Basic fibroblast growth factor (FGF) and keratinocyte growth factor (KGF) are structurally related fibroblast growth factors, yet they exhibit distinct receptor binding specificity. Basic FGF binds with high affinity to FGFR1, FGFR2, and FGFR4, whereas KGF does not interact with these receptors and can only bind an isofrom of FGFR2 known as the KGFR. Basic FGF binds KGFR but with lower affinity than KGF. In order to identify domains that confer this specificity, four reciprocal chimeras were generated between the two growth factors and were analyzed for receptor recognition and biological activity. These chimeras were designated BK1 (bFGF1–54:KGF91–194), BK2 (bFGF1–74:KGF111–194), KB1 (KGF31–90:bFGF55–155), and KB2 (KGF31–110:bFGF75–155). The two BK chimeras similarly interacted with FGFR1 and FGFR4 but differed from each other with respect to KGFR recognition. BK1 displayed a slightly better affinity for KGFR than BK2 and induced a higher level of DNA synthesis in keratinocytes compared with bFGF and BK2. A neutralizing monoclonal antibody directed against bFGF specifically neutralized the biological activity of the BK chimeras. The reciprocal chimeras, KB1 and KB2, exhibited KGFR-like receptor binding and activation properties. However, KB2 displayed higher affinity for KGFR and was significantly more potent mitogen than KB1. Altogether, our results suggest that the amino-terminal part of KGF and bFGF plays an important role in determining their receptor binding specificity. In addition, the results point to the contribution of a segment from the middle part of KGF (residues 91–110) for recognition and activation of the KGFR, as the two chimeras containing these residues (BK1 and KB2) displayed an enhanced interaction with the KGFR.

Basic fibroblast growth factor (bFGF)1 (1) and the keratinocyte growth factor (KGF) (2) belong to the fibroblast growth factor (FGF) family of which nine members have been identified (for review, see Refs. 3 and 4). Unlike bFGF that acts on a wide variety of cells types, the activity of KGF is restricted to cells of epithelial origin (5).

FGFs elicit their biological activities by interacting with cell surface tyrosine kinase receptors. Four closely related receptors, designated FGFR1–FGFR4, have been isolated. In addition, an alternative splicing mechanism gives rise to multiple isoforms of FGFR1–FGFR3 (for review, see Refs. 6 and 7). An important feature of the relationship between FGFs and their ligands is a high degree of cross-reactivity. Thus, all receptors that have been examined interact with more than one type of FGF, and likewise, a given FGF can bind more than one type of FGF. For example, bFGF binds with high affinity to FGFR1 (8, 9), FGFR2 form IIC (10), and FGFR4 (11), whereas acidic FGF (aFGF) binds all four receptors (8–13). KGF is unique among FGFs since it interacts only with one FGFR type. This receptor is an isoform of FGFR2 known as the KGFR or FGFR2 form IIB (10, 14). Basic FGF can also bind this isoform albeit with 10–15-fold lower affinity than KGF (14–16).

Besides binding to FGFRs, FGFs interact with heparin-like molecules. These molecules are heparan sulfate proteoglycans that exist on the surface of many cell types and in the extracellular matrix (17–19). Heparan sulfate (HS) and heparin protect bFGF from heat inactivation and proteolytic degradation (20). In addition, HS modulates the biological activities of FGFs. In cells expressing HS, depending on the cell or FGF receptor type, heparin or HS can either stimulate or inhibit the biological activities of FGFs (5, 16, 21, 22). In cells deficient in HS, the mitogenic activity of aFGF and bFGF is absolutely dependent on the addition of heparin (13, 23–25). In this type of cells, heparin also facilitates the binding of aFGF and bFGF to their receptors (13, 16, 25, 26), whereas the binding of KGF to KGFR is inhibited (16). The mechanism by which HS or heparin exert these multiple effects is not clear. To date, four different mechanisms were proposed (27–30).

