Development 118, 47-59 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993



# Differential expression of multiple fork head related genes during gastrulation and axial pattern formation in the mouse embryo

**Hiroshi Sasaki and Brigid L. M. Hogan**

Department of Cell Biology, Vanderbilt University Medical School, Nashville, Tennessee 37232, USA

## SUMMARY

**Four genes encoding fork-head-domain-containing proteins (FD genes) have been isolated from a mouse 8.5 days post coitum (p.c.) embryo cDNA library. Two are mouse homologues of rat *HNF-3*β and *HNF-3*α. The other two are novel and have been named *MF-1* and *MF-2* (for mesoderm/mesenchyme fork head). Whole-mount in situ hybridization of embryos between 6.5 and 9.5 days p. c. shows that each gene has a unique expression pattern. *HNF-3*β is expressed in the node, notochord, floor plate and gut, while *HNF-3*α is mainly in the definitive endoderm and gut, but also in the floor plate of the midbrain. These results suggest that HNF-3β and HNF-3α, in addition to their known functions as transcriptional activators in adult liver, play a role in body axis formation, neural tube patterning and definitive endoderm formation during gastrulation. *MF-1* RNA is present in non-notochordal mesoderm, and in neural-crest-derived head mesenchyme, while *MF-2* transcripts are found in the sclerotomes of the somites and in head mesenchyme, including that from neural crest. Studies on gastrulation stage embryos suggest that the early temporal and spatial patterns of *HNF-3*β, *MF-1* and *HNF-3*α correlate with populations of cells undergoing commitment to different developmental fates. A model is proposed linking FD gene expression with gastrulation events in the mouse.**

Key words: fork head domain, gastrulation, node, floor plate, notochord, paraxial mesoderm, endoderm, mouse development

## INTRODUCTION

In vertebrate development, the axial body plan is progressively established from the anterior during gastrulation (Sive et al., 1989; for review see Slack and Tannahill, 1992; Kimelman et al., 1992). Most information concerning the molecular and cellular mechanisms underlying gastrulation has been obtained from the experimental manipulation of avian and amphibian embryos (birds, Schoenwolf et al., 1992 and references therein; reviews for amphibians, Slack and Tannahill, 1992; Kimelman et al., 1992). Compared to these two classes, gastrulation in the mammalian embryo is still poorly understood, but at the cellular level at least the process is thought to be similar to that in chick embryos, based on their morphological similarities and on recent lineage tracing experiments with mouse embryos (see Lawson et al., 1991; Lawson and Pederson, 1992).

In order to understand the molecular mechanisms underlying gastrulation in the mouse embryo, it is necessary to identify genes that are expressed at the time of axial patterning. Previous studies have shown that many genes involved in developmental regulation and pattern formation have been highly conserved during evolution. These include genes encoding proteins with motifs such as the homeodomain, POU domain, paired domain, helix-loop-helix motif, zinc finger and fork head domain (Weigel and Jäckle, 1990; for reviews see McGinnis and Krumlauf, 1992; Xi and Rosenfeld, 1991). Recent studies have shown that the fork-head-domain-encoding gene, *XFKH1*, is expressed in Spemann's organizer and midline structures in the *Xenopus* embryo, suggesting a role in axial patterning (Dirksen and Jamrich, 1992). We therefore set out to isolate mouse genes related to *XFKH1* that might be involved in gastrulation events in the mouse.

The fork head domain (FD) is a DNA-binding motif, which was originally identified as a 110 amino acid sequence conserved between the products of two genes; the *Drosophila* homeotic gene, *fork head*, which is required for normal development of terminal regions including foregut and hindgut, and a rat transcription factor, HNF-3α, which is required for liver-specific gene expression (Weigel and Jäckle, 1990; Lai et al., 1991). Recently, other genes encoding FD-containing proteins (FD genes) have been isolated. Of these, the *Drosophila* segmentation genes *sloppy paired -1* and *-2* are involved in the process of segmentation along the anterior-posterior axis (Grossinklaus et al., 1992) and, in *Xenopus*, there is some evidence that the gene *Pintallavis* is involved in the anterior-posterior and dorsoventral patterning of the neural tube (Ruiz i Altaba and Jessel, 1992). These results suggest that FD genes are involved in embryonic pattern formation and regional specification.

Here, we report the cloning and early embryonic expres-

sion of four mouse FD genes. The temporal and spatial expression patterns of these genes suggest their involvement in the specification of notochord, floor plate, definitive endoderm, non-notochordal mesoderm and sclerotome.

## MATERIALS AND METHODS

### Cloning and sequence determination

First, we isolated a 1 kb cDNA fragment of *XFKH1* (nucleotides 22-1010; Dirksen and Jamrich, 1992) by PCR from a *Xenopus* dorsal lip cDNA library (Blumberg et al., 1991). Using this cDNA fragment as a probe, we have screened approximately $1.8\times10^6$ pfu of a lambda gt10 8.5 day post coitum (p.c.) mouse embryo cDNA library (Fahrner et al., 1987) under low-stringency condition as follows. Hybridization was performed in buffer containing 43% formamide, 4× SSC, 0.1 M sodium phosphate buffer (pH 7.2), 0.5% SDS, 200 μg/ml heat-denatured herring sperm DNA and 10% polyethylene glycol 8000 at 37˚C. Final washes were 2× or 0.2× SSC, 0.5% SDS at 37˚C for 20 minutes, twice. With this screening, we obtained a partial cDNA of the mouse homologue of rat *HNF-3*β (Lai et al., 1991), clone c10. Using c10 as a probe, we rescreened another $2.4\times10^6$ pfu of the same cDNA library under both high- and low-stringency conditions. cDNA inserts of positive clones were subcloned into the *Eco*RI site of pBluescript KSII (Stratagene) and screened for FD sequences with degenerate primers corresponding to the conserved motifs KPPYSY, QNSIRH and EKGS(F/Y)W. With this screening, we obtained eight cDNAs for five FD genes: c21 (1.5 kb) and c3β-2 (1.2 kb) for *HNF-3*β; c24 (2 kb) for *HNF-3*α; c52 (1.0 kb) and c58 (1.0 kb) for *MF-1*; c63 (0.95 kb) for *MF-2* and c39 (0.3 kb) and c43 (3 kb), which both encode another novel gene for which the expression pattern has not yet been determined. The cDNAs were sequenced in both strands using Sequenase version 2.0 sequencing kit (USB).

### Mouse embryos and staging

Embryos were obtained from ICR females mated with Swiss Webster males. Noon on the day of plug is 0.5 days p. c. Embryos were classified under the dissecting microscope as early streak when the distal end of the mesodermal wedge extended less than 50% the length of the posterior side of the egg cylinder. Mid- and late streak embryos had mesoderm extending more than 50% of the posterior side.

### Whole-mount in situ hybridization

The whole-mount in situ hybridization protocol described below was based on one kindly provided by Jill McMahon (Roche Institute, Nutley). Embryos were dissected in Dulbecco's modified Eagle's medium (DMEM) containing 10 mM sodium Hepes buffer (pH 7.4) and 5% fetal bovine serum and fixed on ice in 4% paraformaldehyde in calcium/magnesium-free phosphate-buffered saline (PBS) for 45 minutes (stages up to 8.5 day p.c.) or 2 hours (older embryos). Fixed embryos were washed three times in ice-cold PBS containing 0.1% Tween-20 (PBT), followed by dehydration through a series of methanols in PBT (25%, 50%, 75% and 2× 100% methanol). Dehydrated embryos were stored at –20˚C in methanol until use.

All the following procedures were performed at room temperature with rocking unless noted, and the incubation time of each step is approx. 5 minutes unless noted. Embryos were rehydrated into PBT through a methanol series (75%, 50%, 25% methanol and 2× PBT) and were bleached in 4:1 methanol/30% hydrogen peroxide for one hour. Following three PBT washes, embryos were treated with proteinase K (Sigma, 15 μg/ml in PBT) for 5 minutes, washed twice with glycine (2 mg/ml) in PBT and post-fixed in 0.2% glutaraldehyde/4% paraformaldehyde in PBT for 20 minutes. Following three PBT washes, embryos were washed once in prehybridization buffer (50% formamide, 5× SSC (pH 5.0), 50 μg/ml yeast total RNA, 1% SDS, 50 μg/ml heparin) and then prehybridized for 1 hour at 70˚C. After one more wash with prehybridization buffer, embryos were hybridized overnight with digoxigenin-labeled RNA probe (approx. 1 μg/ml) in the hybridization buffer at 70˚C. Digoxigenin-labeled RNA probes were synthesized with T3 or T7 RNA polymerase using the entire cDNAs as templates. For *HNF-3*β, *HNF-3*α, *MF-1* and *MF-2* genes, c21, c24, c52 and c63 were used, respectively. For mouse *goosecoid*, the 909 bp *Pst* I-*Hin*c II genomic DNA fragment (probe 2; Blum et al., 1992) was used. Hybridized embryos were washed twice with solution I (50% formamide, 5× SSC (pH 5.0), 1% SDS) for 30 minutes at 70˚C followed by a 1:1 solution I/solution II (solution II is 0.5 M NaCl, 10 mM Tris-HCl [pH 7.5], 0.1% Tween-20) wash for 10 minutes at 70˚C, three solution II washes, two RNAase A treatments (100 μg/ml in solution II) for 30 minutes at 37˚C, and two solution III (50% formaldehyde, 2× SSC (pH 5.0)) washes for 30 minutes at 65˚C. Following three washes with Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBS-T), embryos were blocked with 10% heat-inactivated lamb serum in TBS-T for 1 hour. To prevent nonspecific binding of antibody, the anti-digoxigenin Fab alkaline phosphatase conjugate (Boehringer Mannheim) was diluted 1:2000 with approx. 0.3% heat-inactivated mouse embryonic powder and 0.1% heat-inactivated lamb serum in TBS-T, and was preabsorbed for 1 hour at 4˚C and centrifuged 15,000 revs/minute at 4˚C. The embryos were incubated overnight in the preabsorbed antibody solution at 4˚C followed by three 5 minute and six 1 hour washes with TBS-T containing 2 mM levamisole. Following two 20 minute washes in NTMT (100 mM NaCl, 100 mM Tris HCl [pH 9.5], 50 mM $MgCl_2$, 0.1% Tween-20) containing 2 mM levamisole, coloring reactions were performed in coloring solution (4.5 μl/ml NBT, 3.5 μl/ml BCIP and 2 mM levamisole in NTMT) in the dark without rocking. The color development reactions were continued for 2-18 hours, depending on the probes. The reaction was stopped by two washes of PBT containing 1 mM EDTA and stained embryos were kept in this solution at 4˚C in the dark. Some embryos were cleared by passing the embryos into 50% and 80% glycerol in PBT containing 1 mM EDTA for 1 hour each.

