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Gene expression pattern

CMIX, a paired-type homeobox gene expressed before and during formation of the avian primitive streak

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Abstract

We cloned a chicken homeobox gene closely related to the *Xenopus Mix.1* gene. *CMIX* is expressed early in embryogenesis in a sickle-shaped area in the posterior zone of the blastoderm. With the beginning of gastrulation, *CMIX* transcripts are found in the primitive streak primordium, then in the young and medium-sized streak, however not in the mesoderm after its emergence. In the fully-extended streak, *CMIX* is restricted to its middle, i.e. the prospective ventral mesoderm. *CMIX* RNA is undetectable by whole-mount in-situ analysis in later stages. We compare *CMIX* expression to the early pattern of the *brachyury* gene. © 1998 Elsevier Science Ireland Ltd. All rights reserved

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In *Xenopus* the two closely-related genes *Mix.1* (Rosa, 1989) and *Mix.2* (Vize, 1996) together with the recently described *Milk* gene (V. Ecochard et al., unpublished data, but see GenBank accession number: AF005999) define a distinct subtype of the paired class homeobox genes. The two, possibly pseudo-allelic *Mix* genes are expressed in indistinguishable patterns, beginning immediately after the midblastula transition in the vegetal half of the embryo, mainly in the presumptive endoderm. Later the expression domain includes part of the prospective mesoderm in the marginal zone and with onset of gastrulation expression is restricted to the ventral mesoderm (see also http://vize222.-zo.utexas.edu). The *Mix* genes respond by immediate early transcriptional activation to signals of TGF-β family members like activin, Vg-1, TGF-β, and BMP4 (Rosa, 1989; Huang et al., 1995; Chen et al., 1996; Vize, 1996). Dorsal mesoderm is re-specified to a ventral fate by the ectopic action of *Mix.1* homodimers, but *Mix.1* misexpression cannot induce secondary axes or mesoderm per se. Thus the Mix homeodomain factors seem to be important determinants necessary for the establishment of ventral mesoderm identity. Despite considerable efforts (e.g. Hermesz et al., 1996) no *Mix* type homeobox gene has been identified from non-amphibian sources so far. Here we report the isolation of cDNAs from early chick embryos, which clearly belong to the *Mix* typus based on sequence and expression analysis.

Mix-related DNA fragments could be amplified with degenerated primers designed to fit the 5′ and 3′ ends of the *Xenopus Mix.1* homeobox from *Xenopus*, but not from chicken embryonic RNA. Therefore, a cDNA library prepared from chicken HH3$^+$/4 nodes was screened under low stringency conditions applying the *Xenopus* PCR fragments as probes. Two overlapping cDNA clones were obtained and sequenced (Accession number: AJ004903; EMBL Nucleotide Sequence Database). The encoded '*CMIX*' homeodomain is 69% identical to *Mix.1*/*Mix.2*, and 57% identical to the *Milk* homeo domain (Fig. 1). Although the name assigned to this gene acknowledges its close relationship to the *Xenopus Mix.1* and *Mix.2* genes, a clear understanding of the degree of functional homology requires further study. The next-closest relatives are the *Otx2* and

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CMIX      QRRKRTSFTAAQLETLELVFQDTMYPDIYLRERLADATQIPESRIQVWFQNRRAKSRRRRL
Mix.1     ......F..Q...DI..QF..TN.....HH..E..RHIY..............V..QGA
Milk      N.....VYSPSD.AL..QY..TN.....HQ..E..RQMGL............S.A..QGS

Xotx2     ...E..T..R...DI..AL.AK.R....FM..EV.LKINL....V....K.....C.QQQQ
Xcart1    K..H..T..SL...E..K...K.H...V.V..Q..LR.ELT.A.V..........W.K.ER
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Fig. 1. Alignment of Mix homeodomains and their closest relatives. Dots represent residues identical to the *CMIX* sequence. Note the methionine at position 24 of the *Mix* homeodomains, which can not be found in any other of the paired class homeodomain proteins at this position. All sequences except *CMIX* are from *Xenopus*. The homeodomain of *Mix.2* is identical to *Mix.1*. *Xcart1* is from GenBank accession number U15276; for further references see text.

the *Cart1* genes (Fig. 1). There is little sequence-similarity of *CMIX* to the other proteins outside the homeodomain. The *CMIX* gene we report is also described in a parallel work (Peale et al., 1998). This cDNA is distinguished by a 221-bp insertion in the coding region, resulting in alternative reading frames in the 3′ end of the gene, involving a replacement of RRL (Fig. 1) at the end of the homeodomain by QRG.

