Functional Evolutionary History of the Mouse *Fgf* Gene Family

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Fibroblast Growth Factors (FGFs) are polypeptides with diverse activities in development and physiology. The mammalian *Fgf* family can be divided into the intracellular *Fgf11/12/13/14* subfamily (iFGFs), the hormone-like *Fgf15/21/23* subfamily (hFGFs), and the canonical *Fgf* subfamilies, including *Fgf1/2/5*, *Fgf3/4/6*, *Fgf7/10/22*, *Fgf8/17/18*, and *Fgf9/16/20*. However, all *Fgfs* are evolutionarily related. We propose that an *Fgf13-like* gene is the ancestor of the *iFgf* subfamily and the most likely evolutionary ancestor of the entire *Fgf* family. Potential ancestors of the canonical and *hFgf* subfamilies, *Fgf4-*, *Fgf5-*, *Fgf8-*, *Fgf9-*, *Fgf10-*, and *Fgf15-like*, appear to have derived from an *Fgf13*-like ancestral gene. Canonical FGFs function in a paracrine manner, while hFGFs function in an endocrine manner. We conclude that the ancestral *Fgfs* for these subfamilies acquired this functional diversity before the evolution of vertebrates. During the evolution of early vertebrates, the *Fgf* subfamilies further expanded to contain three or four members in each subfamily. *Developmental Dynamics 237:18–27, 2008.* © 2007 Wiley-Liss, Inc.

INTRODUCTION

Fibroblast Growth Factors (FGFs) are polypeptides with diverse biological activities in multiple developmental and metabolic processes. The human/mouse *Fgf* gene family comprises 22 members. Most FGFs mediate their biological responses as extracellular proteins by binding to and activating cell surface tyrosine kinase FGF receptors (FGFRs) (Ornitz and Itoh, 2001; Itoh and Ornitz, 2004; Thisse and Thisse, 2005). However, FGFs 11–14 function as intracellular proteins, hereafter referred to as iFGFs, and act in an FGF Receptor (FGFR)-independent manner (Goldfarb, 2005).

The genes encoding FGFs have been identified in multicellular organisms ranging from *Caenorhabditis elegans* to humans (Itoh and Ornitz, 2004). Two *Fgf* genes were found in *C. elegans*, whereas 22 *Fgf* genes have been identified in humans and mice, indicating that the *Fgf* gene family greatly expanded during the evolution of primitive metazoa to vertebrates. The *Fgf* family expanded in two phases (Itoh and Ornitz, 2004; Popovici et al., 2005). In the first phase, during early metazoan evolution, *Fgfs* expanded from two or three to six genes by gene duplications. In the second phase, during the evolution of early vertebrates, the *Fgf* family expanded via two large-scale genome duplications. Phylogenetic and gene location analyses of the human *Fgf* family indicate that it comprises several subfamilies. Ancestors of the subfamilies were generated in the first phase. In the second phase, the subfamilies expanded to contain three or four members. However, their detailed expansion history remains mostly unclear.

According to the "exon theory of genes," introns are expected to be ancient relics of primordial genes (Rogozin et al., 2005; de Roos, 2007; Csuros et al., 2007), indicating that exon/intron organizations suggest the potential evolutionary history of a gene family. In addition, as ontogeny is ex-

pected to correlate with phylogeny (Seitz et al., 2006; Madsen, 2007), functional roles of genes in development also provide useful clues for elucidating the evolutionary history of a gene family. As exon/intron organizations of vertebrate *Fgf* genes and their roles in development have been most studied in mice, we have examined the potential evolutionary history of the mouse *Fgf* gene family based on their gene organization and function in development along with phylogenetic and gene location analyses. In this review, we propose a model for the evolutionary history of the *Fgf* family, using the mouse *Fgf* family as a model.

IDENTIFICATION OF THE MOUSE *FgF* GENE FAMILY

Two FGFs, Acidic FGF (FGF1) and Basic FGF (FGF2), were originally isolated from the brain and pituitary gland as growth factors for fibroblasts (Gospodarowicz, 1974; Gospodarowicz and Morgan, 1974; Gospodarowicz et al., 1975). Thereafter, seven *Fgfs, Fgf3–Fgf9*, were also identified as oncogenes or isolated as growth factors for cultured cells (Dickson et al., 1991; Yoshida et al., 1991; Goldfarb et al., 1991; Coulier et al., 1991; Aaronson et al., 1991; Tanaka et al., 1992; Miyamoto et al., 1993). FGFs 1–9 range in size from ~150 to 260 amino acid residues and have a conserved ~120-amino acid residue core with ~30 to 70% sequence identity (Ornitz and Itoh, 2001; Itoh and Ornitz, 2004). Based on their conserved amino acid sequences, *Fgfs 10–14* and *16–23* were isolated by conducting a homology-based polymerase chain reaction or identified by homology-based searches in nucleotide sequence databases (Yamasaki et al., 1996; Smallwood et al., 1996; Miyake et al., 1998; Hoshikawa et al., 1998; Ohbayashi et al., 1998; Nishimura et al., 1999, 2000; Ohmachi et al., 2000; Nakatake et al., 2001; Yamashita et al., 2000). In addition, *Fgf15* was identified as a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx (McWhirter et al., 1997). *Fgfs 1–23* have been identified in humans and mice. However, *FGF19* is a human ortholog of mouse *Fgf15*. Thus, the mouse/human *Fgf* gene family comprises 22 members (Ornitz and Itoh, 2001; Itoh

and Ornitz, 2004). FGFs 10–23 range in size from ~160 to 250 amino acid residues and have a conserved ~120-amino acid residue core (Ornitz and Itoh, 2001; Itoh and Ornitz, 2004).

