We have identified a cDNA that encodes a variant form of murine syndecan-1. The variant cDNA lacks the sequence corresponding to the first 132 nucleotides of the third exon of the syndecan-1 gene. The corresponding message is rare. The alternative splice respects the reading frame and deletes 44 amino acids from the protein, joining the S45GS47GT sequence to a variant immediate downstream context sequence. This sequence context initiates with alanine instead of glycine as residue 50, reducing the number of SGXG sequence motifs in the protein from two to one. Expression of this variant syndecan-1 in Madin-Darby canine kidney or MOLT-4 cells yielded a recombinant proteoglycan with a reduced number and clustering of the heparan sulfate chains. Both the conversions of Ala50 and of Lys53 into glycine enhanced the heparan sulfate substitution of the variant protein. These findings support the concept that serine-glycine dipeptide signals for glycosaminoglycan/heparan sulfate synthesis depend on sequence context

Syndecan-1 is a transmembrane heparan sulfate proteoglycan that is subject to strong developmental regulations (1). The protein is expressed in spatiotemporal- and cell type-specific patterns and shows, in addition, tissue- and cell-specific differences in glycosylation. This variable glycosylation pertains to the number, size, and fine structure of the glycosaminoglycan chains that substitute the protein (glycanation) and yields a highly polymorphic proteoglycan (2, 3). The mechanisms that regulate this variation are not fully understood, but the differences in glycanation can change the binding properties of the proteoglycan (3, 4). Since heparan sulfate proteoglycans, and the syndecans in particular, are thought to function as co-receptors for various ligands that control cell shape, adhesion, proliferation, and differentiation, these differences in glycanation could be developmentally significant (1).

Analysis of the glycanation of syndecan-1 from mouse mammary epithelial cells has indicated that substitution with heparan sulfate is limited to serines in the N-terminal half of the protein (5). Studies, in Chinese hamster ovary cells, of recombinant chimeric proteoglycans that contained various segments of syndecan-1, and mutations of these segments have identified three serines, occurring in the sequence DGS35GDDS-DNFS3GS57GTG, as the sites that prime heparan sulfate synthesis in this protein (6). The strongest sites for heparan sulfate synthesis in these experiments consisted of the repeat unit S45GS57G, and evidence was obtained for a coupling phenomenon across adjacent Ser-Gly dipeptides that enhances the substitution of these repeats with heparan sulfate. The same studies demonstrated that the synthesis of heparan sulfate on these serines also depended on adjacent clusters of acidic residues and identified the sequence (Ser-Gly)n (n ≥2) and a nearby cluster of acidic residues (D/E)n (n ≥3) within 6 residues as a structural motif that is shared by many of the heparan sulfate proteoglycans (6). This motif incorporates a consensus peptide structure (acidic amino acids closely followed by the tetrapeptide SGXG) identified in prior studies as an efficient acceptor for xylosyltransferase, the enzyme that initiates the assembly of glycosaminoglycan chains (7). In syndecan-1 this acceptor motif is realized twice in the sequence S45GS57GTG, which is encoded by the junction of the second and third exons (TCT GGC TCT GGC ACA (G/G)T) of the syndecan-1 gene (8, 9) and the upstream sequence DDDSNF that immediately flanks the Ser-Gly repeat.

We now report a variant cDNA for murine syndecan-1 that likely results from the use of an alternative splice acceptor site. The alternative splicing process skips part of the third exon but respects the reading frame and, upon translation, is predicted to bring the S45GS47GT sequence in a variant immediate downstream sequence context. Expression of this syndecan-1 cDNA in MDCK1 and MOLT-4 cells indicates that, in comparison to the more common or “standard” form of this protein, the variant protein is less substituted with heparan sulfate and that the clustering of the heparan sulfate chains is disrupted in this proteoglycan. Conversion of the S45GS47GT sequence in the splice variant into S45GS57GTG enhances the glycosaminoglycin.
can substitution of this protein and restores the clustering of the heparan sulfate chains in the proteoglycan. These findings support the concept that the strengths of Ser-Gly dipeptides as signals for heparan sulfate synthesis depend on sequence context and imply that alternative splicing mechanisms may in part control the molecular polymorphism of syndecan-1 and the efficiency and versatility of this protein in its co-receptor functions.

