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Gene expression pattern

Mapping and expression analysis of the mouse ortholog of *Xenopus Eomesodermin*

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Abstract

The T-box gene family has been conserved throughout metazoan evolution. The genes code for putative transcription factors which share a uniquely defining DNA binding domain, known as the T-box (Bollag et al., 1994). They are implicated in the control of diverse developmental processes by their highly specific expression patterns throughout gastrulation and organogenesis in mouse and other species (Chapman et al., 1996) (Gibson-Brown et al., 1998), and by mutations in T-box genes that have profound developmental effects (Papaioannou, 1997; Chapman and Papaioannou, 1998; Papaioannou and Silver, 1998). In this report, we describe the mapping and expression pattern of the mouse ortholog of a gene, *Eomesodermin*, first identified in *Xenopus* (Ryan et al., 1996). The mouse gene was previously reported (Wattler et al., 1998) under the name *MmEomes*. The gene maps to mouse chromosome 9 in a region syntenic with human chromosome 3p. Mouse *eomesodermin* is expressed in the trophoblast of the blastocyst and in its derivative, the chorionic ectoderm. At gastrulation, *eomesodermin* is expressed in the primitive streak and embryonic mesoderm as well, but this expression disappears prior to the end of gastrulation. Later, *eomesodermin* is expressed in the developing forebrain, in a pattern largely overlapping a closely related T-box gene, *Tbr1* (Bulfone et al., 1995), and is also seen in a localized area of each limb.

Keywords: Mouse; Embryogenesis; Organogenesis; T-box genes; *eomesodermin*; *Tbr1*; Extraembryonic ectoderm; Chorion; Telencephalon; Cerebral cortex; Brain; Primitive streak; Mesoderm; Limbs

1. Results

Based on the amino acid sequences of the T-box region of known genes, degenerate PCR primers were designed and used to perform PCR on a mouse blastocyst stage cDNA library (gift of Barbara B. Knowles and Sue-Yun Hwang). The nucleotide sequence of the T-box of a mouse ortholog of *Xenopus Eomesodermin* was compiled from six clones, and found identical to that previously reported (Wattler et al., 1998). It is 82% identical to the T-box of *Xenopus Eomesodermin* at the nucleotide level, with the amino acid sequence 93% identical.

Using whole-mount in situ hybridization, we found evidence of *eomesodermin* transcripts in the trophoblast of embryos at 4.5 days *post coitus* (dpc) (Fig. 1A). A faint but clear signal was seen throughout the polar and lateral trophectoderm, whereas the inner cell mass appeared negative. Signal above background was also seen in the mural trophoblastic giant cells. At the early egg cylinder stage, *eomesodermin* is expressed in the extraembryonic ectoderm which forms the ectoderm of the chorion, a polar trophoblast derivative. At slightly later egg cylinder stages, a second area of expression was seen in the primitive streak and lateral wings of newly formed mesoderm (Fig. 1B,C). Expression in this area is transient, ceasing abruptly between 7.5 and 8.0 dpc (Fig. 1C). *Eomesodermin* transcripts were not seen again in the embryo until 10.5 dpc

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when they appeared in the mantle zone of the telencephalon, persisting at least through 12.5 dpc, the latest stage examined (Fig. 1D–G). Expression in the developing cerebral cortex overlaps *Tbr1* expression (Bulfone et al., 1995), the only other member of this T-box subfamily (Papaioannou and Silver, 1998), but with an additional stripe of *eomesodermin* expression in the anterior/ventral part of the cortex (arrow in Fig. 1F). Starting at 11.5 dpc, expression was seen in the forelimbs and, lagging slightly behind, in the hindlimbs. This was localized to a spot in the mesenchyme of

