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Mechanisms of Development 109 (2001) 427–431

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Gene expression pattern

Cloning and expression of the TALE superclass homeobox Meis2 gene during zebrafish embryonic development

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Received 20 April 2001; received in revised form 3 September 2001; accepted 3 September 2001

Abstract

Meis and Prep/Pknox (MEINOX family) proteins, together with Pbx (PBC family) proteins, belong to the TALE superfamily characterized by an atypical homeodomain containing three additional amino acids between helix 1 and helix 2. Members of the MEINOX and PBC families have been isolated in *Caenorhabditis elegans*, *Drosophila*, *Xenopus*, chick, mouse and human, and play crucial roles in many aspects of embryogenesis. Here, we report the isolation of *meis2* in zebrafish. Expression of *meis2* is first detected at the beginning of gastrulation. Later during embryogenesis, *meis2* transcripts are found in distinct domains of the central nervous system with the strongest expression in the hindbrain. Expression was also detected in the isthmus, along the spinal cord and in the lateral mesoderm. As development proceeds, *meis2* is also expressed in the developing retina, pharyngeal arches, and in the vicinity of the gut tube.

Keywords: Homeobox; TALE; Meis; Zebrafish; Development

1. Results and discussion

The *Drosophila* TALE proteins, Extradenticle (EXD) and Homothorax (HTH), have been implicated in numerous important regulatory processes throughout embryogenesis, including HOM-C dependent regulation of segment identity (Mann, 1995; Wilson and Desplan, 1995), peripheral nervous system (PNS) patterning (Kurant et al., 1998), and establishment of territories in the eye (Pai et al., 1998; Pichaud and Casares, 2000), wing and leg imaginal discs (Morata and Sanchez-Herrero, 1999). In addition, HTH may be involved in determination of antennal identity (Dong et al., 2000; Yao et al., 1999) and salivary gland development (Andrew et al., 2000; Henderson and Andrew, 2000). In vertebrates, members of the PBC and MEINOX families have been shown to play crucial roles in hindbrain patterning (Ferretti et al., 2000; Maconochie et al., 1997; Popperl et al., 1995; Salzberg et al., 1999) and limb outgrowth (Capdevila et al., 1999; Gonzalez-Crespo et al., 1998; Mercader et al., 1999; Mercader et al., 2000). Yet, their function in multiple aspects of vertebrate development is still not completely understood.

We have isolated *meis2*, a member of the TALE superclass of homeodomain proteins. The 2.8 kb *meis2* cDNA contains an open reading frame encoding a protein of 393 amino acids, which exhibits the atypical homeodomain and the MEINOX domain characteristic of Meis proteins (Burglin, 1998). Amino acid sequence comparison with human, mouse, chicken, *Xenopus*, *Drosophila* and *Caenorhabditis elegans* Meis proteins reveals extremely high homology within these conserved domains (the MEINOX and the homeodomain of zebrafish and mouse *meis2* proteins are 97.5 and 98.4% identical, respectively). Phylogenetic analysis suggests that the zebrafish Meis isolated in this study is a true ortholog of chick and mouse Meis2 proteins (Fig. 1B), as it is clustered with the cMeis2 and mMeis2 orthologs.

Zebrafish *meis2* transcripts are first detected during gastrulation, at 60% epiboly, in two lateral domains within the ectoderm (Fig. 2A,B). At 80% epiboly, these expression domains have enlarged (Fig. 2C), now covering the whole area of the prospective hindbrain (Woo and Fraser, 1995). Double labeling with otx2, a marker for the presumptive forebrain and midbrain (Li et al., 1994) confirms that *meis2* expressing cells are restricted to the presumptive hindbrain. Between 100% epiboly and late bud stage (Fig. 2D), a second expression domain appears, presumably in the anterior neural plate (Fig. 2D, arrowhead). During somito-

