Gene Cloning and Nucleotide Sequence of Human Dihydrolipoamide Dehydrogenase-Binding Protein

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ABSTRACT : The pyruvate dehydrogenase complex (PDC), a member of α-keto acid dehydrogenase complex, catalyzes the oxidative decarboxylation of pyruvate with the formation of CO_2, acetyl-CoA, NADH, and H^+. This complex contains multiple copies of three catalytic components including pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3). Two regulatory components (E1-kinase and phospho-E1 phosphatase) and functionally less-understood protein (protein X, E3BP) are also involved in the formation of the complex. In this study, we have partially cloned the gene for E3BP in human. Nine putative clones were isolated by human genomic library screening with 1.35 kb fragment of E3BP cDNA as a probe. For investigation of cloned genes, Southern blot analysis and the construction of the restriction map were performed. One of the isolated clones, E3BP741, has a 3 kb-*Sac*I fragment, which contains 200 bp region matched with E3BP cDNA sequences. The matched DNA sequence encodes the carboxyl-terminal portion of lipoyl-bearing domain and hinge region of human E3BP. Differences between yeast E3BP and mammalian E3BP coupled with the remarkable similarity between mammalian E2 and mammalian E3BP were confirmed from the comparison of the nucleotide sequence and the deduced amino acid sequence in the cloned E3BP. Cloning of human E3BP gene and analysis of the gene structure will facilitate the understanding of the role(s) of E3BP in mammalian PDC. ***(Asian-Aust. J. Anim. Sci. 2001. Vol 15, No. 3 : 421-426)***

Key Words : Pyruvate Dehydrogenase Complex, Dihydrolipoamide Dehydrogenase-Binding Protein, Nucleotide Sequence

INTRODUCTION

The pyruvate dehydrogenase complex (PDC), a member of alpha-keto acid dehydrogenase complex, is a large enzyme complex that has essential roles in biological energy metabolisms. The PDC provides the link between glycolysis and the citric acid cycle and produces acetyl-CoA for the citric acid cycle and acetyl groups for acetylcholine synthesis (Harper, 1989). The complex is composed of thirty copies of a tetrameric ($\alpha 2\beta 2$) pyruvate dehydrogenase (E1; EC 1. 2. 4. 1), sixty copies of monomeric dihydrolipoamide acetyltransferase (E2; EC 2. 3. 1. 61), twelve copies of homodimeric dihydrolipoamide dehydrogenase (E3; EC 1. 8. 1. 4), and twelve copies of monomeric protein X (Roche and Patel, 1989; Patel and Roche, 1990).

These three catalytic components catalyze a series of coordinated reactions involving five coenzymes including thiamin pyrophosphate (TPP), lipoic acid, coenzyme A, FAD, and NAD^+. E1 catalyzes a decarboxylation of pyruvate with production of CO_2 and hydroxyethylthiamine pyrophosphate. The hydroxyethylthiamine pyrophosphate is then oxidized to acetylthiamine pyrophosphate with the reduction of lipoic acid. The acetyl group is then transferred to lipoic acid yielding the 8-S-acyl compound. E2 catalyzes transfer of the acetyl group to coenzyme A yielding the acetyl-CoA, leaving a reduced E2 lipoyl group that E3 uses as an electron source for FAD-dependent reduction of NAD^- to NADH (Patel and Roche, 1990; Patel and Harris, 1995).

Protein X, recently called as E3-binding protein (E3BP) for its binding property, is one of the PDC components which is biochemically and genetically less understood. It has been difficult to establish and characterize the function(s) of E3BP in the complex because E3BP was tightly associated with the E2 core of the complex. The studies using limited proteolysis revealed that protein X contributes to the binding and functioning of E3 (Roche et al., 1989). It has been interesting that E3BP has sequence similarity with E2 and a covalently bound lipoyl moiety (Hodgson et al., 1986; Neagle et al., 1989). The E3BP is not just a tissue specific isozyme of E2 because it is found in liver, kidney, heart, adipose tissue, spleen, skeletal muscle, testes, uterus, red blood cells, and brain of the rat (Hodgson et al., 1988). The conditions for reconstitution of mammalian pyruvate and 2-oxoglutarate dehydrogenase complexes have been established (Sanderson et al., 1996). Reed and his colleagues (Maeng et al., 1996) found that E3BP anchors E3 homodimers inside each of the 12 pentagonal faces of the 60-mer E2. Steric hindrance by the lipoyl and E3 binding domains limits the binding of E2. A

