cDNA, genomic sequence cloning and overexpression of the ribosomal protein S13 gene in the giant panda (*Ailuropoda melanoleuca*)

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ABSTRACT. The cDNA and the genomic sequence of ribosomal protein S13 (*RPS13*) of the giant panda (*Ailuropoda melanoleuca*) was cloned using reverse transcription-polymerase chain reaction (RT-PCR) and touchdown-PCR, respectively. These two sequences were sequenced and analyzed, and the cDNA of the *RPS13* gene was overexpressed in *Escherichia coli* BL21. We compared the nucleotide sequences of the coding region and the amino acid sequences with those of seven other mammalian species retrieved from GenBank. The cDNA fragment of the *RPS13* cloned from the giant panda is 496 bp in size, containing an open-reading frame of 456 bp, encoding 151 amino acids. The length of the genomic sequence is 2277 bp, with five exons and four introns. The coding sequence shows a high degree of homology to those of *Homo sapiens*, *Bos taurus*, *Canis lupus familiaris*, *Macaca mulatta*, *Mus musculus*, *Rattus norvegicus*, and *Pan troglodytes*; the degree of homology was 91.23, 94.30, 94.74, 92.11, 87.94, 87.72, and 91.45%, respectively. The homologies for the deduced amino acid sequences reached as high as 99%. Primary structure analysis revealed that the molecular weight of the puta-

tive RPS13 protein is 17.22325 kDa, with a theoretical pI of 10.42. Based on topology prediction, there is one protein kinase C phosphorylation site, one casein kinase II phosphorylation site, two N-myristoylation sites, and one ribosomal protein S15 signature in the RPS13 protein of the giant panda. The *RPS13* gene can be expressed in *E. coli* and the RPS13 protein fused with the N-terminally GST-tagged form, which gave rise to the addition of an expected 43-kDa polypeptide.

Key words: Giant panda; *Ailuropoda melanoleuca*; *RPS13* gene; cDNA cloning; Sequence analysis; Overexpression

INTRODUCTION

Increasing evidence suggests that many ribosomal proteins are not only involved in the basic machinery of protein synthesis and regulation, but also in various extra-ribosomal activities, including the regulation of cell proliferation, DNA repair, transcription, and RNA processing (Mager, 1988; Wool et al., 1995; Wool 1996; Draper and Reynaldo, 1999; Barakat et al., 2001; Bortoluzzi et al., 2001; Zeng, et al., 2001; Perry, 2005; Ishii, et al., 2006). The ribosomal protein S13 (*RPS13*) gene encodes a protein that is a component of the 40S subunit of the ribosome. This protein belongs to the S15P family of ribosomal proteins. It is located in the cytoplasm and has been shown to bind to 5.8S rRNA in rats (Shi et al., 2004). *RPS13* (along with *rpL23*) has been found to promote multidrug resistance in gastric cancer cells by suppressing drug-induced apoptosis (Shi et al., 2004). *RPS13* also inhibits splicing of its own pre-mRNA due to binding close to the splicing sites in intron 1. This gene is co-transcribed with two small nucleolar U14 RNA genes, which are located in the third and fifth introns (Kumar et al., 1995). As is typical for genes encoding ribosomal proteins, there are multiple processed pseudogenes of this gene dispersed throughout the genome. A large number of studies have been made about *RPS13*, including in *Homo sapiens*, *Bos taurus*, *Canis lupus familiaris*, *Macaca mulatta*, *Mus musculus*, *Rattus norvegicus*, *Pan troglodytes*, and the giant panda, *Ailuropoda melanoleuca* (Sanchez et al., 1996; Malygin et al., 2005).

