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Identification, CDNA Cloning and Characterization of *Pax3* Gene in Chinese Domestic Goose

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Abstract: Paired box (Pax) protein 3 is a member of the Pax family of transcription factors, plays critical roles in muscle development. Many studies showed that *Pax3* gene was a functional candidate gene for production and meat quality. However, the sequence information of goose *Pax3* gene remains unknown. In this study, the comparativegenomicstechnology was used to clone the cDNA sequence of *Pax3* gene from the breast muscle tissue of Zhedong goose. The gene structures were analyzed by the bioinformatics software and the mRNA expression profile of *Pax3* gene in different tissues was measured by the semi-quantitative RT-PCR. The goose Pax3 full-length coding sequence consisted of 1327 bp and encoded 421 amino acids. Sequence analysis displayed that a splicing variant of goose Pax3 absence of a glutamine residue was also identified. Both of the Pax3-a (inclusion of a glutamine residue) and Pax3-b (exclusion of a glutamine residue) isoforms were predicted contain three conserved domains (a paired domain, an octapeptide region and a homeodomain) and were highly conserved (>90%) relative to the known Pax3 proteins from other species. Multiple sequence alignments and phylogenetic analysis displayed that the deduced goose Pax3 proteins have a close genetic relationship and evolutional distance with the Pax3 proteins in other avian species, especially in ducks. The semi-quantitative RT-PCR results revealed Pax3 mRNA was highly expressed in the breast muscle tissue, followed by the lung, leg muscle and brain, little or no expression was observed in heart, liver, spleen, kidney, muscular stomach, intestine and sebum. These findings will help us understand the functions of the *Pax3* gene and the molecular breeding in Chinese domestic goose.

Key words: Goose, Pax3, gene cloning, bioinformatics analysis, alternatively spliced transcript, molecular characterization, RT-PCR, gene expression

INTRODUCTION

The paired box (Pax) is a DNA-binding domain which was originally identified in the Drosophila segmentation gene paired (Frigerio *et al*., 1986; Bopp *et al*., 1986). Subsequently, several genes contained a paired-box and the conserved DNA-binding motifs were found in other species and defined them as the paired-box gene family (Noll, 1993; Loosli *et al*., 1996; Glardon *et al*., 1997). In vertebrates, 9 subtypes (Pax1-Pax9) of the Pax family have been identified. Based on the sequence characteristics and the primary structure of the paired domains the Pax genes family was classified into four major subfamilies: Pax1/9, Pax2/5/8, Pax3/7 and Pax4/6 (Walther *et al*., 1991; Gruss and Walther, 1992; Strachan and Read, 1994; Seo *et al*., 1998). All Pax proteins are transcription factors and share the conserved PD which is the most conserved functional motif and exhibits specific DNA-binding activity (Chalepakis *et al*., 1992). Pax3 is a member of the Pax gene family of evolutionary conserved and developmentally regulated transcription factors (Stuart *et al*., 1993). Pax3 cDNA was first cloned from murine (Goulding *et al*., 1994). Subsequently, it was identified and characterized in different species. Similar to other gene in Pax family, Pax3 has all the typical characteristics of a Pax protein including a Paired Domain (PD), a paired-type Homeodomain (HD) and an Octapeptide region (OP) (Gruss and Walther, 1992). Several studies identified that Pax3 was a key regulator of normal skeletal muscle differentiation where it has a central role in activating the MyoD expression that initiates the myogenic program (Maroto *et al*., 1997; Rawls and Olson, 1997; Tajbakhsh *et al*., 1997).

