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Receptor for acidic fibroblast growth factor is related to the tyrosine kinase encoded by the *fms*-like gene (FLG)

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ABSTRACT **We have previously isolated a human gene from an endothelial cell cDNA library encoding a putative tyrosine kinase; we have designated this gene the *fms*-like gene (FLG). To analyze the gene product(s) of FLG, we have generated rabbit polyclonal antibodies directed against a synthetic peptide from FLG and used it to immunoprecipitate biosynthetically labeled FLG protein from a variety of human cell lines. These antibodies specifically recognized glycoprotein(s) of 100, 120, and 135 kDa with protein cores of 90 and 110 kDa. Acidic fibroblast growth factor (aFGF) stimulated tyrosine kinase activity of FLG *in vitro* and in living cells, suggesting that FLG encodes the membrane receptor for aFGF. Further supporting evidence came from cross-linking experiments on intact cells with the covalent cross-linking agent disuccinimidyl suberate and ^{125}I-labeled aFGF as a specific probe. The cross-linked ^{125}I-labeled aFGF–aFGF receptor complex was specifically immunoprecipitated with FLG anti-peptide antibodies. It appears, therefore, that the receptor(s) for aFGF is related to the FLG gene product.**

Polypeptide growth factors mediate their mitogenic response by binding to and activating specific cell surface receptors. A large family of growth factor receptors possess cytoplasmic domains with intrinsic protein tyrosine kinase activities (for reviews, see refs. 1–3). Mutational analysis has shown that the protein tyrosine kinase activity of these growth factor receptors is essential for signal transduction, mitogenesis, transformation, and normal cellular trafficking (for reviews, see refs. 1 and 2). The receptors containing protein tyrosine kinase activity include the receptors for insulin, insulin-like growth factor 1, epidermal growth factor, platelet-derived growth factor (PDGF), and colony-stimulating factor 1 (CSF-1) (1–3). It was also reported that the binding of either basic or acidic fibroblast growth factor (bFGF and aFGF, respectively) to their cell surface receptors elicited tyrosine phosphorylation of cellular substrates in living cells, suggesting that FGF receptors also belong to the tyrosine kinase receptor gene family (4, 5). FGFs are essentially ubiquitous in nature. High-affinity FGF receptors in the range of 110–150 kDa have been identified on a variety of cell types by chemical crosslinking (6–14). Low-affinity binding sites have also been described and appear to be glycosaminoglycans (14, 15). High-affinity FGF receptors are lost permanently during mouse skeletal muscle-cell terminal differentiation *in vitro* (16) and are reduced at high cell density (17). Further investigation of these phenomena would be greatly facilitated with molecular probes for studies of FGF receptor expression.

In recent years tyrosine kinases were identified by a variety of approaches including the analysis of retroviral oncogenes, transformation of NIH 3T3 cells by transfection with DNA from human or animal tumors and by hybridization at low stringency of either cDNA or genomic libraries with DNA probes of known tyrosine kinases.

By using low-stringency hybridization conditions we have previously described (18) a partial clone isolated from an endothelial cell cDNA library encoding a tyrosine kinase. This gene, which we designated the *fms*-like gene (FLG), encodes a protein tyrosine kinase with a typical kinase insert similar in its location to the kinase inserts of PDGF and CSF-1 receptor kinases (1, 3). We have recently cloned and sequenced cDNA clones encoding the amino-terminal region of FLG (C.D., M.J., and J.S., unpublished results) and shown that the extracellular domain of FLG contains typical signal sequence and is highly homologous to the avian bFGF receptor (19). In fact, 11 of 14 tryptic peptides of the purified chicken bFGF receptor were identified within the sequence of FLG and an oligonucleotide based upon the FLG sequence was used to clone the chicken bFGF receptor (19). Therefore, this receptor represents the avian counterpart of FLG. A similar protein tyrosine kinase designated bek has been isolated from a murine liver cDNA library by using an expression cloning approach with phosphotyrosine-specific antibodies (20). The amino acid sequences upstream of the kinase domain of FLG contain a putative transmembrane domain and several asparagine-linked glycosylation sites in the extracellular domain. The extracellular domain of FLG is similar to the extracellular domain of the interleukin 1 (IL-1) receptor mainly in the positions of its cysteine residues and the presence of structural motifs shared with the immunoglobulin superfamily (21). A weak but significant sequence identity between IL-1 and aFGF was described (22), which is interesting in light of the structural similarities among FLG (18), the avian bFGF receptor (19), and the IL-1 receptor (21).

