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ANALYSIS OF EXPRESSED SEQUENCE TAGS FROM HEPATOPANCREATIC cDNA LIBRARY OF COMMON CARP (CYPRINUS CARPIO) AND CHARACTERIZATION OF LB-Fabp mRNA

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Abstract: A representative cDNA library obtained from hepatopancreas of common carp (*Cyprinus carpio*) was constructed, and 1016 expressed sequence tags were generated assembling into 115 contiguous sequences and 282 singletons. 215 (54.15%) of the assembled sequences were annotated based upon matching known genes in the GenBank public Databases. Functional categorization and comparative analyses of the sequence provided gene identities, expression information and a solid base for further research on common carp hepatopancreas. High expression of liver basic fatty acid binding protein (*Lb-Fabp*) (11/1016 ESTs) in the library was detected. Two types of *Lb-Fabpa* and *Lb-Fabpb* had an open reading frame of 381 bp encoding 126 amino acids. mRNA expression of *Lb-Fabps* in embryos, larva and adult common carp tissues were determined using semi-quantitative RT-PCR followed by Southern Blot analysis. *Lb-Fabp* mRNA was expressed with high levels in hepatopancreas, middle intestine and posterior intestine. Low levels of *Lb-Fabp* mRNA expression were observed in testis and skin, while much lower levels in muscle, brain, ovary, kidney, spleen, gill and heart. No *Lb-Fabp* mRNA expression was observed in fat and anterior intestine. *Lb-Fabp* mRNA was first observed in neurula stage, and expressed higher in the following stages. The expression pattern of *Lb-Fabp* mRNA suggests that common carp Lb-FABPs play an important role in lipid metabolism after the morphogenesis of hepatopancreas and intestine.

Key words: Common carp (*Cyprinus carpio*); cDNA library; Expressed sequence tags; Fatty acid-binding protein; mRNA expression

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The fatty acid binding protein (FABP) super-family is constituted by 14—15 kDa soluble proteins which bind with a high affinity either long-chain fatty acid (LCFA), bile acids or retinoids [1]. Based on the first tissue from which are isolated, FABPs have been classified into several types including heart, intestinal, adipocyte, myelin, ileal, liver, brain and epidermal [2]. The distinctive pattern of tissue expression of the different types of FABPs, as well as the co-expression of different FABPs in a single cell type, shows that each FABP has a specific function in lipid metabolism [3].

In the liver, L-FABP is structurally and functionally different from the other FABP types: L-FABP can bind two fatty acids per molecule but others only have one fatty-acid-binding site [4,5]. The L-FABP is able to bind not only fatty acids but also other hydrophobic ligands, such as acyl-CoA, retinoic acid, bile salts, prostaglandins, heme and peroxisome proliferators [6—8]. In

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function, L-FABP might be preferentially implicated in phospholipids synthesis, membrane protection and gene regulation [9].

The L-FABP type was first reported in mammals, then a basic FABP (PI=9.0) was isolated from chicken (*Gallus gallus*) liver [10]. Subsequently, Lb-FABP has been found in axolotl (*Ambystoma mexicanum*) [8], iguana (*Anolis pulchellus*) (GenBank accession number U28756), toad (*Bufo arenarum*) [11], frog (*Rana catesbeiana*) [12], zebrafish (*Danio rerio*) [13], catfish (*Rhamdia sapo*) [14,15], shark (*Halaetunus bivius*) [16], lungfish (*Lepidosiren paradoxus*) [17], and atlantic salmon (*Salmo salar*) [18]. The two types of FABP in liver are homologous genes most likely arose by a whole-genome duplication event [19,20]. But the function of Lb-FABP, which is restricted to non-mammalian vertebrates, is totally unclear.

Analysis of expressed sequence tags (ESTs) by single pass random sequencing of cDNA library is a powerful tool for rapid and cost-effective gene discovery. Some carp cDNA libraries have been constructed for special application[21,22]. Common carp, one of the most widely cultured fish species, is considered to be a cheap source of proteins. It is essential to research on the efficiency of feed for culturing on a large scale. Many researches have done in this domain, but molecular mechanism on the assimilation, especially lipids metabolism is still not comprehensive. Liver is the most important organ on the lipids metabolism, so constructing the cDNA library and discovering relevant genes on lipids metabolism is indispensable.