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In Zebrafish, several isoforms and splice variants of Pax3 have been identified and study displayed that Pax3 play important roles in adult growth and myofiber maintenance (Seo *et al*., 1998; Kirkpatrick *et al*., 2010). In humans, studies showed that mutations in Pax3 could caused Waardenburg syndrometype 1 (Jalilian *et al*., 2015; Wang *et al*., 2010). In mouse, recent results have underlined the importance of the Pax 3/7 population of cells for skeletal muscle development and regeneration. In chicken, the different expression profiles of *Pax3* gene in postnatal skeletal muscle in lines of chickens divergently selected for high and low weight body weight were identified (Yin *et al*., 2014). In ducks, study revealed that *Pax3* gene played important roles in muscle development and its association with some production traits have been reported (Zhang *et al*., 2014). However, very little is known about *Pax3* gene in goose. The present research aimed to clone the cDNA of the goose *Pax3* gene and phylogenetic analysis was performed. Furthermore, the two alternative spliced variants were identified and the normal expression pattern of this gene in different tissues was carried out. The data obtained from this study will aid in understanding the function of *Pax3* gene in Chinese domestic goose.

MATERIALS AND METHODS

Animal and tissues sampling: Three healthy female Zhedong white geese (12 week old) were collected from Institute of Zhedong white goose (xiang shan) and all geese were set free in an open ground with a free swimming pool and reared under normal management conditions. A total of 11 tissues including heart, liver, spleen, lung, kidney, breast muscle, leg muscle, brain, intestine, muscular stomach and sebum were sampled from each goose and used for tissue distribution analysis. All geese were slaughtered by a ventral cut of the neck blood vessels with 10 sec after the end of the stun. The tissues were collected immediately, frozen in liquid nitrogen and stored at -80°C until RNA extraction. Blood samples were withdrawn from the three Zhedong-White geese, stored at -20°C until genomic DNA was extracted. All animal procedures were handled in compliance with the Law of the People's Republic of China on Animal Protection.

RNA extracted and cDNA synthesis: Total RNA was extracted from the muscle and other tissues using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). DNaseI treatment was used to remove any contaminated genomic DNA from the total RNA. First-strand cDNA was synthesized using a PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Japan) according to the manufacture's instruction. The first strand cDNA from the muscle tissue was used to clone the cDNA of goose *Pax3* gene. The cDNA of each tissue was used for *Pax3* gene expression analysis.

Molecular cloning of *Pax3* gene: Based on the mRNA sequences of Pax3 in duck (GI: 880796889) and chicken (GI: 45383597), 4 pairs of primers (Pax3-F1/Pax3-R1 Pax3-F4/Pax3-R4, Table 1) were designed to amplify the complete coding sequence of goose *Pax3* gene. The PCR products were amplified using a PCR Mix (Trans Gen Biotech Co., Ltd., Beijing, China) following the manufacture's instruction. The PCR procedure was as follows: 94°C for 5 min; 35 cycles of 30 sec at 94°C, 30 sec at annealing temperature and 72°C for 30 sec and 72°C for 5 min.

All PCR products were gel-purified using the Gel Extraction Kit (Trans Gen Biotech, Co., Ltd. Beijing, China) ligated into the PMD19-T vector (TaKaRa, Japan) and transformed into the Trans-T1 competent cells. Positive clones were identified and sequenced by Sangon Biotech, Co., Ltd. (Shanghai, China).

Table 1: Primers used in the present study

Primers purpose	Primer name	Primer sequence (5'→3')	Product size (bp)	Tm (°C)
cDNA cloning	Pax3-F1	ATGACCACGCTGGCCGGGGCCGTC	695	68.2
	Pax3-R1	AGCTCTTCCAGTTGCTCTGCTGT		
	Pax3-F2	CGACAAGAAGGCGAAGCACA	605	58.9
	Pax3-R2	TGGCAGGGTTCATGGGATTT		
	Pax3-F3	CTACCAGCCCACCTCCATAC	478	57.9
	Pax3-R3	TTGTCCGTACTGGTAGCCTG		
	Pax3-F4	AAACTGATTATGCCCTGTCC	236	54.9
	Pax3-R4	TTATGCAATATCTGGCTTCA		
Expression profile	Pax3-RT-F	AGAAAGCAGCGTCGTAGCAG	275	58.0
	Pax3-RT-R	GAGAGCTGGTATGTCGGCAA		
Internal control	β-actin-F	TCCGTGACATCAAGGAGAAG	224	58.0
	β-actin-R	CATGATGGAGTTGAAGGTGG		

