Fibroblast Growth Factor-2 Can Mediate Cell Attachment by Linking Receptors and Heparan Sulfate Proteoglycans on Neighboring Cells*

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The myeloid 32D cell line, which grows in suspension and does not express FGF receptors or heparan sulfate proteoglycans, was transfected with the cDNA encoding FGF receptor-1 (32D-flg cells). When co-cultured with glutaraldehyde-fixed Chinese hamster ovary (CHO) cells, the 32D-flg cells remained in suspension in the absence of FGF-2 but attached to the CHO monolayer in the presence of 10 ng/ml FGF-2. In contrast, 32D cells transfected with the vector alone did not attach to the CHO monolayer in the presence of FGF-2. FGF-2-dependent attachment of 32D-flg cells was prevented by inclusion of 10 μg/ml heparin in the incubation medium and was diminished when CHO mutants in glycosaminoglycan synthesis or wild-type CHO cells treated with heparinase were used, indicating that the attachment occurred through FGF-2 interactions with heparan sulfates on the CHO cells. Attachment of 32D-flg cells to wild-type CHO cells was half-maximal at 0.4 ng/ml FGF-2 and was also observed with FGF-1 but not FGF-4. 32D-flg cells also attached to living CHO cells in a FGF-2-dependent manner, but attachment was transient at 37 °C. Induction of new proteins was not required for FGF-2-dependent attachment, since attachment occurred when the co-cultures were incubated at 4 °C and when the 32D-flg cells were preincubated with cycloheximide. FGF-2-dependent attachment of 32D-flg cells was also observed with Balb/C 3T3, NIH 3T3, and bovine capillary endothelial cells. We conclude that attachment is due to FGF-2 binding simultaneously to receptors on the 32D-flg cells and heparan sulfates on the CHO monolayers; thus, the FGF-2 acts as a bridge between receptor-expressing cells and heparan sulfate-bearing cells. In addition, induction of DNA synthesis in 32D-flg cells in response to FGF-2 was potentiated by the CHO-associated heparan sulfates to the same extent as by soluble heparin, indicating that this interaction has functional significance.

The fibroblast growth factors (FGFs) are a family of nine polypeptides that share sequence homology and a high affinity for heparin (1, 2). The members of the family have a variety of activities in vivo, including stimulation of proliferation, migration, and differentiation (1, 2), and the activities of the members of the family overlap to a considerable extent (1, 2). The two prototypes of the family, acidic and basic FGFs (FGF-1 and FGF-2), were originally identified and purified as factors that induce an angiogenic response in cultured endothelial cells. In vivo, FGF-1 and FGF-2 act as potent angiogenic factors and stimulate the formation of new blood vessels (3). However, these growth factors also seem to have important roles in the development and maintenance of the nervous system, skeletal system, muscle, and blood cells.

FGF-2 interacts with both specific high affinity receptors and heparan sulfate proteoglycans on the cell surface (4). The FGF receptors also constitute a family of transmembrane tyrosine kinases with four known members that have overlapping affinities for the various members of the FGF family (5). At least two of the FGF receptors, FGF receptor-1 (the flg gene product) and FGF receptor-2 (the bkg gene product), are high affinity receptors for FGF-2 (6–8). Binding of FGF-2 to FGF receptor-1 or FGF receptor-2 results in autophosphorylation of the receptor and signaling to the cell (9). Several members of the receptor family also exist in alternatively spliced forms (5). Thus, FGF receptor-1 and FGF receptor-2 can exist in forms containing either two or three immunoglobulin-like domains in the extracellular portion of the molecule. The presence of 2 or 3 immunoglobulin-like domains may alter the affinity of the receptor for its ligands (10). Another splicing variation can occur in the second half of the third immunoglobulin-like domain. Variation in this region affects ligand specificity. Expression of the IIIb exon in this location in FGF receptor-2 generates a receptor that recognizes FGF-1 and keratinocyte growth factor but not FGF-2, whereas expression of the IIIc exon generates a receptor that recognizes FGF-1 and FGF-2 but not keratinocyte growth factor (11). In addition to these regions defined by splicing variations, other regions of the extracellular portion of the FGF receptors that might have functional importance have been identified. These include (i) the acidic box, a sequence of four to eight contiguous acidic amino acids between the first and second immunoglobulin-like domains, (ii) a proposed heparin-binding domain within the second immunoglobulin-like domain (12), and (iii) a region between the first and second immunoglobulin-like domains that bears homology to the cadherin cell adhesion recognition sequence (13).