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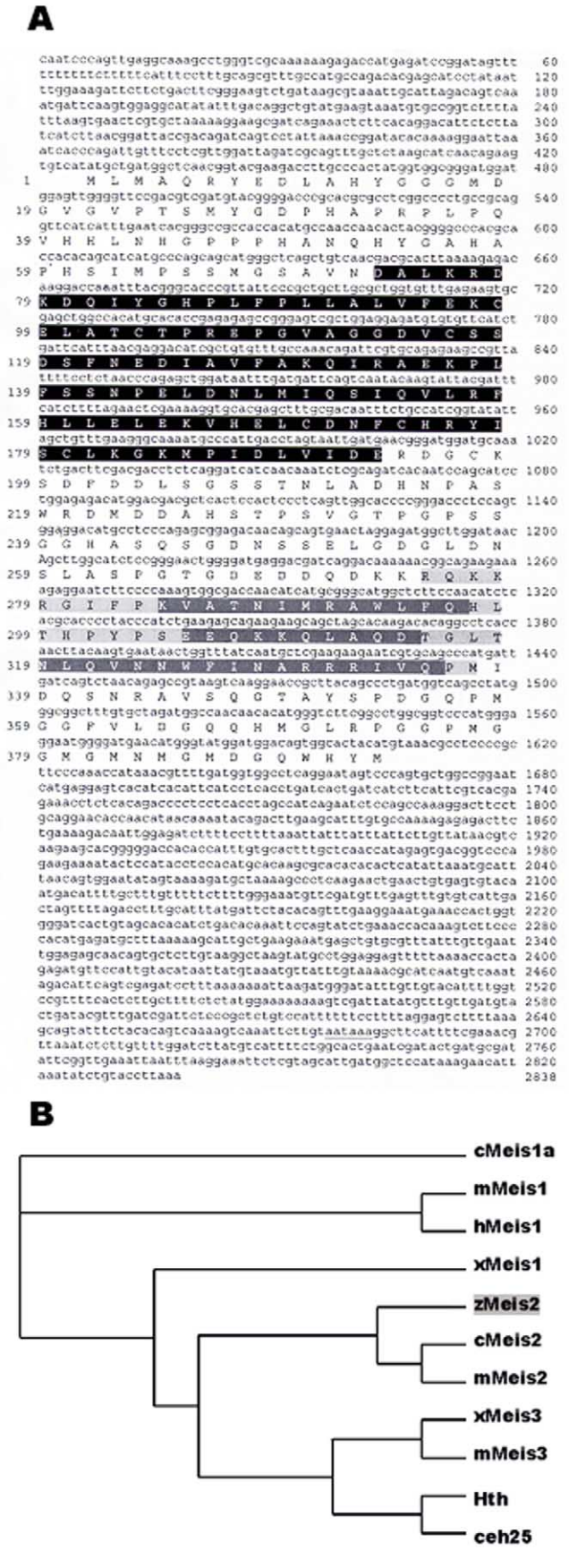
0925-4773/01/$ - see front matter
PII: S0925-4773(01)00554-8



genesis (Fig. 2E–G), expression is detected within the forebrain, posterior midbrain and anterior hindbrain (rhombomeres, r1 through r3; Fig. 2F). In the spinal cord, strong expression is seen anteriorly, that gradually weakens posteriorly (Fig. 2E,F). *meis2* expression is also seen in the somite, but is absent from the presomitic mesoderm. The alternate pattern shown by *meis2* and *myoD* expression indicates that *meis2* is expressed in the anterior border of each somite (Weinberg et al., 1996; Fig. 2G, arrows). *meis2* expression is also found in two lateral mesodermal domains, which extend anteriorly up to the mid–hindbrain junction (Fig. 2E–F).

At 24 h post-fertilization (hpf), *meis2* expression continues in several distinct domains within the developing forebrain, including basal ventral telencephalon and hypothalamus (Fig. 2H). In the midbrain, expression is seen in both the ventral tectum (Fig. 2H, arrowhead) and tegmentum (Fig. 2H, arrow). Interestingly, several clusters of *meis2* positive cells are also detected laterally in the ventral part of the midbrain (Fig. 2J, asterisks), similar to the expression of *hoxa1a* (Shih et al., 2001). Groups of cells at the rostral border, as well as in the caudal dorsal part of the cerebellum, also express *meis2* (Fig. 2H,J). Within the hindbrain (Fig. 2J), r3 and r4 exhibit the strongest expression, while other rhombomeres retain patchy expression ventrally. The nascent heart tube also shows weak levels of *meis2* expression (Fig. 2I,J, arrow). In the trunk, *meis2* is also expressed in the hypaxial musculature (Fig. 2K).

meis2 remains strongly expressed in the zebrafish hindbrain until 77 hpf (Fig. 3A–D). Additional expression is seen in the retina (Fig. 3E–G). Surprisingly, we did not observe expression in the pectoral fin buds at any stages (Fig. 3H–J). We detected *meis2* transcripts in pharyngeal arches at 45 and 55 hpf (Fig. 3K,L), notably in the region of the jaw in the presumptive mandibular and hyoid primordia (Fig. 3L). We also found *meis2* expression in the vicinity of the gut tube, presumably in the pronephric glomerulus or an endodermally-derived organ, such as the liver or pancreas (Fig. 3L).

