

Expression of Fgf Receptors 1, 2, and 3 in the Developing Mid- and Hindbrain of the Mouse

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Fibroblast growth factor 8 (FGF8) mediates the function of the midbrain–hindbrain organizer (MHO). FGF signals are transmitted by means of four known FGF receptors (FGFRs). Studies of *Fgfr* expression in early vertebrate development have shown that *Fgfr1* is expressed along the entire neural tube, whereas *Fgfr2* and *Fgfr3* expression has been shown to spare the tissue adjacent to the MHO. The FGF8 signal from the MHO, therefore, was believed to be transmitted by FGFR1 exclusively. However, incongruent results from conditional mutants of *Fgf8* and *Fgfr1* in the midbrain–hindbrain (MHB) region contradict this hypothesis. Therefore, we reexamined the expression of the *Fgf*s in this region. *Fgfr1* is expressed all over the neural tube. Strikingly, *Fgfr2* is expressed throughout the floor plate of the MHB region. In the basal plate, *Fgfr2* directly abuts the *Fgf8* expression domain at the MHO, anteriorly and posteriorly. *Fgfr3* expression is in contact with the *Fgf8* expression domain only in the rostroventral hindbrain. Based on these findings, we postulate a role for FGFR2 and FGFR3 in FGF signaling in the ventral midbrain and hindbrain. *Developmental Dynamics* 233:1023–1030, 2005. © 2005 Wiley-Liss, Inc.

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INTRODUCTION

Patterning and development of the mid- and hindbrain (MHB) depends on the midbrain–hindbrain organizer (MHO), located at the boundary between these two regions. A variety of transcription factors (e.g., *Otx2*, *Gbx2*, *Pax2*, *Pax5*, *En1*, *En2*) and secreted molecules (e.g., *Fgf8*, *Wnt1*, *Shh*) is expressed at this MHB boundary, and their mutual interactions are responsible for the correct development of the MHB region (for review, see Wurst and Bally-Cuif, 2001; Raible and Brand, 2004).

One of these molecules is the secreted fibroblast growth factor 8 (FGF8), which has organizer activity on its own. Gain-of-function studies have shown that FGF8 is sufficient to induce expression of midbrain/rhombomere 1 (*r1*) genes at ectopic positions (Liu et al., 1999) and is also sufficient to induce the formation of ectopic midbrain and cerebellar structures (Martinez et al., 1999). *Fgf8* conditional knockouts, in which region-specific inactivation of *Fgf8* is achieved by the expression of the Cre recombinase in the *En1* locus, lack the

midbrain and the anterior hindbrain, including ventral structures (Chi et al., 2003). This finding clearly substantiates that FGF8 is not only sufficient but also necessary for normal development of the MHB region. Two other FGFs highly related to FGF8, FGF17 and FGF18, also have been shown to be involved in development of the MHB region. Both FGF17 and FGF18 are involved in the regulation of progenitor cell proliferation (Xu et al., 2000; Liu et al., 2003).

FGFs exert their function by means of high-affinity receptors. These FGF

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receptors (FGFRs) belong to the tyrosine kinase family of receptors and comprise four proteins (FGFR1–4). FGF receptors are single transmembrane glycoprotein receptors containing three Ig-like loops in the extracellular domain and a split tyrosine kinase intracellular domain (for a review, see Powers et al., 2000; Reuss and von Bohlen und Halbach, 2003). It has been shown by *in vitro* studies that FGF8 can bind to the four FGF receptors with different affinities (FGFR4 = FGFR3 > FGFR2 > FGFR1), with FGFR1 showing almost no binding (MacArthur et al., 1995; Blunt et al., 1997; Xu et al., 2000). However, the binding of FGFs to their receptors has been shown to be modified by cell type-specific heparan sulfates *in vivo* (Kan et al., 1999). Thus, the affinity of FGF8 to the four FGF receptors might differ between the *in vitro* and the *in vivo* situation.

Several studies report on the expression patterns of *Fgf* receptors in different species and at different time points of development. There have been studies in the developing *Xenopus* (Golub et al., 2000), zebrafish (Thisse et al., 1995; Carl and Wittbrodt, 1999; Tonou-Fujimori et al., 2002), chicken (Heuer et al., 1990; Orr-Urtreger et al., 1993; Wilke et al., 1997; Walshe and Mason, 2000), and rat (Wanaka et al., 1991) central nervous system (CNS).

In mouse embryos, expression studies concentrated on certain developmental stages or regions of the neural tube (Orr-Urtreger et al., 1991; Yamaguchi et al., 1992; Peters et al., 1993; Ozawa et al., 1996; Bansal et al., 2003), some of them reporting on the expression of *Fgfrs* in the MHB region (Ishibashi and McMahon, 2002; Liu et al., 2003; Trokovic et al., 2003).

Taken together, it was shown in zebrafish, chicken, and mouse embryos that *Fgfr1* is expressed throughout the MHB region, whereas *Fgfr2* is sparing the MHO and surrounding tissues. *Fgfr3* was found in the neuroepithelium of the diencephalon and caudal hindbrain, whereas the midbrain and anterior hindbrain appeared to be completely devoid of *Fgfr3* expression. The gap in expression around the MHO seemed even larger for *Fgfr3* compared with *Fgfr2* expression. *Fgfr4* transcripts were found in the developing

mouse CNS by reverse transcriptase-polymerase chain reaction (RT-PCR; Cool et al., 2002), but no detailed expression analysis is available. Based on these *in vivo* expression patterns, despite the contradictory results of the *in vitro* binding assays, it was believed that the receptor transmitting the FGF8 signal in the MHB region is the FGFR1.

