Syndecans are cell surface proteoglycans with a long evolutionary history. No multicellular animal appears to be without at least one, and in mammals, there are four (1-3). These arose, in common with many other cell surface molecules, through two rounds of gene duplication at the invertebrate-chordate boundary (4). Wherever it has been examined, syndecans are always substituted with heparan sulphate chains, though some have additional galactosaminoglycan, either chondroitin or dermatan sulphate (5). Heparan sulphate is a vital carbohydrate; experiments with *C. elegans*, *Drosophila* and mice show that deletions of key polymerases involved in its synthesis are lethal (6, 7). Therefore, heparan sulphate is essential for tissue function in animals. In a sense this is unsurprising, heparan sulphate has the property of interacting with an immense array of ‘ligands’ that may be growth factors, chemokines, cytokines, extracellular matrix molecules, morphogens, clotting factors, and even some enzymes involved in lipid metabolism (8). A key question is how all these possible interactions are regulated at the cell surface. There are only two major families of cell surface proteoglycans, the transmembrane syndecans and the glypicans, with C-terminal glycosylphosphatidylinositol anchors to the outer membrane leaflet (3, 9). There are other heparan sulphate proteoglycans in the extracellular matrix, and particularly basement membranes, and these are unrelated to each other in terms of protein structure. They include perlcan, agrin and type XVIII collagen (10).

On one level, it is proposed that cell surface heparan sulphate is a mechanism to concentrate ligands on the cell surface (11), and they are well designed for that. Experiments with *Drosophila* for example, show that disruption of heparan sulphate in embryos can interfere with morphogen gradients and thereby disrupt normal differentiation and cell fate decisions (12). However, this does not answer a key point as to why both transmembrane and GPI-anchored HSPGs are present in nearly all nucleated cells. It is rare to find a cell type that has only one HSPG. Likely, the cell surface diffusion kinetics, turnover and dynamics of syndecans and the GPI-anchored glypicans are distinct, and perhaps they occupy different cell surface niches (3, 11, 12). There is evidence that syndecan entry into lipid raft domains is a regulated process. However, where undertaken, single knock-outs of syndecan or glypican genes in mice yield mild phenotypes (1, 3, 14, 15). This suggests redundancy among the cell surface proteoglycans, at least in embryonic development. Glypican-3 might be an exception, since mutations in man give rise to the rare Simpson-Golabi-Behmel overgrowth syndrome and a similar phenotype in the null mouse (16).

In recent years it has become clear that all vertebrate syndecans can link to the actin cytoskeleton, through direct interactions with actin-associated proteins (3, 17). This distinguishes them from the glypicans. Cytosplasmic interactions of invertebrate syndecans are not as well characterised. Syndecan linkage to the cytoskeleton can be a means of localisation and stability on the cell surface. Alternately, the syndecans can partake in the organisation of the cytoskeleton if they can undergo regulated signalling. In this review, the structure and function, as we understand it today, of the syndecan cytoplasmic domains is discussed. Evidence suggests that indeed syndecans can signal, and the molecular detail is now emerging.

**SYNDECAN STRUCTURE**

Syndecans are type I membrane glycoproteins, having three major domains, ectodomain, transmembrane and cytoplasmic. Originally it was thought that the protein ectodomains of syndecans had solely the function of being substituted with...
glycosaminoglycan chains. While there is not much conservation of sequence between syndecans and even between, say, syndecan-4 from different species (3, 14), in fact it is now clear that there is functional activity in the extracellular core protein. Heparan sulphate chains are usually close to the N-terminus, and there appears to be at least three in each syndecan, though the reason is not clear. Between the carbohydrate and the cell surface is a domain that, when isolated or expressed as a GST fusion protein, promotes integrin-mediated adhesion (18-20). This has been demonstrated for syndecans 1, 2 and 4. We have proposed that the interaction between syndecan-4 and integrin is indirect, and the molecular basis for the function is under investigation. In the case of syndecans 2 and 4, which make up a subfamily based on sequence homology, the integrins promoting cell adhesion belong to the $\beta_1$ class, while for syndecan-1, often present on epithelial cells, the integrins are $\beta_3$ and $\beta_5$ (19, 20). Questions still remain about this property. It is not yet understood whether this function is important when syndecans are shed from the cell surface (as they are by a variety of metalloproteases (21)), or whether the ectodomain adhesion function is constitutive, acting either in trans or in cis on a single cell. However, this is a mechanism whereby syndecans can only indirectly signal, since it is independent of contributions from the cytoplasmic domain. Interestingly, it is now also believed that the ectodomains of the glypicans also have protein-protein interaction functions (22), and that once again there is more to cell surface core proteins than being decorated by heparan sulphate.

