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Temporal and spatial expression of FGF ligands and receptors during *Xenopus* development

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Abstract

Fibroblast growth factor (FGF) signalling plays a major role during early vertebrate development. It is involved in the specification of the mesoderm, control of morphogenetic movements, patterning of the anterior-posterior axis and neural induction. In mammals, 22 FGF ligands have been identified which can be grouped into seven subfamilies according to their sequence homology and function. We have cloned 17 *fgf* genes from *Xenopus tropicalis* and have analysed their temporal expression by RT-PCR and spatial expression by whole mount *in situ* hybridisation at key developmental stages. It reveals the diverse expression pattern of *fgf* genes during early embryonic development. Furthermore, our analysis shows the transient nature of expression of several *fgfs* in a number of embryonic tissues. This study constitutes the most comprehensive description of the temporal and spatial expression pattern of *fgf* ligands and receptors during vertebrate development to date.

Keywords

FGF; Xenopus; FGFR; otic vesicle; iFGF; hFGF; vertebrate

Introduction

The mammalian Fibroblast Growth Factor (FGF) family comprises 22 ligands. The first members of the family to be identified were FGF1 and FGF2 based on their ability to induce proliferation of fibroblasts in culture, hence their name (Gospodarowicz and Moran, 1975). Subsequent studies have shown that FGFs can also modulate cell survival, migration and differentiation of cells in culture (Dailey et al., 2005; Xian et al., 2005). Furthermore, deregulation of FGF signalling has been associated with diverse pathologies such as skeletal diseases and cancer (Eswarakumar et al., 2005; Itoh, 2007).

The FGF family consists of three subgroups: the canonical FGFs, the intracellular FGFs and the hormonal FGFs. The canonical FGFs are secreted ligands, with binding sites for acidic glycosaminoglycans, such as heparin and heparan sulfate (Ornitz, 2000). These FGF ligands bind the cell surface Fibroblast Growth Factor Receptors (FGFRs) in combination with heparan sulfate to form a 2:2:2 FGF:FGFR:heparan dimer leading to the activation of the FGFRs (Mohammadi et al., 2005a). In vertebrates, the FGFR family consist of four genes, *FGFR1-4*. Structurally, all FGFRs contain an extracellular ligand-binding domain, a single transmembrane domain and an intracellular tyrosine kinase domain. The extracellular region has two or three immunoglobulin (Ig)-like domains and a heparin-binding domain important for the interaction with the ligand. The extracellular domain of FGFR is subject to multiple

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alternative splicing which modulate the affinity of a receptor for its ligand (Klint and Claesson-Welsh, 1999; Mohammadi et al., 2005b). One such alternative splicing extensively studied is the IgIII domain, which has two alternative exons resulting in receptors with very different ligand affinity properties (Ornitz et al., 1996). However, it has also been shown that the first Ig domain (Ig-I) can be spliced out thereby increasing the affinity of the ligand for its receptor (Wang et al., 1995; Mohammadi et al., 2005b). FGF ligand binding induces dimerisation of the FGFRs resulting in the subsequent phosphorylation of specific intracellular tyrosine residues (Furdui et al., 2006). This triggers the activation of cytoplasmic signal transduction pathways such as the Ras-MAPK, the Akt or the Protein Kinase C (PKC) pathways (Dailey et al., 2005).

The second subgroup of FGFs is the intracellular FGFs (iFGFs) also known as FGF Homologous Factors (FHFs) comprising *fgf11, fgf12, fgf13* and *fgf14*. While they share a common structural core with other FGF ligands, they are not secreted, are unable to bind the FGFR and all contain a nuclear localisation signal (Smallwood et al., 1996; Olsen et al., 2003). Knockout studies show that iFGFs are mainly involved in neuronal functions such as the control of axonal excitability (Goldfarb et al., 2007) but very little is known about their molecular mechanism of action (Goldfarb, 2005).

The third subgroup of FGFs is the hormone-like FGFs (hFGF), which have been shown to have a systemic action rather than a local, paracrine action (Goetz et al., 2007). The hFGFs have lower affinity to heparan sulfate than the canonical FGFs and they require the expression of Klotho, a transmembrane protein with a short intracellular tail, to be able to bind the FGFR (Fukumoto, 2008). Members of this subfamily include *fgf19*, involved in bile acid metabolism, *fgf21*, important for carbohydrate and lipid metabolism and *fgf23*, thought to be necessary for vitamin D metabolism (for review, (Kuro-o, 2008).

It has been demonstrated that FGF signalling plays various roles during early embryonic development. Experiments in chicken, mouse and *Xenopus* have shown that FGF signalling is essential for the specification of the mesoderm, the induction of neural tissue, the control of morphogenetic movements and the setting up of the anterior-posterior axis (Amaya et al., 1991; Partanen et al., 1998; Sun et al., 1999; Nutt et al., 2001; Yang et al., 2002; Bottcher and Niehrs, 2005; Thisse and Thisse, 2005; Maegawa et al., 2006; Stavridis et al., 2007). Understanding the various roles of FGF signalling during embryogenesis requires a full description of the expression pattern of all the FGF ligands and receptors during early development. While partial analyses have been reported for different organism and/or organs, there is no exhaustive report of the expression pattern of *fgfs* and *fgfrs* during early embryogenesis. The most complete published analysis has been done in the mouse embryo (Yaylaoglu et al., 2005), but that study was restricted to only one stage of development, E14.5. We therefore identified, cloned and analysed the pattern of expression of all the *fgf* and *fgfr* genes from *Xenopus tropicalis* and analyse their temporal and spatial expression pattern. It is the most exhaustive study of *fgf* and *fgfr* expression during early development and it gives us insight on the role of the different *fgfs* during embryogenesis.

Results and Discussion

Identification of the *fgf* family members in the genome of *Xenopus tropicalis*

We used both sequence homology and synteny to identify and annotate the *X. tropicalis* orthologues of human and mouse *FGF* genes (http://genome.jgi-psf.org/Xentr4/Xentr4.home.html). Three examples of synteny, which include *fgf2, fgf3, fgf4, fgf6, fgf19* and *fgf23*, are shown in Supp. Fig. S1A, demonstrating the conservation of gene location from *X. tropicalis* to mouse and human. Out of the 22 FGF family members present in the mammalian genome, we have identified and annotated 19 orthologues in the *X. tropicalis*

genome. Out of the three remaining *fgf* genes, we were able to identify *fgf21*, based on homology to *X. laevis* Expressed Sequence Tags (ESTs) and synteny to mammalian genomes. However the region of the *X. tropicalis* genome containing *fgf21* includes many gaps and we were unable to annotate this gene fully (Supp. Fig. S1B). However, we were unable to identify orthologues of mammalian *Fgf17* or *Fgf18* in the *X. tropicalis* genome. We analysed the region of the genome that should contain *fgf17* based on synteny and we failed to find this gene, suggesting that *X. tropicalis* does not contain an orthologue of the mammalian *Fgf17* (Supp. Fig. S1C). We performed a similar analysis for *fgf18*, and we found that the quality of the *X. tropicalis* genome in the region expected to contain *fgf18* is not sufficient to conclude whether this gene is present in the genome or not. Thus we conclude that *X. tropicalis* contains orthologues of 20 out of the 22 mammalian *Fgf* genes. Finally, we found that *fgf23* in *X. tropicalis* has undergone a duplication event, resulting in two *fgf23* paralogous genes next to each other in the genome (Supp. Fig. S1A).

