

Zheng Chen · Long Yu · Hai Wu · Jianqiang Yu  
Lisha Zhang · Daojun Jiang · Lijie Ma · Dan Li  
Shouyuan Zhao

## Identification of a novel liver-specific expressed gene, *TCP10L*, encoding a human leucine zipper protein with transcription inhibition activity

Received: 16 June 2003 / Accepted: 21 August 2003 / Published online: 29 October 2003  
© The Japan Society of Human Genetics and Springer-Verlag 2003

**Abstract** The incidence of hepatoma is high in the Chinese population. Searching for genes involved in the functions of the liver, especially genes specifically expressed in the liver, will facilitate an insight into the molecular basis of normal and abnormal liver functions. Based on a differentially displayed cDNA fragment, which was down regulated in hepatoma tissues, we cloned a novel cDNA of 957 bp, *TCP10L* (T-complex protein 10 like), from the human liver cDNA library. Northern hybridization of this novel gene in 30 adult human tissues was examined. The result revealed that *TCP10L* expressed specifically in the human liver and testis. The *TCP10L* contains a 645-bp open reading frame encoding a deduced protein of 215 amino acids. As the deduced protein was analyzed further, a typical leucine zipper motif was found. We firstly examined the transcriptional function of the TCP10L protein by transfecting recombinant pM-TCP10L into mammalian cells. The subsequent analysis based on the dual luciferase assay system showed that TCP10L significantly inhibited the expression of reporter genes. Compared with that of the negative control, the luciferase activity were down regulated in HEK293 and SK-HEP-1, CHO cells by about 2.6, 9.8, and 5.5 folds respectively. A mutated type of *TCP10L* was also constructed. It showed that the repression of *TCP10L* to the expression of the reporter gene almost completely decreased, suggesting that the leucine zipper structure is critical for

*TCP10L* to play its role in regulation function. Then we transfected the recombinant TCP10L-EGFP into cells. The results indicated that *TCP10L* subcellularly located in nuclei, either in HEK 293 or SK-HEP-1 cells. In addition, human *TCP10L* was found comprised of five exons and four introns, and mapped to chromosome 21q22.11.

**Keywords** Leucine zipper motif · Liver-specific expression · Chromosome 21q22.11 · Transcription inhibition activity

### Introduction

The liver is involved in many important functions such as maintaining the metabolic balance of nutrients including fat, and carbohydrate; synthesizing substances, such as bile acid; and playing a central role in the detoxification of poisonous substances. In addition, the liver is also an important organ in the body's defense mechanisms and a critical hematopoietic organ in the fetal stage (Petti et al. 1985; Houssaint et al. 1988). Liver diseases, especially hepatitis, cirrhosis, and hepatoma, are common in the Chinese population (Yeh et al. 1989; Lee 1983; Sun Z et al. 1991). Therefore, cloning and identification of the genes related to liver functions, especially those specifically expressed in the liver, might help us gain insights into the molecular mechanism of the development, physiological functions, and functional disorders of the liver.

In recent years, the gene expression profile in the human fetal liver has been investigated, and many tissue-specific and developmental-stage-specific genes in the liver were identified (Yu et al. 2001). For instance, cholesterol-7-hydroxylase NADPH: oxygen oxidoreductase (7-hydroxylase) is a liver-specific gene product that controls bile acid synthesis, the major pathway responsible for eliminating cholesterol from the body (Edwards et al. 1996). Besides, some researchers compared the gene expression profiles of hepatocellular

Z. Chen · L. Yu (✉) · H. Wu · J. Yu · L. Zhang · D. Jiang  
L. Ma · D. Li · S. Zhao  
State Key Laboratory of Genetic Engineering,  
Institute of Genetics, School of Life Science,  
Fudan University, 220 Handan Road,  
200433 Shanghai, P.R. China  
E-mail: longyu@fudan.edu.cn  
Tel.: +86-21-65642422  
Fax: +86-21-65643250

Z. Chen  
Department of Biochemistry,  
Medical College of Suzhou University,  
215007 Suzhou, P.R. China

carcinoma with those of corresponding noncancerous liver tissues, and a number of candidate genes altered in their expression levels have been found (Xu et al. 2001). Among these tissue-specifically-expressed genes, the genes encoding transcription factors may be of importance. Through regulating the expression of other genes, transcription factors can influence their target genes and subsequently influence the functions of tissues or organs. Therefore, studies on those transcription-factor genes specifically expressed in the liver may give us clues to the molecular basis of the liver's physiologic functions.

In the present study, a novel gene, human *TCP10-like* gene, which is specifically expressed in the liver and testis, was identified. It was found that the *TCP10-like* gene is located in nuclei of mammalian cells and bears transcription inhibition activity. We propose that the *TCP10-like* gene might be a transcription factor gene and might have roles in the development of the liver.