Although much is known concerning the multiple forms of FGFs and their ligand binding properties, less is known about the structural domains that determine the receptor binding characteristics of FGFs. Because bFGF and KGF exhibit distinct receptor binding specificity, we reasoned that the generation of chimeric molecules between the two growth factors will help to identify determinants that confer receptor binding specificity. In this study, such chimeric molecules were generated, and their biological properties were characterized.

**EXPERIMENTAL PROCEDURES**

**Materials—**Recombinant bFGF and KGF were produced in bacteria as described previously (21, 31). Bovine brain aFGF was purchased from R&D. Na125I (5000 Ci/mmol) was purchased from DuPont NEN. Heparin-Sepharose CL-6B was from Pharmacia Biotech Inc. Bovine serum albumin was purchased from Boehringer Mannheim. Disuccinimidyl suberate was from Pierce. Fetal and newborn calf serum and media were purchased from Biological Industries (kibbutz Beth-Haenek). Heparin from bovine lung and all other chemicals were purchased from Sigma. Fibronectin and Type I antibody were purchased from Colipex Inc.
from Upstate Biotechnology Inc.

Tissue Culture—The generation of L6E9 cell lines expressing high levels of the KGF receptor gene product (designated L6/KGFR cells) FGFR1 (L6/R1) and FGFR4 (L6/R4) was described elsewhere (11). These cell lines were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, NIH/3T3 or NIH/3T3 cells were overexpressing the KGFR (NIH/KGFR cells) (14) were grown in Dulbecco's modified Eagle's medium containing 10% new born calf serum. Balb/MK cells were grown in low calcium media containing 5 ng/ml epidermal growth factor and 10% dialyzed fetal calf serum, as described previously (32).

FGFR1 (L6/R1) and FGFR4 (L6/R4) was described elsewhere (11). The chimeric molecules were generated in two steps by polymerase chain reaction technique (33) as illustrated in Fig. 1B. In step I, each fragment to be recombined was amplified such that it would contain a tail of 21 nucleotides from the region of the reciprocal molecule that was to flank the recombination site. In step II, the amplification products of the first step were annealed to each other (A to D and C to B) and amplified with the appropriate amino and carboxyl-terminal primers (see Fig. 1B). The chimeric molecules were adapted for cloning by adding an NdeI and BglI site in their amino and carboxyl termini, respectively. Each fragment was cloned into the pet3C vector containing and that point mutations were not introduced during the amplification reaction.

Production and Purification of the Chimeric Proteins—Production and purification of the recombinant proteins was done as we described previously (21). BL21 (DE3) pl expression plasmid were grown at 30 °C in terrific broth (36) containing 100 μg/ml ampicillin and 25 μg/ml of chloramphenicol. When A600 reached 0.8, isopropyl-1-thio-D-galactopyranoside (0.2 mM) was added, cultures were incubated for 2.5 h, and cells were collected by centrifugation. The cell pellet was resuspended in TENG buffer (10 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 5 mM MgCl2, pH 7.5) containing protease inhibitors, and the cells were lysed by three successive cycles of freezing and thawing. The lysate was disrupted by sonication and clarified by centrifugation.

Purification of the recombinant proteins was performed by applying the bacterial supernatant to a heparin-Sepharose column. The column was washed with phosphate buffer (50 mM, pH 7.2, containing 0.2 mM NaCl) until the absorbance fell to near base line and then was subjected to a linear step gradient of increasing NaCl concentration. Aliquots from the fractions were analyzed by SDS-PAGE (37) and immunoblotting and tested for mitogenic activity. The recombinant BK chimeras were eluted at 0.45 M NaCl, and the KB chimeras were eluted at 0.85 M NaCl. Selected fractions were concentrated 10–20-fold with a Centricon-10 microconcentrator (Amicon). The concentrated fractions were diluted to 0.2 M phosphate buffer, pH 6.8, and subjected to fast protein liquid chromatography using a gradient of increasing NaCl concentrations.