Except for the iFGFs (FGF11–14) and hFGFs (FGF15/19, 21, 23), all other FGFs activate FGFRs with high affinity and different degrees of specificity (Ornitz et al., 1996; Zhang et al., 2006). We refer to these FGFs as canonical FGFs. Canonical FGFs are secreted (extracellular proteins) that bind to FGFRs and induce their dimerization and the phosphorylation of specific cytoplasmic tyrosine residues (Eswarakumar et al., 2005). Four *Fgfr* genes, *Fgfr1–Fgfr4*, have been identified in humans and mice. These genes encode receptor tyrosine kinases (~800 amino acids) that contain an extracellular ligand-binding domain with three immunoglobulin domains (I, II, and III), a transmembrane domain, and a split intracellular tyrosine kinase domain. *Fgfr1–Fgfr3* encode two different versions of immunoglobin-like domain III (IIIb and IIIc) generated by alternative mRNA splicing that utilizes one of two unique exons. The immunoglobulin-like domain III is an essential determinant of ligand-binding specificity (Johnson and Williams, 1993). Thus, seven FGFR proteins (FGFRs 1b, 1c, 2b, 2c, 3b, 3c, and 4) differing in ligand-binding specificity are generated from four *Fgfr* genes in vertebrates (Ornitz et al., 1996; Zhang et al., 2006). In contrast, although iFGFs bear strong sequence similarity to canonical FGFs, their biochemical and functional properties are largely unrelated to those of canonical FGFs. Current data suggest that iFGFs act in an FGFR-independent manner. They have been shown to interact with intracellular domains of voltage-gate sodium channels and with the neuronal mitogen-activated protein kinase scaffold protein, islet-brain-2 (reviewed in Goldfarb, 2005).

Canonical FGFs and the hFGF subfamily can be further characterized based on the mechanism by which they are released from cells. FGFs 3–8, 10, 15, 17, 18, 21, 22, and 23 are secreted proteins with cleavable amino terminal signal peptides (Ornitz and Itoh, 2001). FGFs 9, 16, and 20 are also secreted proteins, but con-

tain uncleavable bipartite signal sequences (Miyakawa et al, 1999; Revest et al., 2000). By contrast, FGFs 1 and 2 do not have identifiable signal sequences but nevertheless can be found in extracellular locations. FGFs 1 and 2 might be released from damaged cells or by an exocytotic mechanism that is independent of the endoplasmic reticulum-Golgi pathway (Mignatti et al., 1992). It has also been reported that exogenously added FGF2 can be translocated to the nucleus where it can interact with and activate nuclear targets (Bonnet et al., 1996; Bailly et al., 2000). However, the physiological significance of FGF2 action in the nucleus remains unclear.

The canonical FGFs all have binding sites for acidic glycosaminoglycans including heparin and heparan sulfate (Ornitz, 2000). In the presence of heparan sulfate, FGFs stably bind to FGFRs, which lead to the formation of 2:2:2 FGF-FGFR-heparan sulfate dimers (Mohammadi et al., 2005). In addition, acidic glycosaminoglycans in the form of heparan sulfate proteoglycans function to retain these FGFs in the vicinity of FGF-producing sites, such that they primarily act in a paracrine manner. By contrast, the hFGFs have low-affinity heparin-binding sites and have been found to act in an endocrine manner (Tomlinson et al., 2002; Lundasen et al., 2006; Kharitonenkov et al., 2005, 2007; Fukumoto and Yamashita, 2007; Liu and Quarles, 2007).

PHYLOGENETIC AND GENE LOCATION ANALYSES OF THE MOUSE *FgF* GENE FAMILY

Phylogenetic analysis of the mouse *Fgf* gene family identifies seven subfamilies: *Fgf1 (1,2), Fgf4 (4,5,6), Fgf7 (7,10,22), Fgf8 (8,17,18), Fgf9 (9,16,20), iFgfs (11,12,13,14)*, and *hFgfs (15, 21, 23)* (Fig. 1) that are essentially consistent with those of the human *FGF* family (Itoh and Ornitz, 2004). However, phylogenetic analysis alone is not sufficient to determine all evolutionary relationships (Horton et al., 2003). Analysis of gene loci on chromosomes also indicates potential evolutionary relationships within a gene family. We have examined mouse *Fgf* gene loci and conserved chromosomal gene location (syn-

