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Full Length Research Paper

cDNA, genomic sequence cloning and overexpression of ribosomal protein S16 gene (*RPS16*) from the Giant Panda

Wan-ru Hou*, Yi-ling Hou, Yan-zhe Hao,Tian Zhang, Zheng-song Peng, Yan-Song and Guang-fu Wu

College of Life Science, China West Normal University, 44# Yuying Road, 637002, Nanchong, China.

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***RPS16* of eukaryote is a component of the 40S small ribosomal subunit encoded by *RPS16* gene and is also a homolog of prokaryotic *RPS9*. The cDNA and genomic sequence of RPS16 was cloned successfully for the first time from the Giant Panda (*Ailuropoda melanoleuca*) using reverse transcription-polymerase chain reaction (RT-PCR) technology and Touchdown-PCR, respectively, which were both sequenced and analyzed preliminarily. The cDNA of the *RPS16* gene was overexpressed in *Escherichia coli* BL21. The length of cDNA fragment cloned is 448 bp containing an open reading frame of 441 bp encoding 146 amino acids and the length of the genomic sequence is 2510 bp, containing five exons and four introns. Alignment analysis indicates that the nucleotide sequence share a high homology with those of *Bos taurus*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus* and *Danio rerio* by 95.46, 92.97, 89.80, 89.80 and 82.54%, respectively. The deduced amino acid sequence is entirely identical compared with the first four animals and share a high homology with that of *D. rerio* by 96.58%. Topology prediction shows that there is one cAMP- and cGMP-dependent protein kinase phosphorylation site, three protein kinase C phosphorylation sites, one casein kinase II phosphorylation site, two N-myristoylation sites, one amidation site and one ribosomal protein S9 signature in the *RPS16* protein of the Giant Panda . The *RPS16* gene can be readily expressed in *E. coli* and it fused with the N-terminally GST-tagged protein which gave rise to the accumulation of an expected 20.095 kDa polypeptide, in good agreement with the predicted molecular weight. The expression product obtained could be used for purification and further study of its function.**

Key words: cDNA cloning, *RPS16*, the Giant Panda, genomic cloning, overexpression.

INTRODUCTION

Eukaryotic ribosomes are intricate structures containing three to four rRNAs and 70 to 80 distinct proteins. Synthesis of ribosomal precursors, that is, ribonucleo-protein particles, occurs in the nucleus. Processing of these particles to mature ribosomes commences in the nucleus and continues after the particles traverse the nuclear membrane into the cytoplasm (Perry, 1976). Increasing evidence suggests that, in addition to their role in the basic machinery of protein synthesis and its regulation, many ribosomal proteins are involved in various extraribosomal activities, including the regulation of cell proliferation, DNA repair, transcription and RNA processing (Wool et al., 1995; Wool, 1996). The ribosomal protein S16 belongs to the S9P family of ribosomal proteins. The mammalian ribosome contains several other distinct ribosomal proteins whose expression is coordinately regulated with the S16 protein (Surinder et al., 1991). Human ribosomal protein (rp) S16 that is most strongly bound to 18S rRNA in the 40S subunit (Ian'shina et al., 2007) is a homologue of prokaryotic RPS9 that associated with other ribosomal proteins (S4, S8 and S20) to facilitate

*Corresponding author. E-mail: hwr168@yahoo.com.cn. Tel/ Fax: +86-0817-2568653.

Abbreviations: eiF-2; Eukaryotic initiation factor 2, **RP;** ribosomal protein, **DEPC;** diethypyrocarbonate, **IPTG;** isopropyl-b-D-thiogalactopyranoside; **rp;** ribosomal protein; **RT-PCR,** reverse transcription-polymerase chain reaction.

the binding and assembly of the 16s rRNA into the 30s subunit (Kanakari et al., 1992; Stern et al., 1988). It also participates in the binding of eukaryotic initiation factor 2 (eIF-2) to ribosomes and involved in Diamond Blackfan anemia (DBA) pathogenesis (Bommer et al., 1988). *RPS16* is located in the cytoplasm. As is typical for genes encoding ribosomal proteins, there are multiple processed pseudogenes of this gene dispersed through the genome.

