Syndecan-1 Expression Inhibits Myoblast Differentiation through a Basic Fibroblast Growth Factor-dependent Mechanism*

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The process of myogenic development involves an ordered sequence of molecular events that includes commitment of muscle precursor cells, cessation of cell division, myoblast terminal differentiation, and formation of myotubes that express muscle-specific genes involved in the specialized functions of the myofiber (1). These processes are governed by a network of transcription factor of the basic helix-loop-helix family that activates skeletal muscle-specific gene products. Conversely, in cell culture experiments the reduction in the concentration of these growth factors below a critical level results in irreversible withdrawal from the cell cycle and terminal differentiation.

It has been demonstrated that heparan sulfate proteoglycans are necessary for the modulation of terminal myogenesis (12, 13), probably by acting as low affinity receptor for some growth factors such as bFGF (14) and HGF/SF (15). We have shown that the expression of heparan sulfate proteoglycans is regulated during terminal skeletal muscle differentiation of the C2C12 myoblast cell line (16). The expression of glypican increases (17, 18), whereas expression of perlecan decreases during skeletal muscle differentiation (19). Recently, we have shown that the expression of syndecan-1, a transmembrane heparan sulfate proteoglycan (20, 21), is down-regulated during myoblast terminal differentiation. Syndecan-1 mRNA as well as cell-surface syndecan-1 disappear almost completely by 48 h after triggering differentiation (22).

Cell-surface heparan sulfate molecules are critical in the activation of signaling receptors by growth factors (23, 24). It has been shown that, depending on the cellular localization, syndecans can either increase or decrease responsiveness to bFGF. Syndecans potentiate bFGF binding to the signaling receptor when they are associated with the membrane but inhibit bFGF receptor binding when added in soluble form (25).

Overexpression of syndecan-1 in a cell line in which most of the expressed proteoglycan is shed from the membrane also leads to decreased responsiveness to bFGF (26). The potential to regulate growth factor activity by means of heparan sulfate-dependent interactions provides an attractive system for fine-

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tuning cellular responses to growth factors in the extracellular environment. Because its potential to act as a growth factor co-receptor (14, 21, 27), the down-regulation of syndecan-1 might play a critical role in attenuating growth factor activity in myoblasts and thus promote muscle cell differentiation (16, 22, 28).

To investigate the role of syndecan-1 during skeletal muscle terminal differentiation, we transfected C_{2}C_{12} myoblasts with expression plasmids containing rat syndecan-1 cDNA. Constitutive expression of syndecan-1 resulted in a strongly diminished capacity of the transfected myoblasts to differentiate, and a 6-7-fold increase in sensitivity to bFGF-dependent inhibition of myogenesis. Thus the sensitivity of myoblasts to bFGF, an inhibitor of myogenesis, is directly modulated by syndecan-1 expression.

**EXPERIMENTAL PROCEDURES**

**Materials**—The C_{2}C_{12} cell line, BALB/c mouse embryonic fibroblast, and pRSVlacZII phagemid vector (β-galactosidase reporter) were purchased from ATCC. EMSV-Myosin is an EMSV vector containing the human myogenin cDNA (a generous gift of Dr. Eric Olson, University of Texas, Houston). pGREEN LANTERN–1 vector was obtained from Life Technologies, Inc. Trizol LS, LipofectAMINE, DMEM, chicken embryo extract (CEE), horse serum, Opti-MEM I, Hanks’ balanced salt solution, and G418 were obtained from Life Technologies, Inc. Wizard Plus Maxiprep, Prime-a-Gene labeling system, pCAT-basic vector, and n-butyryl-CoA were from Promega, Madison, WI.

