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Molecular Cloning and Sequence Characterization and Tissue Transcription Profile Analyses of Three Novel Common Carp (*Cyprinus carpio*) Genes-*RaB7*, *RaB8a* and *RDH1l*

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Abstract: The complete coding sequences of three common carp genes *RAB7*, *RAB8A* and *RDH1L* were amplified using the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) based on the sequence information of the zebrafish and referenced highly homologous common carp ESTs. The sequence analyses of these three genes revealed that common carp *RAB7* gene encodes a protein of 204 amino acids which has high homology with the RAB family member rab-7 (RAB7) of fifteen species: Atlantic salmon (97%), zebrafish (91%), sheep (90%), western clawed frog (89%), red jungle fowl (90%), African clawed frog (88%), human (89%), dog (89%), horse (89%), *Sumatran orangutan* (89%), cattle (89%), rat (89%), mouse (89%), *Aiptasia pulchella* (88%) and rabbit (87%). The common carp *RAB8A* gene encodes a protein of 207 amino acids which has high homology with the cmember RAS oncogene family rab8a (RAB8A) of eight species: zebrafish (98%), human (92%), dog (92%), cattle (92%), mouse (91%), rat (91%), *Sumatran orangutan* (91%) and chicken (87%). The common carp *RDH1L* gene encodes a protein of 319 amino acids that has high homology with tthe retinol dehydrogenase 1, like (RDH1L) of two species: zebrafish (83%) and Atlantic salmon (65%). Phylogenetic tree analysis revealed that the common carp RAB8A and RDH1L have closer genetic relationships with the zebrafish RAB8A and RDH1L but the common carp RAB7 has a closer genetic relationship with the RAB7 of Atlantic salmon. The tissue transcription profile analyses indicated that that the common carp *RAB7*, *RAB8A* and *RDH1L* genes are generally but differentially expressed in the detected tissues including in tissues including muscle, heart, brain, skin, gills, eye, fin. These data serve as a foundation for further research on these three genes.

Key words: Common carp, RAB7, RAB8A, RDH1L, tissue transcription profile, China

INTRODUCTION

RAB7 is a small Rab GTPase that regulates vesicular traffic from early to late endosomal stages of the endocytic pathway. GTPase Activating Proteins (GAPs) interact with GTP-bound Rab and accelerate the hydrolysis of GTP to GDP.

Guanine nucleotide Exchange Factors (GEFs) interact with GDP-bound Rabs to promote the formation of the GTP-bound state. Rabs are further regulated by Guanine nucleotide Dissociation Inhibitors (GDIs) which facilitate Rab recycling by masking C-terminal lipid binding and promoting cytosolic localization (Rojas *et al*., 2008; Patel *et al*., 2008; Spinosa *et al*., 2008).

RAB8A is a member of the RAS superfamily which are small GTP/GDP-binding proteins with an average size of 200 amino acids. The RAS related proteins of the RAB/YPT family may play a role in the transport of proteins from the endoplasmic reticulum to the Golgi and the plasma membrane (Shisheva *et al*., 1999; Hattula and Peranen, 2000; Hattula *et al*., 2002).

RDH1L is another gene that had been demonstrated to be play an important roles in the aexocrine pancreas development, gut morphogenesis, metabolic process, oxidation reduction, pectoral fin morphogenesis, pectoral fin morphogenesis, retinol metabolic process, skeletal development (Nadauld *et al*., 2005).

Based on these described above these three genes are associated with important biological functions. To date, the common carp *RAB7*, *RAB8A* and *RDH1L* genes have not been reported.

The objective of this study was to clone and analyze the coding sequences of common carp *RAB7*, *RAB8A* and *RDH1L* genes and determine their tissue transcription profiles. The data obtained will serve as a basis for understand these common carp genes.