The giant panda is considered to be precious not only in China but also throughout the world. Its population has been declining for thousands of years, and it is now rare and limited to several provinces of China. Pandas are known as "living fossils" (Yoshihama et al., 2002). Previous studies on the giant panda have concentrated on the macro-level, such as breeding and propagation, ecology, genetic diversity, parentage, and so on; few studies have been made on the functional genome of the giant panda (Montali, 1990; Wu et al., 1990; Mather et al., 1997; Liao et al., 2003; Du et al., 2007; Hou et al., 2007a,b, 2008, 2009a,b). Recently, the giant panda's draft genome sequence has been generated and assembled, with good coverage and completeness for genes with unique sequences (Li et al., 2010). Molecular studies of functional genes such as *RPS13* would further our understanding of this rare species and provide data for formulating more effective conservation strategies.

MATERIAL AND METHODS

Skeletal muscle was collected from a dead giant panda at the Wolong Conservation Center of the Giant Panda, in Sichuan, China. The collected skeletal muscle was frozen in liquid nitrogen and then used for DNA and RNA isolation.

DNA and RNA isolation

The genomic DNA was isolated from muscle tissue (Sambrook et al., 1989). The DNA was dissolved in TE buffer and kept at -20°C.

Total RNA was extracted from about 400 mg muscle tissue using Total Tissue/Cell RNA Extraction Kits (Waton Inc., Shanghai, China) according to manufacturer instructions and then dissolved in RNase-free double-distilled water, and kept at -70°C. DNA and RNA sample quality was checked using Experion (Bio-Rad) and quantification was performed spectrophotometrically.

Primer design, RT-PCR, cloning of the cDNA sequence and sequencing

The polymerase chain reaction (PCR) primers were designed with Primer Premier 5.0, based on the mRNA sequence of *RPS1*3 from *H. sapiens* (NM_001017), *B. taurus* (NM_001025342), *C. lupus familiaris* (XM_846944), *M. mulatta* (XM_001086088), *M. musculus* (NM_026533), *R. norvegicus* (NM_130432), and *P. troglodytes* (XM_508306). The specific primers of the cDNA sequences were: *RPS13*-F: 5'- CGTTGCCTGATCGCCGCCAT-3', *RPS13*-R: 5'-GCTTGAGTACACAGACAAAT-3'.

Total RNAs were synthesized into the first-strand cDNA using a reverse transcription kit with Oligo dT, followed by PCR amplification, according to manufacturer instructions (Promega Shanghai, China).

The synthesized first-strand cDNA was used as a template; reverse transcription reactions were performed in duplicate. After amplification, PCR products were separated by electrophoresis on 1.5% agarose gel with 1X TAE buffer, stained with ethidium bromide and visualized under UV light. The fragments of the PCR products were harvested from the gel and purified using a DNA harvesting kit (Omega bio-tek, USA), and then ligated into pMD19-T vector (TaKaRa, Dalian, China) at 16°C for 2 h. The recombinant molecules were transformed into *Escherichia coli* competent cells (*DH5α*) and then spread on the LB-plate containing 50 μg/mL ampicillin, 200 mg/mL IPTG and 20 mg/mL X-gal. Plasmid DNA was isolated and digested with *Pst*I and *Sca*II to check the insert size. Plasmid DNA was sequenced by the Huada Zhongsheng Scientific Corporation (Beijing, China).

Cloning the genomic sequence of *RPS13*

The PCR primers were designed basing on the cDNA sequence of the *RPS13* from the giant panda. The primers of the genomic sequence were the same as for Pd-*RPS13*-F and Pd-*RPS13*-R, as follows: *RPS13*-F': 5'-CGTTGCCTGATCGCCGCCAT-3', *RPS13*-R': 5'-GCTTGAGTACACAGACAAAT-3'.

The genomic sequence of the *RPS13* gene was amplified using touchdown-PCR under the following conditions: 94°C for 30 s, 61°C for 45 s, 72°C for 3 min in the first cycle and the annealing temperature was decreased 0.5°C per cycle; after 20 cycles, conditions changed to 94°C for 30 s, 51°C for 45 s, 72°C for 4 min for another 20 cycles. The amplified fragment was also purified, ligated into the clone vector and transformed into the *E. coli* competent cells. Finally, the recombinant fragment was sequenced by the Huada Zhongsheng Scientific Corporation.

