Plasticity in Interactions of Fibroblast Growth Factor 1 (FGF1) N Terminus with FGF Receptors Underlies Promiscuity of FGF1*5

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Tissue-specific alternative splicing in the second half of Ig-like domain 3 (D3) of fibroblast growth factor receptors 1–3 (FGFR1 to -3) generates epithelial FGFR1b–FGFR3b and mesenchymal FGFR1c–FGFR3c splice isoforms. This splicing event establishes a selectivity filter to restrict the ligand binding specificity of FGFRb and FGFRc isoforms to mesenchymally and epithelially derived fibroblast growth factors (FGFs), respectively. FGF1 is termed the “universal FGF ligand” because it overrides this specificity barrier. To elucidate the molecular basis for FGF1 cross-reactivity with the “b” and “c” splice isoforms of FGFs, we determined the first crystal structure of FGF1 in complex with an FGFRb isoform, FGFR2b, at 2.1 Å resolution. Comparison of the FGF1–FGFR2b structure with the three previously published FGF1–FGFRc structures reveals that plasticity in the interactions of the N-terminal region of FGF1 with FGFR D3 is the main determinant of FGF1 cross-reactivity with both isoforms of FGFs. In support of our structural data, we demonstrate that substitution of three N-terminal residues (Gly-19, His-25, and Tyr-23) enables the FGF2 triple mutant to bind and activate FGFR2b. These findings taken together with our previous structural data on receptor binding specificity of FGF2, FGF8, and FGF10 conclusively show that sequence divergence at the N termini of FGFRs is the primary regulator of the receptor binding specificity and promiscuity of FGFs.

Fibroblast growth factor (FGF) signaling plays pleiotropic roles in mammalian development and metabolism (1–3). The mammalian FGF family comprises 18 members (FGF1–FGF10 and FGF16–FGF23), which are divided into six subfamilies based on sequence homology and phylogeny. The FGF1 subfamily comprises FGF1 and FGF2; the FGF7 subfamily comprises FGF3, FGF7, FGF10, and FGF22; the FGF4 subfamily comprises FGF4, FGFr5, and FGF6; the FGF8 subfamily comprises FGF8, FGF17, and FGF18; the FGF9 subfamily comprises FGF9, FGF16, and FGF20; and the FGF19 subfamily consists of FGF19, FGF21, and FGF23 (4, 5). In some nomenclatures, FGF homologous factors (FHF1–FHF4) are considered to form an additional FGF subfamily, namely the FGF1 subfamily, because these proteins share strong sequence homology to other FGFs. Biochemical and structural analyses of FHFs, however, have shown that these proteins are functionally distinct from FGFs (6–8). The FGF1, FGF4, FGF7, FGF8, and FGF9 subfamilies act in a paracrine fashion to direct tissue patterning and organogenesis during embryogenesis. In contrast, the FGF19 subfamily members have very low affinity for heparan sulfate (HS) and hence act in an endocrine fashion (9) to regulate important metabolic activities, including bile acid and lipid metabolism (10–13), glucose homeostasis (14–17), phosphate and vitamin D homeostasis (18–20).

The paracrine FGFs carry out their diverse functions by binding and activating the FGF receptor (FGFR) family of tyrosine kinase receptors in an HS-dependent fashion (21–23). The endocrine FGF19 subfamily members require klotho co-receptors to bind and activate their cognate FGFRs (24–26). Whether these endocrine FGFs still require HS for signaling remains to be determined. There are four FGFR genes (FGFR1 to FGFR4) that encode single-pass transmembrane receptors composed of an extracellular ligand-binding region consisting of three immunoglobulin (Ig)-like domains (D1, D2, and D3) connected by flexible linker sequences and a cytoplasmic region that harbors the conserved tyrosine kinase domain (27–29). The D2-D3 segment of the ectodomain is necessary and sufficient for ligand binding (30–34). The D1 and D1-D2 linker regions, albeit dispensable for ligand binding, are implicated in receptor autoinhibition because loss of these regions enhances the FGF and HS binding affinity of the D2-D3 region (35, 36). Upon binding of ligand and HS, FGFRs assemble into a 2:2:2 FGF-FGFR-HS symmetric dimer (32, 37, 38) in which the juxtaposed cytoplasmic kinase domains gain sufficient opportu-

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This article contains supplemental Figs. 1–7.
The atomic coordinates and structure factors (codes 3OJ2, 3OJM, and 3OJV) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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6 The abbreviations used are: FHF, FGF homologous factor; FGFR, FGF receptor; HS, heparan sulfate; PDB, Protein Data Bank; SPR, surface plasmon resonance.
N-terminal Plasticity Underlies FGF1 Promiscuity

N-terminally to transphosphorylate each other on A-loop tyrosines and become activated (39, 40). FGFR kinase activation triggers activation of various downstream signaling pathways, including the RAS-MAPK (41), PI hydrolysis/PKC/Ca<sup>2+</sup> (42, 43), PI3K-AKT (44–46), and RAC1/CDC42 (47) signaling pathways (48, 49).

FGF signaling is tightly regulated by spatial and temporal expression of FGFs, FGFRs, and HS cofactors and, most importantly, by means of FGF-FGFR binding specificity. The tissue-specific alternative splicing in the D3 domain of FGFR1–FGFR3 is the main mechanism in the regulation of FGF-FGFR binding specificity (30, 34, 50, 51). In FGFR1–FGFR3, two alternative exons (IIB and IIC) code for the second half of D3 and are spliced in a mutually exclusive fashion to the common exon IIIa that encodes the first half of D3 (52–55). This splicing event results in the expression of epithelial “b” isoforms (FGFR1b–FGFR3b) or mesenchymal “c” isoforms (FGFR1c–FGFR3c), increasing the number of principal FGFRs to seven, namely FGFR1c, FGFR1b, FGFR2c, FGFR2b, FGFR3c, FGFR3b, and FGFR4. The D3 alternative splicing restricts the ligand binding specificity of FGFR1b–FGFR3b isoforms to mesenchymally expressed FGFs and that of FGFR1c–FGFR3c isoforms to epithelially expressed FGFs (30, 34). For example, FGF2 binds with comparably high affinity to both FGFR1c and FGFR2c but does not bind the remaining five FGFRs (50, 56–58). FGF8b binds FGFR1c–FGFR3c and FGFR4 but does not recognize the “b” isoforms (30, 50, 51, 56, 59). FGF10 and FGF7 are the most specific FGF ligands and activate only FGFR2b (50, 51). FGF1 is the main mechanism in the regulation of FGF-FGFR binding specificity barrier set by alternative splicing and binds equally specific FGF ligands and activate only FGFR2b (50, 51). FGF1 is isoforms (30, 50, 51, 56, 59). FGF10 and FGF7 are the most specific FGF ligands and activate only FGFR2b (50, 51). FGF1 is the main mechanism in the regulation of FGF-FGFR binding specificity barrier set by alternative splicing and binds equally specific FGF ligands and activate only FGFR2b (50, 51). FGF1 is isoforms (30, 50, 51, 56, 59). FGF10 and FGF7 are the most specific FGF ligands and activate only FGFR2b (50, 51). FGF1 is isoforms.