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MATERIALS AND METHODS

Samples collection, RNA extraction and first-strand cDNA synthesis: The tissue samples of muscle, heart, brain, skin, gills, eye, fin were obtained from six (about 3 kg, 3 females and 3 males) local common carps in Yunnan province of China. Total RNA extraction and first strand cDNA synthesis for these tissue samples were performed using methods previously described (Liu *et al*., 2008). Briefly, total RNA was extracted using the TRIzol Reagent Total RNA Extraction Kit (GIBCO, USA). DNase I treatment was done before continuing with the first-strand cDNA synthesis. For each RNA sample, a single reverse transcription reaction was set up and then the efficiency of reverse transcription was checked on 1% agrose/EtBr gel.

Isolation of the common carp *RAB7*, *RAB8A* and *RDH1L* genes: RT-PCR was performed to amplify complete coding sequences of these three common carp genes using the cDNA obtained from the pooled tissues above. The 20 μL reaction system was 2.0 μL cDNA (pooled), 2.0 μL 2 mM mixed dNTPs, 2.0 μL 10×Taq DNA polymerase buffer, 1.2 μL, 25 mM $MgCl_2$, 1.0 μL 10 mM forward primer, 1.0 μL 10 mM reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 μL) and 9.8 μL sterile water. The primers for common carp *RAB7* gene isolation were designed based on the coding sequences from zebrafish *RAB7* gene and their highly homologous common carp EST sequences (GeneBank numbers CA969330, CA970203 and EC392463). Similarly, the primers for common carp *RAB8A* gene isolation were designed based on the coding sequences from zebrafish *RAB8A* gene and their highly homologous common carp EST sequences (Genbank numbers EC393638 and EX882991). The primers for isolating the common carp *RDH1L* gene were designed based on the coding sequences from zebrafish *RDH1L* genes and their highly homologous common carp EST sequences (GenBank numbers CF662451 and EX880878). These primers were all designed to amplify the complete CDS from start codon to the stop codon) and partial flanking sequences for these three genes. These primer sequences and their annealing temperature for RT-PCR reaction are shown in Table 1.

The PCR products for common carp RAB7, RAB8A and RDH1L cDNAs 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 transcription profile: RT-PCR for transcription profile analyses were performed using the cDNA obtained from the specific tissues above as described by Liu and Xiong (2007). The housekeeping gene, *actin* was used as an internal control. The primers and annealing temperature for common carp *actin* gene (GeneBank number: AY309091) amplification is shown in Table 1. The size of the actin PCR fragment is 254 bp. To ensure that no false positive PCR fragments were generated from pseudogenes in the contaminating genomic DNA, actin primers were derived from different exons in the same gene. The primers for common carp *RAB7*, *RAB8A* and *RDH1L* gene which were used to perform the RT-PCR for tissue transcription profile analyses were the same as the primers used for amplification in RT-PCR above. The 25 L reaction system was: 2.0 L pooled cDNA of each tissue (100 ng μL^{-1}), 2.5 L 2 mM mixed dNTPs, 2.5 l 10×Taq DNA polymerase buffer, 2.5 L 25 mM $MgCl_2$, 2.0 L 10 mM forward primer, 2.0 L 10 mM reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 L) and 9.5 L sterile water. The PCR program initially started with a 94°C denaturation for 4 min followed by 30 cycles of 94°C/1 min, Ta°C (Table1)/1 min, 72°C/1 min then 72°C extension for 10 min, finally 4°C to terminate the reaction. Every PCR was repeated 5 times.