Fetal calf serum (FCS), CR-C-30 medium, creatine kinase assay kit, monoclonal anti-α-tubulin, monoclonal anti-myc, alkaline phosphatase-conjugated goat anti-rabbit IgG, and goat anti-mouse IgG were from Sigma. Affinity purified rabbit polyclonal anti-human FGF-1 antibody was from Santa Cruz Biotechnology Inc., Santa Cruz. Human bFGF, bFGF enzyme-linked immunosorbent assay kit, [35S]NaCl carrier-free (1050–1600 Ci/mmol), [14C]chlordane (50 mCi/mmol) RNA Isolation and Northern Blot Analysis—Total RNA was isolated from cell cultures using Trizol. RNA samples (15 μg/lane) were electrophoresed through 1.2% agarose/formaldehyde gels, transferred to Nytan membranes, and hybridized with probes for myogenin and syndecan-1 as described previously (22). Blots were hybridized with random primed labeled probes in a buffer containing 1.0 M NaCl, 1% SDS, 10% dextran sulfate, and 100 μg/ml denatured salmon testes DNA at 65 °C overnight. Hybridized membranes were washed twice at 65 °C in 0.2× SSC, 0.1% SDS for 5 min and exposed to Kodak x-ray film. For quantitative determination of the mRNA levels, the intensity of the hybridization signals was measured by densitometric scanning (GS 300 Scanit). The cells were then washed twice with HBSS and incubated for 2 days in growth medium followed by 1 or 2 days in differentiation medium or 30 h in differentiation medium containing bFGF (0–30 ng/ml). The cells were harvested and assayed for choromophilic acetyltransferase (CAT) and β-galactosidase activities as described previously (22). All transfections were performed at least two times with at least two plasmid DNA preparations.

**Analysis of Creatine Kinase Activity**—Myoblasts and myoblasts induced to differentiate for 2, 3, or 4 days were washed twice with PBS and lysed with PBS containing 0.1% Triton X-100 for 10 min at 25 °C. The cells were scraped, and creatine kinase activity was determined using the creatine kinase assay kit. All data points represent the mean of duplicate determinations from three independent experiments.

**RESULTS**

**Heparan Sulfate Proteoglycans Modulate Myogenin Activation**—The expression of early myogenic regulators like myogenin is strongly inhibited by growth factors such as bFGF and HGF-SF (7, 8). The binding of these growth factors to their signaling receptors and their biological activities are strongly potentiated by binding to heparin or heparan sulfate proteoglycans (15, 34). To determine if heparan sulfate proteoglycans...
Inhibit myogenin expression. C2C12 myoblasts were grown for 30 h in differentiation medium in the presence or absence of 10 ng/ml bFGF, 50 mM sodium chlorate, or 10 ng/ml heparin, as indicated. Total RNA was extracted and subjected to Northern blot analysis with a 32P-labeled myogenin cDNA probe. The resulting autoradiogram (top) and ethidium bromide-stained gel (bottom) are shown. The arrows on the right indicate the sizes of myogenin mRNA and ribosomal RNAs. B, the inhibitory effect of bFGF on myogenin expression is potentiated by heparin during differentiation. C2C12 myoblasts were transiently co-transfected with pMYOCAT and RSV-β-galactosidase plasmids. The cells were incubated for 48 h in growth medium and then an additional 30 h in differentiation medium containing bFGF (5 ng/ml) and the indicated concentrations of heparin. The cells were harvested, and the CAT and β-galactosidase activities were determined. The pMYOCAT/β-galactosidase activities were as follows: after 48 h in growth medium, 2,808; after 30 h in differentiation medium, 35,111; and after 30 h in differentiation medium containing 5 ng/ml of bFGF, 15,800. The values shown in the graph are means of two different experiments done in duplicate. nt, nucleotide.

