The Cytoplasmic Domain of Syndecan-1 Is Required for Cytoskeleton Association but Not Detergent Insolubility

IDENTIFICATION OF ESSENTIAL CYTOPLASMIC DOMAIN RESIDUES*

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David J. Carey†, Katharine M. Bendt, and Richard C. Stahl
From the Sigfried and Janet Weis Center for Research, Geisinger Clinic, Danville, Pennsylvania 17822

Syndecan-1 is a member of a gene family of multifunctional transmembrane heparan sulfate proteoglycans that bind a variety of extracellular ligands and possess highly conserved non-catalytic cytoplasmic domains. It has been shown that antibody-mediated clustering of syndecan-1 causes the proteoglycan to become associated with microfilaments and insoluble in non-ionic detergent. A series of truncation and point mutations of the syndecan-1 core protein was constructed to identify specific structural features that were required for these characteristics. The transmembrane domain but not the cytoplasmic domain was required for cell surface expression of syndecan-1. Deletion of the COOH-terminal 11 amino acids of the cytoplasmic domain had no effect, while deletion of an additional 12 amino acids abolished microfilament association. Mutation of a conserved tyrosine residue within the latter region also abolished microfilament association. In contrast, mutation of 2 tyrosine residues outside this region had no effect. Deletion of the entire cytoplasmic domain (except for a short stop-transfer sequence) did not affect insolubility of the proteoglycan in detergent. Analysis of a form of syndecan-1 that lacked glycosaminoglycan acceptor sites revealed that covalently attached glycosaminoglycans were not required for cell surface expression, microfilament association, or detergent insolubility. These results demonstrate that microfilament association is a function of a subregion within the cytoplasmic domain and suggest that insolubility in detergent is a function of the transmembrane domain.

The syndecans are a gene family of transmembrane heparan sulfate proteoglycans. Syndecans have been proposed to play an important role in tissue morphogenesis by virtue of their ability to bind, via their covalently attached glycosaminoglycan chains, to a variety of extracellular adhesive molecules including fibronectin, thrombospondin, various collagens, and heparin-binding growth-associated molecule (1–3) and growth factors such as basic fibroblast growth factor (4–6). Although nearly all vertebrate cells express at least one of the four known syndecan types (7), the pattern of expression of individual syndecans is highly regulated in both cell type and development specific patterns (8, 9).

The syndecan family core proteins are type I transmembrane proteins with cleavable NH2-terminal signal peptides, extracellular domains containing the glycosaminoglycan acceptor sites, single hydrophobic membrane-spanning domains, and short, non-catalytic COOH-terminal cytoplasmic domains (1, 2). The most highly conserved regions of the syndecan core proteins are the transmembrane and cytoplasmic domains. Overall, these regions of the four mammalian syndecans display >50% amino acid identity. The conservation of the transmembrane and cytoplasmic domain sequences has been maintained during evolution, as revealed by the sequence of a Drosophila syndecan core protein (10). The sequence conservation of the transmembrane and cytoplasmic domains is in striking contrast to the extracellular domains, which vary in their length and in the number and placement of covalently attached glycosaminoglycan chains, and show only limited amino acid sequence similarity among syndecan types.

This modular structural design suggests that individual mammalian syndecans have evolved to carry out specific functions within tissues where they are expressed, most likely related to the nature of their interactions with extracellular ligands. This conjecture is supported by the highly regulated patterns of expression of specific syndecan types. On the other hand, the structural conservation of the transmembrane and cytoplasmic domains strongly suggests that they play a role in an essential intracellular function that may be common to all the syndecans. There is evidence to suggest that this may be related to their ability to associate with and reorganize cytoskeletal structures (11–13).

This aspect of syndecan function has been examined by analysis of rat Schwann cells that have been transfected to stably express syndecan-1. Expression of syndecan-1 results in enhanced spreading of the cells on various adhesive substrata and a marked reorganization of cytoskeletal structures, including microfilaments (12). This occurs despite the lack of an apparent stable association between cell surface syndecan-1 and actin filament or focal adhesions. A transient colocalization of cell surface syndecan-1 and nascent microfilaments is observed during the spreading of these cells but is lost when the cells form stable microfilament bundles. An apparent reassociation of syndecan-1 with microfilaments can be produced by cross-linking syndecan-1 molecules on the cell surface with antibodies (13).