Protein Detection—The fractions containing the recombinant proteins were identified following SDS-PAGE (37) and immunoblotting with bFGF antiserum directed against the native molecule or KGF polyclonal antibodies (21) raised against peptides corresponding to residues 33–44 or 179–194 (residue 1–31 in KGF corresponds to the hydrophobic signal sequence). The purity of the recombinant proteins was determined following SDS-PAGE, fixation of the gel, and silver staining (38) using the reagents and protocol available from Bio-Rad. The final concentrations of purified proteins were determined by Lowry assay (39) and by comparing the concentrations of diluted samples with those of a standard molecule (bFGF or KGF) following SDS-PAGE and Coomassie Brilliant Blue staining.

Immunoprecipitation, when indicated in the text, was done using 200 μl of purified bFGF or KGF. The proteins were incubated for 2–12 h with either a polyclonal antibody directed against KGF or a monoclonal antibody directed against bFGF (Type I antibody) in IP buffer (40). The complex was recovered by protein A-Sepharose and then separated by electrophoresis and blotted onto nitrocellulose filter paper. Immunodetection was done with a polyclonal antibody directed against either bFGF or KGF, and development was done using enhanced chemiluminescence reagents.

Growth Factor Iodination, Receptor Binding Assays, and Mitogenic Activity—Acidic FGF, bFGF, or KGF were labeled with Na125I using the chloramine-T method (41) as described previously (11). The specific activities of iodinated growth factors was in the range of 1.5–2 x 106 cpm. For the binding competition assays, subconfluent cultures in 24-well microtiter plates were washed 3 times with ice-cold phosphate-buffered saline, and then tracer levels of radiolabeled ligand (1–2 ng/ml) were added in HEPES binding buffer (15) in the presence of increasing concentrations of unlabeled competitor (0–2 μg/ml). Incubation was performed for 2 h on ice, and analysis of specifically bound growth factor was done as described previously (16). Covalent cross-linking was performed with subconfluent cultures of cells in 60-mm dishes using 4 ng/mg labeled KGF or bFGF as described previously (11). Equal amounts of protein were subjected to SDS-PAGE on 6% gel, followed by drying and autoradiography.

DNA synthesis was measured by [1H]thymidine incorporation assay. NIH/3T3 or Balb/MK cells, plated in fibronectin (1 μg/cm2) coated 96-well microtiter plates, were serum-starved for 48 h, treated with growth factor, and processed as described elsewhere (42).

RESULTS

Generation and Production of bFGF and KGF Chimeric Molecules—Comparison of the predicted amino acid sequence of FGFs reveals the greatest divergence in their amino-terminal domains. Therefore, the chimera were generated by replacing amino-terminal parts of bFGF and KGF. The polymerase chain reaction technique (33) was utilized to generate four reciprocal chimeric molecules between bFGF and KGF (see Fig. 1, A and B). In order to increase the probability of obtaining functional molecules, the recombination points were designed in regions of high similarity between KGF and bFGF. The 155-amino acid form of bFGF and the mature KGF polypeptide (residues 31–194) were used in this study. The amino acid composition of the chimeric proteins is as follows: bFGF 1–54, KGF 91–194 (designated BK1), bFGF 1–74, KGF 111–194 (BK2), KGF 31–90, bFGF 55–155 (KB1), KGF 31–110; bFGF 75–155 (KB2) (Fig. 1A). The mutants and the parental molecules were expressed in E. coli and purified to homogeneity utilizing heparin-Sepharose affinity chromatography followed by ion exchange as described previously (21). The BK chimeras similar to KGF were eluted from the heparin-Sepharose column at 0.45 M NaCl, while the KB chimeras were eluted at about 0.85 M salt. The identity of the chimeric molecules was confirmed using polyclonal antibodies directed against bFGF or KGF. Thus, the anti-bFGF antibody and an antibody directed against a carboxyl-terminal peptide of KGF immunologically recognized the BK chimeras, whereas an antibody directed against an amino-terminal peptide of KGF recognized only the KB chimeras (data not shown).

