Heparan Sulfate Regulates Targeting of Syndecan-1 to a Functional Domain on the Cell Surface*

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In polarized B lymphoid cells, syndecan-1 is targeted specifically to a discrete membrane domain termed the uropod that is located at the cell's trailing edge. Within this functional domain, syndecan-1 promotes cell-cell adhesion and concentration of heparin binding growth factors. The present study reveals the surprising finding that targeting of syndecan-1 to uropods is mediated by its heparan sulfate chains and that targeting is regulated by cell surface events rather than solely by intracellular mechanisms. The addition of exogenous heparin or the treatment of polarized cells with heparitinase initiates a rapid and dramatic redistribution of uropod syndecan-1 over the entire cell surface, and a mutated syndecan-1 lacking heparan sulfate chains fails to concentrate within uropods. Interestingly, the heparan sulfate-bearing proteoglycans glypican-1 and betaglycan fail to concentrate in uropods, indicating that targeting may require heparan sulfate structural motifs unique to syndecan-1 or that the core protein of syndecan-1 participates in specific interactions that promote heparan sulfate-mediated targeting. These findings suggest functional specificity for syndecan-1 within uropods and, in addition, reveal a novel mechanism for the targeting of molecules to discrete membrane subcellular domains via heparan sulfate.

Heparan sulfate proteoglycans on the surface of cells regulate cell behavior by binding via their heparan sulfate chains to numerous protein ligands (e.g. growth factors, chemokines, and proteases) (1). Heparan sulfate can act in diverse roles as either an inhibitor or a promoter of protein function or, in some instances, it may act simply to sequester and protect certain proteins at the cell surface. This ability to fine tune protein function is dependent on the structure of the heparan sulfate chain, the binding site for heparan sulfate on the protein, and the availability of the protein-heparan sulfate complex to interact with other molecules such as high affinity receptors for growth factors (2–5). Often these high affinity receptors must interact with other molecules such as high affinity receptors for growth factors. The present study reveals the surprising finding that syndecan-1 over the entire cell surface, and a mutated syndecan-1 lacking heparan sulfate chains fails to concentrate within uropods. Interestingly, the heparan sulfate-bearing proteoglycans glypican-1 and betaglycan fail to concentrate within uropods, indicating that targeting may require heparan sulfate structural motifs unique to syndecan-1 or that the core protein of syndecan-1 participates in specific interactions that promote heparan sulfate-mediated targeting. These findings suggest functional specificity for syndecan-1 within uropods and, in addition, reveal a novel mechanism for the targeting of molecules to discrete membrane subcellular domains via heparan sulfate.

Heparan sulfate proteoglycans can exist on the cell surface in discrete subcellular domains, but our knowledge is limited as to how their specific localization is controlled. For example, in neurons syndecan-2 localizes specifically to synapses, whereas syndecan-3 is concentrated in axons (7). In polarized epithelial cells, syndecan-1 localizes to the basolateral surface and is absent from the apical cell surface (8). Intracellular sorting mechanisms apparently regulate localization of syndecans in these cells, because the concentration of syndecan-2 on dendritic spines at the synapse is dependent on regions within the cytoplasmic domain (9), whereas deletion of the last 12 amino acids of the syndecan-1 cytoplasmic domain results in the expression of the mutant proteoglycan at both the apical and basolateral surfaces of Madin-Darby Canine Kidney (MDCK) cells (10). In addition to proteoglycan core protein domains, heparan sulfate apparently can affect intracellular sorting mechanisms, because the amount of glypican-1 present on the apical surface of polarized epithelial cells is inversely related to the heparan sulfate content of the proteoglycan (8). Thus, heparan sulfate on glypicans may interfere with its intracellular sorting to apical compartments, or perhaps heparan sulfate acts to direct sorting specifically to basolateral compartments.