To date, crystal structures of seven different FGF-FGFR complexes have been solved. These structures include three featuring FGF1 (FGF1-FGFR1c (PDB code 1EVT), FGF1-FGFR2c (PDB code 1DJS), and FGF1-FGFR3c (PDB code 1RY7) (31, 33, 35)), two featuring FGF2 (FGF2-FGFR1c (PDB codes 1CVS and 1FQ9) and FGF2-FGFR2c (PDB code 1EVT) (31, 32)), one featuring FGF8b (FGF8b-FGFR2c (PDB code 2FDB) (30)), and one with FGF10 (FGF10-FGFR2b (PDB code 1UNN) (34)). Analysis of these crystal structures has revealed that alternative splicing controls FGF-FGFR binding specificity by switching the primary sequences of key FGF binding sites in the second half of D3, including the βC′-βE and βF-βG loops and βF and βG strands (30, 31, 34). More specifically, analysis of the FGF2-FGFR1c and FGF2-FGFR2c structures has shown that specific hydrogen bonds between Gln-65 in the β4 strand of FGF2, a residue that is unique to FGF2, and an aspartic acid in the βC′-βE loops of FGFR1c and FGFR2c (Asp-320 in FGFR1c and Asp-321 in FGFR2c) restrict binding of FGF2 to these two FGFRc isoforms (31, 32). Similarly, analysis of the FGF10-FGFR2b structure has identified the highly specific hydrogen bonding between Asp-76 in the N terminus of FGF10 with Ser-315 in the βC′-βE loop of FGFR2b as the main determinant of the tight specificity of FGFR10 for the FGFR2b isoform (34). Finally, analysis of the FGF8b-FGFR2c structure has shown that the hydrophobic contacts between Phe-93 (in the β4-β5 loop of FGF8b), Val-36 and Phe-32 (in the N terminus of FGF8b), and residues in the βF and βG strands of receptor narrow the binding specificity/promiscuity of FGF8b to FGFR1c-FGFR3c and FGFR4 (30).

In contrast, the structural basis for indiscriminate binding of FGF1 to both “b” and “c” isoforms of a given FGFR has remained elusive. In the FGF1-FGFR1c structure, the alternatively spliced βC′-βE loop in D3 had poor electron density and could not be modeled. This observation was attributed to the lack of contacts between this inherently flexible loop and FGF1 (31). Because contacts between FGF and the alternatively spliced βC′-βE loop of receptor have been established to comprise one of the key determinants of FGF-FGFR specificity, the observation that FGF1 did not engage this loop to bind FGF1 was harmonious with the promiscuity of this ligand toward both isoforms. Hence, it was proposed that indiscriminate binding of FGF1 to both alternative splice isoforms of FGFRs is due to the fact that FGF1 does not rely on the βC′-βE loop for FGFR binding (31). In the FGF1-FGFR2c structure (Protein Data Bank code 1DJS), however, the βC′-βE loop is ordered and makes specific contacts with the core region of FGF1 (33), arguing against the validity of our proposed model.

The lack of a crystal structure of FGF1 bound to an FGFRb isoform has been a critical barrier to our comprehension of the molecular basis for the unique ability of FGF1 to break the specificity barrier set by alternative splicing. Hence, in this report, we describe the first crystal structure of FGF1 complexed with an FGFRb isoform, FGFR2b, at 2.1 Å resolution. We also revisit the role of the disordering of the alternatively spliced βC′-βE loop of receptor in the promiscuity of FGF1 by solving the crystal structure of FGF1-FGFR1c in a new crystal lattice at 2.6 Å resolution. By comparing the newly solved FGF1-FGFR2b structure with FGF1-FGFRc structures, we show that the remarkable ability of N-terminal residues of FGF1 to adapt to D3 of both “b” and “c” splice isoforms of FGFRs underlies the ability of FGF1 to cross-react with all isoforms of FGFR.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The cDNA fragment encoding full-length wild type human FGF1 (residues 1–155) was subcloned into the expression vector pET30a using the cloning sites NdeI and HindIII. The cDNA for full-length wild type human FGF2 (residues 1–155) was subcloned into pET30a using the Ncol and Xhol cloning sites such that a 47-residue-long N-terminal tag, including a hexahistidine tag, was fused in frame to the N terminus of FGF2. The cDNA fragments for the ligand-binding region (D2-D3 domains) of wild type human FGFR1c (residues 142–365), wild type human FGFR2b (residues 140–369), and wild type human FGFR2c (residues 149–368) were subcloned into pET28a using the Ncol and HindIII cloning sites that such a 47-residue-long N-terminal tag, including a hexahistidine tag, was fused in frame to the N terminus of FGF2. The cDNA fragments for the ligand-binding region (D2-D3 domains) of wild type human FGFR1c (residues 142–365), wild type human FGFR2b (residues 140–369), and wild type human FGFR2c (residues 149–368) were subcloned into pET28a using the Ncol and HindIII cloning sites. The QuikChange XL site-directed mutagenesis kit (Stratagene) was used to sequentially introduce the F26Y, H25N, and G19F mutations into the hexahistidine-tagged wild type human FGF2 expression construct; the A172F or P253R gain-of-function mutations, or the E323A or I257A/Y281A loss-of-function mutations into the expression construct for the ligand-binding region of wild type FGF2b; and the R251Q loss-of-function mutation into the expression construct for wild type FGF2c ligand-binding region (supplemental Fig. 1). FHf1b and the N-terminally truncated FGF1 (FGF1(21–155))
were expressed and purified as described previously (6, 8, 31, 35).

Competent BL21 DE3 Escherichia coli cells were transformed with expression constructs for FGFs and FGFR ligand-binding regions and were cultured at 37 °C to an \( A_{600} \) of \(-0.5\) (Beckman DU530 UV-visible spectrometer) and induced with 1 mM isopropyl 1-thio-\(\beta\)-d-galactopyranoside for 4 h. Cell pellets were lysed in 150 mM NaCl, 10% glycerol, 25 mM Heps-NaOH, pH 7.5, 5 mM EDTA, and 625 \( \mu \)M PMSF using a French press (Thermo Spectronick). The soluble lysate fraction containing FGF1 or FGF2 protein was loaded onto a heparin affinity column, and ligands were eluted with a step gradient ranging from 500 mM to 2 M NaCl in 25 mM Heps-NaOH, pH 7.5. Fractions containing FGF1 or FGF2 were pooled, diluted to 200 mM NaCl, loaded onto a cation exchange column (Source S, Amersham Biosciences), and eluted with a continuous gradient ranging from 200 mM NaCl to 700 mM NaCl in 25 mM Heps-NaOH, pH 7.5. Purified FGF1 and FGF2 proteins were aliquoted and then stored at \(-80^\circ C\) until use.

In contrast to FGFs, the FGFR ligand-binding regions were expressed as insoluble proteins. Inclusion bodies containing the misfolded FGFR ligand-binding regions were isolated and solubilized in 6 M guanidinium hydrochloride, 20 mM EDTA, 20 mM DTT, and either 50 mM Heps-NaOH, pH 7.5 (for FGFR2c and FGFR2b) or 50 mM Tris-HCl, pH 7.5 (for FGFR1c). The solubilized FGFR proteins were dialyzed at 4 °C against a 22.5 mM Heps-NaOH or Tris-HCl, pH 7.5, buffer containing 150 mM NaCl, 7.5% glycerol, 1 mM l-cysteine. The dialysis buffer was then exchanged, and the FGFR proteins were dialyzed for a further 48 h at 4 °C against 100 mM NaCl, 5% glycerol, and 22.5 mM Heps-NaOH or Tris-HCl, pH 7.5. Refolded FGFRs were loaded onto a heparin affinity column, eluted with 1 M NaCl in 25 mM Heps-NaOH, pH 7.5, and then further purified by size exclusion chromatography (HiLoad 16/60 Superdex 75, Amersham Biosciences) using 25 mM Heps-NaOH, pH 7.5, buffer containing 1 M NaCl. FGFRs were also aliquoted and stored at \(-80^\circ C\) until use. The expression and purification of mutated FGFs and FGFR ligand-binding regions were carried out as for the wild type counterparts. Protein concentrations were measured under denaturing conditions at a wavelength of 280 nm using the NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington DE). Extinction coefficients for proteins were derived using the ProtParam software (ExPASy Proteomics Server).

