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Sequence Characterization and Expression Pattern of the *Capra hircus* (Goat) RAB14, RB11b and SOD3 Genes

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Abstract: The complete CDS sequences of three goat genes-goat RAB14, RB11B and SOD3 were amplified using RT-PCR. Sequence analysis of these three genes revealed that the goat RAB14 gene encodes a protein of 215 amino acids and has high homology with the Ras-related protein Rab-14 (¨RAB14) of seven species-rat and pig (100%) human, mouse, chicken and orangutan (99%), dictyostelium discoideum (71%). The goat RB1B gene encodes a protein of 218 amino acids and has high homology with the Ras-related protein Rab-11B (RB11B) of five species-human, rat and bovine (99%), mouse (98%), electric ray (97%). The goat SOD3 has high homology with the superoxide dismutase 3, extracellular (SOD3) of four species-rabbit (74%), human (74%), rat (61%) and mouse (60%). The phylogenetic tree analysis revealed that the goat RAB14 gene has a closer genetic relationship with the RAB14 gene of rat and pig and the goat RB1B gene has a closer genetic relationship with the RB11B of human, rat and bovine. The goat SOD3 gene has a closer genetic relationship with the SOD3 of rabbit. The gene expression profile analysis indicated that the goat RAB14, RB11B and SOD3 genes were differentially expressed in tissues including ovary, pituitary, muscle, kidney, heart, lung, liver and spleen. The experiment established the primary foundation for further research on these three goat genes.

Key words: Goat, RAB14, RB11B, SOD3, gene expression profile, phylogenetic tree, China

INTRODUCTION

RAB14 is an important GTPases which are localized to biosynthetic compartments, including the rough ER, the Golgi complex and the trans-Golgi network and to endosomal compartments, including early endosomal vacuoles and associated vesicles.

RAB14 had been believed to function in both the biosynthetic and recycling pathways between the Golgi and endosomal compartments (Junutula *et al*., 2004; Proikas-Cezanne *et al*., 2006; Kyei *et al*., 2006). RB11B regulates the recycling pathways from endosomes to the plasma membrane and to the trans-Golgi network and is also thought to function in the histamine-induced fusion of tubulovesicles containing H+, K+ATPase with the plasma membrane in gastric parietal cells and in insulin-stimulated insertion of GLUT4 in the plasma membrane of cardiomyocytes (Duman *et al*., 1999; Gromov *et al*., 1998; Bhartur *et al*., 2000; Palmieri *et al*., 2006).

SOD3 encodes a member of the Superoxide Dismutase (SOD) protein family. SODs are antioxidant enzymes that catalyze the dismutation of two superoxide radicals into hydrogen peroxide and oxygen. The product of this gene is thought to protect the brain, lungs and other tissues from oxidative stress. The protein is secreted into the extracellular space and forms a glycosylated homotetramer that is anchored to the Extracellular Matrix (ECM) and cell surfaces through an interaction with heparan sulfate proteoglycan and collagen (Cheng *et al*., 2006; Serra *et al*., 2003; Di Massimo *et al*., 2006).

Based on above described about these three genes, it is necessary to isolate these three genes from goat for they are associated with health, caveolar trafficking, neutrophil respiratory burst, fusion of tubulovesicles and other important functions. These functions are potentially related with the goat production. But until today the goat RAB14, RB11B and SOD3 genes have not been reported yet.

In present experiment, we will isolate the coding sequences of goat goat RAB14, RB11B and SOD3 genes, subsequently perform some necessary sequence analysis and finally conduct the tissue expression analysis for these three genes. These will establish the primary foundation of understanding these three goat genes.

MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis: The tissue samples of ovary, pituitary, muscle, kidney, heart, lung, liver and spleen were derived

from one mature Yunling goat (A Yunnan local black goat breed). Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed as the methods describe by Liu *et al*. (2004).