To test this hypothesis, a conditional knockout of *Fgfr1* was created, leading to an inactivation of *Fgfr1* in the *En1* domain (i.e., in the caudal mid- and rostral hindbrain; Trokovic et al., 2003). Mutant mice lack the inferior colliculi and the vermis of the cerebellum, structures of the dorsal mid- and hindbrain, respectively. However, no loss of ventral tissues was detected. This phenotype is in sharp contrast to the conditional *Fgf8* knockout where *Fgf8* is inactivated in the same region. In this *Fgf8* mutant, the dorsal as well as most of the ventral midbrain and anterior hindbrain are missing, strongly suggesting that the other FGF receptors must be involved in FGF signal transduction at the ventral MHO. Therefore, this study was performed to revisit the expression patterns of the *Fgf* receptors *Fgfr1–4* in early mouse embryonic stages, with special emphasis on the ventral MHB.

RESULTS

The expression of *Fgfr1*, *Fgfr2*, and *Fgfr3* was studied in the MHB region and was analyzed from embryonic day (E) 8.5 until E12.5 in comparison to the expression of *Fgf8* and the MHB marker *En1*. *Fgf8* is known to be expressed in the anterior r1 from E8.0 on, whereas from E9.5 on, it becomes restricted to a sharp band in the anteriormost part of r1 (Crossley and Martin, 1995). *En1* is expressed across the MHB boundary in the caudal midbrain and anterior r1 (Davis and Joyner, 1988). Using both markers on consecutive sections hybridized with the *Fgf* receptors allowed us to map precisely the extent of expression of the *Fgfrs* in this region. *Fgfr4* is not expressed in the developing mouse MHB region (our own data) and, therefore, was not analyzed further.

Fgfr1 Is Expressed Throughout the Neural Tube

At E8.5, *Fgfr1* is weakly expressed all over the CNS, including the floor plate and basal plate of the MHB. The *Fgfr1* expression domain is overlapping with the expression of *En1* (Fig. 1A–C). At all stages examined, *Fgfr1* also overlaps with the expression of *Fgf8* (Fig. 1D–F and data not shown). This widespread expression of *Fgfr1* in the mouse CNS is maintained throughout later stages.

Fgfr2 Is Expressed in the Ventral Midbrain and in the Floor Plate

Fgfr2 expression at E8.5 covers most of the embryonic neuroectoderm (Fig. 2C,G). Using *En1* as a marker for the midbrain and r1, we found that *Fgfr2* overlaps with the *En1* expression domain in the midbrain but not in the anterior hindbrain. Instead, there is a small gap between the *En1* expression in the anterior hindbrain and the *Fgfr2* expression in the caudal hindbrain (Fig. 2B–D). In relation to *Fgf8* expression, we found that the *Fgfr2* anteriorly abuts *Fgf8* expression. Posteriorly, it does not reach the *Fgf8* expression domain (Fig. 2F–H). Therefore, *Fgfr2* at E8.5 is expressed in the midbrain and in the posterior part of the hindbrain, exhibiting a gap in r1.

By E9.5, *Fgfr2* expression still shows a gap. In the basal plate of the caudal midbrain and of the anterior r1, this gap now exactly abuts the *Fgf8* expression domain. In the alar and roof plate of the midbrain, *Fgfr2* expression retracts from the *Fgf8* expression domain. Therefore, dorsally, *Fgfr2* is not in contact with *Fgf8*, which itself has a broader expression domain in the dorsal hindbrain (Fig. 2I–N). In contrast to this dorsal gap in expression, *Fgfr2* is expressed continuously in the floor plate throughout the mid- and hindbrain. Of interest, *Fgfr2* expression in the ventral midline complements the gap in expression of *Fgf8* in the floor plate (Figs. 2L–N, 3). At E9.5, this very specific *Fgfr2* expression is weak and is seen in cells lining the lumen of the neural tube (Fig. 3D,H,L).

At E11.5, expression of *Fgfr2* in the floor plate of the MHO is not restricted to ventricular cells anymore but ex-