Construction of the expression vector and overexpression of recombinant *RPS13*

PCR fragment corresponding to the *RPS13* polypeptide was amplified from the *RPS13* cDNA clone with the following forward and reverse primers, respectively: *RPS13*-F": 5'-ACTGGATCCCAGAGATTCGCCATCA-3' (*Bam*HI), *RPS13*-R": 5'-ACTGCGGCCGCACCCCAGGCTTTACAC-3' (*Not*I) (the underlined letters mean restriction sites).

The PCR was performed at 94°C for 2 min; 35 cycles of 30 s at 94°C, 50 s at 57°C and 1 min at 72°C; with a final extension for 7 min at 72°C. The amplified PCR product was cut and ligated into a corresponding site of the pGEX 4T-1 vector (Stratagen, Shanghai, China). The resulting construct was transformed into *E. coli* BL21 strain (Novagen, Shanghai, China) and used for induction by isopropyl-b-D-thiogalactopyranoside at an OD_{600} of 0.6 and cultured further for 4 h at 37°C, using the empty vector transformed BL21 as a control. The culture was centrifuged at 10,000 *g* for 5 min at room temperature and then induced for 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h, respectively. The culture supernatant was concentrated with methanol and chloroform (3:1, v/v), and SDS-PAGE (SDS polyacrylamide gel electrophoresis) was performed to investigate protein production and purity using slab gels containing 12% (w/v) polyacrylamide on a Mini-Protean II Slab Cell Apparatus (Bio-Rad, Hercules, CA, USA). Protein samples were visualized by Coomassie brilliant blue R-250 staining.

Data analysis

The sequence data were analyzed by the GenScan software (http://genes.mit.edu/GENSCAN.html). Homology research of the giant panda *RPS13* compared with the gene sequences of other species was performed using Blast 2.1 (http://www.ncbi. nlm.nih.gov/blast/). Open-reading frames (ORFs) of the DNA sequence was searched using the ORF finder software (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The protein structure of the cloned *RPS13* sequence was deduced using the Predict Protein software (http://cubic.bioc. columbia.edu/predictprotein/). Multiple sequence alignment was performed with the DNAstar Lasergene software and DNAMAN 6.0.

RESULTS

Analysis of the cDNA of *RPS13* from the giant panda

A cDNA fragment of about 0.5 kb was amplified from the giant panda with primers *RPS13*-F and *RPS13*-R (Figure 1).
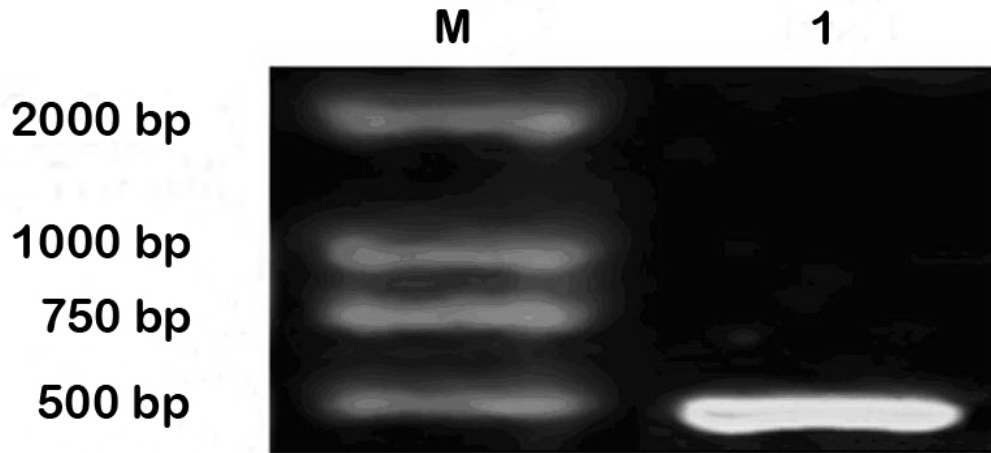
Figure 1. Reverse transcription polymerase chain reaction products of the giant panda *RPS13*. *M* = Molecular marker DL2000; *lane 1* = the amplified *RPS13*.

