Development 124, 4253-4264 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
DEV4917

A role for FGF-8 in the dorsoventral patterning of the zebrafish gastrula

Maximilian Fürthauer, Christine Thisse and Bernard Thisse*

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS, INSERM, ULP, 1 rue Laurent Fries, BP 163, 67404 ILLKIRCH Cedex, France

*Author for correspondence (e-mail: thisse@titus.u-strasbg.fr)

SUMMARY

Signals released from Spemann's organizer, together with ventralizing factors such as BMPs, are necessary to pattern the dorsoventral axis of the vertebrate embryo. We report that a member of the FGF family, *fgf-8*, not secreted by the axial mesoderm but expressed in a dorsoventral gradient at the margin of the zebrafish gastrula, also contributes to the establishment of the dorsoventral axis of the embryo. Ectopic expression of FGF-8 leads to the expansion of dorsolateral derivatives at the expense of ventral and posterior domains. Moreover, FGF-8 displays some organizer properties as it induces the formation of a partial secondary axis in the absence of factors released from Spemann's organizer territory. Analysis of its interaction with the ventralizing factors, BMPs, reveals that overexpression of FGF-8 inhibits the expression of these factors in the ventral part of the embryo as early as blastula stage, suggesting that FGF8 acts upstream of BMP2 and BMP4. We conclude that FGF-8 is involved in defining dorsoventral identity and is an important organizing factor responsible for specification of mesodermal and ectodermal dorsolateral territories of the zebrafish gastrula.

Key words : *fgf-8*, Spemann's organizer, gastrulation, dorsoventral patterning, convergence, BMP2, BMP4, Chordin, Noggin, Goosecoid

INTRODUCTION

Members of secreted protein families including the fibroblast growth factors (FGF), transforming growth factors (TGF), Wnt and hedgehog proteins function together to organize and pattern different tissues throughout embryogenesis and adult life (Christian and Moon, 1993; Echelard et al., 1993; Smith, 1994, and reviewed in Yamaguchi and Rossant, 1995). The FGFs, which currently constitute a family of nine members, FGF1-FGF9, affect target cells via high-affinity receptor tyrosine kinases and act primarily through an evolutionarily conserved ras-dependent intracellular signal transduction pathway (Tanaka et al., 1992; Miyamoto et al., 1993; reviewed by Basilico and Moscatelli, 1992). FGF signal transduction pathways play essential roles in promoting the growth and differentiation of cells of diverse origins. Depending of the target cell, FGFs can stimulate mitogenic, differentiation, migration and survival responses depending on the target cell. FGFs have been also implicated in mesoderm induction and patterning during early *Xenopus* and zebrafish embryogenesis (Kimelman and Kirschner, 1987; Griffin et al., 1995). For example, overexpression of a dominant-negative mutant form of a *FGFR* gene results in a lack of posterior structures, suggesting that FGF signaling is required for the differentiation of mesoderm (Amaya et al., 1991). A similar conclusion was drawn from analyses of mice embryos homozygous for a null allele of the *FGFR1* gene (Yamaguchi et al., 1994).

One of the FGF family members, *Fgf-8*, was identified based on its capacity to mediate the androgen-dependent growth of a mouse mammary tumor cell line (Tanaka et al., 1992). *Fgf-8* is expressed in several regions of the developing mouse that direct the outgrowth and patterning including the limb, the elongating body axis, the face and the midbrain/hindbrain region (Crossley and Martin, 1995). Functional analyses led to the proposal that FGF-8 is an important signaling molecule as it initiates limb bud outgrowth and takes part in the regulation of limb development (Crossley et al., 1996a). FGF-8 was also shown to display an inducing activity in midbrain development of the chick embryo (Crossley et al., 1996b). However, little is known about the potential role of FGF-8 during early embryogenesis. In order to focus on these early events, we have chosen to analyze FGF-8 in zebrafish (*Danio rerio*) and have isolated a full-length cDNA corresponding to the homologous mouse *FGF-8b* isoform also called *AIGFI* (Crossley and Martin, 1995). Zebrafish *fgf-8* is a zygotic gene, expressed as early as the blastula stage. During gastrulation, FGF-8 transcripts accumulate in a dorsoventral gradient at the margin of the embryo. When ectopically expressed, FGF-8 induces dorsolateral structures. It is also able to organize a secondary axis up to the position of the anterior midbrain, even in the absence of the expression of Spemann's organizer factors. We also show that the dorsalization induced by FGF8 is associated with an inhibition of the ventralizing factors BMP2 and BMP4 gene expression as early as the blastula stage.

Taken together, our results strongly suggest that FGF-8 affects the dorsoventral positional identity of cells within the gastrula and that it modifies the convergence movements of cells toward the dorsal midline. We propose that FGF-8 is involved in the control of ventralizing factors expression and that, in concert with dorsalizing Spemann organizer factors, FGF8 acts to specify the dorsolateral structures of the zebrafish embryo.

MATERIALS AND METHODS

Whole-mount in situ hybridization

Whole-mount in situ hybridizations were performed according to Thisse et al. (1993).

Preparation of capped RNA and injections

The *fgf-8* cDNA was inserted into the expression vector pCS2+ (a gift from R. Rupp). Plasmid was linearized with *Not*I and transcribed in vitro using the Stratagene in vitro transcription kit. Injections were performed as described in Wittbrodt and Rosa (1994). 40 pg of FGF-8 synthetic RNA was pressure injected into single blastomeres of cleavage-stage zebrafish embryos. After injection, embryos were allowed to develop until somitogenesis stages and then fixed in 4% paraformaldehyde with their uninjected siblings to examine expression of different marker genes.

In a different series of injections, we used β-galactosidase RNA as a lineage tracer (Vize et al., 1991). Coinjected FGF-8 and β-galactosidase RNAs are expected to be similarly distributed. A mix of 40 pg of FGF-8 RNA and 200 pg of β-galactosidase RNA was injected in cleavage-stage embryos. Embryos were then fixed at early somitogenesis stages and localization of β-galactosidase activity was performed as described by Vize et al. (1991). The defects generated by ectopic expression of FGF-8 were then analyzed by in situ hybridization.

Where indicated, rhodamine coupled to 2 MDa dextran (Molecular probes, USA), a lineage tracer known to be unable to diffuse from one blastomere to another at early cleavage stage (Strehlow et al., 1994), was included at 35 μg/μl in the injection mix.

The zebrafish BMP2 cDNA (a gift from Dr Tada) was subcloned into pCS2+. 60 pg of synthetic RNA was injected into single blastomeres of cleavage-stage embryos. The zebrafish full-length BMP4 was obtained starting from an initial clone kindly given by Dr Chin. The full-length clone was sequenced and subcloned in pCS2+. 4 pg of synthetic BMP4 RNA was injected into blastomeres of 2-cell-stage embryos. 200 pg of *Xenopus* Chordin RNA (Sasai et al., 1995) or 2 pg of synthetic *Xenopus* Noggin RNA (Smith and Harland, 1992) were injected.

RESULTS

Isolation and sequence analysis of zebrafish *fgf-8* cDNA

A *fgf-8* cDNA was isolated during a screen looking for genes expressed during blastula and gastrulation stages. The clone isolated from a segmentation stage cDNA library (a gift from D. Grunwald) was 1.3 kb long. Sequence analysis showed that it encoded a protein sharing sequence similarities with the mouse and chicken FGF-8 proteins (Crossley and Martin, 1995; McArthur et al., 1995). Since this clone did not include the entire coding region, it was used to rescreen the cDNA library. Eleven clones were isolated and sequenced. Among them, three *fgf-8* cDNA clones, contained the same complete protein coding region, the entire 3′ untranslated region and differing portions of the 5′ untranslated leader sequence.

The zebrafish FGF-8 protein is 210 amino acids long. In the amino-terminal portion, 22 amino acids share the characteristic sequence similarities of a potential signal peptide motif, which is known to allow the secretion of the protein after cleavage (reviewed in Fernig and Gallagher, 1994). The amino-acid sequences of different members of the FGF-8 family were aligned and compared (Fig. 1). The zebrafish FGF-8 shares 84.7% identity with the chicken FGF-8 (McArthur et al., 1995) and 79.2% identity with the AIGF1 murine isoform (Crossley and Martin, 1995). All proteins are highly conserved within their internal region, diverging more in their amino and carboxy terminal parts.

