Published in final edited form as:
Cytokine Growth Factor Rev. 2005 April ; 16(2): 215–220. doi:10.1016/j.cytogfr.2005.02.002.

FIBROBLAST GROWTH FACTOR HOMOLOGOUS FACTORS: EVOLUTION, STRUCTURE, AND FUNCTION

Mitchell Goldfarb, Ph.D.
Department of Biological Sciences, Hunter College of City University, New York, NY 10021

Summary

Fibroblast growth factor homologous factors (FHFs) bear strong sequence and structural similarity to fibroblast growth factors (FGFs). However, the biochemical and functional properties of FHFs are largely, if not totally, unrelated to those of FGFs. Whereas FGFs function through binding to the extracellular domains of FGF receptors (FGFRs), FHFs bind to intracellular domains of voltage-gated sodium channels (VGSCs) and to a neuronal MAP kinase scaffold protein, islet-brain-2 (IB2). These findings demonstrate the remarkable functional adaptability during evolution of the FGF gene family. FHF gene mutations in mice result in a range of neurological abnormalities, and at least one of these anomalies, cerebellar ataxia, is linked to FHF mutations in humans. This article reviews the sequences and structure of FHFs, along with our still limited understanding of FHF function.

FHF gene structure and sequence

FHF genes were discovered through searches of cDNA and genomic DNA databases for sequences homologous to known FGFs (Coulier et al., 1997; Hartung et al., 1997; Smallwood et al., 1996). FHF/FGF sequence homology spans a region encoding 120–130 amino acids, a domain which adopts a β-trefoil fold (Murzin et al., 1992; Olsen et al., 2003). There are four FHF genes in many vertebrate species; these FHF genes still retain FGF gene designations: FHF1 = FGF12, FHF2 = FGF13, FHF3 = FGF11, FHF4 = FGF14. (All future reference to “FGFs” shall imply non-FHF members of the FGF gene family.) By contrast, non-vertebrate chordate ascidians harbor one FHF gene (Satou et al., 2002), while insect and worm genomes do not encode for FHFs, but do encode for FGFs. Pairwise sequence homologies between vertebrate FHF genes in the same or different organisms support the evolutionary expansion of the FHF gene family to four members prior to or coincident with the emergence of vertebrates, conferring upon all vertebrates FHF1, FHF2, FHF3, and FHF4 paralogs. The unique FHF gene in ascidia may represent a direct descendent of a founding proto-FHF gene following duplication and divergence of preexistent FGF genes (Figure 1) (Satou et al., 2002).

Each FHF gene has two or more sites of transcription initiation generating multiple messenger RNAs with distinct first exons spliced to common exons II–V, which are translated to produce proteins with different amino-terminal sequences (Figure 2) (Hartung et al., 1997; Munoz-Sanjuan et al., 1999; Munoz-Sanjuan et al., 2000b; Wang et al., 2000; Yamamoto et al., 1998). FHFs share substantial sequence homology to one another across their entire length. The β-trefoil core sequences of FHFs are more similar to one another (~65% sequence identity) than are they to FGFs (30–40% sequence identity). The FHF core includes several conserved signature motifs that are not present in FGFs (Figure 2), and at least some of these motifs contribute to FHF function (see below). The ~40 amino acid residue C-terminal tails of FHFs are highly related to one another (~50% sequence identity) and also contribute to FHF function (see below). The long N-terminal extensions of the four FHF “a” isoforms also bear extensive sequence homology to one another (Figure 2).

Vertebrate FHF gene expression and protein distribution

FHF gene expression has been studied in humans, mice, and chickens. Although none of these investigations have been fully comprehensive, no significant disparities have been noticed in gene expression among vertebrate species. Therefore, this overview shall not make repeated mention of species designations.

The developing and mature nervous system is the tissue showing highest levels of FHF RNAs. FHF expression commences during embryogenesis after the emergence of post-mitotic neurons and continues into adulthood (Hartung et al., 1997; Munoz-Sanjuan et al., 1999; Smallwood et al., 1996; Wang et al., 2002; Wang et al., 2000). Expression is almost exclusively in neuronal cells, although Schwann cells also express FHF2 RNA (C. Pardee and M. Goldfarb, unpublished data). Each FHF gene is expressed in a distinct subset of neurons in the central and peripheral nervous systems, so that different types of neurons have distinguishing FHF expression profiles, some of which are listed in Table 1. Certain neurons have been shown to preferentially express one splice isoform of FHF RNA over another (Munoz-Sanjuan et al., 2000b; Wang et al., 2000).