Crystallography, Data Collection, and Structure Determination—Purified FGFR1c and FGFR2b ligand-binding regions were mixed with a slight molar excess of FGF1(21–155) or full-length FGF1, respectively. The resulting complexes were concentrated to about 4 mg/ml using a Centricron 10 concentrator and applied onto a Superdex 200 size exclusion column equilibrated in 25 mM Heps-NaOH, pH 7.5, containing 1 M NaCl. Fractions containing 1:1 FGF-FGFR complexes were pooled, concentrated to \( \sim 60–70\) mg/ml, and stored at 4 °C until use. Prior to crystallization, the complexes were diluted to 10 mg/ml using 25 mM HEPES, pH 7.5, which brought down the salt concentrations to \( \sim 150\) mM NaCl. The FGF1(21–155)-FGFR1c complex was supplemented with an equimolar amount of fully sulfated heparin octasaccharide prior to crystallization. The FGF-FGFR complexes were crystallized by mixing 1.5 \( \mu \)l of the complex with 1.5 \( \mu \)l of crystallization buffer using the hanging drop vapor diffusion method. The FGF1-FGFR2bP253R complex crystallized in 0.1 M Heps, pH 7.5, 22% monomethyl ether PEG 5000, and 0.2 M ammonium sulfate. The FGF1-FGFR2bA172F complex crystallized in 0.1 M Heps, pH 7.5, 20% PEG 4000, and 0.2 M ammonium sulfate. The FGF1-FGFR1c complex crystallized in 0.1 M Tris, pH 8.5, 15% PEG 4000, and 0.1 M ammonium sulfate. Crystals were transferred into cryoprotectant solution composed of the mother liquor and 15% glycerol and were flash-frozen under a nitrogen stream at \(-196^\circ C\). Diffraction data were collected at NSLS beamline X4A and processed using HKL2000 (60).

The FGF1-FGFR1c complex crystals belong to space group P1 with unit cell dimensions \( a = 53.324, b = 53.421, c = 80.463, \alpha = 106.441, \beta = 106.396, \gamma = 94.436 \). There are two FGF1-FGFR1c complexes per asymmetric unit in the crystal with a solvent content of 50.4%. The dimensions of the “a” and “b” axes as well as the \( \alpha \) and \( \beta \) angles of this P1 unit cell are nearly equal, implying that the crystal may be in monoclinic space group C2 rather than P1. However, the distortion index for the C2 space group was 1.2%, and accordingly the diffraction data could not be integrated and scaled in C2.

Crystals of the FGF1-FGFR2bWT complex diffracted poorly, and attempts to improve their diffraction failed. In contrast, the crystals of the FGF1-FGFR2bA172F and FGF1-FGFR2bP253R mutant complexes diffracted to 2.2 and 2.1 Å, respectively. The FGF1-FGFR2bA172F and FGF1-FGFR2bP253R crystal structures are in space groups P3\( _2 \) and P2\( _1 \), and have two and one FGF1-FGFR2b complex per asymmetric unit, respectively. The solvent contents of the FGF1-FGFR2bA172F and FGF1-FGFR2bP253R crystals are 55.8 and 51.9%, respectively. All three crystal structures were solved by the molecular replacement program AMORE (61) using the published FGF1-FGFR1c (PDB code IEVT) (31) and FGF10-FGFR2b (PDB code 1NUN) (34) structures as search models. The program O (62) was used for model building into the 2\( F_o - F_c \) and \( F_o - F_c \) electron density maps, and CNS was used for rigid body, positional, and \( B \) factor refinements (63). For the FGF1-FGFR1c and FGF1-FGFR2bA172F structures, tight noncrystallographic symmetry restraints were imposed throughout the refinement for the backbone atoms of FGF1, FGFR D2, and FGFR D3 domains. The refined model for the FGF1-FGFR1c structure consists of two FGF1 molecules (residues 21–153), two FGFR1c molecules (residues 147–359), one heparin octasaccharide (of which only six monosaccharide units are modeled), and 32 water molecules. The FGF1-FGFR2bP253R structure in space group P2\( _1 \) contains one FGF1 molecule (residues 10–154), one FGFR2bP253R molecule (residues 151–361), 161 water molecules, and three sulfate ions. The FGF1-FGFR2bA172F structure in space group P3\( _2 \) contains two FGF1 molecules (residues 19–153), two FGFR2bA172F molecules (residues 151–359), 120 water molecules, and five sulfate ions. The FGF1-FGFR2bP253R structure in the P2\( _1 \) space group was used to make the structural figures.

Surface Plasmon Resonance (SPR) Spectroscopy—Real-time biomolecular FGF-FGFR interactions were analyzed with a BIAcore 2000 system (GE Healthcare) in HBS-EP buffer (0.01 M
Crystal Structure of FGF1 in Complex with FGFR2b Ligand-Binding Region—To elucidate the molecular mechanism underlying the unique ability of FGF1 to override the FGFR specificity barrier set by alternative splicing, we chose to solve the crystal structure of FGF1 complexed with the ligand-binding region of FGFR2b. Crystals of full-length wild type FGF1 (FGF1(1–155)) in complex with the ligand-binding region of wild type FGFR2b encompassing D2, D3, and the D2-D3 linker (residues 158–369) (supplemental Fig. 1) diffracted poorly, and attempts to improve their diffraction failed. To overcome this problem, 1:1 complexes of FGF1 with mutated FGFR2b constructs harboring either the P253R or A172F gain-of-function mutations were generated. The P253R FGFR2 mutant maps to the D2-D3 linker region, and we have previously shown that this mutation enhances FGF-FGFR binding, thereby improving crystal growth/diffraction (30, 66, 67). The A172F mutation maps to the receptor-receptor interface in the FGF-FGFR 2:2 dimer, and it also improves crystal growth/diffraction by introducing FGFR-FGFR contacts (68). The FGF1-FGFR2bP253R and FGF1-FGFR2bA172F complexes crystallized in the orthorhombic (P212121) and hexagonal (P32) space groups, respectively. Data collection and refinement statistics are summarized in Table 1. The crystal structures of the complexes were solved by molecular replacement and have been refined to 2.1 Å (in P212121, PDB code 3OJM) and 2.2 Å (in P32, PDB code 3OJ2) resolution. The asymmetric units of the P212121 and P32 crystal forms contain one and two FGF1-FGFR2b complexes, respectively. Data collection and refinement statistics are summarized in Table 1. Superimposition of the Ca traces of residues 20–151 of FGF1 from the FGF1-FGFR2b complexes in the two crystal structures gives a root mean square deviation of only 0.3 Å (supplemental Fig. 2) and shows that the first ordered FGF1 residues in the complex from the P212121 and P32 crystal forms are Thr-10 and Pro-19, respectively (Fig. 1A and supplemental Fig. 2). The observed ordering of the additional nine residues at the FGF1 N-terminal region in the P212121 and P32 crystal forms is due to the fact that these residues interact with receptor D2 (Fig. 1A). How-
TABLE 1

