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Mapping of human and murine genes for latent TGF-β binding protein-2 (LTBP2)

X. Li,[1] W. Yin,[3] L. Pérez-Jurado,[2] J. Bonadio,[3] U. Francke[1,2]

[1]Howard Hughes Medical Institute, Beckman Center, Stanford University Medical Center, Stanford, California 94305-5428, USA
[2]Departments of Genetics and Pediatrics, Stanford University Medical Center, Stanford, California 94305-5428, USA
[3]Department of Pathology, University of Michigan, Ann Arbor, Michigan 48109-0650, USA

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Abstract. A novel gene, isolated because of structural similarities to fibrillin, was called LTBP2 when its 4.6-kb transcript was found to encode a protein sequence related to the latent TGF-β binding protein (LTBP1), which is encoded on human chromosome (Chr) 2, region p12-q22. We have assigned the human and murine LTBP2 loci to regions of conserved synteny on human Chr 11 and mouse Chr 19. By PCR analysis of somatic cell hybrid DNA and fluorescence in situ hybridization, LTBP2 was mapped to human Chr band 11q12 and *Ltbp2* to mouse Chr band 19B. Differences between inbred strains were discovered by single-strand conformation analysis of PCR products from the 3′ untranslated region. Analysis of BXD and AKXL recombinant inbred strains have placed *Ltbp2* between *D19Rp19* and *Ly10* on proximal mouse Chr 19.

Introduction

A family of small related proteins, consisting of transforming growth factor (TGF) β1, β2, β3 and others, have both stimulatory and inhibitory effects on growth of different cell types and play a role in the production and degradation of the extracellular matrix (Roberts and Sporn 1990; Lyons and Moses 1990). TGF-β molecules are secreted in the form of latent large MW complexes that contain other proteins, such as latent TGF-β1 binding protein (LTBP1). LTBP1 is covalently bound to extracellular matrix and, thus, mediates the matrix binding of TGF-β (Taipale et al. 1994). The structure of human LTBP1 resembles fibrillin in that it includes 16 epidermal growth factor-like repeats and three copies of a novel 8-cysteine motif (Kanzaki et al. 1990; Maslen et al. 1991; Pereira et al. 1993). LTBP purified from different tissues may have different sizes owing to either proteolytic cleavage or alternative splicing (Kanzaki et al. 1990). A single LTBP1 locus appears to exist in the human genome that has been mapped to Chr 2, region p12-q22 (Stenman et al. 1994). The gene has not yet been mapped in the mouse.

A structurally and functionally related gene has recently been identified, called LTBP2, that encodes a 4.6-kb transcript in mouse embryo tissues (Yin et al. submitted). The predicted gene product is a secreted protein of 1252 amino acids that binds TGF-β. The mouse Ltbp2 sequence is only about 40% identical to the human LTBP1 sequence, but important domain structures are conserved, including the cysteine spacing in 13 EGF-like repeats.

To gain insight into the evolution of gene families and to identify candidate genes for human disorders and murine mutations, we are systematically mapping interesting genes in both species. Of relevance here, we have previously found that the genes for TGF-β1, TGF-β2, and TGF-β3 are dispersed on different chromosomes in human and mouse (Fujii et al. 1986; Barton et al. 1988) and that genes for fibrillin-1 and fibrillin-2 are on different chromosomes in mouse, in regions of homology with the human loci (Li et al. 1993). Here, we report that the human (HSA) and murine (MMU) LTBP2 genes are asyntenic with LTBP1 (in human) and are located in small regions of conserved synteny on HSA 11q12 and MMU 19B.

Correspondence to: U. Francke

Materials and methods

Somatic cell hybrid panels and PCR amplifications

For primary assignment of the LTBP2 gene to a human chromosome, we performed PCR amplification on a panel of 16 human × Chinese hamster hybrid cell lines derived from several independent fusion experiments (summarized in Francke et al. 1986). To distinguish the human-specific PCR product from amplified hamster sequences, the PCR primers were designed from human genomic sequences. The forward primer was 5′-GTGGGCGGGGAGTTCACAGTT (8954A) and the reverse primer 5′-CCTGTGACCTGAGTGGGACGA (9232A). The PCR conditions were 95°C, 5 min; then 35 cycles of 94°C, 1 min; 62°C, 1 min; 72°C, 1 min; followed by 72°C, 7 min. The specificity of the 233-bp PCR product was verified by restriction analysis with three different enzymes.

