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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.

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activates transcription of an activin responsive reporter in transfection assays (Mario Minuzzo and Monica Beltrame unpublished), like its *Xenopus* and mammalian homologs (Weisberg et al. 1998; Yeo et al. 1999).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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z  .......... .......... MTKHWGGPGL LAPPVITVGE GAQRDHHLDC RIGYSSSKRS CHRSSNPLLE LGGRLDKSTG MAQDSCYRAK ATNQGPWELQ DGNSSGGKKK NYQRYPKPPY  100
x  MRDPSSLYSG FPAGSQYESV EPPSLALLSS IDQEQLPVAT GQSYNHSVQP WPQPWPPLSL YREGGTWSPD RGSMYGLSPG THEGSCTHTH EGPKDSMAGD HTRSRKSKKK NYHRYYKPPY  120
m  .......... .......... .......... .......... .......... ...MASGWDL ASTYTPTTPS PQLALAPAQG YLPCMGPRDN SQLRPPEAES LSKTPKRRKK RYLRHDKPPY   67
h  .......... .......... .......... .......... .......... .......... .......... ....MGPCSG SRLGPPEAES PSQPPKRRKK RYLRHDKPPY   36
                                                                                                                    --------------

z  SYLAMIAMVI QNSPEKKLTL SEILKEISTL FPFFKGNYKG WRDSVRHNLS SYDCFVKVLK DPGKPQGKGN FWTVEVNRIP LELLKRQNTA VSR.....QD ETIFAQDLAP YIFQGYSQPN  215
x  SYLAMIALVI QNSPEKRLKL SQILKEVSTL FPFFNGDYMG WKDSIRHNLS SSDCFKKILK DPGKPQAKGN FWTVDVSRIP LDAMKLQNTA LTR.....GG SDYFVQDLAP YILHNYKYEH  235
m  TYLAMIALVI QAAPFRRLKL AQIIRQVQAV FPFFRDDYEG WKDSIRHNLS SNRCFHKVPK DPAKPQAKGN FWAVDVSLIP AEALRLQNTA LCRRWQNRGA HRAFAKDLSP YVLHG.....  182
h  TYLAMIALVI QAAPSRRLKL AQIIRQVQAV FPFFREDYEG WKDSIRHNLS SNRCFRKVPK DPAKPQAKGN FWAVDVSLIP AEALRLQNTA LCRRWQNGGA RGAFAKDLGP YVLHG.....  151
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z  KSKPLPPESS LPPVPTRHSP PPSEDPYR.. ..PKLDSTFA IDSLLHSLR. .......... PASSAGEGLR ERESWGVGPP .....PHTRS TTPPRPCNAS YNGSSSASSV SPAS......  309
x  NAGAYGHQ.. MPPSHAR.SL SLAEDSQQTN TGGKLNTSFM IDSLLHDLQE VDLPDASRNL ENQRISPAVA MNNMWSSAPL LYTHSKPTRN ARSPGLSTIH STYSSSSSSI STISPVGFQK  352
m  ..QPYQPPSP PPPPRE.... .......... .......GFS IKSLL..... .......... ......GDPG KESTWPQHPG LPGQNTAAQA GTLSKGEERM GTGPSSSSET PLWPLCSLPG  258
h  ..RPYRPPSP PPPPSE.... .......... .......GFS IKSLL..... .......... ......GGSG EGAPW...PG LAPQSSPVPA GTGNSGEEAV PTPPLPSSER PLWPLCPLPG  224

z  ..DFSDEDWR RVTVVGKRSG DRGITSDAYS DSCP...PPN KSSKRGNTPP WELPTSYAKY TPPNAVAPPS MRFNGNPFMP LGGIPFYGYG SAHVTTSHLI GHPYWPILPS GPVS...IQA  421
x  EQEKSGRQTQ RVGHPIKRSR EDDDCSTTSS DPDTGNYSPI EPPKKMPLLS LDLPTSYTKS VAPNVVAPPS V.......LP FFHFPRFTY. .YNYGPSPYM TPPYWGFPHP TNSGGDSPRG  463
m  PTIIEGESSQ GEVIRPSPVT PDQGSWPLHL LEDSADSRGV PRRGSRASLW GQLPTSYLPI YTPNVVMPLA T.......IP TTCCPQCPF. ........SA SPAYWSVGTE SQGS......  356
h  PTRVEGETVQ GGAIGPSTLS PEPRAWPLHL LQGTAVPGGR SSGGHRASLW GQLPTSYLPI YTPNVVMPLA P........P PTSCPQCP.. ........ST SPAYWGVAPE TRGP......  320

