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Cell Type and Tissue Distribution of the Fibroblast Growth Factor Receptor

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A receptor for fibroblast growth factor (aFGF, bFGF) was partially characterized in intact cell cultures, cell plasma membranes, and tissue plasma membrane preparations. Analysis of 24 different cell types from four species identified a 165-kDa FGF receptor present on the cell surface of most mesodermal and neuroectodermal cells. Chemical crosslinking of ^{125}I-aFGF to its cell surface receptor was specifically blocked by a 100-fold molar excess of either aFGF or bFGF. In contrast to the similar molecular weight of FGF receptors, different cell types exhibited significant variations in binding of ^{125}I-aFGF to intact cultures with low values of 8 pM and 700, to high values of 60 pM and 30,000, for the K_d and receptor number per cell, respectively. A binding assay was developed for quantitation of ^{125}I-aFGF binding to cell- and tissue-derived membrane preparations. Membranes prepared from baby hamster kidney cells exhibited a K_d of 55 pM, while a similar K_d of 67 pM was determined for intact baby hamster kidney cells. Although ten different adult bovine tissue membrane preparations and human term placental membranes exhibited no specific binding of ^{125}I-aFGF, FGF receptor was detected in embryonic murine tissues (17 days gestation). These results support the existence, in a variety of cells, of either a common FGF receptor that binds both aFGF and bFGF or closely related FGF receptors that cannot be distinguished by molecular weight. The differential binding of FGF to its receptor in embryonic vs. adult tissues suggests a potentially broad role for FGF in embryonic development and a more restrictive role in the adult.

Key words: proliferation, differentiation, growth factor receptors, embryogenesis

A number of biological activities have been demonstrated for acidic and basic fibroblast growth factors [aFGF, bFGF; reviewed in Gospodarowicz et al., 1986; Baird et al., 1986; Folkman and Klagsbrun, 1987]. The recent identification of oncogenes with extensive homology to both bFGF and aFGF [Dickson and Peters, 1987; Delli-Bovi et al., 1987; Yoshida et al., 1987; Smith et al., 1988] and the discovery that FGF may play a key role in amphibian development [Slack et al., 1987; Kimmelman and Kirschner, 1987], has implicated FGF in a diverse number of biological roles. In contrast, little is known concerning receptors for FGF. A single FGF receptor has been

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identified and partially characterized in a few cell types [Neufeld and Gospodarowicz, 1985; Friesel et al., 1986; Huang et al, 1986; Moenner et al., 1986; Olwin and Hauschka, 1986; Moscatelli, 1987]. Based on results from analysis of FGF receptors in a single cell line, BHK (baby hamster kidney), the existence of two receptors has been proposed [Neufeld and Gospodarowicz, 1986]. In addition, both aFGF and bFGF competed for ^{125}I-FGF binding, suggesting that aFGF and bFGF compete for a single cellular receptor [Olwin and Hauschka, 1986] or two cellular receptors [Neufeld and Gospodarowicz, 1986]. Although these studies suggest aFGF and bFGF mediate their actions through a common receptor, they were performed on a limited number of cell types, and FGF binding to FGF receptor in vivo was not examined.

In this study, we investigated the binding characteristics and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) migration patterns of FGF receptor from 24 different cell types that exhibit biologically diverse responses to FGF. A comparison of ^{125}I-aFGF binding to intact BHK cells and BHK cell membrane preparations was also undertaken to identify optimum conditions for examination of FGF receptors in tissue membrane preparations. Embryonic, but not adult membranes expressed significant numbers of FGF receptor. The results suggest that aFGF and bFGF bind to a common FGF receptor or a group of closely related receptors, which mediate the biological actions of FGF.

MATERIALS AND METHODS

Materials

Na^{125}I was purchased from New England Nuclear, Enzymobeads from BioRad Laboratories, and disuccinimidyl suberate from Pierce Chemical Co. Hydrophilic Durapore filters (HVLP, 0.45 μm) were purchased from Millipore, Inc.

