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Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes-*RAB1A*, *RAB4A* and *RAB5A*

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Abstract: The complete coding sequences of three sheep genes-*RAB1A*, *RAB4A* and *RAB5A* were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Sequence analysis revealed that the sheep *RAB1*A gene encodes a protein of 204 amino acids that shares high homology with the RAB1A, member RAS oncogene family (RAB1A) proteins of nine species-human (98%), mouse (98%), rat (98%), pig (98%), zebrafish (98%), Western clawed frog (97%), Atlantic salmon (96%), disc abalone (92%) and great pond snail (87%). The sheep *RAB4A* gene encodes a protein of 218 amino acids that shares high homology with the RAB4A, member RAS oncogene family (RAB4A) proteins of eight species-cattle (100%), dog (99%), human (99%), rhesus monkey (99%), horse (99%), chicken (99%), rat (98%) and mouse (98%). The sheep *RAB5A* gene encodes a protein of 215 amino acids that shares high homology with the RAB5A, member RAS oncogene family (RAB5A) proteins of fifteen species-cattle (98%), rabbit (98%), dog (98%), horse (98%), pig (98%), sumatran orangutan (98%), human (97%), mouse (97%), rat (97%), chimpanzee (97%), chicken (95%), rhesus monkey (93%), African clawed frog (93%), Western clawed frog (93%) and zebrafish (92%). Finally, these three novel sheep genes were assigned to GeneIDs: 100302065, 100302066 and 100302084. Phylogenetic analysis indicated that the sheep *RAB1A* gene has a closer genetic relationship with the *RAB1A* genes of human, mouse and rat. The sheep *RAB4A* and *RAB5A* genes both have closer genetic relationships with the *RAB4A* and *RAB5A* genes of cattle. Tissue expression profile analysis was also carried out and results demonstrated that sheep *RAB1A*, *RAB4A* and *RAB5A* genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, RAB1A, RAB4A, RAB5A, tissue expression, China

INTRODUCTION

RAB1A, member RAS oncogene family (RAB1A) is a member of Rab1 subfamily. Rab1 is found in eukaryote and is an important regulatory factor for the transport of vesicles from the ER to the Golgi apparatus. Latest researches suggested a novel function for Rab1a in the regulation of cell migration through controlling integrin beta1 recycling and localization to lipid rafts via a specific downstream effector pathway. Researches also revealed that the Rab1a plays a crucial role in mammalian autophagy (Huang *et al*., 2011; Wang *et al*., 2010; Diao *et al*., 2008).

RAB4A, member RAS oncogene family (RAB4A) is a member of Rab4 subfamily. Rab4 has been implicated in numerous functions within the cell. Experimental data revealed that overexpression of Rab4 regulates angiotensin II type I receptor phosphorylation and sensitization. Rab4A had also been identified to be a critical effector of VEGFR1 during branching morphogenesis of the vasculature (Esseltine *et al*., 2011; Schonhoff *et al*., 2009; Kachhap *et al*., 2007).

RAB5A, member RAS oncogene family (RAB5A) is a member of Rab5-related subfamily. This subfamily includes Rab5 and Rab22 of mammals, Ypt51/Ypt52/Ypt53 of yeast and RabF of plants. Recent researches showed that selective upregulation of Rab5 level is associated with mild cognitive impairment, Alzheimer's disease and sporadic motor neuron disease. Rab5a can promote proliferation of ovarian cancer cells (Ginsberg *et al*., 2010; Zhao *et al*., 2010; Matej *et al*., 2010).

As mentioned before, *RAB1A*, *RAB4A* and *RAB5A* genes are three genes which have important functions. Until today, *RAB1A*, *RAB4A* and *RAB5A* genes had

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been reported in human and other animals but the sheep *RAB1A*, *RAB4A* and *RAB5A* genes have not been reported yet.

In present experiment, the researchers will isolate the coding sequences of sheep *RAB1A*, *RAB4A* and *RAB5A* genes based on the coding sequence information of *RAB1A*, *RAB4A* and *RAB5A* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform some necessary sequence analysis and tissue expression profile analysis for these genes. These will establish the primary foundation of understanding these three sheep genes.

MATERIALS AND METHODS

Animals and sample preparation: Five adult Yunnan local sheep were slaughtered. Spleen, skin, lung, fat, muscle, heart, liver, kidney and ovary samples were collected, frozen in liquid nitrogen and then stored at -80°C. The total RNA was extracted using the total RNA extraction kit (Gibco, USA). First-strand cDNA synthesis was performed as that described by Liu *et al*. (2004). These first-strand cDNA samples were used to perform RT-PCR for the isolation of sheep *RAB1A*, *RAB4A* and *RAB5A* genes and for the tissue expression profile analysis.