The Giant Panda (*Ailuropoda melanoleuca*) is a rare species currently found only in China. They are known as a “living fossil”. Studies on the Giant Panda have been concentrated on fields of breeding and propagation, ecology, genetic diversity, parentage, phylogenesis and molecular biology (Du et al., 2007; Hou et al., 2007a, 2007b; Hou et al., 2008, 2009a, 2009b; Jennie et al., 1992; Liao et al., 2003; Montali, 1990; Wu et al., 1990).

This study was conducted using reverse transcription-polymerase chain reaction (RT-PCR) technique to amplify the cDNA of *RPS16* gene from the total RNA and Touchdown-PCR technique to amplify the genomic sequence of the *RPS16* from DNA from the skeleton muscle of the Giant Panda and then analyzed the sequence characteristics of the protein encoded by the cDNA and compared it with those of human and other mammalian species reported. We also overexpressed it in *E. coli* using pET28a plasmids. The study provides scientific data for inquiring into the hereditary traits of the gene from Giant Panda and formulating the protective strategy for the Giant Panda.

MATERIALS AND METHODS

Materials and RNA isolation

Skeletal muscle was collected from a dead Giant Panda at the Wolong Conservation Center of the Giant Panda, Sichuan, China. The collected skeletal muscle was frozen in liquid nitrogen and then used for RNA isolation. Total RNAs were isolated from about 400 mg of muscle tissue using the Total Tissue/Cell RNA Extraction Kits (Waton Inc., Shanghai, China) according to the manufacturer's instructions. The total RNAs extracted were dissolved in diethypyro-carbonate (DEPC) water and kept at -70°C.

Primers design, RT-PCR, cloning of RT-PCR products and sequencing

The PCR primers were designed by Primer Premier 5.0, basing on the mRNA sequence of *RPS16* from *Homo sapiens* (NM_001020), *Mus musculus* (NM_013647) and *Rattus norvegicus* (BC084715). The specific primers of cDNA sequence are as follows:

Pd-*RPS16*-F: 5′- AG[T/C]CATGCCG TCCAAGGGCC- 3′
Pd-*RPS16*-R: 5′- GGCTTATCGG TAGGATTTCT-3′

Total RNAs were synthesized into the first-stranded cDNAs using a reverse transcription kit with Oligo dT as the primers according to the manufacturer’s instructions (Promega). The 20 μL of first-strand cDNA synthesis reaction system included 1 μg of total RNAs, 5 mM of $MgCl_2$, 1 mM of dNTPs, 0.5 μg of Oligo dT_{15}, 10 U/μL of RNase inhibitor and 15 U of AMV reverse transcriptase and was incubated at 42°C for 60 min.

The first-strand of cDNA synthesized was used as a template. The total reaction volume for DNA amplification was 25 μL. Reaction mixtures contained 1.5 mM of $MgCl_2$, 200 μM of each of dATP, dGTP, dCTP and dTTP (Omega), 0.3 μM of each primer, 5.0 units of Taq plus DNA polymerase (Sangon Co., Shanghai, China). DNA amplification was performed using a MJ Research thermocycler, Model PTC-200 (Watertown, MA) with a program of 4 min at 94.0°C, followed by 30 cycles of 1 min at 94.0°C, 0.5 min at 45°C and 1.5 min at 72.0°C and then ended with the final extension for 10 min at 72.0°C. After amplification, PCR products were separated by electrophoresis in 1.5% agarose gel with 1 × TAE (Tris-acetate-EDTA) buffer, stained with ethidium bromide and visualized under UV light. The expected fragments of PCR products were harvested and purified from gel using a DNA harvesting kit (Omega, China) and then ligated into a pET28a vector at 22°C for 12 h. The recombinant molecules were transformed into *E. coli* competent cells (JM109) and then spread on the LB-plate containing 50μg/mL ampicillin, 200mg/mL (IPTG) isopropyl-beta-D-thiogalactopyranoside and 20mg/mL X-gal. Plasmid DNA was isolated and digested by *Pst*I and *Sca*II to verify the insert size. Plasmid DNA was sequenced by Huada Zhongsheng Scientific Corporation (Beijing, China).

Cloning the genomic sequence of *RPS16*

The PCR primers were the same as the Pd-*RPS16* -F and Pd-*RPS16* -R presented above. The genomic sequence of the *RPS16* gene was amplified using Touchdown-PCR with the following conditions: 94°C for 30 s, 62°C for 45 s, 72°C for 4 min in the first cycle and the anneal temperature deceased 0.5°C per cycle; after 20 cycles conditions changed to 94°C for 30 s, 52°C for 45 s, 72°C for 4 min for another 20 cycles. The fragment amplified was also purified, ligated into the clone vector and transformed into the *E. coli* competent cells. Finally, the recombinant fragment was sequenced by Sangon (Shanghai, China).

