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Progressively restricted expression of a new homeobox-containing gene during *Xenopus laevis* embryogenesis

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Summary

We have isolated cDNAs encoding a novel *Xenopus* homeodomain-containing protein homologous to the mouse *Hox-7.1* and the *Drosophila muscle segment homeobox* (*msh*). Northern blot and RNAase protection experiments established that transcripts of the frog gene, termed *Xhox-7.1*, first appear at about the beginning of gastrulation. After a rapid increase, mRNA levels plateau between the neurula and middle-tailbud stages, and decrease steadily thereafter. *In situ* hybridization localized the *Xhox-7.1* message to the dorsal mesodermal mantle of gastrula stage embryos. Comparison of the hybridization patterns of progressively more anterior cross-sections of tailbud stage embryos localized the signal to the dorsal neural tube and neural crest, to specific regions of the lateral plate mesoderm, and to the cardiogenic region. By the tadpole stage, the *Xhox-7.1* message appears only at specific sites in the central nervous system, such as in the dorsal hindbrain. Thus, during embryonic development levels of *Xhox-7.1* expression decrease as the transcript becomes more progressively localized. Finally, evidence is presented of a distinct *msh*-like transcript (provisionally termed *Xhox-7.1'*) which begins to accumulate at early-gastrula stage, as well.

Key words: developmental regulation, homeobox gene, *Xenopus* embryos.

Introduction

Genetic and molecular analyses of the fruit fly, *Drosophila melanogaster*, have led to the identification of genes controlling normal development and body patterning (For recent reviews, see Gehring, 1987; Levine and Hoey, 1988; Ingham, 1988; Akam, 1989; Biggin and Tjian, 1989; Scott *et al.* 1989; Wright *et al.* 1989*a*). Many of these genes share a highly conserved DNA sequence, the homeobox, encoding a 60-amino acid protein domain, the homeodomain (Gehring, 1987; Wright *et al.* 1989*a*; Scott *et al.* 1989). This sequence, also present in several mammalian transcription factors, exhibits structural similarity to the helix-turn-helix DNA-binding motif of regulatory proteins in yeast and prokaryotes (Levine and Hoey, 1988; Scott *et al.* 1989; Biggin and Tjian, 1989; Johnson and McKnight, 1989).

Drosophila homeobox sequences have been used to isolate a large cadre of vertebrate and invertebrate homeobox-containing genes that display defined temporal and spatial patterns of expression during embryogenesis (Akam, 1989; Wright *et al.* 1989*a*, Scott *et al.* 1989). Hence, it has become generally accepted that this phylogenetically conserved family of genes encodes nuclear proteins that regulate crucial developmental programs in a wide variety of organisms. Indeed, the potential role of these genes in modulating vertebrate morphogenesis has been strongly supported by studies in *Xenopus* and the mouse (Harvey and Melton, 1988; Ruiz i Altaba and Melton, 1989*a*; Fritz *et al.* 1989; Wright *et al.* 1989*b*; Wolgemuth *et al.* 1989; Balling *et al.* 1989; Kessel *et al.* 1990).

Based on structural features of the homeodomain and the use of the *Drosophila* genes as prototypes, Scott *et al.* (1989) have grouped eighty-three homeobox genes from various organisms into ten different classes. This number has recently increased to eleven with the addition of the mouse *Hox-7.1* gene and its *Drosophila* prototype, the *muscle segment homeobox* (*msh*) gene (Robert *et al.* 1989; Hill *et al.* 1989). The *Hox-7.1* gene displays a unique pattern of developmentally regulated expression among the known murine homeobox genes (Robert *et al.* 1989; Hill *et al.* 1989), and preliminary data suggest that additional *msh*-like genes may exist in the mouse (Hill *et al.* 1989).

In order to extend the characterization of this novel group of homeobox genes, we have used the *Drosophila msh* gene to isolate several homologs from different vertebrate species. Here we describe the data pertaining to a *msh*-like homeobox gene of *Xenopus laevis*, designated *Xhox-7.1*.

Materials and methods

Embryo cultures and RNA purification and analysis

X. laevis was purchased from *Xenopus* I (Ann Arbor, MI). Fertilization of eggs and development of embryos was according to the published protocols (Newport and Kirschner, 1982; Kimelman and Kirschner, 1987). Staging of embryos was according to Nieuwkoop and Faber (1967). RNA was purified from eggs and different stage embryos using the guanidinium thiocyanate method followed by centrifugation in cesium chloride solutions (Sambrook *et al.* 1989). Approximately 10 μg of total RNA (2–2.5 embryos equivalent) was electrophoresed in a 0.8% agarose gel in the presence of formamide/formaldehyde, transferred onto a Zeta-Probe nylon membrane (Bio-Rad) and hybridized to DNA labeled using the random primer method (Sambrook *et al.* 1989). The control probe was cytoskeletal gamma actin (Mohun *et al.* 1984). RNAase protection was performed according to Krieg and Melton (1985) using 20 μg of total RNA and uniformly ^{32}P-labelled antisense riboprobes generated as described below.