In this paper, we choose hepatopancreas of common carp as the basic material to study the function and mechanism of lipids metabolism. Firstly, we constructed the hepatopancreatic cDNA library, and large-scale EST sequencing was performed. Two cDNAs of *Lb-Fabp* were obtained. To get more information of common carp *Lb-Fabp* mRNA expression, the distribution pattern in adult tissues as well as in different stages of embryos and early larval development was examined.

1 Materials and Methods

1.1 Animals Common carps (*Cyprinus carpio*) with body weight ranging from 500 to 1500g were obtained from Huangsha aquatic market of Guangzhou, and then they were cultured in tanks under the natural conditions in the lab. Hepatopancreas and other tissues were collected after the animals had been anesthetized on ice. Samples were immediately frozen in liquid nitrogen. Mature unfertilized eggs, embryos of different stages and larval carps from 1- to 30-day-old were obtained from February to March, 2007, during reproductive season, and frozen in liquid nitrogen. All samples were stored at -80℃ until assay.

1.2 Construction of the hepatopancreatic cDNA library Total RNA was isolated from common carp hepatopancreas, using Trizol Reagent (Invitrogen, U.S.A) and subsequently the mRNA was purified by PolyATtract Z5300 kit (Promega, U.S.A). cDNA library was made from six micrograms poly(A)$^+$ RNA purified above, using the CloneMiner™ cDNA library construction kit (Invitrogen, U.S.A). The kit contains a cDNA size-fractionation step using Sephacryls-500 HR resin column chromatography to assure the short segments away. Inserts were directionally cloned into *att*P1 and *att*P2 of the pDNOR™ 222 vector. Each constructed plasmid was transformed into DH10B™ cells by electroporation.

1.3 cDNA sequencing Individual transformants from the plasmid library were picked into 96-well microtiter plated containing LB medium with 100μg/mL kanamycin. Plates were incubated overnight at 37℃, 250 *rpm* and frozen at -80℃ after being mixed with same volume of 25% glycerol. Extraction and sequencing of plasmid DNA were done by Invitrogen Bio-Tech, Shanghai of China. cDNA inserts were sequenced from the 5'-end using M13 primer.

1.4 Sequence analysis Raw sequences data were processed to assess quality, remove vector sequence and contaminants, finally aligned and assembled into a unigene set (contig). The software used in the process was SeqMan II module of Lasergene Suite (DNAStar, Madison, WI). The consensus sequence of each contig was subjected to BLAST analysis against the GenBank non-redundant protein and nucleotide databases. Based on the sequence similarity, contigs were grouped as high significant similarity $E \leq 10^{-20}$, moderately signif-

icant similarity $10^{-19} \leq E \leq 10^{-5}$, and low or no significant similarity $E > 10^{-5}$.

1.5 RT-PCR analysis of common carp *Lb-Fabp* **gene** Based on the *Lb-Fabp* cDNA sequences from cDNA library, special PCR primers (Forward: 5′-CGTGGCAGGTTTATGT-3′, Reverse: 5′-CTCCTACTGTCAGGGTCT-3′) were designed in the conservative areas of common carp *Lb-Fabpa* and *Lb-Fabpb*. Then semi-quantitative RT-PCR assay was performed to measure the level of mRNA expression of common carp *Lb-Fabp*. Total RNAs of embryos, larvae and 15 tissues were isolated using Trizol Reagent (Invitrogen, U.S.A), respectively. After digested with DNase I, 1μg of total RNA from each tissue was reverse-transcribed into cDNA using oligo $(dT)_{18}$ primer by RevertAid™ H Minus M-MuLV reverse transcriptase kit (MBI Fermentas, USA).

The PCR conditions consisted of a denaturation cycle 94℃ for 3 min, followed by 32 PCR cycles each consisting of 15 s denaturation (94℃), 15 s annealing (56℃) and 30 s extension (72℃). Extension time of the last cycle was increased to 10 min. Five microliters of each PCR reaction was electrophoresed and stained with EB, and measured. An internal control PCR reaction was performed in a separate tube, using two specific 18S ribosomal RNA (GenBank accession number AF133089) primers (Forward: 5′-CCTGAGAAACGGCTACCACATCC-3′, Reverse: 5′-AGCAACTTTAATATACGCTATTGGAG-3′). The PCR condition for 18S was the same as the *Lb-Fabp* PCR, except for using the 30 cycles instead of 32 cycles.

