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Sequence characterization, polymorphism, and tissue expression profile of an effector immediate-early gene: activity-regulated cytoskeletal associated protein gene (*Arc*/*Arg3.1*) in swamp and river buffalo

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ABSTRACT. The activity-regulated cytoskeletal associated protein (Arc/Arg3.1) has been implicated in experience-dependent synaptic plasticity and memory formation. However, information regarding its coding gene in buffalo remains scarce. In this study, the full-length of *Arc*/*Arg3.1* was isolated and characterized (accession No. JX491649) and genetic variations of six river buffalo and eight swamp buffalo were investigated. A tissue expression profile was obtained using semi-quantitative reverse transcription-polymerase chain reaction.

The coding region sequence of *Arc*/*Arg3.1* contained 1191 nucleotides encoding a putative protein of 396 amino acids with a theoretical isoelectric point (pI) and molecular weight (Mw) of 5.4 and 45.2 kDa, respectively. Four polymorphisms (c.63T>C, c.228T>C, c.558G>A, and c.625G>C) were found in buffalo; however, only substitution c.625G>C was non-synonymous, leading to an amino acid change from Val to Leu at the 209th position of the Arc/Arg3.1 protein sequence. Bioinformatics analysis revealed that this substitution had no significant effect on Arc/Arg3.1 function (subPSEC = -1.4039, $P_{deleterious}$ = 0.1685), which indicated that *Arc*/*Arg3.1* was highly conserved and functionally important in buffalo. Phylogenetic analysis revealed that the gene is closely related to that of *Bos taurus* and *Bos grunniens*. The gene was moderately expressed in the hypophysis and the placenta; it was weakly expressed in the kidney, milk, mammary gland, cerebrum, lung, heart, rumen, fat, and uterus; and it was almost silent in the muscle, liver, and skin. These findings will provide further insights into the structure and function of the immediate-early gene in buffalo.

Key words: Activity-regulated cytoskeletal associated protein; Swamp buffalo; River buffalo; Effector immediate-early gene

INTRODUCTION

The activity-regulated cytoskeletal associated protein (Arc/Arg3.1) is an effector immediate-early gene (IEG), and has been widely implicated in memory formation and experience-dependent synaptic plasticity (Lyford et al., 1995; Steward et al., 1998; Guzowski et al., 2000; Ben et al., 2006; Ploski et al., 2008). *Arc*/*Arg3.1* has been shown to impair the consolidation of a variety of hippocampal and amygdala-dependent memory tasks, including object recognition, spatial learning, and contextual and conditioned taste aversion (Alberini, 2005; Plath et al., 2006). Local knockdown of the *Arc*/*Arg3.1* protein within the amygdala or hippocampus using antisense oligodeoxynucleotides (ODNs) selectively impairs the consolidation of auditory fear conditioning and spatial learning, respectively (Guzowski et al., 2000; Chowdhury et al., 2006; Ploski et al., 2008).

The main subjects of studies concerning the *Arc*/*Arg3.1* gene are humans and rats, and studies in domestic animals are rare. Water buffalo is an important domestic animal in tropical and sub-tropical agriculture. The total global population of buffalo consists of approximately 1.8 x 10^8 individuals, 67% of which are river buffalo and 33% of which are swamp buffalo (Miao et al., 2008). The river buffalo is mainly distributed in India, Pakistan, Egypt, and Italy, and is used as a dairy breed with high milk yield (1800-2300 kg/year). The swamp buffalo is mainly spread throughout South China, Southeast Asia, and other regions, is used primarily in paddy fields, and its milk yield is approximately 500-1000 kg/year (Miao et al., 2008). The two buffalo types exhibit obvious morphological and production performance differences; however, few studies have investigated their genetic differences.

The objective of this study was to isolate the full-length coding sequence of the *Arc*/*Arg3.1* gene, to identify its polymorphisms within buffalo populations, and to clarify its se-

quence characterization and differential expression in a range of buffalo tissues. This study will help to reveal genetic differences in *Arc*/*Arg3.1* between the two types of buffalo and will provide a primary foundation for further research on this gene.

MATERIAL AND METHODS

Sample collection, RNA extraction, and cDNA synthesis

Fresh tissue samples were collected from 14 mature buffalo, which included six river buffalo (two Binglangjiang buffalo from Tengchong county, Yunnan, China; two Murrah and two Nili-Ravi buffalo from Dali, Yunnan, China) and eight swamp buffalo (two Fuzhong buffalo from Fuchuan county, Guangxi, China; two Dehong buffalo from Lianghe county, Yunnan, China; and four Yanjin buffalo samples from Zhaotong region, Yunnan, China). The samples analyzed consisted of kidney, hypophysis, milk, mammary gland, muscle, cerebrum, liver, skin, lung, heart, jejunum, rumen, adipose tissue, placenta, and uterus, which were immediately placed in liquid nitrogen after dissection from slaughtered buffalo and then stored at -80°C until use.