Construction of the expression vector and overexpression of recombinant RPS16

PCR fragment corresponding to the RPS16 polypeptide was amplified from the *RPS16* cDNA clone with the forward primer, 5’-ACTGGATCCATGCCGTCCAAG -3’ (*Bam*HI) and reverse primer, 5’- GACAAGCTTTTATCGGTAGGAT -3’ (*Hind*III), respectively. The PCR was performed at 94°C for 3 min; 35 cycles of 30 s at 94°C, 45 s at 53°C and 1 min at 72°C;10 min at 72°C. 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 induction by adding IPTG (isopropyl-b-D-thiogalactopyranoside) at an OD600 of 0.6 and culturing further for 4 h at 37°C, using the empty vector transformed BL21 (DE3) as a control. The recombinant protein samples were induced after 0, 0.5, 1.5, 2, 2.5, 3 and 3.5 h and then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stained with Commassie blue R 250.

Data analysis

The sequence data were analyzed by GenScan software (http://genes.mit.edu/GENSCAN.html). Homology research of the Giant Panda *RPS16* compared with the gene sequences of other species ware performed using Blast 2.1 (http://www.ncbi.nlm.nih.gov/blast/). Open reading frame (ORF) of the DNA sequence was searched using ORF finder software (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Protein structure of the *RPS16* sequence

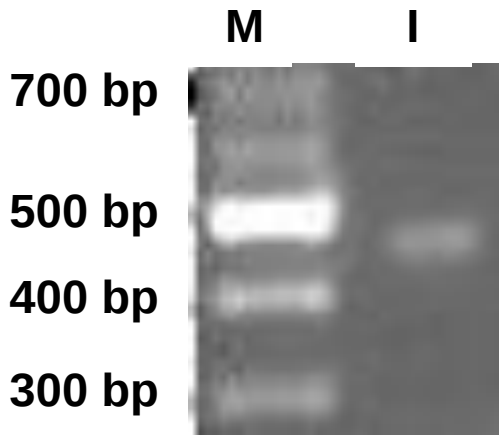

Figure 1. Reverse transcription polymerase chain reaction products of the Giant Panda *RPS16*. M: molecular marker; 1: the amplified *RPS16.*

cloned was deduced using PredictProtein software (http://cubic.bioc.columbia.edu/predictprotein/).

RESULTS

Analysis of the cDNA of *RPS16* from the Giant Panda

About 450 bp of cDNA fragment was amplified from the Giant Panda with the primers *RPS16* -F and *RPS16* -R (Figure 1). The length of the cDNA cloned is 448 bp. Blast research showed that the cDNA sequence cloned is highly homologous with the *RPS16* from *H. sapiens* and some other mammals reported. On the basis of high identity, it was concluded that the cDNA isolated is the cDNA encoding the Giant Panda *RPS16* protein. The *RPS16* sequence has been submitted to Genbank (accession number: FJ849054). An ORF of 441 bp encoding 146 amino acids was found in the cDNA sequence (Figure 2).

Analysis of the genomic sequence of *RPS16* from the Giant Panda

A DNA fragment of about 2500 bp was amplified with primers *RPS16* -F and *RPS16* -R (Figure 3). The length of the DNA fragment cloned is 2510 bp. Comparison between the cDNA sequence and the DNA fragment sequence of the *RPS16* amplified from Giant Panda was performed by software Lasergene. The result indicated that the cDNA sequence is in full accord with three fragments in the DNA fragment, which manifests that the DNA fragment amplified is the genomic sequence of the *RPS16* from Giant Panda. The genomic sequence of the *RPS16* has been submitted to Genbank (accession number: FJ849055)

Prediction and analysis of protein functional sites in *RPS16* protein of the Giant Panda

Primary structure analysis revealed that the molecular weight of the putative *RPS16* protein of the Giant Panda is 16.44532 kDa with a theoretical pI 10.21. Topology prediction shows that there is one cAMP- and cGMP-dependent protein kinase phosphorylation site, three protein kinase C phosphorylation sites, one casein kinase II phosphorylation site, two N-myristoylation sites, one amidation site and one ribosomal protein S9 signature in the *RPS16* protein of the Giant Panda (*A. melanoleuca*) (Figure 5).