RT-PCR products were conformed by Southern Blot analysis. In brief, 5 μL of the products was electrophoresed and stained. Gel with the amplified DNA band was excised, denatured, and then transferred onto a positively charged nylon membrane by electronic transfer. The membrane was then hybridized with the common carp *Lb-Fabp* Dig-labeled cDNA probe prepared by PCR. The hybridization and detection procedure were carried out according to the manufacturer's instructions. Signal was captured in Syngene Genenome (Syngene, England).

2 Results

2.1 cDNA library and cDNA sequence analysis

The cDNA library created from hepatopancreas of common carp contains 1.432×10^7 *cfu* (colony-forming units). Twenty-six clones were randomly selected (according to manipulation manual of CloneMiner™) from the library and the length of each cDNA insert was measured by restriction digestion with *Ban* Ⅱ, which revealed an average insert size of 1.03 kb.

Sequencing reactions were performed for 1074 cDNA clones, and 1016 ESTs were generated after an elimination of inserts shorter than 100 bp, sequence with low quality and vector sequence only. The ESTs were aligned and assembled into 115 contiguous sequence (contigs) and 282 singletons. Contigs varied in size from 2 ESTs to 49 ESTs (Fig. 1) were divided into 5 groups with a span of 10 ESTs. 76% (87/115) contigs contained 2 to 9 ESTs, while 24% (28/115) contig contained 10 or more ESTs. Parts of the highly expression genes were cited in Tab. 1 (non-claim in the databases).

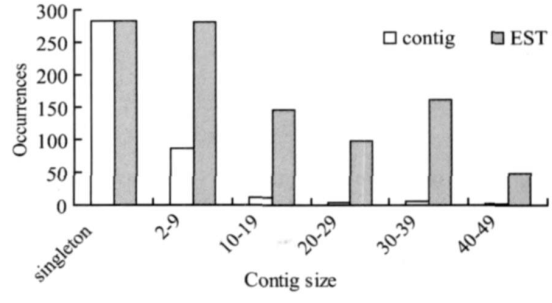

Fig. 1 Prevalence distribution of the contig size

Sequences were grouped according to the similarity with genes in the GenBank Database. 54.15% (215) had highly significant similarity with known genes, 30.73% (122) had moderate significant similarity, including unnamed protein genes and cosmid DNA sequence. 15.11% (60) had low or no significant similarity. The 215 (54.15%) sequences having highly significant similarity with known genes were grouped into functional categories (Tab. 2): 180 sequences consisting of 540 ESTs belonged to class A, which comprised a large fraction of the recognized protein-coded mRNA transcripts, containing structural and enzy-

matic housekeeping proteins associated with the functions of many different cells; class B, consisting of 25 protein-coded sequences from 156 ESTs associated with cell-cell communication; class C contained 10 transcription factors and other gene regulatory protein-coded sequences consisting of 25 ESTs.

Tab. 1　Part of the highly expression genes in the library

Contigs	Expect-value	Gene Description	EST Numbers
Contig 1	$0.00e^{+00}$	Fibrinogen gamma polypeptide	49
Contig 4	$2.00e^{-61}$	14 kDa apolipoprotein	33
Contig 15	$3.00e^{-139}$	Intelectin	33
Contig 180	$2.00e^{-36}$	Haptoglobin precursor isoform	21
Contig 112	$0.00e^{+00}$	Aldolase b, fructose - bisphosphate	13
Contig 5	$2.00e^{-77}$	Apolipoprotein A - I	30
Contig 6	$5.00e^{-27}$	Preprohepcidin	22
Contig 240	$1.00e^{-168}$	Fibrinogen alpha chain	19
Contig 108	$3.00e^{-35}$	Similar to Apoa4 protein	13
Contig 123	$4.00e^{-14}$	Tributyltin binding protein type 1	18
Contig 11	$7.00e^{-120}$	Fatty acid binding protein 10	11
Contig 20	$4.00e^{-170}$	Uncoupling protein 1	10
Contig 78	$7.00e^{-56}$	Microfibrillar-associated protein 4	18
Contig 214	$0.00e^{+00}$	Fibrinogen, B beta polypeptide	31