Fig. 1 A, bFGF requires sulfated heparan sulfate proteoglycans to inhibit myogenin expression. C2C12 myoblasts were grown for 30 h in differentiation medium in the presence or absence of 10 ng/ml bFGF, 50 mM sodium chlorate, or 10 ng/ml heparin, as indicated. Total RNA was extracted and subjected to Northern blot analysis with a 32P-labeled myogenin cDNA probe. The resulting autoradiogram (top) and ethidium bromide-stained gel (bottom) are shown. The arrows on the right indicate the sizes of myogenin mRNA and ribosomal RNAs. B, the inhibitory effect of bFGF on myogenin expression is potentiated by heparin during differentiation. C2C12 myoblasts were transiently co-transfected with pMYOCAT and RSV-β-galactosidase plasmids. The cells were incubated for 48 h in growth medium and then an additional 30 h in differentiation medium containing bFGF (5 ng/ml) and the indicated concentrations of heparin. The cells were harvested, and the CAT and β-galactosidase activities were determined. The pMYOCAT/β-galactosidase activities were as follows: after 48 h in growth medium, 2,808; after 30 h in differentiation medium, 35,111; and after 30 h in differentiation medium containing 5 ng/ml of bFGF, 15,800. The values shown in the graph are means of two different experiments done in duplicate. nt, nucleotide.

Stable Transfection of C2C12 Myoblasts with a Syndecan-1 cDNA—We reasoned if down-regulation of syndecan-1 cDNA expression was responsible for the decrease in bFGF activity, then its constitutive expression would inhibit or delay myogenesis. To test this idea a cDNA coding for full-length rat syndecan-1 was subcloned into the selectable expression vector pCMVneo (Fig. 2A) and used to stably transfect C2C12 myoblasts. Clones were selected under growth conditions in the presence of G418. As a control, myoblasts were transfected with the same vector containing no cDNA insert. A total of 8 syndecan-1-transfected G418-resistant clones were isolated. Several of these clones were chosen for more detailed analysis.

G418-resistant clones were analyzed by Northern blot for expression of the syndecan-1 transgene. Fig. 2B shows the results of this analysis using a full-length syndecan-1 cDNA probe that was hybridized to total RNA isolated from control-transfected myoblasts (lane 1) and four syndecan-1-transfected clones (lanes 2–6). As expected, hybridization signals were detected in all lanes for the endogenous syndecan-1 mRNA.
Increased expression of syndecan-1 during differentiation in syndecan-transfected clones. Clonal lines of stably transfected control cells (C1) and syndecan-1-transfected cells (S2 and S4) were incubated in growth medium (Myoblasts) or in differentiation medium for 4 days (Day 4). The cells were metabolically labeled with $[^{35}S]$Na$_2$SO$_4$, Triton X-100 extracts were prepared and immunoprecipitated with affinity purified rabbit anti-syndecan-1 antibodies. DNA was quantitated by fluorometry. Numbers in parentheses indicate the fold decrease of syndecan-1 synthesis during incubation in differentiation medium. Aliquots of the detergent extracts were also immunoprecipitated with rabbit anti-glypican antibodies (Inset). Results correspond to three independent experiments and are expressed as means ± S.D.

Myogenic Differentiation Is Blocked in Syndecan-1-transfected Cells. Wild type (WT), control-transfected (clone C1), and syndecan-1-transfected (clones S2 and S4) C2C12 cells were grown on coverslips. The cells were photographed with phase-contrast optics as myoblasts (MB) and after incubation for 4 days in differentiation medium (D4). The bar corresponds to 50 μm.

It is well established that skeletal muscle differentiation, including expression of creatine kinase and myosin, is dependent on the expression of myogenin. Expression of myogenin mRNA in control and syndecan-1-transfected C2C12 cells was measured by Northern blot analysis. As shown in Fig. 5, induction of myogenin mRNA was apparent in control-transfected cells (Fig. 5A) by 12 h after initiation of differentiation. In contrast, in syndecan-1-transfected S2 and S4 cells (Fig. 5, B and C), myogenin induction was delayed and reduced in magnitude. Densitometric analyses of Northern blots (Fig. 5D) indicated that myogenin transcripts in syndecan-1-transfected cells were 4–5-fold lower than in control cells at 12, 24, and 48 h after initiation of differentiation.
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TABLE I