Overexpression of the *RPS16* gene in *E. coli*

The *RPS16* gene was overexpressed in *E. coli*, using pET28a plasmids carrying strong promoter and terminator sequences derived from phage T7. For this purpose, the *RPS16* gene was amplified individually by PCR and cloned in a pET28a plasmid, resulting in a gene fusion coding for a protein bearing a GST-tag extension at the N terminus. Expression was tested by SDS-PAGE analysis of protein extracts from recombinant in *E. coli* BL21 strains (Figure 4). The results indicated that the protein *RPS16* fusion with the N-terminally His-tagged form gave rise to the accumulation of an expected 20.095 kDa polypeptide that formed inclusion bodies. Apparently, the recombinant protein was expressed after half an hour of induction and the after 2.5 h reached the highest level. These results suggested that the protein is active and it is just the protein encoded by the *RPS16* from the Giant Panda. The expression product obtained could be used to purify the protein and study its function further.

DISCUSSION

Ribosomes, the organelles that catalyze protein synthesis, consist of a small 40S subunit and a large 60S subunit. Together these subunits are composed of 4 RNA species and approximately 80 structurally distinct proteins (Yoshihama et al., 2002; Hwang et al., 2004). We reported here the identification and characterization of genomic sequence and cDNA clone encoding ribosomal protein S16 from Giant Panda. The genomic sequence of *RPS16* is 2510 bp in size. A comparison of the nucleotide sequences of the genomic and cDNA sequences indicated that the genomic suquence of *RPS16* possesses five exons and four introns, which is also supported by restriction mapping of the genomic and cDNA sequences. Compared with some mammals incluing *H. sapiens, B. taurus, M. musculus*, *C. familims* and *D. rerio*, the five exons, which comprise the cDNA sequence of *RPS16* gene after RNA splicing, is highly conserved and remain essentially the same. The restriction site in the exons is the same in both the cDNA and the

```
1      AGTCATG CCG TCC AAG GGC CCT CTG CAG TCC GTG CAG GTC TTC GGA CGC AAG  AAG
1         M  P  S  K  G  P  L  Q  S  V  Q  V  F  G  R  K  K
56     ACG GCC ACA GCC GTG GCG CAC TGC AAA CGG GGC AAC GGC CTC ATC AAG GTG AAT GGG
18      T  A  T  A  V  A  H  C  K  R  G  N  G  L  I  K  V  N  G
113    CGG CCC CTG GAG ATG ATC GAG CCG CGC ACG CTG CAA TAC AAG CTA CTG GAA CCT GTT
37      R  P  L  E  M  I  E  P  R  T  L  Q  Y  K  L  L  E  P  V
170    CTG CTT CTG GGC AAG GAG CGA TTT GCT GGG GTG GAC ATC CGA GTC CGA GTG AAG GGT
56      L  L  L  G  K  E  R  F  A  G  V  D  I  R  V  R  V  K  G
227    GGT GGT CAC GTG GCC CAG ATT TAC GCA ATC CGC CAG TCC ATC TCC AAA GCC CTG GTG
75      G  G  H  V  A  Q  I  Y  A  I  R  Q  S  I  S  K  A  L  V
284    GCC TAT TAC CAG AAA TAT GTG GAT GAG GCT TCC AAG AAG GAG ATC AAA GAC ATC CTC
94      A  Y  Y  Q  K  Y  V  D  E  A  S  K  K  E  I  K  D  I  L
341    ATC CAG TAT GAC CGG ACC CTG CTG GTA GCT GAT CCC CGG CGC TGC GAA TCC AAA AAG
113     I  Q  Y  D  R  T  L  L  V  A  D  P  R  R  C  E  S  K  K
398    TTT GGC GGT CCT GGT GCC CGT GCT CGC TAC CAG AAA TCC TAC CGA TAA GCC
