Xenopus paraxis Homologue Shows Novel Domains of Expression

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The *paraxis* gene encodes a basic helix-loop-helix transcription factor that is expressed in paraxial mesoderm and whose mutant displays an inability to form epithelial somites. Here, the molecular characterization of *Xenopus paraxis* is reported. *paraxis* is expressed in the paraxial mesoderm and somites but is down-regulated during muscle differentiation. In addition to its paraxial mesodermal expression, described in other organisms, two novel expression domains of *paraxis* were found: the neural tube and the head mesoderm. *paraxis* expression in the neural tube was compared with the expression of the neural markers *Xash* and *Xiro1*, and we concluded that *paraxis* is expressed in a broad band in the prospective sulcus limitans of the neural tube. *Developmental Dynamics 231:609–613, 2004.* © 2004 Wiley-Liss, Inc.

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INTRODUCTION

The *paraxis* gene encodes a basic helix-loop-helix (bHLH) transcription factor identified in mouse (Burgess et al., 1995), chick (Barnes et al., 1997; Sosic et al., 1997; Andree et al., 1998), and zebrafish (Shanmugalingham and Wilson, 1998). *paraxis* is required to maintain the epithelial dermomyotome (Burgess et al., 1996), to regulate the formation of the nonmigratory myoblasts of the lateral dermomyotome (Wilson-Rawls et al., 1999), and to maintain anterior/posterior somite polarity independent of Notch signaling (Johnson et al., 2001).

In mice homozygous for a *paraxis* null mutation, cells from the paraxial mesoderm are unable to form epithelia; therefore, somite formation is disrupted. In the absence of normal somites, the axial skeleton and skeletal muscle form but are improperly patterned (Burges et al., 1996; Barnes et al., 1997). In the medial myotome, where the expression of the myogenic factor Myf5 is required for commitment of myoblasts, the migration pattern of committed myoblasts is altered in the absence of *paraxis*. In contrast, in the lateral myotome and migratory somitic cells, which require the expression of MyoD, expression of the myogenin–lacZ transgene is delayed by several days. This delay correlates with an absence of MyoD expression in these regions, indicating that *paraxis* is required for commitment of cells from the dorsolateral dermomyotome to the myogenic lineage. It has been concluded that *paraxis* is an important regulator of a subset of the myogenic progenitor cells from the dorsolateral dermomyotome that are fated to form the nonmigratory hypaxial muscles (Wilson-Rawls et al., 1999).

Expression of paraxis has been reported in somites (Barnes et al., 1997; Sosic et al., 1997; Andree et al., 1998; Shanmugalingham and Wilson, 1998) as well as in myoblast during limb development (Delfini and Duprez, 2000). We have characterized a *Xenopus paraxis* homologue, and shown that in addition to the mesodermal expression, described in other organisms, *Xenopus paraxis* is also expressed in the neural tube and the head mesoderm.

RESULTS AND DISCUSSION

Molecular Characterization of *Xenopus paraxis*

By performing a homology search in the expressed sequence tag (EST)