Total RNA was extracted using RNAiso Plus (TaKaRa, Dalian, China) according to manufacturer instructions. RNase-free DNase I (TaKaRa) was used to digest RNA to remove any genomic DNA contamination. Three micrograms RNA was reverse transcribed with Maloney-murine leukemia virus (M-MLV) reverse transcriptase and oligo $(dT)_{18}$ primer (Invitrogen, USA). The efficiency of reverse transcription was checked with 1.5 µL cDNA on 2.0% agarose gels containing ethidium bromide.

Isolation and polymorphism detection of the *Arc/Arg3.1* gene

A primer pair was designed to amplify the complete coding sequence of the buffalo *Arc*/*Arg3.1* gene by the Primer Premier 5.0 software with the *Bos taurus Arc*/*Arg3.1* gene sequence (NCBI database accession No. AC_000171). cDNAs obtained from six river buffalo and eight swamp buffalo were employed to isolate the buffalo *Arc*/*Arg3.1* gene and detect its polymorphisms using reverse transcription-polymerase chain reaction (RT-PCR) with the primers 5'-CCCCAAGAGGATTTTGCAGC-3' (forward) and 5'-GGGCGGGCCACTCTAAA GTC-3' (reverse). The total 20-µL reaction system contained 1.5 µL of 50 ng/µL cDNA, 1.8 µL of 2.5 mmol/L mixed dNTPs (TaKaRa), 6.0 µL of GC buffer, 0.4 µL 10 µmol/L forward primer, 0.4 µL of 10 µmol/L reverse primer, 0.2 µL of 5 U/µL Taq DNA polymerase (TaKaRa), and 9.7 µL sterile water. The PCR program initially started with 94°C denaturation for 4 min, followed by 35 cycles of 94°C for 30 s, 59°C for 30 s, 72°C for 45 s, then 72°C extension for 10 min, and finally brought down to 4°C to terminate the reaction. PCR products were then sequenced bidirectionally using the commercial fluorometric method.

Bioinformatics analysis

Sequences were examined and edited using the DNASTAR software (DNAstar Inc.). National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) and the ExPaSy (http://www.expasy.org) software were used to analyze the buffalo *Arc/Arg3.1*

gene sequence. The GenScan software (http://genes.mit.edu/GENSCAN.html) was utilized to predicted the cDNA sequence. Putative protein theoretical molecular weight (Mw), isoelectric point (pI) prediction, and signal peptide prediction were performed using the compute pI/Mw tool (http://us.expasy.org/tools/pi_tool.html), PSort II (http://psort.hgc.jp/form2.html), and TMHMM-2.0 server (http://www.cbs.dtu.dk/services/TMHMM-2.0), respectively. The substitution position-specific evolutionary conservation score (subPSEC) and the probability that a given variant will cause a deleterious effect on protein function ($P_{deleterious}$) were calculated using the PANTHER software (http://www.pantherdb.org/). A web-based microRNA (miRNA) predicting program was used to locate conserved potential miRNA targets (http://www.mirbase.org/). The alignment of the nucleotide sequences and deduced amino acid sequences were computed using the ClustalX and MEGA4.0 software (Kumar et al., 2008) with standard parameters. Secondary structures of deduced amino acid sequences were predicted with SOPMA (http://npsa-pbil.ibcp.fr/). The position and number of single nucleotide polymorphism (SNPs) were exported with the MEGA4.0 software (Kumar et al., 2008).

Semi-quantitative RT-PCR

To further characterize the buffalo *Arc*/*Arg3.1* gene, RT-PCR was conducted to determine its expression in tissues of two types of buffalo. We repeated the RT-PCR five times using 1, 2, 3, 4, and 5 μL cDNA as templates for eliminating the effect of cDNA concentration. The housekeeping gene *18S* rRNA was selected as a positive control. The control primers used were: 5'-GGACGTCTAAGGGCATCAG-3' (forward) and 5'-AATTCCGATAACGAAGAGACT-3' (reverse). The primers used to perform the semi-quantitative RT-PCR tissue expression profile of the *Arc*/*Arg3.1* gene in buffalo were the same as those used for the gene isolation mentioned above. PCR reactions were optimized over several cycles to ensure sufficient product amounts within the linear phase of amplification. Five microliters RT-PCR products of each tissue were required to run electrophoresis on 1% agarose gel.