All three major domains of syndecan core proteins have a tendency to form dimers. Of these, it is a strong self-association of the transmembrane domains that dominate, and are mostly responsible for the ability of syndecan core proteins to be SDS-resistant and resolve as dimers on SDS-PAGE (23, 24). Their GXXXG motif is the vital site for this property (24). All syndecans have very similar transmembrane domain sequences, and it has been suggested, on the basis of synthetic peptide experiments, that heterodimers are possible (24). However, as yet it has not been shown that whole syndecans can form heterodimers, and this will be an interesting, but difficult area, since it may be hard to distinguish heterodimers from hetero-oligomers.

Cytoplasmic domains have three regions, two of which are highly conserved across all syndecans, and are a hallmark of the entire class (Fig. 1). These are the membrane-proximal C1 and membrane distal C2 domains. Between them is a variable (V) region that is unique to each syndecan, yet conserved within each specific syndecan member (1, 3, 17). So, avian syndecan-2 has a V region sequence almost identical to that of mammals. Even where there are conservative changes in sequence, the structure is conserved. Syndecan-4 cytoplasmic domain is amenable to NMR spectroscopy, and forms a stable dimer of unusual characteristics (Fig. 2). It forms a twisted clamp, and some of the key residues that stabilise the structure are at either end of the V region (25). Moreover, although two residues of the zebrafish syndecan-4 are different to all mammals, the structure...
is completely conserved (Fig. 2). Both fish and mammal syndecan-4 V regions bind the membrane lipid, phosphatidylinositol 4,5 bisphosphate (PtdIns4,5P2), and on so doing undergoes a shape change, revealed by NMR spectroscopy (26). Currently it is believed that the inositol-syndecan complex then is able to bind protein kinase Cα, to form a ternary complex. The kinase is then persistently activated (2, 3, 17, 27).

BINDING PARTNERS OF SYNDECAN CYTOPLASMIC DOMAINS- PDZ DOMAIN PROTEINS

The C-terminus of all syndecans has a hydrophobic nature, and can interact with proteins containing a PDZ domain (postsynaptic density 95, discs-large, ZO-1) (28). The first of these to be identified was syntenyin (29, 30), a molecule known also as mda-9 (melanoma differentiation associated gene-9), and known to promote cell migration of some tumour cells. Syntenyin (31) contains two tandem PDZ domains, although their structures are dissimilar. While PDZ domains were believed to be involved in protein-protein interactions, it was suggested by Zimmermann et al. (32) that syntenyin PDZ1 had higher affinity for PtdIns4,5P2, while PDZ2 was the preferred domain for interaction with syndecans. All four mammalian syndecans can interact with syntenyin, not surprising since each terminates in the same EFYA sequence. This fits within the PDZ2 pocket and has been visualised by NMR spectroscopy and crystallography (Weontae Lee et al., unpublished data). The recent structural work precisely confirms the previous work performed by surface plasmon resonance spectroscopy, and shows that PI2 and syndecan-4 (in this case) bind strongly to PDZ1 and 2 respectively. However, discerning the function of syntenyin is complicated by the fact that many cell surface receptors can interact with this protein. This suggests that syntenyin may have a scaffolding function at the cell membrane that is common to many receptor types (28-30). The lipid binding function has been further dissected by Zimmermann et al. (33), and shown to be essential for trafficking of syntenyin/syndecan complexes to the cell membrane from endosomal compartments, in an Arf6-dependent manner. Whether and how syntenyin is released from syndecans at the cell surface remains to be discovered, but our data suggest that syntenyin interaction with syndecan-4 can inhibit its signalling through protein kinase Cα (unpublished data). Syntenyin has been localised to regions of cell attachment to matrix known as focal adhesions (32), in addition to other subcellular compartments. In a very recent report, syntenyin is proposed to interact with c-Src, which in complex with focal adhesion kinase may localise it to these adhesion sites and promote migration (33). However, this report should be regarded as preliminary since no direct interaction between the two molecules was shown. It also overlooks the fact that syndecan-4 is also a focal adhesion component, providing a second mechanism for focal adhesion localisation of syntenyin.