Zebrafish *fgf-8* is expressed in a dorsoventral gradient at gastrulation

Transcripts of FGF-8 first accumulated at sphere stage, soon after the midblastula transition, in cells at the dorsal margin of the embryo. At the beginning of epiboly, marginal expression extended rapidly to additional ventral cells and FGF-8 RNA was localized all around the margin (Fig. 2A). In this marginal region FGF8 RNA was also observed in cells of the enveloping layer, from the blastula stage to the end of gastrulation (not shown). When gastrulation begins, as FGF-8 transcripts became restricted to the dorsal part of the marginal region (Fig. 2B), *fgf-8* appeared to be expressed in a characteristic dorsoventral gradient at the margin of the embryo (Fig. 2C). FGF-8-expressing cells were detected in the superficial epiblast layer cells of the embryonic shield but not in the hypoblastic deep cells that will later form the prechordal plate. As gastrulation proceeds, the *fgf-8* marginal domain of expression was confined to epiblastic cells directly in contact with the underlying hypoblast (Fig. 2D). In addition to this expression, *fgf-8* in the dorsal part of the embryo was also detected in the paraxial hypoblast territory (Fig. 2D). Starting at 70% epiboly and persisting until somitogenesis stages, FGF-

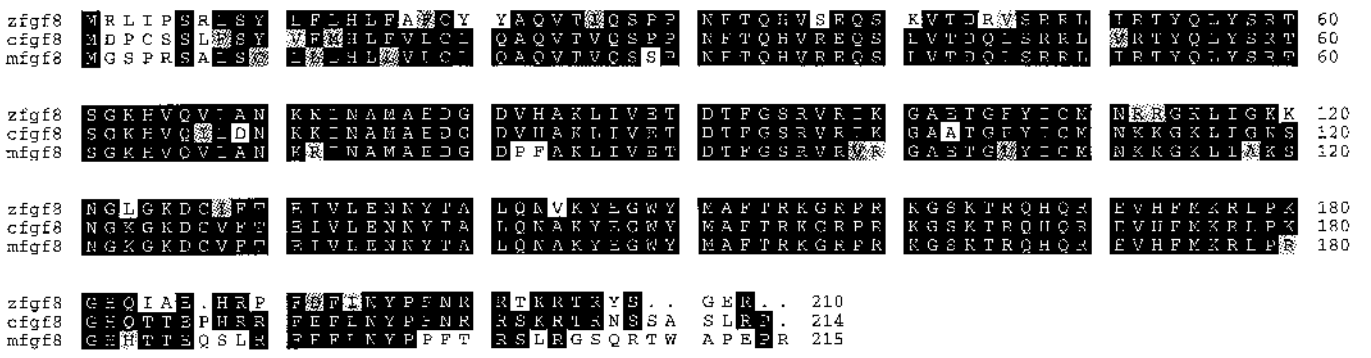

Fig. 1. Comparison of the predicted protein sequence of zebrafish FGF-8 (zfgf8) with the chicken FGF-8 (cfgf8, McArthur et al., 1995) and the AIGF1 murine isoform of FGF-8 (mfgf8, Crossley et al., 1995). Identical amino acids are indicated by black boxes, conservative substitutions by grey. Dots indicate gaps inserted in the sequences to maximize homology.

8 transcripts also appeared in the epiblast layer, as two bands of labeled cells that will become part of the hindbrain (Fig. 2E,F).

During somitogenesis, transcripts were also located in the anterior forebrain, in the epiphysis, in rhombomeres 1, 2, and 4 as well in the anterior border of somites. As in mice and chicken embryos (Crossley and Martin, 1995; Crossley et al., 1996a), *fgf-8* expression was also closely associated with regions thought to be signaling centers such as the facial ectoderm, the mesencephalon-telencephalon junction, the apical ectodermal ridge of the fin bud and the tail bud (data not shown).

Ectopic expression of FGF-8 alters the dorsoventral patterning of the zebrafish embryo

Since FGF family members have been implicated in dorsoventral patterning of vertebrate embryos and as *fgf-8* is expressed in a dorsoventral gradient at gastrulation, we examined whether FGF-8 affected early embryonic development through misexpression studies. First, in vitro synthesized FGF-8 RNA was injected into 2-cell-stage embryos and the resulting phenotypes were analyzed by morphology and by in situ hybridization. At the end of gastrulation, 70% (84/121) of FGF-8-injected embryos displayed an ovoid or an elongated shape, characteristic of dorsalization phenotypes (Mullins et al., 1996). The FGF-8-induced alterations became very striking at beginning of somitogenesis when the somites were expanded more ventrally and, in extreme cases, extended all around the circumference of the embryo fusing at the ventral midline (Fig. 3B,C). Derived embryos were followed until 36 hours of development and to facilitate description, have been grouped into classes, depending on the extent of the generated defects. Weak phenotypes (22.6% or 19/84) consisted of a small shortening in their tail length and the absence of the ventral caudal fin (Fig. 3E). Phenotypes of intermediate strength (23.8% or 20/84) consisted of a shortened, and/or twisted tail and a reduced blood territory (Fig. 3F). A stronger class of defects (16.6% or 14/84) gave rise to embryos for which the trunk axis twisted around itself, the tail was missing and blood was not apparent (Fig. 3G,H). Finally, the strongest class of alterations (17.8% or 15/84) corresponded to embryos for which somites fused on the ventral midline. They lysed without reaching late somitogenesis stages (not shown).

Among the dorsalizing phenotypes that we have obtained, 19% of embryos (16/84) displayed a partially defective secondary axis extending anteriorly up to the midbrain (Fig. 3I-K). In this secondary axis, structures such as otic vesicles (Fig. 3I), a beating heart and a second set of pectoral fin buds were easily identified. In contrast, optic and olfactory placodes, as well as notochord and floor plate, were lacking (Fig. 3J,K). To determine precisely the extent of the structures generated in the cephalic region of the secondary axis, a combination of probes including *Krox20* (Oxtoby and Jowett, 1993) and *Eng3* (Ekker et al., 1992), which label rhombomeres 3 and 5, and the midbrain-hindbrain junction, respectively, revealed that the forebrain and anterior midbrain were absent from this secondary axis. Labeling with *Isl-1* (Inoue et al., 1994) which marks the cranial ganglia, Rohon-Beard neurons and primary motor neurons showed that, in the spinal cord, dorsal sensory neurons were present while ventrally located motor neurons were missing (Fig. 3K). Analysis at gastrulation of the expression of genes specifically expressed in the organizer region such as *goosecoid* (Thisse et al., 1994) or in the axial midline such as *sonic hedgehog* (Krauss et al., 1993) showed that both genes were never induced by the overexpression of FGF-8 (data not shown). This result correlates with the absence of anterior and axial structures in the secondary axis of FGF-8-injected embryos.

To further characterize the effect of ectopic FGF-8, synthetic RNA was injected into single blastomeres of embryos from the 2- to the 16-cell stage. We examined expression of a combination of mesodermal- and ectodermal-specific genes. The embryos were analyzed by in situ hybridization during early somitogenesis stages, with probes for *MyoD* (Weinberg et al., 1996), which labels adaxial cells adjacent to the notochord and the posterior part of each somite, and for the *AP-2* gene. Zebrafish *AP-2* (our unpublished data) is homologous to the mouse *AP-2* gene (Mitchell et al., 1991) and is expressed in cells bordering the neural plate, including presumptive neural crest cells (Fig. 4A). Other sets of probes included both *AP-2* and *AS28*, a zebrafish cDNA whose transcripts are specific of

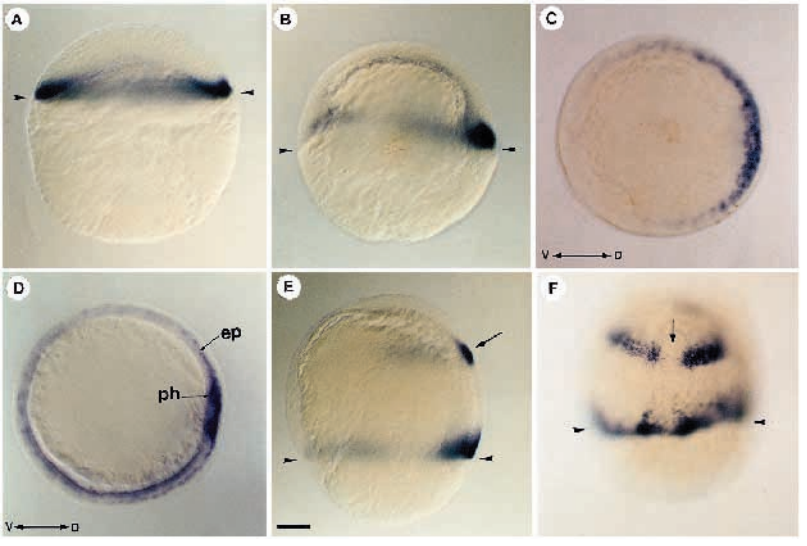

Fig. 2. Localization of FGF-8 RNA during blastula and gastrula stages. (A) At blastula stage (30% epiboly), FGF-8 RNA is detected all around the margin of the blastoderm. (B) At early gastrula (50% epiboly) FGF-8 is restricted to the dorsal part of the marginal region. (C) Animal pole view of an embryo at 50% epiboly showing that *fgf-8* is expressed as a dorsoventral gradient. (D) At midgastrula stage, FGF-8 transcripts are confined to the epiblast cells (ep) in contact with the underlying hypoblast. Dorsally, paraxial hypoblast (ph) territory is also positive for FGF-8 transcripts. (E) In addition to the marginal gradient, *fgf-8* at midgastrula is expressed in the presumptive hindbrain (arrow). (F) Two bands of cells expressing FGF-8 are separated by a non-expressing domain (arrow) which will later give rise to the ventral midline of the hindbrain. (A,B,E) Lateral views; (C) animal pole view; (D) vegetal pole view; (F) dorsal view. V, ventral; D, dorsal. Arrowheads in A,B,E,F indicate the position of the margin. Scale bar : 100 μm

primary neurons (personal data, Fig. 4I-J) or a combination of *Krox 20*, *Eng3* and *AS28*.