In this paper, additional data on the nature of the association of syndecan-1 with microfilaments are presented. Using antibody-induced microfilament colocalization as an assay, this activity has been localized to a specific region within the syndecan cytoplasmic domain and has been shown to require a conserved cytoplasmic tyrosine residue. The data also demonstrate that insolubility of the protein in Triton X-100, which is often interpreted as an indication of cytoskeleton association, does not require the cytoplasmic domain.
MATERIALS AND METHODS

Cell Culture—Schwann cells were cultured from neonatal rat sciatic nerves as described previously (14). For routine culture, the cells were grown on poly-L-lysine-coated tissue culture plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 2 μM forskolin. Media and serum were from Life Technologies, Inc. All other tissue culture reagents were purchased from Sigma.

Vector Construction and Transfection—The preparation of a mammalian expression plasmid coding for full-length rat syndecan-1 and the preparation and characterization of Schwann cells stably transfected to express syndecan-1 have been described previously (12). For the present study, Schwann cells that stably expressed mutant forms of the syndecan-1 core protein were also prepared. These included a set of nested truncations of the cytoplasmic domain plus a truncation that resulted in the synthesis of the extracellular domain without the transmembrane or cytoplasmic domains. These expression constructs were prepared by polymerase chain reaction amplification using the wild type expression plasmid as a template and sense and antisense primers that flanked the regions of interest (see “Results” for specific truncations). The antisense primers contained an in frame stop codon immediately following the terminal amino acid codon. These cDNAs were subcloned into the expression plasmid pCMVneo as described previously (12). Specific point mutations were introduced into the core protein sequence using the unique restriction site elimination method, as carried out with the Transformam site-directed mutagenesis kit (Clontech). These included mutant forms in which individual cytoplasmic tyrosine residues were changed to phenylalanine. An additional mutant form of syndecan-1 was prepared in which all five of the extracellular glycosaminoglycan acceptor site serine residues were changed to alanines (GAG-less). The sequence of the mutant cDNAs was confirmed by DNA sequence analysis using the dye deoxy chain termination method and modified T7 DNA polymerase (Sequenase, U.S. Biochemical Corp.).

These expression constructs were transfected into Schwann cells with the aid of Transfectam (Promega). Stably expressing cells were selected by growth in medium containing G418, exactly as we have described earlier. For the experiments described in this paper, stably expressing populations of cells were used. At least two separate populations of stably expressing cells were analyzed for the wild type and each mutant core protein. Expression of the proteins was monitored by immunoblot and immunofluorescence microscopy as described previously (12). In every case, a high percentage (>90%) of syndecan-1 positive cells was observed.

Characterization of Proteoglycans—Cell surface expression of the syndecan-1 constructs was determined by staining live, unfixed cells (14). The glycosaminoglycan composition of the expressed proteoglycans was determined by digestion of the proteins with purified bacterial heparinase (type A, Seikagaku America, Inc.) and heparitinase ABC (Sekagaku America, Inc.) under conditions described previously (6). The digestion products were analyzed by immunoblot analysis on 7.5% polyacrylamide gels and stained with anti-syndecan-1 antibodies with detection by enhanced chemiluminescence. Signals were quantitated using a scanning laser densitometer (Molecular Dynamics). Collagen type I binding activity was determined by affinity chromatography. Purified collagen type I, obtained by acetic acid extraction of rat tail tendons, was coupled to cyanogen bromide-activated Sepharose. Expressed syndecan-1 molecules were purified by high pressure liquid chromatography on an anion exchange chromatography on a DEAE-SPW column (Beckman Instruments) that was eluted with a linear gradient of 0–1 M NaCl. The syndecan-1 molecules, which were eluted at a NaCl concentration of approximately 0.6–0.7 M, were identified by immunoblot analysis. Syndecan-1-containing fractions were pooled and dialyzed against 0.14 M NaCl, 10 mM Tris-HCl, pH 7.5 (column buffer). Aliquots of syndecan-1 were diluted to 1 ml with column buffer and applied to a 0.5-ml column of collagen type I-Sepharose equilibrated with column buffer. The column was washed twice with column buffer (1 ml each) followed sequentially by buffers consisting of 0.21, 0.32, 0.48, and 0.79 M NaCl in 10 mM Tris-HCl, pH 7.5 (1 ml each) until no additional protein was detectable in the column. The fractions were subjected to immunoblot analysis with anti-syndecan-1 antibodies. Syndecan-1 binding to collagen type I was also measured by affinity co-electrophoresis (19). Anti-syndecan-1 antibodies were produced in rabbits using recombinant rat syndecan-1 ectodomain as the antigen and were affinity purified prior to use. These antibodies have been described previously (15).