FHF genes are expressed to a more limited extent in non-neuronal tissues during development. Two of these sites are similar to neurons inasmuch as they are electrically excitable tissues: embryonic olfactory epithelium and cardiac myocytes (Hartung et al., 1997). Further sites of expression include patches of embryonic limb mesenchyme, some embryonic cartilage condensations, fetal thymus, pancreas, and kidney glomeruli, and basal keratinocytes (Hartung et al., 1997; Kawano et al., 2004; Munoz-Sanjuan et al., 2001; Munoz-Sanjuan et al., 2000a).

The “a” and “b” isoforms of FHF proteins display distinct subcellular distributions. When expression vectors for FHF1a, FHF2a, or FHF4a are transfected into cultured cells, the proteins are predominantly nuclear in distribution (Smallwood et al., 1996; Wang et al., 2000). Nuclear localization is mediated by a bipartite translocation signal sequence located in the amino-terminal region unique to the “a” isoform (Smallwood et al., 1996). By contrast, “b” isoforms of FHFs expressed following transfection are almost exclusively cytoplasmic in distribution (Wang et al., 2000). The subcellular distribution of endogenous neuronal FHF2 isoforms, as studied on Western blots of fractionated tissues, does not readily comply with observations made on transfected cells. FHF2a and FHF2b are both detected abundantly in postnuclear fractions of brain homogenates (Schoorlemmer and Goldfarb, 2002). For both FHF2 isoforms, some of the protein is detected in plasma membranes purified by lectin affinity partitioning, while some of the protein is in the free cytosol, detected in the supernatant after high-speed membrane pelleting (M. Goldfarb, unpublished data). Membrane association of FHFs is due, at least in part, to their association with voltage-gated sodium channels (see below).

Recombinant FHF1b and FHF4b, like their FGF counterparts, behave as monomeric proteins when subjected to gel filtration chromatography (Olsen et al., 2003). Additionally, FHFs and FGFs are similar in their affinity for heparin, which has aided in FHF protein purification (Olsen et al., 2003). While FGFs utilize extracellular heparan sulfate proteoglycan (HSPG) as an integral cofactor in receptor clustering (Rapraeger et al., 1991; Schlessinger et al., 2000; Yayon et al., 1991), FHFs reside in an intracellular environment lacking HSPGs. FHFs have conserved the heparin-binding domain for use in binding one of its protein targets (see below).

FHFs cannot stimulate FGF receptors

FHFs differ from most FGFs in their lack of N-terminal secretion signal sequences, and FHFs are not secreted at detectable levels from transfected cultured cells. However, FHF release by noncanonical physiological mechanisms or by tissue injury, as has been shown for FGF1 and FGF2, is possible in principle. For this reason, potential activation of FGF receptors by extracellular FHF has been put to tests as a potential biochemical function of the FHFs.

Recombinantly expressed human FHF1b and FHF4b were tested for ability to promote survival and growth of seven BaF3 lymphoid cell lines expressing each of the seven mammalian FGF receptors (FGFR1-IIIb, FGFR1-IIIc, FGFR2-IIIb, FGFR2-IIIc, FGFR3-IIIb, FGFR3-IIIc, FGFR4) (Olsen et al., 2003). The FGFR3 and FGFR4 receptors were expressed as chimeric proteins bearing the intracellular domain of FGFR1, which is more potently mitogenic (Ornitz et al., 1996). Neither FHF at concentrations up to 12 nM (~250 ng/ml) had any biological effect on these cells. Additionally, the FHFs could not antagonize through competition the mitogenic activity of FGF1 towards these cells (Olsen et al., 2003). These data demonstrate that FHFs cannot interact with the extracellular domains of FGF receptors in a functionally significant manner.