Bioinformatic and phylogenetic analysis: The obtained sequences were matched using DNAMAN software (Pointe-Claire, Canada). Sequence similarity was analyzed with BLAST. The Open Reading Frame (ORF) analysis was performed using a NCBI ORF Finder. Multiple sequence alignment of the deduced amino acid sequence was performed using clustal omega online software and edited with BOXSHADE. Calculated Molecular weight (Mw) and predicted isoelectric point (pI) were obtained by using the ExPASy online software. The potential domains of amino acid sequences were analyzed by I-TASSER program. Phylogenetic and molecular evolutionary analyses were constructed on the basis of the deduced amino acid sequences of other reported species available at NCBI using MEGA 6.0 and based on the Neighbor-Joining (NJ) method with a bootstrap of 1000 repetitions (Tamura *et al*., 2011).

Expression of Pax3 mRNA in domestic goose: To study the mRNA expression level of goose Pax3, semi quantitative RT-PCR were performed using total RNA isolated from the above 11 tissues. The PCR programs included a denaturation step of 5 min at 94°C, followed by 28-36 cycles of 30 sec at 94°C, 30 sec at 58°C, 30 sec at 72°C and a final step of 5 min. As control, a pair of primers (β-actin-F/β-actin-R, Table) was used under the above conditions. The PCR reactions were optimized for a number of cycles to ensure product intensity within the linear phase of amplification. PCR products were visualized on 2.5% agarose gels stained with ethidium bromide and visualized with ultraviolet light.

RESULTS AND DISCUSSION

Molecular cloning of the goose Pax3 cDNA: Using the comparative cloning and sequence matching techniques, a total of 1449 bp length cDNA sequence was obtained by the four pairs of primers (Pax3-F1/R1-Pax3-F4/R4, Table), clear amplified band of each pair of primer was shown in Fig. 1. Nucleotide sequence analysis showed that the Pax3-F1/Pax3-R1 primers amplified another short product which lacked a 3 bp fragment than the long one. Thus, two complete cDNA sequences, one of 1449 bp ORF and the other 1446 bp ORF were generated due to a 3 bp fragment deletion and resulted in the glutamine absence. In human, murine and zebra fish, Pax3 encoded the same two isoforms that differ in the inclusion or exclusion of a glutamine residue at the end of exon 4 had been reported and characterized (Seo *et al*., 1998; Vogan *et al*., 1996; Parker *et al*., 2004). BLAST analysis showed that the two mRNAs shared high level of homology to the published Pax3 sequences of other species. Therefore, we claimed that we successfully identified the two spliced isoforms of goose Pax3, one variant inclusion of a glutamine residue (Pax3-a, Q+) the other exclusion of a glutamine residue (Pax3-b, Q-). Except the above two Pax3 variants we have not detected the other seven alternatively spliced transcripts which have been identified in human (Parker *et al*., 2004). Unlike chicken and ducks the complete cDNA sequence of goose *Pax3* gene still remain unknown. The obtained cDNA sequences in this study will facilitate the future research on the gene function.

Amino acid sequence analysis of Pax3 proteins: The prediction results from the Swiss Institute of bioinformatics software showed that the Pax3-a variant (Genbank: KP965884) encoded a protein of 482 amino acids (aa) with a Molecular Weight (MW) of about 53.20 kDa and an isoelectric point (pI) of 8.81. The Pax3-b variant (Genbank: KT380623) encoded a protein of 481 aa with a calculated MW of about 53.07 kDa and the same pI. Similar to its prologue Pax7 of Pax gene family the two predicted proteins contained three conserved domains: a Paired Domain (PD) an Octapeptide Region (OP) and a

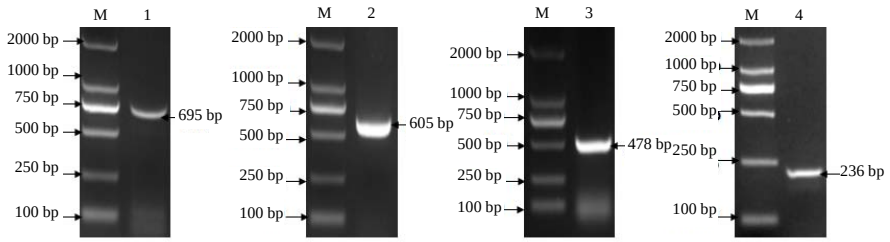

Fig. 1: The PCR amplification results of goose *Pax3* gene; Lane M: DL2000 Marker; Lane 1, 2, 3 and 4, amplification the cDNA fragment used the primers Pax3-F1/Pax3-R1, Pax3-F2/Pax3-R2, Pax3-F3/Pax3-R3 and Pax3-F4/Pax3-R4, respectively

