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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(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).

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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.

**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 presomatic 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.
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. 3C-E).

A transverse section through the trunk region of stage 13 embryo shows paraxis transcripts in the whole 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. 3C-E).

A transverse section through the trunk region of stage 13 embryo shows paraxis transcripts in the whole presomitic 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 expressed in the most dorso-lateral region of somites, which appears to be the dermatoome (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 dermatoome 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 pBlue-script 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). 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 (55°C, 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: (55°C, 26 cycles; U: 5'-CAG ATT GGT GCT GGA TAT GC; R: 5'-ACT GCC TTG ATG ACT CCT AG).

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