Several studies have suggested that the binding of FGF-2 to heparan sulfates may play an important role in the regulation of FGF-2 activity. This is supported by our observations that FGF-2 is bound to heparin and that most heparan sulfate proteoglycans on the cell surface are proteolyzed and denatured, thereby releasing FGF-2. We have shown that the binding of FGF-2 to heparan sulfates confers several biological advantages to the growth factor: (i) FGF-2 bound to heparin or heparan sulfates is protected from proteolysis and thermal denaturation (16, 17); (ii) the heparan sulfate-bound FGF-2 serves as a reserve of growth factor that can support long term responses to FGF-2 after a...
brief exposure to the growth factor (18, 19); (iii) the heparan sulfates of the tissues may provide a means to localize FGF-2 to a particular site, limiting its diffusion (20); (iv) soluble heparan sulfates can act as carriers of FGF-2 and by preventing its interaction with fixed heparan sulfates in the tissues assure its dissemination away from its site of release (20); (v) FGF-2 can be internalized through its interaction with cell-surface heparan sulfates, clearing excess active molecules from the cell surface, perhaps helping to dampen the response to FGF-2 (21–23); and (vi) heparin or heparan sulfates can increase the affinity of FGF-2 for its receptors, by decreasing the dissociation rate of the FGF-2-receptor complex (24–26). This final point suggests that trimolecular complexes of FGF-2, receptor, and heparan sulfate are formed and that these complexes are more stable than complexes of FGF-2 and receptor alone.

The interaction of FGF-2 with heparin or heparan sulfates is reported to be necessary for interaction of the growth factor with its tyrosine kinase receptor (27–29). However, several recent studies have found that heparin or heparan sulfates were not strictly required for binding of FGF-2 to its receptor but increased the affinity of the FGF-2-receptor interaction to a moderate degree (26, 30–33). Some of these results are based on experiments with 32D cells (a myeloid cell line that does not normally express FGF receptors or heparan sulfates) that have been transfected with the cDNA encoding FGF receptor-1 (32D-flg cells). The 32D-flg cells bound FGF-2 both in the presence and in the absence of heparin, but heparin increased the affinity of binding about 4-fold (26).

Although the requirement for heparin or heparan sulfates for binding of FGF-2 to its receptor remains controversial, there seems to be a strong requirement for heparin or heparan sulfates for long term responses to FGF-2 (34). To determine whether the heparan sulfates from one cell were able to potentiate the binding of FGF-2 to its receptor on another cell type, the 32D cells expressing FGF receptor-1, which lack heparan sulfates and grow in suspension, were incubated with CHO cells, which express heparan sulfates but have very low levels of FGF receptors and grow attached to the culture dish. In the co-cultures, the normally suspended 32D-flg cells bound FGF-2 both in the presence and in the absence of heparin, but heparin increased the affinity of binding about 4-fold (26).

**EXPERIMENTAL PROCEDURES**

Cell Lines—CHO K-1 cells and mutants in glycosaminoglycan synthesis derived from them (pgsA-745, pgsB-618, pgsB-650, pgsD-677, and pgsE-606) were a generous gift of Dr. J effrey Esko of the University of Alabama. The CHO cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 7.5% fetal calf serum (Intergen Co., Purchase, NY). 32D cells, a myeloid-derived cell line requiring interleukin-3, were a generous gift of Dr. Jeffrey Esko of the University of Alabama. The 32D cells were grown in DMEM with 7.5% fetal calf serum (Intergen Co., Purchase, NY). 32D cells, a myeloid-derived cell line requiring interleukin-3, were a generous gift of Dr. Jeffrey Esko of the University of Alabama.

