Syndecan-1 Mediates Cell Spreading in Transfected Human Lymphoblastoid (Raji) Cells

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Abstract. Syndecan-1 is a cell surface proteoglycan containing a highly conserved transmembrane and cytoplasmic domain, and an extracellular domain bearing heparan sulfate glycosaminoglycans. Through these domains, syndecan-1 is proposed to have roles in growth factor action, extracellular matrix adhesion, and cytoskeletal organization that controls cell morphology. To study the role of syndecan-1 in cell adhesion and cytoskeleton reorganization, mouse syndecan-1 cDNA was transfected into human Raji cells, a lymphoblastoid cell line that grows as suspended cells and exhibits little or no endogenous cell surface heparan sulfate. High expressing transfectants (Raji-S1 cells) bind to and spread on immobilized thrombospondin or fibronectin, which are ligands for the heparan sulfate chains of the proteoglycan. This binding and spreading is not dependent on the cytoplasmic domain of the core protein, as mutants expressing core proteins with cytoplasmic deletions maintain the ability to spread. The spreading is mediated through engagement of the syndecan-1 core protein, as the Raji-S1 cells also bind to and spread on immobilized mAb 281.2, an antibody specific for the ectodomain of the syndecan-1 core protein. Spreading on the antibody is independent of the heparan sulfate glycosaminoglycan chains and can be inhibited by competition with soluble mAb 281.2. The spreading can be inhibited by treatment with cytochalasin D or colchicine. These data suggest that the core protein of syndecan-1 mediates spreading through the formation of a multimolecular signaling complex at the cell surface that signals cytoskeleton reorganization. This complex may form via intramembrane or extracellular interactions with the syndecan core protein.

SYNDECAN-1, an integral membrane cell surface proteoglycan initially described on mouse mammary epithelial cells (Rapraeger et al., 1985; Rapraeger and Bernfield, 1983; Saunders et al., 1989), is the first characterized member of a syndecan family, which also includes syndecan-2 (fibroglycan) (Marynen et al., 1989; Pierce et al., 1992), syndecan-3 (*N*-syndecan) (Carey et al., 1992; Gould et al., 1992), and syndecan-4 (ryudocan or amphiglycan) (David et al., 1992; Kojima et al., 1992). The unifying features of this family are their highly conserved transmembrane and cytoplasmic domains, and the presence of heparan sulfate glycosaminoglycan chains on their ectodomains. Most cells express at least one member of the syndecan family. However, many cells express more than one member, suggesting that each syndecan may have specific functions at the cell surface (Kim et al., 1994). The syndecans are proposed to have roles in growth factor action, extracellular matrix adhesion, and cytoskeleton organization that controls cell morphology (for review see Bernfield et al., 1992; Jalkanen et al., 1992; Rapraeger, 1993).

Although syndecan-1 is expressed by a variety of cell types, it is most prevalent on epithelia (Kim et al., 1994). It is on the entire surface of cells in transitional or stratified epithelia, but is confined to the basolateral cell borders of simple epithelia (Rapraeger et al., 1986; Sanderson and Bernfield, 1988), suggesting its participation in cell adhesion mechanisms. This role is also suggested during early development of the mouse, where syndecan-1 is localized to sites of cell–cell contact beginning at the blastocyst stage (Sutherland et al., 1991). This role is also suggested by the binding of syndecan-1 to a variety of matrix macromolecules via its heparan sulfate glycosaminoglycan chains, including interstitial collagens (Koda et al., 1985; Ridley et al., 1993), fibronectin (Saunders and Bernfield, 1988), tenascin (Salmivirta et al., 1991), and thrombospondin (Sun et al., 1989).

Syndecan-1 is also expressed within the B cell lineage. The proteoglycan is found on precursor B cells in the bone marrow, but is absent from B cells that are released from the marrow into circulation. Syndecan-1 is expressed once again, however, when these cells differentiate into plasma cells (Sanderson et al., 1989). It has been suggested that

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the expression of syndecan-1 on these cells serves to immobilize them within the extracellular matrix; syndecan-1 expressed on plasma cells or on syndecan-1 transfected ARH-77 B cells has been shown to bind collagen (Ridley et al., 1993; Sanderson et al., 1992) and prevents B cell invasion of collagen matrices (Liebersbach and Sanderson, 1994). Expression of syndecans 1 and 4 in ARH-77 lymphoid cells also causes cell–cell aggregation in a calcium- and heparan sulfate–dependent heterotypic interaction (Stanley et al., 1995).

Several investigators have questioned the role of syndecan-1 by manipulating its expression level. Expression of exogenous syndecan-1 cDNA in rat Schwann cells causes the cells to spread more extensively on fibronectin and laminin (Carey et al., 1994*a*) and to form an increased number of focal adhesions (Hansen et al., 1994). Overexpression of syndecan-1 in 293T renal epithelial cells causes the cells to become more anchorage dependent and less motile, with an increase in serum-dependent proliferation and a decreased proliferation rate in the absence of serum (Numa et al., 1995). Overexpression of syndecan-1 in NIH 3T3 fibroblasts also causes a morphological transition, and the cells become less responsive to basic FGF (bFGF) (Elenius et al., 1992).

There are several examples of the importance of syndecan-1 in epithelial morphogenesis. However, relatively little is known about its mechanism of action. In early development, syndecan-1 is expressed at sites of epithelial–mesenchymal interactions (Boutin et al., 1991; Thesleff et al., 1988; Trautman et al., 1991; Vainio et al., 1989*a*, *b*) and is reportedly downregulated on epithelia during epithelial–mesenchymal transformation (Bernfield et al., 1992). A similar conversion is noted in cell culture. When syndecan-1 expression is reduced by antisense RNA in NMuMG mouse mammary epithelial cells, the cells downregulate E-cadherin, redistribute β_1 integrins, and convert to a fusiform morphology (Kato et al., 1995). This suggests a crucial role for syndecan-1 in the maintenance of epithelial morphology. Interestingly, the heparan sulfate chains of syndecan-1 do not appear to be required for this activity, nor is it clear which domains of the core protein have an important role. In another example, treatment of S115 mammary epithelial cells with steroids causes a reduction in syndecan-1 expression that is accompanied by conversion to a fusiform morphology. If syndecan-1 expression is maintained by transfection of an exogenous syndecan gene, the cells retain their epithelial morphology (Leppä et al., 1992). In this example, however, the activity is dependent upon the heparan sulfate chains of the extracellular domain and does not appear to require either the cytoplasmic or transmembrane domains of the core protein (Mali et al., 1994).

This suggests that each of the domains of the syndecan core protein may have individual roles. In syndecan-1–expressing rat Schwann cells, antibody-induced clustering of the proteoglycan causes cocapping with actin filaments. This cocapping is dependent on the cytoplasmic domain of the core protein of syndecan-1 (Carey et al., 1994*b*). Although this recognition is demonstrated for syndecan-1, it is not clear whether other syndecans would behave identically. For example, syndecan-4 is localized to focal adhesions in a variety of cell types, whereas syndecan-2 on the same cells is not localized to these structures (Woods and Couchman, 1994).

Increasing evidence suggests that cell surface proteoglycans collaborate in activating intracellular signaling pathways. Fibroblasts plated on fibronectin require both the engagement of cell surface proteoglycans and integrins to spread fully, forming focal contacts and stress fibers (Woods et al., 1986). The requirement for proteoglycan engagement can be overcome, however, by treatment of the cells with phorbol ester (Woods and Couchman, 1994), suggesting that the engagement of the proteoglycan generates a signal to establish the fully spread phenotype. This signal can be negated by inhibitors of protein kinase C. Similarly, expression of collagenase by fibroblasts plated on fibronectin is regulated by proteoglycan engagement, acting together with integrins that recognize the fibronectin cell binding domain (Tremble et al., 1994; Werb et al., 1989). The chondroitin-sulfate proteoglycan NG2 also collaborates with $\alpha_4\beta_1$ integrin to promote cell spreading and focal contact formation (Iida et al., 1995). In this system, the proteoglycan and the integrin can be triggered independently to generate the spreading signal, suggesting that the proteoglycan itself can assemble a signaling complex to generate a signal that it has been engaged.

One difficulty in studying specific roles for individual proteoglycans is that cells usually express more than one type, making it difficult to determine the role of a particular proteoglycan. In the present work, we examine human Raji cells transfected with the cDNA for a single cell surface proteoglycan, namely syndecan-1. Raji cells (Pulvertaft, 1965) were chosen for this study because they have tested negative for syndecan-1 by Northern and immunodetection analysis and for cell surface heparan sulfate by their failure to bind ligands with heparin-binding domains, such as bFGF (data not shown). We have obtained cell populations expressing high levels of syndecan-1 and truncated variants of this molecule to study its interactions at the cell surface and its role in cytoskeleton assembly and cell spreading.