Screening of cDNA library and DNA sequencing

Approximately 10^5 recombinant phages of a neurula stage (stage 17) cDNA library (Kintner and Melton, 1987) were initially screened at low stringency using a 700 bp genomic fragment containing the homeobox of the *Drosophila msh* gene using the same conditions detailed by Robert *et al.* (1989). The same number of recombinants were subsequently screened using a 380 bp fragment of the frog cDNA that contains the homeobox sequence under more stringent conditions (40°C in 40% formamide, 6×SSC, 5×Denhart solution, 100 μg ml^{-1} denatured salmon sperm DNA). Filters were washed three times at room temperature in 2×SSC and 1% SDS, and at 42°C in progressively decreasing salt and SDS concentrations (down to 0.25×SSC and 0.25% SDS). Sequencing was carried out according to the dideoxynucleotide chain termination procedure on double-stranded DNA (Zagursky *et al.* 1986). Sequencing of both DNA strands was achieved by generating progressively overlapping deletions with the exonuclease III/mung bean nuclease method (Henikoff, 1984). Sequences were analyzed using the computer program of Mount and Conrad (1987). Nucleotide sequence of the *Xenopus msh*-like cDNAs have been deposited in the EMBL/GeneBank data library under the accession number X-54031.

In situ *hybridization*

For *in situ* hybridization, the 5′ foremost 430 bp fragment of the *Xhox-7.1* cDNA was subcloned into the transcription vector pT7/T3–19 (Bethesda Research Laboratories). ^{35}S-labelled single-stranded sense and antisense riboprobes were synthesized on 2 μg of linearized template as described by Swalla *et al.* (1988). Albino *Xenopus* embryos were treated overnight at 4°C with Bouin's fixative, dehydrated and embedded in paraffin according to the published procedure (Swalla *et al.* 1988). The same protocol was employed for preparation, treatment, hybridization and washing of the embryo sections.

Results

Isolation and structural analysis of Xenopus msh*-like cDNAs*

A cDNA library prepared from neurula stage *Xenopus* embryos (Kintner and Melton, 1987) was screened under low-stringency conditions with a 700 bp genomic fragment that contains the homeobox sequence of the *Drosophila msh* gene (Robert *et al.* 1989). This led to the identification of one positive clone, pSU-1, whose 1520 bp insert was subcloned into a pUC18 plasmid vector and sequenced. This identified a long open reading frame which is in frame with sequences encoding a putative homeodomain (Fig. 1A). When the conceptual translation of this putative homeodomain was compared to those the mouse *Hox-7.1* and the recently described quail *Quox-7* (Hill *et al.* 1984; Takahashi and Le Douarin, 1990), a very high level of sequence homology both at the nucleotide (data not shown) and amino acid levels was noted (Fig. 1A). In addition, the homology extends outside the homeodomain for 9 and 11 amino acids toward the amino- and carboxy-termini, respectively (Fig. 1A). Based on these data, the *Xenopus* cDNA was termed *Xhox-7.1*.

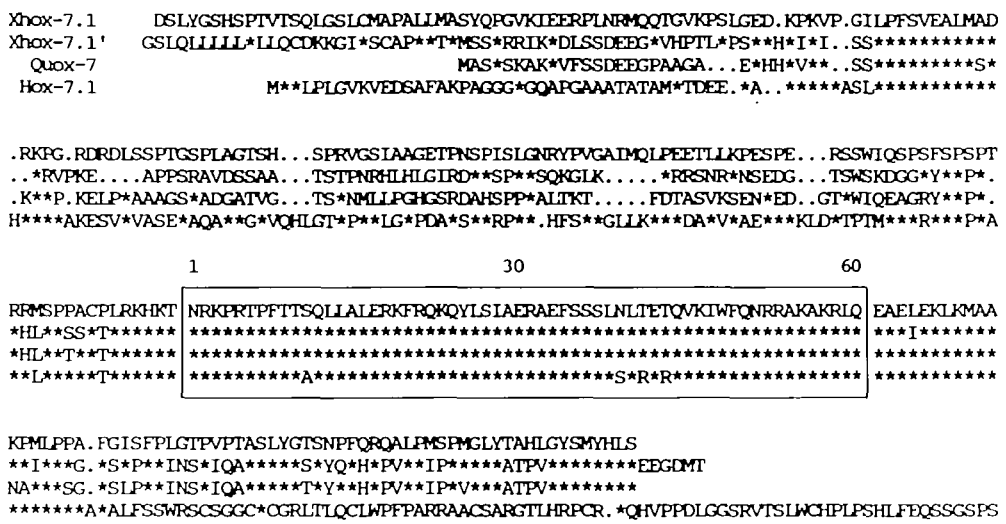
To search for additional *Xhox-7.1* clones, the neurula-stage cDNA library was re-screened under more stringent conditions with the homeobox sequence of pSU-1. Twenty-five positive clones were identified. After partial purification, the phage plaques were hybridized in triplicate to pSU-1 sequences specific for the homeobox and for its 5′ and 3′ flanking regions. All but one of the clones gave a positive signal with the homeobox flanking probes. The negative phage (pSU-32), and three randomly selected positive clones (pSU-54, pSU-64 and pSU-65) were purified further and characterized.

