

FIG. 1. Localization of the PAC clone LLNLP704E17877Q3 by fluorescence *in situ* hybridization. **(a)** Metaphase spread after FISH with PAC LLNLP704E17877Q3 as a molecular probe. Signals are visible on both chromosomes 13 as indicated by arrows. Chromosomes were stained by propidium iodide and the PAC was labeled with FITC. **(b)** Same metaphase spread of lymphocytes with a normal karyotype GTG-banded prior to FISH (both chromosomes 13 are indicated by arrows).

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Localization of Human Transcription Factor TEF-4 and TEF-5 (TEAD2, TEAD3) Genes to Chromosomes 19q13.3 and 6p21.2 Using Fluorescence *in Situ* Hybridization and Radiation Hybrid Analysis

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Functional gene description: Transcriptional enhancer factors (TEFs)-4 and -5 (HUGO/GDB-approved gene symbols TEAD2 and TEAD3, respectively) belong to a family of transcription factors containing the TEA DNA-binding domain (3). This family comprises two other members designated TEF-1 and TEF-3 (renamed TEAD1 and TEAD4) (3, 4). First identified in the simian virus 40 enhancer, TEAD-binding sites have been principally studied in cardiac and skeletal muscle-specific promoters where they are referred to as M-CAT sites. The TEADs are expressed in different but partially overlapping patterns during mouse embryogenesis (3-5), most notably in skeletal and cardiac muscle, mitotic neuroblasts, and placenta. Mouse Tead2 is strongly expressed throughout the embryo from the onset of zygotic gene expression, while Tead3 is expressed in the extraembryonic tissues, notably the trophoblast giant cells and the labyrinthine region of the placenta (5). Human TEAD3 is expressed in the placental syncytiotrophoblast, and it is upregulated during trophoblast differentiation *in vitro* (5).

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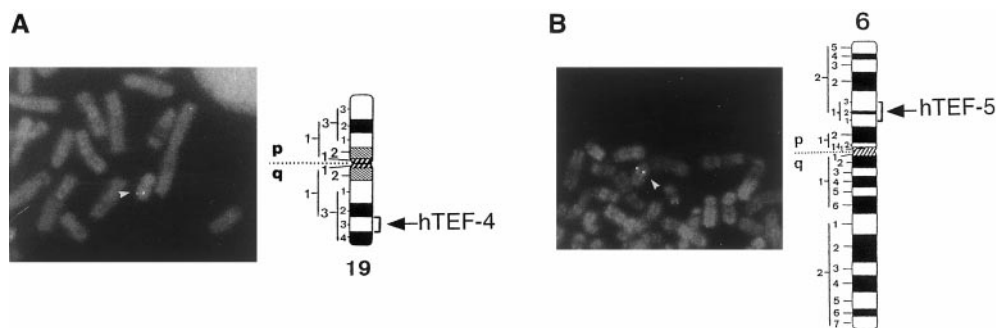


FIG. 1. (A) Fluorescence *in situ* hybridization to human metaphase chromosomes with TEAD2 (left). A chromosome 19 ideogram indicating localization of the TEAD2 gene to 19q13.3 (right). (B) Hybridization to human metaphase chromosomes with TEAD3 (left). A chromosome 6 ideogram indicating localization of the TEAD3 gene to 6p21.2 (right).

Name of clone or DNA source: The TEAD2 genomic probe was isolated as a λ phage from a human genomic DNA library, and the TEAD3 cDNA was isolated from a human placental cDNA library.

Description of clone or DNA: The TEAD2 probe was a λ GEM phage with a >10-kb portion of the TEAD 2 genomic locus. The TEAD3 probe was a 3-kb cDNA clone containing the entire open reading frame along with the 5' and 3' untranslated regions.

Method used to validate gene identity: The GenBank accession numbers for TEAD2 and TEAD3 are X94440 and X94439, respectively.

Methods of mapping: The chromosomal assignment of TEAD2 and TEAD3 was performed by both fluorescence *in situ* hybridization (FISH) and radiation hybrid panel analyses.