**FGF-2-mediated Cell Attachment**

Cell Attachment Assays—CHO cells or nontransfected CHO-K1 cells were trypsinized and replated at subconfluent density. Twenty-four hours later they were washed twice with Ca"++- and Mg"++-free PBS and detached from their dishes after incubation in Ca"++- and Mg"++-free PBS containing 10 mg/ml EDTA. Cells were collected by centrifugation, washed twice with PBS, resuspended in DMEM containing 0.15% gelatin and 25 mM HEPES, pH 7.5, with or without 3 units/ml F. heparan

**Materials—** Recombinant human FGF-2 was a gift from Synergen Inc. (Boulder, CO). Recombinant human FGF-1 was a gift from Dr.
The ability of heparan sulfates from one cell to potentiate the binding of FGF-2 to its receptor on another cell type was examined by co-culturing 32D cells expressing FGF receptor-1 (32D-flg cells), which lack heparan sulfates and grow in suspension, and CHO cells, which express heparan sulfates but have very low levels of FGF receptors and grow attached to the culture dish. To avoid possible confounding effects caused by the metabolism of the test cells, the CHO cells were fixed with glutaraldehyde so that they were not metabolically active but their surface components were preserved. Preservation of heparan sulfates in the fixed cells was confirmed by the fact that the fixed cell bound 80% of the amount of 125I-FGF-2 on low affinity binding sites as parallel cultures of non-fixed cells. In these co-cultures, the normally suspended 32D-flg cells attached to the CHO monolayer in an FGF-dependent manner. 

FIG. 1. FGF-2-dependent binding of 32D-flg cells to cells expressing heparan sulfates. A, five hundred thousand 32D cells transfected with a plasmid containing the cDNA for the two-immunoglobulin-domain form of FGF receptor-1 (32D-flg) or with the vector alone (32D-neo) were added to glutaraldehyde-fixed cultures of CHO-K1 cells (5 × 10⁶ cells) in the absence of FGF-2 (open bars) or in the presence of 10 ng/ml FGF-2 alone (filled bars) or 10 ng/ml FGF-2 and 10 μg/ml heparin (stippled bars). After 2 h at 37 °C, nonattached 32D cells were removed by gentle washing with PBS, and attached cells were recovered by a subsequent more vigorous wash with PBS containing 10 μg/ml heparin. Results are presented as (attached cells)/(unattached cells × 100). B, 32D-flg cells were added to cultures of glutaraldehyde-fixed CHO-K1 wild type cells or CHO mutants pgsE-606, pgsB-618, pgsB-650, pgsD-677, or pgsA-745 in medium containing no addition (open bars), 10 ng/ml FGF-2 (filled bars), 10 μg/ml heparin (hatched bars), or 10 ng/ml FGF-2 and 10 μg/ml heparin (stippled bars). After 2 h at 37 °C, nonattached and attached cells were recovered as described above and counted.

To confirm these results, FGF-2-dependent attachment of 32D-flg cells was assessed using a series of well-defined CHO mutants in the synthesis of glycosaminoglycans (35). As shown above, 32D-flg cells attached to wild type CHO-K1 cells in the presence of FGF-2, but little attachment was observed in the absence of FGF-2 (Fig. 1B). Furthermore, heparin alone did not promote attachment and heparin inhibited the attachment normally observed in the presence of FGF-2. The mutant CHO cell line pgsA-745, which lacks the enzyme xylosyltransferase that initiates glycosaminoglycan synthesis, and pgsB-618, which lacks the enzyme galactosyl transferase I, catalyzing the second step in glycosaminoglycan synthesis, make no glycosaminoglycans and did not support attachment of 32D-flg cells either in the presence or absence of FGF-2 (Fig. 1B). The mutant CHO cell line pgsD-677, which is deficient in heparan sulfate synthesis but makes supernormal levels of chondroitin sulfate, also did not support FGF-2-dependent attachment of 32D-flg cells. The CHO mutant pgsB-650, which has a 3-fold reduction in glycosaminoglycan synthesis, and pgsE-606, which displays diminished sulfation of heparan sulfate, supported lower levels of FGF-2-dependent attachment of 32D-flg cells. The attachment of 32D-flg cells to these CHO cell mutants in the presence of FGF-2 reflected their capacity to bind FGF-2 (Table II). Thus, the ability of CHO cells to support the FGF-2-mediated attachment of 32D-flg cells depends on their expression of normally sulfated heparan sulfate proteoglycans. This conclusion is further supported by the observation that FGF-2-dependent attachment of 32D-flg cells to wild type CHO-K1 cells was eliminated by pretreatment of the CHO cells with heparinase but not by pretreatment with chondroitinase ABC (data not shown).