To assign the *Ltbp2* gene to a mouse chromosome, we analyzed a mapping panel consisting of 14 mouse × Chinese hamster and two mouse × rat somatic cell hybrid lines. The hybrids were derived from four independent fusion experiments as described previously (Francke et al. 1977; Francke and Taggart 1979; Joyner et al. 1985). The PCR primers were derived from the 3′-untranslated region of the murine *Ltbp2* sequences with the forward primer (5′-CACTGAAAGTGGAGACAGACAAGT) (1416C) and the reverse primer (5′-TCCCGGCGGATGTATTTATTGTAC) (1413C). PCR was performed as described above except that the annealing temperature was 60°C.

Fluorescence in situ hybridization

The chromosomal locations of the human and murine LTBP2 genes were independently determined by fluorescence chromosomal in situ hybridization as previously described (Milatovich et al. 1991). Briefly, lambda clones containing human or mouse LTBP2 sequences were labeled with biotin-11-dUTP by nick translation with commercial reagents (Boehringer Mannheim). Labeled probes were hybridized at a concentration of 6 ng/μl (50 μl per slide) for human LTBP2 and 12 ng/μl for murine *LTBP2* to pretreated and denatured metaphase chromosomes from human lymphocyte culture or primary mouse embryo culture. Hybridization was performed in the presence of salmon sperm DNA and cold competitor, either human placental DNA or mouse NIH 3T3 cell DNA.

After washing, the slides were covered with avidin/FITC (Vector Laboratories) and amplified with biotinylated goat anti-avidin D antibody (Vector Laboratories), followed by another round of avidin/FITC treatment. Chromosomes were counterstained with 200 ng/μl propidium iodide in 2 × SSC. Twenty metaphase spreads were analyzed under an Axiophot (Zeiss) epifluorescence microscope. Signals were counted as specific only

when the fluorescent signal was seen on both chromatids of a chromosome. A cooled charge-coupled device (CCD) camera (Photometrics PM512)/ Macintosh computer system was used for digital imaging, with software supplied by Tim Rand (Yale University). Black and white photographs were generated from PICT files.

PCR-SSCP analysis of recombinant inbred mouse strains

The murine *Ltbp2* gene was integrated into the genetic map of mouse Chr 19 by genotyping two independent panels of recombinant inbred (RI) mouse strains, BXD and AKXL (DNA obtained from The Jackson Laboratory). Progenitor strains were screened for DNA variants within the 3′UTR region of LTBP2 by single-strand conformation analysis of PCR products as described (Poduslo et al. 1991). For PCR amplification, the same primers and conditions were employed as described above for the mapping experiments except that 20 μCi of ^{35}S-dATP was added to a 25-μl reaction. The DNA was denatured by mixing 9 μl PCR product with 1 μl of 0.5 M NaOH, 10 mM EDTA, and incubating in a 42°C waterbath for 5 min, followed by cooling on ice. The mixture was loaded with 1 μl of formamide dye on to a 6% nondenaturing polyacrylamide gel containing 5% glycerol in 0.5 × TBE buffer. Electrophoresis was performed at 100 W maximum for 14 h at 8°C constant temperature. The gel was dried on a 3MM paper and exposed to X-ray film for 6–24 h.

Results

Assignment of human LTBP2 gene to Chr band 11q12

Genomic DNA from a panel of 16 human × Chinese hamster hybrid cell lines was analyzed by PCR with primers that specifically amplified the human LTBP2 sequences. Under these PCR conditions no specific amplification was seen from hamster DNA. The LTBP2-specificity of the 233-bp PCR product was verified by endonuclease digestion. Single *Hae*III and *Apa*I sites and two *Msp*I sites were identified within the 233-bp DNA fragment at the expected positions, indicating that the PCR amplification was from human LTBP2 sequences (data not shown). The 233-bp PCR product was obtained only from hybrid cell lines that had retained human Chr 11, and all other human chromosomes were excluded by the presence of discordant hybrids (Table 1a). In addition, the LTBP2-specific PCR amplification was seen in one hybrid cell line containing only 11q but not in another hybrid cell line retaining only 11p. These results indicated that the human LTBP2 gene is located on 11q.