We then analysed the relationship between the different *fgf* genes using CLUSTALW (Supp. Fig. S2). The analysis suggests that, as in mouse and human, *X. tropicalis fgfs* can be divided into seven subfamilies (Itoh and Ornitz, 2008). The tree using the sequences from *Xenopus tropicalis* is remarkably similar to the one shown for the mouse Fgf orthologues (Itoh and Ornitz, 2008) showing the high level of conservation of this family of ligands in vertebrates.

Temporal expression of *fgfs* and *fgfrs*

We have performed a time course of expression on all the *fgfs* identified by RT-PCR. However, we were unable to get amplification products for *fgf5, fgf9, fgf10, fgf11* and *fgf21* either because they are expressed at very low level or because we could not amplify them with our PCR conditions.

Four *fgf* transcripts are expressed maternally (*fgf1, fgf2, fgf13* and *fgf22*, Fig. 2A). During gastrulation stages, when FGF signalling is required for mesoderm specification and morphogenetic movements, *fgf1, fgf2, fgf4, fgf8, fgf20* and to a lesser extent *fgf22* are expressed. While the role of *fgf4* and *fgf8* has been extensively studied at these stages (Isaacs et al., 1994; Fletcher et al., 2006), very little is known about the role of the other ligands. By stage 40, at least 13 different *fgf* genes are expressed (Fig. 1A).

The genes encoding the FGF receptors are expressed throughout early development with the exception of *fgfr3*, which is not expressed during gastrulation (Hongo et al., 1999), Fig. 1B). The *fgfr* genes can be alternatively spliced in their extracellular domain conferring them differential affinity for FGF ligands. Here, we have designed oligonucleotides either side of the exon encoding for the Ig-I domain. Consistent with published data, both *fgfr1* and *fgfr2* have two splice variants in this region (Powers et al., 2000). Interestingly, the long isoforms of *fgfr1*, containing the Ig-I domain, are the main isoforms expressed at early stages, while the short isoforms are mostly expressed after stage 15 (Fig. 1B). This is in contrast to what has been reported in the mouse where only the long isoforms are expressed during early embryonic development (Xu et al., 1999). As shown in mammalian systems (Powers et al., 2000), only the long, IgI-containing, isoforms of *fgfr3* and *fgfr4* are expressed in early *Xenopus* embryos (Fig. 1B).

Spatial expression of fgf receptors and ligands

We have analysed the pattern of expression for all cloned *fgf* genes in *Xenopus tropicalis* by whole mount *in situ* hybridisation (WISH) followed by sectioning to reveal the localisation of the staining in greater detail. We have divided the results by FGF subfamily as defined in (Itoh and Ornitz, 2008).

a. FGF1 subfamily—The FGF1 subfamily comprises *fgf1* (also known as acidic *fgf*) and *fgf2* (basic *fgf*), which both lack canonical signal peptide and are inefficiently secreted (Florkiewicz et al., 1998). RT-PCR data indicate that *X. tropicalis fgf1* is expressed at all stages of development (Fig. 1A). By *in situ* hybridisation, no specific pattern is observed until stage 23 when *fgf1* is very strongly expressed in the notochord (Fig. 2A, 3A). This is very transient, as by stage 28 *fgf1* is no longer detected in the notochord. Instead, *fgf1* is expressed in the forebrain, in the ventricular zone of the neural tube and otic vesicles (Fig. 2A, 3A). At stage 35, it is also expressed in the roof of the anterior neural tube and at stage 40, *fgf1* is detected in the dorsal fin (Fig. 2A).

Fgf2 is also expressed throughout early embryonic development as assayed by RT-PCR with a peak at stage 15 (Fig. 1A). By WISH, *fgf2* is weakly expressed in the mesoderm at st10.5 and then in the presomitic mesoderm at st15, 23 and 28. From st35, *fgf2* is expressed in the branchial arches (possibly neural crest derived) and faintly in the pronephros. The cranial mesoderm is also positive for *fgf2* expression, as is the otic vesicle (Fig. 3B) and the tip of the tailbud.

Even though *fgf1* and *fgf2* belong to the same subfamily, their patterns of expression are very distinct. This suggests that their roles during embryonic development will be very different. The double knockout of *Fgf1* and *Fgf2* has a very mild phenotype in the mouse possibly due to the redundancy with other Fgf ligands rather than redundancy from each other (Miller et al., 2000).

b. The FGF4 subfamily—The FGF4 subfamily comprises *fgf4, fgf5* and *fgf6*. While all of them have been identified in the *Xenopus tropicalis* genome, we were not able to clone *fgf5*. It is absent from the EST databases and our attempts to amplify it from cDNA derived from *X. tropicalis* embryos were unsuccessful. *Fgf4* (previously annotated as eFGF) has long been known as a potent inducer of mesoderm fate and anteroposterior specification in *Xenopus* (Isaacs et al., 1994). In mouse, *Fgf4* knockout is embryonic lethal at E4-5 (Feldman et al., 1995). In *X. laevis* (Isaacs et al., 1994) and in *X. tropicalis* (Fig. 1A, 2B), *fgf4* starts to be expressed during gastrulation stages in the marginal zone and in the posterior mesoderm at stage 15. At later stages (23, 28, 35 and 40) it is expressed in the developing tailbud. Additionally, *fgf4* is weakly expressed in the Midbrain-Hindbrain Boundary (MHB) at stage 35 and in the otic vesicle at stage 28 and 35 (Fig. 2B).

Fgf6 starts to be expressed only at later stages of development (between stage 25 and 30, Fig. 1A). *Fgf6* expression does not show a particular expression pattern until st35 when it is expressed in the somitic mesoderm (Fig. 2B). This is a specific pattern of expression as the sense probe does not stain the somitic mesoderm (data not shown). This pattern of expression is compatible with the phenotype seen in *fgf6* knockout mice, which have a defect in muscle regeneration (Floss et al., 1997).

c. The FGF8 subfamily—We have cloned only one member of the FGF8 subfamily (*fgf8*). Signalling by FGF8 has been involved in numerous developmental processes and the knockout of *Fgf8* in the mouse is embryonic lethal at E8 with gastrulation defects (Meyers et al., 1998; Sun et al., 1999). In *X. laevis, fgf8* has been shown to be important for mesoderm formation and posterior neural tissue induction (Christen and Slack, 1997; Fletcher et al., 2006). Even though the expression pattern of *fgf8* in *X. laevis* (described in (Christen and Slack, 1997) is remarkably similar to the one seen in *X. tropicalis* (Fletcher et al., 2006), our analysis of sectioned embryos shows that, in addition of sites of expression previously reported, *fgf8* is also expressed in the cranial mesoderm dorsal to the cement gland but more ventral to the forebrain (Fig. 2C, 3C).

d. The FGF7 subfamily—The FGF7 subfamily contains *fgf3, fgf7, fgf10* and *fgf22*. In *X. tropicalis, fgf3* starts to be expressed at the end of gastrulation (stage 12), it peaks at stage 15 and then decreases but is still present through stage 40 (Fig. 1A). At stage 15, *fgf3* is expressed in two stripes lateral to the anterior neural tube. These two stripes have been described as being rhombomeres 3-4-5 (Lombardo et al., 1998). At later stages, it is expressed in the ventricular zone of the neural tube and otic vesicle, the branchial arches, the MHB and in the developing tailbud (Fig. 4A and 5A). The later expression pattern (stage 28 onwards) is reminiscent of *fgf8* expression. Indeed, it has been shown that *fgf3* and *fgf8* have unique and redundant functions in the otic placode and forebrain development in zebrafish (Walshe and Mason, 2003).

