

# Ya-fish (*Schizothorax prenanti*) spexin: identification, tissue distribution and mRNA expression responses to periprandial and fasting

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**Abstract** Spexin (SPX) is a novel peptide which was known for its role in physiological homeostasis. A recent study has confirmed that SPX plays an important role in the feeding regulation. However, the reports about SPX are very limited. In the present study, we characterized the structure, distribution and mRNA expression responses to feeding status of SPX in Ya-fish (*Schizothorax prenanti*). The full-length cDNA of Ya-fish SPX was 1330 base pairs (bp), which encoded 106 amino acid residues. These residues contained a 31-amino acid signal peptide region and a 14-amino acid mature peptide. The sequence alignment demonstrated that the Ya-fish SPX showed high conservation with other species. Our data revealed that SPX was widely expressed in all test tissues. The highest expression of SPX mRNA was observed in Ya-fish forebrain. Compared with the Ya-fish SPX mRNA expression in the forebrain between the

preprandial and postprandial groups, the fed group was prominently increased than unfed groups after a meal, while the unfed group at 1 and 3 h substantially decreased than preprandial groups ( $P < 0.01$ ). In addition, SPX mRNA expression in forebrain was significantly decreased ( $P < 0.01$ ) during fasting for a week and sharply increased ( $P < 0.01$ ) after refeeding on the 7th day, and then return to normal level on the 9th day. These results point toward that SPX mRNA expression is regulated by metabolic status or feeding conditions in Ya-fish.

**Keywords** Ya-fish · Cloning · Structure · Tissue distribution · Fasting · Refeeding

## Introduction

The research of peptide hormones has received considerable attention on their role in modulating a wide range of behavioral and somatic responses to environmental stimuli (Kastin 2010). Food intake is one of the most basic needs common to all animals, which is a kind of the behaviors mediated by a complex interaction of central and peripheral-derived endocrine peptide hormones with orexigenic or anorexigenic actions (Gonzalez and Unniappan 2010; Oh-I et al. 2006; Lenard and Berthoud 2008). Ghrelin (Zhou et al. 2014) and neuropeptide Y (Tiesjema et al. 2009; Zhou et al. 2013) can stimulate food intake intensively. Oppositely, some peptides

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have anorexigenic function, for example nesfatin-1 (Lin et al. 2014) and leptin (Scott et al. 2011). Numerous circulating peptides produced in the body influence appetite (Coll et al. 2007; Morton et al. 2006; Nguyen et al. 2011). Thereinto, SPX is one of the important peptides. Compelling evidence suggests that SPX as a satiety molecule is involved in the control of food intake (Wong et al. 2013).

SPX is a novel peptide hormone which first identified in 2007 through bioinformatics search strategy based on a hidden Markov model (Mirabeau et al. 2007). And then, SPX was researched in different vertebrates through evolutionary probabilistic models (Sonmez et al. 2009). The SPX was composed of a signal peptide sequence and two predicted dibasic prohormone cleavage sites (RR and GRR) (Sonmez et al. 2009). The 14-amino acid SPX mature peptide region between dibasic cleavage sites was highly conserved from fish to mammals.

Reports showed that SPX has been determined in brain regions and peripheral tissues in some vertebrates. These results suggest that SPX has multiple physiological functions. But little is known about it. However, it was known to induce gastrointestinal contraction (Mirabeau et al. 2007), inhibit adrenocortical cell proliferation with minor stimulation on corticosteroid release (Rucinski et al. 2010) and play a role in urine production, cardiovascular function and nociception (Toll et al. 2012). The spatiotemporal expression of SPX will be important to define when and where SPX functions are needed. However, only limited data are available concerning the SPX gene and expression of its mRNA or protein product. In situ hybridization identified SPX mRNA in the intermediate lobe of the pituitary, the submucosal layer of esophagus and stomach fundus and the zona glomerulosa of the mouse adrenal gland (Mirabeau et al. 2007). By means of reverse transcription-polymerase chain reaction (RT-PCR), SPX transcripts were found in human and rodent brains. Northern blot analysis suggested the presence of SPX protein product in brain, kidney, pancreas and placenta (Mirabeau et al. 2007; Sonmez et al. 2009). Recently, reports described a broad distribution of SPX mRNA and protein in rat and goldfish, respectively (Porzionato et al. 2010; Wong et al. 2013). These data suggested that SPX may have pleiotropic actions in different tissues. Recently, a report revealed that SPX mRNA levels could be elevated in the hypothalamus and telencephalon of

goldfish after feeding (Wong et al. 2013). Moreover, intracerebroventricular injection of SPX inhibited feeding behavior and food consumption in goldfish (Wong et al. 2013). These results showed the novel role of SPX as a satiety factor in regulating the food intake in teleost.