Isolation of the sheep *RAB1A*, *RAB4A* and *RAB5A* genes: The primers for sheep *RAB1A* gene isolation were designed based on the coding sequence information of human *RAB1A* gene and its highly homologous sheep EST sequences: EE751788 and EE747095. Similarly, the primers for sheep *RAB4A* gene isolation were designed based on the coding sequence information from human *RAB4A* gene and its highly homologous sheep EST sequence: EE794089. The primers for sheep *RAB5A* gene isolation were designed based on the coding sequence information from human and mouse *RAB5A* genes and their highly homologous sheep EST sequences: EE806405 and EE791858. These primer sequences and their annealing temperature for RT-PCR reaction were shown in Table 1. The RT-PCR was performed to isolate these three sheep 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 PCR program initially started with a 94°C denaturation for 4 min followed by 35 cycles of 94°C/50 sec, Ta°C/50 sec, 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction.

Table 1: Primers for sheep *RAB1A*, *RAB4A*, *RAB5A* and *Beta-actin* genes and their annealing temperatures

Genes	Primer sequence	Ta/°C
RAB1A	Forward 5'-ATGTCCAGCATGAATCCC-3'	
	Reverse:5'-TTAGCAGCACCTCCACTT-3'	56
RAB4A	Forward :5'-ATGTCGCAGACGGCCATGT-3	
	Reverse: 5'-TCAGCAGCCGCACTCCTG-3	62
RAB5A	Forward5'-ATGGCTAATCGAGGAGCAG-3'	
	Reverse:5'-TCAGTTGCTGCAGCACTG-3'	57
Beta-actin	Forward: 5'-CTTGATGTCACGGACGATTT -3'	
	Reverse: 5'-CACGGCATTGTCACCAACT-3'	56

These PCR products for sheep *RAB1A*, *RAB4A* and *RAB5A* genes were then cloned into PMD18-T vector and sequenced bidirectionally with the commercial fluorometric method. At least five independent clones were sequenced for every gene.

RT-PCR for tissue expression profile analysis: RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Gao, 2009; Yonggang and Shizheng, 2009; Liu, 2009). We selected the housekeeping gene *beta-actin* (Accession No.: NM_001009784) as a positive control. The primers of sheep *RAB1A*, *RAB4A* and *RAB5A* genes which were used to perform the RT-PCR for tissue expression profile analysis were same as the primers for isolation RT-PCR above. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. The 25 μL reaction system was 1 μL cDNA (100 ng μL^{-1}), 5 pmoles each oligonucleotide primer, 2.5 μL 2 mmol L^{-1} mixed dNTPs, 2.5 μL 10×Taq DNA polymerase buffer, 2.5 μL 25 mmol L^{-1} $MgCl_2$, 1.0 unit of Taq DNA polymerase and finally add sterile water to volume 25 μL. The PCR program initially started with a 94°C denaturation for 4 min followed by 25 cycles of 94°C/50 sec, Ta°C/50 sec, 72°C/50 sec then 72°C extension for 10 min, finally 4°C to terminate the reaction.

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 BLAST tool 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).

RESULTS AND DISCUSSION

RT-PCR results for sheep *RAB1A*, *RAB4A* and *RAB5A* genes: Through RT-PCR with pooled tissue cDNAs for sheep *RAB1A*, *RAB4A* and *RAB5A* genes, the resulting PCR products were 615, 657 and 648 bp (Fig. 1).

Sequence analysis: The cDNA nucleotide sequence analysis using the BLAST software at NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that these three genes were not homologous to any of the known sheep genes and they were then deposited into the GenBank database (Accession No.: FJ943970, FJ943971 and FJ943983). The gene prediction was carried out using the GenScan software and results showed that the 615, 657 and 648 bp cDNA sequences represent three single genes which encoded 204, 218 and 215 amino acids, respectively. Finally, these three novel sheep genes were assigned to GeneIDs: 100302065, 100302066 and 100302084.