Table 1: Primers for common carp *RAB7*, *RAB8A*, *RDH1L*, *actin* genes and their annealing temperatures

Gene	Primer sequence	Ta/°C
RAB7	Forward:5'-GCAGCAGATCGTTAAACC-3'	
	Reverse:5'-GGATCTCTCCCTTTCCCA-3'	57
RAB8A	Forward:5'-GGCTGAAGTACCGGGCGG-3'	
	Reverse:5'-AGCATAGTCCAGTCGTGT-3	56
RDH1L	Forward: 5'-CACTTTTCTTATAAAAACAAT-3'	
	Reverse:5'-CCATCAATATGCTGCATA-3'	51
Actin	Forward: 5'-GAGCGTGGTTATTCTTTCG-3'	
	Reverse:5'-TAGGCGGTCTCATGGATT-3'	54

Sequence analysis: The gene analysis for cDNA sequence was conducted using GenScan software (http://genes.mit.edu/GENSCAN.html). The protein prediction and analysis were performed using the Conserved Domain Architecture Retrieval Tool of Blast at the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov/BLAST) and the ClustalW software (http://align.genome.jp/). The theoretical isoelectric point (pI) and Molecular weight (Mw) of proteins was computed using the Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html).

RESULTS AND DISCUSSION

Isolation results for common carp *RAB7*, *RAB8A* and *RDH1L* gene: Through RT-PCR with pooled tissue cDNAs for common carp *RAB7*, *RAB8A* and *RDH1L* gene, the resulting PCR products were 742, 717 and 1063 bp (Fig. 1).

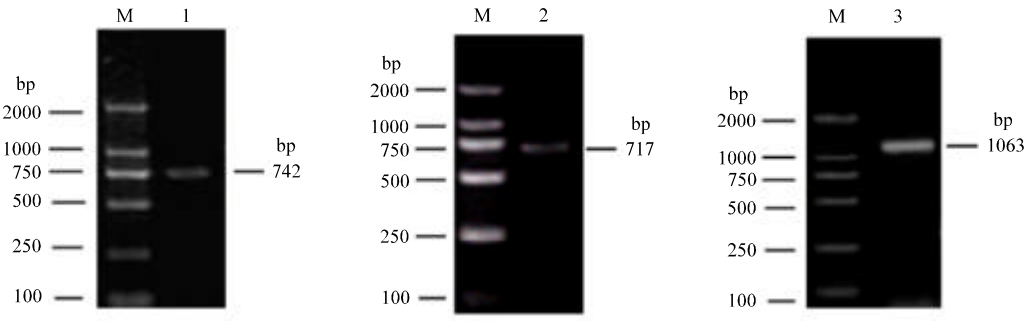

Fig. 1: Isolation results for common carp *RAB7*, *RAB8A* and *RDH1L* genes. M, DL2000 DNA markers; 1) PCR product for common carp *RAB7* gene; 2) PCR product for common carp *RAB8A* gene; 3) PCR product for common carp *RDH1L* gene

Sequence analysis: These cDNA nucleotide sequence analysis using the BLAST software revealed that these genes were not homologous to any of the known common carpgenes and they were then deposited into the GenBank database (Accession number: EU287430, EU287431 and EU295556). The sequence prediction was carried out using the GenScan software and results showed that the 742, 717 and 1063 bp cDNA sequences represented three single genes which encoded 204, 207, 319 amino acids, respectively. The theoretical isoelectric point (pI) and Molecular weight (Mw) of these deduced proteins of these three common carpgenes were computed using the Compute pI/Mw Tool. The pI of common carpRAB7, RAB8A and RDH1L are 6.35, 8.99 and 8.93, respectively. The molecular weights of these three putative proteins are 23089.19, 23667.21 and 36033.34, respectively.