### Sectioning of stained embryos

After rehydration into PBT (when necessary), stained embryos were re-fixed in 4% paraformaldehyde in PBS for 30 minutes at 4˚C. Following dehydration with a methanol series (30%, 50%, 70% and 100% twice, 5 minutes each), embryos were soaked with methanol 2× 1 hour, xylene 2× 1 hour, 1:1 xylene/paraffin (paraplast plus) 1 hour at 60˚C and paraffin wax 3× 2 hours at 60˚C. After oriented embedding in paraffin wax, 7 μm sections were prepared. Sections were dewaxed through 3 changes of xylene (10 minutes each) and mounted with Permount. Alternatively, dewaxed sections were rehydrated into water with an ethanol series, (100% × 2, 95%, 70% and 50%, 5 minutes each) followed by counterstaining with 0.02% eosin solution. Counterstained sections were dehydrated through two changes of 100% ethanol and xylene, and were mounted with Permount.

### Activin treatment of mouse embryos

Mid streak stage (6.5 days p. c.) embryos were incubated in DMEM containing 0.5% bovine serum albumin (added to prevent non-specific absorption of activin) for 3 hours at 37˚C, in an atmosphere of 5% $CO_2$ in air with or without 10 or 200 ng/ml human activin A (kindly provided by Dr Anthony Mason, Genentech). Control experiments showed that 10 ng/ml of the same

activin induced elongation and dorsal mesoderm, such as muscle and notochord, formation in 100% of *Xenopus* animal caps treated. Both 10 and 200 ng/ml concentrations of activin gave similar results. Before incubation, part of the extraembryonic regions of the embryos were removed and, in some experiments, the embryos were pierced several times with a needle through the proamniotic cavity to allow access of the activin to epiblast.

## RESULTS

### Cloning of four mouse fork-head-domain-encoding genes

Using a partial mouse *HNF-3*β cDNA as a probe, we isolated eight independent cDNA clones encoded by five FD genes. They include mouse homologues of rat *HNF-3*β and *HNF-3*α (Lai et al., 1990, 1991). The other three are new. Two of them were named *MF-1*, *MF-2* (for **m**esoderm/**m**esenchyme **f**ork head). The expression pattern of the third, encoding the cDNA c43, has not yet been determined.

The mouse *HNF-3*β cDNA encodes a 459 amino acid protein. The deduced amino acid sequence is almost identical (98.3% identical) to rat HNF-3β (sequence submitted to Genbank). The deduced amino acid sequences of the FDs of all five genes presented here are listed in Fig. 1, together with other reported FDs. Comparison of FDs shows that they can be grouped into at least four classes (Fig. 1). Within each class, every member has more than 75% identity, while between classes the identities are lower (<70%). Moreover, there are short conserved sequences unique to each class, such as amino residues 35-38, 50-53 and 74-77, boxed in Fig. 1. For example, in the case of residues 50-53, sequences of each class are 'NQQR' (class I), 'NKQG' (class II), 'KFPA' (class III) and 'NTQR' (class IV). The other genes cannot yet be classified into distinct classes. The FD of mouse HNF-3α is 99.1% identical to that of rat HNF-3α. MF-1 and MF-2 show highest homologies with XFD4 (91.8% identity) and XFD6 (87.1% identity), respectively (Knöchel et al., 1992). In the case of *MF-2*, our cDNA starts from inside the FD so that its overall identity with other members may be somewhat different. However, it is unlikely that *MF-1* and *MF-2* are the mouse homologues of *XFD4* and *XFD6*, since a much higher level of conservation is seen between the FDs of mouse HNF-3β and XFD3 (one conservative change; Knöchel et al., 1992). Comparison of all of these sequences reveals conserved amino acids, which are shown in the consensus sequence

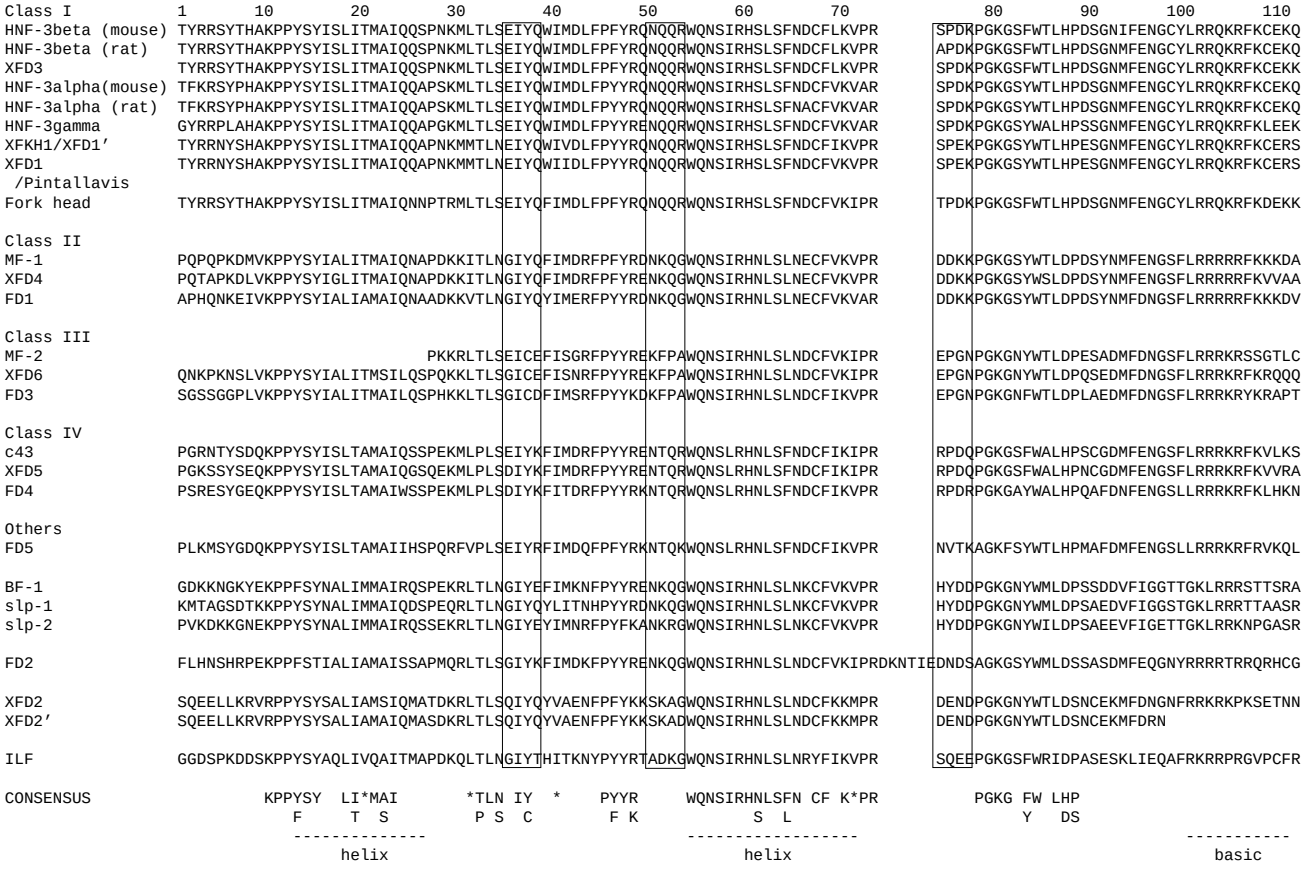


**Fig. 1.** Comparison of fork head domains. FDs are aligned to show maximum similarity. Four classes are indicated. Well conserved amino acids are shown as a consensus sequence. Stars indicate hydrophobic residues. References of sequences are as follows: rat HNF-3α, β, γ, Lai et al. (1991); XFD1-6, Knöchel et al. (1992); XFKH1, Dirksen and Jamrich (1992); Pintallavis, Ruiz i Altaba and Jessel (1992); FD1-5, Häcker et al. (1992); fork head, Weigel et al. (1989); BF-1, Tao and Lai (1992); slp-1 and -2, Grossinklaus et al. (1992); ILF, Li et al. (1991).

in Fig. 1. As well as these amino acids, three previously reported characteristic regions are also conserved (Lai et al., 1991; Häcker et al., 1992). One region is a cluster of basic amino acids in the carboxy terminus (residues 100-110, Fig. 1), thought to be a DNA-binding region by analogy to basic sequences accompanying helix-loop-helix and leucine zipper structures (Murre et al., 1989; Landschulz et al., 1988; for review see Xi and Rosenfeld, 1991). The exact positions and sequences of the basic regions vary between classes. This is also the case with DNA-binding regions of helix-loop-helix and leucine zipper proteins, in which the basic region positions and sequences are thought to be important for DNA-binding specificity. The other two regions are short (residues 13-26) and long (residues 54-71) putative helical structures (Fig. 1). The position of the long helix was originally noted as residues 46-71 by Lai et al. (1991), based on sequence comparisons within Class I genes. However, most members of the other classes have proline or glycine at residue 52 or 53, which are often incompatible with helix formation, suggesting that the putative helix region in these genes is shorter. The positions of the basic residues and two helices are indicated in Fig. 1.

## Developmental expression patterns

To investigate the involvement of these FD genes in mouse gastrulation and axial patterning, we have studied the expression of each gene using whole-mount in situ hybridization of 6.5-9.5 days p. c. mouse embryos. In all cases, the longest cDNAs available were used as templates for probe preparation. It is unlikely that the reported patterns represent cross-hybridization, for the following reasons. (1) All the cDNAs hybridize to unique single bands on genomic DNA Southern blot analysis (data not shown); (2) their expression patterns in the embryo are clearly different and (3) RNAase A treatment was used in addition to stringent washing steps in the hybridization protocol. In all cases, sense RNA probes were used as controls, and these gave no specific staining of embryos before 9.5 days p. c., after which some non-specific background was observed.