Fluorescence in situ hybridization confirmed the LTBP2 gene assignment to Chr 11 and refined the physical map position. A specific fluorescent signal on both chromatids of a Chr 11 was seen in 23/30 metaphase cells analyzed. Nineteen of the 23 positive cells had a specific signal on both homologs. Based on an R-banding pattern produced by the incorporation of BrdU after synchronization and counterstaining with propidium iodide, the human LTBP2 gene was assigned to band 11q12 (Fig. 1A, C).

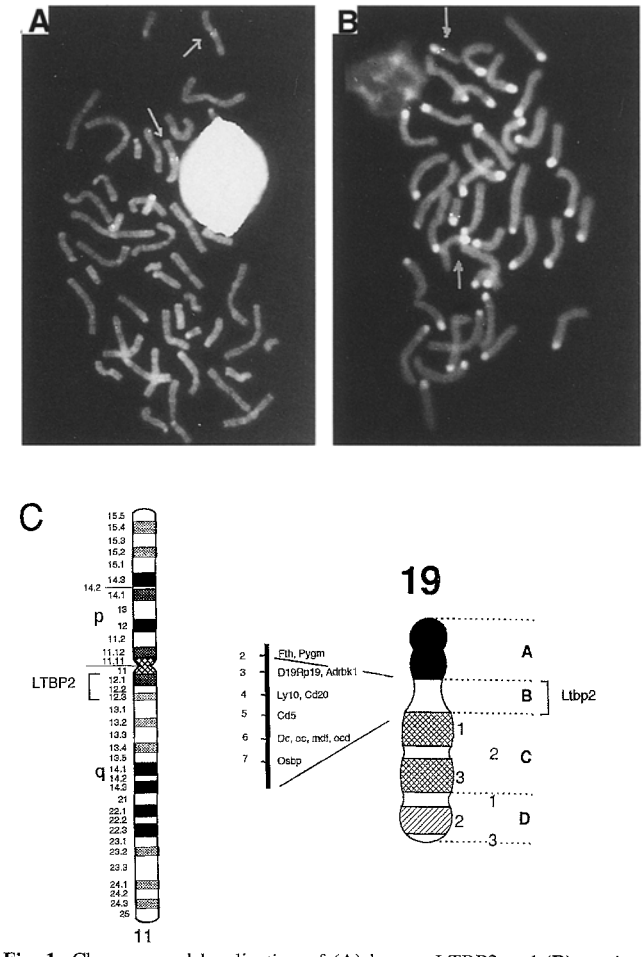

Fig. 1. Chromosomal localization of **(A)** human LTBP2 and **(B)** murine *Ltbp2* by fluorescence in situ hybridization (FISH). Double chromatid signals (indicated by **arrows**) are present on human Chr 11 at band q12 **(A)** and at a site corresponding to band B on mouse Chr 19 **(B). (C)** G-band idiograms of human Chr 11 (Francke 1994) and mouse Chrs 19 (Nesbitt and Francke 1973), illustrating the FISH localizations (indicated by brackets) of the human (LTBP2) and murine (*Ltbp2*) genes and other loci in the conserved region from consensus map of MMU19 (Guénet and Poirier 1993). The human homologous genes FTH1, PYGM, ADRBK1, CD20, CD5, and OSBP have been mapped to Chr 11 bands q12-q13.

Assignment of murine Ltbp2 to Chr band 19B proximal to Ly10

By using primers derived from 3′UTR sequences of the murine *Ltbp2* gene, a 381-bp DNA fragment was amplified from mouse genomic DNA. When the mapping panel of mouse × rodent hybrid cell lines was analyzed, the presence or absence of mouse Chr 19 in hybrid cell lines was in complete concordance with the murine

Table 1a. Comparison of human LTBP2 sequences with human chromosomes in human × hamster somatic cell hybrids