teny). In contrast to the phylogenetic analysis, the conserved gene location analysis indicates that the mouse *Fgf* gene family may more meaningfully be divided into six subfamilies: *Fgf1/2/5, Fgf3/4/6/15/21/23, Fgf7/10/22, Fgf8/ 17/18, Fgf9/16/20,* and *iFgfs (Fgf11/12/ 13/14)* (Fig. 2). The mouse *Fgf* subfamilies derived from the gene location analysis are also essentially consistent with the human *Fgf* subfamilies (Itoh and Ornitz, 2004). Members of the *Fgf7, Fgf8, Fgf9,* and *iFgf* subfamilies from the gene location analysis are consistent with those of the *Fgf7/10/22, Fgf8/ 17/18, Fgf9/16/20,* and *iFgf* subfamilies from the phylogenetic analysis. *Fgf5,* a member of the *Fgf4* subfamily in the phylogenetic analysis (Fig. 1), is however more closely linked to *Fgf2* by gene location analysis. Both *Fgf5* and *Fgf2* are closely linked to *Annexin A3* (*Anxa3*) and *Annexin 5* (*Anxa5*), respectively (Fig. 2). This indicates that *Fgf1, Fgf2,* and *Fgf5* are members of a common subfamily, *FGF1/2/5*.

Conserved gene location is observed among *Fgf4, Fgf6, Fgf15, Fgf21,* and *Fgf23,* indicating that these *Fgfs* belong to the same subfamily (Fig. 2). In addition, although *Fgf3* is a member of the *Fgf7* subfamily according to the phylogenetic and functional analysis, the gene location analysis indicates that *Fgf3* is linked to *Fgf4* and *Fgf6,* indicating that these are members of the same subfamily. However, the phylogenetic analysis and consideration of the biochemical and functional properties described above indicate that the *hFgfs* (*Fgf15, Fgf21,* and *Fgf23*) are distinct from *Fgf3, Fgf4,* and *Fgf6*. Therefore, these *Fgfs* should be divided into the *Fgf3/4/6* and *hFgf* subfamilies. In summary, we propose that the secreted *Fgfs* should be divided into six subfamilies with three members in each; the canonical *Fgfs* (*Fgf1/2/5, Fgf3/4/6, Fgf7/10/22, Fgf8/ 17/18, Fgf9/16/20*) and the *hFgfs* (*Fgf/ 15/21/23*) (Fig. 2).

EXON/INTRON ORGANIZATIONS OF MOUSE *FgF* GENES

It is proposed that introns are relics of primordial genes (Rogozin et al., 2005; de Roos, 2007; Csuros et al., 2007). This leads to the hypothesis that exon/ intron organization can be used to in- fer the evolutionary history of a gene family. To this end, we have examined the exon/intron organizations of mouse *Fgf* genes. The conserved FGF core domain in the coding region of *Fgf4* is divided by two introns (Fig. 3). The intron locations are highly conserved in the core regions of all canonical *Fgfs* and the *hFgfs* (data not shown). These two introns are also conserved in the core regions of the *iFgf* subfamily, indicating that *iFgfs, hFgfs,* and canonical *Fgfs* are evolutionarily related to each other (data not shown). However, two additional introns are also located outside of the core region of *Fgf13* within sequences encoding the amino and carboxy terminal domains of the protein (Fig. 3). The positions of these four introns are also highly conserved among the other *iFGFs* (data not shown).

Several Fgfs have acquired alternatively spliced amino terminal ends. Alternative spliced variants of *Fgf8* and *Fgf17* are generated from distinct splice sites that divide the "first" exon into four (in humans) sub-exons (MacArthur et al., 1995; Gemel et al., 1996; Xu et al., 1999; Olsen et al., 2006). Similarly, the *iFgfs* have acquired alternatively spliced amino terminal variants that are generated by alternative utilization of distinct first exons (reviewed in Goldfarb, 2005). Importantly, none of these alternative splicing events affects the sequences of the conserved FGF core domain.

PHENOTYPES OF *FgF* KNOCKOUT MICE

Biological function of mouse *Fgfs* in development and physiology also provide useful clues to the evolutionary history of the *Fgf* gene family. Most *Fgf* genes have been disrupted by homologous recombination in mice. Phenotypes range from early embryonic lethality to subtle changes in adult physiology (Table 1 and Fig. 4). *Fgf4* and *Fgf8* knockout mice die at early embryonic stages. *Fgf4* and *Fgf8* have essential roles in blastocyst formation and gastrulation, respectively (Feldman et al., 1995; Sun et al., 1999). *Fgf9, Fgf10,* and *Fgf18* knockout mice died shortly after birth. *Fgf10* is critical for epithelial–mesenchymal interactions necessary for the development of epithelial components of multiple organs (Min et al., 1998; Sekine et al., 1999; Ohuchi et al., 2000; Sakaue et al., 2002). *Fgf9* and *Fgf18* have essential roles in the development of mesenchymal components of multiple organs (Colvin et al., 2001a, 2001b, Ohbayashi et al., 2002; Liu et al., 2002; Usui et al., 2004). In contrast, *Fgf15* knockout mice die at variable times during embryonic and postnatal stages of development. FGF15 functions in the development of the cardiac outflow tract (Vincentz et al., 2005) but also acts as an endocrine hormone in postnatal life (Inagaki et al., 2005). Other *Fgf* knockout mice either are viable or die during postnatal stages (Table 1). Conditional knockouts of *Fgfs* that have essential roles early in development have also revealed important functions for these *Fgfs* at later times of development (Moon et al., 2000; Moon and Capecchi 2000; Sun et al., 2000; Chi et al., 2003; Lewandoski et al., 2000).

