Molecular Cloning and Sequence Characterization and Tissue Transcription Profile Analyses of Three Novel Common Carp (Cyprinus carpio) Genes-Rab7, Rab8a and Rdh11

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Abstract: The complete coding sequences of three common carp genes Rab7, Rab8a and Rdh11 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 smember 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 Rdh11 gene encodes a protein of 319 amino acids that has high homology with the retinol dehydrogenase 1, like (RDH11L) of two species: zebrafish (83%) and Atlantic salmon (65%). Phylogenetic tree analysis revealed that the common carp Rab8A and Rdh11 have closer genetic relationships with the zebrafish Rab8A and Rdh11 but the common carp Rab7 has a closer genetic relationship with the Rab7 of Atlantic salmon. The tissue transcription profile analyses indicated that the common carp Rab7, Rab8A and Rdh11 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, Rdh11, 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 Rab5 to promote the formation of the GTP-bound state. Rab5 are further regulated by Guanine nucleotide Dissociation Inhibitors (GDI) 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).

Rdh11 is another gene that has 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 Rdh11 genes have not been reported.

The objective of this study was to clone and analyze the coding sequences of common carp Rab7, Rab8A and Rdh11 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 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 MgCl2, 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 (GenBank 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 (GenBank number: AY306991) 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 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°C denaturation for 4 min followed by 30 cycles of 94°C/1 min, Ta°C (Table 1)/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.

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).
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 RDHIL 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 (ACI67927, 65%), zebrafish (NP_955903, 83%), sheep (NP_00119836, 90%), western clawed frog (NP_0006026, 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_001089531, 92%), mouse (P55528, 91%), rat (NP_446450, 91%), Sumatran orangutan (NP_001127003, 91%) and chicken (Q5F470, 87%). The common carp RDHIL 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 RDHIL, 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 RDHIL have closer genetic relationships with the zebrafish RAB8A and RDHIL 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 RDHIL 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 carp. 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.

GGCTGAAGTAGTACCGGGGCAGATTTTCCGCAATATACTAATTAGATATGCGAAGACATCAGATTATTTGTTAAA
MAKTYDYLF
CTGCTGTTAAATCGGGGATTTCCCGGCTTGGAAGACATCGTGTCGTTCAAGATTTTCCCGGACGTGCC
LLILDGSVGTKTCVLFRRFSEDA
TTATACCTACGCTTATTTTCCGACATAGATTATTTGATCTAAGATCGAAGATAGATGCGG
FNSTFISTIGDFKIERTLIDG
AAGAGAAGATAAAGGTACATATGGAAGAGACGCGACAGAGGATCCCAACAATCACAACCGCGG
KIKILQIQWDTAGGERFRTITA
TATACAGGAGTGCTAATTGGGGATCATGCTGTTTATGATTATACTAATGAAATACATTGGACACAC
YRAGMIGMLVDYDITNEKSFND
ATCAAACATCGGATCAGAAAATAGAGGACATCATACCCGAGATGTAAGAAAATGATTATGGGG
IKNWIRNIEEHSADVEKMLG
AACAAATGGTACATTAAATGAGAAGAGACGCAGCTGCTGAGAAAAAGACCAGAGGGACGCGAGAGCTGCGGTAGAG
NKCDINEKQRVSKDRGEKLA
TATGGCATACTATGGAAGACGACTGCCGAAGCTATATATCATACCGTGATACTACATTGGACACAC
YGIKEMETSACKANINVEFSLT
CTCGCCAGACAGATAAAATCAAAGATGGACACAGAATTGAGGGAAGCACAATTCCCATAGACGACGAAC
LARDIJKSDKMTKLEGNNPQSS
CATGGAGTGAATAATTACCATCGAAGAAGAGCAAGAAGACGCTTATCTCTCCGCTGTGCTACAGTGGA
HGVKITTETQQKKSSSFRCVLL*
AGATCAAGCCCTACTGTCGCCGCTTGAGGCGACAGACGACTGACTATGCTG

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

GGCTGAAGTAGTACCGGGGCAGATTTTCCGCAATATACTAATTAGATATGCGAAGACATCAGATTATTTGTTAAA
MAKTYDYLF
CTGCTGTTAAATCGGGGATTTCCCGGCTTGGAAGACATCGTGTCGTTCAAGATTTTCCCGGACGTGCC
LLILDGSVGTKTCVLFRRFSEDA
TTATACCTACGCTTATTTTCCGACATAGATTATTTGATCTAAGATCGAAGATAGATGCGG
FNSTFISTIGDFKIERTLIDG
AAGAGAAGATAAAGGTACATATGGAAGAGACGCGACAGAGGATCCCAACAATCACAACCGCGG
KIKILQIQWDTAGGERFRTITA
TATACAGGAGTGCTAATTGGGGATCATGCTGTTTATGATTATACTAATGAAATACATTGGACACAC
YRAGMIGMLVDYDITNEKSFND
ATCAAACATCGGATCAGAAAATAGAGGACATCATACCCGAGATGTAAGAAAATGATTATGGGG
IKNWIRNIEEHSADVEKMLG
AACAAATGGTACATTAAATGAGAAGAGACGCAGCTGCTGAGAAAAAGACCAGAGGGACGCGAGAGCTGCGGTAGAG
NKCDINEKQRVSKDRGEKLA
TATGGCATACTATGGAAGACGACTGCCGAAGCTATATATCATACCGTGATACTACATTGGACACAC
YGIKEMETSACKANINVEFSLT
CTCGCCAGACAGATAAAATCAAAGATGGACACAGAATTGAGGGAAGCACAATTCCCATAGACGACGAAC
LARDIJKSDKMTKLEGNNPQSS
CATGGAGTGAATAATTACCATCGAAGAAGAGCAAGAAGACGCTTATCTCTCCGCTGTGCTACAGTGGA
HGVKITTETQQKKSSSFRCVLL*
AGATCAAGCCCTACTGTCGCCGCTTGAGGCGACAGACGACTGACTATGCTG
Fig. 4: The complete coding sequence of common carp RDHIL 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
**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.

