Identification and Characterization of a Fibroblast Growth Factor (FGF) Binding Domain in the Cysteine-rich FGF Receptor*

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Three distinct transmembrane glycoproteins bind fibroblast growth factor (FGF) family members. These include heparan sulfate proteoglycans, the tyrosine kinase-containing FGF receptors (FGFRs), and a cysteine-rich FGF receptor (CFR). The four FGFRs are thought to mediate FGF-signaling events but require the participation of the heparan sulfate proteoglycans to bind FGFs and transduce intracellular signals. However, a number of groups have proposed that FGF action requires events independent of FGFR activation. CFR, a high affinity FGF-binding protein, was first isolated from chicken embryos. To better understand the interactions between CFR and FGFs, we have constructed a series of CFR deletion mutants and CFR fragments. Analysis of these has identified a 200-amino acid domain that constitutes a CFR FGF binding site. A CFR fragment of 450 residues, CFR290–740, binds FGF-2 with an affinity indistinguishable from the full-length molecule, whereas smaller fragments display greatly reduced FGF binding. Although CFR binds heparin, it does not stimulate tyrosine phosphorylation of the FGFRs. Consequently, an analysis of the heparin-CFR interaction failed to identify a linear sequence containing a heparin binding site. Two types of FGF binding sites were identified: an ionic strength and heparin-independent site that represents FGF binding to CFR290–740 and an additional FGF binding site that is heparan sulfate-dependent and sensitive to high ionic strength. This latter site is likely to bind FGF indirectly via heparan sulfate binding to CFR. FGF-2 peptides that encompass a sequence implicated in FGF-2 binding to FGFRs also block FGF-2 binding to CFR. Our data suggest that binding of FGFs to CFR and FGFRs is mutually exclusive, since the CFR FGF binding site does not require heparan sulfate, and similar regions on FGF-2 interact with both FGFRs and CFR.

A diverse array of functions is ascribed to the FGF1 family of polypeptide growth factors. These include repression of skeletal muscle differentiation (1), promotion of neuronal differentiation and survival (2–4), induction of limb outgrowth (5), control of limb outgrowth and patterning (6, 7), induction of mesoderm (8), lung branching (9), control of hair follicle growth (10), inner ear development (11, 12), and induction of angiogenesis (13, 14). Three types of membrane anchored FGF-binding proteins have been identified that are likely to be involved in mediating the diverse functions of the FGF family. These include four tyrosine kinase-containing membrane glycoproteins (FGFRs) (15, 16), a large family of diverse heparan sulfate proteoglycans (17), and a cysteine-rich FGF receptor (CFR) (18). An additional secreted FGF-binding protein has also been characterized (19). Among these, the FGFRs are proposed to mediate the biological functions of the FGFs. However, these receptors require heparan sulfate proteoglycans to bind FGFs with high affinity and to transduce FGF signals (20, 21). Both binding sites for heparan sulfate present on the FGF and the FGFRs are likely to be required for FGF binding and signaling (22–27). The majority of FGF-mediated signaling events appear to require FGFRs, but additional requirements involving internalization and intracellular transport of the FGF ligand have been proposed (28–30). A role for other FGF-binding proteins in mediating FGF signaling is thus expected, since these recent reports have identified FGF receptor tyrosine kinase-independent activities of FGFs that include the stimulation of DNA synthesis (29) and the regulation of plasminogen activator activity (30, 31). In addition, mutants of both FGF-1 and FGF-2 have revealed that the biological activities of these factors do not entirely correlate with their ability to stimulate tyrosine phosphorylation of the FGFRs (32). Thus, it is likely that additional FGF-binding proteins or receptors are involved in mediating some of the biological activities of the FGF family. A number of functions can be envisioned for these additional FGF-binding proteins. If transport of FGF into the cytoplasm is necessary for FGF function, then there are likely to be proteins involved in FGF transport and perhaps in regulation of intracellular FGF levels.

CFR was first isolated as a high affinity FGF-binding protein from chicken embryos (35). Isolation, sequencing, and characterization of the CFR cDNA revealed a protein sequence with no known homologs (18). CFR was found to bind a number of FGFs and to possess an amino-terminal signal sequence, a transmembrane domain, and a basic cytoplasmic region comprising 13 amino acids. Three other groups have since independently isolated CFR based on its identification as an FGF-binding protein (36), a medial Golgi protein (37), and a ligand for the E-selectin receptor (38). Although the function of CFR is not known, we have recently reported a role for CFR in regulat...
lation of intracellular FGF trafficking (39).