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novel E3BP that lacks the amino terminal lipoyl domain has been found in *Ascaris suum* (Klingbeil et al., 1996). The lipoyl domains of E3BP may substitute for the lipoyl domains of E2 in overall complex catalytic activity (Sanderson et al., 1996). However, the unique catalytic function(s) and genetic structure of the E3BP in the complex are still not known.

Here we have cloned and sequenced the genomic clones of the human E3BP partially. The matched sequence corresponding to bases 343 to 542 of E3BP cDNA is the lipoyl-bearing domain and hinge region of the human E3BP.

MATERIALS AND METHODS

Strain, plasmid and culture conditions

Escherichia coli DH5α and XL1-Blue (Stratagene, USA) was used for transformation and propagation of appropriate plasmids. *E. coli* strains were grown in LB medium (1% (w/v) Bactotryptone, 0.5% (w/v) yeast extract, and 1% (w/v) NaCl, pH 7.3) at 37°C by vigorous shaking. For the selection of plasmid-containing transformants, ampicillin (50 μg/ml), tetracycline (12.5 μg/ml), and kanamycin (10 μg/ml) were added into media, respectively. Isopropyl-β-D-thiogalacto-pyranoside (IPTG, 40 μl of 20 mg/ml solution) and X-gal (40 μl of 20 mg/ml solution) were used for selection of transformants harboring insert-containing plasmids (Lim et al., 2000).

Screening of human genomic library

Approximately 1.2×10^6 phages of a human placenta genomic DNA library constructed in the λ EMBL3 SP6/T7 (Clontech, U.S.A.) were screened using a probe from the human E3BP cDNA (Lee et al., 2001). A 1.35 kb *Nco*I/*Bam*HI fragment of E3BP cDNA clone was used as a probe. This DNA fragment was labeled with [α-^{32}P]dCTP by random hexamer labeling kit using nick translation (Sambrook et al., 1989). The labeled probe was purified and confirmed by agarose gel electrophoresis, and used to screen the genomic library by plaque hybridization according to standard procedures (Sambrook et al., 1989). Hybridization was carried out at 50°C for 15 h in hybridization solution (5×SSC, 5×Denhardt's solution, 0.1% SDS, 100 μg/ml sonicated salmon sperm DNA, 50 mM Tris-HCl, pH 7.6) plus the denatured radioactive probe by heating and cooling. After hybridization, the filters were washed twice in wash buffer 1 (2×SSC, 0.5% SDS) at 45°C for 15 min, twice in wash buffer 2 (1×SSC, 0.1% SDS) at 45°C for 15 min, and twice in wash buffer 3 (0.5×SSC) at 45°C for 15 min. Autoradiography was carried out at -70°C for 2 days and/or more depending the radioactive of probe used. Positive plaques were selected and purified by secondary and tertiary screening.

Phage DNA isolation

0.5 ml of Chloroform was added to the lysed culture medium (50 ml) and vigorous shaking incubation was done for more than 30 min. Cell debris were pelleted by centrifugation (4,000×g, 15 min, 4°C), and the phage were precipitated by incubation with 1 M NaCl and 10% (w/v) polyethylene glycol (PEG, M.W. 8,000) for at least 12 h. The phages were collected by centrifugation (10,000×g, 15 min, 4°C). The pellet was dried and remained PEG was removed by paper towel. The pellets were resuspended in 1 ml of λ dilution buffer, and incubated at 37°C for 30 min after the addition of DNaseI (20 μg/ml) and RNaseA (50 μg/ml). The solution was centrifuged at 2,000×g for 5 min and the supernatant was transferred to a fresh tube. Then, the solution was incubated at 37°C for 30 min supplemented with SDS (0.5%), EDTA (10 mM, pH 8.0), and proteinase K (50 μg/ml). One time of phenol/chloroform (1:1) extraction and twice of chloroform/isoamyl alcohol (24:1) extractions were performed to clean up the solution. The phage DNAs were harvested by ethanol precipitation and resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for further analyses (Lee and Clark, 1997).