In contrast to intracellular interactions that regulate proteoglycan targeting to specific domains, much less is known regarding extracellular events at the cell surface that direct proteoglycan distribution. Studies have shown that cross-linking of syndecan-1 or syndecan-4 extracellular domains at the cell surface can promote sequestering of these molecules within cholesterol-rich, detergent-insoluble lipid rafts (11, 12). However, a role for heparan sulfate in proteoglycan targeting at the cell surface has not been described, although such activity seems likely based on heparan sulfate's capacity to bind many proteins resident at the cell surface.

Migration of immune cells involves cell polarization, whereby distinct subcellular domains are formed. The leading edge of the migrating cell is termed the lamellipodium, and the trailing edge is called the uropod (13). Receptors that detect and respond to chemoattractant gradients are located within the lamellipodia, whereas adhesion molecules such as ICAMs, CD44, and P-selectin glycoprotein ligand-1 (PSGL-1) are located within the uropod (14). In addition to normal lymphoid cells, we previously reported that myeloma cells exhibit polarization and that syndecan-1 localizes specifically to the uropod of these cells (15). Within the uropod, syndecan-1 promotes homotypic cell adhesion and adhesion to the extracellular matrix and can also interact with and concentrate heparin-binding growth factors. It was recently shown that osteoprotegerin (OPG) binds specifically to the uropods of myeloma cells

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‡ The abbreviations used are: ICAM, intercellular adhesion molecule; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; GPI, glycosylphosphatidylinositol; TDM, triple deletion mutant.
through interaction with the heparan sulfate chains of syndecan-1 (16). Following binding, the OPG is taken up by the cell and degraded, implying a mechanism by which myeloma cells can enhance bone turnover. Thus, the subcellular localization of syndecan-1 to the uropod has an important and distinct impact on the behavior of tumor cells and, perhaps, on that of other cells in the tumor microenvironment.

The present study reveals that syndecan-1 is targeted to the uropod via its heparan sulfate chains. Surprisingly, neither the cytoplasmic nor the transmembrane domains of syndecan-1 are required for targeting, but the extracellular core protein domain is important because other heparan sulfate-bearing proteoglycans (betaglycan and glypican-1) fail to target specifically to uropods. These results provide further evidence for functional specificity among genetically distinct heparan sulfate proteoglycans and demonstrate that heparan sulfate chains can participate in regulating the localization of proteoglycans to specific sites on the cell surface, thereby compartmentalizing their function.

**EXPERIMENTAL PROCEDURES**

**Antibodies and Reagents**—The following monoclonal antibodies were used: FITC-labeled mouse anti-human syndecan-1 (1D138) antibody (clone HB4, Sorotec, Oxford, UK) (17); FITC-labeled rat anti-mouse syndecan-1 antibody (clone 281.2) (18); biotinylated anti-human ICAM-1 (CD54) (clone HA58, BD Biosciences); rabbit anti-rat glypican-1 polyclonal antibody (kindly provided by Dr. Arthur Lander) (19); and anti-heparan sulfate antibody (clone 10E4, Seikagaku America, Falmouth, MA) (20). Triton X-100, cholera toxin beta-FITC, cytochalasin-D, and heparin isolated from porcine intestinal mucosa were purchased from Sigma.

**Cell Lines and Transfections**—ARH-77 cells (American Type Culture Collection, Manassas, VA) were grown in RPMI 1640 medium supplemented with 5% fetal calf serum. These cells are Epstein-Barr virus-positive lymphoblastoid cells established from a patient with plasma cell leukemia. CAG cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. These cells were established at the Arkansas Cancer Research Center from Histopaque-1077-separated bone marrow aspirates taken from a myeloma patient. Constructs used in transfections were prepared, cloned into expression vectors, and transfected into cells as described previously (Fig. 1). Cells expressing the transfected proteoglycans were enriched and purified either by cell sorting or panning using the appropriate antibody.