Biological Properties of the Chimeric Molecules—To investi-
with the KGFR. Due to the strong inhibitory effect of heparin on the BK chimeras, it was necessary to examine their interaction and receptor binding specificity. FGFR1 and FGFR4, the BK chimera exhibited bFGF-like receptor binding ability and mitogenic activity. The activity of both bFGF and KGF is modulated by heparin (16, 21, 22). Preliminary experiments revealed that the biological activities of the BK chimeras are highly dependent on heparin, whereas those of the KGFR cells were strongly inhibited by heparin (as will be described later). Therefore, analysis of the biological activities of the BK chimeras was done in the presence of heparin, whereas those of the reciprocal, KB chimeras, was carried out in the absence of heparin.

The receptor recognition properties of the chimeras were assessed by examining their ability to compete for the binding of radiolabeled KGF to KGFR and for the binding of bFGF to FGFR1 and FGFR4. The binding experiments were performed in the rat myoblast cell line, L6E9, that lacks detectable high affinity binding sites for FGFs and was engineered to overexpress functional FGFR1, FGFR4, and KGFR (11, 43). Some of the experiments were also carried out in NIH/3T3 cells overexpressing the KGFR. The results are shown in Figs. 2 and 3. The two KB chimeras, which contain the amino-terminal part of KGF and carboxyl-terminal part of bFGF (KB1 and KB2), did not compete with bFGF for binding either to L6/R1 or to L6/R4 cells (Fig. 2, A and B). In contrast, both KB chimeras competed with KGF for binding to the L6/KGFR cells (Fig. 2C). KB2 competed for bound KGF about 6-fold more efficiently than KB1 but about 20-fold less efficiently than KGF. Thus, the two KB chimera displayed the receptor binding specificity of KGF.

In contrast to the results with the KB chimeras, the reciprocal chimeras, BK1 and BK2, competed for the binding of 125I-bFGF to both L6/R1 and L6/R4 cells (Fig. 3, A and B). The efficiency of displacement for both BK chimeras was about 20-fold lower than that obtained with unlabeled bFGF. These findings indicate that with respect to the interaction with FGFR1 and FGFR4, the BK chimera exhibited bFGF-like receptor binding specificity.

In order to complete the assessment of the binding profile of the BK chimeras, it was necessary to examine their interaction with the KGFR. Due to the strong inhibitory effect of heparin on the binding of KGF to L6/KGFR cells (16) and the observed dependence of the BK chimeras on heparin for receptor binding, it was not possible to perform the assay with 125I-KGF on L6/KGFR cells. Therefore, competition was carried out using NIH/KGFR cells. In these cells, heparin inhibits the binding of KGF to its receptor as well, but in heparin concentrations higher than 3 μg/ml (16). Alternatively, the assay was carried out in L6/KGFR cells but on bound 125I-aFGF. Unlike KGF, the binding of aFGF to the KGFR is not inhibited by heparin (16). As shown in Fig. 3C, the binding of KGF to NIH/KGFR cells was inhibited by both BK chimeras, but BK1 was slightly more efficient competitor than BK2. Half-maximal displacement was obtained at about 80 and 250 ng/ml of BK1 and BK2, respectively. It should be noted that a comparison of the efficiency of competition with that of bFGF in this experiment is complicated by the fact that heparin inhibits the binding of bFGF to the KGFR (compare Figs. 2C and 3C). Similar results were obtained when the competition assay was performed in L6/KGFR cells using 125I-aFGF (data not shown).