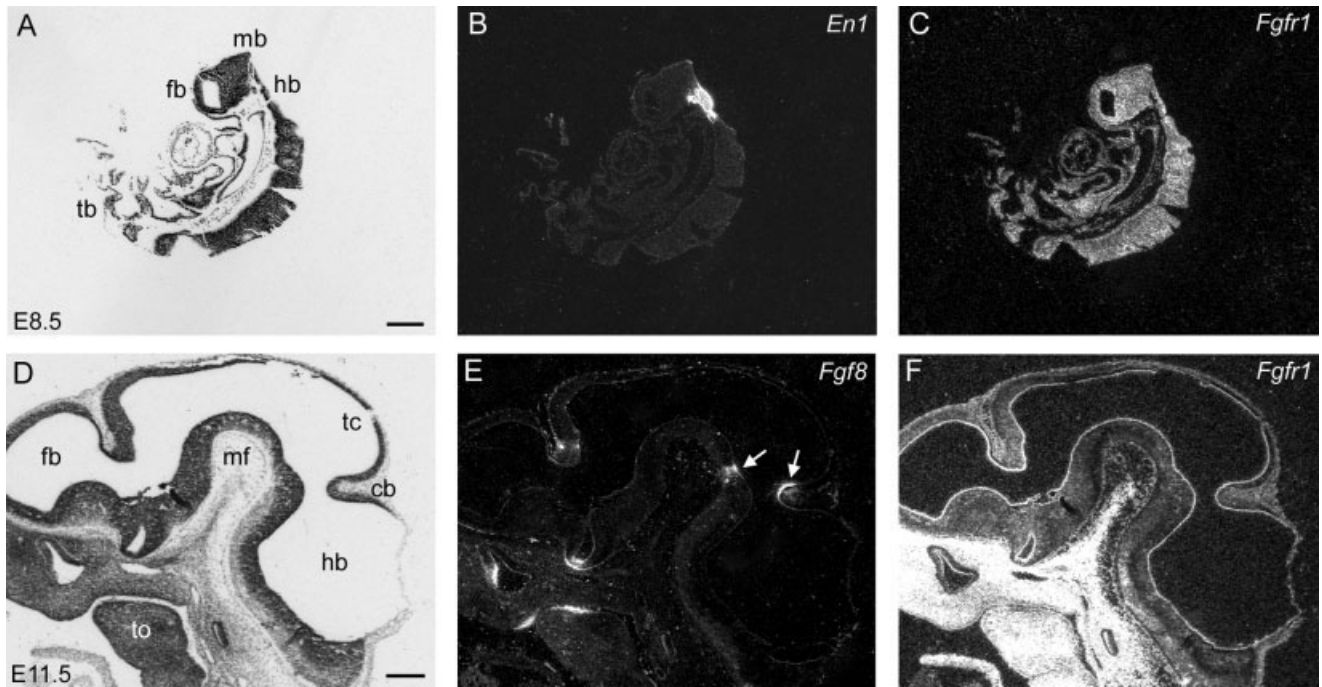


Fig. 1. A–F: *Fgfr1* is ubiquitously expressed in the developing mouse neural tube and overlaps with expression of *En1* and *Fgf8* in the midbrain–hindbrain. In situ analysis was performed on sagittal sections of E8.5 (A–C) and E11.5 (D–F) embryos with radioactive antisense probes for *Fgfr1* (C,F), *En1* (B), and *Fgf8* (E). A,D: Brightfield images of the sections shown in C and F, respectively. E: The arrows indicate the *Fgf8* expression domain at the midbrain–hindbrain organizer. Anterior is to the left. fb, forebrain; hb, hindbrain; mb, midbrain; tb, tail bud; cb, cerebellar anlage; mf, mesencephalic flexure; tc, tectum; to, tongue. Scale bars in A,B = 250 μ m (applies to A–F).

tends throughout the thickness of the neuroepithelium (Fig. 2S,T). *Fgf8* expression at the MHB boundary is restricted to a narrow ring at E11.5, and expression of *Fgfr2* in the basal plate follows this contraction of the *Fgf8* domain, leaving only a small gap at the MHB boundary, which corresponds to the *Fgf8*-positive tissue (Fig. 2O–Q). Also, the extent of the dorsal gap changes. By E12.5, this gap has narrowed, so that only the most caudal part of the midbrain and the rostral part of the cerebellar anlage are free of *Fgfr2* (data not shown).

Fgfr3 Is Expressed in the Ventral Hindbrain

In contrast to *Fgfr2*, *Fgfr3* expression does not overlap with the *En1* expression domain at E8.5 (Fig. 4B–D). The gap formed by the *Fgfr3* expression corresponds to the *En1* expression domain, indicating that *Fgfr3* expression completely spares the midbrain and the anterior hindbrain (Fig. 4D). This finding is supported by the fact that *Fgfr3* does abut the posterior border of *Fgf8* expression in the hindbrain (Fig. 4H).

At E9.5 and E10.5, ventral *Fgfr3* expression closes in and slightly overlaps with the borders of *En1* expression in the midbrain and hindbrain. Caudally, it abuts the *Fgf8* domain in r1, which is contracting at the MHB boundary (Fig. 4M). From this stage on (E10.5–E12.5), expression of *Fgfr3* advances toward the MHB boundary in the ventricular layer of the neuroepithelium, thereby narrowing the gap in ventral expression (Fig. 4K,P). This advancement of *Fgfr3* expression correlates with a progressive exclusion of *En1* from the ventricular zone of the ventral neural tube in the anterior midbrain and in the hindbrain. Thus, between E8.5 and E12.5, *Fgfr3* expression advances from the diencephalon into the midbrain.

By E12.5, only the caudal-most part of the midbrain is devoid of *Fgfr3* in the basal plate and floor plate, leaving a gap of only a few cell diameters anterior to the *Fgf8* signal (Fig. 4N–R). In the hindbrain, expression of *Fgfr3* now overlaps with the *Fgf8* expression domain (Fig. 4O,Q,R). Furthermore, only the ventricular zone of the midbrain and anterior hindbrain ex-

presses *Fgfr3* at E12.5, which corresponds to weaker expression of *En1* in these cells compared with the rest of the neuroepithelium (Fig. 4N–P).