The ability of soluble glycosaminoglycans to inhibit attachment of 32D-flg cells was compared. Half-maximal inhibition of the attachment of 32D-flg cells to wild type CHO-K1 cells was obtained with 10 ng/ml heparin and with approximately 200 ng/ml fucoidin or dermatan sulfate (Fig. 2). Chondroitin 4-sulfate, chondroitin 6-sulfate, and keratan sulfate had no effect on FGF-2-dependent 32D-flg cell attachment to CHO cells (Fig. 2). The ability of glycosaminoglycans to block attachment of 32D-flg cells to CHO cells reflected their ability to block FGF-2 binding to heparan sulfates (4).

The ability of FGFs to promote attachment of 32D-flg cells was investigated in more detail. Half-maximal attachment of 32D-flg cells to fixed wild type CHO-K1 cells was obtained with
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Table I

Stability of attached 32D-flg cells to dislodgment by shaking

Five hundred thousand 32D-flg cells in 1 ml of serum-free medium containing 10 ng/ml FGF-2 were added to a glutaraldehyde-fixed monolayer of 5 x 10^4 CHO-K1 cells on 35-mm dishes. The cells were incubated at 37°C for 2 h to allow the 32D-flg cells to attach. The cultures were then placed on a rotary shaker and shaken at the indicated speeds for 15 min at room temperature. Nonattached and attached 32D-flg cells were recovered as described under “Experimental Procedures” and counted.

| rpm     | 32D-flg cells bound (cpm) |
|---------|---------------------------|
| 0       | 83.1 ± 2.2                |
| 40      | 82.2 ± 1.0                |
| 80      | 80.8 ± 0.5                |
| 120     | 79.1 ± 0.0                |
| 160     | 68.6 ± 1.7                |

Table II

Binding of 125I-FGF-2 to heparan sulfates on CHO cell lines

Cultures of CHO K-1 wild type cells or CHO mutants were incubated with medium containing 10 ng/ml 125I-FGF-2 for 2 h at 4°C. The 125I-FGF-2 bound to low affinity (heparan sulfate) binding sites was removed with two washes with 2 M NaCl in 20 mM HEPES at pH 7.4 and quantitated. Parallel cultures were incubated with 10 ng/ml 125I-FGF-2 and 10 μg/ml heparin to estimate binding not due to interactions with heparan sulfates. The binding in the presence of heparin (approximately 4000 cpm/10^6 cells) was subtracted from the binding in the absence of heparin.

| CHO cell line | 125I-FGF-2 bound to low affinity sites (cpm) |
|---------------|-----------------------------------------------|
| K1            | 28,872 ± 2818                                 |
| 745           | 2318 ± 605                                    |
| 618           | 3290 ± 1043                                   |
| 677           | 1963 ± 544                                    |
| 650           | 5101 ± 470                                    |
| 606           | 11,998 ± 960                                  |

approximately 0.4 ng/ml FGF-2 (Fig. 3A). To determine if other members of the FGF family would support attachment of 32D-flg cells, the cells were incubated at 37°C with fixed CHO-K1 cells in the presence of 10 ng/ml FGF-1, FGF-2, or FGF-4. Significant attachment was obtained in the presence of either FGF-1 or FGF-2, but not FGF-4 (Fig. 3B). Attachment of 32D-flg cells to the CHO cells in the presence of either FGF-1 or FGF-2 could be inhibited by the addition of heparin. The ability of FGF family members to support attachment of 32D-flg cells is consistent with their affinity for FGF receptor-1 (7).

To determine if the relative number of 32D-flg and CHO cells would affect the FGF-2-dependent attachment, varying numbers of 32D-flg and CHO cells were incubated with 10 ng/ml in co-cultures with CHO-K1 cells fixed at different densities. Fig. 4A shows that at high densities of CHO cells, a high percentage of the added 32D-flg cells attached, approaching 100% at the highest densities except when very high numbers of 32D-flg cells were added. At low densities of CHO cells, only a low percentage of the 32D-flg cells attached. At higher ratios, attachment decreased proportionately. This may indicate a saturation of attachment sites on the CHO cells or a physical hindrance between 32D-flg cells crowded over a few CHO cells.