FHF Binding Proteins: I. IB2

Islet brain-2 (IB2) is a putative MAP kinase scaffold protein expressed in developing and mature neurons, olfactory epithelium, and pancreatic islet cells (Negri et al., 2000; Yasuda et al., 1999). FHF-IB2 interaction was originally discovered in a yeast two-hybrid screen for FHF-interacting proteins (Schoorlemmer and Goldfarb, 2001). In cultured cells transfected to express FHFs and IB2, all FHFs tested (FHF1a, FHF1b, FHF2a, FHF4a) formed immunoprecipitable complexes with IB2, but not with the related scaffold IB1 (Schoorlemmer and Goldfarb, 2001)(Q. Wang and D. Ornitz, personal communication). More importantly, FHF1/IB2 native complexes were detected by coimmunoprecipitation in homogenates of rat brain and of a rat insulinoma cell line (Schoorlemmer and Goldfarb, 2001). Whether all FHFs complex with IB2 in neurons is an unresolved question, due to lack of suitable FHF antibodies for this analysis.

The FHF-binding domain on IB2 has been mapped to a 250 residue segment unique to this protein. The related scaffold protein IB1 (also termed JIP1) does not bear this segment and does not bind FHFs (Schoorlemmer and Goldfarb, 2001). The FHF-binding domain of IB2 bears no sequence similarity to the FGF-bindings domains in FGF receptors. FHF-IB2 binding is mediated, in part, by FHF surface residues in the β4–β5 loop and in the β9 strand which are conserved in all FHFs and not found in FGFs (Figure 2) (Olsen et al., 2003).

A potential function of FHFs may be to serve as cofactors for the recruitment of a MAP kinase to the scaffold protein. p38δ is a MAP kinase which preferentially binds IB2 in cells transfected with IB2 and various MAP kinases. FHF1a and FHF2a increase p38δ binding to IB2 (Schoorlemmer and Goldfarb, 2001). The function performed by FHF/IB2 interaction in neurons is still unknown, as is the function of IB2/MAP kinase interaction.

FHF Binding Proteins: II. VGSCs

Voltage-gated sodium channels (VGSCs) are a family of sodium channels expressed in excitable cells (neurons, skeletal muscle, cardiac muscle) which transiently open in response to membrane depolarization. Channels are comprised of one of nine known alpha subunits (termed Nav1.1, Nav1.2, etc.) acting alone or complexed with one of three smaller beta subunits (Caterall, 2000). The pore-forming alpha subunits of VGSCs contain four related

integral membrane domains (I–IV), each of which contains six transmembrane segments (S1–S6) along with a partial membrane reentrant loop. Domains I–IV are linked by three large intracellular loops, and domain IV is followed by a cytoplasmic C-terminal tail ranging from 200–300 amino acid residues. The S4 segment in each domain acts as a voltage sensor undergoing a voltage-dependent conformation change and mediating channel activation following depolarization (Lossin et al., 2003). The III–IV loop plays a critical role in voltage-dependent channel inactivation, thereby limiting channel opening to a 1–2 msec window following depolarization. The C-terminal tail of VGSCs has multiple functions, and is required for both the activation and inactivation of the channel. Deletions and specific missense mutations in this region of Nav1.1 (a brain-specific channel) prevent channel activation and are associated with severe myoclonic epilepsy of infancy (Sugawara et al., 2003). Other missense mutations in the tail of Nav1.5 (the cardiac channel) cause delayed or incomplete channel inactivation and are associated with human long QT cardiac syndrome (Abriel et al., 2001; Abriel et al., 2000; Benhorin et al., 1998; Bezzina et al., 1999; Rivolta et al., 2001).

FHF interaction with VGSCs was first discovered during a yeast two-hybrid screen for proteins that could bind to the C-terminal tail of Nav1.9, which is expressed in nociceptive neurons (Liu et al., 2001). Since then, it has been shown that FHFs can bind to Nav1.5, which is expressed in cardiac myocytes (Liu et al., 2003), and to Nav1.6, the principal channel mediating initiation and propagation of action potentials in most mature neurons of the central and peripheral nervous systems (Shtraizent and Goldfarb, 2005; Wittmack et al., 2004). Native complexes of FHFs with sodium channels have been detected by coimmunoprecipitation from extracts of brain or dorsal root ganglia (M. Goldfarb, unpublished data). FHF2 colocalizes with Nav1.6 at conduction nodes of Ranvier in myelinated sensory axons (Wittmack et al., 2004). Although an early report suggested that only specific sodium channels associate with FHFs, there is now no evidence showing such restriction. Conversely, the potential for selective binding of channels to specific FHFs has not been thoroughly explored. While FHF2b has been detected in complexes with Nav1.9 in dorsal root ganglia (M. Goldfarb, unpublished data), native interaction of FHF “a” isoforms with channels is still unclear.