Materials and Methods

cDNA Constructs

Mouse syndecan-1 cDNA (Saunders et al., 1989) was cleaved from pGEM3Z-Syndecan-1 (4-19b), generously provided by Dr. Merton Bernfield (Joint Program in Neonatology, Harvard Medical School, Boston, MA), and inserted into plasmid 636, generously provided by Dr. Bill Sugden (McArdle Laboratory, University of Wisconsin, Madison, WI). The 636 plasmid contains genes conferring hygromycin resistance for selection in mammalian cells and ampicillin resistance for selection in bacteria. It also contains the origin of plasmid replication, *ori P*, of the Epstein-Barr Virus (EBV)[1] to generate a high copy number of plasmids in EBV-positive transfected cells (Sugden et al., 1985). To generate p636synd1-FL, syndecan cDNA was cleaved from pGEM3Z-Syndecan-1 with EcoRI and HindIII (New England Biolabs, Inc., Beverly, MA); HindIII linkers (New England Biolabs, Inc.) were ligated using T4 DNA ligase (New England Biolabs, Inc.), trimmed with HindIII, and the resulting syndecan-1 cDNA was subcloned into the unique HindIII site in 636. This construct encodes full-length syndecan-1 protein.

The plasmid p636synd1-Δ12 was generated by cleaving p636synd1-FL with BanI, truncating the cytoplasmic domain at nucleotide 1131. A linker

1. *Abbreviations used in this paper*: CMF-PBS, calcium- and magnesium-free PBS; EBV, Epstein-Barr virus; Hb-DME, Hepes-buffered DME.

encoding a stop codon and an NheI cloning site was ligated to the ends of the fragment. This insert was subcloned into parental 636 via its HindIII and NheI cloning sites. This construct encodes a core protein truncated after amino acid 299.

The plasmid p636synd1-Δ33 was generated by cleaving pGEM7zf+-synd1-FL (Promega Corp., Madison, WI) with BsaW1, which cleaves syndecan-1 cDNA to truncate the cytoplasmic domain at nucleotide 1069. A linker encoding a stop codon and an NheI cloning site was ligated to the ends, and this fragment was cleaved with NheI and XhoI. The insert was cloned into p636synd-1-FL, which had been cleaved with XhoI and NheI. XhoI cleaves mouse syndecan-1 cDNA at nucleotide 105 in the 5′ untranslated region. This construct encodes a core protein truncated at amino acid 280 (which is converted from methionine to glycine) due to the insertion of a stop codon. All constructs were analyzed for correct orientation by restriction enzyme mapping and confirmed by sequence analysis using the dideoxy-mediated chain-termination method (Sanger et al., 1977) with Sequenase version 2.0 (United States Biochemical Corp., Cleveland, OH).

Cell Culture

The human Raji cell line is an EBV-positive Burkitt's lymphoma cell that expresses the Epstein-Barr nuclear antigen 1, which is necessary and sufficient for latent replication of DNA containing the EBV *ori P* site of replication (Reisman and Sugden, 1986). Raji cells were grown in RPMI 1640 (Gibco Laboratories, Gaithersburg, MD) supplemented with 10% calf serum (Hyclone Laboratories, Logan, UT). Low passage NMuMG mouse mammary epithelial cells were grown as previously described (David and Bernfield, 1979) in DME (Gibco Laboratories) supplemented with 10% calf serum (Hyclone Laboratories).

Plasmid DNA was introduced into parental Raji cells by electroporation (Neumann et al., 1982), and transfected cells were selected by growth in hygromycin-B (300 μg/ml). High expressing transfectants were selected further by panning on mAb 281.2. Briefly, mAb 281.2 (50–250 μg/ml) or control rat IgG was incubated at 4°C overnight in Hepes-buffered DME (Hb-DME), pH 7.4, on 35-cm^2 tissue-culture dishes. The dishes were washed three times with Hb-DME, and transfected cells were plated in RPMI 1640 containing 10% calf serum. After incubating 2 h at 37°C, nonadherent cells were aspirated, and the plates were rinsed three times in RPMI containing 10% calf serum. Medium was changed on day 3, and the adherent cells were harvested by treatment with trypsin (0.25%) at 37°C on day 5, followed by growth in suspension.

Antibodies

Two antibodies were used to detect syndecan-1: (*a*) a rat monoclonal $IgG2_A$ (mAb 281.2) specific for the ectodomain of syndecan-1 (Jalkanen et al., 1985) and (*b*) an affinity-purified antiserum (S1CD) to the carboxyl-terminal 10 amino acids of the cytoplasmic domain. Control antibodies for spreading assays and immunofluorescence included rat IgG (Jackson Laboratories, West Grove, PA), anti–human IgM (Jackson Laboratories), anti-thy1.1, a rat $IgG2_A$ purified from hybridoma (M5/49.4.1; obtained from the American Type Culture Collection, Rockville, MD) supernatants in the same manner as mAb 281.2, and preimmunization serum from the rabbit injected to produce the S1CD antibody.

Gel Electrophoresis and Immunoblotting of Syndecan-1

Whole cells were lysed and sonified in 8 M Urea, 0.1% Triton X-100, 10 mM Tris, 1 mM Na_2SO_4, pH 8.0. DEAE-Sephacel (Pharmacia LKB, Uppsala, Sweden) was added to the lysate (100 μl per 10^7 cells) and mixed overnight at 4°C. Proteoglycans were eluted from the DEAE beads with 1 M NaCl in heparitinase buffer as described (Yeaman and Rapraeger, 1993). Enzymatic cleavage of heparan sulfate and chondroitin sulfate chains was accomplished by diluting samples twice with dH_2O followed by incubation twice for 2 h at 37°C with either heparitinase III (0.2 U/ml), chondroitin ABC lyase (0.05 U/ml) (ICN Nutritional Biochemicals, Cleveland, OH), or with both enzymes. The samples were fractionated by electrophoresis, electrotrophoretically transferred onto cationic membrane (Zeta-Probe-GT; Bio-Rad Laboratories, Hercules, CA), and probed with mAb 281.2 or S1CD as previously described (Yeaman and Rapraeger, 1993).

Preparation of Protein-coated Surfaces

Nitrocellulose-coated tissue-culture dishes were prepared as described (Lagenaur and Lemmon, 1987). Briefly, 5 cm^2 of nitrocellulose was dissolved in 6 ml 100% methanol overnight and applied to tissue-culture plasticware. The methanol was allowed to evaporate under UV light in a laminar flow hood for 30 min, and the coated surfaces were washed and incubated with dH_2O for 15 min. On a 2-cm^2 surface, 100 μl human thrombospondin (75 μg/ml), mAb 281.2 (250 μg/ml), or rat IgG (250 μg/ml) was applied to the nitrocellulose in Hb-DME, pH 7.4, for 30 min at room temperature. Human thrombospondin was generously provided by Drs. Vishva Dixit (Department of Pathology, University of Michigan, Ann Arbor, MI) and Deane Mosher (Department of Medicine, University of Wisconsin, Madison, WI). The protein solution was then aspirated. The wells were washed twice with dH_2O followed by Hb-DME and then blocked with 1% BSA in Hb-DME for 1 h at 37°C. Proteins were also bound directly to tissue-culture dishes or glass coverslips without nitrocellulose coating by incubation at 4°C overnight at concentrations ranging from 10 μg/ml to 250 μg/ml. They were then washed and blocked in the same manner as above. Plates and coverslips were blocked with either 1% heat-denatured BSA or 10% calf serum.

Fibronectin and fibronectin fragments (50–100 μg/ml) (generous gifts of Dr. Donna P. Peters, Department of Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI) were bound to glass coverslips at 37°C for 2 h, washed four times with Hb-DME, and then blocked for 2 h at 37°C with 1% heat-denatured BSA in Hb-DME. Cells were plated in the same solution.

Immunocytochemistry

For detection of syndecan-1 in fixed cells, cells were plated onto protein-coated coverslips in 1% BSA/Hb-RPMI or in RPMI 1640 including 10% calf serum, incubated 2 h at 37°C, and then fixed for 2 h via the periodate-lysine-paraformaldehyde method (2% paraformaldehyde, 0.1 M lysine, 0.01 M sodium periodate) (McLean and Nakane, 1974). Fixed cells were washed twice in 0.05% sodium borohydride and were incubated 30 min in 0.1 M glycine in calcium- and magnesium-free PBS (CMF-PBS) at 4°C. Cells were permeablized with 0.2% Triton X-100 in PBS for 3 min and incubated for 3 × 10 min in CMF-PBS containing 10% FBS to reduce nonspecific binding. Primary antibody (5 μg/ml for S1CD) was incubated in CMF-PBS containing 10% FBS for 30 min. Cells were washed thoroughly and then incubated with secondary antibody (goat anti–rabbit fluorescein-conjugated IgG) (Jackson Laboratories) used at 25 μg/ml in CMF-PBS containing 10% FBS for 30 min. Rhodamine-conjugated phalloidin (Molecular Probes, Inc., Eugene, OR), was used at a dilution of 1:40 during the secondary antibody incubation. Cells were washed 10 min in CMF-PBS containing 10% FBS and then three times in CMF-PBS. Coverslips were mounted onto glass slides in phosphate-buffered glycerol containing 1 mg/ml *p*-phenylenediamine as an anti-photobleaching agent and viewed on a microscope (Microphot-FX; Nikon Inc., Garden City, NY). Pictures were recorded on film (Tri-X Pan 400; Eastman-Kodak Co., Rochester, NY) exposed at ASA 800.