Ya-fish (*Schizothorax prenanti*) is an endemic cold temperate fish in the southwest of China. However, slow growth rate has been recognized as a major problem in Ya-fish farming (it takes at least 3–5 years to grow into the marketable sizes, about 500 g), so it is important to find ways to increase production of farmed fish. For improving its culture, we used the Ya-fish as an experimental model to understand the genetic control of feeding and growth (Wei et al. 2013a, b; Lin et al. 2014). Hereby we identified and characterized the SPX gene from Ya-fish and investigated the expression levels in different tissues and feeding-related changes of SPX mRNA expression in Ya-fish forebrain.

## Materials and methods

### Animals

Ya-fish were obtained from Runzhao Fisheries Co., Ltd. (Sichuan, China). Fish were acclimated in Sichuan Agricultural University Farm (Ya'an, China) indoor tanks (0.5 m<sup>3</sup>) at least 2 weeks prior to each study and supplied with well-aerated water at  $16 \pm 1$  °C under a 12-h day/light photoperiod. All fish were fed commercial pellet diet (G68, Meishan, China) to satiation according to the one-meal-per-day feeding schedule (11:00, 0 h). The uneaten pellet diet within an hour was removed by using a dip net. All experimental protocols using fish were approved by the Regulations for the Administration of Affairs Concerning Experimental Animals and approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University. Fish were anesthetized in 0.02 % tricaine methane sulfonate (MS-222) before decapitation. Tissues were dissected rapidly and snap-frozen in liquid nitrogen and then stored at  $-80$  °C until total RNA extraction was performed.

For cDNA cloning and analysis of tissue distribution, the tissues (forebrain, pituitary, eye, gill, skin, red muscle, white muscle, heart, hepatopancreas, spleen, head kidney, trunk kidney, foregut, midgut, rectum, testis

and ovary) were collected from Ya-fish ( $236.55 \pm 13.73$  g,  $n = 10$ , 1:1 male-to-female sex ratio).

In order to examine periprandial (pre- and post-feeding) changes of SPX mRNA expression in Ya-fish forebrain, 56 weight-matched Ya-fish juveniles ( $35.81 \pm 3.56$  g,  $n = 8$ /group) were evenly distributed in seven tanks.

For measurement of fasting-induced changes of SPX mRNA in the forebrain, nine groups of weight-matched Ya-fish ( $94.85 \pm 7.68$  g,  $n = 8$ /group) were allowed to acclimate to tank conditions for several weeks before experimental use. Severally, four control groups were fed daily as control, and five groups of fish were fasting for a week. The control samples were collected on the 3rd, 5th, 7th and 9th days, respectively. Among the unfed groups, two were refed from 7th day unto the end. The samples were collected on the 3rd, 5th and 7th days from fasted groups, while from refed groups on the 7th and 9th days.

#### cDNA synthesis and molecular cloning

Total RNA was extracted from Ya-fish tissue samples using the TRIzol<sup>®</sup> RNAiso Plus (Takara, Dalian, China) with the manufacturer's standard protocol. The integrity and concentration of the RNA were assessed by 1.0 % agarose gel electrophoresis and optical density absorption ratio (A260/280). Samples with an absorption ratio (1.8–2.0) were used for subsequent cDNA synthesis.

Primers (Table 1) used for RT-PCR were designed on the basis of regions of high identity among zebrafish (XM\_005164774.1) and goldfish (JX035990.1). PCR

was performed at 94 °C denaturation for 5 min; followed by 32 cycles at 94 °C for 30 s, a gene-specific annealing temperature for 1 min at 55 °C, and 72 °C for 1 min; extension for 7 min at 72 °C; and then cooled to 4 °C using a C1000<sup>™</sup> Thermal Cycler (Bio-Rad, Hercules, CA, USA).

For obtaining the complete SPX mRNA sequence, gene-specific primers, SPX-F1 and SPX-F2 were designed for 3' end amplification, and SPX-R1 and SPX-R2 were designed for 5' end amplification (Table 1). The 3'/(5')RACE was amplified by two rounds of PCR. SPX-F(R)1,2 and Universal Primer Mix (UPM) provided in the RACE Kit (BD Biosciences Clontech, Palo Alto, CA, USA) were used for PCR, respectively. Touchdown PCR (TD-PCR) amplification conditions for 5' and 3'RACE were as follows: initial denaturation for 5 min at 94 °C; 5 cycles at 94 °C for 30 s, 70 °C for 30 s, 72 °C for 2 min; 5 cycles at 94 °C for 30 s, 68 °C for 30 s and 72 °C for 2 min; 25 cycles at 94 °C for 30 s, 66 °C for 30 s and 72 °C for 2 min; a final extension for 10 min at 72 °C; and then cooled to 4 °C.