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ATGACCACGCTGGCCGGGGCCGTCCCCAGGATGATGCGGCCCGGGGCCGGGCAGAACTACCCCCGCAGCG    90
GCTTCCCGCTGGAAGTCCCT
 M T T L A G A V P R M M R P G A G Q N Y P R S G F P L E V P                30
ACTCCTCTAGGCCAGGGCAGAGTCAATCAGCTCGGAGGAGTGTTTATCAATGGCAGGCCTTTACCCAACCA  180
TATCCGACACAAGATAGTG
 T P L G Q G R V N Q L G G V F I N G R P L P N H I R H K I V                60
GAGATGGCCCACCATGGCATAAGGCCCTGTGTCATCTCTCGCCAGCTGCGAGTGTCCCACGGCTGCGTCTC  270
CAAAATTCTCTGCAGGTAC
 E M A H H G I R P C V I S R Q L R V S H G C V S K I L C R Y                90
CAGGAGACGGGCTCCATCCGGCCGGGGGCCATCGGCGGCAGCAAGCCCAAGCAGGTGACGACGCCGGAC  360
GTGGAGAAGAAAATCGAGGAA
 Q E T G S I R P G A I G G S K P K Q V T T P D V E K K I E E                120
TACAAGCGGGAAAACGCCGGCATGTTCAGCTGGGAGATCCGAGACAAGCTGCTGAAGGACGGCGTGTGC    450
GACCGCAACACGGTGCCCTCG
 Y K R E N A G M F S W E I R D K L L K D G V C D R N T V P S                150
GTGAGCTCCATCAGCCGCATCCTGCGGAGCAAGTTTGGGAAAGGCGAGGAGGAGGAGGCCGAGCTGGAG    540
AGGAAGGAGGCGGAGGAAGGC
 V S S I S R I L R S K F G K G E E E E A E L E R K E A E E G                180
GACAAGAAGGCGAAGCACAGCATCGACGGCATCCTCAGCGAGAGAGCACCCCAGTCTGATGAAGGCTCT    630
GACATTGATTCTGAACCAGAC
 D K K A K H S I D G I L S E R A P Q S D E G S D I D S E P D                210
TTACCTTTAAAAAGAAAGCAGCGTCGTAGCAGGACAACCTTCACAGCAGAGCAACTGGAAGAGCTGGAA    720
AGAGCTTTTGAGAGGACACAC
 L P L K R K Q R R S R T T F T A E Q L E E L E R A F E R T H                240
TACCCTGACATTTATACTCGGGAAGAACTTGCGCAAAGAGCCAAACTCACGGAGGCTCGAGTTCAGGTCT    810
GGTTTAGTAATCGTCGTGCT
 Y P D I Y T R E E L A Q R A K L T E A R V Q V W F S N R R A                270
AGATGGAGGAAACAGGCAGGAGCCAACCAACTGATGGCTTTCAACCATCTGATCCCAGGAGGATTTCCAC    900
CCAGTGCCATGCCAACTTTG
 R W R K Q A G A N Q L M A F N H L I P G G F P P S A M P T L                300
CCGACATACCAGCTCTCCGAGCCATCCTACCAGCCCACCTCCATACCGCAAGCCGTGTCTGATCCAAGCAG    990
TACAGTCCATAGACCTCAG
 P T Y Q L S E P S Y Q P T S I P Q A V S D P S S T V H R P Q                330
CCTCTTCCTCCAAGCACTGTACACCAAAGCAGCCTCCCTTCGAATCCAGAGAGCAGCACTGCCTATTGCCT  1080
ACCCAGCACCAGGCATGGA
 P L P P S T V H Q S S L P S N P E S S T A Y C L P S T R H G                360
TTTTCCAGCTATACAGACAGCTTTGTGCCTCCGTCCGGGCCCTCAAATCCCATGAACCCTGCCATTGGCAAT  1170
GGCCTTTCACCTCAGGTA
 F S S Y T D S F V P P S G P S N P M N P A I G N G L S P Q V                390
ATGGGACTCTTGACTAACCATGGTGGTGTGCCCCACCAGCCTCAAACTGATTATGCCCTGTCCCCTTTGACT  1260
GGGGGCCTGGAGCCCACC
 M G L L T N H G G V P H Q P Q T D Y A L S P L T G G L E P T                420
ACCACGGTCTCAGCCAGCTGCAGTCAGCGGCTAGAGCACATGAAGAGTTTAGACAGCCTGCCTACATCCC  1350
AGTCCTACTGCCCACCGACC
 T T V S A S C S Q R L E H M K S L D S L P T S Q S Y C P P T                450
TACAGCACCACAGGTTACAGCATGGACCCTGTCACAGGCTACCAGTACGGACAATATGGACAAAGTGCCTT  1440
TCATTATCTGAAGCCAGAT
 Y S T T G Y S M D P V T G Y Q Y G Q Y G Q S A F H Y L K P D                480
ATTGCATAA                                                                 1449
 I A *                                                                     482
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Fig. 2: Composite nucleotide and deduced amino acid sequences of the goose *Pax3* gene; the letters are underlined indicate the start codon (ATG), the asterisk denotes the stop codon (TAA). The Paired Domain (PD), Octape Ptide region (OP) and paired-type Homeo Domain (HD) are indicated by dotted line, wave line and double line, respectively. The glutamine (Q) in the paired domain is shown in bold