Further Blast analysis of these proteins revealed that common carp RAB7 has high homology with the RAB family member rab-7 (RAB7) of fifteen species: Atlantic salmon (ACI34062, 97%), zebrafish (NP_957222, 91%), sheep (NP_001119836, 90%), western clawed frog (NP_001008026, 89%), red jungle fowl (XP_414359, 90%), African clawed frog (NP_001087006, 88%), human (NP_004628, 89%), dog (NP_001003316, 89%), horse (XP_001488351, 89%), *Sumatran orangutan* (NP_001127416, 89%), cattle (NP_001030253, 89%), rat (NP_076440, 89%), mouse (CAA61797, 89%), aiptasia pulchella (JC8006, 88%) and rabbit (NP_001075503, 87%). The common carp RAB8A has high homology with the member RAS oncogene family rab8a (RAB8A) of eight species: zebrafish (NP_001083031, 98%), human (NP_005361, 92%), dog (XP_855569, 92%), cattle (NP_001098951, 92%), mouse (P55258, 91%), rat (NP_446450, 91%), *Sumatran orangutan* (NP_001127003, 91%) and chicken (Q5F470, 87%). The common carp RDH1L has high homology with the retinol dehydrogenase 1, like (RDH1L) of two species: zebrafish (NP_955903, 83%) and Atlantic salmon (ACI67927, 65%) (Fig. 2-7).

Based on the results of the alignment of RAB7, RAB8A and RDH1L, phylogenetic trees were constructed using the Dendrogram procedure of ClustalW software (http://align.genome.jp/) as shown in Fig. 8-10.

The phylogenetic tree analysis revealed that the common carp RAB8A and RDH1L have closer genetic relationships with the zebrafish RAB8A and RDH1L but the common carp RAB7 has a closer genetic relationship with the RAB7of Atlantic salmon.

Tissue transcription profile: Tissue transcription profile analysis revealed that compared to the expression of common carp *actin* gene, the common carp *RAB7*, *RAB8A* and *RDH1L* genes are generally but differentially expressed in the detected tissues including in tissues including muscle, heart, brain, skin, gills, eye, fin (Fig. 11).

Comparative genomics research has revealed that virtually all (99%) of the protein-coding genes in humans align with homologs in mouse and over 80% are clear 1:1 orthologs (Hardison, 2003). This extensive conservation in protein-coding regions implies that this conservation of protein-coding sequences may be expected in different fish species including zebrafish and common carps. With the completion of zebrafish whole genome sequencing and development of modern bioinformatics, many more specific databases such as NCBI common carp EST database were established.

These along with different convenient analysis tools make it much easier to find the common carp EST sequences highly homologous to some protein-coding sequences of zebrafish. These tools also make it easer to isolate the encoding regions of some common carp genes based on the protein-coding sequence information of zebrafish and the highly homologous common carp EST sequence information.

