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Cdna Cloning, Characterization And Expression Analysis of Ribosomal Protein S24 Gene of Periplaneta Americana (Blattodea: Blattidae)

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Abstract: Ribosomal proteins S24 (RPS24) is one of the small subunit ribosomal proteins, and is likely involved with the initiation and elongation process during protein synthesis. In this study, a full-length of RPS24 cDNA was first cloned from the American cockroach, Periplaneta americana (L.) (Blattodea: Blattidae) by using rapid amplification of cDNA ends (RACE) approaches. The full-length cDNA of the P. americana RPS24 (PaRPS24) was of 572 bp, containing a 5′ untranslated region (UTR) of 33 bp, a 3′ UTR of 137 bp with a poly (A) tail, and an open reading frame of 402 bp encoding a polypeptide of 133 amino acids with the predicted molecular weight of 15.32 kDa and estimated isoelectric point of 10.86 (NCBI accession number: KJ650197). BLAST analysis revealed that amino acids of PaRPS24 shared identity with Harpegnathos saltator 89%, Biphyllus lunatus 89%, Carabus granulatus 89%, Camponotus floridanus 88%, Tribolium castaneum 88%, Solenopsis invicta 88%, and Lygus lineolaris 86%, and so on. Quantitative RT-PCR analysis indicated that PaRPS24 could be detected in all the tested tissues, in which the relative expression levels were 1.01-, 6.19-, 4.99-, 2.62- and 1.24-fold higher in the testis, head, thorax, leg and gut than in the ovary, respectively. The results provided some important information, and may help to understand the evolution of ribosomes and to unravel the function of the proteins in insects.

Key Words: Periplaneta americana, RPS24, RACE, quantitative RT-PCR, cloning

Introduction

Ribosomal proteins are a major component of ribosomes, eukaryotic ribosomes are complex structures that contain 4 species of RNA and about 80 distinct proteins (Xu et al., 1994). Ribosomal proteins play critical roles in protein biosynthesis. Also, it was involved in various cellular processes, such as replication, transcription, RNA processing, and DNA repairs (Kim et al., 2008).

Ribosomal proteins S24 (RPS24) is one of the small subunit ribosomal proteins. Its normal physiological role is largely unknown, but it is likely involved with the initiation and elongation process during protein synthesis. RPS24 has been shown to be associated with several translation factors involved in the initiation and elongation steps of protein synthesis, and thus may have an important function in translation (Xu et al., 1994). The regulation of gene expression at the translational level is important for cell growth. RPS24 participates in the binding of eukaryotic initiation factor 2 (eif-2) to ribosomes (Bommer et al., 1988), and it can be crosslinked to initiation factor eif-3 (Tolan et al., 1983) which is necessary for the binding of mRNA to ribosomes and, hence, it is located at the site where the factor binds and where initiation of translation of mRNA occurs, its function is controlled in an erythroid-specific manner by heme-regulated kinase (Pain, 1996).An increase in ribosomal protein gene expression in general correlates with proliferation seen in tumor cells, where this has been studied.

The expression of RPS24 is decreased during adipocyte differentiation, and its expression is elevated markedly in certain transformed and tumor cell lines (Xu et al., 1994). However, some ribosomal proteins were not found to be induced during cell proliferation, and RPS24 might have a dual role in cell proliferation and growth arrest (Mafune et al., 1991).

The cockroach is one of the oldest winged insects known, and its habits are closely associated with those of humans. The household pest American cockroach Periplaneta americana (L.) (Blattodea: Blattidae) is now found nearly everywhere on the planet. P. americana has been regarded as model for studying insect physiology (Irles et al., 2009; Yoon et al., 2009). However, no information has been available for RPS24 in this evolutionarily important organism so far. The aim of the present study was to characterize RPS24 cDNAs from P. americana, the information may aid understanding the evolution of ribosomes, and unraveling the function of the protein.

Materials and methods

Insect Sampling

Specimens of P. americana were obtained from our laboratory. The colony was maintained at 60-70% RH,12:12 h L:D and 25^0C The cockroaches, which molted to adults within 20 to 30 days were selected. We collected heads, thoraxes, legs and guts from 10 females and 10 males, respectively, and also ovaries from 10 females and testes from 10 males. All dissection and tissue samplings were carried out on carbon dioxide-anaesthetized specimens (Irles et al., 2009).