Tab. 2　The functional categories of genes having high similarity with the known genes

Classes of Gene Function and Gene Annotation	Number of Genes	Number of ESTs
A　Functions that Many Kinds of Cells Use		
Cytoskeleton and membrane proteins	7	14
Transportation and binding proteins	34	190
Cell replication related (histones, cyclins, DNA polymerase, topoisomerases, RNA processing, etc)	15	19
Protein synthesis, degradation related (ribosomal proteins etc)	43	83
Intermediary synthesis, catabolism related	47	79
Stress response, detoxification and cell defense proteins	28	147
Apoptosis-related	6	8
B　Cell-Cell Communication		
Signaling receptors, signal pathway, signal intermediates	22	153
Extracellular matrix proteins and cell adhesion	3	3
C　Transcription Factors and Other Gene Regulatory Proteins	10	25
Total	215	721

2.2　Identification of the common carp Lb-Fabp gene

Common carp *Lb-Fabp* was one of the high expression genes in the hepatopancreatic cDNA library (11/1016 ESTs). Two types of *Lb-Fabp* cDNA were obtained by EST sequencing and assembling. cDNA sequence of *Lb-Fabpa* (GenBank accession number EU363800) was 507 bp long, while *Lb-Fabpb* (GenBank accession number EU363801) was 517 bp long. The major difference was in the 3'-untranslated region. Both had an open reading frame (ORF) of 381 bp encoding 126 amino acids, and a cytoplasmic fatty acid binding proteins signature in the N-terminal. Further secondary structure analysis revealed that both *Lb-Fabps* had 10 sheets and 2 helix, which were conservative in FABP family. The two types of Lb-FABP shared a 96.8% amino acid identity.

In the comparison of the deduced amino acid sequences of FABPs between common carp and other species (Fig. 2, Tab. 3), the two types of carp Lb-

FABP had high homology to 90.5% and 92.1% respectively with Lb-FABP of zebrafish (FABP10). The common carp Lb-FABP exhibited an overall higher sequence identity of 69.8, 72.15, 71.4 to anolis, axolotl, chicken Lb-FABP. The identity was lower (<50%) to L-FABPs

Fig 2 Alignment of amino acid sequences of common carp Lb-FABP and other species FABPs (clustalx 1.83). The identical, highly conserved and less conserved amino acid residues were indicated by (*), (:), (.) respectively

Tab. 3 Amino acid sequence percent identity of common carp Lb-FABP compared to other vertebrates

1	2	3	4	5	6	7	8	9	10	11	12	
	96.8	35.7	44.4	90.5	69.8	40.5	71.4	42.1	71.4	40.5	42.9	1 Carp Lb-FABPa
		34.1	45.2	92.1	69.8	42.1	73.0	42.9	71.4	40.5	41.3	2 Carp Lb-FABPb
			59.8	34.1	34.9	61.1	36.5	63.0	34.9	59.1	63.8	3 Zebrafish FABP1a
				43.7	46.0	61.9	48.4	60.6	44.4	53.5	55.9	4 Zebrafish FABP1b
					67.5	42.1	69.8	40.5	69.8	39.7	42.1	5 Zebrafish FABP10
						42.9	68.3	42.9	72.2	42.1	42.9	6 Anolis Lb-FABP
							42.1	74.6	38.9	70.6	73.8	7 Axolotl L-FABP
								42.9	73.8	38.9	41.3	8 Axolotl Lb-FABP
									39.7	69.3	70.1	9 Chicken L-FABP
										39.7	39.7	10 Chicken Lb-FABP
											84.3	11 Mouse L-FABP
												12 Human L-FABP

Note: The identity was calculated with MegAlign of DNAStar soft

Phylogenetic tree was constructed using alignment of amino acid sequences of FABPs among common carp and other species (Fig 3). The results showed that the two types of common carp Lb-FABP are grouped into the Lb-FABP clade with high bootstrap support