In this report we show that the FLG gene directs the synthesis of glycoproteins of 100, 120, and 135 kDa with protein cores of 90 and 110 kDa. The FLG protein(s) were immunoprecipitated from cultured cells by using a specific rabbit antiserum directed against a synthetic peptide from FLG. The FLG protein was phosphorylated in living cells and aFGF was able to stimulate tyrosine phosphorylation of FLG *in vitro* and in living cells. Moreover, ^{125}I-labeled aFGF was specifically cross-linked to the FLG protein and immunoprecipitated with anti-peptide antiserum to FLG. A similar approach was used to demonstrate that bFGF was also able to interact, albeit more weakly, with FLG protein, indicating that the receptor(s) for the increasing family of growth factors belonging to the FGF family (22–28) are related to FLG gene product(s).

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Abbreviations: aFGF, acidic fibroblast growth factor; bFGF, basic fibroblast growth factor; IL-1, interleukin 1; PDGF, platelet-derived growth factor; CSF-1, colony-stimulating factor 1; FLG, *fms*-like gene.

MATERIALS AND METHODS

Rhabdomyosarcoma (A204) cells, glioblastoma (A172) cells, epidermoid carcinoma (A431) cells, and NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (vol/vol) fetal calf serum (Flow Laboratories). For metabolic labeling, cells were grown in 10-cm dishes to 80% confluency and incubated for 30 min in methionine-free medium. The cells were pulse-labeled by incubating at 37°C in methionine-free medium supplemented with [^{35}S]methionine (200 μCi/ml; 1 Ci = 37 GBq). The cells were washed with isotonic phosphate-buffered saline (PBS) and then lysed by adding buffer containing 20 mM Hepes (pH 7.5), 150 mM NaCl, 10% (vol/vol) glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, aprotinin (1 μg/ml), leupeptin (1 μg/ml), and 1 mM phenylmethylsulfonyl fluoride. The cell lysates were scraped from the plates and incubated for 10 min on ice. The lysate was clarified by centrifugation at 12,000 × *g* for 10 min. Immunoprecipitations were performed by adding 5 μl of either an antiserum against a synthetic peptide from FLG (residues 583–606 in ref. 18) or as a control, preimmune antiserum to the labeled lysates for 1 hr at 4°C. Protein A-Sepharose (Sigma) was then added to each sample for 1 hr at 4°C. The protein A-Sepharose–antibody complex was washed once with HNTG (20 mM Hepes, pH 7.5/150 mM NaCl/0.1% Triton X-100/10% glycerol), three times with HNTG/500 mM NaCl, and once more with HNTG. Sample buffer was added, and the samples were then heated at 95°C for 2 min and analyzed on a 7.5% polyacrylamide/SDS gel. In certain experiments, the medium was supplemented with tunicamycin (20 μg/ml) for 2 hr prior to labeling. The cells were then pulse-labeled with medium containing [^{35}S]methionine and tunicamycin (20 μg/ml).

Covalent Cross-Linking Experiments. Cultured rhabdomyosarcoma cells (A-204) or NIH 3T3 cells were grown to 90% confluency and then serum-starved for 18 hr. The cells were washed for 10 min with binding buffer [DMEM/2% (wt/vol) bovine serum albumin/25 mM Hepes, pH 7.4] containing 5 units of heparin per ml. The cells were then incubated at 4°C for 90 min with either ^{125}I-labeled aFGF or ^{125}I-labeled bFGF (Amersham). The cells were washed three times with binding buffer and then incubated for 20 min in PBS containing 0.3 mM disuccinimidyl suberate, a covalent cross-linking agent. The cells were washed with PBS and incubated for 20 min at 4°C in lysis buffer. The lysates were clarified for 20 min at 4°C in lysis buffer and immunoprecipitated as described above.