RESULTS

Sequence analysis of *Arc*/*Arg3.1*

The RT-PCR product obtained from the buffalo *Arc/Arg3.1* gene in this study was 1324 bp in length (Figure 1). The sequence alignment using the BLAST software (NCBI) revealed that the coding sequence (CDS) of the buffalo *Arc*/*Arg3.1* gene was not homologous to any of the known buffalo genes. This gene was therefore deposited in the NCBI database and was assigned accession No. JX491649. Sequence prediction was carried out using the GenScan software and results showed that the coding region of the *Arc*/*Arg3.1* gene consists of 1191 nucleotides with a base composition of 234 A, 376 C, 425 G, 156 T, and a G+C content of 67.25%, which encodes a protein of 396 amino acid residues. The complete CDS of the buffalo *Arc*/*Arg3.1* gene and its deduced amino acid sequence are presented in Figure 2.

Four SNPs in the complete coding sequence of the *Arc*/*Arg3.1* gene were identified in both types of buffalo: c.63T>C, c.228T>C, c.558G>A, and c.625G>C. Only one substitution, c.625G>C, is non-synonymous, leading to an amino acid residue change from Val to Leu at position 209 (p.V209L) in Arc/Arg3.1. For prediction of protein function changes by the cod-

ing SNP, we calculated the subPSEC and $P_{deleterious}$ with the PANTHER software, which were -1.4039 and 0.1685, respectively. The information of genetic variations for the CDS of the *Arc*/*Arg3.1* gene in river buffalo and swamp buffalo are listed in Table 1. SNP63 and SNP558 were shared between river and swamp buffalo, whereas SNP228 and SNP625 were only detected in river buffalo, and were already homozygous in swamp buffalo.

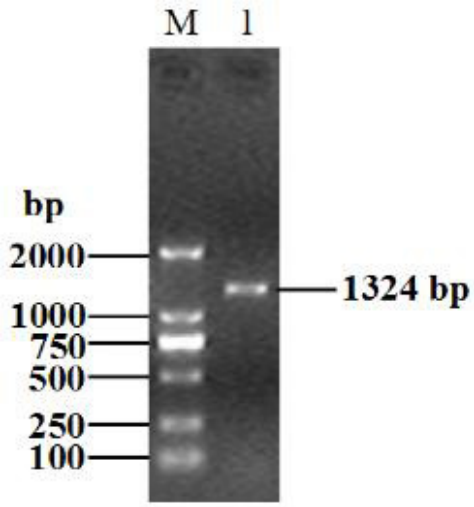

Figure 1. RT-PCR results for buffalo *Arc*/*Arg3.1* gene. *Lane M* = DL2000 DNA marker; *lane 1* = PCR product.