Both Fgf7 (also known as Keratinocyte Growth Factor, KGF) and Fgf10 have been shown to be able to induce proliferation of keratinocytes rather than fibroblast in cell cultures (Rubin et al., 1989; Igarashi et al., 1998). The role of *Fgf7* during wound healing has long been established (Werner et al., 1994; Werner, 1998). It starts to be expressed at stage 20 (Fig. 1A) and its localisation is unique amongst *fgf* genes. It is expressed in the mesenchymal tissue underlying the fin crest. It is faintly visible in this region at stage 23 and the expression becomes stronger at stage 28 until stage 40 (Fig. 4A, 5B). We would postulate that *fgf7* plays an important role during the development and/or maintenance of the fin in *X. tropicalis*, perhaps by inducing proliferation of the overlying keratinocytes of the fin. Additionally, *fgf7* is also expressed in the branchial arches from st28.

In *X. tropicalis, fgf10* is the first *fgf* gene detected in the otic placode as early as stage 23. Furthermore, its expression is specific for the developing ear until stage 28 (Fig. 4A, 5C). This pattern of expression is consistent with findings that *Fgf10* knockout mice have defects in the development of the ear (Ohuchi et al., 2000; Alvarez et al., 2003). From stage 35, *fgf10* starts to be expressed in the branchial arches, and in the ventral side of the otic vesicles. Even though we were able to detect expression of *fgf10* in the developing ear of the embryos as assayed by WISH, we were not able to detect it by RT-PCR, possibly due to the highly localised expression, but overall low level of expression in the embryo.

The last member of this subfamily is *fgf22*, which is expressed at low level maternally, peaks at stage15 and 20, and is faintly expressed at stage 40 (Fig. 1A). By WISH, *fgf22* does not seem to have a particularly localised pattern of expression. The low level of staining seen throughout the head maybe due to some non-specific retention of probe (Fig. 4A, data not shown).

e. The FGF9 subfamily—All three members of the FGF9 subfamily have been identified in the *Xenopus tropicalis* genome. We have obtained *fgf9* from the *X. tropicalis* full-length library and used an IMAGE clone for *fgf20* but were unable to clone *fgf16*. The pattern of expression of *fgf9* is not very defined and we were unable to detect its expression by RT-PCR. *Fgf9* is probably expressed at low levels during early stages of the embryonic development as the sense probe does not give any staining (Fig. 4B, data not shown). It has been previously shown in *Xenopus laevis* that *fgf9* is expressed throughout early development (Song and Slack, 1996). The knockout of *fgf9* in mouse is lethal at birth due to defects in lung development (Colvin et al., 2001). Furthermore, *fgf9* has been shown to be expressed in the developing limbs, a structure not present at the stages of our analysis.

Fgf 20 is expressed in the mesoderm at gastrulation stages, which makes it one of four *fgfs* expressed in the mesoderm at these critical stages of embryonic development (with *fgf2, fgf4* and *fgf8*). It is then expressed in the branchial arches, the tailbud, in the dorsal ventricular zone of the anterior neural tube as well the ventricular zone of the otic vesicle. Additionally, staining can be seen dorsal to the cement gland, possibly in cranial mesoderm

(4B, 5D). So far, the knockout has not been reported in the mouse but the prediction would be that it is embryonic lethal. Overexpression of *fgf20* leads to gastrulation defects in *Xenopus laevis* (Koga et al., 1999) and it has been recently reported that *fgf20* is strongly upregulated upon amputation of the tail in *Xenopus* (Lin and Slack, 2008).

f. The FHF family—Intracellular Fgfs or FHF (for FGF Homology Factors) comprises *fgf11-14*. We have cloned *fgf12, 13* and *14* but were unable to isolate *fgf11* or find an EST for it. By RT-PCR, *fgf12, fgf13* and *fgf14* start to be expressed at around stage 20 (Fig. 1A).

Fgf12 is expressed in the olfactory placodes (Fig. 6A, 7A) at stage 28, 35 and 40. At stage 40, *fgf12* is expressed in different regions, including the anterior neural tube as well as weak staining in the eye.

It has been reported that human *FGF13* undergoes alternative splicing in its first exon resulting in 5 different splice variants. We have cloned the orthologue of *Fgf13.1* and two new variants, named *fgf13.7* (accession number FJ480180) and *fgf13.8* (accession number FJ480181). *Fgf13* has different splice variants in *X. laevis* and is involved in neuronal differentiation (Nishimoto and Nishida, 2007). The murine *Fgf13* has been shown to interact with neuronal sodium channel (Wittmack et al., 2004). In the chick, *fgf13* is expressed in the lateral side of the neural tube (Munoz-Sanjuan et al., 1999). This is consistent with the expression pattern seen in *X. tropicalis*. From stage 23 onwards, *fgf13* is expressed in the trigeminal and sensory neurones and it is expressed in the somites but only transiently at stage 23 (Fig. 6A, 7B).

Fgf14 knockout mice are viable but display neurological defects (Xiao et al., 2007). In *X. tropicalis, fgf14* starts to be expressed at stage 15 in the floor plate of the neural tube (Fig. 6A). From stage 23, it stains very strongly the somites. *Fgf14* also marked the lens very transiently at stage 35 (Fig. 6A, 7C).

g. The Hormone-like FGFs—The last family of FGFs is called the hormone-like FGFs (HFGF) because they are thought to act in a systemic fashion rather than a local action for the canonical FGFs (Goetz et al., 2007). We have cloned *fgf19* and *23* and have identified *fgf21* in the genome by synteny and homology to a partial EST clone for *X. laevis fgf21* (Supp. Fig. S1B), but we were unable to clone it from *X. tropicalis* embryonic cDNAs. *Fgf23* is duplicated in the *X. tropicalis* genome (*fgf23.1* and *fgf23.2*, sharing 70.7% identity in their core region but differing in their N and C-termini). We have cloned *fgf23.1* but we did not get an amplification product with oligonucleotides specific for *fgf23.2*. Neither *fgf19* nor *fgf23.1* show a very defined expression pattern (Fig. 6B).

Spatial expression of the *fgfr* genes

The pattern of expression of the *Xenopus laevis* FGF receptors has been reported in detail elsewhere (Hongo et al., 1999; Golub et al., 2000). However, a few conclusions can be drawn from the comparison of the expression pattern of the *fgf* and *fgfr* genes in *X. tropicalis* (Fig. 8 and 9). Despite the fact that from stage 15, the pattern of expression of *fgfr* genes is very defined, they are expressed in domains where there is no obvious expression of *fgfs*. This is particularly apparent in a region lateral to the neural plate at stage 15 where the domain of expression of the *fgfrs* is much wider than of the *fgfs*.

The expression pattern of the *fgfr* genes is very complex. Even when they are expressed in the same domain such as the eye (Fig. 8), a more detail analysis on sections reveals that they are not expressed in the same cells. While *fgfr3* is strongly expressed in the lens, *fgfr1* and *fgfr4* are expressed in the cells surrounding the lens and *fgfr2* is expressed in the outer epithelium of the eye (Fig. 9, in all cases, the first section is at the level of the eye). This

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suggests that each *fgfr* has a different role in the development of the eye. Other organs where multiple *fgfrs* are expressed include the pronephros (*fgfr1, fgfr2* and to a lesser extent *fgfr4*, Fig. 8 and 9), the neural tube and the otic vesicle (see below). Furthermore, each *fgfr* gene is expressed in different domains such as *fgfr1* in the tailbud or *fgfr2* in the neural tube at stage 15. This suggests a strict transcriptional control of their expression.