To better delineate the roles of CFR in FGF action, we have constructed a series of CFR deletion mutants and CFR fragments, expressed the recombinant proteins in COS cells, and determined their FGF binding properties. In this article, we report that the binding of FGFs to CFR occurs within a 450-amino acid stretch of CFR sequence and that this binding does not require the presence of heparan sulfate. FGF-2 peptides that encompass a sequence implicated in FGF-2 binding to FGFRs also block FGF-2 binding to CFR. This result suggests that a similar region on FGF-2 may interact with both FGFRs and CFR. These data thus define an FGF binding site on CFR that is unlike the heparan sulfate-dependent FGF binding sites on the FGFRs (20, 21) and may be involved in mediating distinct CFR-dependent FGF actions (39).

EXPERIMENTAL PROCEDURES

Materials—Human recombinant FGF-2 was purified as described previously (40). Where indicated, chicken CFR was purified from chick embryos as described previously (35). Affi-Gel-activated agarose and molecular weight standards were purchased from Bio-Rad. Nα-Desthiobiotin and [3H]heparin were purchased from DuPont NEN. Fluoresceinated heparin was a gift from Arthur Lander (University of California, Irvine, CA) and was labeled with Na125I as described (41). The anti-hemagglutinin antibody-I monoclonal antibody was purchased from Babco (Berkeley, CA). COS-1 cells were obtained from the American Type Culture Collection. The COS cell expression vector PHYK with an ICRI, removing the intervening DNA, was constructed by digesting CFR with Smal and HindIII digestion to remove DNA sequences near the amino terminus of the CFR coding sequence. The CFR monoclonal antibody epitope (LA) was inserted into the XhoI site immediately in front of the coding sequence for the HA1 tag by a similar method. The LA epitope tag sequence will be described in a subsequent publication.

Expression of CFR in COS Cells—COS cells were grown and transfected as described (43). After 48 h, they were washed twice with ice-cold phosphate-buffered saline and harvested with a rubber policeman. Cells were centrifuged at 300 × g for 5 min at 4°C and resuspended in solubilization buffer (20 mM Tris, pH 7.4, 100 mM NaCl, 4 mM EDTA, 1 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100). Membranes were solubilized by incubating samples on a rotating wheel for 30 min at 4°C, and detergent-insoluble material was removed by centrifugation at 18,000 × g for 10 min at 4°C. Protein concentrations were determined using the BCA assay per the manufacturer’s instructions (Pierce).

Culture of COS Cells—COS cells were grown and transfected as described (40). 125I-FGF binding to CFR-transfected COS cell extracts was determined by quantitative immunoblot analysis using a Bio-Rad GS363 phosphorimager.

Iodination of FGF-2—Iodination of human recombinant FGF-2 was performed with chloramine T as described previously (40). Specific activities were determined by MM14 cell cycle exit assay (44) and range from 1000 to 5000 cpm/nmol.

Equilibration of FGF-2 with Macromolecular Substrates (RISA) for FGF and Heparin Binding—Anti-CFR monoclonal antibody 15E9, referred to as the anti-LA epitope tag monoclonal antibody, was purified from ascites fluid by caprylic acid precipitation (45), and 0.3 µg/ml (100 µW/m) was bound to the wells of a polystyrene microtiter plate in 20 mM NaHCO3, pH 9.5. Nonspecific sites were blocked with TBST (0.5 mg/ml Tris, pH 7.4, 100 mM NaCl and 0.05% Triton X-100) containing 0.5% bovine serum albumin for 2 h at 4°C. Unbound antibody was removed by washing three times with TBST. COS cell extracts (30 µg/ml) were incubated in the wells for 2 h at 4°C with equivalent amounts of CFR protein adsorbed as determined by quantitative immunoblot analysis. Nonimmunoabsorbed factors were removed by washing three times with TBST. 125I-FGF-2 (200 pM), [3H]heparin (360 ng/ml or 0.2 µCi/well), or [125I]heparin (9.5 ng/ml/mg) was added for 2 h in the presence or absence of FGF-2 peptide competitors at 0.5 mg/ml. Unbound 125I-FGF-2 or [3H]heparin was removed by washing three times with TBST. For 125I-FGF-2 binding analysis, the wells were cut from the plate, and the 125I cpm remaining in the wells were determined by counting in a LKB Clinigamma counter. For [3H]heparin binding analysis, 150 µl/well Hi-Load scintillation fluid (FSA Laboratory Supplies, Loughborough, United Kingdom) was added and counted using a Wallac (Turku, Finland) Microbeta plate counter. Nonspecific binding of both assays was defined as counts per minute using mock-transfected COS cell lysates.