Immunoblotting Experiments. Subconfluent cells in 15-cm dishes were incubated in DMEM with 0.5% calf serum overnight. After serum deprivation, aFGF was added directly to the medium for 5 min. The 15-cm dish was then washed twice with ice-cold PBS and the cells were lysed with 1 ml of 50 mM Hepes, pH 7.5/150 mM NaCl/10% glycerol/1% Triton X-100/1.5 mM $MgCl_2$/1 mM EGTA/aprotinin (10 μg/ml)/leupeptin (10 μg/ml)/1 mM phenylmethylsulfonyl fluoride/200 μM sodium orthovanadate/10 mM pyrophosphate/100 mM sodium fluoride/30 mM *p*-nitrophenyl phosphate. The lysates were incubated on ice for 5 min and then centrifuged for 5 min at 10,000 × *g*. Protein A-Sepharose was incubated for 20 min with FLG anti-peptide antibodies and then washed three times with HNTG. The protein A-Sepharose–antibody complex was then added to the cell lysates for 90 min at 4°C. After three further washes with HNTG, 30 μl of 1× sample buffer was added to the immunoprecipitate and heated to 90°C for 4 min. After separation by SDS/PAGE on 7% polyacrylamide gels, the proteins were transferred to nitrocellulose overnight at 100 mA by using a Trans-Blot apparatus (Bio-Rad). The blot was then incubated with polyclonal antibodies to phosphotyrosine. After washing and incubation with ^{125}I-labeled protein A, the blots were exposed for autoradiography.

***In Vitro* Autophosphorylation Experiments.** Cells were lysed and subjected to immunoprecipitation with FLG anti-peptide antibodies and incubated on ice for 5 min with either aFGF or buffer alone. Then 15 nM $MnCl_2$ plus 15 μCi of [γ-^{32}P]ATP was added for 1 min at 4°C. The reaction was stopped by addition of sample buffer and boiled for 5 min. The samples were electrophoresed on a 7% polyacrylamide/SDS gel. After autoradiography the phosphorylated bands were excised and phospho amino acid analysis was performed according to published procedures (29).

RESULTS

We have demonstrated (18) that FLG is expressed in various cell types including fibroblasts, glioblastomas, rhabdomyosarcomas, and endothelial cells. Fig. 1 depicts a Northern blot with total RNA isolated from human glioblastoma cell line A172, from human epidermoid carcinoma cell line A431, and from a human rhabdomyosarcoma cell line A204 hybridized with a ^{32}P-labeled FLG probe. This experiment showed that FLG is expressed in the glioblastoma and rhabdomyosarcoma cell lines but not in the epidermoid carcinoma cells.

Immunoprecipitation analysis from [^{35}S]methionine-labeled A172 or A204 cells with anti-FLG antiserum revealed two sharp protein bands and one diffuse band at 100, 120, and 135 kDa, respectively (Fig. 2*A*). Preimmune serum from the same rabbit failed to immunoprecipitate these proteins (Fig. 2*A*). Similarly, the appearance of the three protein bands in the immunoprecipitates was specifically blocked by the synthetic peptide used for immunization (Fig. 2*B*). A protein of similar molecular mass was also immunoprecipitated from human foreskin fibroblasts and from 3T3 cells (clone 2.2) but not from A431 cells (data not shown). These results indicate that the anti-FLG antiserum recognizes a heterogeneous population of FLG proteins in these cells.

Inspection of the primary structure of FLG suggests that it contains a single transmembrane region and at least 11 asparagine-linked glycosylation sites in its putative extracellular domain (ref. 18, and C.D., M.J., and J.S., unpublished results). To evaluate the possibility that FLG is a glycoprotein and to reveal the size of its protein core, the rhabdomyosarcoma cells (A204) were pulse-labeled with [^{35}S]methionine in the presence or absence of tunicamycin (Fig. 3). In the presence of tunicamycin, the apparent molecular mass of FLG protein decreased and two new bands of 90 and 110 kDa were observed instead of the 100-kDa and 120-kDa

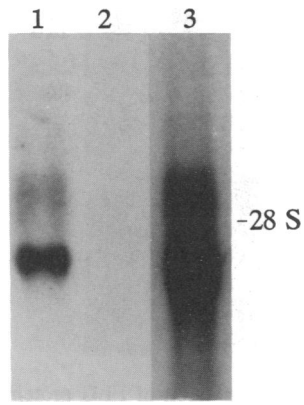

FIG. 1. Analysis of FLG transcripts. Total RNA (10 μg) isolated from various human cell lines was electrophoresed on a 0.8% formaldehyde/agarose gel. After transfer to nitrocellulose, the blot was hybridized with ^{32}P-labeled nick-translated FLG and, after washing, analyzed by autoradiography. RNA was from human glioma A172 (lane 1), human epidermoid carcinoma A431 (lane 2), and human rhabdomyosarcoma A204 (lane 3). The position of 28S rRNA is shown.