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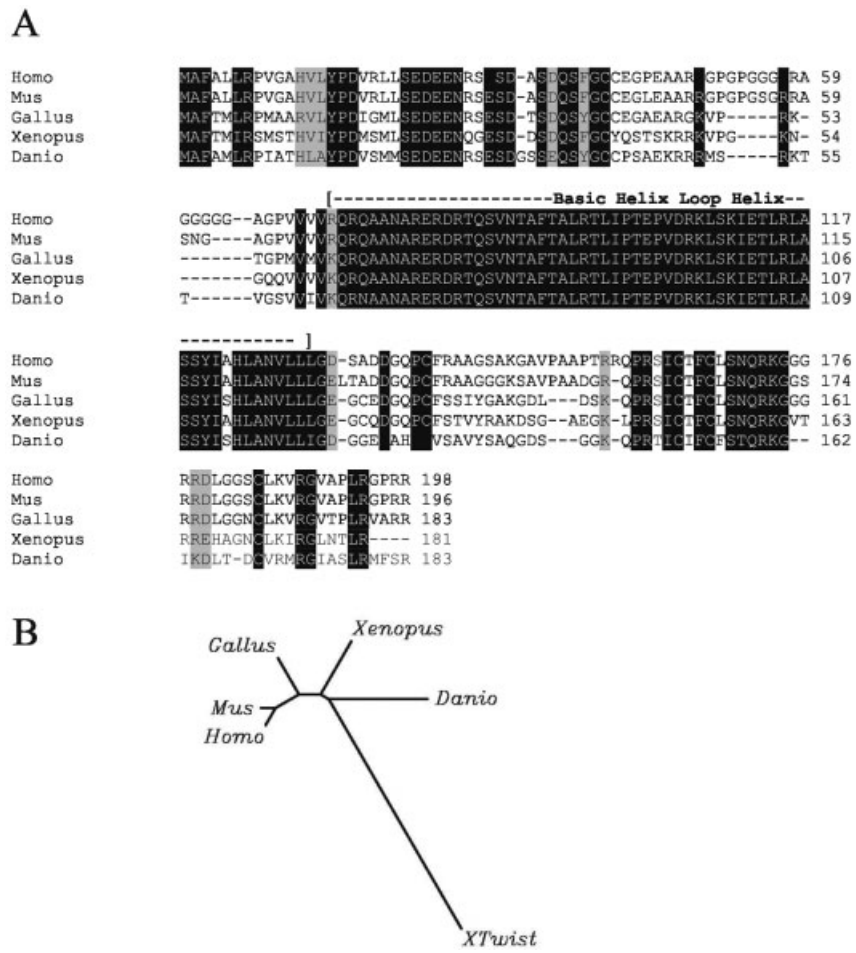

Fig. 1. Sequence and structure of *Xenopus paraxis*. **A:** Deduced amino acid sequences from human, mouse, chick, *Xenopus*, and zebrafish *paraxis* were aligned using the ClustalW algorithm. Identical and similar amino acids are in black and gray boxes, respectively. The helix-loop-helix domain is indicated. **B:** Phylogenetic unrooted tree including different *paraxis* members as well as the *XTwist* protein.

databases at NCBI using the mouse *paraxis* cDNA sequence, we identified a *Xenopus paraxis* gene. The in silico screen yielded one clone originated from a tail bud-stage library with an E-value of 3e-71. *Xenopus paraxis* is a cDNA of more than 1 kb long, which contains an open reading frame of 543 nucleotides, encoding a protein predicted to be 181 amino acids (GenBank accession no. AY599421). The bHLH domain of *Xenopus paraxis* shares approximately 95% amino acid identity to that of chick, mouse, and zebrafish genes (Fig. 1A). The N-terminal region of *Xenopus paraxis* show 57% identity, and the C-terminus domain 47% identity, to equivalent regions of mouse *paraxis*. A phylogenetic unrooted tree including different *paraxis* genes as well as the bHLH *twist* protein sequence clearly indicates that *Xenopus paraxis* is closely related to other *paraxis* proteins (Fig. 1B).

Expression of *Xenopus paraxis*

Xenopus paraxis is first detected at the late gastrula stage in dorsal but not axial mesoderm (Fig. 2A,E). As gastrulation proceeds, the region of *paraxis* expression becomes narrower and moves toward the dorsal midline, indicating the movement of the paraxial mesoderm (Fig. 2B–D,F–H). It can be seen in sections that *paraxis* is expressed in the whole presomitic tissue until the late gastrula stage (Fig. 2E–H). During somitogenesis, the expression of *paraxis* becomes restricted to cells that likely correspond to the dermatome or sclerotome in maturing somites (Fig. 2I,J). Somitogenesis proceeds in an anterior–posterior wave; therefore, it is possible to find paraxial mesoderm that expresses *paraxis* in all cells in more posterior presomitic regions of the same embryo (Fig. 2I,K). A down-regulation of the gene is observed in somites whose cells are differentiated into muscles (Fig. 2L). The pattern of *Xenopus paraxis* expression

Fig. 2. *paraxis* is expressed in somitic mesoderm. **A–D:** Dorsal view of embryos at different stages as indicated in the figure. Anterior (a) is at the top; p, posterior. **E–H:** Sections of embryos shown in A–D, in the region indicated by the line; n, notochord. **I:** Stage 22 embryo showing an anterior–posterior wave of paraxis down-regulation. **J:** Anterior section of embryo shown in I. Arrowhead, somitic expression of *paraxis* is restricted to the dermatome; arrow, initial expression of paraxis in the neural tube; n, notochord. **K:** Posterior section of embryo shown in I. Note that the complete somite is stained; n, notochord. **L:** Stage 26 embryo. Note that, as muscle differentiation is more advanced in this embryo, a down-regulation of *paraxis* is observed, except in the most posterior somites.

