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Fox (forkhead) genes are involved in the dorso-ventral patterning of the *Xenopus* mesoderm

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ABSTRACT ***Fox* (forkhead/winged helix) genes encode a family of transcription factors that are involved in embryonic pattern formation, regulation of tissue specific gene expression and tumorigenesis. Several of them are transcribed during *Xenopus* embryogenesis and are important for the patterning of ectoderm, mesoderm and endoderm. We have isolated three forkhead genes that are activated during gastrulation and play an important role in the dorso-ventral patterning of the mesoderm. *XFKH1(FoxA4b)*, the first vertebrate forkhead gene to be implicated in embryonic pattern formation, is expressed in the Spemann-Mangold organizer region and later in the embryonic notochord. *XFKH7*, the *Xenopus* orthologue of the murine *Mfh1(Foxc2)*, is expressed in the presomitic mesoderm, but not in the notochord or lateral plate mesoderm. Finally, *XFD-13'(FoxF1b)1* is expressed in the lateral plate mesoderm, but not in the notochord or presomitic mesoderm. Expression pattern and functional experiments indicate that these three forkhead genes are involved in the dorso-ventral patterning of the mesoderm.**

KEY WORDS: *Fox genes, mesoderm, the Spemann-Mangold organizer, pattern formation.*

Introduction

Forkhead proteins play an important role in embryonic pattern formation, regulation of tissue specific gene expression, and tumor formation (Dirksen and Jamrich, 1992; Li and Vogt, 1993; Sasaki and Hogan, 1993; Hatini *et al.*, 1994: Dirksen and Jamrich, 1995; Martinez *et al.*, 1997; Kenyon *et al,.* 1999; Brownell *et al.*, 2000 and others; for review see Kaufman and Knochel, 1995). These proteins contain a highly conserved 110 amino acid long DNA binding domain that was originally identified in *Drosophila* mutant *fork head* (Weigel *et al.*, 1989; Weigel and Jackle, 1990) and in the rat hepatocyte nuclear factor HNF-3 (Lai *et al.*, 1990; 1991). Because of their structure, these proteins are also referred to as winged helix proteins (Clark *et al.*, 1993). They bind DNA as monomers and can act as transcriptional activators or repressors. They were recently renamed as Fox genes (Kaestner *et al.*, 2000). Fox genes represent a large gene family with more than 20 members in each of the higher vertebrate species. They show remarkable evolutionary conservation between species as distant as yeast and man. Several Fox genes are expressed during early stages of *Xenopus* development and are regulating diverse aspects of pattern formation of the endoderm, ectoderm and mesoderm. We have isolated three Fox genes that are involved in the dorso-ventral patterning of the mesoderm.

In *Xenopus*, mesoderm arises in the equatorial region of the embryo in response to inductive signals from the vegetal hemisphere. This mesoderm is not uniform, but rather displays a dorso-ventral patterning that is conferred by the vegetal endoderm. The dorsal mesoderm of the gastrula will form the notochord and presomitic mesoderm while the ventral mesoderm will form the lateral plate mesoderm and the blood. Several TGF beta-like signaling molecules have been implicated to be involved in the formation and dorso-ventral patterning of the mesoderm (for review see Stennard *et al.*, 1997; Hogan, 1996). Activin-like signals play a critical role in the formation of the dorsal mesoderm, while BMPs are important for the formation of the ventral mesoderm. Signals from TGF beta-like proteins are transduced to the nucleus by members of a family of intracellular proteins, called Smads (Massague, 1998), where they, in association with other proteins, activate the dorsal and ventral specific genes (Whitman, 1998). Fox proteins have an important role in this activation. They mediate TGF beta signals by directly interacting with Smads. For example, the Fox protein FAST-1 was shown to form a complex

Abbreviations used in this paper: BMP, Bone morphogenetic protein; TGF, Transforming growth factor.