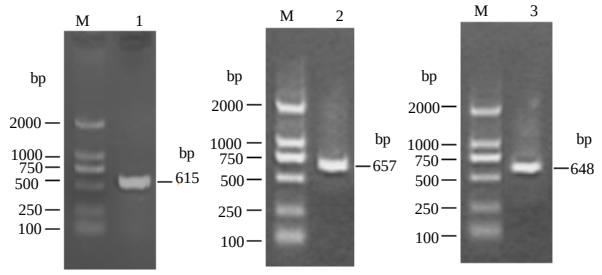

Fig. 1: RT-PCR results for sheep *RAB1A*, *RAB4A* and *RAB5A* genes. M: DL2000 DNA markers; 1: PCR product for sheep *RAB1A* gene; 2: PCR product for sheep *RAB4A* gene and 3: PCR product for sheep *RAB5A* gene

Further BLAST analysis of these deduced proteins revealed that the sheep RAB1A protein has high homology with the RAB1A, member RAS oncogene family (RAB1A) proteins of nine species human (Accession No.: NP_004152; 98%), mouse (Accession No.: NP_033022; 98%), rat (Accession No.: NP_112352; 98%), pig (Accession No.: NP_001026957; 98%), zebrafish (Accession No.: NP_001007162; 98%), Western clawed frog (Accession No.: NP_001004787; 97%), Atlantic salmon (Accession No.: ACN11413; 96%), disc abalone (Accession No.: ABO26625; 92%) and great pond snail (Accession No.: Q05974; 87%) (Fig. 2).

The sheep RAB4A protein has high homology with the RAB4A, member RAS oncogene family (RAB4A) proteins of eight species cattle (Accession No.: DAA14394; 100%), dog (Accession No.: XP_536353; 99%), human (Accession No.: NP_004569; 99%), rhesus monkey (Accession No.: XP_001082985; 99%), horse (Accession No.: XP_001498005; 99%), chicken (Accession No.: XP_419573; 99%), rat (Accession No.

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Human_Mouse_Rat          MSSMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Pig                      MSSMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Sheep                    MSSMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Atlantic salmon          ---MNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Western clawed frog      MSSMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Zebrafish                ---MNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Disc abalone             MSTMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
Great pond snail         MSTMNPDYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTI
                            ***:**************************************************

Human_Mouse_Rat          KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
Pig                      KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQGSFNNVKQWLQEIDRYASENVNKL
Sheep                    KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
Atlantic salmon          KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
Western clawed frog      KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
Zebrafish                KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
Disc abalone             KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
Great pond snail         KLQIWDTAGQERFRTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKL
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Human_Mouse_Rat          LVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATNVEQSFMTMAAEIKKRMGPGA
Pig                      LVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATNVEQSFMTMAAEIKKRMGPGA
Sheep                    LVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATNVEQSFMTMAAEIKKRMGPGA
Atlantic salmon          LVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKSATNVEQAFMTMAAEIKKRMGPGA
Western clawed frog      LVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATNVEQAFMTMAAEIKKRMGPGA
Zebrafish                LVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATNVEQAFMTMAAEIKKRMGPGA
Disc abalone             LVGNKCDLTTKKVVDYTTAKEYADQLGIPFLETSAKNATNVEQAFMTMAAEIKNRMGPVT
Great pond snail         LVGNKSDLTTKKVVDFTTAKEYADQLGIPFLETSAKNATNVEQAFMTMAAEIKNRMGPIT
                         *****.*********:*****:**.***********.******:*********:**** :

Human_Mouse_Rat          TAGGAEKSNVKIQSTPVKQSGGGCC
Pig                      TAGGAEKSNVKIQSTPVKQSGGGCC
Sheep                    TAGGAEKSNVKFRALRSAVKWRCC-
Atlantic salmon          TTGGNEKSNVKIQSTPVKPASGGCC
Western clawed frog      TAGGQEK-NVKIQSTPVKQSSGGCC
Zebrafish                TAGGSEK-TMKIESTPVKPASGGCC
Disc abalone             AASENKPSVKINSSTPVKQGGGGCC
Great pond snail         AASDSKPSVKINSSTPVSANKGGCC
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Fig. 2: The alignment of the protein encoded by sheep *RAB1A* gene and nine other kinds RAB1A proteins