All the PCR amplified products were run on 1.5 % agarose gels. Bands of the expected size were excised from the gel and purified with DNA Purification kit (TIANGEN, Beijing, China). The purified products were connected with pMD19-T vector (Takara, Dalian, China) and then transformed into *E.Coli* DH5a competent cells. All steps are according to manufacturer's instructions. The recombinants were identified through blue-white color selection in ampicillin-containing LB plates and confirmed by PCR. Three positive clones in each PCR fragment were sequenced by Beijing

**Table 1** Primers sequences and function used in this study

Primer name	Primer sequence (5'–3')	Applications
SPX-F	5'ACAACGTCTGACACTATGAA 3'	SPX cloning
SPX-R	5'TGGTTTCAGTAAGGCTCAC 3'	SPX cloning
SPX-F1	5'CGCTTAGAGTCACGCAGCCAAAACACAG3'	SPX 3'RACE outer
SPX-F2	5'GGCAACATTTGTGTCCCATTCCTGGAGT 3'	SPX 3'RACE inner
SPX-R1	5'CGAGACGACTTTAAGCATGAATGTGGAC3'	SPX 5'RACE outer
SPX-R2	5'TCCGACGCTGAAAACCTGCCCTTGGGT 3'	SPX 5'RACE inner
SPX-F3	5'AGGATTTAGGGACTCTTGC GGCTTA3'	SPX qPCR
SPX-R3	5'TCTGTGTTTTGGCTGCGTGACTC3'	
β-actin-F	5'CGAGCTGTCTTCCCATCCA3'	β-actin qPCR
β-actin-R	5'TCACCAACGTAGCTGTCTTTCTG3'	
18S-F	5'ACCACCCACAGAATCGAGAAA3'	18S qPCR
18S-R	5'GCCTGCGGCTTAATTTGACT3'	

β-actin and 18S were used as housekeeping genes

Genomics Institute (BGI, Beijing, China), and these resulting sequences were verified and subjected to cluster analysis in National Center of Biotechnology Information (NCBI).

### Structural and phylogenetic analysis

The presence and location of signal peptide cleavage sites in amino acid sequence were predicted by SignalP 4.1 server (<http://www.cbs.dtu.dk/services/SignalP/>) (Peteresen et al. 2011). Prediction of arginine and lysine propeptide cleavage sites was performed by ProP 1.0 server (<http://www.cbs.dtu.dk/services/ProP/>) (Duckert et al. 2004). Phylogenetic analysis was conducted by using relevant amino acid sequences obtained from the GenBank. Multiple sequence alignments were conducted by using ClustalW (<http://www.ebi.ac.uk/clustalw/>) (Thompson et al. 1994). Based on the amino acid sequences, the phylogenetic tree was constructed by the neighbor-joining method in MEGA 5 program (<http://www.megasoftware.net/index.html>) (Kumar et al. 2008).

### Tissue distribution of SPX mRNA

Quantitative real-time PCR (qPCR) analysis was performed by using the Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Total RNA was extracted, and cDNA was synthesized as described previously. The gene-specific primers are listed in Table 1. Ya-fish-specific  $\beta$ -actin and 18S-rRNA primers served as internal controls to normalize cDNA quantity for each tissue sample. Studies have shown that normalization of qPCR data by geometric averaging of multiple housekeeping genes is more accurate (Vandesompele et al. 2002). Quantification of SPX,  $\beta$ -actin and 18S-rRNA mRNA was performed in triplicate on all samples using SYBR Premix Ex Tap<sup>-2</sup> (Perfect real time) (Takara, Dalian, China) according to the manufacturer's instructions. The qPCR was conducted as follows: heated to 95 °C for 3 min as initial denaturation, followed by 40 cycles of denaturation at 95 °C for 15 s and a gene-specific annealing temperature of 58/60 °C for 30 s. Melting curves were also plotted (60–90 °C) in order to make sure that a single PCR product was amplified for each pair of primers. The PCR data were obtained as CT values and analyzed using the comparative Ct method ( $2^{-\Delta\Delta Ct}$ ) (Livak and Schmittgen 2001).

### Periprandial expression of SPX mRNA in the forebrain

Among tissues that expressed SPX mRNA, the forebrain was of particular interest because of their well-established role in the regulation of food intake and energy homeostasis (Kirkham et al. 2002; Bernier et al. 2004). In this study, we examined periprandial (pre- and postfeeding) changes of SPX mRNA expression in the forebrain that show abundant expression of SPX. Forebrain samples were collected at: 3 h prior to feeding (−3 h), 1 h prior to feeding (−1 h), upon commencement of feeding (0 h), 1 h after feeding (+1 h) and 3 h after feeding (+3 h). Three unfed groups were sampled at 1 and 3 h and served as the unfed group. Tissue extraction, cDNA synthesis and qPCR were carried out as described previously.