In contrast, in the dorsal MHB region (alar- and roof plate) from E9.5 on, *Fgfr3* displays a gap extending from the caudal half of the midbrain until the caudal end of the hindbrain. The anterior to posterior (A/P) extent of this gap in the dorsal midbrain and hindbrain remains unchanged until E12.5. In the dorsal to ventral (D/V) axis, *Fgfr3* expression gradually expands further dorsal into the alar plate of the caudal midbrain between E9.5 and E12.5 (Fig. 5 and data not shown).

Taken together, we show that *Fgfr1* is weakly expressed throughout the MHB region during all stages examined. In contrast, *Fgfr2* and *Fgfr3* show dynamic and distinct expression patterns in this region (Fig. 5).

DISCUSSION

FGF8 is the key signal mediating the activity of the MHO located at the MHB boundary (reviewed in Wurst and Bally-Cuif, 2001). Two other re-

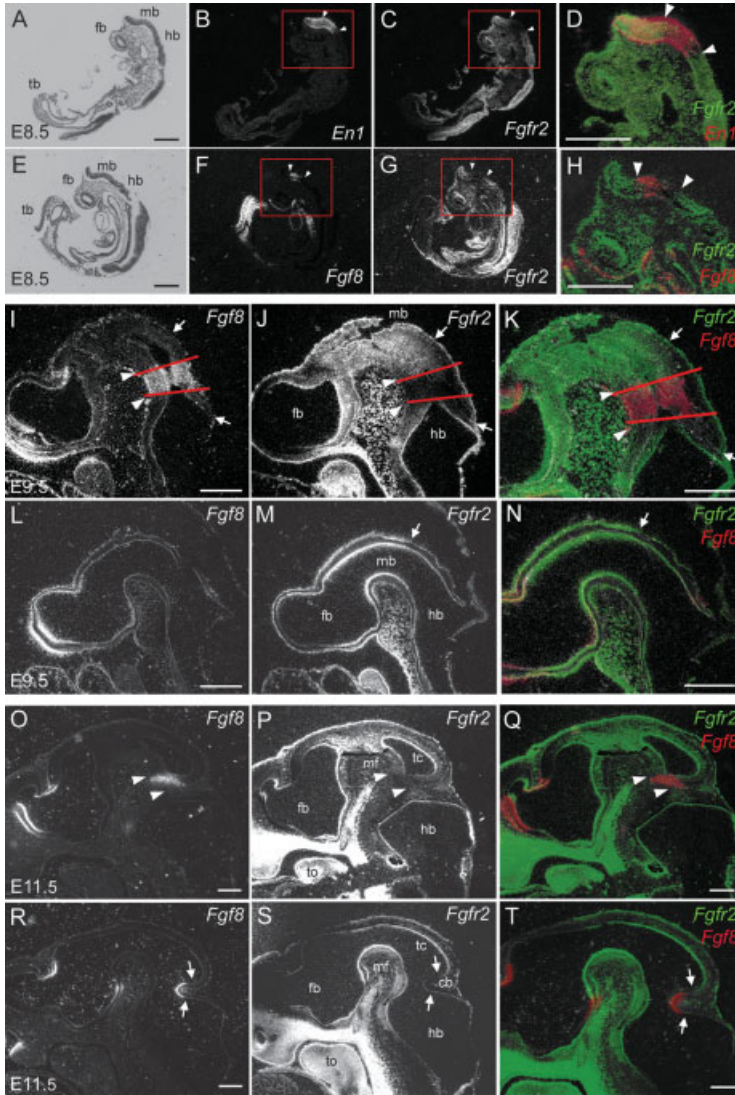


Fig. 2.

Fig. 2. A–T: *Fgfr2* expression is excluded from the *Fgf8*-positive tissue of the midbrain–hindbrain organizer but is continuous in the floor plate of the midbrain–hindbrain region. In situ hybridization on sagittal sections of embryonic day (E) 8.5 (A–H), E9.5 (I–N), and E11.5 (O–T) embryos was performed with antisense probes directed against *Fgfr2* (C,G,J,M,P,S), *En1* (B), and *Fgf8* (F,I,L,O,R). D,H,K,N,Q,T: False-color overlays of *Fgfr2* (green) and *En1* or *Fgf8* (red) were made from adjacent sections to compare expression domains. Insets in B, C, F, and G frame the area from which overlays were made. I,J,K: Lines demarcate the borders of the *Fgf8* expression domain. Arrowheads mark the ventral borders of *Fgfr2* expression, arrows mark the borders of dorsal *Fgf8* expression. I,J,O,P: Parasagittal sections. L,M,R,S: Midsagittal sections. Anterior is to the left. fb, forebrain; hb, hindbrain; mb, midbrain; tb, tail bud; cb, cerebellar anlage; mf, mesencephalic flexure; tc, tectum; to, tongue. Scale bars in A (applies to B,C),D,E (applies to F,G),H,I (applies to J),K,L (applies to M),N,O (applies to P),Q,R (applies to S),T = 250 μ m.

Fig. 3. A–L: At embryonic day (E) 9.5, *Fgfr2* is weakly expressed in cells close to the lumen of the neural tube in the floor plate of the midbrain–hindbrain organizer (MHO). Coronal sections of an E9.5 embryo were hybridized with antisense probes for *Fgfr2* (C,G,K) and *Fgf8* (B,F,J). D,H,L: False-color overlays of *Fgfr2* (green) and *Fgf8* (red) were made from adjacent sections to compare the expression domains. A,E,I: Brightfield images of the sections in B, F, and J, respectively. Upper row is posterior midbrain, middle row is at the level of the ventral MHO, lowest row is anterior hindbrain. rp, roof plate; ap, alar plate; bp, basal plate; fp, floor plate. Scale bars in A (applies to B,C),D,E (applies to F,G),H,I (applies to J,K),L = 150 μ m.