Ectopic expression of FGF-8 led to a range of pattern abnormalities along the dorsoventral embryonic axis, which could be distinguished into four classes. The first class of defects was characterized by an expansion of the dorsolateral territories, visualized by a lateral extension of the somitic furrows labeled with *MyoD* (Fig. 4B) and an enlargement of the neural plate revealed by *AP-2* staining further from the midline than in wild type (Fig. 4A,B). The second class of alterations consisted of a fragmentation of the paraxial territory (Fig. 4C). These embryos displayed a partial formation of a secondary axis, containing paraxial but not axial or adaxial mesoderm. In the third class (Fig. 4D,E), the secondary axis was located in a ventral position (Fig. 4E). In the anterior part of the primary axis (Fig. 4D), *AP-2* expression in premigratory neural crest extended up to the midbrain-hindbrain junction and left and right domains of *AP-2* expression remained separated. In contrast, left and right domains of *AP-2* expression fused in the anterior part, reflecting the lack of presumptive midbrain and forebrain in the secondary axis (Fig. 4E). The last class of alterations consisted of embryos showing extremely severe dorsalization, visualized by the circularization of somites all around the embryo (Fig. 4F). Except for the axial and adaxial territories, all mesodermal cells acquired a paraxial identity. This severely dorsalized class of embryos was also probed with *snail2*, which is expressed at the end of gastrulation in cephalic mesoderm and posterior prechordal plate (Thisse et al., 1995a). In these embryos displaying a strong phenotype (Fig. 4H), Snail2 staining revealed that lateral cephalic mesoderm was also completely circularized. Thus in the mesodermal territory, dorsolateral domains were expanded ventrally while axial and adaxial structures were not affected by FGF-8 overexpression.

Embryos presenting mesodermal defects displayed corresponding alterations in the ectodermal layer. Analysis of FGF-8-injected embryos with *AS28* and *AP-2* allowed defects on the dorsoventral patterning of the ectodermal layer to be precisely defined. During early somitogenesis of wild-type

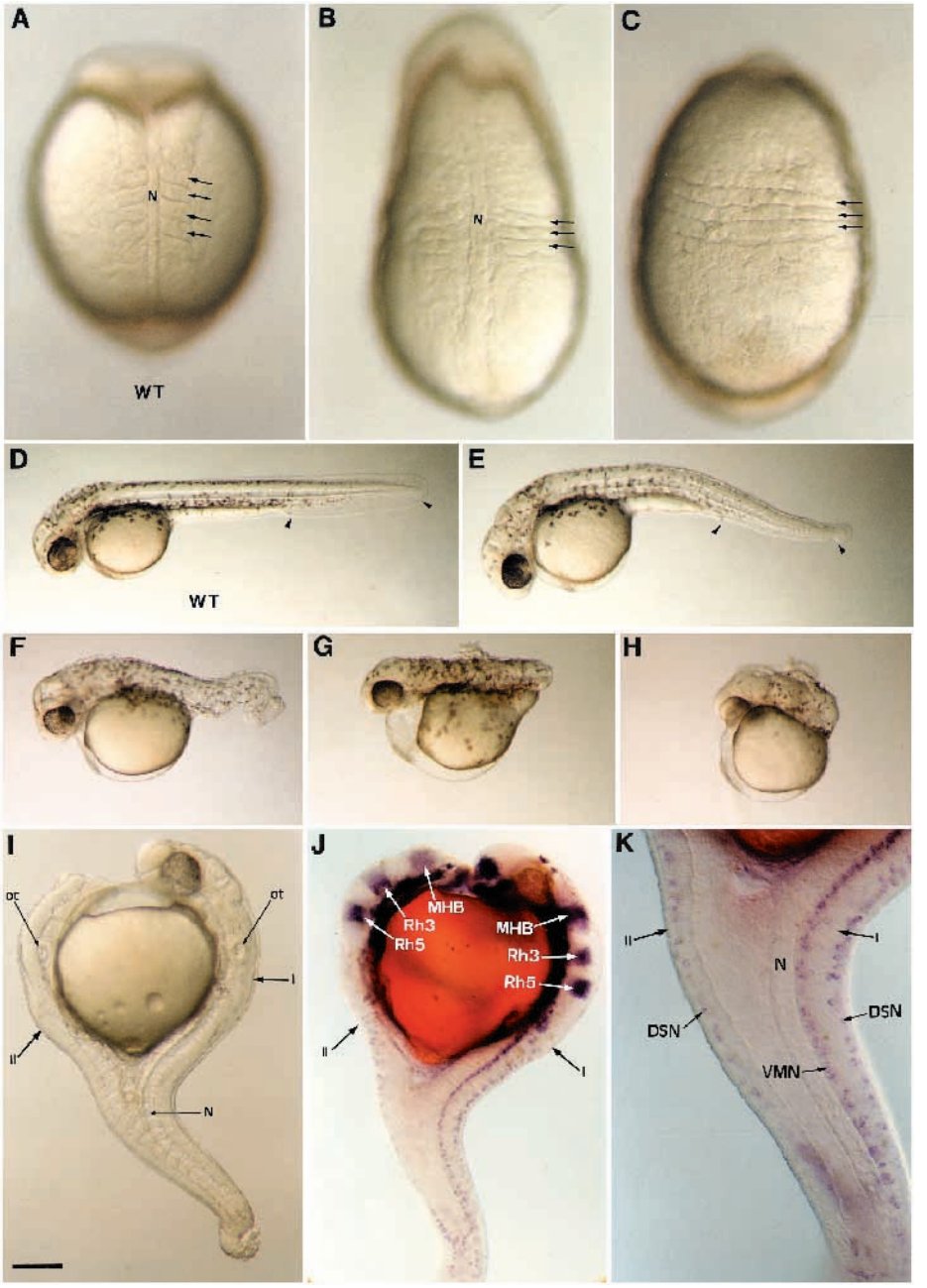

Fig. 3. Morphological defects generated by ectopic expression of FGF-8. A range of dorsalization phenotypes is obtained after FGF-8 RNA injection. At the beginning of somitogenesis, the embryos display an elongated shape and somites (arrows) expand laterally (B) or even fuse ventrally (C) compared to wild-type control in A. At 36 hours, weak phenotype is apparent as a small reduction in the tail region delimited with arrowheads (E), compared to wild type in (D). Phenotype of intermediate strength are represented by a shortened and twisted tail (F). Strong phenotypes are characterized by a deletion of the tail (G) or by a deletion of both trunk and tail (H). (I-K) A 36 hours old dorsalized embryo presenting a double axis (I, primary axis; II, secondary axis). (I) Anteriorly the secondary axis forms opposite to the primary axis while both are fused in the caudal portion. (J) Using *Krox20* (Oxtoby and Jowett, 1993) and *Eng3* (Ekker et al., 1992) reveal that the secondary axis develops up to the posterior midbrain. Anterior to this position, forebrain, anterior midbrain, olfactory placodes and optic vesicules are missing in the secondary axis. (J,K) The secondary axis is defective in notochord and floor plate. Ventral motor neurons (VMN) are missing while dorsal sensory neurons (DSN) labeled with *Isl-1* (Inoue et al., 1994) are present. MHB, midbrain-hindbrain boundary labeled with *Eng3*; N, notochord; ot, otic vesicles, rh3, rh5, rhombomeres 3 and 5 labeled with *Krox20*. (A,B) Dorsal views; (C) ventral view; (D-K) lateral views. Scale bar, 100 μm in A-C,K; 250 μm in D-J.