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ATGGAGTTGGACCACAGGACGACCGGCGGCCTCCACGCCTACCCCGGGCCCCGCAGCGGTCCTGCCGCCAAGCCCAACGTGATCTTGCAG    90
 M  E  L  D  H  R  T  T  G  G  L  H  A  Y  P  G  P  R  S  G  P  A  A  K  P  N  V  I  L  Q     30
ATCGGTAAGTGCCGGGCGGAGATGCTGGAGCACGTGCGGAGGACCCACCGGCACCTGCTCGCTGAAGTGTCCAAGCAGGTGGAGCGGGAG    180
 I  G  K  C  R  A  E  M  L  E  H  V  R  R  T  H  R  H  L  L  A  E  V  S  K  Q  V  E  R  E     60
CTGAAGGGGCTGCACCGGTCCGTGGGCAAGCTGGAGAGCAACCTGGATGGGTACGTGCCCACCAGCGACTCGCAGCGCTGGAAGAAGTCC    270
 L  K  G  L  H  R  S  V  G  K  L  E  S  N  L  D  G  Y  V  P  T  S  D  S  Q  R  W  K  K  S     90
ATCAAGGCCTGCCTGAGCCGCTGCCAGGAGACCATCGCCAACCTGGAGCGCTGGGTCAAGCGGGAGATGCACGTGTGGCGGGAGGTCTTC    360
 I  K  A  C  L  S  R  C  Q  E  T  I  A  N  L  E  R  W  V  K  R  E  M  H  V  W  R  E  V  F     120
TACCGGCTGGAGAGGTGGGCTGACCGCCTGGAGTCCGGGGGCGGCAAGTACCCGGTGGGCAGCGACCCAGCGCGCCACACCGTCTCCGTG    450
 Y  R  L  E  R  W  A  D  R  L  E  S  G  G  G  K  Y  P  V  G  S  D  P  A  R  H  T  V  S  V     150
GGCGTCGGGGGTCCCGAGAGCTACAGCCAGGAGGCAGACAACTACGACTACACTGTCAGCCCCTATGCCATTACCCCTCCACCGGCCGCC    540
 G  V  G  G  P  E  S  Y  S  Q  E  A  D  N  Y  D  Y  T  V  S  P  Y  A  I  T  P  P  P  A  A     180
GGCCAGCTGCCCGGGCAGGAGGAAGTGGAAGCCCAGCAGTACCCGCCCTGGGGGCCGGGGGAGGACGGGCAGCTGAGCCCCGGCGTGGAC    630
 G  Q  L  P  G  Q  E  E  V  E  A  Q  Q  Y  P  P  W  G  P  G  E  D  G  Q  L  S  P  G  V  D     210
ACACAGGTCTTCGAGGACCCGCGGGAGTTCCTCAGCCACCTGGAGGACTACCTGCGCCAGGTGGGTGGCTCCGAGGAGTACTGGCTGTCT    720
 T  Q  V  F  E  D  P  R  E  F  L  S  H  L  E  D  Y  L  R  Q  V  G  G  S  E  E  Y  W  L  S     240
CAGATCCAGAACCACATGAACGGGCCGGCCAAGAAGTGGTGGGAGTTCAAGCAGGGCTCGGTGAAGAACTGGGTGGAGTTCAAGAAGGAG    810
 Q  I  Q  N  H  M  N  G  P  A  K  K  W  W  E  F  K  Q  G  S  V  K  N  W  V  E  F  K  K  E     270
TTCCTGCAGTACAGCGAGGGCACGCTGTCCCGGGAGGCCATCCAGCGGGAGCTGGACCTGCCGCAGAAGCAGGGCGAGCCGCTGGACCAG    900
 F  L  Q  Y  S  E  G  T  L  S  R  E  A  I  Q  R  E  L  D  L  P  Q  K  Q  G  E  P  L  D  Q     300
TTCCTGTGGCGCAAGCGGGACCTGTACCAGACGCTGTATGTGGACGCCTGAGGAGGAGGAGATCATCCAGTACGTGGTGGGCACTCTGCAG    990
 F  L  W  R  K  R  D  L  Y  Q  T  L  Y  V  D  A  E  E  E  E  I  I  Q  Y  V  V  G  T  L  Q     330
CCCAAGCTCAAGCGTTTCCTAAGGCCCCCGCTGCCCAAGACCCTGGAGCAGCTCATCCAGAAGGGCATGGAGGTGCAGGATGGCCTGGAG   1180
 P  K  L  K  R  F  L  R  P  P  L  P  K  T  L  E  Q  L  I  Q  K  G  M  E  V  Q  D  G  L  E     360
CGGGCGGCCGAGCCCACTGGCCCCCACCCGCCCGCTGAGGAAGAGGCCGAGGCCCTCACGCCCGCCCTCACCAACGAGTCCGTAGCCAGC   1170
 R  A  A  E  P  T  G  P  H  P  P  A  E  E  E  A  E  A  L  T  P  A  L  T  N  E  S  V  A  S     390
GACCGGACTCAGCCCGAGTAG                                                                        1191
 D  R  T  Q  P  E  *                                                                          396
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Figure 2. Complete CDS of buffalo *Arc*/*Arg3.1* gene and its deduced amino acid sequence. ATG = start codon; TAG = stop codon; capital letters, complete CDS and amino acid sequence.

Table 1. Information of genetic variations for the CDS of the *Arc*/*Arg3.1* gene.

Buffalo	SNP	Genotype/individual number			Gene frequency	
		ww	wm	mm	w	m
River buffalo	c.63T>C	5	1	0	0.917	0.083
	c.228T>C	5	0	1	0.833	0.167
	c.558G>A	5	1	0	0.917	0.083
	c.625G>C	5	1	0	0.917	0.083
Swamp buffalo	c.63T>C	7	1	0	0.938	0.062
	c.228T>C	8	0	0	1.000	0.000
	c.558G>A	7	1	0	0.938	0.062
	c.625G>C	8	0	0	1.000	0.000

w = wild-type allele; m = mutant-type allele.