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Dog                     MSQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
Chicken                 MSQAAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
Sheep_Cattle            MSQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
Human_Rhesus monkey     MSQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
Horse                   MSQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
Rat                     MAQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
Mouse                   MAQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGK
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Dog                     YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
Chicken                 YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
Sheep_Cattle            YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
Human_Rhesus monkey     YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
Horse                   YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
Rat                     YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
Mouse                   YVKLQIWDTAGQERFRSVTRSYYRGAAGALLVYDITSRETYNALTNWLTDARMLASQNIV
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Dog                     IILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFVQCARKILNKIES
Chicken                 IILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFVQCARKILNKIES
Sheep_Cattle            IILCGNKKDLDTDREVTFLEASRFAQENELMFLETSALTGENVEEAFVQCARKILNKIES
Human_Rhesus monkey     IILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFVQCARKILNKIES
Horse                   IILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFVQCARKILNKIES
Rat                     IILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFMQCARKILNKIES
Mouse                   LILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFMQCARKILNKIES
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Dog                     GELDPERMGSGIQYGDAALRQLRSPRRAQAPSAQECGC
Chicken                 GELDPERMGSGIQYGDAALRQLRSPRRAQAQSAQECGC
Sheep_Cattle            GELDPERMGSGIQYGDAALRQLRSPRRAQAPSAQECGC
Human_Rhesus monkey     GELDPERMGSGIQYGDAALRQLRSPRRAQAPNAQECGC
Horse                   GELDPERMGSGIQYGDAALRQLRSPRRTQAPNAQECGC
Rat                     GELDPERMGSGIQYGDAALRQLRSPRRTQAPSAQECGC
Mouse                   GELDPERMGSGIQYGDAALRQLRSPRRTQAPSAQECGC
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Fig. 3: The alignment of the protein encoded by sheep *RAB4A* gene and eight other kinds of RAB4A proteins

NP_037151; 98%), mouse (accession number: NP_033029; 98%) (Fig. 3). The sheep RAB5A protein has high homology with the RAB5A, member RAS oncogene family (RAB5A) proteins of fifteen species-cattle (Accession No.: NP_001069654; 98%), rabbit (Accession No.: XP_002716250; 98%), dog (Accession No.: NP_001003317; 98%), pig (Accession No.: NP_001116652; 98%), horse (Accession No.: XP_001495368; 98%), sumatran orangutan (Accession No.: XP_002814060; 98%), human (Accession No.: NP_004153; 97%), mouse (Accession No.: NP_080163; 97%), rat (Accession No.: NP_073183; 97%), chimpanzee (Accession No.: XP_516319; 97%), chicken (Accession No.: NP_001006363; 95%), rhesus monkey (Accession No.: XP_001086669; 93%), African clawed frog (Accession No.: NP_001080535; 93%), Western clawed frog (Accession No.: NP_001008068; 93%) and zebrafish (Accession No.: NP_958893; 92%) (Fig. 4).

Based on the results of the alignment of RAB1A, RAB4A and RAB5A proteins, three phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software (http://align.genome.jp/) as shown in Fig. 5-7.

The phylogenetic analysis revealed that the sheep *RAB1A* gene has a closer genetic relationship with the *RAB1A* genes of human, mouse and rat. The sheep *RAB4A* and *RAB5A* genes both have closer genetic relationships with the *RAB4A* and *RAB5A* genes of cattle.

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *RAB1A*, *RAB4A* and *RAB5A* genes are all generally but differentially expressed in tissues including spleen, lung, muscle, kidney, ovary, skin, liver, heart and fat (Fig. 8).

In the current study, the researchers firstly get the coding sequences of sheep *RAB1A*, *RAB4A* and *RAB5A* genes by RT-PCR. With the development of modern bioinformatics and establishment of specific sheep NCBI EST database, researchers can easily find the useful ESTs which were highly homologous to the coding sequences of human genes. Based on these sheep EST sequences, the researchers can obtain the complete coding sequences of some novel sheep genes through the some experimental methods such as RT-PCR. From the clone and sequence analysis of sheep *RAB1A*, *RAB4A* and *RAB5A* genes, it could be seen that this is an effective method to isolate some novel sheep genes.