To further confirm the receptor binding specificities of the chimeric molecules, we examined the pattern of cross-linking of 125I-bFGF to L6/R1 and 125I-KGF to NIH/KGFR cells in the absence or presence of unlabeled chimeric molecules. As shown in Fig. 4, a band corresponding to 125I-bFGF cross-linked to FGFR1 (Fig. 4A) and of 125I-KGF to KGFR (Fig. 4B) was observed in the absence of competitors. Excess of bFGF, BK1 and BK2 efficiently competed for the binding of radiolabeled bFGF to FGFR1 and of KGF to KGFR (Fig. 4, A and B). The KB chimeras, similar to KGF, could only compete for the binding of 125I-KGF to the KGFR but not for the binding of 125I-bFGF to FGFR1 (compare Fig. 4, A and B, lanes 6–10).

We next examined whether the receptor binding properties of the chimeras correlated with the known specificities of bFGF and KGF in eliciting a mitogenic response. To this end, we performed [3H]thymidine incorporation assay in NIH/3T3 and Balb/MK cells. Basic FGF is highly mitogenic to NIH/3T3 cells and, to a lesser extent, to Balb/MK cells, while KGF induces a mitogenic response only in Balb/MK cells (5, 42). As shown in Fig. 5, the two BK chimera induced an efficient and identical
level of DNA synthesis in NIH/3T3 cells (Fig. 5A). The two BK chimeras were also mitogenic to Balb/MK cells, but in these cells BK1 induced a higher level of DNA synthesis than BK2 or even bFGF (Fig. 5B). To exclude the possibility that the lower biological activity of BK2 in Balb/MK cells was due to instability, we incubated equal amounts of BK1 and BK2 at 37° C in Dulbecco’s modified Eagle’s medium in the presence and absence of Balb/MK cells for 16 h. The medium was then removed, clarified by centrifugation, and tested for mitogenic activity on NIH/3T3 cells. Both chimeras exhibited identical activity, indicating that BK2 is not more labile than BK1. The lack of instability problem and the fact that both chimeras exhibited an identical activity in NIH/3T3 cells, suggests that the additional 20 residues of KGF (residues 91–110), which are present only in BK1, confer on this molecule a better ability to move, clarified by centrifugation, and tested for mitogenic activity. The biological activity of BK2 in Balb/MK cells was due to instability, as judged by binding competition and immunoprecipitation experiments (Fig. 5D). Taken together, the results of the mitogenic assay correlated well with the receptor recognition properties of the chimeric molecules.

A Neutralizing Monoclonal Antibody Directed against bFGF Specifically Inhibits the Biological Activity of the BK Chimeras—If receptor recognition determinants are present in the amino-terminal part of bFGF, it may be expected that neutralizing antibodies directed against this molecule will recognize and neutralize the BK chimeras. The results obtained with a neutralizing anti-bFGF monoclonal antibody (designated Type I) are shown in Fig. 6. This antibody did not cross-react with KGF, as judged by binding competition and immunoprecipitation experiments (Fig. 6, A and B). The antibody neutralized the mitogenic activity of bFGF and of the two BK chimeras but not that of KGF and the KB2 chimera (Fig. 6C). The extent of neutralization was 55 and 85% for BK1 and BK2, respectively. Apparently, three different preparations of polyclonal antibodies against native bFGF also neutralized the biological activities of bFGF and the BK chimeras, suggesting that the amino-terminal part of bFGF contains a strong antigenic determinant (data not shown).

The Effect of Heparin on the Biological Activities of the Chimeric and Parental Molecules—Heparin modulates the biological activities of bFGF and KGF (16, 21, 22). Depending on the cell type, it can either enhance or inhibit the biological activity of bFGF. For example, in NIH/3T3 cells that express FGRF1, heparin is not necessary for bFGF-induced mitogenic response, but it potentiates the biological activity of this growth factor (3). In contrast, heparin inhibits the mitogenic activity of bFGF in Balb/MK cells that naturally express KGFR (22). The binding of KGF to the KGFR and its biological activity are inhibited by heparin (16, 21).

