the carbohydrate portion. The Cys residues in the central domain of the core protein are probably sometimes S-nitrosylated, sometimes free and sometimes forming disulfide bonds. The latter should be common after zinc-supported, deaminative cleavage of HS (see above). Endoproteolytic cleavage of Gpc core protein has been described earlier [1, 87], giving rise to shedding of Gpc from the cell surface or to the formation of core protein subunits held together by disulfide bonding. The latter phenomenon takes place when Gpc-3 is processed by a furin-like convertase and thereby induced to participate in growth factor signalling [88].

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