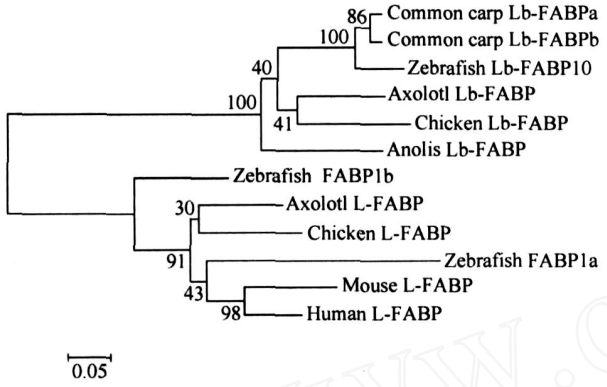

Fig 3 Phylogenetic tree based on the amino acid sequences alignment of carp Lb-FABP and reported FABPs (liver) in other species. Phylogenetic analysis was carried out by Mega 3.1 based on the Neighbor-jointing method with 1000 bootstrap replicates. The number shown at each branch indicates the bootstrap values (%). The FABPs and their GenBank accession numbers are: human (*Homo sapiens*) L-FABP, AAA52419; mouse (*Mus musculus*) L-FABP, AAH09812; chicken (*Gallus gallus*) L-FABP, AAP84705; chicken (*Gallus gallus*) Lb-FABP, p80226; anolis (*Anolis pulchellus*) Lb-FABP, Q90239; axolotl (*Ambystoma mexicanum*) L-FABP, P81399; axolotl (*Ambystoma mexicanum*) Lb-FABP, P81400; zebrafish (*Danio rerio*) FABP1a, AAZ08575; zebrafish (*Danio rerio*) FABP1b, AAZ08576; zebrafish (*Danio rerio*) FABP10, NP694492; common carp (*Cyprinus carpio*) Lb-FABPa, EU363800; Lb-FABPb, EU363801

2.3 Tissue distribution of Lb-Fabp gene

The expression of Lb-FABP mRNA in different adult tissues was analyzed by semi-quantitative RT-PCR followed by Southern Blot analysis, and the results are shown in Fig 4. *Lb-Fabp* mRNA was found in the hepatopancreas, middle intestine and posterior intestine by RT-PCR, and the hepatopancreas had the strongest signal, while other tissues also had lower signals of *Lb-Fabp* after Southern Blot analysis, such as heart, red muscle, white muscle, brain, ovary, testis, kidney, spleen, gill and skin.

2.4 Expression of Lb-Fabp mRNA during embryonic and larval development

The temporal expression patterns of Lb-FABP during early development were analyzed by semi-quantitative RT-PCR followed by Southern Blot analysis, and

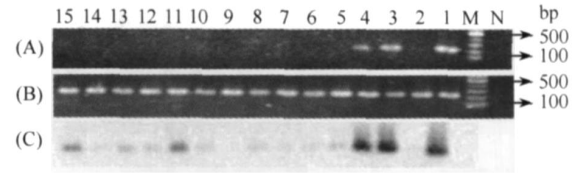

Fig 4 RT-PCR/Southern Blot analysis of the expression of *Lb-Fabp* transcript in adult common carp tissues. (A) RT-PCR analysis of the expression of *Lb-Fabp* transcript in adult common carp tissues. (B) 18S ribosomal RNA transcript of tissues of adult common carp amplified as the internal control by RT-PCR. (C) Simultaneous Southern Blot analysis of the same gel using DIG-labeled probe. M: 100bp DNA ladder; N: Negative control; 1: Hepatopancreas; 2: Anterior intestine; 3: Middle intestine; 4: Posterior intestine; 5: Heart; 6: Red muscle; 7: White muscle; 8: Brain; 9: Fat; 10: Ovary; 11: Testis; 12: Kidney; 13: Spleen; 14: Gill; 15: Skin

the results are shown in Fig 5 and Fig 6. No *Lb-Fabp* mRNA expression was observed in the unfertilized eggs, cell cleavage stage, blastula stage and gastrula stage. The expression level of *Lb-Fabp* mRNA increased gradually from optic bubble formation stage to newly hatched larva (Fig 5). After hatching *Lb-Fabp* mRNA could be obviously detected by RT-PCR, and the level increased firstly and then maintained relatively high levels till 30-day-old post hatching (Fig 6).