| Data collection and refinement statistics | FGF1-FGFR2b<sup>P2,121</sup> | FGF1-FGFR2b<sup>A17,28</sup> | FGF1-FGFR1c-heparin octasaccharide |
|------------------------------------------|-----------------|-----------------|-----------------------------------|
| **Data collection**                      |                 |                 |                                   |
| Space group                              | P2, 2121        | P32             | P1                                |
| Unit cell parameters                      |                 |                 |                                   |
| \(a\)                                    | 66.740          | 77.945          | 53.324                            |
| \(b\)                                    | 72.349          | 77.945          | 53.421                            |
| \(c\)                                    | 91.912          | 137.555         | 80.463                            |
| \(\alpha\)                                | 90              | 90              | 106.841                           |
| \(\beta\)                                 | 90              | 90              | 106.396                           |
| \(\gamma\)                                | 90              | 120             | 94.436                            |
| Solvent content (%)                       | 51.9            | 55.8            | 50.4                              |
| Wavelength (Å)                            | 0.92018         | 1.277           | 0.97912                           |
| Resolution (Å)                            | 30.0-2.1        | 30.0-2.2        | 50-2.6                            |
| Reflections (total/unique)                | 330055/27015    | 162990/47189    | 90699/24626                       |
| Completeness (%)                          | 99.5 (98.6)<sup>a</sup> | 99.3 (99.6)<sup>b</sup> | 97.8 (91.0)<sup>c</sup>          |
| \(R_{sym}\) (%)                          | 5.4 (10.3)      | 4.8 (26.5)      | 6.4 (32.7)                        |
| Signal ((\(I/\sigma_I\))                  | 40.5 (35)       | 25.4 (5.9)      | 16.6 (5.5)                        |
| Redundancy                                | 12.2 (10.1)     | 3.5 (3.4)       | 3.7 (3.5)                         |
| **Refinement**                            |                 |                 |                                   |
| Resolution (Å)                            | 25-2.1          | 25-2.2          | 25-2.6                            |
| Reflections (total/test)                  | 26221/2605      | 45665/2287      | 23714/2349                        |
| \(R_{cryst}/R_{free}\) (%)               | 22.9/26.3       | 26.2/29.4       | 25.1/30.9                         |
| Bond (Å)                                  | 0.005           | 0.008           | 0.008                             |
| Angle (degrees)                           | 1.35            | 1.42            | 1.49                              |
| \(B\)-factor (Å<sup>2</sup>)             | 1.05            | 0.888           | 1.2                               |
| No. of atoms                              | 2774            | 5372            | 5329                              |
| Protein                                   | 12              | 20              | 0                                 |
| Sulfate                                   | 161             | 120             | 32                                |
| Water                                     | 0               | 0               | 105                               |
| Heparin octasaccharide                    | 25              | 48              | 45                                |
| Average \(B\)-factor (Å<sup>2</sup>)     | 24              | 48              | 45                                |
| Protein                                   | 26              | 48              | 46                                |
| Angle (degrees)                           | 27              | 40              | 27                                |
| \(B\)-factor (Å<sup>2</sup>)             | 44              | 88              | NA<sup>e</sup>                    |
| Number of atoms                           | 25              | 48              | 45                                |
| Heparin octasaccharide                    | 24              | 48              | 46                                |
| Number of atoms                           | 27              | 40              | 27                                |
| Ramachandran plot                         | 271 (89.4%)     | 516 (88.4%)     | 477 (81.1%)                       |
| Residues in most favored region           | 31 (9.9%)       | 65 (11.1%)      | 96 (16.3%)                        |
| Residues in generously allowed region     | 1 (0.3%)        | 3 (0.5%)        | 8 (1.4%)                          |
| Residues in disallowed region             | 1 (0.3%)        | 0               | 7 (1.2%)                          |
| PDB code                                  | 3OJM            | 3OJ2            | 3OJV                              |

<sup>a</sup> Values in parentheses in this column are for the highest resolution shell: 2.18–2.1 Å.
<sup>b</sup> Values in parentheses in this column are for the highest resolution shell: 2.28–2.20 Å.
<sup>c</sup> Values in parentheses in this column are for the highest resolution shell: 2.69–2.6 Å.
<sup>e</sup> NA, not applicable.

However, because these contacts are not seen in the P3<sub>2</sub> crystal form, they were deemed crystal-favored and will not be discussed. Aside from the contacts between this distal N-terminal region of FGF1 and the FGFR2b D2 domain, the remaining interactions at the interface between FGF1 and FGFR2b are essentially identical between the two crystal forms. Significantly, in both crystal forms, the alternatively spliced \(\beta'\)-\(\beta\) loop in receptor D3, which is a key determinant of the ligand binding specificity of FGF1, is ordered and is engaged by the ligand (Fig. 1A). Hence, our two new FGF1-FGFR2b structures, taken together with our previously published FGF1-FGFR3c structure (PDB code 1RY7) (Fig. 1D) (35) and the FGF1-FGFR2c structure solved by the Hendrickson laboratory (PDB code 1DJ5) (Fig. 1C) (33), argue against our initial hypothesis that FGF1 achieves indiscriminate binding to both isoforms by not utilizing the alternatively spliced \(\beta'\)-\(\beta\) loop of receptor D3 (31).

In New FGF1-FGFR1c Structure, Alternatively Spliced \(\beta'\)-\(\beta\) Loop Is Ordered and Interacts with FGF1—Because in both of our newly solved FGF1-FGFR2b structures FGF1 engages the \(\beta'\)-\(\beta\) loop of the receptor, it became imperative to reassess the role of \(\beta'\)-\(\beta\) loop disordering in our previous FGF1-FGFR1c structure (PDB code 1EVT) (31). To this end, we decided to solve the crystal structure of FGF1 bound to FGFR1c in a crystal lattice different from that of our first FGF1-FGFR1c structure (PDB code 1EVT) (31). For recrystallization of the FGF1-FGFR1c complex, we used the same N-terminally truncated FGF1 protein (FGF1(21–155)), which we originally used to generate PDB entry 1EVT. FGF1(21–155) and full-length FGF1 bind FGFR1c with identical affinities (69). To encourage crystallization of the FGF1-FGFR1c complex in a crystal lattice different from that of 1EVT, we intentionally supplemented the complex in a 1:1 ratio with a homogeneously sulfated heparin in order to induce formation of a 2:2:2 FGF1-FGFR1c-heparin octasaccharide dimer. The crystal structure of the FGF1-FGFR1c-octasaccharide complex was solved using molecular replacement with 1EVT as the search model. The asymmetric unit of this new FGF1-FGFR1c structure (PDB code 3OJV) contains two complexes, and the structure has been refined to 2.6 Å.
FIGURE 1. Plasticity at the FGF1-D3 interface. **A**, left, \( \alpha \) trace of FGF1 from the FGF1-FGFR2b\(^{2253}\) structure in space group P2,\( _1\)2,\( _1\)2 (PDB code 3OJM) (in orange) is superimposed onto that of FGF1 from the FGF1-FGFR2b\(^{172}\) structure in space group P3\( _2\) (PDB code 3OJ2) (in black). The first ordered N-terminal residue in each FGF1 molecule is labeled. FGFR2b is shown as a surface, with D2 colored green, D2-D3 linker in gray, the common region of D3 in cyan, and the alternatively spliced portion of D3 in purple. This color scheme for the receptor is used throughout the paper including in supplemental Figs. 3, 4, 5, and 7. The FGF1-FGFR2b\(^{2253}\) structure in space group P2,\( _1\)2,\( _1\)2 (PDB code 3OJM) is used for comparison with other FGF1-FGFR structures. **Right**, a close-up view of selected N-terminal and hydrophobic patch contacts of FGF1 with D3 of receptor in the FGF1-FGFR2b\(^{2253}\) crystal structure. FGF1 is shown as a ribbon diagram, and selected residues are rendered as sticks, with oxygen colored red and nitrogen colored blue. Water molecules are indicated as red spheres. This color scheme for atoms is used throughout the paper including in supplemental Figs. 3, 4, 5, and 7. The location of the hydrophobic patch between FGF core and the D3 loop is indicated by an arrow. **B**, the new FGF1-FGFR1c structure (PDB code 3OJV), showing an ordered D3 loop. **C**, selected N-terminal and hydrophobic patch contacts of FGF1 with D3 of receptor in the FGF1-FGFR2c crystal structure (PDB code 1DJS). **D**, selected N-terminal and hydrophobic patch contacts of FGF1 with D3 of receptor in the FGF1-FGFR3c structure (PDB code 1RY7). Note that Ala-314 in D3 of FGFR3c cannot make a strong hydrophobic contact with Pro-94, Tyr-79, and Tyr-70 in the FGF1 core, and as a result, the \( \beta \) -\( \beta \) loop falls away from the core of the ligand.