Isolation of coding sequences for the goat RAB14, RB11B and SOD3 genes: The RT-PCR was performed to isolate these three goat genes using the pooled cDNAs from different tissues above. The 25 μL reaction system was: 2.0 μL cDNA, 2.5 μL 2 mM mixed dNTPs, 2.5 μL 10×Taq DNA polymerase buffer, 2.5 μL 25 mM $MgCl_2$, 2.0 μL 10 μM forward primer, 2.0 μL 10 μM reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 μL) and 9.5 μL sterile water.The primers for goat RAB14 gene isolation were designed based on the conserved CDS sequences information from rat and mouse RAB14 genes. Similarly, the primers for goat RB11B gene isolation were designed based on the conserved CDS sequences information from human, rat and mouse RB11B genes.

The primers for goat SOD3 gene isolation were designed based on the conserved CDS information from human and mouse SOD3 genes and the highly homologous goat EST sequences: EV444219, EV437585 and EV443277. These primer sequences and their annealing temperature for RT-PCR reaction were shown in Table 1.

Semi-quantitative RT-PCR: Semi-quantitative RT-PCR was performed as previously described elsewhere (Fehr *et al*., 2000; Daigo *et al*., 2006; Liu *et al*., 2005). About 25 μL volumes consisting of the following reagents: 100 ng cDNA; 10×PCR buffer (Sangon, Shanghai, China); 1.5 mM $MgCl_2$; 25 μM each of dATP, dCTP, dGTP and dTTP; 1.0 U Taq polymerase (Sangon, Shanghai, China) and 10 pmol of each primer (Table 1). Amplification was done using a MJ Research PTC100 thermocycler (Watertown, MA, USA) under the following conditions: 4 min at 94°C followed by 25 cycles of 45 sec at 94°C, 45 sec at annealing temperature (Table 1), 1 min at 72 °C and a final extension of 10 min at 72°C. Amplification of β-actin (Accession no: OAU39357) was performed as a positive control. About 7 μL PCR products were used to detect the expression pattern. Reaction products were electrophoresed through 1.5% agarose gels.

Sequence analysis: The cDNA sequence prediction was conducted using GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using the Conserved Domain Architecture Retrieval Tool of BLAST at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://www.ebi.ac.uk/ clustalw).

Table 1: Primers for goat RAB14, RB11B, SOD3 and β-actin genes and their annealing temperatures

Gene	Primer sequence	Tm/°C
RAB14	Forward: 5-ATGGCAACTACACCGTACAAC-3	58
	Reverse: 5-CTAGCAGCCACAGCC TTC-3	
RB11B	Forward: 5'-ATGGGCACCCGCGACGAC -3	54
	Reverse: 5'-TTAGATGTTCTG ACAGCACTG -3	
SOD3	Forward: 5'-ATGCCGGCGCTGCTCTGT -3'	54
	Reverse: 5'-TCAGACGTCCTTACACTC -3'	
β-actin	Forward: 5'-CTTGATGTCACGGACGATTT -3'	56
	Reverse: 5'-CACGGCATTGTCACCAACT -3'	

RESULTS AND DISCUSSION

RT-PCR results for goat RAB14, RB11B and SOD3 gene: Through RT-PCR with pooled tissue cDNAs from ovary, pituitary, muscle, kidney, heart, lung, liver and spleen, for goat RAB14, RB11B and SOD3 gene, the resulting PCR products were 648, 657 and 735 bp (Fig. 1).

Sequence analysis: The cDNA nucleotide sequence analysis for these sequenced PCR products using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that these genes were not homologous to any of the known goat genes and they were then deposited into the GenBank database (Accession number: EU244431, EU244432, EU559622). The sequence prediction was carried out using the GenScan software and results showed that these 648, 657 and 735 bp cDNA sequences represented three single genes which encoded 215, 218 and 244 amino acids, respectively. The complete coding sequences of these genes and the encoded amino acids were shown in Fig. 2-4.

Further BLAST analysis of these proteins revealed that goat RAB14 has high homology with the Ras-related protein Rab-14 (RAB14) of seven species-rat and pig (100%), human, mouse, chicken and orangutan (99%), dictyostelium discoideum (71%). The goat RB1B has high homology with the Ras-related protein Rab-11B (RB11B) of five species-human, rat and bovine (99%), mouse (98%), electric ray (97%).

