Journal of Animal and Veterinary Advances 10 (21): 2772-2778, 2011
ISSN: 1680-5593

Molecular Cloning, Sequence Identification and Tissue Expression Profile of Three Novel Sheep (*Ovis aries*) Genes-*ARL2*, *ARL3* and *ARL4C*

[1]Jiang Caode, [2]Liu Yonggang and [3]Xia Xueshan
[1]School of Life Science, Southwest University, 400715 Chongqing, China
[2]College of Animal Science and Technology, Yunnan Agricultural University, 650201 Kunming, China
[3]Faculty of Life Science and Technology,
Kunming University of Science and Technology, 650224 Kunming, China

Abstract: The complete coding sequences of three sheep genes-*ARL2*, *ARL3* and *ARL4C* were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Sequence analysis revealed that sheep *ARL2* gene encodes a protein of 184 amino acids that shares high homology with the ADP-Ribosylation factor-like 2 (ARL2) proteins of 9 species-cattle (100%), pig (99%), human (99%), dog (98%), mouse (96%), rat (95%), western clawed frog (90%), African clawed frog (89%) and channel catfish (85%). The sheep *ARL3* gene encodes a protein of 182 amino acids that shares high homology with the ADP-Ribosylation factor-like 3 (ARL3) proteins of 9 species-cattle (100%), pig (99%), horse (98%), human (98%), mouse (98%), rat (97%), African clawed frog (97%), red jungle fowl (96%) and zebrafish (94%). The sheep *ARL4C* gene encodes a protein of 192 amino acids that shares high homology with the ADP-Ribosylation factor-like 4C (ARL4C) proteins of 11 species-cattle (100%), human (100%), chimpanzee (100%), mouse (100%), taeniopygia guttata (98%), gray short-tailed opossum (98%), green anole (98%), atlantic salmon (96%), rainbow smelt (96%), zebrafish (95%) and African clawed frog (91%). Finally, these three novel sheep genes were assigned to GeneIDs: 100302310, 100302311 and 100302312. The phylogenetic analysis revealed that the sheep *ARL2* and *ARL3* genes both have closer genetic relationships with the *ARL2* and *ARL3* genes of cattle. The sheep *ARL4C* gene has a closer genetic relationship with the *ARL4C* genes of cattle, human, chimpanzee and mouse. Tissue expression profile analysis was also carried out and results demonstrated that sheep *ARL2*, *ARL3* and *ARL4C* genes were all generally but differentially expressed in detected tissues.

Key words: Sheep, ARL2, ARL3, ARL4C, tissue expression, China

INTRODUCTION

ADP-Ribosylation factor-like 2 (ARL2) encodes a small GTP-binding protein of the RAS superfamily which functions as an ADP-Ribosylation Factor (ARF). The encoded protein is one of a functionally distinct group of ARF-like genes. In its GTP bound form, Arl2 interacts with the protein binder of Arl2 (BART) and the complex is believed to play a role in mitochondrial adenine nucleotide transport. In its GDP bound form, Arl2 interacts with tubulin-folding Cofactor D; this interaction is believed to play a role in regulation of microtubule dynamics that impact the cytoskeleton, cell division and cytokinesis (Shultz *et al*., 2008; Renault *et al*., 2001; Hanzal-Bayer *et al*., 2002; Beghin *et al*., 2007).

ADP-Ribosylation factor-like 3 (ARL3) is a member of the ADP-ribosylation factor family of GTP-binding proteins. ARL3 binds guanine nucleotides but lacks ADP-ribosylation factor activity. Arl3 is an Arf family protein that differs from most Arf family members in the N-terminal extension. In mice, the absence of Arl3 is associated with abnormal epithelial cell proliferation and cyst formation (Grayson *et al*., 2002; Evans *et al*., 2010; Veltel *et al*., 2008; Schrick *et al*., 2006).

ADP-Ribosylation factor-like 4C (ARL4C) is also a member of the ADP-ribosylation factor family of GTP-binding proteins. ARL4C is closely similar to ARL4A and ARL4D and each has a nuclear localization signal and an unusually high guanine nucleotide exchange rate. This protein may play a role in cholesterol transport (Lim *et al*., 2006; Wei *et al*., 2009; Hofmann *et al*., 2007; Low *et al*., 2010). *ARL2*, *ARL3* and *ARL4C* genes are 3 genes which have important functions. Until today, *ARL2*, *ARL3* and *ARL4C* genes had been reported in human and other animals but the sheep *ARL2*, *ARL3* and *ARL4C* genes have not been reported yet.