132     F  G  G  P  G  A  R  A  R  Y  Q  K  S  Y  R  *
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Figure 2. Nucleotide and deduced amino acid sequences of cDNA encoding the Giant Panda *RPS16*.

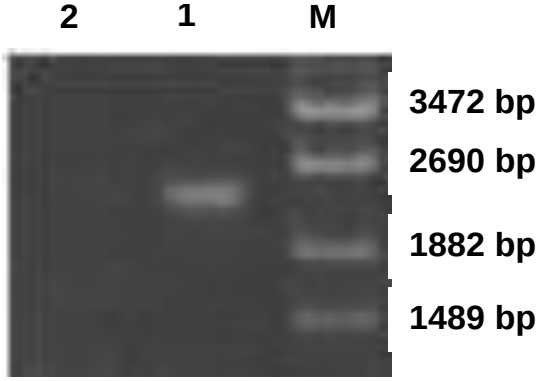

Figure 3. Polymerase chain reaction products of genomic sequence of *RPS16* from Giant Panda M: molecular marker, 1: the amplified *RPS16* genomic sequence and 2: check.

gennomic sequences. On the contrary, the four introns are different in length (Table 1). The largest intron of the *RPS16* gene from Giant panda is 1695 bp, while the length ranges from 1083 bp (*M. musculus*) to 2143 bp (*C. familims*). The variations in lengths of the introns determine the lengths of the *RPS16* genes.

The length of cDNA fragment cloned is 448 bp with a 441 bp open reading frame which contains 22.8% A, 28.8% C, 29.5% G and 19.0% T. Further analysis of base usage bias revealed that the *RPS16* gene bias some kind of bases. Specifically, the first base of codon biases G had a average content of 33.0%, the second base of codon biases A also had a average content of 33.0% and the third base of codon obviously biases G had a average content of 35.0%, while the site have A average content was merely 13.0%. Statistic of base substitution indicated that substitution rate of the third site (15.6%) is far superior to the rate of the first (0.91%) and the second site (0.0%), which illuminates that base substitution rate of the third site is far faster than the rate of the first and the second site and this may be the main way for the evolution of *RPS16* gene.

The deduced amino acid sequence encodes a protein of 146 residues with a molecular mass of 16.44532 kDa. As with most ribosomal proteins, S16 is highly basic, containing a combined 32 Arg, Lys and His residues and only 12 Asp and Glu residues, which results in an estimated isoelectric point of 10.21. The basic amino acids are not evenly distributed, but rather are relatively concentrated in three regions; for example, 8 of the 22 amino acids at the C terminus are basic. Another notable feature of the protein is its high content of hydrophobic amino acids (46.6%), with many of these grouped into uninterrupted blocks of four to five residues. The similarities in sequence among the Giant Panda, human, rat and mouse S16 proteins were not surprising since most mammalian ribosomal proteins examined previously have shown a high degree of sequence conservation (Surinder et al., 1991). Alignment analysis of *RPS16* among the Giant Panda and those of *H. sapiens*, *M. musculus*, *R. norvegicus* and *B. taurus*, indicated that both the nucleotide sequence and the deduced amino acid sequence are highly conserved. There is no deletion and insertion of nucleotide and amino acid residue. As determined by BLAST analysis, the nucleotide sequence *RPS16* cloned from the Giant Panda share a high homology with those of *B. taurus*, *H. sapiens*, *M. musculus*, *R. norvegicus* and *D. rerio* by 95.46, 92.97, 89.80, 89.80 and 82.54%, respectively. The deduced amino acid sequence is entirely

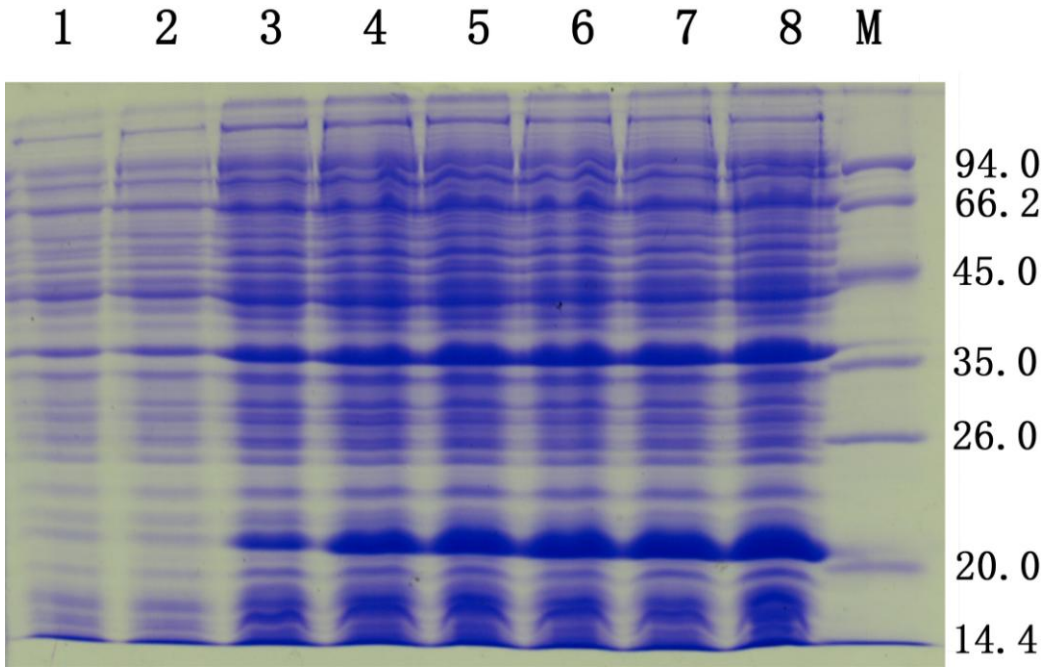