z  PPLLMDLDSM LQSVPPNKSV FDALGSNN.Q TVHPSPNQYA LQNGPSLCKY SL     472
x  PQSPLDLDNM LRAMPPNKSV FDVLTSHPGD LVHPSFLSQC LGSSGSPYPS RQGLM  518
m  QDLLCDLDSF FQGVPPNKSI YDVWVSHPRD LAAPAPGWLL SWYSM            401
h  PGLLCDLDAL FQGVPPNKSI YDVWVSHPRD LAAPGPGWLL SWCSL            365
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Fig. 1. Alignment of FAST protein sequences from zebrafish (z), *Xenopus* (x), mouse (m), and human (h). Amino acid sequences were derived from GenBank nucleotide sequences under accession numbers AF264751, U70980, AF177770, NM_003923, respectively, and aligned using the PileUp and eclustalw programs from the UWGCG Package. The numbering on the right refers to the last amino acid shown in each line. Dots mark spacings inserted to maximize alignment. Identical residues in all four sequences are marked in red; overall identity is 41% between zFAST and xFAST, 35–36% between xFAST or zFAST and hFAST or mFAST, 75% between hFAST and mFAST. The two known functional domains of FAST proteins, the winged helix and the Smad interacting motif (SIM) (aa 88–199 and 432–449 of zFAST, respectively, underlined in the figure), are highly conserved in the zebrafish protein. As for the SIM (Germain et al. 2000), the final P present in x, m, h appears to be substituted by a double N in the zebrafish sequence, that can be structurally equivalent in breaking an α-helix. In the forkhead domain region, the zebrafish protein shows a higher degree of similarity to the *Xenopus* than to the mammalian counterparts (75 vs. 54% identity).

extends along the antero-posterior (AP) axis (Fig. 3F–H); the staining is far from uniform: a faint triangular shape is evident. Around tailbud stage (Fig. 4A,B) the staining appears as a midline deeper signal and two lateral stripes,

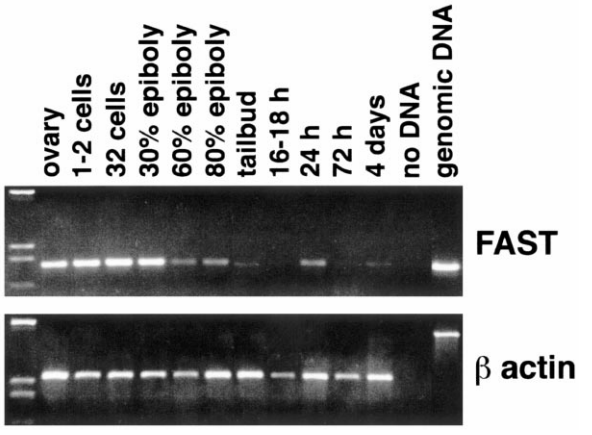

Fig. 2. Developmental expression of zebrafish FAST. Total RNAs were treated with DNaseI, retrotranscribed using an oligo-dT primer, and PCR amplified (35 cycles). FAST transcripts are present in the ovary and in embryos before zygotic transcription. The transcripts level is reduced during gastrulation and the amplified fragment is barely detectable at later developmental stages. Equal aliquots of cDNA were amplified with oligonucleotides specific for FAST (upper panel; 349 bp product; primers 5′-TCAGATGCATACAGTGACTCC-3′ and 5′-TTGGATCCAAGCGC-ATCAAAC-3′) or β-actin (lower panel; primers 5′-TGTTTTCCCCT-CCATTGTTGG-3′ and 5′-TTCTCCTTGATGTCACGGAC-3′). The β-actin fragment amplified from cDNA (560 bp) differs in size from that amplified from a genomic DNA control (last lane), due to the presence of an intron. Size of marker bands (shown on the left): 1.4, 0.5, 0.4, 0.2 kb, from top to bottom.

which are larger and extend more rostrally. A dorsal diencephalic band is visible, with two intensely stained spots at the sides of the midline. During somitogenesis, the signals along the AP axis retract. At 14–16 h post fertilization (hpf), the midline signal is confined to the tail, where the notochord is clearly stained (Fig. 4D). The two lateral stripes become thinner and do not extend any more along the entire axis but are limited to the trunk region. A fainter signal marks bilaterally the lateral plate mesoderm (LPM). The rostral band is now composed of two lateral plus one midline areas and is located dorsally (Fig. 4C,E). After 24 hpf, staining becomes undetectable.