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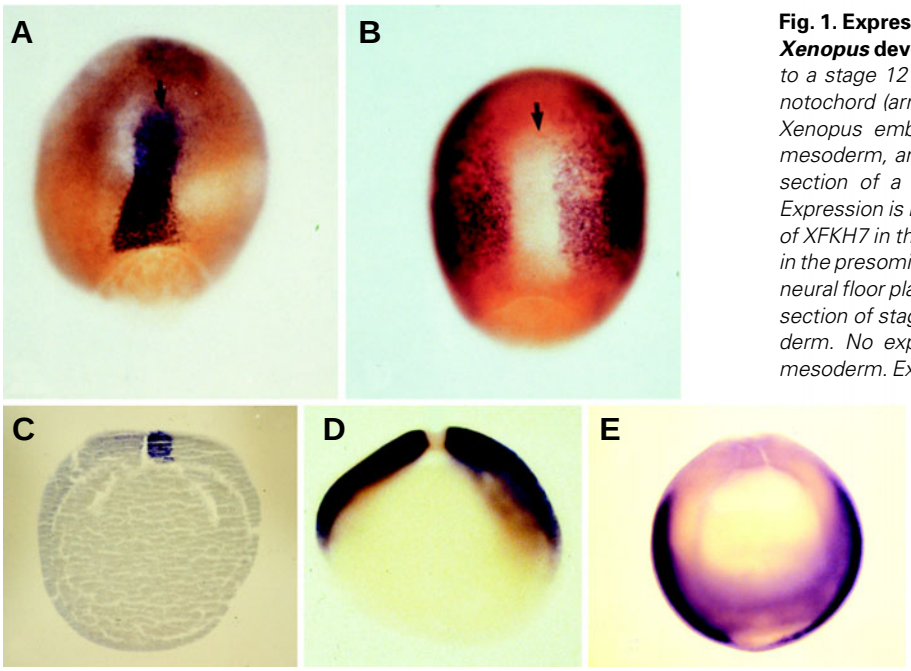

Fig. 1. Expression of three different forkhead genes during early *Xenopus* development. (A) *In situ hybridization of XFKH1(FoxA4b) to a stage 12 Xenopus embryo (dorsal view). Expression is in the notochord (arrow).* **(B)** *In situ hybridization of XFKH7 to a stage 12 Xenopus embryo (dorsal view). Expression is in the presomitic mesoderm, and is absent in the notochord (arrow).* **(C)** *Transverse section of a stage 15 embryo hybridized with XFKH1(FoxA4b). Expression is in the notochord and neural floor plate.* **(D)** *Expression of XFKH7 in the trunk section of the stage 14 embryo. Expression is in the presomitic mesoderm, but it is absent in the notochord and the neural floor plate.* **(E)** *Expression of the XFD-13'(FoxF1b) in the trunk section of stage 15 embryo. Expression is in the lateral plate mesoderm. No expression is visible in the notochord and presomitic mesoderm. Expression is also absent in the most ventral mesoderm.*

with Smad2 that activates transcription of the *Mix.2* homeobox gene (Watanabe and Whitman, 1999). FAST-2, another Fox protein, directly interacts with Smad2 and forms a multimeric complex on the activin response elements of activin response genes (Liu *et al*., 1999).

In addition to being mediators of signal transduction, some Fox genes are also the early targets of signaling molecules involved in the dorsoventral patterning of the mesoderm. We have shown previously that the forkhead gene *XFKH1(FoxA4b)* is preferentially expressed in the Spemann-Mangold organizer and later in the notochord (Dirksen and Jamrich, 1992). This gene is an immediate early target of activin induction and Xsmad2 is directly involved in activation of *XFKH1(FoxA4b)* via its intronic activin response element (Howell and Hill, 1997). In this paper we present two other Fox genes that are activated in *Xenopus* gastrula and might play an important role in the formation of the presomitic mesoderm and lateral plate mesoderm.

Results

XFKH1(FoxA4b)

A few years ago we isolated the *Xenopus* Fox gene, *XFKH1(FoxA4b),* and showed that this gene is likely to be involved in pattern formation of *Xenopus* embryos (Dirksen and Jamrich, 1992). Ruiz i Altaba and Jessel, (1992) and Knochel *et al*., (1992) isolated the "b" form of this gene (*Pintallavis* and *XFD1*, respectively). Both genes have a very similar expression pattern. *XFKH1(FoxA4b)* is first activated in the Spemann-Mangold organizer (Spemann and Mangold, 1924) and later is expressed in the dorsal mesoderm and the neural floor plate (Dirksen and Jamrich, 1992). In the dorsal mesoderm, *XFKH1(FoxA4b)* is expressed in the notochord, but not in the presomitic mesoderm (Fig. 1 A,C). This expression is of functional significance, since Ruiz i Altaba and Jessel, (1992) showed that the overexpression of *pintallavis* in *Xenopus* embryos leads to enlargement of the notochord and the neural floor plate. Overexpression of *HNF-3 beta*, the murine functional homologue of *XFKH1(FoxA4b),* in mice induces formation of ectopic neural floor plate (Sasaki and Hogan, 1994). Targeted elimination of *HNF-3 beta* results in embryos that do not form a distinct node and lack a notochord (Ang and Rossant, 1994; Weinstein *et al*., 1994). *XFKH1(FoxA4b)* is activated in *Xenopus* animal caps by activin even in the presence of cycloheximide. This shows *that XFKH1(FoxA4b)* is an immediate early response gene to activin induction (Dirksen and Jamrich, 1992).