Fig. 3. *paraxis* is expressed in the paraxial mesoderm and neural tube. a, anterior; p, posterior. **A:** Stage 23 embryo stained for *paraxis* (purple) and the 12/101 antigen (brown) that is expressed in differentiated muscles. Note that *paraxis* expression is not found concurrent with markers of muscle differentiation. **B:** Stage 31 embryo stained for *paraxis* and 12/101. Note that, at this stage, most of the somitic muscles have already differentiated and no *paraxis* expression is observed, except in the tip of the tail bud. No expression in the neural tube is observed. **C:** Section of embryo shown in A. Note that, in the somite, *paraxis* is restricted to the dermatome (arrowhead). In addition, a clear expression in the neural tube is observed (arrows); n, notochord. **D:** Equivalent section of a stage 23 embryo showing expression of *Xash* in the sulcus limitans of the neural tube (arrows); n, notochord. **E:** Equivalent section of a stage 23 embryo showing expression of *Xiro1* in the sulcus limitans of the neural tube (arrows); n, notochord. **F:** Summary of *paraxis* expression in the neural tube (nt) and somites (s), compared with sulcus limitans markers. n, notochord. Scale bars = 125 μm in C–E.

observed in the somites is similar to that which has been described for mouse, chick, and zebrafish, suggesting that *Xenopus paraxis* may also play a role in somite formation as has been shown in chick and mouse (Burgess et al., 1996, Socsic et al., 1997). To analyze the correlation between *paraxis* expression and somite differentiation, we performed a double staining of *paraxis* and 12/101, an antigen that is expressed in differentiated muscle (Jen et al., 1997). We observed that *paraxis* expression is not found concurrent with the marker of muscle differentiation 12/101 (Fig. 3A), which recognize myofiber found in differentiated muscular cells (Griffin et al., 1987). Sections of these embryos clearly show that the muscle cells that have developed in the myotome do not express *paraxis,* while the remaining expression of *paraxis* is detected only in the external cells of the somite that are likely to be dermatome (Fig. 3C, arrowhead). At the tail bud stage, when most of the somites have already differentiated into muscle, *paraxis* expression is detected only in the tip of the tail bud (Fig. 3B).

