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Screening and Identification of The Proteins Interacting with The Transcription Factor X-box Binding Protein 1*

GUO Feng-Jin[1], SONG Fang-Zhou[2]**, Yi Fa-Ping[2], Cheng Hai-En[2]
([1]*Department of Cell Biology and Genetics, Chongqing Medical University, Chongqing* 400016, China;
[2]*Department of Biochemistry and Molecular Biology,Chongqing Medical University, Chongqing* 400016, China)

Abstract X-box binding protein1 (XBP1) is an important transcription factor, which participates in many signal transduction procession.To investigate the biological function of XBP1, yeast two-hybrid system to screen proteins interacting with XBP1 in hepatocytes was performed. The XBP1 coding sequence was amplified by polymerase chain reaction (PCR) method, and was cloned in pGEM-T vector. After the target region was sequenced, it was subcloned into the bait plasmid pGBKT7, then was transformed into yeast AH109(a type). After the expression of bait plasmid pGBKT7-XBP1 in AH109 yeast strains were proved by Western blot. The transformed yeast AH109 was mated with yeast Y187(α type) containing hepatocyte cDNA library plasmids pACT2 in 2×YPDA medium. Diploid yeast was plated on synthetic dropout nutrient medilium (SD/-Trp-Leu-His-Ade) containing X-α-gal for selection and screening. After sequencing and verifying ORF of positive colonies,7 different kinds of proteins were obtained.In order to further testify the interaction between the screened proteins and XBP1, one of positive colonies MT1E was cloned.The interaction between MT1E and XBP1 *in vitro/in vivo* were examined successfully with GST pulldown and coimmunoprecipitations.It was shown that MT1E would be a new regulatory protein of XBP1. These screened proteins by yeast two-hybird system were closely correlated with liver fundamental metabolism,protein synthesis and transport,cell proliferation and apoptosis. The results mentioned above contributed to reveal the XBP1 biological function, and brought some new clues for further exploration of the expressing and regulating mechanism of XBP1.

Key words X-box binding protein1, yeast two-hybrid system, transcription factor, bait

Human X-box binding protein1, a basic region-leucine zipper (bZIP) protein, is an important transcription factor of CREB/ATF family. XBP1 is ubiquitously expressed in adult tissues. It is a sequence-specific DNA-binding protein, and can regulate the gene expression connecting with its binding *cis*-element factor[1,2]. XBP1-binding sites are also selected *in vitro* to generate a consensus 8 bp core motif that is very similar to CREB sites and identical to the ATF6-binding site. Kanemoto *et al*.[3] found that XBP1 activated the transcription of its target genes *via* an ACGT core sequence under ER stress. In mammalian cell, the transmembrane protein kinase/endoribonuclease IRE1 is activated by endoplasmic reticulum stress and subsequently processes XBP1 mRNA to generate the spliced form of XBP1 protein (XBP1-S)[4,5]. In IRE1-XBP1 pathway, XBP1, as a signaling molecule downstream of IRE1 and ATF6 in the UPR, participates in the IRE1α-mediated UPR signaling transmittion, coordination of IRE1α-independent ATF6 processing and IRE1α-dependent XBP1 mRNA splicing[6,7]. Nuclear-localized IRE1α and cytoplasmic-localized ATF6 signaling pathways merge through regulation of XBP1 activity to induce downstream gene expression. The IRE1-XBP1 pathway directs both protein refolding and degradation in response to ER stress. ER stress results from the accumulation of unfolded or misfolded proteins in the ER, and cells can alleviate this stress by degrading or refolding these proteins. Sriburi *et al*.[8] found that XBP1 links the mammalian UPR to phospholipid biosynthesis and biogenesis of the

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**Corresponding author . Tel: 86-23-60935289, Fax: 86-23-68485806,
E-mail: guofengjin919@sohu.com; xwx_999@263.net
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endoplasmic reticulum(ER). XBP1 is concerned with the procession of unfolded or misfolded proteins in the ER as the first transcription suppressor during ER stress stimulus.