## Early expression of *HNF-3*β

The expression of the mouse *HNF-3*β gene is first detected in a localized region at the anterior of the primitive streak at the mid streak stage (Fig. 2A; for definition of gastrulation stages, see Materials and Methods). Sectioning of the stained embryos shows that the expression is restricted to the epiblast cells which are just beginning to delaminate (Fig. 2B). After the node has formed, *HNF-3*β expression is localized in the node region and also extends along the anterior midline in the notochord and overlying neural plate (Fig. 2C-F). The mouse node consists of two layers. One is a dorsal layer that is continuous with the epiblast and the other is a ventral layer that is continuous laterally with the endoderm. *HNF-3*β signal can be seen in both layers of the node (data not shown). The node is positive for as long as it can be seen as a discrete structure (up to approx. 8.0 day p.c.).

The expression domain of *HNF-3*β in the mid streak stage embryo appears to be the same as that of mouse *goosecoid* (data not shown; Blum et al., 1992). Previous studies have shown that the expression domain of *goosecoid* was expanded after 3 hours exposure of mid- to late streak stage embryos (stated as 6.4 day p.c. in Blum et al., 1992) to human recombinant activin A (30 mesoderm induction units/ml, equivalent to 200 ng/ml; Blum et al., 1992). To test whether a similar induction mechanism exists for *HNF-3*β, we investigated the effect of human recombinant activin A on *HNF-3*β gene expression. In some experiments, the extraembryonic regions were completely removed, or embryos were pierced to allow the activin access to the epiblast (see Materials and Methods). In our hands, however, activin A did not result in overexpression of either *HNF-3*β or *goosecoid* (data not shown). Moreover, there was no effect on the overall morphology of the treated embryos compared with controls. The experiment was repeated six times for *HNF-3*β and three times for *goosecoid.*

## Late expression of *HNF-3*β

At the head fold stage (approx. 7.5 day p.c.), *HNF-3*β expression is seen in the invaginating foregut endoderm (Fig. 2E). In 8.5 and 9.0 days p. c. embryos, high levels of *HNF-3*β expression are seen along the midline and in the foregut and hindgut (Fig. 3A-C). Examination of sections showed that the midline expression is not only localized to the notochord but is also seen in the ventral midline cells of the neural tube (Fig. 3D-F). This region gives rise to the floor plate, which is important for dorsoventral patterning and axon orientation in the neural tube (Yamada et al., 1991; Hatta et al., 1991; Hatta, 1992). The expression in the floor plate starts temporally and spatially in the anterior (with the midbrain as the anterior limit: Fig. 3B,C) and extends progressively caudally (Fig. 3F,G; data not shown). Therefore, by 8.5 days p. c., the hybridization signal in the anterior to middle midline (head to trunk level) represents expression in both floor plate and notochord, while in the posterior midline (tail), it is only in the notochord. The domain of *HNF-3*β-positive cells in the midbrain is not restricted to ventral midline cells but also spreads more dorsally than in the posterior part of the neural tube (Fig. 3E). Likewise, expression in the gut is not uniform, but is stronger in both anterior and posterior regions compared to the middle (Fig. 3A,C). Expression is also seen in the liver primordium of 9.5 day p.c. embryos (arrowhead in Fig. 3C). This embryonic gut expression correlates with the later expression of *HNF-3*β reported in adult endoderm-derived tissues, i. e. liver, lung and intestine, and its known function as a liver transcription factor (Lai et al., 1990, 1991). The embryonic expression in the node, notochord and floor plate, on the other hand, suggests an additional role in axis formation and neural tube patterning.

## *HNF-3*α

*HNF-3*α expression is first detected at the anterior part of the primitive streak in the late primitive streak stage embryo (Fig. 4A). At the early head fold stage, expression is seen over the entire surface of the embryo (Fig. 4B,C). Examination of sections shows expression is restricted to the definitive endoderm (Fig. 4D), including the anterior notochord, which is still continuous with the endoderm at this stage (data not shown). No expression was seen in the node

(both dorsal and ventral layers), posterior notochord, non-notochordal mesoderm or ectoderm (epiblast), even in the primitive streak where endodermal cells arise (Fig. 4D, and data not shown). By 9.0 days p.c., strong expression is seen in the entire gut, including the liver primordium, and the floor plate of the midbrain (Fig. 4E,G). Moreover, weaker signals are detected in the posterior notochord and in the floor plate of the posterior neural tube (Fig. 4F; not shown). As in the case of *HNF-3β*, the early expression of *HNF-3α* in the entire definitive endoderm and in the midbrain floor plate suggests that the role of this gene is not restricted to the function of adult endoderm-derived organs, e. g. liver, lung and intestine, in which expression has been previously reported (Lai et al., 1991), but that it has additional roles

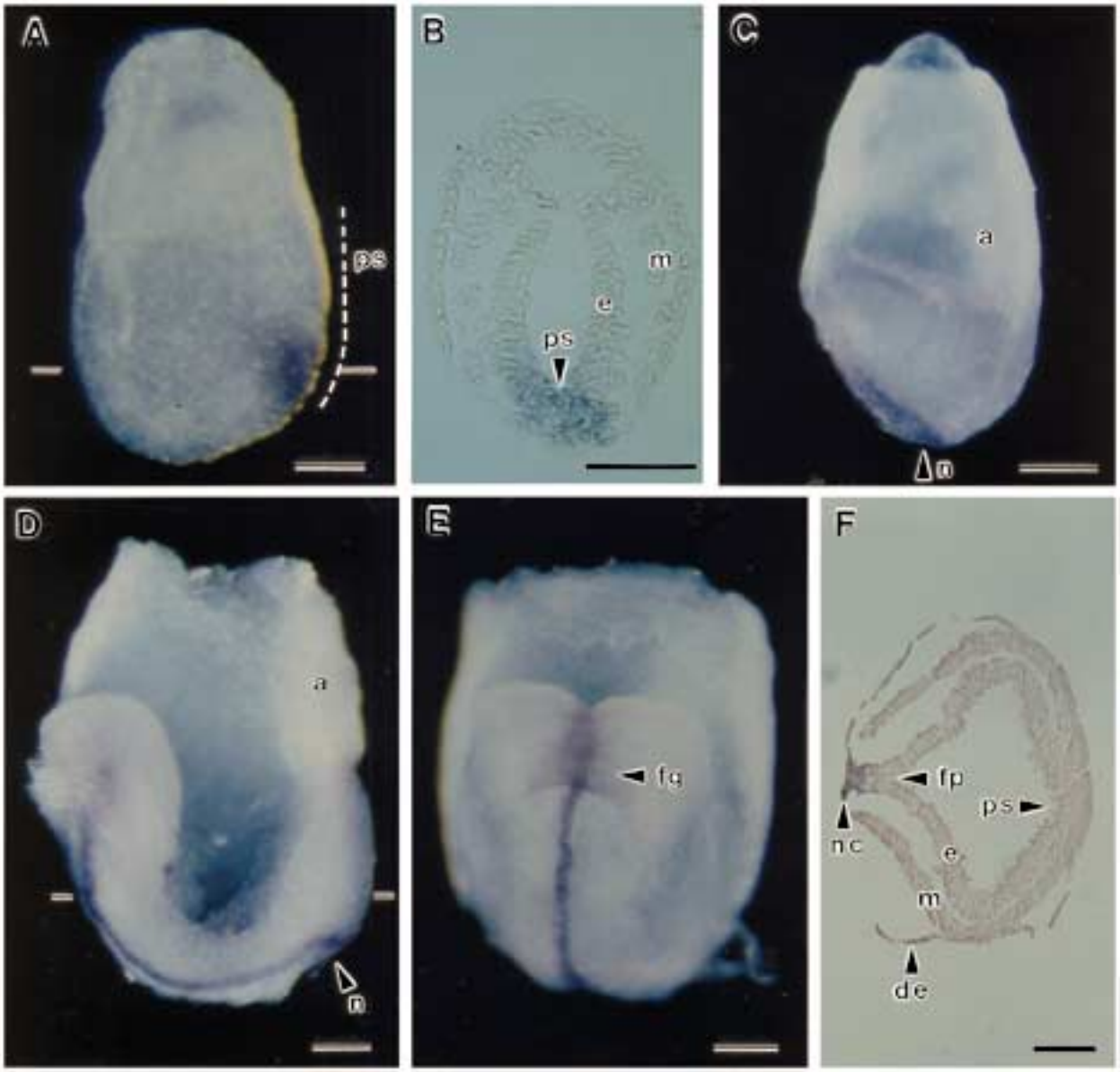


**Fig. 2.** Localization of HNF-3β RNA in 6.5-7.5 days p. c. mouse embryos. In Figs 2-7, RNA was detected by whole-mount in situ hybridization, and unless otherwise noted embryos are oriented so that anterior is to the left and dorsal is to the top. (A) Lateral view of mid- to late streak stage embryo. Signal is localized to the anterior part of the primitive streak. (B) A section of a similar stage embryo to A. The sectioning plane is indicated in A and anterior is to the top. Signal is seen in delaminating epiblast cells. The slightly deformed shape of the epiblast is an artifact due to processing. (C) Lateral view of an early head fold stage embryo showing transcripts extending anteriorly from the node. (D,E) Lateral and anterior views of a three-somite stage embryo. Signal is detected in the node, anteriorly along the midline and in the foregut. (F) A section of a similar stage embryo as in panel D. The sectioning plane is indicated in panel D. Signal can be seen in both the notochord and the presumptive floor plate region of the neural plate. Scale bars indicate 50 μm (A, B) and 100 μm (C-F). All abbreviations used in the figures of this paper are as follows: a, allantois; am, amnion; b, branchial arch; de, definitive endoderm; e, epiblast; ev, eye vesicle; fg, foregut; fp, floor plate; h, heart; hb, hindbrain; hg, hindgut; l, liver primordium; lp, lateral plate mesoderm; m, non-notochordal mesoderm; mb, midbrain; n, node; nc, notochord; ov, otic vesicle; pp, prechordal plate; ps, primitive streak; s, somite; sc, sclerotome.