To examine the effect of temperature on 32D-flg cell attachment, the 32D-flg cells were incubated at 4°C with fixed CHO-K1 cells in the presence or absence of 10 ng/ml FGF-2. The same number of 32D-flg cells attached to CHO-K1 cells in the presence of FGF-2 if the co-cultures were incubated at 37 or 4°C (Fig. 5), indicating that cell metabolism is not required for attachment. There was little attachment to the CHO 618 heparan sulfate mutants at either temperature. In addition, treatment of the 32D-flg cells with the protein synthesis inhibitor cycloheximide for 30 min prior to exposure to FGF-2 and throughout the attachment assay had no effect on their ability to attach to CHO-K1 cells, demonstrating that expression of new proteins is not required for attachment. Some cytokines can cause a rapid increase in integrin activity on the cell surface (36). Since attachment through integrins and cadherins is Ca2+-dependent (36), the ability of the divalent ion chelators EDTA and EGTA to inhibit attachment was investigated. Addition of 10 mM EDTA or EGTA during the 2-h assay had no effect on FGF-2-dependent 32D-flg cell attachment (data not shown). Furthermore, addition of the protein-tyrosine kinase inhibitor genistein did not inhibit the FGF-2-dependent attachment of 32D-flg cells to CHO-K1 cells, suggesting that signaling through the receptor is not involved (data not shown). Finally, addition of antibodies to FGF-2 to attached cells resulted in a rapid detachment of the 32D-flg cells (data not shown), suggesting that attachment directly involves FGF-2 and does not require the induction of other attachment molecules.

The 32D-flg cells attached to living CHO cells as well as glutaraldehyde fixed CHO cells, but the cell-cell association was transient when measured at 37°C. Fig. 5 shows that at 4°C approximately equal numbers of 32D-flg cells attached to fixed or living CHO-K1 cells in the presence of 10 ng/ml FGF-2. However, at 37°C substantially fewer 32D-flg cells attached to living CHO cells than fixed CHO cells. The number of 32D-flg cells attached to living CHO cells in the presence of FGF-2 varied with time. At 37°C, 32D-flg cell attachment to living CHO-K1 cells reached a peak at 2 h, approaching 70% of the level of attachment observed with fixed CHO-K1 cells (Fig. 6). The number of 32D-flg cells attached to living CHO-K1 cells declined after that, reaching values only slightly above control by 24 h. Low levels of attachment to the CHO-pgsB-618 heparan sulfate-deficient mutants were observed independent of whether the cells were fixed or living.

To determine whether this FGF-2-dependent attachment of 32D-flg cells was limited to CHO cells, attachment to bovine capillary endothelial cells, NIH 3T3 cells, and Balb/C 3T3 cells...
was investigated. At 4 °C in the absence of FGF-2, only small numbers of 32D-flg cells attached to either glutaraldehyde-fixed CHO-K1 cells in medium containing the indicated concentrations of FGF-2 (bFGF). After 2 h at 37 °C, nonattached and attached cells were recovered as described under "Experimental Procedures." B, 32D-flg cells were incubated with glutaraldehyde-fixed CHO-K1 cells in medium containing no addition or 10 ng/ml FGF-2, FGF-1, or FGF-4 (filled bars). Parallel cultures were incubated under the same conditions with the addition of 10 μg/ml heparin (stippled bars). After 2 h at 37 °C, nonattached and attached cells were recovered as described under "Experimental Procedures" and counted.

The ability of cells expressing both FGF receptors and heparan sulfates to participate in FGF-2-dependent attachment was examined. CHO cells expressing transfected FGF receptor-1 containing two immunoglobulin-like domains (CHO-flg) were detached from their culture dishes with EDTA and were incubated at 4 °C in suspension over a glutaraldehyde-fixed monolayer of CHO-K1 cells in the presence of EDTA. Untreated CHO-flg cells did not attach to the fixed CHO cells either in the presence or absence of FGF-2 (Fig. 7, column a). However, if the CHO-flg cells were treated with heparinase prior to their incubation with the glutaraldehyde-fixed CHO-K1 cells, they attached to the monolayer in the presence of 10 ng/ml FGF-2 (Fig. 7, column b). No attachment of heparinase-treated CHO-flg