FHFs bind to the C-terminal tail region of sodium channel alpha subunits. The FHF binding domain has been mapped to the proximal segment of the tail in Nav1.5 and Nav1.9 (Liu et al., 2001; Liu et al., 2003), and to the region between residues 16–147 in the tail of Nav1.6 (Shtraizent and Goldfarb, 2005). Hence, FHFs bind to a region of the channel dedicated to activation (Sugawara et al., 2003), inactivation (Abriel et al., 2001; Abriel et al., 2000; Benhorin et al., 1998; Bezzina et al., 1999; Rivolta et al., 2001), and subcellular distribution (Garrido et al., 2001). There is evidence to support a role of FHFs in the regulation of multiple channel properties. When expressed ectopically by transfection, FHF1b induces a significant hyperpolarizing shift in the voltage dependence of Nav1.5 channel inactivation, without affecting channel activation (Liu et al., 2003). This effect was not observed in similar studies on Nav1.6. By contrast, ectopic expression FHF2b increased the maximal activatable whole cell Nav1.6 sodium currents by 2-fold (Wittmack et al., 2004). This may be attributable to either increased surface channel density or to more efficient activation of available surface channels.

FHF Genetics

Mice harboring experimentally disrupted FHF genes show a range of behavioral anomalies that are believed to reflect impaired neurological function. One of these deficits, cerebellar ataxia, has also been linked to inherited FHF mutations in humans. *Fhf1*$^{-/-}$ mice, *Fhf4*$^{-/-}$ mice, and animals lacking both of these FHFs have been generated, but the *Fhf4*$^{-/-}$ mouse

phenotype is the only one to have been investigated thoroughly and findings published (Wang et al., 2002).

Fhf4$^{-/-}$ knockout mice display several behaviors indicative of impaired central nervous system function (Wang et al., 2002). One of these phenotypes is paroxysmal hyperkinetic dyskinesia, manifested as episodic locomotor freezing with limb hyperextension. This behavior is not epileptic seizure, and is mostly likely the consequence of aberrant basal forebrain activity. FHF4 is expressed in non-dopaminergic neurons of the putamen and globus pallidus, and the mutant mice show impaired locomotor responses to psychostimulants cocaine and amphetamine, which target these basal forebrain areas. A second phenotype in *Fhf4*$^{-/-}$ mice is ataxia, manifested as a shuffling gait with poor coordination of forelimb/hindlimb positional correspondence during walking. Ataxia reflects impaired cerebellar function, and FHF4 is normally expressed in cerebellar granule neurons, with FHF4 protein likely transported into the parallel fiber network (Wang et al., 2002). In humans, spinocerebellar ataxia (SCA) is a genetically inherited syndrome, and many different SCA loci have been genetically mapped. One SCA locus spans the FHF4 gene, and coding missense or frameshift mutations have been identified in a few families transmitting SCA (Dalski et al., 2005; van Swieten et al., 2003). The third *Fhf4*$^{-/-}$ phenotype is muscle weakness, as manifested by reduced ability to hang on to tilted or inverted grids and reduced climbing ability. This phenotype may reflect normal expression of FHF4 in motoneurons. Despite the extent of behavioral phenotypes, the nervous system of *Fhf4*$^{-/-}$ mice appears normal by histology and by immmunocytochemical detection of specific cell types, including dopaminergic neurons which innervate the basal forebrain (Wang et al., 2002).

Fhf1$^{-/-}$ knockout mice have little demonstrable phenotype (J. Schoorlemmer and M. Goldfarb, unpublished data). These animals also display muscle weakness, although less severe than that in *Fhf4*$^{-/-}$ mice. Strikingly, *Fhf1*$^{-/-}$ *Fhf4*$^{-/-}$ double mutant mice show enhanced and novel phenotypes not seen in mice with either individual gene mutated (J. Schoorlemmer, Q. Wang, D.M. Ornitz, and M. Goldfarb). Muscle weakness is more pronounced in *Fhf1*$^{-/-}$ *Fhf4*$^{-/-}$ mice and is accompanied by substantial limb muscle atrophy. Ataxia is also more severe, as judged by a wobbly gait and occasional loss of balance while walking. These enhanced phenotypes may reflect the normal coexpression of FHF1 and FHF4 genes in motoneurons and cerebellar granule neurons. The *Fhf1*$^{-/-}$ *Fhf4*$^{-/-}$ mice also display hyperexcitability, manifested as prolonged pacing and circling when animals are disturbed or placed in a new environment. This behavior is somewhat paradoxical, in light of accompanying motor weakness, and can result in prolonged periods of inactivity following such excitement possibly brought on by fatigue. No histological or immunohistochemical abnormalities have been detected in the nervous system of *Fhf1*$^{-/-}$ *Fhf4*$^{-/-}$ mice (J. Schoorlemmer, Q. Wang, D.M. Ornitz, and M. Goldfarb).