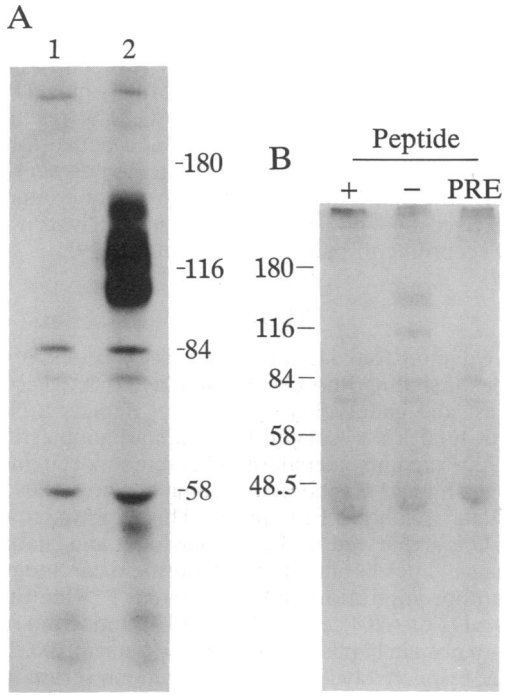

FIG. 2. Analysis of FLG-encoded proteins by immunoprecipitation from [^{35}S]methionine-labeled cells by using FLG anti-peptide antiserum. Cultured A204 cells were labeled for 3 hr with [^{35}S]-methionine. After solubilization the cells were subjected to immunoprecipitation analysis with antibodies generated against a synthetic peptide from FLG. This was followed by SDS/PAGE analysis and autoradiography. (*A*) Lanes: 1, preimmune antiserum; 2, FLG anti-peptide antiserum. (*B*) Immunoprecipitation from biosynthetically labeled glioma A172 cells with FLG anti-peptide antiserum in the absence (lane −) or presence (lane +) of synthetic peptide used for immunization or with preimmune antiserum (lane PRE).

bands and the diffuse band of 135 kDa observed in the absence of tunicamycin treatment. It is possible that anti-FLG recognizes two glycoproteins whose protein cores are 90 and 110 kDa. Alternatively, the 100-kDa polypeptide may represent a partially glycosylated form of FLG or its degradation product. The rhabdomyosarcoma cells were also pulse-labeled with [^{3}H]glucosamine and the cellular lysates were immunoprecipitated with anti-FLG antiserum. Incorporation of [^{3}H]glucosamine into similar 100-, 120-, and 135-kDa proteins further confirmed that FLG is a glycoprotein (data not shown).

The similarity between IL-1 and aFGF (22) and between the putative extracellular domains of the IL-1 receptor (21) and FLG (18) prompted us to assess the possibility that FLG is related to FGF receptors. Moreover, the avian bFGF receptor is related to FLG (19), also suggesting that FLG may recognize aFGF or other members of the FGF family (22–28). The cell surface receptors for either aFGF or bFGF were identified (6–14) using bifunctional cross-linking agents and ^{125}I-labeled FGF molecules as specific probes. By using this approach it was demonstrated that aFGF and bFGF recognize similar or closely related receptor molecules (8). Hence, we used this approach to analyze the nature of FLG protein expressed in the rhabdomyosarcoma and in NIH 3T3 cells. Two lines of evidence support the proposal that FLG protein is endowed with the capacity to specifically bind and respond to FGFs. These cells were incubated with ^{125}I-labeled aFGF for 90 min at 4°C and then for an additional 20 min with the covalent cross-linking agent disuccinimidyl suberate. After washing and solubilization, the samples were subjected to immunoprecipitation analysis with FLG anti-peptide antibodies and, as a control, with preimmune antibodies. Fig. 4 shows that FLG anti-peptide antibodies recognize two cross-linked polypeptides of 135 and 150 kDa, respectively. The radiolabeled protein band was barely visible when competed out by the addition of unlabeled aFGF in the ^{125}I-labeled aFGF reaction mixture, exhibiting the specificity of the cross-linking approach. Similar but less pronounced cross-linked products were observed when the covalent cross-linking experiment was performed with ^{125}I-labeled bFGF (Fig. 4*A*). The molecular mass of the cross-linked proteins, after subtraction of the molecular mass of either ^{125}I-labeled