**EXPERIMENTAL PROCEDURES**

Isolation of a Variant Syndecan-1 cDNA—Poly(A)+ RNA was isolated from whole 14-day-old NMRI mouse embryos, annealed to oligo(dT), and used as template for the synthesis of cDNA by Moloney murine leukemia virus reverse transcriptase (10). Sequences coding for mouse syndecan-1 were amplified from this cDNA by PCR. The PCR mixture contained 2 units of Taq DNA polymerase, 1× Taq buffer (16.6 mM ammonium sulfate, 67 mM Tris/HCl, pH 8.8, 6.7 mM MgCl2, 10 mM β-mercaptoethanol, 6.7 μM EDTA, 170 μg/ml of BSA), 10% MeSO2, 1 ng of cDNA, 1.5 mM dNTP, and 1.4 mM primers. The sense and antisense primers (Fig. 1) were 42-mers identical, respectively, to the murine leukemia virus reverse transcriptase (10). Sequences coding for mouse syndecan-1 were amplified from this sequence (from residue 1131 to 1172), as reported by Saunders et al. (11). After 40 thermal cycles (1 min denaturation at 94°C, 1 min annealing at 55°C, and 1 min extension at 68°C), the amplification products were analyzed in 1.1% agarose gels and detected by ethidium bromide staining. The amplification products were analyzed. Variant insert of only 800 bp (MoSyn-1/6) and its corresponding standard size of 933 bp (MoSyn-1/6) were further characterized by restriction site mapping and completely sequenced by the dyeoxy chain termination method (12), using supercoiled plasmid, a modified T7 DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden), T7 and SP6 primers, and both dGTP and C7-deaza-dGTP.

**Characterization of the Syn-1s and Syn-1v Proteoglycan—** Transfected MOLT-4 cells were analyzed for cell-surface expression of syndecan-1 protein and heparan sulfate by flow immunocytochemistry, using the antibodies 281-2, 10E4, and 3G10 as described before (16). The analyses were performed on a FACSort (Becton Dickinson, Mountain View, CA), and data were analyzed with the program Lysis II.

Cell Binding Assay—Cell binding to type I collagen from rat tails (Roche Molecular Biochemicals, Mannheim, Germany) was performed in polyvinyl 96-well U-bottom plates (Corning Costar Europe, Badhoevedorp, Netherlands), using a modified version of the method of Koda et al. (17). Collagen coats were made by overnight incubation at 4°C (100–300 μg of collagen in 1 ml of a 2:1 (v/v) mixture of 10 mM acetic acid/PBS, pH 4.4, per well). After removal of the non-absorbed collagen solution, the wells were rinsed twice with 0.3% BSA (Sigma, Bornem, Belgium) in Dulbecco’s PBS (Life Technologies, Inc., Merelbeke, Belgium) and overcoated for 1 h at room temperature with 1% heat-denatured BSA in PBS.

MOLT-4 cell cultures were split at a concentration of 3 × 105 cells/ml, 24 h before the cell binding assays. Just prior to the assay, the cells were labeled with the vital cytoplasmic fluorescent dye calcein AM (Molecular Probes Europe, Leiden, Netherlands), according to Braut-Boucher et al. (18). Cells, washed twice with DMEM/F12 (1:1) medium (Life Technologies, Inc.), were suspended at 2 × 106 cells/ml in Dulbecco’s PBS, 1% BSA and incubated for 30 min at 37°C with 25 μM (from a 1 mM stock in MeSO2) calcein AM in PBS, 0.5% BSA. Labeled cells were added to the coated microtiter wells (100 μl) at 5 × 104 cells/ml (in PBS, 1% BSA) and allowed to settle for 30 min at room temperature. Then, the microtiter plates were centrifuged at 1000–4000 rpm for 5–15 min in a Heraeus Minifuge 2 and scanned by a Fluorimeter. The effect of heparin on cell adhesion was tested by preincubating the collagen-coated wells for 30 min with 100 μg/ml porcine intestinal heparin (Sigma) in PBS and adding 100 μg/ml heparin to the medium during the adhesion assay.