Spreading Assays

Cells were plated on protein-coated surfaces in Hb-DME, pH 7.4, containing 1% BSA and were incubated for 2 h at 37°C. The percentage of spread cells was then determined by counting total cells vs. spread cells in three 1-cm^2 areas present in each of the duplicate or triplicate wells. A spread cell was defined as one having at least one filopodium visible at a magnification of 25 and was less refractive than unspread cells.

Glycosaminoglycan chains were removed from the cell surface by enzymatic cleavage. Cells were pretreated for 30 min with 0.2 U/ml heparitinase III, 0.05 U/ml chondroitin ABC lyase, or both enzymes, and plated in the spreading assay in the continued presence of these enzymes.

Cells were preincubated for 1 h at 37°C in either 10 μM cytochalasin D (Sigma Chemical Co., St. Louis, MO) or 50 μM colchicine (Sigma Chemical Co.) in Hb-DME containing 1% BSA. Cells were pretreated with cycloheximide (25 μg/ml) for 2 h before plating. Inhibition of protein synthesis was monitored by Tran^{35}S-label (ICN Pharmaceuticals, Inc., Irvine, CA) labeling into TCA-precipitable fractions of cells after a 2-h preincubation period. All cells were plated in the spreading assays in the continued presence of the inhibitors. Each assay was repeated at least three times.

Antibody Inhibition of Cell Spreading

Cells were plated on mAb 281.2 in RPMI 1640 containing 10% calf serum. After 10 min, unbound cells were aspirated, and bound cells washed with

medium. Competing antibodies, mAb 281.2 or nonspecific rat IgG, were added to the medium at 100 μg/ml. The cells were incubated at 37°C for 2 h, and the percentage of spread cells determined. Alternatively, the cells were incubated for 2 h at 37°C before addition of competing antibody. The cells were washed three times, and mAb 281.2 was added at concentrations of 10 μg/ml and 100 μg/ml. Rat IgG was added to control wells at 100 μg/ml. After incubating 4 h at 37°C, the cells were counted and the percent spread determined.

Results

Expression of Syndecan-1 in Human Raji Cells

The Raji cell line transfected with the cDNA for syndecan-1 is a good candidate for exploring roles of this cell surface proteoglycan. The parental cells (*a*) contain little or no cell surface heparan sulfate, assessed by the failure to bind ligands such as bFGF and thrombospondin, (*b*) are negative for syndecan-1 mRNA, assessed by Northern analysis (data not shown), and (*c*) are negative for syndecan-1 protein, assessed by immunoblotting analysis (Fig. 1). Thus, transfection of Raji cells with the 636 plasmid containing the mouse syndecan-1 cDNA, which is replicated at high levels in these cells (see Materials and Methods), allows high level expression of the syndecan-1 core protein and analysis of whether or not any changes in cell behavior accompany this expression.

Cells were transfected with the plasmid p636-Synd1-FL and selected by resistance to hygromycin B. Hygromycin-resistant cells were further selected by panning on mAb 281.2, a syndecan-1–specific mAb. These cells were confirmed to be a population of cells expressing high levels of heparan sulfate–decorated syndecan-1 by immunoblot analysis with mAb 281.2 (Fig. 1). The syndecan-1–expressing cells (Raji-S1) are compared to parental cells (Raji) and to NMuMG epithelial cells that are known to express cell surface syndecan-1. The parental Raji cells are negative for syndecan-1 protein, whereas the Raji-S1 cells contain immunoreactive proteoglycan that runs in a heterodisperse distribution on polyacrylamide gels with a modal molecular weight of 134,000. This is smaller than the form detected in NMuMG cells, which has a modal molecular weight of 198,000. The level of expression in these cells is similar to that of the NMuMG cells, which are known to express abundant levels of syndecan-1.

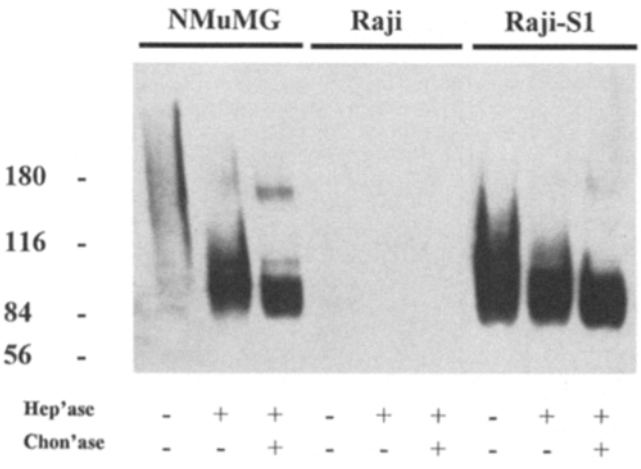

Figure 1. Expression of syndecan-1 in Raji cells transfected with p636-Synd1-FL. Syndecan-1 was batch adsorbed to DEAE Sephacel from lysates of Raji parental cells or Raji-S1 cells transfected with p636-Synd1-FL. NMuMG mouse mammary epithelial cells, known to express syndecan-1, are shown as a positive control. Proteoglycans were isolated as described in Materials and Methods. Samples were electrophoresed on a 3.5–15% Tris-borate SDS–polyacrylamide gradient gel (2.5 million cell equivalents per lane) after digestion without or with heparitinase III (*Hep'ase*), or heparitinase III together with chondroitin ABC lyase (*Chon'ase*). The gel was electrophoretically transferred to Zeta Probe-GT cationic membrane, which was then probed with mAb 281.2, as described in Materials and Methods. Protein molecular weight standards are indicated (*left*) in kD.

Decoration of the syndecan-1 core protein with glycosaminoglycan chains was analyzed by digestion with heparitinase III, which specifically cleaves heparan sulfate, and chondroitin ABC lyase, which specifically cleaves chondroitin sulfate. Syndecan-1 expressed by the Raji-S1 cells is largely decorated with heparan sulfate, as treatment with heparitinase III reduces the size of the proteoglycan to ~80 kD. The proteoglycan may also be decorated with minor amounts of chondroitin sulfate as additional reduction in size is seen if the heparitinase is augmented with chondroitin ABC lyase. This size and composition is consistent with syndecan-1 expressed as a heparan sulfate proteoglycan in B lymphocytes (Sanderson et al., 1989). The Raji-S1 cells may also be producing core protein that does not contain glycosaminoglycan chains, as staining is seen at 80 kD even in the absence of enzyme treatment.

Syndecan-1 Transfected Raji Cells Bind to and Spread on Thrombospondin and Fibronectin

To examine the potential influence of syndecan-1 on cell attachment, the Raji-S1 cells were plated on thrombospondin, a ligand for the heparan sulfate chains of syndecan-1 (Sun et al., 1989). The Raji parental cells fail to bind to thrombospondin (Fig. 2 *A*). However, as anticipated, the Raji-S1 cells bind to the ligand. The binding occurs within 5 min of plating and involves the majority of the plated cells. In addition to binding the thrombospondin, however, the cells also spread on this ligand and acquire a bipolar morphology when fully spread (Fig. 2 *B*).

To test the ability of Raji-S1 cells to spread on another syndecan-1 ligand, Raji cells were plated on fibronectin (Saunders and Bernfield, 1988). In contrast to the results observed with thrombospondin, the Raji parental cells bind to fibronectin in the absence of syndecan-1, although only a small percentage of the cells (~10%) spread on this ligand (Fig. 2 *C*). This suggests that the Raji parental cells express a cell adhesion molecule, presumably an integrin, that binds to fibronectin. When compared to the parental cells, however, a much greater proportion of the Raji-S1 cells spread and to a much greater extent (Fig. 2 *D*). The additional spreading response appears due to the binding of heparan sulfate on syndecan-1 to the heparin-binding domain of the fibronectin because only a small percentage of the Raji-S1 cells spread if plated on a fragment of fibronectin lacking this domain (data not shown). The cells behave identically to parental cells that are plated either on intact fibronectin (Fig. 2 *C*) or on the fragment with the heparin-binding domain removed (data not shown).