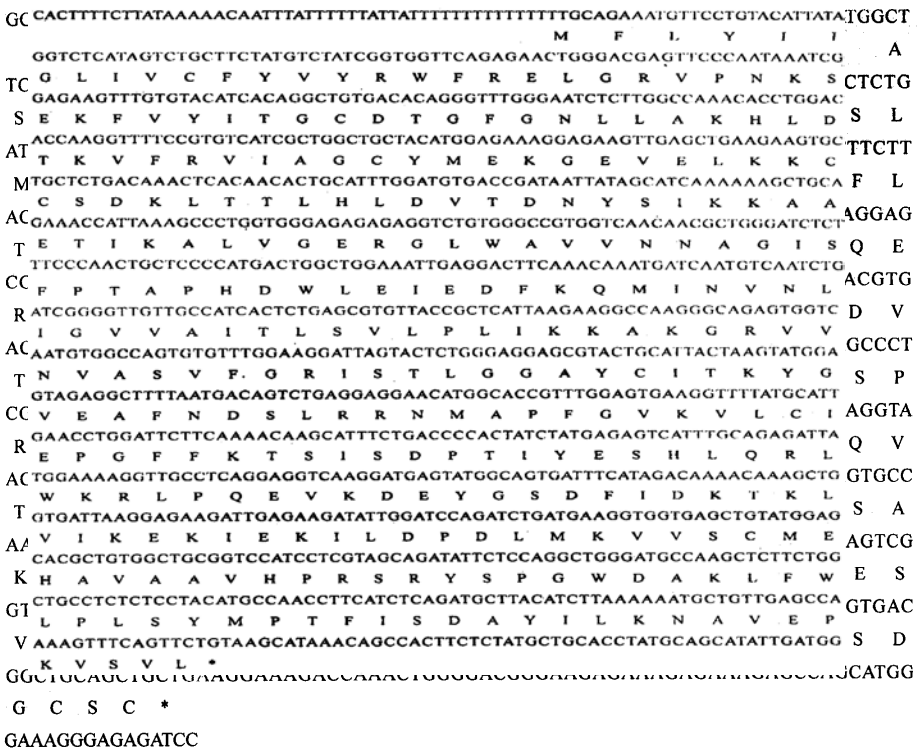

Fig. 2: The complete coding sequence of common carp *RAB7* gene and its encoding amino acids * indicates the stop codon

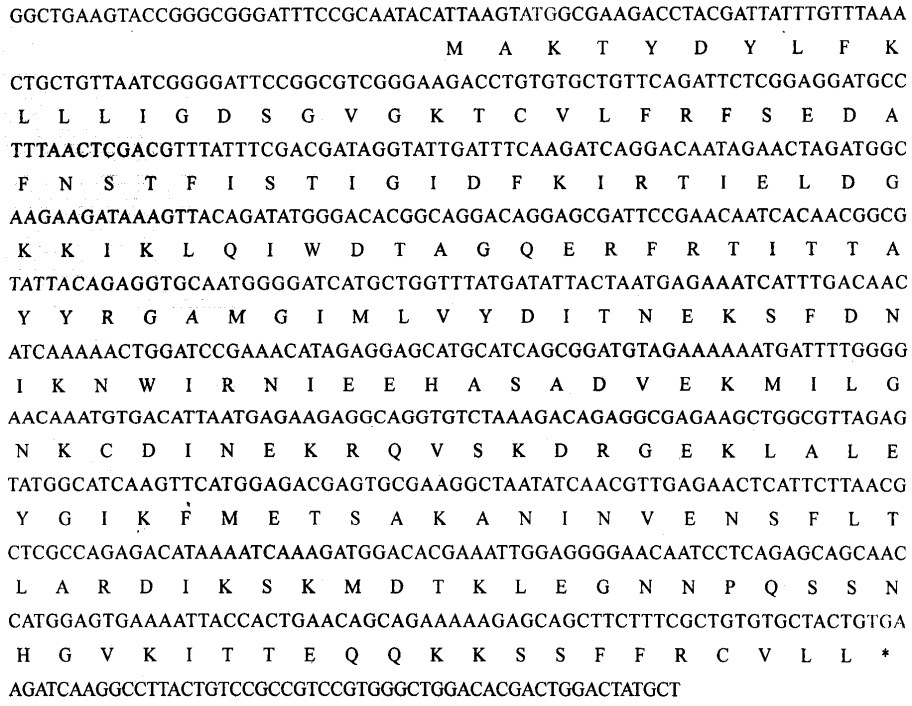

Fig. 3: The complete coding sequence of common carp *RAB8A* gene and its encoding amino acids *indicates the stop codon

Fig. 4: The complete coding sequence of common carp *RDH1L* gene and its encoding amino acids *indicates the stop codon