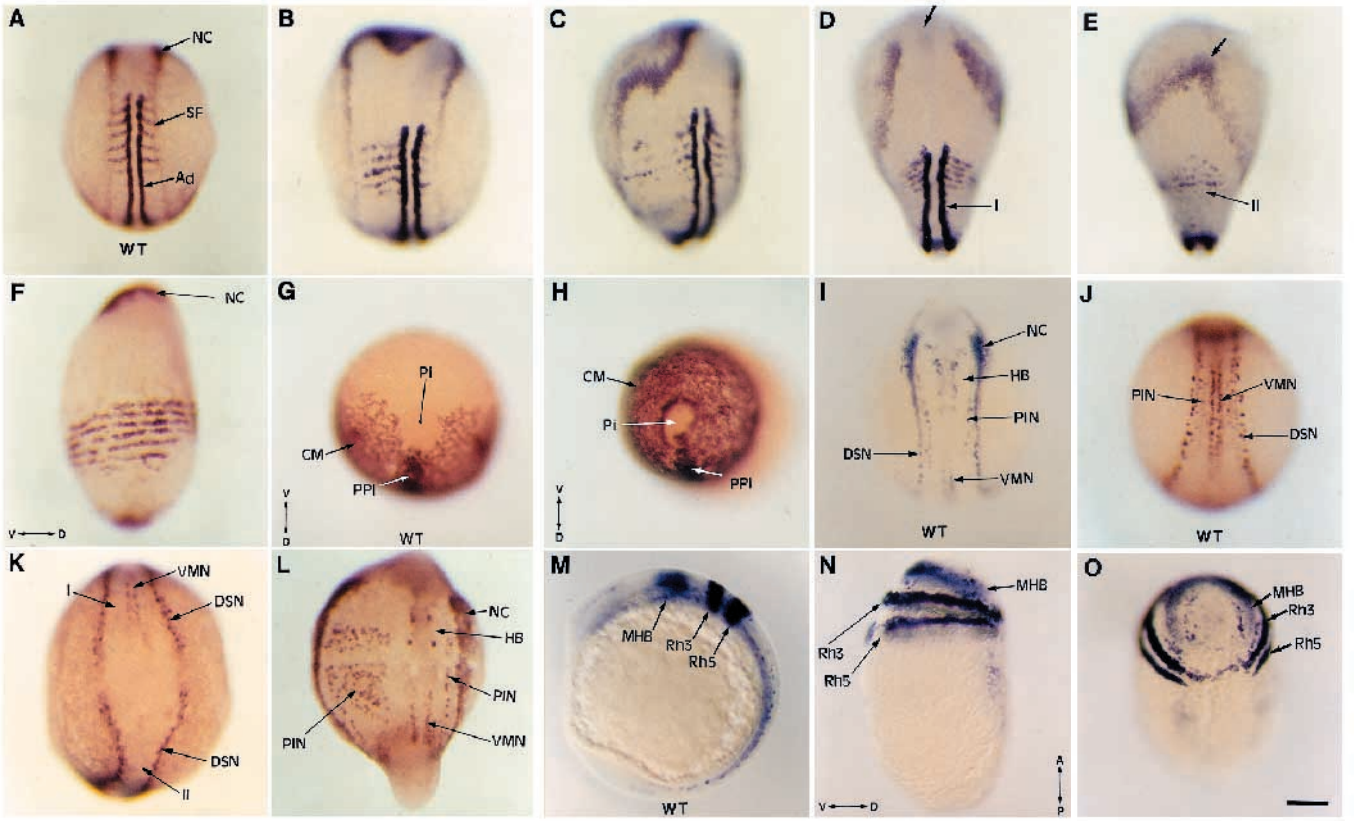

Fig. 4. FGF-8 affects dorsoventral patterning of both mesoderm and ectoderm. After injection of FGF-8 RNA, embryos and their uninjected siblings were fixed at the beginning of somitogenesis, and probed using *MyoD* and *AP2* in (A-F), *snail2* (G,H), *AS28* and *AP2* (I-L), *Krox20*, *Eng3* and *AS28* in (M-O). Wild-type siblings in A,G,I,J,M). Labeling with *MyoD* and *AP2* shows the gradual effects induced by FGF-8 from an enlarged paraxial territory (B) to a fragmented paraxial territory (C), or to the formation of a defective secondary axis on the ventral side of the embryo (E, compared to its dorsal wild-type side in D) or in extreme cases to a complete circularization of the paraxial territory (F). The cephalic mesoderm of these extreme dorsalized embryos, probed with *snail2* (H compared to its wild-type control in G) is also circularized. The neural plate (the borders of which are labeled with *AP2*) is also expanded (B,C compared to A). When a secondary axis is formed (E), because of the absence of presumptive forebrain and midbrain, *AP2* domain fuses anteriorly (arrow), while it remains separated in the primary axis (D). Analysis with *AS28*, *Krox20* and *Eng3* probes reveals the dorsolateralization effect of FGF-8 on the ectodermal layer. When a secondary axis (II) is formed as in K, dorsal sensory neurons (DSN) are present while ventral motor-neurons (VMN) are lacking. In embryos showing a dorsolateral expansion, as in L, the number of primary interneurons (PIN) on the affected side strongly increases (compared to the unaffected side or wild-type siblings in I,J). Strong dorsalized phenotypes (N,O) display a complete circularization of the neural plate visualized by *Krox20* and *Eng3* expression all around the yolk sac compared to wild-type control in (M). (A-D,I,J,L) Dorsal views; (E) ventral view; (G,H,O) animal pole views; (F,M,N) lateral views; (K) caudal view. (D,E) The dorsal and the ventral view of the same embryo. For references of the probes used, please see text**.** Ad, adaxial cells; CM, cephalic mesoderm; HB, hindbrain; MHB, midbrain-hindbrain boundary; NC, neural crest; Pi, pillow; PIN, primary interneurons; PPI, prechordal plate; Rh3, Rh5, rhombomeres 3 and 5; SF, somitic furrow; V, ventral; D, dorsal; A, anterior; P, posterior. Scale bar, 150 μm.

embryos, *AS28* is expressed by primary neurons, including dorsal sensory neurons, ventral motor neurons in the truncal region, as well as primary interneurons (Fig. 4I-J). When a complete secondary axis was induced (Fig. 4K), staining using the *AS28* marker revealed that, in the primary axis, ventral motor neurons and dorsal sensory neurons were present as in wild type. In contrast, the secondary axis of these embryos appeared thinner and lacked all ventral motor neurons (Fig. 4K). For embryos showing a dorsolateral expansion, as shown with mesodermal probes in Fig. 4B, the number of primary interneurons labeled with *AS28* largely increased on the affected side (Fig. 4L) whereas, on the unaffected side, *AS28* expression was detected in a single line of primary interneurons identical to wild type (Fig. 4I). The pattern of expression of *AS28* appeared unchanged along the anteroposterior axis suggesting that FGF-8 overexpression does not affect the anteroposterior patterning. In the case of the strongest phenotypes, as illustrated with mesodermal probes in Fig. 4F, *Krox20* and *Eng3* expression was located all around the cephalic part of the embryo, showing that the neural plate was completely circularized (Fig. 4N-O).

Correlation between the effect and the position of the FGF-8 secreting clone

To correlate the position of cells secreting ectopic FGF-8 with the sites of defects generated after misexpression, single blastomeres of cleavage-stage embryos were coinjected with FGF-8 and β-galactosidase RNA as a lineage tracer of the descendants of injected cells. Embryos were allowed to develop until somitogenesis stages, then processed for the detection of β-galactosidase activity and in situ hybridization. Control exper-

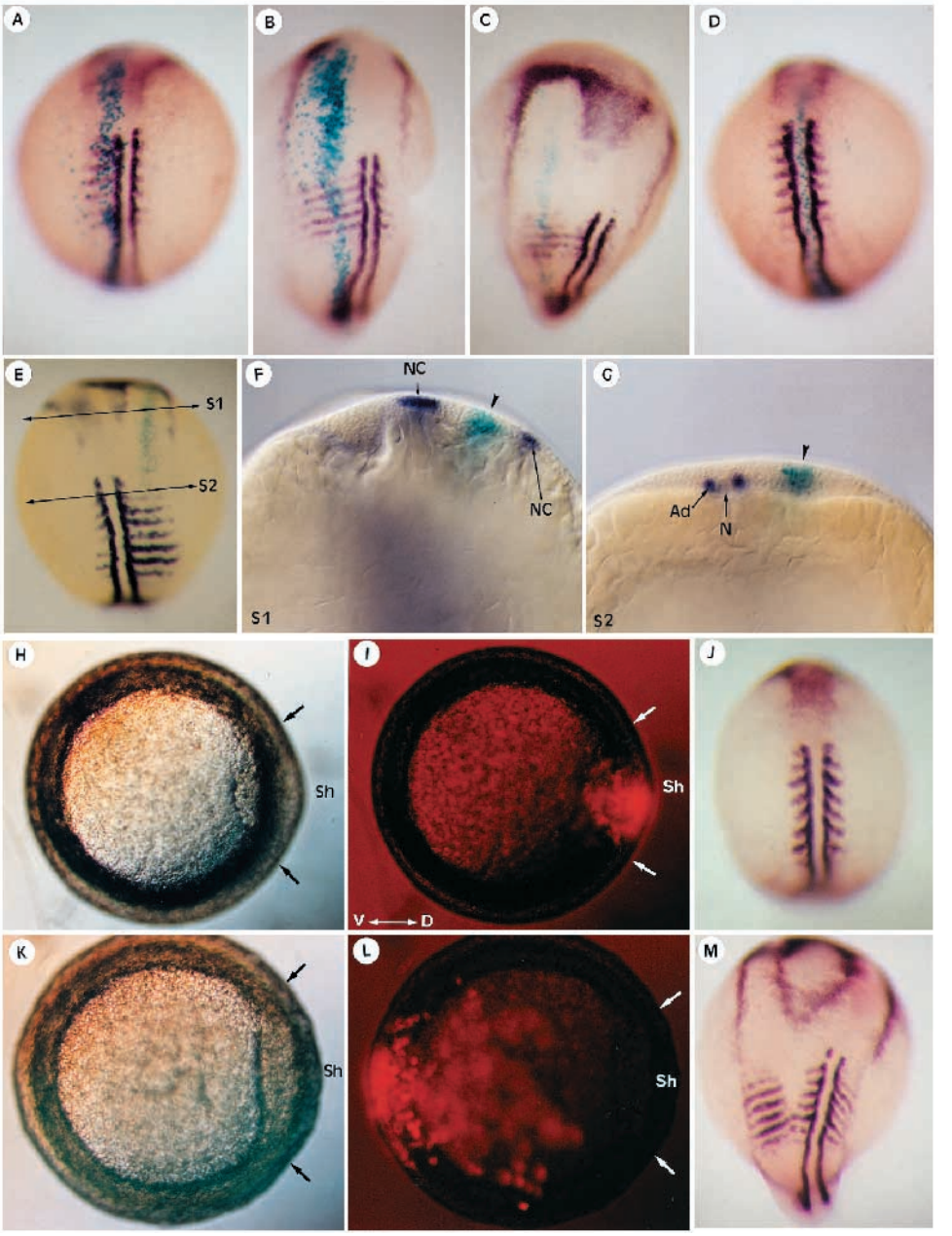

Fig. 5. Correlation between the effect and localization of the clone secreting FGF-8. FGF-8 and β-galactosidase RNA were coinjected at the cleavage stage, and presence of β-galactosidase expression was revealed at early somitogenesis stages, using X-gal staining. The embryos then underwent in situ hybridization using *MyoD* and *AP2* probes. β-galactosidase staining is always seen in the midline of the expanded dorsolateral territory resulting from misexpression of FGF-8 (A-C). When located in the axial midline (D), the FGF-8-secreting clone has no effect on the dorsoventral patterning of the embryo. (E) Dorsal view of an embryo displaying a partial secondary axis phenotype. Optical cross sections through plane S1 (F) or through S2 (G) showing that cells surrounding the FGF-8-secreting clone converge towards this position (arrowhead) instead of converging normally towards the dorsal side of the embryo. (G) The FGF-8 secreting cells are found in both mesoderm and ectoderm. (H,I) Embryo injected with FGF-8 and rhodamine coupled to 2 MDa dextran, visualized in bright field in H at gastrulation. (I) In this embryo, strong fluorescence is seen in the embryonic shield (Sh, delimited by two arrows). (J) In situ hybridization of the same embryo as in H,I using *MyoD* and *AP2* probes at early somitogenesis showing that when located in the axial midline of the embryo at gastrulation, the FGF-8 clone has no effect on the dorsoventral patterning of the embryo. (K,L) When the FGF-8 clone is located opposite to the shield (Sh), the resulting phenotype consists of the formation of a secondary axis (M). (A-E,J,M) Dorsal views; (H,I,K,L) Animal pole views. Ad, Adaxial cells; N, notochord; NC, neural crest.

iments, in which β-galactosidase was injected alone, gave rise to clones of labeled cells aligned along the anteroposterior axis, as a result of the convergence-extension movements occurring in the embryo (Kimmel et al., 1994) but did not exhibit defects in dorsoventral patterning. In contrast, embryos derived from the coinjection of FGF-8 and β-galactosidase RNA showed a range of pattern alterations that depended on the position of the labeled clone of cells. When the clone secreting FGF-8 was located close to the midline, the defects consisted of a slight enlargement of the dorsolateral domains of the embryo (Fig. 5A), while for a clone positioned more laterally, dorsolateral territories were much more expanded (Fig. 5B). When farther distant from the midline, the clone led to a second axis formation (Fig. 5C). These secreting FGF-8 clones were always found in the medial part of the structure that was generated (Fig. 5A-C). In contrast, when the clone of cells ectopically secreting FGF-8 was located exactly in the axial midline, including the notochord, prechordal plate and ventral CNS (Fig. 5D), the embryos were morphologically normal.