Basic FGF and KGF display different affinities for heparin. Dissociation of bFGF from heparin-Sepharose or cell-associated heparan sulfates occurs at 1.5–2 mM NaCl, while KGF displacement from these glycosaminoglycans occurs at 0.5 mM salt (17, 21). Apparently, the BK chimeras displayed KGF-like affinity for heparin, whereas the affinity of the KB chimeras for this glycosaminoglycan was about 1.6-fold higher than that of KGF (eluted at 0.85 mM NaCl). The results shown in Fig. 7 demonstrate the effect of heparin on the biological activities of the parental and chimeric molecules. Similar to KGF, the mitogenic activity of KB2 was inhibited by about 10-fold in the presence of heparin (Fig. 7A). The mitogenic activity of bFGF was enhanced by heparin in NIH/3T3 cells but inhibited in Balb/MK cells (Fig. 7, B and C), in agreement with previous report (22). In contrast to the situation with bFGF, heparin enhanced the biological activity of BK1 in both cell types (Fig. 7, B and C). Similar results were obtained with the other two chimeras (BK2 and KB1), and similar modulation of heparin was observed on receptor binding (data not shown).

DISCUSSION

In the present study, we generated chimeric molecules between bFGF and KGF in order to find out how their unique receptor binding characteristics will be segregated. The BK chimeras containing the amino-terminal part of bFGF (BK1,
bFGF1–54:KGF91–194; BK2, bFGF1–74:KGF111–194) interacted of the binding of 125I-bFGF (m) by a type I antibody against bFGF. Panel A, inhibition of the binding of 125I-bFGF (●) to L6/R1 and 125I-KGF (●) to L6/KGFR cells by type I antibody; panel B, lack of cross-reactivity between KGF and type I antibody. Purified KGF or bFGF were first immunoprecipitated (IP) by either a type I antibody or a monoclonal antibody against KGF. The complex was recovered using protein A-Sepharose, separated on 12% SDS-PAGE, and immunoblotted with polyclonal antisera against KGF or bFGF. Panel C, neutralization of the biological activity of the BK chimeras by type I antibody. The indicated growth factors were added in the absence (closed bars) or in the presence of 5 μg/ml of Type I antibody (shaded bars) or nonspecific IgG (open bars). The mitogenic assay was carried out as described in the legend to Fig. 5.

bFGF1–54:KGF91–194, BK2, bFGF1–74:KGF111–194) interacted similarly with FGFR1 and FGFR4 and exhibited an identical mitogenic activity in fibroblasts that responds to bFGF but not to KGF. Since only bFGF but not KGF can bind and activate FGFR1 and FGFR4, these findings strongly suggest that the first 54 residues of bFGF confer bFGF-like receptor recognition. The BK chimera, like bFGF, interacted with the KGFR. The affinity of BK1 to this receptor was only slightly higher than that of BK2. However, BK1 induced a significantly higher level of mitogenic response in keratinocytes that naturally express the KGFR and at high concentrations reached a maximal response that is similar to that obtained with KGF. In these cells, BK1 (at concentrations ≥10 ng/ml) also induced higher level of DNA synthesis than bFGF. These results point to the importance of residues 91–110 of KGF for the activation of the KGFR since these residues are only present in BK1 but not in BK2.

The reciprocal chimeras, KB1 and KB2, displayed KGF-like receptor recognition and did not recognize FGFR1 and FGFR4. Therefore, residues 59–110 of KGF contain a determinant that is involved in conferring KGF-like receptor recognition (residues 31–58 are not required for receptor binding as we have previously shown (21)). The finding that KB2 (KGF 31–110; bFGF 75–155) has higher affinity for KGFR and is a much more potent mitogen than KB1 (KGF 31–90; bFGF 55–155) points again to the importance of residues 91–110 of KGF for KGFR receptor recognition and activation. Apparently, the corresponding 20-residue segment of bFGF (amino-acids 55–74) does not play a similar role in the interaction with FGFR1 and FGFR4, as both BK chimeras similarly interacted with these two receptors.

