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Developmental Expression Pattern

Identification and developmental expression of *Xenopus paraxis*

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ABSTRACT During vertebrate embryogenesis, the paraxial mesoderm becomes segmented in a rostro-caudal progression and gives rise to the somites. In this paper we report the isolation of a *Xenopus* orthologue of *paraxis*, a member of a family of basic helix-loop-helix proteins, which has been suggested to play a role in paraxial mesoderm development. *Xenopus paraxis* is initially expressed in the presomitic paraxial mesoderm and later in the dorsal portion of the developing somites. Finally, *paraxis* expression becomes restricted to the most dorso-lateral region of mature somites.

KEY WORDS: *paraxis, bHLH, somite, paraxial mesoderm, dermatome*

The metameric organization of the vertebrate body is first revealed during embryogenesis by the segmentation of the presomitic mesoderm into somites. In all vertebrates, segmentation proceeds in a rostral-to-caudal wave and gives rise to somites which bilaterally flank the notochord and neural tube. Mature somites are differentiated into three compartments: dermatome, myotome and sclerotome, which form the dermis, trunk musculature and axial skeleton of the adult structures. Although the initial and completed states of somitogenesis are similar in all of the vertebrates, the morphogenetic processes vary from one species to another. In *Xenopus*, blocks of mediolaterally elongated cells form somites by simultaneously rotating 90 degrees to lie with their long axes parallel to the anterior-posterior axis (Hamilton, 1969; Youn and Malacinski, 1981). In addition, the small dermatomal and sclerotomal cell population are so inconspicuous that little is known about the patterning of the somites into dermatome, myotome and sclerotome (Keller, 2000). Therefore, one of the major challenges in this experimental system is to develop molecular markers for major components of the developing somite.

paraxis is a basic helix-loop-helix (bHLH) transcription factor expressed in paraxial mesoderm and later localized in the dermatome compartment of the somites, first cloned in mouse and chick (Burgess *et al.*, 1995; Barnes *et al.*, 1997). In the absence of *paraxis* function, the axial skeleton and skeletal muscles form, but are not correctly patterned (Burgess *et al.*, 1996). *paraxis* is nearly identical to another bHLH gene, *scleraxis* (Cserjesi *et al.*, 1995; Brown *et al.*, 1999), within the bHLH region and shares 84% nucleotide identity in the entire coding region in mouse. As the somites compartmentalize in the developing mouse embryo, *paraxis* expression is maintained in the dermatome and sclerotome. At this stage, *paraxis* and *scleraxis* are co-expressed in the sclerotome, but *paraxis* expression declines after sclerotome formation whereas *scleraxis* expression increases (Burgess *et al.*, 1995). Since *paraxis* and *scleraxis* are structurally highly related, we used the mouse *scleraxis* cDNA to screen for both genes in a *Xenopus* genomic library. This screening resulted in the isolation of the *Xenopus* orthologue of *paraxis*, which is the topic of this paper. We found no evidence that a *scleraxis* orthologue is present in the *Xenopus* genome.

Results and Discussion

Identification of a Xenopus paraxis *orthologue*

Screening a *Xenopus* genomic library with the mouse full-length *scleraxis* led to isolation of five positive clones. Sequence analysis of these clones revealed that these clones encode the *Xenopus* orthologue of *paraxis*. *Xenopus paraxis* contains an open reading frame of 579 nucleotides encoding a protein predicted to be 193 amino acids.

Comparison of the deduced amino acid sequence of *Xenopus paraxis* with chick (Barnes *et al.*, 1997), zebrafish (Shanmugalingam and Wilson, 1998), mouse (Burgess *et al.*, 1995) and human

Abbreviations used in this paper: bHLH, basic helix-loop-helix.