XFKH7

XFKH7 is a novel *Xenopus* Fox gene that is also activated during gastrulation. In contrast to *XFKH1*, it is expressed in the presomitic mesoderm, but not in the notochord. *XFKH7* appears to be the orthologue of the murine Fox gene *Mfh1(Foxc2)* (Miura *et al*., 1993). Comparison of amino acid sequences of the *XFKH7 and Mfh1(Foxc2)* gene are presented in Fig. 2.

XFKH7 is first activated during early stages of gastrulation. By stage 12 its RNA can be easily visualized by whole mount *in situ* hybridization. Expression is in the presomitic mesoderm, and is absent in the notochord (Fig. 1B). During neurulation expression of *XFKH1(FoxA4b)* and *XFKH7* is mutually exclusive in the dorsal mesoderm. While *XFKH1(FoxA4b)* is expressed in the notochord and floor plate (Fig. 1C), expression of *XFKH7* is present in the presomitic mesoderm and remains excluded from the notochord and floor plate (Fig. 1D). As shown in the transverse section of stage 15 embryo (Fig. 3A), *XFKH7* is strongly and uniformly expressed throughout the entire somite forming mesoderm. A dorsal view of a stage 14 embryo shows that *XFKH7* expression is quite uniform along the antero-posterior axis (Fig. 3B). Only the most anterior and posterior regions are devoid of *XFKH7* transcripts. A few hours later, at stage 18, *XFKH7* displays an intricate expression pattern along the antero-posterior axis (Fig. 3C). A distinct repetitive pattern in the dorsal mesoderm can be observed. This pattern is indicative of differentiation of the presomitic mesoderm along the antero-posterior axis of the embryo. This antero-posterior differentiation becomes more pronounced by stage 25. A lateral view of a stage 25 embryo hybridized with *XFKH7* probe demonstrates the complex expression pattern of this gene at this stage (Fig. 3D). Strong expression is present in the head as well as in the trunk mesoderm. Sections though this

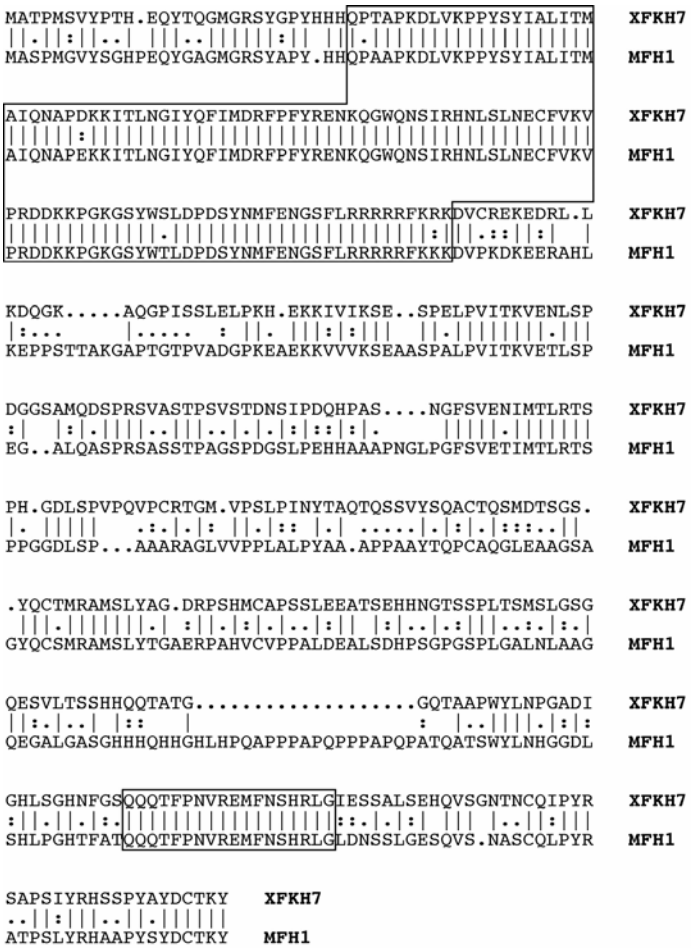

Fig. 2. **Comparison of amino acid sequences of the *XFKH7* and *Mfh1(Foxc2)* genes.** *Amino acids are in the single-letter IPAC code. Vertical bars indicate identities. The forkhead domain is in the upper box, the lower box indicates a highly conserved domain of unknown function.*

