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Developmental Expression Pattern

Isolation, genomic structure and developmental expression of *Fgf8* in the short-tailed fruit bat, *Carollia perspicillata*

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ABSTRACT Fibroblast growth factor-8 *(Fgf8)* encodes a secreted protein which was initially identified as the factor responsible for androgen-dependant growth of mouse mammary carcinoma cells (Tanaka *et al.*, 1992). *Fgf8* has been subsequently implicated in the patterning and growth of the gastrulating embryo, paraxial mesoderm (somites), limbs, craniofacial tissues, central nervous system and other organ systems during the development of several vertebrate model animals. Consistent with these findings, *Fgf8* is expressed in a complex and dynamic pattern during vertebrate embryogenesis. Here we report the isolation and characterization of a bat *(Carollia perspicillata) Fgf8* orthologue. Compared with those of other model vertebrates, *Carollia Fgf8* is conserved with respect to genomic structure, sequence and many domains of developmental expression pattern. Interestingly, the expression domain marking the apical ectodermal ridge of the developing limb shows a striking difference compared to that of mouse, consistent with evolutionary diversification of bat limb morphology.

KEY WORDS: *Chiroptera, Carollia perspicillata, Fgf8, genomic structure, gene expression*

The fibroblast growth factors (FGFs) are a family of related polypeptides that act to regulate growth, motility and differentiation of a diverse variety of cell-types and tissues. In mammals, 22 members of the FGF gene family (*Fgf1-Fgf23*) have been described to date (Ornitz and Itoh, 2001). The FGF gene family encodes proteins that share an internal core region containing 28 conserved and 6 identical amino acids, but vary in both sequence and size of their N- and C- termini. Most FGFs encode secreted ligands that signal to target cells through a family of high-affinity receptor tyrosine kinases, which act primarily through a conserved MAP Kinase-dependant signal transduction pathway (Eswarakumar *et al.*, 2005).

Fgf8 is expressed in a complex and dynamic pattern during embryogenesis and regulates growth and patterning of the brain, limbs, heart, kidney, ear, nose and eye (Boulet *et al.*, 2004, Crossley *et al.*, 1996a, Crossley *et al.*, 1996b, Kawauchi *et al.*, 2005, Lewandoski *et al.*, 2000, Meyers *et al.*, 1998, Perantoni *et al.*, 2005, Reifers *et al.*, 1998, Reifers *et al.*, 2000, Shanmugalingam *et al.*, 2000, Storm *et al.*, 2006, Sun *et al.*, 1999, Trumpp *et al.*, 1999). *Fgf8* activity is further regulated by alternative splicing and has been shown to be alternatively spliced in human, mouse, chick and Xenopus (Crossley and Martin, 1995, Fletcher *et al.*, 2006, Gemel *et al.*, 1996, Ghosh *et al.*, 1996, Haworth *et al.*, 2005, MacArthur *et al.*, 1995). The alternatively spliced mRNAs give rise to protein isoforms, differing in the length and/or sequence of the N terminus, several of which are known to have distinct activities *in vivo* (Blunt *et al.*, 1997, Fletcher *et al.*, 2006, MacArthur *et al.*, 1995, Olsen *et al.*, 2006, Song *et al.*, 2000).

A consequence to the rise of interest in evolution and development (Evo-Devo) is the realization that the use of a small number of traditional animal models is insufficient to gain a thorough understanding of the evolution of morphological diversity (Behringer *et al.*, 2006, Carroll, 2004, Eakin and Behringer, 2004). Making use of morphologically divergent alternative models is critical for elucidating the molecular mechanisms that underlie morphological differences between species. The over 1000 extant species of bats comprise the mammalian order Chiroptera (Neuweiler, 2000, Nowak, 1999, Simmons, 2001). Bats are unique among mammals because their limbs are strikingly modified into wings, making them the only mammals capable of powered flight.

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 # Note: NISC comparative sequencing program refers to a group author. The staff of the NISC comparative sequencing program are acknowledged for their construction of the Carollia genomic BAC library and sequencing of the Fgf8-containing BAC insert.

