

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 the 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% agarose/EtBr gel.

Isolation of the common carp *RAB7*, *RAB8A* and *RDHIL* 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 μ L reaction system was 2.0 μ L cDNA (pooled), 2.0 μ L 2 mM mixed dNTPs, 2.0 μ L 10 \times Taq DNA polymerase buffer, 1.2 μ L, 25 mM MgCl₂, 1.0 μ L 10 mM forward primer, 1.0 μ L 10 mM reverse primer, 2.0 units of Taq DNA polymerase (1 U/1 μ L) and 9.8 μ L 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 *RDHIL* gene were designed based on the coding sequences from zebrafish *RDHIL* 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 *RDHIL* 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

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

Gene	Primer sequence	Ta/ $^{\circ}$ C
<i>RAB7</i>	Forward: 5'-GCAGCAGATCGTTAAACC-3' Reverse: 5'-GGATCTCTCCCTTCCCA-3'	57
<i>RAB8A</i>	Forward: 5'-GGCTGAAGTACCGGGCGG-3' Reverse: 5'-AGCATAGTCCAGTCGTGT-3	56
<i>RDHIL</i>	Forward: 5'-CACTTTCTTATAAAAACAAT-3' Reverse: 5'-CCATCAATATGCTGCATA-3'	51
<i>Actin</i>	Forward: 5'-GAGCGTGGTATTCTTCG-3' Reverse: 5'-TAGGCCTCTCATGGATT-3'	54

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 *RDHIL* 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 μ L $^{-1}$), 2.5 L 2 mM mixed dNTPs, 2.5 1 10 \times Taq DNA polymerase buffer, 2.5 L 25 mM MgCl₂, 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 $^{\circ}$ C denaturation for 4 min followed by 30 cycles of 94 $^{\circ}$ C/1 min, Ta $^{\circ}$ C (Table1)/1 min, 72 $^{\circ}$ C/1 min then 72 $^{\circ}$ C extension for 10 min, finally 4 $^{\circ}$ C to terminate the reaction. Every PCR was repeated 5 times.

Sequence analysis: The gene analysis for cDNA sequence was conducted using GenScan software (<http://genes.mit.edu/GENS CAN.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 *RDHIL* gene: Through RT-PCR with pooled tissue cDNAs for common carp *RAB7*, *RAB8A* and *RDHIL* gene, the resulting PCR products were 742, 717 and 1063 bp (Fig. 1).

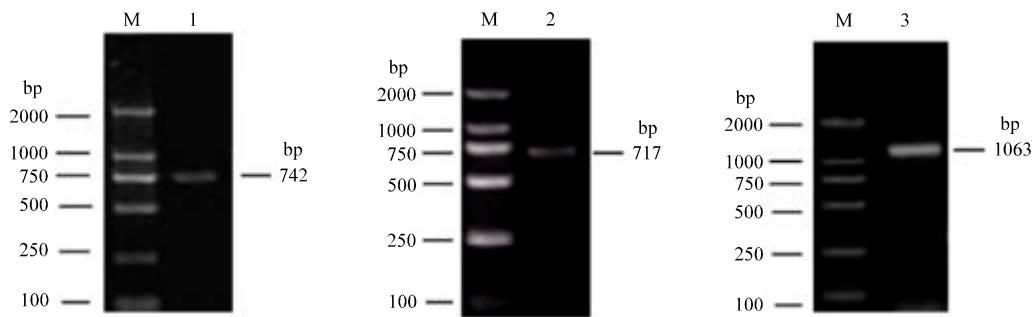


Fig. 1: Isolation results for common carp *RAB7*, *RAB8A* and *RDH1L* genes. M, DL2000 DNA markers; 1) PCR product for common carp *RAB7* gene; 2) PCR product for common carp *RAB8A* gene; 3) PCR product for common carp *RDH1L* gene

Sequence analysis: These cDNA nucleotide sequence analysis using the BLAST software revealed that these genes were not homologous to any of the known common carp genes 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 carp genes were computed using the Compute pI/Mw Tool. The pI of common carp *RAB7*, *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 *RAB7* of 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 easier 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