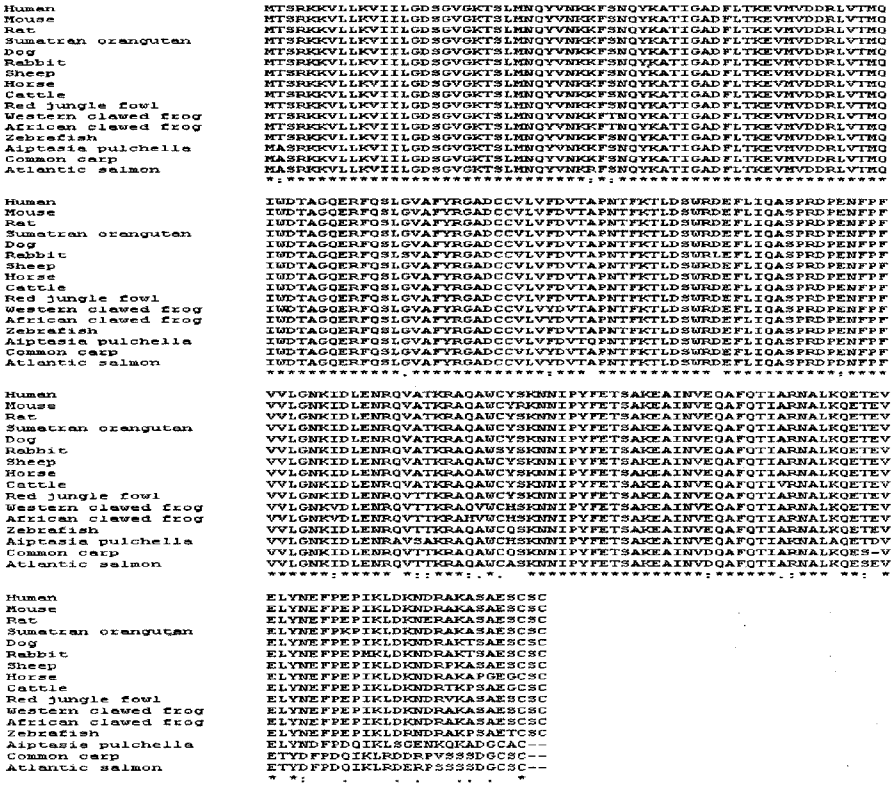

Fig. 5: The alignment of the protein encoded by common carp *RAB7* gene and other fifteen kinds of RAB7 from Atlantic salmon, zebrafish, sheep, western clawed frog, red jungle fowl, African clawed frog, human, dog, horse, *Sumatran orangutan*, cattle, rat, mouse, *Aiptasia pulchella* and rabbit

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Human_Dog               MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRIKLQ
Sumatran orangutan      MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRIKLQ
Cattle                  MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRIKLQ
Chicken                 MAKTYDYLFKLLLIGDSGVGKTCALFRFSEDAFNATFISTIGIDFKIRTIELDGKRIKLQ
Mouse_Rat               MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRIKLQ
Common carp             MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKKIKLQ
Zebrafish               MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKKIKLQ
                        ************************.**********:********************:****

Human_Dog               IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Sumatran orangutan      IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Cattle                  IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Chicken                 IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFENIRNWVRNIEEHASPDVEKMILG
Mouse_Rat               IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Common carp             IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFDNIKNWIRNIEEHASADVEKMILG
Zebrafish               IWDTAGQERFRTITTAYYRGAMGIMLVYDITNEKSFDNIKNWIRNIEEHASADVEKMILG
                        ************************************:**:**:********.********

Human_Dog               NKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFFTLARDIKAKMDKKLEGN
Sumatran orangutan      NKRDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFFTLARDIKAKMDKKLEGN
Cattle                  NKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFYTLARDIKAKMDKKLEGN
Chicken                 NKCDANDKRQVSREQGEKLAASFGIKFMETSAKANINIENAFFTLARDIKAKMDKKLEGN
Mouse_Rat               NKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFFTLARDIKAKMDKKLEGN
Common carp             NKCDINEKRQVSKDRGEKLALEYGIKFMETSAKANINVENSFLTLARDIKSKMDTKLEGN
Zebrafish               NKCDINEKRQVSKDRGEKLALEYGIKFMETSAKANINVENAFLTLARDIKAKMDTKLEGN
                        ** * *:*****:::***** .:*************:**:* *******:***.*****