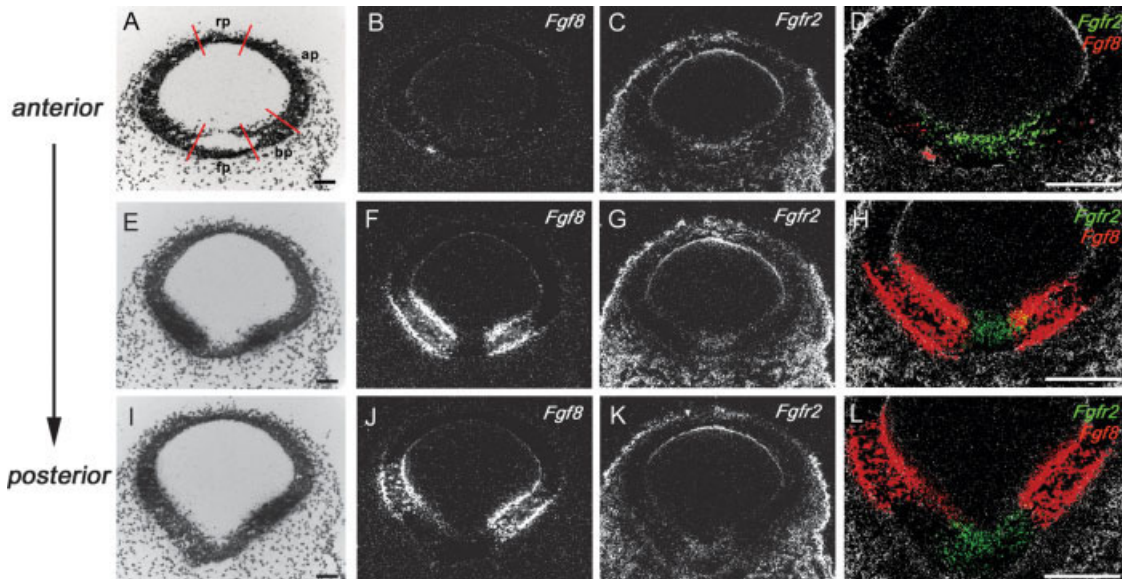


Fig. 3.

lated *Fgfs*, *Fgf17* and *Fgf18*, are also expressed at the MHB boundary but are probably not involved in patterning of the MHB rather in proliferation (Xu et al., 2000; Liu et al., 2003).

It has been reported previously that, in mouse, the *Fgf* receptors 2 and 3 are not expressed in tissues adjacent to the MHO (Ishibashi and McMahon, 2002; Liu et al., 2003; Trokovic et al., 2003). In contrast, *Fgfr1* has been shown to be expressed along the entire neural tube at E8.5, and this expression pattern of *Fgfr1* persists throughout embryonic development.

Therefore, FGFR1 was believed to transmit the FGF8 signal in the embryonic midbrain and hindbrain, leading to the proper development of the structures emanating from this region. However, this hypothesis could not be verified by a conditional knockout for this receptor, in which the *Fgfr1* gene becomes inactivated under the control of the *En1* promoter. Although, dorsally, there is a loss of the cerebellum and the inferior colliculi, there is no loss of tissue arising from the ventral part of the midbrain and hindbrain. Furthermore, there is no loss of ventral neuronal populations residing in this region (Trokovic et al., 2003). In contrast, conditional mutagenesis of *Fgf8* in the *En1* expression domain results in a severe loss of midbrain and hindbrain tissue, including loss of markers for ventral midbrain dopaminergic neurons (Chi et al., 2003). Therefore, it has to be assumed that other FGFRs are involved in transmitting the FGF8 signal in the ventral midbrain and hindbrain.

We show here that *Fgfr1* is continuously expressed along the neural tube from E8.5 onward, whereas *Fgfr2* and *Fgfr3* exhibit differences in expression and have distinct dynamics of expression at the MHB boundary. In contrast to earlier studies in mouse and chicken, we can show the presence of *Fgf* receptors 2 and 3 in the region around the ventral MHB boundary, as for example in the floor plate of the neural tube (*Fgfr2*), using a very sensitive radioactive in situ hybridization method, which enabled us to detect very low expression levels.

At E8.5, expression of *Fgfr2* does

not reach the *Fgf8* expression domain in the hindbrain, but abuts it in the midbrain. From E9.5 on, there is only a small gap in expression of *Fgfr2* in the basal plate of the neural tube, which corresponds to the area of *Fgf8* expression at the MHO. *Fgf8* is not expressed in the floor plate of the MHB boundary (Crossley and Martin, 1995). One remarkable finding is that *Fgfr2* is continuously expressed along the A/P axis of the floor plate from E9.5 onward, even at the level of the MHO, exactly where the *Fgf8* expression displays a gap. Dorsal expression of *Fgfr2* retracts from the *Fgf8* expression at the MHB boundary at E9.5. This dorsal gap gradually closes down until E12.5. *Fgfr3* initially, at E8.5, displays a gap in expression in the midbrain and anterior hindbrain. From then on, it gradually progresses from the diencephalic/mesencephalic border toward the *Fgf8* expression domain at the MHO but does not abut it in the ventral midbrain until E12.5, the latest stage examined. At E12.5, the posterior *Fgfr3* expression even overlaps with the *Fgf8* domain (summarized in Fig. 5). *Fgfr3* exhibits a broader gap in the dorsal MHB region. The A/P extent of this gap does not change until E12.5, but *Fgfr3* expression expands further dorsal, shifting from the basal plate into the alar plate from E9.5 until E12.5. Although a gap in expression of *Fgfr2* and *Fgfr3* in the MHB region has been described before (Ishibashi and McMahon, 2002; Liu et al., 2003; Trokovic et al., 2003), our results clearly show that the extent of this gap is much smaller than reported, especially in the ventral part of the region.