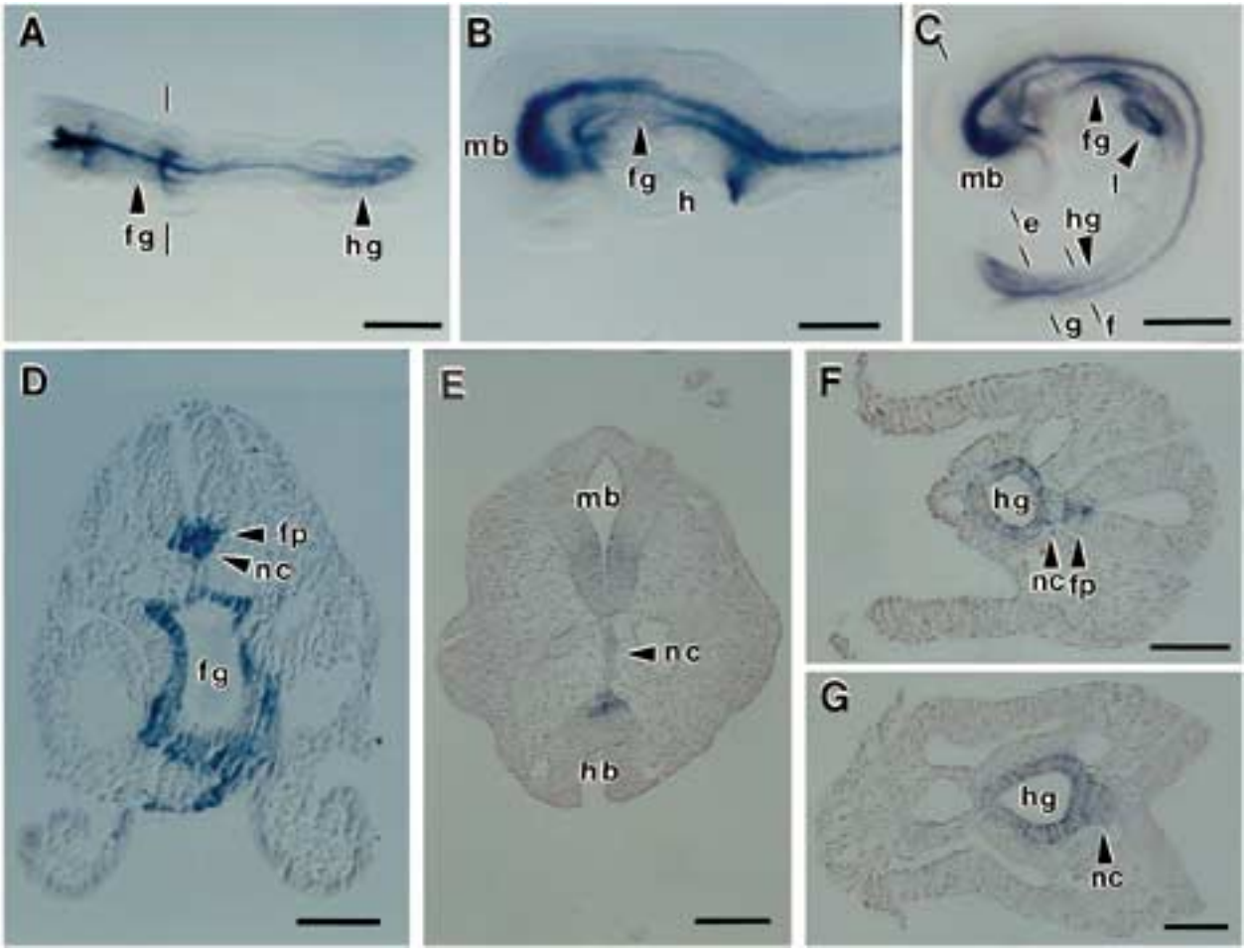


**Fig. 3.** Localization of HNF-3β RNA in 8.5-9.0 days p. c. mouse embryos. (A,B) Dorsal and lateral views of an 8.5 days p. c. embryo. Hybridization signals are seen in the midline and in both the foregut and hindgut. The anterior limit of the midline signal is in the midbrain. (C) Lateral view of a 9.0 days p. c. embryo. Expression is seen in the midline, foregut and hindgut, and liver primordium. (D) A section through the trunk region of an 8.5 days p. c. embryo. A strong hybridization signal is detected in the floor plate of the neural tube, notochord and in the foregut epithelium. Sectioning plane is indicated in A. (E-G) Sections of 9.0 days p.c. embryos. Sectioning planes are indicated as e, f and g in panel C. The expression domain in the midbrain extends laterally (E), and is not restricted to the ventral midline cells as seen in the hindbrain (E) and in the spinal cord (F). The floor plate expression extends almost the entire body length (E, F) except in the posteriormost part (G) of the spinal cord. Signal is also seen in both notochord (E-G) and gut epithelium (F, G). Scale bars indicate 500 μm (A, C), 250 μm (B), 100 μm (E) and 50 μm (D, F, G). Abbreviations are listed in Fig. 2.

in the establishment of the definitive endoderm and in neural tube patterning.

### *MF-1*

A very low level of *MF-1* transcripts is first detected as a wedge-shaped hybridization signal on both sides of the streak of mid- to late primitive streak stage embryos (Fig. 5A). Later, in the early head fold stage embryo (7.5 days p.c.), the expression is seen as two faint bands either side of the primitive streak and notochord, with the strongest expression adjacent to the node (data not shown). Increasing the time of color development, and sectioning of the stained embryos, shows that expression of *MF-1* is in the entire non-notochordal mesoderm, including the prechordal plate, which is thought to be derived from early (pre-node formation) primitive streak (Figs 5C,D, 6A; background staining is shown in Fig. 5B) (Lawson et al., 1991; Lawson and Pederson, 1992). No expression is seen in either the notochord or the node. Furthermore, no specific staining is seen in either the endoderm or the epiblast, even at the level of the primitive streak, where non-notochordal mesoderm cells are delaminating from the epiblast (Fig. 6A). Therefore, *MF-1* expression is restricted to the entire non-notochordal mesoderm, with highest levels in the paraxial region and lower levels in lateral regions.

In 7.5-9.0 days p. c. embryos, expression is seen in two continuous wide bands running parallel to the neural plate/tube and primitive streak. This expression extends from the presomitic mesoderm anteriorly to the head mesoderm. Posterior to the node, the width of the hybridization band gradually narrows caudally and expression ceases around the middle of the primitive streak (Fig. 5E-G). This pattern suggests that cells expressing *MF-1* in the trunk paraxial mesoderm originate from the anterior part of the

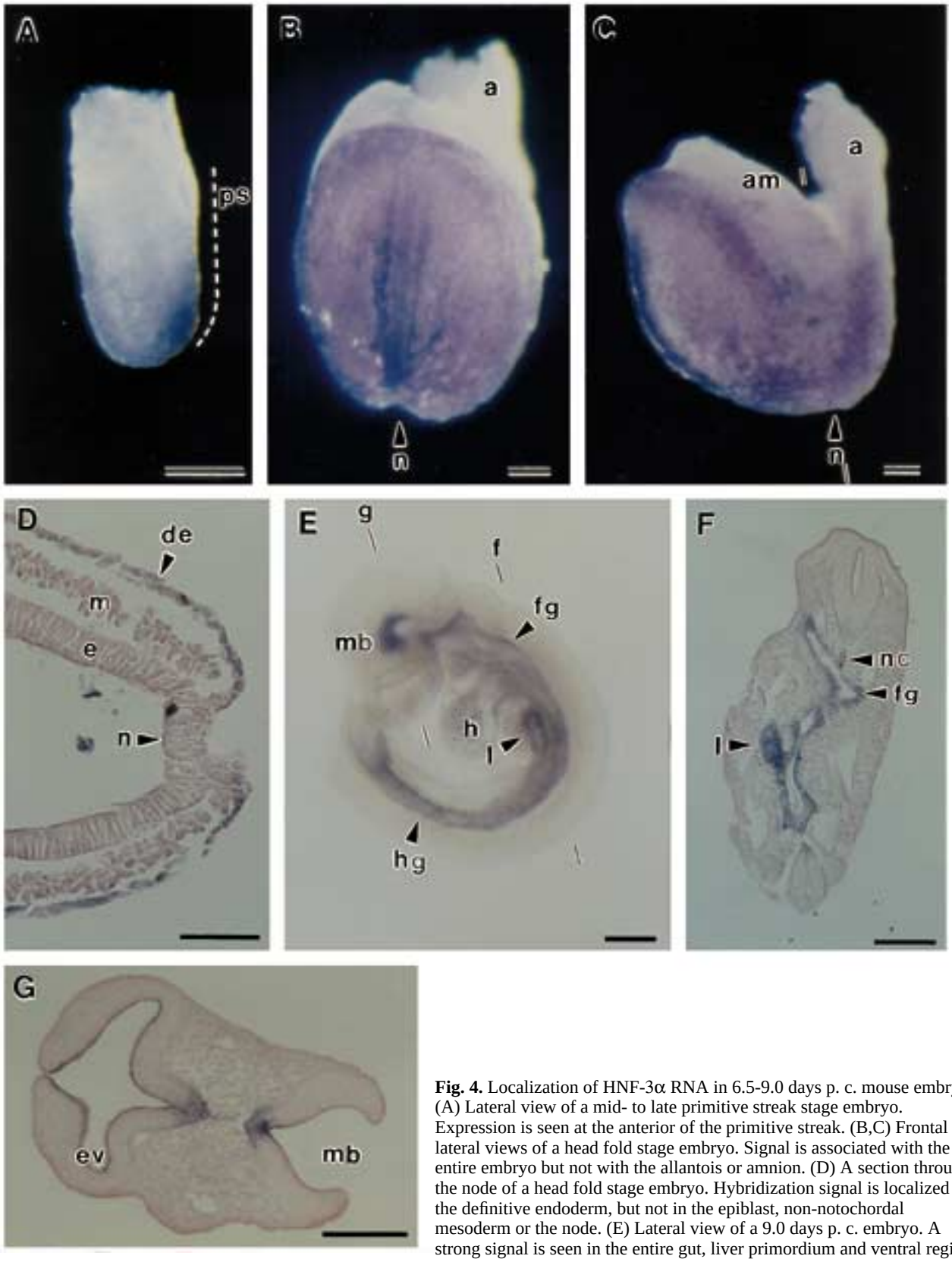


**Fig. 4.** Localization of HNF-3α RNA in 6.5-9.0 days p. c. mouse embryos. (A) Lateral view of a mid- to late primitive streak stage embryo. Expression is seen at the anterior of the primitive streak. (B,C) Frontal and lateral views of a head fold stage embryo. Signal is associated with the entire embryo but not with the allantois or amnion. (D) A section through the node of a head fold stage embryo. Hybridization signal is localized in the definitive endoderm, but not in the epiblast, non-notochordal mesoderm or the node. (E) Lateral view of a 9.0 days p. c. embryo. A strong signal is seen in the entire gut, liver primordium and ventral region of the midbrain. Staining in the heart is non-specific. (F,G) Sections of a 9.0 days p. c. embryo. The sectioning planes are indicated as f and g in E. Strong expression is in the gut epithelium and the liver primordium (F), and in the floor plate of the brain (G). Weaker signals are also seen in the notochord (F). Scale bars indicate 50 μm (B-D), 100 μm (A, F), 200 μm (G) and 250 μm (E). Abbreviations are listed in Fig. 2.