A completely new domain of ex-

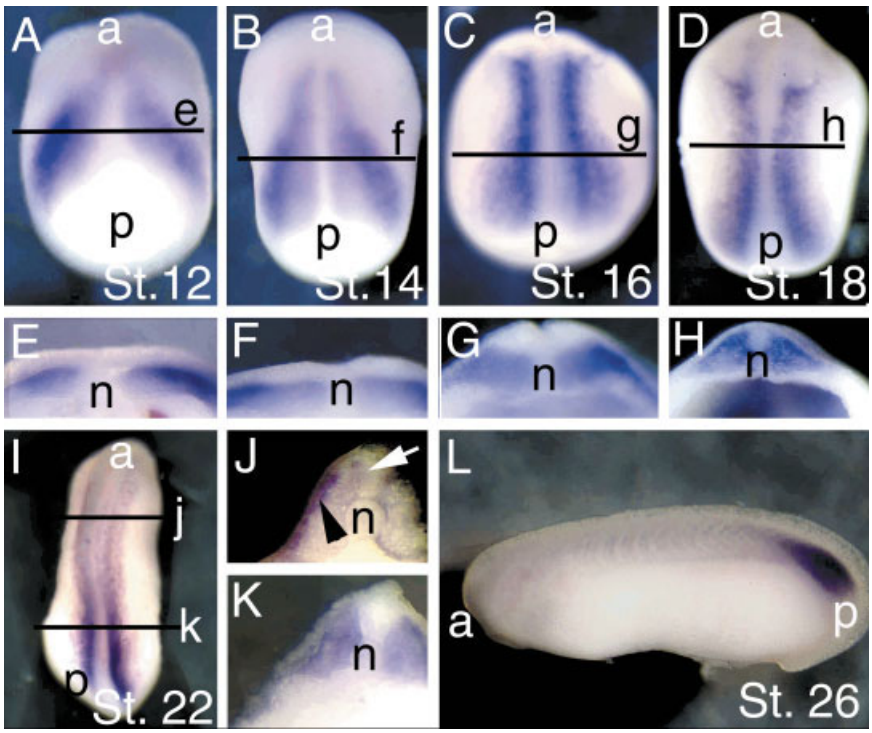

Fig. 2.

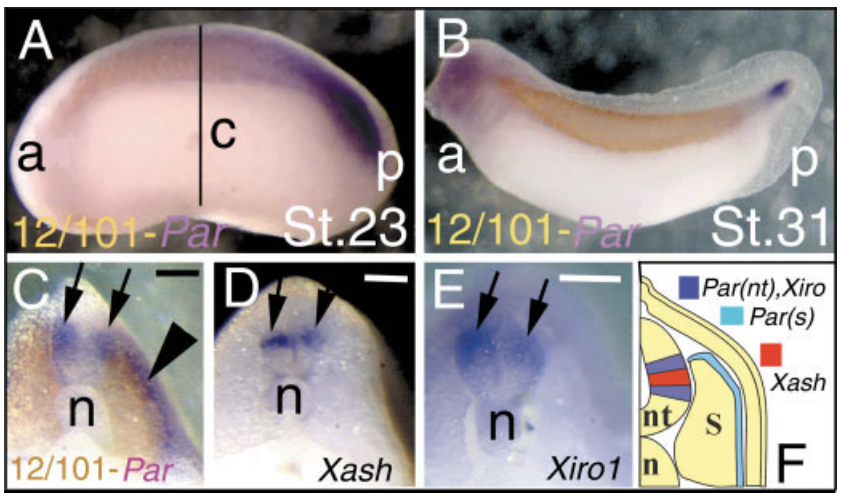

Fig. 3.

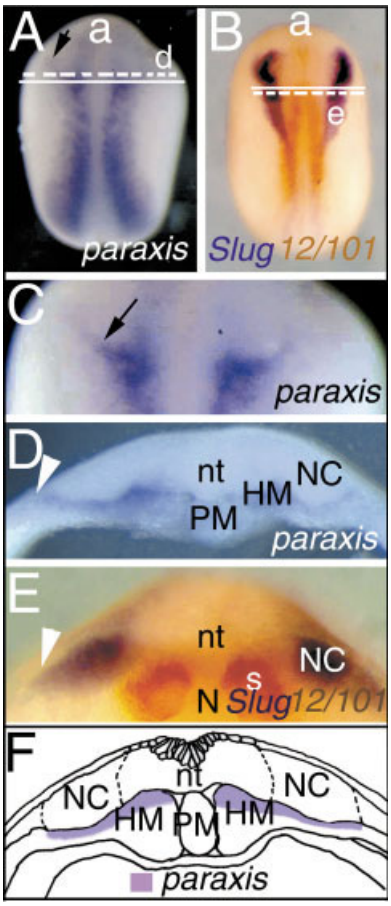

Fig. 4. Paraxis is expressed in the head mesoderm underlying the neural crest territory. **A:** Dorsal view of a stage 18 embryo stained for *paraxis*. Arrow, expression in a region anterior to the paraxial mesoderm. Continuous line, anterior limit of paraxial mesoderm; dashed line, section shown in D. **B:** Dorsal view of a stage 18 embryo stained for *Slug* (purple) and 12/101 (brown). Continuous line, anterior limit of paraxial mesoderm; dashed line, section shown in E. **C:** Higher magnification of anterior region of A. **D:** Section of embryo shown in A. The section was made just anterior to the somite expression. Note a broad band of expression in the head mesoderm (HM) and no expression in the prechordal mesoderm (PM). NC, neural crest; nt, neural tube. **E:** Section of embryo shown in B. The section was made at the border of somite expression, to include somite labeling as a landmark. s, somite. **F:** Summary of *paraxis* expression in the head mesoderm (HM); PM, prechordal mesoderm; NC, neural crest; nt, neural tube.