Clone pSU-64 encodes the same product as the original recombinant pSU-1. This cDNA extends 18 additional bp in the 5′ direction without interrupting the open reading frame of clone pSU-1. At the 3′ end, pSU-64 extends for approximately 100 bp, half of which constitutes the poly(A) tail of the mRNA. This finding and the size of the *Xhox-7.1* transcript (see next section) strongly suggest that the two overlapping cDNAs approximate the full-length message.

Clones pSU-54 and pSU-65 contain identical, overlapping sequences. In the coding region, they are highly homologous to pSU1 and pSU-64 (both at the nucleotide (92%) and amino acid levels (93%)), while in the 3′ untranslated region they exhibit a lesser level of sequence homology (87%) (data not shown). Like other homeobox genes in *Xenopus*, the two cDNAs are likely to be the products of duplicated *Xhox-7.1* genes rather than alleleic forms of one gene (Fritz *et al.* 1989). Indeed, our preliminary data seem to corroborate the former hypothesis.

The conceptual amino acid translation of the sequences of clone pSU-32 revealed that this cDNA encodes a polypeptide containing the *Xhox-7.1* homeodomain flanked, however, by significantly divergent sequences (Fig. 1A). To be precise, pSU-32 displays 87% nucleotide sequence homology with pSU-1 in the homeodomain coding region, whereas the level of nucleotide sequence similarity decreases to 51% and 34% in the 3′ and 5′ coding regions, respectively. These data strongly suggest that pSU-32 represents the

A



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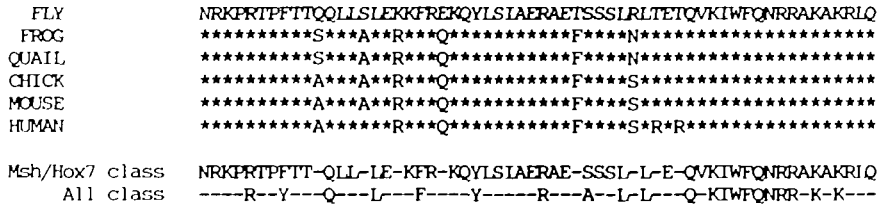

Fig. 1. (A) Comparison of the deduced amino acid sequences of *Xhox-7.1*, *Xhox-7.1'*, *Quox-7* and *Hox-7.1* (Hill *et al.* 1989; Robert *et al.* 1989; Takahashi and Le Douarin, 1990). The *Xhox-7.1* sequence includes the additional six amino acids encoded by clone pSU-64. Asterisks signify identity, while dots indicate gaps inserted to give best alignment. The region comprising the sixty residues of the homeodomain is boxed. (B) Comparison of the homeodomain residues of the *Drosophila msh* and the vertebrate *msh*-like genes. *Drosophila* sequences are from Robert *et al.* (1989); human sequences are from our laboratories (unpublished data) and from Ivens *et al.* (1990); chick sequences are from Suzuki *et al.* (manuscript in preparation). Also shown is the derived consensus sequence for the *msh*/hox7 class of homeodomains compared to the proposed consensus sequence for all eukaryotic homeodomains (Scott *et al.* 1989). Only absolutely conserved residues are shown for the *msh*/hox7 consensus sequence, while the overall consensus includes highly conserved residues that are not absolutely conserved.

transcript of a distinct *msh*-like gene or an alternatively spliced product of the *Xhox-7.1* gene. Experiments are currently in progress to determine the exact nature of the pSU-32 clone which, short of more compelling evidence, is provisionally designated *Xhox-7.1'*.

In their recent survey, Scott *et al.* (1989) have derived consensus sequences for each of ten proposed classes of homeodomains, as well as a general homeodomain consensus sequence. Here the latter is compared with that derived from the comparison of the *Drosophila msh* gene and several vertebrate *msh*-like genes (Fig. 1B). From this analysis, it is readily apparent that the highest conservation is seen in the putative recognition helix of the helix-turn-helix motif centered around the fiftieth residue of the homeodomain (Fig. 1B) (Scott *et al.* 1989). An important difference was, however, noted between the predicted frog and mouse proteins in the region carboxy-terminal to the homeodomain (Hill *et al.* 1989). Unique amongst the mouse homeobox genes, this region of *Hox-7.1* is characterized by the presence of an unusual cluster of seven cysteines. This finding prompted Hill *et al.* (1989) to suggest that these residues may be involved in intramolecular and/or intermolecular interactions with other proteins or in stabilizing DNA binding. This structural feature is not, however, present in the frog and quail counterparts (Fig. 1B), or in the chick and human gene products (data not shown). This may indicate the lack of functional significance of the cysteine cluster in the mouse polypeptide.