EVOLUTIONARY HISTORY OF THE MOUSE *FgF* GENE FAMILY

The coding regions of the *iFgfs* are divided by four introns, while the coding regions of canonical *Fgfs* and *hFgfs* are divided by two introns. Consistent with the idea that introns are relics of primordial genes (Rogozin et al., 2005; de Roos, 2007), these organizations indicate that the *iFgf* subfamily is the likely ancestor of the canonical *Fgf* families.

Numerous examples exist in which phylogenetic relationships appear to correspond with gene function at very early developmental stages (Seitz et al., 2006; Madsen, 2007). This model suggests that FGF family members with very early functions in development may represent the prototype genes for their subfamily. By examining early developmental phenotypes of *Fgf* knockout mice as a guide, one can therefore make predictions of prototype members of *Fgf* subfamilies. The *iFgf* subfamily comprises *Fgf11– Fgf14. Fgf12* and *Fgf14* knockout mice are viable and have neurological phenotypes (Wang et al., 2002; Xiao et al., 2007; Goldfarb et al., 2007; Laezza et al., 2007). Although *Fgf11* and *Fgf13* knockout mice have not been re-
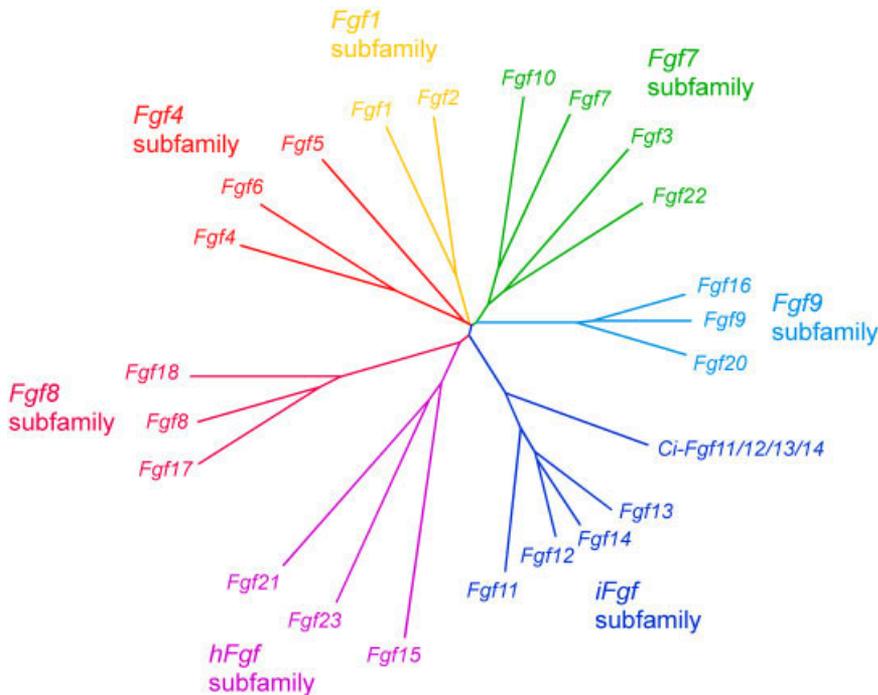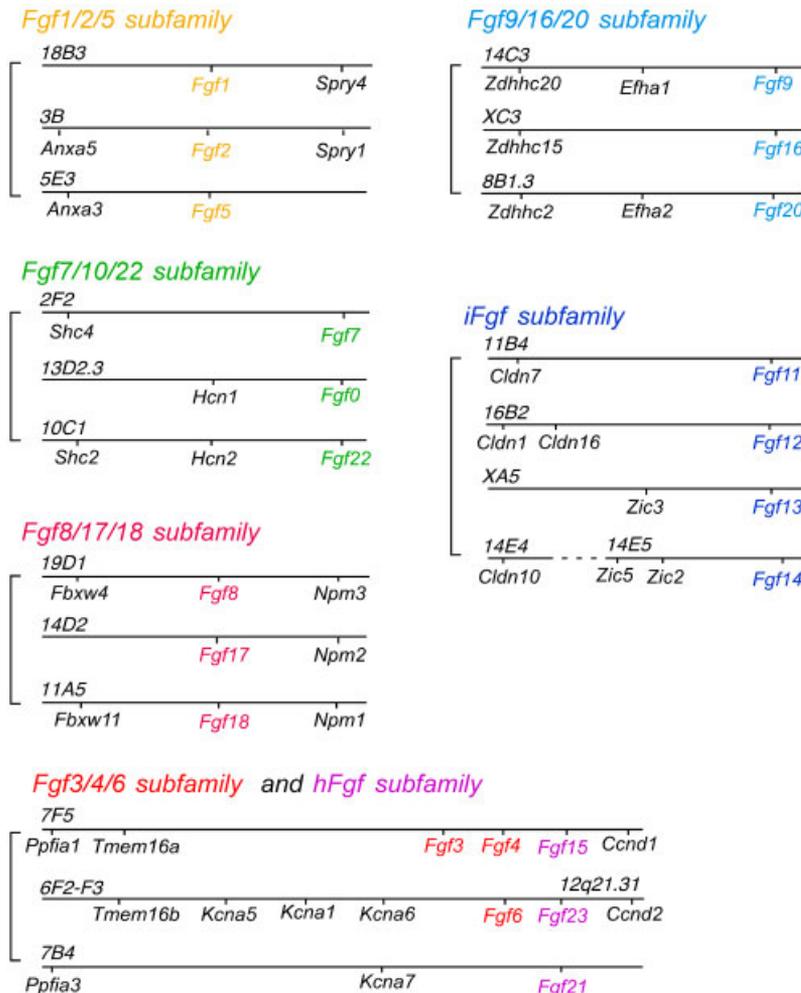
Fig. 1.