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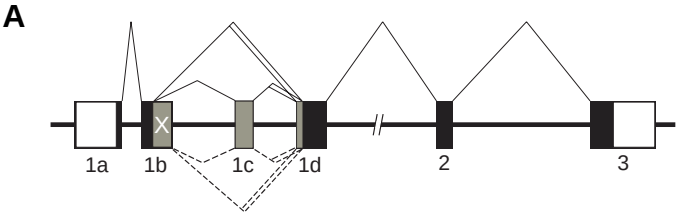
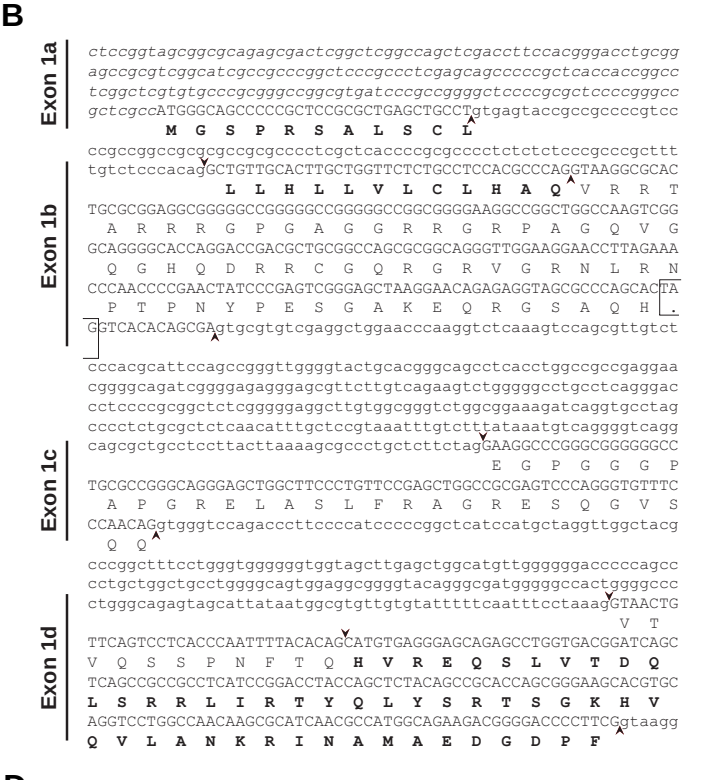
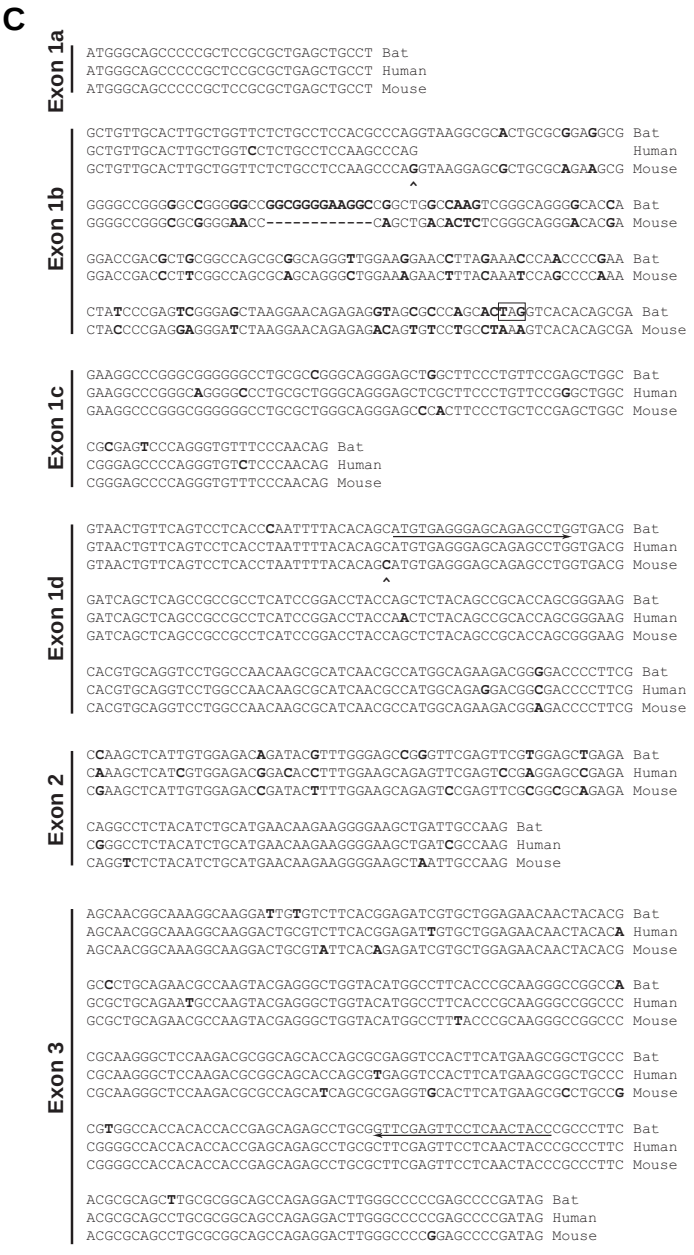
Many bat species are also unusual in that they navigate in darkness using echolocation. Echolocation in bats likely required modification of facial structures to emit focused ultrasonic sounds, modification of ears to receive sound reflections and modification of neural auditory processing centers to translate aural input into spatial information. Since *Fgf8* has been shown to be an important regulator of limb, craniofacial, ear and CNS development as well as a useful molecular marker for all of these tissues, we chose to identify and analyze the *Fgf8* gene of our bat model *Carollia perspicillata* (henceforth *Carollia*).

As a first step in isolating the bat *Fgf8* orthologue, we PCR amplified a *Carollia* embryo cDNA template using a set of primers designed against regions of near identity among mammalian *Fgf8* cDNA sequence alignments. One primer pair, FGF8-F3 and -R1, gave a robust product at the expected size of ~480 bp. Sequence analysis of the cloned product, CpFgf8F3R, revealed 94.6% and 94.2% identity to the corresponding region of human and mouse *Fgf8* cDNAs, respectively. This fragment corresponds to portions







of mouse and human *Fgf8* exons 1d, 2 and 3 which are common to all known mRNA isoforms (Crossley and Martin, 1995, Ghosh *et al.*, 1996, MacArthur *et al.*, 1995).

