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Of Worms and Men: An Evolutionary Perspective on the Fibroblast Growth Factor (FGF) and FGF Receptor Families

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Abstract. FGFs (fibroblast growth factors) play major roles in a number of developmental processes. Recent studies of several human disorders, and concurrent analysis of gene knock-out and properties of the corresponding recombinant proteins have shown that FGFs and their receptors are prominently involved in the development of the skeletal system in mammals. We have compared the sequences of the nine known mammalian FGFs, FGFs from other vertebrates, and three additional sequences that we extracted from existing databases: two human FGF sequences that we tentatively designated FGF10 and FGF11, and an FGF sequence from *Cœnorhabditis elegans.* Similarly, we have compared the sequences of the four FGF receptor paralogs found in chordates with four non-chordate FGF receptors, including one recently identified in *C. elegans.* The comparison of FGF and FGF receptor sequences in vertebrates and nonvertebrates shows that the FGF and FGF receptor families have evolved through phases of gene duplications, one of which may have coincided with the emergence of vertebrates, in relation with their new system of body scaffold.

Key words: FGF — FGF receptor — Phylogeny — Vertebrate — Invertebrate — Gene duplication

Introduction

> *A man may fish with the worm that hath eat of a king, and eat of the fish that hath fed of that worm.*
> Shakespeare, *Hamlet*

Comparative genomic and developmental analyses may provide clues for understanding the origin of genes as well as help in linking macro-evolutionary morphological transformations to modifications in embryonic patterns of expression of specialized genes (Sordino et al. 1995; Coates 1995). We compiled available data on the FGF (historically, fibroblast growth factors) and FGF receptor (FGFR) gene families in various organisms, and we review these data in an evolutionary context. In mammals, the FGF family currently comprises 11 members which interact with membrane-associated tyrosine kinase receptors (FGFR1 to FGFR4), and with heparan-sulfate proteoglycans. FGFs/FGFRs interactions play major roles in various developmental processes involving formation of mesoderm during gastrulation, integration of growth, budding and patterning during early postimplantation, and development of various tissues such as ear, limb, hair, and the skeletal system (Johnson and Williams 1993; Mason 1994; Wilkie et al. 1995; Yamaguchi and Rossant 1995). These roles have been established through study of patterns of expression, gene invalidations, physiological experiments using application of beads soaked with recombinant proteins, and analysis of human hereditary skeletal disorders (Johnson et al. 1994; Niswander et al. 1994; Muenke and Schell 1995; Tanaka

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and Gann 1995; Tickle 1995; Yamaguchi and Rossant 1995). Two new human *FGF* genes, and for the first time an invertebrate (*C. elegans*) sequence that has the potential to code for an FGF-related molecule, have been identified in databases (Wilson et al. 1994; Hodgkin et al. 1995), confirmed by more extensive DNA sequencing, and added to the existing list of FGF members. Recently, a *C. elegans* sequence encoding an FGF receptor was reported (DeVore et al. 1995). The discovery of FGF and FGF receptor–encoding genes in worms allows for speculation both on the role of such factor-receptor interactions in nonvertebrates and on the evolution of the families.

In line with the proposed function of FGF/FGFR interactions in the development of the skeletal system, we suggest that an important increase in the number of *FGF* genes might be associated with the period of macroevolutionary change that coincided with the origin of vertebrates and might have thus provided information in the making of the skeletal system.

Methods

Sequences. Protein sequences were obtained directly from EMBL, NCBI (Genbank and dbEST), or Swissprot databases. When needed, nucleotide sequences (also obtained from databases) were translated using the PCGene (Intelligenetics) package. Additional sequencing was done by automated methods using Applied Biosystem 373A instrument.

Species abbreviations are as follows: bt: *Bos taurus* (bovine); cc: *Coturnix coturnix* (quail); ce: *Caenorhabditis elegans* (nematode); dm: *Drosophila melanogaster* (fruit fly); dr: *Danio rerio* (zebrafish); gd: *Gallus domesticus* (chicken); hs: *Homo sapiens* (human); ma: *Mesocricetus auratus* (golden hamster); md: *monodelphis domestica* (short-tailed opossum); mm: *Mus musculus* (mouse); nv: *Notophtalmus viridescens* (newt); oa: *Ovis aries* (sheep); ol: *Oryzias latipes* (medaka fish); pw: *Pleurodeles waltl* (Iberian ribbed newt); m: *Rattus norvegicus* (rat); sp: *Stroxyglocentrotus purpuratus* (purple urchin); ss: *Sus scrofa* (pig); xl: *Xenopus laevis* (African clawed frog).

Accession numbers for FGF and FGFR sequences are as follows: bt-FGF1: P03968; bt-FGF2: P03969; bt-FGF4: P48803; cc-FGFR4: X76885; ce-FGF: U00048.; ce-FGFR: U39761; dm-FGFRa: Q07407; dm-FGFRb: Q09147; dr-FGF3: P48802; dr-FGFR4: U23839; gd-FGF1: A60130; gd-FGF2: P48800; gd-FGF3: P48801; gd-FGF4: P48804; gd-FGFR1: M24637; gd-FGFR2: M35196; gd-FGFR3: M35195; hs-FGF1: P05230; hs-FGF2: P09038; hs-FGF3: P11487; hs-FGF4: P08620; hs-FGF5: P12034; hs-FGF6: P10767; hs-FGF7: P21781; hs-FGF8: g999172; hs-FGF9: P31371; hs-FGF10: Z70275, T27215, H15590; hs-FGF11: Z70276, H19128, H62672, R58169, H28811; hs-FGFR1: P11362; hs-FGFR2: P21802; hs-FGFR3: P22607; hs-FGFR4: P22455; hs-IL1b: P01584; hs-IL1R: P14778; hs-SRC: P12931; ma-FGF1: P34004; md-FGF2: P48798; mm-FGF2: P15655; mm-FGF4: P11403; mm-FGF5: P15656; mm-FGF6: P21658; mm-FGF7: P36363; mm-FGF8: P37237; nv-FGFR2: L19870; oa-FGF2: P20003; oa-FGF7:P48808; ol-FGFR1: D13550; ol-FGFR2: D13551; ol-FGFR3: D13552; ol-FGFR4: D13553; pw-FGFR1: X59380; pw-FGFR2:X74332; pw-FGFR3: X75603; pw-FGFR4: X65059; m-FGF1: P10935; rn-FGF2: P13109; rn-FGF5: P48807; rn-FGF7: Q02195; rn-FGF9: P36364; sp-FGFR: U17164; ss-FGF1: JH0476; xl-FGF2: P12226; xl-FGF3: P36386; xl-FGF4-I: P48805; xl-FGF4-II: P48806; xl-FGFR1: M61687; xl-FGFR2: X65943; xl-FGFR4: D31761.

The hs-FGF10 sequence was obtained by assembling EMBL database sequences H15590 and T27215, along with extensions and corrections from additional sequencing of the corresponding clones, ym27b06 and MT0120, respectively.

The hs-FGF11 sequence was obtained by assembling EMBL database sequences H19128, H62672, R58169, and H28811, along with extensions and corrections from additional sequencing of clone ym44e12 corresponding to H19128.

The hs-FGF10 and hs-FGF11 core sequences have been submitted to the EMBL database and assigned accession numbers Z70275 and Z70276, respectively.

Sequence Alignment. All protein sequences were aligned using the Clustal W program (Thompson et al. 1994).

FGF ‘‘core’’ sequences corresponding to hs-FGF1 amino acids 28–151, after removing internal insertions in FGF1 (corresponding to amino acids 120 and 121 of hs-FGF1), FGF3 (corresponding to amino acids 137–152 of hs-FGF3), FGF5 (corresponding to amino acids 181–186 of hs-FGF5), FGF7 (corresponding to amino acids 159–162 of hs-FGF7), FGF9 (corresponding to amino acids 156–161 of hs-FGF9), hs-FGF10 (amino acids 92–97 of the putative partial sequence), hs-FGF11 (amino acids 92–97 of the putative partial sequence), and ce-FGF (amino acids 92–95 of the putative sequence), were used for the alignment.