Expressions of creatine kinase and myosin heavy chain are inhibited in syndecan-1-expressing cells

|          | Creatine kinase | Myosin/tubulin, (Day 4) |
|----------|-----------------|-------------------------|
| **WT**   | 5 ± 1           | 1.06 ± 0.06             |
| **C1**   | 4 ± 1           | 1.00 ± 0.04             |
| **S2**   | 5 ± 1           | 0.14 ± 0.02             |
| **S4**   | 3 ± 1           | 0.12 ± 0.02             |

The effect of syndecan-1 transfection on myogenin expression was confirmed using the pMYOCAT reporter construct. Fig. 6a shows the β-galactosidase-normalized CAT activities in control (C1) and syndecan-1-transfected (S2) myoblasts that were transiently transfected with pMYOCAT and induced to differentiate for 1 or 2 days. The expression of pMYOCAT was inhibited by approximately 65% in syndecan-1-transfected cells compared with control cells.

To demonstrate that the inhibition of myogenin expression seen in syndecan-1-transfected cells was not caused by a defect in the ability of the cells to activate myogenin expression, we measured the induction of myogenin reporter activity and myosin expression by a mechanism that is independent of growth factor-dependent regulation. Myogenin can activate its own expression by a positive feedback loop (31). We determined whether exogenous myogenin could activate myogenin reporter transcriptional activity to normal levels in syndecan-1-transfected cells. Control (clone C1) and syndecan-1-transfected (clone S2) cells were transiently co-transfected with 5 µg of pMYOCAT and RSV-β-galactosidase plasmids. After transfection the cells were incubated in growth medium for 48 h and then switched to differentiation medium. The cells were harvested at the indicated times, and CAT and β-galactosidase activities were determined. The results shown are the means from two different experiments performed in duplicate. B, myogenin transfection induces pMYOCAT expression to similar levels in control and syndecan-1-expressing cells. Control-transfected (clone C1) and syndecan-1-expressing (clone S2) C2C12 cells were transiently co-transfected with 5 µg of pMYOCAT and RSV-β-galactosidase plasmids without or with EMSV/Myo8. After transfection the cells were grown for 3 days and then harvested for determinations of CAT and β-galactosidase activities. The CAT/β-galactosidase activity ratios obtained in the cells transfected with EMSV/Myo8 (Myo8) are expressed relative to the values obtained from cells not transfected with EMSV/Myo8 (C1 and S2). The values shown are means of results obtained from two experiments performed in duplicate.

![Table 1](image)

![Fig. 5](image)

**Fig. 5.** Myogenin expression is inhibited in syndecan-1-expressing clones. Myogenin expression in control-transfected cells (A, clone C1) and syndecan-1-transfected cells (B, clone S2, and C, clone S4) was evaluated by Northern blot analysis. 15 µg of total RNA isolated from myoblasts (lane 1) and myoblasts incubated in differentiation medium for 12 h (lane 2), 24 h (lane 3), or 48 h (lane 4) were separated by electrophoresis, blotted to nylon membranes, and hybridized with a 32P-labeled myogenin cDNA probe. The transcript size is indicated in each panel. The EtBr-stained gel is shown in the lower half of each panel, and the ribosomal RNAs are indicated. D shows the results of quantitation of the myogenin hybridization signals; clone C1, open bars; clone S2, gray bars; clone S4, black bars. The densitometric values were normalized to the amount of 18 S rRNA. nt, nucleotide.