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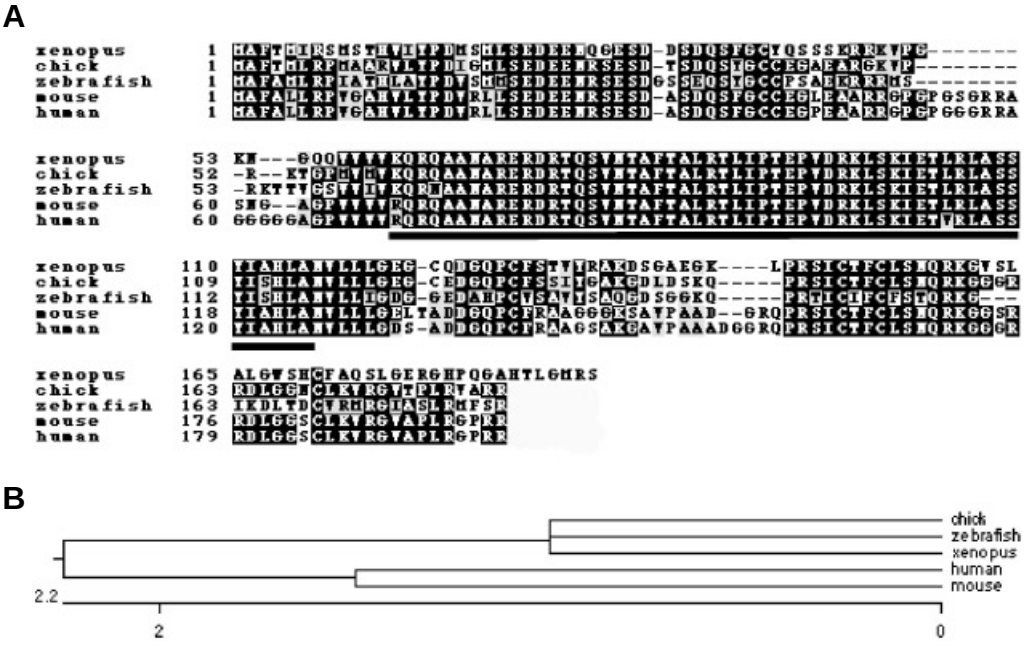
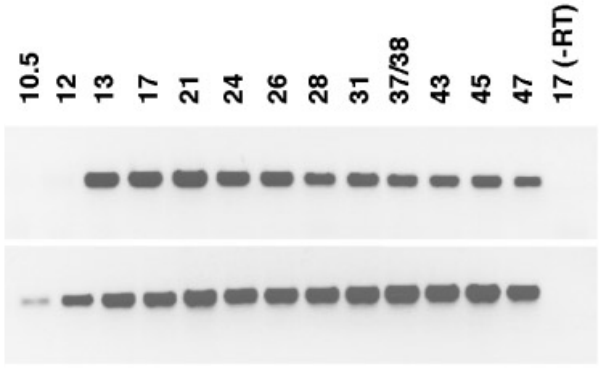


Fig. 1. Analysis of paraxis protein sequences. (A) *Amino acid sequence comparison between* Xenopus *paraxis and the orthologues in other species. Identical amino acids are shaded black and conserved changes are shaded gray. The absence of residues at the corresponding region is indicated by dashes. The basic helix-loop-helix domain is underlined.* **(B)** *Phylogenetic tree for* paraxis *in* Xenopus*, zebrafish, chick, mouse and human prepared using the DNAstar program using the J. Hein method with PAM250 matrix (Hein, 1990). The scale shown is the divergence calculated in the distance matrix.*

(Quertermous *et al.*, 1994) Paraxis protein reveals 77, 69, 68, 66% sequence identity, respectively (Fig. 1A). Alignment of all currently available Paraxis sequences from different species shows that the basic helix-loop-helix region is highly conserved and that the carboxy-terminus is most divergent across species (Fig. 1A). A phylogenetic tree based on amino acid comparisons within the bHLH region indicates that the Paraxis orthologue of chick, zebrafish and *Xenopus* form a group distinct from mammalian orthologues of Paraxis (Fig. 1B). In this subgroup, *scleraxis* was only found in chick (Schweitzer *et al.*, 2001), implying a differential regulation of somite development between lower and higher vertebrates (Morin-Kensicki and Eisen, 1997; Keller, 2000).