Thus, although the zebrafish *meis2* expression pattern resembles that of the mouse *Meis2* ortholog, especially in the central nervous system (CNS), our study points out some striking differences. Firstly, no expression was observed in the pectoral fin buds, as described for the mouse and chick (Capdevila et al., 1999; Mercader et al., 1999). Secondly, the restriction of zebrafish *meis2* expression in the anterior portion of the somites was not observed for mouse *Meis2*. In addition, *Meis2* is expressed in the segmental plate in the mouse, but not in zebrafish (Cecconi et al., 1997; Oulad-

Fig. 1. (A) Nucleotide sequence and open reading frame of the zebrafish *meis2* gene. The conserved MEINOX domain is highlighted in black. The homeodomain is highlighted in light grey and the three helixes in dark grey. (B) A phylogenetic tree established with TREEVIEW shows the evolutionary relationships between zebrafish Meis2 and Meis proteins of other species.

Abdelghani et al., 1997). Finally, whereas expression of *Meis1* but not *Meis2* was reported in the retina in mice (Toresson et al., 2000), we do find expression of *meis2* in the zebrafish retina at 36 hpf onwards.

2. Materials and methods

A 246 bp fragment, corresponding to a highly conserved region of *meis* genes, was isolated using a degenerate PCR-based approach (forward primer, 5′-ATHTTYGARAART-GYGAR-3′; and reverse primer, 5′-RTGRCARAARTT-RTCRCA-3′) from a 15–19 hpf zebrafish cDNA library (a generous gift from Dr Bruce Appel). The amplified fragment was subsequently used as a probe to obtain a full-length cDNA from a shield stage library available from RZPD (http://www.rzpd.de). One positive clone was isolated, sequenced in both directions and deposited in GenBank (accession number, AF170065).

Whole-mount in situ hybridizations was performed as described (Hauptmann and Gerster, 1994).

3. Note added in proof

While this manuscript was in preparation, the cloning of the same gene and its expression during embryogenesis was reported by T. Zerucha and V.E. Prince (Zerucha and Prince, 2001).