Methods

Purification of aFGF and bFGF was as previously described [Olwin and Hauschka, 1986, 1988] with modifications described as follows. After separation of aFGF and bFGF by heparin affinity chromatography, each polypeptide was isolated by FPLC on a mono-S column (0.5 × 5 cm; Pharmacia, Inc.) developed with a 0–1 M sodium chloride gradient in 0.05 M sodium phosphate, pH 7.0. High-performance liquid chromatography (HPLC) analysis [Olwin and Hauschka, 1986] confirmed that detectable crosscontamination of FGF in either preparation was less than 1% of the total FGF present. As an added control, recombinant human bFGF, kindly supplied by Zymogenetics, Inc., was used to confirm results obtained with purified bovine bFGF. ^{125}I-aFGF was prepared as previously described [Olwin and Hauschka, 1986] except that FPLC-purified bovine aFGF was used for ^{125}I-labeling. Intact cell binding and crosslinking of ^{125}I-aFGF using 0.15 mM disuccinimidyl suberate were as described previously [Olwin and Hauschka, 1986].

Mouse myoblast MM14, MM14 DD-1, C2C12, and rat myoblast L6E9 cells were grown as described by Olwin and Hauschka [1988]. Rat pheochromocytoma (PC-12) cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% horse serum, 5% fetal bovine serum, and 1% antibiotics (10,000 IU/ml penicillin, 0.5 mg/ml streptomycin sulfate). Mouse hybridoma cells were grown in RPMI 1640

buffered with 10 mM Hepes, pH 7.4, and containing 10% horse serum, 5% fetal bovine serum, and 1% antibiotics. All other cells were grown in DMEM supplemented with 10% defined calf serum (Hyclone laboratories) and 1% antibiotics.

BHK cell membranes were prepared by rinsing three confluent 15-cm plates three times with sodium phosphate, pH 7.4, containing 0.1 M NaCl (PBS), scraping the cells in 3 ml of HB (homogenization buffer: 20 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, pH 7.4, 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol bis(-amino-ether-)-N,N,N′,N′-tetraacetic acid, 1 μg/ml leupeptin, 20 Kallikrein inhibitory Aprotinin units/ml) including 1 mM phenylmethylsulfonylflouride, and centrifuging for 5 min at 3,000*g*. The cell pellet was resuspended in 3 ml HB and homogenized 20 strokes by hand in a dounce homogenizer with a tight-fitting pestle, centrifuged at 100,000*g* for 30 min, homogenized, and centrifuged again. The membrane pellet was then resuspended in 0.2 ml HB, frozen in liquid N_2 and stored at −70°C. ^{125}I-aFGF binding on freshly prepared and frozen membrane preparations demonstrated no loss of binding activity upon freezing (unpublished data). Similar procedures were used to prepare tissue membrane samples except that Polytron homogenization was utilized to disrupt samples instead of dounce homogenization. Both cell and tissue membranes were adjusted to a protein concentration of 10 mg/ml before they were frozen in liquid N_2 and stored at −70°C.

Detection of particulate membrane FGF receptor was performed as follows. First, membranes were resuspended in PBSMB (PBS containing 2 mM $MgCl_2$ and 0.2% bovine serum albumin) by trituration through a 25 gauge needle. Unless otherwise specified, binding assays included 200 pM ^{125}I-aFGF and 20 μg membrane protein in a final volume of 100 μl of PBSMB for 30 min at 22°C. To terminate the assay, samples were diluted with 2.5 ml PBSMB and applied to a Durapore filter (Millipore, Inc.) under vacuum; the tubes were rinsed twice with 2.5 ml of PBSMB, and the filters were rinsed three times with the same volume of PBSMB. The filters were then removed, and ^{125}I-aFGF bound to the particulate membrane fraction was determined by γ-counting. Typically, 0.5% of the total applied ^{125}I-cpm bound irreversibly to the filter and was subtracted from the total cpm to determine the percent of nonspecific binding.

Protein concentrations were determined by the method of Bradford [1976] using Pentex Grade V bovine serum albumin (Miles Scientific) as a standard.

