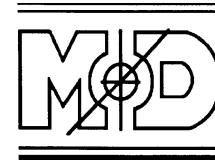




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Gene expression pattern

Cloning and expression pattern of a zebrafish homolog of forkhead activin signal transducer (FAST), a transcription factor mediating Nodal-related signals

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Abstract

Forkhead activin signal transducer (FAST) is a member of the winged-helix family of DNA-binding proteins that has been implicated in mesoderm induction and left-right axis specification during embryonic development in *Xenopus* and mouse. We have cloned and characterized a zebrafish FAST homolog. Zebrafish *fast* is expressed maternally and zygotically. Transcripts start regionalizing and decline in level during gastrulation. During somitogenesis, *fast* is expressed bilaterally in the lateral plate mesoderm, like its mouse homolog. In addition, zebrafish *fast* is also expressed bilaterally in the dorsal diencephalon, where the nodal-related *cyclops* gene is only expressed on the left side. It remains to be demonstrated whether FAST expression in the brain can mediate Nodal-induced asymmetric development. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Forkhead activin signal transducer; Maternal genes; Activin; Nodal; *cyc*; *oep*; Left–right asymmetry; Brain; Epithalamus; Habenula; Winged helix; Forkhead domain; Smad

1. Results and discussion

The TGF- β family of secreted polypeptides, including the Activins and Nodals, act on target cells by modifying the expression of key target genes. The transcription factor FAST associates with Smad2 and Smad4 proteins, which are activated by activin/nodal-related receptors upon ligand binding and translocate into the nucleus. The FAST/Smad complex binds to activin/nodal responsive elements (ARE/NRE) and activates homeobox genes implicated in mesoderm induction in both *Xenopus* and mouse (reviewed in Massagué and Wotton, 2000).

We cloned a zebrafish cDNA with high homology to *Xenopus* FAST-1 (Chen et al. 1996). By 5'-RACE, we obtained the complete coding region (GenBank AF264751). The overall zebrafish and *Xenopus* sequences are slightly more related to each other than to the mouse

(Weisberg et al. 1998) and human (Zhou et al. 1998) counterparts (Fig. 1). The similarity is higher in the winged-helix and Smad interaction domains. The zebrafish protein activates transcription of an activin responsive reporter in transfection assays (Mario Minuzzo and Monica Beltrame unpublished), like its *Xenopus* and mammalian homologs (Weisberg et al. 1998; Yeo et al. 1999).

Zebrafish *fast* is expressed maternally, and the transcript level drops during gastrulation (Fig. 2), as in *Xenopus* and mouse (Chen et al. 1996; Weisberg et al. 1998). *fast* mRNA is ubiquitous in pre-gastrulating embryos (Fig. 3A,B). During gastrulation it starts regionalizing: most is in the ventral portion of the embryo, but some accumulation at the presumptive shield (the zebrafish organizer) appears around 50% epiboly (Fig. 3C,D). While all cells are stained on the ventral side, only the external shield cells are marked in an equatorial section (not shown). Zebrafish fate maps show that dorsal shield cells involute to form the notochord and the mesendoderm of the prechordal plate (Shih and Fraser, 1995). At later gastrulation stages, the dorsal signal

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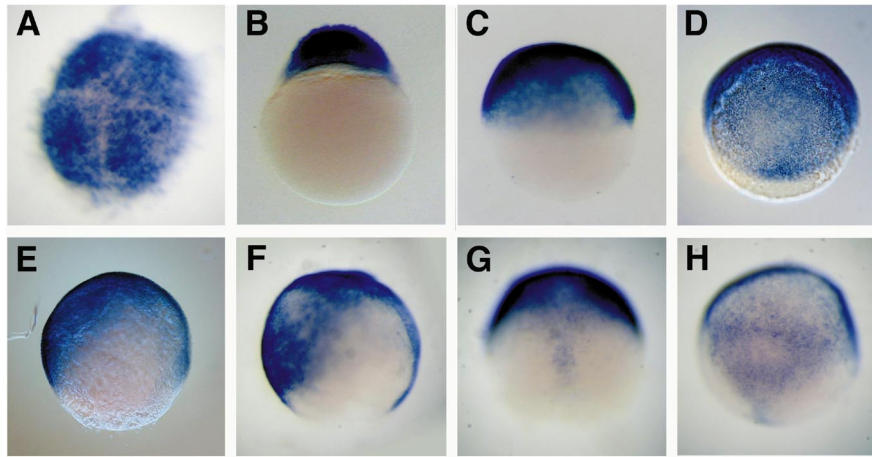