**RESULTS**

Isolation of a Variant Murine Syndecan-1 cDNA—By using primers corresponding to the published N-terminal and C-terminal amino acid sequences of murine syndecan-1 (11), we isolated two distinctive reverse transcription-PCR products from 14-day total mouse embryo RNA: the expected standard product of 933 bp (corresponding to residues 240–1172 of the published murine cDNA sequence) and a “variant” shorter product. Sequencing indicated that the shorter product was missing 132 bp, corresponding to residues 388–519 of the published cDNA sequence (Fig. 1). The boundaries of the missing sequence complied with the so-called GT-AG rule for splice site selection, suggestive of alternative splicing of the syndecan-1 gene transcript. The putative splice variant lacked the nucleotide sequence located just downstream of the nucleotides encoding the S+G5+G7 GT amino acid sequence, which contains the two serines identified as the major attachment sites for heparan sulfate in this protein (6). The removal of these 132 bases respects the reading frame, creating a novel S+G5+G7 GT sequence (Ala94 to Ala94 in the original sequence) instead of glycine as residue 50, followed by the sequence GEEPEG. Thus, the splice reduced the number of SGXG motifs in the protein and generated a novel immediate downstream context for the Ser-Gly repeat, rich in acidic amino acids but also containing a charged residue.

Expression of the Variant Mouse Syndecan-1 Message—To
assess the relative abundance of the variant message, we performed ribonuclease protection assays on total and poly(A) RNAs from different embryonic and adult mouse tissues. By using a 446-nt in vitro antisense transcript for the standard form of mouse syndecan-1, we observed a major protected band with the size of a 430-nt fragment, consistent with the prediction for the standard syndecan-1 mRNA. Alignment of the probe with the variant cDNA predicted that protection by the alternatively spliced RNA should yield fragments of 200 and 101 nucleotides. Fragments of this size were present in 15-day mouse embryonic lung and adult lung RNA but remained nearly undetectable in 15-day total mouse embryo or adult liver RNA, even after prolonged exposures (Fig. 2). The ratio of the higher (430) versus the lower (200 + 101) bands was at least 10:1, indicating that the alternatively spliced message represented at most a minor population of the total syndecan-1 message in these tissues and a fortiori the whole embryo. The long exposure autoradiograms revealed several additional discrete protected bands that could not be accounted for by the currently identified syndecan-1 cDNAs. These bands might represent additional splice variants or unspliced RNAs, but this possibility was not further investigated.

In Northern blot experiments, cDNA probes for the standard and the alternatively spliced forms of mouse syndecan-1 and also a 131-bp probe corresponding to the fragment that was missing in the splice variant, all recognized both the 2.6- and 3.4-kb messages for this proteoglycan, in a similar 3:1 relative abundance as described previously (not shown).

Fig. 1. Sequence of the murine syndecan-1 variant. Nucleotide sequence (upper line) of the variant syndecan-1 cDNA (upper case) and standard syndecan-1 cDNA (upper lower case), and predicted syndecan-1v (upright) and syndecan-1s (upright + italics) amino acid sequences (lower line). The nucleotide sequences corresponding to the primers used in the reverse transcription-PCR are indicated in bold. The nucleotide sequences corresponding to the probes used in the ribonuclease protection assay (see Fig. 2) are underlined. The serine residues in syndecan-1s that support the synthesis of heparan sulfate (6) are highlighted by shading. Acidic residues in syndecan-1s and syndecan-1v that occur upstream or downstream of these serines within a distance of six residues are indicated in bold. The GenBank™ accession number for the variant cDNA sequence is AF134897.
Synecan-1 Splice Variant

FIG. 3. Expression of syndecan-1 in wild-type and transfectant MDCK cells. MDCK cells were transfected with pRc/RSV/Syn-1s or pRc/RSV/Syn1-v encoding, respectively, the standard and variant form of murine syndecan-1. Total proteoglycan extracts from wild-type MDCK cells (SYN-1E) and from the transfectants (SYN-1S and SYN-1V) were digested (+) or not digested (−) with heparitinase (Hase) and chondroitinase ABC (Case) and were fractionated by electrophoresis in SDS gels. The Western blots of these gels were stained with mAb 2E9, which reacts with the evolutionarily conserved cytoplasmic domain of syndecan-1.