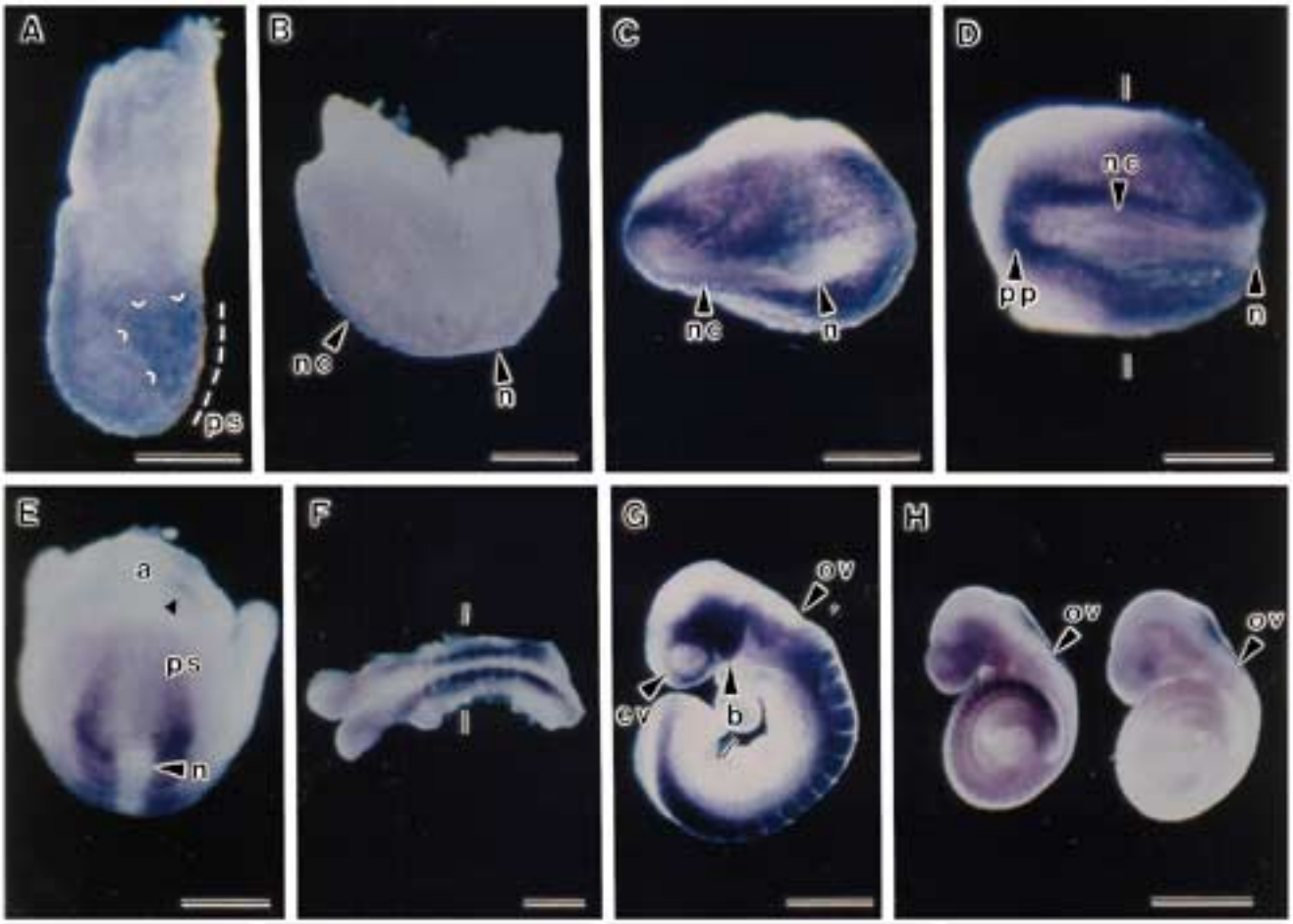


**Fig. 5.** Localization of MF-1 RNA in 6.5-9.5 days p.c. mouse embryos. (A) Lateral view of a mid- to late primitive streak stage embryo showing wedge-shaped staining on either side of the primitive streak (white marks). At this stage, the background staining is high due to the long color reaction time (18 hours) necessary to reveal low expression. (B) Lateral view of an early head fold stage embryo showing background staining caused by the extended color reaction time (18 hours). MF-2 anti-sense probe was used. (C,D) Ventral and frontal views of an extended color reaction time (18 hours) early head fold stage embryo. In D, top is to the left. Because of the extended color reaction time, the hybridization signal has saturated in the highly expressing regions and the expression seems to be rather uniform. Little staining is seen in the node or notochord. With shorter color reaction time (4 hours), signals are detected on either side of the midline, and are strongest around the node (data not shown). (E) Posterior view of a head fold stage embryo. *MF-1* expression is continuous from the anterior paraxial mesoderm to approximately the middle of the primitive streak (between the two black triangles). (F) Dorsal view of an 8.5 days p. c. embryo. Hybridization signals are present on either side of the midline, from head to tail. (G) Lateral view of a 9.0 days p. c. embryo. In the head region, signal is seen both in the neural-crest-derived cells of the first branchial arch and in somitomere-derived cells including those adjacent to rhombomere 5 which is opposite the otic vesicle. (H) Lateral views of two 9.5 days p. c. embryos. At this stage, *MF-1* expression gradually decreases from the anterior (left embryo). A control embryo hybridized with sense probe (right) is also shown for background staining. Scale bars indicate 100 μm (A-D), 500 μm (E-G) and 1000 μm (H). Abbreviations are listed in Fig. 2.

primitive streak. As seen in sections of these embryos, in the trunk region, signal is detected in paraxial and medial mesoderm and in the medial part of the lateral mesoderm. In the lateral mesoderm, expression gradually fades laterally, with no distinct expression boundary (Fig. 6B). No signal is detected in the notochord or neural tube. In the head region, gene expression is not restricted to the paraxial region (Fig. 6C) and the distribution of hybridization signal suggests that two intermixed populations of cells express *MF-1*; those derived from paraxial mesoderm (somitomeres) and others from the neural crest. First, high levels of signal are seen in cells filling the first branchial arch (Fig. 5G). These cells are probably of neural crest origin, as are the *MF-1*-positive cells in the frontonasal area anterior of the eye (Lumsden et al., 1991; Serbedzija et al., 1992). A second *MF-1*-positive population of cells extends along the side of the head, from the level of the otic vesicle to the optic vesicle. Since this population is part of a continuous band throughout the head and trunk, it is probably derived from paraxial mesoderm (somitomeres) rather than from neural crest, which is absent from regions adjacent to rhombomeres 3 and 5 (Fig. 5G, rhombomere 5 is opposite the otic vesicle; Lumsden et al., 1991; Serbedzija et al., 1992). By 9.5 day p. c., *MF-1* expression decreases from the anterior, so that expression now appears as an anterior-posterior gradient with the highest level posteriorly

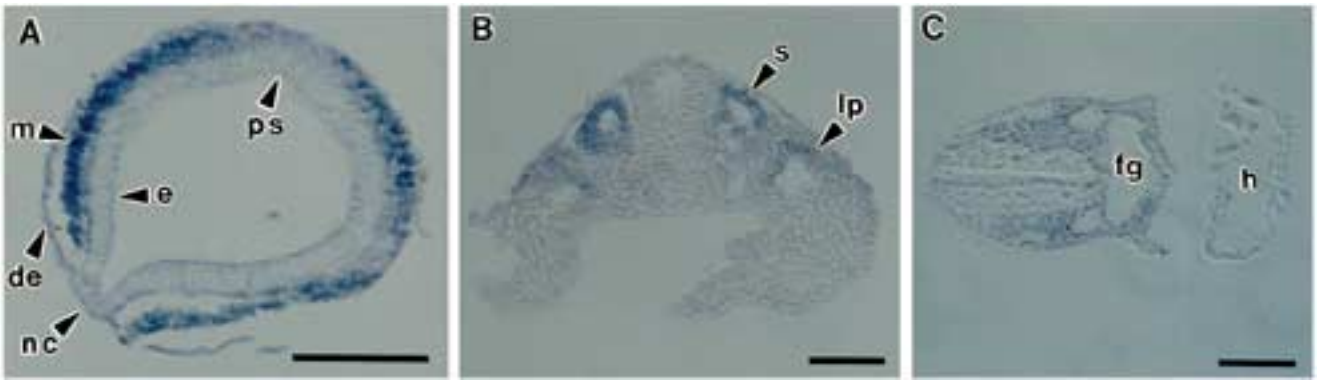


**Fig. 6.** Localization of MF-1 RNA in 7.0-9.0 days p.c. mouse embryos. (A) A section of an early head fold stage embryo stained for 18 hours. The sectioning plane is shown in Fig. 5D. Signal is localized to non-notochordal mesoderm. No expression is seen in the epiblast, even in the primitive streak. (B) Section through the trunk region of a 8.5 days p. c. embryo. Signal is present in the somite and in the lateral mesoderm. There is no distinct boundary but only a gradual decline in intensity in the lateral mesoderm. The sectioning plane is shown in Fig. 5F. (C) A section through the head of a 9.0 days p. c. embryo. Dorsal is to the left. Staining is seen uniformly in the non-notochordal mesoderm except in the heart. The sectioning plane is shown in Fig. 5G. Scale bars indicate 100 μm (A,B) and 250 μm (C). Abbreviations are listed in Fig. 2.

(Fig. 5H). Since no expression is seen in adult tissues (data not shown), *MF-1* expression is transient.