![Fig. 6](image)

**Fig. 6.** A, pMYOCAT expression is inhibited in syndecan-1-expressing cells. Control-transfected (clone C1) and syndecan-1-transfected (clone S2) C2C12 cells were transiently co-transfected with 5 µg of pMYOCAT and RSV-β-galactosidase plasmids. After transfection the cells were incubated in growth medium for 48 h and then switched to differentiation medium. The cells were harvested at the indicated times, and CAT and β-galactosidase activities were determined. The results shown are the means from two different experiments performed in duplicate. B, myogenin transfection induces pMYOCAT expression to similar levels in control and syndecan-1-expressing cells. Control-transfected (clone C1) and syndecan-1-expressing (clone S2) C2C12 cells were transiently co-transfected with 5 µg of pMYOCAT and RSV-β-galactosidase plasmids without or with EMSV/Myo8. After transfection the cells were grown for 3 days and then harvested for determinations of CAT and β-galactosidase activities. The CAT/β-galactosidase activity ratios obtained in the cells transfected with EMSV/Myo8 (Myo8) are expressed relative to the values obtained from cells not transfected with EMSV/Myo8 (C1 and S2). The values shown are means of results obtained from two experiments performed in duplicate.

In contrast, transfection with myogenin cDNA was not able to induce fusion of syndecan-1-transfected cells. Transiently transfected cells were visualized...
by their expression of green fluorescent protein (Fig. 8, A–C). These results demonstrate that intracellular mechanisms for activation of myogenin and myosin expression are intact in syndecan-1-transfected cells. In contrast, myoblast fusion was not restored by myogenin expression in cells that constitutively express syndecan-1.

Syndecan-1 Expression Increases the Sensitivity of Myoblasts to bFGF-dependent Inhibition of Myogenin Expression—To determine whether the diminished expression of myogenin observed in syndecan-1-transfected cells resulted from a higher sensitivity to bFGF-dependent inhibition, we measured the concentration dependence of bFGF inhibition of pMYOCAT expression. Wild type, control-transfected (clone C1), and syndecan-1-transfected (clone S2) C2C12 myoblasts were transiently transfected with pMYOCAT and incubated for 30 h in differentiation medium supplemented with different amounts of bFGF. As shown in Fig. 9 exposure of the cells to bFGF resulted in a significant inhibition of pMYOCAT expression. Wild type, control-transfected (clone C1), and syndecan-1-transfected (clone S2) C2C12 myoblasts were transiently transfected with pMYOCAT and incubated for 30 h in differentiation medium supplemented with different amounts of bFGF. As shown in Fig. 9 exposure of the cells to bFGF resulted in a significant inhibition of pMYOCAT expression. The syndecan-1-transfected cells, however, showed a marked shift in the dose-response curve, from an IC_{50} of approximately 2 ng/ml for wild type and control cells to approximately 0.2 ng/ml for S2 cells (Table II). This shift in sensitivity to bFGF did not appear to result from differences in the levels of expression of the FGFR. Western blot analysis of detergent lysates of wild type, control-transfected, and syndecan-1-transfected myoblasts showed similar amounts of anti-FGFR-1 staining (data not shown).

To determine whether this 6–7-fold shift in the sensitivity of the cells to bFGF might be relevant to conditions encountered by the cells during in vitro differentiation, we measured the amount of bFGF present in myoblast-conditioned medium by an enzyme-linked immunosorbent assay (Table II). Conditioned medium obtained after 30 h of culture under differentiation conditions contained bFGF at a concentration of approximately 0.2 ng/ml. This value and the bFGF dose-response curve of wild type and control-transfected cells (Fig. 9) are consistent with the ability of these cells to undergo differentiation. In contrast, this concentration of bFGF should be sufficient to maintain the syndecan-1-transfected cells in an undifferentiated state, as was observed.

Heparan Sulfate Proteoglycans Increase during Skeletal Muscle Differentiation—Heparan sulfate proteoglycan synthesis during skeletal muscle terminal differentiation was evaluated. Myoblasts and myoblasts induced to differentiate were labeled for 18 h with $^{35}$SO_{4} and extracted with Triton X-100. As shown in Table III there was an increase in the relative amount of cell-associated heparan sulfate, during differentiation, in both detergent-soluble and insoluble forms. This increase likely corresponds to the increase in glypican reported previously by us (18).