### Fasting-induced changes of SPX mRNA in the forebrain

Fasting induces several neuroendocrine changes affecting the expression of appetite regulatory peptides in fish (Wan et al. 2012; Gonzalez and Unniapan 2010). The objective of this study was to determine whether fasting changes SPX mRNA expression in the forebrain of Ya-fish during fasting conditions over a 1-week period. Total RNA extraction and quantification were performed as described above.

### Statistical analysis

Statistical analysis was carried out using Student's *t* test and two-way ANOVA followed by Student–Newman–Keul's post hoc test with the SPSS Statistical 18.0 software package (SPSS Inc., Chicago, IL, USA). All data are expressed as mean  $\pm$  SEM. The threshold for significance was set at  $P < 0.05$ .

## Results

### Molecular cloning of Ya-fish SPX

By means of the RACE PCR, Ya-fish full-length cDNA sequence of SPX was obtained. The Ya-fish

SPX (GenBank accession number: KJ561306) nucleotide sequence was 1330 bp in length and contained a 479 bp of 5'-untranslated region (5'-UTR), a 530 bp of 3'-UTR and a 321 bp of open reading frame (ORF).

The deduced SPX protein is composed of 106 amino acid residues, with a 31-amino acid signal region followed by a 14-amino acid mature peptide flanked by two dibasic processing sites (RR and GRR; Fig. 1). The amino acid sequence of the Ya-fish SPX showed high identity with fish species (Fig. 2), such as zebrafish (93 %) and goldfish (91 %). It showed 54.9–72.3 % identity with birds, relatively low level (38.2–45.1 %) in mammals. This result was in line with the laws of the evolution. Also, it was confirmed in the phylogenetic analysis that the Ya-fish SPX was grouped with the fish SPX subfamily and teleost SPX was divided from the mammal model furthest (Fig. 3).



**Fig. 1** Nucleotide and predicted propeptide cleavage sites in amino acid sequences of Ya-fish SPX mRNA. The mature peptide region is shaded in gray. The gray highlighted character

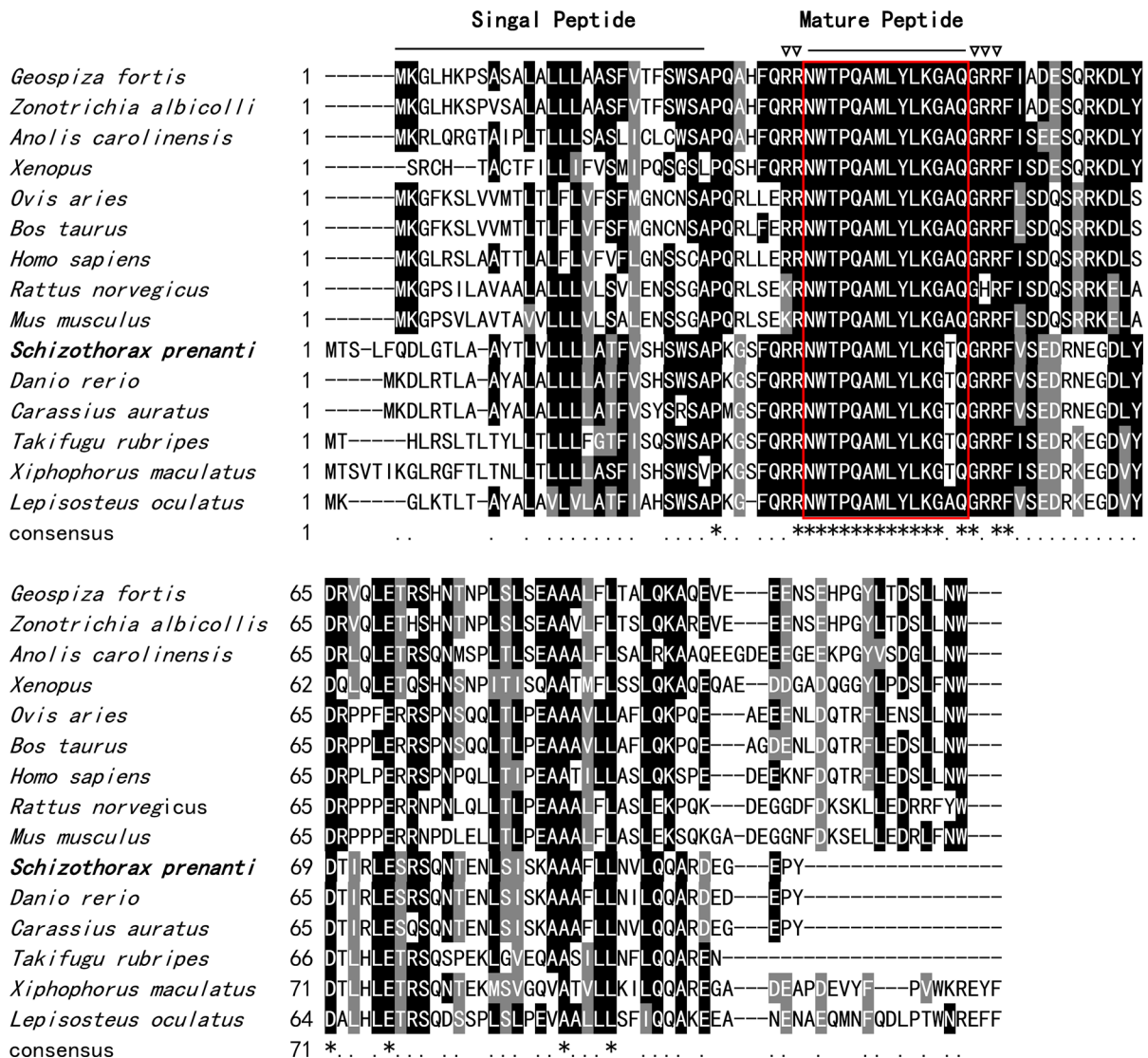
### Tissue expression of Ya-fish SPX mRNA