Fig. 2.

ported, the expression of these genes in the central and peripheral nervous system suggests that these genes will also be found to function in neurodevelopment or neurophysiology. Experiments with *Xenopus* embryos indicate that *Fgf13* is essential for neural differentiation in early embryonic development (Nishimoto and Nishida, 2007). In addition, the *C. intestinalis Fgf11/12/13/14*, an ancestor of the vertebrate *iFgf* subfamily (Satou et al., 2002), is most homologous (57% amino acid identity in the core region) to *Fgf13*, although phylogenetic analysis between the mouse *iFgf* subfamily and the *C. intestinalis Fgf11/12/13/14* has not clearly indicated their relationship (Fig. 1). Thus, *Fgf13* appears most similar to an ancestral member of the *iFgf* subfamily.

All canonical *Fgf* subfamilies and the *hFgfs* comprise three members. Phenotypes of knockout mice for all *Fgf3/4/6, Fgf7/10/22, Fgf8/17/18,* and *hFgf* subfamily members have been reported. Knockout studies indicate that *Fgf4, Fgf10, Fgf8,* and *Fgf15* have the most severe phenotypes at early developmental stages within their corresponding families and can thus be considered as the ancestral members of their subfamilies (Fig. 4). Of the members of the *Fgf9/16/20* subfamily, *Fgf9* is essential during embryonic stages, whereas *Fgf16* knockout mice are viable (N. Itoh et al., unpublished data). Although phenotypes of *Fgf20*

Fig. 1. Evolutionary relationships within the mouse *Fgf* gene family and *C. intestinalis Fgf11/12/13/14*. Twenty-two *Fgfs* have been identified in mice. The apparent evolutionary relationships of the mouse *Fgf* family and *C. intestinalis Fgf11/12/13/14* were examined by CLUSTRALW (http://align.genome.jp/). Phylogenetic analysis suggests that the *Fgfs* can be divided into seven subfamilies containing two to four members each. Branch lengths are proportional to the evolutionary distance between each gene.

Fig. 2. Chromosomal gene loci maps for mouse *Fgf*s. Gene loci maps were constructed by examining mouse *Fgf* gene loci using the Ensembl Genome Browser (http://www.ensembl.org/). *Fgf* gene loci and closely linked genes are shown. The bar lengths are not proportional to the distances between genes. Gene symbols are described according to the browser. The conservation of gene orders in the *Fgf* subfamilies supports a model for large-scale genome duplication events.
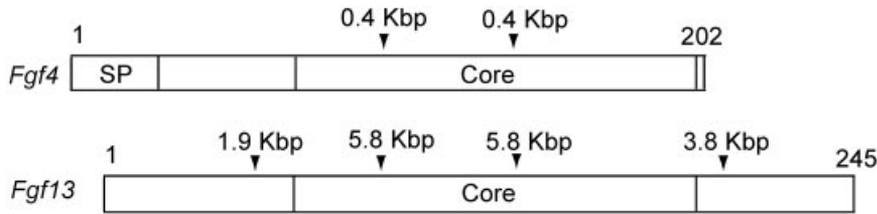
Fig. 3. Schematic representations of *Fgf4* and *Fgf13* intron locations. *Fgf4* and *Fgf13* are shown as representatives of the canonical *Fgf* subfamilies and *iFgf* subfamily, respectively. Arrowheads indicate the positions of introns. The positions of two introns in *Fgf4* and four introns in *Fgf13* are conserved among canonical *Fgfs* and *iFgfs*. SP and Core indicate a secreted signal sequence and core region, respectively. The numbers refer to the positions of amino acid residues. The introns are 0.4–5.8 Kbp long.