Equilibrium Binding and Determination of the Kd Values for 125I-FGF-2 Binding—For equilibrium binding analysis, the RISA was performed as described, except COS cell extracts containing 10 µg of protein were used. The specifically bound 125I-FGF-2 was defined as the 125I-FGF-2 bound to CFR-containing COS cell extracts. Unspecific binding was defined as the amount bound to mock-transfected COS cell extract. The free concentration of 125I-FGF-2 was determined by removing an aliquot of the incubation mixture and counting it just prior to termination of the assay. The data were plotted according to the method of Scatchard (46), and curve fitting was performed using the Ultrafit program (Biosoft, Cambridge, UK).
FGF-agarose was prepared by coupling purified human recombinant FGF-2 to Affi-Gel 10 according to the manufacturer’s instructions. Control Affi-Gel beads were made by blocking undervatized beads with ethanolamine. The beads were then equilibrated with HBT containing 0.15 M NaCl.

FGF-agarose was washed twice with 2 M NaCl and then equilibrated with TBST prior to their use. COS cell lysates were diluted in 100 μl of HBT containing 0.15 M NaCl and mixed with 20 μl of FGF-agarose and control agarose. After incubating at room temperature for 2 h on a rotator, the beads were washed three times with TBST. 20 μl of SDS-PAGE buffer was added to each sample, and the samples were boiled for 2 min. The supernatants were removed and subjected to SDS-PAGE. The amount of CFR present was quantified as described previously for the CFR deletion mutants by phosphorimage analysis.

RESULTS

The CFR protein binds affinity matrices containing covalently attached FGF-1, FGF-2, or heparin, suggesting that CFR binds not only to FGFs but also to heparin (Fig. 1). Although the CFR-associated proteins of 70 and 45 kDa are adsorbed to the affinity matrices, we have previously demonstrated that CFR binds FGFs directly (35). However, these data do not determine whether FGF binding to CFR requires the presence of heparin or heparan sulfate, as is observed for the FGFRs. Therefore, we performed a detailed analysis of the CFR interaction with FGFs and heparin to further characterize the binding sites for the individual ligands.

Five deletion mutants were constructed that maintained the open reading frame of CFR and an intact signal peptide sequence (Fig. 2A). Detection of the deletion mutants was initially accomplished using the anti-LA monoclonal antibody. However, deletion of carboxyl-terminal regions including

![Fig. 1. CFR binds to FGF-1, FGF-2, and heparin-agarose.](image)

![Fig. 2. Analysis of 125I-FGF-2 binding to CFR and its deletion mutants in COS cells.](image)
amino acids 740–1138 or 219–1138 removed the anti-LA antibody epitope. Therefore, an HA1-tag was inserted into the mutants following the signal peptide sequence. Although the HA1 tag was satisfactory for Western analysis, it was unsuitable for analysis of 125I-FGF binding to CFR by RISA. Since the anti-LA antibody was originally used for RISA analysis for wild type CFR (47), we identified the 17-amino acid epitope and inserted the LA tag immediately preceding the coding sequence of the HA tag (Fig. 2A).

Each deletion mutant was inserted into a COS cell expression vector and transiently transfected into COS cells. The expression levels were determined by Western blot analysis of COS cell extracts using the anti-LA antibody (Fig. 2B). The Western blot analysis of CFR deletion mutants demonstrated that similar levels of protein were present, with no apparent differences in degradation of any individual mutant compared with wild type CFR (CFRwt). To normalize the levels of CFR protein present for determination of 125I-FGF binding, extracts were subjected to immunoblot analysis with both the anti-LA and anti-HA epitope tag monoclonal antibodies. The relative levels of CFR protein were determined by chemiluminescence quantitation of phosphorimages. The relative levels of protein detected by both antibodies were used to normalize the expression of CFR deletion mutants and CFR fragments to the level of wild type CFR expression. This procedure eliminated any potential artifacts due to differences in the expressed proteins or differences in the recognition of individual antibody epitopes.

Equivalent amounts of wild type CFR and CFR deletion mutants were analyzed for 125I-FGF-2 binding by RISA. Three of the deletion mutants bound 125I-FGF-2 similarly to wild type CFR, whereas two mutants exhibited low levels of 125I-FGF-2 binding (Fig. 2C). Typically, bound counts averaged 4000 cpm, whereas the nonspecific binding averaged 500 cpm. These data suggest that the FGF binding site on CFR lies within residues 219–496 or 624–740.