Southern blot analysis

DNAs of putative clones were digested by restriction endonucleases and electrophoresed through 0.8% agarose gel. After electrophoresis, the gel was soaked in denaturation solution (0.5 N NaOH, 0.1 N NaCl) for 1hr with slowly shaking, and then washed with deionized water for 30 min. The denatured gel was dehydrated by covering with the paper towels and putting a heavy substance on, and dried for 30 min at 60°C using vacuum gel dryer. These agarose gel membrane was directly used for hybridization. Southern hybridization was carried out in as same procedure mentioned above (Sambrook et al., 1989).

Subcloning and sequence analysis

The appropriate restriction endonucleases were used to identify the clone by Southern blot and to construct the physical map. The restriction fragments of phage clones were cloned into appropriate sites of pBluescript SK- vector (Stratagene, USA) using T4 DNA ligase (24°C, 3 h). Plasmid DNAs were isolated by alkaline lysis procedure. The plasmids were sequenced on both strands, and sequence data were analyzed using DNASIS (Hitachi Software Engineering Co., Ltd., Japan) and BLAST Searching program of National Center for Biotechnology Information (National Library of Medicine, Bethesda, MD, USA).

RESULTS AND DISCUSSION

Partial cloning of human E3BP gene

Because the PDC complex plays central roles in intermediary metabolism, aberrations in metabolic steps resulting from genetic defects in the PDC have been recognized for the past few decades. The advent of modern molecular biological techniques has facilitated the identification at the genetic level of inherited disorders of this complex in affected patients. Furthermore, knowledge and understanding of the genetic structures, organization, and regulation of this complex has also emerged in recent years. In this study, the procedures of genomic library screening, isolation of E3BP clones, and nucleotide sequencing have been performed to investigate the gene structure and characterization of human E3BP. A human placenta genomic DNA library constructed in the λ EMBL3 SP6/T7 vector was screened using probe from human E3BP cDNA. The Probe DNA was prepared from 1.35 kb *Nco*I/*Bam*HI fragment of human E3BP cDNA. By repeated plaque hybridization, nine positive plaques were selected and purified through secondary and tertiary screening. The phage DNAs of these clones were isolated by PEG precipitation. The isolated DNAs were digested with several restriction enzymes and separated on 0.8% agorose gel, and then subjected to Southern blot analysis for Exon-containing fragments (figure 1) A few DNA fragments digested with restriction endonucleases were hybridized strongly to probe DNA. The DNA fragments showing significantly positive signal were cloned to pBluescript SK- digested with appropriate restriction enzymes. The cloned DNAs were used for further analysis by treatment with restriction enzymes. One of these clones, the E3BP741, has an insert DNA of 15 kb in length and several restriction enzyme sites, including *Eco*RI, *Sac*I, *Kpn*I, *Bam*HI, *Pst*I, *Sma*I. Restriction map of the clone E3BP741 was constructed and nucleotide sequence was determined (figure 2).

Nucleotide sequence analysis of human E3BP gene

For nucleotide sequencing, large fragments of clones were digested with other restriction enzymes and cloned in pBluescript SK- vector. These subclones were sequenced and aligned with BLAST database and the E3BP cDNA sequence. In 3 kb-*Sac*I fragment of the cloned E3BP741,

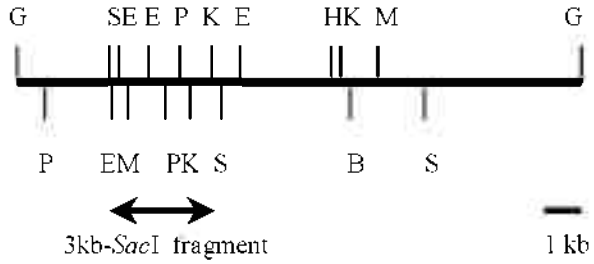

Figure 2. Restriction map of the genomic clone of human E3BP, E3BP741. It spans about 15 kb DNA fragment and was digested with several restriction enzymes. About 3 kb-*Sac*I fragment region matched with human E3BP cDNA was shown as bi-directional arrow. B: *Bam*HI, E: *Eco*RI, G: *Bgl*II, H: *Hin*dIII, K: *Kpn*I, M: *Sma*I, P: *Pst*I, S: *Sac*I,