A probe generated from this cloned fragment was used to screen *Carollia* cDNA library and a *Carollia* genomic BAC library. Partial *Fgf8* cDNAs were isolated from the former, whose 5' ends were within the sequence contained in CpFgf8F3R1 but extended 3' an additional ~300 bp to a poly-A tail. Five *Fgf8*-containing BAC clones were identified from the latter and one clone was selected for sequencing (106,727 bp insert, Genbank accession # AC147851). Comparisons with available vertebrate genomes revealed that in addition to *Fgf8*, the BAC insert also contained two syntenic loci, *NPM3* and *MGEA5*. These comparisons were also used to determine the structure of the bat *Fgf8* coding region and to predict exon–intron junctions (Fig. 1).

Like those of other mammals, the bat *Fgf8* locus consists of 6 exons, designated exon 1a-d, 2 and 3 (Fig. 1A, B, C). Alignment of the DNA sequence of the predicted bat exons with those of human and mouse revealed a high degree of sequence conservation, 95% and 94% identity to human and mouse, respectively, for all of the potential coding sequences combined (Fig. 1C). The 3' untranslated region (UTR) sequence obtained from *Carollia Fgf8* partial cDNA clones shows 73% and 68% identity to human and mouse 3' UTRs, respectively and alignment of the *Carollia* genomic sequence immediately upstream of exon 1a with human and mouse*Fgf8* 5' UTRs reveals 76% and 69% identity, respectively (data not shown).

Predicted exons 1a, 1d, 2 and 3 of *Carollia Fgf8* encode amino acids that are 100% identical to the corresponding human and mouse proteins (Fig. 1D). *Carollia* exon 1c shows 3 differences in 29 amino acids encoded compared to human exon 1c and 5 differences compared to mouse exon 1c (Fig. 1D). *Carollia* and human exon 1b show a marked divergence compared to the corresponding mouse exon. Mouse exon 1b splices to downstream exons from two potential slice donors (indicated by arrowheads below the DNA sequence in Fig. 1B). If the first conserved splice donor in *Carollia Fgf8* is utilized, 11 of 12 amino acids are identical to human or mouse, but if the second splice donor is used the reading frame is blocked by an in-frame stop codon (boxed in Fig. 1B, C) and no functional FGF8 protein would be produced (Fig. 1D). In human *FGF8,* the reading frame of the longer alternative form of exon 1b is similarly blocked by in-frame stop codons (Gemel *et al.*, 1996). This data, combined with a lack of human cDNAs containing the long form of exon 1b, led to the conclusion that the human *FGF8* isoforms corresponding to mouse *Fgf8c, d, g* and *h* are not made (Gemel *et al.*, 1996, Ghosh *et al.*, 1996). In addition to the in-frame stop codon, the *Carollia* genomic sequence that corresponds to the longer form of mouse exon 1b is markedly less conserved than the rest of the gene, showing only 61% identity to mouse at the amino acid level, compared to ~98% amino acid identity for remaining coding sequence. It therefore seems likely that *Carollia*, like human, lacks the 4 potential *Fgf8* isoforms that include the longer form of exon 1b (dashed lines below the exons in Fig. 1A).

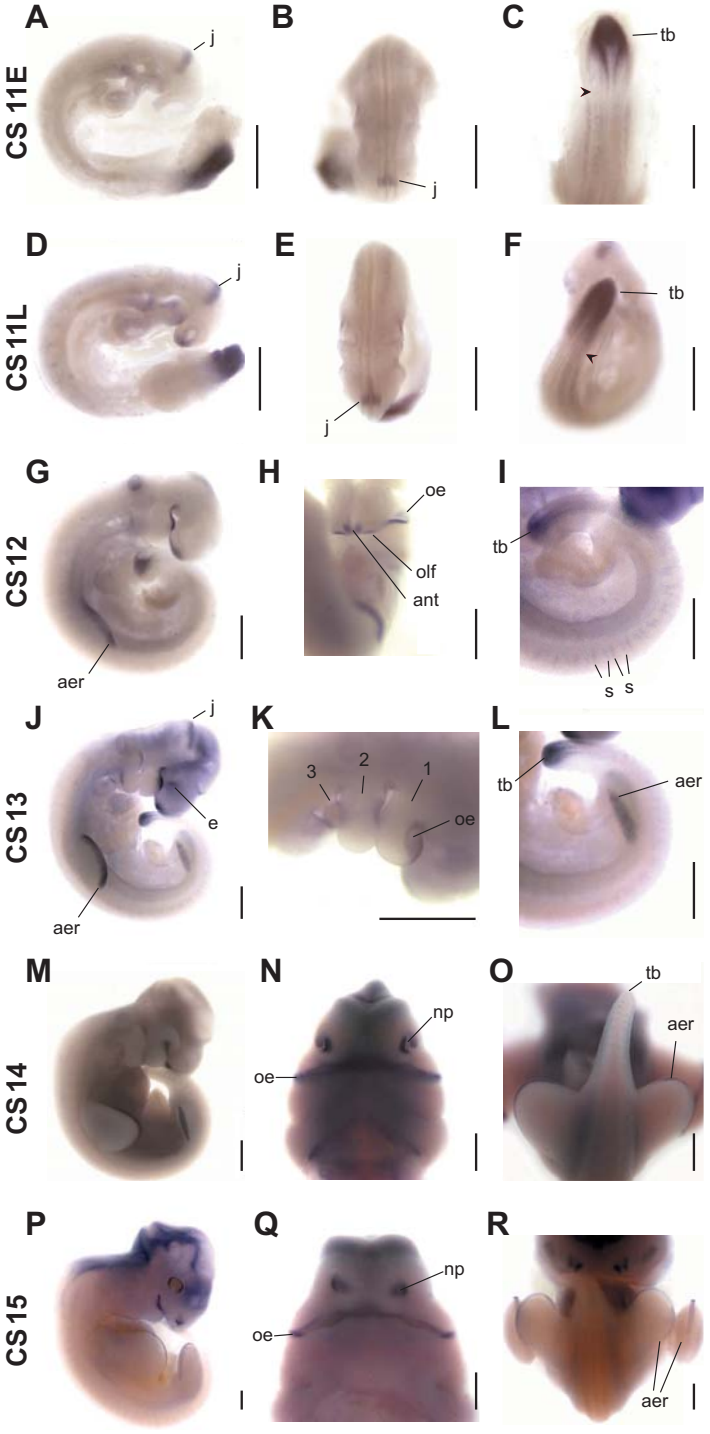
Fgf8 expression was examined in *Carollia* stage (CS) 11–15 embryos (Figs. 2, 3). The CS11 bat embryo is morphologically equivalent to a ~ 9 day (E9.0) mouse embryo. During the course of CS11, the developing bat embryo goes from 13 to 20 pair of trunk somites, the ventricles of the brain expand while the brain bends ventrally at the level of the midbrain to form the cranial flexure and the first two pharyngeal arches form (Cretekos *et al.*, 2005). The CS15 bat embryo is morphologically equivalent to a ~ E11.5 mouse embryo. During the course of CS15, the forelimb and hindlimb footplates form sequentially and the distal parts of the right and left first pharyngeal arch fuse at the midline to form the upper and lower jaws (Cretekos *et al.*, 2005).