The cDNA clone is 496 bp long. Blast research showed that the cloned cDNA sequence shares a high homology with the *RPS13* from some other mammals, including *H. sapiens* (NM_001017), *B. taurus* (NM_001025342), *C. lupus familiaris* (XM_846944), *M. mulatta* (XM_001086088), *M. musculus* (NM_026533), *R. norvegicus* (NM_130432), and *P. troglodytes* (XM_508306). On the basis of the high level of identity, we concluded that we had cloned the cDNA encoding the giant panda RPS13 protein. The *RPS13* cDNA sequence was submitted to Genbank (accession No. HM996917).

The 496 bp of the giant panda *RPS13* sequence contains the 5'-untranslated sequence that is 20 bp and a 20-bp 3'-unstranslated region. An ORF of 456 bp, which contains 25.9% A, 26.1% C, 23.9% G, and 24.1% T, encoding 151 amino acids was found in this cDNA (Figure 2).

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1    CGTTGCCTGATCGCCGCCATATG GGT CGC ATG CAC GCT CCC GGG AAG GGC CTG TCC CAG TCT GCT CTG CCT TAC CGC
1                           M   G   R   M   H   A   P   G   K   G   L   S   Q   S   A   L   P   Y   R
78   CGC AGC GTC CCC ACC TGG CTG AAG TTG ACT TCT GAC GAC GTG AAG GAG CAG ATC TAC AAA CTG GCC AAG AAG
20   R   S   V   P   T   W   L   K   L   T   S   D   D   V   K   E   Q   I   Y   K   L   A   K   K
150  GGC CTT ACT CCC TCA CAA ATC GGT GTG ATC CTG AGA GAC TCC CAT GGT GTA GCA CAA GTA CGT TTT GTG ACA
44   G   L   T   P   S   Q   I   G   V   I   L   R   D   S   H   G   V   A   Q   V   R   F   V   T
222  GGC AAT AAA ATC TTG CGA ATT CTT AAG TCC AAA GGA CTT GCT CCT GAT CTT CCT GAG GAT CTC TAC CAT TTG
68   G   N   K   I   L   R   I   L   K   S   K   G   L   A   P   D   L   P   E   D   L   Y   H   L
297  ATT AAG AAA GCT GTT GCT GTT CGA GAG CAT CTT GAG AGG AAC AGA AAG GAT AAG GAT GCT AAA TTC CGA CTG
92   I   K   K   A   V   A   V   R   E   H   L   E   R   N   R   K   D   K   D   A   K   F   R   L
366  ATT CTG ATC GAG AGC CGT ATT CAT CGG TTG GCT CGA TAT TAC AAG ACC AAA AGA GTC CTC CCC CCC AAT TGG
116  I   L   I   E   S   R   I   H   R   L   A   R   Y   Y   K   T   K   R   V   L   P   P   N   W
438  AAA TAC GAG TCA TCC ACA GCC TCT GCC CTG GTT GCA TAA ATTTGTCTGTGTACTCAAGC
140  K   Y   E   S   S   T   A   S   A   L   V   A   *
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Figure 2. Nucleotide sequence of cDNA encoding the giant panda *RPS13* and the amino acid sequence deduced from its open-reading frame. *Indicates the stop cordon.