**Immunostaining**—Cells (5 × 10⁴) were harvested from culture and gently fixed in pre-warmed (37 °C) formaldehyde (final concentration, 3.7%) and fixed for 5 min. Cells were washed twice by adding 1 ml of PBS and centrifuged at 800 rpm for 5 min, followed by resuspension in 100 μl of either FITC-conjugated or unconjugated primary antibodies at room temperature for 45 min. When unconjugated primary antibodies were used following removal of the primary antibody and washing, FITC-labeled secondary antibodies were incubated with cells for 30 min at room temperature. Cells were washed and resuspended in one drop of Vectashield mounting medium (Vector Laboratories, Burlingame, CA), mounted on glass slides, and analyzed for proteoglycan distribution to uropods as described previously (15) by fluorescence and phase contrast microscopy using an Olympus BX60 microscope equipped with a BX-FLA reflected light fluorescence attachment (Olympus America, Melville, NY) and a Nikon digital camera.

**Disruption of Syndecan-1 Targeting to Uropods**—For disruption of syndecan-1 targeting using heparin, exogenous heparin (110 μg/ml) was added to cells for 30 min at 37 °C followed by fixation and immunostaining. In some studies following incubation of cells for 30 min in heparin, the heparin was washed out, and the cells were resuspended in PBS followed by incubation in PBS at 37 °C. Analysis of syndecan-1 localization was performed at 1, 2, and 4 h after removal of the heparin. To remove heparan sulfate chains from the cell surface, cells were sequentially treated twice with heparitinase (1 milliunit/ml; Seikagaku America) at 37 °C for 30 min and analyzed for syndecan-1 localization.

**RESULTS**

**Targeting of Syndecan-1 to Uropods Is Dependent on Heparan Sulfate Chains**—Polarized myeloma cells exhibit a clearly defined morphological protrusion known as the uropod (Fig. 2A). Immunofluorescence staining of polarized myeloma cells demonstrates that syndecan-1 concentrates on uropods on the surface of myeloma cells (Fig. 2B). Staining of these cells with antibody to ICAM-1, a well characterized marker for uropods (21), demonstrates co-localization of syndecan-1 and ICAM-1 on uropods and confirms that syndecan-1 indeed is localized predominantly on the uropod (Fig. 2, C and D). This, together with our previous study, confirms that under physiological conditions syndecan-1 is a marker for uropods present on polarized myeloma cells (15). However, following exposure of the cells to exogenous heparin, a dramatic redistribution of syndecan-1 occurs, resulting in relatively equal distribution of syndecan-1 over the entire cell surface (Fig. 2E) rather than concentration predominantly on uropods (note uniform cell surface staining of syndecan-1 in Fig. 2E compared with intense staining on uropods in Fig. 2, B and C). Removal of heparan sulfate chains from the cell surface with heparitinase also promotes redistribution of syndecan-1 over the entire cell surface (Fig. 2F). This redistribution occurs even though the cells clearly retain their uropods and polarized morphology. Dual staining with antibodies to syndecan-1 and ICAM-1 confirms that uropod integrity is maintained in the presence of heparin and that, although syndecan-1 becomes distributed over the entire cell surface, ICAM-1 is retained predominantly on uropods (Fig. 2, G and H). The effect of heparin or heparitinase on syndecan-1 distribution is not limited to a few cells; rather, almost all of the cells treated exhibit a loss of syndecan-1 concentration within the uropod (Table I). To confirm that heparan sulfate is required for syndecan-1 targeting to the uropod, ARH-77 cells were employed. These cells are often polarized, although we have previously established that the uropods on ARH-77 cells
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FIG. 2. Targeting of syndecan-1 to the uropod requires heparan sulfate chains. Phase microscopy (A) and immunofluorescent staining for syndecan-1 (B) show that syndecan-1 is concentrated in the uropod (arrows) with little staining over the remaining cell surface. Dual staining for syndecan-1 (C) and ICAM-1 (D), a marker for uropods, confirms that syndecan-1 localization is predominantly within uropods. Dual staining was performed using a FITC-labeled antibody to syndecan-1 and a biotinylated antibody to ICAM-1 followed by incubation of cells with avidin-Texas Red. Syndecan-1 redistributes over the entire cell surface following the addition of 10 μg/ml heparin to the culture media for 30 min (E) or when cells are treated with heparitinase, an enzyme that strips heparan sulfate chains from the syndecan-1 core protein (F). Note that, although the syndecan-1 redistributes, the cells remain polarized with distinct uropods (arrows). Dual staining of syndecan-1 (G) and ICAM-1 (H) on cells treated with heparin confirms that syndecan-1 localizes over the entire cell surface, whereas ICAM-1 remains concentrated on uropods. In ARH-77 cells transfected with murine syndecan-1, the proteoglycan localizes to uropods (J). However, when ARH-77 cells are transfected with a form of the syndecan-1 core protein unable to bear heparan sulfate chains (SynTDM), the core protein localizes over the entire cell surface rather than concentrating solely on uropods (J).