An optical cross section of embryos affected by ectopic FGF-8 expression suggested strongly that a convergence movement occurred toward the group of cells secreting this factor (Fig. 5E-G). This convergence movement resulted in a local thickening of the embryo and the formation of a secondary axis (Fig. 5F,G). Nevertheless, this movement of convergence of cells was less pronounced than in the primary axis and, in consequence, the secondary axis appeared thinner than its wild-type counterpart (Fig. 5F).

Further analysis was done in order to correlate the phenotype induced by FGF-8 ectopic expression and the position of FGF-8-secreting cells at the onset of gastrulation. Embryos were coinjected with FGF-8 mRNA and rhodamine coupled to high

molecular weight dextran, then later fixed at early somitogenesis stages and analyzed by in situ hybridization. When the clone of fluorescent cells was located at the onset of gastrulation in the dorsal marginal region, which corresponds to the location of the strongest endogenous *fgf-8* expression, embryos developed in a wild-type fashion (Fig. 5H-J). In contrast, clones located ventrally, in a territory devoid of endogenous FGF-8 transcripts, gave rise to a secondary axis formation (Fig. 5K-M). Ventrolateral clones gave expanded dorsolateral structures whereas clones covering the animal pole region produced complete circularization of both dorsolateral mesoderm and ectoderm (data not shown). This study reveals that the alterations generated by FGF-8 misexpression strongly depend on the position of the clone secreting this factor at the onset of gastrulation. Moreover, the alterations are much more pronounced when the clone is located in a territory devoid of endogenous FGF-8.

In summary, the results presented here strongly suggest that FGF-8 contributes to the establishment of the dorsoventral patterning of the embryo. FGF-8 overexpression leads to the expansion of dorsolateral territories at the expense of ventral and caudal domains but does not affect dorsal or axial territories.

FGF-8 acts upstream of BMP2 and BMP4

The ventralizing factors BMP2/4 have been shown to be implicated in the dorsoventral patterning of the gastrula embryo (review in Hogan, 1995; Sasai et al., 1995) and are known to be antagonized by the dorsalizing activity of the organizer factors Noggin and Chordin (Zimmerman et al., 1996; Piccolo et al., 1996). FGF-8 and BMP2/4 are localized in complementary expression domains (Nikaido et al., 1997; our own unpublished data) and induce opposite phenotypes when overexpressed (Jones et al., 1992; Fainsod et al., 1994; Graff et al., 1994; own observations), suggesting that FGF-8 may also antagonize ventralizing BMP signals. In order to investigate the potential interaction between these molecules, we performed additional FGF-8 overexpression experiments in which we analyzed the expression pattern of *bmp2* and *bmp4* at blastula and gastrula stages. We injected 300 embryos with FGF-8, then probed 150 injected embryos with BMP2 and the other 150 with the BMP4 RNA probe. We show that, when overexpressed, FGF-8 led to a lack of expression of *bmp2* (148/150 embryos) and *bmp4* (147/150 embryos) in their ventral domain both at blastula and gastrula stages (Fig. 6A-D). *bmp2* expression remained detectable in the yolk syncytial layer and at the margin but was absent from the ventralmost territory (Fig. 6A,B). In the case of *bmp4*, all ventral staining was lacking while the prechordal plate expression was unaffected (Fig. 6C,D). As *bmp2* and *bmp4* ventral expression never appeared when FGF8 was overexpressed, this suggests that FGF-8 prevents the initiation of the expression of *bmp2* and *bmp4* in the ventral territory. In a reverse experiment, we tested the effect of BMP2 and BMP4 on the transcription of *fgf-8*. 200 embryos were injected with BMP2 RNA and 210 embryos with BMP4 RNA. When BMP2 or BMP4 were overexpressed, the expression pattern of *fgf-8* (194/200 embryos in BMP2 overexpression experiments and 206/210 embryos in BMP4 overexpression experiments) remained unaffected at blastula and early gastrula stages (Fig. 6E,F). Later on, at late gastrula, as the result of the ventralization induced by BMP2/4,

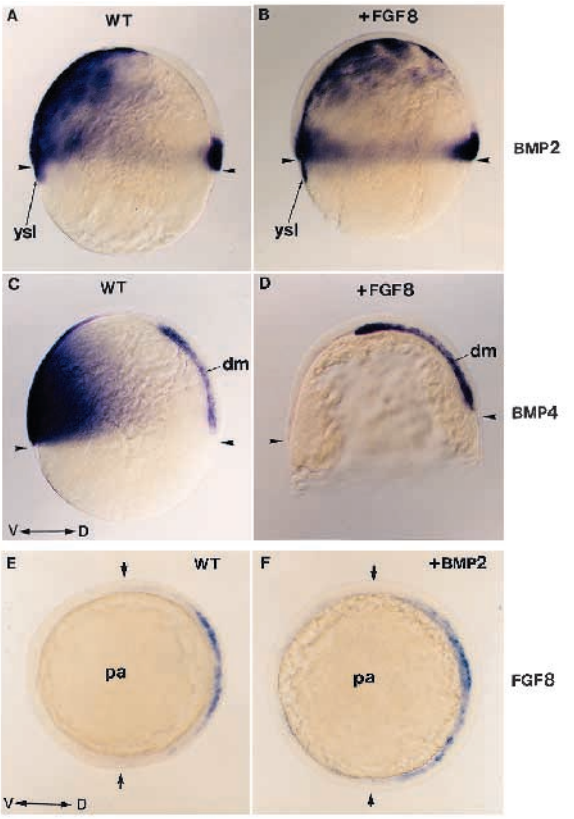

Fig. 6. FGF-8 acts upstream of BMP2 and BMP4. (A-D) Expression of BMP2 and BMP4 at gastrula stage (60% epiboly) in wild type (A,C) and in embryos injected with FGF-8 RNA (B,D) showing that BMP labeling is absent from the ventral domain of the injected embryos. (A,B) BMP2 expression in wild type (A) and in FGF-8-injected embryos in B. (C,D) BMP4 expression in wild-type (C) and in FGF-8-injected embryos (D). (E,F) Expression at early gastrulation stage of FGF-8 in an embryo injected with BMP2 RNA (F) and its corresponding sibling (E). FGF8 expression is not affected by BMP2. (A-D) Lateral view; (E,F) animal pole view. dm, dorsal mesendoderm; pa, animal pole; ysl, yolk syncytial layer; WT, wild type; D, dorsal; V, ventral. Arrowheads in A-D show the position of the margin. Arrows in E-F indicate the limits of FGF8 gradient of expression.

the *fgf-8* marginal gradient of expression was restricted to the dorsalmost part of the embryo (not shown). We then examined the effect of the inhibition of the BMP signaling pathway on the expression of *fgf-8*. This was done by overexpressing Chordin and Noggin, which are known to antagonize BMPs. In both cases, overexpression of Noggin or Chordin did not affect the early expression of *fgf-8* at blastula. Later on, at late gastrula, as a consequence of the dorsalization induced by these factors, the gradual expression of *fgf-8* appeared enlarged at the margin (not shown). In a parallel study, we examined the expression of *fgf-8* in embryos carrying the spontaneous mutation *aubergine* (our own data), which display a phenotype identical to *swirl* (Mullins et al., 1996) and are also defective in the BMP signaling pathway. Mutants in *aubergine* are dorsalized and show an enlargement of the neurectoderm and dorsolateral mesoderm and a reduction of ventral and ventrolateral mesoderm. As for *swirl* (Hammerschmidt et al., 1996b), in *aubergine* mutant embryos, *bmp4* expression is lost every-

where except in the dorsal mesendoderm (own unpublished results). *bmp2* expression in these mutant embryos is detected only in the yolk syncytial layer and at the dorsal margin. Looking at the expression of *fgf-8* in *aubergine* mutant embryos, it appears that the initial expression of *fgf-8* was unaffected. During gastrulation the marginal gradient of *fgf-8* did not form and transcripts were homogeneously distributed at the margin of the embryos (not shown). We then analyzed the epistatic relationship between FGF-8 and BMP2/BMP4 using coinjection experiments. 95% (171/180) of embryos injected with both FGF-8 and BMP2 or 98% (206/210) of embryos injected with both FGF-8 and BMP4 displayed a ventralization phenotype at the end of gastrulation. As BMP2 or BMP4 overexpression is able to rescue the dorsalization phenotype mediated by FGF-8, this shows that the ventralizing phenotype mediated by BMP2 and BMP4 is epistatic on the dorsalization phenotype induced by FGF-8. This observation, in addition to the inhibition of BMP2 or BMP4 expression mediated by the overexpression of FGF-8, shows that this factor acts upstream of BMP2 and BMP4 and may be involved in the control of the establishment of BMPs expression pattern at blastula and gastrulation stages.