Expression of *fgf* and *fgfr* genes in the otic vesicle

While it has been known for a long time that multiple *fgfs* are expressed in the otic placode in chick and mouse embryos (for review, see Schimmang, 2007), in *Xenopus* only *fgf3* and *fgf8* have been shown to be expressed in the otic vesicle (Christen and Slack, 1997; Lombardo et al., 1998; Fletcher et al., 2006). Here we show that *fgf1, 2, 3, 4, 8, 10* and *20* are expressed in different structures of the otic placode (Table 1, Fig. 10A). *Fgf10* is the first *fgf* gene detected in the developing otic vesicle at stage 23 (Fig. 4A, 5C). By stage 28, *fgf8* and *10* are expressed in the mesenchyme underlying the otic vesicle. While in the mouse, it has been proposed that *Fgf8* induces *Fgf10* expression during the formation of the otic vesicle (Ladher et al., 2005), the timing of expression of these two genes in *X. tropicalis* would suggest this might be the other way round. *Fgf1* is the first *fgf* gene detected in the ventricular zone of the vesicle (stage 28, Fig. 10A). By stage 35, *fgf1* expression is restricted to the distal region of the otic vesicle, *fgf10* is expressed in a group of cells posterior to the placode, and *fgf3, 4* and *20* are faintly expressed in the ventricular zone. *Fgf2* is expressed only from stage 40 in cells located ventral to the otic vesicle. *X. tropicalis*, therefore, displays a similar expression pattern of *fgf* genes to the one described in the mouse and chick (Schimmang, 2007). The combinatorial expression of the different *fgf* genes is further complicated by the complex expression pattern of their receptors (Fig. 10B). The first receptor to be expressed in the otic vesicle is *fgfr2* as early as st28 (and possibly st23, Fig. 9), followed by *fgfr1* at stage35 and finally *fgfr4* from st40. The challenge will now be to knockdown the expression of each *fgf* genes singularly and in combination to understand their role during the otic vesicle development in *Xenopus*.

Conclusions

Three themes emerged from our spatial and temporal analysis of the expression of the *fgfs* and *fgfrs* genes. Firstly, *fgf* and *fgfr* genes display a wide variety of expression patterns. This has also been shown in other organisms, but our extensive analysis of their expression at different stages of development strongly reinforces this concept. Secondly, multiple *fgfs* are expressed in the same developing organs in the embryos (Table 1). Finally, and perhaps the most striking finding of this study is the dynamic nature of their expression with bursts of expression in a particular region or tissue at a particular stage but which is then quickly switched off. For example, *fgf1* is expressed very strongly in the notochord only at stage 23. Similarly, *fgf4* and *fgf14* are expressed in the otic vesicle and in the lens respectively only at stage 35. It is therefore crucial to have a detailed time course of expression for each of the *fgf* genes to be able to understand their role during embryonic development. Taken all together, the data presented in this study highlight the complexity of the pattern of expression of *fgf* and *fgfr* genes during early embryonic development. Such a resource gives us the means to understand better the pleiotropic roles of FGF signalling during development.

Experimental Procedures

Cloning of *fgfs* and RT-PCR

Total RNA was extracted from *X. tropicalis* embryos from the indicated stages according to the Niewkoop and Faber table (Nieuwkoop and Faber, 1994) using Trizol (Invitrogen).

cDNAs were synthesized using Superscript II (Invitrogen), and PCR reactions were performed using *Taq* polymerase (Roche) according to established protocols. For the RT-PCR analysis, the oligonucleotides used for each *fgf* and the conditions of the PCR are indicated in Supp. Table 1. Control primers for *ornithine decarboxylase* (ODC) have been previously described (Sivak et al., 2005).

For *fgfs* without an EST in the *X. tropicalis* full-length library (Gilchrist et al., 2004) or an IMAGE clone, we amplified the coding sequence using the same oligonucleotides as described for the RT-PCR (Supp. Table 1) with the exception of *fgf1*, for which we used the following primers fwd 5′-ATGGCAGAGGGAGACATCAC-3′, rev 5′-CTAGTCAGGTGATGCTGGCAG-3′ and *fgf22* rev 5′-TTACATGGGAAAAGGTAAAAAGTGTGCTG-3′. After amplification, the PCR products were purified and TA cloned using either pCR2.1 or PCRII (both from Invitrogen) according to the manufacturers' instructions. All clones were verified by sequencing.

Whole-mount *in situ* hybridisation and histology

Whole-mount *in situ* hybridisations were performed essentially as previously described in (Harland, 1991) using DIG-labelled antisense probes and anti-DIG AP conjugated antibodies (Roche). BM Purple (Roche) was used as the substrate for the alkaline phosphatase. The constructs used to generate probes are described in Supp. Table 2. Once a satisfactory signal had been obtained the embryos were post-fixed in Bouin solution (without picric acid) and bleached in 69.5% formamide, 30% MetOH, 0.5% H2O2. Embryos with a specific expression pattern were then embedded in a gelatin/albumin mixture and solidified with glutaraldeyhyde. The embryos were subsequently sectioned with a 25-30 μm thickness using a Leica VT1000M vibratome. The sections were then mounted in 90% glycerol. Images were taken using an Olympus IX70 inverted microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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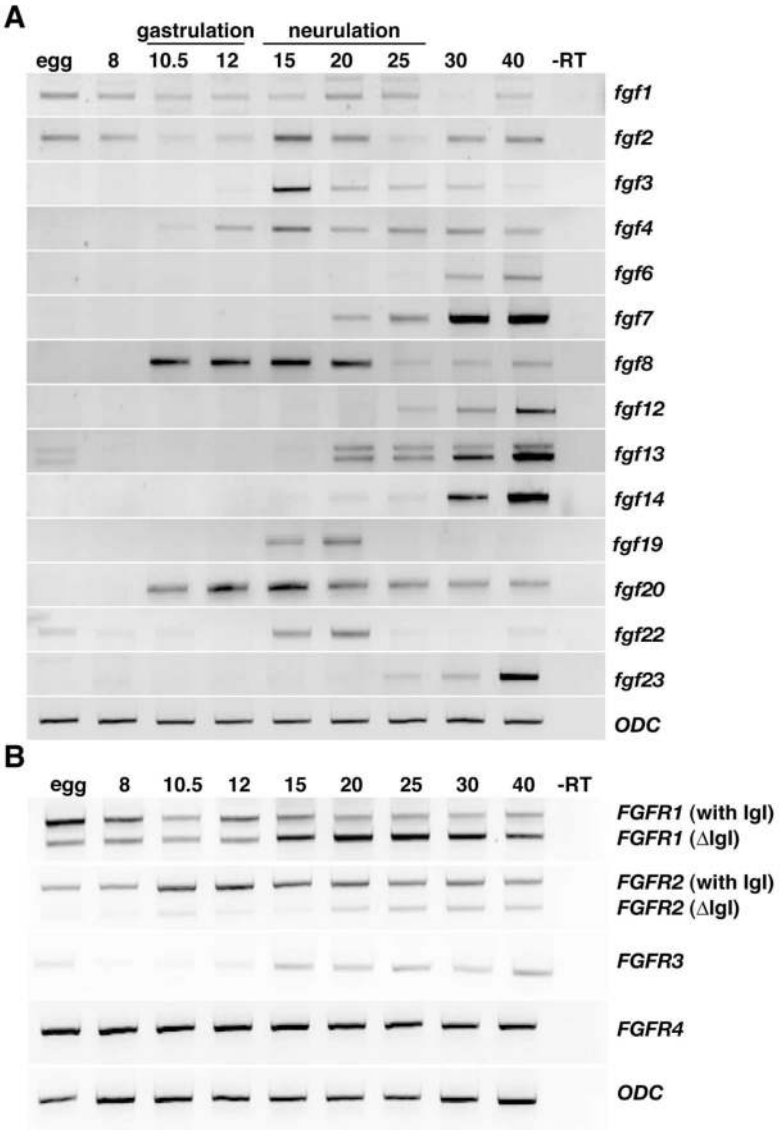

Figure 1.