TABLE I
Syndecan-1 redistributes following the addition of exogenous heparin or treatment of cells with heparitinase

| Treatment        | Pattern of syndecan-1 expression |
|------------------|----------------------------------|
|                  | Uropod  | Partial uropod | Entire cell surface |
| None             | 92      | 1              | 7                   |
| Heparin          | 1       | 9              | 90                  |
| Heparitinase     | 0       | 7              | 93                  |

are not as prominent as those of CAG cells (15). ARH-77 cells lack syndecan-1 expression, but, following transfection with a cDNA coding for syndecan-1, the proteoglycan localizes in the uropod (Fig. 2f). In contrast, when ARH-77 cells are transfected with a cDNA coding for a mutated form of the syndecan-1 core protein lacking all three heparan sulfate attachment sites (SynTDM; triple deletion mutant) (22), the core protein does not concentrate in uropods but is distributed over the entire cell surface (Fig. 2f). Together, these findings demonstrate that heparan sulfate chains are required for targeting of syndecan-1 to uropods.

To determine whether the effects of heparin on the distribution of syndecan-1 at the cell surface are reversible, cells were incubated with heparin for 30 min, washed three times in PBS to remove exogenous heparin, resuspended in PBS at 37°C, and analyzed for syndecan-1 distribution. Two hours after the removal of heparin, an accumulation of syndecan-1 is detected within the uropod and, by 4 h after removal of heparin, the majority of syndecan-1 is detected solely in the uropod (Fig. 3, A–C). The finding that syndecan-1 relocates to the uropod when cells are suspended in PBS in the absence of serum suggests that targeting to the uropod is not mediated by serum proteins that cross-link syndecan-1 to each other or to other cell surface molecules. It is possible that some or all of the syndecan-1 that concentrates in the uropod following removal of exogenous heparin is actually newly synthesized rather than redistributed syndecan-1 (i.e. the syndecan-1 that exits the uropod in the presence of exogenous heparin could be shed or recycled, and the syndecan-1 that accumulates in the uropod over 4 h could be newly synthesized). Thus, to further examine syndecan-1 redistribution to uropods following incubation of cells with exogenous heparin, FITC-labeled antibody to syndecan-1 was added to cells as a tracer of the cell surface molecule. As expected, immediately after labeling with antibody, the syndecan-1 is seen over the entire cell surface (Fig. 3D). However, after only 30 min almost all of the labeled syndecan-1 is detected within uropods (Fig. 3E). Interestingly, the presence of antibody bound to syndecan-1 appears to accelerate the targeting process as compared with relocalization to uropods in the absence of antibody (compare Fig. 3, E and B). A similar acceleration of targeting to the uropod is seen with CD43 when it is engaged by antibody (23). Together, the results support the conclusion that syndecan-1, once on the cell surface, is actively

Fig. 3. Redistribution of syndecan-1 by addition of exogenous heparin is reversible and dynamic. Heparin was added to CAG cells for 30 min and washed out, and the cells were fixed and stained for syndecan-1 immediately (A) or placed back in the incubator at 37°C for 2 (B) or 4 h (C) and then fixed and stained for syndecan-1. In a separate experiment, following treatment of cells with heparin, cells were washed and stained for syndecan-1 at 37°C and observed either immediately (D) or 30 min after staining with antibody (E). In the presence of cytochalasin D (10 μM for 60 min at 37°C), syndecan-1 does not accumulate within the uropod at 4 h (F) after removal of heparin. Staining of uropods with cholera toxin beta-FITC demonstrates that the addition of heparin to CAG cells does not disrupt lipid rafts within the