In present experiment, researchers will isolate the coding sequences of sheep *ARL2*, *ARL3* and *ARL4C* genes based on the coding sequence information of *ARL2*, *ARL3* and *ARL4C* genes from human or other mammals and their highly homologous sheep ESTs sequence information, subsequently perform some

Corresponding Author: Xia Xueshan, Faculty of Life Science and Technology, Kunming University of Science and Technology, 650224 Kunming, China

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 *ARL2*, *ARL3* and *ARL4C* genes and for the tissue expression profile analysis.

Isolation of the sheep *ARL2*, *ARL3* and *ARL4C* genes: The primers for sheep *ARL2* gene isolation were designed based on the coding sequence information of human *ARL2* gene and its highly homologous sheep EST sequences: EE777072 and EE774064. Similarly, the primers for sheep *ARL3* gene isolation were designed based on the coding sequence information from human *ARL3* gene and its highly homologous sheep EST sequences: DY479155 and DY519198.

The primers for sheep *ARL4C* gene isolation were designed based on the coding sequence information from human and mouse *ARL4C* genes and their highly homologous sheep EST sequences: EE827413 and EE805580. 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 (1U/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. These PCR products for sheep *ARL2*, *ARL3* and *ARL4C* 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.

Table 1: Primers for sheep *ARL2*, *ARL3*, *ARL4C* and *Beta-actin* genes and their annealing temperatures

Genes	Primer sequence	Ta/°C
ARL2	Forward: 5'-ATGGGGCTTCTGACCATA-3'	55
	Reverse: 5'-TCAGTCGGCCATGAAGAT-3'	
ARL3	Forward: 5'-ATGGGCTTACTCTCAATT-3'	50
	Reverse: 5'-TTACTTCTTCTTTGCGCT-3'	
ARL4C	Forward: 5'-ATGGGCAACATCTCCTCC -3'	57
	Reverse: 5'-TTACCGCTTCTTCTTCTGC-3'	
Beta-actin	Forward: 5'- CTTGATGTCACGGACGATTT-3'	56
	Reverse: 5'- CACGGCATTGTCACCAACT -3'	

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). Researchers selected the housekeeping gene *β-actin* (Accession no: NM_001009784) as a positive control. The primers of sheep *ARL2*, *ARL3* and *ARL4C* 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 *ARL2*, *ARL3* and *ARL4C* genes: Through RT-PCR with pooled tissue cDNAs for sheep *ARL2*, *ARL3* and *ARL4C* genes, the resulting PCR products were 555, 549 and 579 bp (Fig. 1).

Sequence analysis: These 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 number: FJ969412, FJ969414 and FJ969415). The sequence prediction was carried out using the GenScan software and results showed that the 555, 549 and 579 bp cDNA sequences represent three single genes which encoded 184, 182 and 192 amino acids, respectively. Finally, these 3 novel sheep genes were assigned to GeneIDs: 100302310, 100302311 and 100302312.

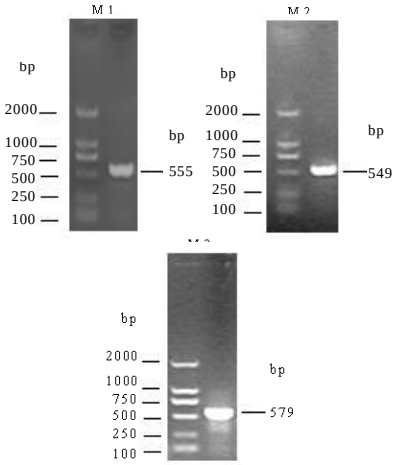

Fig. 1: RT-PCR results for sheep *ARL2*, *ARL3* and *ARL4C* genes. M, DL2000 DNA markers; 1, PCR product for sheep *ARL2* gene; 2, PCR product for sheep *ARL3* gene; 3, PCR product for sheep *ARL4C* gene

Further BLAST analysis of these proteins revealed that the sheep ARL2 protein has high homology with the ADP-Ribosylation factor-like 2 (ARL2) proteins of 9 species-cattle (accession number: NP_001033168; 100%), pig (accession number: XP_003122597; 99%), human (accession number: AAM12602; 99%), dog (accession number: XP_540874; 98%), mouse (accession number: NP_062696; 96%), rat (accession number: NP_113899; 95%), western clawed frog (accession number: NP_989148; 90%), African clawed frog (accession number: NP_001088984; 89%) and channel catfish (accession number: NP_001187733; 85%) (Fig. 2).