The association of FHFs with VGSCs raises the possibility that some neurons in FHF mutant mice are impaired in their ability to initiate action potential spikes or in their ability to conduct these spikes along axons to nerve terminals. It is anticipated that data on this important matter shall be forthcoming soon.

FHF Protein Structure and Basis for Target Protein Recognition

The sequence of the FHF core homology region had strongly suggested that FHFs adopt a β-trefoil configuration, as do FGFs. The recently reported crystal structure of human FHF1b confirmed this prediction, and further demonstrated that the surfaces of FHFs are also surprisingly similar to the FGFs (Olsen et al., 2003). This study did not address potential tertiary structure of the 40-residue tail, as this segment was proteolyzed prior to protein crystallization.

Structures of FHFs in complex with known protein targets (IB2 and VGSCs) have not been reported as of yet. However, FHF mutagenesis in conjunction with binding studies has provided some important insights. Arginine-52 in the β4–β5 loop and valine-95 in the β9 strand are FHF1b surface residues conserved in all FHFs and not present in FGFs (Figure 2). Mutation of either residue to its most common FGF counterpart (R52G or V95N) prevented FHF1b binding to either IB2 or to VGSCs (Olsen et al., 2003; Shtraizent and Goldfarb, 2005). The FHF1b C-terminal tail is also required for binding either target. Therefore, FHF uses a similar surface for binding its two proteins of unrelated sequence and function.

The heparin-binding domain of FHF1b is similarly positioned to that of FGFs, as demonstrated by sites of sulfate ion chelation within the FHF crystal structure (Olsen et al., 2003). Mutation of surface residues in this region abolishes FHF1b's ability to bind the tail of Nav1.6 (Shtraizent and Goldfarb, 2005). It is possible that the FHF heparin-binding motif serves the function of binding one of several highly acidic "heparin-like" segments in VGSC tails (Cormier et al., 2002).

Future Directions

FGFs and their roles in development, disease, and therapies have been studied extensively for over 25 years. By contrast, the study of FHFs is an emerging field. We now know that FHF deficits can induce neurological syndromes in mice and humans. Furthermore, several proteins targeted by FHFs have been identified. The major challenge in the immediate future is to gain an appreciation for how FHF-target interactions affect the developmental or electrical behavior of neurons and to provide a description of FHF mutant phenotypes at the cellular and physiological levels. Additionally, we may have only scratched the surface of neuronal dysfunctions accompanying FHF gene deficits. Since most vertebrate neurons express several FHFs (Table 1) and since the biochemical functions of these FHFs are quite similar, functional redundancy may have limited the breadth and severity of deficits in the FHF mutants so far described. A fuller description of FHF-mediated neurological functions should accompany the future characterization of FHF2 and FHF3 mutant animals and of more combinations of mutations among FHF genes. Analyses on such mutant animals may also reveal biological functions served by FHFs outside of the nervous sytem.

The evolution of FHF genes and the sequence and structural similarities between FHFs and FGFs has raised questions for which there are no easy answers. First, given the functional importance of FHFs in vertebrates, why are FHFs not found in invertebrate genomes? Second, although the known functions of FGFs and FHFs are completely different, why are these proteins so structurally similar? Is there an unidentified common target and shared function for FHFs and FGFs?