Mouse FAST2 is implicated in the signalling pathway that induces the left-right asymmetric expression of *lefty2* and *nodal* in the LPM (Saijoh et al. 2000). *Fast2* is bilaterally expressed when *nodal* and *lefty2* are expressed on the left side. Nodal, in the presence of an EGF-CFC protein, can activate the binding of FAST2 to a left side-specific enhancer controlling the asymmetric expression of both *nodal* itself and *lefty2*. Although the complete left-right signalling pathway remains to be solved in zebrafish, counterparts of the mammalian players have been identified: two nodal-related molecules are encoded by *cyclops* (*cyc*) and *squint* (*sqt*) (Rebagliati et al. 1998; Feldman et al. 1998; Sampath et al. 1998), *antivin/lefty1* (*lft1*) is a *lefty* homolog (Thisse and Thisse, 1999; Bisgrove et al. 1999; Meno et al., 1999), *one-eyed pinhead* (*oep*) encodes an EGF-CFC Nodal coreceptor (Gritsman et al. 1999). The zebrafish *smad*s have recently been described (Dick et al. 2000) and we now report the cloning of zebrafish FAST.

Interestingly, several of the above-mentioned genes show

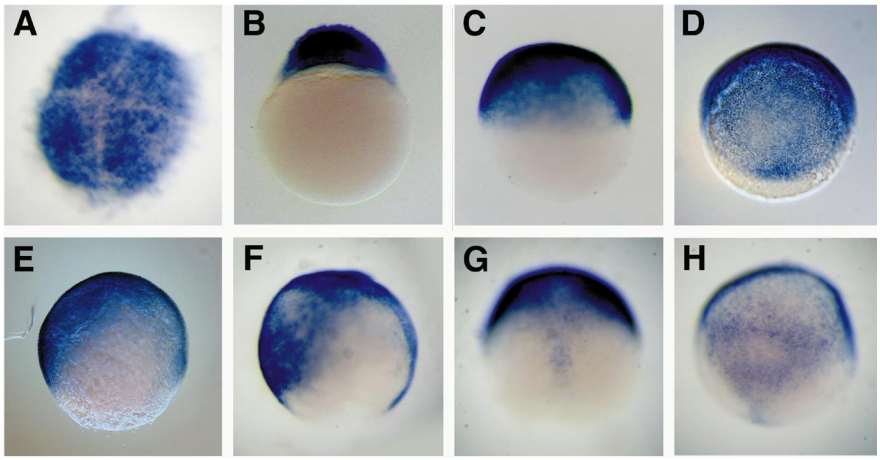

Fig. 3. Localization of zebrafish FAST transcripts in early developmental stages. The mRNAs are initially ubiquitously expressed ((A) 4-cells stage; (B) blastula). At 50% epiboly ((C) lateral view; (D) dorsal view) most of the signal is ventral, but the presumptive shield is also marked. As gastrulation 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

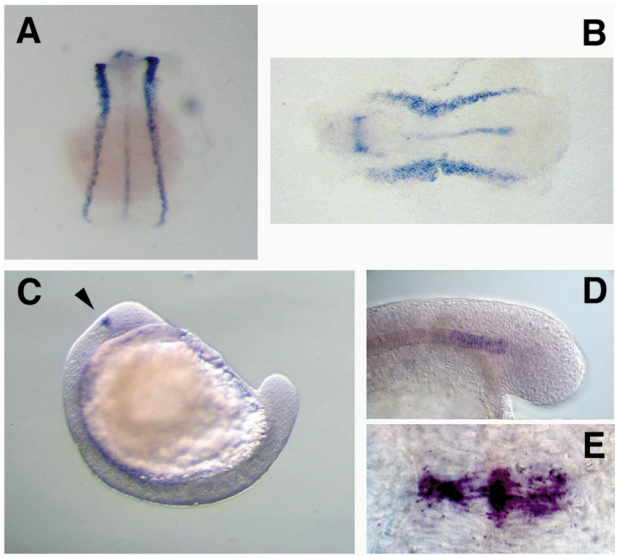

Fig. 4. Localization of zebrafish FAST transcripts during somitogenesis. (A,B) at tailbud-early somites transition a midline signal and two lateral stripes are visible along the AP axis; a dorsal rostral band is also evident. (A) Dorsal view, anterior to the top. (B) Flat-mount embryo, anterior to the 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.

(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.

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′-GGAATTCGGTTCACTTCCACAGTCC-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 *Nsi*I-cut pBluescript-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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