The sheep ARL3 protein has high homology with the ADP-ribosylation factor-like 3 (ARL3) proteins of nine species-cattle (accession number: NP_001033656; 100%), pig (accession number: NP_001026955; 99%), horse (accession number: XP_001498643; 98%), human (accession number: NP_004302; 98%), African clawed frog (accession number: AAH87495; 97%), red jungle fowl (accession number: XP_421730; 96%), zebrafish

```
Western         MGLLTILKKMKQKEREVRLLMLGLDNAGKTTILKKFNGEDINTISPTLGFNIKTLEHRGF
African         MGLLTILKKMKQKEREVRLLMLGLDNAGKTTILKKFNGEDINTISPTLGFNIKTLEHRGF
Mouse           MGLLTILKKMKQKERELRLLMLGLDNAGKTTILKKFNGEDVDTISPTLGFNIKTLEHRGF
Rat             MGLLTILKKMKQKERDVRLLMLGLDNAGKTTILKKFNGEDVDTISPTLGFNIKTLEHRGF
Sheep           MGLLTILKKMKQKERELRLLMLGLDNAGKTTILKKFNGEDIDTISPTLGFNIKTLEHRGF
Cattle          MGLLTILKKMKQKERELRLLMLGLDNAGKTTILKKFNGEDIDTISPTLGFNIKTLEHRGF
Pig             MGLLTILKKMKQKERELRLLMLGLDNAGKTTILKKFNGEDIDTISPTLGFNIKTLEHRGF
Human           MGLLTILKKMKQKERELRLLMLGLDNAGKTTILKKFNGEDIDTISPTLGFNIKTLEHRGF
Dog             MGLLTILKKMKQKERELRLLMLGLDNAGKTTILKKFNGEDIDTISPTLGFNIKTLEHRGF
Channel         MGLLTILKKLKHKEREMRLLMLGLDNAGKTTILKKFNGEDVSTISPTLGFNIKTLEHRGF
                *********:*:***::**********************:.******************

Western         KLNMWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRARLQDCAQELAGLLLEERLAGATL
African         KLNMWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRARLQDCAQELSGLLLEERLAGATL
Mouse           KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQSLLVEERLAGATL
Rat             KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQSLLVEERLAGATL
Sheep           KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQNLLVEERLAGATL
Cattle          KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQNLLVEERLAGATL
Pig             KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQSLLVEERLAGATL
Human           KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQSLLVEERLAGATL
Dog             KLNIWDVGGQKSLRSYWRNYFESTDGLIWVVDSADRQRMQDCQRELQSLLVEERLAGATL
Channel         KLNIWDVGGQKSLRSYWRNYFESTDGLVWVVDSADRLRLEDCRKELCALLLEERLAGATL
                ***:**********************:******** *::** :**  **:*********

Western         LVFANKQDLPGALSKDAIKEALELDNIKTHHWCIQGCSAVTGENLLTGIDWLLDDISSRI
African         LVFANKQDLPGALSKDAIKEAMELDNIKTHHWCIQGCSAVTGENLLTGIDWLLDDISSRI
Mouse           LIFANKQDLPGALSCNAIQEALELDSIRSHHWRIQGCSAVTGEDLLPGIDWLLDDISSRV
Rat             LIFANKQDLPGALSCNAIQEALELDSIRSHHWRIQGCSAVTGEDLLPGIDWLLDDISSRV
Sheep           LIFANKQDLPGALSSNAIREALELDSIRSHHWCIQGCSAVTGENLLPGIDWLLDDISSRI
Cattle          LIFANKQDLPGALSSNAIREALELDSIRSHHWCIQGCSAVTGENLLPGIDWLLDDISSRI
Pig             LIFANKQDLPGALSSKAIREALELDSIRSHHWCIQGCSAVTGENLLPGIDWLLDDISSRI
Human           LIFANKQDLPGALSSNAIREALELDSIRSHHWCIQGCSAVTGENLLPGIDWLLDDISSRI
Dog             LIFANKQDLPGALSSNAIREALELDSIHSHHWCIQGCSAVTGENLLPGIDWLLDDISSRI
Channel         LVFANKQDLPGALSKDAIREALALDDIKTHHWCILGCSAVTGENLLTGVDWLLDDIAARI
                *:************ .**:**: **.*:.*** * ********:**.*:********:.*:
Western         FTAD
African         FTAD
Mouse           FTAD
Rat             FTAD
Sheep           FMAD
Cattle          FMAD
Pig             FMAD
Human           FTAD
Dog             FTAD
Channel         FTAD
                * **
```