Temporal pattern of Xhox-7.1 *expression*

To ascertain the temporal pattern of expression of the *Xhox-7.1* gene, RNA isolated from various stages of *Xenopus* development was analyzed by northern blot hybridization using a non-homeobox sequence. This 430 bp probe covers the highly divergent 5' region of the frog *msh*-like mRNAs. In these experiments, cytoskel-

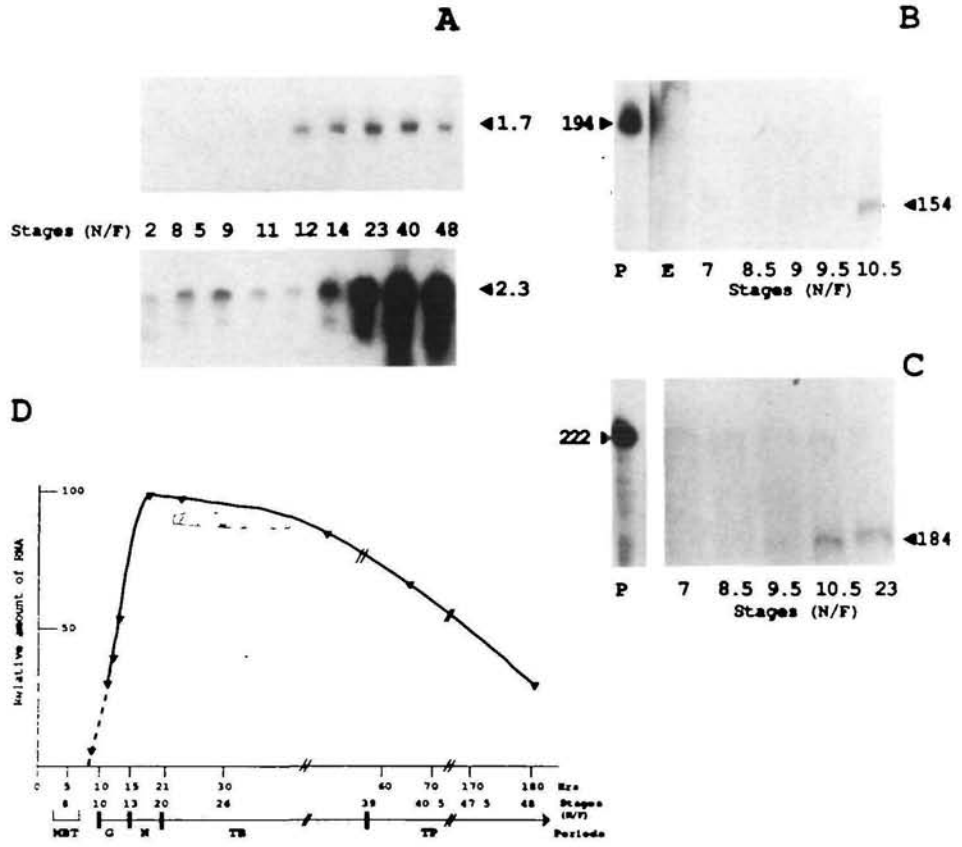

Fig. 2. (A) A northern blot of total RNA hybridized first to a *Xhox-7.1* probe and then to cytoskeletal gamma actin, top and bottom respectively. Total RNA samples (10 μg in each track) are from embryos of stages 2 to 48. (N/F) denotes the numerical stage according to Nieuwkoop and Faber (1967). Autoradiographic exposure was 4 days for *Xhox-7.1* and 1 day for actin. Size of transcripts, determined by parallel running of RNA markers, are indicated in kb. (B and C) RNAase protection of total RNA samples (20 μg in each track) from *Xenopus* eggs (E) and embryos of stages 7 to 23 hybridized to *Xhox-7.1* (B) and *Xhox-7.1'* (C) riboprobes. Exposure of the autoradiograms was for 4 days. In each panel, the autoradiography on the left is a shorter exposure (12 h) of the same gel showing the undigested labelled riboprobe (P). Note that the shortening of the antisense riboprobes after RNAase treatment is due to elimination of vector sequence. Sizes of fragments are indicated in bp. (D) Profile of *Xhox-7.1* mRNA accumulation plotted after quantitation of signals observed in the northern (A) and slot-blot hybridizations (not shown) of total RNA (5 μg) prepared at hourly intervals from unfertilized eggs and stages 8 to 40 embryos. Values are normalized for embryo number. Developmental periods and (N/F) stages are shown below the graph. MBT, midblastula transition; G, gastrulation; N, neurulation; TB, tailbud and TP, tadpole stages. The dotted line denotes the period when *Xhox-7.1* expression is not well defined.

etal gamma actin was used as an internal control (Mohun *et al.* 1984) (Fig. 2A). At all stages of development, the *Xhox-7.1* probe hybridized to a single RNA species whose size (1.7 kb) approximates the combined length of clones pSU-1 and pSU-64 (Fig. 2A). *Xhox-7.1* transcripts were first detected in gastrula stage samples; steady-state mRNA levels increased thereafter reaching maximal expression between neurula and tailbud stages, and then progressively decreased later in development (Fig. 2A). The more sensitive RNAase protection assay, performed with the 5′ foremost segment of pSU-1, detected *Xhox-7.1* mRNA in early-gastrula embryos (stage 10.5) (Fig. 2B). Moreover, a very faint band (not visible in the photographic reproduction of Fig. 2B) seemed to be present also in samples from stage 9.5 embryos. This suggested that the onset of zygotic *Xhox-7.1* transcription might initiate just before gastrulation. The low level of sequence homology between the 5′ foremost regions of pSU-1 and pSU-32 enabled us to establish the onset of *Xhox-7.1'* transcription by the RNAase protection assay. This demonstrated that, like *Xhox-7.1*, also the *Xhox-7.1'* mRNA begins to accumulate in the developing *Xenopus* embryos at early-gastrula stage (Fig. 2C).