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resolution. Data collection and refinement statistics are given in Table 1. Importantly, although both 3OJV and 1EVT are in triclinic space group P1, the crystal lattice contacts are very different between these two structures (supplemental Fig. 3, A and B). The interface between FGF1 and FGFR1c in 3OJV is similar to that in our previous FGF1-FGFR1c structure (PDB code 1EVT) (31) with the notable difference that the electron density for the alternatively spliced βC′-βE loop in D3 was sufficient to confidently build the entire loop (Fig. 1B). The loop, however, has a high average temperature factor of 80 Å² (compared with an average temperature factor of 45 Å² for the whole structure), reflecting the fact that it makes only very few contacts with the ligand (Fig. 1B). In contrast, FGF1 interacts more with the βC′-βE loop in the FGF1-FGFR2c (Fig. 1C) and FGF1-FGFR2b (Fig. 1A) structures, accounting for the lower average temperature factors of the βC′-βE loop in the FGF1-FGFR2c (31 Å²) and FGF1-FGFR2b structures (22 Å²). Based on our new FGF1-FGFR2b and FGF1-FGFR1c structures, we can conclude that the promiscuity of FGF1 toward FGFR isoforms cannot be attributed to the fact that FGF1 does not rely on the alternatively spliced βC′-βE loop of FGFR for binding as we initially proposed (31).

Plasticity at Interface between FGF1 N Terminus and FGFR D3—Comparison of our newly solved FGF1-FGFR2b and FGF1-FGFR1c structures with previous FGF1-FGFR2c (PDB code 1DJS) (33) and FGF1-FGFR3c (PDB code 1RY7) (35) structures reveals that cross-reactivity of FGF1 with both “b” and “c” splice isoforms of FGFRs stems from the fact that the N terminus of FGF1 has a remarkable ability to adapt to D3 of either of the two FGFR splice isoforms (Fig. 1, A–D). Notably, the nine N-terminal residues (Phe-16 to Lys-24) preceding the variable interactions with D3 of FGFR across different FGF1-FGFR structures. FGF1 employs a different number of residues from this N-terminal region to engage the C-terminal end of the alternatively spliced βC′-βE loop (the most divergent region in D3) and the constant region of D3 in different FGF1-FGFR complexes (Fig. 1, A–D). Moreover, the same residue from this N-terminal region of FGF1 interacts differently with D3 of receptor in different FGF1-FGFR complexes. Starting with Lys-24 and moving N-terminally toward Phe-16, the contacts between FGF1 and D3 are as follows. Lys-24 of FGF1 makes hydrogen bonds with a highly conserved glutamate at the C-terminal end of the βC′-βE loop of the receptor in FGF1-FGFR1c (Glu-324), in FGF1-FGFR2b (Glu-323), and in FGF1-FGFR3c (Glu-322) but not in FGF1-FGFR2c (Glu-325) (Fig. 1, A–D). The hydroxyl group of Tyr-23 directly hydrogen-bonds with the carboxylate side chain of this conserved glutamate of receptor in FGF1-FGFR2b and FGF1-FGFR3c but not in our new FGF1-FGFR1c structure or in FGF1-FGFR2c (Fig. 1, A–D). Tyr-23 of FGF1 also engages in a water-mediated hydrogen bond with a backbone amide of an FGFR-invariant valine (Val-280 in FGF2b) in the βB′ strand of FGFR in all structures except FGF1-FGFR3c. In addition to these receptor-specific contacts, in all four FGF1-FGFR structures, Tyr-23 engages in conserved hydrophobic contacts with a FGFR-invariant proline in the βB′-βC loop and an FGFR-invariant valine (Val-280 in FGF2b) in the βB′ strand. Moreover, the backbone atoms of Tyr-23 make hydrogen bonds with a highly conserved glutamate in the βB′-βC loop of the receptor in all four structures (Fig. 1, A–D). As for Asn-22 of FGF1, in FGF1-FGFR2c and FGF1-FGFR3c, its side chain directly hydrogen-bonds to the backbone atoms of D3 of receptor, whereas in FGF1-FGFR2b, Asn-22 makes a water-mediated hydrogen bond with D3. In the FGF1-FGFR1c complex, Asn-22 does not participate in receptor binding at all (Fig. 1, A–D). In the FGF1-FGFR2b structure, the backbone amide of Gly-21 of FGF1 makes a hydrogen bond to the backbone carbonyl oxygen of Val-280 of receptor (Fig. 1A). However, in FGF1-FGFR2c, the carbonyl oxygen of Gly-21 engages in a water-mediated hydrogen bond with the backbone carbonyl oxygen of Asp-283 of receptor (Fig. 1C). In FGF1-FGFR3c, the backbone amide of Gly-21 directly hydrogen-bonds to the backbone of Glu-320 of receptor (Fig. 1D), whereas in our new FGF1-FGFR1c structure, Gly-21 is not involved in receptor binding (Fig. 1B). Last, in the FGF1-FGFR3c structure, Phe-16 and Leu-18 of FGF1 engage in hydrophobic contacts with Ile-254 and Tyr-278 and in van der Waals contacts with Gln-256 and Lys-276 of receptor that are not seen in the other three FGF1-FGFR complexes (Fig. 1D). Hence, based on our detailed structural analysis, plasticity in the interactions of residues Phe-16 to Lys-24 at the FGF1 N terminus with FGFRs imparts receptor binding promiscuity to FGF1.

N-terminal Residues of FGF1 Are Principal Determinants of FGF1 Promiscuity—Sequence alignment of the N termini of human FGFs (Fig. 2B) clearly shows that no other FGF would be capable of recapitulating the plastic interactions that this 9-amino acid-long N-terminal region of FGF1 makes with FGFRs in the four different FGF1-FGFR structures. Even FGF2, the other FGF1 subfamily member, shares limited sequence homology with FGF1 at this region. Only four residues in this region are conserved in FGF2 (Fig. 2A). Of the non-conserved, Phe-26 of FGF2 cannot provide the direct or water-mediated hydrogen bonds that Tyr-23 of FGF1 makes with Glu-323 of FGF2b or with Glu-320 of FGF3c in the FGF1-FGFR2b and FGF1-FGFR3c structures (supplemental Fig. 4 and Fig. 1, A and D). Similarly, Gly-19 of FGF2, which corresponds to Phe-16 of FGF1, is incapable of making the hydrophobic contacts or van der Waals contacts with Ile-254, Gln-256, Lys-276, and Tyr-278 of FGF3c that are seen in the FGF1-FGFR3c structure (Fig. 1D). His-25 of FGF2 would not be able to make the hydrogen bonds that the corresponding Asn-22 of FGF1 makes with FGFRs in the FGF1-FGFR2b, FGF1-FGFR2c, and FGF1-FGFR3c structures (Fig. 1, A, C, and D). Hence, based on the structural data and sequence analysis, residues 16–24 of FGF1 should play a major role in endowing FGF1 with the ability to bind indiscriminately to either splice isoform of FGFR2.