FIG. 3. Dependence of 32D-flg cell attachment on FGF-2 concentration. A, 32D-flg cells were incubated with glutaraldehyde-fixed CHO-K1 cells in medium containing the indicated concentrations of FGF-2 (bFGF). After 2 h at 37 °C, nonattached and attached cells were recovered as described under "Experimental Procedures." B, 32D-flg cells were incubated with glutaraldehyde-fixed CHO-K1 cells in medium containing no addition or 10 ng/ml FGF-2, FGF-1, or FGF-4 (filled bars). Parallel cultures were incubated under the same conditions with the addition of 10 μg/ml heparin (stippled bars). After 2 h at 37 °C, nonattached and attached cells were recovered as described under "Experimental Procedures" and counted.

FIG. 4. Effect of cell density on FGF-2-dependent 32D-flg cell attachment. A, CHO-K1 cells were plated in the indicated numbers on 35-mm dishes. Sixteen hours later, they were fixed with glutaraldehyde and washed thoroughly with PBS. Two milliliters of medium containing 10 ng/ml FGF-2 and 48 × 10^5 (squares), 19.7 × 10^5 (diamonds), 6.9 × 10^5 (circles), or 2.5 × 10^5 (triangles) 32D-flg cells were added. After 2 h at 37 °C, nonattached and attached cells were recovered as described under "Experimental Procedures" and counted. B, the ratio of 32D-flg cells to CHO-K1 cells was calculated for each point in the experiment of A, and the data were replotted.

FIG. 5. Effect of temperature and fixation on attachment of 32D-flg cells to CHO cells. Living 32D-flg cells were incubated at 4 or 37 °C with glutaraldehyde-fixed or living CHO-K1 cells in medium containing no FGF-2 (open bars), 10 ng/ml FGF-2 (filled bars), or 10 ng/ml FGF-2 with 10 μg/ml heparin (stippled bars). Parallel experiments were performed with glutaraldehyde-fixed or living CHO-pgsB-618 mutants in heparan sulfate synthesis. After 2 h, nonattached and attached 32D-flg cells were recovered as described under "Experimental Procedures" and counted.
cells was detected in the absence of FGF-2 or if FGF-2 and soluble heparin were added together. Nontransfected CHO cells treated with heparinase did not attach in the presence or absence of FGF-2 (Fig. 7A, column d), showing that attachment was dependent on the presence of FGF receptors on the CHO cells. No attachment of heparinase-treated CHO-flg cells to glutaraldehyde-fixed CHO-pgsB-618 glycosaminoglycan mutant cells was observed either in the presence or absence of FGF-2, showing that attachment was dependent on the presence of heparan sulfates (Fig. 7A, column f). The percentage of cells that attached was variable in these experiments, perhaps because of incomplete digestion of heparan sulfates. However, treatment of CHO-flg cells with heparinase rather than heparinase did not improve their ability to participate in FGF-2-dependent attachment (Fig. 7B). Thus, expression of heparan sulfates by the same cell type that expresses FGF receptors limits the interaction of the FGF-2-receptor complex with heparan sulfates on neighboring cells.

To determine whether the interaction of FGF-2 with heparan sulfates on the CHO cells could potentiate its activity, the effect of co-culture on incorporation of $^{125}$I-deoxyuridine into DNA was assessed. When 32D-flg cells were cultivated alone, the addition of FGF-2 at concentrations up to 20 ng/ml stimulated incorporation of $^{125}$I-deoxyuridine into DNA to a minor extent (Fig. 8, open squares). Addition of 10 μg/ml heparin along with the FGF-2 increased the stimulation significantly, resulting in a dose-dependent increase in $^{125}$I-deoxyuridine incorporation, with a maximal stimulation about 3.5-fold above control levels.
with 10 to 20 ng/ml (Fig. 8, filled squares). When 32D-flg cells were cultivated in co-culture with glutaraldehyde-fixed CHO pgsB-618 glycosaminoglycan mutant, they responded to FGF-2 in a manner similar to the cells cultivated alone. Less than 2-fold stimulation of 125I-deoxyuridine incorporation was obtained with concentrations of FGF-2 up to 20 ng/ml (Fig. 8, open triangles). Addition of heparin along with the FGF-2 resulted in a dose-dependent response to FGF-2 with maximal stimulation at 10 to 20 ng/ml (Fig. 8, filled triangles). However, addition of FGF-2 to 32D-flg cells co-cultured with glutaraldehyde-fixed wild type CHO-K1 cells resulted in a dose-dependent stimulation DNA synthesis in the absence of added heparin with a maximum 3.5-fold increase at 10–20 ng/ml (Fig. 8, open circles). Addition of soluble heparin along with the FGF-2 did not significantly increase this stimulation (Fig. 8, filled circles). Thus, the heparan sulfates of the wild type CHO-K1 cells were able to substitute for soluble heparin in the potentiation of FGF-2 bioactivity.