RNA Isolation and cDNA Preparation

Total RNA was isolated from female or male adult P. americana using RNeasy® Plant Mini Kit (Qiagen), following the manufacturer's protocols (Irles et al., 2009). Considerable care was taken to ensure that all total RNA samples used for RACE were of high quality (A260/A280 >1.8 in nuclease-free water) with minimal degradation (Geng et al., 2009). To obtain a complete cDNA sequence, 5′ and 3′ RACE were conducted according to the manufacturer's instructions, using the SMARTERTM RACE cDNA Amplification Kit (Clontech, USA) following the manufacturer's protocols.

Cloning the Full-Length cDNA of P. americana RPS24 (PaRPS24)

A putative RPS24 expressed sequence tag (EST) sequence in P. americana was obtained from our previous study (Chen et al., 2013). Based on the partial sequence obtained, specific primers, RPS24-F: 5′-GTTCAAACTTCATTGCGTAGTCCA-3′ and RPS24-NF: 5′-ACCACGCACCTTCTTCATTCTGTTC-3′ for 5′ RACE, and RPS24-R: 5′-CATAGGTTATCTAGGCACGGGCTCTT-3′ and RPS24-NR: 5′-GAACAGAATGAAGAAGGTGCGTGG-3′ for 3′ RACE, were designed. The PCR fragments were subjected to electrophoresis on 1.5 % agarose gel to determine length differences, and the target band was purified by PCR purification kit (Promega, USA). The amplified fragments were cloned into PMD® 19-T Simple Vector (Takara) and sequenced.

Sequence Analysis

Open Reading Frame Finder in NCBI http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used for distinguishing initiation codon, coding region, termination codon, and 5′ and 3′ UTR of these RPS24 transcript sequences. The nucleotide and deduced amino acid sequences of RPS24 cDNA were analyzed and compared using Basic Local Alignment Search Tool (BLAST) http://www.blast.ncbi.nlm.nih.gov/blast.cgi). The deduced protein sequence of PaRPS24 for homology analysis was compared with those of the other known RPS24 proteins from various insects, such as Biphyllus lunatus (Coleoptera: Biphyllidae), Carabus granulatus (Coleoptera: Carabidae), Tribolium castaneum (Coleoptera: Tenebrionidae),Camponotus floridanus (Hymenoptera: Formicidae), Harpegnathos saltator (Hymenoptera: Formicidae), Solenopsis invicta (Hymenoptera: Formicidae), Lygus lineolaris (Hemiptera: Miridae) and so on (Table 1), in the GenBank nr database. Molecular weight and isoelectric point of these proteins were predicted by using ProtParam http://web.expasy.org/protparam/The

signal peptide was predicted by SignalP 4.1 (Bendtsen et al. 2004). N-linked glycosylation site was predicted by using NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc/).. Transmembrane domains were predicted by the TMHMM program http://www.cbs.dtu.dk/services/TMHMM/).

Phylogenetic Analysis

Phylogenetic analysis of the PaRPS24 gene was performed via multiple steps. Putative orthologues were identified by a PSI-BLAST (Schäffer et al., 2001) search of the GenBank nr database and compiled within a FASTA file. Sequences were aligned using stand-alone ClustalX software (Thompson et al., 1997). Alignments of amino acid sequences were performed using BioEdit 7.0.5 (Hall, 1999). Phylogenetic trees were performed using MEGA 6.0 program (Tamura et al., 2013) in which the robustness of the nodes was assessed by bootstrap proportion analysis computed from 1,000 replicates.

