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Full Length Research Paper

cDNA, genomic sequence cloning and over-expression of ribosomal protein P2 gene (*RPLP2*) from the Giant Panda (*Ailuropoda melanoleuca*)

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RPLP2 is a component of the 60S large ribosomal subunit encoded by *RPLP2* gene and directly participate in protein synthesis, which is located in the cytoplasm. The cDNA and genomic sequence of *RPLP2* were cloned successfully from the Giant Panda using RT-PCR and Touchdown-PCR technology, respectively. The results showed that the cDNA fragment cloned is 394 bp in size, containing an open reading frame of 348 bp. The length of the genomic sequence is 1838 bp, with four exons and three introns. The deduced protein is composed of 115 amino acids with 11.66 KD of estimated molecular weight and 4.14 of pI. Alignment analysis indicated that the nucleotide sequence and the deduced amino acid sequence are highly conserved to other four species reported, including *Homo sapiens*, *Bos taurus*, *Mus musculus* and *Rattus norvegicus*. The homologies for nucleotide sequences of Giant Panda *PRLP2* are 90.80, 87.64, 88.79 and 87.64% to those of the four species, while the homologies for amino acid sequences are 88.27, 85.20, 86.31 and 85.20%. The *RPLP2* gene was overexpressed in *Escherichia coli* BL21, and the result indicated that fusing RPLP2 with the N-terminally His-tagged form gave rise to the accumulation of an expected 17.5 KD polypeptide, in accordance with the predicted protein, which could be used to purify and investigate the function of this protein.

Key words: cDNA cloning, overexpression, ribosomal protein P2 gene (*RPLP2*), Giant Panda (*Ailuropoda melanoleuca*).

INTRODUCTION

Ribosome, a compact ribonucleoprotein (RNP) grain that catalyzes protein synthesis, consists of 4 RNA species and approximately 80 structurally distinct proteins (Yoshihama et al., 2002; Hwang et al., 2004). It can be dissociated into a small subunit and a large one, whose shape and structure are irregular and asymmetric. The large ribosomal subunit has a distinct lateral protuberance called the stalk, which is an important and essential structure involved directly in the interaction of the elongation factors with ribosome during protein synthesis (Rodriguez-Gbriel et al., 1999; Krokowski et al., 2006). Acidic ribosomal phosphoprotein with pH 3 to 5 of isoelectric point, named P protein, including RPLP0, RPLP1 and RPLP2 in large ribosomal subunit in eukaryotic cell, has been reported directly participate in protein synthesis (Remacha et al., 1995; Qiu et al., 2006). RPLP2, together with RPLP0, RPLP1 and the conserved domain of 28S rRNA, constitutes a major part of the GTPase-associated center in eukaryotic ribosomes (Hagiya et al., 2005). In addition, it can be attached to ubiquitin and assist the latter in regulating numerous important cellular processes including apoptosis, transcription, and the progression of cell cycle (Archibald et al., 2003).

Giant Panda is a rare species currently found only in China. For many years, studies on the Giant Panda have been mainly concentrated on fields such as breeding and

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propagation, ecology, morphology, taxology, physiology and pathological biochemistry (Hou et al., 2007a, b, 2008, Du et al., 2007, 2008). And recently, functional gene analysis is one of the hot issues in current Giant Panda research (Wu et al., 1990; Liao et al., 2003; Zhang et al., 2009; Hou et al., 2009; Sun et al., 2011). There are few reports about *RPLP2* gene of Giant Panda. This study was conducted to amplify the cDNA and genomic sequence of *RPLP2* gene from Giant Panda, and then analyzed the sequence characteristics of the cDNA and the deduced protein. This gene was over-expressed in *Escherichia coli* using pET28a plasmids.

MATERIALS AND METHODS

The skeletal muscle tissue were collected from a dead Giant Panda at the Wolong Conservation Center of the Giant Panda, Sichuan, China, and kept in liquid nitrogen. A total of 500 mg muscle tissue from Giant Panda was ground in liquid nitrogen to a fine powder, and the powder was suspended completely in 15 ml lysis buffer containing 10 mM Tris-HCl, pH 8.0, 100 mM EDTA and 0.5% SDS. After treatment with proteinase K (100 mg/ml, final concentration) at 55℃ for 3 h, the mixture was then cooled to room temperature and mixed with an equal volume of saturated phenol (pH 8) before being centrifuged at 5000 g at 4℃ for 20 min. The supernatant was pooled and then mixed with an equal volume of 1:1 (v:v) phenol-chloroform and then centrifuged as above and the supernatant collected, from which the DNA was precipitated by ethanol. The DNA obtained was then dissolved in TE buffer and kept at -20℃.