RESULTS

Analysis of FGF Receptor in Cultured Cells

The distribution and partial characterization of a receptor for FGF in a variety of cultured cell lines, primary cell cultures, and tissues was examined by identification of high-affinity FGF binding sites. FGF receptors were initially identified by covalently crosslinking specifically bound ^{125}I-aFGF to intact cells and analyzed by SDS-PAGE and autoradiography. Further FGF receptor characterization included the determination of the K_d and the relative FGF receptor number by Scatchard analysis of equilibrium binding of ^{125}I-aFGF to intact cells or tissue membrane preparations.

Examination of FGF receptor in 24 different cell lines from human, mouse, hamster, and rat revealed a strikingly similar pattern of migration on SDS polyacryl-

TABLE I. Characteristics of FGF-Receptor in Cell Lines*

Cell type	FGFR per cell	K^d(pM)	FGFR MW (kDa)
Mouse myoblast MM14	700	8	165
Mouse myoblast MM14 DD-1	12,000	11	165
Mouse myoblast C2C12	~5,000	nd	165
Mouse myoblast BC3H1	7,000	15	165
Mouse swiss 3T3	20,000	20	165
Mouse C3H10T1/2	30,000	20	165
Mouse neuroblastoma N-18	nd	nd	165
Mouse endocarcinoma F-9	nd	nd	165
Mouse fibroblast L-cell	nd	nd	165
Mouse hybridoma MF-20	—	—	—
Mouse hybridoma FOX/NY	—	—	—
Human A431	10,000	30	165
Human smooth muscle	nd	nd	165
Human endothelial	nd	nd	140
Human fibroblast (SK-5)	nd	nd	165
Human osteosarcoma MG-63	nd	nd	165
Human fibroblast W1-38	nd	nd	165
Human astrocytoma 1321N1	nd	nd	165
Human melanoma A875	nd	nd	165
Human HeLa	nd	nd	165
Rat myoblast L6	—	—	—
Rat neuronal PC-12	3,000	20	165
Hamster BHK	13,000	60	165
Hamster CHO	nd	nd	165

*Data for K_d and FGF receptor number per cell were obtained by extrapolation of Scatchard plots, and FGF receptor MW was determined by relative mobility of ^{125}I-aFGF-FGF receptor complexes on SDS-polyacrylamide gels (15 kDa was subtracted from the labeled complex for the estimation of FGF receptor MW). nd = Not determined; —= not detectable.

amide gels, suggesting the existence of a single, or closely related FGF receptors (Table I; Fig. 1). Furthermore, in all cell types examined, both unlabeled aFGF and bFGF competed specifically for the binding of ^{125}I-aFGF to a FGF receptor. Although the MW of the FGF receptor was nearly identical for most cells, the K_d for ^{125}I-aFGF binding and receptor number per cell varied significantly (Fig. 1; Table I). Most mesoderm and neuroectoderm-derived cell lines as well as primary cultures expressed significant specific binding for ^{125}I-aFGF. The rat L6 skeletal muscle cell line (Table I), was the only mesodermal cell type tested that did not bind ^{125}I-aFGF. In addition, cell lines of hematopoeitic origin, including two myeloma lines (Sp2/0 and FOX/NY) and two different hybridoma cell lines, did not bind FGF (Table I).

In all cell lines examined, the presence of a high MW band is seen on the gel, which runs at the interface of the 4% stacking gel and the 7.5% separating gel. Some of these bands are visible in the autoradiograms illustrated in Figure 1. Presumably, they represent higher-order crosslinked polypeptides incapable of entering the separating gel. In addition, a few cell lines, notably the F-9, C3H10T1/2, and 132 1N1, exhibit a band migrating at less than 180 kDa that may represent a proteolytic degradation product (see "Discussion").