Tissue Expression of PaRPS24

Ovary, testis, head, thorax, leg and gut were dissected from P. americana. The mRNA expressions of RPS24 in different tissues were determined by quantitative RT-PCR. Total RNA was extracted as described above. The cDNA synthesis was performed with 500 ng total RNA in a 10 µL reverse transcription reaction system, using PrimeScript® RT reagent Kit (Takara) for the genes following the manufacturer's protocols. The RT-PCR reactions were performed with Rotor-Gene Q RT-PCR system. The SYBR Premix Ex Taq kit (Takara) was used according to the manufacturer's protocol. In each PCR reaction, the gene-specific primers of RPS24, 5′-TTTTCTGTTTTGGTTTCCGT-3′ and 5′-TGGTGGTTACTTCTTGCCTG-3′, were designed to amplify a product of 242 bp. The housekeeping gene, β-actin gene of P. americana was used as internal control for normalizing the expression level of PaRPS24. Two β-actin gene-specific primers, 5′-TTACCACCACTGCCGAACGA-3′ and 5′-CCTCTGGACAACGGAACCTC-3′, were designed to amplify a product of 180 bp. RT-PCR was performed in a total volume of 15 µL containing 7.5 µL of 2 × SYBR GREEN™ Realtime PCR mastermix, 1.5 µL of cDNA, 0.3 µL of each primer and 5.4 µL of double-distilled water. The reaction conditions were as follows: 95^0C for 30 s, followed by 40 cycles of 95^0C for 5 s and 50^0C for 30 s. The melt curve has to be checked to prove that the primers are amplifying the amplicon, so the values reflect the increase in amplicon, not primer dimers or other unrelated nucleotides. The calculated efficiency values for PaRPS24 and β-actin rRNA gene amplicons were always within the range of 95% to 100%; therefore, no correction for efficiency was used in further calculations. The relative expression values were calculated from 3 biological replicates using a modified $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Data from the quantitative RT-PCR were subjected to LSD test in analysis of variance (ANOVA) (SPSS 17.0 for Windows).

Results

Sequence Characterization of PaRPS24

Based on the 375 bp fragment obtained from our previous study, a 296 bp and a 204 bp fragment were amplified by 5′-RACE and 3′-RACE, respectively. These 3 fragments (i.e., 375 bp, 296 bp and 204 bp fragment) were assembled by overlapping to be a 572 bp nucleotide sequence representing the complete PaRPS24 cdna. The complete PaRPS24 cdna was further verified by end to end PCR. The PaRPS24 cdna consisted of an open reading frame (ORF) of 402 nucleotides encoding 133 amino acids, and included a 5′-UTR located 33 bp upstream of the start codon (ATG) and 3′-UTR of 137 nucleotides that ended in a poly (A) tail (Fig. 1). A polypyrimidine sequence, CTTTC, was at the 5′ noncoding sequence. A possible consensus polyadenylation signal, AATAAA, was at position 515-520, 17 bases upstream of the poly(A) site. The polyadenylation signal is required for post-translational cleavage-polyadenylation of the 3′ end of the pre-mRNA (Proudfoot and Brownlee, 1976). The calculated molecular mass of PaRPS24 was 15.32 kDa, and the estimated isoelectric point was 10.86. The PaRPS24 cDNA sequence has been submitted to GenBank (accession number: KJ650197).