Although there is strong evidence that XBP1 transcription factor plays an important role in the regulation of responses to ER stress stimulus, little is known about the modulation and physiological significance of XBP1 induction. Clearly, in order to understand the significance of XBP1 induction by stress signals, it is important to elucidate which target gene it regulates and how many target genes it connects with. It is significant to study the XBP1 binding protein to show the regulating mechanism of XBP1. In this study, we designed a bait plasmid including the XBP1 sequence, using yeast two-hybird system and a series of testify experiments in order to screen out XBP1 binding proteins in hepatocytes. It is reasonable to expect that these binding proteins would provide some new clues for the research of the physiological function of XBP1.

1 Materials and methods

1.1 Materials

All of the yeast strains and plasmids for yeast two-hybrid experiments,including Matchmaker GAL4 two -hybrid system 3 and vector pACT2 containing the human liver cDNA library, were obtained from Clontech Co. USA[9]. X-α-gal, 3-AT and all kinds of selection media were from Clontech Co.

pGEX-4T-2 vector was from Amersham Pharmacia Co, and pGEM T vector was from Promega Co. All of the restriction endonuclease were from TaKaRa Co; RT-PCR kit and TNT® Quick Coupled Transcription/ Translation Systems were from Promega Co; the MATCHMAKER Co-IP kit was from ClontechCo; GST Gene Fusion system, IPTG and [^{35}S] -methionine were from Amersham Pharmacia Co. Both anti-XBP1 monoclonal antibody and anti-ME1T polyclonal antibody were generated by Beijing Biosynthesis Biotechnology Co, and Zymoprotein inhibitor was from RocheCo.

1.2 Amplification of the XBP1 region and construction of "bait" plasmid

XBP1 sequence [10] was generated by PCR amplification of human L02 cDNA.L02 cell is the normal liver cell. The sequence of the primers containing the *Eco*R Ⅰ and *Bam*H Ⅰ restriction enzyme sites was the following: sense primer, 5′ *GAATTC* ATGGTGGTGGTGGCAG 3′, antisense primer, 5′ *GGATCC* GTTCATTAATGGCTTCCAG 3′. The PCR conditions were at 94℃ for 1 min, 63℃ for 1 min and 72℃ for 1 min. 10 ng of PCR products were cloned with pGEM-T vector. The primary structure of insert was confirmed by direct sequencing. β-actin was the positive control. The primers were: sense primer, 5′ GTGGGGCGCCCCAGGCACCA 3′, antisense primer, 5′ CTTCCTTAATGTCACGCAC-GATTTC 3′. XBP1 sequence was released from pGEMT-XBP1 by digestion with *Eco*R Ⅰ and *Bam*H Ⅰ, and subcloned into pGBKT7. Plasmid pGBKT7-XBP1 containing full-length XBP1 ORF could directly express DNA binding domain, c-myc and XBP1 fusion protein.The construction was verified by restriction enzyme digestion and sequencing.

1.3 Western blotting analysis

The bait plasmid was transformed into yeast strain AH109 by lithium acetate method, in which XBP1 cDNAs was inserted in-frame with a Myc-epitope. After transformed, a single isolated colony (1 ～2 mm in diamete) of yeast AH109 transformed with pGBKT7-XBP1 was selected and the yeast fusion protein was extracted according to Urea/SDS method. Western blotting was performed to confirm the expression of the fusion protein using c-myc monoclonal antibody. The yeast AH109 cells with transformed pGBKT7 were used as positive control and the untransformed yeast AH109 cells were used as negative control.

1.4 Screening of the hepatocyte cDNA library by the yeast two-hybrid system

The yeast AH109 containing pGBKT7-XBP1was cultured on SD/-Trp/X-gal, SD/-Trp/-His/X-gal and SD/-Trp/-Ade/X-gal to exclude the autonomous transcriptional activity. Then the transformed AH109 mated with yeast Y187 containing pACT2 with human liver cDNA library for 22 ～24 h. The cells were spread on 50 large(150 mm) plates containing 100 ml of SD/-Leu/-Trp (low-stringency), SD/-His/-Leu/-Trp (medium-stringency) and SD/-Ade/-His/-Leu/-Trp (QDO, high-stringency). 2.5 mmol/L 3-AT was added into the selection media to reduce background. After growing for 7 ～20 days, the yeast colonies were transferred onto the plates containing X-α-gal to check for expression of the MEL1 reporter gene (blue colonies) to exclude false positives.The yeast AH109 containing pGBKT7-53 mated with yeast strain Y187 containing pGADT7-T provided a positive control for

interacting proteins. The yeast AH109 containing pGBKT7-53 mated with yeast strain Y187 containing pGBKT7-Lam provided a negative control[9].