FISH experiments were performed essentially as described by Pinkel *et al.* (8). Metaphase spreads were prepared from phytohemagglutinin-stimulated human lymphocytes, cultured at 37°C for 72 h. 5-Bromodeoxyuridine was added for the final 7 h of culture (60 μ g/ml of medium) to ensure good chromosomal R-banding. The TEAD2 probe was prepared by nick-translation of a purified phage containing a >10-kb insert of the TEAD2 gene in λ GEM with biotin-16-dUTP (outlined by the Boehringer Mannheim protocol). The biotinylated TEAD3 probe comprised a 3-kb cDNA fragment in pEXlox. Hybridized probe was detected by fluorescence isothiocyanate-conjugated avidin (Vector Laboratories). Chromosomes were counterstained and R-banded with propidium iodide diluted in antifade solution, pH 11.0, as described (6).

To refine further the localization of the TEAD2 and TEAD3 genes, we used the GeneBridge 4 radiation hybrid panel (Whitehead Institute, Research Genetics, Inc.). For TEAD2, PCR amplifications were performed using a primer pair in exon 8, generating a 154-bp product (forward primer 5'-CCTGTTTCGTGCACATCAGC-3' and reverse primer 5'-GAC-CAGGAAGAAGGCATGG-3'), and for TEAD3, a primer pair in the 3' untranslated region generating a 366-bp product was used (forward primer 5'-AGCCTCGCGTGACCTCAGA-3' and reverse primer 5'-CCCTGGATGAGAACTCCAG-3'). Thirty-five cycles of polymerase chain reaction for TEAD2 were performed at 94°C for 45 s, 63°C for 45 s, and 72°C for 45 s. For TEAD3, annealing was performed at 58°C for 45 s.

Results: A total of 30 metaphase cells were analyzed for each probe. For TEAD2, 90% of the cells showed specific fluorescent spots on the subdistal band of the long arm of chromosome 19, band q13.3 (Fig. 1A). The TEAD2 gene therefore maps close to the ERCC1, ERCC2, R-RAS, and SNRP70 genes. A search for linkage homology between the

mouse chromosome 7 region where the Tead2 gene was located and the human 19q13.3 region revealed the presence of the murine equivalents of the above four markers, indicating that these two regions are indeed syntenic. For TEAD3, specific fluorescent spots were present on 90% of the cells on the 6p21.2 band (Fig. 1B). Thus, TEAD3 maps close to the oncogene PIM1 and cyclin-dependent kinase inhibitor 1A (CIP1/WAF1), genes both present on 6p21.2.

The results of the radiation hybrid experiments using the sequence-tagged site database at the Whitehead Institute confirmed the localization obtained by FISH. TEAD2 was localized between the anonymous markers D19S412 and D19S210, 2.33 cR telomeric from NIB1805 (lod score >3). The marker D19S412 (1) previously mapped to 19q13.3, is located 5.4 cR centromeric to TEAD2. TEAD3 was localized 2.74 cR centromeric from NIB 1566 between D6S291 and D6S271 (lod score >3). The marker D6S273, which is localized in the interval 6p21.2–p11 (7) is 26.6 cR telomeric to the TEAD3 locus, confirming that TEAD3 is located on 6p21.2. Further confirmation of this localization came from the mapping of the murine Tead 3 gene to chromosome 17 in a region syntenic to human chromosome 6p21.2 (manuscript in preparation).

Additional comments: The related TEAD1 and TEAD4 genes have been mapped to chromosomes 11p15.4 and 12p13.2–p13.3, respectively (9), paralogous regions of the human genome resulting from an ancient chromosome duplication, where the myogenic MyoD, myf5, and myf6 genes are also located.

Homologies: Mouse Tead2 (also called ETF) and Tead4 are located on chromosomes 7 and 6, respectively (2, 10). Linkage homology between human and mouse indicates that the Tead1 gene may also be present on mouse chromosome 7.

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