Fig. 2: The alignment of the protein encoded by sheep *ARL2* gene and nine other kinds ARL2 proteins. Western clawed frog; African clawed frog; Channel catfish

```
Sheep           MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Cattle          MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Pig             MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Horse           MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Human           MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Mouse           MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Rat             MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Red             MGLLSILRKLKSTPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
African         MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Zebrafish       MGLLSILRKLKSTPDQEVRILLLGLDNGGKTTLLKQLASEDITHITPTQGFNIKSVQSQG
                ***********:*************.*************:*****************

Sheep           FKLNVWDIGGQRKIRPYWRNYFENTDILIYVIDSADRKRFEETGQELAELLEEEKLSCVP
Cattle          FKLNVWDIGGQRKIRPYWRNYFENTDILIYVIDSADRKRFEETGQELAELLEEEKLSCVP
Pig             FKLNVWDIGGQRKIRPYWRNYFENTDILIYVIDSADRKRFEETGQELAELLEEEKLSCVP
Horse           FKLNVWDIGGQRKIRPYWRNYFENTDILIYVIDSADRKRFEETGQELAELLEEEKLSCVP
Human           FKLNVWDIGGQRKIRPYWKNYFENTDILIYVIDSADRKRFEETGQELAELLEEEKLSCVP
Mouse           FKLNVWDIGGQRKIRPYWRSYFENTDILIYVIDSADRKRFEETGQELTELLEEEKLSCVP
Rat             FKLNVWDIGGQRKIRPYWRSYFENTDILIYVIDSADRKRFEETGQELTELLEEEKLSCVP
Red             FKLNVWDIGGQRKIRPYWRNYFENTDILIYVIDSADRKRFEETGQELAELLDEEKLGGVP
African         FKLNVWDIGGQRKIRPYWRNYFENTDVLIYVIDSADRKRFEETGQELAELLDEEKLSGVP
Zebrafish       FKLNVWDIGGQRKIRPYWRNYFENTDVLIYVIDSADRKRFEETGQELAELLDEEKLSGVP
                *****************:.******:*******************:***:****. **

Sheep           VLIFANKQDLLTAAPASEIAEGLNLHTIRDRFWQIQSCSALTGEGVQDGMNWVCKNVSAK
Cattle          VLIFANKQDLLTAAPASEIAEGLNLHTIRDRFWQIQSCSALTGEGVQDGMNWVCKNVSAK
Pig             VLIFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Horse           VLIFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGIQDGMNWVCKNVNAK
Human           VLIFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Mouse           VLIFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Rat             VLVFANKQDLLTAAPAAEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Red             VLIFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALSGEGVQDGMNWVCKNVNAK
African         VLIFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Zebrafish       VLVFANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKSVNAK
                **:*************:**************.*********:***:*********.*.**
Sheep           KK
Cattle          KK
Pig             KK
Horse           KK
Human           KK
Mouse           KK
Rat             KK
Red             KK
African         KK
Zebrafish       RK
                :*
```

Fig. 3: The alignment of the protein encoded by sheep *ARL3* gene and nine other kinds of ARL3 proteins. African clawed frog; Red jungle fowl

(accession number: NP_001038373; 94%), mouse (accession number: NP_062692; 98%) and rat (accession number: NP_073191; 97%) (Fig. 3).