1.5 Plasmids isolation from yeast and PCR identification

Yeast plasmid was isolated from positive yeast colonies by the beading method, and thirteen positive plasmids were obtained, which could be amplified by PCR.Because the cDNA libraries were inserted into the pACT2 plasmid, the current primer was the following: sense, 5′ CTATTCGATGATGAAGATACCCCACCAAACCC 3′, antisense, 5′ GTGAACTTGCGG GGTTTTTCAGTATCTACGA 3′. Then many different bands ranging from 200 bp to 800 bp were observed by 0.9% agarose gel electrophoresis. These diffenent PCR products were analyzed by sequencing in ShangHai JiKang Biological Corporation.

1.6 Bioinformatic analysis and part screening gene clone

After sequencing of the positive colonies, the sequences were blasted with GenBank to analyze the function of the genes. On the basis of liver cDNA library of genes of proteins interacting with XBP1 protein, the coding sequence of MT1E was obtained by bioinformatics methods. The sequence of the primers containing the *Eco*R Ⅰ and *Bam*H Ⅰ restriction enzyme sites were the following, sense, 5′ *GAATTC* ATGGACCCCAACTG CTC 3′, antisense, 5′ *GGATCC* CTAATAGCAAGCCTGGCTC 3′. The PCR conditions were: 95℃ for 60 s, 60℃ for 60 s, and 72℃ for 60 s, altogether for 35 cycles. The MT1E PCR product was cloned with pGEM-T vector. The primary structure of insert was confirmed by direct sequencing. The gene fragment was subcloned into plasmids pGADT7.The construction was verified by restriction enzyme digestion and sequencing.

1.7 Confirmation of the true interaction in yeast

To confirm the true protein-protein interaction and to exclude false positives, the plasmids of positive colonies pGADT7-MT1E were reintroduced into yeast strain Y187, and then mating experiments were carried out by mating yeast strain AH109 containing pGBKT7-XBP1 with yeast strain Y187 containing pGADT7-MT1E. The yeast AH109 containing pGBKT7-53 mated with yeast strain Y187 containing pGADT7-T provided a positive control for interacting proteins. The yeast AH109 containing pGBKT7-53 mated with yeast strain Y187 containing pGBKT7-Lam provided a negative control [9]. After mating, the diploids yeast were plated on SD/-Ade-His-Leu-Trp (QDO) and then lined on SD/-Ade-His-Leu-Trp covered with X-α-gal to test the specificity of interactions. pCL1 encoded the full-length, wild-type GAL4 protein and provided a positive control for α-galactosidase assays.

1.8 Interaction between XBP1 and MT1E *in vitro*

The pGEX-4T-2 and pGEX-4T-2-XBP1 plasmid were transformed into BL21 *Escherichia coli*, respectively. After induced in 0.1 mmol/L IPTG at 30℃ for 4 h, the bacteria were collected, broken and harvested. After added into the glutathione Sepharose 4B, the harvested bacteria were rocked at room temperature for 30 min, discarded the supernatant. Then added into reductive glutathione wash buffer, rocked at room temperature for 20 min, harvested the supernatant, which was the purified GST and GST-XBP1.

MT1E was associated specifically with GST-XBP1 in a GST pull-down assay. MT1E was translated *in vitro* by the TNT ® T7 Coupled Reticulocyte Lysate System, and marked with [^{35}S]. Then MT1E was mixed with either GST-XBP1 or GST alone in a 1∶10 ratio. The proteins were allowed to interact for 30 min at room temperature before added to 400 μl of PBS containing ～25 μl of glutathione beads. After rocked overnight at 4℃, the beads were washed, and the proteins were eluted from the beads by boiling in 2× sample loading buffer. The proteins were separated by SDS-PAGE, then the gel was dried and X-ray film was developed by standard techniques.