Cytoskeleton Association Assay—Antibody-mediated cytoskeleton association of cell surface syndecan-1 was assayed by immunofluorescence microscopy as described previously (13). The cells were grown on polycl-lysine-coated glass slide chamber (Lab-Tek, Thomas Scientific). In the standard assay, the cells were incubated with anti-syndecan-1 antibodies on ice for 30 min and then incubated in medium containing affinity-purified goat anti-rabbit IgG antibodies conjugated to either rhodamine or fluorescein (Sigma) for 20 min at 37 °C. After removing the antibody solution, the cells were fixed with 3% paraformaldehyde. Control cells for syndecan-1 clustering were fixed with paraformaldehyde before addition of the secondary antibodies. Actin filaments were visualized by staining with phalloidin conjugated to either fluorescein or rhodamine (Molecular Probes, Inc.). In some experiments, following antibody-mediated clustering of syndecan-1, the cells were extracted with 1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 5 mM MgCl2, 2 mM EGTA for 2 min at room temperature. The distribution of syndecan-1 and actin filaments in the cells was visualized by fluorescence light microscopy.

RESULTS

Antibody-mediated Microfilament Association of Syndecan-1 Is Temperature-dependent—As was described previously, when syndecan-1 expressing Schwann cells are incubated with antisyndecan-1 antibodies at 37 °C (see “Materials and Methods”), the cell surface syndecan-1 molecules become organized into a punctate filamentous pattern that co-aligns with a subset of microfilaments (13) (see Fig. 1). Evidence has been presented that this filamentous pattern results from an association of syndecan-1 molecules with microfilaments (13). As shown in Fig. 1, when the cells were incubated with the antibodies at 4 °C, the rearrangement of cell surface syndecan-1 molecules into filament-like strands was almost totally inhibited. This observation demonstrates that the organization of cell surface syndecan-1 into filaments, which reflects an association of the proteoglycan core protein with microfilaments, is an active process and is not due to nonspecific entrapment of cross-linked syndecan molecules as they diffuse within the membrane.

The Cytoplasmic Domain Is Not Required for Cell Surface Expression or Glycanation of Syndecan-1—To determine whether specific cytoplasmic domain sequences and/or the glycosaminoglycan chains of syndecan-1 were required for the association of the proteoglycan with microfilaments, a series of syndecan-1 molecules with specific truncation and point mutations was expressed and analyzed. A diagram summarizing the mutant constructs used is shown in Fig. 2. Immunoblot analysis revealed that the mutant proteins were synthesized by stably transfected Schwann cells and that all but one of the mutant syndecans migrated as high molecular weight smears on SDS-polyacrylamide gels, characteristic of glycanated proteoglycans (Fig. 3). The exception was the GAG-less mutant (described below).

Because one of the main goals of this study was to characterize syndecans with mutations in the cytoplasmic domain, it was necessary to determine whether the cytoplasmic domain played a role in the cell surface expression or glycanation of syndecan-1. The construct Δ3, which lacked the entire cytoplasmic domain except for a 4-amino acid basic sequence that was retained to provide a stop-transfer sequence for proper membrane insertion, was expressed on the cell surface of stably transfected Schwann cells (Fig. 4). Its distribution was indistinguishable from that of wild type syndecan-1. In contrast, the cytoplasmic construct, which lacked the transmembrane and cytoplasmic domains (see Fig. 2), was not detectable on the surface of transfected Schwann cells (Fig. 4). A glycanated form of this protein was detected in conditioned medium of stably transfected Schwann cells (Fig. 3, lane 8). These results demonstrate that the transmembrane domain but not the cytoplasmic domain is required for cell surface expression of the proteoglycan.