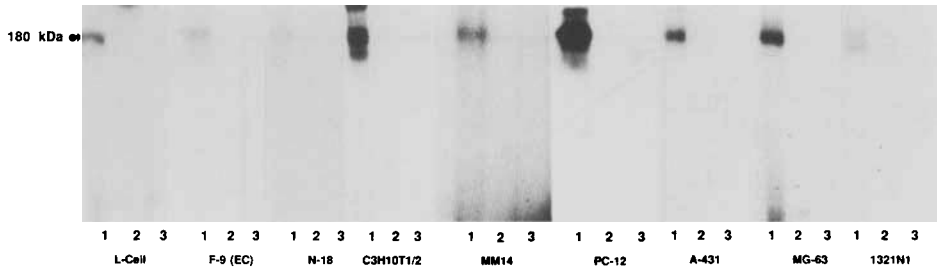

Fig. 1. Crosslinking of ^{125}I-aFGF to intact cells. Intact cell cultures were incubated for 1 h at 22°C with 100 pM ^{125}I-aFGF in the absence (**1**) and presence of a 100-fold molar excess of either unlabeled aFGF (**2**) or bFGF (**3**) and then processed as described in "Materials and Methods." Names of cell types correspond to those given in Table I. These are composites of several 7.5% SDS-polyacrylamide gels. The arrow marks a relative molecular weight of 180,000 as determined by the R_f of the crosslinked product in each gel. The bands appearing at the top of some of the lanes represent material that did not enter the running gel and is presumably composed of aggregated, crosslinked complexes. The SDS-polyacrylamide gels were analyzed by autoradiography at −70°C between 4 and 14 days.

Diverse Biological Actions of FGF Appear to Be Mediated by a Common Cell Surface Receptor

Addition of FGF to murine C3H10T1/2 cells [Reznikoff et al., 1973], rat pheochromocytoma (PC-12) cells [Schubert et al., 1974], and murine skeletal muscle (MM14) myoblasts [Hauschka et al., 1979] causes cell division in C3H10T1/2 cells (unpublished observations), stimulation of neurite outgrowth in PC-12 cells [Togari et al., 1985; Wagner and D'Amore, 1986; Walikie et al., 1986], and repression of myogenic differentiation in mouse MM14 myoblasts [Linkhart et al., 1981], respectively. Examination of FGF receptor by crosslinking of ^{125}I-aFGF to these three intact cells identified a similar 165-kDa receptor (Fig 1). However, significant variations of both the affinity for ^{125}I-aFGF and the receptor number per cell are observed from equilibrium binding studies (Fig 2; Table I). Consistent with our previous observations, both aFGF and bFGF competed for specific binding of ^{125}I-aFGF to intact C3H10T1/2, PC-12, and MM14 cells, respectively (Fig. 1).

Comparison of ^{125}I-aFGF Binding to Intact BHK Cells and BHK Membranes

Because the binding and crosslinking of ^{125}I-bFGF has been well documented in BHK cells [Neufeld and Gospodarowicz, 1985; Moscatelli, 1987], these cells were chosen for a comparison of ^{125}I-aFGF binding to intact cells and cell membrane preparations. Scatchard analysis of ^{125}I-bFGF binding to intact BHK cells has identified the presence of two distinct binding sites, a low-affinity site sensitive to ionic strength and heparinase and a high-affinity site insensitive to these treatments [Moscatelli, 1987]. Our analysis of ^{125}I-aFGF binding to intact BHK cells identified only a single class of noninteracting sites (Fig. 3a), possibly because of the reduced affinity of aFGF for heparin when compared to bFGF. To determine if high-affinity ^{125}I-aFGF binding