Temporal expression of *fgf* ligands and receptors. **A** Temporal expression of *fgfs* genes by RT-PCR. **B** Temporal expression of *fgfr* by RT-PCR. In **A** and **B**, embryos were harvested for RNA extraction at the indicated stages, and RT-PCR analysis was performed using the oligonucleotides and conditions described in Supp. Table 1. The house keeping gene *ornithine decarboxylase* (*ODC*) was used as a control for equal loading. −RT lane is a negative control using RNA from st40 without the addition of reverse transcriptase.

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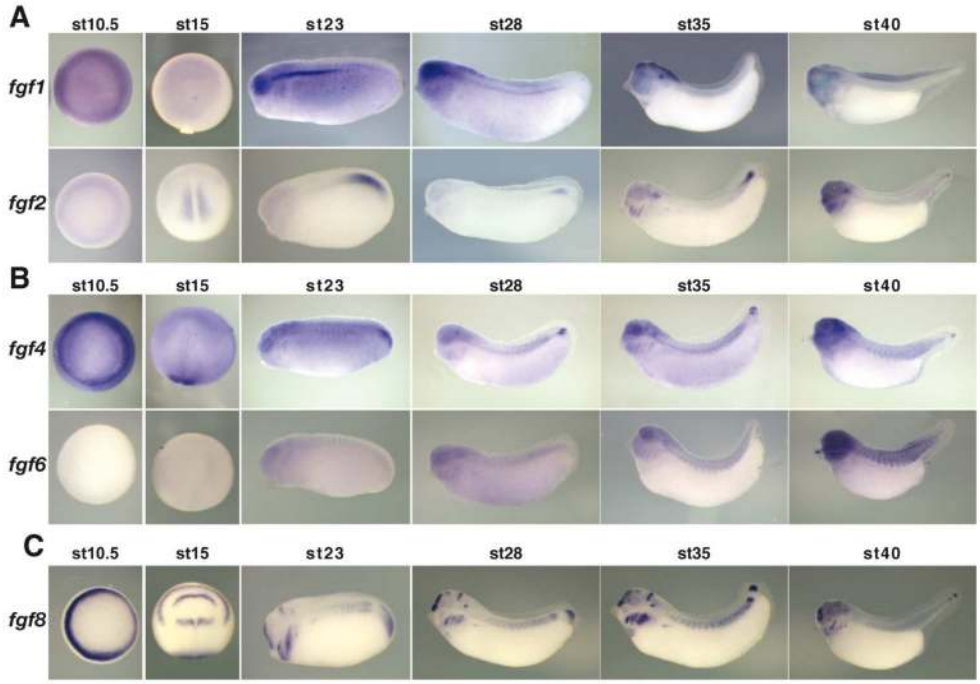

Figure 2.

Analysis of the spatial expression patterns of the *fgf1, fgf4* and *fgf8* subfamilies. **A** Whole mount *in situ* hybridisations of *fgf1* and *fgf2* at the indicated stages. **B** Whole mount *in situ* hybridisations of *fgf4* and *fgf6* at the indicated stages. **C** Whole mount *in situ* hybridisations of *fgf8*. For stage 10.5, the images are vegetal views, for stage 15 the images are dorsal views and for stage 23, 28, 35 and 40 the images are lateral views (anterior is left, posterior is right, dorsal is up and ventral down). A schematic representation of a *Xenopus* embryo at stage 28 with the different embryonic tissues labelled is presented in Supp. Fig. S3.

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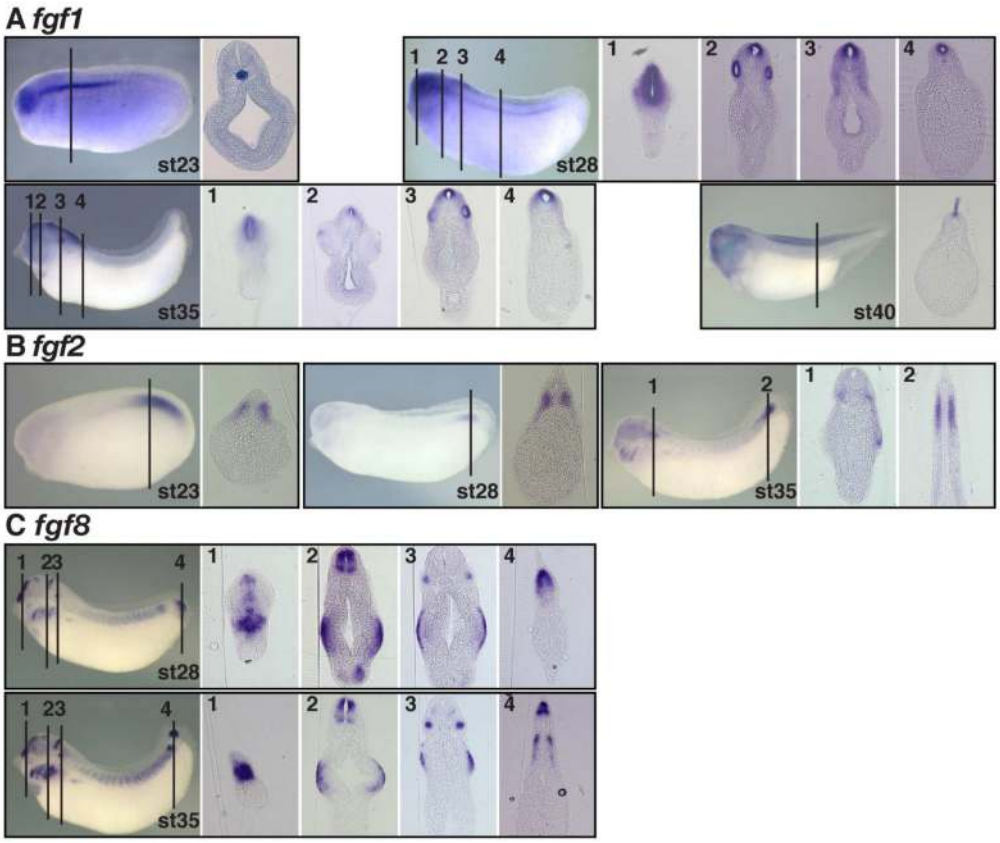

Figure 3.

Analysis of the spatial expression patterns of the *fgf1, fgf4* and *fgf8* subfamilies. Coronal sections of indicated *fgfs* from the whole mount *in situ* hybridisations shown in Fig. 2. The black lines indicate the position of the sections shown, when multiple sections are shown numbers correspond to the appropriate black line and therefore the section's position on the embryo. In all sections, dorsal is up and ventral is down.