The goat SOD3 has high homology with the superoxide dismutase 3, extracellular (SOD3) of four species-rabbit (74%), human (74%), rat (61%) and mouse (60%) (Fig. 5-7). Based on the results of the alignment analyses of goat RAB14, RB11B and SOD3 genes, the phylogenetic trees were constructed using the ClustalW software (http://www.ebi.ac.uk/clustalw), as shown in (Fig. 8).

The phylogenetic tree analysis revealed that the goat RAB14 has a closer genetic relationship with the RAB14 gene of rat and pig and the goat RB11B has closer genetic relationships with the RB11B of human, rat and bovine. The goat SOD3 has closer genetic relationships with the SOD3 of rabbit.

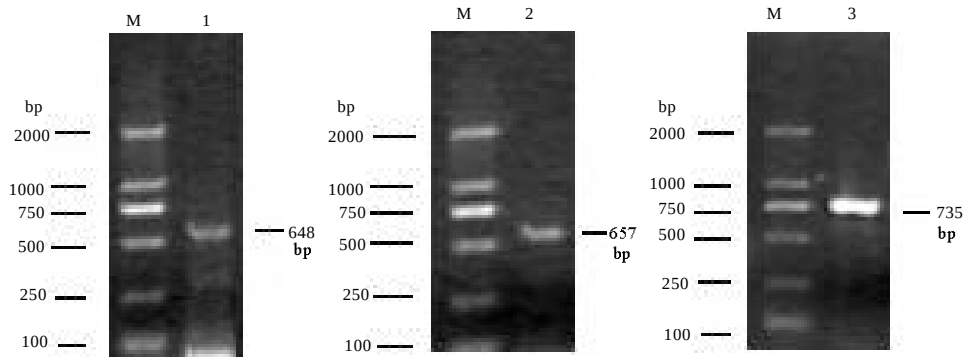

Fig. 1: RT-PCR results for goat RAB14, RB11B and SOD3 gene. M, DL2000 DNA markers; 1, PCR product for goat RAB14 gene ; 2, PCR product for goat RB11B gene; 3, PCR product for goat SOD3 gene

```
ATGGGCACCCGCGACGACGAGTACGACTACCTATTCAAAGTGGTGCTCATCGGGGAC
 M  G  T  R  D  D  E  Y  D  Y  L  F  K  V  V  L  I  G  D
TCGGGCGTGGGGAAGAGCAACCTGCTGTCCCGCTTCACCCGCAACGAGTTCAACCTG
 S  G  V  G  K  S  N  L  L  S  R  F  T  R  N  E  F  N  L
GAGAGCAAGAGCACCATTGGTGTGGAATTTGCCACCCGCAGCATCCAGGTGGACGGC
 E  S  K  S  T  I  G  V  E  F  A  T  R  S  I  Q  V  D  G
AAGACCATCAAGGCGCAAATCTGGGACACTGCTGGCCAGGAGCGCTACCGCGCCATC
 K  T  I  K  A  Q  I  W  D  T  A  G  Q  E  R  Y  R  A  I
ACCTCGGCGTACTACCGTGGCGCAGTGGGCGCCCTGCTGGTATATGACATTGCCAAG
 T  S  A  Y  Y  R  G  A  V  G  A  L  L  V  Y  D  I  A  K
CACCTGACGTATGAGAACGTGGAGCGCTGGCTGAAGGAGCTTCGGGACCATGCTGAC
 H  L  T  Y  E  N  V  E  R  W  L  K  E  L  R  D  H  A  D
AGCAACATCGTCATCATGCTGGTGGGCAACAAGAGCGACCTGCGCCACCTGCGAGCT
 S  N  I  V  I  M  L  V  G  N  K  S  D  L  R  H  L  R  A
GTGCCCACGGACGAGGCCCGCGCCTTCGCAGAAAAGAACAACTTGTCCTTCATTGAG
 V  P  T  D  E  A  R  A  F  A  E  K  N  N  L  S  F  I  E
ACCTCAGCCCTGGATTCCACCAACGTGGAGGAAGCGTTTAAGAACATCCTCACAGAG
 T  S  A  L  D  S  T  N  V  E  E  A  F  K  N  I  L  T  E
ATCTATCGCATCGTGTCACAGAAGCAGATTGCGGACCGCGCAGCACACGACGAGTCC
 I  Y  R  I  V  S  Q  K  Q  I  A  D  R  A  A  H  D  E  S
CCCGGAAACAACGTTGTGGACATCAGCGTGCCGCCCACCACCGACGGACAGAAACCC
 P  G  N  N  V  V  D  I  S  V  P  P  T  T  D  G  Q  K  P
AACAAGCTGCAGTGCTGTCAGAACATCTAA
 N  K  L  Q  C  C  Q  N  I  □
```