TABLE 1. Phenotypes in *Fgf* Knockout Mice

Gene	Phenotype	
iFgf subfamily		
Fgf11	—	—
Fgf12	Viable	Neuromuscular function
Fgf13*	—	—
Fgf14	Viable	Neurological function
Fgf3/4/6 subfamily		
Fgf3	Viable	Inner ear, tail and CNS development
Fgf4*	Lethal, E4–5	Blastocyte formation
Fgf6	Viable	Subtle, muscle regeneration
Fgf1/2/5 subfamily		
Fgf1	Viable	None identified
Fgf2	Viable	Cardiovascular, skeletal, and neuronal development
Fgf5*	Viable	Hair development
Fgf8/17/18 subfamily		
Fgf8*	Lethal, E8	Gastrulation development
Fgf17	Viable	Cerebellar development
Fgf18	Lethal, PD0	Skeletal and lung development
Fgf9/16/20 subfamily		
Fgf9*	Lethal, PD0	Lung, heart, vascular, GI tract, and testis development
Fgf16	Viable	Heart development
Fgf20	—	—
Fgf7/10/22 subfamily		
Fgf7	Viable	Subtle, muscle regeneration
Fgf10*	Lethal, PD0	Multiple organ development
Fgf22	Viable	—
hFgf subfamily		
Fgf15*	Lethal, E13.5-PD21	Heart development and bile acid metabolism
Fgf21	Viable	—
Fgf23	Lethal, PW4-13	Phosphate and vitamin D metabolism

Phenotypes of most *Fgf* knockout mice have been published (reviewed in Ornitz and Itoh, 2001; Itoh and Ornitz, 2004). *Fgf16* and *Fgf21* knockout mice are viable (N. Itoh et al., unpublished data). *Fgf22* knockout mice are also viable (H. Umemori, personal communication). Phenotypes of *Fgf11, Fgf13,* and *Fgf20* knockout mice remain unclear. E, embryonic day; PD, postnatal day; PW, postnatal week. Asterisks indicate potential prototypes of the *Fgf* subfamilies. —, indicates unknown phenotypes.

knockout mice have not been reported, we tentatively have assigned an *Fgf9-like* gene as the ancestor of the *Fgf9/16/20* subfamily. Knockout mice for the *Fgf1/2/5* subfamily are all viable with relatively subtle phenotypes (Miller et al., 2000; Hebert et al., 1994). FGF1 and FGF2 lack signal peptides while FGF5 functions as a typical secreted protein. As the ancestral members of other canonical *Fgf* subfamilies are typical secreted proteins, we assign an *Fgf5-like* gene as the ancestor of the *Fgf1/2/5* subfamily.

No conserved gene order was observed among ancestors of the *Fgf* subfamilies, indicating that the ancestors were generated by gene duplications, not by genome duplications. The ancestral gene of the *Fgf* family, which is tentatively assigned to *Fgf13-like*, encodes an intracellular protein without a secretory signal sequence, whereas the ancestral members of the other *Fgf* subfamilies encode secreted proteins with identifiable secretory signal sequences. In addition, although *Fgf13* has four introns within its coding regions, all other ancestral *Fgfs* have only two introns. These results indicate that the ancestral members of the canonical *Fgf* subfamilies and *hFgfs* were derived from an *Fgf13-like* ancestral gene accompanied by loss of two introns. Of the canonical *Fgfs*, *Fgf4* is required at the earliest stages of development, indicating that an *Fgf4-like* gene may be the ancestor of the canonical *Fgf* subfamilies (Fig. 4). From these results, we propose a model for the evolutionary history of the *Fgf* family (Figs. 5 and 6) with an *Fgf13-like* gene as the ancestral *Fgf*. An *Fgf4-like* gene was then generated from *Fgf13-like* by a gene duplication event followed by gene translocation. During this evolution, *Fgf4-like* lost two introns in the first and third exons of its coding region and acquired a cleavable amino terminal signal sequence in the first exon. *Fgf5, Fgf8, Fgf9*, and *Fgf10* were then generated from *Fgf4* by gene duplications followed by gene translocations. Cleavable secreted signal sequences and the intron positions were conserved in *Fgf4, Fgf5, Fgf8*, and *Fgf10* expansions. The *Fgf9/16/20* subfamily appears to have formed next with the
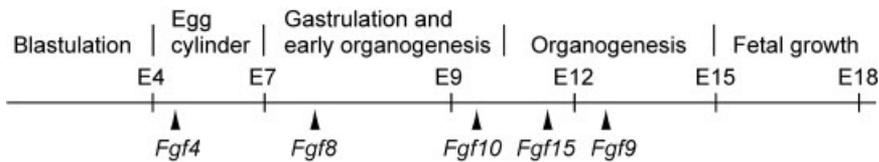
Fig. 4. Earliest identified roles for ancestral *Fgf*s in mouse embryonic development. *Fgf4* and *Fgf8* have essential roles in blastocyte formation and gastrulation, respectively. *Fgf10* is essential for the development of multiple organs and tissues including the limbs, lungs, and white adipose tissue. *Fgf9* is essential for the development of the lungs, heart, cecum, and testes. *Fgf15* functions in cardiac outflow tract development. E, embryonic day. Arrowheads indicate the stage of earliest identified phenotypes of null mutants.

evolution of an uncleaved bipartite signal sequence in an *Fgf9-like* ancestral gene.

All canonical FGFs have high-affinity heparin-binding sites and act in a paracrine manner. However, the hFGFs have low-affinity heparin-binding sites. We thus propose that an *Fgf15-like* gene was derived from *Fgf4-like* by a cis-gene duplication accompanied by loss of its high-affinity heparin-binding capacity, thus allowing it to function as an endocrine molecule. The hFGF family also acquired affinity for alternative co-factors, Klotho and βKlotho, required for signaling in target tissues (see below).