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ARH-77 cells expressing either glypican-1 (A and B) or betaglycan (C and D) were immunostained to determine the location of the proteoglycan core proteins (A and C) or the cell surface heparan sulfate (B and D). Neither proteoglycan core protein concentrates in the uropod (arrows), and heparan sulfate is localized over the entire cell surface. Syndecan-1 bearing a single heparan sulfate chain at amino acid position 47 (Syn-1^{1 HS47}) targets predominantly to the uropod (E).

targeted to the uropod and that the heparin-mediated disruption of syndecan-1 localization to uropods is dynamic and reversible.

To examine how the actin cytoskeleton affects syndecan-1 localization on these cells, we employed cytochalasin D, which blocks actin polymerization. When cells were exposed to cytochalasin D after removal of heparin, the syndecan-1 remains distributed over the entire cell surface even 4 h after removal of heparin (Fig. 3F). Cytochalasin D is known to disrupt the polarized morphology of lymphocytes, causing cells to round up and abolish uropods. Thus, this result indicates that, in the absence of uropods, syndecan-1 fails to concentrate within any specific region of the cell surface.

Recent studies show that lipid rafts comprise subdomains within uropods of T lymphocytes (24). To determine whether the addition of heparin disrupts lipid rafts within the uropod, cells were stained with the FITC-labeled cholera toxin beta. Staining of uropods with cholera toxin remained prominent in the presence of heparin, indicating that lipid rafts remained unperturbed, even though the syndecan-1 escapes the uropod (Fig. 3G). Thus, heparin apparently does not alter uropod morphology or disrupt lipid raft organization within the uropod.

Specificity of Syndecan-1 Targeting to Uropods via Heparan Sulfate—To determine whether other heparan sulfate proteoglycans would target specifically to the uropod, ARH-77 cells transfected with a cDNA for either glypican-1 or betaglycan were examined. Neither of these proteoglycans share significant homology to the syndecan-1 core protein, yet both bear heparan sulfate chains (25, 26). Surprisingly, neither of these heparan sulfate-bearing proteoglycans localize specifically to uropods (Fig. 4, A and C), rather, they are detected over the entire cell surface, even on those cells with clearly distinguishable uropods (arrows). In fact, some of the cells appear to have proteoglycan concentrated in areas distinct from uropods. Staining with antibody specific for heparan sulfate chains confirms that these proteoglycans bear heparan sulfate and that this heparan sulfate is not concentrated within uropods (Fig. 4, B and D). Moreover, the lack of detection of any focal concentration of heparan sulfate in the uropods and the lack of intense staining over the entire cell surface (Fig. 4, B and D) indicate that the heparan sulfate-dependent targeting mechanism to the uropod has not been saturated.

One possible explanation for the difference between syndecan-1 and these other proteoglycans in their localization could be the number of heparan sulfate chains present on the core protein. Glypican-1 has three closely grouped heparan sulfate attachment sites in a region of the core protein close to the cell membrane (27). It is not known how many heparan sulfate chains are actually present on each glypican-1 molecule. Beta-glycan bears only one heparan sulfate chain (28). In contrast, syndecan-1 has three sites for heparan sulfate attachment and is known to have multiple heparan sulfate chains on each core protein (29). To determine whether localization of syndecan-1 to the uropod requires multiple heparan sulfate chains, we utilized a mutated form of syndecan-1 having two of its three heparan sulfate attachment sites mutated. This mutated syndecan-1 is composed of a core protein having a single heparan sulfate chain attachment site at amino acid 47 (22, 30). Although it is not as strongly associated with the uropod as wild-type syndecan-1, most of the proteoglycan does appear to concentrate in uropods (Fig. 4E). This suggests that the failure of glypican-1 and betaglycan to localize specifically to uropods is not due to the fact that they may bear only one heparan sulfate chain.