FGFR extracellular domain sequences (acidic box, Ig loops II and III) corresponding to amino acids 119–359 of hs-FGFR1 were aligned after removing internal insertions in ce-FGFR (amino acids 459–465) and sp-FGFR (amino acids 441–444 and 453–481).

FGFR intracellular domain sequences (kinase subdomains II–VII; Hanks et al. 1988 corresponding to amino acids 492–663 of hs-FGFR1) were aligned after removing internal insertions in ce-FGFR (amino acids 747–750 and762–773), dm-FGFRa (amino acids 519–522), and dm-FGFRb (amino acids 823–829).

Phylogeny Inference. Phylogenetic trees were constructed using the distance matrix (Dayhoff PAM matrix) and neighbor-joining algorithms of the Phylogeny Inference Package of J. Felsenstein (Felsenstein 1989). Human Interleukin 1β (hs-IL1b), human interleukin 1 β-receptor (hs-IL1R), and human c-SRC (hs-SRC) sequences were used as outgroups in the constructions of trees for the FGFs and the FGFRs ecto- and kinase domains, respectively.

Bootstrapping is a resampling technique that allows one to calculate confidence limits on trees (Felsenstein 1985). The bootstrap value (in percentages) indicates the number of times a given branching occurs among the bootstrapped samples and is a measure of the significance of a grouping with respect to the particular data set and to the method used for drawing the tree (Higgins et al. 1991). To test the validity (robustness) of branching, a total of 2,000 bootstrapped data sets were subjected to analysis, and a consensus tree was obtained using the Consense program (Felsenstein 1989).

Results

Identification of New FGF Genes FGF10, FGF11, and ce-FGF

Database searches with known FGF sequences allowed us to identify FGF-related human cDNA clones (ym27b06, yr45d03, ym44e12 (Hillier et al. 1996), and MTO120 (Brody et al. 1995)), isolated as Expressed Sequence Tag (EST), and a cosmid clone (CO5D11) (Wilson et al. 1994), isolated from a *C. elegans* genomic library. Based on nucleic acid and amino acid alignments of the partial sequences, we were able to identify the

Table 1. Amino acid identity score between FGF sequences

	ce-FGF	hs-FGF11	hs-FGF10	rn-FGF9	hs-FGF9	mm-FGF8	hs-FGF8	rn-FGF7	oa-FGF7	mm-FGF7	hs-FGF7	mm-FGF6	hs-FGF6	rn-FGF5	mm-FGF5	hs-FGF5	xl-FGF4-II	xl-FGF4-I	mmFGF4	gd-FGF4	bt-FGF4	hs-FGF4	xl-FGF3	gd-FGF3	br-FGF3	hs-FGF3	xl-FGF2	rn-FGF2	oa-FGF2	mm-FGF2	md-FGF2	gd-FGF2	bt-FGF2	hs-FGF2	ss-FGF1	rn-FGF1	ma-FGF1	gd-FGF1	bt-FGF1
hs-FGF1	31	35	35	42	42	24	24	38	40	39	40	32	34	40	40	40	37	39	36	36	38	39	40	40	43	38	55	57	57	57	56	57	57	57	98	97	98	91	93
bt-FGF1	32	36	34	40	40	24	24	36	37	37	37	32	34	40	40	40	37	39	36	37	38	39	40	40	42	38	53	56	57	56	55	57	57	56	90	90	91	88	■
gd-FGF1	30	34	35	42	42	23	23	38	39	39	39	32	34	40	40	40	37	39	37	36	40	40	40	41	44	38	54	57	57	57	56	57	57	57	90	89	89	■	
ma-FGF1	32	36	34	42	42	25	25	38	40	39	40	33	35	41	41	40	37	39	36	37	39	40	40	40	43	38	56	57	58	57	57	57	58	57	97	99	■		
rn-FGF1	32	36	35	42	42	25	25	38	40	40	40	33	35	41	41	40	37	39	36	37	39	40	40	40	43	38	55	57	57	57	56	57	57	57	97	■			
ss-FGF1	33	35	35	42	42	24	24	37	39	38	39	32	34	40	40	40	36	38	36	36	38	39	40	40	42	39	54	56	57	56	55	56	57	56	■				
hs-FGF2	30	34	33	41	41	30	30	39	40	40	40	45	45	45	45	44	40	41	42	45	44	45	43	44	45	44	86	98	98	98	94	92	98	■					
bt-FGF2	31	34	33	41	41	30	30	38	39	39	39	44	44	45	45	44	40	41	42	45	44	45	43	44	45	44	86	98	100	98	94	93	■						
gd-FGF2	32	31	32	40	40	28	28	38	39	39	39	44	45	44	44	43	40	42	42	43	43	44	40	41	43	42	85	93	93	93	92	■							
md-FGF2	29	33	32	40	40	30	30	39	40	40	40	44	45	43	43	42	40	42	40	44	41	43	42	43	45	43	84	95	94	95	■								
mm-FGF2	30	34	33	41	41	30	30	39	40	40	40	44	45	44	44	43	40	42	41	44	43	44	43	44	45	44	85	100	98	■									
oa-FGF2	31	34	33	41	41	30	30	38	39	39	39	44	44	45	45	44	40	41	42	45	44	45	43	44	45	44	86	98	■										
rn-FGF2	30	34	33	41	41	30	30	39	40	40	40	44	45	44	44	43	40	42	41	44	43	44	43	44	45	44	85	■											
xl-FGF2	28	33	34	39	39	26	26	36	36	36	36	41	40	42	42	41	40	40	40	41	42	43	40	40	41	41	■												
hs-FGF3	36	36	37	47	47	35	35	45	47	46	47	42	42	51	51	50	40	40	34	42	36	36	87	89	78	■													
br-FGF3	34	36	35	46	46	33	33	45	46	45	46	43	43	50	50	50	42	42	34	43	37	37	83	83	■														
gd-FGF3	38	37	37	50	50	35	35	47	49	48	49	42	42	51	51	50	40	40	34	41	35	35	95	■															
xl-FGF3	36	37	36	50	50	35	35	46	48	47	48	41	41	49	49	48	39	39	33	40	34	34	■																
hs-FGF4	31	43	44	44	44	32	32	34	35	36	36	68	69	51	51	51	75	76	91	80	90	■																	
bt-FGF4	28	40	43	42	42	30	30	32	32	33	32	69	70	51	51	51	69	70	89	75	■																		
gd-FGF4	31	43	39	43	43	36	36	35	36	37	36	76	78	49	49	50	84	85	77	■																			
mm-FGF4	27	41	41	41	41	32	32	32	32	33	33	66	67	48	48	48	71	73	■																				
xl-FGF4-I	30	44	42	43	43	36	36	35	36	37	36	72	74	48	48	49	97	■																					
xl-FGF4-II	29	43	40	42	42	35	35	35	36	37	36	73	75	48	48	49	■																						
hs-FGF5	35	37	38	47	47	31	31	42	44	43	44	52	51	97	97	■																							
mm-FGF5	36	36	38	48	48	31	31	43	45	44	45	51	50	100	■																								
rn-FGF5	36	36	38	48	48	31	31	43	45	44	45	51	50	■																									
hs-FGF6	31	40	38	43	43	36	36	36	38	37	38	94	■																										
mm-FGF6	31	41	39	43	43	35	35	34	36	35	36	■																											
hs-FGF7	36	34	35	43	43	31	31	93	98	98	■																												
mm-FGF7	35	35	36	43	43	31	31	95	97	■																													
oa-FGF7	36	34	35	43	43	31	31	93	■																														
rn-FGF7	34	33	35	43	43	31	31	■																															
hs-FGF8	28	22	27	36	36	100	■																																
mm-FGF8	28	22	27	36	36	■																																	
hs-FGF9	38	39	40	100	■																																		
rn-FGF9	38	39	40	■																																			
hs-FGF10	32	66	■																																				
hs-FGF11	30	■																																					

human clones as two new *FGF* sequences, tentatively called *hs-FGF10* (ym27b06 and MTO120) and *hs-FGF11* (yr45d03 and ym44e12). Further sequencing of these clones allowed us to derive the peptide sequences for the complete core region (Coulier et al. 1991, 1994). *hs-FGF10-* and *hs-FGF11*-related genomic sequences appear to exist in the mouse (data not shown).