**DISCUSSION**

These results suggest that FGF-2, a potent growth factor, can also act as an attachment factor for suspension cells that express FGF receptors. There have been previous reports that FGF-2 can promote cell attachment (37, 38). In these experiments, the attachment of PC-12 cells or endothelial cells to FGF-2 coated on a plastic surface was measured. In addition, PC-12 cells plated on heparin-coated dishes aggregated in the presence of FGF-2 (37). Both adhesion and aggregation could be inhibited by FGF-2 antagonists, suggesting that the receptor was involved in these processes. Furthermore, the ability of cells to bind to FGF-2-coated plastic dishes has also been used as an assay for the cloning of FGF-2-binding molecules (39). In these experiments, a cDNA library from baby hamster kidney cells was introduced into the human lymphoblastoid cell line W1-L2-729 H2. The parental cells did not bind to FGF-2-coated dishes, and transfected cells that gained the capacity to bind to FGF-2-coated dishes were selected. Transfected cells that gained the ability to bind were found to express the heparan sulfate proteoglycan, syndecan. Together, these earlier studies showed that both FGF receptors and heparan sulfates could participate in FGF-2-mediated adhesion events. Our observations provide one mechanism by which both FGF receptors and heparan sulfates are involved directly in cell to cell adhesion interactions.

Other growth factors, including macrophage colony-stimulating factor, kit ligand, and transforming growth factor-α, have also been proposed to act as attachment factors (40–42). The primary translation products of these growth factors are anchored in the plasma membrane by hydrophobic transmembrane sequences. It is proposed that a membrane-anchored growth factor on one cell type can interact with its transmembrane receptor on a second cell type, promoting cell-to-cell interactions. Indeed, the transmembrane forms of transforming growth factor-α and macrophage colony-stimulating factor can mediate the attachment of cells bearing specific receptors for those growth factors (40, 42). Thus, with these growth factors, there is a two-component linkage, in which a growth factor that is a membrane constituent of one cell binds to a receptor expressed in the membrane of a second cell. The model proposed here for FGF-2 is novel in that it is composed of three components: a binding molecule on one cell (heparan sulfate), a nominally soluble growth factor, and a transmembrane receptor on a second cell type. The model we propose is shown in Fig. 9.

The use of heparan sulfate synthesis mutants of CHO cells and digestion of cell surface heparan sulfates on wild-type CHO cells demonstrated that the 32D-flg cell attachment is heparan sulfate-dependent. The addition of small amounts of soluble heparin inhibited attachment of the 32D-flg cells to wild-type CHO cells, presumably by competing with the cell surface heparan sulfates for binding of FGF-2, thereby preventing FGF-2-mediated bridging between the cells. These results suggest that this type of attachment may be limited to cells like the 32D-flg cells that express FGF receptors but do not produce heparan sulfates. If cell surface heparan sulfates are present on the same cells that are expressing the receptors, their relatively high concentration in the vicinity of the receptors may effectively displace heparan sulfates on other cells from interactions between FGF-2 and receptors. Indeed, when the same receptors were expressed in wild type CHO cells, which do produce heparan sulfates, FGF-2-dependent attachment could not be observed unless the heparan sulfates were removed by heparinase or heparitinase treatment.

However, since many leukemia-derived cells do not produce heparan sulfates (43), natural equivalents of the transfected 32D-flg cells may exist in the primitive blood cell population. Recent evidence that FGF-2 can promote hematopoiesis in
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