attachment of glycosaminoglycans to proteins (7), whereas clusters of acidic residues adjacent to coupled Ser-Gly dipeptides appear to be part of the consensus structures for heparan sulfate glycosaminoglycan attachment (6). The variant syndecan-1 cDNA coded for S45GS47GTA instead of S45GS47GTG (one instead of two partially overlapping SGX tetrapeptide sequences), flanked by a more acidic downstream context, suggesting that the splice potentially influenced the number, type, or structure of the glycosaminoglycan (chondroitin or heparan sulfate) chains that were bound to the core protein. We have analyzed this possibility by constructing Syn-1s and Syn-1v pRc/RSV expression vectors encoding, respectively, the standard and variant (alternatively spliced) forms of murine syndecan-1 (see “Experimental Procedures”) and expressing both these proteins in MDCK cells. Figs. 3 and 4 show results of Western blotting experiments with these cells.

Non-transfectant MDCK cells expressed a syndecan-1-related proteoglycan, as indicated by the mouse monoclonal antibody 2E9, which is specific for the evolutionarily highly conserved (and structurally very similar) cytoplasmic domains of the syndecans-1 and -3. This syndecan carried some chondroitin sulfate but mostly heparan sulfate. It yielded a core of ~74 kDa after heparitinase treatment and slightly higher yields of this protein after combined heparitinase and chondroitinase ABC digestions, behaving as a heparan sulfate or heparan sulfate-rich hybrid proteoglycan (Fig. 3). This canine syndecan was not detected by the rat anti-mouse syndecan-1 antibody 281-2, which is directed against the evolutionarily less conserved ectodomain of this protein (not shown), but most likely represents syndecan-1 (syndecan-3 is not expressed in these cells).

MDCK cells transfected with Syn-1s cDNA (encoding the standard form of murine syndecan-1) produced both 281-2-reactive (Fig. 4) and 2E9-reactive proteoglycans (Fig. 3). The properties of the 281-2-reactive (transfectant) and the 2E9-reactive (transfectant and endogenous) proteoglycan species were quite similar (although not completely identical) and reminiscent of the properties of the 2E9-reactive endogenous syndecan expressed by non-transfectant cells. Chondroitinase ABC reduced the amount of syndecan trapped at the interface of the stacking and separating gels but yielded no discrete immunoreactive bands in the separating gels, indicating that these chondroitinase-digested syndecans were still polydisperse. The effect of the chondroitinase ABC treatment, however, was more marked on the 281-2-reactive (transfectant) forms than on the 2E9-reactive (transfectant and endogenous) forms. Heparitinase-treated syndecans still migrated as a smear or stagger of bands, with small yields of ~74-kDa core protein. Clearly, in relative terms, the heparitinase digestion of the transfectant syndecan (281-2-reactive) yielded less ~74-kDa core protein than the heparitinase digestion of the endogenous syndecan (2E9-reactive) in the non-transfectant cells. Combined heparitinase and chondroitinase ABC digestions yielded single 2E9- or 281-2-reactive core proteins of ~74 kDa. These results indicated that transfectant syndecan-1s was primarily a heparan sulfate/chondroitin sulfate hybrid proteoglycan with a core protein of ~74 kDa.