At CS11, *Fgf8* mRNA is detected primarily in a posterior domain of the main body axis and in the anterior neural tube. In the posterior region, *Fgf8* is expressed in a graded fashion extending from the tailbud to an area about 2 somite-widths posterior to the nascent somite (nascent somite indicated by arrowhead in Fig. 2C, F). Expression in this domain is detected at least through late CS13 (Fig 2I, L). We have shown previously that the process of somitogenesis continues until 40 pairs of somites have formed at CS14 (Cretekos *et al.*, 2005), thus downregulation of expression in this domain is concurrent with the end of somite formation. *Fgf8* is not detected in newly-formed somites, but once the somites have differentiated *Fgf8* mRNA is detected in narrow stripes of lateral myotome cells located at the anterior and posterior margins of each somite (Fig. 2D, I).

Fgf8 expression was detected in four distinct domains in the developing anterior neural tube. From anterior to posterior, the first is at the anterior end, the second is in the region of the developing eyes, the third is along the ventral midline of the of the fore- and mid-brain and the last is in the border between the prospective midbrain and hindbrain (Fig. 2A, B, D, E, H, J). This latter expression domain is restricted to a narrow band of expressing cells in the region referred to as the midbrain-hindbrain

Fig. 1. Genomic structure and sequence of the *Carollia Fgf8* gene. (A) *Diagram of the predicted genomic structure; boxes represent exons, with open boxes indicating non-coding sequence, gray-filled boxes indicating variable isoform-specific coding sequence and black-filled boxes indicating coding sequence common to all known isoforms. Exon 1a and 1b are separated by a 92 bp intron; 1b and 1c by a 328 – 532 bp intron, depending on which of two possible splice donors is used; 1c and 1d by a 226 – 259 bp intron, depending on which of two possible splice acceptors is used. Exon 1d and 2 are separated by a ~3.2kb intron, whereas exon 2 and 3 are separated by a 866 bp intron.* **(B)** *The variable N-terminal region of mammalian FGF8 proteins is encoded by alternative splicing among exons 1b, 1c and 1d. The intron-exon junctions of* Carollia *exon 1a-d shown here were predicted by comparisons with published human and mouse genome sequences. The coding sequence is indicated by UPPERCASE, 5' untranslated sequence by* italic lowercase *and introns are in plain lowercase. Deduced amino acid sequence is shown below the coding sequence; amino acid sequence that is common to all known isoforms is indicated by* ***bold*** *text. Conserved splice donor and splice acceptor sites are indicated, respectively, by arrowheads below and above the DNA sequence. An in-frame stop codon in the putative long form of exon 1b is boxed.* **(C)** *The coding sequence of the 6 predicted exons compared with those of human and mouse. Sequence divergence is indicated by* ***bold*** *text. The conserved first splice donor site in exon 1b and alternative second splice acceptor site in exon 1d of mouse and human* Fgf8 *are indicated by carot symbols (^) below the sequence. An in-frame stop codon in the putative long form of exon 1b is boxed. The positions of PCR primers FGF8-F3 and R1 are indicated by arrow underlined text.* **(D)** *The deduced amino acid sequence of the 6 predicted exons is shown compared with those of human and mouse. Sequence divergence is indicated by* ***bold*** *text and the alternative longer forms of exons 1b and 1d are indicated by* italics.

junction or isthmus. This region has been shown to be an important organizing center for brain pattering and *Fgf8* expression is known to be critical for this function (Olsen *et al.*, 2006, Reifers *et al.*, 1998, Storm *et al.*, 2006). Expression of *Fgf8* in the midbrain-hindbrain junction was detected until at least CS14 (Fig. 2J and data not shown).