In situ hybridizations were performed to trace the expression of the *CMIX* gene in chick embryogenesis (Fig. 2). Embryos were staged according to Eyal-Giladi and Kochav (1976) and Hamburger and Hamilton (1951). The first transcripts are detectable before formation of the primitive streak in the posterior marginal zone in a crescent-shaped domain (Fig. 2A). *CMIX*-expressing cells reside not only in the epiblast but also in deeper layers (Fig. 2I,J). During the following stages the laterally-extending expression domain narrows to the embryonic midline and the primitive streak primordium. Concomitantly the population of *CMIX*-expressing cells becomes more densely packed than during the earlier stages (Fig. 2B,C). In stage HH3 to HH3$^+$ the entire primitive streak except for its very posterior part is strongly positive for *CMIX* (Fig. 2D,E). Transverse sections show that it marks the epiblast as well as the streak mesoderm, while most of the mesoderm that already emerged from the streak, and the endoderm does not express (Fig. 2K–M). Shortly before the primitive streak reaches its full extension, *CMIX* RNA becomes restricted to prospective ventral mesoderm in middle-to-posterior portions of the streak, leaving out the anterior part including Hensen's node (Fig. 2G). During further development *CMIX* transcription is rapidly downregulated also in the middle of the streak and after completion of head-process formation (HH6) *CMIX* expression is no longer detectable (Fig. 2H; examined up to the 22-somite stage, not shown).

The principal similarities between *MIX* gene expression in chicken and *Xenopus* become obvious especially in comparison to other genetic markers. In early *Xenopus* embryos, *Mix* characterizes the late, i.e. post-MBT vegetal cells, and *brachyury* becomes induced at the interphase to the *Xotx2*