To validate our structural conclusion, we generated three FGF2 mutants in which the N-terminal residues Phe-26, Asn-25, and Gly-19 of FGF2 are progressively replaced with the corresponding residues of FGF1 (Tyr-23, Asn-22, and Phe-16) (supplemental Fig. 1). The ability of these FGF2 mutants, namely FGF2F26Y, FGF2H25N/F26Y, and FGF2G19F/H25N/F26Y, to bind and activate FGF2b was tested in vitro and in living cells. We reasoned that if these three N-terminal residues of FGF1 were responsible for promiscuous binding of FGF1 to either
FGFR2 isoform, then the FGF2 mutants should acquire the ability to bind and activate FGFR2b. SPR spectroscopy and size exclusion chromatography were used to compare binding of FGF1, FGF2, FGF2F26Y, FGF2H25N/F26Y, and FGF2G19F/H25N/F26Y to wild type FGFR2b and FGFR2c ligand-binding regions in vitro. For SPR studies, the ligand-binding regions of wild type FGFR2b and FGFR2c were coupled to biosensor chips, and various concentrations of FGF1, FGF2, and FGF2 mutants were passed over the chips. Consistent with the well known promiscuity of FGF1 toward either of the FGFR isoforms, the SPR data show that FGF1 binds with comparable affinity to both FGFR2c and FGFR2b (Fig. 3A). Similarly, in agreement with our structural data, FGF2 binds with high affinity to FGFR2c and exhibits negligible binding to FGFR2b (Fig. 3A). Interestingly, the affinity of FGF2 for FGFR2c is nearly 5-fold greater than that of FGF1 for FGFR2c (KD of 18 nM versus 86 nM; Fig. 3A), emphasizing the specificity of FGF2 for FGFR2c. The FGF2F26Y, FGF2H25N/F26Y, and FGF2G19F/H25N/F26Y mutants bound to FGFR2b with progressively increased affinity (Fig. 3A). In fact, FGF1 and the FGF2G19F/H25N/F26Y mutant bound FGFR2b with comparable affinity (KD of 44 nM versus 31 nM; Fig. 3A), demonstrating that residues 16, 22, and 23 of FGF1 have imparted to FGF2 the ability to bind FGFR2b (Fig. 3A). All three FGF2 mutants bound to FGFR2c with the same affinity as their parent wild type FGF2 ligand. Size exclusion chromatographic analysis of 1:1 mixtures of FGF1, FGF2, and the FGF2G19F/H25N/F26Y mutant with the ligand-binding region of FGFR2b validates the SPR data (Fig. 3B). The FGF2G19F/H25N/F26Y mutant clearly forms a stable complex with FGFR2b in solution and elutes at a retention time comparable with that of the FGF1-FGFR2b complex (Fig. 3B). In contrast and as expected, wild type FGF2 failed to form a complex with the FGFR2b ligand-binding region. These in vitro data show that residues 16–24 of FGF1 are the main determinants of the ability of FGF1 to override the D3 specificity barrier mediated by alternative splicing (Fig. 3A and B).

To provide further biochemical support for our finding that plasticity in the interactions of the N-terminal region of FGF1 with receptor D3 is responsible for the promiscuity of FGF1, we introduced mutations into FGFR2b to specifically ablate the interactions observed in the FGF1-FGFR2b structure between FGFR2b and this N-terminal region. Two FGFR2b ligand-binding region constructs were made, one carrying the E323A single mutation (FGFR2bE323A) and the other carrying the I257A/H252P double mutation (FGFR2bI257A/H252P). As expected, FGF1 bound with comparable affinity to both FGFR2bE323A and FGFR2bI257A/H252P (Fig. 3C). In contrast, wild type FGF2 bound with comparable affinity to FGFR2b and exhibited negligible binding to FGFR2bE323A and FGFR2bI257A/H252P, emphasizing the specificity of FGF2 for FGFR2c. These in vitro data show that residues 16–24 of FGF1 are the main determinants of the ability of FGF1 to override the D3 specificity barrier mediated by alternative splicing (Fig. 3, A and B).
A double mutation (FGFR2bI257A/Y281A). Based on the FGFR1-FGFR2b structure (Fig. 1), the E323A mutation should cause a reduction in FGFR2b binding affinity by eliminating the hydrogen bonding between Tyr-23 of FGFR1 and Glu-323 of FGFR2b. The I257A/Y281A double mutation should also reduce the binding affinity of FGFR2b for FGFR1 because Tyr-281 engages in a hydrophobic interaction with Pro-20 of FGFR1, and Ile-257 helps orient the side chain of Tyr-281 for optimal interaction with Pro-20 of FGFR1 (Fig. 1A). Consistent with the FGFR1-FGFR2b crystal structure, the SPR data show that relative to the wild type FGFR2b ligand-binding region, the FGFR2bI257A/Y281A and FGFR2bE323A mutants sustained a 3-fold and 5-fold loss in FGF1 binding affinity, respectively (Fig. 3C). Size exclusion chromatography analyses of 1:1 mixtures of wild type and mutated FGFR2b ligand-binding regions with FGF1 support the SPR data. The FGF1-FGFR2b, FGF1-FGFR2bI257A/Y281A, and FGF1-FGFR2bE323A complexes eluted two and four fractions later than the wild type FGF1-FGFR2b complex, respectively (Fig. 3D). Taken together, these receptor mutagenesis experiments further confirm that interactions between the FGF1 N terminus and FGFR are crucial for the promiscuity of FGF1 (Fig. 3, C and D).

Last, to validate the biological relevance of our structural and biochemical findings, we examined the ability of FGF1, FGF2, and the three FGF2 mutants to induce proliferation of BaF3 cell lines ectopically expressing FGFR2b or FGFR2c. FGF10, a ligand that is highly selective for FGFR2b, was included as a control in these experiments. BaF3 cells were deprived of interleukin-3 for 3–4 h and then treated with these FGFs (100 ng/ml) in the presence of 5 μg/ml heparin. FGFR2b-expressing cells exhibited a 4-fold and 10-fold increase in cell proliferation in response to FGF1 and FGF10, respectively. In contrast, wild type FGF2 induced a modest (1.5-fold) proliferation of these cells (Fig. 4A). FGFR2c-expressing cells proliferated robustly in response to FGF1 and FGF2 (~7-fold greater than control), whereas FGF10 had no effect (Fig. 4B). Importantly, relative to the parent FGF2 molecule, the FGF2I26Y, FGF2I25N/F26Y, and FGF2G19F/I25N/F26A mutants all induced greater proliferation.
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FIGURE 4. Substitution of three N-terminal residues of FGF2 for the analogous ones in FGF1 confers upon FGF2 the ability to induce proliferation of BaF3 cells ectopically expressing FGFR2b. A, a comparison of the ability of FGF1, FGF2, and FGF2 mutants to induce proliferation of FGFR2b-expressing BaF3 cells. FGF10, a BaF3-specific ligand, was used as a positive control. Experiments were done in triplicate, and error bars reflect one S.D. The data presented are a representative example of seven independent experiments. As expected, FGF2 induced only minimal proliferation of cells expressing FGFR2b. Compared with wild type FGF2, the FGF2G19F/H25N/F26Y, FGF2H25N/F26Y, and FGF2G19F/H25N/F26Y mutants elicit a 2.1-, 2.4-, and 2.8-fold greater response, respectively. The increase in cell proliferation induced by FGF2G19F/H25N/F26Y, FGF2H25N/F26Y, or FGF2G19F/H25N/F26Y relative to FGF2 was statistically significant (p < 0.01). B, a comparison of the ability of FGF1, FGF2, and FGF2 mutants to induce proliferation of FGFR2c-expressing BaF3 cells. The FGF2 mutants retain the ability to induce proliferation of FGFR2c-expressing cells. As expected, FGF10 fails to promote proliferation of FGFR2c-expressing cells.

Comparison of the four FGF1-FGFR structures show that FGF1 core also engages in plastic interactions with the alternatively spliced BC’-βE and BF-βG loops in D3, which are the least conserved regions of FGFRs (supplemental Fig. 6D). At the interface between FGF1 and the BC’-βE loop, Glu-64 of FGF1 makes direct and water-mediated hydrogen bonds with the backbone atoms of Ile-317 and Asn-318 of receptor in the FGF1-FGFR2b structure (Fig. 1A), whereas in the FGF1-FGFR2c structure, it only makes a water-mediated hydrogen bond to the backbone of Val-317 of receptor (Fig. 1C). Ser-62 of FGF1 makes a direct hydrogen bond to a backbone atom of Val-317 of receptor in FGF1-FGFR2c (Fig. 1C), whereas in the FGF1-FGFR1c and FGF1-FGFR2b structures, Ser-62 does not contact the loop at all (Fig. 1, A and B). Uniquely in FGF1-FGFR2c, Lys-72 and Tyr-79 of FGF1 directly hydrogen-bond with the side chain of Asn-318 of receptor (Fig. 1C).