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ttctttccctgcgctgaccgacgaggtggcacgATGAGTGAAGGAACAGCTACTATCAGAACTAGAAAGTTTATGACGAACCGGC
                                  M  S  E  G  T  A  T  I  R  T  R  K  F  M  T  N  R
TTTTGTGCCGGAAACAAATGGTTGTGGATGTTTTGCATCCGGGACAACCATCGGTTAAGAAGACTGAGATCAGAGAGAAACTAGC
L  L  C  R  K  Q  M  V  V  D  V  L  H  P  G  Q  P  S  V  K  K  T  E  I  R  E  K  L  A
AAAGATGTACAAAGTTACATCAGATTTAGTTTTCTGTTTTGGTTTCCGTACAGCTTTTGGTGGTGGAAAATCAACTGGGTTCGCC
  K  M  Y  K  V  T  S  D  L  V  F  C  F  G  F  R  T  A  F  G  G  G  K  S  T  G  F  A
CTAATATATGATACAATGGACTACGCAATGAAGTTTGAACCTAAACATAGGTTATCTAGGCACGGGCTCTTCGAGAAGCAAAAGG
 L  I  Y  D  T  M  D  Y  A  M  K  F  E  P  K  H  R  L  S  R  H  G  L  F  E  K  Q  K
CCACACGCAAGCAGCGTAAAGAACGTAAGAACAGAATGAAGAAGGTGCGTGGTACCAGAAAGTCGAAAGTAGGAGCAGCTTCAGG
A  T  R  K  Q  R  K  E  R  K  N  R  M  K  K  V  R  G  T  R  K  S  K  V  G  A  A  S  G
CAAGAAGTAAccaccatacttcaacatggagacaacaaaatcttaacatcttcggcgtcaaatgtattataatttgatatttacg
  K  K  *
tggcaataaagtcctcatatatttgtgaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
```

Fig1. *Nucleotide and deduced amino acid sequence of RPS24 cDNA from Periplaneta americana. The start codon is indicated with bold and the stop codon is indicated both with bold and an asterisk. The polypyrimidine sequence is boxed. The polyadenylation signal is underlined. Sequence analyses using ExPASy translation software.*

Table1. *Homology analysis of PaRPS24. Comparison of the deduced protein sequence of PaRPS24 the sequences in the GenBank database of other known RPS24 proteins from various insect species.*

Protein	Organism	Accession No.	Amino acids	Total score	Identity
HsRPS24	Harpegnathos saltator	EFN78087.1	183	227	89%
BlRPS24	Biphyllus lunatus	CAJ01864.1	132	226	89%
CfRPS24	Camponotus floridanus	EFN71100.1	137	226	88%
CgRPS24	Carabus granulatus	CAJ01865.1	132	224	89%
TcRPS24	Tribolium castaneum	XP_967577.1	132	224	88%
LlRPS24	Lygus lineolaris	ABQ18248	133	223	86%
SiRPS24	Solenopsis invicta	EFZ09298.1	131	223	88%
AeRPS24	Acromyrmex echinatior	EGI62641.1	156	223	88%
CtRPS24	Chrysomela tremula	ACY71267.1	132	221	85%
McRPS24	Meladema coriacea	CAJ01866.1	132	221	87%
AmRPS24	Apis mellifera	XP_006566005.1	134	218	84%
AdRPS24	Apis dorsata	XP_006619668.1	134	218	84%
SlRPS24	Scarabaeus laticollis	CAJ01868.1	136	218	84%
BmRPS24	Bombyx mori	NP_001093077.1	132	216	84%
HmRPS24	Heliconius melpomene	ABS57445.1	132	216	84%
BbRPS24	Biston betularia	ADO33052.1	132	214	84%
DmRPS24	Drosophila melanogaster	NP_611693.1	131	203	82%
AaRPS24	Aedes aegypti	XP_001648354.1	130	201	82%
ApRPS24	Acyrthosiphon pisum	XP_001943982.1	135	204	81%
PhcRPS24	Pediculus humanus corporis	XP_002432126.1	155	188	83%

PaRPS24 is a rather hydrophobic protein with 44 hydrophobic amino acids out of 133 residues. PaRPS24 lacks tryptophan and has a large excess of basic amino acids (21 lysine acids, 14 arginine acids and 3 histidine acids) mostly located in the amino-terminal half of the protein, and a low percentage of acidic amino acids (4 aspartic acids and 6 glutamic acids) mostly situated in the carboxy-terminal half. The strong basic character of S24 including PaRPS24 may be instrumental for its binding to rRNA in the 40S subunit of eukaryotic ribosomes (Ulbrich et al., 1979; Dudov and Perry, 1984; Wiedemann and Perry, 1984). A signal peptide scan using the