The data showed that the Ya-fish SPX mRNA was widely distributed among the tissues, with the highest level in forebrain (Fig. 4). The expression of Ya-fish SPX was abundant relatively in pituitary, foregut and midgut. Weaker SPX mRNA expression was found in the skin, ovary, testis, red muscle and white muscle (Fig. 4).

### Periprandial expression of Ya-fish SPX mRNA

In the case of Ya-fish SPX mRNA expression at different time points (−3, −1, 0, 1 and 3 h) in the periprandial experiment, two-way ANOVA was conducted with time and treatment as the two variables for

indicates the putative signal peptide. The predicted propeptide cleavage sites required for processing are boxed



**Fig. 2** Multiple alignment of SPX amino acid sequences. The colored amino acids highlight the differences in conservation of the amino acids between species of SPX. Identical amino acid is indicated by an asterisk. In addition, the predicted mature SPX peptide is boxed in red. Species names and GenBank accession numbers used in the alignment were as follows: *Geospiza fortis* (XP\_005429821.1), *Zonotrichia albicollis* (XP\_005492779.1), *Anolis carolinensis* (XP\_003220804.1), *Xenopus* (Ref. Wong et al. 2013), *Ovis aries* (NP\_0012

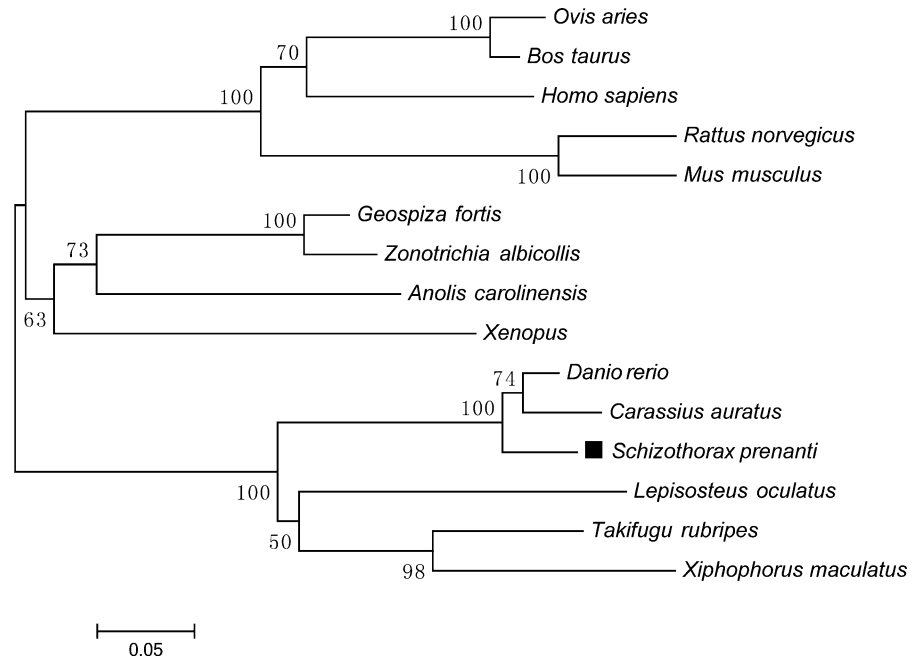
74398.1), *Bos taurus* (NP\_001068875.1), *Homo sapiens* (NP\_085049.1), *Rattus norvegicus* (NP\_001077402.2), *Mus musculus* (NP\_001229274.1), *Schizothorax prenanti* (KJ561306), *Danio rerio* (XP\_005164831.1), *Carassius auratus* (AFK29204.1), *Takifugu rubripes* (XP\_003979286.1), *Xiphophorus maculatus* (XP\_005805513.1), *Lepisosteus oculatus* (XP\_006633257.1), positions of the two dibasic protein cleavage sites (RR and GRR), which are essential for the release of the 14-amino acid mature peptide

