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Divergent spatial regulation of duplicated fatty acid-binding protein (*fabp*) genes in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

The increased use of plant oil as a dietary supplement with the resultant high dietary lipid loads challenges the lipid transport, metabolism and storage mechanisms in economically important aquaculture species, such as rainbow trout. Fatty acid-binding proteins (Fabp), ubiquitous in tissues highly active in fatty acid metabolism, participate in lipid uptake and transport, and overall lipid homeostasis. In the present study, searches of nucleotide sequence databases identified mRNA transcripts coded by 14 different fatty acid-binding protein (*fabp*) genes in rainbow trout (*Oncorhynchus mykiss*), which include the complete minimal suite of seven distinct *fabp* genes (*fabp1*, *2*, *3*, *6*, *7*, *10* and *11*) discovered thus far in teleost fishes. Phylogenetic analyses suggest that many of these extant *fabp* genes in rainbow trout exist as duplicates, which putatively arose owing to the teleost-specific whole genome duplication (WGD); three pairs of duplicated *fabp* genes (*fabp2a.1*/*fabp2a.2*, *fabp7b.1*/*fabp7b.2* and *fabp10a.1*/*fabp10a.2*) most likely were generated by the salmonid-specific WGD subsequent to the teleost-specific WGD; and *fabp3* and *fabp6* exist as single copy genes in the rainbow trout genome. Assay of the steady-state levels of *fabp* gene transcripts by RT-qPCR revealed: (1) steady-state transcript levels differ substantially between *fabp* genes and, in some instances, by as much as 30×10^4-fold; (2) some *fabp* transcripts are widely distributed in many tissues, whereas others are restricted to one or a few tissues; and (3) divergence of regulatory mechanisms that control spatial transcription of duplicated *fabp* genes in rainbow trout appears related to length of time since their duplication. The suite of *fabp* genes described here provides the foundation to investigate the role(s) of fatty acid-binding proteins in the uptake, mobilization and storage of fatty acids in cultured fish fed diets differing in lipid content, especially the use of plant oil as a dietary supplement. These nutritional dietary supplements may well lead to high lipid loads with the resultant challenges to lipid homeostasis and, thus, health of cultivated fish which may be mediated by appropriate transcriptional control of *fabp* genes.

1. Introduction

For the past decade, aquaculture is the fastest growing food producing sector in the world. Global aquaculture production in 2012 reached 66.6 million tonnes, an increase of 6.5% compared to 2011 (62.0 million tonnes) (FAO, 2014). Fish oil (FO) is traditionally used as a main energy source in aquaculture diets, and its production has remained constant during this period because of increasing fishing pressure on wild fish stocks. Fish feed manufacturers, therefore, have decreased FO content and increased economic vegetable oil (VO) sources in fish diets to improve profitability (Bayir et al., 2011).

FO is an abundant source of n-3 highly unsaturated fatty acids (n-3 HUFA), such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which are essential for normal growth and reproduction in fish (Sargent et al., 1999). VOs, however, are rich in linoleic (LA; 18:2n-6) and alpha linolenic (LNA; 18:3n-3) acid and many freshwater fish, including salmonids, can convert these fatty acids to longer-chain n-3 HUFA by elongation and desaturation activities (Tocher, 2003). Previous studies in salmonids reported that FO can be totally or partially replaced by VOs without any adverse effects on their growth performance and survival rate (Torstensen et al., 2000; Bell et al., 2001; Fonseca-Madrigal et al., 2005; Bayir et al., 2011). VO-rich fish diets, however, are often detrimental to the nutritional quality of fish meat, especially n-3 HUFA content, FAs which are beneficial to human health (see for example, Masiha et al., 2013).

Fatty acid-binding proteins (FABPs) belong to the large multigene family of intracellular lipid-binding proteins (iLBPs). Other members

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Table 1
GenBank accession numbers for the longest expressed sequence tags (EST) coded by rainbow trout *fabp* genes, zebrafish Fabp sequences used as queries in BLAST searches, and two rainbow trout ESTs used as reference genes in the assay of steady-state levels of *fabp* transcripts.