An overall profile of *Xhox-7.1* mRNA accumulation was derived by quantitative scanning of northern and slot-blot hybridizations of RNA extracted at hourly intervals from unfertilized eggs to stage 48 embryos (Fig. 2D).

Spatial pattern of Xhox-7.1 *expression*

The spatial pattern of *Xhox-7.1* expression during *Xenopus* development was determined by *in situ* hybridization to sections of embryos at gastrula (stage 11), tailbud (stage 21) and tadpole (stage 46) stages.

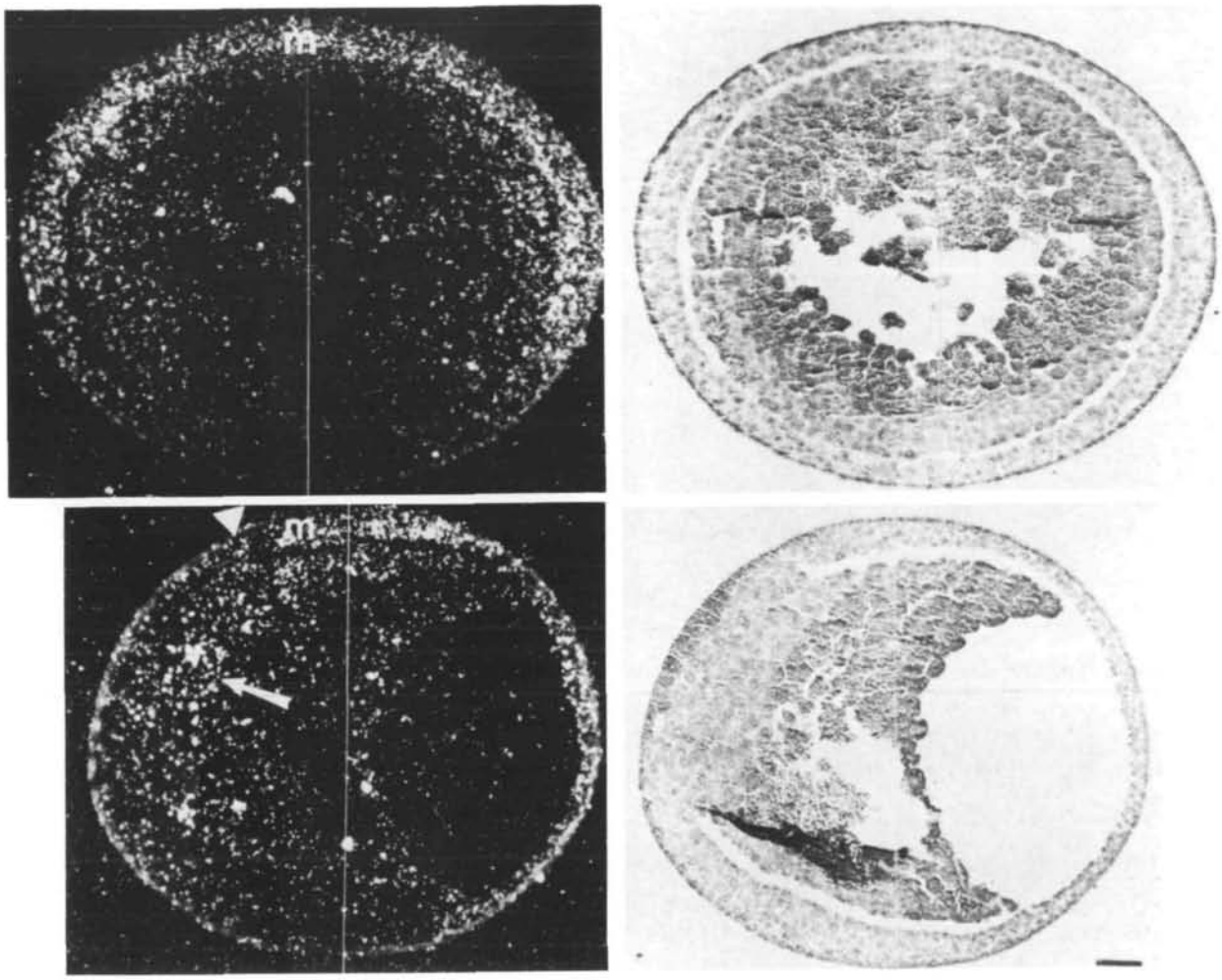

Fig. 3. Expression of *Xhox-7.1* at gastrula stage. On the top, cross-section at stage 11; dark-field (left) and bright-field (right) micrographs. Note the strong signal for *Xhox-7.1* in the dorsal mesodermal mantle (m). In serial cross-sections, this distribution can be observed in the mesoderm all along the anterior–posterior axis. On the bottom, mid-sagittal section of stage 11 embryo; dark-field (left) and bright-field (right) micrographs. The localization of *Xhox-7.1* message to the dorsal mesodermal mantle (m) is observed in this mid-sagittal section as well. A strong signal is detected along the whole anterior–posterior axis in the chordamesoderm from the level of the dorsal lip (arrowhead) (including both the deep and superficial layers of the mesoderm), along the dorsal side and extending around the anterior end towards the ventral surface of the embryo. In serial parasagittal sections, localization in the anterior chordamesoderm is more pronounced than in this mid-sagittal section (not shown). There is also signal in cells of the yolk plug near the dorsal lip (arrow). No significant signal was detected with the sense probe (not shown). Bar=100 microns.