1.9 Interaction between XBP1 and MT1E *in vivo*

The mouse fetal liver tissue was collected, cut with scissors and then rinsed with 500 ml 0.7% NaCl-0.04% Triton X-100, followed by 500 ml 0.7% NaCl. Then the tissues were suspended in 5 ml/g of buffer Ⅰ, and homogenized. After filtered, the nuclei were harvested by centrifuging at 2 000 *g* for 10 min, resuspended in 5 ml/g buffer Ⅰ, and the suspension was overlaid on an equal volume of buffer Ⅱ. The suspension was centrifuged at 2 000 *g* for 10 min, and the nuclear pellet was resuspended in low-salt buffer. High-salt buffer was added to make the final concentration of salt 0.8 mol/L. The mixture was placed on a shaker for at least 30 min at 4℃ and then spun for 1 h at 40 000 *g*. After the lipid layer was removed, the supernatant was the nuclear extract

which was ready for the coimmunoprecipitations *in vivo*.

XBP1 monoclonal antibody and a commercially available β-galactosidase monoclonal antibody were cross-linked to a mixture of protein A/G agarose beads. Approximately 25 μl of the cross-linked beads were added to 100 μl of liver nuclear extract in 400 μl of Co-IP buffer. The beads containing nuclear extracts were rocked overnight at 4℃. The beads were washed six times with Co-IP buffer lacking magnesium. 50 μl of 2× sample loading buffer was added to the beads, and the mixture was boiled.The extracts were then separated by SDS-PAGE. The proteins were transferred to PVDF membranes, which were probed with XBP1 antibody.

2 Results

2.1 Identification of recombinant plasmids

The full length sequences of XBP1 and MT1E were generated by PCR amplification of L02 cDNA. Analysis of the PCR reaction products by agarose gel electrophoresis showed the clear bands with the expected size (789 bp of XBP1, 185 bp of MT1E and 540 bp of β-actin). β-actin was the positive control. Sequences of the PCR products were correct.Then they were cloned in pGEM-T vector respectively. After sequenced and analyzed by comparing with Vector NTI 8 and BLAST database homology search, the XBP1 fragment was in-frame ligated into pGBKT7 plasmid at the *Eco*R Ⅰ/*Bam*H Ⅰ site and MT1E fragment was in-frame ligated into pGADT7 plasmid at the *Eco*R Ⅰ/*Bam*H Ⅰ site. The results of restriction enzyme analysis of pGBKT7-XBP1 and pGADT7-MT1E with *Eco*R Ⅰ/*Bam*H Ⅰ were: 7 300 bp empty pGBKT7 and 789 bp XBP1; 7 900 bp empty pGADT7 and 185 bp MT1E.

2.2 Expression of the fusion protein

Yeast strain AH109 transformed with pGBKT7-XBP1 and yeast strain Y187 transformed with pGADT7-MT1E could stably express the fusion protein at high level. The XBP1 fusion protein was 50 ku and the MT1E fusion protein was 28 ku (Figure 1a and 1b).

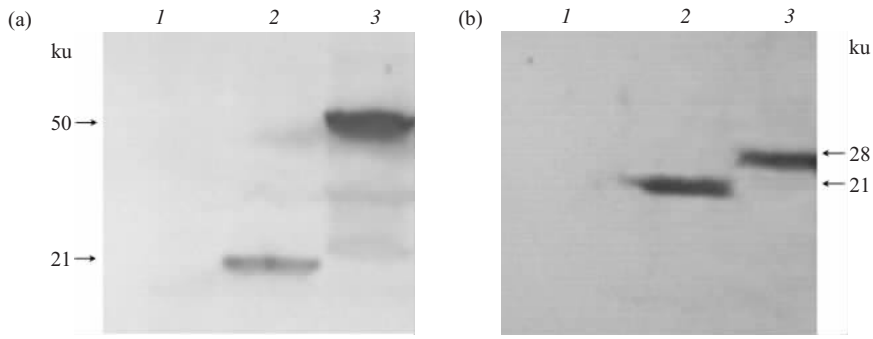

Fig. 1 Western blotting showed the expression of XBP1 (a) and MT1E (b) in yeast
(a) *1*: Negative control; *2*: pGBKT7 protein(positive control); *3*: pGBKT7-XBP1 fusion protein. (b) *1*: Negative control; 2 : pGADT7 protein(positive control); *3*: pGADT7-MT1E fusion protein.