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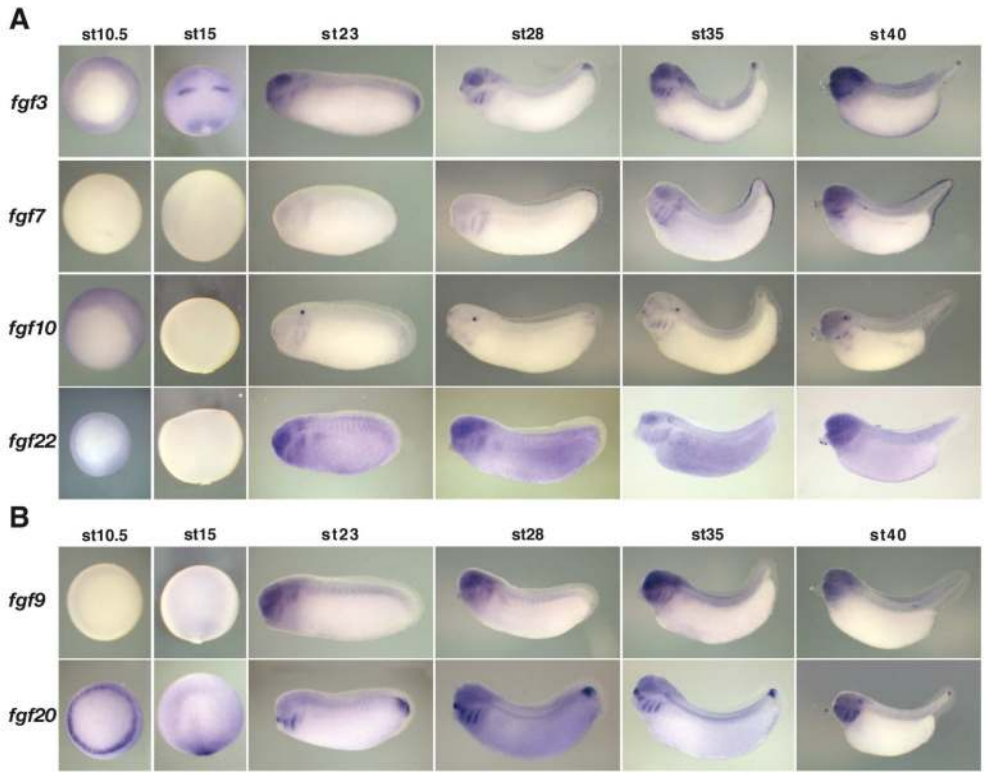

Figure 4.

Analysis of the spatial expression patterns of the *fgf7* and *fgf9* subfamilies. **A** Whole mount *in situ* hybridisations of *fgf3, fgf7, fgf10* and *fgf22* at the indicated stages. **B** Whole mount *in situ* hybridisations of *fgf9* and *fgf20* at the indicated stages. For stage 10.5, the images are vegetal views, for stage 15 the images are dorsal views and for stage 23, 28, 35 and 40 the images are lateral views (anterior is left, posterior is right, dorsal is up and ventral down). A schematic representation of a *Xenopus* embryo at stage 28 with the different embryonic tissues labelled is presented in Supp. Fig. S3.

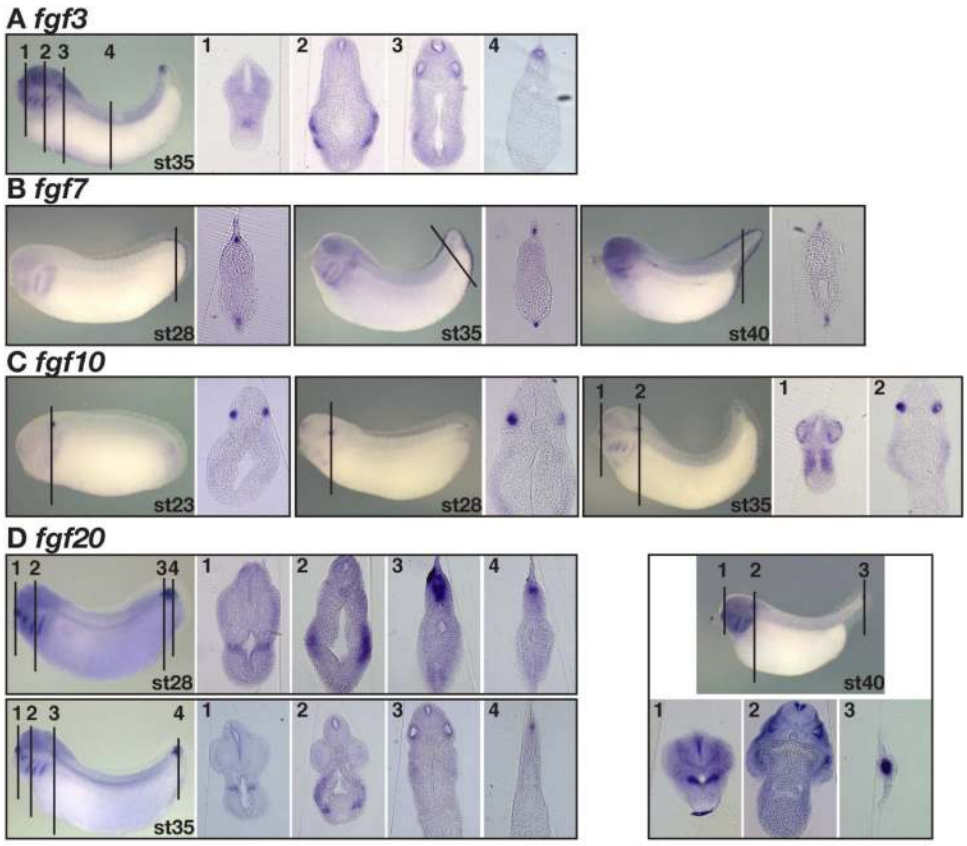

Figure 5.

Cross-section analysis of the spatial expression patterns of the *fgf7* and *fgf9* subfamilies. Coronal sections of the indicated *fgfs* from the whole mount *in situ* hybridisations shown in Fig. 4. The black lines indicate the position of the sections shown, when multiple sections are shown numbers correspond to the appropriate black line and therefore the section's position on the embryo. In all sections, dorsal is up and ventral is down.

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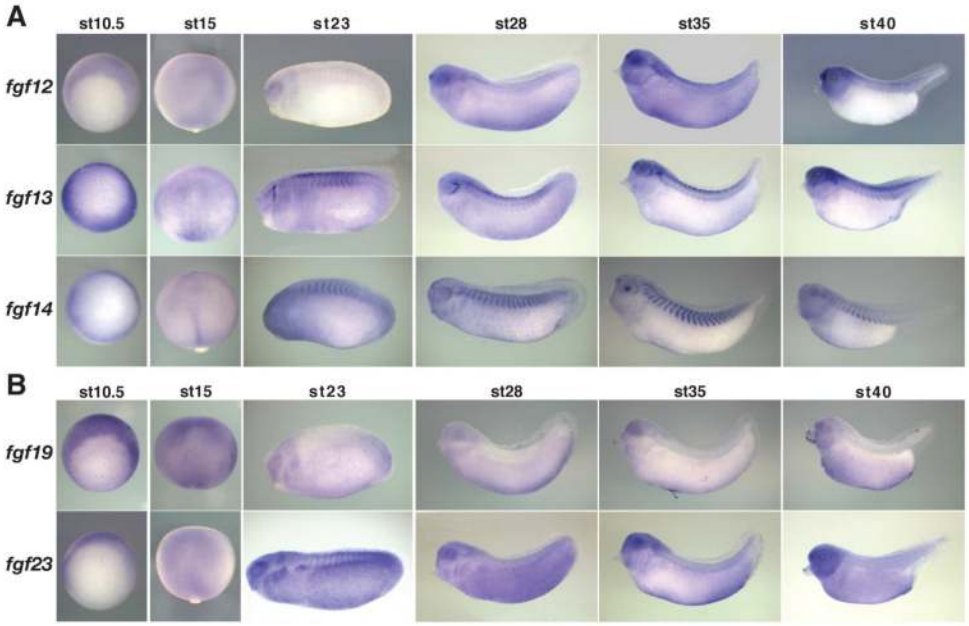

Figure 6.