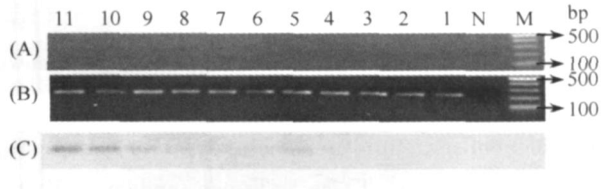

Fig 5 RT-PCR/Southern Blot analysis of the expression of *Lb-Fabp* transcript in the common carp embryos. (A) RT-PCR analysis of the expression of *Lb-Fabp* transcript in different embryonic stages. (B) 18S ribosomal RNA transcript of common carp embryos amplified as the internal control by RT-PCR. (C) Simultaneous Southern Blot analysis of the same gel using DIG-labeled probe. M: 100bp DNA ladder N: Negative control; 1: Unfertilized eggs; 2: cell cleavage stage; 3: Blastula stage; 4: Gastrula stage; 5: Neurula stage; 6: Optic bubble formation stage; 7: Lens formation stage; 8: Heart-beating stage; 9: Blood cycling stage; 10: Hatching stage; 11: Newly hatched larva

3 Discussion

It is possible to find a EST of a new gene by screening 1000 clones [23]. So the cDNA library we constructed from the common carp hepatopancreas consisted of 1016 ESTs can reflect the hepatic gene ex-

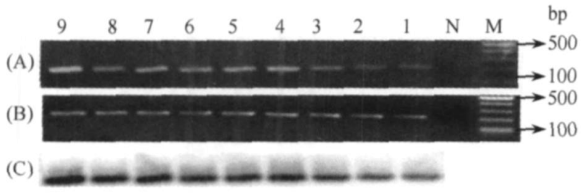

Fig. 6 RT-PCR/Southern Blot analysis of the expression of *Lb-Fabp* transcript in the common carp larval stages. (A) RT-PCR analysis of the expression of *Lb-Fabp* transcript in different larval stages. (B) 18S ribosomal RNA transcript of common carp larvae amplified as the internal control by RT-PCR. (C) Simultaneous Southern Blot analysis of the same gel using DIG-labeled probe. M: 100bp DNA ladder; N: Negative control; 1: 1-day-old post hatching (1dph); 2: 3 dph; 3: 5 dph; 4: 7 dph; 5: 10 dph; 6: 15 dph; 7: 20 dph; 8: 25 dph; 9: 30 dph

pression pattern. The ESTs which are more than 150 bp long contain more information, and are very suit for searching the homologous products [24]. In the present study, before sequencing we chose and identified the clones which were 200 bp long to ensure the quantity of the library, and got more information of the mRNAs in common carp hepatopancreas. When searching the homologous products in GenBank Database, our criterion identifying the Blast result was meaningfulness was: the value of Score exceeding 80 [25] and the E value under e^{-10} [22, 26]. By screening the ESTs, 215 sequences had highly significant similarity with known genes. These genes contained enzymes related to protein metabolism, lipid-transfer and binding Proteins, and immune factors. These ESTs can fundamentally reflect the expression patterns of common carp hepatopancreas genes.

In contrast with the co-expression of different FABPs in the liver, lungfish liver expresses only Lb-FABP. Using antibody with specificity to rat L-FABP, H-FABP, A-FABP, and I-FABP failed to recognize such FABP types in lungfish liver [17]. The expression pattern resembles that of tetrapods, which only expresses the L-FABP. In the 1016 ESTs, there were 11 ESTs representing common carp Lb-FABP. But we did not get the L-FABP. So we suppose that in the carp liver Lb-FABP plays more important roles than L-FABP in the lipid metabolism, or their expression is relevant to the developmental stages, or in the carp it is possible that there is only one type FABP.

The homology analysis based on the amino acid sequences identities further indicates that the common carp Lb-FABP is more closely related to the Lb-FABPs than to L-FABPs, averagely 75.8% and 41.1% identity, respectively. A further comparison of the known L- and Lb-FABP sequences indicates that there are 24 residues conserved in these proteins (Fig. 2). This suggests that these residues are important to those functions shared by both liver FABP types. The Arg-121 and Ser-123 that stabilize the fatty acid carboxyl group [19, 27] are also present in the common carp Lb-FABP.