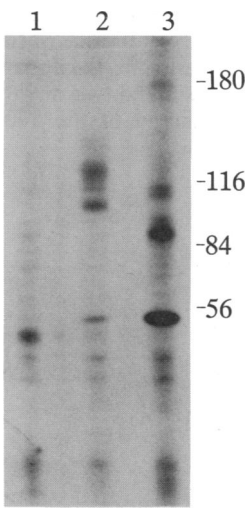

FIG. 3. Immunoprecipitation of FLG protein from [^{35}S]methionine-labeled cells treated with tunicamycin. Cultured A204 cells were biosynthetically labeled with [^{35}S]methionine after treatment in the presence or absence of tunicamycin (20 μg/ml) for 2 hr at 37°C. After solubilization, the samples were subjected to immunoprecipitation analysis with FLG anti-peptide antiserum and then analyzed by SDS/PAGE and autoradiography. Lanes: 1, preimmune antiserum; 2, FLG anti-peptide antiserum; 3, tunicamycin-treated and FLG anti-peptide antiserum. A low molecular mass 50-kDa contaminant was observed in some of the immunoprecipitates.

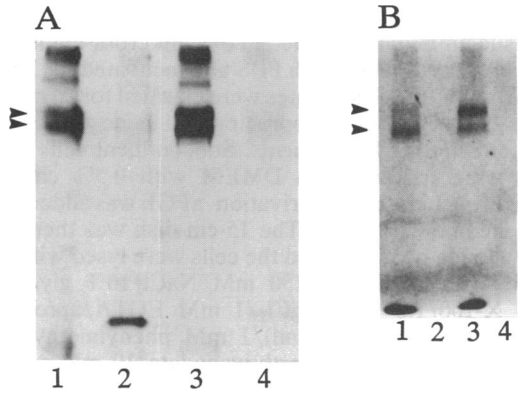

FIG. 4. FLG anti-peptide antibodies immunoprecipitate aFGF or bFGF cross-linked to its receptor. (*A*) Autoradiogram of 7.5% polyacrylamide/SDS gel. Lanes: 1, total cross-linked product with ^{125}I-labeled aFGF in 3T3 2.2 cells; 2, total cross-linked product with ^{125}I-labeled bFGF in 3T3 2.2 cells; 3, immunoprecipitate of the material shown in lane 1; 4, immunoprecipitate of the material shown in lane 2. The arrows indicate the position of ^{125}I-labeled growth factor-crosslinked product with apparent molecular masses of 135 and 150 kDa. (*B*) Autoradiogram of 7.5% polyacrylamide/SDS gel containing immunoprecipitates of ^{125}I-labeled aFGF cross-linked to human rhabdomyosarcoma A204 cells (lanes 1 and 2) or 3T3 2.2 cells (lanes 3 and 4). The cell extracts were immunoprecipitated with FLG anti-peptide antiserum (lanes 1 and 3) or normal rabbit serum (lanes 2 and 4). The arrowheads indicate the positions of the cross-linked species with apparent molecular masses of 135 and 150 kDa.

aFGF or ^{125}I-labeled bFGF (≈15 kDa), is consistent with the biosynthetically labeled 135-kDa and 120-kDa species but is inconsistent with the 100-kDa biosynthetically labeled species. It appears, therefore, that the 100-kDa protein does not covalently cross-link to ^{125}I-labeled aFGF. We do not know yet whether the low molecular mass species is transported to the cell surface and whether it represents a partially glycosylated form of FLG that is retained in an intracellular compartment. Alternatively, it may represent an additional product encoded by a distinct FLG-related gene. Finally, although the solubilization buffer contains numerous inhibitors of proteolytic enzymes, it is impossible to completely rule out proteolytic degradation.