References

Abriel H, Cabo C, Wehrens XH, Rivolta I, Motoike HK, Memmi M, Napolitano C, Priori SG, Kass RS. Novel arrhythmogenic mechanism revealed by a long-QT syndrome mutation in the cardiac Na(+) channel. Circ Res. 2001; 88:740–745. [PubMed: 11304498]

Abriel H, Wehrens XH, Benhorin J, Kerem B, Kass RS. Molecular pharmacology of the sodium channel mutation D1790G linked to the long-QT syndrome. Circulation. 2000; 102:921–925. [PubMed: 10952963]

Benhorin J, Goldmit M, MacCluer JW, Blangero J, Goffen R, Leibovitch A, Rahat A, Wang Q, Medina A, Towbin J, Kerem B. Identification of a new SCN5A mutation, D1840G, associated with the long QT syndrome. Mutations in brief no. 153. Online. Hum Mutat. 1998; 12:72. [PubMed: 10627139]

Bezzina C, Veldkamp MW, van Den Berg MP, Postma AV, Rook MB, Viersma JW, van Langen IM, Tan-Sindhunata G, Bink-Boelkens MT, van Der Hout AH, et al. A single Na(+) channel mutation

causing both long-QT and Brugada syndromes. Circ Res. 1999; 85:1206–1213. [PubMed: 10590249]

Caterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron. 2000; 26:13–25. [PubMed: 10798388]

Cormier JW, Rivolta I, Tateyama M, Yang AS, Kass RS. Secondary structure of the human cardiac Na + channel C terminus: evidence for a role of helical structures in modulation of channel inactivation. J Biol Chem. 2002; 277:9233–9241. [PubMed: 11741959]

Coulier F, Pontarotti P, Roubin R, Hartung H, Goldfarb M, Birnbaum D. Of worms and men: an evolutionary perspective on the fibroblast growth factor (FGF) and FGF receptor families. J Mol Evolution. 1997; 44:43–56.

Dalski A, Atici J, Kreuz FR, Hellenbroich Y, Schwinger E, Zuhlke C. Mutation analysis in the fibroblast growth factor 14 gene: frameshift mutation and polymorphisms in patients with inherited ataxias. Eur J Hum Genet. 2005; 13:118–120. [PubMed: 15470364]

Garrido JJ, Fernandes F, Giraud P, Mouret I, Pasqualini E, Fache MP, Jullien F, Dargent B. Identification of an axonal determinant in the C-terminus of the sodium channel Na(v)1.2. Embo J. 2001; 20:5950–5961. [PubMed: 11689435]

Hartung H, Feldman B, Lovec H, Coulier F, Birnbaum D, Goldfarb M. Murine FGF-12 and FGF-13: expression in embryonic nervous system, connective tissue and heart. Mech Dev. 1997; 64:31–39. [PubMed: 9232594]

Kawano M, Suzuki S, Suzuki M, Oki J, Imamura T. Bulge- and basal layer-specific expression of fibroblast growth factor-13 (FHF-2) in mouse skin. J Invest Dermatol. 2004; 122:1084–1090. [PubMed: 15140207]

Liu C, Dib-Hajj SD, Waxman SG. Fibroblast growth factor homologous factor 1B binds to the C terminus of the tetrodotoxin-resistant sodium channel rNav1.9a (NaN). J Biol Chem. 2001; 276:18925–18933. [PubMed: 11376006]

Liu CJ, Dib-Hajj SD, Renganathan M, Cummins TR, Waxman SG. Modulation of the cardiac sodium channel Nav1.5 by fibroblast growth factor homologous factor 1B. J Biol Chem. 2003; 278:1029–1036. [PubMed: 12401812]

Lossin C, Rhodes TH, Desai RR, Vanoye CG, Wang D, Carniciu S, Devinsky O, George AL Jr. Epilepsy-associated dysfunction in the voltage-gated neuronal sodium channel SCN1A. J Neurosci. 2003; 23:11289–11295. [PubMed: 14672992]

Munoz-Sanjuan I, Cooper MK, Beachy PA, Fallon JF, Nathans J. Expression and regulation of chicken fibroblast growth factor homologous factor (FHF)-4 during craniofacial morphogenesis. Dev Dyn. 2001; 220:238–245. [PubMed: 11241832]

Munoz-Sanjuan I, Fallon JF, Nathans J. Expression and regulation of chicken fibroblast growth factor (FHF)-4 at the base of the developing limbs. Mech Dev. 2000a; 95:101–112. [PubMed: 10906454]

Munoz-Sanjuan I, Simandl BK, Fallon JF, Nathans J. Expression of chicken fibroblast growth factor homologous factor (FHF)-1 and of differentially spliced isoforms of FHF-2 during development and involvement of FHF-2 in chicken limb development. Development. 1999; 126:409–421. [PubMed: 9847253]