After the original finding of syntenyin as a PDZ domain partner for syndecan, others have been described. These include CASK, synbindin and GIPC/synectin (1-3, 35-37). GIPC/synectin has been shown to interact with the cytoplasmic domain of syndecan-4, but its ability to bind other syndecans is unclear. As with syntenyin, synectin is not restricted to binding a syndecan, but also interacts with unrelated receptors such as megalin and neurotrophin receptors (38). When over-expressed in endothelial cells, GIPC/synectin suppresses cell migration in a syndecan-4 dependent manner (37), yet the same outcome is also seen in cell expressing syndecan-4 mutants unable to bind syntenyin, or synectin null cells (39). This mirrors results comparing syndecan-4 over-expression with knock-out fibroblasts, where cell migration is compromised in both cases (3, 15, 40). A regulated expression of these molecules is apparently required for optimal cell migration. GIPC/synectin seems to be involved with syndecan removal from the cell surface, for which its interaction with myosin VI and the endocytic vesicle may be relevant (38). Moreover, GIPC/synectin interacts with the Rho GEF syx-1 and a recent report shows that knock-down of this protein in zebrafish compromises vascular branching (41). Similarly, GIPC/synectin knock-out mice have decreased arterial length and volume densities, together with reduced numbers of arteries and altered pattern of arterial branching (41). The venous system however, was normal. This implicates syndecan-4 and GIPC/synectin with important roles in the vascular system, yet syndecan-4 null mice do not show the range of defects seen in GIPC/synectin null mice. Potentially, other syndecans may take over in the absence of syndecan-4, but no other C2 binding protein can replace GIPC/synectin.