The pI and the Mw of buffalo *Arc*/*Arg3.1* were 5.4 and 45.2 kDa, respectively. With a hidden Markov model algorithm, transmembrane topology prediction showed that the buffalo *Arc*/*Arg3.1* gene was not a potential membrane protein. Then, the putative Arc/Arg3.1 protein was analyzed using the Prosite software (http://expasy.org/prosite). Four types of sites were found: *N*-myristoylation sites (9-GGlhAY-14, 63-GLhrSV-68, 140-GSdpAR-145, 153-GGpeSY-158, 181-GQlpGQ-186, 259-GSvkNW-264, 358-GLerAA-363), protein kinase C phosphorylation sites (45-ThR-47, 84-SqR-86, 90-SiK-92, 260-SvK-262, 390-SdR-392), casein kinase II phosphorylation sites (73-SnlD-76, 222-ShlE-225, 393-TqpE-396), and N-glycosylation sites (385-NESV-388). The prediction of secondary structure by SOPMA indicated that the deduced buffalo Arc/Arg3.1 contains 202 alpha helices, 29 extended strands, 15 beta turns, and 150 random coils (Figure 3).

Figure 3. Secondary structure of the buffalo Arc/Arg3.1 protein predicted by SOPMA. h = Alpha helix; e = extended strand; t = beta turn; c = random coil.

Analysis of sequence identity and evolutionary relationships of *Arc*/*Arg3.1*

The deduced protein sequence of buffalo *Arc*/*Arg3.1* was submitted to generate BLAST reciprocal best hits, and similarity comparison revealed that the buffalo Arc/Arg3.1 protein has high homology with those of 12 other species: *Bos grunniens* (99%), *B. taurus* (99%), *Capra hircus* (98%), *Cavia porcellus* (93%), *Papio anubis* (92%), *Otolemur gamettii* (92%), *Gorilla*

gorilla (92%), *Homo sapiens* (92%), *Pongo abelii* (92%), *Mus musculus* (92%), *Pan paniscus* (92%), and *Macaca mulatta* (92%) (Figure 4). To evaluate the evolutionary relationship of buffalo *Arc/Arg3.1* with other species, we constructed a phylogenetic tree on the basis of the *Arc*/*Arg3.1* amino acid sequences of buffalo and the other 12 species. Phylogenetic analysis revealed that the water buffalo *Arc*/*Arg3.1* gene is most closely related to the *Arc*/*Arg3.1* genes of *B. taurus* and *B. grunniens* than to that of the other 10 species (Figure 5).

