A Role of Secreted Glycosaminoglycans in Cell-Substratum Adhesion*

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An adhesion-deficient variant, designated M3A, was derived from the anchorage-dependent L6 skeletal muscle myoblast line (Schubert, D., and La Corbiere, M. (1980) J. Biol. Chem. 255, 11557-11563). To investigate the defect in the M3A variant, adhesion to various substrata was studied. M3A adhered rapidly to substrate-attached material (SAM) prepared from L6 cultures and serum, but adhered slowly to SAM derived from M3A itself. The role of collagen and fibronectin in the adhesion of M3A cells to L6- and M3A-derived SAMs was ruled out, but several experiments suggested that glycosaminoglycans play a rate-limiting role in the adhesion process. The adhesive interaction of the M3A cells with the different substrata is specific with respect to the glycosaminoglycans involved, since the type and concentration of purified glycosaminoglycans required to inhibit the interaction is unique to each surface. An alteration in glycosaminoglycan synthesis by M3A cells may account for the difference in the adhesive properties of SAM derived from L6 and from the M3A variant.

A variant of Yaffe’s L6 skeletal muscle myoblast cell line was isolated which has lost the anchorage-dependent growth characteristics of the parental cells (1-3). The variant clonal cell line, designated M3A, shares some of the muscle-specific properties of L6 (4), but grows only loosely associated with the substratum. A comparison of the macromolecules released into the culture medium showed that L6 secreted complete (pro) α-collagen chains, while the M3A variant secretes a collagen-related protein of M₆ ≈ 56,000 and very little high molecular weight α-chain. There are also differences in the types of glycosaminoglycans synthesized by the two cell lines.

To characterize the defect in cell-substratum adhesion of M3A relative to L6, an assay was required which measured the affinity between cell and substratum. A modification of Stallcup’s procedure (5) was used to measure the amount of isotopically labeled cells adhering to SAM-coated Petri dishes. Using this assay, it was shown that L6 adheres rapidly to SAM derived from L6, but M3A cells adhere poorly to SAM derived from M3A. Neither collagen nor fibronectin is involved in these adhesions; glycosaminoglycans appear to play the primary role.

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The abbreviations used are: SAM, substrate-attached material; Heps, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; PBS, phosphate-buffered saline, 0.01 M sodium phosphate, pH 7.1, 0.15 M sodium chloride; EGTA, ethylene glycol bis[β-aminoethyl ether]N,N,N',N'-tetraacetic acid; Heps-BSA, Heps medium containing 0.2% BSA; PBS-EGTA, PBS containing 5 × 10⁻⁵ M EGTA.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—The L6 clonal rat skeletal muscle myoblast line and its M3A variant were grown as described elsewhere (2, 3).

**Adhesion Assays**—To assay cell-substratum adhesion, early stationary phase (1 to 2 × 10⁶ cells/100-mm culture dish) M3A cells were labeled overnight in complete, unchanged growth medium with 10 μCi/mL of [¹³C]glucose (New England Nuclear, Boston, MA). The cells were then washed 3 times with Heps-buffered modified Eagle’s medium (Heps medium) containing 0.2% bovine serum albumin (Calbiochem, San Diego, CA), and resuspended in the same medium. The 0.2-ml aliquots were then pipetted into 35-mm Falcon plastic Petri dishes coated with the indicated SAM or other compounds, and containing 2 ml of Heps medium with BSA (Heps-BSA). At the indicated times, the dishes were swirled by hand 10 times, the medium aspirated, and the remaining cells dissolved in 3% Triton X-100 and counted in a scintillation counter. Variation between duplicate samples was less than 5%, and the number of cells remaining, if the medium was removed immediately after the addition of the cells, was less than 0.5% of the input. The data are presented as the per cent of the input cells remaining attached to the surface as a function of time. All experiments were repeated at least twice with similar results.