Analysis of the spatial expression patterns of the intracellular *fgfs* and hormone-like *fgfs* subfamilies. **A** Whole mount *in situ* hybridisations of *fgf12, fgf13* and *fgf14* at the indicated stages. **B** Whole mount *in situ* hybridisations of the intracellular *fgfs* and hormone-like *fgfs* at the indicated stages. For stage 10.5, the images are vegetal views, for stage 15 the images are dorsal views and for stage 23, 28, 35 and 40 the images are lateral views (anterior is left, posterior is right, dorsal is up and ventral down). A schematic representation of a *Xenopus* embryo at stage 28 with the different embryonic tissues labelled is presented in Supp. Fig. S3.

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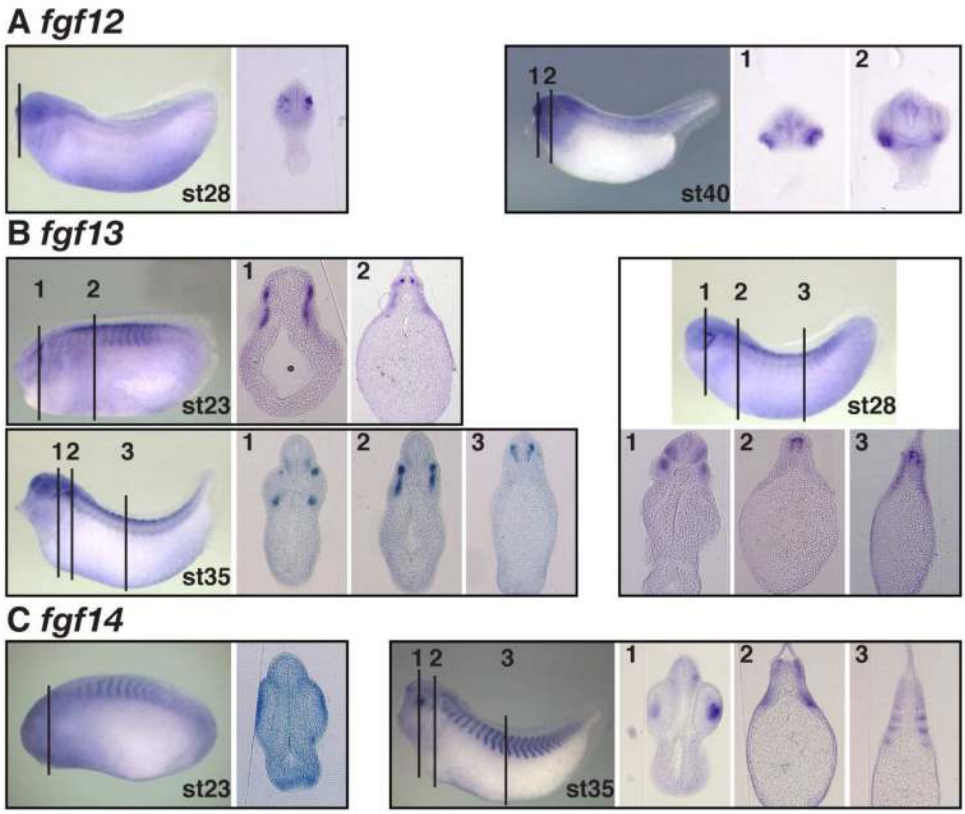

Figure 7.

Cross-section analysis of the spatial expression patterns of the intracellular *fgfs* and hormone-like *fgfs* subfamilies. Coronal sections of indicated *fgfs* from the whole mount *in situ* hybridisation shown in Fig. 6. The black lines indicate the position of the sections shown, when multiple sections are shown numbers correspond to the appropriate black line and therefore the section's position on the embryo. In all sections, dorsal is up and ventral is down.

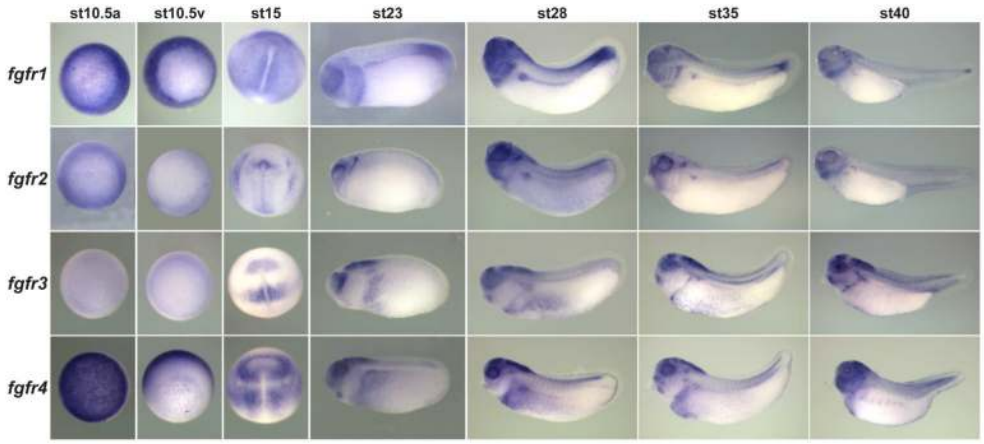

Figure 8.

Spatial expression of the *fgfr* genes during early *Xenopus tropicalis* development. Antisense probes specific for each receptor were generated from the constructs indicated in Supp. Table 2 and *in situ* hybridisations were performed. 10.5a are animal views and 10.5v are vegetal views of gastrulating embryos, and stage15 are dorsal views of a neurulating embryo. Stage 23, 28, 35, 40 are lateral views with anterior left and dorsal up. A schematic representation of a *Xenopus* embryo at stage 28 with the different embryonic tissues labelled is presented in Supp. Fig. S3.