In addition to the expression domains in the developing brain, *Fgf8* mRNA is also detected in several other regions of the head. *Fgf8* is expressed in the pharyngeal arches, which give rise to many craniofacial tissues including jaws, teeth, tongue and outer ears. *Fgf8* expression is detected in the lateral regions of the pharyngeal arches, along the grooves that separate each arch (Fig. 2K). High level expression is found in the oral ectoderm of the first pharyngeal arch (Fig. 2H, K, N, Q; Fig. 3 G, H). *Fgf8* expression was also detected in the ectoderm covering the anterior forebrain in the region of the prospective olfactory placodes by late CS11 (Fig. 2D). By CS14, expression in this region is localized in the ectoderm of the nasal processes bracketing the nasal pits (Fig. 2N, Q; Fig. 3 G, H). At morphologically similar stages of mouse development, *Fgf8* expression in this region is continuous around the nasal pits (Bachler and Neubuser, 2001), whereas in *Carollia* we see separate medial and lateral domains (Fig. 3 G, H).

The appearance of the forelimb buds in a developing *Carollia* embryo marks the onset of CS12 and hindlimb buds appear one stage later (Cretekos *et al.*, 2005). *Fgf8* is detected in the presumptive apical ectodermal ridge (AER) of the forelimb buds during early CS12 and in the presumptive AER of the hindlimb buds in early CS13 (Fig. 2G, J, L). Expression in the AER persists until at least late CS15 (Fig. 2R). The AER has long been known to be a source of signals required for proximal-distal growth of the limb bud and *Fgf8* expression in the AER has been shown to be required, along with *Fgf4*, for survival and proliferation of the underlying mesoderm during limb bud outgrowth (Lewandoski *et al.*, 2000; Moon and Capecchi, 2000; Boulet *et al.*, 2004). The *Fgf8* expression domain in the bat forelimb AER appears relatively wide in the dorsal-ventral axis compared to that of mouse *Fgf8* at comparable stages (Fig. 3A, B). Transverse sections through forelimb buds of bat embryos at CS12 and 14 and mouse embryos at E9.5 and 10.5 reveal that the AER expression domain in bat is 2.7 times wider in CS12 bat embryos compared to E9.5 mouse embryos and 3 times wider in CS14 bat vs. E10.5 mouse (Fig. 3C, D, E, F and data not shown). The overall size of

Fig. 2. Analysis of *Fgf8* expression during *Carollia* development by whole-mount RNA *in situ* hybridization. *Lateral views of CS11 – 15 embryos are shown in A,D,G,J,M and P. Dorsal is to the left and anterior to top-right.* **(A,B,C)** *CS11 embryo with 14 somites shown in lateral view (A), dorsal view of the head (B) and tail (C), highlighting the midbrain-hindbrain junction expression stripe (j) and graded expression in the posterior (tb).* **(D,E,F)** *Lateral view (D) and dorsal view of the head (E) and tail (F) of an 18-somite CS11L (late) embryo.* **(G,H)** *CS12 embryo with 25 somites in lateral (G) and face-on view (H) showing expression at the anterior end of the neural tube (ant), olfactory (olf) and oral (oe) ectoderm.* **(I)** *A lateral view of the trunk and tail of a CS12 embryo with 26 somites, showing expression in narrow bands at the lateral edges of the differentiating somites (s),* **(J, K, L)** *CS13 embryo. (K) A higher magnification lateral view of the head at CS13 showing expression in the oral ectoderm (oe) and in the grooves between all of the pharyngeal arches (1, mandibular arch; 2, hyoid arch; 3, glossopharyngeal arch). (L) Lateral view of the trunk and tail of a CS13 embryo with 31 somites, showing expression in the apical ectodermal ridge (aer) of the hindlimb bud and the posterior domain of the tailbud (tb).* **(M,N,O)** *CS14 embryo. (N) Face-on view of the head at CS14 showing expression bracketing nasal pits (np) and oral ectoderm (oe). (O) High magnification view of the tail at CS14 showing down-regulation of posterior expression as the last somites form.* **(P,Q,R)** *CS15 embryo. (Q) Face-on view of the head at CS15 showing expression in nasal pits (np) and oral ectoderm (oe). (R) High magnification view of the tail at CS15 showing persistence of expression in fore- and hindlimb apical ectodermal ridges (aer). Scalebar, 0.5 mm in all panels.*

bat and mouse forelimb buds at CS12/E9.5 is similar, while at CS14/E10.5 the bat forelimb bud is somewhat larger in all dimensions. A novel domain of *Fgf8* expression in the interdigital mesenchyme between forelimb digits 2 - 5 during later stages of *Carollia* development has been previously described and been proposed to play a role in the persistence of these tissues for the formation of the chiropatagium (handwing) wing membrane (Weatherbee *et al.*, 2006).

Experimental Procedures

Identification of a Carollia Fgf8 *probe*

A probe for *Carollia Fgf8* was generated by PCR using primers FGF8-F3 (5'ATG TGA GGG AGC AGA GCC TG 3') and FGF8-R1 (5' GGT AGT TGA GGA ACT CGA AGC 3'), designed from regions of near identity in cDNA sequence alignments of human, mouse, rat and cow *Fgf8*. The 484 bp product was cloned into EcoRV-digested and T-tailed pBluescriptII KS (Stratagene) and confirmed by sequencing (CpFgf8F3R1, Genbank accession # EF035451).

