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Molecular cloning, expression and single nucleotide polymorphisms of protein phosphatase 1 (PP1) in mandarin fish (*Siniperca chuatsi*)

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ABSTRACT

In the wild, mandarin fish (*Siniperca chuatsi*) only feed on live prey fish, refusing dead prey. When reared in ponds, training will result in some mandarin fish accepting artificial diets. However, little is currently known about the molecular mechanism of the individual difference. Serine/threonine protein phosphatase 1 (PP1) is a suppressor of learning and long-term memory (LTM) in mammals. In the present study, the relationship between PP1 and the individual difference in acceptance of artificial diets in mandarin fish was investigated. The complete CDS (coding sequence) of four PP1 isoforms (*PP1caa*, *PP1cab*, *PP1cb* and *PP1cc*) were cloned in mandarin fish. The amino acid sequences of these PP1 isoforms are highly conserved in different species. The mRNA expressions of *PP1caa* and *PP1cb* in brain of artificial diet feeders were significantly higher than those in nonfeeders, suggesting the deficiency in the maintenance of long-term memory of its natural food habit (live prey fish). The SNP loci in *PP1caa* and *PP1cb* were also found to be associated with the individual difference in acceptance of artificial diets in mandarin fish. These SNPs of *PP1caa* and *PP1cb* genes could be useful markers for gene-associated breeding of mandarin fish, which could accept artificial diets. In conclusion, different mRNA expression and SNPs of *PP1caa* and *PP1cb* genes in feeders and nonfeeders of artificial diets might contribute to understanding the molecular mechanism of individual difference in acceptance of artificial diets in mandarin fish.

1. Introduction

Memory is the storage of the information from the past (Lucon-Xiccato and Dadda, 2014), and learning is the acquisition of memories (Okano et al., 2000; Pearce and Bouton, 2001). Learning and memory is one of the most fundamental and important abilities of humans and other animals, which must adapt to changing environments (Sclafani, 1997; Higgs, 2005). Learning and memory could affect food intake, appetite, feeding strategies and behavior in mammals (Higgs, 2002, 2005, 2008). Like mammals, learning and memory systems in fish also play important roles in foraging processes (Lieberman, 2000; Rodriguez et al., 2002; Warburton, 2003). Several studies have reported that fish are capable of performing well in a range of learning tasks such as olfactory conditioning, shuttle box active appetitive conditioning, appetitive choice discrimination, and visual discrimination learning (Clifton et al., 1998; Liang et al., 1998; Bilotta et al., 2005; Colwill et al., 2005; Braubach et al., 2009; Pather and Gerlai, 2009; Guttridge and Brown, 2014). However, little is known about the genes involved in learning and memory of nocturnal piscivorous mandarin fish.

The omnipresent protein phosphatases (PPs) regulate several essential cellular processes, such as protein synthesis, transcription and neuronal signaling (Cohen, 2002). A balance between protein kinases (PKs) and PPs determine memory formation and synaptic plasticity (Martin et al., 2000; Munton et al., 2004; Rahman et al., 2012). The PP family consists of PP1, PP2A, PP3, PP4, PP5, PP6 and PP7, with PP1 and PP2A being the best studied and most abundant enzymes (Shi, 2009; Peti et al., 2013). The function of PP1 is conserved from *Drosophila* to mammals (Asztalos et al., 1993). Mutant fruit flies (*Drosophila*) lacking PP1 have defective habituation and associative learning (Asztalos et al., 1993). In the human (*Homo sapiens*) brain, PP1 accounted for about 10% of the total tau phosphatase activity (Liu et al., 2005). PP1 regulates synaptic transmission and plasticity, hence affecting learning and memory (Bennett et al., 2001; Terry-Lorenzo et al., 2002; Monti et al., 2005; Mansuy and Shenolikar, 2006; Yamashita et al., 2006). Increased PP1 activity in the mammalian hippocampus is associated with impairment of learning and LTM (Koshibu et al., 2009; Lee and Silva, 2009; Graff et al., 2010; Haege et al., 2010; Genoux et al., 2011; Koshibu et al., 2011;

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Rahman et al., 2012). The dysfunction of PP1 enzyme may cause cognitive deficits, neurodegenerative diseases, learning and memory impairment (Bennett et al., 2001; Mansuy and Shenolikar, 2006).