To further delineate the FGF binding domain, a number of CFR fragments were constructed (Fig. 3A). CFR fragments were expressed in COS cells, and extracts were isolated and quantitated for CFR protein content as described for the CFR deletion mutants. Western analysis of all four CFR fragments demonstrated that the proteins were stably expressed in COS cells (Fig. 3B). Binding of 125I-FGF-2 to CFRwt and to the CFR fragments identified only one fragment (CFR290–740) that was capable of binding significant levels of 125I-FGF-2 (Fig. 3C). Although the fragments appeared stable when analyzed in COS cell extracts, more than one freeze-thaw cycle abolished 125I-FGF-2 binding to CFR290–740. Similar treatments did not affect binding to CFRwt. The four CFR fragments constructed were expected to narrow an FGF binding domain on CFR to approximately 200 residues. Data from the deletion analyses (see Fig. 2) suggested that the FGF binding domain was localized to a region encompassing either residues 219–496 or 624–740. However, only the CFR290–740 fragment bound 125I-FGF-2 (Fig. 3C).

Several possible hypotheses could explain the lack of 125I-FGF-2 binding to CFR fragments smaller than CFR290–740-
First, the RISA requires that a monoclonal antibody interacts with and retains the CFR fragment. It is possible that smaller CFR fragments exhibit reduced FGF binding due to steric hindrance following antibody binding. Alternatively, the fragments may not possess sufficient structural stability to maintain an FGF binding site or may not be folded properly, or the binding site for FGFs may comprise non-linear amino acid sequences contained in residues 219–496 and 624–740. To distinguish between these two possibilities, an FGF binding assay independent of the RISA was developed. COS cell extracts containing equal amounts of CFR fragments or wild type CFR were adsorbed to FGF-agarose. The amount of CFR or CFR fragment adsorbed to the affinity matrix was determined by boiling the washed beads in SDS-PAGE sample buffer, separating the fragments by SDS-PAGE, and visualizing by immunoblot analysis. The amount of CFR present was quantified as described previously for the CFR deletion mutants. These data were consistent with the RISA data in that CFRwt and the CFR290–740 fragment were retained on FGF-agarose, whereas other CFR fragments were not retained (Fig. 3D). Thus, the inability of the smaller CFR fragments to bind to the affinity matrix is likely to arise from improper folding or instability of CFR fragments, resulting in proteins incapable of FGF binding.

Scatchard analysis of $^{125}$I-FGF-2 equilibrium binding to CFRwt and CFR290–740 revealed $K_D$ values that were virtually identical and comparable with those previously determined by our group (35). Equilibrium binding data from three independent experiments yielded $K_D$ values of $1.4 \pm 0.8$ and $1.8 \pm 0.9$ nM for CFR290–740 and CFRwt, respectively. Curve fitting identified only a single class of sites when plotted according to Scatchard. Thus, the CFR domain encompassing residues 290–740 contains a complete and intact FGF binding site. We hypothesize that CFR higher order structure is necessary for the formation of an FGF binding site, as: 1) bacterially produced CFR lacking a signal peptide and transmembrane domain does not bind FGFs; 2) denaturation of CFR by boiling abolishes FGF binding; and 3) CFR290–740 possesses intact disulfide bonds, a characteristic of the wild type protein. $^2$

Next, we examined the heparin binding to CFR to determine whether CFR bound heparin directly and to identify putative heparin binding domains. The specificity of heparin binding to the CFR complex was determined by RISA, examining $^{125}$I-heparin binding and competition with heparin or chondroitin sulfate. CFR expressed in COS cells and partially purified CFR specifically bound heparin, since unlabeled heparin but not chondroitin sulfate competed for $^{125}$I-heparin binding (Fig. 4). CFR expressed in COS cells does not contain bound CFR-associated proteins (47), suggesting a direct interaction of CFR with heparin.

Heparin binding to CFR deletion mutants and CFR fragments was performed analogously to the previously described FGF binding experiments. CFR deletion mutants and fragments bind heparin but exhibit a complex pattern (Fig. 5A). At least one heparan sulfate binding site appears to be present. Binding of CFR deletion mutants and fragments to heparin-agarose also presents a complex pattern (Fig. 5B). Both assays demonstrate that alterations of CFR structure and primary sequence affect heparin binding. These data do not allow unambiguous identification of a heparin binding domain within the CFR primary amino acid sequence.