Fig. 2: The complete CDS of goat RAB14 gene and its encoding amino acids *indicates the stop codon

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that goat RAB14 gene was moderately expressed in ovary, muscle, heart, lung and liver, hardly expressed in pituitary, kidney and spleen.

The goat RB1B gene was highly expressed in kidney, heart, lung and hardly expressed in ovary, pituitary, muscle, liver, spleen. The goat SOD3 gene was highly expressed in ovary, pituitary, muscle and spleen, hardly expressed in kidney, heart, lung and liver (Fig. 9). Comparative genomics is the analysis and comparison of genomes from different species. Researchers have learned a great deal about the function of human genes by examining their counterparts in simpler model organisms such as the mouse and some results has revealed that virtually all (99%) of the protein-coding genes in humans align with homologs in mouse and over 80% are clear 1:1 orthologs (Hardison *et al*., 2003). This extensive conservation in protein-coding regions implied that this

```
ATGGCAACTACACCGTACAACTACTCCTACATCTTTAAGTACATCATCATCGGGGAC
 M  A  T  T  P  Y  N  Y  S  Y  I  F  K  Y  I  I  I  G  D
ATGGGAGTGGGGAAGTCCTGCCTGCTTCACCAGTTCACTGAGAAGAAGTTTATGGCT
 M  G  V  G  K  S  C  L  L  H  Q  F  T  E  K  K  F  M  A
GACTGTCCTCACACAATTGGTGTTGAATTTGGTACAAGAATAATTGAAGTTAGTGGC
 D  C  P  H  T  I  G  V  E  F  G  T  R  I  I  E  V  S  G
CAAAAAATCAAATTGCAGATCTGGGATACAGCAGGACAGGAGAGGTTCAGAGCTGTC
 Q  K  I  K  L  Q  I  W  D  T  A  G  Q  E  R  F  R  A  V
ACACGAAGCTACTACAGAGGAGCCGCGGGAGCGCTGATGGTGTATGACATCACTAGG
 T  R  S  Y  Y  R  G  A  A  G  A  L  M  V  Y  D  I  T  R
AGAAGCACATATAATCACCTGAGCAGCTGGCTGACGGACGCCAGGAACCTCACCAAC
 R  S  T  Y  N  H  L  S  S  W  L  T  D  A  R  N  L  T  N
CCAAACACTGTGATAATCCTCATAGGGAATAAAGCAGATCTGGAGGCTCAGAGGGAT
 P  N  T  V  I  I  L  I  G  N  K  A  D  L  E  A  Q  R  D
GTGACGTATGAGGAAGCCAAACAGTTTGCTGAAGAAAATGGTTTATTGTTCCTTGAA
 V  T  Y  E  E  A  K  Q  F  A  E  E  N  G  L  L  F  L  E
GCAAGTGCAAAAACGGGAGAGAACGTAGAAGATGCTTTCCTTGAGGCTGCCAAGAAG
 A  S  A  K  T  G  E  N  V  E  D  A  F  L  E  A  A  K  K
ATCTATCAGAACATTCAGGATGGAAGCTTGGATCTGAACGCTGCCGAGTCTGGTGTA
 I  Y  Q  N  I  Q  D  G  S  L  D  L  N  A  A  E  S  G  V
CAGCACAAACCTTCAGCCCCACAGGGGGGCCGGCTAACCAGCGAGCCCCAGCCCCAG
 Q  H  K  P  S  A  P  Q  G  G  R  L  T  S  E  P  Q  P  Q
AGGGAAGGCTGTGGCTGCTAG
 R  E  G  C  G  C  □
```