## Materials and methods

### cDNA cloning and sequencing

In a differential display analysis between hepatoma tissues and their adjacent nontumor tissues from a female Chinese patient suffering from hepatocellular carcinoma, a 350-bp cDNA fragment down regulated in hepatoma tissues was found. Afterward, the cDNA fragment was sequenced and used as an information probe to search the human EST division in GenBank with the BLASTn program. A series of ESTs (AA758217, AA832234, AA927467, AI183985, AI004575, H94895, AA621014, AA621071, F11677, and AA563930) were obtained and assembled into a 957-bp EST contig. These published cDNA sequences provided the basis for designing the following two primers: JY-A, 5'-ATG ATC TGA TCC TGG CAG TAG TG -3'; and JY-B, 5'-CAG TGT AGA GTG ACA CAG GTG TC-3'. The primers were custom-made by ShengGong Inc (Shanghai, China) and were used to amplify the full-length cDNA from human  $\lambda$ gt11 liver cDNA library (Clontech). PCR conditions were as follows: 1  $\mu$ l template (>108 plaque-forming U/ml) was amplified in a final volume of 50  $\mu$ l containing 5  $\mu$ l of 10 $\times$ PCR buffer, 1  $\mu$ l of 20 mM dNTPs, 1.5  $\mu$ l of 2.5 mM MgCl<sub>2</sub>, 2 U Taq polymerase (Promega) and 1  $\mu$ l of 25 mM of each specific primer. PCR reactions were run on a PTC-200 DNA engine (MJ Research, Watertown, MA, USA) for 34 cycles (1 min at 94°C, 1 min at 61°C, and 1 min at 72°C) after an initial denaturation at 94°C for 3 min, and were followed by incubation at 72°C for 10 min. The amplification product was cloned into vector pGEM-T and five independent clones were sequenced using an ABI PRISM 377 DNA sequencer subsequently.

### Plasmid construction and analysis of transcriptional function

The full-length ORF of the TCP10L cDNA was inserted in frame into mammalian cell expression plasmid pM (Clontech) with EcoRI/XbaI restriction enzyme sites to generate fusion genes encoding PM-TCP10L. The primers used in this construct were PM-TCP10LA 5'-GCG GAA TTC ATG CTG GCA GGT CAA CTC -3', PM-TCP10LB 5'-GCG TCT AGA CCT TTC CAT CTT CAG AC-3'. The constructs were verified by DNA sequencing. Human HEK293, CHO, SK-Hep-1 cells were, respectively, grown in 24-well plates. The FuGENE transfection reagent (Roche) was used to perform the transfections as described in the manufacturer's protocol. The recombinant PM-TCP10L, the empty pM, and the positive control pM3-VP16, pM-ZNF191 were cotransfected into cells respectively with reporter plasmid pGAL4<sub>5</sub>tkLUC containing

five consensus GAL4 binding sites and thymidine kinase minimal promoter upstream of the luciferase. We then used 0.6  $\mu$ g of mixed plasmids in each experiment. The plasmid pRL-SV40 encoding Renilla luciferase was used as the internal control in each transfection. The day before transfection, about 2~6 $\times$ 10<sup>4</sup> cells were seeded per well of a 24-well plate in appropriate growth medium with serum. The cells were incubated at 37°C with 5% CO<sub>2</sub>.

Forty-eight hours after the transfection, the adherent cells were washed with 1 $\times$  phosphate-buffered saline and lysed in 1 ml passive lysis buffer (Promega). The dual-luciferase reporter assay system (Promega) was used to assay luciferase activity of the samples. The cell lysate was firstly assayed for the firefly luciferase activity using 100  $\mu$ l of the substrate LARII. Then 100  $\mu$ l of the Stop & Glo reagent was added to quench the firefly luciferase activity and activate the Renilla luciferase activity. Each set of transfections was repeated three times. The relative light units were measured by luminometer (Lumat LB9507).

### Intracellular localization assay

Two primers were designed to amplify the TCP10L cDNA. EGFP-TCP10LA: 5'-CCC GAA TTC TCA TGC TGG CAG GTC-3', EGFP-TCP10LB: 5'-ATA GGA TCC CGG ACA CCC CCC CG -3'. The acquired fragment digested with EcoRI/BamHI was inserted into the corresponding sites of enhanced green fluorescent protein (pEGFP-N1, Clontech) vector. Two lines of cells (HEK293 and SK-Hep-1) were seeded in 35-mm tissue culture plates and grown in suitable medium supplemented with 10% fetal bovine serum in a 5% CO<sub>2</sub> incubator at 37°C. Cells were transfected the next day with EGFP-TCP10L using FuGENE transfection reagent (Roche). Each transfection was performed in duplicate. The transfected cells were grown for an additional 48 h and then examined using a Leica confocal microscope.

### Northern blot analysis

Northern hybridizations were performed on multiple-tissue Northern membranes with mRNA samples from adult human tissues. The probes were prepared by labeling the cDNA fragments with [ $\alpha$ -<sup>32</sup>P]dATP (Amersham) using the PCR method and purified on a Sepharose-G50 column. The membranes were prehybridized in hybridization/prehybridization solution {5 $\times$ SSPE [1 $\times$ SSPE = 0.15 M NaCl/10 mM sodium phosphate (pH 7.4)/1 mM EDTA], 50% formamide, 10 $\times$ Denhardt's (1 $\times$ Denhardt's = 0.02% Ficoll 400/0.02% polyvinylpyrrolidone/0.002% BSA), 2% (w/v) SDS, 100  $\mu$ g/ml calf-thymus DNA} at 42 °C for 16 h, and hybridized in a hybridization oven (Hybaid Interactive, Teddington, Middx, UK) with the labeled probe for a further 24h continuous shaking. The membranes were then washed several times with 0.1 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl/0.015 M sodium citrate) containing 0.1% SDS at 42°C and were exposed to X-ray film at -80°C for 5 days.