As most ancestors of the *Fgf* subfamilies have been identified in the ascidian, *C. intestinalis*, *Fgf* family expansion must have occurred before the evolution of vertebrates (Itoh and Ornitz, 2004). Ancestors of the canonical *Fgf* subfamilies have distinct receptor-binding specificity (Zhang et al., 2006). However, the *Fgfr* family expanded during the evolution of early vertebrates (Itoh and Ornitz, 2004), indicating that canonical FGFs acquired diversity in receptor specificity during the evolution of early vertebrates.

Conserved gene orders are observed among members of each *Fgf* subfamily, indicating that each subfamily expanded into three or four members via two large-scale genome duplications during the evolution of early vertebrates (Itoh and Ornitz, 2004). Although each canonical FGF has distinct receptor binding specificity, each member of an FGF subfamily has similar receptor specificity (Zhang et al., 2006). The most notable example of this is the ability of FGF9, FGF16, and FGF20 to bind FGFR3b in addition to c splice forms of FGFRs 1–3. In contrast, other canonical FGF subfamilies bind either b or c splice forms of FGFRs but not both.

PERSPECTIVES

The human/mouse *Fgf* gene family comprises 22 members with high-sequence similarity (Itoh and Ornitz, 2004). However, their roles and mechanisms of action are highly diverse. FGF11–FGF14 (iFGFs) function as intracellular proteins in an FGFR-independent manner (Wang et al, 2000; Goldfarb, 2005). In contrast, canonical FGFs function as extracellular proteins in an FGFR-dependent manner.

Canonical FGFs mediate their biological responses by binding to and activating FGFRs. Seven FGFR proteins (Fgfrs1b, 1c, 2b, 2c, 3b, 3c, and 4) with differing ligand-binding specificity are generated from four *Fgfr* genes in vertebrates (Ornitz et al., 1996; Zhang et al., 2006). Canonical FGFs have heparin-binding sites that bind to extracellular acidic glycosaminoglycans. Acidic glycosaminoglycans function as FGF cofactors to facilitate efficient activation of FGFRs (Ornitz, 2000). In addition, acidic glycosaminoglycans limit the diffusion of FGFs, localizing their activity to the vicinity of FGF-producing cells (Flaumenhaft et al., 1990). In contrast, hFGFs have poor heparin-binding affinity and act on target cells far from their site of production in an endocrine manner (Tomlinson et al., 2002; Lundasen et al., 2006; Kharitonenkov et al., 2005, 2007; Fukumoto et al., 2007; Liu et al., 2007). In addition, FGF15, FGF21, and FGF23 require co-receptors, Klotho or βKlotho, to activate FGFRs (Ogawa et al., 2007; Urakawa et al., 2006). These co-receptors are specifically expressed in target cells and are required for the specific actions of these FGFs in target tissues. These

findings indicate that hFGFs have evolved as novel metabolic regulators along with corresponding novel mechanisms of regulation.

Loss-of-function studies in mice have identified many other functions of FGFs. Most members of the *Fgf* family have been targeted in mice. Phenotypes range from early embryonic lethality to subtle changes in the physiology of adult mice, to no identifiable phenotype (*Fgf1*). Most canonical FGFs have essential roles as proliferation or differentiation factors in the development of various organs and tissues. In contrast, hFGFs have important roles as hormones that regulate bile acid metabolism, phosphate and vitamin D metabolism, and energy metabolism at postnatal stages (Shimada et al., 2004; Inagaki et al., 2005; Inagaki et al., 2007; Badman et al., 2007). Several of the *Fgfs* that exhibit early embryonic lethality as null mutations have also been studied later in development by conditional gene targeting and have been found to have many additional activities. Additionally, redundancy between FGFs, both within subfamilies and across subfamilies, has been identified. For example, redundancy between *Fgf4* and *Fgf8* in limb bud development was revealed by conditional, limb bud–specific, targeting of *Fgf4* and *Fgf8* (Moon et al., 2000; Moon and Capecchi, 2000; Sun et al., 2000, 2002; Lewandoski et al., 2000; Boulet et al., 2004). Redundancy between *Fgf3* and *Fgf8* occurs in otic vesicle and hindbrain development (Maroon et al., 2002; Walshe et al., 2002).