The sheep ARL4C protein has high homology with the ADP-Ribosylation factor-like 4C (ARL4C) proteins of eleven species-cattle (accession number: NP_001095818; 100%), human (accession number: NP_005728; 100%), chimpanzee (accession number: XP_003309579; 100%), mouse (accession number: NP_796279; 100%), Atlantic salmon (accession number: NP_001133949; 96%), zebrafish (accession number: NP_998413; 95%), African clawed frog (accession number: NP_001089936; 91%), taeniopygia guttata (accession number: XP_002189927; 98%), gray short-tailed opossum (accession number: XP_001362360; 98%), green anole (accession number: XP_003215191; 98%) and rainbow smelt (accession number: ACO09249; 96%) (Fig. 4). Based on the results of the alignment of ARL2, ARL3 and ARL4C 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 *ARL2* and *ARL3* genes both have closer genetic relationships with the *ARL2* and *ARL3* genes of cattle. The sheep *ARL4C* gene has a closer genetic relationship with the *ARL4C* genes of cattle, human, chimpanzee and mouse.

```
Atlantic        MGNSFSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Rainbow         MGNSFSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Zebrafish       MGNSFSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Taeniopygia     MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIRLSNGTAK
Sheep           MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Human           MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Cattle          MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Mouse           MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Chimpanzee      MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Green           MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIKLSNGTAK
Gray            MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTEKIRLSNGAAK
African         MGNISSNISSFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTERIRLSNGAAK
                ***  ****:****************************************:*:****:**

Atlantic        GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Rainbow         GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Zebrafish       GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Taeniopygia     GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Sheep           GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Human           GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Cattle          GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Mouse           GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Chimpanzee      GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Green           GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Gray            GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDVDRLEEAKTELHKVTKFAENQGT
African         GISCHFWDVGGQEKLRPLWKSYSRCTDGIIYVVDSVDSDRLEEAKTELYKVTKFAENLGT
                ************************************ **********:******** **

Atlantic        PLLVIANKQDLPKSLPVADIEKQLALHELTPSTTYHVQPCCAIIGEGLHEGMDKLYEMIV
Rainbow         PLLVIANKQDLPKSLPVADIEKQLALHELTPSTTYHVQPACAIIGEGLHEGMDKLYEMIV
Zebrafish       PLLVIANKQDLPKSLSVADIEKQLALQELTPATTYHIQPACAIIGEGLHEGMDKLYEMIV
Taeniopygia     PLLVIANKQDLPKSLPVAEIEKQLALHELTPSTTYHIQPACAIIGEGLTEGMDKLYEMIL
Sheep           PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAIIGEGLTEGMDKLYEMIL
Human           PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAIIGEGLTEGMDKLYEMIL
Cattle          PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAIIGEGLTEGMDKLYEMIL
Mouse           PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAIIGEGLTEGMDKLYEMIL
Chimpanzee      PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAIIGEGLTEGMDKLYEMIL
Green           PLLVIANKQDLPKSLPVTEIEKQLALPELSPSTTYHVQPACAIIGEGLTEGMDKLYEMIL
Gray            PLLVIANKQDLPKSLPVAEIEKQLALPELVPATTYHVQPACAIIGEGLTEGMDKLYEMIL
African         PLLVIANKQDLPKSMAVSDIERQLALQELSPSTPYHVQPACAIIGEGLTEGMDKLYEMIL
                **************:.*::**:**** ** *:*.**:**.******** **********:

Atlantic        KRRKSLKQKKKR-
Rainbow         KRRKSLKQKKKR-
Zebrafish       KRRKSLKQKKKR-
Taeniopygia     KRRKSLKQKKKR-
Sheep           KRRKSLKQKKKR-
Human           KRRKSLKQKKKR-
Cattle          KRRKSLKQKKKR-
Mouse           KRRKSLKQKKKR-
Chimpanzee      KRRKSLKQKKKR-
Green           KRRKSLKQKKKR-
Gray            KRRKSLKQKKKR-
African         KRRKTLKQKKKQR
                ****:******:
```

Fig. 4: The alignment of the protein encoded by sheep *ARL4C* gene and eleven other kinds of ARL4C proteins. African clawed frog; Atlanticsalmon; taeniopygia guttata; gray short-tailed opossum; green anole; rainbow smelt

Tissue expression profile: Tissue expression profile analysis was carried out and results revealed that the sheep *ARL2*, *ARL3* and *ARL4C* 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, we firstly get the coding sequences of sheep *ARL2*, *ARL3* and *ARL4C* genes by RT-PCR. With the development of modern bioinformatics, establishment of specific sheep NCBI EST database and different convenient analysis tools, researchers can easily find the