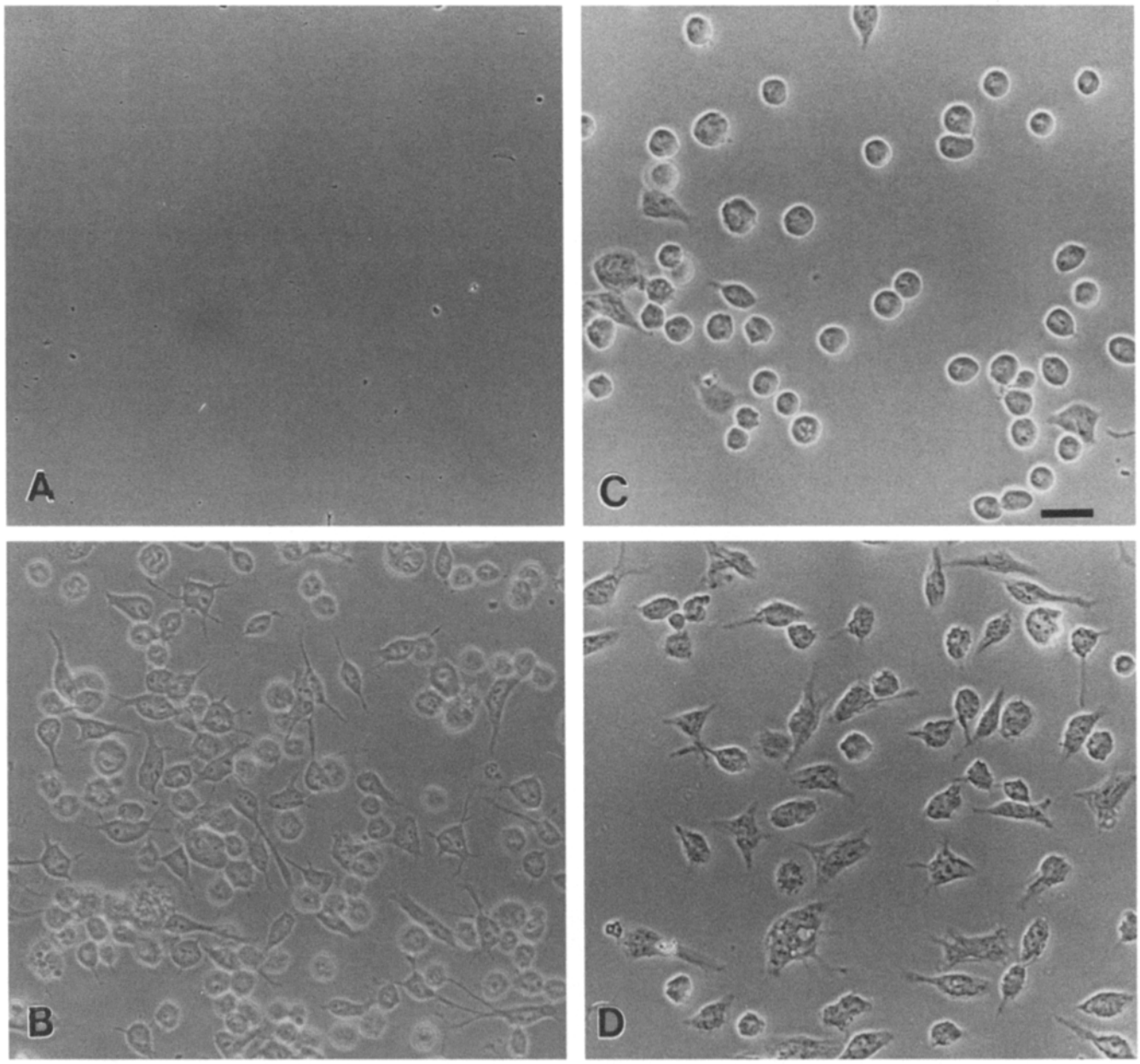

Figure 2. Binding and spreading of Raji parental and Raji-S1 cells on thrombospondin and fibronectin. Raji parental cells (*A* and *C*) and Raji-S1 cells (*B* and *D*) were plated on glass coverslips coated with 55 μg/ml thrombospondin (*A* and *B*) or 100 μg/ml fibronectin (*C* and *D*) and blocked with heat-denatured BSA. The cells were incubated at 37°C for 2 h before fixation. Bar, 25 μm.

As suggested for fibronectin, the spreading on thrombospondin requires the heparan sulfate glycosaminoglycan chains on syndecan-1. Raji-S1 cells were pretreated with heparitinase III, chondroitin ABC lyase, or both enzymes to remove heparan sulfate and/or chondroitin sulfate glycosaminoglycans before plating on thrombospondin (Fig. 3 *A*). Treatment of the Raji-S1 cells with heparitinase reduces their spreading to 4%, similar to that of Raji parental cells used as controls. This is in contrast to the untreated Raji-S1 cells, of which 86% are spread within 2 h of plating. Treatment with chondroitin ABC lyase is without effect (87% spread), whereas treatment with the combined enzymes is equivalent to treatment with heparitinase alone (2% spread).

Spreading of Raji-S1 Cells Occurs Rapidly and Does Not Require New Protein Synthesis

The Raji-S1 cells attach and spread rapidly. Within 20 min of plating, 54% of the cells plated on thrombospondin are beginning to spread, and 93% of the cells are fully spread within 60 min after plating (Fig. 4). These data suggest that the cells are binding to the supplied ligand (in this case, thrombospondin), rather than assembling their own extracellular matrix. This was further tested by performing spreading studies after incubation of the cells in cycloheximide to block new protein synthesis. Cells were treated with 25 μg/ml cycloheximide for 2 h, blocking incorporation of [^{35}S]methionine and cysteine into TCA-insoluble

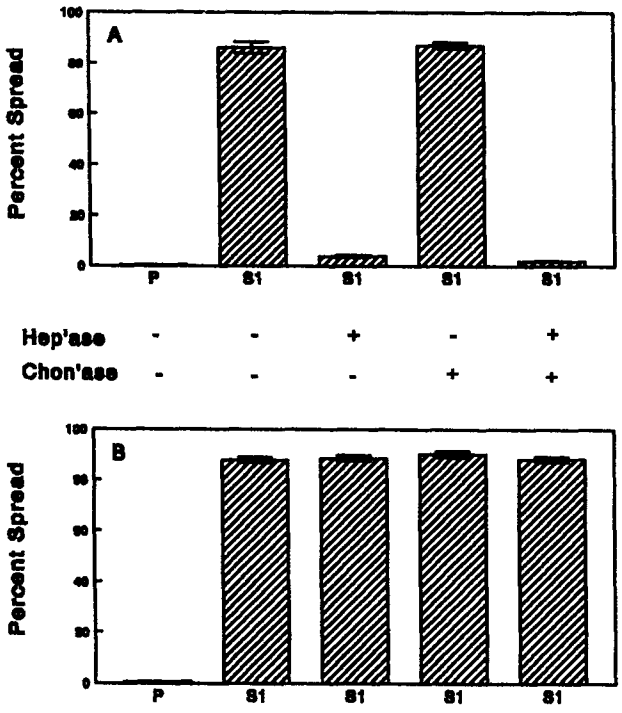

Figure 3. Analysis for the requirement of heparan sulfate chains for spreading on thrombospondin or mAb 281.2. Raji-S1 cells (*S1*) were pretreated for 30 min at 37°C with heparitinase III, chondroitin ABC lyase, or both enzymes to selectively remove GAG chains at the cell surface. The cells were plated in 1% BSA in Hb-DME onto nitrocellulose-coated dishes incubated with thrombospondin (*A*) or mAb 281.2 (*B*). Spreading of Raji parental cells (*P*) is shown as a control in each panel.

material by 88.7% (SE 3.9%). These cells are not inhibited in their ability to spread (91% spread relative to control, untreated cells) (see Fig. 10).

The Cytoplasmic Domain of Syndecan-1 Is Not Required for the Syndecan-1–dependent Spreading

The cytoplasmic domain of syndecan-1 has been implicated in its interaction with the cytoskeleton. For example, in Schwann cells expressing full-length syndecan-1, antibody-induced capping of syndecan-1 also caps actin filaments to these sites, whereas in cells expressing a core protein truncated in its cytoplasmic domain, actin fails to cocap with syndecan-1 (Carey et al., 1994*b*). To assess whether the cytoplasmic domain of the syndecan-1 core protein is required for the syndecan-1–dependent spreading observed here, truncation mutants were expressed in Raji parental cells and assayed for their ability to spread on heparan sulfate ligands. Raji-S1Δ12 cells express a form of the proteoglycan in which the last 12 amino acids of the cytoplasmic domain have been deleted. Raji-S1Δ33 cells express a truncated core protein in which all but one amino acid of the cytoplasmic domain has been removed, but a second amino acid (glycine) is added, leaving a two–amino acid cytoplasmic domain. The cells express these truncated forms of syndecan-1 as heparan sulfate proteoglycans at the cell surface, and they both mediate cell adhesion to thrombospondin and fibronectin (see below).