**DISCUSSION**

In these studies we have shown that the constitutive expression of syndecan-1 in myoblasts induces a strong inhibition of skeletal muscle differentiation, marked by an inhibition of
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**FIG. 9.** Titration of myogenin inhibitory activity of bFGF in control and syndecan-1-expressing cells. Wild type (open circles), control-transfected (clone C1, open squares), and syndecan-1-transfected (clone S2, filled circles) C2C12 cells were transiently co-transfected with 5 μg of pMYOCAT and RSV-β-galactosidase plasmids. After transfection the cells were incubated for 48 h and then for 30 h in differentiation medium containing the indicated concentrations of bFGF. Cells were harvested, and the CAT and β-galactosidase activities were determined and normalized to protein levels. The resulting values (CAT/β-galactosidase activities per microgram of protein) are expressed as percentages of the values determined in cells incubated in the absence of bFGF. The results shown were obtained from two experiments performed in duplicate.

**TABLE II**

IC₅₀ for bFGF inhibitory activity on myogenin expression and bFGF concentration in differentiation medium

|  | ng/ml |
|---|-------|
| IC₅₀ WT | 1.51 ± 0.24 |
| IC₅₀ C1 | 1.48 ± 0.33 |
| IC₅₀ S2 | 0.26 ± 0.06 |
| b-FGF | 0.21 ± 0.06 |

**TABLE III**

Relative amount of HSPG associated to C₂C₁₂ cells during skeletal muscle terminal differentiation

|  | Triton X-100 soluble | Triton X-100 insoluble |
|---|----------------------|-----------------------|
| MB | %                    | %                     |
| Day 2 | 23 ± 4                | 24 ± 6                |
| Day 5 | 53 ± 1                | 56 ± 9                |

myotube fusion and low levels of expression of the muscle-specific proteins creatine kinase and myosin heavy chain. More importantly, we have shown that the constitutive expression of syndecan-1 resulted in decreased and delayed expression of myogenin, an early master gene that governs the expression of several skeletal muscle-specific genes. Analysis of a myogenin reporter revealed that constitutive syndecan-1 expression resulted in a 6–7-fold increase in sensitivity to bFGF-dependent inhibition of myogenin expression.

Previous studies have shown that soluble heparin/heparan sulfate and heparan sulfate proteoglycans modulate bFGF activity (14, 34–36). Heparan sulfate-heparin interactions can either increase or decrease growth factor activity, depending on the specific manner in which the proteoglycan interacts with the growth factor and with the cell surface (21). The dependence of skeletal muscle cell differentiation on heparan sulfate proteoglycans was first reported by Rapraeger and co-workers (12), who demonstrated a decrease in myosin expression in myoblasts treated with chlorate, an inhibitor of proteoglycan sulfation. In this work we have extended these observations, and we demonstrate that the inhibitory effect of bFGF on myogenin expression is lost when myoblasts are treated with chlorate and that exogenous heparin potentiates the inhibitory effect of bFGF on myogenin expression in myoblasts induced to differentiate in the presence of a subsaturating concentration of bFGF. These two observations suggest that in myoblasts bFGF activity is dependent on interactions with heparan sulfate proteoglycans and that after induction of skeletal muscle cell differentiation bFGF activity is limited by the amount of available heparan sulfate proteoglycan (heparin).

Syndecan-1 appears to be a good candidate for this function, because it is located on the plasma membrane and its expression decreases after differentiation is triggered. In the present study we determined the effect of syndecan-1 expression on myogenesis by isolating stably transfected cells that constitutively express this proteoglycan. Cells that exhibited increased expression of cell-associated syndecan-1 under conditions that would normally induce differentiation showed a reduced ability to differentiate, as evaluated by the extent of myoblast fusion as well as the induction of creatine kinase activity and myosin expression. Syndecan-1-transfected clones also expressed less myogenin compared with control cells after induction of differentiation. Despite the inability to differentiate exhibited by the syndecan-1-expressing myoblasts, these cells were able to induce myogenin transcriptional activity and the expression of myosin following transfection with a myogenin expression plasmid (31). These results indicate that the intracellular machinery for the induction of myogenin and skeletal muscle-specific markers is intact in these cells and support the conclusion that the developmental defect in these cells results from a change in their response to growth factors.