LTBP2 signal/chromosome	Human chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Discordant hybrids																							
+/–	4	5	3	4	5	2	6	4	6	7	0	3	5	6	4	3	0	3	4	0	5	3	1
–/+	2	1	4	4	0	4	1	3	2	0	0	2	5	3	5	4	7	4	1	2	2	2	2
Concordant hybrids																							
+/+	4	3	5	3	4	6	1	5	1	2	7	6	4	6	4	5	2	5	7	7	6	7	2
–/–	5	6	2	3	7	3	6	3	5	7	7	5	1	1	3	2	7	4	3	7	2	3	2
Informative hybrids	15	15	14	14	16	15	14	15	14	16	14	16	15	16	16	14	16	16	15	16	15	15	7
% Discordance	40	40	50	57	31	40	50	47	57	44	0	31	67	56	56	50	44	44	33	13	47	37	43

Table 1b. Comparison of mouse *Ltbp2* sequences with mouse chromosomes in rodent × mouse somatic cell hybrids

Ltbp2 product/chromosome	Mouse chromosome 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X
Discordant hybrids																				
+/–	3	2	0	0	0	1	1	0	1	2	2	1	2	1	1	1	2	0	0	2
–/+	3	1	3	3	4	2	2	3	7	7	11	4	6	6	2	4	1	5	0	3
Concordant hybrids																				
+/+	7	10	8	7	5	7	8	8	4	3	0	7	4	5	9	6	10	5	10	7
–/–	2	3	5	5	5	4	4	5	4	3	3	4	3	4	4	4	3	5	5	3
Informative hybrids	15	16	16	15	14	14	15	16	16	15	16	16	15	16	16	15	16	15	15	15
% Discordance	40	19	19	20	29	21	20	19	50	60	81	31	53	44	19	33	19	33	0	33

Data for chromosomes with rearrangements or present at low copy (<0.1) were excluded. The % discordance is calculated as the sum of discordant over total informative hybrids for each chromosome.

Table 2. Strain distribution patterns of *Ltpb2* allele in recombinant inbred strains compared with neighboring loci

	AKR/J × C57L/J (AKXL)																			
Strain no.	5	6	7	8	9	12	13	14	16	17	19	21	24	25	28	29	37	38		
D19Rp19	A	A	A	A ×	A	A	L	A	L	L	L	L	A	A	A	A	A	L		
Xmmv 42	A	A	A	L	A	A	L	A	L	L	L	L	A	A	A	A	A	L		
Ltbp2	A	A	A	L	A	A	L	A	L	L	L	L	A	A	A	A	A	L		
Ly 10	A	A	A	L	A	U	L	A	L	L	L	L	A	A	A ×	A	A	L		
Cd5	A	A	A	L	A	A	A	A	L	L	L	L	A	A ×	L	A	A	L		
Fcerlb	A	A	A	L	A	A	A	A	L	L	L	L	A	L	L	A	A	L		

Strain no.	C57BL/6J × DBA/2J (BXD) 1	2	3	4	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
D19Byu2	D	B			B	D	D	B	B	B	D	B	B	B	B	B	D	B	B	D	D ×	B	D	B	B	D	D	B
D19Rp19	D	B			B	D	D	B	B	B	D	B	B	B	B	B	D ×	B	B	D	D	B	D	B	B	B	D	B
D19Byul	D ×	B			B	D	D	B	B	B	D ×	B	B	B	B	B	B	B	B	D	D	B	D	B	B	B	D ×	B ×
Ltbp2	B ×	B			B	D	D	B	B	B	B ×	B	B	B	B	B	B ×	B	B	D	D	B	D	B	B	B	B ×	D ×
Ly10	D ×	B			B	D	D	B	B	B	D	B	B	B	B	B	D	B	B	D	D	B	U	B	B	B ×	D	B
D19Mit22	B	B	B	D	B	D	D	B	B	B	D	B	B	B	B	B	D	B	B	D	D	B	D	B	B	D	D	B
Cd5	B	B	B	D	B	D	D	B	B ×	B	D	B	B	B	B	B	D	B	B	D	D	B	D	B	B	D	D	B
Osbp	B	B	B	D	B	D	D	B	D	B	D	B	B	B	B	B	D	B	B	D	D	B	D	B	B	D	D	B

Ltbp2-specific amplification. All other mouse chromosomes were excluded by at least 19% discordant hybrids (Table 1b).