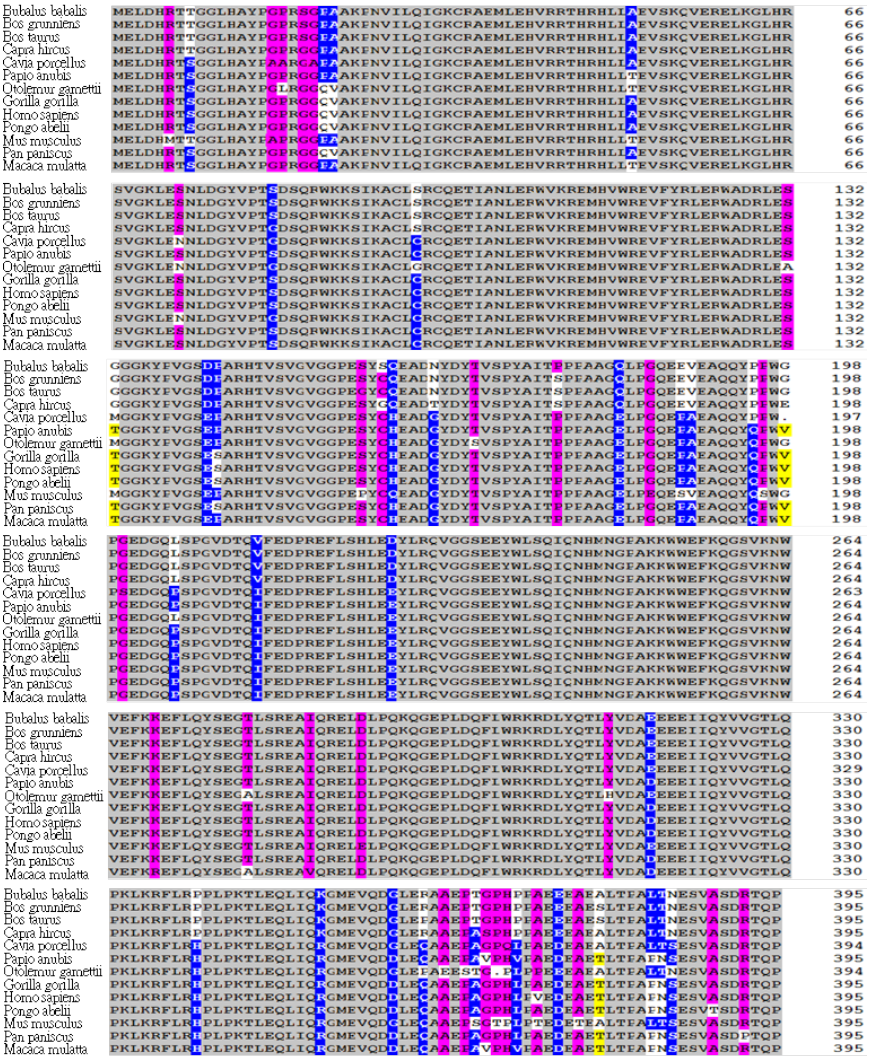

Figure 4. Alignment of the protein encoded by the *Bubalus bubalis Arc*/*Arg3.1* and other twelve kinds of *Arc*/*Arg3.1* from *Bos grunniens* (AFV31113), *Bos taurus* (NP_001193336), *Capra hircus* (AFV31115), *Cavia porcellus* (XP_003473338), *Papio Anubis* (XP_003903261), *Otolemur gamettii* (XP_003803326), *Gorilla gorilla* (XP_004047647), *Homo sapiens* (NP_056008), *Pongo abelii* (XP_002819540.2), *Mus musculus* (NP_061260), *Pan paniscus* (XP_003819534), and *Macaca mulatta* (AFE66548).

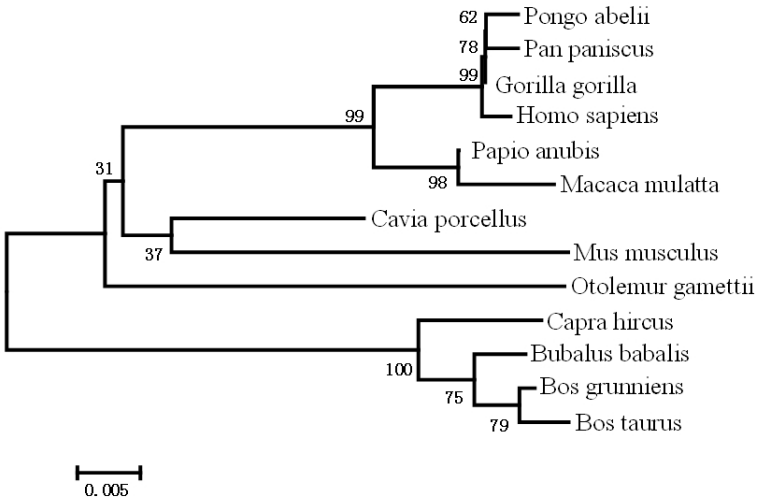

Figure 5. Phylogenetic tree for thirteen kinds of Arc/Arg3.1 protein from *Bubalus babalis* (AFV31112), *Bos grunniens* (AFV31113), *Bos Taurus* (NP_001193336), *Capra hircus* (AFV31115), *Cavia porcellus* (XP_003473338), *Papio Anubis* (XP_003903261), *Otolemur gamettii* (XP_003803326), *Gorilla gorilla* (XP_004047647), *Homo sapiens* (NP_056008), *Pongo abelii* (XP_002819540.2), *Mus musculus* (NP_061260), *Pan paniscus* (XP_003819534), and *Macaca mulatta* (AFE66548).

Location of potential miRNA targets

We used a web-based miRNA predicting program (miRBase; http://www.mirbase.org/) to locate conserved potential miRNA targets. Results showed that nine *B. taurus* miRNAs (bta-miR-490, bta-miR-2890, bta-miR-193b, bta-miR-328, bta-miR-665, bta-miR-671, bta-miR-2330-5p, bta-miR-2416, and bta-miR-6517) were found to have their target sites in the buffalo *Arc*/*Arg3.1* sequence. They were 666-ccaccuggaggacuaccugc-685, 1087-ggccuggagc gggcgg-1072, 1096-acuggccccccacccgcccgcu-1116, 1068-cagaagggcauggaggugcag-1048, 1124-aggccgaggccc-1135, 1067-aggauggccuggagcgggcgg-1087, 1032-gcccccgcugcccaagacc-1014, 1110-gcccgcugaggaagagg-1126, and 1140-gccgaggcccucacg-1126.

mRNA tissue-differential expression profile

To evaluate relative expression levels of buffalo *Arc*/*Arg3.1* mRNA in various buffalo tissues, semi-quantitative RT-PCR was performed in the 15 types of buffalo tissues mentioned above. The continuously expressed gene *18S rRNA* served as an endogenous reference for determination of targeted mRNA profiles. Results revealed similar expression patterns in the two types of buffalo. The buffalo *Arc*/*Arg3.1* gene was moderately expressed in the hypophysis and placenta, weakly expressed in kidney, milk, mammary gland, cerebrum, lung, heart, rumen, fat, and uterus, and almost silent in muscle, liver, and skin (Figure 6).