GGCTGAAGTACCGGGCGGGATTCCGCAATACATTAAGTATGCCAAGAACCTACGATTATTGTTAA
M A K T Y D Y L F K
CTGCTGTTAACGGGATTCCGGCGTCGGGAAGACCTGTGCTGTTAGATTCTGGAGGATGCC
L L L I G D S G V G K T C V L F R F S E D A
TTAACCTCGACGTTTATTCGACGATAGGTATTGATTCAAGATCAGGACAATAGAACTAGATGCC
F N S T F I S T I G I D F K I R T I E L D G
AAGAAGATAAAAGTTACAGATATGGGACACGGCAGGACAGGAGCGATTCCGAACAATCACACGGCG
K K I K L Q I W D T A G Q E R F R T I T T A
TATTACAGAGGTGCAATGGGATCATGCTGGTTATGATATTACTAATGAGAAATCATTGACAAC
Y Y R G A M G I M L V Y D I T N E K S F D N
ATCAAAAACCTGGATCCGAAACATAGAGGAGCATGCATCAGCGGATGTAGAAAAAAATGATTTGGGG
I K N W I R N I E E H A S A D V E K M I L G
AACAAATGTGACATTAATGAGAAGAGGCAGGTGCTAAAGACAGAGGCAGAGCTGGCGTTAGAG
N K C D I N E K R Q V S K D R G E K L A L E
TATGGCATCAAGTTCATGGAGACGAGTGCAGGCTAATATCACCGTTGAGAACTCATTCTAACG
Y G I K F M E T S A K A N I N V E N S F L T
CTGCCAGAGACATAAAATCAAAGATGGACACGAAATTGGAGGGAAACAATCCTCAGAGCAGCAAC
L A R D I K S K M D T K L E G N N P Q S S N
CATGGAGTGAAAATTACCACTGAACAGCAGAAAAAGAGCAGCTTCTCGCTGTGCTACTGTGA
H G V K I T T E Q Q K K S S F F R C V L L *
AGATCAAGGCCTTACTGTCCGCGTCCGTGGGCTGGACACGACTGGACTATGCT

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

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CACTTTCTTATAAAAACAATTATTTTATTATTTTTTGAGAAATGTCCTGTACATTATA
M F I Y I I
GGTCTCATAGTCGTTCTATGCTATCGGGGGTCAGAGAACTGGGACGAGTCCCATAATCG
G L I V C F Y V Y R W F R E L G R V P N K S
GAGAAAGTTGTCACATCACAGGCTGTGACACAGGGTTGGAAATCTTGGCAAACACCTGGAC
E K F V Y I T G C D T O F G N L L A K H L D
ACCAAGGTTCCGTTCATCGCTGGCTGACATGGAGAAAGGAGAAGTTGAGCIGAAAGTGC
T K V F R V I A G C Y M E K G E V E L K K C
TGCTCTGACAAACTCACAACACTGCATTTGGATGTGACCATAATTAGCATCAAAAGCTGA
C S D K L T T L H L D V T D N Y S I K K A A
GAAACATTAAAGCCCATGGTGGAGAGAGAOGTCTGTGGGGCGTGGTCAAACACGCTGGGATCTCT
E T I K A L V G E R G L W A V V N N A G I S
TTCCAACGTCCCCATGACTGGCTGGAAATTGAGGACTCAAAACAAATGATCAATGCAATCTG
F P T A P H D W L E I E D F K Q M I N V N L
ATCGGGGTTGTCACACTGAGCGTGTACCGCTCATTAAGAAGGCCAAGGGCAGAGTGGC
I G V V A I T L S V L P L I K K A K G R V V
AATGCGGCACTGTTGGAAAGGATTAGTACTCTGGGAGGAGCGTACTGCAITACTAAGTATGGA
N V A S V F . O R I S T L G G A Y C I T K Y G
GTAGAGGCTTTAATGACAGTCAGGAGGAACATGCCACCGTGGAGTGAAGGTTTATGCAATT
V E A F N D S L R R N M A P F G V K V L C I
GAACCTGGATTCTCAAAACAGCATTCTGACCCACTATCTATGAGAGTCATTGAGAGATA
E P G F F K T S I S D P T I Y E S H L Q R L
TGGAAAAGGTTGCGCTCAGGAGGTCAAGGATGAGTATGGCAGTGATTCATAGACAAAAGCTG
W K R L P Q E V K D E Y G S D F I D K T K L
GTGATTAAGGAGAAGATTGAGAAGATATTGGATCCAGATCTGATGAAGGTTGAGCTGTATGGAG
V I K E K I E K I L D P D L M K V V S C M E
CACGCTGTGGCTCGGTCCATCTCGTAGCAGATATTCTCAGGCTGGGATGCCAAGCTTCTGG
H A V A A V H P R S R Y S P G W D A K L F W
CTGCTCTCTCTACATGCCAACCTTCATCTAGATGCTTACATCTTAAAAATGCTGTTGAGCCA
L P L S Y M P T F I S D A Y I L K N A V E P
AAAGTTCACTGTTCTAAGCATAAACAGCCACTCTCATGCTGACCTATGCAAGCATATTGATGG
K V S V L *