## Results

### Cloning of human TCP10L

Using the primers JY-A,B derived from the correlated EST sequences, we applied PCR to produce a 729-bp of JY-AB fragment from human  $\lambda$ gt11 liver cDNA library. The automated sequencing showed that the sequences of all the clones in pGEM-T vector selected independently, are consistent with that of the EST contig (nt49-777), which we assembled formerly. Searching the GenBank nonredundancy database with the assembled 957-bp contig, it was found that this cDNA sequence had not

been reported in the known databases. The gene was submitted to GenBank under the accession number of AF115967. We searched GenBank with the nucleotide sequence of the novel gene and found that this gene is similar to some members of human T-complex protein 10 (*TCP10*) gene family, such as TCP10A (U03399) (Islam et al. 1993). When human TCP10A was aligned with the novel putative protein, an identity about 39.7% was discovered in their amino acid sequences. We thereby submitted our gene to the HUGO gene nomenclature committee, and this novel gene was symbolized as *TCP10L*, *TCP10-like* gene.

Using the ORF Finder analysis tool in NCBI, we found the TCP10L cDNA contains an intact open reading frame encoding a putative polypeptide of 215 amino acids (nt 88–735)(Fig. 1A). In the upstream of the initial codon ATG of TCP10L cDNA, there are three stop codons (TGA, nt 54–56; TAG, nt 67–69; TGA, nt70–72); while in its 3'-UTR, there are a typical polyadenylation signal (AATAAA, nt822–827) and an atypical one (ATATAA, nt866–871). The deduced TCP10L is predicted to have an approximate molecular mass of 52KD and an acidic PI of 5.13 by ExPASy program (<http://www.expasy.ch>). Additionally, a leucine zipper motif was found near the middle part of TCP10L by 2ZIP program (<http://www.dkfz-heidelberg.de/tbi/services/2zip>) (Bornberg et al. 1998). The leucine zipper motif spans 25 amino acids from the residue 72–96, and contains four periodic repeats of leucine residues (residue 75, 82, 89, 96) (Fig. 1C). Moreover, the leucine zipper motif exists in a coiled coil conformation predicted by 2ZIP program.

To further explore the function of TCP10L, this leucine zipper motif was compared to the leucine zipper motifs in other 13 proteins containing leucine zipper (Fig. 1B and C), which are BATF (GenBank accession number: XM\_041063), TBZF (AB032478), LAZipII (AF408398.1), MafK (AF059194), c-FOS (V01512), GCN4 (K02205), KRM12 (AF109780), AP-1 (J04111), NRL (XM\_033338), LOC90183 (XM\_029709), C/EBP (X52560), BACH2 (XM\_031521), c-MYC (X00364). Structure comparison of these proteins revealed that the structure of the leucine zipper motif in TCP10L is reliable, although the position of the leucine zipper might appear near the N-terminus or the C-terminus or in the middle part of proteins (Fig. 1B). Besides, we still noticed that more than half the proteins (eight out of 14) have leucine zippers near C-termini. It is also observed that in leucine zippers, the number of leucine residues is variable from four repeats to six, which may govern stability and orientation of dimerization (Fig. 1C).

#### Assay of transcriptional function

Several protein motifs, which are essential to DNA recognition and binding, have been identified in transcription factors, such as the leucine zipper, zinc finger, helix-loop-helix, and helix-turn-helix (Busch et al.

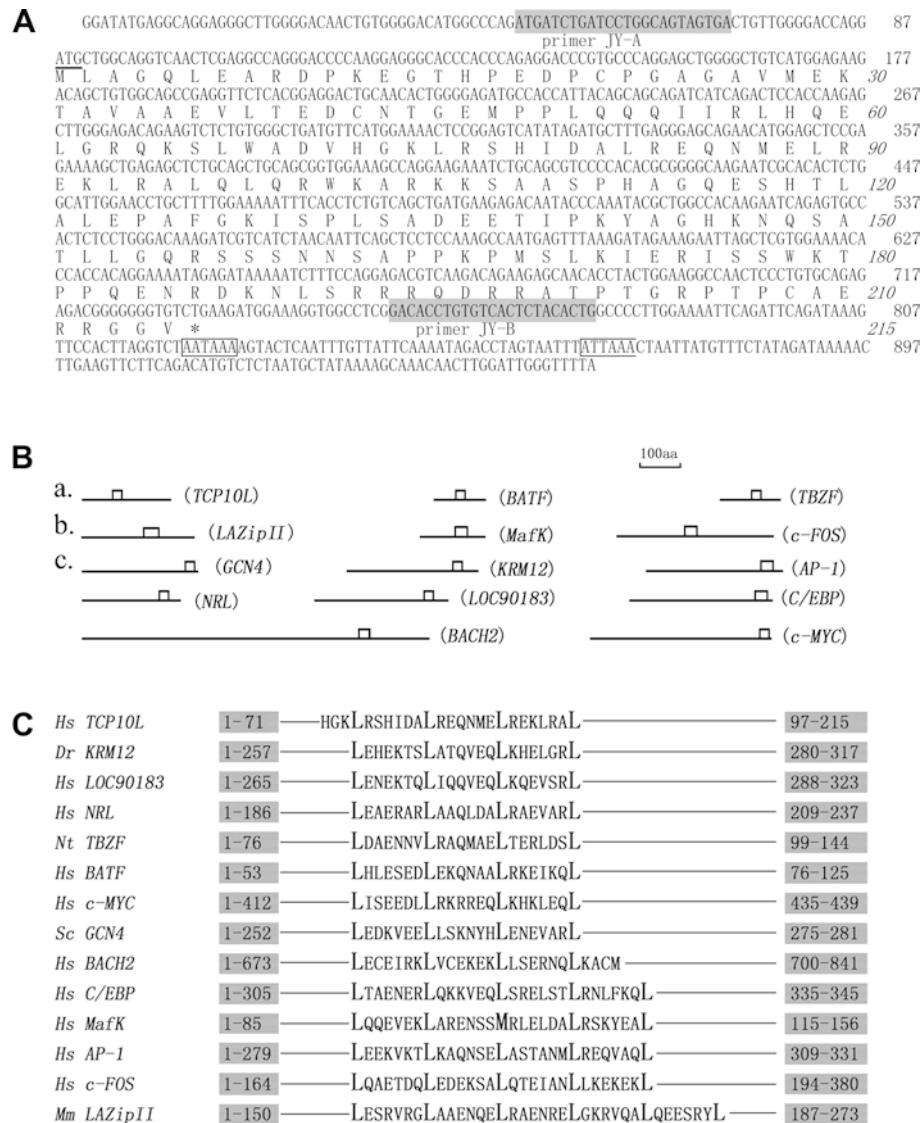
1990). Accumulating data show that the leucine zipper motif, first postulated by Landschulz et al. (1988), exists in numerous transcription factors such as c-Myc (protooncoproteins) (Amati et al. 1993; Luscher et al. 1999), AIF3, Jun and Fos (bZip family members of transcription factors) (Perez et al. 2001; Smith et al. 1990), and GCN4 (Sellers et al. 1989; Kouzarides et al. 1989). The leucine zipper is indispensable to the biological functions of these transcription factors through dimerization.