Rainbow trout gene	Rainbow trout EST	Zebrafish Fabp
fabp1a	CR371215.2	NP_001038177 (Fabp1a)
fabp1b	CR370172.2 and CU063180.1	NP_001019822 (Fabp1b.1)
fabp2a.1	MMSRT107A_scaff_3056_1[a]	NP_571506 (Fabp2)
fabp2a.2	BX317932.2	NP_571506 (Fabp2)
fabp2b	BX860658.3	NP_571506 (Fabp2)
fabp3	CA381377.1	NP_694493 (Fabp3)
fabp6	CR942998.1	NP_001002076 (Fabp6)
fabp7b.1	BX082627.2	NP_999972 (Fabp7b)
fabp7b.2	CA369721.1	NP_999972 (Fabp7b)
fabp10a.1	DR696714.1	NP_694492 (Fabp10a)
fabp10a.2	BX871393.3	NP_694492 (Fabp10a)
fabp10b	BX912954.3	XP_003200455 (Fabp10b)
fabp11a	CF752694.1	NP_001004682 (Fabp11a)
fabp11b	CU065876.1 and CX723086.1	NP_001018394 (Fabp11a)
ß-actin	AJ438158.1	
ef1	NM_001124339.1	

[a] Sequence retrieved from MMSRT107A_scaff_3056_1 at National Animal Genome Research Program (NAGRP) database (www.animalgenome.org).

Table 3
Isoelectric point (p*I*) of Fabp10 polypeptides from teleost fishes and chicken.

Species	Protein	p*I*
Rainbow trout	Fabp10a.1	8.89
Rainbow trout	Fabp10a.2	7.70
Zebrafish	Fabp10a	8.87
Zebrafish	Fabp10b	5.94
Atlantic salmon	Fabp10a.1	8.52
Atlantic salmon	Fabp10a.2	8.53
Medaka	Fabp10a	8.40
Medaka	Fabp10b	7.77
Tilapia	Fabp10a	7.74
Tilapia	Fabp10b	8.31
Catfish	Fabp10	9.10
Chicken	Fabp10	9.00

of this conserved multigene family are the cellular retinol acid-binding proteins and the cellular retinoic acid-binding proteins (Bernlohr et al., 1997; Schroeder et al., 2008). Thus far, eighteen paralogous iLBPs have been identified in vertebrates. FABPs were originally named according to the initial tissue of isolation, e.g. heart-type fatty acid-binding protein (H-FABP), brain-type fatty acid-binding protein (B-FABP), etc. Since each FABP is not specific to a single tissue and many FABPs show overlapping patterns of tissue distribution, this nomenclature is confusing (Parmar et al., 2012a, 2012b; Parmar and Wright, 2013). We, therefore, use here the nomenclature of Hertzel and Bernlohr (2000) where each Fabp and its gene are given an Arabic number approximating the order in which they were discovered, e.g., FABP3 (heart-type), FABP7 (brain-type), etc. The multigene family of FABPs is augmented in teleost fishes owing to a whole genome duplication (WGD) early in the teleost fish lineage approximately 230–400 million years ago (reviewed in Braasch and Postlethwait, 2012). Duplicate *fabp* genes that arose via the teleost-specific WGD are given suffixes "a" and "b", e.g., *fabp7a* and *fabp7b*. Subsequent to the teleost WGD, a second WGD event occurred in salmonids no later than 88 million years ago, and 40–50 million years ago before subsequent salmonid subfamilies diverged, such that all living salmonids are of autotetraploid origin (Braasch and Postlethwait, 2012; Macqueen and Johnston, 2014). Duplicate genes of the salmonid-specific WGD are given numerical suffixes, e.g. *fabp7b.1* and *fabp7b.2* (Lai et al., 2009; Lai et al., 2012; see http://zfin.org/ for gene nomenclature conventions).