MDCK cells transfected with Syn-1v (the variant murine syndecan-1) cDNA also produced 2E9 and 281-2-reactive proteoglycan, but the properties of the 281-2-reactive (Fig. 4) and the 2E9-reactive (Fig. 3) syndecans were dissimilar and different from the syndecans expressed in non-transfectant cells or in cells transfected with Syn-1s cDNA. The 281-2-reactive (transfectant) proteoglycan in Syn-1v cells entered the separating gels to a larger extent than the 2E9-reactive (transfectant + endogenous syndecan-1) proteoglycan in the same cells, the 2E9 or 281-2-reactive proteoglycan in the cells transfected with Syn-1s, or the 2E9-reactive proteoglycan (endogenous syndecan-1) in non-transfectant. After treatment with only chondroitinase ABC, a substantial fraction of the 2E9-reactive syndecan-1 and an even larger fraction of the 281-2-reactive syndecan-1 migrated as a broad band of ~70–120 kDa and small amounts as a discrete ~56-kDa band. Single heparitinase digestion yielded a broad band of ~65–100 kDa and a sharp band of ~56 kDa that reacted with the mAbs 2E9 and 281-2, but the 56-kDa band was more conspicuous in the 281-2-staining than in the 2E9-staining reaction. Combined heparitinase and chondroitinase ABC digestions yielded two sharp bands of ~74 and ~56 kDa that reacted with mAb 2E9, and only one band of ~56 kDa that reacted with mAb 281-2. Single enzyme digestions produced also more important band shifts and resulted in more quantitative transfers of immunostainable proteoglycan than observed for the endogenous synde-
The immunopurified transfectant syndecans were also analyzed by Western blotting, using the mAbs 281-2 (anti-core protein) and 3G10 (anti-ΔHSH) as immunoprobes (Fig. 4). Doubly heparitinase and chondroitinase ABC-digested recombinant syndecan-1s yielded ~74-kDa core protein that was strongly stained by both 281-2 and 3G10. After a similar treatment, recombinant syndecan-1v yielded ~56-kDa core protein that was strongly stained by mAb 281-2 but much more weakly by mAb 3G10. Since the heparitinase treatment leaves only one Δ4,5-glucuronate (3G10 epitope) per chain in association with the core protein, these results indicated a lower HS chain number/protein ratio in the syndecan-1v than in the syndecan-1s proteoglycans.

When the purified HS chains were digested with heparinase prior to gel filtration over Sepharose CL6B, two discrete populations of N-acetylated heparinase-resistant regions, one with a size ~9.3 kDa and a second with a size of ~3.6 kDa, were observed in all three preparations. The ratios between these two peaks were similar in all three samples. This indicated similar spacings between highly sulfated (heparinase-sensitive) domains in all these chains (not shown). The heparan sulfate chains from endogenous syndecan-1 and from recombinant syndecans also eluted at near identical positions from ion-exchange columns, suggesting similar average charge densities or sulfations (not shown).

Recognition Signals for Glycosaminoglycan Attachment in Variant Syndecan-1—The variant splice reduces the number of SGXG tetrapeptide sequences in the protein, but it also brings the direct Ser-Gly repeat sequence in proximity to a variant and somewhat unusual (see "Discussion") downstream sequence. To determine the significance of these differences for the glycanation of the proteoglycan, we converted the S\textsuperscript{45}GS\textsuperscript{50}GTA\textsuperscript{59} sequence in the variant into S\textsuperscript{45}GS\textsuperscript{57}GTG\textsuperscript{56}, restoring the SGXG consensus for the second serine in the Ser-Gly repeat sequence. In a separate construct, we also substituted a glycine for the lysine that interrupts the acidic downstream sequence (GER\textsuperscript{59}PHE-GER\textsuperscript{59}PHE). The constructs (Syn-1vA→G and Syn-1vK→G) were used in transfection experiments, and the sizes and glycanations of the recombinant proteoglycans were analyzed as described above. Converting Ala\textsuperscript{50} into glycine increased the apparent size of the variant proteoglycan up to that of syndecan-1s (>200 kDa), the recombinant standard form of syndecan-1 (Fig. 7). Moreover, syndecan-1v with an Ala → Gly substitution yielded clusters of heparan sulfate chains (compare the size of the HS moieties after alkali release and after proteinase K digestion, in Fig. 7), and
after a combined heparitinase and chondroitinase ABC digestion, it stained more intensely for the 3G10 epitope than syndecan-1v (not shown). Changing Lys53 in syndecan-1v into glycine had no effect on the size of the intact variant proteoglycan (not shown) but increased the ratio of the 3G10 epitope (21). To assess whether the variant form of this proteoglycan harbors the serines that were identified as sites for heparan sulfate attachment (6) and approximates a region rich in acidic amino acids to these sequences. At first sight, the variant therefore still reproduces a consensus pattern (repetitive Ser-Gly sequences, flanked by both upstream and downstream clusters of acidic amino acids) that can be observed in the other vertebrate syndecans and the Drosophila syndecan (Fig. 10), and in several other heparan sulfate proteoglycans (6).

