

Giant panda ribosomal protein S14: cDNA, genomic sequence cloning, sequence analysis, and overexpression

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ABSTRACT. RPS14 is a component of the 40S ribosomal subunit encoded by the RPS14 gene and is required for its maturation. The cDNA and the genomic sequence of RPS14 were cloned successfully from the giant panda (Ailuropoda melanoleuca) using RT-PCR technology and touchdown-PCR, respectively; they were both sequenced and analyzed. The length of the cloned cDNA fragment was 492 bp; it contained an open-reading frame of 456 bp, encoding 151 amino acids. The length of the genomic sequence is 3421 bp; it contains four exons and three introns. Alignment analysis indicates that the nucleotide sequence shares a high degree of homology with those of *Homo sapiens*, *Bos taurus*, Mus musculus, Rattus norvegicus, Gallus gallus, Xenopus laevis, and Danio rerio (93.64, 83.37, 92.54, 91.89, 87.28, 84.21, and 84.87%, respectively). Comparison of the deduced amino acid sequences of the giant panda with those of these other species revealed that the RPS14 of giant panda is highly homologous with those of *B. taurus*, *R.* norvegicus and D. rerio (85.99, 99.34 and 99.34%, respectively), and is 100% identical with the others. This degree of conservation of RPS14 suggests evolutionary selection. Topology prediction shows that there are two N-glycosylation sites, three protein kinase C phosphorylation sites, two casein kinase II phosphorylation sites, four N-myristoylation sites, two amidation sites, and one ribosomal protein S11 signature in the RPS14 protein of the giant panda. The *RPS14* gene can be readily expressed in *Escherichia coli*. When it was fused with the N-terminally His-tagged protein, it gave rise to accumulation of an expected 22-kDa polypeptide, in good agreement with the predicted molecular weight. The expression product obtained can be purified for studies of its function.

Key words: cDNA cloning; *RPS14*; Giant panda; Overexpression; *Ailuropoda melanoleuca*; Genomic cloning