Accordingly, we speculated that the TCP10L might possess transcription-regulating activity because of its leucine zipper motif. To elucidate the significance of the leucine zipper in TCP10L, the experiment based on the dual luciferase assay system was performed to assay the transcription function. The ORF of *TCP10L* was inserted into mammalian cell expression plasmid pM, and the recombinant plasmid pM-*TCP10L* was constructed to express the fusion protein Gal4-TCP10L. Plasmid pM-*TCP10L* was cotransfected into different cell lines with reporter plasmid pGAL45tkLUC encoding firefly luciferase and the plasmid pRL-SV40 encoding Renilla luciferase, respectively.

One negative control, mock pM, and two positive control, pM3-*VP16* and pM-*ZNF191*, were introduced in this experiment. Among them, VP16 is a known transactivator and ZNF191 is known as a transcriptional repressor (Han et al.1999). The luciferase activities of samples were determined by the Dual-Luciferase Assay System (Promega). Fig. 2 shows that recombinant TCP10L could significantly inhibit the expression of the reporter gene. Compared with that of the negative control, the luciferase activity were down-regulated by about 2.6, 9.8, and 5.5 folds in HEK293, SK-HEP-1, and CHO cells, respectively ( $p < 0.001$ ,  $n = 4$ ). As shown in Figs. 2B and C, the inhibition activity of TCP10L is much more remarkable in SK-HEP-1, a hepatoma-derived cell line, than in HEK293 cells.

Further, we examined whether transcription inhibition of the pGAL45tkLUC reporter gene activity was proportioned to the used amount of *TCP10L*. In this experiment, we used a fixed amount of pGAL45tkLUC reporter gene with varying amounts of *TCP10L* to transfect SK-HEP-1 cells, and the total amount of DNA was equalized by adding 10 ng pRL-SV40. In Figs. 2D and E, we showed that TCP10L gave dose-dependent expression inhibition of the pGAL45tkLUC reporter gene ( $p < 0.001$ ,  $n = 4$ ) and that ZNF191 as a control also gave dose-dependent repression to the expression of the reporter gene ( $p < 0.001$ ,  $n = 4$ ).

As we know, the leucine zipper consists of a periodic repetition of leucine residues at every seventh position, and regions containing them appear to span eight turns of alpha-helix. The leucine side chains that extend from one helix interact with those from a similar helix, hence facilitating dimerization in the form of a coiled-coil. Thus, the zipper is important for the protein to bind to a specific sequence of DNA.