The Cytoplasmic and Transmembrane Domains of Syndecan-1 Are Not Required for Targeting of the Proteoglycan to Uropods—The syndecan-1 core protein is composed of a cytoplasmic, transmembrane, and heparan sulfate-bearing extracellular domain. To determine whether the cytoplasmic and/or transmembrane domains are required for targeting of syndecan-1 to the uropod, cells were transfected with either a mutated form of syndecan-1 lacking the coding region for the cytoplasmic domain (Syn^{779}, see Fig. 1) or with a chimeric proteoglycan composed of the syndecan-1 extracellular domain linked to the GPI anchor of glypican-1 (Syn/glyp). Both of these proteoglycans are known to bear heparan sulfate chains (22). When expressed in either ARH-77 cells or CAG cells, the Syn^{779} and Syn/glyp proteoglycans are found concentrated in uropods (Fig. 5). Moreover, a chimera composed of the glypican-1 extracellular domain linked to the transmembrane and cytoplasmic domains of syndecan-1 (Glyp/syn) fails to concentrate in uropods. Staining with antibody to heparan sulfate confirms that heparan sulfate chains are present on the Glyp/syn chimeric
proteoglycan (Fig. 5G). Thus, the cytoplasmic and transmembrane domains of syndecan-1 are not required for targeting syndecan-1 to the uropod, and the addition of these domains to glypican-1 does not promote its concentration within uropods.

**Syndecan-1 within Uropods Resists Detergent Extraction**—When cells are extracted with Triton X-100 at 37 °C, syndecan-1 is retained within the uropod (Fig. 6, A and B). We have previously demonstrated that cooling cells on ice for 30 min results in the loss of syndecan-1 from the uropod, with subsequent redistribution of the proteoglycan over the entire cell surface (15). Upon brief cooling on ice for 5 min, some of the syndecan-1 remains in the uropod, whereas some is lost to surrounding membrane surfaces (Fig. 6C). Extraction of these cells with Triton X-100 results in specific removal of only the non-uropod syndecan-1 (Fig. 6D). Thus, at 4 °C, syndecan-1 loses its association with the uropod and becomes easily extractable with detergent. Similarly, following the addition of exogenous heparin and redistribution of syndecan-1, the proteoglycan is readily extracted with detergent, as is the form of syndecan-1 that lacks heparan sulfate chains (Fig. 6, E and F). These results indicate that the loss of syndecan-1 localization to uropods is associated with a change in the way that syndecan-1 is anchored to the cell.

**DISCUSSION**

**Targeting of Syndecan-1 to Uropods Requires Heparan Sulfate**—Localization of syndecan-1 to the uropods of B lymphoid cells is functionally important, because it promotes both tight cell adhesion and concentration of growth factors (15). In the present study we describe the surprising finding that this localization requires the heparan sulfate chains of syndecan-1. Either the addition of exogenous heparin or the removal of heparan sulfate from the cell surface results in the loss of specific localization of syndecan-1 to the uropod and the rapid redistribution of the proteoglycan over the entire cell surface. In addition, when a mutated form of syndecan-1 lacking heparan sulfate chains is expressed on cells, it fails to target specifically to uropods. Although little is known regarding mechanisms that direct targeting of heparan sulfate proteoglycans to discrete subcellular domains, our findings clearly establish that heparan sulfate can play a determining role in this process.

Intracellular sorting of proteoglycans to distinct cell surface compartments can be influenced by both core protein structure and heparan sulfate chains (8, 10). However, intracellular sorting mechanisms do not explain targeting to, and retention of, syndecan-1 within the uropods because, when dispersed by exogenous heparin, the syndecan-1 relocates to the uropod once heparin is removed. Moreover, if the syndecan-1 is tagged with antibody after it is dispersed by heparin, the labeled syndecan-1 rapidly relocates to the uropod. Together, these results indicate that targeting of syndecan-1 to uropods involves events occurring at the cell surface and is not solely due to targeted delivery of intracellular syndecan to the uropod compartment. Furthermore, in parallel to what we have demonstrated here for B lymphoid cells, Triton X-100 extraction of syndecan-1 from Chinese hamster ovary cells is also facilitated by removal of heparan sulfate chains or addition of exogenous heparin (10). Thus, our discovery that syndecan-1 organization on the cell surface is regulated by heparan sulfate may apply to non-lymphoid cells as well.