Human_Dog               SPQGSNQGVKITPDQQKRSSFFRCVLL
Sumatran orangutan      SPQGSNQGVKITPDQQKRSSFFRCVLL
Cattle                  SPQGSNQGVKITPDQQKRSSFFRCVLL
Chicken                 SPQGSNQGVKITPDQQKKSSFFRCVLL
Mouse_Rat               SPQGSSHGVKITVEQQKRTSFFRCSLL
Common carp             NPQSSNHGVKITTEQQKKSSFFRCVLL
Zebrafish               NPQGSNHGVKITTEQPKKSSFFRCVLL
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Fig. 6: The alignment of the protein encoded by common carp *RAB8A* gene and other eight kinds of RAB8A from zebrafish, human, dog, cattle, mouse, rat, *Sumatran orangutan* and chicken

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Common carp             MFLYIIGLIVCFYVYRWFRELGRVPNKSEKFVYITGCDTGFGNLLAKHLDTKVFRVIAGC
Zebrafish               MYLYIAGLVVLFYVYRWFRELGRVSNKSEKFVYITGCDTGFGNLLARHLDTKGFRVIAGC
Atlantic salmon         MFLYLLGLVVFYYLYRWFREIPRVPDKGDKYVYITGCDSGFGNLLARHLDELGFCVIASC
                        *:**: **:* :*:******: **.:*.:*:*******:*******:***   * ***.*

Common carp             YMEKGEVELKKCCSDKLTTLHLDVTDNYSIKKAAETIKALVGERGLWAVVNNAGISFPTA
Zebrafish               YSEKGEDELKKICSDRLITLHLDVTDNENVKKAAETIKSLVGQKGLWAVVNNAGIAFPTA
Atlantic salmon         FTEKGEEDLRKACSDRFTTLHLDVKKNESVDKAAALIKDKVGARGLWAVVNNAGVAIPSA
                        : **** :*:* ***:: ******..* .:.***  **  ** :**********:::*:*

Common carp             PHDWLEIEDFKQMINVNLIGVVAITLSVLPLIKKAKGRVVNVASVFGRISTLGGAYCITK
Zebrafish               PNDWLEIEDFTPMINVNLIGVIAVTLSVLPLIKKAKGRVVNVASVFGRISTLGGAYCITK
Atlantic salmon         PCDWLTIDDYKSMLDVNLNGVIAVTLSVLPLIKKARGRVVNVASVFGRISPVGGPYTVSK
                        * *** *:*:. *::*** **:*:**********:**************.:**.* ::*

Common carp             YGVEAFNDSLRRNMAPFGVKVLCIEPGFFKTSISDPTIYESHLQRLWKRLPQEVKDEYGS
Zebrafish               YGVEAFNDALRRQMAPFGVKVLCIEPGFFKTIVTDFNIVESTLHRLWNKLPQEVKDEYGS
Atlantic salmon         YGVEAFNDSLRRNMAPFGVKVLCIEPGFFKTTVTDSVVLGKSIKQLWEKMPQQVRDDYGP
                        ********:***:****************** ::*  :  . :::**:::**:*:*:**.

Common carp             DFIDKTKLVIKEKIEKILDPDLMKVVSCMEHAVAAVHPRSRYSPGWDAKLFWLPLSYMPT
Zebrafish               DYVDKTKLTAKELLEKLADGDLMKVVSCMEHAVAAVHPRTRYSPGWDAKFFWLPLSYMPT
Atlantic salmon         DYLDKVDVMMREKLAKMSDTDLMKVVSCMEHAISAVRPRTRYSPGWDAKLFWIPMSYMPT
                        *::**..:  :* : *: * ************:::**:**:*********:**:*:*****

Common carp             FISDAYILKNAVEPKVSVL
Zebrafish               FISDALLLKKAVQPKASIL
Atlantic salmon         GFADWLLLKEAILPAKAVN
                         ::*  :**:*: *  ::
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Fig. 7: The alignment of the protein encoded by common carp *RDH1L* gene and other two kinds of RDH1L from zebrafish and Atlantic salmon