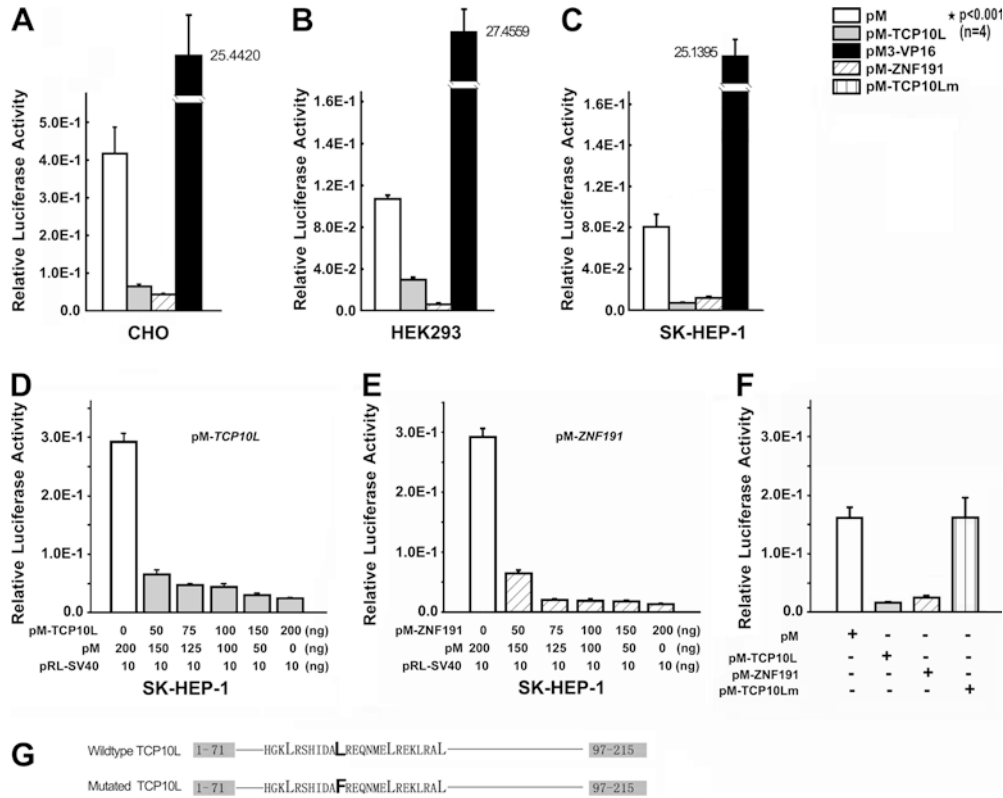


**Fig. 1A–C** The cDNA sequence, the putative protein, and the leucine zipper motif of human *TCP10L*. **A** The sequences of human *TCP10L* cDNA and its putative *TCP10L* protein. The sequences shaded in *gray* are the two primers, JY-A and JY-B, to amplify the full-length cDNA from the human  $\lambda$ gt11 liver cDNA library; the letters *underlined* are the start codon, the *asterisk* indicates the stop codon; two poly (A) signal are *boxed*. In addition, the *regular numbers* are the numbers of the nucleotides, and the *italic numbers* are the numbers of amino acids. The GenBank accession numbers of the *TCP10L* gene is AF115967. **B** The structure models of the *TCP10L* protein and other 13 proteins containing the leucine zipper motif. The *boxes* are the position of the leucine zipper motifs in these proteins. The other 13 proteins are BACH2 (Genbank accession number: XM\_031521), c-MYC (X00364), C/EBP (X52560), KRM12 (AF109780), LOC90183 (XM\_029709), NRL (XM\_033338), LAZipII (AF408398.1), c-FOS (V01512), GCN4 (K02205), TBZF (AB032478), BATF (XM\_041063), MafK (AF059194), AP-1 (J04111). **C** Comparison of the leucine zipper motifs of the 14 proteins. All of the protein sequences are displayed in *single-letter code*. The abbreviations of *Hs*, *Dr*, *Mm*, *Sc* and *Nt* represent *Homo sapiens*, *Danio rerio*, *Mus musculus*, *Saccharomyces cerevisiae*, *Nicotiana tobacum* respectively. The digits shaded in *gray* represent the non-leucine-zipper region of the 13 proteins. The *letters enlarged and bolded* show the periodical arrays of leucine or methionine residues

Therefore, we constructed a mutated type of *TCP10L* where the second leucine residue (UUG) of the zipper was replaced instead of phenylalanine (UUC) (Fig. 2G). It showed that the repression of *TCP10L* to the expression of the reporter gene almost completely decreased ( $p < 0.001$ ,  $n = 4$ ) (Fig. 2F). Another mutation, the third leucine residue (CUC) of the zipper replaced by arginine (CGC), also lead to the inhibitory activity loss of *TCP10L* (data not shown). The results suggest that the leucine zipper structure is critical for *TCP10L* to play its role in the regulatory function.

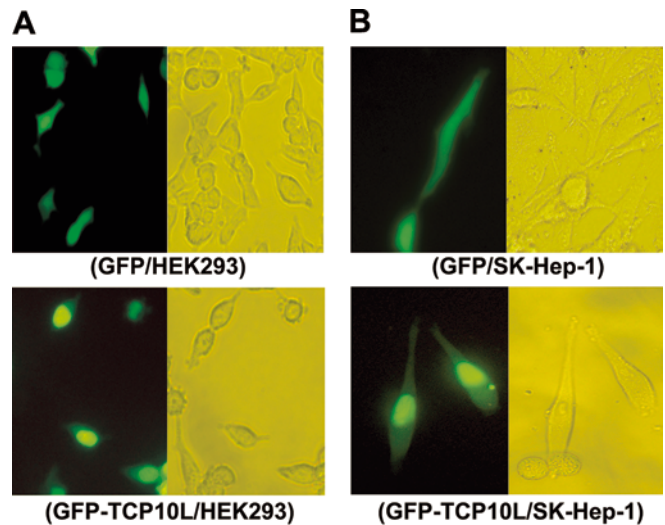
#### Subcellular localization of human *TCP10L* fused with EGFP

After we indicated that the *TCP10L* have a transcription repression function, we further explored the cellular localization of *TCP10L*. We hypothesize that, if *TCP10L* is a transcription factor, it must localize in



**Fig. 2A–G** Transcription function analysis of human TCP10L protein. By cotransfecting recombinant plasmids pM-TCP10L with reporter plasmid pGAL45tkLUC, determination of luciferase activity was performed in CHO (A), HEK293 (B), and SK-HEP-1 (C) cells respectively. One negative control, pM, and two positive controls (a transcriptional activator pM3-VP16, a transcriptional repressor pM-ZNF191) were introduced into this assay system. The plasmid pRL-SV40 encoding Renilla luciferase was used as the internal control in each transfection. **D** Dose-dependent inhibition of pM-TCP10L to the activity of the reporter gene pGAL45tk-LUC. A fixed amount of reporter gene with varying amounts of TCP10L was used; the total amount of DNA was equalized by adding 10 ng pRL-SV40. **E** Dose-dependent inhibition of pM-ZNF191 to the activity of the reporter gene as control. A fixed amount of reporter gene with varying amount of ZNF191 was used. **F** Comparison of the luciferase activity of wild type TCP10L and its mutated type TCP10Lm in SK-HEP-1 cell line. The positive control, pM-ZNF191 is also shown. **G** The leucine zipper sequence of wild type TCP10L and its mutated type TCP10Lm, where the second leucine residue (UUG) of the zipper was replaced instead of phenylalanine (UUC). In this figure, the blank columns, the gray columns, the black columns, and the diagonal columns indicate transfection of pM, pM-TCP10L, pM3-VP16, and pM-ZNF191 respectively. Each value in this figure represents the mean of the enzyme activities determined in four assays. The error bars indicate standard deviation from means