Yet, in MDCK and in MOLT-4 cells, this variant syndecan-1 protein is less efficient at priming the synthesis of heparan sulfate than the full-length form of this protein. A first indication is provided by the marked size differences of the heparan sulfate proteoglycans (after chondroitinase ABC digestion) but not of their constituent heparan sulfate chains. The second evidence is the clustering of heparan sulfate chains in the standard form of the proteoglycan but not in the variant form. The final and direct indication is the lowering of the ΔHS: protein epitope ratio in the variant form.

The precise structures that determine the glycanation of a protein remain enigmatic. Both heparan sulfate and chondroitin sulfate are O-linked via a galactose-galactose-xylose linkage to serine residues in the core proteins of proteoglycans. Sequence alignments indicate that the substituted serines are followed by a glycine residue, and peptides containing a Ser-Gly dipeptide can serve as acceptors for xylosyltransferase-catalyzed transfer of xyloside (31). However, additional signals are involved in the biosynthetic recognition of the core proteins by the xylosyltransferase, since most proteins that contain Ser-Gly sequences are not proteoglycans and not all Ser-Gly sequences of proteoglycan core proteins are substituted with glycosaminoglycan. Testing synthetic polypeptides for the ability to accept xylose has indicated that acceptor activity could be enhanced by the presence of a doublet or triplet of acidic residues preceding the Ser-Gly sequence and the occur-
rence of the Ser-Gly dipeptide in the sequence context of Ser-Gly-Xaa-Gly (where Xaa stands for any amino acid) (7). This consensus motif can be found in many of the known core proteins, but it is clear that it does not encompass all the sites used for glycosaminoglycan attachment. The reduced glycanation of syndecan-1v when its Ala50 is converted into glycine, is consistent with the \textit{in vitro} xylosyltransferase acceptor activity studies which indicated that peptide containing the sequence Ser-Gly-Xaa-Ala retained only 10% of the activity measured for the tetrapeptide Ser-Gly-Xaa-Gly in an otherwise similar context (7).

All this evidence and prior findings on syndecan-1v (6) would be consistent with a mostly three-heparan sulfate chain model for the standard form of syndecan-1, one chain occurring on Ser35 and the clustered chains occurring on the (protease K-resistant) S45GS47G tetrapeptide that has been identified as the major heparan sulfate-glycanation site (6), and a mostly two-heparan sulfate chain model, one chain on Ser35 and one on Ser45 (Ser47 remaining unprimed), for the variant from of syndecan-1. The consistency of the results in MDCK and MOLT-4 cells suggests that the findings are not accidental and due to interferences between the protein expression levels and the capacity of the glycanation machinery of the cells (16, 22). The findings do, however, not imply that the natural equivalents of syndecan-1s (or syndecan-1v) in different cell types and stages of differentiation invariably correspond to the substitution model identified here in these two cell types.

Attempts to define core protein features that designate a Ser-Gly dipeptide for heparan sulfate rather than chondroitin sulfate attachment have revealed a coupling phenomenon between adjacent Ser-Gly dipeptides that leads to a preferential substitution of these sites with heparan sulfate, and sequence alignments indicate that most heparan sulfate proteoglycan core proteins contain repetitive (Ser-Gly)\textsubscript{n} segments and a nearby cluster of acidic residues (6). This incorporates the consensus motif defined above but with Xaa standing for serine. However, also this motif does not cover all sites known to be substituted with heparan sulfate, and it is clear that sequences in non-glycosaminoglycan binding regions of the core protein (e.g., the SEA module from perlecarn) can influence the utilization of the attachment sites for heparan sulfate synthesis (32). From the mutagenesis of fusion proteins (6) and the analysis of the structural motifs represented in all the known heparan proteoglycans (6), one might also suspect that the assembly of HS chains on the variant could be influenced by the proximity of the acidic cluster downstream of the Ser-Gly repeat. Proximal downstream acidic sequences are common in heparan sulfate proteoglycans, but decreasing the number of amino acids between the downstream acidic cluster and the Ser-Gly attachment site in betaglycan, for example, progressively reduces the priming of heparan sulfate by this protein (33). On the other hand, the novel flanking region in variant syndecan-1 reads as AGKEPK EEGE, and scrutiny of the published heparan sulfate core protein sequences also indicates that positively charged residues invariably correspond to the sequences that flank the Ser-Gly dipeptides. The follow-up mutagenesis experiments on the splice variant, converting the lysine residue in the AGKEPK EEGE sequence to glycine, supported the suggestion that the positive charge may suppress HS priming. The substitution increased the AHS:protein ratio after heparitinase digestion but not the size of the intact proteoglycan, suggesting it favors extending linkage regions initiated on Ser35 and Ser45 with HS rather than chondroitin sulfate but does not stimulate the initiation of a third glycosaminoglycan chain on Ser47. Substituting glycine for Ala50 in variant syndecan-1 enhanced heparan sulfate synthesis and restored heparan sulfate chain clustering but still yielded a mixture of heparan sulfate and chondroitin sulfate proteoglycan (approximately 40% chondroitin sulfate). The gain in size noticeable for the intact proteoglycan persisted after single heparitinase or single chondroitinase digestions (results not shown), indicating syndecan-1vA-G carried an excess of both heparan sulfate and chondroitin sulfate chains in comparison to syndecan-1v and an excess of chondroitin sulfate in comparison to syndecan-1s. Indirectly, this suggests that not only the reduction of Ser-Gly-Xaa-Gly consensus sequences but also the novel downstream sequence context in syndecan-1v

