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EXPRESSION NOTE

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Mbx, a novel mouse homeobox gene

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Abstract The homeobox gene *Mbx* is thought to play important roles in the development of eyes and tectum in zebrafish. We isolated mouse *Mbx* cDNA and analyzed its expression pattern during early mouse embryogenesis. Expression is predominantly restricted to the midbrain region at E9.5. At subsequent stages of development, *Mbx* transcripts were also found in the forebrain in addition to midbrain. Thus, the *Mbx* gene provides a useful molecular marker for early mouse midbrain development and may play a critical role in brain development.

Keywords Mbx · Homeobox · Midbrain

Homeobox genes play critical roles in early CNS patterning (Beddington and Robertson 1998). Among these, paired-type homeobox genes, including members of the Otx, Rpx and Emx families, show region-specific expression in anterior regions of the developing brain (Martinez-Morales et al. 2001; Mathers et al. 1997; Suda et al. 2001). Recently, we have identified a novel paired-type homeobox gene, *Mbx,* in the human and zebrafish genomes. In zebrafish, *Mbx* is expressed predominantly in the midbrain between gastrulation and the 26-h stage (A. Kawahara and I. Dawid, unpublished results).

In an effort to study the function of *Mbx* in mouse development, we made use of the Advantage PCR cloning kit (Clontech) to isolate a 520-base-pair fragment that encompasses the homeodomain from an E7.0 mouse embryo cDNA library. The following Mbx-specific primers were designed based on homologies between human and zebrafish *Mbx* cDNAs: F1 5′-ACTACCCAGATGTGGTGATGCG-3′ and R1 5′-TGCTCCTGCAGACGGAAGAGGCTCAG-3′. The resulting DNA fragments were inserted into the pGEM-T Easy cloning vector (Promega), and sequenced. Thereafter, RACE (5′- and 3′- rapid amplification of cDNA ends) was performed using the SMART RACE cDNA amplification kit (Clontech). The following primers were used: 5RA1, 5′-CGGCACGGGAGTCCTCTTCACGGTCCAG-3′; 5RA2, 5′-AGGATCACCACTGG GCAAACCAGGAGGC-3′; RA1, 5′-CTGGACCGTGAAGAGGACTCCCGTGCCG-3′; 3RA2, 5′-CGGGCTGCAAGAGGGGCAGCCCTAAG-3′. The resulting DNA fragments were inserted into the pGEM-T Easy cloning vector and sequenced. All sequence analyses were carried out in both directions. We thus obtained a full-length mouse *Mbx (*midbrain homeobox) cDNA whose predicted amino-acid sequence is shown in Fig. 1A. The N- and C-terminal regions are highly conserved between human and mouse (95% and 72% identity, respectively). In human and zebrafish, two distinct splicing variants produce long and short forms which differ by only five amino acids (Kawahara et al., unpublished results). The sequence shown here corresponds to the long form, whereas tentative short-form-specific primer sets did not yield any results (data not shown). The homeodomain of Mbx is identical among mouse, human and zebrafish, and has some similarity to that of Aristaless and Pax family proteins (Fig. 1B). The Aristaless and Pax proteins display glutamine and serine, respectively, at the critical position 50 in their homeodomains whereas Mbx has a lysine residue at this position. We conclude that Mbx is a novel homeodomain protein that is highly conserved between mouse, human and zebrafish.