Analysis of the genomic sequence of *RPS13* from the giant panda

A DNA fragment of about 2000 bp was amplified with primers *RPS13*-F' and *RPS13*-R' (Figure 3). The length of the cloned DNA fragment is 2277 bp, which was found to possess five exons and four introns. A comparison between the cDNA sequence and the DNA fragment sequence of the *RPS13* amplified from the giant panda was made with DNAMAN version 6.0. The result revealed that the cDNA sequence is in full accord with five fragments in the DNA fragment, which confirms that the amplified DNA fragment is the genomic sequence of the *RPS13* from the giant panda. The genomic sequence of *RPS13* has been submitted to Genbank (accession No. HM996918).
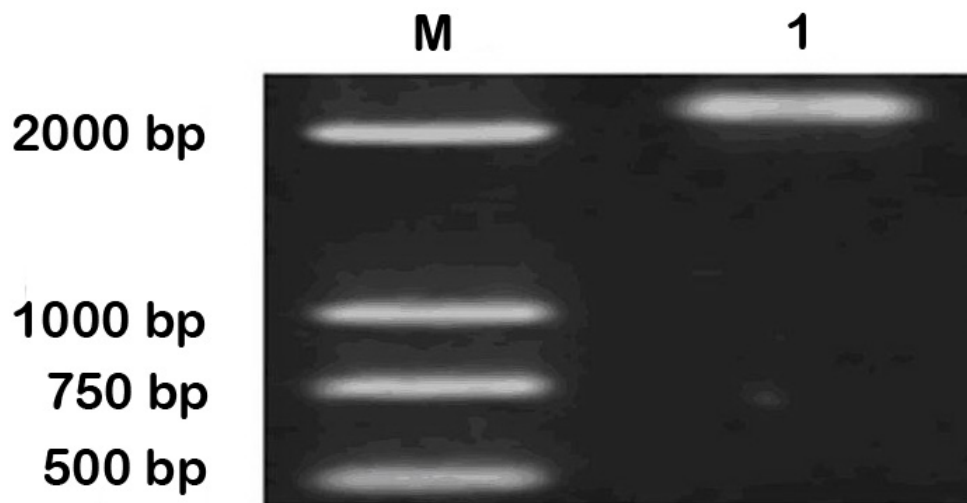

Figure 3. Polymerase chain reaction products of complete genomic sequence of *RPS13* from the giant panda. *M* = Molecular marker DL2000; *lane 1* = the amplified *RPS13* genomic sequence.

Prediction and analysis of protein functional sites in RPS13 protein of the giant panda

Primary structure analysis showed that the molecular weight of the putative RPS13 protein of the giant panda is 17.22325 kDa, with a theoretical isoelectric point (pI) of 10.42. Topology prediction shows that there is one protein kinase C phosphorylation site, one casein kinase II phosphorylation site, two N-myristoylation sites, and one ribosomal protein S15 signature in the RPS13 protein of the giant panda (Figure 4).

Overexpression of the *RPS13* gene in *E. coli*

The *RPS13* gene was overexpressed in *E. coli* and amplified individually by PCR, then cloned in a pGEX 4T-1 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 *E. coli* BL21 (Figure 5).

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PD*  MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
PD#  MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
HM   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
BT   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
CL   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
MM   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
MS   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
RN   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76
PT   MGRMHAPGKGLSQSALPYRRSVPTWLKLTSDDVKEQIYKLAKKGLTPSQIGVILRDSHGVAQVRFVTGNKILRILK  76