Z. Du and R. Waterston (unpublished) predicted the existence of a gene encoding a heparin-binding growth-factor-related peptide within cosmid CO5D11 derived from chromosome III of *C. elegans.* Alignment of this peptide sequence, tentatively called ce-FGF, with that of known FGFs, revealed 27–37% of amino acids identity with the other members of the family. We have determined that mRNA corresponding to this gene can be found in larval stages of *C. elegans* (data not shown).

FGF Sequence Comparisons Show Conserved and Variable Stretches of Amino Acids

The core amino acid sequences of 39 identified vertebrate FGFs (corresponding to 11 mammalian, four avian, one fish, and three amphibian FGF paralogs) and the *C. elegans* FGF (ce-FGF) were aligned for comparison (Table 1; Fig. 1). The core sequence was obtained by deletion of N- and C-terminal extensions as well as specific internal sequences. It represents about 120 amino acid residues in length. Twelve positions (10%) were found conserved in all sequences. The conservation among the other residues varies along the core sequence, with scattered clusters of highly conserved positions (for example, 79–89) and regions with limited similarity (for example, 97–114 or 138–146).

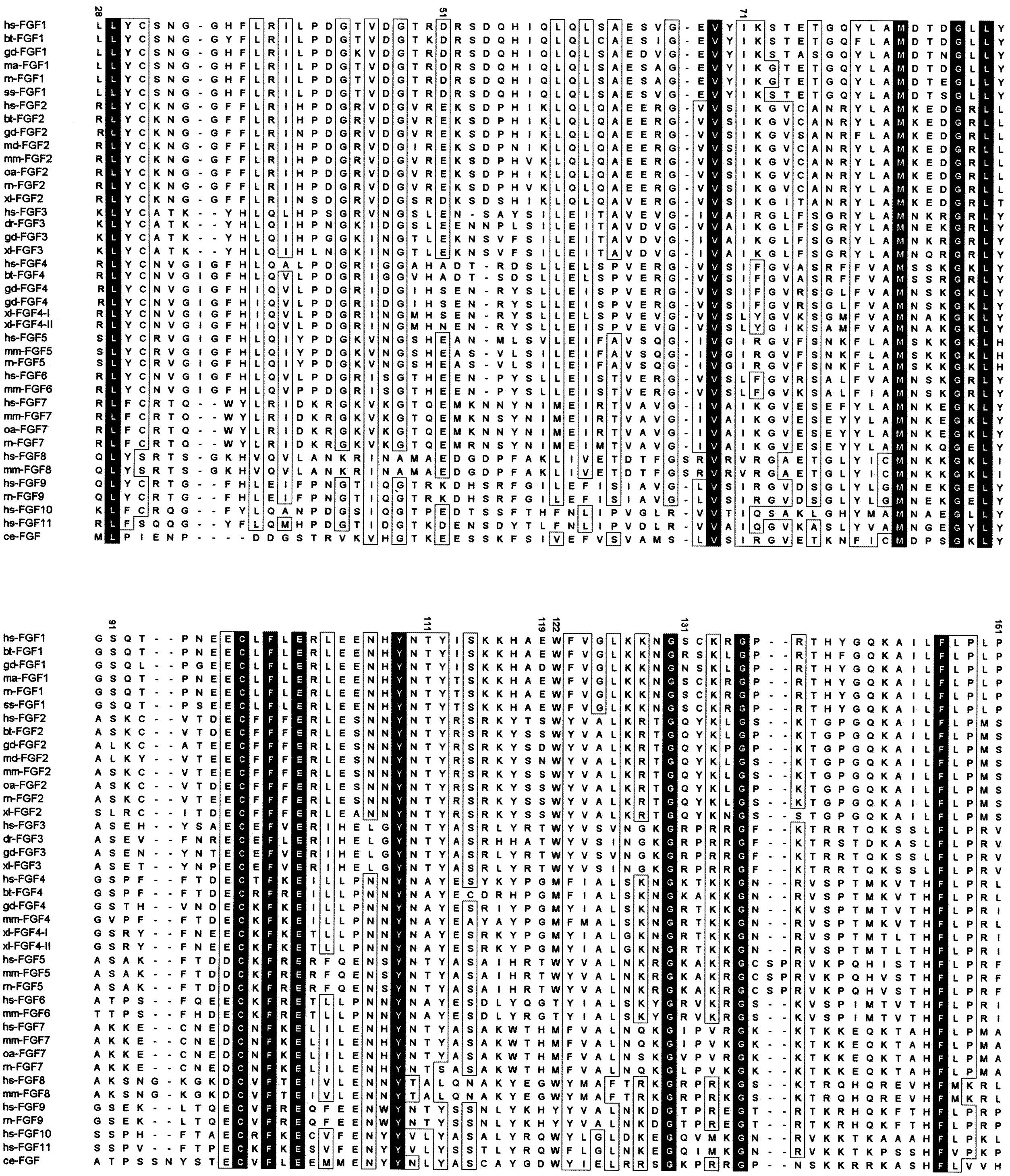

Fig. 1. Alignment of FGF sequences. Sequences derived from 40 known FGF proteins were aligned, allowing for gaps (−) in order to optimize the alignment. Positions of perfect identity are indicated (*black boxes*), while *open boxes* contain amino acids that are identical or similar in at least eight orthologous sequences. Amino acid numbering is according to human FGF1 sequence.

Earlier studies on FGFs have tried to define structure–function relationships using mutants or protein fragments (Jaye et al. 1986; Presta et al. 1992). The determination of the three-dimensional structure of FGF1 and FGF2 has brought clues about the importance of some amino acid residues (Eriksson et al. 1991; Zhang et al. 1991; Zhu et al. 1991; Faham et al. 1996). These include the role of the cysteine residues and the position of the heparan-sulfate– and receptor-binding sites. The two cysteines consistently present in FGF sequences do not seem

Table 2. Amino acid identity score between FGFR sequences

Kinase domain

	sp-FGFR	dm-FGFRb	dm-FGFRa	ce-FGFR	xl-FGFR4	pw-FGFR4	ol-FGFR4	hs-FGFR4	dr-FGFR4	cc-FGFR4	pw-FGFR3	ol-FGFR3	hs-FGFR3	gd-FGFR3	xl-FGFR2	pw-FGFR2	ol-FGFR2	nv-FGFR2	hs-FGFR2	gd-FGFR2	xl-FGFR1	pw-FGFR1	ol-FGFR1	hs-FGFR1	gd-FGFR1
gd-FGFR1	66	58	60	54	76	74	81	73	79	76	85	84	82	87	78	84	85	85	86	85	92	93	90	97	■
hs-FGFR1	66	58	60	54	75	73	79	72	77	75	84	83	82	85	76	82	84	83	84	83	90	92	90	■	96
ol-FGFR1	65	56	60	54	73	69	76	70	76	72	80	80	77	81	77	80	83	80	83	81	88	92	■		
pw-FGFR1	67	58	59	54	74	70	77	72	77	73	82	83	79	84	77	80	84	81	83	82	92	■		86	87
xl-FGFR1	67	57	58	54	76	72	77	72	76	72	81	83	78	83	77	79	83	80	82	81	■	78		79	81
gd-FGFR2	65	58	61	51	77	74	81	74	79	78	88	86	87	90	90	97	91	97	99	■	71	74		74	75
hs-FGFR2	65	58	61	51	76	74	80	73	78	77	88	86	85	90	89	95	92	96	■	93	70	75		73	74
nv-FGFR2	65	58	61	51	75	73	80	73	78	77	87	86	85	89	87	98	90	■	86	87	69	71		72	73
ol-FGFR2	65	57	59	52	77	76	83	74	81	78	87	87	84	89	86	89	■								
pw-FGFR2	64	58	61	51	75	73	79	73	77	77	87	85	85	88	87	■		94	86	85	69	72		73	73
xl-FGFR2	62	58	60	52	76	71	77	73	77	77	81	83	83	83	■	83		83	82	81	66	68		68	69
gd-FGFR3	66	58	62	55	78	78	86	77	84	80	97	95	94	■	70	74		75	79	79	70	76		74	75
hs-FGFR3	66	59	63	54	79	76	82	77	81	78	91	91	■	88	68	71		71	74	74	67	69		70	71
ol-FGFR3	66	58	61	54	77	76	83	74	83	78	94	■													
pw-FGFR3	65	58	62	55	77	77	85	74	83	78	■		84	87	66	70		70	73	72	66	69		69	70
cc-FGFR4	63	57	57	50	80	82	83	78	82	■	73		76	76	65	69		67	69	70	62	64		65	64
dr-FGFR4	62	55	60	50	80	81	95	74	■	75	69		72	73	66	69		68	68	69	64	62		63	64
hs-FGFR4	62	56	60	52	76	74	75	■	71	80	70		70	72	64	66		65	66	69	63	63		64	64
ol-FGFR4	63	55	60	50	82	83	■																		
pw-FGFR4	59	53	55	49	83	■		78	76	84	72		77	75	64	68		67	68	69	63	64		65	65
xl-FGFR4	64	56	58	52	■	79		77	75	83	73		76	76	66	69		67	70	70	64	64		66	67
ce-FGFR	51	48	50	■	30	31		32	31	29	32		31	34	32	34		34	34	34	34	32		33	32
dm-FGFRa	56	77	■	30	35	34		34	33	36	35		36	38	33	33		33	34	35	34	33		33	33
dm-FGFRb	54	■	33	31	29	29		30	29	30	33		31	33	31	30		30	33	31	30	31		29	29
sp-FGFR	■	28	28	27	29	28		31	31	29	35		33	35	33	32		34	33	34	32	34		34	34