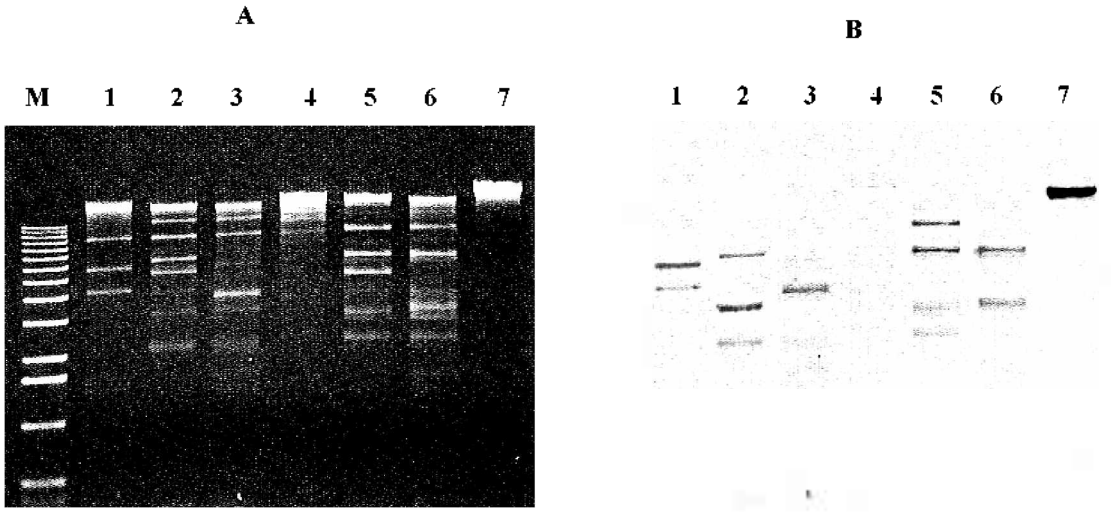

Figure 1. Southern blot analysis of the genomic clones of human E3BP. The hybridization was performed with the 1.35 kb fragment of human E3BP. Cloned DNA was digested with *Eco*RI, *Sac*I, *Kpn*I, *Bam*HI, *Pst*I, *Sma*I, respectively (lane 1-6). The undigested cloned DNA was shown in lane 7. Agarose gel electrophorsis patterns are shown in panel A, and their Southern blot analyses are shown in panel B