2.3 Screening of the hepatocyte cDNA library

The XBP1 protein was used as the bait for screening human hepatocyte cDNA library. Thirty-seven clones grew in the absence of tryptophan, leucine, histidine, adenine. The clones were processed for β-galactosidase assay, and blue colonies were picked.Yeast plasmid was isolated from positive yeast colonies by the beading method and received twenty-one positive plasmids. After the plasmids were amplified by current primer, the different fragments were obtained whose length ranged from 200 bp to 800 bp. Figure 2 showed some positive yeast strain PCR products after X-α-gal identification.

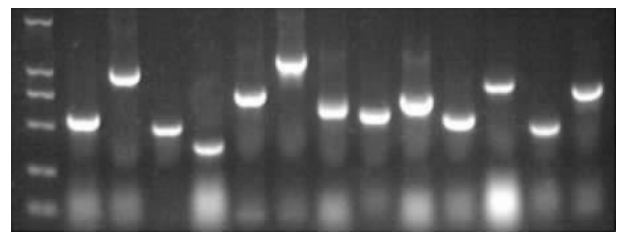

Fig. 2 Some positive yeast strain PCR products after X-α-gal identification
(1% agarose gel electrophoresis).

To confirm the true interaction with XBP1 protein in yeast, the plasmids of twenty-one independent positive colonies were transformed into yeast strain Y187, and were further tested for specificity of β-galactosidase expression. In Figure 3, twenty-one positive clones, which grew on media lacking leucine, tryptophan, histidine and adenine,were lined on the media covered with X-α-gal and all became blue. After confirmation of the true interaction with XBP1 protein in yeast, twenty-one independent positive clones were identified and sequenced.

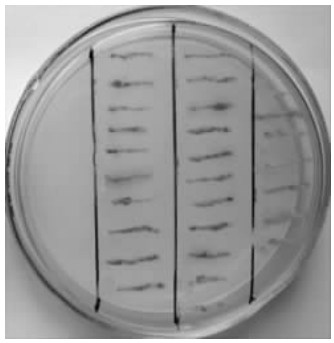

Fig. 3 Positive clones interactive with XBP1 protein lining on QDO containing X-α-gal

2.4 Analysis of coding sequence of positive clones

The nucleotide sequences of 21 clones from this cDNA library were sequenced and analyzed, but only the sequencing results of 13 clones revealed ORF in frame with the AD coding region.The presence of an open reading frame (ORF) fused with the GAL4 AD sequence was verified, and the sequence was compared with those in GenBank. The mRNA full length sequences were obtained with Vector NTI 8.0 and by searching BLAST database (http://www.ncbi.nlm.nih.gov/). These colonies were prescreened by PCR to make sure that only colonies with different products were subjected to sequencing. Summary of the data is presented in Table 1.

Table 1 Comparison between positive clones and similar sequences in GenBank

High similarity to known genes (mRNA)	Number of similar	GeneBank number	Site of ORF	Homolog/%
Homo sapiens haplotype M*2 mitochondrion	3	AF382012	3331-3566 (cds:3307～4263)	100%
Homo sapiens syntaxin 11	2	AF071504	67-543 (cds: 55～918)	100%
Homo sapiens metallothionein 1E	2	NM_175617	192-321 (cds: 180～365)	100%
Homo sapiens metallothionein 2A	1	BC007034	85-205 (cds:76～261)	100%
Homo sapiens alanine-glyoxylate aminotransferase (AGT1)	2	NM_000030	841-1066 (cds:123～1301)	100%
Homo sapiens dicarbonyl/L-xylulose reductase (DCXR)	1	NM_016286	64-598 (cds:16～750)	99%
Homo sapiens transferrin (TF)	2	NM_001063	408-834 (cds:51～2147)	99%

2.5 Interaction between XBP1 and MT1E *in vitro*

After GST-XBP1 and GST were transformed in *E. coli* BL21, GST was mostly expressed in the supernatant. Only 20% GST-XBP1 was expressed in the supernatant, and the rest was in the inclusion bodies. The purified GST and GST-XBP1 were mixed with MT1E, respectively, which was translated *in vitro* and marked with [^{35}S]. The result showed the interaction between XBP1 and MT1E *in vitro* (Figure 4).