Plasticity is also evident in the interactions of FGF1 core with the alternatively spliced BF-βG loop in D3 of the receptors. In particular, interactions between FGF1 and the BF-βG loop of receptor in the FGF1-FGFR2b complex are markedly different from those observed in FGF1-FGFRc structures (supplemental Fig. 7). At this interface in the FGF1-FGFR2b structure, the phenyl ring of Tyr-345 in the BF-βG loop of receptor engages in a π-cation interaction with Arg-103 of FGF1, whereas the hydroxyl group of this Tyr-345 is directly hydrogen-bonding to the backbone amide of Leu-104 of FGF1 (supplemental Fig. 7A). In FGFRc isoforms, Tyr-345 is replaced by a serine (supplemental Fig. 6D) that is engaged in water-mediated hydrogen bonds with Leu-104 and Glu-102 of ligand in the FGF1-FGFR1c and FGF1-FGFR2c structures (supplemental Fig. 7B).

Structural and sequence-based analyses indicate, however, that plasticity in the interactions of FGF1 core region with D3 of FGFRs plays a minor role in conferring promiscuity upon FGF1. There is significantly greater sequence similarity at the core region between FGFRs, suggesting that other FGFRs should be capable of recapitulating the contacts that the core region of FGF1 makes with different FGFRs. For example, the Ser-62 in...
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β4 of FGF1 that engages in variable contacts with the βC’-βE loop is conserved in FGF4 and FGF6, which are known to be specific toward FGFR1c and FGFR2c (Fig. 2D). Furthermore, at the position analogous to Glu-64 in β4 of FGF1, FGF2 also possesses a glutamic acid (Glu-67). In the FGF2-FGFR1c and FGF2-FGFR2c structures, Glu-67 of FGF2 makes hydrogen bonds with the βC’-βE loop of FGFR (supplemental Fig. 4) that are reminiscent of those seen between Glu-64 of FGF1 and this loop in the FGF1-FGFR2b and FGF1-FGFR2c structures (Fig. 1, A and C). FGF2 also possesses a tyrosine (Tyr-72) at the position corresponding to Arg-103 of FGF1, yet FGF2 is FGF10-FGFR2b specificity. Notably, FGF2 also has an arginine (Kuro-o, M., ed) Landes Bioscience, Austin, TX

REFERENCES

1. Beenken, A., and Mohammadi, M. (2009) Nat. Rev. Drug Discov. 8, 235–253
2. Kuro-o, M. (2008) Trends Endocrinol. Metab. 19, 239–245
3. Ornitz, D. M. (2005) Cytokine Growth Factor Rev. 16, 205–213
4. Itoh, N., and Ornitz, D. M. (2004) Trends Genet. 20, 563–569
5. Popovici, C., Roubin, R., Coulier, F., and Birnbaum, D. (2005) BioEssays 27, 849–857
6. Goetz, R., Dover, K., Laeza, F., Shtraizent, N., Huang, X., Tchetchik, D., Eliseenkova, A. V., Xu, C. F., Neubert, T. A., Ornitz, D. M., Goldfarb, M., and Mohammadi, M. (2009) J. Biol. Chem. 284, 17883–17896
7. Goldfarb, M. (2005) Cytokine Growth Factor Rev. 16, 215–220
8. Olsen, S. K., Garbi, M., Zampieri, N., Eliseenkova, A. V., Ornitz, D. M., Goldfarb, M., and Mohammadi, M. (2003) J. Biol. Chem. 278, 34226–34236
9. Beenken, A., and Mohammadi, M. (2012) in Versatile Biological Functions of Kuro-o, M., ed) Landes Bioscience, Austin, TX