**Preparation of Secreted and Substrate-attached Materials**—Substrate attached materials from cells were prepared in two ways. The first was described by Culp (8). Cells were grown to confluency on 35-mm Petri dishes, washed once with 2 ml of 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.1 (PBS), once with 2 ml of 5 × 10⁻⁴ M EGTA in PBS (EGTA-PBS), and the cells were removed by EGTA-PBS by shaking at 37°C for 30 min. The plates were then washed twice with EGTA-PBS, 3 times with water, and once with Heps medium. Finally, the adhesion buffer, Heps medium containing 0.2% BSA, was added. The second procedure involved the use of growth-conditioned medium for the preparation of the adhesion substratum. Exponentially dividing L6 or M3A cells were washed 3 times in serum-free Heps medium, and resuspended in the same medium for 15 h in a humidified air incubator at 37°C. The conditioned medium was collected and centrifuged at 700 × g for 5 min. Then 2 ml of this material was placed in 35-mm Falcon Petri dishes for 18 h at 37°C in a humidified air incubator. The conditioned medium was removed and the dishes were washed with PBS-EGTA and incubated for 30 min at 37°C with PBS-EGTA. They were then washed with water and Heps medium exactly as described with SAM deposited directly onto the culture dish by cells. Except for Fig. 1A, all of the cell-derived SAM used in the following experiments was prepared from conditioned serum-free medium. Another type of SAM was prepared by incubating dishes with Heps medium containing 10% fetal calf serum and treating the dishes as described with L6 and M3A SAM. The SAM made from serum-containing medium will be called “serum SAM.”

**RESULTS**

**Adhesion Assay**—M3A is a variant of the anchorage-dependent L6 skeletal muscle myoblast cell line which was selected for growth in suspension culture (2, 3). To study the alterations in cellular biochemistry which lead to anchorage-independent growth, an assay was used which measures cell-
substratum adhesion. Advantage was taken of the fact that M3A cells do not attach to plastic bacteriological Petri dishes which have not been exposed to cells. M3A does, however, attach to varying extents to Petri dishes on which cells have been grown. To assay for cell-substratum adhesion, M3A cells were isotopically labeled and plated onto 35-mm Petri dishes. When the initial kinetics of adhesion were followed for cells plated onto Petri dishes, less than 0.5% of the input cells were attached to the dish over a period of 1 h (Fig. 1). If, however, the cells were placed into Petri dishes in which L6 cells were grown for 1 week and then removed by EGTA, they rapidly adhered. They adhered poorly, however, to Petri dishes which had previously contained M3A cells (Fig. 1A).

The conditions for the assay were critical, for if highly sulfonated Falcon plastic tissue culture dishes were used in place of Petri dishes, M3A cells adhered rapidly (Fig. 1A). When the Petri dishes were incubated at 37°C for 18 h with 0.2% BSA, there was no increase in the normal "background" adhesion kinetics. If, however, the dishes were incubated overnight with 10% fetal calf serum, treated with EGTA, and washed with water and Hepes as in the preparation of cell-derived SAM, M3A cells adhered to the dish (Fig. 1A). Thus, under the standard assay conditions, M3A cells adhered rapidly to cell-derived L6 SAM, serum SAM, tissue culture dishes, and less well to cell-derived M3A SAM.

To further study these adhesive responses, it would be advantageous to have a system where the material responsible for the cell-substratum adhesion is in a soluble form in the absence of serum proteins. This was accomplished by briefly growing cultures of M3A or L6 cells in serum-free Hepes medium. The "conditioned" culture medium was then incubated in 35-mm Petri dishes and the SAM-coated dishes were prepared as described above. This substrate-attached material prepared from culture supernatants was used in all of the following experiments employing SAM. Fig. 1B shows that when isotopically labeled M3A cells were plated into dishes containing SAM from equal numbers of L6 and M3A cells, the kinetics of adhesion were very similar to those using cell-derived SAM.

The adhesive properties of M3A negated the requirement for treatment with proteolytic enzymes or chelating agents for dissociating the cells, thus permitting a valid evaluation of their adhesive characteristics. The adhesive properties of the cells did, however, vary as a function of the culture growth curve. M3A cells were plated such that cells at different densities could be labeled simultaneously and assayed for their ability to adhere to Petri dishes coated with L6 SAM. Fig. 2 shows that exponentially dividing cells were much less adhesive than stationary phase cells. Only stationary phase cells at densities between 1 and 2 x 10^7/100-mm dish were used as test cells in the following experiments. In contrast to the test cells, SAMs were prepared only from exponentially dividing M3A and L6 cells, since L6 myoblasts fuse at high cell density and the secretory properties of myotubes are different from mononucleate myoblasts (9). To obtain a valid comparison between the SAMs of these two cell lines, cells in the same (exponential) growth state must be used.