embryo demonstrate the correlation between the differentiation of the presomitic mesoderm and the expression of *XFKH7*. A transverse section through the anterior trunk region (Fig. 3E) of the embryo in figure 3D show strong reduction of the expression in the developing anterior somite. The dermatome and myotome are devoid of *XFKH7* expression; the only area of expression appears to be in the sclerotome. In the mid-trunk region (Fig. 3F) of the embryo, there is also a strong reduction of expression in the somites. Only the most ventral part of the dorsal mesoderm is expressing this gene. While we have not performed a double hybridization with markers of kidney development, some of this mesoderm expressing *XFKH7* in the anterior and mid-trunk region is likely to be nephrogenic mesoderm. In the posterior trunk region (Fig. 3G) the presomitic mesoderm is still undifferentiated and the expression of *XFKH7* is present across the entire presomitic mesoderm.

XFD-13'(FoxF1b)

XFD-13'(FoxF1b) is a Fox gene that appears to be the frog orthologue of the human forkhead gene *FREAC-1* (Clevidence *et al*., 1996; Hellquist *et al*., 1996; Larsson *et al*., 1995), murine and chick *HFH-8* (Peterson *et al*., 1997; Mahlapuu *et al*., 1998; Funayama *et al*., 1999) and is likely to be the same gene as *XFD-13'* by Koster *et al*. (1999). At the amino acid level, *XFD-13'(FoxF1b)* and *FREAC1* are 80% identical and they share almost complete conservation in the fork head domain with mouse and chick *HFH8* genes. *XFD-13'(FoxF1b)* is activated during gastrulation in the presumptive ventrolateral mesoderm. During neurulation, the strongest expression is in the lateral plate mesoderm. The most ventral mesoderm has either no or much less expression than the lateral plate mesoderm (Fig. 1C). From neural tube stages on, *XFD-13'(FoxF1b)* is increasingly expressed in the lateral plate mesoderm. The expression appears to progress from anterior to posterior and from dorsal to ventral (not shown). In tadpoles this gene is expressed in the lateral plate mesoderm, the ventral mesoderm and the migrating neural crest cells of the head (Fig. 4C). Most of the expression in the head is ventral to the eyes, in the branchial arches. Figure 4A shows expression of *XFD-13'(FoxF1b)* in the head of a stage 35 embryo. Figure 4B shows the expression of *XFD-13'(FoxF1b)* in the trunk of the same embryo. Expression is in the lateral plate mesoderm and ventral mesoderm. The circulatory system, including blood cells, blood and blood vessels, is derived from the lateral plate mesoderm and ventral mesoderm. However, *XFD-13'(FoxF1b)* is not expressed in the heart anlage, as is demonstrated by double *in situ* hybridization with *troponin*, a marker specific for heart development (Drysdale *et al*., 1994) and *XFD-13'(FoxF1b*). As shown in Fig. 4D, there is no overlap between the two signals. This pattern is very similar to the expression of *HFH-8* in mouse and confirms the expression analysis of *XFD-13* by Koster *et al*., (1999). BMP4 is the most potent activator of *XFD-13'(FoxF1b)* in animal caps, but activin and FGF also activate this gene to a lesser degree (not shown).

Discussion

The molecular analysis of pattern formation and lineage specification has experienced major growth during the last two decades. This was partially due to the discovery that many genes controlling pattern formation are members of multigene families. These gene families frequently contain conserved DNA-binding domains that can be used to isolate additional members of a family. Homeobox genes are probably the best known example of such a family, but Fox genes have received a lot of attention lately. Several Fox genes expressed during early *Xenopus* development are involved in development of the embryonic mesoderm.

Xenopus mesoderm that arises in the equatorial region of the embryo is not uniform, but rather displays a dorso-ventral patterning. The dorsal mesoderm of the gastrula will later form the notochord and presomitic mesoderm and the ventral mesoderm will form the lateral plate mesoderm and the blood. The dorso-ventral patterning is conferred by the vegetal endoderm and is mediated by TGF beta-like signaling molecules. Activin-like signals are believed to be important in formation of the dorsal mesoderm while BMPs are involved in formation of the ventral mesoderm. These TGF beta superfamily members activate heteromeric complexes of serine-threonine kinase receptors that are located on the plasma membrane. These receptors activate signal transducers, Smads, by phosphorylation (Derynck and Feng, 1997; Massague, 1998). Different TGF family members utilize different receptors and receptor-phosphorylated Smads. These Smads form complexes