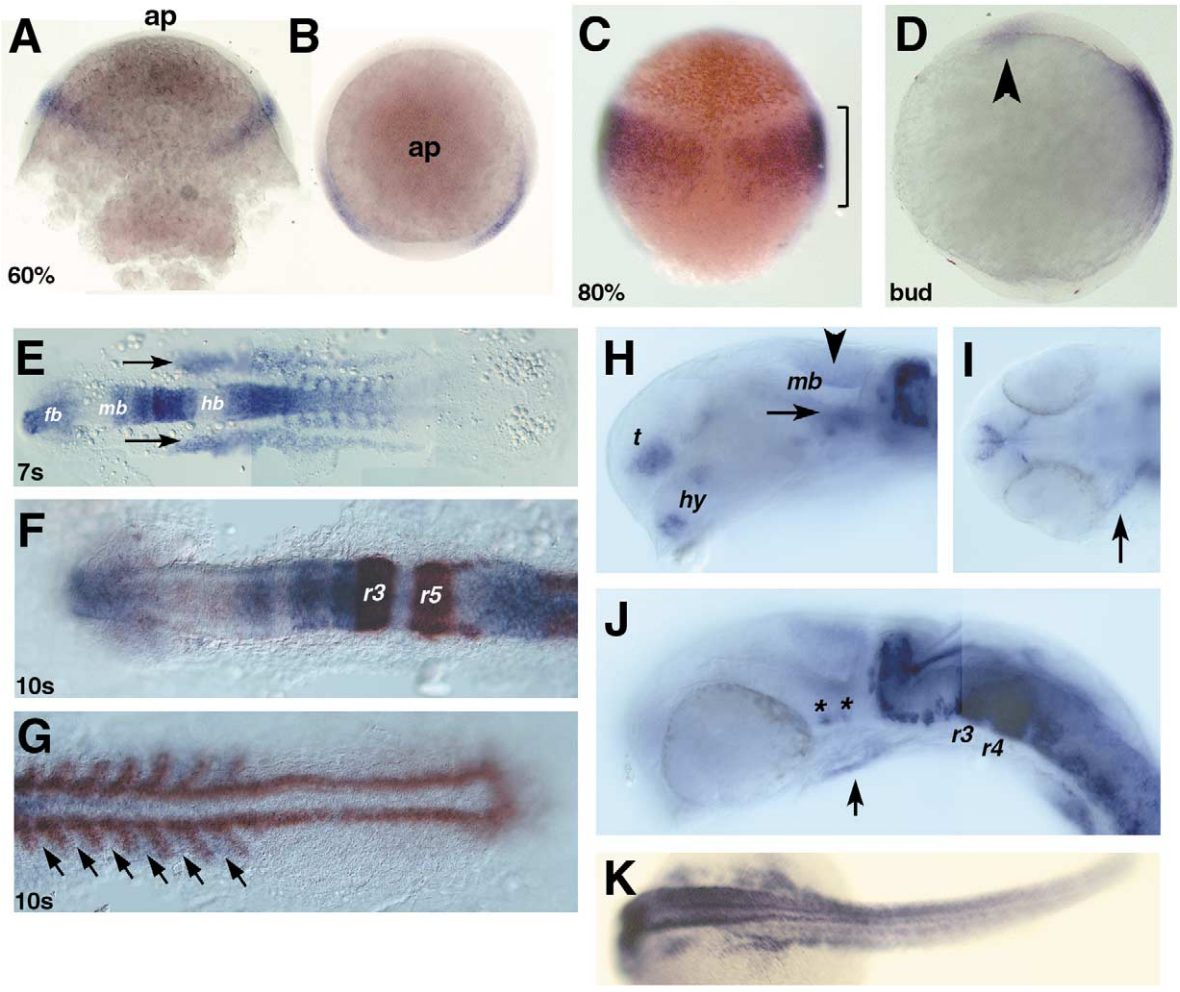


Fig. 2. Expression of *meis2* in wildtype zebrafish embryos from gastrulation to 24 h post-fertilization (hpf). Dorsal (A) and animal pole (B) views at 60% epiboly. (C) Eighty percent epiboly, dorsal view; and (D), bud stage, lateral view. Arrowhead points at the new expression in the presumptive anterior neural plate. (E) Seven-somite stage, dorsal, flat-mounted view. Arrows point at the bilateral mesodermal domains. (F,G) Ten-somite stage, dorsal, flat-mounted view showing expression of *meis2* (in blue), *Krox20* (F) and *myoD* (G) (in brown). Arrows point at the anterior border of each somite. (H–K) expression of *meis2* at 24 hpf. (H) Lateral view, anterior to the left, dorsal to the top of the fore- and midbrain regions. Arrowhead points at the ventral tectum, and arrow points at the tegmentum. (I) Dorsal view, anterior to the left of a flat-mounted embryo showing expression in the forming heart tube. (J) Lateral view, anterior to the left, dorsal to the top of the mid- and hindbrain regions. Asterisks show patchy expression in the ventral midbrain. (K) Dorsal view, anterior to the left showing the expression in the hypaxial muscles. fb, forebrain; hb, hindbrain; mb, midbrain; hy, hypothalamus; r(3, 4, 5), rhombomere (3, 4, 5); sc, spinal cord; t, telencephalon.

