

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

¹Jiang Caode, ²Liu Yonggang and ³Xia Xueshan

¹School of Life Science, Southwest University, 400715 Chongqing, China

²College of Animal Science and Technology, Yunnan Agricultural University, 650201 Kunming, China

³Faculty 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

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 MgCl₂, 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.

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 MgCl₂, 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.

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

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

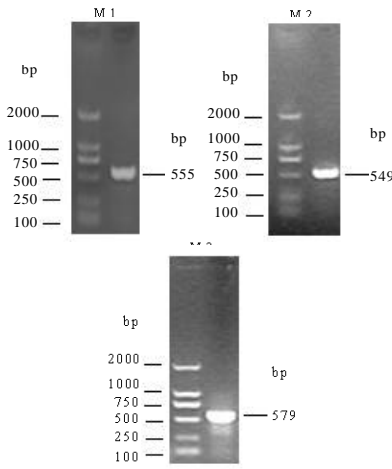


Fig. 1: RT-PCR results for sheep *ARL2*, *ARL3* and *ARLAC* genes. M, DL2000 DNA markers; 1, PCR product for sheep *ARL2* gene; 2, PCR product for sheep *ARL3* gene; 3, PCR product for sheep *ARLAC* gene

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

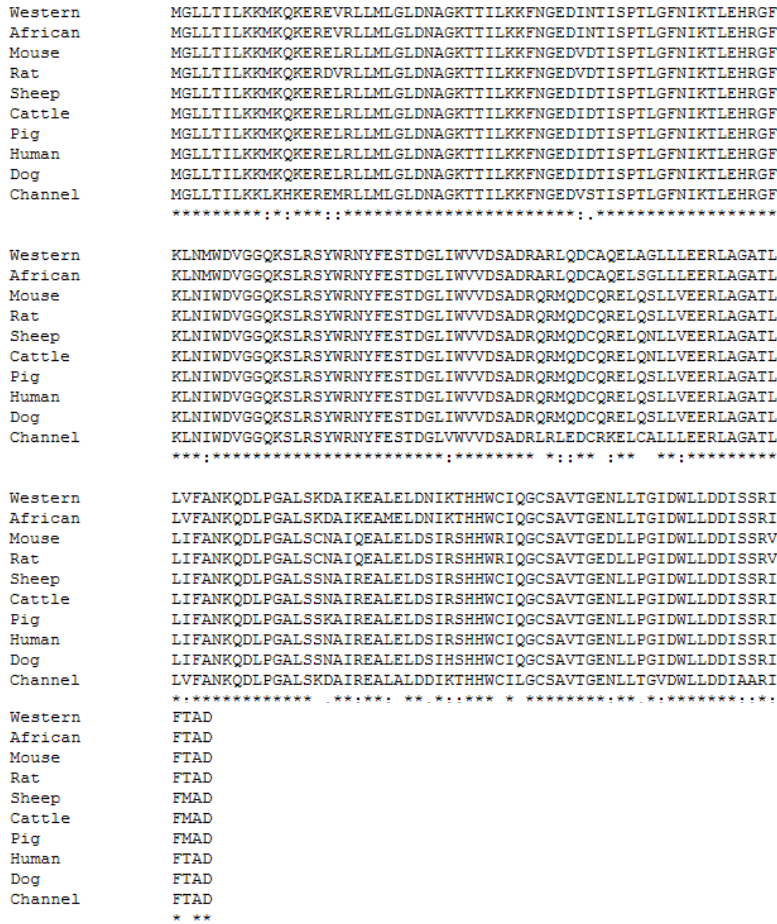


Fig. 2: The alignment of the protein encoded by sheep *ARL2* gene and nine other kinds *ARL2* proteins. Western clawed frog; African clawed frog; Channel catfish

```

Sheep      MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Cattle     MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Pig        MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Horse      MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Human      MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Mouse      MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Rat        MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Red        MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
African    MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
Zebrafish  MGLLSILRKLKLSAPDQEVRIILLGLDNAGKTTLLKQLASEDISHITPTQGFNIKSVQSQG
*****:*****.*****:*****

Sheep      FKLNVWDIGGQRKIRPYWRNYFENTDILYVIDSADRKRFEETGQELAEELLEEKLSCVP
Cattle     FKLNVWDIGGQRKIRPYWRNYFENTDILYVIDSADRKRFEETGQELAEELLEEKLSCVP
Pig        FKLNVWDIGGQRKIRPYWRNYFENTDILYVIDSADRKRFEETGQELAEELLEEKLSCVP
Horse      FKLNVWDIGGQRKIRPYWRNYFENTDILYVIDSADRKRFEETGQELAEELLEEKLSCVP
Human      FKLNVWDIGGQRKIRPYWRNYFENTDILYVIDSADRKRFEETGQELAEELLEEKLSCVP
Mouse      FKLNVWDIGGQRKIRPYWRSYFENTDILYVIDSADRKRFEETGQELTELEEEKLSCVP
Rat        FKLNVWDIGGQRKIRPYWRSYFENTDILYVIDSADRKRFEETGQELTELEEEKLSCVP
Red        FKLNVWDIGGQRKIRPYWRNYFENTDILYVIDSADRKRFEETGQELAEELDEEKLGSVP
African    FKLNVWDIGGQRKIRPYWRNYFENTDVLIVVIDSADRKRFEETGQELAEELDEEKLGSVP
Zebrafish  FKLNVWDIGGQRKIRPYWRNYFENTDVLIVVIDSADRKRFEETGQELAEELDEEKLGSVP
*****:*****:*****:*****

Sheep      VLI FANKQDLLTAAPASEIAEGLNLHTIRDRFWQIQSCSALTGEGVQDGMNWVCKNVSAK
Cattle     VLI FANKQDLLTAAPASEIAEGLNLHTIRDRFWQIQSCSALTGEGVQDGMNWVCKNVSAK
Pig        VLI FANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Horse      VLI FANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGIQDGMNWVCKNVNAK
Human      VLI FANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Mouse      VLI FANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Rat        VLVFANKQDLLTAAPAAEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