To determine if L6 and M3A have saturated the culture dish with the molecules mediating cell-substratum adhesion, increasing amounts of M3A and L6 serum-free Hepes-conditioned media were added to Petri dishes, the SAM was prepared, and the adhesion of M3A cells was determined. Fig. 3 shows that the adhesion of M3A cells to L6 and M3A SAM was directly proportional to the amount of conditioned medium incubated in the dish, and that neither curve was saturated with up to 8 ml of conditioned medium. These data show that SAM prepared from L6 and M3A did not saturate the assay dishes with respect to binding of M3A cells. However, since growth-conditioned media from both cell lines deposit similar amounts of protein and carbohydrate upon the
were washed with EGTA, incubated with EGTA for 1 h, washed 3 times with water, and 2 ml of Hepes-BSA were added. The adhesion of M3A cells was then assayed at 15-min intervals over a period of 1 h. A smooth curve was drawn through the data (Fig. 1), and the per cent of the input cells adhered at 40 min is presented. Dishes were coated with rat tail collagen according to the procedure of Bornstein and Piez (30) and with gelatin according to Yaffe (31). Fibronectin A was human colc-insoluble globulin obtained from Dr. S. J. Singer (University of California, San Diego) and the 35-mm Petri dishes were coated with a solution of 44 μg/ml in Hepes medium for 18 h at 37°C. Fibronectin B was human CIG obtained from Collaborative Research, Waltham, Mass., and the dishes were treated with 50 μg/ml as above. SAM-coated dishes were treated with 20 μg/ml of highly purified collagenase for 20 h at 37°C. L6 and M3A cell-conditioned Hepes medium and Hepes medium containing 10% fetal calf serum were also treated with 20 μg/ml of the collagenase for 24 h at 37°C without changing the adhesive properties of the SAM prepared from them (data not shown).

To assay the effectiveness of collagenase treatment, two experiments were done. M3A- and L6-secreted protein and SAMs were prepared from cultures isotopically labeled with [14C]proline. Following enzyme treatment, dishes were washed with EGTA-PBS and 6 N HCl was added. The dishes were incubated in a nitrogen atmosphere for 8 h at 37°C, the HCl was removed, and the remaining proteins were hydrolyzed at 110°C for 12 h. The samples were dried and assayed for 4-hydroxyproline and proline by column chromatography (9). Greater than 95% of the acid-extractable 4-hydroxyproline was removed from the collagenase-treated dishes relative to controls. The effectiveness of collagenase in removing soluble collagens from secreted protein was greater than 90% as determined by gel electrophoresis (9). To remove fibronectin from serum, 30 ml of serum-containing medium were passed through a gelatin-Sepharose (0.6 X 20 cm) or Sepharose column, the eluants placed in 35-mm Petri dishes, incubated overnight at 37°C, washed, and incubated with EGTA-PBS, washed with water, and the adhesion assays done by the usual procedures. The gelatin-derivitized column removed fibronectin from the serum defined by gel diffusion against rabbit anti-human cold-insoluble globulin.

![Fig. 3](attachment:image.png)

**Fig. 3. Dependence of M3A adhesion kinetics on SAM concentration.** L6 and M3A cells of the same density were incubated for 15 h in serum-free Hepes medium at 37°C, the medium harvested, the cells removed by centrifugation, and 4 ml of the conditioned medium added to a series of 35-mm Petri dishes. 18 h later, these media were removed and replaced by an additional 4 ml of the same type. Another series of Petri dishes were made containing 3 ml, 2 ml, 1 ml, and 0.5 ml of the conditioned media, all made up to a final volume of 4 ml and the plates were incubated at 37°C. The next day, the dishes were washed with EGTA, incubated with EGTA for 1 h, washed 3 times with water, and 2 ml of Hepes-BSA were added. The adhesion of M3A cells was then assayed at 15-min intervals over a period of 1 h, and the data plotted as in Fig. 1. The per cent of input cells adhered at 20 min to SAMs from M3A and L6 was then plotted as a function of the amount in milliliters of conditioned medium added to the assay dishes. x—x, L6 SAM; o——o, M3A SAM.