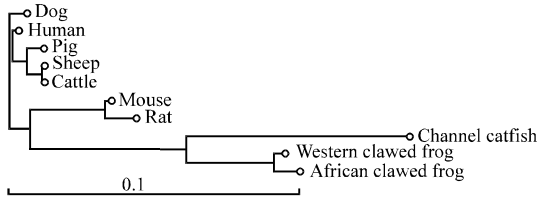

Fig. 5: The phylogenetic analysis for ten kinds of *ARL2* genes

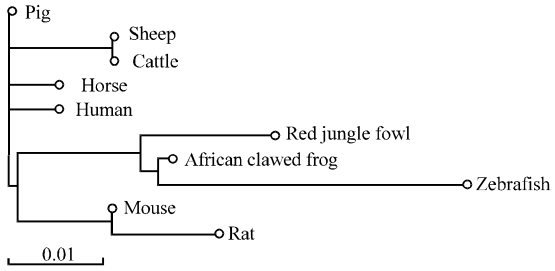

Fig. 6: The phylogenetic analysis for ten kinds of *ARL3* genes

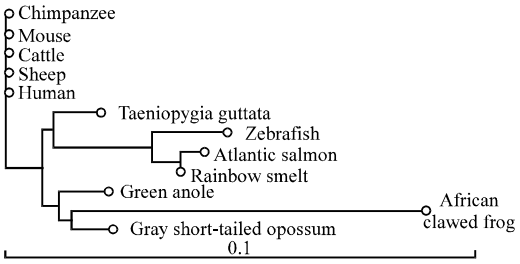

Fig. 7: The phylogenetic analysis for twelve kinds of *ARL4C* genes

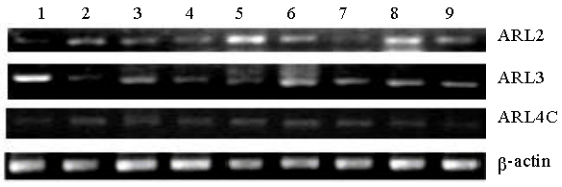

Fig. 8: Tissue expression distribution of sheep *ARL2*, *ARL3* and *ARL4C* genes. The beta-actin expression is the internal control, spleen, skin, lung, muscle, heart, fat, liver, kidney and ovary

useful ESTs which were highly homologous to the coding sequences of human genes. Based on these sheep EST sequences, we 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 *ARL2*, *ARL3* and *ARL4C* genes, it could be seen that this is an effective method to isolate some novel sheep genes. Through sequence analysis, we found that the encoding protein of the sheep *ARL2*, *ARL3* and *ARL4C* genes are highly homologous with ARL2, ARL3 and ARL4C proteins of human and some other animals. This implied that the *ARL2*, *ARL3* and *ARL4C* genes were highly conserved in some species and the sheep *ARL2*, *ARL3* and *ARL4C* genes will have similar functions as the *ARL2*, *ARL3* and *ARL4C* genes of human and other animals. We also found that the sheep ARL2, ARL3 and ARL4C proteins do not show complete identity to other animals. This implied that the sheep *ARL2*, *ARL3* and *ARL4C* genes will have some differences in functions to those of other animals.

The phylogenetic analysis revealed that the sheep *ARL2* and *ARL3* genes both have closer genetic relationships with the *ARL2* and *ARL3* genes of cattle. This implied that we can use cattle as a model organism to study the sheep *ARL2* and *ARL3* genes or use sheep as a model organism to study the cattle *ARL2* and *ARL3* genes. The sheep *ARL4C* gene has a closer genetic relationship with the *ARL4C* genes of cattle, human, chimpanzee and mouse so that we can use cattle, human, chimpanzee and mouse as model organisms to study the sheep *ARL4C* gene or use sheep as a model organism to study the *ARL4C* genes of cattle, human, chimpanzee and mouse.

From the tissue distribution analysis in the experiment, it can be seen that the sheep *ARL2*, *ARL3* and *ARL4C* genes were obviously differentially expressed in some tissues. As we did not study functions at protein levels yet there might be many possible reasons for differential expression of sheep *ARL2*, *ARL3* and *ARL4C* 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 *ARL2*, *ARL3* and *ARL4C* genes were presented diversely in different tissues.