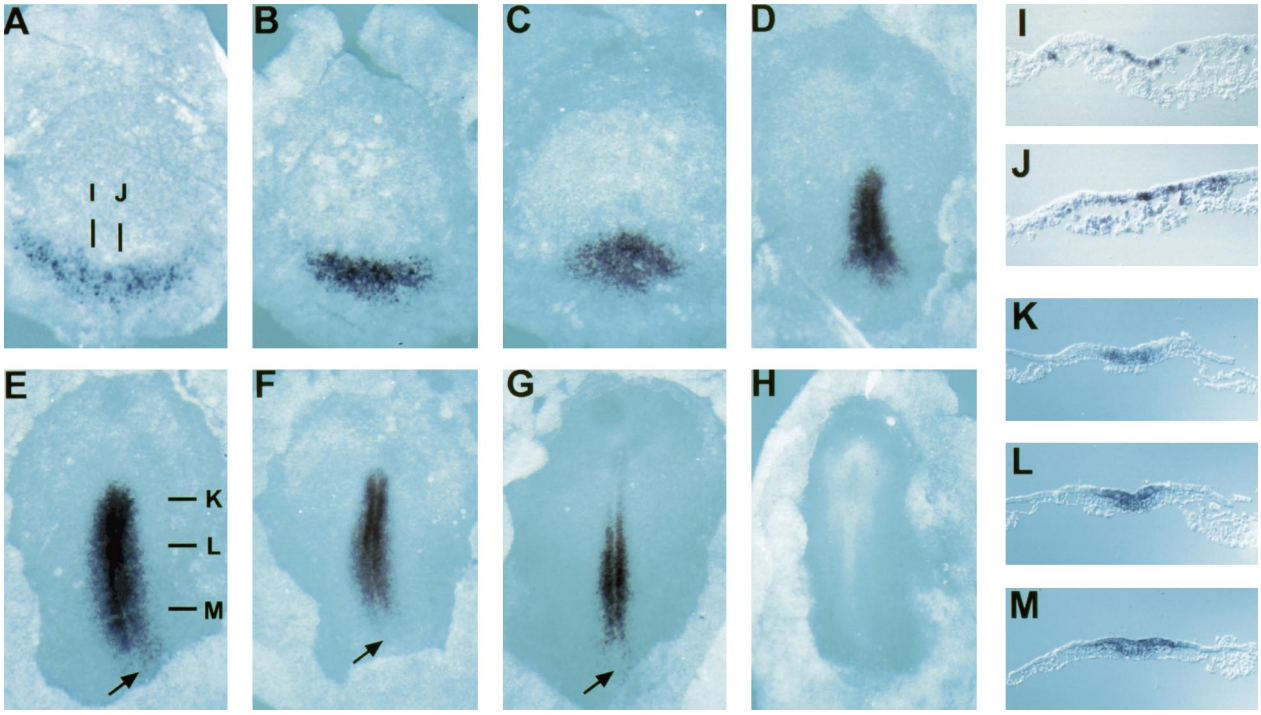

Fig. 2. Distribution of *CMIX* transcripts during chick embryogenesis. For description and discussion see text. (A) EK stage XII. (B) stage HH1. (C) HH2$^-$. (D) HH3. (E) HH3$^+$. (F) HH4$^-$. (G) HH4. (H) HH6. Arrows indicate lack of expression in the posterior streak, lines indicate direction and levels of sections shown in I–M.

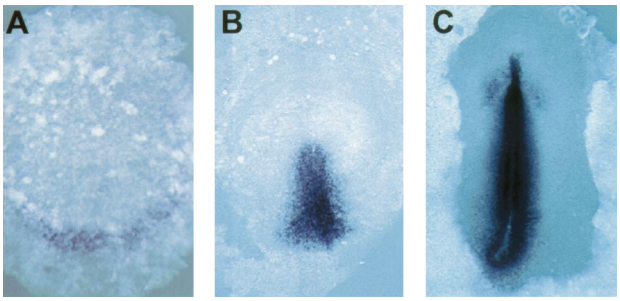

Fig. 3. Early pattern of the chicken *brachyury* gene. *Brachyury* expression (Kispert et al., 1995; Knezevic et al., 1997). (A) EK stage XII. (B) HH3. (C) HH5.

expressing animal half (Smith et al., 1991; Pannese et al., 1995). A comparison of the homologs of the same three genes in chick embryos reveals a quite similar situation. *C-otx2* characterizes the majority of the epi- and hypoblast (Bally-Cuif et al., 1995), *CMIX* lies at the opposing posterior marginal zone (Fig. 2A) and *brachyury* at the interphase or the overlap (Fig. 3A). During primitive streak formation, *brachyury* appears first as a panmesodermal marker similar to *CMIX* (Fig. 3B), but then is maintained in the streak and in the late dorsal mesoderm, the notochord, after ingression (Fig. 3C). *C-otx2* becomes restricted to dorsal/anterior structures, the node and the prospective fore- and midbrain (Bally-Cuif et al., 1995). The earliest *brachyury* domain detected in chick embryos (Knezevic et al. (1997) Fig. 3A) confirms the recent finding of *brachyury* expression between the extraembryonic and embryonic part of the mouse egg cylinder (Thomas and Beddington, 1996), and may identify the earliest site of mesoderm induction in these two amniota. An intriguing similarity exists between the early expression patterns of *CMIX* and the chicken *cVg1* gene (Seleiro et al., 1996; Shah et al., 1997), a homolog of the vegetally expressed frog factor *Vg1* (Huang et al., 1995). Processed Vg1 is sufficient for ectopic induction of *Mix*-genes in *Xenopus* animal cap explants, and ectopic cVg1 induces primitive streak formation (Huang et al., 1995; Shah et al., 1997). In conclusion, based on sequence and expression, we have isolated the first non-amphibian *Mix*-type homeobox gene.

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