The differential expression of the three *Fgf* receptors examined may be the result of a repressor activity triggered by FGF8. This activity may change in a spatiotemporal manner, probably mediated by other downstream factors. Indeed, it has been shown in vitro that FGF8 represses both *Fgfr2* and *Fgfr3* (Liu et al., 2003). The gap in expression of *Fgfr2* at the MHO, exactly where *Fgf8* is expressed, and its continuous expression in the floor plate, where *Fgf8* is not expressed, substantiate a repression of *Fgfr2* by FGF8 in vivo. *Fgfr1*, which is expressed at the MHO, could medi-

ate the repressive effect of FGF8 on *Fgfr2* and *Fgfr3*.

However, in contrast to *Fgfr2*, which obviously follows the contraction of the *Fgf8* expression domain at the MHB boundary, *Fgfr3* in the caudal midbrain never does contact the *Fgf8* expression domain at the MHO. Instead, in the hindbrain, expression of *Fgfr3* shows an overlap with the *Fgf8* expression at later stages. Furthermore, *Fgfr3* expression is not continuous in the floor plate at the MHO. This finding hints toward another factor regulating *Fgfr3* in vivo during early MHB development. We are well aware that this notion seems to be in contrast to a study in the zebrafish *ace* mutant, a hypomorphic mutant of *Fgf8*, in which the *Fgfr3* expression domain is expanded into the caudal midbrain upon loss of *Fgf8* (Sleptsova-Friedrich et al., 2001). However, this expansion of the *Fgfr3* domain might be a secondary effect due to a reduction of *engrailed* expression in the MHB region of the *ace* mutant (Reifers et al., 1998). Of interest, we show that *Fgfr3* expression at E8.5 complements that of *En1*. Later, the expression of *En1* is gradually excluded from the ventricular zone in the posterior midbrain and in the anterior hindbrain, whereas *Fgfr3* expression expands into the ventricular zone in these regions. These mutually exclusive expression patterns of *Fgfr3* and *En1* indicate a potential interdependence in the regulation of *En1* and *Fgfr3*.

The differences in expression of the three *Fgf* receptors at the MHB boundary might reflect specific roles for each of them in the development and maintenance of the MHB territory. *Fgfr1* is the only *Fgf* receptor expressed throughout the neural tube at early stages of development. Therefore, FGFR1 could be involved early in general patterning of the neural tube. Expression of *Fgfr2* and *Fgfr3* in the ventral but not in the dorsal part of the MHB could indicate a role for FGF signaling in D/V patterning. Indeed, FGF8 has been shown to be involved in D/V patterning in medaka (Carl and Wittbrodt, 1999). In zebrafish, on the other hand, *Fgfr1* has been shown to be necessary for FGF8 signaling in dor-