Through sequence analysis, the researchers found that the encoding protein of the sheep *RAB1A*, *RAB4A* and *RAB5A* genes are highly homologous with RAB1A, RAB4A and RAB5A proteins of human and some other

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Mouse                   -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Rat                     -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Cattle                  -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Pig                     -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Sheep                   -MANRGAARPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Rabbit                  -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Human                   -MASRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Chimpanzee              -MASRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Sumatran orangutan      -MASRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Dog_Horse               -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Rhesus monkey           -MANRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Chicken                 -MANRGATRPNGPNAGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
African clawed frog     MANRGGATRPNGPNAGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Western clawed frog     MANRGGATRPNGPNAGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
Zebrafish               MANRGGATRPNGSNAGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLT
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Mouse                   QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Rat                     QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFSRAKNWVKELQ
Cattle                  QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Pig                     QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Sheep                   QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Rabbit                  QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Human                   QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Chimpanzee              QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Sumatran orangutan      QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Dog_Horse               QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Rhesus monkey           QTVCLDDTTVKFEIRDTAGQERYHSLAPMYYRGAQAVIVVYDITNEESFARAKNWVKELQ
Chicken                 QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
African clawed frog     QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Western clawed frog     QTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
Zebrafish               QTLCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIVVYDITNEESFARAKNWVKELQ
                        **:*********** ********************.************;**********

Mouse                   RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Rat                     RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Cattle                  RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Pig                     RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Sheep                   RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Rabbit                  RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Human                   RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Chimpanzee              RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Sumatran orangutan      RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Dog_Horse               RQASPNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
Rhesus monkey           RQASPNIVIALAGNKANLANKRAVNFQEAQSFADDNSLLFMETSAKTSMDVNEIFMAIAK
Chicken                 RQASPNIVIALAGNKADLANKRAVDFQEAQAYADDNSLLFMETSAKTSMNVNEIFMAIAK
African clawed frog     RQASPNIVIALSGNKADLSTKRAVDFQEAQAYADDNSLLFMETSAKTSVNVNEIFMAIAK
Western clawed frog     RQASPNIVIALSGNKADLASKRAVDFQEAQAYADDNSLLFMETSAKTSVNVNEIFMAIAK
Zebrafish               RQASPNIVIALSGNKADLANKRAVDFQDAQSYADDNSLLFMETSAKTSMNVNEIFMAIAK
                        ****** ****:****:*:.****:**:**::****************::**********

Mouse                   KLPKNEPQNPGANSARGRG-VDLTEPAQPARSQCCSN
Rat                     KLPKNEPQNPGANSARGRG-VDLTEPAQPARSQCCSN
Cattle                  KLPKNEPQNPGANSTRGRG-VDLTEPTQPTRSQCCSN
Pig                     KLPKNEPQNPGINCTRGRG-VDLTEPTQPTRSQCCSN
Sheep                   KLPKNEPQNPGAIPPRGRG-VDLTEPTQPTRSQCCSN
Rabbit                  KLPKNEPQNPGGNSARGRG-VDLTEPTQPTRSQCCSN
Human                   KLPKNEPQNPGANSARGRG-VDLTEPTQPTRNQCCSN
Chimpanzee              KLPKNEPQNPGANSARGRGGVDLTEPTQPTRNQCCSN
Sumatran orangutan      KLPKNEPQNPGANSARGRG-VDLTEPTQPTRSQCCSN
Dog_Horse               KLPKNEPQNPGANSARGRG-VDLTEPTQPTRSQCCSN
Rhesus monkey           KLPKDEPQNPGANSARGRG-VDLTEPTRPTRSQCCSN
Chicken                 KLPKNEPQNTGASSARGRG-VDLTEPTQPPKSQCCSN
African clawed frog     KLPKTEPQAGASNTIRGRG-VDLTETAQPTKSQCCSN
Western clawed frog     KLPKTEPQAGGSNTIRGRG-VDLTETAQPTKSQCCSN
Zebrafish               KLPKSEPQAAGANSGRSRG-VDLTETAQPTKAPCCSN
                        **** ***  .    *.** *****.:::*.:  ****
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Fig. 4: The alignment of the protein encoded by sheep *RAB5A* gene and fifteen other kinds of RAB5A proteins

animals. This implied that the *RAB1A*, *RAB4A* and *RAB5A* genes were highly conserved in some species and the sheep *RAB1A*, *RAB4A* and *RAB5A* genes will have similar functions as the *RAB1A*, *RAB4A* and *RAB5A* genes of human and other animals. The researchers also found that the sheep RAB1A, RAB4A and RAB5A proteins do not show complete identity to human or other animals. This implied that the sheep *RAB1A*, *RAB4A* and *RAB5A* genes will have some differences in functions to those of human or other mammals.