Homeodomain (HD) which is consistent with the structure of human, mouse and zebra fish Pax3 as reported by Seo *et al.* (1998). Meanwhile, both of the deduced proteins lacked an apparent signal peptide sequence and no obvious transmembrane helices (Fig. 2). This also reveals the Pax3 and Pax7 are generally highly conserved in different species.

Homology and phylogenetic analysis: To evaluate the relationship between the deduced goose Pax3 and orthologs in other species, a phylogenetic tree was constructed on the known Pax3 amino acid sequences. The tree showed three major branches with strong bootstrap support, the avian species clustered into one branch separate from mammals and zebra fish which occupied the other two branches (Fig. 3 and 4). The phylogenetic analysis demonstrated that the goose Pax3 exhibited a closer genetic relationship with those of other birds. The Pax3 phylogeny is consistent with the studies on other genes in goose (Wang *et al*., 2014; Zhang *et al*., 2016).

Comparison of the deduced goose Pax3 sequence with the known Pax3 proteins of other birds, it shared fairly high homology with that of *Anas platyrhynchos*

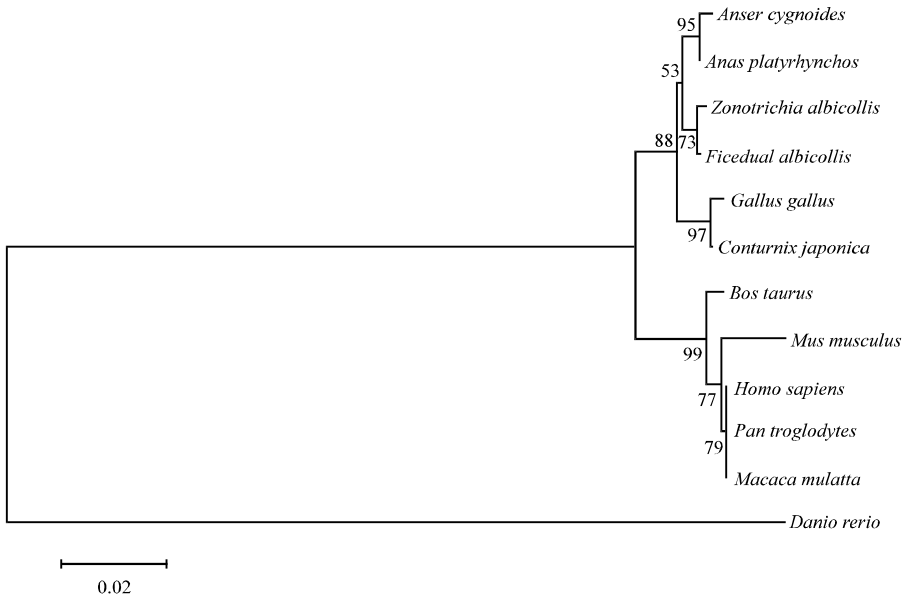

Fig. 3: Phylogenetic analysis of Pax3 proteins; Neighbor-Joining (NJ) analysis based on the Jone-Taylor-Thorton (JTT) model with 1000 bootstrap replicates was performed by the MEGA 6.0 Software. Numbers at each branch denote the bootstrap majority consensus values on 1000 replicates. The branch lengths represent the relative genetic distance among these species

Paired Domain (PD)

A. cygnoides	MTTLAGAVPRMMRPGAGQNYPRSGFPLEVPTPLGQGRVNQLGGVFINGRPLPNHIRHK	110