Immunoblot analysis revealed that construct Δ3 was processed by the cells to a high molecular weight proteoglycan form (Fig. 3, lane 4), demonstrating that the cytoplasmic domain was not required for glycanation of syndecan-1. Synde-
can-1 is a hybrid proteoglycan in most cells and contains both heparansulfate and chondroitinsulfate (16–18). To determine whether the presence of the cytoplasmic domain influenced the type of glycosaminoglycan added, the glycosaminoglycan composition of wild type syndecan-1 and construct D3 was analyzed by specific enzyme digestion and immunoblot analysis. As expected, digestion of wild type syndecan-1 with heparitinase resulted in a large reduction in apparent molecular weight so that the protein migrated as a broad smear that was somewhat larger in size than the core protein (Fig. 5A). Digestion with chondroitinase ABC produced a barely perceptible shift in the migration of the proteoglycan. Digestion with both enzymes resulted in the nearly complete conversion of the proteoglycan to a band with an apparent molecular mass of about 70 kDa.

**Fig. 2.** Syndecan-1 mutant constructs. Syndecan-1 truncation and point mutants used in the study are illustrated. The top section illustrates the full-length wild type (wt) core protein that includes a signal peptide (dark hatch), extracellular domain (open), transmembrane domain (filled), and COOH-terminal cytoplasmic domain (light hatched). Solid vertical lines indicate positions of glycosaminoglycan attachment sites. Also illustrated are the truncation mutant that lacks the transmembrane and cytoplasmic domains (ecto) and the construct that contains point mutations that render the glycosaminoglycan attachment sites inactive (GAG-less). In the lower section, the wild type cytoplasmic domain sequence is shown, along with the three COOH-terminal truncations (Δ1–3) indicated by the horizontal lines. Also shown are three site-directed mutants in which single tyrosine (Y) residues were changed to phenylalanine (F) residues at the positions indicated. Filled circles above the wild type sequence indicate the positions of the conserved cytoplasmic tyrosine residues.

**Fig. 3.** Immunoblot analysis of wild type and mutant syndecan-1 molecules synthesized by stably transfected Schwann cells. Proteins from conditioned media of stably transfected Schwann cells were detected by immunoblot analysis with affinity-purified anti-syndecan-1 antibodies. See Fig. 2 for a description of the constructs. Lane 1, wild type; lane 2, construct Δ1; lane 3, construct Δ2; lane 4, construct Δ3; lane 5, construct Y2F; lane 6, construct Y3F; lane 7, construct Y4F; lane 8, ecto; lane 9, GAG-less; lane 10, medium from control vector-transfected Schwann cells. Numbers to the left indicate positions of migration of molecular mass markers (in kDa).

**Fig. 1.** Antibody-mediated association of cell surface syndecan-1 with microfilaments is temperature-dependent. Syndecan-1 expressing Schwann cells were subjected to antibody-mediated clustering of cell surface syndecan-1 as described under “Materials and Methods.” The cultures were incubated with the secondary antibodies at 4 or 37 °C: a, control cells incubated at 4 °C; b, antibody-clustered cells incubated at 4 °C; c, control cells incubated at 37 °C; d–f, antibody-clustered cells incubated at 37 °C. The micrographs in panels a–d and f show the pattern of cell surface anti-syndecan-1 immunofluorescence; panel e shows phalloidin staining of the same area shown in panel f. Bars = 50 μm.
...results were obtained with construct Δ3. As shown in Fig. 5B, wild type syndecan-1 binding to the affinity column could be demonstrated. A portion of the syndecan-1 that was initially bound to the column was eluted in the wash buffer. The remainder was eluted by step increases in the NaCl concentration of the elution buffer. A virtually identical pattern of binding and elution was observed with construct Δ3 (Fig. 5B). When binding to collagen type I was assayed by affinity co-electrophoresis, dissociation constants of 6 and 9 nM were determined for wild type syndecan-1 and construct Δ3 (not shown). Together, these results demonstrate that the cytoplasmic domain has no apparent effect on cell surface expression, glycosaminoglycan composition, or collagen type I binding activity of syndecan-1 synthesized by Schwann cells.