In order to investigate the Lb-FABP physiological functions in the common carp, the *Lb-Fabp* mRNA expression in the different tissues was analyzed. The result showed that the *Lb-Fabp* mostly expressed in the liver, followed in middle and posterior intestine. The *Lb-Fabp* expression pattern in common carp is different from that reported previously in zebrafish [13, 20]. In situ hybridization analysis of adult male and female zebrafish sections revealed that the Lb-FABP-specific probe hybridized to mRNA only in the liver [13]. But using highly sensitive RT-PCR technique, a *fabp*10-specific product was generated from total RNA extracted from liver, intestine and testis of adult zebrafish [20]. Using rabbit anti-(catfish Lb-FABP) did not detect the protein in any catfish tissue other than liver [15]. Similarly using rabbit anti-(axolotl Lb-FABP) did not detect the protein in any axolotl tissue other than liver [8]. Common carp *Lb-Fabp* is not highly restricted in the liver suggests that it has more functions and plays important roles in liver and intestine. The expression pattern in tissues of the adult common carp extends the *Lb-Fabp* expression areas known species. However, we did not distinguish their expression pattern of *Lb-Fabpa* and *Lb-Fabpb*. In one tissue, whether one of *Lb-Fabps* expressed or co-expressed is still unkown.

Previous study reported that the expression of FABP10 was detected in the liver of the zebrafish embryos at 48 hpf [20]. Abundant *fabp*10 transcripts were also detected in the liver of 5-day-old larva. The present results showed that no *Lb-Fabp* mRNA expression was observed in the unfertilized eggs, cell cleavage stage, blastula and gastrula stages. Weak detection was observed in neurula stage and increased gradually

to 30-day-old larval stage. This may be link to the development of the hepatocytes.

In conclusion, the present study constructs the cDNA library of common carp hepatopancreas and analyzes the ESTs. From the ESTs, we get two types of common carp *Lb-Fabp* cDNAs, and the amino acids alignment shows that there is a high degree of homology with other species Lb-FABP. The expression pattern of *Fabp* mRNA in adult tissues suggests that Lb-FABP plays an important role in liver and intestine. The presence of *Lb-Fabp* mRNA at the late embryonic and larval developmental stages of common carp indicates that the level of *Lb-Fabp* mRNA increases with the development of the digestive organs (liver and intestine). The further study should focus on the function and mechanism of the Lb-FABP on the lipid metabolism.

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鲤鱼肝胰脏 cDNA 文库的表达序列标签分析及 Lb-Fabp mRNA的特征

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摘要:本文构建了鲤鱼肝胰脏 cDNA 文库,共获得了 1016条有效的表达序列标签。拼接组装成 115个 contigs和 282个 singletons。其中 215个拼接序列在 GenBank公共数据库中寻找到相对应的基因。对它们进行功能性分类和比较分析为鲤鱼肝胰脏的研究提供了基因表达信息的基础。文库中 1016条表达序列标签有 11条代表了鲤鱼肝基本型脂肪酸结合蛋白 (Lb-FABP)。通过序列比较我们获得了两个具有相同开放阅读框长度的 *Lb-Fabp* cDNAs。开放阅读框全长 381bp,编码 126个氨基酸。半定量 RT-PCR结合 Southern blot技术研究了 *Lb-Fabp* mRNA 在成鱼不同组织以及早期发育不同时期的表达图式。结果表明,*Lb-Fabp* mRNA 在肝胰脏、中肠和后肠中表达量较高。同时在精巢和皮肤中有低水平的表达。脑、肌肉、卵巢、肾脏、脾脏、鳃和心脏等组织中其表达量更低。而在脂肪和前肠中则没有检测到 *Lb-Fabp* mRNA 表达。*Lb-Fabp* mRNA 最早在胚体形成期检测到有低水平表达,随后的发育阶段中表达量逐渐升高。鲤鱼 *Lb-Fabp*基因的表达图式提示在肝脏和肠等器官开始发育后,它可能在脂肪代谢中具有重要作用。

关键词:鲤鱼; cDNA 文库;表达序列标签;脂肪酸结合蛋白; mRNA 表达