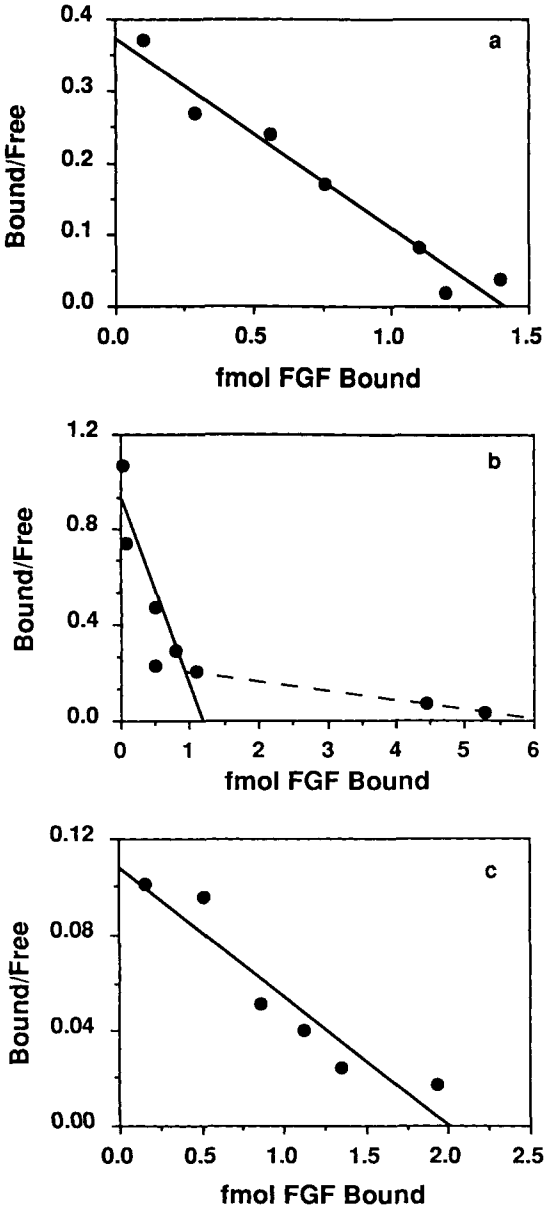

Fig. 2. Equilibrium binding and crosslinking of ^{125}I-aFGF to intact C3H10T1/2, PC-12, and MM14 cells. Increasing concentrations of ^{125}I-aFGF were incubated with intact PC-12 (**a**), C3H10T1/2 (**b**), or MM14 (**c**) cell cultures and processed as described previously [Olwin and Hauschka, 1986]. The data are plotted according to Scatchard [1949]. Cell numbers for each point were 2.5×10^5, 3×10^4, and 1.4×10^6 for PC-12 (a), C3H10T1/2 (b), and MM14 (c), respectively. Each point represents the mean from triplicate determinations. Nonspecific binding at saturation did not exceed 20% of the total bound ^{125}I-cpm for all three cell types. These experiments were repeated two times with similar results.

was retained in plasma membrane preparations, an assay was developed for membrane-associated FGF receptor. Scatchard analysis of equilibrium binding to intact BHK cells and BHK plasma membranes yielded similar K_d values of 67 pM for intact cells and 55 pM for membranes with 13,000 receptors per cell and 45 fmol of ^{125}I-aFGF bound per mg membrane protein, respectively (Fig. 3).

Analysis of FGF Receptor From Tissue Membrane Preparations

Detection of BHK membrane-associated high-affinity ^{125}I-aFGF binding suggested that a similar assay may prove successful for analysis of FGF receptor in tissue-derived membranes. Specific binding of ^{125}I-aFGF was not detected in ten different adult bovine tissues or human term placental membranes (Table II). Estimations of the sensitivity for the membrane binding assay demonstrate that the limit of FGF receptor detection in these assays is at least 0.05–0.1 fmol per 20 μg membrane protein. The FGF receptor content of these membrane preparations is thus less than 2.5–5 fmol per mg membrane protein (see "Discussion").

In contrast to adult tissues, embryonic murine tissues bound significant amounts of ^{125}I-aFGF (Table II). Binding of ^{125}I-aFGF to murine embryonic FGF receptor was specifically blocked by a 100-fold molar excess of unlabeled aFGF and bFGF. The FGF receptor in embryonic membranes binds FGF with high affinity, exhibits a 180-kDa crosslinked ^{125}I-aFGF-FGF receptor complex (manuscript in preparation), and thus is analogous to a previously identified cellular receptor [Olwin and Hauschka, 1986], which is distinct from low-affinity heparin binding sites [Moscatelli, 1987].