### *MF-2*

Expression of *MF-2* is first detected in the 8.0 days p. c. embryo. By 9.0 days p. c., *MF-2*, like *MF-1*, is expressed in the head mesenchyme, including first and second branchial arches, and in the non-notochordal mesoderm of the trunk (Fig. 7A). No expression is detected in the notochord or neural tube. However, the patterns of *MF-1*and *MF-2* expression are clearly different. First, *MF-2* expression starts from the posterior of newly forming somites and no expression is seen in the presomitic mesoderm (Fig. 7A). Second, as shown in sections, expression is localized to the sclerotome region and not the entire paraxial mesoderm (Fig. 7B). In the head region, the hybridization signal suggests that *MF-2* is expressed in both mesoderm (somitomeres) and neural-crest-derived cells, for the same reasons as outlined for *MF-1*. However, its expression pattern is slightly different from that of *MF-1*. For example, *MF-2* expression around the eye vesicle is more obvious than that of *MF-1*. In addition, no expression is seen in mesenchymal cells lying on the ventral side of the brain, which are not derived from neural crest (data not shown) (Serbedzija et al., 1992). In the 9.5 days p. c. embryo, the level of expression of *MF-2* does not decrease, as in the case of *MF-1*, but is uniformly high along the entire body length (Fig. 7C).

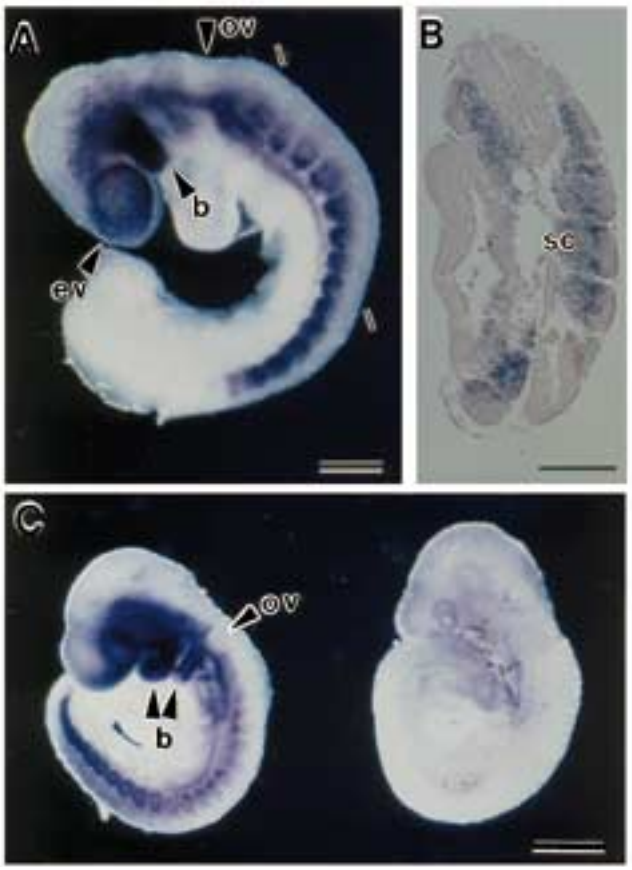


**Fig. 7.** Localization of MF-2 RNA in 9.0-9.5 day p.c. mouse embryo. (A) Lateral view of 9.0 days p. c. embryo. The *MF-2* expression pattern is similar to that of *MF-1* (Fig. 5G). However, no expression is seen in the presomitic mesoderm. (B) A section of a 9.0 day embryo. The sectioning plane is indicated in panel A. Anterior is to the top. *MF-2* expression in the trunk is localized in the sclerotome. (C) A lateral view of two 9.5 days p. c. embryos. *MF-2* expression is uniformly distributed along the anterior-posterior axis at this stage. A control embryo hybridized with sense probe (right) is also shown for background staining. Scale bars indicate 200 μm (B), 250 μm (A) and 500 μm (C). Abbreviations are listed in Fig. 2.

## DISCUSSION

Here we report the developmental expression of four mouse FD genes, each exhibiting a unique expression pattern during gastrulation stages. *HNF-3*β is expressed in the

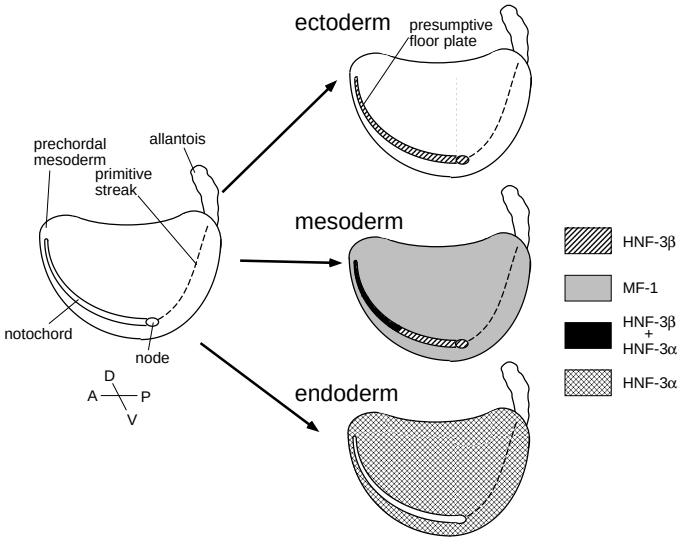


**Fig. 8.** Schematic representation of the expression patterns of FD genes in the head fold stage mouse embryo. Slightly ventral side view of a head fold stage embryo is shown schematically in the left panel. In the right panels, the expression domains of *HNF-3*β, *MF-1* and *HNF-3*α are shown in the three germ layers. From the top, each panel represents ectoderm, mesoderm and endoderm. At this stage, the notochord and node are continuous with the endoderm so that there are no endoderm cells in the notochord and the node regions. *HNF-3*β expression is restricted to the node and extends anteriorly in the notochord and overlying neural plate (ectoderm). *MF-1* is expressed in the entire non-notochordal mesoderm, which is derived from the primitive streak. In this figure, the medial-lateral difference of expression of *MF-1* is not presented. *HNF-3*α is expressed in the entire definitive endoderm, and in the anterior notochord.

node, notochord, floor plate and gut. *HNF-3*α is detected in the definitive endoderm and gut, but also in the anterior notochord and the floor plate of the midbrain. *MF-1* is expressed in non-notochordal mesoderm, including head mesenchyme and *MF-2* in the sclerotomal region of the somites and in the head mesenchyme.

### *HNF-3*β, *MF-1* and *HNF-3*α expression and early gastrulation events in the mouse embryo

Fate mapping studies in the mouse, following horse radish peroxidase injection into single epiblast cells (Lawson et al., 1991; Lawson and Pederson, 1992), suggest that the processes of gastrulation and axis formation are basically similar to those proposed from more detailed studies in the chick embryo, using dye injection and cell grafting techniques. Based on these studies, it is possible to correlate the spatial and temporal expression patterns of *HNF-3*β, *MF-1* and *HNF-3*α (summarized in Fig. 8) with gastrulation events as follows. (1) Notochord cells are derived (probably exclusively) from the anterior portion of Hensen's node (Selleck and Stern, 1991a; Schoenwolf et al., 1992) and, at the time of node formation, cells in the node are already committed to a notochord fate (Selleck and Stern, 1991b; Garcia-Martinez and Schoenwolf, 1992). *HNF-3*β expression is first detected in the presumptive notochord region of the mouse epiblast prior to node formation and subsequently is found in the dorsal and the ventral layers of the node and in the notochord. This suggests that *HNF-3*β expression correlates with the commitment of epiblast cells to a node/notochord cell fate. (2) Non-notochordal mesoderm cells, which include prechordal, paraxial (somites), intermediate and lateral mesoderm cells, are derived from lateral regions of the node and from the primitive streak. Anterior and posterior primitive streak cells give rise to medial and lateral mesoderm, respectively, and these primitive streak cells are not committed to their developmental fate before gastrulation (Selleck and Stern, 1991a,b; Schoenwolf et al., 1992; Garcia-Martinez and Schoenwolf, 1992). *MF-1* expression is thus associated with the commitment of newly formed mesoderm cells to a paraxial, intermediate and medial-lateral cell fate. The reduced expression of *MF-1* in more lateral mesoderm correlates with its lower expression in cells adjacent to the posterior compared with the anterior streak (Figs 5E, 6B). (3) Although information is rather restricted even in chick, definitive endoderm cells are thought to be derived from the very early node region (giving rise to foregut cells) and from the entire primitive streak (Lawson et al., 1991; Lawson and Pederson, 1992; Selleck and Stern, 1991a). *HNF-3*α expression thus appears to coincide with the commitment of definitive endoderm cells. From these correlations alone, it is not possible to ascertain whether *HNF-3*β, *MF-1* and *HNF-3*α play an active role in determining cell fate or whether their expression is a consequence of the committed state of different cell populations. To distinguish between these alternatives, further analysis will be necessary, such as generating transgenic and/or null mutant mice for these genes.

In addition to these correlations between the expression of FD genes and gastrulation events, recent studies in the mouse, and more extensively in *Xenopus*, have identified a number of other genes encoding known or putative nuclear proteins which are expressed in restricted populations of cells during gastrulation. Therefore, it is likely that FD genes are part of a complex network of regulatory elements controlling cell fate. In order to place FD gene expression in the context of these other studies, we propose a model relating gene expression to gastrulation in the mouse embryo. This model is summarized in Fig. 9.