To investigate the role of the extracellular glycosaminoglycan chains, a construct (GAG-less, see Fig. 2) in which all 5 of the serine residues that were within potential glycosaminoglycan acceptor sites (i.e. Ser-Gly) were mutated to alanine residues was expressed. As expected, the expressed protein was not modified by glycanation, as indicated by its migration on SDS-polyacrylamide gels (Fig. 3). Indirect immunofluorescent staining of Schwann cells that stably expressed the GAG-less construct revealed that the protein was expressed on the cell surface (Fig. 4). These results demonstrate that the glycosaminoglycan chains are not required for stable expression on the Schwann cell surface.

Microfilament Association but Not Triton X-100 Insolubility Requires the Cytoplasmic Domain of Syndecan-1—The ability of wild type syndecan-1 and construct Δ3 to associate with microfilaments following antibody-induced clustering of the proteoglycans on the cell surface was determined. A consequence of microfilament association for many transmembrane proteins is their insolubility in non-ionic detergents. It was shown previously that antibody cross-linked syndecan-1 is insoluble in buffer containing Triton X-100 (13). It has also been shown that at low pH levels, syndecan-1 forms complexes that are also resistant to detergent extraction but that this property does not require the cytoplasmic domain (20). Thus, the detergent solubility of wild type syndecan-1 and construct Δ3 after antibody-induced clustering was also determined.

Antibody cross-linking of construct Δ3 caused it to form punctate clusters on the cell surface, but these did not align into a filamentous pattern (Fig. 6). These clusters of immuno-
reactions were still present after extraction of the cells with buffer containing 1% Triton X-100 (Fig. 6) and formed an apparently random pattern. This was in contrast to the striking filamentous pattern that was observed when cells expressing full-length wild-type syndecan-1 were subjected to antibody cross-linking and detergent extraction (Fig. 6). These results demonstrate that the cytoplasmic domain is needed for microfilament association under these conditions but not detergent insolubility. Thus, these appear to be distinct characteristics that are dependent on different structural features of the proteoglycan.

Identification of Structural Features within the Cytoplasmic Domain Required for Microfilament Association—To identify structural features within the syndecan-1 cytoplasmic domain that were required for association with microfilaments, Schwann cells that stably expressed syndecan-1 molecules with additional truncation or point mutations in the cytoplasmic domain were prepared. These are illustrated schematically in Fig. 2.

When cells expressing construct Δ1, which lacked the COOH-terminal 11 amino acids of the core protein, were subjected to antibody-mediated cross-linking, these syndecan-1 molecules associated with microfilaments, as indicated by alignment into filaments, as effectively as the full-length syndecan-1 molecules (Fig. 7). In contrast, deletion of an additional 12 amino acids (construct Δ2) abolished the ability of the protein to associate with microfilaments (Fig. 7). Deletion of this region has no effect on insolubility in Triton X-100. The construct Δ2 molecules were indistinguishable from construct Δ3, which lacked nearly the entire cytoplasmic domain.

These data suggest that the ability to associate with microfilaments is dependent on amino acids located within the central third of the cytoplasmic domain. This region contains 1 of the 4 tyrosine residues that are conserved in all syndecan sequences. To determine whether this tyrosine residue was critical for this functional activity, syndecan-1 constructs, in which the second (Y2F), third (Y3F), or the fourth (Y4F) tyrosine residue of the cytoplasmic domain was changed to phenylalanine (see Fig. 2), were stably expressed. The tyrosine residue mutated in the Y3F construct was within the region that by deletion analysis was found to be required for microfilament association. The tyrosine residue mutated in the Y4F construct was within the region that could be deleted with no apparent effect on this activity.

FIG. 6. The cytoplasmic domain of syndecan-1 is required for antibody-induced microfilament association but not detergent insolubility. Schwann cells expressing either full-length wild-type syndecan-1 (panels a and b) or syndecan-1 lacking the cytoplasmic domain (construct Δ3, panels c and d) were subjected to antibody-mediated clustering of cell surface syndecan-1. The cultures shown in panels b and d were extracted with buffer containing 1% Triton X-100 following the antibody treatment as described under “Materials and Methods.” The micrographs show the pattern of cell surface syndecan-1 immunofluorescence. Bars = 50 μm.