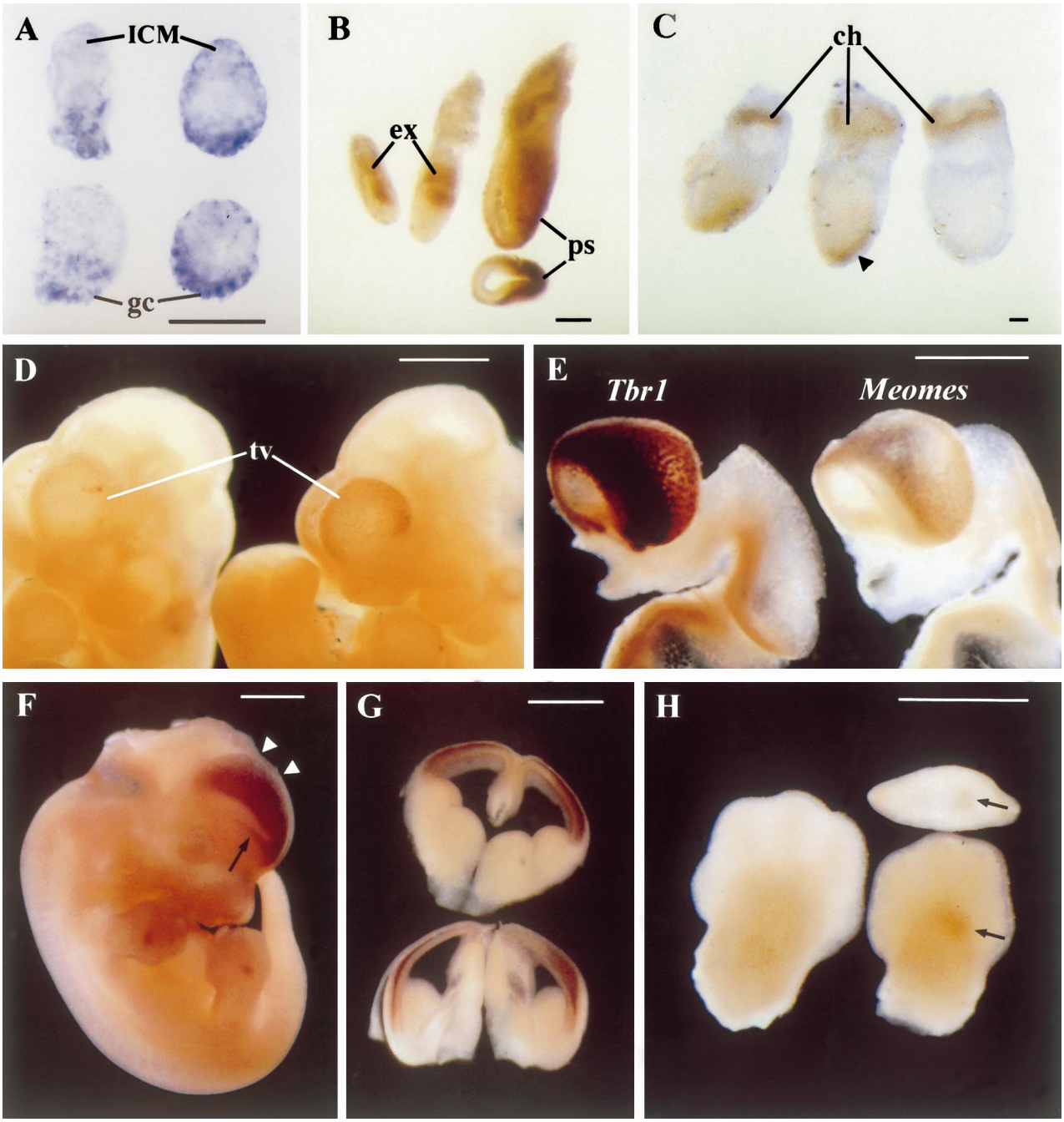

Fig. 1. Expression of *eomesodermin* in mouse embryos as shown by in situ hybridization. (A) 4.5 dpc blastocysts, inner cell mass toward the top, sense probe on left, antisense on right; (B) 6.5 dpc embryos, posterior on right. The thick section at bottom is through the embryonic region of an advanced egg cylinder. (C) 7.5 dpc embryos, posterior on right. Arrowhead indicates expression in the primitive streak and mesoderm which is not seen in the developmentally more advanced embryo on the right. (D) 10.5 dpc embryos, sense on left, antisense on right. (E) Dissected brains from 11.5 dpc embryos comparing *Tbr1* with *eomesodermin*. (F,G) 12.5 day embryos: arrowheads indicate the positions of the thick slices in (G). Note spots of expression in both limbs. (G) Rostrally directed view of thick slices of the head through the telencephalon. (H) 12.5 dpc isolated forelimbs, sense on the left, antisense on the right, and a thick section through the autopod (dorsal surface on top). ch, chorion; ex, extraembryonic ectoderm; gc, trophoblastic giant cells; ICM, inner cell mass; ps, primitive streak; tv, telencephalic vesicle. Scale bar, 100 mm in (A–C); 1 mm in (D–H).

the autopod, at the base of the fourth digit (Fig. 1F and arrows in H). *In situ* hybridization at 11.5 dpc using a probe from the 3′ end of the *eomesodermin* cDNA, showed expression identical to that observed using the *eomesodermin* T-box probe (data not shown). This result and the unique areas of expression detected by the *eomesodermin* probe rules out cross hybridization of the T-box probes for the closely related genes, *eomesodermin* and *Tbr1*.