SignalP 4.1 program was performed, but no signal peptide was identified. And PaRPS24 had no potential N-linked glycosylation site detected by using protparam. Also the protein did not have any transmembrane domain predicted by the TMHMM program.

Homology Analysis of PaRPS24

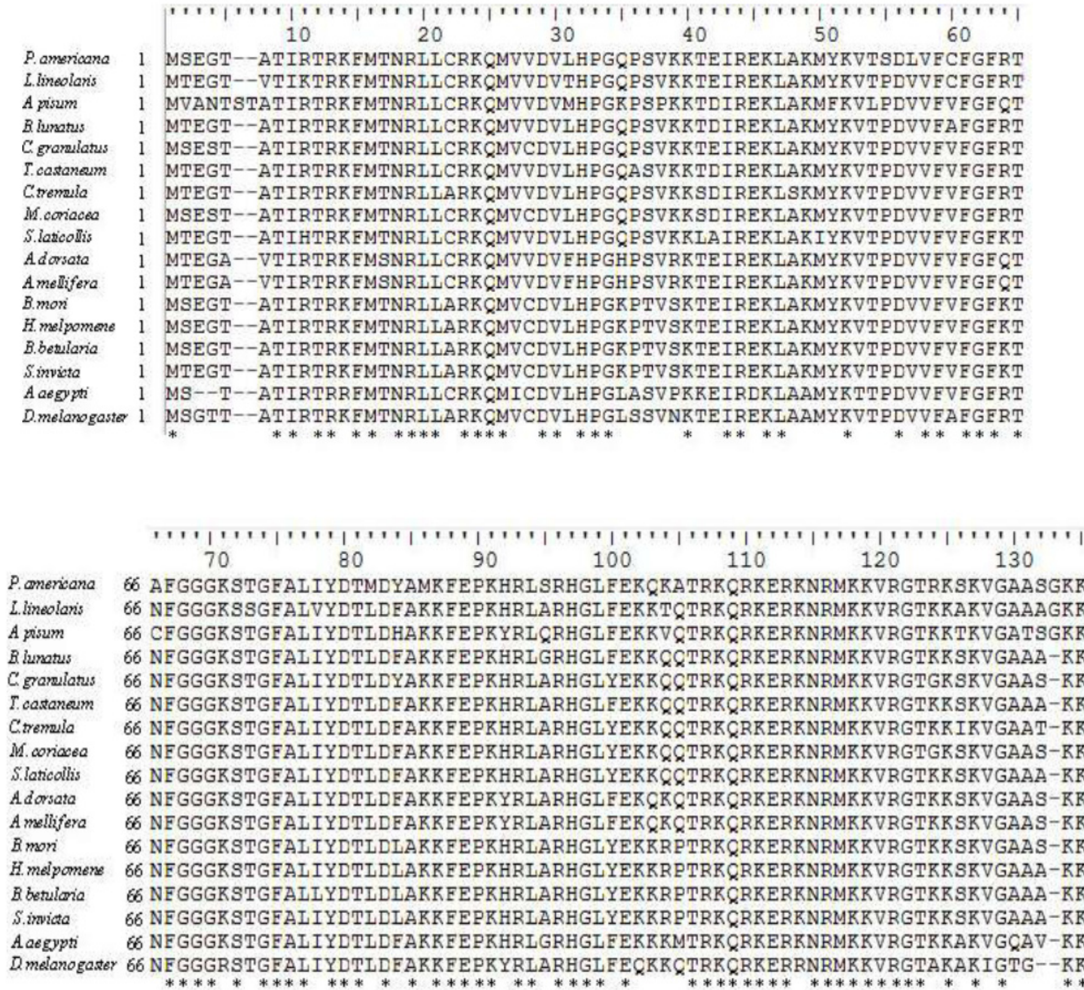

Fig.2. *Comparative analysis of the deduced amino acid sequence of PaRPS24 ORF with other known insects. All the other known insects are listed in Table 1. Sequences were aligned using ClustalX software.*

The deduced protein sequence of PaRPS24 was compared with those of the other known RPS24 proteins from various animals in the GenBank nr database (Table 1). The comparisons that yielded the closest identities were with insect RPS24. PaRPS24 is related to H. saltator (EFN78087.1) with a total score of 227 and in an alignment of the amino acid sequences with 108 identities in 122 possible matches (89% identity); to B. lunatus (CAJ01864.1) with a total score of 226 and in an alignment with 108 identities in 122 possible matches (89% identity); to C. floridanus (EFN71100.1) with a total score of 226 and in an alignment with 107 identities in 122 possible matches (88% identity); to C. granulatus (CAJ01865.1) with a total score is 224 and in an alignment with 108 identities in 121 possible matches (89% identity); to T. castaneum (XP_967577.1) with a total score of

224 and in an alignment with 107 identities in 122 possible matches (88% identity); to L. lineolaris (ABQ18248) with a total score of 223 and in an alignment with 105 identities in 122 possible matches (86% identity); to S. invicta (EFZ09298.1) with a total score of 223 and in an alignment with 106 identities in 121 possible matches (88% identity) , and so on (Table 1). The sequence alignment of PaRPS24 with known insect RPS24 amino acids were showed in Fig. 2. It was suggested that RPS24 is conserved among species.

Phylogenetic Tree

A phylogenetic tree using the neighbor-joining algorithm was inferred from the amino acid sequences of different RPS24 proteins using MEGA 6.0 program. The bootstrap values from 1,000 resamplings were given at each node and the branch lengths were drawn to scale (Fig. 3). Additionally, tree topologies assessed by the unweighted pair-group method with an arithmetic average (UPGMA), minimum evolution (ME) and maximum parsimony (MP) methods were substantially similar to the neighbor joining tree (data not shown). These observations suggested that the deduced amino acid sequence of RPS24 from P. americana was in the subgroup of Hemimetabola (Fig. 3). These findings suggest that the PaRPS24 has closer genetic relationships with the RPS24 of other Hemimetabola than with Holometabolous species as revealed by the difference of genetic distance.

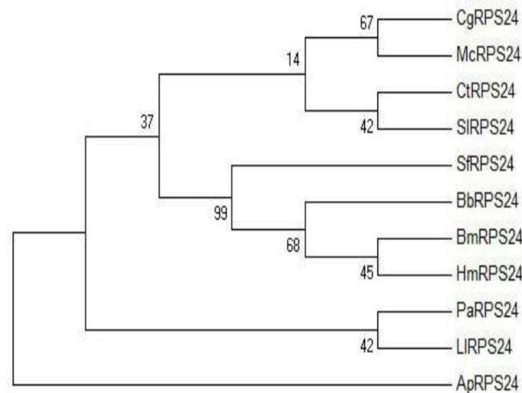