Extracellular domain

to form intramolecular bonds. Indeed, one of the cysteines (position 98 in Fig. 1) is conserved in all FGFs, but the other (position 31) is present in most FGFs but not in FGF8, FGF11, and ce-FGF. The presumed receptor-binding site of FGF2 has been located in the region delimited by residues 106–121. The differences in amino acid sequences within this stretch (which includes insertions of up to 16 residues in FGF3) could account for the discrimination between different receptors. Basic residues K32, K127, R128, K133, K137, and K143 (as numbered in Fig. 1), are thought to constitute a potential binding site for the sulfate group of heparin and other sulfated substrates (Eriksson et al. 1991; Zhang et al. 1991; Zhu et al. 1991; Faham et al. 1996). Other residues possibly involved in saccharide binding include N33, N107, and Q142 (Faham et al. 1996). They are relatively well conserved in all FGF sequences except for K32, N33, K127, and K143.

FGFR Sequence Comparisons

The sequences of 25 FGF receptors identified in various species, including recently published FGFR sequence from *C. elegans* (DeVore et al. 1995), were aligned for comparison (Table 2 and Fig. 2). Comparisons were done separately to the extracellular and kinase domains but led for similar conclusions. In vertebrates, FGFR1, FGFR2, and FGFR3 orthologous sequences have more than 80% identity. FGFR4 sequences are slightly more different. The four identified invertebrate sequences, including the two *Drosophila* FGFR, share an average of 30% identity in the extracellular domain, and from 49 to 67% in the kinase domain, with FGFR1 to FGFR4 vertebrate sequences, and thus could not be identified as orthologs of any of them.

The extracellular regions of the FGF receptors contain 22 amino acid residues conserved throughout all sequences (Fig. 2A). This includes cysteine residues involved in the tertiary structure of immunoglobulin-like domains. Some of these residues, such as C278, Y340, C342, and S347 of FGFR2, are targets of mutations found in human inherited skeletal disorders leading to receptor dysfunction (Muenke and Schell 1995; Mulvihill 1995; Wilkie et al. 1995; Yamaguchi and Rossant 1995). However, several mutations occur in residues which are not conserved in invertebrates. One residue that is not conserved in invertebrate FGFRs is a proline

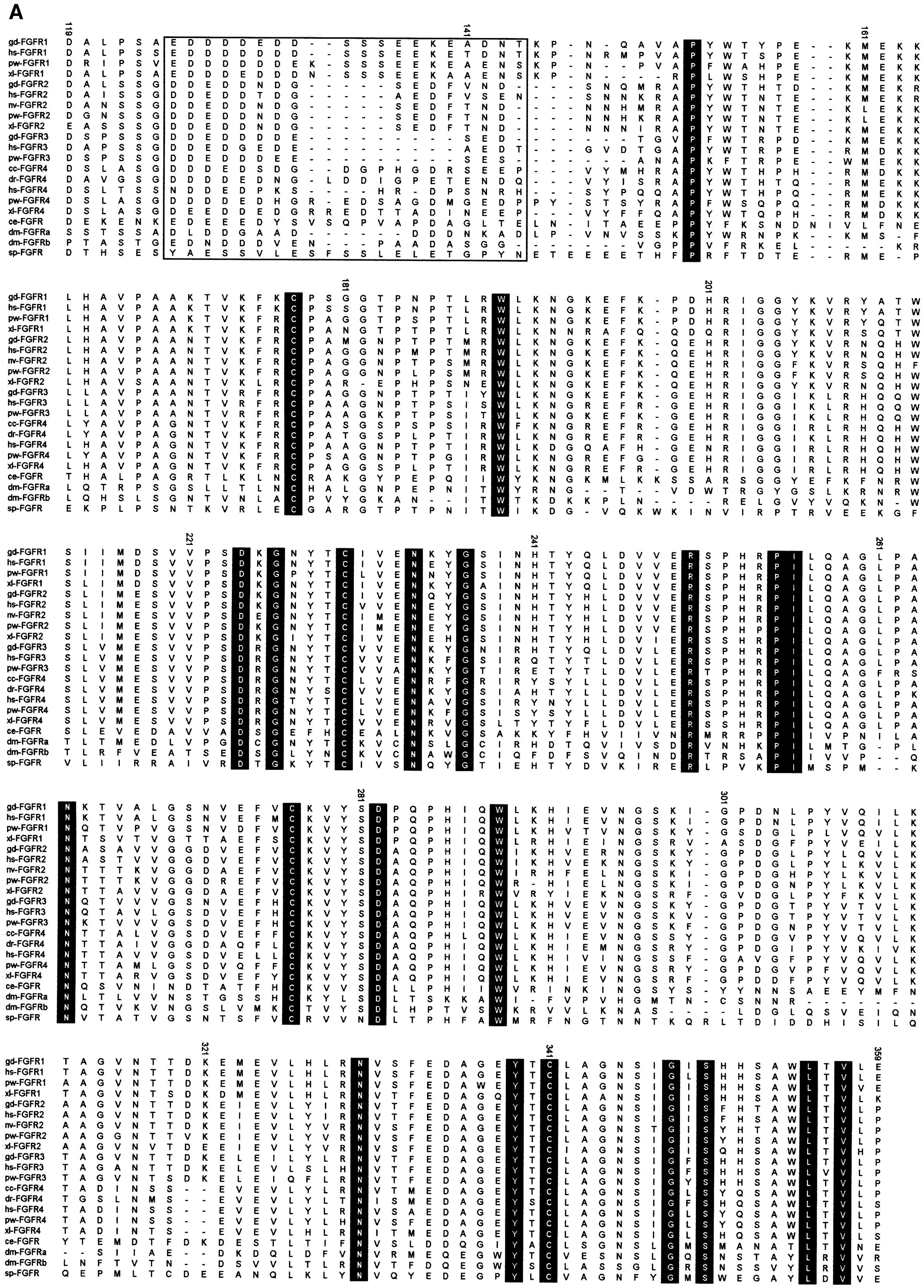

Fig. 2. Alignment of FGFR sequences. **A** Alignment of extracellular domains. Sequences derived from the extracellular domain of 21 known FGF-Receptor proteins were aligned, allowing for gaps (−) in order to optimize the alignment. Portions of sequences used for the alignment include the acidic box and the two C-terminal Ig loops. Positions of perfect identity are indicated (*black boxes*). *Open box* indicates the position of the acidic domain. Amino acid numbering is according to human FGFR1 sequence.**B** Alignment of kinase domains. Sequences derived from the kinase domain of 25 known FGF-Receptor proteins were aligned, allowing for gaps (−) in order to optimize the alignment. Portions of sequences used for the alignment include kinase subdomains II–VII (Hanks et al. 1988). Positions of perfect identity are indicated (*black boxes*). Amino acid numbering is according to human FGFR1 sequence.