2.6 Interaction between XBP1 and MT1E *in vivo*

The results described in the previous sections demonstrated that XBP1 and MT1E proteins could interact with each other *in vitro*. We expected to confirm that these two proteins also interact with each other *in vivo*. For this purpose, we immunoprecipitated nuclear extracts prepared from mouse fetal liver tissue with the XBP1 monoclonal antibody or as a control with an antibody directed against bacterial β-galactosidase. The proteins recovered in the

immunoprecipitate were then analyzed by gel electrophoresis and blotting.

As shown in Figure 5, the blot was probed with XBP1 monoclonal antibody. In the lane labeled "nuc ext", nuclear extract was loaded and used as the positive control for XBP1. The lane labeled MT1E IP contains the proteins immunoprecipitated from nuclear extracts with polyclonal MT1E antibody. Comparing with the "nuc ext" lane, the same protein species XBP1 was observed in the lane labeled MT1E IP. The XBP1 protein was not, however, observed in the lane labeled LacZ IP, which contains the proteins immunoprecipitated from nuclear extract with β-galactosidase antibody. These results indicated XBP1 and MT1E were in an immunoprecipitable complex in mouse fetal liver nuclear extracts. However, there was an unknown protein in MT1E IP lane whose molecular mass was close to MT1E, which might correspond to protein associated with the MT1E and be recognized by the secondary antibody, as we suspected.

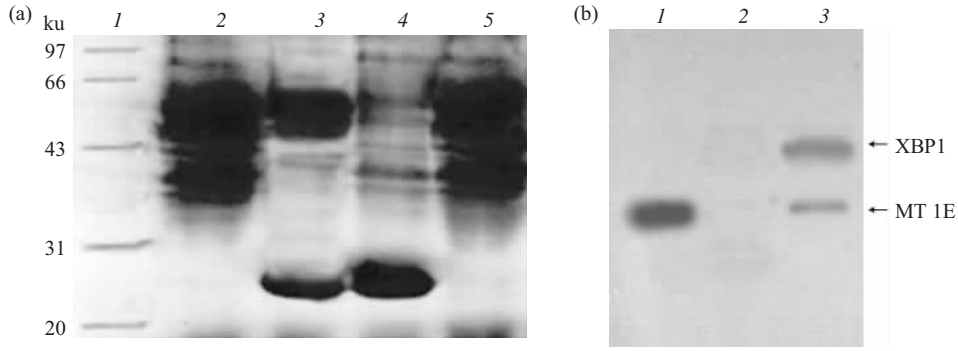

Fig. 4 XBP1 interacts with MT1E *in vitro*

(a) SDS-PAGE analysis of GST-XBP1 fusion protein and GST expressed in *E. coli* BL21. *1*: Mid-range protein molecular mass marker; *2*: Total protein of *E. coli* BL21 transformed by control vector pGEX-4T-2; *3*: Purified GST-XBP1 fusion protein; *4*: Purified GST protein; *5*: Total protein of *E. coli* BL21 transformed by pGEX-4T-2-XBP1. (b) SDS-PAGE and autoradiography represents that XBP1 binds MT1E *in vitro*. *1*: The protein MT1E full length translated *in vitro*; *2*: GST can not pull down MT1E; *3*: GST-XBP1 can pull down MT1E full length.

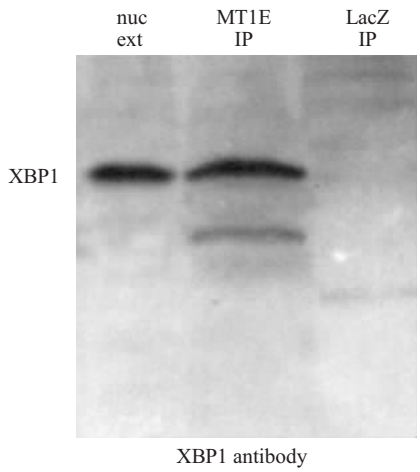

Fig. 5 Endogenous XBP1-MT1E complex were co-immunoprecipitated with the anti-XBP1 monoclonal antibody

The nuc ext lane and the LacZ IP lane were served as controls.