Fig. 3: The complete CDS of goat RB11B gene and its encoding amino acids *indicates the stop codon

```
ATGCCGGCGCTGCTCTGTGCCTCTCTGCTCCTGGTGGCCTGTGCCTCGGCCGCCTCG
 M  P  A  L  L  C  A  S  L  L  L  V  A  C  A  S  A  A  S
GCCGACCAGGTCCAGCAGCAGATGGGCTCCAACACGGAGGAGCAGATCCGCGACATG
 A  D  Q  V  Q  Q  Q  M  G  S  N  T  E  E  Q  I  R  D  M
CACGCCAAGGTGACGGAGATCTGGCAGGAGATGATGCAGCGGCAGGCGGCGGCCATC
 H  A  K  V  T  E  I  W  Q  E  M  M  Q  R  Q  A  A  A  I
GACCCGGACGCGGCGCTCCATGCGGTCTGCCGGGTGCTGCCGTCGGCCACGCTGGAG
 D  P  D  A  A  L  H  A  V  C  R  V  L  P  S  A  T  L  E
GCGGAGCAGCCCCGGGTCAGCGGCCTCGTGCTCTTCCGGCAGCTCCGGCCTGGCGCC
 A  E  Q  P  R  V  S  G  L  V  L  F  R  Q  L  R  P  G  A
CTGCTGGAGGCCTTCTTCCACCTTGAGGGCTTCCCGAACGAGCCCAACGGCACAAGC
 L  L  E  A  F  F  H  L  E  G  F  P  N  E  P  N  G  T  S
CGCGCCATCCACGTGCACCAGTTTGGGGACCTGAGCCAGGGCTGCGACTCCACCGGG
 R  A  I  H  V  H  Q  F  G  D  L  S  Q  G  C  D  S  T  G
CCGCACTACAACCCGATGTCCGTGCTGCACCCGCAGCACCCGGGCGACTTTGGCAAC
 P  H  Y  N  P  M  S  V  L  H  P  Q  H  P  G  D  F  G  N
TTCGCGGTGCGCGATGGCCAGGTCTGGAAGTACCGCTCCAACCTGGCTGCCTCGCTC
 F  A  V  R  D  G  Q  V  W  K  Y  R  S  N  L  A  A  S  L
ACCGGCCCGCACTCGATCGCGGGCCGTGCTGTGGTGGTCCACGCGGGCGAGGACGAC
 T  G  P  H  S  I  A  G  R  A  V  V  V  H  A  G  E  D  D
CTGGGCCGCGGCGGCAATCAGGCCAGTCTGGAGAACGGTAACGCGGGACGCCGGCTT

 L  X  R  G  G  N  Q  A  S  L  E  N  G  N  A  G  R  R  L
GCCTGCTGTGTGGTGGGTCTGTGCGGCCCCGGGCCCTGGGCGCACCAGGCGCAGGAG
 A  C  C  V  V  G  L  C  G  P  G  P  W  A  H  Q  A  Q  E
AACGCGGAGCGCAAGAAGCGACGGCGCGAGAGCGAGTGTAAGGACGTCTGA
 N  A  E  R  K  K  R  R  R  E  S  E  C  K  D  V
```

Fig. 4: The complete CDS of goat SOD3 gene and its encoding amino acids *indicates the stop codon