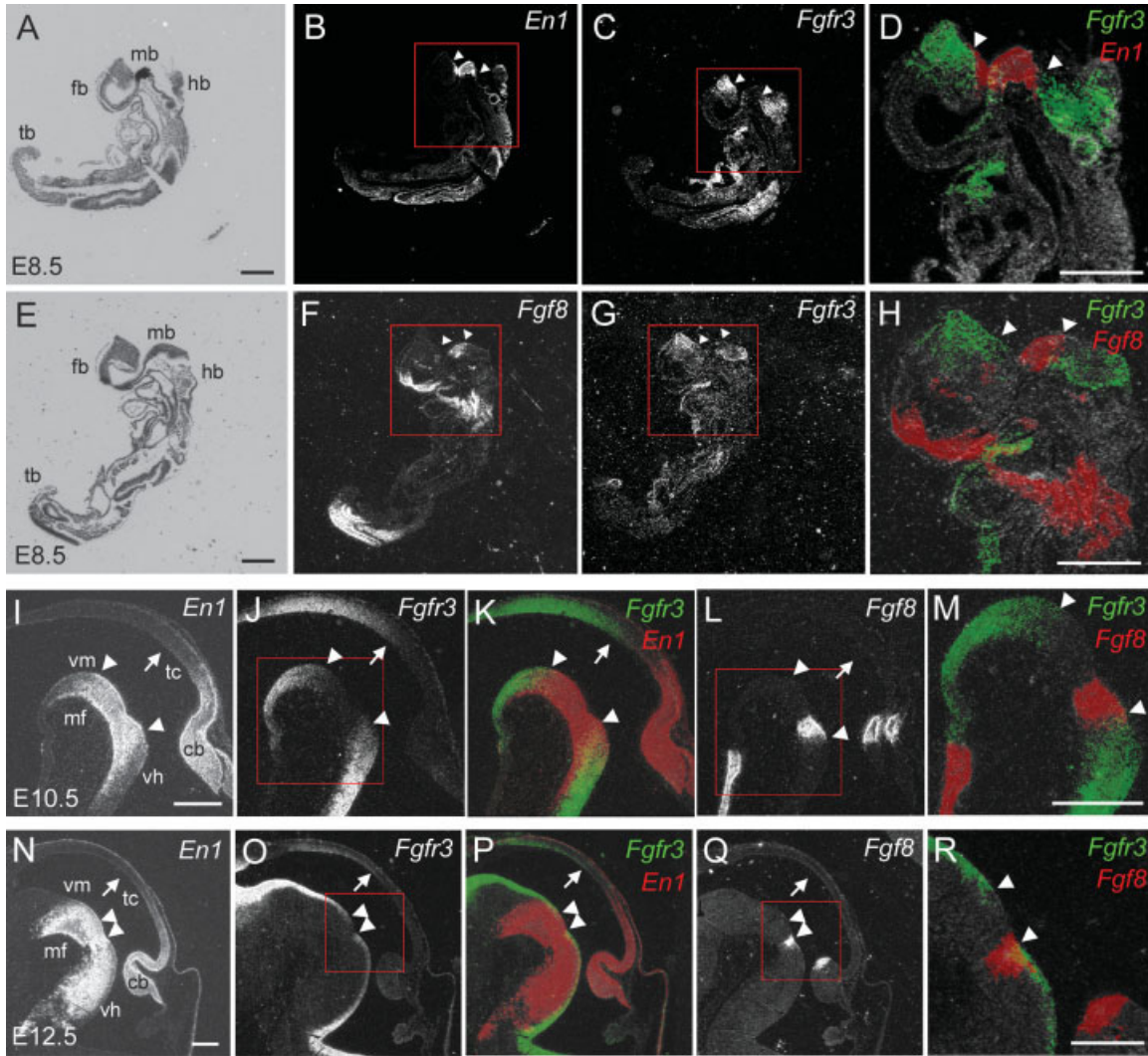


Fig. 4. A–R: *Fgfr3* expression abuts the *Fgf8* expression domain in the hindbrain but is excluded from *En1*-positive tissue. In situ analysis on sagittal sections of embryonic day (E) 8.5 (A–H), E10.5 (I–M), and E12.5 (N–R) embryos was performed using antisense probes for *Fgfr3* (C,G,J,O), *En1* (B,I,N), and *Fgf8* (F,L,Q). A,E: Brightfield images of the sections shown in B and F, respectively. D,H,K,M,P,R: False-color overlays of *Fgfr3* (green) and *En1* or *Fgf8* (red) were made from adjacent sections to compare expression domains. B,C,F,G,J,L,O,Q: Insets frame the area from which the overlays in D, H, M, and R were prepared. Overlays in K and P were made from I, J, N, and O. Arrowheads demarcate ventral expression borders of *Fgfr3*; arrows mark the borders of dorsal *Fgfr3* expression. Anterior is to the left. fb, forebrain; hb, hindbrain; mb, midbrain; tb, tail bud; cb, cerebellar anlage; mf, mesencephalic flexure; tc, tectum; vh, ventral hindbrain; vm, ventral midbrain. Scale bars in A (applies to B,C), D, E (applies to F,G), H, I (applies to J,K,L), M, N (applies to O,P,Q) R = 250 μ m.

sal as well as ventral aspects. Despite the adjacent expression of *Fgfr2* as well as *Fgfr3* at the MHB boundary (Tonou-Fujimori et al., 2002), knockdown morphants for *Fgfr1* in zebrafish phenocopy the *ace* mutant, which is a FGF8 loss-of-function mutant (Scholpp et al., 2004). However, species differences between zebrafish and mouse have been discussed before (Scholpp et al., 2004). Therefore, in mouse, the differences between the phenotypes of the conditional *Fgfr1* and *Fgf8* knockouts (Liu et al., 2003; Trokovic et al., 2003) could be explained by

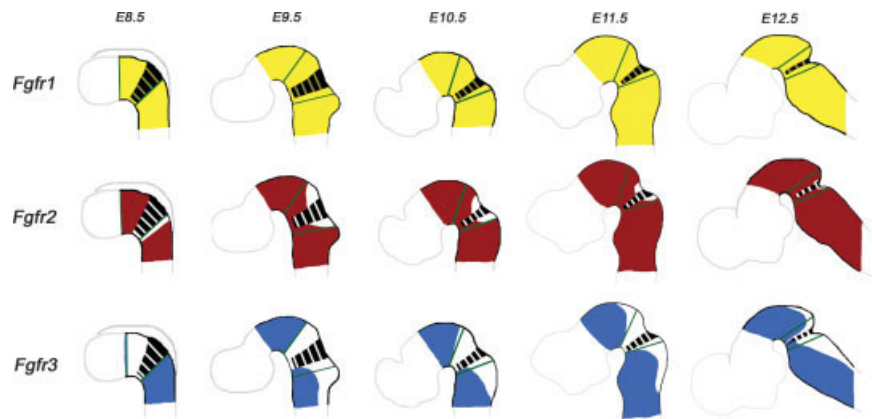


Fig. 5.

the fact that, dorsally, there is only expression of *Fgfr1*, which may be necessary for patterning of the dorsal MHB. In addition to *Fgfr1*, *Fgfr2* is expressed in the ventral midbrain from E8.5 on and *Fgfr3* in the ventral hindbrain. Both receptors may compensate for the loss of *Fgfr1* in the ventral MHB in the conditional *Fgfr1* mutant.