Our findings strongly suggest that the low levels of myogenin expression exhibited by the syndecan-1-transfected cells and their inability to differentiate are caused by changes in bFGF activity. Cells that constitutively express syndecan-1 are about 6–7-fold more sensitive to bFGF than wild type or control-transfected cells. The presence of bFGF in myoblast-conditioned medium at a concentration of approximately 0.2 ng/ml, a bFGF concentration required to exert 50% inhibition of myogenin expression in syndecan-1-transfected cells, provides an attractive explanation for the occurrence of this inhibition in syndecan-1-transfected cells but not in wild type or control-transfected cells.

Previous studies have provided evidence that a factor that is critical for heparan sulfate proteoglycan-dependent modulation of bFGF activity is the location of the proteoglycan with respect to the plasma membrane. It has been shown in hematopoietic cells transfected with FGFR-1 that co-transfection with syndecan-1, syndecan-2, syndecan-4, or glypicans produces an increase in binding of bFGF to the receptor (25). Interest-
ingly, the presence of equimolar amounts of soluble syndecan-4 ectodomain has no effect on the binding, suggesting that the heparan sulfate proteoglycans can support bFGF binding to the receptor only when they are located on the cell surface (25). In other cells overexpression of syndecan-1 has been shown to suppress bFGF activity (26). In these cells, however, most of the expressed proteoglycan was shed into the culture medium where it accumulated in a soluble form. In our experiments the syndecan-1-transfected cells expressed syndecan-1 at levels comparable to those in myoblasts, and most of the proteoglycan was associated with the cells.

Because of the ability of syndecan-1 to interact with other polypeptides, one cannot exclude the possibility that sustained expression of syndecan-1 in the transfected cells directly affects myoblast fusion. One of the molecules that participates in myoblast fusion is N-CAM, which co-localizes with N-cadherin. Their expression increases during myoblast differentiation and activates myoblast fusion (43–45). It has been shown that homophilic N-CAM binding is inhibited by heparan sulfate (46). Therefore, it is possible to speculate that the presence of syndecan-1 might inhibit myoblast fusion. We did not observe any fusion of syndecan-1-transfected myoblasts even after transfection with myogenin cDNA, which induced the expression of myogenin and myosin. It has been shown that fusion is independent of myogenin expression (47–49). Thus, the inabil-

The REFERENCES section is not included in the given text.
43. MacMalman, C. D., Bardeesy, N., Holland, P. C., and Blaschuk, O. W. (1992) 
 *Dev. Dyn.* **195**, 127–132
44. Mège, R. M., Goudou, D., Díaz, C., Nicolet, M., Garcia, L., and Rieger, F. (1992) 
 *J. Cell Sci.* **103**, 897–906
45. Dickson, G., Peck, D., Moore, S. E., Barton, C. H., and Walsh, F. S. (1990) 
 *Nature* **344**, 348–351
46. Reyes, A. A., Akeson, R., Brezina, L., and Cole, G. J. (1990) *Cell Regul.* **1**, 
 567–576
47. Delain, D., and Wahrmann, J. P. (1975) *Exp. Cell Res.* **93**, 495–498
48. Hu, J. S., and Olson, E. N. (1990) *J. Biol. Chem.* **265**, 7914–7919
49. Russo, S., Tomatis, D., Collo, G., Tarone, G., and Tato, F. (1998) *J. Cell Sci.* 
  **111**, 691–700
50. Brandan, E., Fuentes, M. E., and Andrade, W. (1991) *Eur. J. Cell Biol.* **55**, 
  209–216
51. Pacifici, M., and Molinaro, M. (1983) *Exp. Cell Res.* **126**, 143–152
52. Brandan, E., and Inestrosa, N. C. (1987) *J. Neurobiol.* **18**, 271–282