The studies of human diseases also have identified many potential functions of *FGFs*. Human hereditary disorders in FGF signaling result in diverse diseases consistent with the wide range of action of FGFs. Autosomal dominant hypophosphataemic rickets are caused by mutations in *FGF23* that stabilize the FGF23 protein (ADHR Consortium, 2000; White et al., 2001). FGF23 also functions as a humoral phosphaturic factor responsible for tumor-induced osteomalacia (Shimada et al., 2001). Both aplasia of lacrimal and salivary glands and lacrimo-auriculo-dento-digital syndrome are caused by mutations in *FGF10* (Entesarian et al., 2005, 2007; Milunsky et al., 2006; Rohmann et al.,
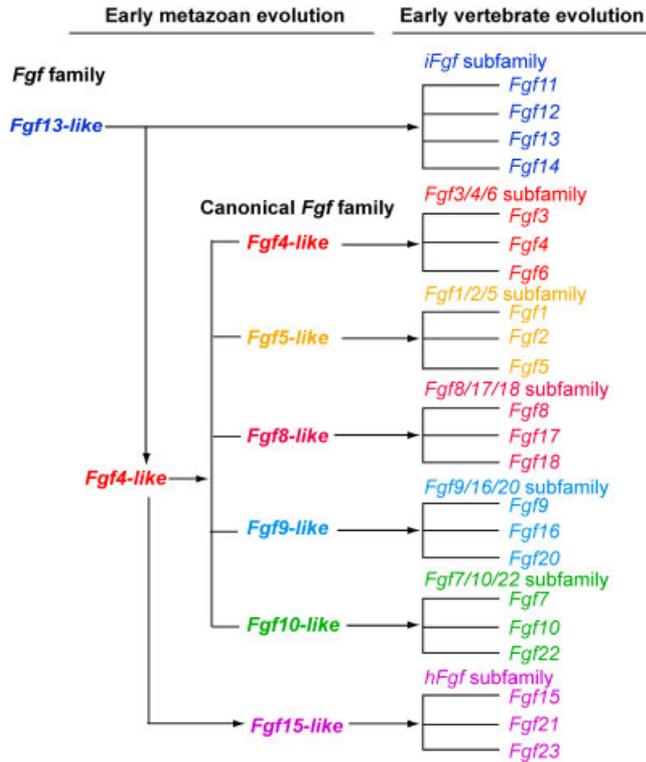
Fig. 5. Evolutionary history of the mouse *Fgf* gene family. The *Fgf* family comprises 22 members. *Fgf13-like* is an ancestral gene of the *Fgf* family. *Fgf4-like* is an ancestral gene of the canonical *Fgf* family. *Fgf4-like* was generated from *Fgf13-like* by a gene duplication. *Fgf5-like, Fgf8-like, Fgf9-like, Fgf10-like*, and *Fgf15-like* were generated from *Fgf4-like* by gene duplications. These expansions occurred before the evolution of vertebrates. Each *Fgf* subfamily expanded to contain three or four members via two large-scale genome duplications during the evolution of early vertebrates.
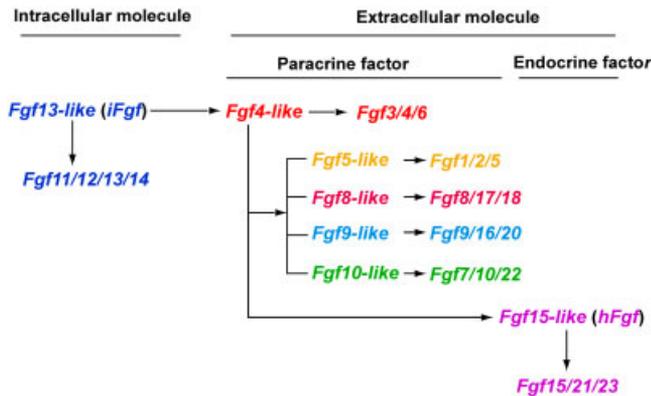

Fig. 6. Functional evolutionary history of ancestors of the mouse *Fgf* gene family. *Fgf13-like* encoding an intracellular molecule is the ancestral gene of the *Fgf* family. *Fgf4-like* was generated from *Fgf13-like* by a gene duplication. During this evolution, *Fgf4-like* acquired a cleavable secreted signal sequence. *Fgf5-like, Fgf8-like, Fgf9-like*, and *Fgf10-like* were generated from *Fgf4-like* by gene duplications. Cleavable secreted signal sequences were conserved in *Fgf-like5, Fgf8-like*, and *Fgf10-like*. A cleavable secreted signal sequence also evolved into an uncleaved bipartite signal sequence in *Fgf9-like*. These *Fgf*s with heparin-binding sites function as proliferation or differentiation factors in a paracrine manner. *Fgf15-like*, generated from *Fgf4-like* by a cis-gene duplication, lost its high-affinity heparin-binding capacity and acquired affinity for an alternative co-factor (βKotho). These changes facilitated acquisition of a new function as an endocrine metabolic regulatory molecule.

2006). Michel aplasia is caused by mutations in *FGF3* (Tekin et al., 2007). *FGF20* is a potential risk factor for Parkinson's disease (van der Walt et al., 2004; Satake et al., 2007). *FGF13* is a candidate gene for Börjeson-Forssman-Lehmann syndrome (Gecz et al., 1999) and a hereditary spinocer- ebellar ataxia syndrome (SCA27) is caused by mutations in *FGF14* (van Swieten et al., 2003; Dalski et al, 2005).

Since all human *FGF* gene loci have been identified, it is likely that mapping studies will uncover other genetic diseases that involve additional members of the FGF family. Additionally, an abundance of mutations in *Fgfrs* result in a variety of skeletal dysplasia syndromes (reviewed in Ornitz and Marie, 2002), suggesting that mutations in *Fgfs* will also be found to affect skeletal development or homeostasis.

As described above, FGF signaling is critical for many developmental and metabolic processes in vertebrates, and disorders in Fgf signaling result in various human diseases. The proposed functional evolutionary history of the *Fgf* family will be useful to elucidate additional functions, redundancies, and networks in development and metabolism.

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