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# Genomic structure and chromosomal mapping of the mouse transcription factor TEF-5 (*Tead3*) gene

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Four members of the Transcriptional Enhancer Factor (TEF) family of transcription factors have been identified in human and mouse (TEF-1, TEF-3, TEF-4, and TEF-5; approved gene symbols TEAD1, TEAD4, TEAD2, and TEAD3 respectively; Yasunami et al. 1995, 1996; Jacquemin et al. 1996, 1997; Yockey et al. 1996; Hsu et al. 1996; Kaneko et al. 1997; reviewed in Jacquemin and Davidson 1997). These factors possess the TEA DNA binding domain which recognizes degenerate sites in the enhancers and promoters of several viral and cellular genes. The TEFs comprise a short variable N-terminal region preceding the highly conserved TEA domain, a variable hydrophobic region immediately after the TEA domain, and a large, well-conserved C-terminal domain (see Fig. 1A).

Expression of the murine (m)Tead factors is differentially regulated during development, the principal sites of expression being mitotic neuroblasts, skeletal and cardiac muscle, the placenta, lung, and several other viscera (see Jacquemin and Davidson 1997). Insertional mutagenesis showed that *Tead1* is essential for cardiac development (Chen et al. 1994). *Tead3* is first expressed in extraembryonic tissues such as the giant trophoblastic cells, and expression persists in the giant cells and labyrinthine region of the placenta throughout gestation (Jacquemin et al. 1998). *Tead3* expression is limited to the extraembryonic layers until around 9.5 days post coitum, when further expression in epithelia and developing viscera is also observed.

As gene targeting experiments will be required to further define the role of *Tead3* in placental development, we report here as a first step the isolation and characterization of the *Tead3* gene and the determination of its chromosomal location.

A λEMBL3 mouse genomic DNA library (kindly provided by J.-M. Garnier) was screened with a mix of the cDNAs encoding the Tead1, -2, -3 and -4 TEA domains as probes. After isolation, phages containing Tead3 were identified by PCR using *Tead3*-specific primers, and their DNA was analyzed by Southern blotting with the full-length *Tead3* cDNA. Following digestion with *Pvu*II, 4 hybridizing fragments ranging from 1.1 to 3.2 kb were cloned. These fragments were entirely sequenced and comparison with the Tead3 cDNA allowed identification of the introns and exons. Junctions between the fragments, and sequences upstream and downstream of the coding region were obtained by direct sequencing on the phage DNA. A total sequence of 12,622 base pairs (bp) was obtained.

Analysis of this sequence indicates that the *Tead3* gene comprises 13 exons (the smallest is 12 bp) and 12 introns (from 91 bp to 3582 bp; Fig. 1A). All of the intron-exon junctions conform with the GT/AG rule. The TEA DBD is encoded by three exons (II–IVA), each coding for one of the three putative α-helices. In chicken (c) two *Tead3* isoforms have been characterized with differences in the C-terminal α-helix of the TEA DBD encoded by exon IV. These isoforms may be generated by differential use of a duplication of this exon. Indeed, downstream of exon IVA, we found a variant copy of this exon (IVB, Fig. 1B). So far only exon IVA has been found in mammalian Tead3 cDNAs. In contrast, in TEAD1, only exon IVB has been found (Fig. 1B). However, in one exceptional case a TEAD1 cDNA from HeLa cells that we characterized previously contained a duplicated region at the end of the TEA domain corresponding to a splice variant in which both exons IVA and IVB were present (our unpublished data and see Fig. 1B). This TEAD1 variant was still able to specifically bind DNA in electrophoretic mobility shift assays (our unpublished data).

Alternative splicing of the four amino acids encoded by exon V was also observed. This exon was present in all the isolated mouse or human TEAD3 cDNAs, but was variable in TEAD1.

Comparison of the *Tead3* locus with that of *Tead2* (Suzuki et al. 1996) showed that the overall organization of the genes is conserved. The location of the intron-exon junctions for *Tead3* relative to the peptide sequence is shown in Fig. 2A. All the junctions are in analogous position to those of *Tead2* with the exception of the upstream and downstream borders of exon VII. A comparison of the exon VII frontiers for *Tead2* and *-3* is shown in Fig. 2B. This exon encodes part of the most variable region of the TEAD family.