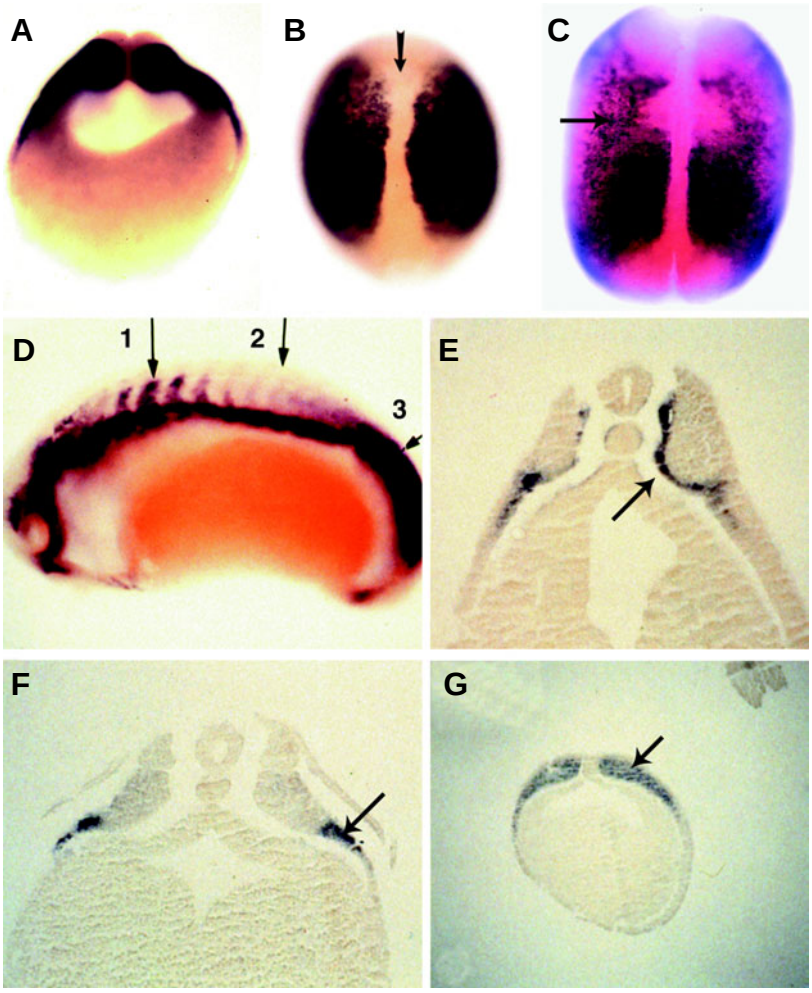

Fig. 3. **Expression of *XFKH7* during *Xenopus* development. (A)** *Transverse section of a stage 15 embryo showing a robust and uniform expression throughout the entire somite forming mesoderm.* **(B)** *A dorsal view of this stage 14 embryo demonstrates the expression of XFKH7 in the presomitic mesoderm and the absence of its expression in the notochord (arrow).* **(C)** *Whole mount in situ hybridization of a stage 18 embryo with the XFKH7 probe, demonstrates an intricate expression pattern of this gene at this stage. Although morphologically visible somites are not yet present, the expression of this gene clearly demonstrates a repetitive pattern of gene expression present in the presomitic mesoderm (arrow).* **(D)** *Whole mount in situ hybridization of a stage 25 embryo with XFKH7 (lateral view) demonstrating the complex expression pattern of this gene. Strong expression is present in the head mesoderm as well as in presomitic trunk mesoderm. Numbers with arrows indicate the position of transverse sections in Figs. 3E, 3F and 3G.* **(E)** *Transverse section through the anterior trunk region (1) of the embryo in Fig. 3D. This section demonstrates the strong reduction of the expression in this part of the embryo in the somites. The remaining expression appears to be in the sclerotome (arrow).* **(F)** *Cross section through the mid-trunk region (2) of the embryo in Fig. 3D displaying a strong reduction of the expression in the somites. Only the most ventral part of the dorsal mesoderm is expressing this gene (arrow).* **(G)** *Transverse section through the posterior trunk region (3) of the embryo in Fig. 3D. Presomitic mesoderm in the tail region is not yet differentiated and we can observe expression of XFKH7 in the entire presomitic mesoderm (arrow).*

with maternally expressed forkhead proteins such as FAST-1 and FAST-2 and activate transcription of tissue specific genes. Some forkhead genes are direct targets of this transcriptional activation, indicating that zygotically activated Fox genes might play a critical role in the dorso-ventral patterning of the embryo.