At the primitive streak stage, in response to some as yet unknown signal(s), epiblast cells at the anterior of the primitive streak are committed as organizing cells and express *HNF-3*β and *goosecoid* (Blum et al., 1992). It is likely that the establishment of the identity of the organizing center depends on the interactions of these genes and others expressed in the node. These include *Brachyury (T)* (Wilkinson et al., 1990; Herrmann, 1991), the mouse homologue of *Xenopus Xlim-1* (Taira et al., 1992; Joseph Barnes and B. L. M. H., unpublished observations) and as yet unidentified mouse homologues of *XFKH1* (Dirksen and Jamrich, 1992) and *Pintallavis*/*XFD-1* (Ruiz i Altaba and Jessel, 1992; Knöchel et al., 1992), which are expressed in the Spemann's organizer region of *Xenopus* embryos. The organizing center cells migrate from the epiblast and form the node. According to our model, *HNF-3*β-expressing node cells divide clonally as a stem cell population and generate *HNF-3*β-expressing notochord cells which move

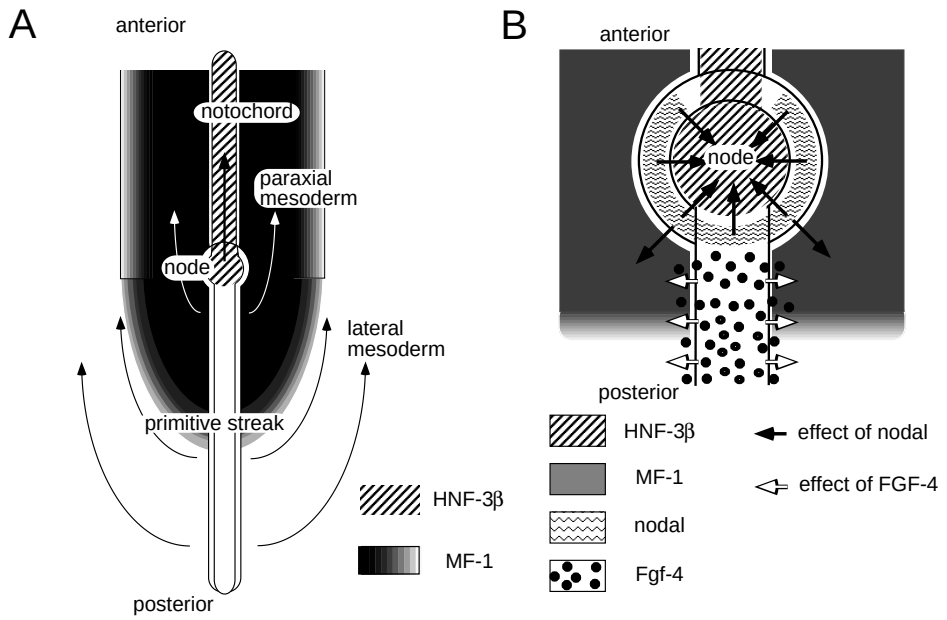


**Fig. 9.** Models of gastrulation in the mouse embryo: mesodermal cell movement and FD gene expression (A), and possible gene interactions around the node (B). (A) A model of mesodermal cell movement and FD gene expression in the head fold stage mouse embryo which is represented as flattened and viewed from the dorsal side. Cell movements are indicated by arrows. The node, which expresses *HNF-3*β, provides *HNF-3*β-expressing notochord cells anteriorly. The anterior primitive streak provides *MF-1*-expressing mesodermal cells. It is likely that mesodermal cells derived from the posterior primitive streak express *MF-1* at a very low level. As development proceeds, anterior and posterior primitive streak give rise to paraxial and lateral mesoderm cells, respectively. This results in the higher MF-1 expression seen in paraxial mesoderm in the trunk region (Fig. 6B). (B) Summary of gene expression patterns and their possible interactions around the node. A TGF-β superfamily gene, *nodal*, is expressed in cells surrounding the node (except the anterior edge) in the endoderm layer and a limited part of the immediately adjacent mesoderm layer (Zhou et al., 1993). *HNF-3*β is expressed in the node and the notochord. *Fgf-4* is expressed in the primitive streak in both the ectoderm and mesoderm layers (Niswander and Martin, 1992). *MF-1* is expressed in the non-notochordal mesoderm in the anterior half of the primitive streak. Therefore, it is possible that the node cell phenotype (including *HNF-3*β expression) is maintained by nodal (solid arrows), and that *MF-1* expression is regulated by both nodal (solid arrows) and FGF-4 (open arrows). It is also possible that other FGFs and putative mesoderm-inducing factors also play a part in *HNF-3*β and *MF-1* expression (see text for details).

towards the anterior (Fig. 9A). At present, the signalling molecules involved in node induction and *HNF-3*β*/goosecoid* expression are unknown. However, it is possible that the phenotype of node cells is induced and/or maintained by a TGF-β superfamily protein encoded by the gene, *nodal*, which is expressed in a crescent of cells around the node (Zhou et al., 1993) (Fig. 9B). Other signalling factors, such as FGFs, activin, the product of *wnt* related gene(s) (reviewed in Slack and Tannahill, 1992) and the putative mouse homologue of the product of the *Xenopus noggin* gene (Smith and Harland, 1992) may also be involved.

Non-notochordal mesoderm cells, including prechordal, paraxial, medial and lateral plate mesoderm, are all formed by gastrulation through the primitive streak (Schoenwolf et al., 1992). Primitive streak cells are initially not committed as non-notochordal mesoderm and are developmentally labile until the onset of *MF-1* expression, which coincides with their commitment to a paraxial, intermediate and medial-lateral mesoderm fate. The expression of *MF-1* is not uniform throughout the newly formed mesoderm since only very low levels of expression are seen around the posterior primitive streak. *MF-1* expression may thus be regulated by at least two diffusible signals, one from the primitive streak and another from the node. All cells receive an equal amount of primitive streak signal. However, the cells proximal to the node receive a strong signal from the node, while distal cells receive a weaker signal (Fig. 9B). As a result of cell migration, anterior and posterior cells make medial and lateral mesoderm, respectively, and this is reflected in the spatial expression of *MF-1* in the trunk (Fig. 9A; Schoenwolf et al., 1992). Again, it is possible that members of the FGF family and, especially FGF-4, which is exclusively expressed in the primitive streak (Niswander and Martin, 1992), and nodal, which is expressed around the node (Zhou et al., 1993), are at least some of the signaling molecules involved (Fig. 9B). It is likely that the establishment of non-notochordal mesodermal fate also involves the action of the gene *Brachyury (T)* (Herrmann, 1991; Cunliffe and Smith, 1992). Since *MF-1* expression is transient, it is possible that other putative transcription factors such as *MF-2* (Fig. 7), *M-twist* (Wolf et al., 1991), *Sna* (Nieto et al., 1992), *Mox-1, -2* (Candia et al., 1992), *Msx-1 (Hox-8)* (Yokouchi et al., 1991) and myogenic basic HLH factors, including *myogenin*, *MyoD1* and *Myf5* (Sassoon et al., 1989, Ott et al., 1991), are important for later mesoderm patterning and differentiation.

Although the timing of commitment of definitive endoderm cells is not yet known, it is possible that presumptive endoderm cells in the epiblast are not committed until gastrulation, after *HNF-3*α is activated. The further regional specification and differentiation of definitive endodermal cells probably involves a combination of other putative DNA-binding transcription factors. These include *HNF-3*β, which is expressed in the gut epithelium after the late head fold stage, *Cdx-1*, which is expressed in the epithelial cells of the embryonic intestine after 14.5 days p. c. (Duprey et al., 1988), and as yet unidentified mouse homologues of chick *CHox-cad*, which is expressed in the early endodermal lineage with posterior dominance (Frumkin et al., 1991), *Xenopus XlHbox 8*, which is expressed as a narrow

band in the endoderm (Wright et al., 1988), and rat *LFB3*, which is expressed in epithelial cells of many organs in the 14.5 days p. c. embryo (De Simone et al., 1991).

### Expression of FD genes in the floor plate of the neural tube

Following expression in the notochord, *HNF-3*β transcripts are detected in the ventral midline cells of the neural tube. This expression domain, whose anterior limit is the midbrain, corresponds to the floor plate. Floor plate is induced by the notochord along the ventral midline of the embryonic neural tube except in the forebrain (Placzek et al., 1990; Yamada et al., 1991; Hatta et al., 1991). It has ventral-pattern-inducing activity within the neural tube and is also important for axon guidance (Yamada et al., 1991; Hatta, 1992). Recently, the expression of two other FD genes, *XFKH1/XFD-1′* and *XFD-1/Pintallavis*, which are very similar to each other (93% identical in their entire amino acid sequences) was reported in the notochord and floor plate in the *Xenopus* embryo (Dirksen and Jamrich, 1992; Häcker et al., 1992; Ruiz i Altaba and Jessel, 1992). *HNF-3*β has some similarities with these two *Xenopus* FD genes. First, the amino acid sequences of the FDs are similar (more than 90% identical) and they belong to the same class, Class I (see Fig. 1). However, it is unlikely that *HNF-3*β is the mouse homologue of *XFKH1/XFD-1′* and/or *XFD-1/Pintallavis*, and is more likely to be the homologue of *XFD-3*, which has an almost identical FD (one conservative change). Second, while the early expression patterns of *XFKH1* and *Pintallavis* are similar to *HNF-3*β, the later patterns are not. The expression of the two *Xenopus* genes starts in the dorsal side of the late blastula, becomes localized to the dorsal lip region (Spemann's organizer), and then to the notochord and in the midline cells of the neural plate. Later, however, the expression of *XFKH1* and *XFD-1/Pintallavis* is restricted to anterior and posterior parts of embryo, respectively. In contrast, *HNF-3*β expression is seen along the entire length of the embryo (at least until 9.5 days p. c.; data not shown). Experiments in *Xenopus* have been interpreted to show that when *Pintallavis* is overexpressed by injecting mRNA into one cell of a 2-cell stage embryo, there is a reduction of forebrain, an increase of posterior neural tissues and fewer dorsal spinal cord sensory neurons in the part of the embryo derived from the injected cells (Ruiz i Altaba and Jessel, 1992). This suggests that *Pintallavis* plays a role in establishing posterior and ventral positional values in the central nervous system. By analogy with this result, it is likely that *HNF-3*β plays a role in the dorsoventral patterning of the CNS in the mouse embryo as a floor plate determinant and that, together, *HNF-3*β, *HNF-3*α (which is strongly expressed in the floor plate of the midbrain) and the homologues of *XFKH1* and *XFD1/Pintallavis* coordinately regulate the anterior-posterior patterning of the part of the CNS that is posterior to the forebrain.

We are grateful to Jill McMahon for unpublished in situ hybridization protocol, Chris Wright for critical reading of this manuscript and for the *Xenopus* dorsal lip cDNA library, Mike Jones for comments on the manuscript and technical advice. Anthony Mason (Genentech) for recombinant human activin A, Eddy De Robertis for the mouse *goosecoid* genomic clone, and Drew Noden and Gary Schoenwolf for comments on the expression of *MF-1* and *MF-2*. H. S. is supported by a long-term fellowship from the Human Frontier Science Program.

## REFERENCES

**Blum, M., Gaunt, S. J., Cho, K. Y., Steinbeisser, H., Blumberg, B., Bittner, D. and De Robertis, E. M.** (1992). Gastrulation in the mouse: the role of the homeobox gene *goosecoid*. *Cell* **69**, 1097-1106.