**Human_Dog**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Sumatran orangutan**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Cattle**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Chicken**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Mouse_Rat**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Common carp**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Zebrafish**
MAKTYYDLFKLILLIGOSGGVKTCLFRSSEADAFTSSTIGFDFIKRTIEDGKRIKLG

**Human_Dog**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Sumatran orangutan**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Cattle**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Chicken**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Mouse_Rat**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Common carp**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Zebrafish**
IWTAQGERFRITIIATAYRAGMIIVLTIDTNEK53DNIRKTNVWNSIIH3ADNVEKMLG

**Common carp**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Sumatran orangutan**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Cattle**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Chicken**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Mouse_Rat**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Common carp**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Zebrafish**
MYCVDNEKDQVRSDKERKLLALYGIKFNMTSARKANIVYFLVYKLRKAKKLELNLGN

**Common carp**
SPGQ90QV7TPDFQRKSSFRICVLL

**Sumatran orangutan**
SPGQ90QV7TPDFQRKSSFRICVLL

**Cattle**
SPGQ90QV7TPDFQRKSSFRICVLL

**Chicken**
SPGQ90QV7TPDFQRKSSFRICVLL

**Mouse_Rat**
SPGQ90QV7TPDFQRKSSFRICVLL

**Common carp**
SPGQ90QV7TPDFQRKSSFRICVLL

**Zebrafish**
NPQSN9H74TKETPQKRSSFRICVLL

**Common carp**
MHFLYLIGVLCFTFYRYRFRELRGRVPNKEKFWTSTIQGTCDFG5LONLGKHLILDVTYRVIAGC

**Zebrafish**
MHFLYLIGVLCFTFYRYRFRELRGRVPNKEKFWTSTIQGTCDFG5LONLGKHLILDVTYRVIAGC

**Atlantic salmon**
MHFLYLIGVLCFTFYRYRFRELRGRVPNKEKFWTSTIQGTCDFG5LONLGKHLILDVTYRVIAGC

**Common carp**
YMEKEOEVEKLKCSDKDLTLHVDTVNHYSIKAELTDLVSEQLWWNQ55FTTA

**Zebrafish**
YMEKEOEVEKLKCSDKDLTLHVDTVNHYSIKAELTDLVSEQLWWNQ55FTTA

**Atlantic salmon**
YMEKEOEVEKLKCSDKDLTLHVDTVNHYSIKAELTDLVSEQLWWNQ55FTTA

**Common carp**
FTEKEOEVELRLACSDFEVTVLFJQNNVSEQVDRAAALIKVDQAPSLNLWAVNNGAIFTPA

**Zebrafish**
FTEKEOEVELRLACSDFEVTVLFJQNNVSEQVDRAAALIKVDQAPSLNLWAVNNGAIFTPA

**Atlantic salmon**
FTEKEOEVELRLACSDFEVTVLFJQNNVSEQVDRAAALIKVDQAPSLNLWAVNNGAIFTPA

**Common carp**
PMDLWIETFQMNIVNLGIVATLVSFLILQALKGARVVNASVFRGISTLGAYCITK

**Zebrafish**
PMDLWIETFQMNIVNLGIVATLVSFLILQALKGARVVNASVFRGISTLGAYCITK

**Atlantic salmon**
PMDLWIETFQMNIVNLGIVATLVSFLILQALKGARVVNASVFRGISTLGAYCITK

**Common carp**
YQVEAFLKLSWRRMRAPFGVWVLCIEGPFHCTISDPTITYESLQLRKLHRLLQPEQVEQDYGS

**Zebrafish**
YQVEAFLKLSWRRMRAPFGVWVLCIEGPFHCTISDPTITYESLQLRKLHRLLQPEQVEQDYGS

**Atlantic salmon**
YQVEAFLKLSWRRMRAPFGVWVLCIEGPFHCTISDPTITYESLQLRKLHRLLQPEQVEQDYGS

**Common carp**
DFIDEKTVLIIKKEIEKEDLFLDILKVVSDKMHEAVAAVHFRSRYSFUGAKLFLWPLSLYPT

**Zebrafish**
DFIDEKTVLIIKKEIEKEDLFLDILKVVSDKMHEAVAAVHFRSRYSFUGAKLFLWPLSLYPT

**Atlantic salmon**
DFIDEKTVLIIKKEIEKEDLFLDILKVVSDKMHEAVAAVHFRSRYSFUGAKLFLWPLSLYPT

**Common carp**
FISDAILKNNVAFKVSYL

**Zebrafish**
FISDAILKNNVAFKVSYL

**Atlantic salmon**
GADWLLKEAEILKAVN

**Fig. 7:** The alignment of the protein encoded by common carp *RDHIL* gene and other two kinds of RDHIL 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.

REFERENCES