We further determined the chromosomal localization of *Tead3*, using an interspecific backcross DNA panel (The Jackson Laboratory; Bar Harbor, Me; Rowe et al. 1994). The panel was generated by using DNA from 94 backcross animals from the reciprocal cross (C57BL/6JEi × SPRET/Ei)$F_1$ × SPRET/Ei ("Jackson BSS" backcross panel). Southern blot analysis with a Tead3 cDNA probe detected a restriction fragment length variation (RFLV) between DNAs of the C57BL/6J and *M. spretus*. The probe detected a 2-kb *Bam*HI fragment in C57BL/6 DNA and a 5-kb fragment in *M. spretus* DNA (data not shown). Therefore, the RFLV identified was used to follow the segregation of the *Tead3* locus in the Jackson BSS backcross panel.

The resulting data show that *Tead3* is 1.1 centimorgans (cM) distal to the *D17WSU92e* locus, 2.1 cM and 3.2 cM proximal to the *Tff3* (intestinal trefoil factor 3) or *D17Mit16* locus respectively, near the proximal end of Chr 17 (Fig. 3). The *Fkbp5* gene (FK506 binding protein 5) is also present in this region of Chr 17. In fact, *Tead3* co-segregates with *Fkbp5* gene in all 94 animals. Thus, we

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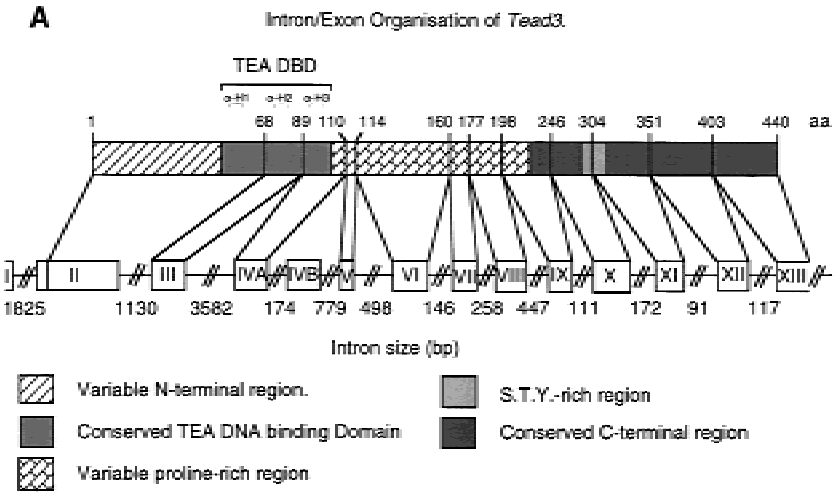


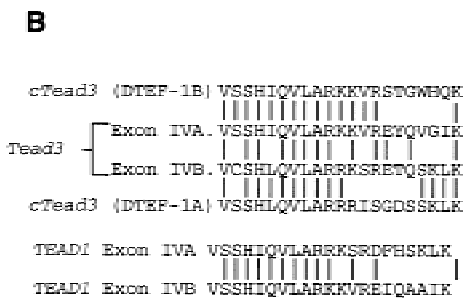


**Fig. 1. (A)** Genomic structure of *Tead3*. Complete exon-intron organization of *Tead3* gene relative to the amino acid (aa) sequence is indicated. The three putative α-helices of the TEA DNA-binding domain are shown. **(B)** Comparisons of the sequences of the alternative exons encoding the putative α-helix 3 of the TEA domains of chicken (c) and mouse (m) *Tead3* and human TEAD1.