Total RNA was isolated using the Total Tissue/Cell RNA Extraction Kits, dissolved in DEPC water, and kept at -70℃. The quality and quantity of the products were detected by electrophoresis and spectrophotometry.

The PCR primers were designed by Primer Premier 5.0, according to the mRNA sequence of *RPLP2* gene from *Homo sapiens* (NC_000011), *Bos Taurus* (AC_000186), *Mus musculus* (NC_000073) and *Rattus norvegicus* (NC_005100), as following:

Forward: 5′-GAGGCTTCTCCGCCGCCGAG-3′;
Reverse: 5′-CAGGGGAGCAGGAACCTAAT-3′.

The cDNAs was synthesized using a reverse transcription kit, and the genomic sequence was amplified using Touchdown-PCR. The PCR products were analyzed by electrophoresis and purified using a DNA harvesting kit. The products were ligated into vector plasmid pUC18 which has been digested by restriction endonuclease *SmaI* and then transformed into *E. coli* competent cells (JM109). The insert size was verified by digesting with *PstI* and *ScaII*. Recombinant pUC18 was sequenced by Huada Zhongsheng Scientific Corporation. The sequence data were analyzed by GenScan software, Blast 2.1, ORF finder software, DNAMAN 6.0, Predict Protein software. Software ExPASy Proteomics Server and SWISS-MODEL software respectively.

The genomic sequence was amplified using Touchdown-PCR, and the primers were the same as following:

Forward: 5′-ATGCGCTACG TTGCCTCCTA-3′;
Reverse: 5′-CTAATCGAACA AGCCGAATC-3′.

The amplification conditions were: 94℃ for 30 s, 62℃ for 45 s, 72℃ for 4 min in the first cycle and the anneal temperature deceased 0.5℃ per cycle; after 20 cycles conditions changed to 94℃ for 30 s, 52℃ for 45 s, 72℃ for 4 min for another 20 cycles. The fragment amplified was also purified, ligated into the clone vector and transformed into the *E. coli* compence cells. Finally, the recombinant fragment was sequenced by Sangon (Shanghai, China).

PCR fragment corresponding to the RPLP2 polypeptide was amplified from the *RPLP2* cDNA clone with the forward primer, 5'-GAGGAATTC ATGCGCTACG TTG-3' (*EcoRI)* and reverse primer, 5'-AACCTCGAG CTAATCGAAC AAG-3'(*XhoI*), respectively. The amplified PCR product was cut and ligated into corresponding site of pET28a vector (Stratagen). The resulting construct was transformed into *E. coli* BL21 (DE3) strain (Novagen) and used for the induction by adding isopropyl-b-D-thiogalactopyranoside (IPTG) at an OD600 of 0.6 and culturing further for 4 h at 37℃, using the empty vector transformed BL21 (DE3) as a control. The recombinant protein production was induced after 0, 0.5, 1, 1.5, 2, 2.5, 3 and 4 h and then protein bands were separated by SDS-PAGE and stained with Commassie blue R250.

RESULTS

About 400 bp of cDNA fragment was amplified from the Giant Panda (Figure 1). The exact length of the Cloned cDNA was 394 bp (accession number: HQ318036) by sequencing analysis. Blast research showed that the cDNA sequence shares a high homology with the *RPLP2* gene from other mammals reported, including *H. sapiens*, *B. taurus*, *M. musculus* and *R. norvegicus.* On the basis of the high identity, it could be concluded that the cDNA isolated is just the cDNA encoding *RPLP2* protein. The *rpLP2* CDS sequence contains an ORF of 348 bp encoding 115 amino acids, 31 bp of 5′-untranslated sequence and the 15 bp of 3′-untranslated region in length.

A DNA fragment of about 2000 bp was amplified and the sequenced length was1838 bp (accession number: HQ318037). Comparison of the cDNA with the amplified genomic DNA fragment indicated that the cDNA sequence is in full accord with four fragments in the genomic DNA fragment, which manifests that the DNA fragment amplified is the genomic sequence of the *rpLP2* gene from Giant Panda (Figure 2).

Primary structure analysis revealed that the molecular weight of the putative *RPLP2* protein is 11.66 KD with a theoretical pI of 4.14. Topology prediction showed there are 3 different patterns of functional sites in the *RPLP2* protein of Giant Panda: one protein kinase C phosphorylation site, two casein kinase II phosphorylation sites, and two N-myristoylation sites (Figure 3). Further study found that the protein is composed of 21 negatively charged residues (Asp and Glu), 12 positively charged residues (Arg and Lys) and 82 uncharged residues.

The secondary structure prediction of the RPLP2 protein sequence indicated that 8.7% of the protein sequence is strand, 49.57% is helix and 41.74% is coil for Giant Panda. Further comparison was conducted to understand the secondary structure of protein subunit RPLP2 in five mammals is consistent or not, the results showed that there is very little difference in the secondary structure of protein subunit RPLP2 between the five mammalian species (Table1).