GATGTGTATT AAGTTATCTG TTTTGTTACA GAACAGACAA AATTGAGCTC TAGTCTGTGC CGGAAAGTCA TAATGCAGAC TAGTTTGAAA
ACGAATGCAT GTTAAAACAT GAATTCTATT TTATATCAGA TAGACATTTG AGCTTGGAAT TCCTGTAACA GTAATTAGAA CTCCATAAAT
GGGAACAGAG TATCAAGTTT GGTATATTGA ATACCATTTT AGACTTCTAA AACCTAATTC AACCTCAACA TGGACACATA CTGTTTGGTT
TTCCATTGTG CAAGCAGGGC TTTTAGAGTA CCTGGAACAT AGCAGATATT CAGTAACTAT TTATTTACTG AATGAACAAT GATGGTTAAA
AAAAAAAACA CACACTCTAA GGTCTGAGTG TGCTGAGAAC AGCAACTTCC TACGTGATGT ACAACGATGT GCGAATATGA TAATGATTAC
AATTCCATAA GAACTGCCAT AACTTTTTTT TTTTACATCA TCCTTTGAAT AAATATATAC TCACCGTAGT GTCCCGGGTA TGTGTTCCTT
CTTGACAGGG ATGGAAATCT GTGGTTCTGG TGAGGGGCGA GGCTCTGAAG GTTTTGAAAC TGGTGGTGGA GGACCTACGT CTTTGGGAAT
TTCAACATGT TTCCAATCTT CTCCTTCTTC TACTATCAAA CCAATTAGTG AACCTAGCCG TATATTTTTA CTTCCTTCTT CAACCTATAT
TTAAAAAAGA AGTAACTGTA GAGAAGTAAA ACCCCATGAC TGCATTAAGT AGTTTGTTCT TTTGTTGGGT AATTCTCCCC TTAAGATATC
TTAATTTGAG CTAAAAGCTG CTTCCAAGAC AGTTTGCCTC TGCCTTCCAC CTGTCACCTT CTCCACTTTC TCCCACTTAT GCCTTCCTTC
ACCATTCTCC TCTTCCCACT TGCTAATGCT ACCTTCTGAA ATAAAGGATA AATCTAGCCT GTATCCTCTT TCTGAGAGCC TTCAAATAAA
CATGTAAGAT CCATCATTAT CATCTTCTTT TTGATGTCAA TACAAATCCA AACAGGAGCC TGTGGCTACA TATCTTAACC ATTTGTCTTT
CCCAGAGAGT TTCTATGCAA TGGCTTTAGA ATTCTAAAAG CTCAGAAGGC ATTAACTCCC TCAACTTAGT CCAATCTACT AAAATTTACC
TAATGCAAGA ATCTTTTCAG TAAACTTAGT CTGAAAAGAT GAACATTCTA ATGTATAGAT AGAACTGGTT AACTATTCCA GTTAATGTTA
AATATAGTCT ACTTCTTTAT ATTTAATTAT TTCCCTGTAT ATCTATTTAC AAAACCTGAA AAATAAAATA AAACCTCATC AGTTTTTTTA
TAAGTATATC AGAAACACAG TGGTGTCTCA GTTAATAAAG CATTTCTTCC ACAGATAATG CCAATTAAAC ACTTAAATAA AATCAAAGAT
AATGTTTTTA AAAGTTAAGT ATATTTTAAG CATTTCAGCA GCATTAATTT ATAAAGAAAG GGCTAATTCT AATTTACTTA CTAAAAAGAA
GTCCCTAGAA AACCTTCAAA TAACAATATT TTATAAGTTC ACTCCTGGAA GCCCCTGTCA TTCCCTTAAA ATCTTCTATT ATTCCCTTTC
AATTACTCTA AATAAGGTTC ATTAGCTGTA TTTAATGAAC AATAGAATGA TAGGCTGCAG GACGTCCACT AATACAGTAA TGGTAAGGAA
AAGCATAACT TCTAGAATGA GTTTGACATA TCAGAGTAAC ACTAATTTCA ATGCTTACTG GTATGTTCAC TATCGTTAAT AAGGCATAAG
TTATAAGGGG TTAGGAAGCT AAGTTTACTA ATTACTGATG AAGAAGAAAA GCAGCAATGC TTTCATGAAG CAAAATACAT ATATATATAT
ATGAAATTAG TATAGAAAGC TGAATAGGAA TGTTTAATGA ATTACAAGCT ATGGAAGAAT ACTAAGAAAG CCAAGAAAAA TCTAGTTGCT
TAGTTGTTTG CCACATACTA AAAAATTTTG CTTCTGCAGA GTTCCTTTTT CTTTACTATG TCAAAAAGAT GAGTTAAGAT TATTATTAGA
CCAATATTTT TCTTCTAAAA GTGTTTAATT ACCTGCTTGT GAATTGCTAA CTCAAAAATC AAAGTAAAAT GACTAATTTT CTTTAAAAAT
GTATACAAAA AAGCACTGTT AAGTGACATG AAGTCCACTT GATGTTAATT CCTTTCAAAA CTGAATCAGA AATAGGATCA ATATCCAAGT
ACAAAGAACA TCATTCAAAT GTAAAGCATT AAAAAAAGTT TTGCTTTAAA AATATCAATA GATTTCTAAA ATCTGAGTCA GACAACTGTA
CTACATCTGA AAGTGGTACC TCACCTTCTG TTTCATTTAC TGTAAATCAT ATAAATGATT CCATATATAC TCTACCAACC TAACATTGAC
ACCTTCACAA GTAGGCAATA AACAGCATTT ATCATCATTT CAATTTTTAC CATTATCTAA ATATCAAATA AAATTTATTT TTAACTATTC
ACATTTTTTT CTATTTAAAA AAGTGACACC ATGAAGCATG AAACCCAAAT ACAATGGACA AGGACAAGTG ATATACAACC ATACAACTTG
AATATATAAC AGAAGATAAA ATACAGTTGA TTATAATTCC ATTTAAAGTC ATTAAAATCT CTATATGAAT TTAAGATAAT TTCTTCAACC
TTTTCTATTC CTTTGGTGCA GACATCTTTC AGAGGTGTGC TAATTTCCTG TAAGCATTTG CTTACACTGT AGTAGGCAAA TACTCAAAAC
TGGTGTAGAC TTAGTATAAA TACGTAAATA ATACTCTTTG CAAAACTGTT TAAAAACCAA GCAGGCCTCT GTTACTGTTC CCTTTCCTCT
CTGAGCTACC TCCATGGTAG CTCATGGTAC CTCCATGGAT ACACTCTGCC CTACCAAAGC ATCTGGAGCT AAGGACACCT CAAATGCCAG
AGTTTTCTTA TGCACTCAGC TTACTTGACC AAAGCCCTCT CTTACTCTTC TGCCAATGAT TTCTTAATAT TAAATGCAGC GTTTTTACAC
ATAGCTCTAT CAACTTAGAG CTCATCGATA CTGAAATATT GAATGTTGAG TCTATCATTT CAAATATTAG CCTTCCCTCC CAGTTGTGTA