Isolation of a Carollia Fgf8 *partial cDNA*

The above primer pair was also used to screen a fractionated *Carollia* embryo cDNA library. This library (CPMGE) was generated in λ-ZAPII (Stratagene) from oligo-dT primed cDNA prepared from poly-A selected RNA purified from 5 whole *Carollia* (CS14, 14L, 15, 16 and 17) embryos. The primary library was titered at 7.9 X 10^6 pfu/μg λ-arms. Two million recombinants of CPMGE were simultaneously amplified and fractionated into 94 sublibraries (CPMGE A1 - H10), each initiated with ~20,000 independent recombinant clones. Phagemid DNA was prepared from each sublibrary and arrayed into 96-well microtiter plates, leaving 2 wells open for positive and negative control DNA, such that the library can be initially screened by 96-well PCR. Sublibrary CPMGE D8 produced an ~480 bp product when amplified using FGF8F3/R1 primer pair. This sublibrary was plated and screened by plaque hybridization using a radiolabeled probe prepared from CpFgf8F3R1. Two hybridizing phagemid were plaque purified, "popped-out" into plasmid clones using eXassist helper phage (Stratagene) and sequenced (Genbank accession #s EF035452 and EF035453)

Isolation and sequencing of the Carollia Fgf8 *genomic locus*

The CpFgf8F3R1 radiolabeled probe was also used to screen an arrayed *Carollia* genomic BAC library (E.D.G., unpublished). 5 independent hybridizing BAC clones were confirmed by PCR with the FGF8DF3/R1 primer pair and subjected to insert end sequencing. BAC-end sequences were aligned to the human genome using BLAST (www.ncbi.nlm.nih.gov) and BAC clone 3O14 was selected for shotgun sequencing by the NIH Intramural Sequencing Center (www.nisc.nih.gov) (106,727 bp insert, Genbank accession # AC147851).

In situ *hybridization and histology*

Whole-mount *in situ* hybridization (ISH) was performed as described previously (Chen *et al.*, 2005) on *Carollia* embryos with the following modifications: *Carollia* embryos were treated with proteinase K (Roche) 2 min for CS11, 3 min for CS11L, 4 min for CS12, 6 min for CS13, 15 min for CS14 and 20 min for CS15. An antisense riboprobe against *Carollia Fgf8* was generated by digesting CpFgf8F3R1 with EcoRI followed by transcription with T7 RNA polymerase in the presence of digoxigenin-11-UTP (Roche). This probe was used at a final concentration of 0.5 - 1 μg/ml. Hybridization and post-hybridization washes were carried out at 65 - 70° C. The *Carollia* embryos used in this study were collected from a wild population on the island of Trinidad, as previously described (Chen *et al.*, 2005, Cretekos *et al.*, 2005) and staged according to Cretekos *et al.* (2005). Whole-mount *in situ* hybridization (ISH) on mouse embryos was performed as described previously (Wilkinson, 1992), using an antisense riboprobe transcribed from a mouse *Fgf8* cDNA template (provided by R. L. Johnson). Some specimens were embedded and processed for cryostat sectioning after whole-mount ISH (Nagy *et al.*, 2003).