Figure 4. Protein extracted from recombinant *E. coli* strains were analyzed by SDS-PAGE gel stained with Commassie blue R 250. Numbers on right shows the molecular weight and the site of 20.095 indicates the recombinant protein bands induced by IPTG with 0, 0.5, 1.5, 2, 2.5, 3 and 3.5 h (lane 2 - 8), respectively. The lane 1 represents the products of the *E. coli* strains with the empty vectors.

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Pd   1 MPSKGPLQSV QVFGRKKTAT AVAHCKRGNG LIKVNGRPLE MIEPRTLQYK LLEPVLLLGK
Bos  1 MPSKGPLQSV QVFGRKKTAT AVAHCKRGNG LIKVNGRPLE MIEPRTLQYK LLEPVLLLGK
Homo 1 MPSKGPLQSV QVFGRKKTAT AVAHCKRGNG LIKVNGRPLE MIEPRTLQYK LLEPVLLLGK
Rat  1 MPSKGPLQSV QVFGRKKTAT AVAHCKRGNG LIKVNGRPLE MIEPRTLQYK LLEPVLLLGK
Mus  1 MPSKGPLQSV QVFGRKKTAT AVAHCKRGNG LIKVNGRPLE MIEPRTLQYK LLEPVLLLGK
Dan  1 MPAKGPLQSV QVFGRKKTAT AVAHCKRGNG LIKVNGRPLE MIEPVTLQYK LLEPLLLLGK

Pd   61 ERFAGVDIRV RVKGGGHVAQ IYAIRQSISK ALVAYYQKYV DEASKKEIKD ILIQYDRTLL
Bos  61 ERFAGVDIRV RVKGGGHVAQ IYAIRQSISK ALVAYYQKYV DEASKKEIKD ILIQYDRTLL
Homo 61 ERFAGVDIRV RVKGGGHVAQ IYAIRQSISK ALVAYYQKYV DEASKKEIKD ILIQYDRTLL
Rat  61 ERFAGVDIRV RVKGGGHVAQ IYAIRQSISK ALVAYYQKYV DEASKKEIKD ILIQYDRTLL
Mus  61 ERFAGVDIRV RVKGGGHVAQ IYAIRQSISK ALVAYYQKYV DEASKKEIKD ILIQYDRTLL
Dan  61 ERFAGVDIRV RVKGGGHVAQ VYAIRQAISK ALVAYYQKYV DEASKKEIKD ILIQYDRTLL

Pd   121 VADPRRCESK KFGGPGARAR YQKSYR
Bos  121 VADPRRCESK KFGGPGARAR YQKSYR
Homo 121 VADPRRCESK KFGGPGARAR YQKSYR
Rat  121 VADPRRCESK KFGGPGARAR YQKSYR
Mus  121 VADPRRCESK KFGGPGARAR YQKSYR
Dan  121 VADPRRCESK KFGGPGARAR YQKSYR
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Figure 5. Functional sites in RPS16 the Giant Panda, *H. sapiens*, *M. musculus*, *R. norvegicus*, *B.Taurus* and *D. rerio*. Pd: the Giant panda; *Bos*: *B. taurus*; homo: *H. sapiens*; *Mus*: *M. musculus*; Rat: *R. norvegicus*; Dan: *D. rerio;* ___: cAMP- and cGMP-dependent protein kinase phosphorylation site; ___: protein kinase C phosphorylation sites; ☐: casein kinase II phosphorylation site; ___: N-myristoylation sites; ☐: amidation site; ☐: ribosomal protein S9 signature; ▒: polymorphic sites.

Table 1. Comparison of *RPS16* genomic among 6 mammal species.

Species	Length of genome	Number of exons	Number of introns	Length of 5′-untranslated sequence	Length of 3′-untranslated sequence	Length of first intron	Length of second intron	Length of third intron	Length of fourth intron
A. melanoleuca	2510	5	4	4	3	132	1695	106	129
H. sapiens	2772	5	4	83	66	139	1845	95	103
M. musculus	1947	5	4	54	57	130	1083	89	83
B. Taurus	2467	5	4	53	68	136	1548	101	110
C.familims	3026	5	4	0	63	124	2143	132	126
D. rerio	3624	5	4	123	46	910	1891	119	48

identical compared with the first four animals and shared high homology with that of *D rerio* by 96.58%. Primary structure analysis revealed that the molecular weight of the putative *RPS16* protein of the *D. rerio* is 16.35626 kDa with a theoretical pI 10.14.