FABPs are 15 kDa polypeptides of 125–135 amino acids in length. FABP genes exhibit a highly conserved gene organization consisting of four exons separated by three introns (Veerkamp and Maatman, 1995; Bernlohr et al., 1997; Storch and Corsico, 2008) with the exception of *fabp1a* in zebrafish (Sharma et al., 2006), *FABP3* in desert locust (Wu et al., 2001) and *fabp11a* in medaka (Parmar et al., 2012b).

Numerous reports suggest that iLBPs have important, perhaps vital, roles in cell physiology, such as: (1) uptake, transport and utilization of fatty acids (FAs), retinoids and other hydrophobic ligands; (2) interaction with other transport and enzymes systems; (3) development, growth and reproduction; and (4) regulation of gene transcription via FA signaling through peroxisome proliferator-activated receptors (Bernlohr et al., 1997; Zimmerman and Veerkamp, 2002; Schroeder et al., 2008; Storch and Corsico, 2008). Studies on the relationships between dietary vegetable oils and *fabp* gene expressions in fish are rare and limited to one study in rainbow trout (Venold et al., 2012). Since Fabps play such crucial roles in fatty acid transport, sequestering and metabolism (Veerkamp and Maatman, 1995; Bernlohr et al., 1997; Storch and Corsico, 2008), their potentially critical functioning in the mobilization and tissue storage of fatty acids provided by VO diets, we investigated the *fabp* genes in rainbow trout, the second most produced salmonid species in the world (Davidson et al., 2010; FAO, 2014). By searches of the National Center for Biotechnology Information sequence databases (http://blast.ncbi.nlm.nih.gov), we identified transcripts coded by 14 distinct *fabp* genes in rainbow trout that represents the complete suite of *fabp* genes found in teleost fishes, thus far. Many of the extant rainbow trout *fabp* genes are the product

Table 2
RT-qPCR primers with optimal annealing temperatures and qPCR efficiencies.

Rainbow trout genes	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)	Tm (°C)	qPCR efficiency
fabp1a	GAGCTGGAGACGTTGACTGG	GGCCCAGCATCATTGTAGCA	57.8	0.97
fabp1b	GAAGGTCAATTCTGTGGTGACGAG	ACATTCGTTTGCTGGTCCTC	54.6	0.98
fabp2a.1	TTGTCGTGAAGGAGGCCAG	ATCTCTGGGCTTCGACTCCATCG	58.9	0.93
fabp2a.2	CGTGAAGGAGGCCAGTTCTT	TTCTCTTGGCTTCGACTCCATCA	62.6	0.99
fabp2b	GTACCTGGGAGATGGATGGA	GCATCCACCCCATCGTAGTT	54.6	0.89
fabp3	ATGAAGGCTCTGGGTGTGG	TCCTTGCCATCCCACTTCTG	54.8	1.04
fabp6	GGGAAAAAGTTCAAGGCCAC	GCTGGTTCTTTTCAGCACGA	57.4	0.94
fabp7b.1	GCACTTGGTGTTGGTTTTGC	CACTTCTTGGCTGTAGTCC	57.2	0.96
fabp7b.2	GCACTTGGTGTTGGTTTTGC	TAAACATTGGCTGTCTCCCAG	58.1	0.99
fabp10a.1	GGGCCATCTCACTCCCAGAAG	CCTGGATGTGTGTGAATTTG	53.1	1.08
fabp10a.2	GGCCATCTCCCTCCCAGAA	CCTGGACGCTGGAGAATTTA	53.2	0.87
fabp10b	AGTTTAAGTGTACTGCCAGACT	GGTGTCAGGAAAAGCCATCC	50.6	0.89
fabp11a	CGACAGAAAAACAATGACCGTT	TATGTCCTCACCGCAACCAC	53.6	0.95
fabp11b	TGCGAAATGTGTCATGGA	CATGGTAGGTACTGAACAGAT	57.8	0.90
ß-actin	CTTCTACAACGAGCTGAGGGT	GGTCTCAAACATGATCTGGGT	57.0	0.93
ef1	AAGCAGCTGAGATGGGCAAG	CCTCAAACTCACCCACACCA	58.2	0.97