The covalent cross-linking experiments indicated that aFGF is able to specifically interact with the FLG protein. Therefore, we examined the capacity of aFGF to stimulate the kinase activity of FLG *in vitro* and in living cells. For the *in vitro* studies, we utilized the standard immunoprecipitation/autophosphorylation analysis in which aFGF was added to washed immunoprecipitates obtained with FLG anti-peptide antibodies. After incubation with [γ-^{32}P]ATP and $MnCl_2$, the samples were analyzed by SDS/PAGE and autoradiography. Fig. 5 shows that aFGF induces the phosphorylation of 120-kDa and 135-kDa proteins that are specifically immunoprecipitated with FLG anti-peptide antibodies. Phospho amino acid analysis of the phosphorylated FLG protein excised from the gel indicated that the aFGF-induced autophosphorylation occurred mainly on tyrosine residues (data not shown).

We have also examined the capacity of aFGF to stimulate autophosphorylation of FLG protein in living cells. For this experiment, cultured rhabdomyosarcoma A204 cells were incubated with aFGF for 5 min at 37°C, then lysed, and subjected to immunoprecipitation analysis with FLG anti-peptide antibodies. After analysis by SDS/PAGE, the samples were immunoblotted with specific antibodies against phosphotyrosine, incubated with ^{125}I-labeled protein A, and autoradiographed. Fig. 5 shows the result of this experiment demonstrating that aFGF induces tyrosine phosphorylation of FLG protein in living cells. Our results clearly demonstrate that aFGF binds to FLG protein and stimulates kinase activity leading to autophosphorylation *in vitro* and in intact cells.

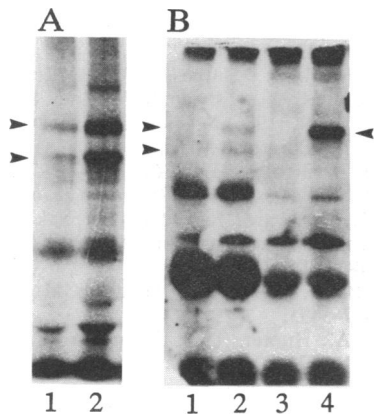

FIG. 5. Analysis of aFGF-activated protein tyrosine kinase activity by using FLG anti-peptide antiserum. (*A*) *In vitro* kinase analysis. Autoradiogram of a 7% polyacrylamide/SDS gel containing 3T3 cell extracts that were immunoprecipitated with FLG anti-peptide antiserum and then subjected to an *in vitro* kinase assay in the absence (lane 1) or presence (lane 2) of aFGF (10 ng/ml). The arrowheads show the positions of proteins with apparent molecular masses of 120 and 135 kDa that are phosphorylated in response to aFGF. (*B*) *In vivo* kinase analysis. Immunoblots probed with anti-phosphotyrosine antibodies and ^{125}I-labeled protein A. Lanes: 1 and 2, extracts of human A-204 cells that had been incubated in the absence and presence, respectively, of aFGF and immunoprecipitated with FLG anti-peptide antiserum; 3 and 4, same extracts immunoprecipitated with anti-phospholipase Cγ antibodies. The arrowheads on the left identify two proteins of 120 and 135 kDa that show increased phosphotyrosine content in response to aFGF. The arrowhead on the right shows that aFGF also increases the phosphotyrosine content of phospholipase Cγ, which served as a positive control for aFGF activation of the cells (W.B., C.D., J.S., and M.J., unpublished results).