PD*  SKGLAPDLPEDLYHLIKKAVAVREHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
PD#  SKGLAPALPEDLYRWIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWK             140
HM   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
BT   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
CL   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
MM   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
MS   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
RN   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
PT   SKGLAPDLPEDLYHLIKKAVAVRKHLERNRKDKDAKFRLILIESRIHRLARYYKTKRVLPPNWKYESSTASALVA  151
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Figure 4. The functional sites of the amino acid sequence encoded by *RPS13* gene of the giant panda. PD* = *Ailuropoda melanoleuca* (we cloned); PD# = *A. melanoleuca* (published); HM = *Homo sapiens*; BT = *Bos taurus*; CL = *Canis lupus familiaris*; MM = *Macaca mulatta*; MS = *Mus musculus*; RN = *Rattus norvegicus*, and PT = *Pan troglodytes*. Open boxes = protein kinase C phosphorylation site; gray boxes = N-myristoylation site; single-underlined letters = ribosomal protein S15 signature; double-underlined letters = casein kinase II phosphorylation site.
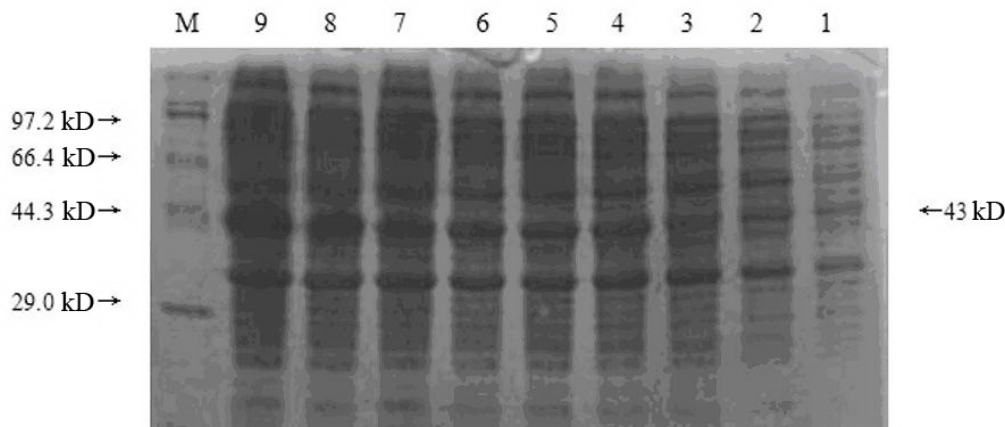

Figure 5. Protein extracted from recombinant *Escherichia coli* BL21 strains analyzed by SDS-PAGE gel stained with Coommassie blue R250. *M* on left shows the molecular weight, and the arrows indicate the recombinant protein bands induced by IPTG after 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h (*lanes 1-9*), respectively.

The results showed that the protein RPS13 fusion with the N-terminally GST-tagged form lead to the accumulation of an expected 43-kDa polypeptide that formed inclusion bodies. The recombinant protein was expressed after half an hour of induction, and the output of the induction continued.

DISCUSSION

Ribosomal protein is an important component of the ribosome, it plays an important role in the ribosomal assembly and formation of the initiation complex and regulates cell division, proliferation, differentiation, and other processes. The RPS13 protein belongs to the S15P family of ribosomal proteins, and the protein is a homolog of prokaryotic *rpS15*, which binds to the central domain of the 16S rRNA and promotes the binding of neighboring proteins in the 30S ribosomal subunit (Jagannathan and Culver, 2003). Here, we report the identification and characterization of the genomic sequence, the cDNA clone and overexpressed encoding ribosomal protein S13 from the giant panda.

Comparison of nucleotide sequences and amino acid sequences of *RPS13* of the giant panda with those of seven other mammals

Alignment analysis of the cDNA sequence of *RPS13* between the giant panda and *H. sapiens* (NM_001017), *B. taurus* (NM_001025342), *C. lupus familiaris* (XM_846944), *M. mulatta* (XM_001086088), *M. musculus* (NM_026533), *R. norvegicus* (NM_130432), and *P. troglodytes* (XM_508306) was performed with the DNAstar Lasergene software. The homologies in the coding sequence between the giant panda and the seven other mammals were 91.23, 94.30, 94.74, 92.11, 87.94, 87.72, and 91.45%, respectively. The homologies for deduced amino acid sequences reached as high as 99%. These results indicated that both the nucleotide sequence and the deduced amino acid sequence of *RPS13* are highly conserved.