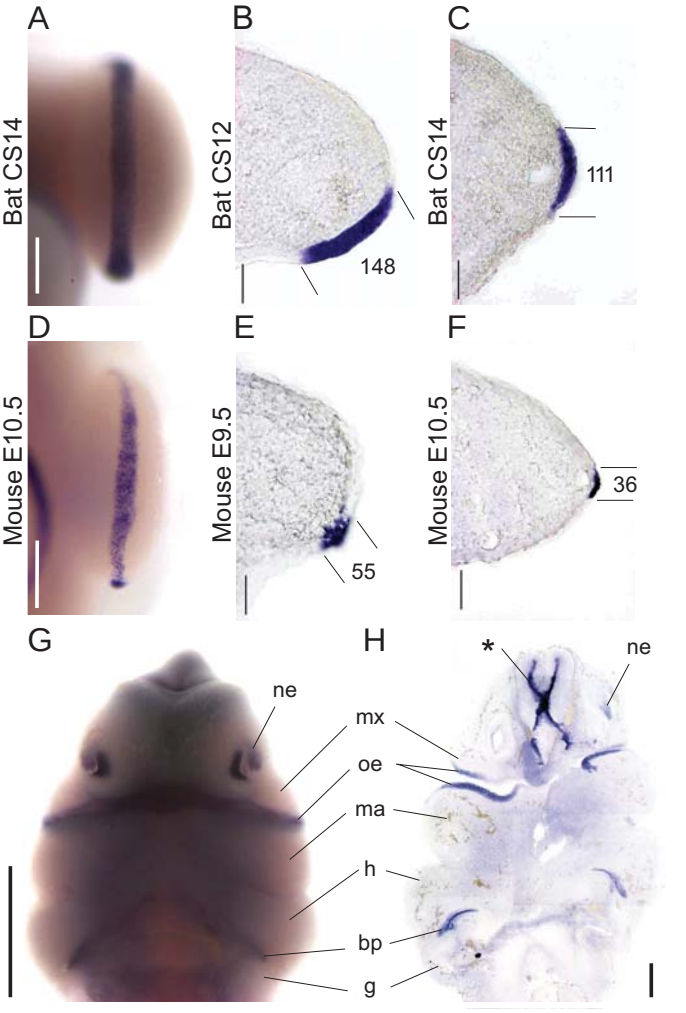


Fig. 3. Detailed analysis of *Fgf8* expression. (A,B,C) Fgf8 in situ *of* Carollia *forelimb buds;* **(D,E,F)** *comparable views of* Fgf8 in situ *of mouse forelimb buds at similar stages.* **(A,D)** *Edge-on views of bat CS14 (A) and mouse E10.5 (D) distal forelimb buds, anterior is to the top and scalebar = 0.5 mm.* **(B,E)** *Transverse sections through the central region of bat CS12 (B) and mouse E9.5 (E) forelimb buds, dorsal is to the top and anterior is into the page, scalebar = 50 μm.* **(C,F)** *Transverse sections through the distal tip of bat CS14 (C) and mouse E10.5 (F) forelimb buds, dorsal is to the top and anterior is into the page; scalebar, 50 μm. Measured width of the AER is shown in panels (B, C, E and F).* **(G,H)** *Face-on view (G) and montage image of frontal sections (H), showing* Fgf8 *expression in craniofacial tissues at CS14. Anterior is to the top, bp, branchial pouch; g, glossopharyngeal arch; h, hyoid arch; ma, mandible; mx, maxilla; ne, nasal epithelium; oe, oral ectoderm; * indicates a probe trapping artifact in the forebrain ventricle. Scalebar, 0.5 mm in panel (G) and 200 μm in panel (H).*

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References

BACHLER, M. and NEUBUSER, A. (2001). Expression of members of the fgf family and their receptors during midfacial development. *Mech. Dev.* 100: 313-6.

BEHRINGER, R.R., EAKIN, G.S. and RENFREE, M.B. (2006). Mammalian diversity: Gametes, embryos and reproduction. *Reprod. Fert. Dev.* 18: 99-107.

BLUNT, A.G., LAWSHE, A., CUNNINGHAM, M.L., SETO, M.L., ORNITZ, D.M. and MACARTHUR, C.A. (1997). Overlapping expression and redundant activation of mesenchymal fibroblast growth factor (fgf) receptors by alternatively spliced fgf-8 ligands. *J. Biol. Chem.* 272: 3733-8.

BOULET, A.M., MOON, A.M., ARENKIEL, B.R. and CAPECCHI, M.R. (2004). The roles of fgf4 and fgf8 in limb bud initiation and outgrowth. *Dev. Biol.* 273: 361-72.

CARROLL, S.B., GRENIER, J. K. & WEATHERBEE, S. D. (2004). *From DNA to diversity: Molecular genetics and the evolution of animal design*. Blackwell Science, Malden, Massachusetts.

CHEN, C.H., CRETEKOS, C.J., RASWEILER, J.J. 4th and BEHRINGER, R.R. (2005). Hoxd13 expression in the developing limbs of the short-tailed fruit bat, carollia perspicillata. *Evo. Develop.* 7: 130-41.

CRETEKOS, C.J., WEATHERBEE, S.D., CHEN, C.H., BADWAIK, N.K., NISWANDER, L., BEHRINGER, R.R. and RASWEILER, J.J. 4th (2005). Embryonic staging system for the short-tailed fruit bat, carollia perspicillata, a model organism for the mammalian order chiroptera, based upon timed pregnancies in captive-bred animals. *Dev. Dynam.* 233: 721-38.

CROSSLEY, P.H. and MARTIN, G.R. (1995). The mouse fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. *Development* 121: 439-51.

CROSSLEY, P.H., MARTINEZ, S. and MARTIN, G.R. (1996a). Midbrain development induced by fgf8 in the chick embryo. *Nature* 380: 66-8.

CROSSLEY, P.H., MINOWADA, G., MACARTHUR, C.A. and MARTIN, G.R. (1996b). Roles for fgf8 in the induction, initiation and maintenance of chick limb development. *Cell* 84: 127-36.

EAKIN, G.S. and BEHRINGER, R.R. (2004). Diversity of germ layer and axis formation among mammals. *Semin. Cell & Dev. Biol.* 15: 619-29.

ESWARAKUMAR, V.P., LAX, I. and SCHLESSINGER, J. (2005). Cellular signaling by fibroblast growth factor receptors. *Cytokine & Growth Factor Reviews* 16: 139-49.

FLETCHER, R.B., BAKER, J.C. and HARLAND, R.M. (2006). Fgf8 spliceforms mediate early mesoderm and posterior neural tissue formation in xenopus. *Development* 133: 1703-14.

GEMEL, J., GORRY, M., EHRLICH, G.D. and MACARTHUR, C.A. (1996). Structure and sequence of human fgf8. *Genomics* 35: 253-7.

GHOSH, A.K., SHANKAR, D.B., SHACKLEFORD, G.M., WU, K., T'ANG, A., MILLER, G.J., ZHENG, J. and ROY-BURMAN, P. (1996). Molecular cloning and characterization of human fgf8 alternative messenger rna forms. *Cell Growth & Differentiation* 7: 1425-34.

HAWORTH, K.E., HEALY, C. and SHARPE, P.T. (2005). Characterisation of the genomic structure of chick fgf8. *DNA Sequence* 16: 180-6.

KAWAUCHI, S., SHOU, J., SANTOS, R., HEBERT, J.M., MCCONNELL, S.K., MASON, I. and CALOF, A.L. (2005). Fgf8 expression defines a morphogenetic center required for olfactory neurogenesis and nasal cavity development in the mouse. *Development* 132: 5211-23.

LEWANDOSKI, M., SUN, X. and MARTIN, G.R. (2000). Fgf8 signalling from the aer is essential for normal limb development. *Nat. Genetics* 26: 460-3.