data analysis. Between-subjects effects showed that the levels of SPX mRNA were affected by time and treatment, and the treatment had a larger impact. Simultaneously, there was no interaction between the two factors ( $P = 0.26$ ).

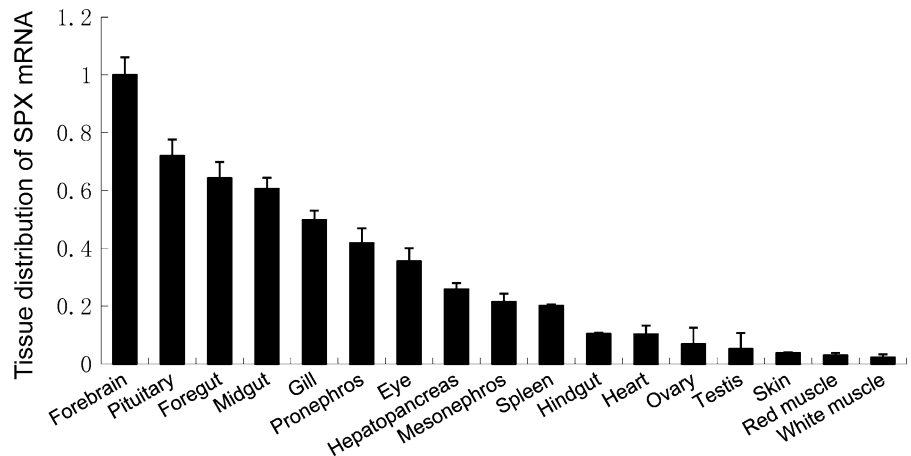
The SPX expression of unfed fish significantly decreased at 1 and 3 h postfeeding ( $P < 0.01$ ). Data

showed that the levels of SPX mRNA were detected at -3-, -1- and 0-h fluctuant expression, and however, there was no significant difference in forebrain SPX mRNA levels among these preprandial groups. Moreover, no significant changes were detected among unfed groups after 0 h (Fig. 5). Expression levels between fed and fasted fish (1- and 3-h groups) were

**Fig. 3** Phylogenetic analysis of SPX amino acid sequences. *Scale bar* indicates the substitution rate per residue. *Numbers at nodes* indicate the bootstrap value, as percentages, obtained for 1000 replicates



**Fig. 4** Tissue distribution of Ya-fish SPX mRNA. The results were expressed as relative expression levels after standardization by  $\beta$ -actin and 18S gene. *Error bars* represent standard error of the mean ( $n = 10$  fish, 1:1 male-to-female sex ratio)



compared using Student's *t* tests. As data shown in Fig. 5, Ya-fish SPX mRNA expression of fed fish significantly increased than that of unfed groups in the forebrain at 1 and 3 h, respectively ( $P < 0.01$ ).

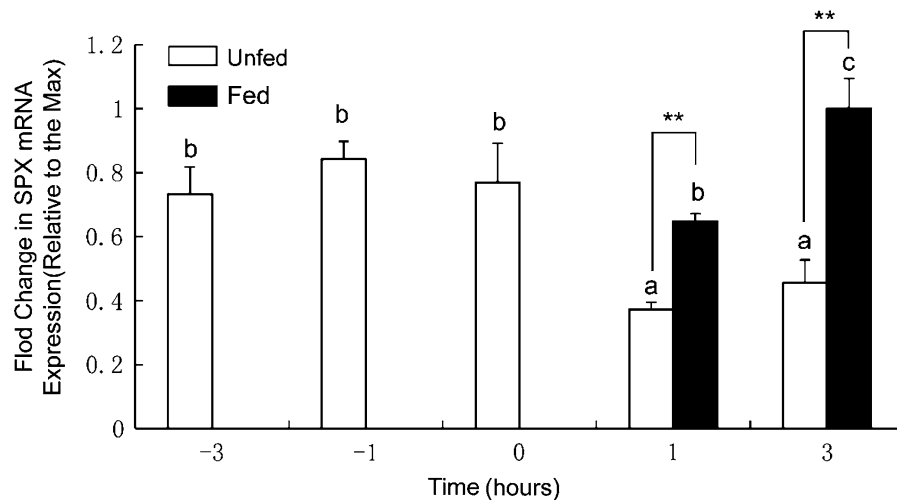
#### Fasting-induced changes of SPX mRNA expression in Ya-fish forebrain