DISCUSSION

We have previously isolated (18) a protein tyrosine kinase from a human endothelial cell cDNA library. Initially, it was not apparent that FLG encoded a growth factor receptor, as its putative transmembrane domain was not as prominent as the typical transmembrane domains of PDGF, CSF-1, epidermal growth factor, and insulin receptors (1, 3). We have cloned the 5′ region of FLG and shown that FLG encodes a transmembrane receptor tyrosine kinase with a typical signal sequence and numerous putative asparagine-linked glycosylation sites in its extracellular domain (C.D., M.J., and J.S., unpublished results). Hence, FLG encodes a receptor tyrosine kinase. The putative extracellular domain of FLG is similar to the extracellular domain of the IL-1 receptor (21). The IL-1 receptor is composed of an extracellular domain that can be subdivided to three immunoglobulin-like domains, a single transmembrane region, and a short cytoplasmic region without tyrosine kinase activity (Fig. 6). The overall sequence identity between the extracellular domains of FLG and IL-1 receptor is only 22%, yet the distribution of cysteine residues and other motifs are conserved. This suggests that FLG belongs to a family of receptors that includes PDGF and CSF-1 receptors, which, similarly, contain immunoglobulin-like domains in their extracellular portions (for reviews, see refs. 1 and 3).

IL-1 and aFGF show weak but significant sequence identity (22). Hence, we examined the possibility that FLG is related to the membrane receptor for aFGF. The conclusions from this study are as follows: (*i*) Covalent cross-linking experiments reveal specific interaction between aFGF and the FLG gene product. (*ii*) aFGF stimulates tyrosine phosphorylation of FLG *in vitro* and in living cells. (*iii*) Immunoprecipitation of biosynthetically labeled cells with FLG anti-peptide antibodies reveals three species of 100, 120, and 135 kDa. Two proteins of 90 and 100 kDa were immunoprecipitated from tunicamycin-treated cells.

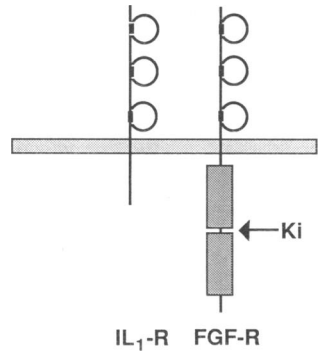

FIG. 6. Model for the structure of FGF receptor(s). This model is based on the primary structures of FLG (ref. 18; C.D., M.J., and J.S., unpublished results), avian bFGF receptor (FGF-R) (19), and IL-1 receptor (IL_1-R) (21). The putative extracellular domains of both receptors contain three motifs belonging to the immunoglobulin superfamily. The cytoplasmic domain of FLG (FGF receptor) contains a protein tyrosine kinase domain with a typical kinase insert (Ki) (1, 3).

The heterogeneity of the proteins immunoprecipitated with anti-FLG antibodies from biosynthetically labeled cells could represent differences in glycosylation patterns as well as differences in the primary structure of the FLG protein(s). Thus, the FLG anti-peptide antibodies may recognize multiple FLG or "FLG-related" proteins that are encoded by distinct genes and expressed in these cells. It is noteworthy that most cells with cross-linked aFGF or bFGF exhibit multiple receptor species (6–14). These proteins may serve as receptors for various members of the FGF family (22–28).

aFGF was used in most of the experiments described in this study; however, we have also shown that bFGF is able to bind to and activate the FLG kinase leading to autophosphorylation *in vitro* and in living cells (data not shown). Moreover, covalent cross-linking experiments reveal a specific, albeit weaker, interaction between ^{125}I-labeled bFGF and FLG protein (Fig. 5). These results may indicate that FLG encodes the aFGF receptor and that bFGF is able to cross-react with FLG. Yet, the avian bFGF receptor appears to be the avian counterpart of FLG (19). We did not attempt to quantitate the binding of either ^{125}I-labeled aFGF or ^{125}I-labeled bFGF to these cells. Such experiments should be performed with cells lacking endogenous FLG, which express transduced FLG molecules. This experimental system should allow unequivocal well-controlled determinations of the binding properties of FLG toward aFGF, bFGF, and other members of the FGF family of growth factors. Nevertheless, on the basis of the present experiments, it is clear that FLG encodes a receptor tyrosine kinase that specifically interacts with and can be activated by either a FGF or bFGF. It has been reported that FLG is related to the avian bFGF receptor (19). It is noteworthy that oligonucleotides based on our published FLG sequence (18) were used to clone the avian bFGF receptor (19) and, therefore, this receptor represents the avian counterpart of FLG. However, it is not clear yet whether the avian bFGF receptor also binds aFGF or any of the five members of the FGF superfamily. Nevertheless, it is reasonable that multiple receptors for the various FGFs will be identified and that these receptors will be probably related to FLG.

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