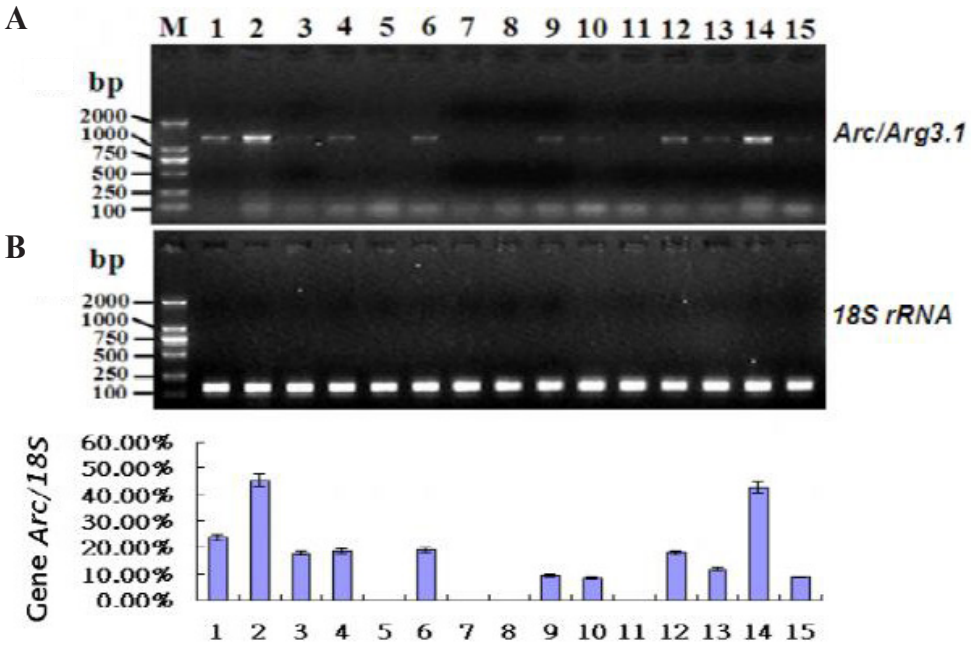

Figure 6. Tissue expression profile of buffalo Arc/Arg3.1 gene. The 18S expression level is used for the internal control. *Lane M* = DL2000 DNA marker; *lane 1* = kidney; *lane 2* = hypophysis; *lane 3* = milk; *lane 4* = mammary gland; *lane 5* = muscle; *lane 6* = cerebrum; *lane 7* = liver; *lane 8* = skin; *lane 9* = lung; *lane 10* = heart; *lane 11* = jejunum; *lane 12* = rumen; *lane 13* = fat; *lane 14* = placenta; *lane 15* = uterus.

DISCUSSION

In the present study, we isolated the *Arc*/*Arg3.1* gene from river buffalo and swamp buffalo. The obtained coding sequences of the *Arc*/*Arg3.1* gene from both buffalo types are 1191 bp in length and encode a 396 amino acid protein. Sequences of the two types of buffalo showed almost identical base compositions. Three synonymous substitutions and one non-synonymous substitution were found in the buffalo populations. The synonymous substitution rate was greater than the non-synonymous substitution rate, which implied that the *Arc*/*Arg3.1* gene was highly conserved and evolution of the gene was limited by its function in buffalo. The values of subPSEC and $P_{deleterious}$ (subPSEC = -1.4039, $P_{deleterious}$ = 0.1685) indicated that SNP625 had no significant effect on Arc/Arg3.1 function.

Among the four SNPs found in this study, SNP63 and SNP558 were shared between river and swamp buffalo with almost the same gene frequency pattern. In contrast, SNP228 and SNP625 were detected only in river buffalo, which were already homozygous in swamp buffalo. Therefore, the genetic compositions in both buffalo populations appear to be different for these genetic variations. Considering the small sample size used in this study, it is necessary to expand the sample size to confirm this result further.

The alignment analyses showed that the buffalo *Arc*/*Arg3.1* gene was highly homologous with *Arc*/*Arg3.1* of *B. taurus*, *B. grunniens*, and other mammals. The phylogenetic analysis further revealed that the buffalo *Arc*/*Arg3.1* gene was most closely related with that of *B.*

taurus and *B. grunniens*. This implies that the Arc/Arg3.1 proteins of bovine species have few functional differences.