When cells expressing these proteoglycans were subjected to antibody-mediated cross-linking, both Y4F and Y2F syndecan-1 molecules exhibited microfilament association in a manner that was indistinguishable from full-length wild-type syndecan-1 (Fig. 8), and the proteoglycans were insoluble in Triton X-100. In contrast, microfilament association of Y3F syndecan-1 was severely impaired, as indicated by its failure to produce a filamentous pattern (Fig. 8). Insolubility in Triton X-100 was not affected. These data strongly suggest that this cytoplasmic domain tyrosine residue is essential for microfilament association of syndecan-1.

Glycosaminoglycan Chains Are Not Required for Microfilament Association or Detergent Insolubility of Syndecan-1—To investigate the role of glycosaminoglycan chains in antibody-induced microfilament association and detergent insolubility, the GAG-less syndecan-1 construct (see Fig. 2) was analyzed. When cells expressing this protein were subjected to antibody-mediated cross-linking, the GAG-less protein assumed a punctate fibrillar pattern, indicating association with microfilaments. This pattern was still apparent after extraction of the cells with Triton X-100 (Fig. 9). These results demonstrate that neither microfilament association nor detergent insolubility is dependent on the presence of the glycosaminoglycan chains of the proteoglycan.

DISCUSSION

The findings presented here extend the earlier observations on the association of syndecan-1 with the cytoskeleton and...
This was based on the observation that addition of heparin or mediated by the heparan sulfate chains of the proteoglycan. 

The investigators concluded, however, that detergent insolubility needed for glycanation of the core protein. 

A somewhat surprising finding was that, whereas the cytoplasmic domain was required for microfilament association, this domain was not needed for detergent insolubility of the proteoglycan. Thus, detergent insolubility is not a direct consequence of association of syndecan-1 with the cytoskeleton. The transmembrane domain was found to be essential for stable association of the proteoglycan with the plasma membrane, as indicated by the lack of cell surface staining of cells expressing syndecan-1 molecules that contained only the extracellular domain. Neither the cytoplasmic nor the transmembrane domains were needed for glycanation of the core protein.

These findings agree in part with the conclusions reached by Miettinen and Jalkanen (20), who also found that the cytoplasmic domain of syndecan-1 was not required for association of the proteoglycan with Triton X-100 insoluble material. These investigators concluded, however, that detergent insolubility was caused by the binding of syndecan-1 to other cell surface or extracellular matrix molecules through interactions that were mediated by the heparan sulfate chains of the proteoglycan. This was based on the observation that addition of heparin or heparan sulfate to the extraction buffer, or treatment of cells with heparan sulfate degrading enzymes, caused syndecan-1 to be released into the detergent-soluble fraction. This pool of proteoglycans may correspond to syndecan-1 molecules, which can be identified by immunoblot analysis, that are solubilized by extraction with phytic acid or heparin. These appear to result from proteoglycans that are shed from the cell surface and associate with the culture substratum or extracellular matrix via interactions that utilize the heparan sulfate chains. This conclusion is supported by the observation that this form of proteoglycan is observed with the syndecan-1 ecto construct that is secreted directly into the culture medium but not with the GAG-less construct. This pool of syndecan-1 molecules is clearly distinct from the proteoglycans we observed by immunofluorescence microscopy on the cell surface. As shown by analysis of the various syndecan-1 mutants, the presence of these proteoglycans on the cell surface is dependent on the transmembrane domain but not the cytoplasmic domain or glycosaminoglycan chains. This finding is consistent with the recent report that the transmembrane domain but not the cytoplasmic domain of CD44 is required for detergent insolubility in fibroblasts (21). These authors concluded that detergent insolubility of CD44 does not result from association with the cytoskeleton but from interactions with detergent-insoluble plasma membrane lipids mediated by the transmembrane domain.