MACARTHUR, C.A., LAWSHE, A., XU, J., SANTOS-OCAMPO, S., HEIKINHEIMO, M., CHELLAIAH, A.T. and ORNITZ, D.M. (1995). Fgf-8 isoforms activate receptor splice forms that are expressed in mesenchymal regions of mouse development. *Development* 121: 3603-13.

MEYERS, E.N., LEWANDOSKI, M. and MARTIN, G.R. (1998). An fgf8 mutant allelic series generated by cre- and flp-mediated recombination. *Nat. Genetics* 18: 136-41.

MOON, A.M. and CAPECCHI, M.R. (2000). Fgf8 is required for outgrowth and patterning of the limbs. *Nat Genet.* 26: 455-9.

NAGY, A., GERTSENSTEIN, M., VINTERSTEN, K. and BEHRINGER, R. (2003). *Manipulating the mouse embryo: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

NEUWEILER, G. (2000). *The biology of bats*. Oxford University Press, New York.

NOWAK, R. (1999). *Walker's mammals of the world*. The Johns Hopkins University Press, Baltimore.

OLSEN, S.K., LI, J.Y., BROMLEIGH, C., ELISEENKOVA, A.V., IBRAHIMI, O.A., LAO, Z., ZHANG, F., LINHARDT, R.J., JOYNER, A.L. and MOHAMMADI, M. (2006). Structural basis by which alternative splicing modulates the organizer activity of fgf8 in the brain. *Genes Dev.* 20: 185-98.

ORNITZ, D.M. and ITOH, N. (2001). Fibroblast growth factors. *Genome Biology* 2: REVIEWS3005.

PERANTONI, A.O., TIMOFEEVA, O., NAILLAT, F., RICHMAN, C., PAJNI-UNDERWOOD, S., WILSON, C., VAINIO, S., DOVE, L.F. and LEWANDOSKI, M. (2005). Inactivation of fgf8 in early mesoderm reveals an essential role in kidney development. *Development* 132: 3859-71.

REIFERS, F., BOHLI, H., WALSH, E.C., CROSSLEY, P.H., STAINIER, D.Y. and BRAND, M. (1998). Fgf8 is mutated in zebrafish acerebellar (ace) mutants and is required for maintenance of midbrain-hindbrain boundary development and somitogenesis. *Development* 125: 2381-95.

REIFERS, F., WALSH, E.C., LEGER, S., STAINIER, D.Y. and BRAND, M. (2000). Induction and differentiation of the zebrafish heart requires fibroblast growth factor 8 (fgf8/acerebellar). *Development* 127: 225-35.

SHANMUGALINGAM, S., HOUART, C., PICKER, A., REIFERS, F., MACDONALD, R., BARTH, A., GRIFFIN, K., BRAND, M. and WILSON, S.W. (2000). Ace/fgf8 is required for forebrain commissure formation and patterning of the telencephalon. *Development* 127: 2549-61.

SIMMONS, N. (2001). Reassessing bat diversity: How many species are there in the world? *Bat Res News* 179-180.

SONG, Z., POWELL, W.C., KASAHARA, N., VAN BOKHOVEN, A., MILLER, G.J. and ROY-BURMAN, P. (2000). The effect of fibroblast growth factor 8, isoform b, on the biology of prostate carcinoma cells and their interaction with stromal cells. *Cancer Res.* 60: 6730-6.

STORM, E.E., GAREL, S., BORELLO, U., HEBERT, J.M., MARTINEZ, S., MCCONNELL, S.K., MARTIN, G.R. and RUBENSTEIN, J.L. (2006). Dose-dependent functions of fgf8 in regulating telencephalic patterning centers. *Development* 133: 1831-44.

SUN, X., MEYERS, E.N., LEWANDOSKI, M. and MARTIN, G.R. (1999). Targeted disruption of fgf8 causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev.* 13: 1834-46.

TANAKA, A., MIYAMOTO, K., MINAMINO, N., TAKEDA, M., SATO, B., MATSUO, H. and MATSUMOTO, K. (1992). Cloning and characterization of an androgen-induced growth factor essential for the androgen-dependent growth of mouse mammary carcinoma cells. *Proc. Natl. Acad. Sci. USA* 89: 8928-32.

TRUMPP, A., DEPEW, M.J., RUBENSTEIN, J.L., BISHOP, J.M. and MARTIN, G.R. (1999). Cre-mediated gene inactivation demonstrates that fgf8 is required for cell survival and patterning of the first branchial arch. *Genes Dev.* 13: 3136-48.

WEATHERBEE, S.D., BEHRINGER, R.R., RASWEILER, J.J. 4th and NISWANDER, L.A. (2006) Interdigital webbing retention in bat wings illustrates genetic changes underlying amniote limb diversification.*Proc Natl Acad Sci USA.* 103(41):15103-7.

WILKINSON, D.G. (1992). *In situ hybridization: A practical approach*. Oxford University Press, New York.

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"I do not understand my images and anyone is free to understand them as he wishes. I have only tried to depict my own dreams..." J.M. Folon. This neo-surrealist painting entitled *"Invasion"* by Jean-Michel Folon (1934, Brussels, Belgium), with its swirling components against a stable background can be considered as a representation of avid cells breaking through the basement membrane to invade the adjacent stroma. With kind permission from J.-M. Folon (2004)

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