Most protein functions are regulated by phosphorylation/dephosphorylation and glycosylation/deglycosylation. In this study, several types of functional sites (such as glycosylation sites, phosphorylation sites, myristoylation sites, and ribokinase protein domains) were found in the Arc/Arg3.1 protein of buffalo, which suggests that the buffalo Arc/Arg3.1 protein may play important functional roles through these sites and domains.

MicroRNAs are small noncoding single-stranded RNAs that play vital roles in gene expression regulation by binding or regulating the translation of their target mRNAs. Nine *B. taurus* microRNAs were found to have their predicted target sites in the buffalo *Arc*/*Arg3.1* coding sequence, and further investigation is needed to confirm whether the corresponding microRNA molecules can regulate *Arc*/*Arg3.1* gene expression in buffalo.

Tissue expression profile analysis revealed that the *Arc*/*Arg3.1* gene was obviously differentially expressed in various tissues, although it demonstrated similar expression patterns in the two types of buffalo. As functions at protein levels were not investigated, there are several possible reasons for differential expression of this immediate-early gene. One suitable explanation given the conditions of the experiment, is that the biological activities associated with the functions of the gene were required to a different extent in different tissues at the same time.

The immediate-early gene was named *Arc* by Worley and *Arg3.1* by Dietmar Kuhi in 1995, and there has been much progress in understanding this important gene within the last decade. Most *Arc*/*Arg3.1* studies have focused on the acquisition and consolidation phases of a variety of memory tasks in rats (Guzowski, 2002; Stephanie and Schafe, 2011), and the role of Arc/Arg3.1 in memory reconsolidation processes in humans (Kida et al., 2002; Gusev et al., 2005; Tronson et al., 2006; Messaoudi et al., 2007). However, little information about the structure and function of this protein is available for buffalo and other domestic animals. Due to the absence of clear structural data, the molecular function of this protein remains an unresolved problem for biochemists. Therefore, the results of this study will be extremely important for elucidating the structure and essential physiological function of the Arc/Arg3.1 protein in buffalo and other bovines in the future.

ACKNOWLEDGMENTS

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REFERENCES

Alberini CM (2005). Mechanisms of memory stabilization: are consolidation and reconsolidation similar or distinct processes? *Trends Neurosci.* 28: 51-56.

Ben MC, Gamache K and Nader K (2006). NMDA receptors are critical for unleashing consolidated auditory fear memories. *Nat. Neurosci.* 9: 1237-1239.

Chowdhury S, Shepherd JD, Okuno H, Lyford G, et al. (2006). Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. *Neuron* 52: 445-459.

Gusev PA, Cui C, Alkon DL and Gubin AN (2005). Topography of Arc/Arg3.1 mRNA expression in the dorsal and ventral hippocampus induced by recent and remote spatial memory recall: dissociation of CA3 and CA1 activation. *J. Neurosci.* 25: 9384-9397.

Guzowski JF (2002). Insights into immediate-early gene function in hippocampal memory consolidation using antisense oligonucleotide and fluorescent imaging approaches. *Hippocampus* 12: 86-104.

Guzowski JF, Lyford GL, Stevenson GD, Houston FP, et al. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J. Neurosci.* 20: 3993-4001.

Kida S, Josselyn SA, Pena de OS, Kogan JH, et al. (2002). CREB required for the stability of new and reactivated fear memories. *Nat. Neurosci.* 5: 348-355.

Kumar S, Nei M, Dudley J and Tamura K (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief. Bioinform.* 9: 299-306.

Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, et al. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* 14: 433-445.

Messaoudi E, Kanhema T, Soule J, Tiron A, et al. (2007). Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus *in vivo*. *J. Neurosci.* 27: 10445-10455.

Miao YW, Li DL, Huo JL and Zhang CX (2008). A review on genetic diversity and origin of Chinese buffalo. *China Cattle Sci.* 34: 16-20.

Plath N, Ohana O, Dammermann B, Errington ML, et al.(2006). Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron* 52:437–444.

Ploski JE, Pierre VJ, Smucny J, Park K, et al. (2008). The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for memory consolidation of pavlovian fear conditioning in the lateral amygdala. *J. Neurosci.* 28: 12383-12395.

Stephanie AM and Schafe GE (2011). The activity-regulated cytoskeletal-associated protein (Arc/Arg3.1) is required for reconsolidation of a Pavlovian fear memory. *J. Neurosci.* 31: 7073-7082.

Steward O, Wallace CS, Lyford GL and Worley PF (1998). Synaptic activation causes the mRNA for the IEG Arc to localize selectively near activated postsynaptic sites on dendrites. *Neuron* 21: 741-751.

Tronson NC, Wiseman SL, Olausson P and Taylor JR (2006). Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. *Nat. Neurosci.* 9: 167-169.