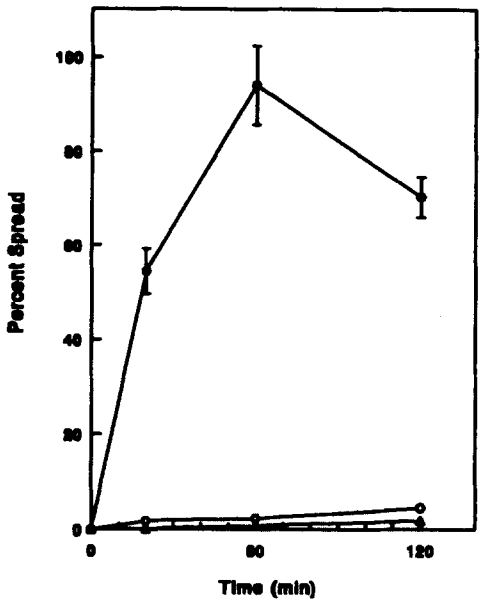

Figure 4. Time course of Raji-S1 cell spreading on thrombospondin. Raji-S1 cells (*closed circles*) and Raji parental cells (*open circles*) were plated on thrombospondin or BSA-blocked nitrocellulose (data shown for Raji-S1 cells; *closed triangles*). At time points of 20, 60, and 120 min, the cells were counted, and the percentage spread was determined. A spread cell was defined as one having at least one filopodium visible at ×25 magnification and was less refractive than unspread cells.

The glycosaminoglycan chains can be removed enzymatically to generate a core protein similar in size to that seen in the Raji-S1 cells (Fig. 5). These core proteins are immunoreactive with mAb 281.2, but not with a polyclonal antibody (SICD) generated against the COOH-terminal 10 amino acids of the native cytoplasmic domain, confirming that the COOH terminus of the cytoplasmic domain has been truncated in these mutants.

Like the Raji-S1 cells, the Raji-S1Δ12 and Raji-S1Δ33

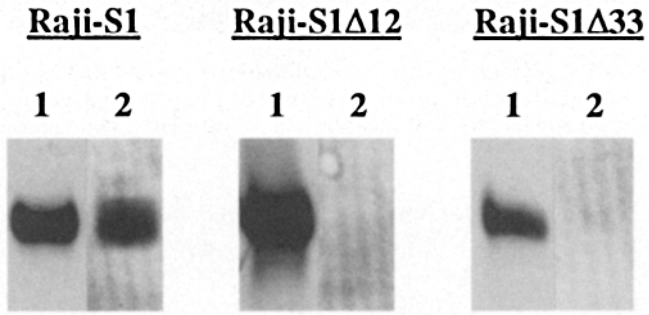

Figure 5. Expression of truncated syndecan-1 in Raji cells. Syndecan-1 was batch adsorbed to DEAE Sephacel from lysates of Raji-S1, Raji-S1Δ12, or Raji-S1Δ33 cells. Proteoglycans were isolated as described in Materials and Methods. Samples were electrophoresed on a 3.5–15% Tris-borate SDS–polyacrylamide gradient gel (1 million cell equivalents per lane) after digestion with heparitinase III and chondroitin ABC lyase. The gel was electrophoretically transferred to Zeta Probe-GT cationic membrane and probed with either mAb 281.2 (lanes *1*) or S1CD (lanes *2*) as described in Materials and Methods.

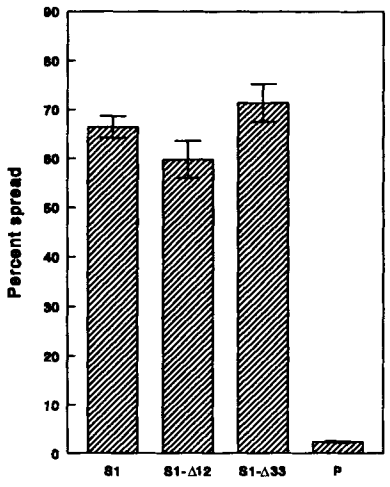

Figure 6. Spreading of syndecan-1 truncation mutants on thrombospondin. Raji-S1, Raji-S1Δ12, Raji-S1Δ33, and parental cells were plated on glass coverslips coated with thrombospondin, blocked with heat-denatured BSA, and incubated at 37°C for 2 h before fixation. Very few of the parental cells bind to the thrombospondin (see Fig. 2), while the majority of the syndecan-1–expressing cells bind to the thrombospondin and spread. Relatively equal proportions of the cells expressing the truncation mutants spread, and the morphology of the cells is indistinguishable when compared to the Raji-S1 cells.

cells spread on thrombospondin or fibronectin. When plated on thrombospondin and assayed for the percentage of cells spread after 2 h, the cells behave similarly (Fig. 6). The morphology of the truncation mutants is indistinguishable from that of the cells expressing the intact core protein on both thrombospondin and fibronectin (not shown). In subsequent assays tested in this manuscript, these truncation mutants behaved similarly to the full-length syndecan-1 protein.

Cell Spreading Is Mediated by the Syndecan-1 Core Protein

The ability of the Raji-S1Δ33 cells to spread on heparan sulfate ligands suggests that the interactions required for spreading in a syndecan-1–dependent manner occur via a binding partner(s) present in the membrane or at the cell surface. Such interactions have previously been described for other proteoglycans. For example, K1735 mouse melanoma cells adhere to fragments of fibronectin via a glypican-like heparan sulfate proteoglycan (Drake et al., 1992). In this case, the heparan sulfate chains of the proteoglycan are proposed to mediate initial cell adhesion to fibronectin, thereby allowing lateral association with other cell adhesion molecules that stabilize the adhesion and promote spreading. The Raji syndecan-1–dependent spreading that is observed here may be due to such a mechanism, and if so would depend on binding interactions of the heparan sulfate chains. Another possibility, however, is that the interaction is through the core protein directly rather than the heparan sulfate chains, presumably via the transmembrane or extracellular domain.

To determine if the spreading can be triggered by engagement of the syndecan-1 core protein alone, cell spreading was examined on mAb 281.2, which recognizes an epitope on the extracellular domain of the syndecan-1 core protein. Cells were plated on coverslips incubated with either mAb 281.2 or nonspecific rat IgG (Fig. 7, *A–D*) and blocked with 1% native BSA in Hb-DME. Whereas the Raji cells do not bind nonspecifically to coverslips blocked with denatured BSA, both the parental and Raji-S1 cells adsorb when native BSA is used as the blocking agent, as has been reported (Ballard et al., 1991). Despite their ability to adhere to the coverslip, however, the cells fail to spread. Thus, when the native BSA mixture is used to block either nonspecific rat IgG or mAb 281.2–coated coverslips, both the parental and Raji-S1 cells adhere. However, the parental cells do not spread on either antibody. In contrast, the Raji-S1 cells rapidly become fully spread on mAb 281.2 but fail to spread on nonspecific rat IgG. Similarly, both the Raji parental cells, which are known to express surface immunoglobulin (Polliack et al., 1983), and Raji-S1 cells bind to immobilized anti-IgM antibody but fail to spread (data not shown). The adsorption of parental and Raji-S1 cells to native BSA-coated or anti-IgM–coated coverslips provide important controls for the syndecan-1–mediated spreading, e.g., simple adhesion to the substratum is not sufficient for cytoskeletal reorganization required for spreading. Rather, adhesion must utilize a receptor that can initiate reorganization of the cytoskeleton. In this case, the syndecan-1 core protein appears to be such a receptor.

To verify that the cells were binding solely to mAb 281.2, and to rule out the contribution of other surface receptors such as the molecules that bind the BSA, cells were plated on coverslips coated with mAb 281.2 and blocked such that nonspecific binding did not occur. This is accomplished either with heat-denatured BSA (Fig. 2 *A*) or 10% calf serum. This abolishes nonspecific binding, and adhesion occurs only by the syndecan-1 core protein. As expected, the Raji-S1 cells rapidly bind to mAb 281.2–coated coverslips and spread (Fig. 7 *F*). These cells fail to bind to nonspecific rat IgG (Fig. 7 *E*). Thus, binding to the syndecan-1 core protein alone is sufficient to promote the spreading.

It is important to rule out the contributions of the glycosaminoglycan chains in the spreading of Raji-S1 cells, even on mAb 281.2. Raji-S1 cells were pretreated with heparitinase III, chondroitin ABC lyase, or both enzymes to remove heparan sulfate and/or chondroitin sulfate glycosaminoglycans before plating on mAb 281.2 (see Fig. 3 *B*). Spreading of the Raji-S1 cells on mAb 281.2 is independent of the glycosaminoglycan chains attached to the core protein. Nearly all of the Raji-S1 cells spread on mAb 281.2, regardless of digestion of either the heparan sulfate, chondroitin sulfate, or both glycosaminoglycans.