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

Human	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Mouse	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Rat	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Sumatran orangutan	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Dog	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Rabbit	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Sheep	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Horse	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Cattle	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Red jungle fowl	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Western clawed frog	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
African clawed frog	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Zebrafish	MTSRKGVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
<i>Aiptasia pulchella</i>	MASRGKVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Common carp	MASRGKVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Atlantic salmon	MASRGKVLKKVIIILGDGSVGKGTSLMNVQVNNGKFNSNOYKATIGADFLTKEVHMVDDRLVITMO
Human	**
Mouse	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Rat	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Sumatran orangutan	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Dog	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Rabbit	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Sheep	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Horse	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Cattle	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Red jungle fowl	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Western clawed frog	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
African clawed frog	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Zebrafish	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
<i>Aiptasia pulchella</i>	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Common carp	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Atlantic salmon	IWD TAGGERFQSLGVAFYRGAADCCLVLFDTA PNT FKLDSWREDFLIGQASPRODPEPF
Human	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Mouse	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Rat	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Sumatran orangutan	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Dog	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Rabbit	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Sheep	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Horse	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Cattle	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Red jungle fowl	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Western clawed frog	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
African clawed frog	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Zebrafish	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
<i>Aiptasia pulchella</i>	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Common carp	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Atlantic salmon	VVLCNKKIDLENRQVATIGAQCNYKNNIPYFETSAKEAINVEQAFOTIARNALKQETTEV
Human	ELYNNEFPEPIKLDKNDRAKASAESCS
Mouse	ELYNNEFPEPIKLDKNDRAKASAESCS
Rat	ELYNNEFPEPIKLDKNDRAKASAESCS
Sumatran orangutan	ELYNNEFPEPIKLDKNDRAKASAESCS
Dog	ELYNNEFPEPIKLDKNDRAKASAESCS
Rabbit	ELYNNEFPEPIKLDKNDRAKASAESCS
Sheep	ELYNNEFPEPIKLDKNDRAKASAESCS
Horse	ELYNNEFPEPIKLDKNDRAKASAESCS
Cattle	ELYNNEFPEPIKLDKNDRAKASAESCS
Red jungle fowl	ELYNNEFPEPIKLDKNDRAKASAESCS
Western clawed frog	ELYNNEFPEPIKLDKNDRAKASAESCS
African clawed frog	ELYNNEFPEPIKLDKNDRAKASAESCS
Zebrafish	ELYNNEFPEPIKLDKNDRAKASAESCS
<i>Aiptasia pulchella</i>	ELYNNEFPEPIKLDKNDRAKASAESCS
Common carp	ELYNNEFPEPIKLDKNDRAKASAESCS
Atlantic salmon	ETYDFFDOIKLDRDPRV2323DGCS--
	ETYDFFDOIKLDRDPRV2323DGCS--