Expression of *Xhox-7.1* is complex and not restricted to any single germ layer or morphogenetic process. At the gastrula stage, transcripts were detected in the chordamesodermal mantle (Fig. 3). Cross-sections of gastrula stage embryos revealed that *Xhox-7.1* mRNA is localized throughout the dorsal half of the mesodermal mantle (Fig. 3, top panel). Mid-sagittal sections, on the other hand, revealed transcripts in the chordamesoderm along the whole anterior–posterior axis on the dorsal side of the embryo, as well as in some endodermal cells in the vicinity of the dorsal lip (Fig. 3, lower panel).

At the tailbud stage, *Xhox-7.1* RNA was detected in the presumptive and definitive neural crest cells, and other cells in the dorsal region of the neural tube. Transcripts were also observed in a subpopulation of lateral plate mesodermal cells, and in the heart (Fig. 4). By comparing more posterior sections with progressively more anterior ones, the sequence of developmental expression can be appreciated. In the tailbud, *Xhox-7.1* message was seen throughout the forming neural tube (Fig. 4A). More anteriorly (observed in the same section), message was more restricted, localized in the dorsal region of the neural folds (Fig. 4A). Still more anteriorly, the signal was detected in the forming neural crest cells, as well as in the dorsal neural tube and the lateral plate mesoderm (Fig. 4B). In more posterior regions of the trunk, *Xhox-7.1* transcripts were observed throughout the lateral plate mesoderm (not shown), while more anteriorly they were localized to the dorsal half of the lateral plate mesoderm (Fig. 4B). In more anterior sections, message was detected in