Red        VLI FANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALSSEGVQDGMNWVCKNVNAK
African    VLI FANKQDLLTAAPASEIAEGLNLHTIRDRVWQIQSCSALTGEGVQDGMNWVCKNVNAK
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      MGNISSNISAFQSLHIVMLGLDSAGKTTVLYRLKFNEFVNTVPTIGFNTERIRLSNGAAK
***      ****:*****:*****:*****:***

Atlantic      GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Rainbow      GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Zebrafish    GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Taeniopygia  GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Sheep        GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Human        GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Cattle       GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Mouse        GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Chimpanzee  GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Green        GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
Gray         GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDVDRLEEAKTELHKVTKFAENQGT
African      GISCHFWDVGGQEKLRPLWKSYSRCTDGI IYVVDSVDSRLEEAKTELYKVTKFAENLGT
*****:***** **

Atlantic      PLLVIANKQDLPKSLPVADIEKQLALHELTPSTTYHVQPACAI IGEGLHEGMDKLYEMIV
Rainbow      PLLVIANKQDLPKSLPVADIEKQLALHELTPSTTYHVQPACAI IGEGLHEGMDKLYEMIV
Zebrafish    PLLVIANKQDLPKSLPVADIEKQLALQELTPATTYHIQPACAI IGEGLHEGMDKLYEMIV
Taeniopygia  PLLVIANKQDLPKSLPVAEIEKQLALHELTPSTTYHIQPACAI IGEGLTEGMDKLYEMIL
Sheep        PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAI IGEGLTEGMDKLYEMIL
Human        PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAI IGEGLTEGMDKLYEMIL
Cattle       PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAI IGEGLTEGMDKLYEMIL
Mouse        PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAI IGEGLTEGMDKLYEMIL
Chimpanzee  PLLVIANKQDLPKSLPVAEIEKQLALHELIPATTYHVQPACAI IGEGLTEGMDKLYEMIL
Green        PLLVIANKQDLPKSLPVAEIEKQLALPELSPSTTYHVQPACAI IGEGLTEGMDKLYEMIL
Gray         PLLVIANKQDLPKSLPVAEIEKQLALPELVPATTYHVQPACAI IGEGLTEGMDKLYEMIL
African      PLLVIANKQDLPKSMAVSDIERQLALQELSPSTPYHVQPACAI IGEGLTEGMDKLYEMIL
*****:***** **

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

```

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

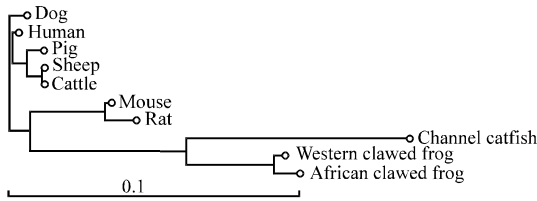


Fig. 5: The phylogenetic analysis for ten kinds of *ARL2* genes

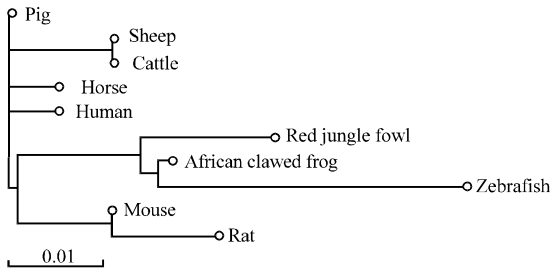


Fig. 6: The phylogenetic analysis for ten kinds of *ARL3* genes

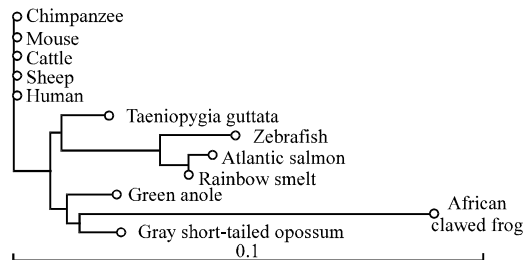


Fig. 7: The phylogenetic analysis for twelve kinds of *ARL4C* genes

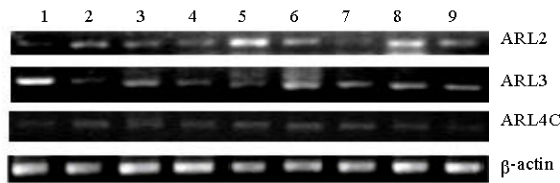


Fig. 8: Tissue expression distribution of sheep *ARL2*, *ARL3* and *ARL4C* genes. The beta-actin expression is the internal control, spleen, skin, lung, muscle, heart, fat, liver, kidney and ovary

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. Messina, 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.
- Vetel, 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.