Fig3. *Evolutionary relationships of deduced amino acid sequence of PaRPS24 with other insects constructed using the neighbor-joining method. Bootstrap values with 1000 trials are indicated on branches. Phylogenetic trees were performed using MEGA 6.0 program.*

Tissue Expression of PaRPS24

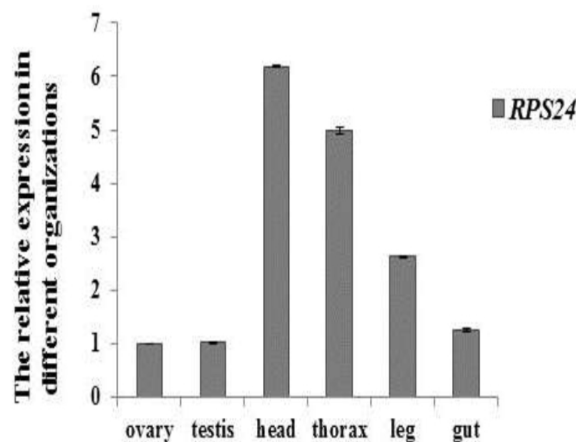

Fig4. *The relative expression levels of the PaRPS24 in different tagmata of adult Periplaneta americana (bar graph represents Mean ± SE; P < 0.05, LSD in ANOVA). As determined using quantitative RT-PCR.*

In order to examine whether PaRPS24 was ubiquitously expressed, quantitative RT-PCR was employed to investigate the distribution of RPS24 mRNA in different tissues (Fig. 4), The RPS24 transcript was expressed in all tissues, indicating that it has a role in various tissue development and insect life cycle. The lowest mRNA level was found in the ovary, and the relative expression levels of RPS24 were 1.01-, 6.19-, 4.99-, 2.62- and 1.24-fold higher in the testis, head, thorax, leg and gut than in the ovary, respectively.

Discussion

In the present study, a novel RPS24 gene was cloned from the Americana cockroach, P. americana. Before this study, no full-length cDNA of RPS24 has been isolated in P. americana, and our study is the first report on cloning of the full-length cDNA of cockroach RPS24. The full length of PaRPS24 was 572 bp, which consisted of an ORF of 402 bp, a 5′-UTR of 33 bp and a 3′-UTR of 137 bp. The ORF of PaRPS24 encoded a polypeptide of 133 amino acids with a calculated molecular weight of about 15.32 KD and an isoelectric point of 10.86. This result is similar to that of Cornish-Bowden (Cornish-Bowden, 1980) in which S23 and S24 had similar compositions with the same molecular weight of 16 KD. The difference index pI calculated for this pair is 10.4, which indicates a close relationship between the two proteins. A polypyrimidine sequence, CTTTC, was at the 5′ noncoding sequence. The 5′ end of most, if not all, eukaryotic ribosomal protein mRNAs has a pyrimidine sequence (Wool, 1996), which is presumed to have a role in the regulation of their translation (Levy et al., 1991).

Homology analysis revealed that the deduced amino acid sequence of PaRPS24 had more than 80% similarity with other insect RPS24 (89 % with H. Saltator, 89% with B. Lunatus, 89% with C. Granulatus, 88% with C. Floridanus, 88% with T. Castaneum, 88% with S. Invicta, and so on). It was suggested that RPS24 is conserved among species.

In animals, ribosomal proteins have been widely used for phylogenetic analysis as they are highly conserved and offer useful comparisons between distant lineages. However, because of lacking sequence information for P. americana, a phylogenetic analysis between this species and other insects based on the RPS24 protein sequences was never performed before. In this study, the results showed that the PaRPS24 has closer genetic relationship with other Hemimetabola (Hemiptera) RPS24 than with Holometabola (Lepidoptera, Hymenoptera and Coleoptera) species, as revealed by the differences in genetic distance.