SPX mRNA expression of unfed fish had 2.6-, 2.7-, 1.8-fold decrease compared to 3th-, 5th- and 7th-day fed animals, respectively ( $P < 0.01$ ). On the 7th day, another food-deprived group was refed, and SPX

mRNA expression significantly increased about 1.2-fold higher than that of normal feeding group ( $P < 0.01$ ). Subsequently, the expression of SPX mRNA declined to control levels on the 9th day. Among normal feeding groups, there were no significant differences in levels of SPX mRNA expression (Fig. 6).

#### Discussion

In this study, we firstly characterized the structure, tissue distribution and mRNA expression responses to



**Fig. 5** Periprandial changes of SPX mRNA expression in the forebrain of Ya-fish. The mRNA expression of SPX was normalized to  $\beta$ -actin and 18S gene. The preprandial (–3, –1 and 0 h) and postprandial groups (3, 1 h) that differ significantly are indicated by *different letters* above bars. *Bars with dissimilar superscripts* indicate groups that differ significantly (two-way

ANOVA followed by a Student–Newman–Keul’s post hoc test,  $P < 0.05$ ). *Asterisks* represent significant differences between the fed and unfed groups at the same time point (Student’s *t* test,  $**P < 0.01$ ). *Error bars* represent standard error of the mean ( $n = 8$  fish/group)

feeding status of the SPX in Ya-fish, an important commercial fish species endemic to China. The sequence and gene structure were well conserved through evolution, in particular among teleost species. Molecular cloning and phylogenetic analysis confirmed that the newly cloned Ya-fish SPX is a member of the SPX gene family in fish models.

Regarding the deduced protein sequence, encoded by the newly cloned Ya-fish SPX is 106 amino acid in size, with a N-terminal signal peptide and a 14-amino acid SPX mature peptide flanked by two well-conserved dibasic cleavage sites (RR and GRR), followed by a relatively long C-terminal region (Fig. 1). The presence of a glycine residue at the end of this putative peptide suggests that it is processed and amidated, a common feature of peptide hormones (Rholam and Fahy 2009; Eipper et al. 1992). Since the GRR motif is known to be a signature sequence as  $\alpha$ -amidation during peptide processing, SPX mature peptide is an  $\alpha$ -amidate which disposed at its C-terminus, such as goldfish (Wong et al. 2013), human (Mirabeau et al. 2007) and rat SPX (Porzionato et al. 2010; Toll et al. 2012).

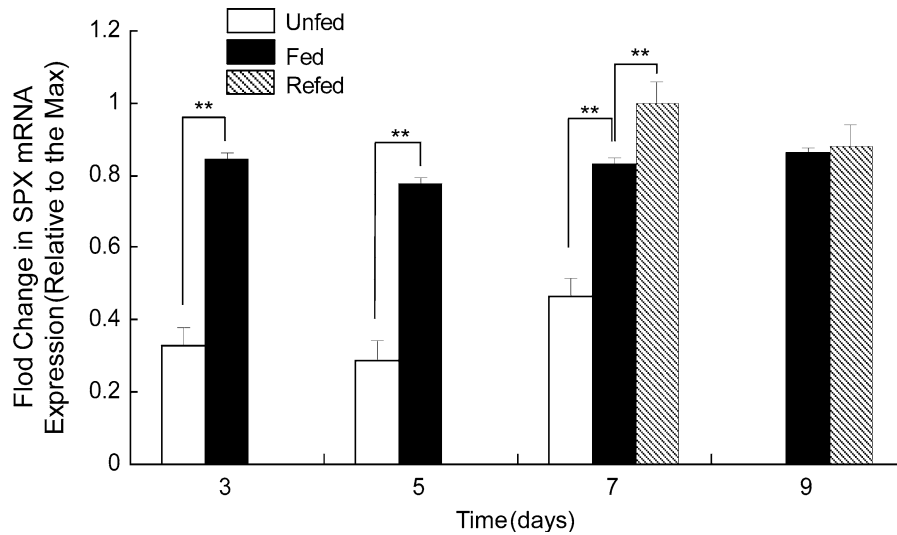
Peptide and/or receptor families usually depended on local duplications before and after two rounds of whole-genome duplication to expand their family members (Hwang et al. 2013), which was closely