The Morphology of the Raji-S1 Spread Cells Changes over Time

To examine the reorganization of the cytoskeleton more closely, the morphology of the Raji-S1 cells spread on mAb 281.2 was examined over time (Fig. 8). Spreading occurs very rapidly on the antibody; within 5 min, smooth lamellipodium formation can be observed (as in Fig. 8, *A* and *B*). The spreading appears to be a biphasic process,

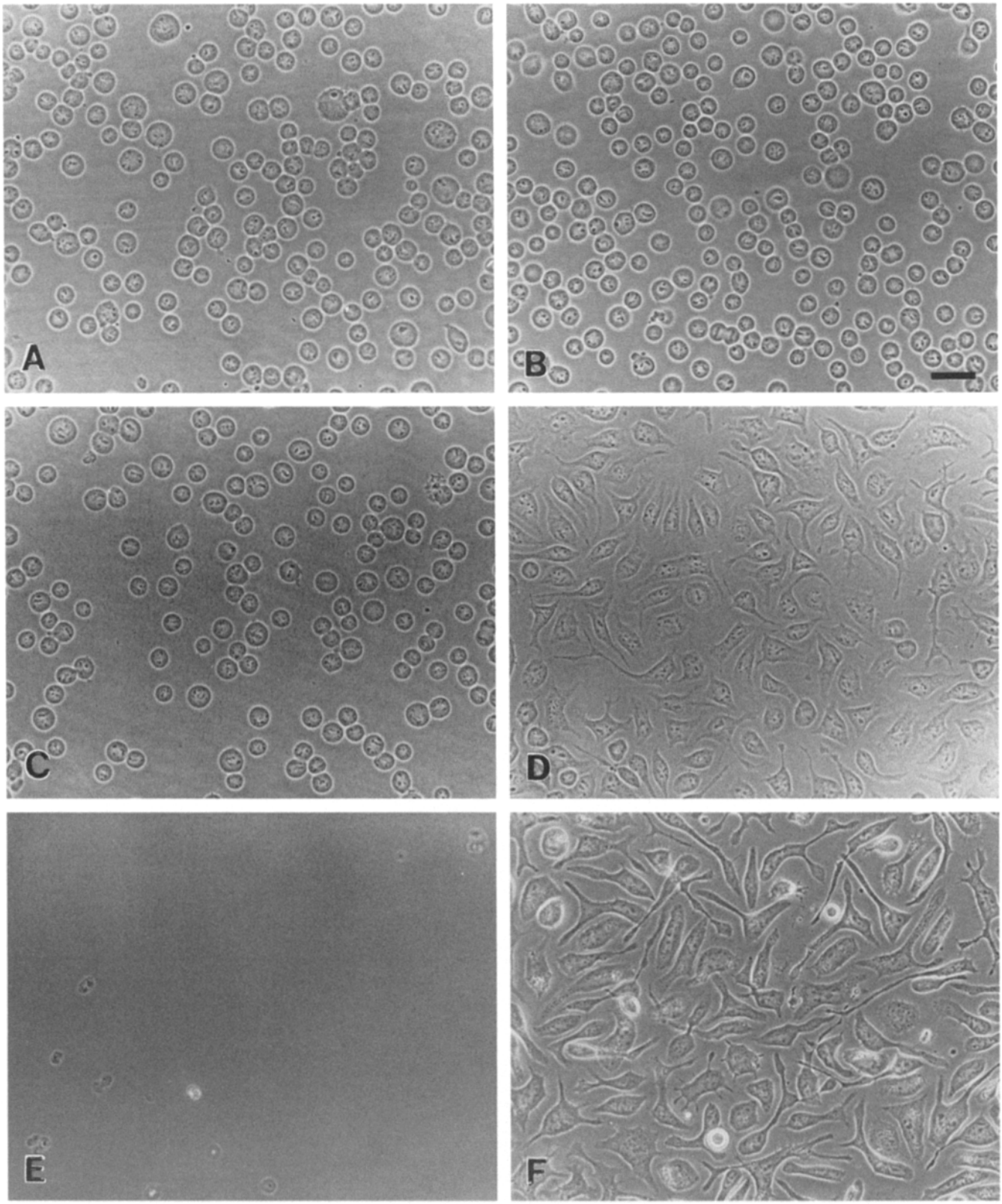

Figure 7. Binding and spreading of Raji-S1 cells on mAb 281.2. Raji parental cells (*A* and *B*) or Raji-S1 cells (*C–E*) were plated on coverslips coated either with rat IgG (*A*, *C*, and *E*) or mAb 281.2 (*B*, *D*, and *F*). The coverslips in panels *A–D* were blocked with native BSA, whereas coverslips in panels *E* and *F* were blocked with 10% calf serum. The cells were plated and incubated at 37°C for 2 h before fixation. Bar, 25 μm.

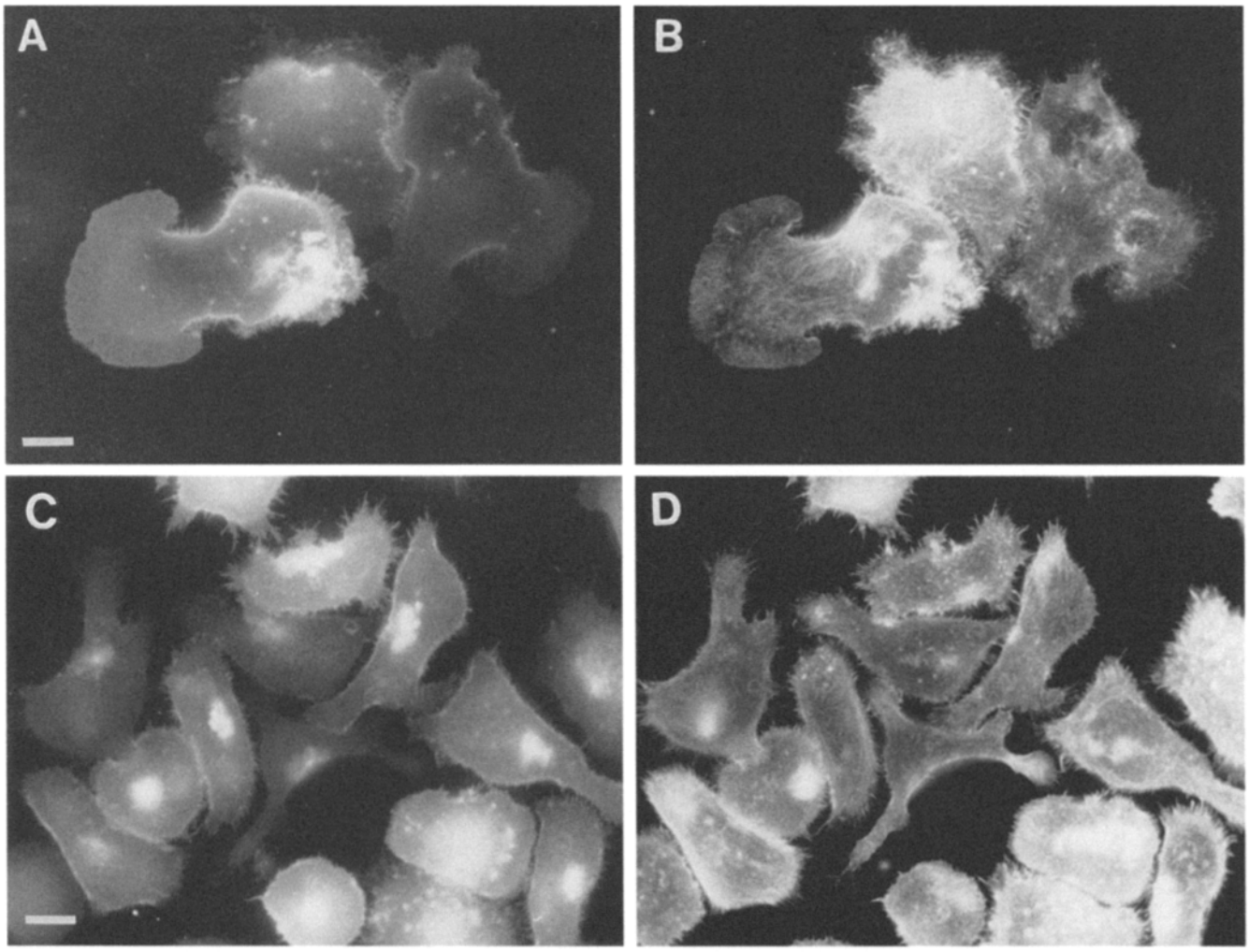

Figure 8. Change in morphology over time of the Raji-S1 cells plated on mAb 281.2. Raji-S1 cells were plated on mAb 281.2 and allowed to spread for either 15 min (*A* and *B*), or 2 h (*C* and *D*) before fixation. Cells were permeabilized with 0.2% Triton X-100 and stained with S1CD polyclonal antiserum (*A* and *C*) and rhodamine-conjugated phallodin (*B* and *D*). The cells send out smooth lamellipodia early in the spreading process (*A* and *B*), and then progress to a more fully spread, bipolar morphology (*C* and *D*). Bars, 7 μm (*A* and *B*), 10 μm (*C* and *D*).

however, as the cells acquire a more elongated, bipolar morphology between 30 min and 1 h after plating (Fig. 8, *C* and *D*). Many of the cells maintain the lamellipodia after the elongation has occurred. The distribution of syndecan-1 on these cells was analyzed by staining with an affinity-purified rabbit antiserum that recognizes the last 10 amino acids of the syndecan-1 core protein (S1CD). Syndecan-1 is distributed evenly at the cell surface and is not localized to distinct structures, such as focal adhesions or stress fibers. The filamentous actin in the lamellipodia (Fig. 8 *B*) is comprised of an orthogonal array of actin as has been described in lamellipodia of other cells (for review see Condeelis, 1993).