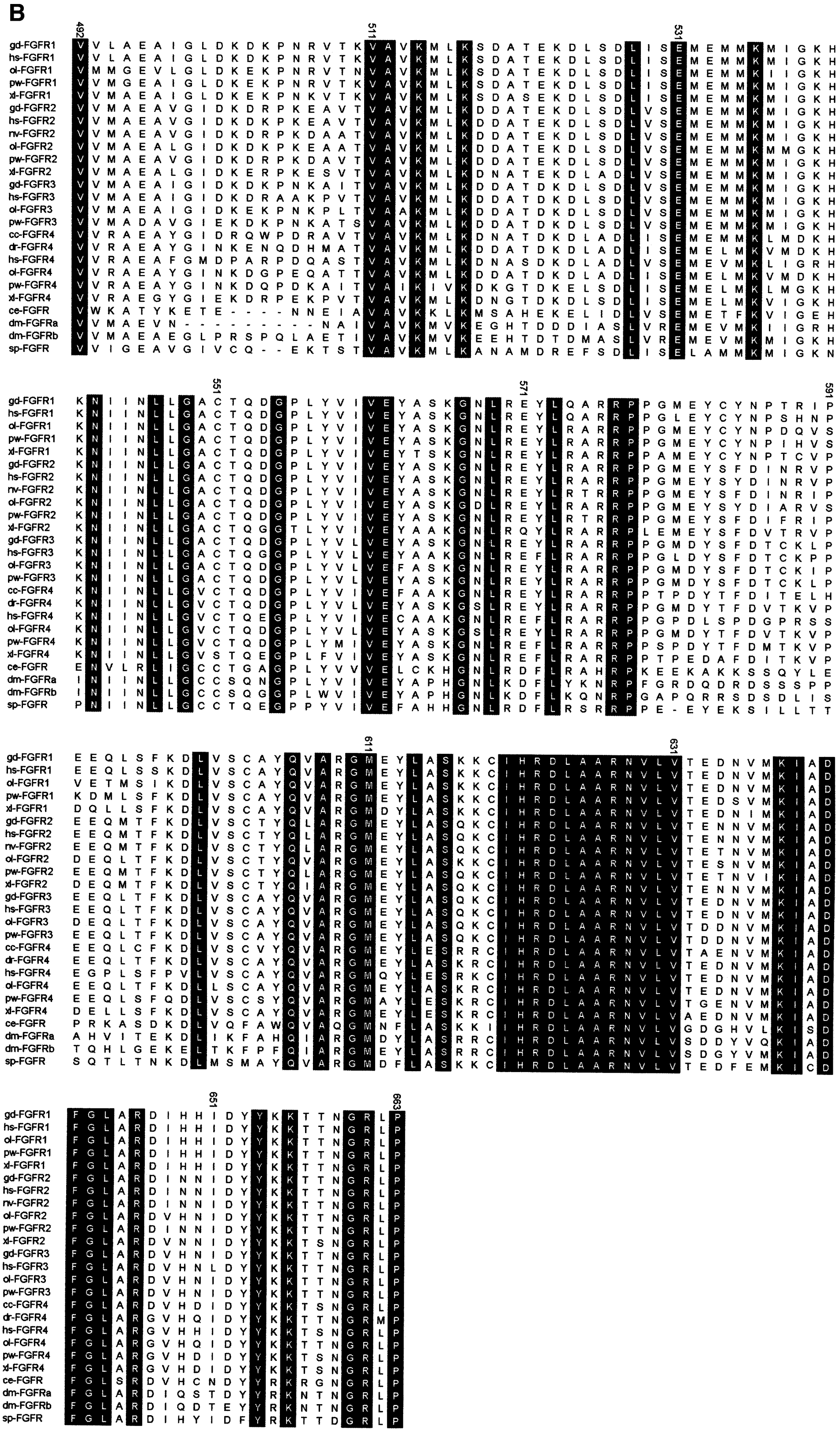

Fig. 2. Continued.

(position 252 in FGFR1 and 253 in FGFR2) (Fig. 2). Substitution of this proline by arginine in FGFR1 and FGFR2 is found in two craniosynostosis syndromes, the Pfeiffer and Apert syndromes, respectively. Interestingly, an arginine residue is found at the corresponding position in the *C. elegans* FGFR sequence. The point mutations found in human disorders are dominant and are presumed to activate constitutively the receptor. It

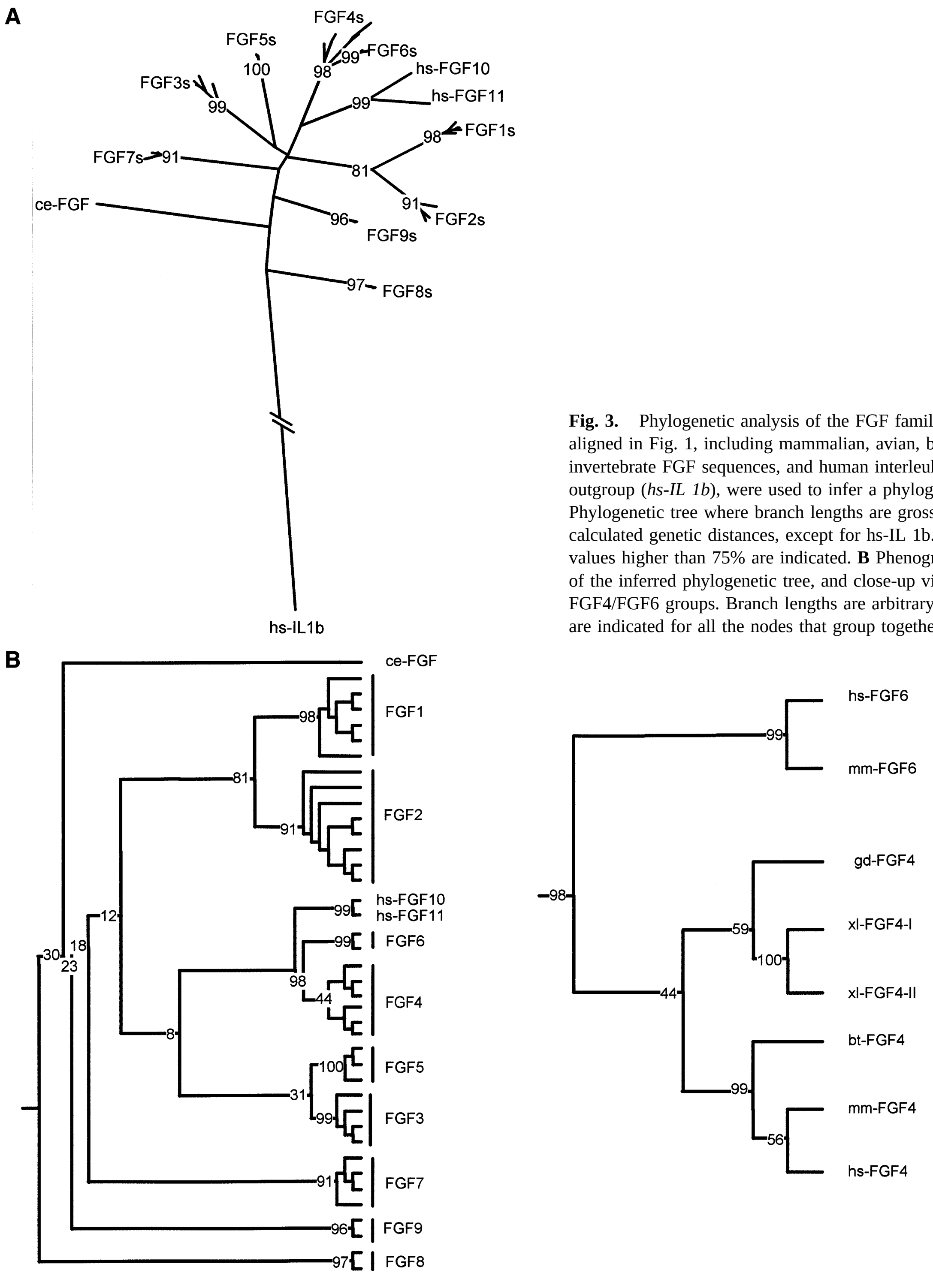

Fig. 3. Phylogenetic analysis of the FGF family. The 40 sequences aligned in Fig. 1, including mammalian, avian, batracian, and invertebrate FGF sequences, and human interleukin 1-β as an outgroup (*hs-IL 1b*), were used to infer a phylogenetic tree. **A** Phylogenetic tree where branch lengths are grossly proportional to calculated genetic distances, except for hs-IL 1b. Only the bootstrap values higher than 75% are indicated. **B** Phenogram representation of the inferred phylogenetic tree, and close-up view of the FGF4/FGF6 groups. Branch lengths are arbitrary. Bootstrap values are indicated for all the nodes that group together FGF paralogs.