Whole mount in situ hybridization (Lowe and Kuehn 2000) was carried out at early stages (E7.5 to E11.5) of development to determine the spatio-temporal expression pattern of *Mbx* mRNA. RNA probes corresponded to the *Mbx* coding region. Transcripts of *Mbx* were first

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Fig. 1 **A** Predicted amino acid sequence of the mouse *Mbx* gene product. The homeodomain is *underlined*. **B** Sequence alignment of the *Mbx* homeodomain with those of *Alx4* (Qu et al. 1997), *Cart1* (Zhao et al. 1993), *Pax7* (Jostes et al. 1990) and *Ptx2* (Arakawa et al. 1998). *Hyphens* indicate identity

A

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MQHYGVNGYSLHAMNSLSAMYNLHQQAAQQAQHAPDYRPS      40
VHALTLAERLAGCTFQDIILEARYGSQHRKQRRSRTAFTA      80
QQLEALEKTFQKTHYPDVVMRERLAMCTNLPEARVQVWFK     120
NRRAKFRKKQRSLQKEQLQKQKEAEGSHGEGKVEAPASDT     160
QLETEQPPGLPSGDPPAELQLSLSEQSASESAPEDQLDRE     200
EDSRAGEPKAEKSPGSESKVPGCKRGSPKADSPGSLAITP     240
AAPGGGLLGPSHSYSSSPLSLFRLQEQFRQHMAATNNLMH     280
YSSFEVGGPAPAAAAAAAAAVPYLGVNMAPLSSLHCQSYY     320
QSLSAAAAAHQGVWGSPLLPAPPTGLAPASAALNSKTTSI     360
ENLRLRAKQHAASLGLDTLPN 381
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B