Figure 3. Nucleotide sequence of the 3 kb-*Sac*I fragment in the genomic clone. E3BP741. The putative clone of E3BP741 was digested by restriction enzyme *Sac*I and cloned into the pBluescript SK- for sequencing. Nucleotide sequences of *Sac*I sites are shown in bold. The matched and homologous region to E3BP cDNA sequence is shown in bold and shaded box

the homologous sequence to E3BP cDNA was found (figure 3) The 3 kb-*Sac*I fragment contained the matched region which was 200 nucleotides long corresponding to bases 343 to 542 of E3BP cDNA (figures 3 and 4). This region corresponds with 62-128 amino acid residues of human E3BP. The exon-intron boundaries which were determined by sequencing and aligning the sequences with the E3BP cDNA sequence are reasonable and acceptable according to the universal feature of the exon-intron junction. GU-AG rule (Tan and Patel, 1999). This region seems to be a very conserved and consensus sequence for lipoyl domain, and similar with lipoyl domain 2 of mammalian E2 as well as lipoyl domain of yeast E3BP (figure 5). This matched sequence region also includes hinge region that shows sequence variety in other PDC components including human E2 and yeast E3BP. Several important and distinct regions of human E3BP compared with human E2 and yeast E3BP are shown at figure 5.

Human E2 has two lipoyl-bearing domains corresponding amino acids 7 to 87 and 135 to 213. Residues 6-83 of human E3BP shows 44% sequence identity with the first lipoyl-bearing domain and 46% identity with the second lipoyl-bearing domain of E2 (Harris et al., 1997). That E3BP should have a lipoyllysine residue in its amino-terminal region is expected from several previous studies demonstrating acetylation of this protein upon incubation of PDC with either radioactive pyruvate or acetyl-CoA (Hodgson et al., 1986; Neagle et al., 1989). Lipoyllysine