Temporal expression of Xenopus paraxis

The temporal expression of *paraxis* was analyzed by RT-PCR using total RNAs isolated from different developmental stages (Fig. 2). The expression of *paraxis* begins during late gastrulation and continues throughout the tadpole stages. *paraxis* transcripts are most abundant during the period when the mesoderm forms somites in *Xenopus* development (stages 17-24).



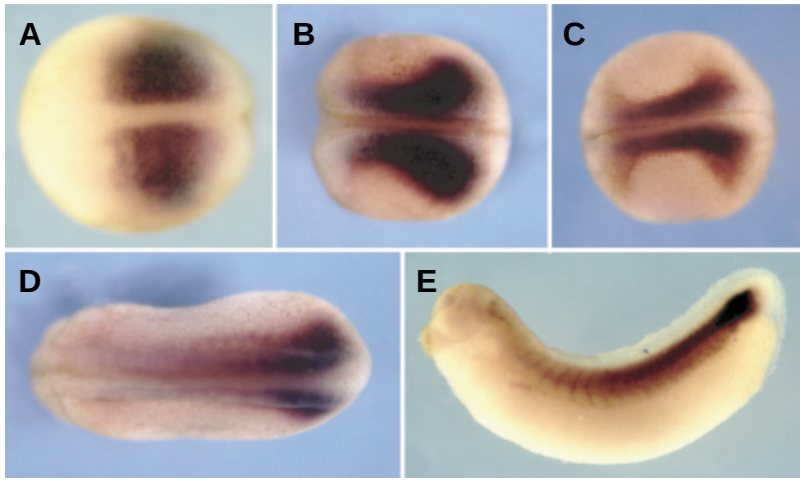


Fig. 2 (Left). Temporal expression pattern of *Xenopus paraxis* (top row) by RT-PCR analysis. *RT-PCR was performed with total RNA from different developmental stages. The last lane was a negative control without adding reverse transcriptase. EF-1α (bottom row) served as a loading control.*

Fig. 3 (Right) . Whole mount *in situ* hybridization analysis of *Xenopus paraxis* mRNA expression. *(A-D) Dorsal view. (E) Lateral view. All embryos are oriented with anterior to the left.* **(A)** *At stage 13,* paraxis *expression is in the presomitic mesoderm.* **(B)** *At stage 17,* paraxis *expression is reduced in the anterior region where somitogenesis begins.* **(C)** *At stage 19,* paraxis *becomes progressively more reduced in the anterior paraxial mesoderm.* **(D)** *At stage 24,* paraxis *transcripts are present at high levels only in the most caudal region where the somites are not yet compartmentalized.* **(E)** *At stage 28,* paraxis *expression is strong in the tip of the tail and in the dorsal half of somites from mid-trunk to hind-trunk.*