We then performed a series of experiments to determine whether the binding of CFR to FGF was dependent on heparan sulfate. CHO-K1 cells (wild type) and CHO cell mutants that do not synthesize heparan sulfate and display low levels of heparan sulfate proteoglycans (CHO677) (48) were stably transfected with CFRwt expression vectors. A CHO-K1 clone and a CHO677 clone were selected for similar levels of CFR protein expression (Fig. 6A) and subjected to $^{125}$I-FGF-2 binding by RISA (Fig. 6B). The CHO677 cells exhibited 50% less $^{125}$I-FGF-2 binding than the CHO-K1 cells (Fig. 6B). To confirm that the assay conditions did not alter the levels of CFR present, the cells were solubilized in SDS-PAGE buffer following the assay, and the products were analyzed by Western blotting (not shown). This confirmed that equivalent levels of CFR were present in all wells.

Heparin appears to augment FGF binding to CFR. Since a high ionic strength wash disrupts the interaction of CFR with heparin (Fig. 1), it should also abrogate heparin-dependent increases in FGF binding. Analysis of $^{125}$I-FGF-2 binding by RISA in the presence or absence of heparin revealed that heparin-dependent increases in FGF binding occur (Fig. 7). Addition of heparin to extracts from CHO-K1 and CHO677 cells expressing CFR increased $^{125}$I-FGF-2 binding to equivalent levels (Fig. 7). A 2 M NaCl wash following incubation with heparin and ligand reduced $^{125}$I-FGF-2 binding 2–3-fold, resulting in equivalent binding to CHO-K1 and CHO677 extracts. In the absence of

$^2$ Z. Zhou and B. B. Olwin, unpublished data.
heparin or heparan sulfate, we observed a single class of FGF binding sites on CFR that were not affected by high ionic strength and displayed a $K_D$ near 1 nM. In the presence of heparin, additional FGF binding was observed that is ionic strength dependent (Fig. 7). Scatchard analysis of CFR and CFR treated with heparin followed by a high ionic strength wash revealed an increase in the number of FGF binding sites but no detectable change in affinity. Thus, it is likely that heparin binding to CFR stabilizes higher order CFR structure.

The interactions of CFR with FGFs appear to differ from those observed for the FGFR tyrosine kinases. FGF-2 peptides have been used previously to identify a critical region of FGF-2 that has been proposed to interact with the FGFR tyrosine kinases (49). FGF-2 peptides containing a loop sequence (residues 106–115) bind to an FGFR site that is proposed to be involved in promoting dimer formation and signaling. The peptides containing this loop sequence block FGF-2 binding to FGF tyrosine kinase receptors, whereas peptides lacking the sequence do not (49). A similar analysis was performed for $^{125}$I-FGF-2 binding to CFR. Peptides containing the loop sequences that inhibit FGF binding to FGFRs also inhibited binding of $^{125}$I-FGF-2 to CFR, whereas a FGF-2 peptide that did not contain the loop sequence did not block $^{125}$I-FGF-2 binding to CFR (Fig. 8). Although our data suggest that the interactions of CFR with FGFs are different than the interac-

Fig. 5. Heparin binding to CFR deletion mutants and CFR fragments. A, CFR deletion mutants and CFR fragments from COS cell extracts were used for heparin binding by RISA. Equal amounts of CFR mutants and fragments were used for each assay. Nonspecific binding was defined as the amount of [3H]heparin bound to the mock-transfected COS cell extract. The values shown were derived by subtracting the nonspecific binding from the total binding. B, CFR fragments expressed in COS cells were incubated with heparin-agarose. The extracts adsorbed to heparin-agarose were washed and then boiled in SDS-PAGE sample buffer, separated by SDS-PAGE, and analyzed by immunoblotting with the anti-LA monoclonal antibody. The amount of CFR present was quantified by phosphorimage analysis.

Fig. 6. FGF binding to CFR expressed in CHO-K1 and CHO.677 cells. A, Western analysis of CFR in CHO cell extracts. Shown here are representative clones isolated following transfection with the parent vector (lane 1), CHO-K1 cells transfected with the CFR$_{wt}$ expression vector (lane 2), and CHO.677 cells transfected with the CFR$_{sa}$ expression vector (lane 3). Equal amounts of extract protein were loaded in each lane. B, extracts from the indicated cell types were used to determine their relative ability to bind $^{125}$I-FGF-2 in a RISA. Equivalent amounts of CFR were added from CHO-K1 and CHO.677 extracts. An immunoblot analysis of the proteins remaining in the wells following the RISA confirmed that equivalent amounts of CFR were immunoadsorbed (not shown).