would be interesting to test whether the counter mutation in *C. elegans* (i.e., arginine to proline) leads to a change in ce-FGFR activity.

Evolution of the FGF Family

The 40 sequences aligned in Fig. 1 were used to infer a phylogenetic tree (Fig. 3) by using the neighbor-joining algorithm (Felsenstein 1989). The sequence of human interleukin-1β, a peptide regulatory factor distantly related to FGFs (Gimenez-Gallego et al. 1985; Eriksson et al. 1991; Zhang et al. 1991; Zhu et al. 1991), was used as an outgroup. Use of the parsimony algorithm led to a similar topology.

All paralogs were grouped together; bootstrap values exceeded 90%.

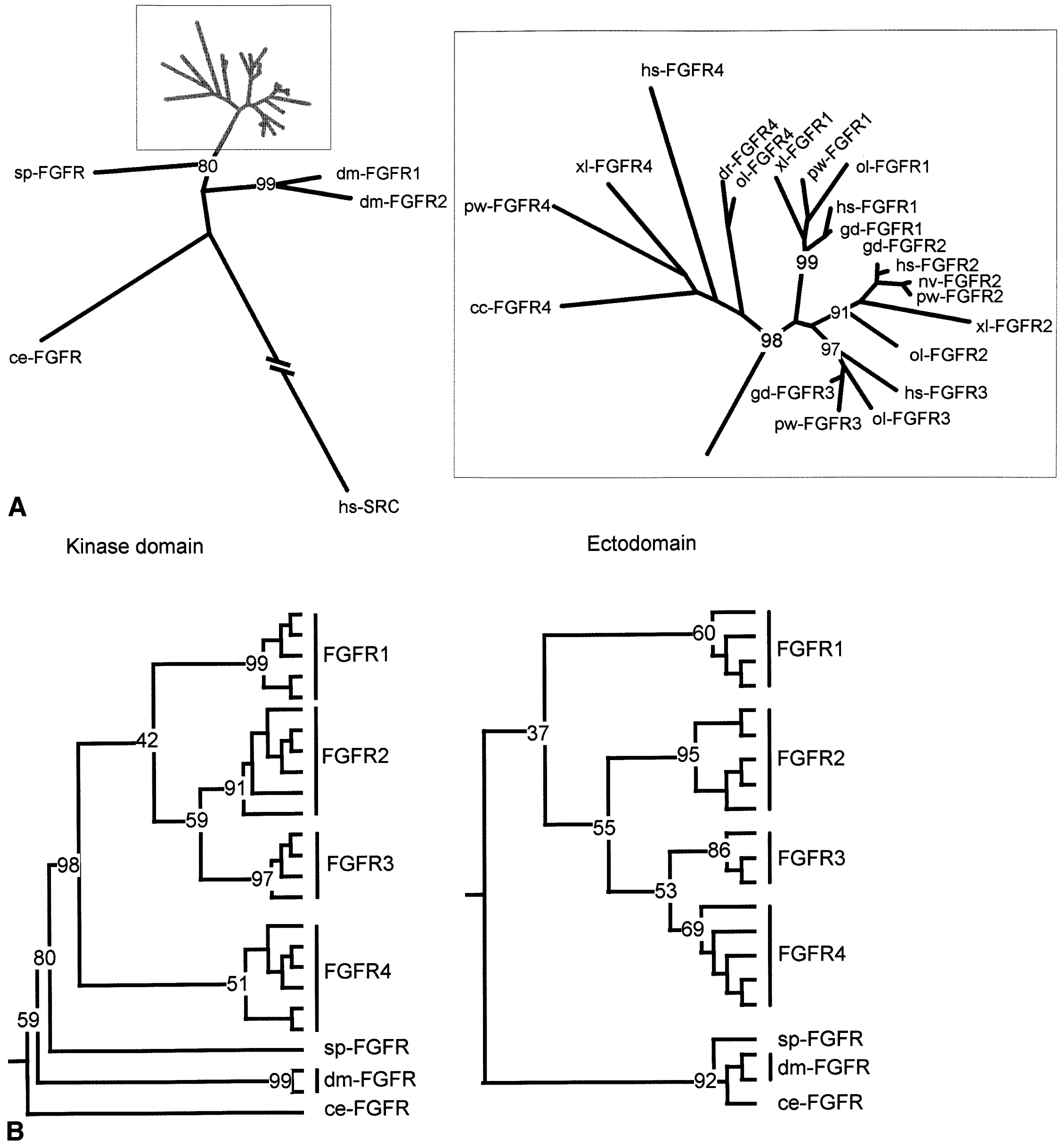

Fig. 4. Phylogenetic analysis of the FGFR family. The 25 kinase domains sequences aligned in Fig. 2B, including mammalian, avian, amphibian, fish, and invertebrate FGFR sequences, and human c-SRC as an outgroup (*hs-SRC*), were used to infer a phylogenetic tree. **A** Phylogenetic tree where branch lengths are proportional to calculated-genetic distances, except for hs-SRC.A closer view of the vertebrate FGFR subtree is shown on the right. Only the bootstrap values higher than 75% are indicated. **B** Phenogram representation of inferred phylogenetic tree using either FGFR kinase or ectodomains. Branch lengths are arbitrary. Bootstrap values are indicated for all the nodes that group together FGFR paralogs.

Studying the branching between paralogs showed that the only branchings with high confidence intervals were between FGF1 and FGF2 (bootstrap value of 81%, see Materials and Methods section), FGF4 and FGF6 (98%), and FGF10 and FGF11 (99%). None of the other branchings between paralogs could exceed a bootstrap value of 31%. FGF1/FGF2, FGF4/FGF6, and FGF10/FGF11 are pairs of FGFs that show the highest similarity score (Table 1), suggesting the corresponding pairs of genes duplicated more recently as compared to the other *FGF* genes.

Evolution of the FGF Receptor Family

A similar analysis was conducted with FGF receptor sequences (Fig. 4). Separate analyses of the extracellular region and intracellular kinase domain were performed using both neighbor-joining and parsimony algorithms, and yielded similar topologies (Fig. 4B). Comparison with the corresponding sequence alignments (Table 2) showed that changes in the kinase domain have occurred at a much slower rate.