Using the interspecific backcross panel, BSS (Rowe et al., 1994), *eomesodermin* was localized to centimorgan position 64 on chromosome 9, between markers D9Mit17 and Cpu3, co-segregating with marker D9Ert428e. Three candidate mutations with neurological defects, *ducky* (*du*), *tippy* (*tip*), and *spinner* (*sr*), also map to this region (Fig. 2).

2. Methods

PCR using vector primers T7 (5′-GTAATACGACTCACTATAGGGC-3′) and SP6 (5′-ATTTAGGTGACACTATAG-3′) was performed on approximately 2×10^7 bacterial cells of the blastocyst stage cDNA library. Cycling parameters were 15 s at 94°C, 15 s at 45°C, and 2 min at 72°C for 20 cycles. PCR using primers FOR1 (5′-ACIGA{AG}ATG{AG}IT{AG}TIAC-3′ where I = inosine) and REV1 (5′-C{GT}{AG}AAICC{TC}TTIGC{GA}AA-3′) was performed on products of the initial reaction. Cycling parameters were 15 s at 94°C, 15 s at variable annealing temperature, and 1 min at 72°C for 40 cycles. Annealing temperature started at 55°C, decreased by 0.5°C per cycle for 19 cycles, and was maintained at 45°C for the remaining 20 cycles. PCR using the degenerate primers FOR2 (5′-{GCA}GI{CA}GIATGTT{TC}CC-3′) and REV2 (5′-CC{TC}TTIGC{AG}AAIGG{AG}TT}-3′) was performed on products of the second reaction using the same parameters. PCR using the primers XBA-FOR2 (5′-GTCTAGA{GCA}GI{CA}GIATGTT{TC}CC-3′) and X-HO-REV2 (5′-ACTCGAGCC{TC}TTIGC{AG}AAIGG-{AG}TT}-3′) was performed on the products of the third reaction. Cycling parameters were 15 s at 94°C, 15 s at 45°C, and 1 min at 72°C for 20 cycles. Products of this reaction were directionally cloned into the *Xba*I and *Xho*I sites of pBluescript SK+. Sequencing of clones was performed with an ABI Prism Model 373 Automated DNA Sequencer.

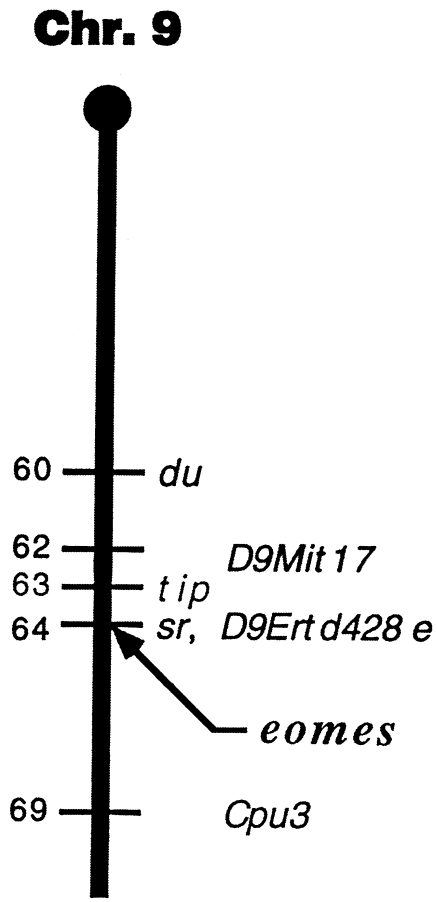

Fig. 2. Chromosomal map position for the mouse *eomesodermin* gene. Linkage of chromosome 9 is shown with some reference loci.

Whole-mount in situ hybridization on paraformaldehyde-fixed, random-bred mouse embryos of different stages was performed essentially according to Wilkinson (1992) using sense and antisense probes from the T-box region of *Tbr1*, *eomesodermin* and the *eomesodermin* 3′ EST. At later stages the yolk sac and placenta were removed prior to *in situ* hybridization.

To map *eomesodermin*, we analyzed the BSS interspecific backcross panel from the Jackson Laboratory (Rowe et al., 1994). Southern blots containing electrophoresed *Bgl*II-digested genomic DNA from 94 backcross animals were hybridized to a 3′ *eomesodermin* probe (EST clone 556226, accession number AA104395) at stringent conditions. Map position was determined by comparison of the strain distribution patterns obtained in this experiment with the database held at the Jackson Laboratory (www.jax.org/resources/documents/emdata/).

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