DISCUSSION

FGF-8 influences the dorsoventral pattern formation at gastrulation

In zebrafish, *fgf-8* is expressed in a dorsoventral gradient at gastrulation, consistent with a potential role in early patterning of the dorsoventral axis. In order to test this hypothesis, misexpression studies were performed. Our results show that ectopic FGF-8 induces a range of defects that resemble the dorsalization mutant phenotypes described in Mullins et al. (1996). The weak class of alterations obtained after FGF-8 injection is similar to mutations carrying the recessive alleles of *lost-a-fin* and *mini fin*. Intermediate strength of defects have the same characteristics described for the *piggytail* mutation. The strongest class of FGF-8 alterations is similar to the phenotypes described for the strongest dorsalized phenotypes of *swirl*, *somitabun* and *snailhouse* mutations. These mutations cause an expansion of structures normally derived from dorsolateral regions of the blastula at the expense of ventrally derived regions. The similar phenotypes of the mutations affecting the dorsolateral territories and defects generated by FGF-8 misexpression strongly suggest that *fgf-8* and the genes affected by these mutations function in a common pathway and are involved in the establishment of the dorsoventral patterning of the embryo.

The expansion of dorsolateral structures in FGF-8-injected embryos is clearly accompanied by a reciprocal reduction of ventral and caudal regions. In contrast, zebrafish ventralizing mutations (Hammerschmidt et al., 1996a; Fisher et al., 1997) or phenotypes obtained after injection of the ventralizing factors BMP2/BMP4 (Hammerschmidt et al., 1996b; Nikaido et al., 1997, our own data) lead to an expansion of the ventral and posterior territories, associated with a deletion of the anterior region. These observations show that, in zebrafish, formation of the anteroposterior and dorsoventral axes is coupled and suggests that, rather than developing along two independent perpendicular dorsoventral and anteroposterior axes, the zebrafish embryo develops along an anterodorsal to posteroventral axis.

Our misexpression study shows that FGF-8 induces dorsolateral truncal and caudal territories but is not able to influence the development of dorsal or axial structures. Moreover, structures generated by ectopic FGF-8 are identical to those formed when the embryonic shield is surgically ablated, removing the Spemann's organizer territory (Shih and Fraser, 1996). As the formation of a partial secondary axis by overexpression of FGF8 occurs without the induction of the expression of the *goosecoid* gene, this suggests that the formation of dorsolateral truncal territories does not depend on this Spemann organizer's factor, which appears only to be required for the formation of the anterior and dorsalmost structures.

These results suggest that the ability to organize the embryo is not restricted to the anterodorsal territories, but is also distributed along the anterodorsal/posteroventral axis and influenced by molecules expressed in the dorsolateral territories of the embryo, such as FGF-8.

FGF-8 contributes to the positional information of the cells within the gastrula

As first proposed by H. Driesch at the end of the last century (reviewed in Wolpert, 1996), one mechanism by which spatial pattern of cell differentiation could be specified during embryonic development is based on positional information. The basic idea is that there is a cell parameter (positional value) that is related to a cell's position in the developing system. The cells then interpret their positional value by differentiating in a particular way.

In zebrafish, each cell differentiates into derivatives of four dorsoventral domains (Fig. 7A,B) depending on its own dorsal positional value (DPV), which is the result of complex interactions occurring between different factors, including ventralizing and dorsalizing factors. Results from our overexpression studies strongly support the idea that FGF-8 contributes to the establishment of dorsoventral positional values that will confer a specific dorsoventral identity to each cell within the gastrula. We propose a model in which the DPV of cells increases continuously from the ventral to the dorsal side of the gastrula (Fig. 7D). The contribution of endogenous FGF-8 to the dorsoventral positional information (green curve, Fig. 7D) depends, first, on the dorsoventral extent of its territory of expression (Fig. 7C) and, second, on its ability to induce the formation of different structures along the dorsoventral axis. Ectopic FGF-8 RNA changes this dorsoventral organization (Fig. 7E-H). Addition of ectopic FGF-8 adjacent to its wild-type domain of expression leads to a reduced ventral domain and an expansion of the dorsolateral territory (Fig. 7E). When localized more ventrally (Fig. 7F), FGF8 injection gives a more dramatic enlargement and even a fragmentation of the paraxial mesoderm. Ectopic FGF-8 at the ventral side of the embryo (Fig. 7G) gives rise to a secondary axis, containing somitic and neural tissue, but as FGF-8 does not contribute to the formation of axial structures, which probably requires the highest DPV, this secondary axis does not form any dorsal mesoderm or ventral central nervous system. When ectopic FGF-8 is distributed in the entire embryo (Fig. 7H), all cells, except for the axial domain, are assigned a DPV corresponding to the positional value of dorsolateral cells. Finally, when the clone of cells secreting FGF-8 is located in the axial midline, which cor-

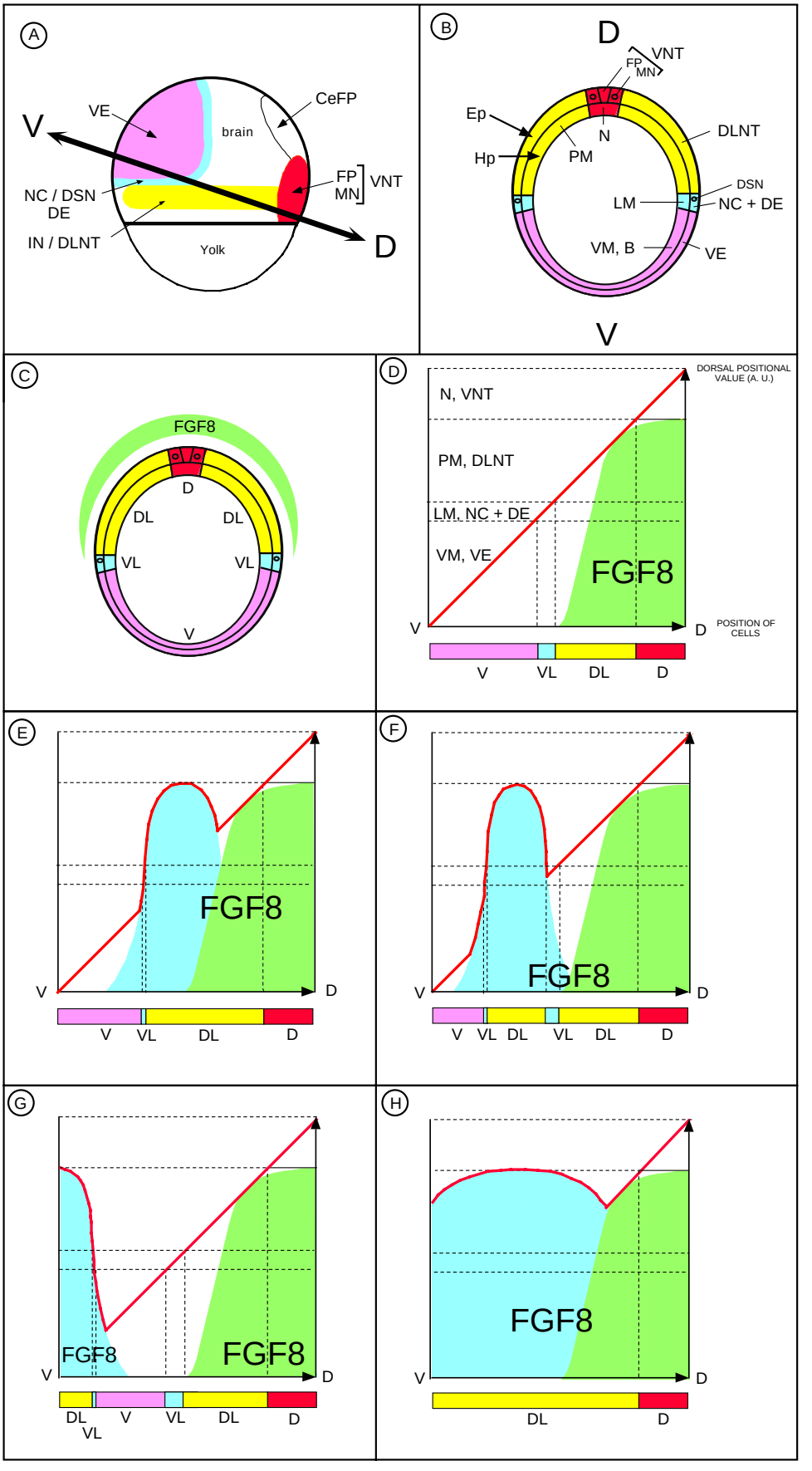

Fig. 7. FGF-8 changes dorsoventral identity at gastrulation. (A) Lateral view and (B) corresponding schematic section along the dorsoventral axis shown in A of a gastrula embryo that indicates the position of dorsal (red, N, notochord; FP, floor plate; MN, motor-neurons; VNT, ventral neural tube), dorsolateral (yellow, IN, interneurons; DLNT, dorsal neural tube; PM, paraxial mesoderm), ventrolateral (blue, LM, lateral mesoderm; DSN, dorsal sensory neurons, NC, neural crest; DE, dorsal epiderm) and ventral territories (pink, VM, ventral mesoderm; B, blood territory; VE, ventral epiderm). CeFp, cephalic floor plate; D, dorsal; Ep, epiblast; Hp, hypoblast; ; V, ventral. (C) Position of the endogenous expression of *fgf-8* (green) in the zebrafish gastrula, extending as a gradient from dorsal to dorsolateral territories. (D) Dorsal positional value (DPV, red curve) of cells in the zebrafish gastrula (ordinate, in arbitrary units AU) in function of their position (abscissa) along the dorsoventral axis. Depending of their DPV, cells differentiate in one of the four dorsoventral domains indicated at the bottom (V, ventral; VL, ventrolateral; DL, dorsolateral; D, dorsal). The green curve represents the contribution of endogenous FGF-8 to the red curve. (E) Ectopic FGF-8 RNA (blue) located next to its wild-type domain of expression (green) leads to an extension of DL and reduction of V compared to the wild-type situation in D. See also embryo Fig. 4B,L. (F) Ectopic FGF-8 RNA localized more ventrally leads to a fragmentation of DL (see embryo Fig. 4C). (G) When ectopic FGF-8 RNA is ventral, a secondary axis is induced, containing DL, VL, V but not dorsal (D) territories (see embryos in Figs 3I-K, 4D-E,K). (H) When ectopic FGF-8 RNA is localized in the whole embryo, except for the axial territory, all cells get a DL identity (see also Fig. 4F,H,N,O).