Fig. 7. Effects of heparin and a 2 M NaCl wash on FGF-2 binding to CFR expressed in CHO-K1 and CHO.677 cells. Binding of $^{125}$I-FGF-2 to CFR expressed in CHO-K1 (□, wild type) and CHO.677 (□, heparan sulfate-deficient) CHO cells was performed in the absence or presence of heparin. Where noted, the binding of $^{125}$I-FGF-2 was followed by two 2 M NaCl washes.
by 2–3-fold. The heparin-dependent increase in FGF-2 binding to cells prior to incubation with FGF-2 increased FGF-2 binding. However, addition of heparin to CFR expressed in CHO677 cells that do not synthesize heparan sulfate provides conclusive evidence that FGF-2 does not require the carbohydrate to bind to CFR. Expression of CFR in a mutant CHO cell line that does not synthesize heparan sulfate provides conclusive evidence that FGF-2 does not require the carbohydrate to bind to CFR. However, addition of heparin to CFR expressed in CHO677 cells prior to incubation with FGF-2 increased FGF-2 binding by 2–3-fold. The heparin-dependent increase in FGF-2 binding is sensitive to 2 M NaCl whereas the heparin-independent FGF binding is not sensitive to high ionic strength. Moreover, the level of FGF-2 binding in the presence of heparin can be reduced to the level of FGF binding in CHO677 cell extracts by treatment with high ionic strength. The salt treatment releases bound heparin from CFR, suggesting the presence of two types of FGF binding sites on CFR (Fig. 9). One site, identified by mutational analysis, is ionic strength-independent and resides between residues 290–740, whereas an additional site is sensitive to high ionic strength and is proposed to be bound indirectly via heparan sulfate interaction with CFR (Fig. 9). We therefore propose that these two types of FGF binding sites are distinct as presented in our model (Fig. 9). In contrast to CFR, FGFRs are incapable of binding FGFs in the absence of heparan sulfate (20, 21, 26, 27). Heparan sulfate binding to both FGF and the FGFR appear necessary to form a functional complex (50, 51). FGFRs contain a heparan sulfate binding site that is dependent on divalent cations (27). These data demonstrate that the FGF “binding site” on FGFRs is likely to recognize a complex of FGF and heparan sulfate, whereas CFR binds FGFs independently of heparan sulfate.

To further delineate the characteristics of the FGF binding site on CFR, we examined the ability of FGF-2 peptides to block 125I-FGF-2 binding to CFR. A similar study analyzing FGF-2 binding to intact cells revealed that a peptide corresponding to residues 106–115 of FGF-2 efficiently blocked FGF-2 activity, FGF-2 cross-linking, and FGF-2 binding (49). The efficacy of inhibition by the peptides on FGF-2 activity and FGF-2 cross-linking was indistinguishable from that observed for 125I-FGF-2 binding to CFR. Thus, the site on FGF-2 that interacts with CFR is likely to include the site that binds to FGFRs. Thus, the FGF binding site on CFR represents the only known FGF binding site that is independent of heparan sulfate. We predict that FGF binding to CFR and to FGFRs would likely be mutually exclusive. These data are consistent with an activity of CFR we have observed in CHO cells. CFR reduces the levels of intracellular FGF-2 and FGF-1 that have been internalized from the extracellular environment (39). Import of these FGFs into the cytoplasm has been observed independently by three laboratories (28, 52–54). Two of these groups have demonstrated that import into the cytoplasm and nuclear localization of FGFs is critical for cell growth. If CFR acts to reduce the levels of intracellular FGFs, and the binding of FGFs to CFR and FGFRs is mutually exclusive as our data indicate, then CFR may modulate FGF functions that require internalized FGFs.

Analysis of CFR deletion mutants and CFR fragments for FGF binding has identified a new type of FGF binding domain that is distinct from the heparan sulfate-dependent binding site present on FGFRs. Further examination of this FGF binding site should reveal additional details of the CFR and FGF interactions. A comparison of the crystal structures for FGFR and CFR bound to FGFs will aid in the elucidation of the role(s) of CFR in FGF action.

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![Fig. 8. Inhibition of FGF binding to CFR by FGF-2 peptide.](Image 86x543 to 270x729)

![Fig. 9. A model for FGF binding to CFR.](Image 339x568 to 532x729)
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