Syndecan-1–induced Cell Spreading Can Be Inhibited by mAb 281.2

To further demonstrate that cell spreading is mediated solely by the binding of the syndecan-1 core protein to immobilized mAb 281.2, competing concentrations of soluble antibody were used in an attempt to inhibit the spreading process. Raji-S1 cells were plated on tissue-culture wells coated with mAb 281.2, allowed to adhere for 10 min, and then the wells were washed with Hb-DME to remove nonadherent cells. When medium containing 100 μg/ml mAb 281.2 is added to the wells, cell spreading over a 2-h period is reduced (16%) compared to 100 μg/ml nonspecific rat IgG (89%), or in the absence of competing antibody (91%) (Fig. 9 *A*). This competition by the antibody is specific for the interaction of the core protein with mAb 281.2 because the attachment and spreading of the cell on thrombospondin is not disrupted by 100-μg/ml concentrations of the antibody (data not shown). Additionally, cells that are already spread on mAb 281.2 become rounded when exposed to competing amounts of mAb 281.2 and lose adherence to the dish. After 4 h, the percentage of spread cells is reduced to 42% in 10 μg/ml mAb 281.2, and to 8% in 100 μg/ml mAb 281.2 (Fig. 9 *B*). In contrast, >90% of the cells remain spread in 100 μg/ml nonspecific rat IgG. This suggests that even 6 h after plating, the Raji-

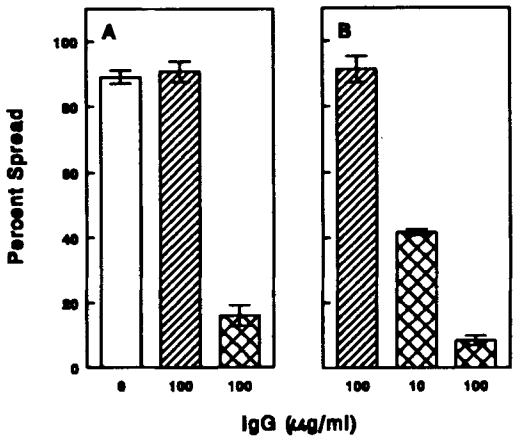

Figure 9. Inhibition of syndecan-1 induced cell spreading by soluble mAb 281.2. Raji-S1 cells were plated onto tissue-culture wells coated with mAb 281.2. (*A*) 10 min after plating, the wells were washed three times with Hb-DME, removing nonadherent cells. Cells bound to the antibody remained but were not yet spread. Medium was added containing antibodies to compete for binding sites: no competing antibody (*open bar*), 100 μg/ml rat IgG (*hatched bar*), or 100 μg/ml mAb 281.2 (*double-hatched bar*). The cells were incubated 2 h at 37°C at which time the cells were counted, and the percentage of spread cells determined. (*B*) Raji-S1 cells were plated on mAb281.2 as above. The cells were allowed to adhere and spread 2 h at 37°C at which time the wells were washed three times with Hb-DME. Competing antibodies were then added: 100 μg/ml rat IgG (*hatched bar*), 10 μg/ml mAb 281.2, or 100 μg/ml mAb 281.2 (*double-hatched bars*). The cells were incubated 4 h in the presence of these competing antibodies. After 4 h, the cells were counted, and the percentage that remained spread was determined.

S1 cells are adhering solely to the substratum through the interaction of mAb 281.2 and the syndecan-1 core protein. The Raji-S1 cells also bind to and spread on Fab fragments of mAb 281.2, demonstrating that the binding is specific for the Fab rather than the Fc portion of the antibody. The mAb 281.2 Fab fragments also compete for spreading on the bivalent mAb 281.2 (data not shown), demonstrating that the Fab is acting to compete with protein binding to the antibody rather than clustering or otherwise disarranging the proteoglycan on the cell surface.

Intracellular Signaling Is Required for the Syndecan-dependent Spreading

Cell spreading is a response to signals initiating the reorganization of the cytoskeleton. To determine whether the observed spreading is dependent on the polymerization of either actin filaments or microtubules, Raji-S1 cells were pretreated for 1 h and plated on glass coverslips coated with mAb 281.2 in the presence of 50 μM colchicine or 10 μM cytochalasin D, respectively. Compared to untreated cells, cell spreading is drastically inhibited in cells treated with either colchicine or cytochalasin D (96 and 93% inhibition, respectively) (Fig. 10). The cells recover and spread if the drugs are removed (data not shown). The inability of these cells to spread is not due to a loss of syndecan-1 at the cell surface, as the cells bind the antibody and remain bound upon washing, and immunocytochemistry shows

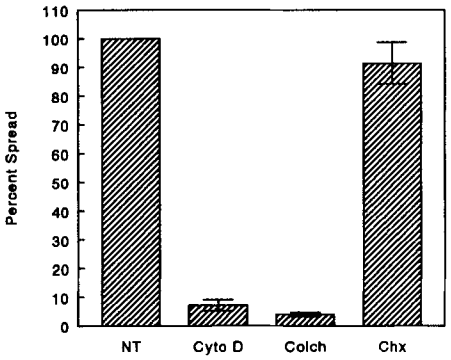

Figure 10. Analysis of syndecan-1 induced spreading in colchicine, cytochalasin D, and cycloheximide. Raji-S1 cells were pretreated with 10 μM cytochalasin D, 50 μM colchicine, or 25 μg/ml cycloheximide and plated onto tissue-culture plastic coated with mAb 281.2 in the continued presence of the inhibitors and incubated for 2 h at 37°C. The cells were then counted, and the percentage of spread cells determined. This graph represents normalized results of three independent experiments for each of the inhibitors (control, untreated samples set at 100%).

syndecan-1 is still present at the surface of the rounded cells (data not shown).

Discussion

The present study examines changes in cell behavior due to the acquisition of cell surface syndecan-1. Raji cells expressing mouse syndecan-1 bind to fibronectin or thrombospondin, ligands for the heparan sulfate chains of the proteoglycan, and spread. This spreading does not appear to be dependent on the cytoplasmic domain of syndecan-1, as cells expressing a truncation mutant lacking all but one amino acid of the cytoplasmic domain also bind to and spread on these ligands. This suggests that a signaling complex is being formed at the membrane by engagement of syndecan-1. Formation of this complex is mediated by the core protein of the proteoglycan, as spreading occurs on mAb 281.2 in the absence of glycosaminoglycan chains. Soluble mAb 281.2 blocks the spreading on the antibody and induces rounding and detachment of cells that are already spread. The spreading is dependent on intracellular signaling mechanisms regulating the cytoskeleton as cell spreading is inhibited by treatment with cytochalasin D or colchicine. These data suggest that syndecan-1 is acting through a multimolecular complex at the cell surface to induce the signaling events required for spreading.

The syndecan-1–mediated adhesion and spreading is dependent on the engagement of the core protein of the proteoglycan. This engagement can be achieved by the binding of the glycosaminoglycan chains to immobilized ligands for heparan sulfate, or through the direct immobilization of the core protein by binding to mAb 281.2. When the cells are plated on ligands for the heparan sulfate chains, the contributions of other cell adhesion molecules, such as integrins that are known to bind to these ligands, must also be considered. For example, it is likely that the parental cells' ability to bind to fibronectin is due to the binding of an integrin, of which two candidates are the $\alpha_4\beta_1$ (VLA-4) or $\alpha_3\beta_1$ (VLA-3) (Takada et al., 1987, 1988;

Wayner et al., 1989). These integrins are known to be present on the Raji cells (Ballard et al., 1991). Analysis of the spreading using mAb 281.2, a substratum specific for the core protein of syndecan-1, avoids this potential complication and demonstrates that cell spreading is seen when adhesion is accomplished solely by the syndecan core protein. Syndecan-1 remains the primary means of attachment even 6 h after plating, as soluble, competing mAb 281.2 can cause rounding and detachment of fully spread cells at this time. Spreading on the antibody also allows demonstration that spreading is independent of the syndecan-1 glycosaminoglycan chains, which can be removed by enzymatic treatment without effect on the adhesion or spreading response. This is an important observation, as it rules out the notion that the heparan sulfate chains serve to aggregate syndecan-1 and other receptors into a signaling complex.