Munoz-Sanjuan I, Smallwood PM, Nathans J. Isoform diversity among fibroblast growth factor homologous factors is generated by alternative promoter usage and differential splicing. J Biol Chem. 2000b; 275:2589–2597. [PubMed: 10644718]

Murzin AG, Lesk AM, Chothia C. beta-Trefoil fold. Patterns of structure and sequence in the Kunitz inhibitors interleukins-1 beta and 1 alpha and fibroblast growth factors. J Mol Biol. 1992; 223:531–543. [PubMed: 1738162]

Negri S, Oberson A, Steinmann M, Sauser C, Nicod P, Waeber G, Schorderet DF, Bonny C. cDNA cloning and mapping of a novel islet-brain/JNK-interacting protein. Genomics. 2000; 64:324–330. [PubMed: 10756100]

Olsen SK, Garbi M, Zampieri N, Eliseenkova AV, Ornitz DM, Goldfarb M, Mohammadi M. Fibroblast growth factor (FGF) homologous factors share structural but not functional homology to FGFs. J Biol Chem. 2003; 278:34226–34236. [PubMed: 12815063]

Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, Gao G, Goldfarb M. Receptor specificity of the fibroblast growth factor family. J Biol Chem. 1996; 271:15292–15297. [PubMed: 8663044]

Rapraeger AC, Krufka A, Olwin BB. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. Science. 1991; 252:1705–1708. [PubMed: 1646484]

Rivolta I, Abriel H, Tateyama M, Liu H, Memmi M, Vardas P, Napolitano C, Priori SG, Kass RS. Inherited Brugada and long QT-3 syndrome mutations of a single residue of the cardiac sodium channel confer distinct channel and clinical phenotypes. J Biol Chem. 2001; 276:30623–30630. [PubMed: 11410597]

Satou Y, Imai KS, Satoh N. Fgf genes in the basal chordate Ciona intestinalis. Dev Genes Evol. 2002; 212:432–438. [PubMed: 12373588]

Schlessinger J, Plotnikov AN, Ibrahimi OA, Eliseenkova AV, Yeh BK, Yayon A, Linhardt RJ, Mohammadi M. Crystal structure of a ternary FGF-FGFR-heparin complex reveals a dual role for heparin in FGFR binding and dimerization. Mol Cell. 2000; 6:743–750. [PubMed: 11030354]

Schoorlemmer J, Goldfarb M. Fibroblast growth factor homologous factors are intracellular signaling proteins. Curr Biol. 2001; 11:793–797. [PubMed: 11378392]

Schoorlemmer J, Goldfarb M. Fibroblast growth factor homologous factors and the islet brain-2 scaffold protein regulate activation of a stress-activated protein kinase. J Biol Chem. 2002; 277:49111–49119. [PubMed: 12244047]

Shtraizent N, Goldfarb M. FHF interaction with voltage-gated sodium channel Nav1.6: biochemical and mutational analyses. 2005 Manuscript submitted.

Smallwood PM, Munoz-Sanjuan I, Tong P, Macke JP, Hendry SH, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J. Fibroblast growth factor (FGF) homologous factors: new members of the FGF family implicated in nervous system development. Proc Natl Acad Sci U S A. 1996; 93:9850–9857. [PubMed: 8790420]

Sugawara T, Tsurubuchi Y, Fujiwara T, Mazaki-Miyazaki E, Nagata K, Montal M, Inoue Y, Yamakawa K. Nav1.1 channels with mutations of severe myoclonic epilepsy in infancy display attenuated currents. Epilepsy Res. 2003; 54:201–207. [PubMed: 12837571]

van Swieten JC, Brusse E, de Graaf BM, Krieger E, van de Graaf R, de Koning I, Maat-Kievit A, Leegwater P, Dooijes D, Oostra BA, Heutink P. A mutation in the fibroblast growth factor 14 gene is associated with autosomal dominant cerebral ataxia. Am J Hum Genet. 2003; 72:191–199. [PubMed: 12489043]

Wang Q, Bardgett ME, Wong M, Wozniak DF, Lou J, McNeil BD, Chen C, Nardi A, Reid DC, Yamada K, Ornitz DM. Ataxia and paroxysmal dyskinesia in mice lacking axonally transported FGF14. Neuron. 2002; 35:25–38. [PubMed: 12123606]