3 Discussion

Yeast two-hybrid system provides a sensitive method for detecting relatively weak and transient protein-protein interactions. This system takes advantage of the properties of the GAL4 protein of the yeast *Saccharomyces cerevisiae*. GAL4 - yeast two-hybrid assay uses two expression vectors, of which uses GAL4 DNA-binding domain (BD) and another uses GAL4 transcription-activation domain (AD). Expression vector pGBKT7 is fused with amino acids 1～147 of the GAL4 DNA BD and the GAL4-BD fuse with a protein 'X', expression vector pGADT7 is fused with amino acids 768～881 of the GAL4 DNA AD and the GAL4-AD fuse with a protein 'Y', respectively. A selection of host cells with different reporter genes and different growth selection markers provide a means to detect and confirm protein-protein interactions. The yeast strain AH109, using three reporters——ADE2, HIS3, and MEL1 (or lacZ), virtually eliminates false positives[11,12]. In this study, the "bait" plasmid pGBKT7-XBP1 was transformed into yeast strain AH109. XBP1 gene was expressed in yeast cells. After the "bait" plasmid pGBKT7-XBP1

yeast strain AH109s mated with liver cDNA library yeast strain Y187, the diploid yeast cells were plated on QDO media containing X-α-gal, 21 true positives were obtained. By sequencing analysis of isolated library plasmids, we obtained the sequences of 13 genes with known functions. In order to further testify the interaction between the screened proteins and XBP1, one of positive colonies MT1E was cloned. The interaction between MT1E and XBP1 *in vitro*/*in vivo* were verified successfully by GST pulldown and coimmunoprecipitations. Besides, 7 kinds of putative proteins interacting with XBP1 were found to include the following: mitochondria, syntaxin 11, metallothionein 1E and 2A, alanine : glyoxylate aminotransferase (AGT1), dicarbonyl/L-xylulose reductase (DCXR) and transferrin (TF).

In our screened proteins, Homo sapiens metallothionein 2A (MT2A) and MT1E were the important proteins in liver. We have testified the interaction between MT1E and XBP1 *in vitro* and *in vivo*. So we concluded that the interaction between them was important to XBP1. Metallothioneins (MTs), a group of ubiquitous metalloproteins, comprise isoforms encoded by ten functional genes in humans. Metallothionein (MT) was expressed to a certain extent in almost all mammalian tissues. Current knowledge of MT was juxtaposed with our understanding of the pathogenesis of disease. MT was known to modulate three fundamental processes: (1) the release of gaseous mediators such as hydroxyl radical or nitric oxide, (2) apoptosis, and (3) the binding and exchange of heavy metals such as zinc, cadmium or copper. The biological significance of MT was related to its various forms MT-1, MT-2, MT-3 and MT4. Different MT isoforms possibly played different functional roles during development or under various physiological conditions such as proliferation and apoptosis [13,14]. For MT-1 several isoforms of the protein existed and it was likely that these isoforms were related to various functions involved in developmental processes occurring at various stages of gestation. MT1E lies in 16q13, which is a small stress response protein induced by exposure to heavy metal cations, oxidative stressors, and acute phase cytokines that mediate inflammation. Human X-box binding protein1 (XBP1) was also a member of the ER stress-response genes during the unfolded protein response (UPR) [15,16], which was essential for survival of all eukaryotic cells under conditions of ER stress and was also essential for differentiation and/or survival of eukaryotic cells. Therefore, we concluded that both XBP1 and MT1E were protective proteins in abnormal environmental stress. The interaction between them could help the cell to bear and relieve the unfavourable influence due to the abnormal surroundings. If the associations between MT1E and XBP1 became abnormal, some diseases, including cancer, circulatory and septic shock, coronary artery disease, and Alzheimer's disease would develop.