	IVEMAHHGIRPCVISRQLRVSHGCVSKILCRYQETGSIRPGAIGGSKPKQVT	
A. platyrhynchos	MTTLAGAVPRMMRPGAGQNYPRSGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHK	110
	IVEMAHHGIRPCVISRQLRVSHGCVSKILCRYQETGSIRPGAIGGSKPKQVT	
Z. albicollis	MTTLAGAVPRMMRPGAGQNYPRSGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHK	110
	IVEMAHHGIRPCVISRQLRVSHGCVSKILCRYQETGSIRPGAIGGSKPKQVT	
F. albicollis	MTTLAGAVPRMMRPGAGQNYPRSGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHK	110
	IVEMAHHGIRPCVISRQLRVSHGCVSKILCRYQETGSIRPGAIGGSKPKQVT	
G. gallus	MTTLAGAVPRMMRPGAGQSYPRGGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHK	110
	IVEMAHHGIRPCVISRQLRVSHGCVSKILCRYQETGSIRPGAIGGSKPKQVT	
C. japonica	MTTLAGAVPRMMRPGAGQSYPRGGFPLEVSTPLGQGRVNQLGGVFINGRPLPNHIRHK	110
	IVEMAHHGIRPCVISRQLRVSHGCVSKILCRYQETGSIRPGAIGGSKPKQVT	

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Octapeptide region (OP)

A. cygnoides	TPDVEKKIEEYKRENAGMFSWEIRDKLLKDGVCDRNTVPSVSSISRILRSKFGKGEEEEA	
	ELERKEAEEGDKKAKHSIDGILSERA--PQSDEGSDIDSEPDLPLKRKQR	218
A. platyrhynchos	TPDVEKKIEEYKRENAGMFSWEIRDKLLKDGVCDRNTVPSVSSISRILRSKFGKGEEEEA	
	ELERKEAEEGDKKAKHSIDGILSERA--PQSDEGSDIDSEPDLPLKRKQR	218
Z. albicollis	TPDVEKKIEEYKRENAGMFSWEIRDKLLKDGVCDRNSVPSVSSISRILRSKFGKGEEEEA	
	ELERKEVEEGDKKAKHSIDGILSERA--PQSDEGSDIDSEPDLPLKRKQR	218
F. albicollis	TPDVEKKIEEYKRENAGMFSWEIRDKLLKDGVCDRNSVPSVSSISRILRSKFGKGEEEEA	
	ELERKEVEEGDKKAKHSIDGILSERA--PQSDEGSDIDSEPDLPLKRKQR	218
G. gallus	TPDVEKKIEEYKRENAGMFSWEIRDRLLKDGVCDRNTVPSVSSISRILRSKFGKGEEEEA	
	ELERKEAEEGDKKAKHSIDGILSERASAAQSDEGSDIDSEPDLPLKRKQR	220
C. japonica	TPDVEKKIEEYKRENAGMFSWEIRDRLLKDGVCDRNTVPSVSSISRILRSKFGKGEEEEA	
	ELERKEAEEGDKKAKHSIDGILSERA--PQSDEGSDIDSEPDLPLKRKQR	218