The association of syndecan-1 with microfilaments was abolished when a 12-amino acid segment from the central region of the cytoplasmic domain was deleted. A comparison of the amino acid sequences of the four rat syndecans reveals that this is the most variable part of the otherwise highly conserved cytoplasmic domain sequences (Fig. 10). Interestingly, the core protein that diverges most from the syndecan-1 sequence in this region is syndecan-4. This syndecan may associate differently with cytoskeletal components, based on the fact that it is the only syndecan that has been reported to be associated with focal adhesions (22). As was reported previously, syndecan-1 expressed in Schwann cells is not present in focal adhesions and may, in fact, be excluded from these structures (12).

The observation that mutation of the conserved tyrosine residue that is located within this segment to phenylalanine abolished microfilament association suggests that tyrosine phosphorylation may be required for microfilament association. A number of cytoplasmic proteins that contain conserved phosphotyrosine binding domains have been identified (23, 24). As yet, however, there is no direct evidence that tyrosine phosphorylation is required for any functional activity of a syndecan. Preliminary results indicate that syndecan-1 core protein can be immunoprecipitated by anti-phosphotyrosine antibodies; the site of phosphorylation and a correlation between phosphorylation state and microfilament association remain to be determined. Indirect support for the idea that tyrosine phosphorylation is involved is the additional finding that treatment of Schwann cells with the tyrosine kinase inhibitor herbimycin A prevents the association of syndecan-1 with microfilaments. However, this treatment produces additional changes in microfilament distribution that are independent of syndecan-1. Thus, it is not possible to conclude from these results that inhibition of phosphorylation of syndecan-1 is responsible for the observed lack of microfilament association. The regular spacing of cytoplasmic tyrosine residues that is conserved among all syndecan sequences is strikingly similar to an activation motif that has been described in T cell antigen receptor subunits (25). These receptors possess short, non-catalytic do-

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1. D. J. Carey, R. C. Stahl and K. M. Bendt, unpublished results.
The pattern of distribution of cell surface anti-syndecan-1 immunofluorescence.

TritonX-100; cells that also bind to the cytoplasmic domains of syndecans. The existence of actin-binding proteins. The clustering of the proteoglycan is mediated by intermediates such as cadherins and integrins (29, 30), it is possible that syndecan binding to microfilaments is mediated by intermediate actin-binding proteins. The existence of actin-binding proteins that also bind to the cytoplasmic domains of syndecans has not been demonstrated as yet. The localized tethering of actin filaments to the plasma membrane would cause a redistribution of microfilaments and actin-binding proteins, with corresponding changes in cellular structure and functional activity.

This model also provides a potential explanation for the functional significance of the spontaneous membrane shedding of syndecan ectodomains that has been demonstrated to occur for all syndecans (7). This shedding results from proteolytic cleavage of the core protein ectodomain at a site near the membrane attachment site (31). This would result in the separation of the extracellular ligand binding domain from the rest of the core protein and cause the disassembly of the transmembrane proteoglycan-cytoskeleton complexes, thus providing a mechanism for terminating this functional activity.

Fig. 9. Syndecan-1 glycosaminoglycan chains are not required for antibody-induced microfilament association or detergent insolubility. Schwann cells stably expressing the syndecan-1 GAG-less construct were subjected to antibody-mediated syndecan-1 clustering and detergent extraction as described under “Materials and Methods.” Panel a, control cells; b, control cells after extraction with buffer containing 1% Triton X-100; c, antibody-clustered cells; d, antibody-clustered cells after extraction with buffer containing 1% Triton X-100. The micrographs show the pattern of distribution of cell surface anti-syndecan-1 immunofluorescence. Bar = 50 μm.

Fig. 10. Comparison of cytoplasmic domain amino acid sequences of rat syndecans. The deduced amino acid sequences of the four rat syndecan core proteins are shown. Gaps have been introduced (-) to maximize alignments of identical residues. Syndecan (syn)-1 and syn-3 appear to form a subfamily, based on a higher degree of sequence conservation. The boxed residues indicate the region that when deleted abolished microfilament association of syndecan-1. Note that most of these residues are not conserved in the syn-4 cytoplasmic domain, which appears to have a different mode of cytoskeleton association. The filled arrowhead indicates the position of the tyrosine residue that, when mutated to a phenylalanine, abolished microfilament association. Open arrows indicate tyrosines that could be mutated with no effect on microfilament association.
Syndecan-1-Microfilament Association

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