CONCLUSION

In this study, we first isolated the sheep *ARL2*, *ARL3* and *ARL4C* genes and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further insight into these novel sheep genes.

ACKNOWLEDGEMENTS

This research was supported by the Candidates of the Young and Middle Aged Academic and Technical Leaders of Yunnan province.

REFERENCES

Beghin, A., S. Honore, C. Messana, E.L. Matera and J. Aim *et al*., 2007. ADP ribosylation factor like 2 (Arl2) protein influences microtubule dynamics in breast cancer cells. Exp. Cell Res., 313: 473-485.

Evans, R.J., N. Schwarz, K. Nagel-Wolfrum, U. Wolfrum, A.J. Hardcastle and M.E. Cheetham, 2010. The retinitis pigmentosa protein RP2 links pericentriolar vesicle transport between the Golgi and the primary cilium. Hum. Mol. Genet., 19: 1358-1367.

Grayson, C., F. Bartolini, J.P. Chapple, K.R. Willison and A. Bhamidipati *et al*., 2002. Localization in the human retina of the X-linked retinitis pigmentosa protein RP2, its homologue cofactor C and the RP2 interacting protein Arl3. Hum. Mol. Genet., 11: 3065-3074.

Hanzal-Bayer, M., L. Renault, P. Roversi, A. Wittinghofer and R.C. Hillig, 2002. The complex of Arl2-GTP and PDE delta: From structure to function. EMBO J., 21: 2095-2106.

Hofmann, I., A. Thompson, C.M. Sanderson and S. Munro, 2007. The Arl4 family of small G proteins can recruit the cytohesin Arf6 exchange factors to the plasma membrane. Curr. Biol., 17: 711-716.

Lim, J., T. Hao, C. Shaw, A.J. Patel and G. Szabo *et al*., 2006. A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. Cell, 125: 801-814.

Liu, G.Y. and S.Z. Gao, 2009. Molecular cloning, sequence identification and tissue expression profile of three novel sheep (*Ovis aries*) genes-BCKDHA, NAGA and HEXA. Biol. Res., 42: 69-77.

Liu, G.Y., 2009. A novel HADHA gene differentially expressed in muscle and other tissues from black-boned vs. ordinary sheep. Anim. Sci. Pap. Rep., 27: 127-137.

Liu, Y.G., Y.Z. Xiong, C.Y. Deng, B. Zuo and J.H. Zhang, 2004. Comparison of gene expression patterns in *Longissimus dorsi* of sheeps between the high-parent heterosis cross combination landrace large white and the mid-parent heterosis cross combination large white Q Meishan. Asian-Aust. J. Anim. Sci., 17: 1192-1196.

Low, S.K., A. Kuchiba, H. Zembutsu, A. Saito and A. Takahashi *et al*., 2010. Genome-wide association study of pancreatic cancer in Japanese population. PLoS One, 5: e11824-e11824.

Renault, L., M. Hanzal-Bayer and R.C. Hillig, 2001. Coexpression, copurification, crystallization and preliminary X-ray analysis of a complex of ARL2-GTP and PDE delta. Acta Crystallogr. D. Biol. Crystallogr., 57: 1167-1170.

Schrick, J.J., P. Vogel, A. Abuin, B. Hampton and D.S. Rice, 2006. ADP-ribosylation factor-like 3 is involved in kidney and photoreceptor development. Am. J. Pathol., 168: 1288-1298.

Shultz, T., M. Shmuel, T. Hyman and Y. Altschuler, 2008. Beta-tubulin cofactor D and ARL2 take part in apical junctional complex disassembly and abrogate epithelial structure. FASEB J., 22: 168-182.

Veltel, S., A. Kravchenko, S. Ismail and A. Wittinghofer, 2008. Specificity of Arl2/Arl3 signaling is mediated by a ternary Arl3-effector-GAP complex. FEBS Lett., 582: 2501-2507.

Wei, S.M., C.G. Xie, Y. Abe and J.T. Cai, 2009. ADP-ribosylation factor like 7 (ARL7) interacts with α-tubulin and modulates intracellular vesicular transport. Biochem. Biophys. Res. Commun., 384: 352-356.

Yonggang, L. and G. Shizheng, 2009. A novel sheep gene, MMP7, differentially expressed in muscles from black-boned sheep and local common sheep. J. Applied Genet., 50: 253-256.