Synbindin is a syndecan-2 interacting protein that has not received much attention. It was identified by yeast two-hybrid assay as a neuronal protein that interacts with the C-terminal EFYA motif of syndecan-2, and appears to be involved with postsynaptic membrane trafficking (36). Syndecan-2 expression promotes dendritic spine maturation in neurons, for which the C2 domain is required (42). Together the data suggested that syndecan-2 functions in concert with synbindin to recruit intracellular vesicles to postsynaptic sites. More recent work now shows that synbindin (also known as trs23) is a component of the transport protein particle (TRAPP) 1, involved in endoplasmic reticulum-to-Golgi transport (43). The crystal structure reveals a PDZ domain most similar to syntenyin PDZ2, consistent with syndecan interaction. Interestingly, the PDZ domain is absent in the yeast trs23 homolog, indicating that its insertion is a metazoan-specific protein binding module, and correspondingly yeast have no syndecan (43). In contrast to synbindin, the Ca2+/calmodulin associated serine/threonine kinase (CASK) is receiving renewed attention as a result of some recent studies. CASK is a membrane-associated guanylate kinase (MAGUK) associated with intercellular junctions. In rat brain it was identified as a protein that binds all syndecans and neurexin (34, 44), but importantly CASK's dual life has been revealed. The C-terminal guanylate kinase domain is a pseudokinase involved in targeting to the nucleus where it interacts in neural cells with the transcription factor T-brain (TBR1) (44). Very recently human mutations of CASK have been reported that lead to X-linked brain malformation, including microcephaly and hypoplasia of the brainstem and cerebellum (45). The Tbr1 mouse mutant, and the reelin mouse mutant have similar phenotypes. Reelin is a brain extracellular matrix molecule whose expression is regulated by CASK-TBR1 (46). In addition, CASK is not restricted to the central nervous system, as it has been shown to be concentrated in nuclei of basal keratinocytes of interfollicular and follicular epidermis (47). In newborn mouse skin this is its primary location, while in adult skin CASK relocates to the cytoplasm and cell periphery. Knock-down by siRNA amplifies responses to growth factors, and accelerates keratinocyte adhesion to collagen, as well as focal adhesion assembly. The balance of CASK distribution seems to be regulated by its binding partner; over-expression of syndecan-3 leads to a predominantly cytoplasmic distribution, while increased Tbr1 has the effect of concentrating CASK in the nucleus (47). In a further twist to the syndecan-3 connection, it has been shown that the proteoglycan is a target for the presenilin/γ-secretase complex, leading to intramembrane cleavage and the loss of the cytoplasmic domain (48). In turn this leads to a reduction in membrane targeting of CASK. Therefore, a dual role of CASK at the cell surface and as
nuclear protein is apparent, the former perhaps related to interactions with syndecans.

BINDING PARTNERS OF SYNDECAN CYTOPLASMIC DOMAINS - THE C1 AND V REGIONS

The other conserved region of syndecan cytoplasmic domains is the membrane-proximal C1. It contains a cationic sequence, common to many transmembrane molecules, and at least with syndecan-2, there are interactions with ezrin, an actin-associated cytoskeletal protein (49). Presumably such interactions take place in other vertebrate and perhaps invertebrate syndecans, since the region is so highly conserved. This region of syndecan-3 has also been reported to bind c-Src, and a substrate of this kinase, cortactin (50). This is the only report of a tyrosine kinase that can be potentially activated through a syndecan, but the regulation of this interaction and the conditions under which c-Src are activated are unclear.

The central V region has provided some interesting information but also some challenges. Only with syndecan-4 has substantial information been obtained, and there is a dearth of information regarding the V regions of invertebrate syndecans, which can be quite divergent in primary sequence. The V region of syndecan-4, as stated above, binds PIP2 and also protein kinase Cα (1-3, 27, 51, 52). It is anticipated that clustering of the syndecan may drive the signalling process, as is common in cell surface receptors. Such clustering occurs, for example, when syndecan-4 incorporates into focal adhesions. Recent work from our laboratory suggests that one substrate of the PKCα is RhoGDIα, which is phosphorylated on serine 34 (Dovas et al., unpublished data). As a result its affinity for GDP-RhoA decreases, allowing the GTPase to become activated by one or more GEFs. Levels of GTP-RhoA then rise, commensurate with actin microfilament bundle contraction and focal adhesion assembly (Fig. 3). Other work suggests that syndecan-4 can act in concert with integrin to regulate p190RhoGAP (53, 54). It is known that this GAP becomes tyrosine phosphorylated in the early stages of cell adhesion (55), and this is integrin-dependent. Syndecan-4 appears to contribute by controlling the distribution of the GAP, in a process that is PKC-dependent, although the substrate in this case is unknown. This also would lead to directed increases in GTP-RhoA. At the same time, further work from the Humphries group implicates syndecan-4 with a regulation of Rac GTase, which when activated can promote ruffling, protrusion and migration. Work with syndecan-4 null fibroblasts suggests that GTP-Rac1 levels are considerably elevated, and that persistence of cell migration is compromised (53). Clearly the connections between GTases and syndecan-4 have further to go, but can be of importance in wound repair, where cell migration is impaired, as shown by slower granulation tissue angiogenesis in the syndecan-4 null mouse (15). There is increasing evidence that syndecan-4 has important roles in vascular responses to injury, though not in development, since in the mouse there is no obvious phenotype. This contrasts to zebrafish where syndecan-4 knock-down compromises neural crest migration (56), and Xenopus where syndecan-4 is required for convergence and extension movements (57).