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mMbx:   QRRSRTAFTAQQLEALEKTFQKTHYPDVVMRERLAMCTNLPEARVQVWFKNRRAKFRKKQ  %
hMbx:   ------------------------------------------------------------ 100
zMbx:   ------------------------------------------------------------ 100
Alx4:   K--N--T--SY---E---V----------YA--Q---R-D-T---------Q-----W--RE 71.6
Cart1:  K--H--TF-SL---E---V----------YV--Q--LR-E-T---------Q-----W--RE 68.3
Pax7:   ------T---E---E---A-ER-----IYT--E--QR-K-T---F----S----RW--QA 66.7
Ptx2:   ---Q--H--S---QE--A---RNR---MST--EI-VW-N-T----R---------W--RE 63.3
```

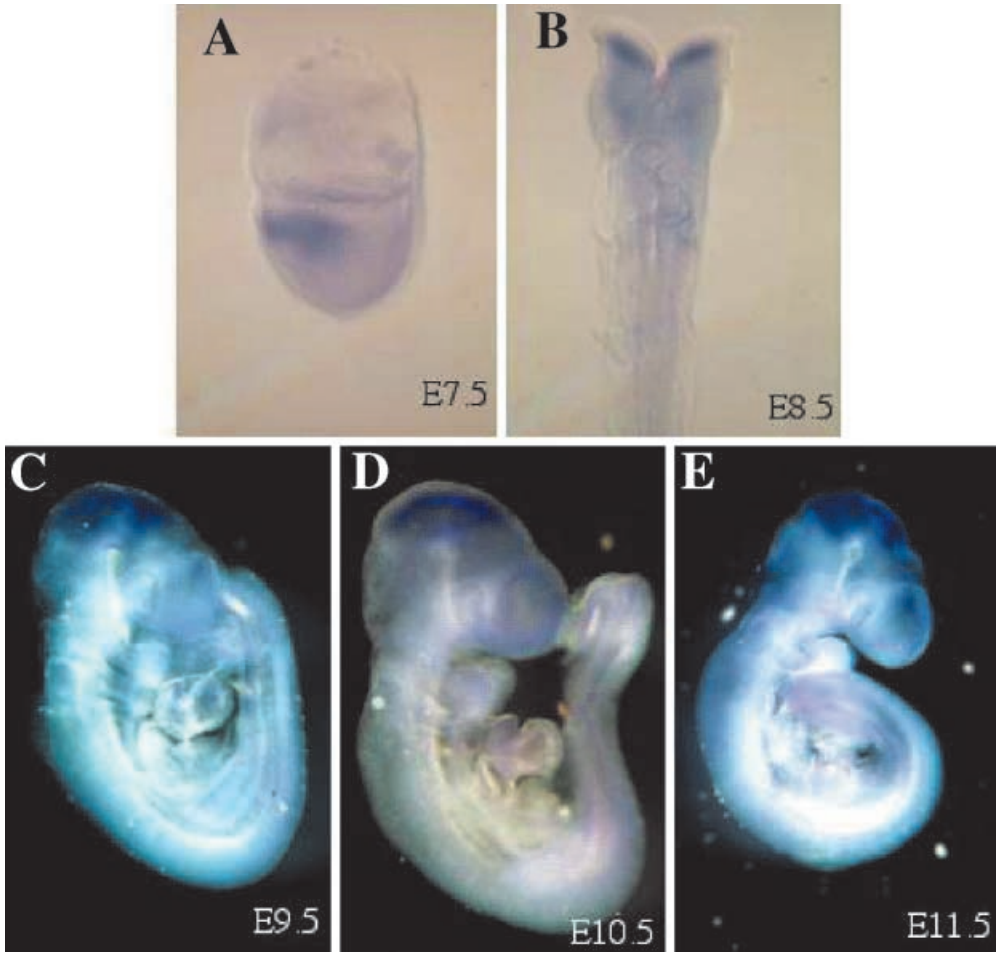

Fig. 2A–E Expression patterns of *Mbx* during early embryonic development. Developmental stages are indicated in the photographs. Expression of *Mbx* is detected from E7.5 onward by whole-mount in situ hybridization. **A** E7.5. Expression is seen in the region of anterior neural induction in the gastrulating embryo. **B** E8.5. *Mbx* transcripts are detected in the anterior neural folds. **C** E9.5. Expression is mainly seen in the midbrain region. **D** E10.5 and **E** E11.5. The expression pattern has expanded to include hindbrain, midbrain and forebrain regions

detected in the anterior region of the E7.5 embryo (Fig. 2A). Expression was restricted to the anterior neuroectoderm at E8.5 (Fig. 2B). A predominant signal was seen in the prospective midbrain region at E9.5 (Fig. 2C) where it persisted through E10.5 and E11.5 (Fig. 2D, E). However, from E9.5 onward, the expression of *Mbx* was also observed in the forebrain region. For the analysis of expression beyond E11.5, we carried out section in situ hybridization (Robinson and Romero 1991). Sagittal sections (5 μm) of wax-embedded embryos were hybridized

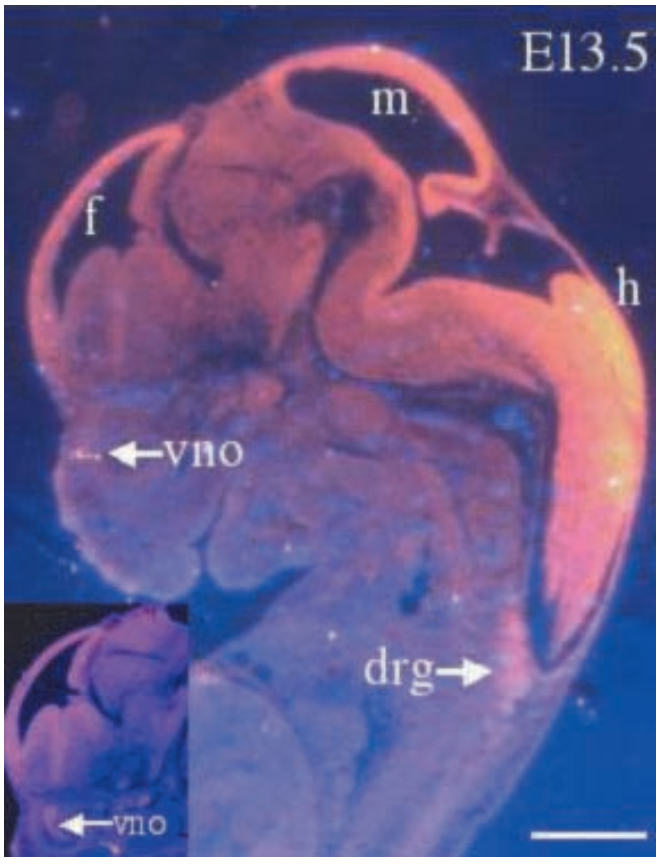

Fig. 3 Expression of *Mbx* detected by in situ hybridization. Sagittal sections of the head at E13.5 were hybridized with antisense probes. No significant signals were obtained by hybridization with a sense probe (data not shown). Expression was detected in the forebrain (*f*), midbrain (*m*) and hindbrain (*h*) regions. In addition, it was detected in the vomeronasal organ (*vno*, Jacobson's organ) and dorsal root ganglions (*drg*). The *white bar* indicates 350 μm

to ^{33}P-labeled riboprobes. RNA probes were prepared using T7 polymerase (Ambion) according to the manufacturer's instructions. Exposure time for radioactive signal detection was 21 days. Riboprobes corresponded to the coding region that encompasses the homeodomain. The result is shown in Fig. 3. There are strong signals over the hindbrain, midbrain and forebrain regions, including the vomeronasal organ (Jacobson's organ) and dorsal root ganglions (marked by arrows). Thus, the expression patterns of *Mbx* in early mouse development, as detected by our probe, are more complex than those described for zebrafish.