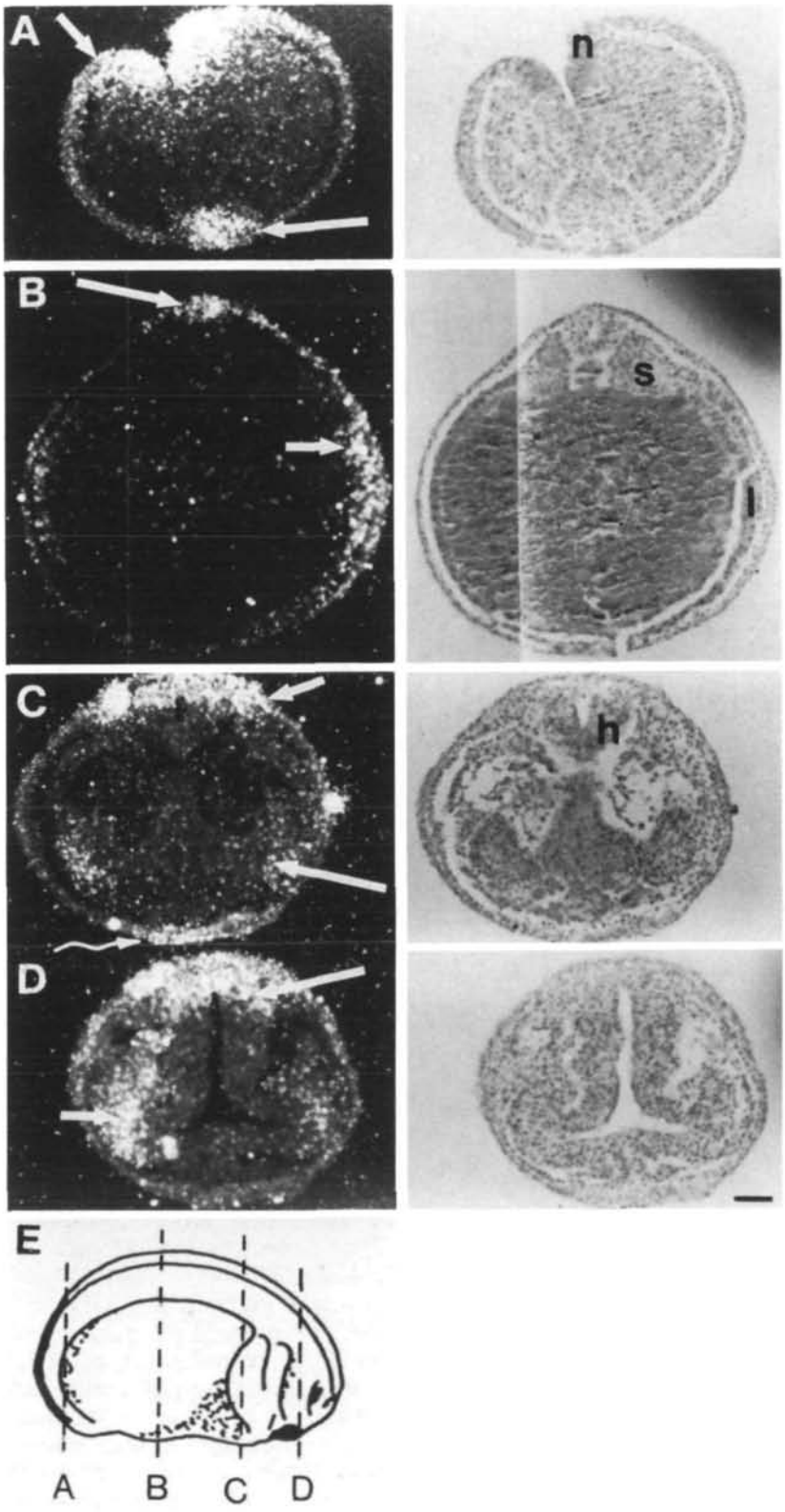

Fig. 4. Expression of *Xhox-7.1* at tailbud stage. Cross sections at different levels of a stage 21 embryo; dark-field (left) and bright-field (right) micrographs. It is possible to appreciate progressively later stages of development by comparing progressively more anterior sections (see diagram at the bottom). (A) Cross section through the curved tail bud. In this section, the signal is localized to the dorsal region of the forming neural tube (long arrow) and the dorsal region of the neural folds (short arrow). (B) Cross section cut at the level of the mid gut. Note that the signal is now detected in the neural crest cells (long arrow) and in the dorsal-most regions of the neural tube and lateral plate mesoderm (short arrow). (C) Cross section cut at the level of the hind brain. Note the intense signal in the dorsal region of the hind brain, the adjacent cranial neural crest cells (short arrow), as well as neural crest adjacent to the pharynx (long arrow) and cells in the cardiogenic region, ventrally (curved arrow). (D) Section through the head. Note the localization of message in the neural crest around the diencephalon (short arrow) and midbrain (long arrow), as well as in the same adjacent neural structures. No distinctive signal was detected with the sense probe (not shown). (E) Diagram indicating the approximate level of the sections illustrated. Bar=100 microns, neural fold (n), somite (s), lateral plate (l), hind brain (h).

cranial neural crest cells, as well as adjacent neural tissue. In panels C and D of Fig. 4, for example, *Xhox-7.1* mRNA is detected in the dorsal side of the hindbrain and adjacent neural crest cells, as well as neural crest adjacent to the forebrain and midbrain. Message was also seen in the region of the developing heart (Fig. 4C). By the tadpole stage, *Xhox-7.1* message was only detected in localized regions of the central nervous system, such as the dorsal part of the hindbrain (Fig. 5).

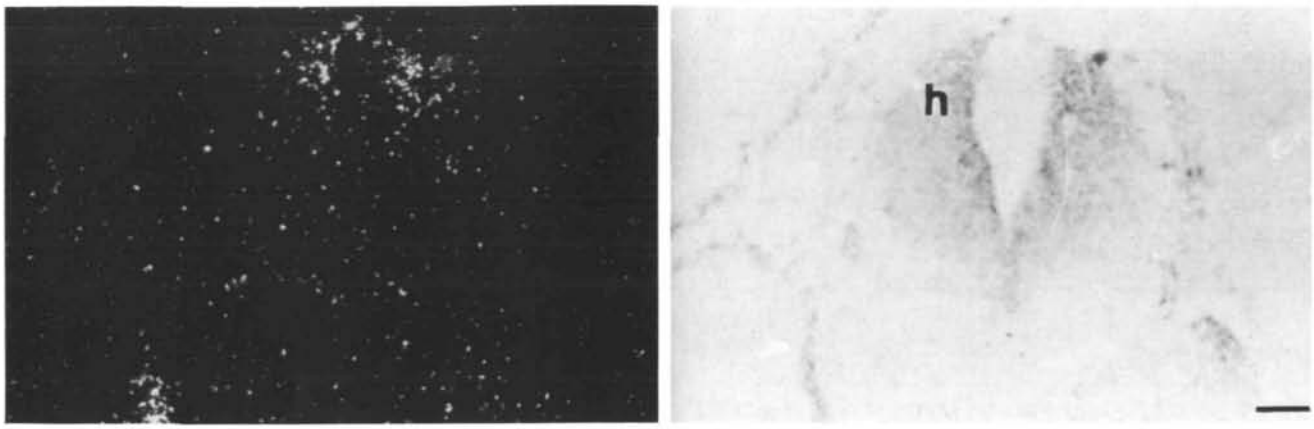

Fig. 5. Expression of *Xhox-7.1* at tadpole stage. Section through the head of a stage 46 embryo; dark-field (left) and bright-field (right) micrographs. By this stage, the message is detected only at specific sites of the central nervous system, such as cells in the dorsal region of the hindbrain (h). No significant signal was detected with the sense probe (not shown) Bar=100 microns.