A notable phenotype of syndecan-4 null cells is a lack of stress fibre incorporation of α smooth muscle actin (26, 58, 59). This contrasts to the wild type equivalent cells, and can be restored in the null cells by syndecan-4 cDNA. In all probability the reason is that the small focal adhesions/contacts formed by the null cells cannot exert sufficient tension on the substrate. This is known to be a requirement for α smooth muscle actin incorporation into stress fibers (60), and indicates a potential importance for the proteoglycan in wound repair. Additionally, it supports the notion that syndecan-4 contributes to focal adhesion assembly, consistent with its localisation to these sites (3, 53, 61). Other work has shown that null fibroblasts spread on the integrin-binding central portion of fibronectin cannot respond to addition of the more C-terminal HepII domain of fibronectin, which in normal cells leads to focal adhesion assembly (3, 62). A detailed examination of cytoskeletal organisation in null cells may be highly informative.

Besides PKCα, there are two other interacting partners of the syndecan-4 cytoplasmic domain. Syndesmos emerged from a yeast 2-hybrid screen, a 40kD protein that binds a combination of V and C1 regions, and consistent with this, is reported to be syndecan-4 specific (63). Its functions remain largely unknown, beside a further interaction with the focal adhesion component, paxillin and the related Hic5 (63). How this may contribute to focal adhesion or turnover is not known. A very recent report suggests that the Xenopus protein Nudt16 is a closely related paralogous protein (64). This protein is involved in nuclear RNA decapping, a property not shared with syndesmos. However, syndesmos does retain RNA binding ability, but whether this is a functional attribute in vivo is unclear.

The final protein interacting with syndecan-4 V region is the actin-bundling protein α-actinin (65, 66). This provides a second direct link to the cytoskeleton, but the site of interaction in α-actinin has not been identified. It is not clear either what precise role this interaction has. It may be regulated by phosphorylation of the syndecan, since it has been suggested that phosphorylation of the single serine residue of syndecan-4 cytoplasmic domain (at the C1-V junction) increases the affinity of the interaction (67). The kinase may be protein kinase Cδ (68). We have established that there is a substantial shape change of syndecan-4 cytoplasmic domain when it is phosphorylated, consistent with a sharply decreased affinity for PIP2 and PKCα sharply decreased (69). This suggests that PKCα and α-actinin may be alternate binding partners for syndecan-4. However, if that the interaction between α-actinin and syndecan-4 is dependent on the latter's phosphorylation then it is presumably transient rather than stable, since most phosphorylation events are concerned with information relay and amplification. This area certainly deserves more attention.

V REGION INTERACTIONS: STILL A WAY TO GO

Syndecan-2 is also implicated in zebrafish vascular biology, since knock-down of expression leads to defective
branching morphogenesis (70). In Xenopus, syndecan-2 appears to regulate left-right asymmetry, for example of the heart looping. In this case a role for protein kinase Cγ was shown (71), but whether this kinase directly associates with the V region of syndecan-2 cytoplasmic domain is not known. Indeed binding partners for the V regions of all syndecans except syndecan-4 have been difficult to come by, perhaps in part because a dimeric structure is required which is hard to replicate in yeast 2-hybrid experiments, for example. There are suggestions that the cytoplasmic domain of syndecan-2 signals through protein kinase A (72), in which case there is a common theme of serine/threonine kinase associations with syndecans. No interacting partners of the syndecan-1 or -3 V region have been identified, and there is a similar dearth of syndecans. No interacting partners of the syndecan-1 or -3 V region have been identified, and there is a similar dearth of information from the invertebrates. Genetic experiments reveal that the syndecan of invertebrates is a regulator of axonal growth and targeting (73, 74). However, not a single V region interaction has been identified, but is surely an interesting prospect for the future.