TABLE II. FGF-Receptor Content in Mammalian Membrane Preparations

Tissue	FGFR content (fmol ^{125}I-aFGF bound per mg protein)
Bovine brain	<2.5[a]
Bovine pituitary	<2.5
Bovine atrium	<2.5
Bovine ventricle	<2.5
Bovine uterine smooth muscle	<2.5
Bovine adrenal	<2.5
Bovine kidney	<2.5
Bovine liver	<2.5
Bovine lung	<2.5
Bovine testis	<2.5
Mouse 17 day embryo body	6.5 ± 1.8
Mouse 17 day embryo head	10 ± 2
Mouse 17 day embryo placenta	11 ± 4
Human term placenta	<2.5

[a]Where measurable, values are reported as the mean ± standard deviation from three independent experiments. When not detectable, the values are reported as less than 2.5 fmol/mg membrane protein, a conservative detection estimate. Membranes (20 μg) prepared as described in "Materials and Methods" were incubated 1 h at 22°C with 200 pM ^{125}I-aFGF. Nonspecific binding did not exceed 15% of the total specifically bound ^{125}I-cpm for those values reported as measurable.

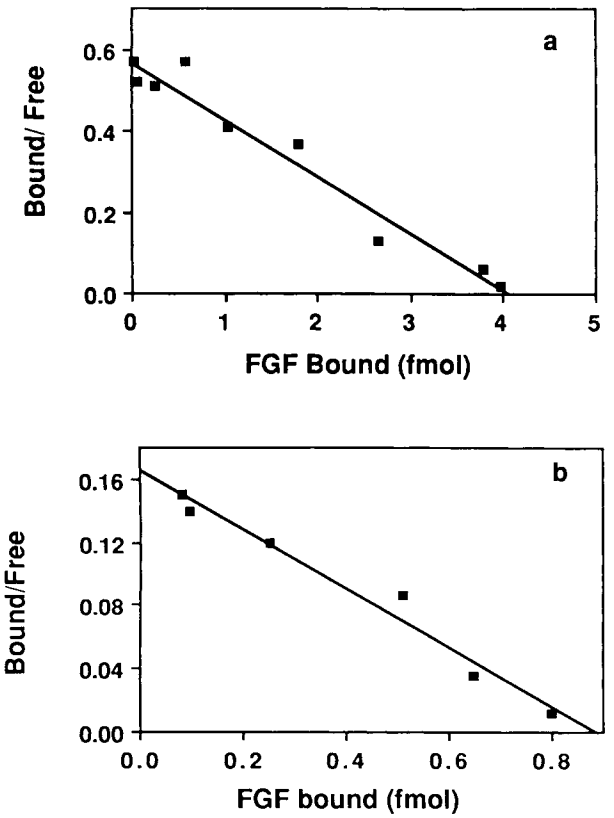

Fig. 3. Comparison of ^{125}I-aFGF binding to intact cells and cell plasma membrane preparations. **a:** Scatchard analysis of ^{125}I-aFGF binding to intact BHK cultures was as described previously [Olwin and Hauschka, 1986]. Each data point is the mean of triplicate determinations. For each point, 2.5×10^5 cells were present. The experiment was performed three times with similar results. **b:** Analysis of FGF receptor in BHK membrane preparations was performed as described in "Materials and Methods." The equilibrium binding data from a single representative experiment was plotted according to Scatchard [1949]. Each data point is the mean of triplicate determinations. At saturation, the nonspecific binding to the membrane preparation did not exceed 10%. These experiments were repeated two times with similar results.

DISCUSSION

High-affinity FGF binding sites are present on a wide variety of mesoderm, neuroectoderm, and tumor-derived cells. In many cell types, FGF functions primarily as a mitogen. All cells examined that express high-affinity ^{125}I-aFGF binding sites also express a 165-kDa FGF receptor. Furthermore, in all cell types examined, bovine aFGF, bFGF, and recombinant human bFGF compete for ^{125}I-aFGF binding to a cell surface receptor (Fig. 1) (unpublished data). Thus, FGF receptors identified by cross-linking of ^{125}I-aFGF to intact cells appear to interact with both acidic and basic forms of FGF. It is unlikely that the competition observed for either aFGF or bFGF is due to contamination of these preparations with the corresponding FGF. The contamination of bFGF in the aFGF preparation is 1% or less (see "Materials and Methods"), and