Further analysis of the nucleotide sequences showed that there are some differences in the coding region between the giant panda and other species. Alignment analysis showed that the changes in the nucleotide coding sequence of the *RPS13* gene involve transition and transversion, but no insertions, deletions or inversions. The stop codon TAG is used in *B. taurus*, while the giant panda and the other six mammals terminated with TAA. These differences in the coding nucleotide sequence of the giant panda compared to other species show that these mammals have a high degree of conservation at the nucleotide sequence of the *RPS13* gene, but there are differences. This will provide a theoretical basis for the research of the origin and evolutionary relationships between the giant panda and the other seven species.

The amino acid sequences of these eight mammals show that the giant panda is only slightly different from the other seven mammals. Site 100 is located in the ribosomal protein S15 signature; there is a glutamic acid at position 100 in the giant panda, but the other mammals have a lysine at this site. Also, most base transitions of the gene coding sequence in these mammals were synonymous mutations, such as at positions 42, 82, 171, and 207. These synonymous mutations did not result in any changes in the corresponding DNA information and did not change the amino acid in the expression product; consequently the spatial structure of the corresponding protein would not be affected.

Furthermore, comparing the amino acid sequence of *RPS13* of the giant panda that we cloned and the giant panda draft sequence counterpart; aspartic acid, histidine, leucine, and glutamic acid were located at positions 83, 90, 91, and 100, respectively, of the amino acid sequence we cloned, while alanine, arginine, tryptophan, and lysine are found in the published sequence. The published sequence has 11 fewer amino acids than in the other mammals, including the sequence of the giant panda that we cloned. The amino acid sequence of the giant panda that we cloned is closer to that of the other seven mammals.

Comparison of the *RPS13* sequence among eight mammal species, including the giant panda

The genomic sequence of *RPS13* has 2277 bp. A comparison of the nucleotide sequences of the genomic and cDNA sequences indicated that the genomic sequence of *RPS13* possesses five exons and four introns; the lengths of the five exons are 23, 49, 79, 170, and 135 bp, respectively, the length of the four introns are 147, 174, 998, and 462 bp, respectively, and the junction sequence of exons and introns is in accordance with the law of gene composition, which is also supported by restriction mapping of the genomic and cDNA sequences.The other seven mammals have six exons, which comprise the cDNA sequence of *RPS13* gene after RNA splicing; this sequence is highly conserved. The restriction sites in the exons are the same in both the cDNA and the genomic sequences. When we compare the genomic sequence of the giant panda with those of the seven other mammals, the first to the fourth exons of these eight mammals are exactly the same, but the fifth and the sixth exons of the giant panda are next to each other, so the giant panda has only five exons and four introns. This is why pandas have fewer exons and introns than other mammals. Also, the genomic, the intron, the 5'-untranslated and the 3'-untranslated sequences differ in length (Table 1). The lengths of *C. lupus familiaris*, *M. mulatta* and *P. troglodytes*'s genomic sequences differ considerably compared to those of the other mammals, because of the variations in the lengths of the introns in the *RPS13* gene. Furthermore, when we compare our data with the giant panda's draft genome sequence (accession No. GL193225), not only the genome sequence but also the number of exons are different. The length of the genomic sequence of the draft is 76 bp shorter than what we found. Our exon 5 had 135 bp; the corresponding exon 1 of the draft is only 99 bp. The other corresponding exons did not differ. We conclude that exon 1 of the draft sequence is truncated; our data supplement information on *RPS13*.

Prediction and analysis of functional protein sites in RPS13 protein

Topology prediction shows that there is one protein kinase C phosphorylation site, one casein kinase II phosphorylation site, two N-myristoylation sites, and one ribosomal protein S15 signature in the RPS13 protein of the giant panda. Alignment analysis of *RPS13* among those proteins revealed that the functional sites are identical in RPS13 proteins of *A. melanoleuca* (that we cloned), *H. sapiens*, *Bos taurus*, *C. lupus familiaris*, *M. mulatta*, *M. musculus*, *R. norvegicus*, and *P. troglodytes* (Figure 4). We can see that although they there are small differences in the amino acid sequences, the functional sites in these eight mammals are identical. This shows that the variation in nucleotide sequences has no effect on the structure and the function of the RPS13 protein. In conclusion, in the giant panda and in the seven other mammalian species, the *RPS13* gene has a high level of nucleotide and amino acid conservation.