**Table I**

| Condition                  | Per cent |
|----------------------------|----------|
| No addition                | 0.4      |
| L6 SAM                     | 72       |
| M3A SAM                    | 19       |
| Serum SAM                  | 36       |
| Rat tail collagen          | 0.6      |
| Gelatin                    | 0.5      |
| Fibronectin A              | 0.8      |
| Fibronectin B              | 1.6      |
| L6 SAM + collagenase       | 68       |
| M3A SAM + collagenase      | 23       |
| Serum SAM + collagenase    | 31       |
| Collagenase alone          | 1.3      |
| Gelatin-Sepharose serum eluant | 25     |
| Sepharose serum eluant     | 27       |

Inhibition of M3A Adhesion by Glycosaminoglycans—A classical method for determining the specificity of a ligand-receptor interaction is by testing the efficiency of a variety of ligand-related compounds to inhibit the reaction. If the interaction between M3A cells and the various SAMs involves different carbohydrate specificities, then individual sugars or glycosaminoglycans may inhibit adhesion to the different substrates to different degrees. To test this, isotopically labeled M3A cells were plated into adhesion medium containing several concentrations of sugars or protein-free glycosaminoglycan polymers. The adhesion kinetics were determined over a period of 1 h, and the inhibition of binding relative to control combined data strongly suggest that neither collagen nor fibronectin is involved in the adhesion of M3A cells to substrate-attached material.
Glycosaminoglycans in Cell-Substratum Adhesion

Inhibition of M3A adhesion to SAM by glycosaminoglycans

Serum SAM and SAMs from L6 and M3A were prepared on 35-mm Petri dishes as described under "Experimental Procedures." Two ml of Hepes-BSA medium were added to the dishes, some containing varying amounts of carbohydrate, and the kinetics of M3A adhesion determined. For each concentration of carbohydrate, the percentage of input cells attached to the dish was determined over the period of 1 h. Representative data are shown in Fig. 4. From these data, curves were constructed which plotted the percentage of input cells attached at 30 min against the carbohydrate concentration, and the concentration of carbohydrate which gave 50% inhibition was determined and presented in Table II. The concentrations are presented as the molarity of glucuronic acid, assuming it were a free molecule.

Inhibition (50%)

| Reagent                  | L6     | M3A    | Serum  | Tissue culture |
|--------------------------|--------|--------|--------|----------------|
| Chondroitin sulfate      | 5 x 10^{-4} | >1 x 10^{-3} | >1 x 10^{-4} | >1 x 10^{-3} |
| Chondroitin sulfate A    | 1 x 10^{-6} | 5 x 10^{-4} | >1 x 10^{-3} | 2 x 10^{-3}  |
| Chondroitin sulfate B    | 6 x 10^{-4} | >1 x 10^{-3} | >1 x 10^{-4} | >1 x 10^{-3} |
| Chondroitin sulfate C    | >1 x 10^{-3} | >1 x 10^{-3} | >1 x 10^{-3} | >1 x 10^{-3} |
| Heparin                  | >1 x 10^{-4} | 2 x 10^{-4} | >1 x 10^{-4} | 1 x 10^{-4}  |
| Heparan sulfate          | >1 x 10^{-4} | 2 x 10^{-4} | >1 x 10^{-4} | 1 x 10^{-4}  |

Table II

Incubation of M3A cells with purified glycosaminoglycans prior to adhesion assay

M3A cells were labeled overnight with [3H]leucine, washed 3 times with Hepes medium, and incubated at 1 x 10^5 cells/ml of medium for 4 h at 4°C with serum-free Hepes medium containing 0.5 mg/ml of the indicated polysaccharides. The cells were washed twice with serum-free Hepes medium and the kinetics of adhesion to the indicated substrata were determined over 1 h, a smooth curve was drawn through the data, and the percentage of input cells which adhered at 30 min was determined. The data are presented as the per cent of cells which adhere in the experimental condition relative to control cells incubated with serum-free medium.

Table III

Adhesion assay, M3A binding to tissue culture dishes was inhibited. This glycosaminoglycan was less efficient in inhibiting the interaction of M3A cells to serum and L6 SAMs, and had no effect on their binding to M3A SAM. Heparin was the only glycosaminoglycan which affected the adhesion of M3A cells to M3A SAM, while both heparin and heparan sulfate effectively blocked the interaction with tissue culture dishes as well as L6 SAM. Finally, chondroitins A and B partially reduced adhesion to L6 SAM and chondroitin sulfates B and C increased the binding of cells to tissue culture dishes. These data show that the adhesion of M3A cells to L6 SAM is much more susceptible to inhibition by glycosaminoglycans than the equivalent interaction with M3A SAM as defined by the interaction of glycosaminoglycans directly with cells.