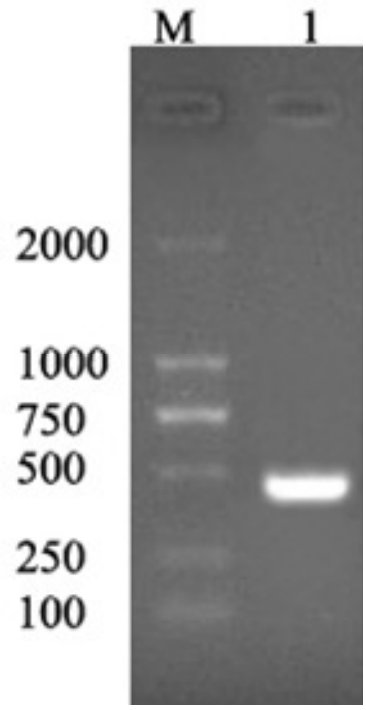

Figure 1. Reverse transcription polymerase chain reaction products of the Giant Panda *RPLP2*. M: Molecular ladder DL2000; 1: The amplified *RPLP2*.

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1     GTCGCACGCGTGAGGCTTCTCCGCCGCCGAG ATG CGC TAC GTT GCC TCC TAC CTG CTG GCC GCC
1                                     M  R  Y  V  A  S  Y  L  L  A  A
65    CTC GGG GGC AAC GCC TCC CCC AGT GCC AAG GAC ATC AAG AAG ATT CTG GAC AGC GTG
12     L  G  G  N  A  S  P  S  A  K  D  I  K  K  I  L  D  S  V
122   GGC ATC GAG GCG GAT GAC GAC CGG CTC AAC AAG GTC ATC AGT GAG CTG AAC GGA AAA
31     G  I  E  A  D  D  D  R  L  N  K  V  I  S  E  L  N  G  K
179   AAC ATT GAA GAC GTC ATC GCC CAG GGT ATT GGC AAG CTG GCC AGT GTA CCT GCT GGT
50     N  I  E  D  V  I  A  Q  G  I  G  K  L  A  S  V  P  A  G
236   GGG GCT GTC ACC GTC TCT GCC GCC CCA GGG TCT GCA GCT CCT GCT GCT GGC GCT GCC
69     G  A  V  T  V  S  A  A  P  G  S  A  A  P  A  A  G  A  A
293   CCC GCT GCA GCA GAG GAA AAG AAA GAC GAG AAG AAG GAG GAG TCG GAG GAG TCA GAC
88     P  A  A  A  E  E  K  K  D  E  K  K  E  E  S  E  E  S  D
350   GAC GAC ATG GGA TTC GGC TTG TTC GAT TAG GTT CCT GCT CCC CTG
107    D  D  M  G  F  G  L  F  D  *
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Figure 2. Nucleotide sequence of cDNA of Giant Panda *RPLP2* gene and the amino acid sequence deduced (* representing for the terminator code).

The *RPLP2* gene was overexpressed in *E. coli*, and the results indicated that the fusion of *RPLP2* with the N-terminally His-tagged form gave rise to the accumulation of an expected 17.5 kDa polypeptide that formed inclusion bodies (Figure 4). Apparently, the recombinant protein was expressed after half an hour of induction and

Figure 3. Comparison of the RPLP2 amino acid sequences among the different species (N-myristoylation site: protein kinase C phosphorylation site: asein kinase II phosphorylation site:__).

Table 1. Comparison of secondary structure of RPLP2 protein among 5 mammal species.

Species	Amino acid sites					
	11	32-36	46	51	149	81-86
A. melanoleuca	H	C	C	C	C	H
H. sapiens	C	C	C	C	C	C
B. taurus	C	C	H	H	H	C
M. musculus	C	C	C	C	C	C
R. norvegicus	H	H	C	C	C	C

Note: H: Alpha helix; C: Random coil.

the after 2 h reached the highest level. These results suggested that the protein is active and it is just the protein encoded by the *RPLP2* from *Ailuropoda melanoleuca*. The expression product obtained could be used to purify the protein and to study its function further.

DISCUSSION

Recently, the studies on ribosomal protein have made much progress in Giant Panda (Hou et al., 2009a, b), however, there is no reports on RPLP2 protein in this species. In this study, we cloned genomic sequence and cDNA encoding RPLP2 from Giant Panda. The genomic sequence of *rpLp2* is 1838 bp in size. Compared with some mammals including *H. sapiens*, *B. taurus, M. musculus* and *R. norvegicus,* there are four exons and three introns. Further study indicated that the genomic, the introns, the 5′-untranslated sequence and the 3′-untranslated sequence are different in length (Table 2). The variations in lengths of the introns determine the lengths of the *rpLP2* gene.