Quantitative RT-PCR revealed that PaRPS24 was expressed in all the tested tissues, and the highest expression level occurred in the head and the lowest mRNA level was found in the ovary. The relative expression levels were 1.01-, 6.19-, 4.99-, 2.62- and 1.24-fold higher in the testis, head, thorax, leg and gut than in the ovary, respectively. The differential expression of PaRPS24 in various P. americana tissues showed that it is an important gene that could effectively be involved in a number of physiological activities, such as regulating protein synthesis (McMAHON and Landau, 1982) and functioning during retinoic acid induced neuronal differentiation (Bévort and Leffers, 2000). However, the significance of the relative high expression level of PaRPS24 in the head is unknown, need further investigation.

In summary, the determination of the sequence of amino acids in cockroach RPS24 is a contribution to a set of data which it is hoped to eventually include the structure of all the proteins in the ribosomes of this insect species. Although it may be unsufficient, the accumulation of these data are necessary for a solution of the structure of the organelle (Chan et al., 1990). Meanwhile, the information may also help to unravel the function of this protein, to understand the evolution of ribosomes, to define the rules that control the interaction of the proteins and rRNAs, and to reveal the amino acid sequences that direct the proteins to the nucleolus for assembly on nascent rRNA. Future work should focus on the understanding of the roles of RPS24 on regulating the organelles of P. americana.

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References cited

[1] BÉVORT, M., AND LEFFERS, H. 2000. Down regulation of ribosomal protein mRNAs during neuronal differentiation of human NTERA2 cells. Differentiation 66(2): 81-92.

[2] BOMMER, U.A., STAHL, J., HENSKE, A., LUTSCH, G. AND BIELKA, H. 1988. Identification of proteins of the 40S ribosomal subunit involved in interaction with initiation factor eIF-2 in the quaternary initiation complex by means of monospecific antibodies. FEBS Lett. 233(1): 114-118.

[3] Chan, Y.L., Paz, V., Olvera, J. and Wool, I.G. 1990. The primary structure of rat ribosomal protein S24. FEBS Lett. 262(2): 253-255.

[4] CHEN, W., JIANG, G. F., SUN, S. H., LU, Y., MA, F., AND LI, B. 2013. Identification of differentially expressed genes in American cockroach ovaries and testes by suppression subtractive hybridization and the prediction of its miRNAs. Mol. Genet. Genomics 288(11): 627-638.

[5] CORNISH-BOWDEN A. 1980. Critical values for testing the significance of amino acid composition indexes. Anal. Biochem. 105(1): 233-238.

[6] DUDOV, K. P., AND PERRY, R. P. 1984. The gene family encoding the mouse ribosomal protein L32 contains a uniquely expressed intron-containing gene and an unmutated processed gene. Cell 37(2): 457-468.

[7] GENG, Y. J., GAO, S. T., HUANG, D. N., ZHAO, Y. R., LIU, J. P., LI, X. H., AND ZHANG, R. L. 2009. Differentially expressed genes between female and male adult Anopheles anthropophagus. Parasitol. Res. 105(3): 843-851.

[8] HALL, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95-98.

[9] IRLES, P., BELLES, X., AND PIULACHS, M. D. 2009. Identifying genes related to choriogenesis in insect panoistic ovaries by Suppression Subtractive Hybridization. BMC Genomics 10: 206.