thus inclusion of a 100-fold molar excess of bFGF would contain at most aFGF concentrations equivalent to the ^{125}I-aFGF concentration in the binding assay. Since a 100-fold molar excess of FGF is required for complete reduction of ^{125}I-aFGF binding sites, the contaminating FGF concentration is not sufficient for complete competition. Furthermore, as demonstrated in previous studies, equivalent concentrations of aFGF and bFGF compete for ^{125}I-aFGF binding to Swiss 3T3 cells [Olwin and Hauschka, 1986] and to a similar extent in BHK cells [Neufeld and Gospodarowicz, 1986], suggesting that both factors bind to a common receptor. Other growth factor receptors also bind multiple ligands. For example, epidermal growth factor receptor binds both epidermal growth factor and transforming growth factor α [reviewed in Carpenter, 1987].

The lower MW band present in F-9, C3H10T1/2, and 1321N1 cells most likely represents a proteolytic fragment of the FGF receptor. Similar bands are occasionally seen in other cell types, including BHK cells. These results are consistent with those originally observed for the epidermal growth factor receptor [reviewed in Carpenter, 1987], which is rapidly degraded by a Ca^{2+}-activated protease. Although the existence of more than one polypeptide cannot be absolutely discounted, the presence of lower MW bands most likely represents proteolytic degradation of the FGF receptor from cells possessing greater endogenous protease activity. Experiments designed to address this question were performed by coculturing BHK cells exhibiting two distinct crosslinked complexes with A431 cells exhibiting one product. The cocultures and control cultures of each individual cell type were crosslinked to ^{125}I-aFGF and analyzed by SDS-PAGE and autoradiography. These experiments invariably revealed only one crosslinked product in the cocultures, one in the A431 cultures, and two in the BHK cultures (unpublished data). Until further molecular detail is available for the FGF receptor, the existence of multiple receptor types will remain unresolved.

The biological activities of FGF in vitro are extensive [for reviews, see Gospodarowicz et al., 1986; Baird et al., 1986; Folkman and Klagsbrun, 1987]. Actions of FGF distinct from mitogenesis include stimulation of angiogenesis [for a review, see Folkman and Klagsbrun, 1987], stimulation of neuronal differentiation [Togari et al., 1985; Walickie et al., 1986; Wagner and D'Amore, 1986; Morrison et al., 1986], and repression of skeletal muscle differentiation [Linkhart et al., 1981; Lathrop et al., 1985; Clegg et al., 1987]. Previous studies indicated that the 165-kDa FGF receptor, identified by homobifunctional crosslinking of ^{125}I-aFGF to intact Swiss 3T3 cultures and murine MM14 myoblasts, binds FGF with affinities consistent with its function as a FGF receptor and mediator of FGF's biological activities [Olwin and Hauschka, 1986, 1988]. To determine whether the biological actions of FGF in this study were mediated by distinct cell surface receptors, we examined the binding characteristics and FGF receptor MW in these three cell lines. Although the K_d and FGF receptor number per cell varies between these cell types, they were within the range exhibited by other cells (Table I). Also consistent with other cell types, an apparent receptor MW of 165,000 is observed for all three cell lines (Fig. 1). Both aFGF and bFGF are biologically active for all three cell types, and both forms of FGF effectively compete for ^{125}I-aFGF binding (Fig. 1). Similar results have been observed using ^{125}I-bFGF (manuscript in preparation). These data suggest that the diverse biological actions of FGF do not originate via interaction of either aFGF or bFGF with different cell surface receptors specific for either growth factor. Although the low-affinity FGF bind-

ing sites seen in C3H10T1/2 cultures were not examined further, similar low-affinity sites have been characterized in detail for ^{125}I-bFGF binding to BHK cells [Moscatelli, 1987]. Since the low affinity BHK binding sites do not correlate with the biological activity of FGF, and since the sites are sensitive to both high ionic strength and treatment with heparinase [Moscatelli, 1987], the C3H10T1/2 low-affinity sites most likely represent cell-associated heparin binding sites for FGF.