Many organisms have multiple PP1genes, and the structural nature is extremely conserved in the nucleotide and amino acid levels from invertebrates to vertebrates, such as *paramecium* (Friderich et al., 1992), sea urchin (*Echinoidea*) (Byrum et al., 2006), fruit fly (*Drosophila*) (Dombrádi et al., 1987a,b, 1989, 1990a,b), medaka (*Oryzias latipe*) (Kasahara et al., 2007), turbot (*Psetta maxima*) (Qi et al., 2008), zebrafish (*Danio rerio*) (Jayashankar et al., 2013), and mammals (Ceulemans et al., 2002; Moorhead, 2007; Peti et al., 2013). The mammal genomes contain three different genes that encode four distinct catalytic subunits of PP1: PP1α (*PP1ca*), PP1β/δ (*PP1cb*), and PP1γ (*PP1cc*, *PP1cc-2*), with the last two being spliced isoforms (Ceulemans et al., 2002; Peti et al., 2013). In teleost, the zebrafish has five PP1 isoforms: two genes similar to PP1α (*PP1caa* and *PP1cab*), two genes similar to PP1β (*PP1cba* and *PP1cbb*), and one gene related to PP1γ (*PP1cc*) (Jayashankar et al., 2013). In this study, we had found four PP1 genes in mandarin fish.

Mandarin fish (*Siniperca chuatsi*), a typical carnivorous fish, has very peculiar food preference. In the wild, as soon as they begin to feed, they feed exclusively on live fry of other fish species (Liu and Cui, 1989; Liang et al., 2008). In rearing conditions, many mandarin fishes also only accept live prey fish, refusing dead prey fish or artificial diets (Liang et al., 1998). However, Liang et al. (2001) designed a specific training procedure for this fish resulting in some mandarin fish (feeders) eventually feeding on minced fish prey, even artificial diets, but some (nonfeeders) still refused (He et al., 2013). However, little is currently known about the molecular mechanism of the difference. To investigate the association between PP1 and the individual difference in acceptance of artificial diets in mandarin fish, we cloned the complete CDS of all PP1 isoforms, and analyzed SNPs (single nucleotide polymorphisms) and mRNA expression of PP1 in feeders and nonfeeders, contributing to understanding the molecular mechanism of individual difference in acceptance of artificial diets in mandarin fish.

Table 1
PCR primer sequences for PP1 genes.

Primers	Primer sequence (5′–3′)
Primers for PP1cab clone	
PP1cab 3′RACE-RT	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTTT
PP1cab 3′RACE-F	TGAGCCAGCCAATACTGCTTGAACT
PP1cab 3′RACE-R	GGCCACGCGTCGACTAGTAC
Primers for PP1cc splice	
PP1cc F	TGTGCGATTTGCTCTGGTCT
PP1cc R	TTGCTTTCATCGGGTAGGAG
Primers for sequence the complete CDs	
PP1caa F	GGAGTTATTAGAAACCGACC
PP1caa R	TCCTGCTAACAAGGTTCATC
PP1cab F	CATCATAGGAAGACTGTCGTAA
PP1cab R	TTGGTTTTCAGAAGGGAAGAGA
PP1cb F	TTTGGCATTATCGGTCGC
PP1cb R	TACAGACGGGGGTAAGGT
PP1cc F	AGCAGGGTCACACCAACA
PP1cc R	GCTTTCATCGGGTAGGAG
Primers for SNP screening	
PP1caa F	CGACCGTGGTGTCTCCTTCA
PP1caa R	GCACGCACTCACTCAGACAAT
PP1cb F	CAGCTCCCAATTACTGTGGC
PP1cb R	ATCGTTTCTTTGGAGGTTGG
Primer sequences for real-time RT-PCR	
PP1caa F	ACTGCGGTGAGTTCGACAAT
PP1caa R	CGAGGGCAAACTACTGGTGT
PP1caa F	ACTGCGGTGAGTTCGACAAT
PP1caa R	CGAGGGCAAACTACTGGTGT
PP1cb F	AACCCTGATGTGCTCCTTCC
PP1cb R	TTCTTTGGAGGTTGGGCTGT
PP1cc F	ATCTTTCTCAGTCAACCCATTC
PP1cc R	CCCTGTCCACATAGTCACCC