Physical and chemical analysis showed that the molecular weight of the putative protein among the five mammalians is very close and that the theoretical pI is exactly identical (Table 3). Secondary structure analysis showed that although the amino acid sequence of the 11, 32 to 36, 46, 51, 149, 81 to 86 site in five kinds of mammals have different structure, this does not cause changes in their functional genes, subsequently the corresponding functional sites has not changed (Table 1).

From the alignment analysis of the cDNA sequence of *rpLP2* gene and the deduced amino acid sequence between Giant Panda and other mammals reported

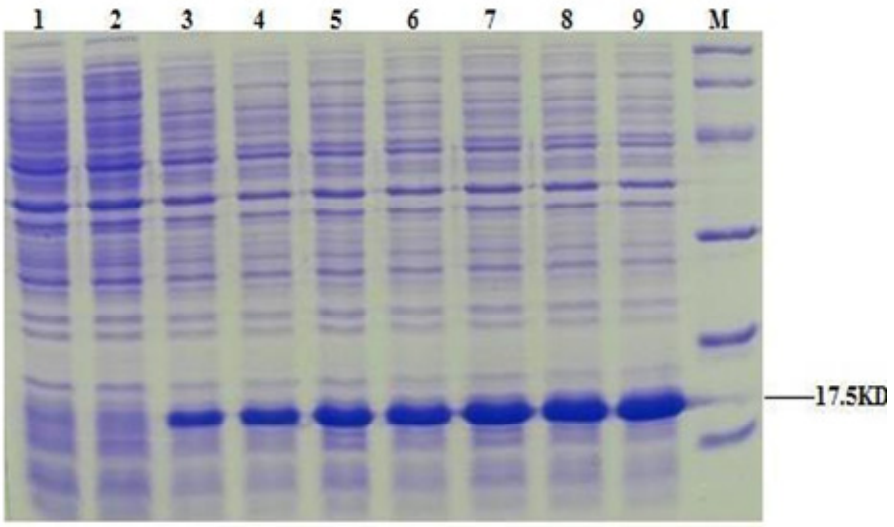

Figure 4. Overexpress of RPLP2 protein in recombinant *E. coli* BL21. Lane 1: control; Lines 2 to 9: overexpressing for 0, 0.5, 1, 1.5, 2, 2.5, 3 and 4 h; Line M: marker; Arrow: indicating recombinant protein.

Table 2. Comparison of *RPLP2* genomic sequence among 5 mammalian species.

Species	Size bp	No. of exons	Join sites in the CDS	Accession No.
A. lanoleucame	1838	4	1..123, 598..646, 1578..1676, 1762..1838	HQ_318037
H. sapiens	2941	4	300..422, 1662..1710, 2600..2698, 2825..2901	NC_000011
B. taurus	2139	4	258..380, 1195..1243, 1822..1920, 2026..2102	AC_000186
M. musculus	3693	4	260..382, 1077..1125, 3355..3453, 3583..3659	NC_000073
R. norvegicus	2272	4	247..369, 1047..1095, 1942..2040, 2160..2236	NC_005100

Table 3. Comparison of RPLP2 in *A. melanoleuca* with other 4 mammals.

Items	Species			
	H. sapiens	***B. taurus***	***R. norvegicus***	***M. musculus***
Cds similarity (%)	90.8	87.64	88.79	87.64
Aa Similarity (%)	97.39	97.39	97.39	97.39
Molecular weight (KD)	11.66	11.70	11.69	11.65
PI	4.14	4.23	4.15	4.14

including *H. sapiens, B. taurus, M. musculus* and *R. norvegicus,* it was found that Giant Panda shares high homology in nucleotide sequence with those mammals. Among them, the Giant Panda shares the highest homology in nucleotide sequence with *H. sapiens*, and shares the same high homology in amino acid sequences with other 4 mammals (Table 3). Comparison of RPLP2 genetic coding sequence of Giant Panda with these species, some variable sites exist among them, parts of which are degeneration sites and others are single variable sites. Further analysis indicated that those variable sites are caused by transformation or transition of bases, which do not result to changes in the amino acid sequences encoded. So, protein functional sites in RPLP2 protein of the five mammalians have the same positions and numbers. The cDNA and the genomic sequence of *RPLP2* were cloned successfully from the Giant Panda, and the cDNA of the *RPLP2* gene was also overexpressed in *E. coli* BL21 strains, which confirms the gene cloned was the *RPLP2* gene from giant panda. The data will enrich and supplement the information about *RPLP2.* In addition, it will contribute to the protection for gene resources and the discussion of the genetic polymorphism. Also, it will provide a reliable basis for the

study on the evolution of species.

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