Adhesion alone does not induce spreading; rather, a signal must be generated to initiate the reorganization of the cytoskeleton required for spreading. There are a number of examples of this in other cells. Melanoma cells plated on anti-β_3 integrin antibody adhere and spread. However, mutations within the cytoplasmic domain of β_3, which do not affect adhesion to the antibody, disrupt spreading (Filardo et al., 1995). Similarly, K562 leukocytes transfected with CD21 bind avidly to complement ligand C3bi but fail to spread. However, transfection with $\alpha_M\beta_2$, which also promotes efficient binding to C3bi, leads additionally to spreading and intracellular phosphorylation events (Graham et al., 1994). In this study, we find that Raji cells strongly adhere to nondenatured BSA-coated plates or coverslips, (see also Ballard et al., 1991) but do not spread. Nonetheless, if given an appropriate stimulus such as treatment with phorbol esters, Raji cells do have the capacity to spread on this substratum; PMA-stimulated cells become activated, exhibiting ruffled membranes and enhanced microspikes. The PMA-induced response can be inhibited by 100 nM staurosporin, an inhibitor of protein kinase C. The PMA-activated cells fail, however, to exhibit smooth lamellipodia or the bipolar phenotype observed on syndecan-1 ligands. Also, the syndecan-1–mediated spreading does not appear to be dependent on protein kinase C activity, as treatment of Raji-S1 cells plated on mAb 281.2 is not inhibited by staurosporin (data not shown).

A primary response to the engagement of the syndecan-1 core protein is the rearrangement of the cytoskeleton required for spreading to occur. This response is tightly regulated by second messengers that stimulate the severing of existing actin filaments, polymerization of G- into F-actin, and the cross-linking and bundling of actin filaments (for review see Stossel, 1989). The syndecan-1–dependent spreading is inhibited by treatment of the cells with cytochalasin D, which blocks actin polymerization. It is also blocked by colchicine, which blocks microtubule polymerization. This is an unexpected result, as microtubule extension is not reported to be required for cytoskeletal assembly associated with cell spreading (Arkett et al., 1994, and others). Rather, microtubules have a role in the distribution and movement of intracellular organelles and vesicles. For example, pseudopodial activity is decreased in migrating fibroblasts treated with colcemid (Bershadsky et al., 1991); presumably, microtubules are required to transport vesicles and organelles to the leading edge, thereby positioning them to respond further to a spreading signal. The effect of colchicine on the Raji cells may suggest that transport of molecules to the leading edge may be important for spreading to occur. It is unlikely that such molecules include syndecan-1 itself, as cell adhesion via syndecan is not impaired and surface staining for this protein appears undiminished upon colchicine treatment.

In preliminary experiments we have discovered that treatment of the cells with compactin, a competitive inhibitor of the isoprenylation of monomeric GTPases similar to lovastatin, adhere to mAb 281.2 but fail to spread. Although the action of this inhibitor remains to be clarified, it provides further demonstration that adhesion alone is insufficient to promote spreading. It is tempting to speculate that the block to isoprenylation serves to deactivate the rho family of G-proteins, including rac, rho, and cdc42 that are required for formation of lamellipodia, stress fibers, and filopodia, respectively (Kozma et al., 1995; Nobes and Hall, 1995; Ridley and Hall, 1992; Ridley et al., 1992).

Although the syndecan-1–dependent spreading occurs without its cytoplasmic domain, this domain is likely nonetheless to have an important role in the biology of syndecan-1. The cytoplasmic domain provides localization determinants for basolateral targeting of the proteoglycan (Miettinen et al., 1994) and is required for actin filaments to cluster with antibody-induced aggregates of syndecan-1 (Carey et al., 1994*b*). We have recently determined that the cytoplasmic domain of syndecan-1 is phosphorylated on tyrosine in pervanadate-stimulated NMuMG or Raji-S1 cells (Reiland, J., C. Lebakken, C. Yeaman, V. Ott, J. McCarthy, and A. Rapraeger, manuscript submitted for publication). The identity of the kinase that carries out this phosphorylation remains unknown.

The conclusion that the cytoplasmic domain is not required for the syndecan-1–dependent spreading suggests that signaling is mediated through another, or several, binding partners. Truncation of the cytoplasmic domain suggests that the interaction with binding partners is occurring through either the transmembrane domain or the extracellular domain of the core protein. Recently, a structural motif that mediates self-association of syndecan-3 was defined that contains portions of both the transmembrane and ectodomain of the core protein (Asundi and Carey, 1995). This motif is similar to those found in other transmembrane proteins that also homodimerize, including the platelet-derived growth factor receptor, β_3 integrin, and glycophorin. The motif is suggested to maintain an extended conformation within the NH_2-terminal half of the transmembrane region, rather than the more typical helical conformation. Syndecan-1 contains a similar motif, suggesting a mechanism for homodimerization at the cell membrane. This domain might also be used to generate heterotypic interactions with syndecan-1 and other proteins with similar motifs.

Intracellular signals can be generated by a number of cell surface receptors, ranging from growth factor receptors to membrane channels. Recently, cell adhesion molecules have been implicated more clearly in this process. Integrins, for example, are now known to activate kinases

such as focal adhesion kinase and src (for review see Richardson and Parsons, 1995), leading to activation of other downstream signaling pathways. Lymphoid cells such as the Raji line are known to express the integrin LFA-1 ($\alpha_L\beta_2$) and the β_1 integrins $\alpha_3\beta_1$ and $\alpha_4\beta_1$ (Ballard et al., 1991; Rincon et al., 1992). Syndecan-1 may generate a signal by forming an assembly with these receptors or with other membrane-associated signaling molecules.

Syndecan-1 appears to be involved in the maintenance of epithelial morphology. In NMuMG mammary epithelial cells, syndecan-1 colocalizes with the actin cytoskeleton at the basal and lateral borders of cells (Rapraeger et al., 1986). Also expressed at the lateral borders are E-cadherin and β_1 integrins, which have major roles in cell adhesion. Surprisingly, expression of syndecan-1 antisense mRNA in the NMuMG cells, which substantially reduces syndecan-1 protein expression, results in the loss of epithelial morphology (Kato et al., 1995). This suggests a role for syndecan-1 in the maintenance of cell–cell adhesion in these cells, although this proteoglycan is apparently not a cell–cell adhesion molecule itself. This role is also independent of the heparan sulfate chains. Treatment of NMuMG cells with heparinases or with chlorate, which blocks sulfation of the heparan sulfate and thus its ability to bind to its ligands, does not lead to a change in their epithelial character (data not shown). Thus, the activity of the proteoglycan seems to be generated through the core protein, as described herein. The reduction in syndecan-1 protein expression correlates with downregulation of E-cadherin and redistribution of β_1 integrins (Kato et al., 1995). These results suggest that syndecan-1 protein may regulate cell–cell adhesion by binding to these receptors directly or by forming a signaling complex that regulates their distribution and/or expression.

It is becoming well documented that proteoglycans participate as coreceptors in the adherence and spreading of cells. The glypican-like heparan sulfate proteoglycan in K1735 mouse melanoma cells mediates initial cell adhesion to fibronectin, thereby allowing lateral association with other cell adhesion molecules that stabilize the adhesion and promote spreading (Drake et al., 1992). Schwann cells transfected with syndecan-1 also exhibit enhanced spreading on heparan sulfate ligands and form more focal contacts, although syndecan-1 is not localized to these structures (Carey et al., 1994*a*; Hansen et al., 1994). Similarly, formation of focal adhesions and stress fibers in fibroblasts requires the participation of proteoglycan together with integrins (Woods et al., 1986). Where explored in more detail in several types of cells, the generation of focal adhesions containing $\alpha_5\beta_1$ integrins appears to require the colocalization of syndecan-4 (Woods and Couchman, 1994). A model is proposed whereby syndecan-4 generates intracellular signals necessary for forming these adhesion complexes. In another example of signaling, NG2, a chondroitin sulfate proteoglycan on melanoma cells, acts in concert with the $\alpha_4\beta_1$ integrin to achieve a fully spread morphology, including the presence of focal contacts. Either of these receptors can be engaged independently to generate the required signals, which appear to involve downstream tyrosine kinases (Iida et al., 1995).

These findings demonstrate that proteoglycans have more than a structural role in adhesion, participating as active signaling complexes at the cell surface. The ability of syndecan-1 to mediate cell spreading in Raji cells suggests a similar mechanism. The ability of the protein to convey this information without a cytoplasmic domain suggests that the signaling mechanism must use other molecules in the plasma membrane. Identification of this signaling mechanism is likely to provide insight into how syndecan-1 and other syndecans participate in the regulation of cell behavior.

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