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Fig. 4: Continue

Homeodomain (HD)

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A. cygnoides          RSRTTFTAEQLEELERAFERTHYPDIYTREELAQRAKLTEARVQVWFSNRRARWR 328
                      KQAGANQLMAFNHLIPGGFPPSAMPTLPTYQLSEPSYQPTSIPQAVSDPSSTVHR
A. platyrhynchos      RSRTTFTAEQLEELERAFERTHYPDIYTREELAQRAKLTEARVQVWFSNRRARWR 328
                      KQAGANQLMAFNHLIPGGFPPSAMPTLPTYQLSEPSYQPTSIPQAVSDPSSTVHR
Z. albicollis         RSRTTFTAEQLEELERAFERTHYPDIYTREELAQRAKLTEARVQVWFSNRRARWR 328
                      KQAGANQLMAFNHLIPGGFPPSAMPTLPTYQLSEPSYQPTSIPQAVSDPSSTVHR
F. albicollis         RSRTTFTAEQLEELERAFERTHYPDIYTREELAQRAKLTEARVQVWFSNRRARWR 328
                      KQAGANQLMAFNHLIPGGFPPSAMPTLPTYQLSEPSYQPTSIPQAVSDPSSTVHR
G. gallus             RSRTTFTAEQLEELERAFERTHYPDIYTREELAQRAKLTEARVQVWFSNRRARWR 330
                      KQAGANQLMAFNHLIPGGFPPSAMPTLPTYQLSEPSYQPTSIPQAVSDPSSTVHR
C. japonica           RSRTTFTAEQLEELERAFERTHYPDIYTREELAQRAKLTEARVQVWFSNRRARWR 328
                      KQAGANQLMAFNHLIPGGFPPSAMPTLPTYQLSEPSYQPTSIPQAVSDPSSTVHR

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A. cygnoides          PQPLPPSTVHQSSLPSNPESSTAYCLPSTRHGFSSYTDSFVPPSGPSNPMNPAIGNGL 428
                      SPQVMGLLTNHGGVPHQPQTDYALSPLTGGLEPTTTVSASCSQRLEHMKSLD
A. platyrhynchos      PQPLPPSTVHQSSLPSNPESSTAYCLPSTRHGFSSYTDSFVPPSGPSNPMNPAIGNGL 428
                      SPQVMGLLTNHGGVPHQPQTDYALSPLTGGLEPTTTVSASCSQRLEHMKSLD
Z. albicollis         PQPLPPSTVHQSSLPSNPESSSAYCLPSTRHGFSSYTDSFVPPSGPSNPMNPAIGNGL 428
                      SPQVMGLLTNHGGVPHQPQTDYALSPLTGGLEPTTTVSASCSQRLEHMKSLD
F. albicollis         PQPLPPSTVHQSSLPSNPESSSAYCLPSTRHGFSSYTDSFVPPSGPSNPMNPAIGNGL 428
                      SPQVMGLLTNHGGVPHQPQTDYALSPLTGGLEPTTTVSASCSQRLDHMKSLD
G. gallus             PQPLPPSTVHQSSLPSNPESSSAYCLPSTRHGFSSYTDSFVPPSGPSNPMNPAIGNGL 440
                      SPQVMGLLTNHGGVPHQPQTDYALSPLTGGLEPTTTVSASCSQRLDHMKSLD
C. japonica           PQPLPPSTVHQSSLPSNPESSSAYCLPSTRHGFSSYTDSFVPPSGPSNPMNPAIGNGL 428
                      SPQVMGLLTNHGGVPHQPQTDYALSPLTGGLEPTTTVSASCSQRLDHMKSLD