related to evolutionary history of a gene family. Phylogeny analyses are useful to understand the evolutionary history. Based on the analysis of the SPX study of evolution, they found a SPX paralog (SPX2) in some vertebrate chromosomes, which was detected by using bioinformatics tools (Kim et al. 2014), such as zebrafish (Kim et al. 2014) and goldfish (Wong et al. 2013), but not in human (Mirabeau et al. 2007). We try to study the SPX2 in Ya-fish, but failed. Maybe there is not any SPX2 in Ya-fish. It may be caused by species difference.

Sequence comparative analysis attested to that the Ya-fish SPX mature peptide was highly conserved from fish to mammals (Fig. 2). Also, it was confirmed in the phylogenetic analysis. These results suggested that SPX may be involved in important functions which have constrained its structural change during evolution (Phillips 2008).

In mammalia, SPX has been found to be expressed in many different tissues (Porzionato et al. 2010; Rucinski et al. 2010). Similarly, the main expression of Ya-fish SPX mRNA was observed in the forebrain (Fig. 4), which is consistent with the pattern in other animals, such as rat (Porzionato et al. 2010) and goldfish (Liu et al. 2013). In human, SPX mRNA is abundantly detected in several brain regions, stomach muscle and kidney (Sonmez et al. 2009; Mirabeau





**Fig. 6** Effects of fasting and refeeding on SPX mRNA expression in Ya-fish forebrain. Food deprivation reduced expression of SPX mRNA in the Ya-fish forebrain, and refeeding increased and then restored the normal level. The mRNA expression of SPX was normalized to  $\beta$ -actin and 18S

gene. Asterisks represent significant differences between treatment groups and fed groups at the same time point (Student's *t* test,  $**P < 0.01$ ). Error bars represent standard error of the mean ( $n = 8$  fish/group)

et al. 2007). Interestingly, SPX mRNA abundantly expresses in the neuronal and reproductive tissues of sexually matured goldfish (Liu et al. 2013). Also, it is related to follicular stages in zebrafish (Liu et al. 2013). In this study, we have demonstrated that the levels of SPX mRNA in Ya-fish gonads were scanty, which may be caused by the immature experimental model of Ya-fish.

Based on the limited reports in the rat model, SPX is known to induce stomach contraction (Sonmez et al. 2009). Moreover, gastrointestinal peristalsis contributes to gastric emptying (Anvari et al. 1995). One of the most important reasons is food consumption that effects gastric emptying rate (Moran et al. 2005; Chen et al. 2005). However, feeding behaviors are mediated by a complex interaction of many peptide hormones (Lenard and Berthoud 2008). A number of studies have shown that the forebrain appears to play an essential role in controlling appetite through stimulating neuronal circuits, which produce activating appetite and inhibiting appetite (Bouret 2009, 2010). In addition, Ya-fish SPX mRNA is prolifically expressed in forebrain and foregut. Thus, the high expression of Ya-fish SPX mRNA in forebrain and gut indicates a probable function for SPX in the regulation of food intake and metabolic absorption.

However, there is scarce information about the forebrain SPX expression responded to the feeding status change. Then, in order to get more information about Ya-fish SPX in the feeding regulation, we also compared the forebrain SPX mRNA expression between fed and fasted fish. In our study, the Ya-fish SPX mRNA levels significantly decreased in the forebrain after feeding. This is similar to goldfish SPX mRNA levels which decreased at 1 h postfeeding (Wong et al. 2013). So far, there is not any related report about SPX mRNA expression concerning refeeding. Moreover, during a week fasting, the expression of Ya-fish SPX decreased in the fasting treatment and boosted quickly after refeeding at the 7th day. SPX mRNA expression had a significant decrease compared to control groups at 3rd, 5th and 7th days of fasted fish (Fig. 6). The Ya-fish SPX changing pattern was parallel to other appetite peptides responded to feeding status (Yuan et al. 2014). In conclusion, SPX mRNA expression is related to feeding conditions of Ya-fish. Maybe the changes are regulated by metabolic status in Ya-fish.

In summary, the Ya-fish SPX has been identified and characterized with respect to their expression in various tissues. The expression of SPX in the forebrain is affected by different feeding status, indicating that

SPX mRNA expression is regulated by metabolic status or feeding conditions in Ya-fish. These findings in this study could be useful for future research to further understand the functions and mechanisms of actions of SPX.

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#### Compliance with ethical standards

**Conflict of interest** The authors have nothing to disclose.

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