responds to the position of strong endogenous FGF-8 expression, this overexpression does not affect the dorsoventral patterning of the embryo. This last result suggests that, in this region, at the early gastrula stage, endogenous FGF-8 probably already saturates its specific receptors such as the potential candidate *fgf receptor 4* (Thisse et al., 1995b) or *fgf receptor 1* which is expressed in overlapping territories with *fgf-8* (unpublished observations).

Changes in the DPV induced by FGF8 also alters the convergence-extension movements within the gastrula. When in a ventral or ventrolateral position, ectopic FGF-8 creates locally a high DPV in a region where surrounding cells share a low DPV (Fig. 8C,D). The FGF-8 clone behaves as a local attractive center towards which cells converge and then will be organized to form a secondary axis (Fig. 8C,D compared to A,B). When ectopic FGF-8 spreads over the entire embryo (Fig. 8E,F), except for the dorsal midline, every cell within the gastrula shares the same DPV as its surrounding environment. This results in a loss of local polarity of the embryo and, in consequence, cells do not converge towards a particular location. This inhibition of convergence movement occurs despite the higher DPV of the axial midline territory. This shows that axial cells are not able to attract distant cells suggesting that the nature of the signal that drives convergence movement toward dorsal is not a long-range diffusible signal. We propose that at least a part of the positional information depends on chemoattractant molecules asymmetrically distributed within the gastrula and that cells measure the surrounding concentration in these chemoattractant molecules and migrate toward the position of the highest concentration.

Interaction of FGF-8 with the ventralizing factors BMPs

To explain how pattern formation occurs during early embryogenesis, the model that is generally adopted (reviewed in Graff, 1997) proposes that active BMP signaling establishes ventral fates in both ectoderm and mesoderm. However, it is likely that dorsal-inducing properties of the organizer are in part mediated by inhibiting BMP signaling. The Spemann organizer signals, Follistatin, Noggin and Chordin (reviewed in Tanabe and Jessell, 1996) function by binding to BMP4, preventing it from activating its receptor on the dorsal side of the embryo. Therefore, patterning of the germ layers is an interplay of active BMP signaling on the ventral side and blocking BMP signaling on the dorsal side. Our study shows that, in addition to the known organizer factors, FGF-8 is a dorsalizing factor that affects the BMP signaling pathway by negatively regulating the expression of the *bmp2* and *bmp4* genes. The ability of BMP2 and BMP4 to rescue the dorsalization induced by FGF-8 strongly suggests that at least a part of the effect of FGF-8 is the result of the inhibition of *bmp2* and *bmp4* expression.

Therefore, in addition to being executed by diffusion of

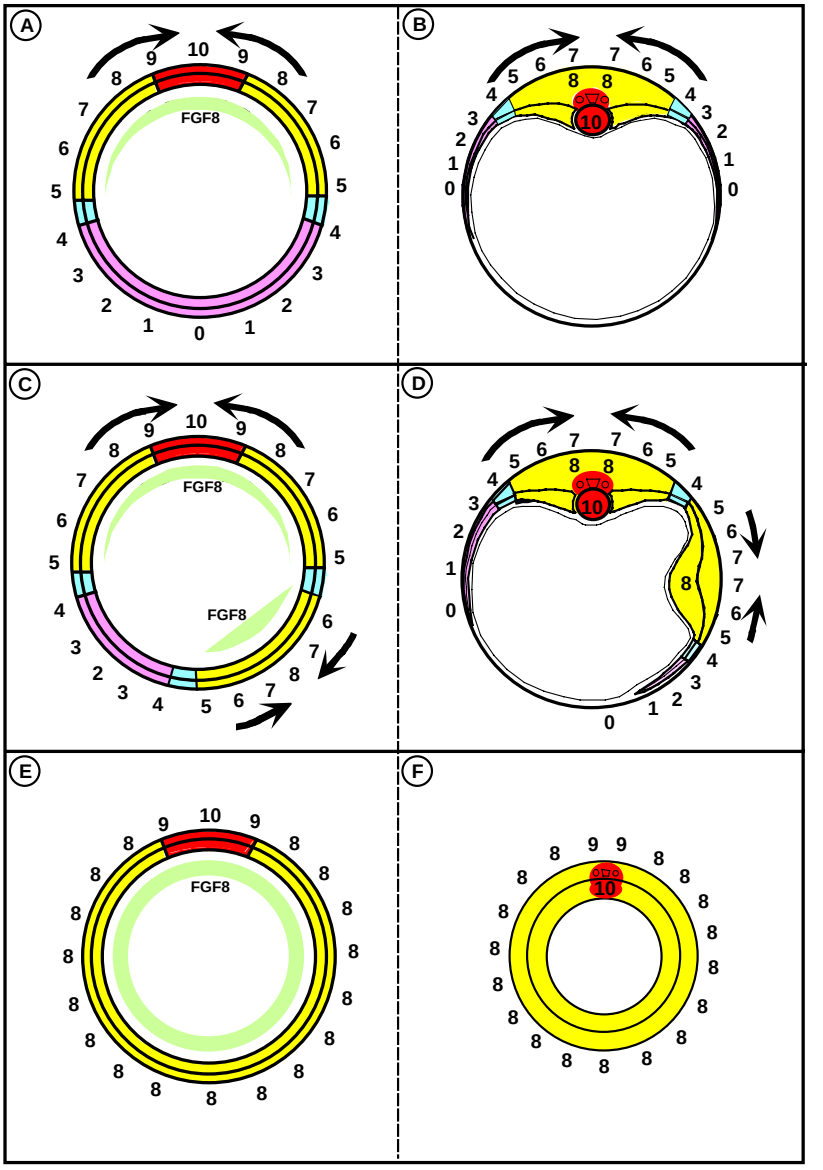

Fig. 8. FGF-8 mediates change in the dorsoventral identity alters convergence-extension movements within the gastrula. Schematic section of an embryo at gastrulation (A) and at early somitogenesis (B). The DPV was arbitrary chosen from 0, ventrally, to 10, dorsally. At early somitogenesis (B), convergence movements occurring during gastrulation (arrows) have driven the cells toward the dorsal part of the embryo where the embryonic axis is formed. The dorsalmost cells at gastrulation (notochord, ventral CNS, in red) have sunk into the embryo and are now covered with cells that were in a dorsolateral position at gastrulation (yellow). Ventrolateral, blue; ventral, pink; gradient of endogeneous FGF-8, green. (C) When the FGF-8 secreting clone is ventral at gastrulation, cells surrounding this clone get a higher DPV than their neighbours. This territory acts as a local attracting center towards which the cells of lower DPV converge. (D) As a result, a secondary axis is formed. (E) When ectopic FGF-8 is distributed in the whole embryo, all gastrula cells get the same DPV, except for the most dorsal part. (F) As a result, at beginning of somitogenesis, ventral cells do not converge towards the dorsal midline. As the epiboly movement still occurs in these embryos, the shape of the embryo is elongated rather than spherical.

secreted molecules that directly bind to and antagonize the ventral BMP signals at gastrulation, the dorsoventral patterning of the zebrafish embryo depends also on the effect of FGF-8 on the BMP expression at the blastula stage.

We thank Dr Masazumi Tada for kindly providing the zebrafish *Bmp-2* full-length cDNA clone prior to publication, Dr Alvin Chin and Dr Matthias Hammerschmidt for a zebrafish cDNA BMP4 clone, Dr E. DeRobertis for the *Xenopus* chordin cDNA and Dr R. Harland for the *Xenopus* noggin cDNA. We thank Drs P. Dollé, M. Halpern and P. Simpson for their comments on the manuscript, V. Heyer for his excellent technical assistance, and D. Biellmann and O. Nkundwa for maintenance of fish. This work was supported by funds from the INSERM, CNRS, the Centre Hospitalier Universitaire Régional.

REFERENCES

Amaya, E., Musci, T. J. and Kirschner, M. W. (1991). Expression of a dominant negative mutant of the *FGF receptor* disrupts mesoderm formation in *Xenopus* embryos. *Cell* **66,** 257-270.

Basilico, C. and Moscatelli, D. (1992). The FGF family of growth factors and oncogenes. *Adv. Cancer Res.* **59**, 115-165.

Christian, J. L. and Moon, R. T. (1993). Interactions between *Xwnt-8* and Spemann organizer signaling pathways generate dorsoventral pattern in the embryonic mesoderm of *Xenopus*. *Genes Dev.* **7**, 13-28.