**Targeting of Syndecan-1 to the Uropod Requires Determinants within the Syndecan-1 Core Protein**—Interestingly, glypican-1 and betaglycan, two heparan sulfate-bearing proteoglycans unrelated to syndecan-1, fail to localize to uropods. Thus, the presence of heparan sulfate chains alone is not sufficient for targeting heparan sulfate proteoglycans to uropods. The finding that syndecan-1 targets to uropods, and other heparan sulfate-bearing proteoglycans do not, suggests that the core protein of syndecan-1 participates in localization of the proteoglycan, perhaps by complexing with other molecules. This could occur via interactions between the syndecan-1 ectodomain and other cell surface molecules. In support of this is evidence that both syndecan-1 and syndecan-4 ectodomain core proteins have domains that can interact with other molecules (22, 31). It is speculated that these ectodomains interact with ligands on the cell surface as part of the formation of molecular complexes, thereby generating signaling events that influence cell adhesion and motility. If this is the case in uropods, syndecan-1 could play a pivotal role in the formation of multi-molecular complexes. This could be critical in triggering specific signaling pathways as a result of syndecan-1-mediated adhesion that occurs via the uropod interaction with adjacent cells or the extracellular matrix.

The localization of syndecan-1 within uropods appears not to be dependent on a direct interaction between syndecan-1 and the cytoskeleton. This is supported by the finding that syndecan-1 lacking its cytoplasmic and transmembrane domains (Syn/glyp) localizes to the uropod. Nonetheless, when wild-type syndecan-1 is localized within uropods, it apparently does associate with the cytoskeleton. This is indicated by the finding that syndecan-1 within the uropod is not extracted with detergent at 37 °C. Further analysis is necessary to determine the functional relevance of syndecan-1 interactions with the cytoskeleton within uropods and the nature of the associations between wild-type syndecan-1 and the cytoskeleton (and whether these associations occur directly or via molecular complexes within the membrane or intracellular domains).

An alternative explanation for the difference in localization between syndecan-1 and the other proteoglycans (glypican-1 and betaglycan) is that these other proteoglycans bear heparan sulfate chains that lack structural features necessary to promote their localization to uropods. Heparan sulfates are linear polysaccharides containing highly sulfated domains. The exact pattern of sulfation within a sulfated domain and the number and spacing of sulfated domains along a heparan sulfate chain are highly variable, thereby generating vast structural and functional heterogeneity among heparan sulfate chains (3, 32).
Although the structure and function of heparan sulfate chains on identical core proteins can differ between cell types (33, 34), little is known regarding differences in heparan sulfate among distinct proteoglycans within the same cell type. A recent study showed only slight structural differences between the heparan sulfates of syndecan-4 and glypican-1 isolated from rat embryo fibroblasts but no difference in the ability of the heparan sulfates to bind the Hep II domain of fibronectin (35). Our previous studies have shown that, when expressed in B lymphoid cells, betaglycan, like syndecan-1, can mediate cell adhesion via its heparan sulfate chains (36). These findings support the notion that different proteoglycan core proteins on the same cell type have heparan sulfate chains that are functionally similar. However, it remains to be determined if structural differences in heparan sulfate account for differences between the localization of syndecan-1 and other heparan sulfate proteoglycans.

In conclusion, the present study establishes that the targeting of syndecan-1 to uropods requires the presence of heparan sulfate chains on the syndecan-1 core protein. Targeting of syndecan-1 to uropods is reversible and dynamic and requires the syndecan-1 ectodomain core protein but not the syndecan-1 cytoplasmic and transmembrane domains. The finding that betaglycan and glypican-1 fail to target to uropods provides further rationale for the existence of multiple genetic forms of heparan sulfate-bearing proteins and suggests a unique function of syndecan-1 within this subcellular membrane domain.

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