**Blumberg, B., Wright, C. V. E., De Robertis, E. M. and Cho, K. W. Y.** (1991). Organizer-specific homeobox genes in *Xenopus laevis* embryos. *Science* **253**, 194-196.

**Candia, A. F., Hu., J. C., Crosby, J., Lalley, P. A., Noden, D., Nadeau, J. H. and Wright, C. V. E.** (1992). *Mox-1* and *Mox-2* define a novel homeobox gene subfamily and are differentially expressed during early mesodermal patterning in mouse embryos. *Development* **116**, 1123-1136.

**Cunliffe, V. and Smith, J. C.** (1992). Ectopic mesoderm formation in *Xenopus* embryos caused by widespread expression of a *Brachyury* homologue. *Nature* **358**, 427-430.

**De Simone, V., de Magistris, L., Lazzaro, D., Gerstner, J., Monaci, P., Nicosia, A. and Cortese, R.** (1991). LFB3. a heterodimer-forming homeoprotein of the LFB1 family, is expressed in specialized epithelia. *EMBO J.* **10**, 1435-1443.

**Dirksen, M. L. and Jamrich, M.** (1992). A novel, activin-inducible, blastopore lip-specific gene of *Xenopus laevis* contains a *fork head* DNA-binding domain. *Genes Dev.* **6**, 599-608.

**Duprey, P., Chowdhurry, K., Dressler, G. R., Balling, R., Simon, D., Guenet, J.-L. and Gruss, P.** (1998). A mouse gene homologous to the *Drosophila* gene *caudal* is expressed in epithelial cells from the embryonic intestine. *Genes Dev.* **2**, 1647-1654.

**Fahrner, K., Hogan, B. L. M. and Flavell, R. A.** (1987). Transcription of H-2 and Qa genes in embryonic and adult mice. *EMBO J.* **6**, 1265-1271.

**Frumkin, A., Rangini, Z., Ben-Yehuda, A., Gruenbaum, Y. and Fainsod, A.** (1991). A chicken *caudal* homologue, *CHox-cad*, is expressed in the epiblast with posterior localization and in the early endodermal lineage. *Development* **112**, 207-219.

**Garcia-Martinez, V. and Schoenwolf, G. C.** (1992). Positional control of mesoderm and fate during avian gastrulation and neurulation. *Dev. Dynamics* **193**, 249-256.

**Grossinklaus, U., Pearson, R. K. and Gehring, W. J.** (1992). The *Drosophila sloppy paired* locus encodes two proteins involved in segmentation that show homology to mammalian transcription factors. *Genes Dev.* **6**, 1030-1051.

**Häcker, U., Grossinklaus, U., Gehring, W. J. and Jäckle, H.** (1992). Developmentally regulated *Drosophila* gene family encoding the fork head domain. *Proc. Natl. Acad. Sci. USA* **89**, 8754-8758.

**Hatta, K.** (1992). Role of the floor plate in axonal patterning in the Zebrafish CNS. *Neuron* **9**, 629-642.

**Hatta, K., Kimmel, C. B., Ho, R. K. and Walker, C.** (1991). The cyclops mutation blocks specification of the floor plate of the zebrafish central nervous system. *Nature* **350**, 339-341.

**Herrmann, B. G. (**1991). Expression pattern of the *Brachyury* gene in whole-mount $T^{wis}/T^{wis}$ mutant embryos. *Development* **113**, 913-917.

**Kimelman, D., Christian, J. L. and Moon, R. T.** (1992). Synergistic principles of development: overlapping patterning systems in Xenopus mesoderm induction. *Development* **116**, 1-9.

**Knöchel, S., Lef, J., Clement, J., Klocke, B., Hille, S., Köster, M. and Knöchel W.** (1992). Activin A induced expression of a fork head related gene in posterior chordamesoderm (notochord) of *Xenopus* embryos. *Mech. Dev.* **38**, 157-165.

**Lai, E., Prezioso, V. R., Smith, E., Livitin, H., Costa, R. H. and Darnel, J. E.** (1990). HNF-3α, A hepatocyte-enriched transcription factor of novel structure is regulated transcriptionally. *Genes Dev.* **4**, 1427-1436.

**Lai, E., Prezioso, V. R., Tao, W., Chen, W. S. and Darnel, J. E.** (1991). Hepatocyte nuclear factor 3α belongs to a gene family in mammals that is homologous to the *Drosophila* homeotic gene *fork head*. *Genes Dev.* **5**, 416-427.

**Landshulz, W. H., Johnson, P. E. and McKnight, S. L.** (1988). The Leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* **240**, 1759-1764.

**Lawson, K. A. and Pederson, R. A.** (1992). Clonal analysis of fate during

gastrulation and early neurulation in the mouse. In *Postimplantation development in the Mouse* (Ciba Found. Symp. 165), pp. 3-26 Wiley: Chichester.

**Lawson, K. A., Meneses, J. J. and Pederson, R. A.** (1991). Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. *Development* **113,** 891-991.

**Li, C., Lai, C., Sigman, D. S. and Gaynor, R. B.** (1991). Cloning of a cellular factor, interleukin binding factor, that binds to NFAT like motifs in the human immunodeficiency virus long terminal repeat. *Proc. Natl. Acad. Sci. USA* **88**, 7739-7743.

**Lumsden, A., Sprawson, N. and Graham, A.** (1991). Segmental origin and migration of neural crest cells in the hindbrain region of the chick embryo. *Development* **113**, 1281-1291.

**McGinnis, W. and Krumlauf, R.** (1992). Homeobox genes and axial patterning. *Cell* **68**, 283-302.

**Murre, C., McCaw, P. S. and Baltimore, D.** (1989). A new DNA binding and dimerization motif in immunoglobin enhancer binding, *daughterless*, *MyoD* and *myc* proteins. *Cell* **56**, 777-783.

**Nieto, M. A., Bennett, M. F., Sargent, M. G. and Wilkinson, D. G.** (1992). Cloning and developmental expression of Sna, a murine homologue of the Drosophila snail gene. *Development* **116**, 227-237.

**Niswander, L. and Martin, G. R.** (1992). *Fgf-4* expression during gastrulation, myogenesis, limb and tooth development in the mouse. *Development* **114**, 755-768.

**Ott, M.-O., Bober, E., Lyons, G., Arnold, H. and Buckingham, M.** (1991). Early expression of the myogenic regulatory gene, *myf-5*, in precursor cells of skeletal muscle in the mouse embryo. *Development* **111**, 1097-1107.

**Placzek, M., Tessier-Lavigne, M., Yamada, T., Jessel, T. and Dodd, J.** (1990). Mesodermal control of neural cell identity: floor plate induction by notochord. *Science* **250**, 985-988.

**Ruiz i Altaba, A. and Jessel T. M.** (1992). *Pintallavis*, a gene expressed in the organizer and midline cells of frog embryos: involvement in the development of the neural axis. *Development* **116,** 81-93.

**Sassoon, D., Lyons, G., Wright, W. E., Lin, V., Lasser, A., Weintraub, H. and Buckingham, M.** (1989). Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis. *Nature* **341**, 303-307.

**Schoenwolf, G. C., Garcia-Martinetz, V. and Dias, M. S.** (1992). Mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dynamics* **193,** 235-248.

**Selleck, M. A. J. and Stern C. D.** (1991a). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development* **112**, 615-626.

**Selleck, M. A. J. and Stern C. D.** (1991b). Commitment of mesoderm cells in Hensen's node of the chick embryo to notochord and somite. *Development* **114**, 403-415.

**Serbedzija, G. N., Bronner-Fraser, M. and Fraser S. E.** (1992). Vital dye analysis of cranial neural crest cell migration in the mouse embryo. *Development* **116**, 297-307.

**Sive, H. L., Hattori, K. and Weintraub, H.** (1989). Progressive determination during formation of the anteroposterior axis in *Xenopus laevis*. *Cell* **58**, 171-180.

**Slack, J. M. W. and Tannahill, D.** (1992). Mechanism of antero posterior axis specification in vertebrates. Lessons from the amphibians. *Development* **114**, 285-302.

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

**Taira, M., Jamrich, M., Good, P. J. and David, I.** (1992). The LIM domain-containing homeobox gene *Xlim-1* is expressed specifically in the organizer region of *Xenopus* gastrula embryos. *Genes Dev.* **6,** 356-366.

**Tao, W. and Lai, E.** (1992). Telencephalon-restricted expression of BF-1, a new member of the HNF-3/*fork head* gene family, in the developing rat brain. *Neuron* **8**, 957-966.

**Weigel, D. and Jäckle, H.** (1990). The fork head domain: a novel DNA binding motif of eukaryotic transcription factors? *Cell* **63**, 455-456.

**Weigel, D., Jürgens, G., Kütter, F., Seifert, E. and Jäckle, H.** (1989). The homeotic gene *fork head* encodes a nuclear protein and is expressed in the terminal regions of the Drosophila embryo. *Cell* **57**, 645-658.

**Wilkinson, D. G., Bhatt, S. and Herrmann, B. G.** (1990). Expression pattern of the mouse *T* gene and its role in mesoderm formation. *Nature* **343**, 657-659.

**Wolf, C., Thisse, C., Stoetzel, C., Thisse, B., Gerlinger, P. and Perrin-Schmitt, F.** (1991). The *M-twist* gene of *Mus* is expressed in subsets of mesodermal cells and is closely related to the *Xenopus X-twi* and the *Drosophila twist* genes. *Dev. Biol.* **143,** 363-373.

**Wright, C. V. E., Schnegelsberg, P. and De Robertis, E. M.** (1988). XlHbox 8: a novel *Xenopus* homeo protein restricted to a narrow band of endoderm. *Development* **104**, 787-794.

**Xi, H. and Rosenfeld, M. G.** (1991). Mechanism of complex transcriptional regulation: implications for brain development. *Neuron* **7**, 183-196.

**Yamada, T., Placzek, M., Tanaka, H., Dodd, J. and Jessel, T. M.** (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell* **64**, 635-647.

**Yokouchi, Y., Ohsugi, K., Sasaki, H. and Kuroiwa, A.** (1991). Chicken homeobox gene *Msx-1*: structure, expression in limb buds and effect of retinoic acid. *Development* **113**, 431-444.

**Zhou, X., Sasaki, H., Lowe, L., Hogan, B. L. M. and Kuehn, M. R.** (1993). *Nodal*, a novel TGF-β-like gene expressed in the mouse node during gastrulation. *Nature* **361**, 543-547.

(Accepted 3 February 1993)