conservation of protein-coding sequences may be expected in different mammals such as including goat, dogs, cats, rabbits, monkeys and apes. This provides us a useful method to isolate the functional regions of

```
Goat                              MATTPYNYSYIFKYIIIGDMGVGKSCLLHQFTEKKFMADCPHTIGVEFGT
Rat_Pig                           MATTPYNYSYIFKYIIIGDMGVGKSCLLHQFTEKKFMADCPHTIGVEFGT
Human_Mouse_Chicken_Orangutan     MATAPYNYSYIFKYIIIGDMGVGKSCLLHQFTEKKFMADCPHTIGVEFGT
Dictyostelium discoideum          ---MSFPYEYIFKYIIIGDMGVGKSCLLHQFTENKFVPDSPHTIGVEFGT
                                     .: *.************************:**:.*.**********

Goat                              RIIEVSGQKIKLQIWDTAGQERFRAVTRSYYRGAAGALMVYDITRRSTYN
Rat_Pig                           RIIEVSGQKIKLQIWDTAGQERFRAVTRSYYRGAAGALMVYDITRRSTYN
Human_Mouse_Chicken_Orangutan     RIIEVSGQKIKLQIWDTAGQERFRAVTRSYYRGAAGALMVYDITRRSTYN
Dictyostelium discoideum          RIVDVNNKKIKLQIWDTAGQERFRAVTRSYYRGAAGALLVYDITRRITYN
                                  **::*..:*********************************:******* ***

Goat                              HLSSWLTDARNLTNPNTVIILIGNKADLEAQRDVTYEEAKQFAEENGLLF
Rat_Pig                           HLSSWLTDARNLTNPNTVIILIGNKADLEAQRDVTYEEAKQFAEENGLLF
Human_Mouse_Chicken_Orangutan     HLSSWLTDARNLTNPNTVIILIGNKADLEAQRDVTYEEAKQFAEENGLLF
Dictyostelium discoideum          HLTTWLTDARNLTNPNTVIMLIGNKKDLEGQRDVTYEEASAFAKQNGLIF
                                  **::****************:***** ***.*********. **::***:*

Goat                              LEASAKTGENVEDAFLEAAKKIYQNIQDGSLDLNAAESGVQHKPSAPQGG
Rat_Pig                           LEASAKTGENVEDAFLEAAKKIYQNIQDGSLDLNAAESGVQHKPSAPQGG
Human_Mouse_Chicken_Orangutan     LEASAKTGENVEDAFLEAAKKIYQNIQDGSLDLNAAESGVQHKPSAPQGG
Dictyostelium discoideum          VESSAKTGENVEEAFLRTAKLIFQSVQEGNVDLIPDGGITKNPP------
                                  :*:*********:***.:** *:*.:*:*.:** .  . .:: *

Goat                              RLTSEPQPQREGCGC
Rat_Pig                           RLTSEPQPQREGCGC
Human_Mouse_Chicken_Orangutan     RLTSEPQPQREGCGC
Dictyostelium discoideum          QTITDKPQDASKCSC
                                  :  ::   : . *.*
```

Fig. 5: The alignment of the protein encoded by goat RAB14 with the selected RAB14 proteins from other species

```
Human_Rat_Bovine      MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFATRSIQVDGKTI
Mouse                 MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFATRSIQVDGKTI
Goat                  MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFATRSIQVDGKTI
Electric ray          MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFATRSIQVDGKTI
                      ************************************************************

Human_Rat_Bovine      KAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYENVERWLKELRDHADSNIVIM
Mouse                 KAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYENVERWLKELRDHADSNIVIM
Goat                  KAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYENVERWLKELRDHADSNIVIM
Electric ray          KAQIWDTAGQERYRAITSAYYRGAVGALLVYDIAKHLTYENVERWLKELRDHADNNIVIM
                      ********************************************************.*****

Human_Rat_Bovine      LVGNKSDLRHLRAVPTDEARAFAEKNNLSFIETSALDSTNVEEAFKNILTEIYRIVSQKQ
Mouse                 LVGNKSDLRHLRAVPTDEARAFAEKNNLSFIETSALDSTNVEEAFKNILTEIYRIVSQKQ
Goat                  LVGNKSDLRHLRAVPTDEARAFAEKNNLSFIETSALDSTNVEEAFKNILTEIYRIVSQKQ
Electric ray          LVGNKSDLRHLRAVPTDEARAFAEKNNLSFIETSALDSTNVEEAFKNILTEIYRIVSQKQ
                      ************************************************************

Human_Rat_Bovine      IADRAAHDESPGNNVVDISVPPTTDGQKPNKLQCCQNL
Mouse                 IADRAAHDESPGNNVVDISVPPTTDGQRPNKLQCCQSL
Goat                  IADRAAHDESPGNNVVDISVPPTTDGQKPNKLQCCQNI
Electric ray          ISDRSAHDESPGNNVVDISVPPTTDGQKSNKLQCCQNM
                      *:**:**********************:.*******.:
```

Fig. 6: The alignment of the protein encoded by goat RB11B gene with the selected RB11B proteins from other species

different genes for goats based on the conserved sequence information of the mouse-human or other mammals and predict what those functions are. In this experiment, the complete coding sequences of the goat RAB14, RB11B and SOD3 genes were isolated based on the conserved coding sequence information of the goat RAB14, RB11B and SOD3 genes from mouse and other mammals.