10. Fu, L., John, L. M., Adams, S. H., Yu, X. X., Tomlinson, E., Renz, M., Williams, P. M., Soriano, R., Corpuz, R., Moffatt, B., Vanden, R., Simmons, L., Foster, J., Stephan, J. P., Tsai, S. P., and Stewart, T. A. (2004) Endocrinology 145, 2594–2603
11. Holt, J. A., Luo, G., Billin, A. N., Bisi, J., McNeill, Y. Y., Kozarsky, K. F., Donahee, M., Wang, D. Y., Mansfield, T. A., Klierer, S. A., Goodwin, B., and Jones, S. A. (2003) Genes Dev. 17, 1581–1591
12. Lundåsen, T., Galman, C., Angelin, B., and Rudling, M. (2006) J. Intern. Med. 260, 510–536
13. Tomlinson, E., Fu, L., John, L., Hultgren, B., Huang, X., Renz, M., Stephan, J. P., Tsai, S. P., Powell-Braxton, L., French, D., and Stewart, T. A. (2002) Endocrinology 143, 1741–1747
14. Coskun, T., Bina, H. A., Schneider, M. A., Dunbar, J. D., Hu, C. C., Chen, Y., Moller, D. E., and Kharitonov, A. (2008) Endocrinology 149, 6018–6027
15. Kharitonov, A., Shiyanova, T. L., Koester, A., Ford, A. M., Micanovic, R., Galbreath, E. J., Sandusky, G. E., Hammond, L. J., Mojey, J. S., Owens, R. A., Gromada, J., Brozinick, J. T., Hawkins, E. D., Wroblewski, V. J., Li, D. S., Mehrbod, F., Jaskunas, S. R., and Shnafael, A. B. (2005) J. Clin. Invest. 115, 1627–1635
16. Kharitonov, A., Wroblewski, V. J., Koester, A., Chen, Y. F., Cluttering, C. K., Tigno, X. T., Hansen, B. C., Shnafael, A. B., and Eitgen, G. J. (2007) Endocrinology 148, 774–781
17. Xu, X., Lloyd, D. J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., Vonderfecht, S., Hecht, R., Li, Y. S., Lindberg, R. A., Chen, J. L., Jung, D. Y., Zhang, Z., Ko, H. J., Kim, J. K., and Véniant, M. M. (2009) Diabetes 58, 250–259
18. Inoue, Y., Segawa, H., Kaneko, I., Yamana, S., Kusano, K., Kawakami, E., Furutani, J., Ito, M., Kuwahata, M., Saito, H., Fukushima, N., Kato, S., Kanayama, H. O., and Miyamoto, K. (2005) Biochem. J. 390, 325–331
19. Saito, H., Kusano, K., Kinosaki, M., Ito, H., Hira, M., Segawa, H., Miyamoto, K., and Fukushima, N. (2003) J. Biol. Chem. 278, 2206–2211
20. Segawa, H., Kawakami, E., Kaneko, I., Kuwahata, M., Ito, M., Kusano, K., Saito, H., Fukushima, N., and Miyamoto, K. (2003) Pflugers Arch. 446, 585–592
21. Ornitz, D. M., Yayon, A., Flanagan, J. G., Svahn, C. M., Levi, E., and Leder, P. (1992) Mol. Cell Biol. 12, 240–247
22. Rapraeger, A. C., Kruftka, A., and Olwin, B. B. (1991) Science 252, 1705–1708
23. Yayon, A., Klagsbrun, M., Esco, J. D., Leder, P., and Ornitz, D. M. (1991) Cell 64, 841–848
24. Goetz, R., Beenken, A., Ihihimi, O. A., Kalinina, J., Olsen, S. K., Eliseenkova, A. V., Xu, C. F., Neubert, T. A., Zhang, F., Linhardt, R. J., Yu, X., White, K. E., Inagaki, T., Kliewer, S. A., Yamamoto, M., Kurosu, H., Ogawa, Y., Kuro-o, M., Lanske, B., Razaque, M. S., and Mohammadi, M. (2007) Mol. Cell Biol. 27, 3417–3428
25. Kurosu, H., Ogawa, Y., Miyoshi, M., Yamamoto, M., Nandi, A., Rosenblatt, K. P., Baum, M. G., Schiavi, S., Hu, M. C., Moe, O. W., and Kuro-o, M. (2006) J. Biol. Chem. 281, 6120–6123
26. Urakawa, I., Yamazaki, Y., Shimada, T., Iijima, K., Hasegawa, H., Okawa, K., Fujita, T., Fukushima, S., and Yamashita, T. (2006) Nature 444, 770–774
27. Givol, D., and Yayon, A. (1992) FEASEB J. 6, 3362–3369
28. Johnson, D. E., and Williams, L. T. (1993) Adv. Cancer Res. 60, 1–41
29. Lee, P. L., Johnson, D. E., Cousens, L. S., Fried, V. A., and Williams, L. T. (1989) Science 245, 57–60
30. Olsen, S. K., Li, J. Y., Bromleieh, C., Eliseenkova, A. V., Ihihimi, O. A., Lao, Z., Zhang, F., Linhardt, R. J., Joyner, A. L., and Mohammadi, M. (2006) Genes Dev. 20, 185–198
31. Plotnikov, A. N., Hubbard, S. R., Schlessinger, J., and Mohammadi, M.
(2000) Cell 101, 413–424
32. Plotnikov, A. N., Schlessinger, J., Hubbard, S. R., and Mohammadi, M. (1999) Cell 98, 641–650
33. Stauber, D. J., DiGabriele, A. D., and Hendrickson, W. A. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 49–54
34. Yeh, B. K., Igarashi, M., Eliseenkova, A. V., Plotnikov, A. N., Sher, I., Ron, D., Aaronson, S. A., and Mohammadi, M. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 2266–2271
35. Olsen, S. K., Ibrahimi, O. A., Raucci, A., Zhang, F., Eliseenkova, A. V., Yeyon, A., Basilo, L., Linhardt, R. J., Schlessinger, J., and Mohammadi, M. (2004) Proc. Natl. Acad. Sci. U.S.A. 101, 935–940
36. Wang, F., Kan, M., Yan, G., Xu, J., and McKeenan, W. L. (1995) J. Biol. Chem. 270, 10231–10235
37. Mohammadi, M., Olsen, S. K., and Ibrahimi, O. A. (2005) Cytokine Growth Factor Rev. 16, 107–137
38. Schlessinger, J., Plotnikov, A. N., Ibrahimi, O. A., Eliseenkova, A. V., Yeh, B. K., Yeyon, A., Linhardt, R. J., and Mohammadi, M. (2000) Mol. Cell 6, 743–750
39. Chen, H., Xu, C. F., Ma, J., Eliseenkova, A. V., Li, W., Pollock, P. M., Pitteloud, N., Miller, W. T., Neubert, T. A., and Mohammadi, M. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 19660–19665
40. Mohammadi, M., Dickey, I., Sorokin, A., Burgess, W. H., Jaye, M., and Schlessinger, J. (1996) Mol. Cell Biol. 16, 977–989
41. Souhara, H., Hadari, Y. R., Spivak-Kroizman, T., Schilling, J., Bar-Sagi, D., Lax, I., and Schlessinger, J. (1997) Cell 89, 693–702
42. Huang, J., Mohammadi, M., Rodrigues, G. A., and Schlessinger, J. (1995) J. Biol. Chem. 270, 5065–5072
43. Mohammadi, M., Dionne, C. A., Li, W., Li, N., Spivak, T., Honegger, A. M., Jaye, M., and Schlessinger, J. (1992) Nature 358, 681–684
44. Carballada, R., Yau, H., and Lemaire, P. (2001) Development 128, 35–44
45. Hadari, Y. R., Gozol, N., Coulier, F., Guy, G. R., Schlessinger, J., and Lax, I. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 8578–8583
46. Ong, S. H., Hadari, Y. R., Gozol, N., Gey, G. R., Schlessinger, J., and Lax, I. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 6074–6079
47. Seo, J. H., Suenaga, A., Hatakeyama, M., Tajii, M., and Imanoto, A. (2009) Mol. Cell 29, 3076–3087
48. Dailey, L., Ambrostetti, D., Mansukhani, A., and Basilo, C. (2005) Cytokine Growth Factor Rev. 16, 233–247
49. Eswarakumar, V. P., Lax, I., and Schlessinger, J. (2005) Cytokine Growth Factor Rev. 16, 139–149
50. Ornitz, D. M., Xu, J., Colvin, J. S., McEwen, D. G., MacArthur, C. A., Coulier, F., Gao, G., and Goldfarb, M. (1996) J. Biol. Chem. 271, 15292–15297
51. Zhang, X., Ibrahimi, O. A., Olsen, S. K., Umemori, H., Mohammadi, M., and Ornitz, D. M. (2006) J. Biol. Chem. 281, 15694–15700
52. Chellaiah, A. T., McEwen, D. G., Werner, S., Xu, J., and Ornitz, D. M. (1994) J. Biol. Chem. 269, 11620–11627
53. Johnson, D. E., Lu, J., Chen, H., Werner, S., and Williams, L. T. (1991) Mol. Cell Biol. 11, 4627–4634
54. Miki, T., Bottaro, D. P., Fleming, T. P., Smith, C. L., Burgess, W. H., Chan, A. M., and Aaronson, S. A. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 246–250
55. Yoon, A., Zimmer, Y., Shen, G. H., Avivi, A., Yarden, Y., and Givol, D. (1992) EMBO J. 11, 1885–1890
56. Chellaiah, A., Yuan, W., Chellaiah, M., and Ornitz, D. M. (1999) J. Biol. Chem. 274, 34785–34794
57. Ron, D., Reich, R., Chedid, M., Lengel, C., Cohen, O. E., Chan, A. M., Neufeld, G., Miki, T., and Tronick, S. R. (1993) J. Biol. Chem. 268, 5388–5394
58. Vainikka, S., Partanen, J., Bellosta, P., Coulier, F., Birnbaum, D., Basilo, C., Jaye, M., and Alitalo, K. (1992) EMBO J. 11, 4273–4280
59. MacArthur, C. A., Lawless, A., Xu, J., Santos-Ocampo, S., Heikinheimo, M., Chellaiah, A. T., and Ornitz, D. M. (1995) Development 121, 3603–3613
60. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
61. Navaza, J. (1994) Acta Crystallogr. A 50, 157–163
62. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. A 47, 110–119
63. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gos, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. D Biol. Crystallogr. 54, 905–921
64. Gartsdie, M. G., Chen, H., Ibrahimi, O. A., Byron, S. A., Curtis, A. V., Wellsens, C. L., Bengstson, A., Yudt, L. M., Eliseenkova, A. V., Ma, J., Curtin, J. A., Hyder, P., Harper, U. L., Riedesel, E., Mann, G. J., Trent, J. M., Bastian, B. C., Metzger, P. S., Mohammadi, M., and Pollock, P. M. (2009) Mol. Cancer Res. 7, 41–54
65. Byron, S. A., Gartsdie, M. G., Wellsens, C. L., Goodfellow, P. J., Birrer, M. J., Campbell, I. G., and Pollock, P. M. (2010) Glycobiol Oncol. 117, 125–129
66. Ibrahimi, O. A., Eliseenkova, A. V., Plotnikov, A. N., Yu, K., Ornitz, D. M., and Mohammadi, M. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 7182–7187
67. Ibrahimi, O. A., Zhang, F., Eliseenkova, A. V., Itoh, N., Linhardt, R. J., and Mohammadi, M. (2004) Hum. Mol. Genet. 13, 2313–2324
68. Ibrahimi, O. A., Yeh, B. K., Eliseenkova, A. V., Zhang, F., Olsen, S. K., Igarashi, M., Aaronson, S. A., Linhardt, R. J., and Mohammadi, M. (2005) Mol. Cell Biol. 25, 671–684
69. Luo, Y., Gabriel, J. L., Wang, F., Zhan, X., Maciag, T., Kan, M., and McKeehan, W. L. (1996) J. Biol. Chem. 271, 26876–26883
70. Wu, X., Ge, H., Lemon, B., Vonderfecht, S., Baribault, H., Weisssmann, J., Gupte, J., Gardner, J., Lindberg, R., Wang, Z., and Li, Y. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 14158–14163