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

Human_Dog	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRKILQ
Sumatran orangutan	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRKILQ
Cattle	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRKILQ
Chicken	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNATFISTIGIDFKIRTIELDGKRKILQ
Mouse_Rat	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRKILQ
Common carp	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRKILQ
Zebrafish	MAKTYDYLFKLLLIGDSVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRKILQ
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
Human_Dog	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Sumatran orangutan	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Cattle	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Chicken	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Mouse_Rat	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Common carp	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
Zebrafish	IWDTAGQERFRITTAYYRGAMGIMLVYDITNEKSFDNIRNWIRNIEEHASADVEKMILG
*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
Human_Dog	NKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFTLARDIKAKMDKKLEGN
Sumatran orangutan	NKRDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFTLARDIKAKMDKKLEGN
Cattle	NKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFTLARDIKAKMDKKLEGN
Chicken	NKCDANDKRQVSREQGEKLAASFGIKFMETSAKANINVIENAFYTLLARDIKAKMDKKLEGN
Mouse_Rat	NKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFTLARDIKAKMDKKLEGN
Common carp	NKCDINEKRQVSQSKDRGEKLALEYGIKFMETSAKANINVENTFLTLLARDIKSKMDTKLEGN
Zebrafish	NKCDINEKRQVSQSKDRGEKLALEYGIKFMETSAKANINVENAFTLlardIKAKMDTKLEGN
:**:*****:*****:*****:*****:*****:*****:*****:*****:	
Human_Dog	SPQGSNQGVKITPDQQKRSSFFRCVLL
Sumatran orangutan	SPQGSNQGVKITPDQQKRSSFFRCVLL
Cattle	SPQGSNQGVKITPDQQKRSSFFRCVLL
Chicken	SPQGSNQGVKITPDQQKRSSFFRCVLL
Mouse_Rat	SPQGSSHGVKITVEQQKRSTSFFRCSSL
Common carp	NPQGSNNHGVKITTEQQKKSSFFRCVLL
Zebrafish	NPQGSNNHGVKITTEQPKKSSFFRCVLL
	:**:*****:*****:*****:

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

Common carp	MFYIIGLIVCFYVYRWFRELGRVPNKSEKFVYITGCDTGFGNLLAKHLDTKVFRVIAGC
Zebrafish	MYLYIAGLVLVLFYVYRWFRELGRVSNKSEKFVYITGCDTGFGNLLARHLDTKGFRVIAGC
Atlantic salmon	MFLYLLGLVVVFYLYRWFREIPRVPFDKGDKYVYITGCDSGFGNLLARHLDLGFCVIAASC
Common carp	*:***: *::*****: *.*.:*****:*****:*****:*** * ***.*
Zebrafish	YMEKGEGEVELKKCCSDKLTTLHLDVTDNYSIKAAETIKALVGERGLWAVVNNAAGISFPFTA
Atlantic salmon	YSEKGEEDEKKICSDRLITLHLDVTDNENVKAETIKSLVGQKLWAVVNNAAGIAFPFTA
Common carp	FTEKGEEDLRKACSDRFITLHLDVVKNESVDKAAALIKDKVGARGLWAVVNNAAGVAIPS
Zebrafish	: **** :*: * ****: *****..* .:*** ** ** * :*****:*****:***:*
Atlantic salmon	PHDWLEIEDFKQMIVNVLIGVVAITLSVLPLIKKAKGRVVNVASVFGRISTLGgayCITK
Common carp	PNDWLEIEDFTPMLIVNVLIGVIAVTLSQLPLIKKAKGRVVNVASVFGRISTLGgayCITK
Zebrafish	PCDWLTIDDYKSMLDVNLNGVIAVTLSQLPLIKKARGRVVNVASVFGRISPVGGPYTVSK
Atlantic salmon	* *** :*: . *:*** ***:*****:*****:*****:***.* :*
Common carp	YGVEAFNDSLRRNMAPFGVKVLCIEPGFFKTSISDPTIYESHLQRLWKRLPQEVKDEYGS
Zebrafish	YGVEAFNDAIRRQMAPFGVKVLCIEPGFFKTIIVDFNIVESTLHRLWNLKPQEVKDEYGS
Atlantic salmon	YGVEAFNDSLRRNMAPFGVKVLCIEPGFFKTIIVDSVVLGKSIQLWEKMPQQVRDDYGP
Common carp	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:
Zebrafish	DFIDKTKLVIKEKIEKILDPLMKVVS CMEHA VAVHPRSR YSPGWD A KLFWLPLSYMPT
Atlantic salmon	DYVDKTKLTAKEL LEKLA DGLM KVVS CMEHA VAVHPRTR YSPGWD A KFFWLPLSYMPT
Common carp	DYLDKVDVMMREKLA KMS DTL MKVVS CMEHA ISAVR PRTR YSPGWD A KLFWIPMSYMPT
Zebrafish	*:***: . : * : * *****:*****:*****:*****:*****:*****:*****:
Atlantic salmon	
Common carp	FISDAYILKNAVEPKVSVL
Zebrafish	FISDALLLKKAVQPKASIL
Atlantic salmon	GFADWLLKAEAILPAKAVN
Common carp	:***:***: * : :