We analyzed the functional sites of the amino acid sequences encoded by *RPS16* genes and found that there is one cAMP- and cGMP-dependent protein kinase phosphorylation site, three protein kinase C phosphorrylation sites, one casein kinase II phosphorylation site, two N-myristoylation sites, one amidation site and one ribosomal protein S9 signature in the *RPS16* protein of the Giant Panda, *B. taurus, H. sapiens*, *R. norvegicus*, *M. musculus* and *D. rerio* (Figure 5). That is to say, the functional sites are entirely identical in *RPS16* proteins of these mammalians. However, the identity among the Giant Panda, *Bos*, Rat, *Mus* and human s16 sequence and their slight difference from the *D. rerio* S16 sequence were somewhat more enigmatic. Further analysis detected five polymorphic sites in the amino acid sequences of the five species compared. In particular, the nonconservative substitution of alanine for serine at position 3 and 87, valine for arginine at position 45, leucine for valine at position 55 and valine for isoleucine at position 81, respectively, of the Giant Panda, *Bos*, Rat, *Mus* and human sequence in the *D. rerio* suggest that these 5 residues are not critical for the function of this molecule. These polymorphic sites are located irregularly in the amino acid sequences all of which result from the transversion or transition of the corresponding codons without any deletion and insertion of base. Among these polymorphic sites, sites 81 and 87 are located in ribosomal protein S9 signature, but it does not result in any differences from Giant Panda and other four mammalian species in the functional site. Alternatively, any one of the sequence differences at these five positions in the *D. rerio* may serve to compensate for the others in preserving the structural and functional integrity of the S16 protein. The fact shows that the variation of sites has no affect on the structure and function of *RPS16* protein and it may have resulted during the evolution of these species. However, what changes caused by other mutations outside the functional sites in the structure and the function of *RPS16* need further studies.

The *RPS16* gene obtained is expressed efficiently in prokaryotic organism using pET28a plasmids and the gained fusion protein is in accordance with the expected 20.095 kDa polypeptide (Figure 4). These results suggest that the protein is active and it is just the protein encoded by the *RPS16* from the Giant Panda. The protein possesses extensive secondary structure. A quantitative estimate of the content of α-helices and β-strands in the protein secondary structure shows that 38.4% of the protein sequence is folded in α-helices and 18.5% in β-strands. The expression product obtained could be used for purification and further study of its function. The complete coding sequence of *RPS16* gene from Giant Panda has been cloned through RT-PCR technique and expressed in *E. coli* successfully. This is the first report on the *RPS16* gene from the Giant Panda.

The characterization of genomic sequence and cDNA clones encoding ribosomal proteins would be beneficial in the study of ribosomal biogenesis and would allow the elucidation of structure, organization and regulation of genes encoding ribosomal proteins in eukaryote. These data will enrich and supplement the information about *RPS16*. In addition, it will contribute to the protection of gene resources and the discussion of genetic polymorphism.

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