The reciprocal experiment was done by incubating SAMs from L6 or M3A, serum SAM, or tissue culture dishes with purified glycosaminoglycans, washing the dish twice with serum-free Hepes medium, and adding normal Hepes-BSA me-

cultures with no additive was determined. When M3A cells were plated in 0.1 M concentrations of lactose, N-acetyl-D-galactosamine, thiodigalactoside, D-glucuronic acid, galactosamine, glucosamine, galactose, or N-acetyl-D-glucosamine, there was no inhibition of cell-substratum adhesion. However, Fig. 4 shows typical inhibition data for a mixture of chondroitin sulfates, and such data were used to calculate the data presented in Table II. These data are tabulated as the concentration of carbohydrate, expressed in terms of the molar glucuronic acid content in the polymers, which inhibited M3A binding by 50% relative to control cultures after 30 min of incubation.

The data indicate that none of the glycosaminoglycans inhibited the adhesion of M3A cells to serum SAM, and only very high concentrations of hyaluronic acid and heparans blocked adhesion of the cells to tissue culture dishes. Hyaluronic acid and chondroitin sulfate A and C inhibited the M3A-L6 SAM interaction; heparin, heparan sulfate, and chondroitin sulfate B (dermatan sulfate) were ineffective. In contrast to L6, the adhesion of M3A to its own SAM was inhibited only by hyaluronic acid and by very low amounts of heparin and heparan sulfate. These data suggest that there is a great deal of specificity in the interaction between M3A cells and the four different substrata in the sense that different classes of molecules on the cell and substratum are involved in the adhesive interactions.

If molecules on the surface of cells or in SAM interact specifically with glycosaminoglycans in the cell-conditioned medium, then it should be possible to bind exogenous glycosaminoglycans to the cells or SAM and inhibit the adhesion of cells to the substratum. Thus, isotopically labeled M3A cells were washed 3 times in serum-free Hepes medium, and incubated at 4°C for 4 h with 6 different mucopolysaccharides. The cells were then washed twice and the kinetics of adhesion to several substrata were determined. Table III shows that when cells were exposed to hyaluronic acid prior to the adhesion assay, M3A binding to tissue culture dishes was inhibited. This glycosaminoglycan was less efficient in inhibiting the interaction of M3A cells to serum and L6 SAMs, and had no effect on their binding to M3A SAM. Heparin was the only glycosaminoglycan which affected the adhesion of M3A cells to M3A SAM, while both heparin and heparan sulfate effectively blocked the interaction with tissue culture dishes as well as L6 SAM. Finally, chondroitins A and B partially reduced adhesion to L6 SAM and chondroitin sulfates B and C increased the binding of cells to tissue culture dishes. These data show that the adhesion of M3A cells to L6 SAM is much more susceptible to inhibition by glycosaminoglycans than the equivalent interaction with M3A SAM as defined by the interaction of glycosaminoglycans directly with cells.

The reciprocal experiment was done by incubating SAMs from L6 or M3A, serum SAM, or tissue culture dishes with purified glycosaminoglycans, washing the dish twice with serum-free Hepes medium, and adding normal Hepes-BSA me-

![Fig. 4. Inhibition of cell-substratum adhesion by chondroitin sulfates. SAM was prepared on 35-mm Petri dishes as described under "Experimental Procedures." Two ml of Hepes-BSA were added to the dishes, some containing different amounts of chondroitin sulfate (Sigma), expressed in terms of the molar content of glucuronic acid assuming it was a free molecule. Isotopically labeled M3A cells were added, and the percentage of input cells which adhered to the dish followed as a function of time. O--O, 10^{-5} M; ▽--▽, 10^{-4} M; △--△, 10^{-3} M; □--□, 10^{-2} M; ×--×, control without additive.](image-url)
The release of an adhesion promoting activity into the culture medium has, however, been described in some fibroblast-like cells (19, 20). Glycoproteins released by fibroblast-like cells also stimulate lectin-induced agglutination (21), and those released by high density cultures enhance the attachment of cells plated at low density (22). These results suggested that both adhesion and the sensitivity to lectin agglutination were mediated by secreted material which can be transferred between cells.