Wang Q, McEwen DG, Ornitz DM. Subcellular and developmental expression of alternatively spliced forms of fibroblast growth factor 14. Mech Dev. 2000; 90:283–287. [PubMed: 10640713]

Wittmack E, Rush AM, Craner MJ, Goldfarb M, Waxman SG, Dib-Hajj SD. Fibroblast growth factor homologous factor 2B: Association with Nav1.6 and selective colocalization at nodes of Ranvier of dorsal root axons. J Neurosci. 2004; 24:6765–6775. [PubMed: 15282281]

Yamamoto S, Mikami T, Ohbayashi N, Ohta M, Itoh N. Structure and expression of a novel isoform of mouse FGF homologous factor (FHF)-4. Biochim Biophys Acta. 1998; 1398:38–41. [PubMed: 9602045]

Yasuda J, Whitmarsh AJ, Cavanagh J, Sharma M, Davis RJ. The JIP group of mitogen-activated protein kinase scaffold proteins. Mol Cell Biol. 1999; 19:7245–7254. [PubMed: 10490659]

Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. Cell. 1991; 64:841–848. [PubMed: 1847668]

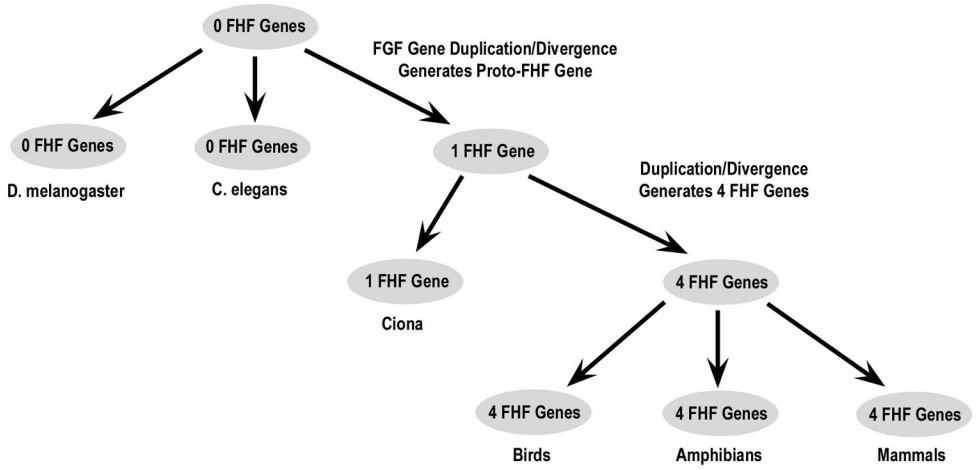
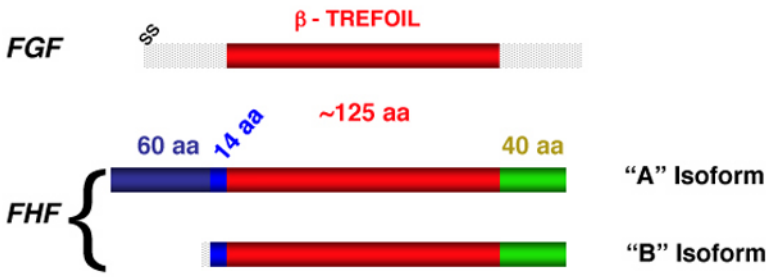


Figure 1.



β-Trefoil Region	Common FGF Residue(s)	FHF Residue(s)
β1 Strand	X(K/R/Q)LΩ(C/S)	TRLΩ(C/S)
β4 Strand	L(Q/E)ΦX(A/S)(D/Ψ)X	FNLIPVG
β4 - β5 Loop	ΦG	L*R*
β9 Strand	YN(A/T)YSXS	Y*V*ΨY(A/S)SX
β11 Strand	X(K/R)(K/R)G	XMKG

Figure 2.

Table 1

Some neuronal sites of FHF gene expression

Site	FHF1	FHF2	FHF3	FHF4
Cerebral cortical neurons	YES	YES	YES	YES
Hippocampal pyramidal neurons	YES	YES	YES	YES
Cerebellar granule neurons	YES	NO	YES	YES
Spinal motor neurons	YES	NO	not tested	YES
Peripheral sensory neurons	YES	YES	not tested	NO