nuclei. In order to test our assumption, we fused TCP10L with EGFP (enhanced green fluorescent protein) to explore its intracellular localization. When TCP10L-EGFP plasmid was transfected into cells, it showed that the fusion protein accumulated in nuclei both in the HEK293 and in SK-HEP-1 cell lines, while EGFP protein, the control, diffused all over the cell (Fig. 3). Thus, the nuclear accumulation experiments of TCP10L proved our hypothesis.



**Fig. 3** The subcellular localization of TCP10L. Two cell lines, HEK293 and SK-HEP-1 cells, were transfected with recombinant plasmid EGFP-TCP10L, and plasmid EGFP was used as control. Adherent cells were mounted on glass slides and examined with confocal microscope (Leica)

Tissue expression patterns of human TCP10L

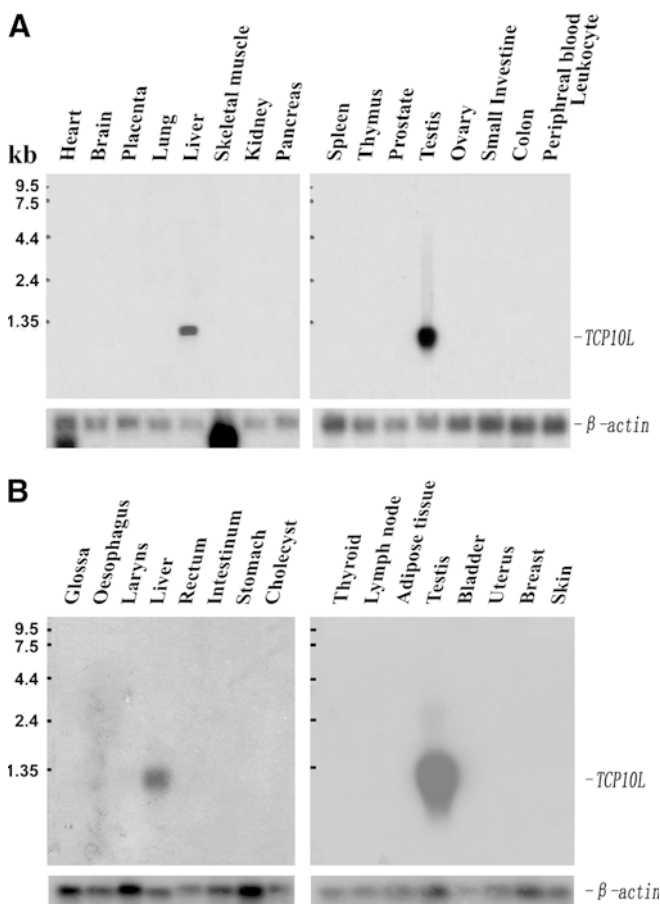
In order to further explore TCP10L function, the expression pattern of human TCP10L in adult human tissue was also examined by Northern hybridization. Using isotope-labeled JY-AB fragment as a probe, the hybridization to the membrane with blotting mRNA from human tissues showed that a transcript about

1.0 kb abundantly expressed in the liver and testis, but cannot be found in 28 other tissues (Fig. 4). In the female, *TCP10L* only expresses in the liver. Metabolic reactions including synthesis and detoxification in human liver cells are very active, and many transcription factors are involved in the expression regulation of various genes participating in these reactions (Schiaffonati et al. 1997). We suggested *TCP10L* might be one of those candidates.

#### Chromosome localization and genomic structure of human *TCP10L*

To understand the genomic characters of human *TCP10L*, we searched the on-line database in the Web site of the Human Genome Project Working Draft (<http://genome.ucsc.edu>), using the full length *TCP10L*

cDNA as the query sequence. With the BLAT program (Kent 2002), the sequence of *TCP10L* was aligned to the sequences in the database, and the information of the localization and genomic structure of *TCP10L* was obtained. The result showed that *TCP10L* is localized at chromosome 21p22.11, and its genomic size is 8955 bp (Fig. 5A). As shown in Fig. 5B, human *TCP10L* comprises of five exons and four introns. The sizes of each intron and exon are shown in Fig. 5C. An STS marker, RH103054, is localized in the fifth exon, and another STS marker, SHGC-170092 is around exon 4 (Fig. 5B). In addition, we noticed that three genes, *GCFC* (AF153208), *SYNJI* (Cremona et al. 2000, Saito et al. 2001), and *KIAA0539* (Nagase et al. 1998) are in the vicinity of the *TCP10L* and that two disease-related genes, *SOD1* (Munch et al. 2001) and *TIAM1* (Ives et al. 1998), have also been mapped to the same chromosome segment, 21p22.11.