A variety of interactions between macromolecules which appear both extracellularly and on the cell surface have been described. These include adhesive interactions between collagen and fibronectin (11), glycosaminoglycans and collagen (23), glycosaminoglycans and fibronectin (24), and between classes of glycosaminoglycans (see, for example, Ref. 25). Since the adhesion of M3A cells to SAM from L6, M3A, or serum does not involve collagen and fibronectin (Table I), the possibility existed that glycosaminoglycans played a major role. Essentially all classes of glycosaminoglycans have been found associated with the surface of nonchondrocytic cultured cells, and exogenous glycosaminoglycans bind to cultured cells in a saturable and temperature-dependent manner (26, 27).

Several studies have implicated glycosaminoglycans in cell-cell interactions. The aggregation of SV-3T3 fibroblast-like cells was blocked by treating the cells with Streptomyces hyaluronidase, and the spontaneous aggregation was also inhibited by hyaluronate, but not by the sulfated glycosaminoglycans (28). These data suggest that there is an adhesive interaction between hyaluronate on one cell and specific hyaluronate receptors on the other. These types of interactions can be used to explain the binding specificity exhibited by M3A cells to SAM prepared from L6, M3A, and serum.

The role of glycosaminoglycans in the various cell-substratum interactions of M3A cells will be discussed in order of increasing apparent complexity. The initial adhesion of M3A cells to tissue culture dishes was 50% inhibited by 2 $\times$ 10^{-5} hyaluronic acid and by lower concentrations of heparin and heparan sulfate, but not by the other glycosaminoglycans (Table I). When cells were exposed to glycosaminoglycans prior to the adhesion assay, hyaluronate and the heparins were also inhibitory (Table III). This suggests that the cellular surface sites, which interact with the very adhesive plastic substratum of tissue culture dishes, can also bind certain glycosaminoglycans, thus blocking the interaction with the dish. This could also explain the increase in adhesion of M3A to tissue culture dishes previously exposed to heparin and heparan sulfate (Table IV) because if those glycosaminoglycans bind to the culture dish, they could then interact with appropriate receptors on the cell surface and increase the binding kinetics to the dish. Since chondroitin sulfates were ineffective, it is unlikely that the interactions are simply electrostatic.

The binding of M3A cells to serum SAM was not altered when the assay was done in the presence of glycosaminoglycans, nor did prior incubation of serum SAM with glycosaminoglycans alter adhesion. Adhesion was partially blocked by the heparin and hyaluronic acid, which are serum constituents (29). However, due to the overall unresponsiveness of the binding assay to glycosaminoglycans, it is likely that factors in serum SAM and on the cell surface other than glycosaminoglycans are responsible for the adhesive interaction.

The more dominant role of glycosaminoglycans in cell-substratum adhesion becomes evident when the adhesion of M3A cells to M3A SAM was examined. When adhesion was measured in the presence of heparin or heparan sulfate, fairly low concentrations block the cell-substratum interaction, while a
higher concentration of hyaluronic acid was the only other inhibitory glycosaminoglycan (Table II). Since the incubation of cells with the purified glycosaminoglycans was relatively ineffective in blocking adhesion, while heparin and heparan sulfate bound to M3A SAM and blocked M3A adhesion (Tables III and IV), it follows that the heparin and heparan sulfate must be binding to "receptors" in the SAM, not vice versa. Thus, M3A cells have on their surface heparin and/or heparan sulfate which interacts with molecules in the SAM of M3A to promote cell-substratum adhesion. Since adhesion can be completely blocked by low concentrations of heparin some extent (Table IV), it follows that the heparin and heparan sulfates have a great deal of specificity. Since most of the material released into culture dishes have on their surface heparin and/or heparan sulfate, which are both "sticky" in a nonspecific way (Fig. 2). Since most of the material released into conditioned medium is derived from the cell surface, it is likely that these glycosaminoglycan-mediated interactions are also involved in cell-cell adhesion. The release of glycosaminoglycans or glycosaminoglycan-protein complexes from cells which are both "sticky" in a nonspecific way (e.g. to the culture dish) and with the capability of mediating the adhesion of specific cell types, may be involved in the construction of adhesive gradients as well as a means of keeping homologous cell types together within a limited environment.

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