Fluorescence in situ hybridization was used to sublocalize the *Ltbp2* gene on mouse Chr 19. Among 30 metaphase cells analyzed, a specific fluorescent signal on both chromatids of a Chr 19 was seen in 19 metaphases, 12 of which carried signal on both homologs. The site of hybridization was just distal to the centromeric heterochromatin region, and, therefore, the *Ltbp2* gene was assigned to Chr band 19B (Fig. 1B, C).

PCR-SSCA revealed a biallelic polymorphism with distinguishable patterns between AKR/J and C57L/J, and between C57BL/6J and DBA/2J, indicating that both AKXL and BXD panels of recombinant inbred strains should be informative (data not shown). When the panels were typed and the *Ltbp2* strain distribution patterns compared with all SDPs in Ben Taylor's database, the localization to Chr 19 was confirmed. Comparison with SDPs of the AKXL panel, published in Guénet and Poirier (1993), places *Ltbp2* into the *D19Rp19–Cd5* interval with no recombinants between *Ltbp2* and *Xmmv42* or *Ly10* (0/18 and 0/17, respectively; Table 2). Analysis of the BXD RI strains supports a position of *Ltbp2* in the *D19Rp1–Ly10* interval, on either side of *D19Byul,* even though double-crossovers are present in strains 1, 13, 31, and 32 (Table 2). When *Ltbp2* typing of the BXD strains was repeated twice, consistent results were obtained.

Discussion

In this study we have assigned the human and murine LTBP2 genes to specific chromosomal positions by several independent approaches, including PCR analysis of interspecies somatic cell hybrid panels, fluorescence in situ hybridization, and recombinant inbred strain mapping in the mouse. The results were completely consistent and are further supported by the existing comparative map. The fact that the loci were assigned to regions of conserved synteny is also consistent with the notion that the LTBP2 genes cloned from both species are likely to be true homologs. With only a small part of the human gene sequenced to date, this is not a trivial point given the existence of a family of genes with EGF and 8-cysteine repeats. The human gene for the first latent TGF-β1 binding protein discovered (LTBP1), which is related to LTBP2, was assigned to human Chr 2 by study of human × rodent somatic cell hybrid lines and was further sublocalized to 2p12-q22 by a regional mapping panel of three hybrid cell lines which retain

partially overlapping regions of Chr 2 (Stenman et al. 1994). No LTBP genes have previously been mapped in the mouse.

Here we have assigned the human LTBP2 gene to Chr 11 band q12. The murine *Ltbp2* gene was mapped to mouse Chr 19, band B, that approximately corresponds to map units 3-10 on the genetic map (Lyon and Kirby 1994). The most proximal 10 cM of the maps of Lyon and Kirby (1994) and Guénet and Poirier (1993) contain about ten genes that are homologous to human genes assigned to human Chr bands 11q12-q13, including those depicted in Fig. 1c: β-adrenergic receptor kinase-1 (*Adrbk1*), ferritin heavy chain (*Fth*), antigens *Cd5* and *Cd20*, ciliary neurotrophic factor (*Cntf*), and oxysterol binding protein (*Osbp*).

To integrate the murine *Ltbp2* gene into the genetic map of mouse Chr 19, we have searched for a polymorphism by PCR-SSCP and have analyzed two panels of recombinant inbred strains. Although more double crossovers than expected were observed in the BXD panel, it is still possible to place *Ltbp2* proximal to *Ly10* in the center of the conserved homologous region. There are four mutant loci in this region, *Dc, oc, mdf* and *ocd*. *Dc*, dancer, is characterized by circling behavior in heterozygotes and by cleft lip and cleft palate in homozygotes that die at birth (Deol and Lane 1966). The recessive *oc* phenotype consists of osteosclerosis with abnormal osteoclasts (Marks et al. 1985). Atrophy of muscle fibers leading to marked reduction of muscle mass characterizes the *mdf*, muscle deficient, homozygotes (Womack et al. 1980). *ocd*, osteochondrodystrophy, arose in the C3H/HeSn strain in 1980 and is a recessive disorder. Homozygotes have reduced body size, a short, slightly domed head, supination of the forefeet, disproportionately shortened long bones of the limbs, and a short thickened tail (Sweet and Bronson 1991). Given that LTBP2 may play a role in the regulation of connective tissue and extracellular matrix, as well as in maintaining TGF-β present in tissues in a latent state, the *Ltbp2* gene could be considered as a potential candidate for the *ocd* or the *oc* mutations.

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