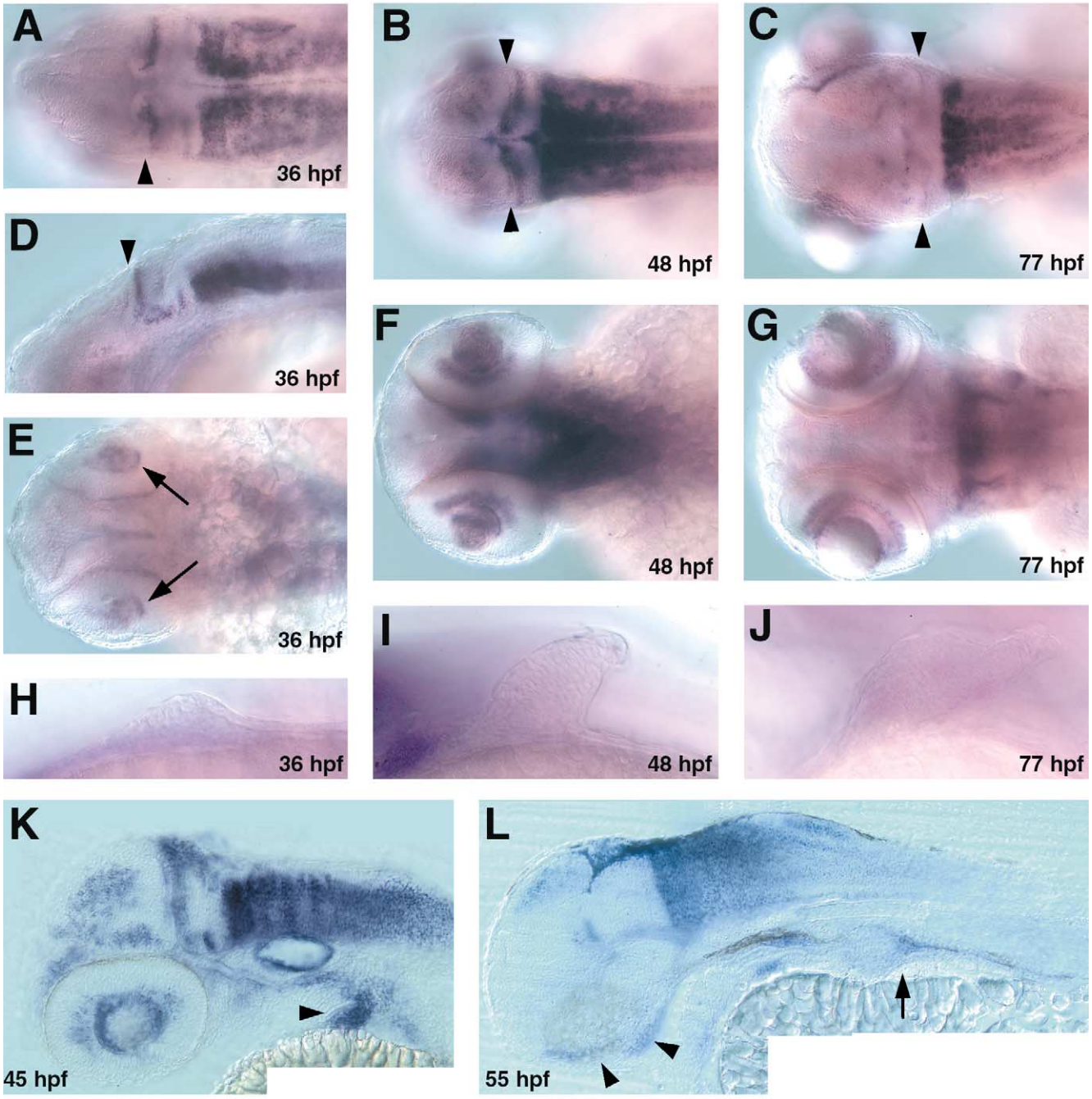


Fig. 3. Expression of *meis2* in late embryonic stages at: (A,D,E,H), 36; (K), 45; (B,F,I), 48; (L), 55; and (C,G,J), 77 hpf. (A–C) Dorsal views, anterior to the left showing overall expression in the brain region at: (A), 36; (B), 48; and (C), 77 hpf. Arrowhead points at the expression in the rostral part of the cerebellum. (D) lateral view of the embryo depicted in (A). Arrowheads point at the expression in the rostral part of the cerebellum. (E–G) Dorsal views, anterior to the left showing expression in the retina at: (E), 36 (E, arrows); (F), 48; and (G), 77 hpf. (H–J) Lateral views, anterior to the left of a pectoral fin bud at: (H), 36; (I), 48; and (J), 77 hpf. (K) Sagittal section of the head region at 45 hpf showing overall brain expression. Arrowhead points at expression in the posterior pharyngeal arch. (L) Sagittal section at 55 hpf showing expression in the presumptive mandibular and hyoid primordium (arrowhead) and gut region (arrow).

Acknowledgements

The authors wish to thank Dr Bruce Appel and the RZPD for providing cDNA libraries, Zoltan Varga for helpful discussions, Karen Lunde and Marianne Voz for critical reading of the manuscript. F.B. holds a doctoral fellowship from the 'Fonds pour la Formation à la Recherche dans l'industrie et dans l'Agriculture (FRIA)'. B.P. is 'Chercheur Qualifié' from the 'Fonds National pour la Recherche Scientifique (FNRS)'. This work was supported by a grant from the EC Fifth Framework (QLRT-1999-00149 to B.P. and W.D.).

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