pression for *paraxis* in *Xenopus* was found in the neural tube. Sections of neurula embryo show a clear expression domain of *paraxis* in the region that corresponds to the sulcus limitans of the neural tube as compared with the expression of markers for this area: *Xash* and *Xiro1* (Fig. 3C–E). The sulcus limitans divides the neural tube along the dorsoventral axis into the basal and alar plate. The expression of *paraxis* in the neural tube is broader than the expression of *Xash* in that region and more similar to the expression of *Xiro1* (Fig. 3F). This new domain of *paraxis* expression is transient; onset is at stage 22 (arrow in Fig. 2J), becoming stronger at stage 23 (arrow in Fig. 3B), and then is extinguished after stage 26. *paraxis* expression in the neural tube is restricted to the third anterior region of the prospective spinal chord. We do not know if the expression of *paraxis* in the neural tube has not been described in other species, because it is not expressed there or because its expression is transient. The role of *paraxis* in the neural tube has yet to be investigated.

Finally, we show that *Xenopus paraxis* is expressed in the head mesoderm of early neurula embryos. We noticed that anterior to the strong paraxial mesodermal expression, a faint staining of *paraxis* was observed (arrows in Fig. 4A,C). Sections of this region show a clear expression of the gene in a broad region that seems to underline the cephalic neural crest (Fig. 4D). We compared the expression of *paraxis* in that region with the expression of the neural crest marker *Slug* and 12/101 to use neural crest and somites as landmarks to define *paraxis* expression (Fig. 4B). Approximately equivalent sections from embryos in Figure 4A and B were compared, but the section of the embryo in Figure 4B was slightly more posterior to see the somite labeling. *paraxis* expression is observed in the most dorsal aspect of the head mesoderm (Fig. 4D). When the lateral limit of its expression is compared with the neural crest marker (Fig. 4E), it becomes evident that this limit corresponds to the limit of neural crest cells (arrowhead in Fig. 4D,E). A summary of *paraxis* expression in the head mesoderm and its relationship with other tissues is shown in Figure 4F. This finding suggests that *paraxis* plays a role in both head and trunk mesoderm development, although different regulatory cascades have been proposed for these two tissues (Mootoosamy and Dietrich, 2002). Therefore, the common and divergent roles of *paraxis* in mesoderm formation need to be investigated.

EXPERIMENTAL PROCEDURES

Molecular Characterization of *paraxis*

The NCBI database was screened by using high stringency Tblastx against a pool of *Xenopus laevis* ESTs constructed from approximately 40 different *Xenopus laevis* embryonic libraries containing around 230,000 ESTs. We used the sequence of the mouse *paraxis* as a probe, and we identified several interesting ESTs. The clones were kindly provided by Dr. Naoto Ueno (NIBB, Okazaki, Japan). These were fully sequenced in both directions, revealing an open frame for *Xenopus paraxis* protein, which was deposited in GenBank (AY599421). Alignments of amino acid sequences were done using the ClustalW algorithm (http://www.ebi.ac.uk/clustalw/index.html). The phylogenetic tree was drawn by using the Phylodendron application (http://iubio.bio.indiana.edu/treeapp/treeprint-form.html) from data generated with ClustalW.

Whole-Mount In Situ Hybridization and Immunohistochemistry

Staining and in situ hybridization were performed as previously described (Glavic et al., 2004). Antisense RNA probes for *Xiro1* (Gómez-Skarmeta et al., 1998), *Xslug* (Mayor et al., 1995), *Xash* (Zimmerman et al., 1993), and *paraxis* were synthesized from cDNAs incorporating digoxigenin (Boehringer Mannheim) tags. Embryo specimens were prepared, hybridized, and stained according to the method of Harland (1991). The alkaline phosphatase substrates used were nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP). Antibody staining after in situ hybridization of the embryos was performed according to the method described by Turner and Weintraub (1994) by using a mouse anti-Myc monoclonal antibody from BabCo. The 12/101 polyclonal antiserum from the Developmental Studies Hybridoma Bank was used to label somites (Griffin et al., 1987). Between 30 and 50 embryos were analyzed in each stage.

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