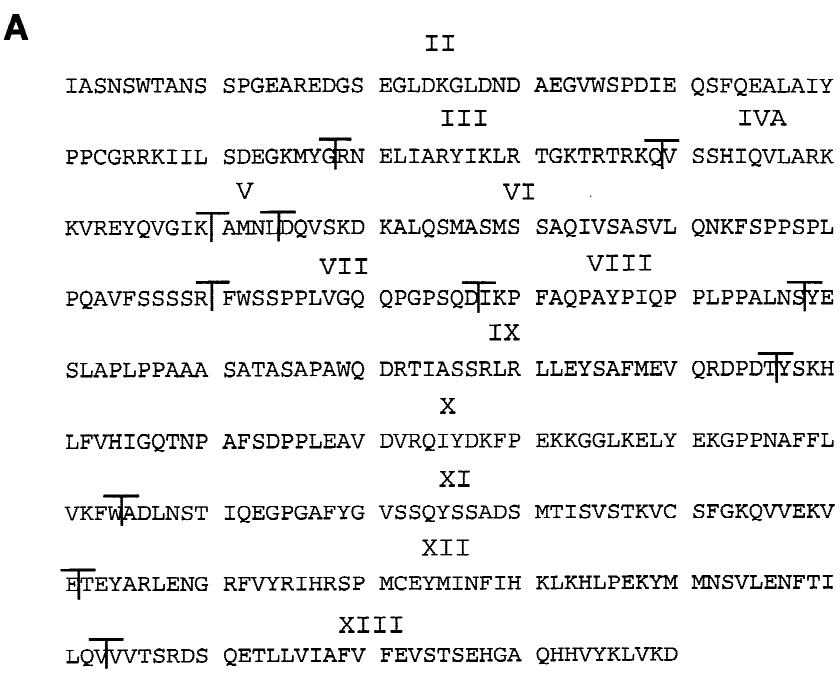



**Fig. 2. (A)** Location of the different exons on the *Tead3* amino acid sequence. Transition between the different exons is indicated. **(B)** Comparison of the exon VII frontiers for *Tead3* and *Tead2*. Exon VII is located in the most variable region between the different Teads.

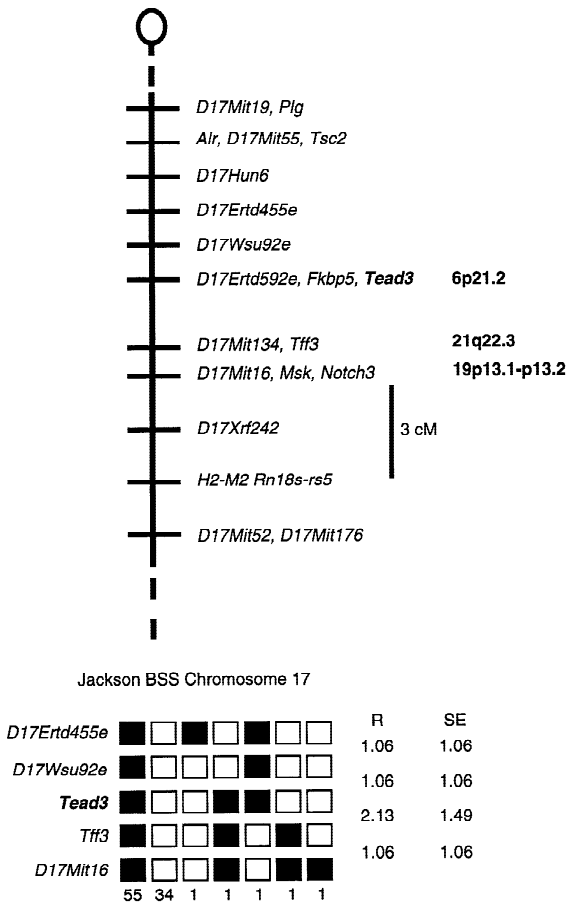


**Fig. 3.** The *Tead3* locus maps in the proximal region of mouse Chr 17. *Tead3* was mapped to mouse Chr 17 by interspecific backcross analysis with the Jackson BSS panel. A partial Chr 17 linkage map showing the location of *Tead3* in relation to linked genes or physical markers is shown. A scale bar of 3 cM is shown to the right of the chromosome. Where known, the chromosomal locations of human homologs of neighboring genes is indicated also on the right. The haplotype analysis, along with the number of animals used, the recombination frequency (R), and the standard error (SE), is shown below the figure.

conclude that *Tead3* is in close proximity to *Fkbp5* near the proximal end of Chr 17.

The location of *Tead3* is syntenic to that of TEAD3, which we previously mapped to 6p21.2 of Chr (Jacquemin et al. 1999). However, *Tff3*, located closed to *Tead3*, is located on human Chr 21. Consequently it appears that the end of syntenicity between human Chr 6 and mouse Chr 17 must lie between these two loci.

In conclusion, these results indicate that the genomic organisation of four TEAD genes is most probably very well conserved. These data could be useful for designing a common scheme for knock-out strategies of the different TEAD genes.

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