[10] KIM, H. L. 2003. Comparison of oligonucleotide-microarray and serial analysis of gene expression (SAGE) in transcript profiling analysis of megakaryocytes derived from CD34^+ cells. Exp. Mol. Med. 35(5): 460-466.

[11] LEVY, S., AVNI, D., HARIHARAN, N., PERRY, R. P., AND MEYUHAS, O. 1991. Oligopyrimidine tract at the 5' end of mammalian ribosomal protein mRNAs is required for their translational control. PNAS. 88(8): 3319-3323.

[12] LIVAK, K. J., AND SCHMITTGEN, T. D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25(4): 402-408.

[13] MAFUNE, K.I., WONG, J.M., STANIUNAS, R.J., LU, M.L., RAVIKUMAR, T., CHEN, L.B. AND STEELE, G.D. 1991. Ubiquitin hybrid protein gene expression during human colon cancer progression. Arch. Surg. 126(4): 462-466.

[14] MCMAHON, G., AND LANDAU, J. V. 1982. Effect of S12 ribosomal mutations on peptide chain elongation in Escherichia coli: a hydrostatic pressure study. J. Bacteriol. 151(1): 516-520.

[15] PAIN, V.M. 1996. Initiation of protein synthesis in eukaryotic cells. Eur. J. Biochem. 236(3): 747-771.

[16] PROUDFOOT, N., AND BROWNLEE, G. 1976. 3′ non-coding region sequences in eukaryotic messenger RNA. Nature 263: 211-214.

[17] SCHÄFFER, A. A., ARAVIND, L., MADDEN, T. L., SHAVIRIN, S., SPOUGE, J. L., WOLF, Y. I., KOONIN, E. V., AND ALTSCHUL, S. F. 2001. Improving the accuracy of PSI-BLAST protein database searches with composition-

based statistics and other refinements. Nucleic. Acids. Res. 29(14): 2994-3005.

[18] TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., AND KUMAR, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol. Biol. Evol. 30: 2725-2729.

[19] THOMPSON, J. D., GIBSON, T. J., PLEWNIAK, F., JEANMOUGIN, F., AND HIGGINS, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic. Acids. Res. 25(24): 4876-4882.

[20] TOLAN, D.R., HERSHEY, J.W. AND TRAUT, R.T. 1983. Crosslinking of eukaryotic initiation factor eIF3 to the 40S ribosomal subunit from rabbit reticulocytes. Biochimie 65(7): 427-436.

[21] ULBRICH, N., LIN, A., AND WOOL, I. G. 1979. Identification by affinity chromatography of the eukaryotic ribosomal proteins that bind to 5.8 S ribosomal ribonucleic acid. J. Biol. Chem. 254(17): 8641-8645.

[22] WIEDEMANN, L. M., AND PERRY, R. P. 1984. Characterization of the expressed gene and several processed pseudogenes for the mouse ribosomal protein L30 gene family. Mol. Cell Biol. 4(11): 2518-2528.

[23] WOOL, I. G. 1996. Extraribosomal functions of ribosomal proteins. Trends Biochem. Sci. 21(5): 164-165.

[24] XU, L., HE, G.P., LI, A. AND RO, H.S. 1994. Molecular characterization of the mouse ribosomal protein S24 multigene family: a uniquely expressed intron-containing gene with cell-specific expression of three alternatively spliced mRNAs. Nucleic Acids Res. 22(4): 646-655.

[25] YOON, C., KANG, S. H., YANG, J. O., NOH, D. J., INDIRAGANDHI, P., AND KIM, G. H. 2009. Repellent activity of citrus oils against the cockroaches Blattella germanica, Periplaneta americana and P. fuliginosa. J. Pestic. Sci. 34(2): 77-88.

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