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**, 439-451.

Crossley, P. H., Minowada, G., McArthur, C. A. and Martin, G. R. (1996a). Roles for FGF8 in the induction, initiation and maintenance of chick limb development. *Cell* **84**, 127-136.

Crossley, P. H., Martinez, S. and Martin, G. R. (1996b). Midbrain development induced by fgf8 in the chick embryo. *Nature* **380**, 66-68.

Echelard, Y., Epstein, D. J., St-Jacques, B., Shen, L., Molher, J., McMahon, J. A. and McMahon, A. P. (1993). *Sonic Hedgehog*, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. *Cell* **75**, 1417-1430.

Ekker, M., Akimenko, M. A., Bremiller, R. and Westerfield, M. (1992). Coordinate embryonic expression of three zebrafish *engrailed* genes. *Development* **116**, 001-1010.

Fainsold, A., Steinbeisser, H. and DeRobertis, E. M. (1994). On the function of BMP-4 in patterning the marginal zone of the *Xenopus* embryo. *EMBO J.* **13**, 5015-5025.

Fernig, D. G. and Gallagher, J. T. (1994). Fibroblast growth factors and their receptors : an information network controlling tissue growth morphogenesis and repair. *Prog. Growth Factor Res.* **5(4)**, 353-377.

Fisher, S., Amacher, S. L. and Halpern, M. E. (1997). Loss of *cerebum* function ventralizes the zebrafish embryo. *Development* **124**, 1301-1311.

Graff, J. M., Thies, R. S., Song, J. J., Celeste, A. J. and Melton, D. A. (1994). Studies with a *Xenopus BMP receptor* suggest that ventral mesoderm-inducing signals override dorsal signals in vivo. *Cell* **79**, 169-179.

Graff, J. M. (1997). Embryonic patterning : to BMP or not BMP, that is the question. *Cell* **89**, 171-174.

Griffin, K., Patient, R. and Holder, N. (1995). Analysis of FGF function in normal and *no tail* zebrafish embryos reveals separate mechanisms for formation of the trunk and the tail. *Development* **121**, 2983-2994.

Hammerschmidt, M., Pelegri, F., Mullins, M. C., Kane, D. A., Van Eeden, J. M., Granato, M., Brand. M., Furutani-Seiki, M., Haffner, P., Heisinberg, C. P., Jiang, Y. J., Kelsh, R. N., Odenthal, J., Warga, R. M. and Nüsslein-Volhard, C. (1996a). *dino* and *mercedes*, two genes regulating dorsal development in the zebrafish embryo *Development* **123**, 95 -102.

Hammerschmidt, M., Serbedzija, G. N. and McMahon, A. P. (1996b). Genetic analysis of dorsoventral pattern formation in the zebrafish : requirement of a BMP-like ventralizing activity and its dorsal repressor. *Genes and Dev.* **10**, 2452-2416.

Hogan, B. L. M. (1995). Bone morphogenetic proteins : multifunctional regulators of vertebrate development. *Genes Dev.* **10**, 1580-1594.

Inoue, A., Takahashi M., Hatta, K., Hotta, Y. and Okamoto, H. (1994). Developmental regulation of *islet-1* mRNA expression during neuronal differentiation in embryonic zebrafish. *Dev. Dyn.* **199**, 1-11.

Jones, C. M., Lyons, K. M., Lapan, P. M., Wright, C. V. and Hogan, B. L. (1992). DVR-4 (bone morphogenetic protein-4) as a posterior-ventralizing factor in *Xenopus* mesoderm induction. *Development* **115**, 639-647.

Kimelman, D. and Kirschner, M. (1987). Synergistic induction of mesoderm by FGF and TGFβ and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell* **51**, 869-877.

Kimmel, C. B., Warga, R. M. and Kane, D. A. (1994). Cell cycles and clonal strings during formation of the zebrafish central nervous system. *Development* **120 (2)**, 265-276.

Krauss, S., Concordet, J. P. and Ingham, P. W. (1993). A functionally conserved homolog of the *Drosophila* segment polarity gene *hh* is expressed in tissues with polarizing activity in zebrafish embryos. *Cell* **75**, 1431-1444.

McArthur, C. A., Lawshé, A., Xu, J., Santos-Ocampo, S., Heikinheimo, M., Chellaiah, A. and Ornitz, D. M. (1995). FGF-8 isoforms activate receptor splice forms that are expressed in mesenchymal regions of mouse development. *Development* **121**, 3603-3613.

Mitchell, P. J., Timmons, P. M., Hebert, J. M., Rigby, P. W. and Tijan, R. (1991). Transcription factor *AP-2* is expressed in neural crest cell lineages during mouse embryogenesis. *Genes Dev.* **5(1)**, 105-119.

Miyamoto, M., Naruo, K. I., Seko, C., Matsumoto, S., Kondo, T. and Kurokawa, T. (1993). Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family, which has a unique secretion property. *Mol. Cell. Biol.* **13**, 4251-4259.

Mullins., M., Hammerschmidt, M., Kane, D. A., Odenthal, J., Brand, M., Van Eeden, F. J. M., Furutani-Seiki, M., Granato, M., Haffner, P., Heisenberg, C. P., Jiang Y. J., Kelsh, R. N. and Nüsslein-Volhard, C. (1996). Genes establishing dorsoventral pattern formation in the zebrafish embryo : the ventral specifying genes. *Development* **123**, 81-93.

Nikaido, M., Tada, M., Saji, T. and Ueno, N. (1997). Conservation of BMP signaling in zebrafish mesoderm patterning. *Mech. Dev.* **61**, 75-88.

Oxtoby, E. and Jowett, T (1993). Cloning of the zebrafish *Krox-20* gene (*Krx-20*) and its expression during hindbrain development. *Nucleic Acids. Res.* **21**, 1087-1095.

Piccolo, S., Sasai, Y., Lu, B. and DeRobertis, E. M. (1996). Dorsoventral patterning in *Xenopus* : inhibition of ventral signals by direct binding of *chordin* to *BMP-4*. *Cell* **86**, 589-598.

Sasai, Y., Lu, B., Steinbeisser, H. and De Robertis, E. M. (1995). Regulation of neural induction by the *chd* and *Bmp-4* antagonistic patterning signals in *Xenopus*. *Nature* **376**, 333-336.

Shih, J. and Fraser, S., E. (1996). Characterizing the zebrafish organizer : microsurgical analysis at early-shield stage. *Development* **122**, 1313-1322.

Smith, W. C. and Harland, R. M. (1992). Expression cloning of noggin, a new dorsalizing factor localized in the Spemann organizer in *Xenopus* embryos. *Cell* **70**, 829-840.

Smith, J. C. (1994). Hedgehog, the floor plate and the zone of polarizing activity. *Cell* **76**, 193-196.

Strehlow, D., Heinrich, G. and Gilbert, W. (1994). The fate of the blastomeres of the 16 cell zebrafish embryo. *Development* **120**, 1791-1798.

Tanabe, Y. and Jessell, T. M. (1996). Diversity and pattern in the developing spinal cord. *Science* 274, 1115-1123.

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**, 8928-8932.

Thisse, C., Thisse, B., Schilling, T. F. and Postlethwait, J. H. (1993). Structure of the zebrafish *snail1* gene and its expression in wild-type, *spadetail* and *no tail* mutant embryos. *Development* **119**, 1203-1215.

Thisse, C., Thisse, B, Halpern, M. E. and Postlethwait, J. H. (1994). *goosecoid* expression in neurectoderm and mesendoderm is disrupted in zebrafish *cyclops* gastrulas. *Dev. Biol.* **164**, 420-429.

Thisse, C., Thisse, B. and Postlethwait, J. H. (1995a). Expression of *snail2*, a second member of the zebrafish snail family, in cephalic mesendoderm and presumptive neural crest of wild-type and spadetail mutant embryos. *Dev. Biol.* **172**, 86-99.

Thisse, B., Thisse, C. and Weston, J. A. (1995b). Novel FGF receptor (Z-FGFR4) is dynamically expresseed in mesoderm and neurectoderm during early zebrafish embryogenesis. *Dev. Dyn.* **203**, 377-391.

Vize, P. D., Hemmati-Brivanlou, A., Harland, R. M. and Melton, D. A. (1991). Assays for gene function in developing *Xenopus* embryo. *Meth. Cell. Biol.* **36**, 367-387.

Weinberg, E. S., Allende, M. L., Kelly, C. S., Abdelhamid, A., Murakami, T., Andermann, P., Duerre, O. G., Grunwald, D. J. and Riggelman, B. (1996). Developmental regulation of zebrafish *MyoD* in wild-type, *notail* and *spadetail* embryos. *Development* **122**, 271-280.

Wittbrodt, J. and Rosa, F. (1994). Disruption of mesoderm and axis formation

in fish by ectopic expression of *activin* variants : the role of maternal *activin*. *Genes Dev.* **8**, 1448-1462.

Wolpert, L. (1996). One hundred years of positional information. *Trends in Genet.* **12**, 359-364.

Yamaguchi, T. P., Harpal, K., Henkemeyer, M. and Rossant, J. (1994). fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev.* **8**, 3032-3044.

Yamaguchi, T. P. and Rossant, J. (1995). Fibroblast growth factors in mamalian development. *Current Op. in Gen. Dev*. **5**, 485-491.

Zimmerman, L. B., De Jesus-Escobar, J., M. and Harland, R. M. (1996). The Spemann organizer signal *noggin* binds and inactivates *bone morphogenetic protein 4*. *Cell* **86**, 599-606.

(Accepted 25 August 1997)