Sequence identification further validated that comparative genomics method is one useful tool to isolate the unknown genes especially the conserved coding region of genes for goat or other mammals.

From the results we can see that goat goat RAB14, RB11B and SOD3 genes are highly homologous with goat RAB14, RB11B and SOD3 genes of human, mouse or other mammals. This implied goat RAB14, RB11B and SOD3 will have similar functions as goat RAB14, RB11B and SOD3 of human, mouse or other mammals. We also find goat RAB14, RB11B and SOD3 do not show complete identity to RAB14, RB11B and SOD3 of some mammals.

This implied that goat RAB14, RB11B and SOD3 will have some differences in functions. This is deserved to study further. In the experiment, we not only isolated the complete coding sequence of goat RAB14, RB11B and SOD3 genes but also performed the sequence analysis and tissue expression profile analysis.

From the tissue expression analysis it can be seen that these genes were obviously differentially expressed in different tissues. The suitable explanation for this is

```
Goat        MPALLCASLLLVACASAASAD-------------QVQQQMGSNTEEQIRDMHAKVTEIWQ
Rabbit      MLALVCSCLLLAALPADTWSG-------------PAAVELGSDTVEQIRDTHAKVTEIWQ
Human       MLALLCSCLLLAAGASDAWTG-------------EDSAEPNSDSAEWIRDMYAKVTEIWQ
Rat         MVAFLFCNLLLVACGSVTWTMSDTG--------ESGVDLADRLDLVEKIGDTHSKDLEIWM
Mouse       MLAFLFYGLLLAACGSVTMSNPGESSFDLADRLDPVEKIDRLDLVEKIGDTHAKVLEIWM
            * *::    ***.*  : : :                          :  * * * ::*  ***

Goat        EMMQRQAAAIDPDAALHAVCRVLPSATLEAEQPRVSGLVLFRQLRPGALLEAFFHLEGFP
Rabbit      ALTQQRAAQGEPAGALHAVCRVQPSATLDAAQPRVSGLVVFRQLGPGAQLEAFFDLEGFP
Human       EVMQRRDD----DGALHAACQVQPSATLDAAQPRVTGVVLFRQLAPRAKLDAFFALEGFP
Rat         ELGKQREAD---AREMHAVCRVQPSAMLPPDQPQITGLVLFRQLGPSSRLEASFNLEGFP
Mouse       ELGRRREVD---AAEMHAICRVQPSATLPPDQPQITGLVLFRQLGPGSRLEAYFSLEGFP
             : :::          :** *:* *** * . **::::*:*:**** * :  *:* * *****

Goat        NEPNGTSRAIHVHQFGDLSQGCDSTGPHYNPMSVLHPQHPGDFGNFAVRDGQVWKYRSNL
Rabbit      VEANLSSRAIHVHQFGDLSQGCDSTGAHYNPLAVQHPQHPGDFGNFAVRDGRLWKYRSGL