Three Fox genes, *XFKH1(FoxA4b), XFKH7* and *XFD-13'(FoxF1b),* are initially activated in early gastrula in a partially overlapping pattern that becomes later mutually exclusive. *XFKH1(FoxA4b)* and *XFKH7* are expressed in the dorsal mesoderm, *while XFD-13'(FoxF1b)* is expressed in the lateral and ventral mesoderm. A schematic diagram of expression of these three forkhead genes in *Xenopus* embryos is presented in Fig. 5. The diagram indicates the expression of these three genes after a mutually exclusive pattern has been established. The initial expression of these genes in gastrulae shows some overlap (not shown) that is eliminated only during neurulation. This expression pattern indicates the progressive nature of the establishment of the dorso-ventral patterning of the mesoderm.

XFKH1(FoxA4b) is initially expressed in the Spemann-Mangold organizer and later in the notochord and neural floor plate (Dirksen and Jamrich, 1992). Ruiz i Altaba and Jessel (1992) has shown that the overexpression of *pintallavis* (a "b" form of *XFKH1*) in *Xenopus* embryos leads to enlargement of the notochord and the neural floor plate. Overexpression of HNF-3 beta, the functional homologue of *XFKH1(FoxA4b)* in mice, induces formation of ectopic neural floor plate (Sasaki and Hogan, 1994). Targeted elimination of *HNF-3 beta* in mice results in embryos that do not form a distinct node and lack a notochord (Ang and Rossant, 1994; Weinstein *et al.*, 1994).

XFKH1(FoxA4b) is activated in *Xenopus* animal caps by activin even in the presence of cycloheximide, demonstrating that no protein synthesis is required for the activation of this gene (Dirksen and Jamrich, 1992). Signal transduction from activin through its receptor involves phosphorylation of Smad2 that, in turn, heterodimerizes with Smad4. This heterodimeric DNA binding complex binds the maternally expressed forkhead protein FAST-1 and activates *XFKH1(FoxA4b)* via its intronic activin response element (Howell and Hill, 1997; Watanabe and Whitman, 1999). Expression pattern and functional characteristics of this gene show that *XFKH1(FoxA4b)* plays an important role in the formation of the notochord.

XFKH7, a Fox gene that appears to be the orthologue of the murine forkhead gene *Mfh1(Foxc2)* (Miura *et al.*, 1993), is first activated during late blastula/early gastrula stage of *Xenopus* embryos. Expression is in the in the paraxial mesoderm, and is absent in the notochord. During neurulation *XFKH1(FoxA4b)* and *XFKH7* expression is mutually exclusive in the dorsal mesoderm. While *XFKH1(FoxA4b)* is expressed in the notochord and floor plate, *XFKH7* is expressed in the presomitic mesoderm and is absent from the notochord and the neural floor plate. During neurulation *XFKH7* expression is robust and uniform throughout the entire somite. Later in development *XFKH7* displays an intricate expression pattern along the antero-posterior axis that coincides with differentiation of somites into a dermatome, myotome and sclerotome. Since the somites are in different stages of differentiation along the anteroposterior axis, the expression of *XFKH7* is different when viewed in cross sections. In the tail region, where somites are still

undifferentiated, the *XFKH7* is expressed in the entire presomitic mesoderm. In the anterior somites, *XFKH7* is expressed only in the sclerotome and is absent from the dermatome and myotome. It appears that the maximal expression of *XFKH7* correlates with undifferentiated state of presomitic mesoderm. This is interesting since several forkhead genes have been shown to have anti-differentiation properties (Xu *et al.*, 1998; Mariani and Harland, 1998; Bourguignon *et al.*, 1998; Kenyon *et al.*, 1999). In addition to the somites strong expression is present in the head. This expression is particularly prominent in the head mesoderm surrounding the eyes.

The early expression pattern of *XFKH7* in *Xenopus* is similar to that of *Mfh1(Foxc2)* in mouse. *Mfh1(Foxc2)* is also expressed in the paraxial mesoderm, is absent in the notochord and shows significant modulation of expression during differentiation of somites (Winnier *et al.*, 1997; Hiemish *et al.*, 1998; Furumoto *et al.*, 1999). The targeted elimination of *Mfh1(Foxc2)* leads to multiple craniofacial and vertebrate column defects. Most importantly, there is a marked reduction of proliferation of sclerotome-derived cells (Winnier *et al.*, 1997; Iida *et al.*, 1997). In addition these animals display aberrant ocular development and renal development (Smith *et al.*, 2000; Kume *et al.*, 2000). These defects are in good agreement with the expression of these gene in nephrogenic mesenchyme and mesenchyme surrounding the eyes. The expression pattern and functional characteristics of this gene show that *XFKH7* plays an important role in the formation and differentiation of the paraxial mesoderm. *XFKH7*, like *XFKH1(FoxA4b),* is activated in animal caps by activin (our unpublished observation). However the exact activation pathway of this gene has not yet been established.