Specific combinations of FGFR1 with FGFR2 and FGFR3 in the ventral midbrain and hindbrain could also be necessary for patterning along the A/P axis, triggering different steps in the specification, differentiation, and maintenance of ventral neural cell populations. Indeed, FGF8, together with the secreted factor Sonic Hedgehog, has been shown to be required for the specification of midbrain dopaminergic (DA) and hindbrain serotonergic (5-HT) neurons, which arise in ventral positions anterior and posterior to the MHB boundary, respectively (Ye et al., 1998).

Several mechanisms could mediate the different functions of the FGF receptors in patterning along the A/P and D/V axes in the MHB region. Differences in expression levels and binding activities of the receptors (MacArthur et al., 1995; Blunt et al., 1997; Xu et al., 1999; Walshe and Mason, 2000; Ishibashi and McMahon, 2002), as well as the fact that FGFRs can build heterodimers (Bellot et al., 1991) may lead to activation of different downstream signaling cascades, such as the MAP kinase pathway or the PI3/

Akt pathway (reviewed in Pawson and Nash, 2000). Thereby, different sets of target genes may become activated, depending on the combination of the *Fgf* receptors expressed. In combination, FGFR1 and FGFR2 in the ventral midbrain could be involved in patterning, specification, and differentiation of ventral mesencephalic cell populations, such as the midbrain dopaminergic neurons. In the hindbrain, FGFR1, FGFR2, and FGFR3 together could be responsible for patterning and specification of ventral hindbrain cell populations, including the serotonergic neurons of the ventral hindbrain.

Our results on the expression of *Fgfr1-3* in the MHB illustrate a possible mechanism to convert one signal, here FGF8, into different outcomes in patterning and specification of neuronal subtypes of the midbrain and hindbrain. To address the precise role of each FGF receptor in the functions of FGF signaling at the MHB boundary, the next step would be to create conditional mouse mutants lacking different combinations of the *Fgf* receptors.

EXPERIMENTAL PROCEDURES

Animals

CD1 mice were purchased from Charles River. Noon of the day of vaginal plug detection was designated E0.5. Embryos were dissected and staged according to Theiler (1989). Embryos were immersion-fixed in 4%

paraformaldehyde and embedded in paraffin.

In Situ Hybridization

Embryos were sectioned at 5–8 μ m in two planes (coronal and sagittal). In situ hybridization was performed according to a modified version of Dagerlind et al. (1992). A detailed version of the protocol is given at <http://www.eumorphia.org/servlet/ECFLP.Frameset>. Plasmids used for transcription of antisense probes contained fragments of *En1* (Davis and Joyner, 1988) and *Fgf8* (Martinez et al., 1999). Plasmids containing fragments of *Fgf* receptors (specific for both isoforms) were gifts from Roland Lauster (*Fgfr1*; nt1966-2369 in NM_010206), Clive Dickson (*Fgfr2*; De Moerloose et al., 2000), and David Ornitz (*Fgfr3*; Peters et al., 1993). The probe against *Fgfr4* was transcribed from a plasmid cloned by A.A.B. (nt550-1375 in BC033313).

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Fig. 5. *Fgf* receptors 1–3 show distinct expression patterns during early development of the midbrain–hindbrain (MHB) in the mouse. The diagrams represent a sagittal view of the rostral neural tube. Anterior is to the left. Stages embryonic day (E) 8.5–E12.5 are shown. Expression patterns in the midbrain and hindbrain are highlighted in colors. First row (in yellow): *Fgfr1* is expressed throughout the neural tube from E8.5 until E12.5 and overlaps with the *En1* (green lines) and *Fgf8* (broken black field) expression domains. Second row (red): *Fgfr2* is expressed in the midbrain abutting the *Fgf8* domain at the MHB boundary but not in the anterior hindbrain at E8.5. From E9.5 on, *Fgfr2* is expressed in the ventral and lateral MHB immediately adjacent to the *Fgf8*-positive domain. *Fgfr2* can also be found along the anterior to posterior (A/P) axis of the floor plate, which is free of *Fgf8* expression. The dorsal part of the caudal midbrain and the anterior hindbrain is devoid of *Fgfr2* at this stage. This gap in dorsal expression narrows until E12.5, when only tissue close to the dorsal midbrain–hindbrain organizer (MHO) does not express *Fgfr2*. Third row (blue): *Fgfr3* is expressed in the hindbrain but not in the midbrain at E8.5. At E9.5, the ventral part of the hindbrain and the anterior midbrain express *Fgfr3*. The dorsal parts of the caudal midbrain and of rhombomere 1 are free of *Fgfr3*. The gap in ventral expression of *Fgfr3* narrows down until E12.5, when only the caudal-most part of the midbrain does not express *Fgfr3*. In the hindbrain, *Fgfr3* expression overlaps with *Fgf8* expression in the ventral MHO at this stage. The dorsal gap in *Fgfr3* expression does not alter in its A/P extent (caudal half of midbrain until rhombomere 7), but in the dorsal to ventral (D/V) axis, it becomes restricted to the dorsal alar plate and the roof plate.

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