Invertebrate FGFRs from three species appeared in separate branches which were all distinct from the vertebrate sequences (Fig. 4A). FGFR paralogs from vertebrate species were grouped together with high bootstrap values. The two FGFRs from *D. melanogaster* were distinct from vertebrate FGFRs and branched together with a very high value of bootstrap (99%), suggestive of a very recent duplication. This topology is in support of the

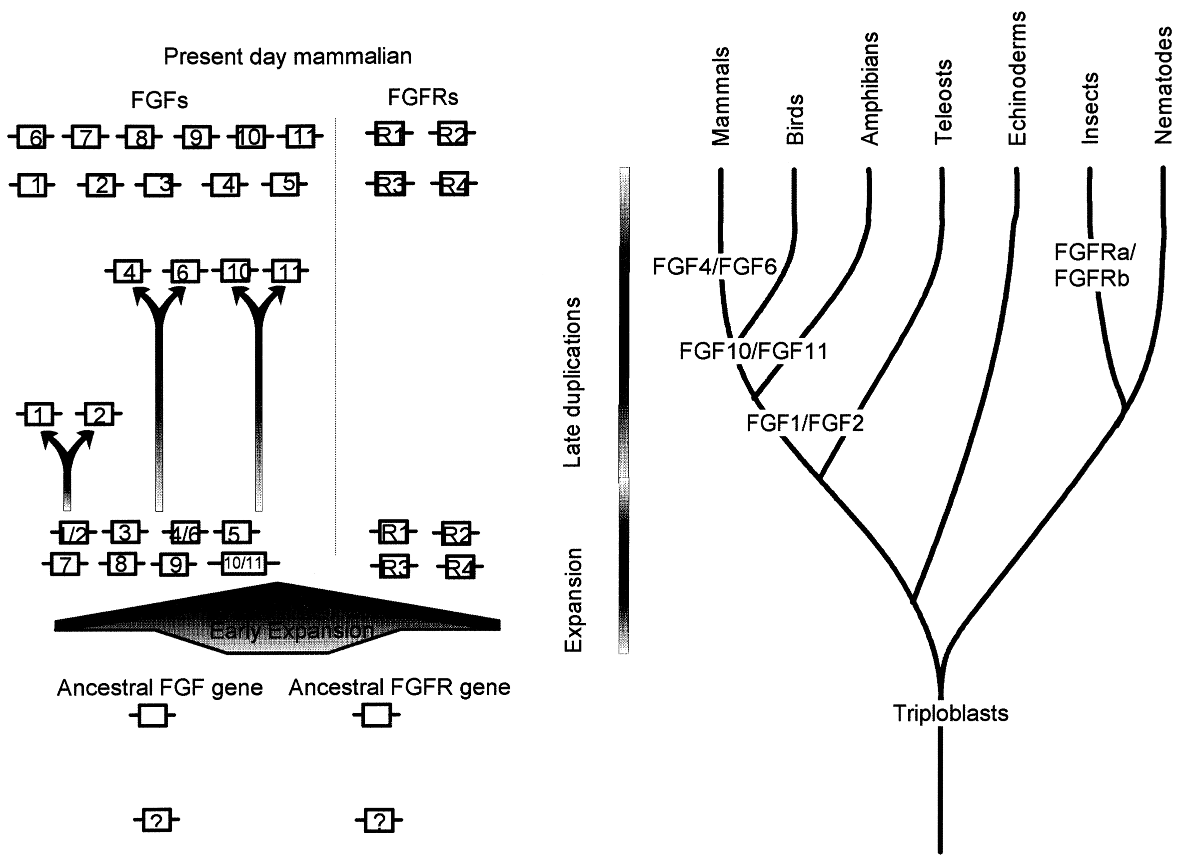

Fig. 5. Representation of a hypothetical FGF and FGFR evolution scheme. This evolutionary scheme is based on the available information on FGF and FGFR sequences in various species. Putative phases of gene duplications (shown on the left) are individualized, and are tentatively related toa phylogenetic tree of Metazoa (shown on the right). FGF and FGFR expansion is shown to occur after the origin of nematodes and echinoderms, but the timing of this expansion will only be precisely fixed upon determination of the number of FGF and FGFR sequences in these species.

existence of one FGFR gene (or a recently duplicated one like in *D. melanogaster*) in invertebrates and of an expansion to four members in vertebrates.

Discussion

This paper reports the presence of an FGF gene in an invertebrate species, the identification of two new human FGF, and the comparison of their deduced amino acid sequences with the known members of the FGF family. A similar analysis was done with FGFR sequences from invertebrate and vertebrate species. Phylogenetic trees inferred from the calculated genetic distances allow for hypotheses concerning the timing and functional significance of *FGF* and *FGFR* gene-family expansion and divergence.

Hypotheses for the Origin of the Topologies

Several hypotheses may explain the observed topologies. It is possible to individualize a few steps in a series of duplications from a common ancestor at the origin of the present-day members of the *FGF* family. The most recent steps gave rise to *FGF4* and *FGF6* and to *FGF10* and *FGF11. FGF1* and *FGF2* may have been created at a similar period, or slightly before *FGF4/FGF6* and *FGF10/FGF11* divergences. We call this step the phase of late duplications (Fig. 5). A more ancient series of duplications, occurring over a limited time period, led to the emergence of eight members—namely, *FGF3, FGF5, FGF7, FGF8, FGF9,* and the *FGF1/FGF2, FGF4/FGF6,* and *FGF10/FGF11* putative ancestors. The topology of the tree suggests that these eight members could have derived from a single gene; the emergence of several FGFs would have occurred during a phase of major genome expansion. The topology of the FGFR tree suggests the existence of an ancestral FGFR gene and only one phase of expansion, leading to four FGFR members as identified in vertebrates.

When did the FGF expansion occur with respect to the origin of the branch leading to nematodes?

The expansion could have occurred before the separation between protostomia and deuterostomia, and up to eight FGFs may be represented in nematodes. If this is the case, it could mean that other putative *ce-FGF* genes may exist and be related to a particular mammalian FGF. Interestingly, a number of genes from chromosome 17 in humans and chromosome III in *C. elegans* (Ruddle et al. 1994) are presumed to be homologs, suggesting that *ce-FGF* and *hs-FGF10* might be paralogs.

FGF/FGFR Expansion May Be Associated with the Origin of Vertebrates

Alternatively, the FGF expansion could have occurred after the protostomia/deuterostomia separation (Fig. 5). One can assume, from the fraction of sequenced genomic DNA and cDNAs from *C. elegans,* that about 30% of the total number of its genes are currently known (Wilson et al. 1994; Berks and The C. elegans Genome Mapping and Sequencing Consortium 1995; Hodgkin et al. 1995). Assuming both a random distribution of FGF-encoding genes throughout the *C. elegans* genome and good efficiency in the search strategies used here, this restricts the number of potential *FGF* genes in this species. It is thus unlikely that eight genes, orthologous to each *FGF* and to the *FGF1/FGF2, FGF4/FGF6,* and *FGF10/FGF11* ancestors, are present in *C. elegans.* A likely hypothesis, sufficient to explain the evolution of the FGF family, is that FGF expansion occurred after the separation of deuterostomia and protostomia and was contemporaneous with a phase of global gene duplications that took place during the period leading to vertebrate emergence (Holland et al. 1994). Confirmation of this hypothesis will await identification of more FGF sequences in the invertebrates.

The topology of the FGFR tree is strongly in support of this hypothesis if one assumes the two families have coevolved. The full complexity of the FGF receptor system already exists in amphibia (Thisse et al. 1995) and bony fish (Emori et al. 1992) as a probable coevolution with its ligand family, but not in insects or echinoderms. Current failure to identify any FGF in *Drosophila* could be due to technical reasons, or, alternatively, be due to an evolutionary process which has resulted in the loss or absence of this type of gene in insects. Insects are known to have evolved as a separate branch of the metazoan tree distant from chordates (Fig. 5). The fact that FGF receptors were found in *Drosophila* (Klämbt et al. 1992; Shishido et al. 1993) would appear to argue against the latter hypothesis but does not constitute definitive proof. In a similar manner, tyrosine kinase neurotrophin receptors have been identified in the fly, but their activation occurs by way of homophylic interaction and is independent of ligands (Pulido et al. 1992). In any case, the FGFRs characterized in *Drosophila* are different from the vertebrate FGFRs (Shishido et al. 1993). It is probable that the full complexity of the family, as it exists in mammals, is not developed in this species. Thus the low number or even complete absence of FGFs in insects is consistent with our hypothesis of FGF expansion associated with the origin of vertebrates.