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ACCTTAGATGCAAGTGATGATGGAATCTTGGCCAAAATCGTGGTTGAAGAAGGAAGTAAA      360
 T  L  D  A  S  D  D  G  I  L  A  K  I  V  V  E  E  G  S  K
AATATACGGCTAGGTTCACTAATTGGTTTGATAGTAGAAGAAGGAGAAGATTGGAAACAT      420
 N  I  R  L  G  S  L  I  G  L  I  V  E  E  G  E  D  W  K  H
GTTGAAATTCCCAAAGACGTAGGTCCTCCACCACCAGTTTCAAAACCTTCAGAGCCTCGC      480
 V  E  I  P  K  D  V  G  P  P  P  P  V  S  K  P  S  E  P  R
CCCTCACCAGAACCACAGATTTCCATCCCTGTCAAGAAGGAACACATACCCGGGACACTA      540
 P  S  P  E  P  Q  I  S  I  P  V  K  K  E  H  I  P  G  T  L
CGGTTCCGTTTAAGTCCAGCTGCCCGCAATATTCTGGAAAAACACTCACTGGATGCTAGC      600
 R  F  R  L  S  P  A  A  R  N  I  L  E  K  H  S  L  D  A  S
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Figure 4. The nucleotide and amino acid sequence of the matched region between 3 kb-*Sac*I fragment of E3BP741 clone and human E3BP cDNA. The matched nucleotide and deduced amino acid sequences are shown in bold and shaded box

A. Lipoyl-bearing domain

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Human E2     135 VLLPALSPTMTMGTVQRWEKKVGEKLSEGDLLAEIETDKA 174
Human E3BP     6 ILMPSLSPTMEEGNIVKWLKKEGEAVSAGDALCEIETDKA  45
Yeast E3BP     5 FSMPAMSPTMEKGGIVSWKYKVGEPFSAGDVILEVETDKS  44
                                                       *
Human E2     175 TIGFEVQEEGYLAKILVPEGTRDVPLGTPLCIIVEKEAD 213
Human E3BP    46 VVTLDASDDGILAKIVVEEGSKNIRLGSLIGLIVEEGED  84
Yeast E3BP    45 QIDVEALDDGKLAKILKDEGSKDVDVGEPIAYIADVDD  83
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B. Hinge region

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Human E2     214 ISAFADYRPTEVTDLKPQVPPPTPPPVAAVPPTPQPLAPT 253
Human E3BP    85 WKHVEIPKDVGP----------PPPVSKPSEPRPSPEPQ 113
Yeast E3BP    84 LATIKLPQEANTANAKSIEIKKPSADSTEATQQHLKKATV 123

Human E2     254 PSAPCPATPAGPKGR 268
Human E3BP   114 ISIPVKKEHIPGTLR 128
Yeast E3BP   124 TPIKTVDGSQANLEQ 138
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Figure 5. Comparison of the deduced amino acid sequences of human E2, human E3BP, and *S. cerevisiae* E3BP. The matched region of 3-kb *Sac*I fragment of E3BP741 clone was corresponding to 62-128 amino acid residues of human E3BP. Conserved amino acids of all three proteins are shown by the gray shading, and conserved amino acids of human E2 and human E3BP are shown by underline. Lypoylation site lysine residues are indicated by asterisks.

residues are typically found to occur 43-44 residues from the amino terminus of mammalian E2 proteins. A lysine residue is located at position 44 amino acid in the deduced sequence of mature E3BP. This lysine occurs in a typical consensus sequence for a lipoyl-attachment site (Harris et al., 1997), and is, therefore, highly likely to be the site of lipoylation (figure 5).

The hinge regions separating the lipoyl-bearing domain and inner core region in E3BP and E2 show the least sequence identity (15%) of any region of these proteins.

The hinge region of E2 that connects the second lipoyl-bearing domain with the peripheral subunit-binding domain is rich in both Pro and Ala residues. The corresponding region of E3BP is rich in Pro but contains no Ala residues (Harris et al., 1997; Lee et al., 2001). Human E3BP has no sequence identity with E2 in the hinge region or with that of yeast E3BP.

It is clear that there are some differences between yeast and mammalian E3BP. However, there is a remarkable similarity of amino acid sequence between mammalian E2 and mammalian E3BP. It is suggested that anchoring E3 to E2 is not the only function of mammalian E3BP. It may be possible that this protein is mediated or regulated by various isoenzymes of pyruvate dehydrogenase kinase (Harris et al., 1997). Useful for the further understanding of the function of human E3BP will be increased availability of recombinant human E3BP, capability for reconstitution of PDC, and more investigation of the genetic structure of the protein's components.

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