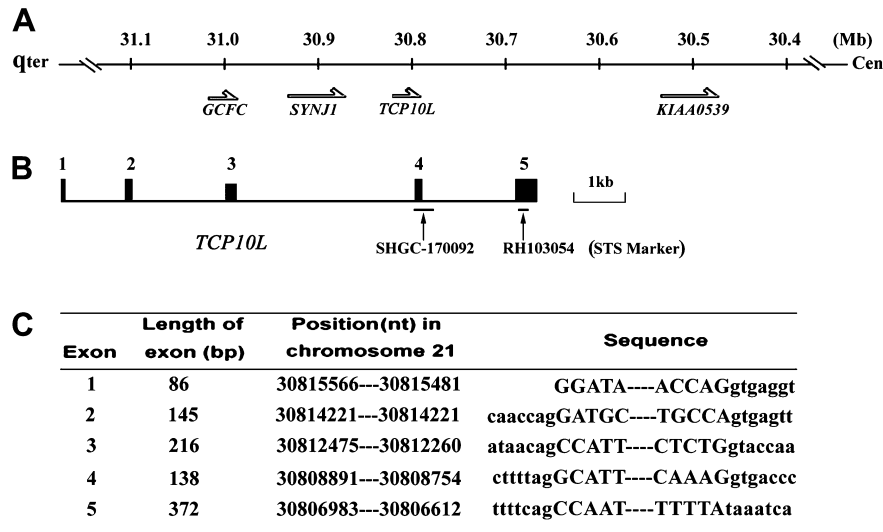
**Fig. 4A,B** The expression pattern of the *TCP10L* in human tissues. **A** Multiple-tissue Northern blots (MTN I and MTN II, Clontech) containing total RNA from 16 adult human tissues were hybridized with the isotope-labeled *TCP10L* cDNA, and the  $\beta$ -actin was used as control. **B** MHB1004 and MHB1006 containing total RNA from 16 adult human tissues were hybridized with the isotope-labeled *TCP10L* cDNA, and the  $\beta$ -actin was used as control. With the Megaprime DNA labeling system (Amersham), the probes were prepared by labeling the cDNA fragments with [ $\alpha$ - $^{32}$ P]dATP and were purified on a Sepharose-G50 column

#### Discussion

The liver is an essential organ bearing many physiological functions. Northern hybridization results in our study showed that *TCP10L* is a novel gene specifically expressed in the liver and testis. The gene structure showed that it was composed of five exons and four introns and spanned about 9.0 kb. Although the flanking sequence of the initial codon ATG is not completely consistent with Kozak rule, there are three stop codons before ATG, indicating the cDNA we cloned is nearly full length. The *TCP10L* contains the representative character of the leucine zipper motif, with a leucine residue in every seventh position. The leucine zipper motif has been observed in a number of proteins thought to function as eukaryotic transcription factors. We hypothesized human *TCP10L* is also a transcription factor. In order to know whether it is a transcriptional activating or repressing factor, we used the dual luciferase assay system. It showed that the *TCP10L* fusion gene inhibited significantly the expression of reporter plasmid pGAL45tk-LUC in CHO, HEK293, SK-Hep-1 cell lines.

It is reported that the leucine residues could be replaced with Met or Ile or Val in some leucine zippers (Bornberg et al. 1998). Take human MafK(AF059194) protein as an example. There is a methionine residue among the periodical repeated leucine residues. In our experiment, when the critical leucine zipper motif in *TCP10L* was demolished by mutation, its transcriptional inhibition was almost disappearing. Our experimental results indicated that human *TCP10L* is a kind of transcription repressing factor. The subcellular localization that the gene is accumulated in the nuclei further proved our hypothesis to some extent.

In recent years, more and more researches have indicated that there are strong associations between oncogenesis and transcription factors possessing the leucine zipper motif (Amati et al. 1993, Luscher et al. 1999, Smith et al. 1990, Cremona et al. 2000). Based on



**Fig. 5A–C** Chromosome locus and genomic structure of the *TCP10L*. **A** Genomic localization of the *TCP10L* gene. The *TCP10L* gene is localized at chromosome 21q22.11. Three genes, *GCFC*, *SYNJ1*, *KIAA0539*, which are adjacent to the *TCP10L* gene, are also shown. The hollow arrows represent the transcription direction of each gene. **B** Genomic organization of human *TCP10L* gene. It consists of five exons and four introns. The black blocks numbered and drawn to scale represent the exons. There are two STS markers, SHGC-170092 and RH103054, near the fourth and fifth exon. **C** The size and position of the exons of the *TCP10L* gene. The sequences flanking the exon/intron boundaries are given

the abundant expression of *TCP10L* in the liver and testis, we suggested that human *TCP10L*, with its transcription inhibition activity, might also play roles in the processes of cell proliferation and differentiation in the liver and testis. Further experiments are being done in our lab to prove our suggestion.

In conclusion, human *TCP10L*, a novel gene specifically expressed in the liver and testis, was cloned and characterized, and its protein product has been testified to have transcription inhibition activity. The present study is of potential value to help us understand the molecular mechanisms of liver physiological and pathological functions.