INDIRECT SIGNALING THROUGH ACCESSORY MEMBRANE RECEPTORS

There is considerable evidence that syndecans co-operate with other receptors to mediate effective signalling. Prime examples for vertebrate syndecans are the fibroblast growth factor receptors, the frizzled receptors for wnt proteins (75), receptors for hedgehog family members (76) and transforming growth factor-β receptors (77). However, this is probably not a syndecan-specific function, since evidence supports similar roles for glypicans that lack a cytoplasmic domain. For Shh and Indian hedgehog, important in skeletal development, another proteoglycan of interest may be perlecan (9). The details are still mostly sketchy, but much effort has been placed on studying the fibroblast growth factor/receptor/heparan sulphate ternary complex, that enhances signalling and has been reviewed extensively (1, 78). While it is suggested that syndecan signalling contributes to FGF regulation of cell behaviour, it is equally the case that the frequent involvement of glypicans suggests that the regulation may depend mostly on heparan sulphate modification of growth factor interactions and clustering of the high affinity tyrosine kinase receptors. Vertebrate syndecans, but apparently not invertebrate syndecans, can influence integrin mediated cell adhesion. This may derive from one or more of three sources, and so far no glypican has been shown to regulate this process. First, heparan sulphate can interact with many extracellular matrix glycoproteins and collagens, at distinct sites from integrins (1, 3). This may cause clustering events of both syndecans and integrins. Second, while undoubtedly the case that integrin signalling can trigger many networks, including focal adhesion kinase, src, MAP kinases etc, syndecans may independently contribute, either by signalling themselves (e.g. PKCα from syndecan-4) or by providing cytoskeletal linkage, such as α-actinin (27, 51, 52, 65). Integrins are well known to interact with the actin associated proteins talin and kindlin, as well as α-actinin. Third, the syndecan extracellular domain separately triggers integrin-mediated signalling but the basis is unclear (18-20). Probably it is indirect, and our preliminary data suggest a role for one or more tyrosine phosphatases.

Drosophila integrin also interacts with a transmembrane tyrosine phosphate, LAR, which interacts with heparan sulphate (79). This may be a very important facet of syndecan biology, since LAR-heparan sulphate interactions promote neuromuscular junction growth and active zone morphogenesis. Interestingly while syndecan promotes LAR function, the glypican (Dallylike protein) inhibits it (80). In addition, syndecan is a receptor for slit ligand, and is required for slit-repellent signalling at the midline of the CNS in development (81). Here, however, there is an apparent functional redundancy with Dallylike, suggesting that heparan sulphate is the key common denominator that controls slit-robo signalling (82). All this only emphasises the lack of information on invertebrate syndecan signalling. It is a common theme that invertebrate syndecans are neural regulators, perhaps a clue to their ancestral roles. In C. elegans, it is required for egg laying (83) and also for neuronal pathfinding. Again slit-robo signalling is a target for syndecan regulation in this invertebrate (84).

PERSPECTIVE

 Syndecans have a long evolutionary history, and originally may have had roles restricted to neural development. The function involves a combination of morphogen sensing and regulation of cell migration. In mammals this function is still seen in syndecan-3 at least. Gene duplications of vertebrates have led to a wider range of functions, but some of these also relate to cell migration. The cytoplasmic motifs of all syndecans still need more analysis, since they are the hallmark of all family members, and yet little is known of their roles, with the possible exception of syndecan-4. Potential redundancy among the syndecan core proteins is not understood. Teleost fish have secondarily lost syndecan-1 (4) and knock-down of syndecans-2 and -4 have phenotypes in development. In mice, however, syndecan-1 and -4 nulls have no obvious developmental phenotype, but more importance in postnatal tissue repair. There is still much to learn, including whether in vivo, the heparan sulphates of syndecans and glypicans differ in fine structure and interactions, and to what extent they regulate distinct microdomains on the cell surface.

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