Taken together, our results indicate that the amino-terminal part of bFGF and KGF is involved in determining their receptor binding specificities. Additionally, a segment from the middle part of KGF also has an important contribution for KGF receptor recognition. The amino-terminal part of other FGFs is likely to play a similar role, since a chimera between aFGF and KGF (aFGF1–34:KGF111–194) displays an aFGF receptor recognition pattern (data not shown). The high degree of amino-acid sequence divergence within the amino-terminal part of FGFs further supports a role for this region in determining their distinct receptor binding characteristics.

In addition to the differential receptor recognition, the chimeric molecules exhibited distinct behavior with respect to the modulation of their activities by heparin. The biological activities of the KB chimeras were inhibited, whereas those of the BK chimeras were enhanced by heparin. Because heparin inhibits the biological activities of KGF (16, 21) and in general potentiates the activities of bFGF (3), it seems that the heparin dependence of the chimeric molecules resembled that of the parent molecule that displayed similar receptor binding specificities. An exception from this general behavior was the effect of heparin on the interaction of bFGF with the BK chimeras with the KGFR. While heparin inhibited the KGFR-mediated biological activities of bFGF, it enhanced those of the BK chimeras. The reason for this is not clear, but it might be the consequence of the significantly lower affinity of the BK chimeras for heparin compared with bFGF (as judged from the salt concentration required for elution from heparin-Sepharose).

The receptor binding site of bFGF was recently mapped by site-directed mutagenesis (30). It was proposed that bFGF contains two separated binding sites. A primary site that is responsible for high affinity interaction of bFGF with its receptor and is comprised of six discontinuous residues that are localized on the same face in the three-dimensional structure (44,
The other binding site plays only a secondary role in receptor binding since its affinity to FGFR1 is extremely low and is located within a loop structure between the ninth and tenth β-strands in the carboxyl-terminal part of bFGF (45, 46). Out of the six residues comprising the proposed primary binding site, four are identical in bFGF and KGF. The residues that differ (Tyr-24 and Arg-44 in bFGF, Phe-71 and Gln-91 in KGF), are located within the region that is suggested by the present study to be important for receptor binding specificity. Although not yet proven, we believe that these two different residues are unlikely to be responsible for the distinct receptor binding characteristics of KGF and bFGF because they represent a conservative change (30). Moreover, the proposed primary binding site of bFGF (except for a conservative change in the carboxyl-terminal tail) aligns with identical residues in aFGF, yet the two growth factors display distinct receptor binding characteristics or tissue specificity (13, 15). Therefore, other residues within the amino-terminal part of FGFs are likely to be involved in the determination of their receptor recognition characteristics. Distinct determinants conferring receptor recognition and high affinity binding were reported for ligands of the integrins (46).

The proposed secondary binding site of FGFs is suggested to play a role in receptor dimerization (30). Because the amino acid sequence of this site shows a high degree of diversity among FGFs, it was also suggested that it might provide their distinct receptor binding characteristics or tissue specificity (30, 47). The ability of the BK chimera (in which the secondary binding site of bFGF was replaced with that of KGF) to induce a biological activity in fibroblasts that do not bind or respond mitogenically to KGF, strongly suggest that, albeit the diversity, the secondary binding site can be exchanged among FGFs without affecting their receptor binding and target cell specificity. If this site, indeed, plays a role in receptor dimerization, we predict that our chimeric molecules will induce the formation of FGFR1 and KGFR heterodimers. Studies are currently under way to address this question.

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Chimeric Molecules between Keratinocyte Growth Factor and Basic Fibroblast Growth Factor Define Domains That Confer Receptor Binding Specificities
Ronit Reich-Slotky, Ester Shaoul, Bluma Berman, Grazia Graziani and Dina Ron

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