The published amino acid sequence of the giant panda has one more N-myristoylation site than the sequence of the giant panda that we cloned, and this functional protein site is located at position 78-84 of the amino acid sequence (Figure 4). The other mammals also have no functional site at this location. This further supports our conclusion, because of the high degree of consistency with the sequence in the seven other mammals.

Table 1. Comparison of the *RPS13* genomic sequence among the 8 mammal species.

	Length of genome (bp)	Number of exons	Number of introns	Length of 5'-untranslated sequence (bp)	Length of 3'-untranslated sequence (bp)	Joint sites in the CDS	GenBank accession Nos.
*A. melanoleuca**	2277	5	4	20	20	21-43,191-239,414-492,1491-1660, 2123-2257	HM996918
A. melanoleuca#	2201	5	4	0	0	1..99,562..731,1730..1808,1983..2031, 2179..2201	GL193225
H. sapiens	3282	6	5	32	41	33..55,197..245,428..506,2051..2220, 2477..2577,3208..3241	NC_000011.8
B. taurus	2725	6	5	24	45	25..47,194..242,417..495,1450..1619, 1936..2036,2647..2680	NC_007313.3
C. lupus familiaris	8281	6	5	75	56	76..98,253..301,466..544,2084..2253, 2660..2760,3355..3388	NC_006603.2
M. mulatta	9547	6	5	62	59	63..85,226..274,457..535,2072..2241, 2479..2579,3116..3149	NC_007871.1
M. musculus	2623	6	5	27	38	28..50,187..235,361..439,1037..1206, 1656..1756,2552..2585	NC_000073.5
R. norvegicus	2426	6	5	26	41	27..49,187..235,360..438,1057..1226, 1633..1733,2352..2385	NC_005100.2
P. troglodytes	9356	6	5	90	53	91..113,255..303,486..564,2115..2284, 2549..2649,3251..3284	NC_006478.2

*The genomic sequence of the giant panda we cloned. #Represents the counterpart published in the draft genome sequence.

Prediction of the physical and chemical features of RPS13 protein

Physical and chemical analysis revealed that the molecular weight of the putative RPS13 protein is 17.22325 kDa and its theoretical pI is 10.42. It also shows that the molecular weight and the theoretical pI of the putative RPS13 protein among the eight mammalians are quite similar (Table 2).

Table 2. Molecular weight (MW) and isoeletric point (pI) of *RPS13* of the giant panda and seven other mammals.

	A. melanoleuca	*H. sapiens*	*B. taurus*	*C. lupus familiaris*	*M. mulatta*	*M. musculus*	*R. norvegicus*	*P. troglodytes*
MW (kDa)	17.22325	17.22231	17.22231	17.22231	17.22231	17.22231	17.22231	17.22231
pI	10.42	10.53	10.53	10.53	10.53	10.53	10.53	10.53

In summary, we can confirm that the sequence that we amplified is that of *RPS13* from the giant panda. All of these homologies prove the highly conserved nature of the giant panda genome. Also, the *RPS13* gene in the giant panda and in seven other mammalian species has a high degree of functional protein site conservation. This is distinct from the published sequence information for this species (Li et al., 2010).

The *RPS13* gene that we obtained is expressed efficiently in prokaryotic organisms such as *E. coli* using pGEX 4T-1 plasmids, and the fusion protein that was obtained is in accordance with the expected 43-kDa polypeptide. These results suggest that the protein is active and is the protein encoded by *RPS13* from the giant panda. The expression product could be purified for further study of its function.

In summary, we cloned the complete coding sequence of *RPS13* gene using RT-PCR. This information will contribute to our understanding of this endangered "Treasure of China".

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