The present study cloned and analyzed the CDS sequences for three common carp genes. The data show that these common carp genes are highly similar to these zebrafish genes making the zebrafish a potential

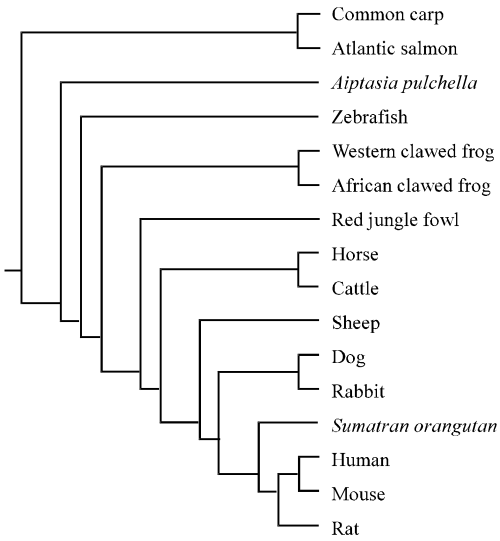

Fig. 8: The phylogenetic tree for five kinds of RAB7 from common carp, Atlantic salmon, zebrafish, sheep, western clawed frog, red jungle fowl, African clawed frog, human, dog, horse, *Sumatran orangutan*, cattle, rat, mouse, *Aiptasia pulchella* and rabbit

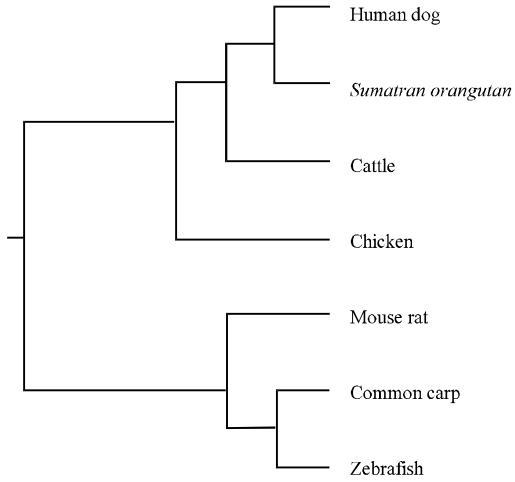

Fig. 9: The phylogenetic tree for five kinds of RAB8A from common carp, zebrafish, human, dog, cattle, mouse, rat, *Sumatran orangutan* and chicken

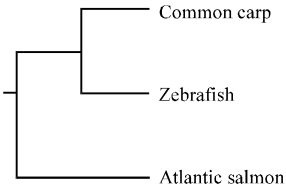

Fig. 10: The phylogenetic tree for four kinds of RDH1L from common carp, zebrafish and Atlantic salmon

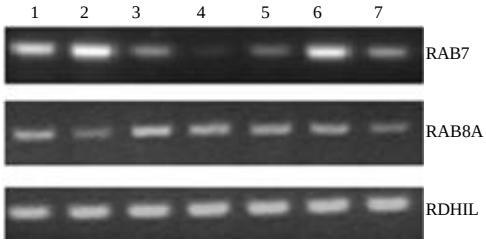

Fig. 11: Tissue transcription profile of common carp *RAB7*, *RAB8A* and *RDH1L* gene. The actin expression is the internal control; 1) muscle; 2) heart; 3) brain; 4) skin; 5) gills; 6) eye; 7) fin

non-primate model for studying these common carp genes. From the tissue transcription profile analysis in the experiment it can be seen that these genes were obviously differentially expressed in some tissues.

Would there be some associations between the functions and mRNA differential expression in some tissues for these common carp genes? This needs to study furtherly.

CONCLUSION

In the study, we first isolated the common carp *RAB7*, *RAB8A* and *RDH1L* genes and performed necessary sequence analysis and tissue transcription profile analysis. This established the primary foundation for further research on these three common carp genes.

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