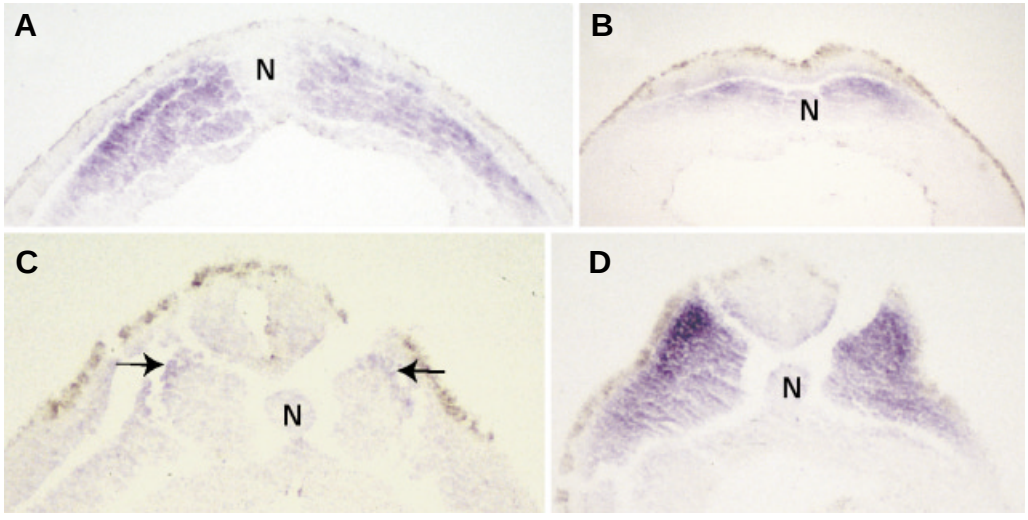


Fig. 4. Analysis of *Xenopus paraxis* expression in embryo sections. *All sections are positioned with dorsal at the top and ventral at the bottom.* **(A)** *A stage 13 transverse section shows* paraxis *transcripts in the whole presomitic mesoderm, but not in the notochord.* **(B)** *A stage 17 transverse section through the anterior trunk shows expression in the dorsal region of somitic mesoderm.* **(C)** *A stage 24 transverse section through the anterior trunk shows weak expression in the dorso-lateral region of somites (arrows).* **(D)** *In contrast, a transverse section through the posterior trunk of the same embryo as in (C) shows strong expression of* paraxis *in the entire undifferentiated somite. N, notochord.*

Spatial expression of Xenopus paraxis

The spatial expression of *paraxis* was determined by whole mount in situ hybridization. At late gastrulae, *paraxis* is strongly expressed in the presomitic mesoderm, but not in the notochord (Fig. 3A). As somitogenesis commences, the *paraxis* transcripts localize to a narrower paraxial region, lateral to the involuting neural tube (Fig. 3B). After the somites are formed, *paraxis* expression is progressively downregulated in the anterior paraxial mesoderm, but a high level of expression remains in the tail-bud region, where the somites are not yet differentiated (Fig. 3 C-E).

A transverse section through the trunk region of stage 13 embryo shows *paraxis* expression in the entire somitogenic mesoderm flanking the midline notochord (Fig. 4A). At stage 17, *paraxis* transcripts are preferentially localized to the dorsal portion of maturing somites (Fig. 4B). Before diminishing to undetectable levels in the rostral somites, *paraxis* is expressed in the most dorso-lateral region of somites, which appears to be the dermatome (Fig. 4C) (Hausen, 1991). In contrast, strong expression persists in the entire undifferentiated somites located in more caudal trunk region (Fig. 4D). Because of its expression pattern, *paraxis* can be used as a marker of dermatome differentiation in *Xenopus*. Together with the forkhead gene FoxC2 (XFKH7) (El-Hodiri *et al.*, 2001), which is a marker of sclerotome differentiation, these two genes can be used for analysis of differentiation of the paraxial mesoderm in *Xenopus*.

Experimental Procedures

Xenopus *genomic library screening*

The full-length mouse *scleraxis* cDNA was used to screen the *Xenopus* genomic library. Positive clones were plaque purified, subcloned into pBluescript SK and sequenced.

Whole mount in situ *hybridization and histology*

Xenopus embryos were staged according to (Nieuwkoop, 1994). Whole mount in situ hybridizations were performed as described by (Harland, 1991). The chromogenic reaction was modified using BM purple substrate (Roche). Probes were synthesized from EcoRI linearized plasmid using T7 RNA polymerase and digoxigenin-UTP (Roche). For histological analysis, embryos were dehydrated in ethanol and embedded in paraffin after whole mount in situ hybridization. Transverse sections were cut at 12 μm intervals, de-waxed in xylene and mounted with Permount (Fisher). Sections were not counterstained to retain visibility of labeled RNA transcripts.

RNA isolation and RT-PCR assay

Preparation of total RNA from animal caps using TRIzol reagent (Invitrogen) was carried out according to manufacturer's instructions. RT-PCR was performed by using the following primers and cycling conditions. For: *paraxis* (55ºC, 30 cycles; forward, 5'-GCG TAA ACA CCG CTT TCA CCG; reverse, 5'-CCT TGG CTC TGT AGA CGG TAC). For EF1alpha that was used as a positive control: (55ºC, 26 cycles; U: 5'-CAG ATT GGT GCT GGA TAT GC; R: 5'-ACT GCC TTG ATG ACT CCT AG).

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