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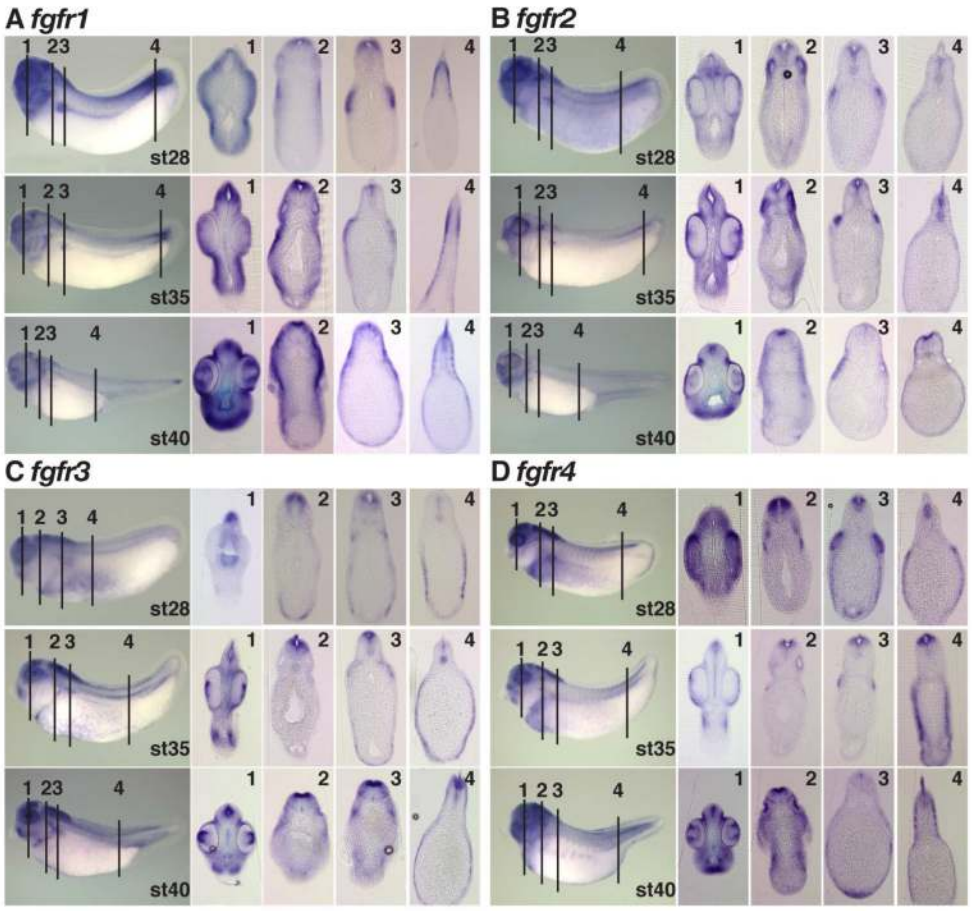

Figure 9.

Cross-section analysis of the spatial expression patterns of the *fgfrs*. Coronal sections of indicated *fgfs* from the whole mount *in situ* hybridisation shown in Fig. 8. The black lines indicate the position of the sections shown. The sections labelled “1” are all at the level of the eye, the sections labelled “2” are through the otic vesicle, the sections labelled “3” are at the level of the pronephros and the section labelled “4” are in the tail of the embryo. In all sections, dorsal is up and ventral is down.

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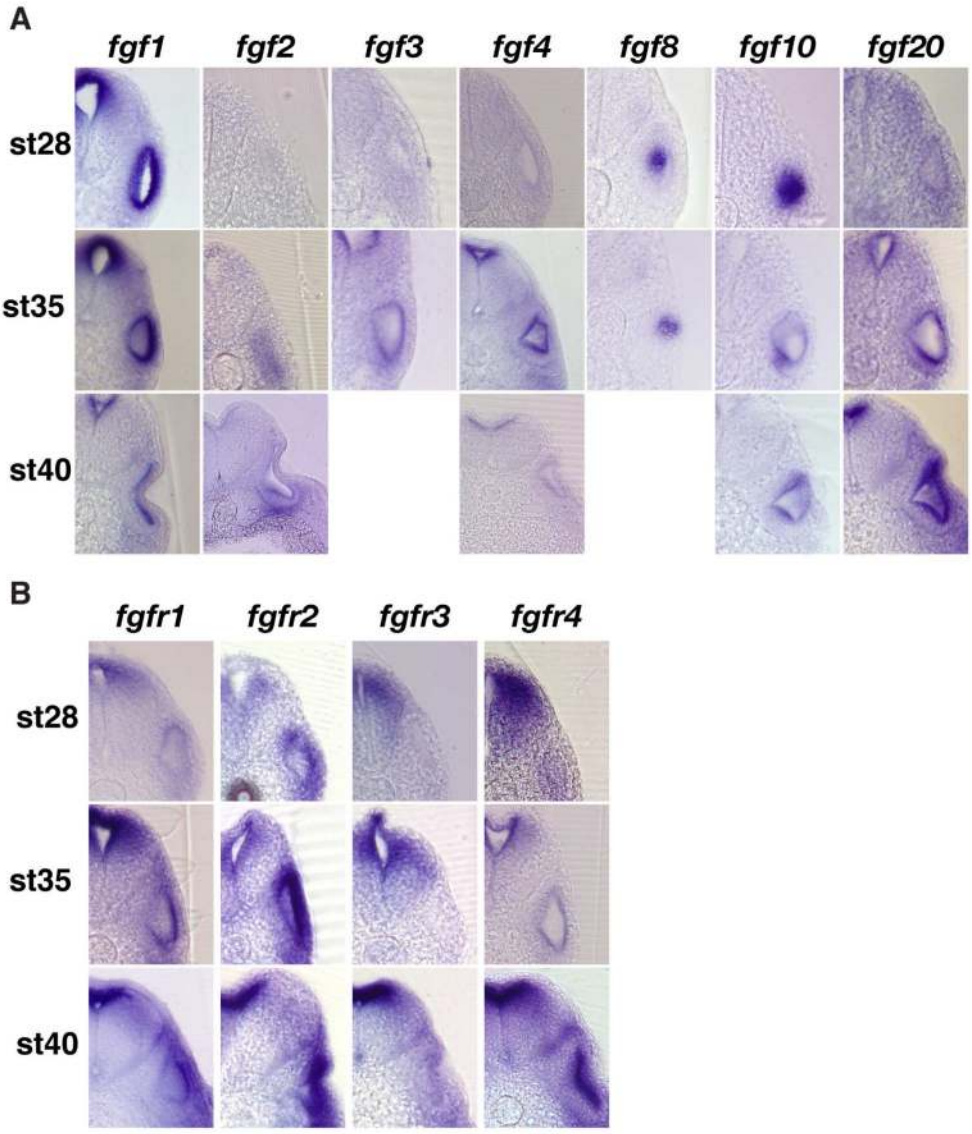

Figure 10.

Detail analysis of the expression of the different *fgf* and *fgfr* genes in the otic vesicle. **A** Coronal sections (dorsal up) of whole mount *in situ* hybridisations staining within the otic vesicle for *fgf1, fgf2, fgf3, fgf4, fgf8, fgf10* and *fgf20* at stages 28, 35 and 40. **B** Coronal sections (dorsal up) of whole mount *in situ* hybridisations staining within the otic vesicle for *fgfr1, fgfr2, fgfr3* and *fgfr4* at stages 28, 35 and 40.

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Table 1

Expression of the *fgf* genes in different organs in *Xenopus* embryos. MHB, midbrain-hindbrain boundary.

forebrain	*fgf1, fgf2, fgf8, fgf20*
tailbud	*fgf2, fgf3, fgf4, fgf8, fgf20*
olfactory placode	*fgf1, fgf12*
otic vesicle	*fgf1, fgf2, fgf3, fgf4, fgf8, fgf10, fgf20*
fin	*fgf1, fgf7*
eye	*fgf1, fgf3, fgf13, fgf14, fgf20*
marginal zone	*fgf2, fgf4, fgf8, fgf20*
branchial arches	*fgf2, fgf3, fgf7, fgf8, fgf10, fgf20*
somites	*fgf6, fgf8, fgf14*
MHB	*fgf3, fgf4, fgf8*
neural tube	*fgf1, fgf3, fgf13, fgf20*