Discussion

In this paper, we report the isolation, partial structure and expression pattern of a novel *Xenopus* homeobox-containing gene. This frog gene, isolated using the *Drosophila msh* homeobox sequence, has been termed *Xhox-7.1* by analogy to its mouse counterpart, *Hox-7.1* (Robert *et al.* 1989; Hill *et al.* 1989). Moreover, evidence is presented for the existence of another *msh*-like related gene, *Xhox-7.1'*, which is produced at about the same time that *Xhox7-1*. The data presented here, as well as a recent report on the quail gene (Takahashi and Le Douarin, 1990) and our own unpublished results establish that the homeodomains of the *msh*-class of genes display an average level of amino acid sequence homology greater than 90% in four vertebrate species. In line with the structural analysis of Scott *et al.* (1989), a consensus sequence for the *msh*/hox7 class of homeobox-containing genes is proposed. Accordingly, *Xhox-7.1* can be classified separately from the seven homeobox genes hitherto identified in the frog. Based on the structural features of the homeodomain, these genes belong to the *Drosophila Antennapedia* (*Antp*) class (*Xhox-36*, *Xlhbox1*, *Xlhbox2*, and *Xlhbox5*), the *deformed* (*Dfd*) class (*Xhox-1a*, and *Xhox-1b*) and the *even-skipped* (*eve*) class (*Xhox-3*) (Scott *et al.* 1989).

The availability of the *Xhox-7.1* probe has enabled us to elucidate the pattern of developmental expression of this gene during amphibian embryogenesis. In this respect, *Xhox-7.1* expression is distinct from that of other *Xenopus* homeobox genes (Muller *et al.* 1984; Carrasco *et al.* 1984; Harvey *et al.* 1986; Carrasco and Malacinski, 1987; Condie and Harland, 1987; Sharpe *et al.* 1987; Oliver *et al.* 1988; Ruiz i Altaba and Melton, 1989*b*; Ruiz i Altaba, 1990). Activation of zygotic *Xhox-7.1* transcription seems to coincide with the period at which the frog embryo begins to gastrulate. Thereafter, *Xhox-7.1* transcripts accumulate rapidly reaching maximal levels by the end of gastrulation. No significant changes in steady-state mRNA levels are seen between early-neurula and middle-tailbud stages. As the embryo approaches the tadpole stage, *Xhox-7.1* mRNA levels begin to decline steadily.

The early period of expression is characterized by a rather broad distribution of the *Xhox-7.1* mRNA, whereas at later stages a more restricted localization in distinct embryonic tissues is seen. This sequence is observed in the dorsal mesodermal mantle, where the message becomes more restricted to the lateral plate mesoderm and then to the dorsal region of the lateral plate mesoderm. Similarly, this progression is also noted in the forming neural tube where expression becomes more restricted to the dorsal neural folds and the neural crest. By the tadpole stage, and parallel to the decrease in mRNA levels, transcripts are only seen at specific sites of the central nervous system.

The tissue distribution of the *Xhox-7.1* mRNA during the post-gastrula stages of *Xenopus* embryogenesis is highly reminiscent of, if not identical to, that reported for the mouse and quail counterparts (Robert *et al.* 1989; Hill *et al.* 1989; Takahashi and Le Douarin, 1990). For example, transcripts of the *Hox-7.1* gene have been shown to accumulate in the neural tube and cephalic neural crest of the mouse embryo, as well as in the lateral mesoderm and the developing embryonic heart (Robert *et al.* 1989; Hill *et al.* 1989). Similar patterns of expression have been also observed for *Quox-7*, the quail *msh*-like gene (Takahashi and Le Douarin, 1990). However, there are presently no data on the expression of *Quox-7* and *Hox-7.1* during early stages of embryogenesis, notably at gastrulation. Recently, our work on the homologous chick gene has indicated that this *msh*-like gene also begins to be transcribed around gastrula stage with a spatial pattern of expression comparable to that of the *Xenopus* counterpart (Suzuki *et al.* manuscript in preparation).

The structural conservation of the homeodomain sequences, as well as the similarity in temporal and spatial patterns of developmental expression in three different species lend support to the notion that the *msh*-class of homeobox genes may play a role in

vertebrate morphogenesis. In this respect, it will be of interest to extend these descriptive analyses to later stages of *Xenopus* embryogenesis. This will be particularly enlightening in regard to the selective accumulation of mouse *Hox-7.1* transcripts in the interdigital mesenchymal tissues of fore- and hind-limbs of 13.5 day embryos, and in the endothelial cells lining the lumen of the heart of 12 day embryos (Robert *et al.* 1989; Hill *et al.* 1989). Likewise, it will also be important to establish whether *Xhox-7.1'* represents an alternatively spliced product of *Xhox-7.1* or the transcript of a distinct *msh*-like gene. Finally, immunohistochemical studies are needed to correlate the pattern of gene expression with that of gene function during *Xenopus* embryogenesis. These studies, together with ongoing analyses of gene expression in artificially perturbed embryos, will shed some light on the possible role that the *Xhox-7.1* gene(s) play(s) during vertebrate embryogenesis.

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