A comparison of FGF receptors from intact BHK cells and BHK plasma membrane preparations indicates the presence of high-affinity ^{125}I-aFGF binding sites in the membrane fraction (Fig. 2b). The affinity of BHK membranes for ^{125}I-aFGF is virtually identical with the affinity of receptors for ^{125}I-aFGF in intact cell cultures (Fig. 2). In addition, the concentration of added unlabeled aFGF required for displacement of 50% of bound ^{125}I-aFGF from either intact cells or membrane preparations was equal to the concentration of ^{125}I-aFGF added to the incubation (unpublished data), suggesting the presence of a single class of noninteracting binding sites.

Retention of high-affinity ^{125}I-aFGF binding in BHK membranes allowed the development of efficient assays for the detection of FGF receptor in tissue-derived membrane preparations. However, an initial examination of ^{125}I-aFGF binding to adult bovine tissues and human term placental membranes failed to reveal specific ^{125}I-aFGF binding (Table II). In contrast to adult membranes, embryonic murine membranes express high-affinity ^{125}I-aFGF binding sites. Crosslinking of embryonic murine membranes identifies a FGF receptor of 165 kDa, which binds both aFGF and bFGF (manuscript in preparation).

One potential artifact that may reduce the apparent number of high-affinity FGF binding sites is the presence of high concentrations of endogenous FGF in the membrane preparations. To test for such artifacts, the FGF content in the membrane preparations was estimated using a repression of differentiation assay for MM14 myoblasts [Olwin and Hauschka, 1986]. Since MM14 cells are absolutely dependent on FGF for cell division [Linkhart et al., 1981; Clegg et al., 1987], the assay is specific for unbound FGF in crude extracts and has a detection limit of 0.3 fmol bFGF [Seed et al., 1988]. Although the initial supernatants from the membrane preparations exhibited significant FGF biological activity, analysis of several washed, membrane preparations did not detect significant levels of biologically active FGF. Additional support for the absence of detectable FGF receptor is the observation that unlabeled aFGF or bFGF compete for bound ^{125}I-aFGF. Therefore, significant exchange should occur between the added excess of ^{125}I-aFGF and the endogenous bound FGF present. Finally, we have attempted to dissociate bound FGF from membrane preparations by treatment with acidic buffers. Such treatment, which does not reduce binding affinity, yields increases in specific ^{125}I-aFGF binding of less than 20% (unpublished data). Thus, the inability to detect FGF receptor in adult tissues does not appear to be due to the presence of endogenous FGF in the membrane preparations, and therefore we conclude that the FGF receptor content of these adult bovine membranes is less than 2.5 fmol per mg protein, the detection limit of the assay.

Since aFGF and bFGF have been implicated as mesoderm-inducing factors in the early development of the amphibian embryo [Slack et al., 1987; Kimmelman and Kirschner, 1987], a distribution of FGF receptor might be present in the developing embryo. Analysis of high-affinity sites for ^{125}I-aFGF binding to embryonic membranes reveals low levels of specific binding (Table II). It is thus tempting to speculate that the presence of relatively high levels of FGF receptor in the embryo signifies the

importance of FGF for embryonic development. The relative lack of widespread FGF receptor in adult tissues examined in this study may indicate more restrictive roles for FGF action in the adult.

Cultured cell lines and embryonic tissues, but not several adult bovine tissues, express high-affinity FGF binding sites. Partial characterization of FGF receptors from several species suggests the existence of a common 165-kDa receptor that is capable of interacting with both aFGF and bFGF. Related FGF receptors are also present on cells that exhibit divergent biological responses upon addition of exogenous FGF. The recent identification of oncogenes possessing approximately 40–50% homology with bFGF [Dickson and Peters, 1987; Delli-Bovi et al., 1987; Yoshida et al., 1987] suggests the existence of a FGF gene family. Although bFGF and aFGF appear to bind to a common cell surface FGF receptor, it is unlikely that an entire FGF gene family exists for which there is only one cell surface receptor. If more than one receptor subtype exists, then the current methods used to identify FGF receptor may not distinguish between related FGF receptor subtypes. It is likely that the receptor that binds both aFGF and bFGF may not bind other members of the FGF family. Identification of such receptor subtypes, if they exist, will require further biochemical characterization of FGF receptor(s).

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