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

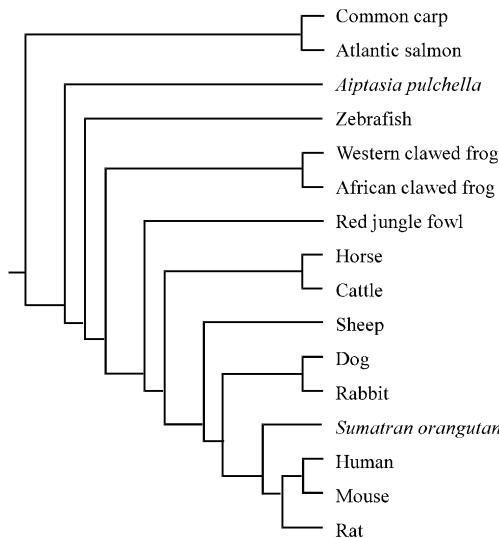


Fig. 8: The phylogenetic tree for five kinds of RAB7 from common carp, Atlantic salmon, zebrafish, sheep, western clawed frog, red jungle fowl, African clawed frog, human, dog, horse, *Sumatran orangutan*, cattle, rat, mouse, *Aiptasia pulchella* and rabbit

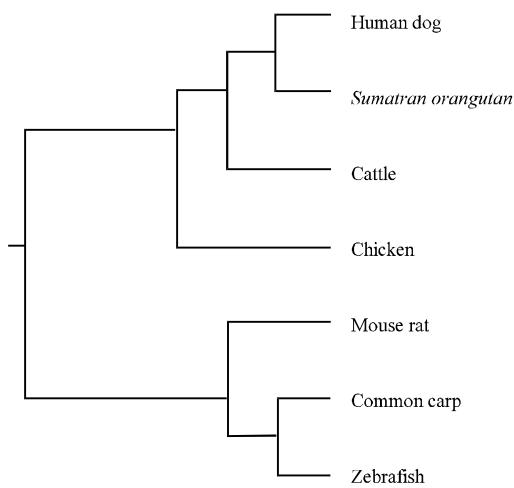


Fig. 9: The phylogenetic tree for five kinds of RAB8A from common carp, zebrafish, human, dog, cattle, mouse, rat, *Sumatran orangutan* and chicken

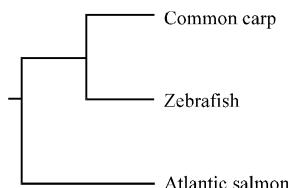


Fig. 10: The phylogenetic tree for four kinds of RDHIL from common carp, zebrafish and Atlantic salmon

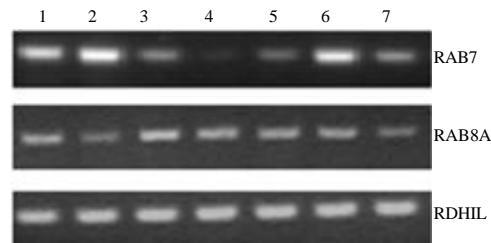


Fig. 11: Tissue transcription profile of common carp *RAB7*, *RAB8A* and *RDHIL* gene. The actin expression is the internal control; 1) muscle; 2) heart; 3) brain; 4) skin; 5) gills; 6) eye; 7) fin

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 *RDHIL* 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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