It is interesting to note that FGFs play important roles in the development of the skeletal system, as shown by the characterization of mutations in their receptors in inherited human diseases. Mutations of FGFR1, FGFR2, and FGFR3 lead to disorders of the long bones and of the flat bones of the skull associated with achondroplasia and craniosynostosis, respectively (Muenke and Schell 1995; Wilkie et al. 1995). (It is interesting to note that FGFR4 behaves slightly differently from the other three receptors in several ways, including its topology in the tree and its noninvolvement in human inherited diseases.) Moreover, studies of limb bud growth and sclerotome formation have demonstrated the important role of FGFs in this process (Tickle 1995; Tanaka and Gann 1995; Grass et al. 1996). It is therefore tempting to speculate that expansion of the FGF/FGFR families is associated with the emergence of the vertebrate systems of motricity.

Possible Role for the Late Duplication Events

When did the phase of late duplications take place? Identification of FGF4 and FGF6 or of their ancestor in fishes, amphibia, and birds could set the time for this step, providing they represent all phyla, without loss through extinction.

The crucial role of FGF4 in limb bud development could suggest that the *FGF4–FGF6* duplication event originated as a consequence of the generation of the morphological novelty that is the tetrapod limb during the fin-to-limb transition (Nelson and Tabin 1995), and that it took place after the origin of the fish lineage. This hypothesis suggests that *FGF4* and *FGF6* orthologs may actually exist in amphibia, but this is not firmly established. The identification of four *FGF* genes in frog and bird and the analysis of the phylogenetic tree suggest that *FGF* genes orthologous to the mammalian *FGFs* exist in these species. Are there amphibian or avian genes orthologous to each mammalian gene or to only eight of them? In other words, did the last step of duplication, which created *FGF1* and *FGF2, FGF4* and *FGF6,* and *FGF10* and *FGF11,* occur before or after the origin of amphibia and birds? Four *FGF* genes have been isolated in frog (Isaacs et al. 1992). Two of these sequences, designated *xl-FGF4-I* and *xl-FGF4-II* and corresponding to pseudo-alleles, are highly related to both *FGF4* and *FGF6* (75–76% and 74–75% amino acid identity with hs-FGF4 and hs-FGF6, respectively). A growth factor, related to both FGF4 and FGF6, has been characterized in chicken (Niswander et al. 1994). Like the above-mentioned *Xenopus FGF, gd-FGF4* seems only slightly more related to *FGF4* than to *FGF6* (Table 1—80% and 78% identity with hs-FGF4 and hs-FGF6, respectively). Thus *FGF4* and *FGF6* orthologs may not exist in birds. It is thus possible to speculate that the FGF4/FGF6 duplication may have occurred after the separation of the bird/reptile branch. While the inferred tree groups together mammal and nonmammal FGF4 (Fig. 3A,B), it should be noted that this grouping has a very low bootstrap value (44%) and may not be significant. Interestingly, FGF4 function is essential for early postimplantation events in the mouse (Feldman et al. 1995), a role not relevant to birds or amphibia.

Table 3. Chromosomal localization of genes of the *FGF* family in humans and the mouse

	Chromosomal localization in:		
Gene	Humans	Mouse	References
FGF1	5q31–33	18	(Jaye *et al.* 1986; Cox *et al.* 1991)
FGF2	4q26–27	3 A2–B	(Lafage-Pochitaloff *et al.* 1990; Mattéi *et al.* 1992; Cox *et al.* 1991)
FGF3	11q13	7 F	(Casey *et al.* 1986; Peters *et al.* 1984)
FGF4	11q13	7 F	(Adélaïde *et al.* 1988; Peters *et al.* 1989)
FGF5	4q21	5 E1–F	(Nguyen *et al.* 1988; Mattéi *et al.* 1992)
FGF6	12p13	6 F3–G1	(Marics *et al.* 1989; deLapeyrière *et al.* 1990)
FGF7	15	2 F–G	(Kelley *et al.* 1992; Mattéi *et al.* 1995a)
FGF8	10q25–q26	19 C3–D	(White et al. 1995; Mattéi *et al.* 1995a)
FGF9	13q12	–	(Mattéi *et al.* 1995b)
FGF10	–	–	
FGF11	–	–	

The presence of chick *FGF1* and *FGF2* genes indicates that the duplication of *FGF1/FGF2* occurred before the origin of the bird/reptile lineage. *xl-FGF2* sequence is more related to hs-FGF2 (86%) than to hs-FGF1 (55%), and the presence of FGF1 protein has been reported in *X. laevis* (Shiurba et al. 1991). The *FGF1/FGF2* duplication may therefore have occurred even before the origin of amphibia. The late duplication phase could thus be subdivided into two events (Fig. 5), the *FGF1/FGF2* duplication having occurred before the origin of amphibia and the *FGF4/FGF6* duplication as late as after the separation between birds and mammals.

As only the human *FGF10* and *FGF11* genes have been identified, there are no data upon which to speculate as to the timing of the *FGF10/FGF11* duplication. The failure to have detected either of these closely related *FGFs* prior to the search of sequence databases may be more than coincidental and reflect somewhat different functions of these two new *FGFs.*

In humans and in the mouse, two species in which chromosomal localization of the *FGF* genes has been determined, *FGFs* are located on different chromosomes. As an exception, *FGF3* and *FGF4* are tandemly linked on chromosomal band 11q13 in humans and on chromosome 7 in the mouse (Table 3). It could be noted also that *FGF4* and *FGF6* on chromosomes 11 and 12 on the one hand, and *FGF1* and *FGF2* on chromosomes 4 and 5 on the other hand, are, respectively, on pairs of chromosomes that appear to contain paralogous genes and are thought to derive from each other (Lundin 1993). The identification of remnants of genome evolution may be explained by late events of duplication. These events could be related to the *FGF* late duplication phase.

An Integrated View of FGF/FGFR Function and Evolution

The presence of an *FGF* gene in *C. elegans* allows for the hypothesis of a scheme of evolution before and after the protostomia/deuterostomia separation.

The biological role of an FGF protein in nematodes is open to investigation. It could be involved in mesoderm induction and mesoderm development or positioning. Studies of mutant *FGFR* gene in *C. elegans* suggest *FGF/FGFR* could be associated with development or positioning of the muscle system (DeVore et al. 1995). It is tempting to speculate that *FGFs* are contemporary of triploblast phyla and may not exist in diploblast species (Fig. 5).

Among various possible roles during embryogenesis, recent studies have shown that FGFs are involved in migration and patterning during the formation of the skeletal system of mammals. Failure to infer a robust phylogenetic tree from available data suggests the *FGF* genes have evolved considerably since their separation from a common ancestor and may be explained by a series of duplications occurring early in evolution. This expansion in the number of *FGF* genes may have been an important determinant of skeletal system formation in vertebrates. A second and late phase of *FGF* duplications may be related to the establishment of improved signaling networks—also involving *BMP*, *WNT*, *Hedgehog*, and *HOX* family members—responsible for the fin-to-limb transition or to the split between ray- and lobe-finned bony fish. This scheme is reminiscent of the presumed evolution of the homeobox-containing *HOX* genes, during which the amplification of a single cluster was coincidental with the transition from invertebrate chordates to vertebrates and was followed by the acquisition of extra *HOX* genes in relation to the appearance of vertebrate head and limb. The *FGF* and *HOX* gene families could have coevolved, leading ultimately to a higher complexity. More generally, the expansion of families of genes is presumed to have coincided with metazoan radiation. Further identification of *FGF* and *FGFR* genes in other organisms and comparison of their protein sequences are necessary to help confirm or refute the above hypothesis.

Note Added in Proof

While this manuscript was in press, Itoh and co-workers reported the isolation of a new FGF gene in rat, which they called *Fgf10* [Yamasaki et al., *J. Biol Chem* (1966) **271**:15918–15921]. The two new human FGF genes described in our paper should therefore be designated *FGF11* and *FGF12*. FGF homologous factor (FHF)-1 to -4 genes have also been reported recently [Smallwood et al., *Proc Natl Acad Sci USA* (1996) **93**:9850–9857]. *FHF-3* and *FHF-1* correspond to *FGF11* and *FGF12* described here. We propose *FHF-2* and *FHF-4* be designated *FGF13* and *FGF14*, in agreement with the recommendation of the Nomenclature Committee [Baird et al., *Ann NY Acad Sci* (1991) **638**:xiii–xxvi].

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