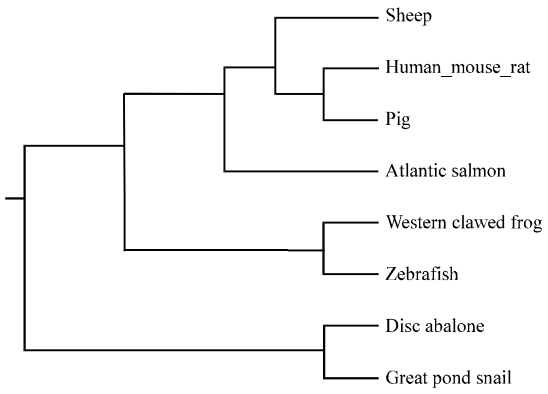

Fig. 5: The phylogenetic analysis for ten kinds of *RAB1A* genes

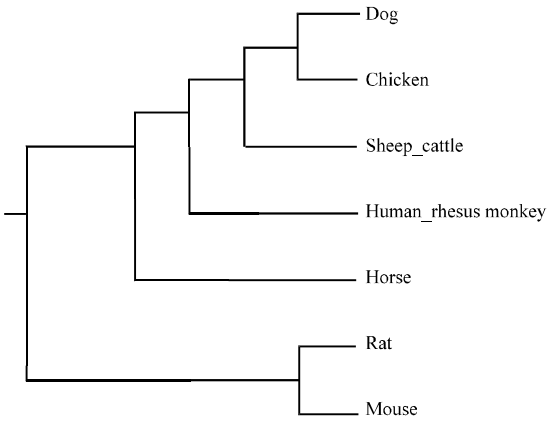

Fig. 6: The phylogenetic analysis for nine kinds of *RAB4A* genes

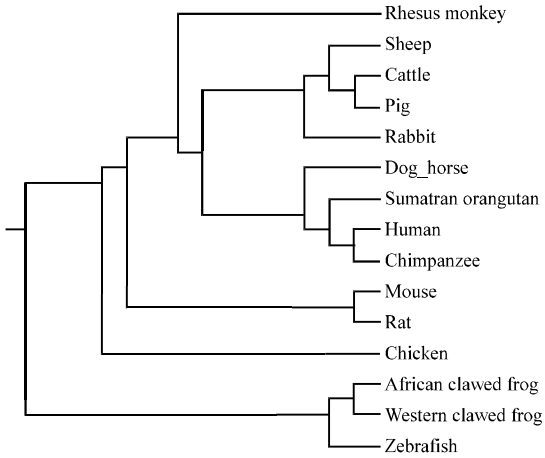

Fig. 7: The phylogenetic analysis for sixteen kinds of *RAB5A* genes

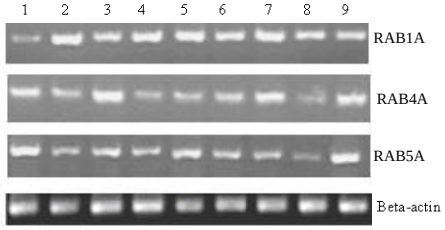

Fig. 8: Tissue expression distribution of sheep *RAB1A*, *RAB4A* and *RAB5A* genes. The beta-actin expression is the internal control. 1: Spleen; 2: Skin; 3: Lung; 4: Muscle; 5: Heart; 6: Fat; 7: Liver; 8: Kidney and 9: Ovary

The phylogenetic analysis revealed that the sheep *RAB1A* gene has a closer genetic relationship with the *RAB1A* genes of human, mouse and rat. This implied that the researchers can use human, mouse and rat as model organisms to study the sheep *RAB1A* gene or use sheep as model organism to study the human, mouse and rat *RAB1A* genes.

The sheep *RAB4A* and *RAB5A* genes both have closer genetic relationships with the *RAB4A* and *RAB5A* genes of cattle. Similarly, we can use cattle as a model organism to study the sheep *RAB4A* and *RAB5A* genes or use sheep as a model organism to study the cattle *RAB4A* and *RAB5A* genes.

From the tissue distribution analysis in the experiment it can be seen that the sheep *RAB1A*, *RAB4A* and *RAB5A* genes were obviously differentially expressed in some tissues. As the researchers did not study functions at protein levels yet, there might be many possible reasons for differential expression of sheep *RAB1A*, *RAB4A* and *RAB5A* genes. The suitable explanation for this under current conditions is that at the same time those biological activities related to the mRNA expression of sheep *RAB1A*, *RAB4A* and *RAB5A* genes were presented diversely in different tissues.

CONCLUSION

In this study, the researchers first isolated the sheep *RAB1A*, *RAB4A* and *RAB5A* genes and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into these novel sheep genes.

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