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9}
\caption{Adhesion to collagen of syndecan-1 transfectant MOLT-4 cells. MOLT-4 cells transfected with pLo/RSV (O), pLo/RSV/Syn-1s (S), or pLo/RSV/Syn-1v (V) were labeled with calcein AM and left to adhere to U-shaped wells coated with bovine serum albumin (BSA) or type I collagen (COL) (duplicate assays). After a spin, non-adherent cells collected in the center of the wells. The cells were detected by fluorimaging. The syn-1s and syn-1v transfectant cells shown expressed similar (moderate) levels of cell-surface syndecan-1 protein as detected by fluorescence-activated cell sorter. The cells expressing syndecan-1s adhered more strongly (larger diameter of the pellet) to collagen than MOLT-4 cells. Adding heparan sulfate (H) to the cell suspension during the adhesion assay abolished this difference.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig10}
\caption{Sequences flanking the SG repeats in the syndecans. Alignment of the sequences that flank the SG repeats in the N-terminal domain of the variant form of murine syndecan-1 (S1v), in syndecan-1 (S1), (11, 23, 24), syndecan-2 (S2) (10, 25, 26), syndecan-3 (S3) (27, 28), and syndecan-4 (S4) (24, 29) from human (h), mouse (m), rat (r), and chick (c) origin, and in the syndecan from \textit{Drosophila melanogaster} (dr) (30). Acidic residues are indicated in \textbf{bold}. The SG sequences are indicated by shaded boxes.}
\end{figure}
negatively affects the substitution of the Ser-Gly repeat with heparan sulfate.

Although the precise structural features that determine the lesser efficiency of the syndecan splice variant as proteoglycan remain unresolved, the present data extend the repertoire of mechanisms that may account for the molecular polymorphism of the heparan sulfate proteoglycans and their effects on cell behavior. It is generally accepted that this polymorphism largely determines the various receptor or co-receptor functions that have been ascribed to cell-surface proteoglycans. In this context it had previously been shown that syndecan-1 from mouse mammary epithelial cells binds with high affinity to type I collagen, through the intermediate of its heparan sulfate chains, but that the protein-free heparan sulfate chains of this proteoglycan do not bind collagen, suggesting that binding of the proteoglycan is based on chain cooperation (21). Although it remains formally unproven that the relative failure of syndecan-1v to support or assist the adhesion of MOLT-4 cells to collagen is due to its reduced glycanation, this failure appears consistent with this binding model. It is also consistent with the evidence that all three sites of heparan sulfate attachment in syndecan-1 are needed for optimal activity of this syndecan in mediating cell-cell and cell-matrix adhesion of myeloma cells and inhibiting the invasion of these cells in collagen gels (34). These findings impart functional importance to the chain valency of the proteoglycans and to the mechanisms that may determine or affect this valency, and encourage further investigations that should help determine whether alternative splicing represents a biologically significant means of regulating the functional versatility of the syndecans and their effects on cell behavior.

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