XFD-13′(FoxF1b) is a Fox gene activated during gastrulation in the lateral and ventral mesoderm. *XFD-13′(FoxF1b)* appears to be the frog orthologue of the human forkhead gene *FREAC-1* (Clevidence *et al.*, 1996; Hellquist *et al.*, 1996) and the murine *HFH-8* (Peterson *et al.*, 1997; Mahlapuu *et al.*, 1998) and is likely to be the same gene as *XFD-13′* by Koster *et al.* (1999). At the amino acid level, *XFD-13′(FoxF1b)* and *FREAC1* are 80% identical and they share almost complete conservation in the fork head domain with mouse and chick *HFH8* genes.

During neurulation, the strongest expression of *XFD-13′(FoxF1b)* is in the lateral plate mesoderm. The most ventral mesoderm has either no expression or much less than the lateral plate mesoderm. In tadpoles, this gene is expressed in the lateral plate mesoderm and in the migrating neural crest cells of the head. Most of the expression in the head is ventral to the eyes, in the branchial arches. In addition, at this stage *XFD-13′(FoxF1b)* transcription is also detectable in ventral mesoderm. The circulatory system including blood cells, blood and blood vessels are derived from the lateral plate and ventral mesoderm. However, *XFD-13′(FoxF1b)* is not expressed in the heart anlage, as is demonstrated by double *in situ* hybridization with *XFD-13′(FoxF1b)* and *troponin*, a marker specific for heart development (Drysdale *et al.*, 1994). By PCR, *XFD-13′(FoxF1b)* is expressed in the adult lung and intestine (unpublished observation). This pattern is very similar to the expression of *HFH-8* in mouse (Peterson *et al.*, 1997) and confirms the expression analysis of the *XFD-13* by Koster *et al.*, (1999). *HFH-8* expression is restricted to mesenchyme derived from the lateral mesoderm. *HFH-8* is expressed in the splachnic layer of the lateral plate mesoderm that contributes to the mesodermal components of the visceral organs. It is not expressed in the developing heart. By Northern blot analysis *HFH-8* is expressed in the adult lung and intestine. Its human orthologue, *FREAC-1*, can act as a transcriptional activator that binds to several lung-specific genes (Hellquist *et al.*, 1996; Clevidence *et al.*, 1994).