Homo sapiens transferrin (TF) was the major iron-binding protein in vertebrate serum, which was responsible for the iron transportation [17]. It was reasonable to conclude that XBP1 might participate in the iron transport procession, and maintain Fe ion homeostasis in cell on basis of our research. Homo sapiens syntaxins 11 was an important target-membrane-associated SNARE (t-SNARE), which was found to serve as targets for the assembly of protein complexes important in regulating membrane fusion [18,19]. Syntaxin11, binding with XBP1, might coordinate each other and finished the proteins folding, trafficking, docking and transportation. Besides, another sort of proteins interacting with XBP1 protein from liver cDNA library was a series of liver-specific intermediary metabolic enzymes, including human alanine:glyoxylate aminotransferase (AGT1) and human dicarbonyl/L-xylulose reductase (DCXR). AGT1 catalysed the irreversible transamination of glyoxylate to glycine with L-alanine as the amino-group donor, and it was targeted to different organelles (mitochondria and/or peroxisomes) in different species[20]. Human L-xylulose reductase[21] was an enzyme of the glucuronic acid/uronate cycle of glucose metabolism. It was well known that XBP1 was essential for liver growth. Mice lacking XBP1 displayed hypoplastic fetal livers [22]. These liver-specific intermediary metabolic enzymes interacting with XBP1 participated in all kinds of pathologic and physiologic procession in liver. In our research, we found that XBP1 was also related with the liver metabolism procession *via* these enzymes, and XBP1 could regulate this procession as transcription factor. The interaction between XBP1 and these metabolic enzymes need to be studied deeply and it will help to clarify the regulating mechanism of XBP1.

The screened proteins are closely correlated with proteins transportation, redox balance in cells, ion

homeostasisand and fundamental metabolism, which are connected with XBP1 function. It may provide a new research clue for revealing biological functions of XBP1, liver pathogenesis and causes of malignancy conversion. How the interactions between XBP1 and the above-mentioned interacting proteins affect the occurrence and development of chronic hepatitis, hepatic fibrosis and hepatocarcinoma needs to be further studied.This study is of significance to clarify the XBP1 gene transcriptional regulation mechanism, the connection between cis-acting element and its binding trans-acting factor within the XBP1 regulation region.

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转录因子 X-box 结合蛋白 1 相互作用蛋白的筛选和鉴定 *

郭风劲 [1)]　宋方洲 [2)**]　易发平 [2)]　成海恩 [2)]

([1)]重庆医科大学细胞生物学与遗传学教研室，重庆 400016；
[2)]重庆医科大学生物化学与分子生物学教研室，重庆 400016)

摘要　X-box 结合蛋白 1 是一种重要的转录因子，参与体内多项信号转导过程. 为进一步研究 XBP1 的生物学功能，运用酵母双杂交技术在肝细胞文库中筛选 XBP1 的结合蛋白. 首先运用 PCR 技术扩增获得 XBP1 的编码序列，克隆至 pGEM-T 载体，经测序鉴定后，亚克隆至诱饵载体 pGBKT7 中，转化酵母 AH109(a type). 免疫印迹检测诱饵质粒 pGBKT7-XBP1 在 AH109 酵母中的表达之后，含有诱饵质粒的酵母 AH109 与含有肝细胞 cDNA 文库质粒 pACT2 的酵母 Y187(α type)配合，配合后的二倍体酵母生长在含有 X-α-gal 的营养缺陷型培养基上 (SD/-Trp-Leu-His-Ade) 进行选择和筛选，经测序和序列比对确定阳性克隆的开放读码框 ORF，得到 7 种不同的蛋白质. 为了进一步验证这些筛选蛋白质与 XBP1 的相互作用，克隆其中一种蛋白质 MT1E，并运用 GST pulldown 和免疫共沉淀技术成功检测了 MT1E 和 XBP1 的相互作用(体外 / 体内)，结果提示，MT1E 可能是 XBP1 的一个新的调节蛋白. 通过酵母双杂交技术筛选得到的 7 种蛋白质分别与肝细胞基础代谢、蛋白质的合成与运输、细胞的增殖与凋亡密切相关. 上述结果有助于揭示 XBP1 的生物学功能，为进一步探讨 XBP1 的表达和调控机制提供新线索.

关键词　X-box 结合蛋白 1，酵母双杂交，转录因子，诱饵
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** 通讯联系人. Tel: 023-60935289, Fax: 023-68485806, E-mail: guofengjin919@sohu.com; xwx_999@263.net
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