## References

- Amati B, Brooks MW, Levy N, Littlewood TD, Evan GI, Land H (1993) Oncogenic activity of the c-Myc protein requires dimerization with Max. *Cell* 72:233–245
- Bornberg-Bauer E, Rivals E, Vingron M (1998) Computational approaches to identify leucine zippers. *Nucleic Acids Res.* 26:2740–2746
- Busch SJ, Sassone-Corsi P (1990) Dimers, leucine zippers and DNA-binding domains. *Trends Genet.* 6:36–40
- Cremona O, Nimmakayalu M, Haffner C, Bray-Ward P, Ward DC, De Camilli P (2000) Assignment of *SYNJ1* to human chromosome 21q22.2 and *Synj12* to the murine homologous region on chromosome 16C3–4 by in situ hybridization. *Cytogenet Cell Genet.* 88:89–90
- Edwards PA, Davis RA (1996) *Biochemistry of lipids, lipoproteins and membranes*, 3rd edn. New Comprehensive Biochemistry 31, Elsevier, Amsterdam, pp 341–362
- Han ZG, Zhang QH, Ye M, Kan LX, Gu BW, He KL, Shi SL, Zhou J, Fu G, Mao M, Chen SJ, Yu L, Chen Z (1999) Molecular cloning of six novel krüppel-like zinc finger genes from hematopoietic cells and identification of a novel transcriptional domain KRN. *J Biol Chem* 274:35741–35748
- Houssaint E, Hallet MM (1988) Inability of adult circulating haemopoietic stem cells to sustain haemopoiesis in mouse fetal liver microenvironment. *Immunology* 64:463–467
- Islam SD, Pilder SH, Decker CL, Cebra-Thomas JA, Silver LM (1993) The human homolog of a candidate mouse t complex responder gene: conserved motifs and evolution with punctuated equilibria. *Hum Mol Genet* 2:2075–2079
- Ives JH, Dagna-Bricarelli F, Basso G, Antonarakis SE, Jee R, Cotter F, Nizetic D (1998) Increased levels of a chromosome 21-encoded tumour invasion and metastasis factor (*TIAM1*) mRNA in bone marrow of Down syndrome children during the acute phase of AML (M7) *Genes Chromosomes Cancer* 23:61–66
- Kent WJ (2002) BLAT <PIC 001> <PIC 002> The BLAST-like alignment tool. *Genome Res* 12:656–664
- Kouzarides T, Ziff E (1989) Leucine zippers of fos, jun and GCN4 dictate dimerization specificity and thereby control DNA binding. *Nature* 340:568–571
- Landschulz WH, Johnson PF, McKnight SL (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. *Science* 240:1759–1764
- Lee YT (1983) Primary carcinoma of the liver: diagnosis, prognosis, and management. *J Surg Oncol* 22:17–25
- Luscher B, Larsson LG (1999) The basic region/helix-loop-helix/leucine zipper domain of Myc proto-oncoproteins: function and regulation. *Oncogene* 18:2955–2966
- Munch C, Ludolph AC (2001) Pharmacological treatment of ALS. *Neurol Neurochir Pol* 35 [Suppl 1] 41–50
- Nagase T, Ishikawa K, Miyajima N, Tanaka A, Kotani H, Nomura N, Ohara O (1998) Prediction of the coding sequences of unidentified human genes. IX. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Res* 5:31–39
- Perez S, Vial E, van Dam H, Castellazzi M (2001) Transcription factor ATF3 partially transforms chick embryo fibroblast by promoting growth factor-independent proliferation. *Oncogene* 20:1135–1141
- Petti S, Testa U, Migliaccio AR, Mavilio F, Marinucci M, Lazzaro D, Russo G, Mastroberardino G, Peschle C (1985) Embryonic hemopoiesis in human liver: morphologic aspects at sequential stages of ontogenic development. *Prog Clin Biol Res* 193:57–71
- Saito T, Guan F, Papolos DF, Lau S, Klein M, Fann CS, Lachman HM (2001) Mutation analysis of *SYNJ1*: a possible candidate

- gene for chromosome 21q22-linked bipolar disorder. *Mol Psychiatry* 6:387–395
- Schiaffonati L, Tiberio L (1997) Gene expression in liver after toxic injury: analysis of heat shock response and oxidative stress-inducible genes. *Liver* 17:183–191
- Sellers JW, Struhl K (1989) Change fos oncoprotein to a jun-independent DNA binding protein with GCN4 dimerization specificity by swapping “leucine zipper”. *Nature* 341:74–76
- Smith MJ, Charron-Prochownik DC, Prochownik EV (1990) The leucine zipper of c-Myc is required for full inhibition of erythroleukemia differentiation. *Mol Cell Biol* 10:5333–5339
- Sun Z, Zhu Y, Stjernsward J, Hilleman M, Collins R, Zhen Y, Hsia CC, Lu J, Huang F, Ni Z, et al (1991) Design and compliance of HBV vaccination trial on newborns to prevent hepatocellular carcinoma and 5-year results of its pilot study. *Cancer Detect Prev* 15:313–318
- Xu XR, Huang J, Xu ZG, Qian BZ, Zhu ZD, Yan Q, Cai T, Zhang X, Xiao HS, Qu J, Liu F, Huang QH, Cheng ZH, Li NG, Du JJ, Hu W, Shen KT, Lu G, Fu G, Zhong M, Xu SH, Gu WY, Huang W, Zhao XT, Hu GX, Gu JR, Chen Z, Han ZG (2001) Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding noncancerous liver. *Proc Natl Acad Sci* 98:15089–15094
- Yeh FS, Yu MC, Mo CC, Luo S, Tong MJ, Henderson BE (1989) Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. *Cancer Res* 49:2506–2509
- Yu Y, Zhang C, Zhou G, Wu S, Qu X, Wei H, Xing G, Dong C, Zhai Y, Wan J, Ouyang S, Li L, Zhang S, Zhou K, Zhang Y, Wu C, He F (2001) Gene expression profiling in human fetal liver and identification of tissue- and developmental-stage-specific genes through compiled expression profiles and efficient cloning of full-length cDNAs. *Genome Res* 11:1392–1403