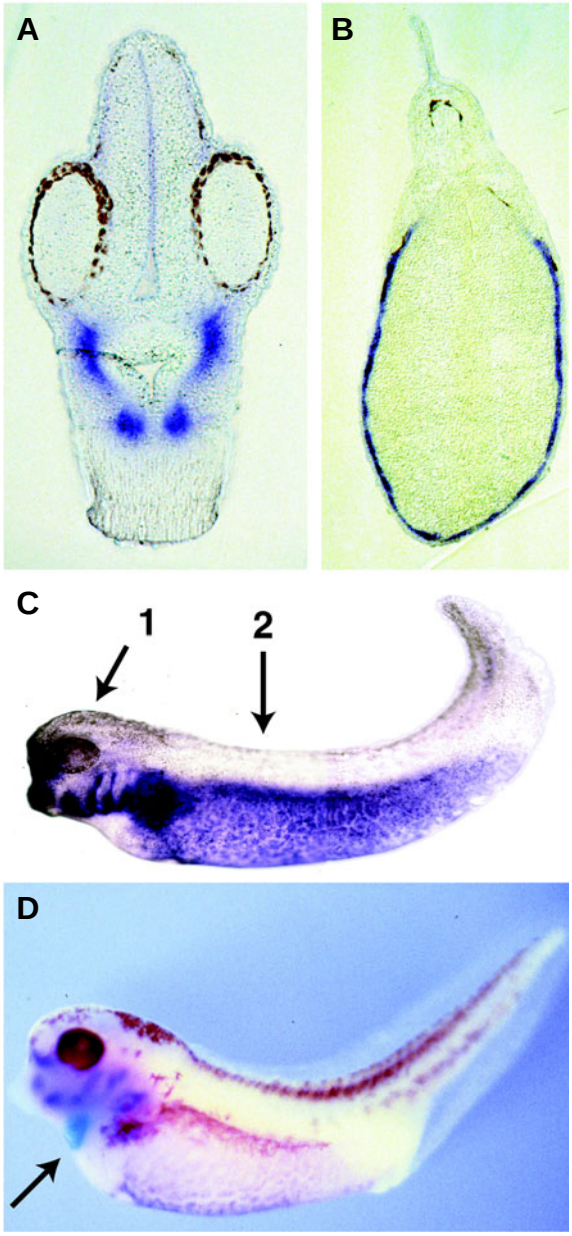

Fig. 4. Expression of *XFD-13′(FoxF1b)* during *Xenopus* development. **(A)** *Transverse section through the head of a stage 35 embryo hybridized with an XFD-13′(FoxF1b) probe. Expression is in the neural crest cells. The plane of section is indicated in the Fig. 4C (number 1).* **(B)** *Transverse section through the trunk of a stage 35 embryo hybridized with an XFD-13′(FoxF1b) probe. Expression is in the lateral and ventral regions of the embryo. The plain of section is indicated in the Fig. 4C (number 2).* **(C)** *Whole mount in situ hybridization of a stage 35 embryo with the XFD-13′(FoxF1b) probe. Numbers indicate the plane of section in Figs. 4 A,B.* **(D)** *Whole mount in situ hybridization of a stage 40 embryo with XFD-13′(FoxF1b) and troponin probes. Light pink staining indicates XFD-13′(FoxF1b) expression, whereas the blue staining indicates troponin expression in the heart. An arrow indicates this area of expression. There is no XFD-13′(FoxF1b) expression in the heart.*

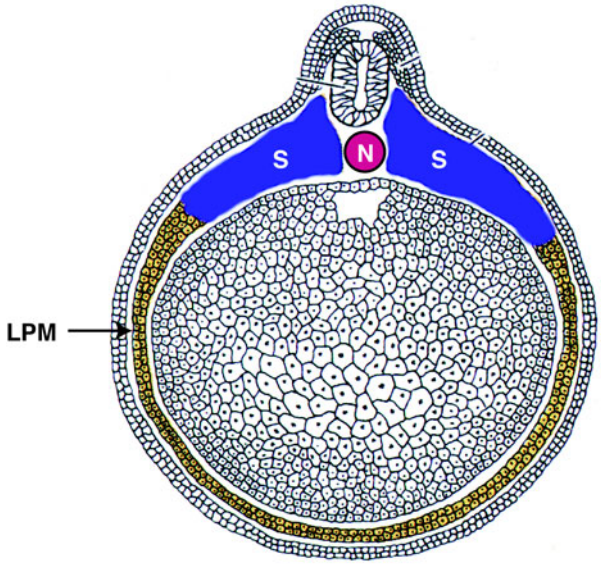

Fig. 5. Schematic diagram of the expression of three forkhead genes in *Xenopus* embryo. *XFKH1(FoxA4b) is expressed in the notochord (N-red); XFKH7 is expressed in the presomitic mesoderm (S-blue) and XFD-13'(FoxF1b) is expressed in the lateral plate mesoderm and ventral mesoderm (LPM-yellow).*

The expression of *XFD-13'(FoxF1b)* in the lateral plate mesoderm is dynamic. During organogenesis, the lateral plate mesoderm splits into the dorsal somatic (parietal) mesoderm, which underlies the ectoderm, and the ventral splachnic (visceral) mesoderm, which overlies the endoderm. The separation of these two mesodermal sheets forms the coelom or body cavity. Recently, Funayama *et al.*, (1999) have shown that the process of lateral plate separation in chicken embryos occurs in an anterior to posterior direction. This progression is very similar to expression of *XFD-13'(FoxF1b)* in the lateral plate mesoderm. In addition, Funayama *et al.*, (1999), showed that chick *HFH-8* expression becomes restricted to the splachnic mesoderm and is not found in the somatic mesoderm. Based on this information, it is likely that the spatial progression of *XFD-13'(FoxF1b)* expression in the lateral plate mesoderm corresponds to the temporal differentiation of lateral plate separation into two layers. However, our data can not confirm this since it is difficult to distinguish the two layers in *Xenopus* without molecular markers. BMP4 is the most potent activator of *XFD-13'(FoxF1b)* in animal caps, but activin and FGF also activate this gene to a lesser degree (not shown). We are currently investigating the signaling pathway leading to the activation of this gene.

It is possible that *XFKH1*, *XFKH7* and *XFD-13'(FoxF1b)* were initially expressed in the entire mesodermal layer of vertebrate embryos and only later became specialized in the mediation of dorsoventral patterning of the mesoderm. Study of expression of their orthologues in species with a simpler organization of mesoderm might provide additional information about the correctness of this hypothesis.

Materials and Methods

XFKH7 and *XFD-13'(FoxF1b)* were isolated from a stage 32 head cDNA library using PCR and degenerate forkhead primers. *In situ* hybridization were performed as described by Harland (1991). Developmental stages of embryos were determined according to Nieuwkoop and Faber (1967).

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