2. Materials and methods

2.1. Fish and sample preparation

Mandarin fish were propagated by artificial reproduction using broodstock from river, and reared using live fry of Indian mrigal (*Cirrhinus mrigala*). The dead prey fish for mandarin fish was prepared by freezing. Minced prey was prepared in a fish cutting machine using grass carp purchased from a local market. The artificial diet was prepared based on the formula of Liang et al. (2001). Training was initiated when the fish reached about 180–200 g. The juvenile mandarin fish were trained in net-cages during the experimental at Guangdong Freshwater Fish Farm (Panyu, Guangdong Province, China) (Liang et al., 2001). About 600 fishes were trained, and 15 fishes with a uniform size were randomly distributed in each of the net-cages (2 m × 2 m × 1 m). The square-shaped net-cages were made of knotless polythene netting.

The specific training procedures followed the methods reported by Liang et al. (2001). The training period lasted about 20 days. On the first day, live prey fish were fed in excess at dusk. On days 2–4, the feeding level was gradually reduced day-by-day. On day 5, the mandarin fish were fed to satiation with live prey fish only, and they were observed capturing the live prey fish immediately by darting at the beneath water surface. On days 6–8, live prey fish were gradually replaced with dead prey fish day-by-day, and more and more mandarin fish were observed accepting dead prey fish. On day 9, only dead prey fish were offered and some of the mandarin fish were observed capturing dead prey fish immediately by darting at the beneath water surface. On days 10–12, minced prey gradually replaced dead prey fish day-by-day, and more and more mandarin fish were observed accepting minced prey. On day 13, only minced prey was fed and some mandarin fish were observed immediately capturing the prey. On days 14–16, the artificial diet was gradually replaced with minced prey day-by-day, and we observed more and more mandarin fish accepting artificial diet. On days 17–20, only the artificial diet was offered and we observed that some of them can capture artificial diet immediately by darting at the beneath water surface. After training, these fish were visually sorted into feeders (those that accepted the artificial diet successfully) and nonfeeders (those that did not accept the artificial diet) on the basis of plumpness or emaciation, respectively (Liang et al., 2001; He et al., 2013). The training success ratio between feeders and nonfeeders was more than 70%. Because of the relatively short time and large size of the fish, hunger did not lead to mortality in the nonfeeders. Before sampling, the feeders and nonfeeders were fed with live prey fish for 2 days to eliminate the effect of hunger on mRNA expression level. During the rearing period, water temperature ranged from 20 to 32 °C, dissolved oxygen content was over 5 mg L^{-1}, and water transparency was between 100 and 200 cm. The water depth of the rearing area was 10-15 m.

After the training, we have successfully obtained two groups: 124 feeders and 124 nonfeeders. Genomic DNA was extracted from the caudal fin ray using the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to manufacturer's directions. In order to detect the mRNA expression of genes, we were taken to a uniform size of five feeders

Table 2
Primers for genotyping *PP1caa* and *PP1cb* SNPs.

Gene	SNPs locus	Genotyping primer
PP1caa	G1416A	AGCTGTTCTATGGTGGTGGA
		CACGGGAAGAGTGGTAAGAG
PP1cb	C1285G	TCCTGAAGCCATCTGAGAAGAAATC
		CTCCCTCTGTCCTTCATCGTTTCTTT