Human       TEPNSSSRAIHVHQFGDLSQGCESTGPHYNPLAVPHPQHPGDFGNFAVRDGSLWRYRAGL
Rat         AEQNTSNHAIHVHEFGDLSQGCESTGPHYNPLGVPHPQHPGDFGNFVVRDGRLWKHRMGL
Mouse       AEQNASNRAIHVHEFGDLSQGCDSTGPHYNPMEVPHPQHPGDFGNFVVRNGQLWRHRVGL
             * * :.:*****:*********:***.****: * ***********.**:* :*:** .*

Goat        AASLTGPHSIAGRAVVVHAGEDDLR-GGNQASLENGNAGRRLACCVVGLCGPGPWAHQAQ
Rabbit      AASLAGPHSIVGRAVVVHAGEDDLGRGGNAASVENGNAGPRLACCVVGASGPAPWARQAQ
Human       AASLAGPHSIVGRAVVVHAGEDDLGRGGNQASVENGNAGRRLACCVVGVCGPGLWERQAR
Rat         ATSLAGPHSILGRAVVVHAGEDDLGKGGNQASVQNGNAGRRLACCVVGTSNSEAWESQTK
Mouse       TASLAGPHAILGRSVVVHAGEDDLGKGGNQASLQNGNAGRRLACCVVGTSSSAAWESQTK
            ::**:***:* **:**********  *** **::***** ******** ...  *  *::

Goat        ENAERKKRRRESECKDV
Rabbit      EHAERKKRRRESECKAA
Human       EHSERKKRRRESECKAA
Rat         E---RKKRRRESECKTT
Mouse       E---RKKRRRESECKTT
            *   *********** .
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Fig. 7: The alignment of the protein encoded by goat SOD3 gene with the selected SOD3 proteins from other species

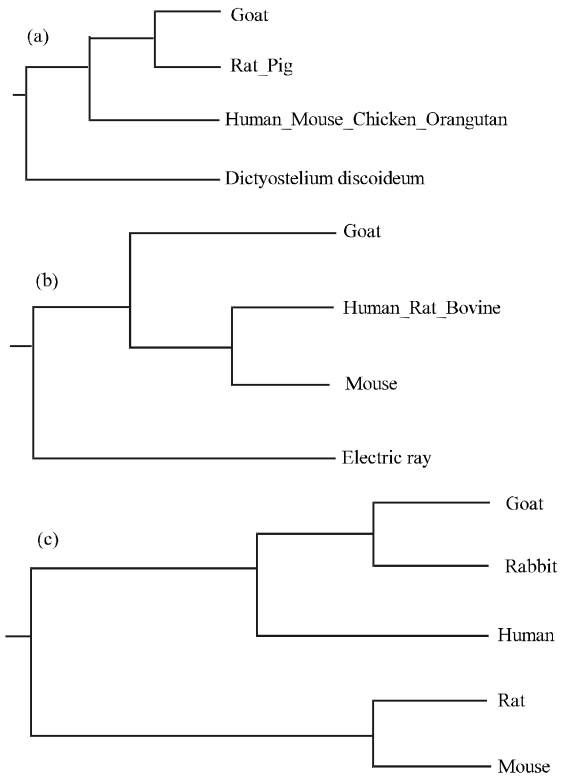

Fig. 8: The phylogenetic trees for selected goat RAB14, RB11B and SOD3 proteins. (a) phylogenetic tree analysis for selected RAB14 proteins; (b) phylogenetic tree analysis for selected RB11B proteins; (c) phylogenetic tree analysis for selected SOD3 proteins

that at the same time the biological activities of these three genes were presented diversely in different tissues.

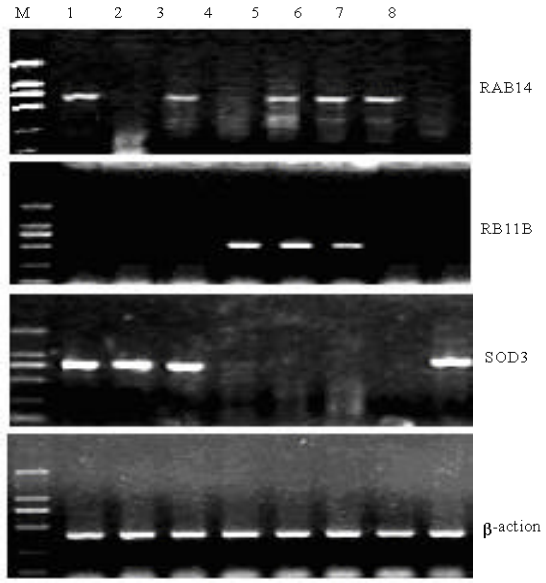

Fig. 9: Tissue expression distribution of the goat RAB14, RB11B and SOD3 gene. M, DL2000 markers; 1, ovary; 2, pituitary; 3, muscle; 4, kidney; 5, heart; 6, lung; 7, liver; 8, spleen

CONCLUSION

In this study, we first isolated encoding regions of the goat RAB14, RB11B and SOD3 genes, performed necessary sequence analysis and tissue expression profile analysis for these three goat genes. This established theprimary foundation for further research on these goat genes.

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