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A. cygnoides          A SLPTSQSYCPPTYSTTGYSMDPVTGYQYGQYGQSAFHYLKPDIA 482
A. platyrhynchos      SLPTSQSYCPPTYSTTGYSMDPVTGYQYGQYGQSAFHYLKPDIA 482
Z. albicollis         SLPTSQSYCPPTYSTTGYSMDPVTGYQYGQYGQSAFHYLKPDIA 482
F. albicollis         SLPTSQSYCPPTYSTTGYSMDPVTGYQYGQYGQSAFHYLKPDIA 482
G. gallus             SLPTSQSYCPPTYSTTGYSMDPVTGYQYGQYGQSAFHYLKPDIA 484
C. japonica           SLPTSQSYCPPTYSTTGYSMDPVTGYQYGQYGQSAFHYLKPDIA 482
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Fig. 4: Alignment of the deduced amino acid sequence of goose Pax3 with those of the other birds; the asterisk indicates residues that are identical among all birds. Dashes indicate gaps introduced to facilitate alignment. The dotted lines highlight the Paired Domain (PD), the wave lines highlight the Octapeptide region (OP) and the double lines highlight the paired-type Homeodomain (HD)

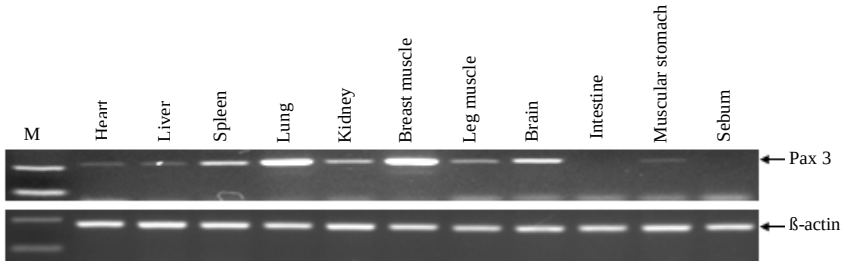

Fig. 5: Tissue distribution of the goose *Pax3* gene in different tissues

(NP-001297282, 99%), *Gallus gallus* (NP-989600, 98%) *Coturnix japonica* (XP-015726824, 99%), *Ficedula albicollis* (XP-005051031, 99%) and *Zonotrichia albicollis* (XP_014124146, 99%). Furthermore, PD, HD and OP domains were highly conserved in all birds sequences analyzed.

In mammals, the Pax3 protein of human consists of 483 aa that of monkey, mouse and cattle consists of 484 aa. In avians, the Pax3 protein of goose, duck, Japanese quail, white-throated sparrow and *Ficedula albicollis* all consist of 482 aa which also indicated that the Pax3 was highly evolutional conservation in avian species. The closer relationship in evolution and highly conservative structure suggest that the signifcance and conservatism of their biological functions during evolution in avian species.

Expression pattern of *Pax3* gene in different tissues: To investigate the tissue distribution of Pax3 mRNA, semi-quantitative RT-PCR was carried out with total RAN from the 11 tissues of Zhedong white goose. As shown in Fig. 5 the high Pax3 mRNA was found in the breast muscle tissue, followed by the lung, leg muscle and brain, little or no expression was observed in heart, liver, spleen, kidney, muscular stomach, intestine and sebum (Fig. 5).

In mouse, Kuang *et al.* (2006) showed that bothPax3 and Pax7 were required for postnatal skeletalmuscle developmentand regeneration (Kuang *et al.*, 2006). In poultry, breast muscle and leg muscle are the two main productive muscle organs as a model to analyze the mechanisms ofmuscle development. In Peking duck the breast muscle and leg muscle showed significantly different muscle mass gain and study reported that the breast muscle had a stronger capacity for both protein synthesis and protein degradation compared with leg muscle (Zhang *et al.*, 2014). In our study, the high expression of goose *Pax3* gene in the breast muscle tissue reveals that this gene may have a key role in goose muscle development. However, the main function and its detailed regulatory mechanism still need to be elucidated in our following research.

CONCLUSION

In study, we first cloned and characterized the cDNA sequences of goose *Pax3* gene and identified two alternatively spliced isoforms (Pax3-a and Pax3-b). The present study also measures a basal mRNA expression of *Pax3* gene in different tissues. All these results will be helpful to understand the functions of *Pax3* gene in Chinese goose.

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