In conclusion, the molecular structure of Mbx is highly conserved among mouse, human and zebrafish. During early embryonic development *Mbx* is predominantly expressed in the prospective midbrain region, at least until E9.5. Thus, at these stages, *Mbx* is a useful midbrain marker. Subsequently, additional expression domains of *Mbx* include forebrain as well as hindbrain regions.

As this study goes to press, we note that the mouse *Mbx* gene has just been independently described by Ohtoshi et al. (2002).

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References

Arakawa H, Nakamura T, Zhadanov AB, Fidanza V, Yano T, Bullrich F, Shimizu M, Blechman J, Mazo A, Canaani E, Croce CM (1998) Identification and characterization of the *ARP1* gene, a target for the human acute leukemia *ALL1* gene. Proc Natl Acad Sci USA 95:4573–4578

Beddington RSP, Robertson EJ (1998) Anterior patterning in mouse. Trends Genet 14:277–284

Jostes B, Walther C, Gruss P (1990) The murine paired box gene, *Pax7*, is expressed specifically during the development of the nervous and muscular system. Mech Dev 33:27–37

Lowe LA, Kuehn MR (2000) Whole-mount in situ hybridization to study gene expression during mouse development. Methods Mol Biol 137:125–137

Martinez-Morales JR, Signore M, Acampora D, Simeone A, Bovolenta P (2001) *Otx* genes are required for tissue specification in the developing eye. Development 128:2019–2030

Mathers PH, Grinberg A, Mahon KA, Jamrich M (1997) The Rx homeobox gene is essential for vertebrate eye development. Nature 387:603–607

Ohtoshi A, Nishijima I, Justice MJ, Behringer RR (2002) *Dmbx1*, a novel evolutionarily conserved paired-like homeobox gene expressed in the brain of mouse embryos. Mech Dev 110:241–244

Qu S, Li L, Wisdom R (1997) Alx-4: cDNA cloning and characterization of a novel paired-type homeodomain protein. Gene 203:217–223

Robinson DJ, Romero J (1991) Sensitivity and specificity of nucleic acid probes for potato leafroll luteovirus detection. J Virol Methods 34:209–219

Suda Y, Hossain ZM, Kobayashi C, Hatano O, Yoshida M, Matsuo I, Aizawa S (2001) Emx2 directs the development of diencephalon in cooperation with Otx2. Development 128:2433–2450

Zhao GQ, Zhou X, Eberspaecher H, Solursh M, de Crombrugghe B (1993) Cartilage homeoprotein 1, a homeoprotein selectively expressed in chondrocytes. Proc Natl Acad Sci USA 90:8633–8637