Fig. 3. Localization of zebrafish FAST transcripts in early developmental stages. The mRNAs are initially ubiquitously expressed ((A) 4-cells stage; (B) blastula). At 50% epiboly ((C) lateral view; (D) dorsal view) most of the signal is ventral, but the presumptive shield is also marked. As gastrulation proceeds, the signal is more regionalized and unstained regions are more pronounced. (E) Lateral view around 70% epiboly. (F–H) Lateral (F), dorsal (G), and animal pole views (H) of a single embryo around 80% epiboly. A signal along the developing AP axis (G) and two lateral ones are emerging; a faint triangular shape staining is evident (H). In all lateral views, dorsal is to the right.

expression patterns similar to *fast*. During somitogenesis, *oep* is expressed in the notochord, in two lateral stripes and in a dorsal diencephalic region that includes the anlage of the epiphysis (Zhang et al. 1998). *cyc* and *lft1* are reported to be expressed unilaterally in the vicinity of the habenula

(Sampath et al. 1998; Bisgrove et al. 1999), a diencephalic structure with marked left-right size asymmetry in many vertebrates. An isoform of *pitx2*, a *bicoid*-homeoprotein involved in the regulatory network including *cyc* and *oep*, is also expressed asymmetrically in the left dorsal diencephalon (Essner et al. 2000). We interpret the three rostral FAST signals as corresponding to the area of the developing habenulae. Zebrafish FAST appears to be expressed bilaterally in the same cephalic area where *cyc* is unilaterally expressed, in strong analogy with what happens with *Fast2* and *nodal* in mouse LPM.

Zebrafish is the first organism in which FAST expression has been described in the brain, in a region with strong left-right asymmetry.

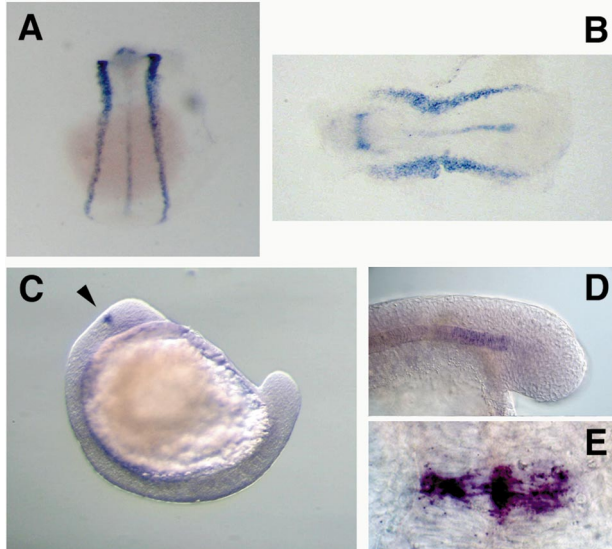


Fig. 4. Localization of zebrafish FAST transcripts during somitogenesis. (A,B) at tailbud-early somites transition a midline signal and two lateral stripes are visible along the AP axis; a dorsal rostral band is also evident. (A) Dorsal view, anterior to the top. (B) Flat-mount embryo, anterior to the left. (C–E) 16 hpf. The overall signal is reduced; the arrowhead in (C) (lateral view) points to the dorsal diencephalic signal, in the region of the future epithalamus, which is blown up in (E) (flat-mount embryo, anterior to the top). The signal comprises three intensely stained areas: two lateral ones of unequal size and a midline one that is extending deeper into the embryo, as revealed by transverse sections (data not shown). (D) Detailed lateral view of the tail region, showing that the final portion of the notochord is stained.

2. Methods

2.1. cDNA cloning

A cDNA library from zebrafish gastrulating embryos was generated in pB42AD for yeast two-hybrid screening. Six clones corresponding to the C-terminal two-thirds of a putative zebrafish FAST homolog (1.8 kb) were isolated from four library equivalents. We performed 5' RACE with the oligonucleotide 5'-GGAATTCGGTTCACCTCCACAGTCC-3'.

2.2. In situ hybridization

Whole-mount in situ hybridization (Thisse et al. 1993) was done with an antisense digoxigenin-labelled probe transcribed with T3 RNA polymerase from an *NsiI*-cut pBlue-script-KS+ subclone containing nt 533–2177 of the cDNA.

Note added in proof

While our work was in progress the groups of D. Meyer and W. Talbot identified *schmalspur* as the gene encoding zebrafish FAST. Their work is published in Current Biology, Vol. 10, pp. 1041–1049 and 1051–1054, respectively.

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