Developmental expression of steroidogenic factor-1, cyp19a1a and cyp19a1b from common carp (Cyprinus carpio)

Bin Tang, Wei Hu, Jun Hao, Zuoyan Zhu

College of Life Science, Wuhan University, Wuhan 430072, China
State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

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ABSTRACT

Steroidogenic factor-1 (SF-1), cyp19a1a and cyp19a1b play pivotal roles in vertebrate steroidogenesis and reproduction. In this study, a SF-1 cDNA (EU022463) was cloned from common carp (Cyprinus carpio). The transcript contains a 1509 base pair (bp) open reading frame (ORF) encoding a 503 amino acid sequence. Comparisons of deduced amino acid sequences demonstrated that carp SF-1 is highly homologous with those of other vertebrates. Tissue specific expressions of SF-1, cyp19a1a and cyp19a1b mRNA were analyzed in 10-month-old carp. SF-1 was abundant in the hypothalamus, pituitary, gonad, spleen and liver (females only). Cyp19a1b was preferentially expressed in the brain of both sexes but also was present at much lower levels in testis, ovary and kidney (females only). Although cyp19a1a expression was preferentially expressed in ovaries, it was also present at much lower levels in brain, testis, kidney and spleen (males only). Northern blot analysis revealed that testes and brains of both sexes expressed a transcript of about 2.8 kb in size. The expression pattern of SF-1, cyp19a1a and cyp19a1b in carp gonads suggested their involvement in sexual development. In 3-month-old carp, SF-1 and cyp19a1b were expressed highly in testes but were at much lower levels in ovaries, while the opposite pattern was observed with cyp19a1a expression. In 10-month-old carp, SF-1 expression was much higher in testes than in ovaries, while the opposite pattern was observed with cyp19a1a expression. These developmental expression patterns in carp gonads suggest important roles of SF-1 and cyp19a1b in testis development and of cyp19a1a in ovary development.

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1. Introduction

In mammals, once sex is determined, gonads usually differentiate in a single developmental pathway until full maturity into testes or ovaries. However, teleosts have the ability to change sexes, which is affected by factors such as sex steroids, temperature and light intensity (Devlin and Nagahama, 2002). With exogenous hormone treatments, Yamamoto found that androgens are responsible for testicular development, and estrogens are responsible for ovarian development in medaka (Oryzias latipes) (Yamamoto, 1968; Yamamoto and Kajishima, 1969). Although it is clear that sex steroids are important for gonadal development in fish, more studies are needed to understand the complex molecular mechanism underlying fish sex determination and differentiation and the tight regulation of the synthesis of sex steroids. Except for a DM-domain gene found in the Y chromosome of medaka (Matsuda et al., 2002), no sex-linked gene has been identified in other fish species. However, some autosomal genes are associated with sex determination and differentiation. Although these genes are not sex-specific, their expression and regulatory mechanisms indicate that they are components of a sex determination and differentiation signaling pathway in which SF-1, and P450 aromatase control synthesis of the sex steroids (von Hofsten and Olsson, 2005).

SF-1 is a tissue-specific orphan receptor located in the nucleus. As a transcriptional factor, SF-1 promotes expression of steroidogenic enzymes. SF-1 is involved in regulating expression of steroidogenic acute regulatory protein (StAR) (Sugawara et al., 1996), cytochrome P450 side chain cleavage enzyme (SCC) (Hu et al., 2001), 3β-hydroxysteroid dehydrogenase (3β-HSD) (Leers-Sucheta et al., 1997), and aromatase cytochrome P450 (P450arom) (Lynch et al., 1993). Furthermore, SF-1 can also regulate several genes involved in gonadal development, such as anti-mullerian hormone (AMH) (Shen et al., 1994), gonadotropin releasing hormone (GnRH) (Lynch et al., 2000), luteinizing hormone β-subunit (LH β-subunit) (Halvorson et al., 1996) and GnRH receptor (Duval et al., 1997). Mice null for SF-1 are born without gonads, adrenal glands or pituitary gonadotropes (Ikeda et al., 1995). The gonadotropes of SF-1 knockout mice lack a number of genes that regulate reproduction, including LH β-subunit, FSH β-subunit, α-subunit of glycoprotein...
hormones (GTH-α) and GnRH receptor (Ingraham et al., 1994). SF-1 is also important for testis differentiation, as it has been found to be highly expressed in developing testes of mice and slider turtles (Trachemys scripta) (Ingraham et al., 1994; Fleming et al., 1999).

P450 aromatase is an oxygenase of the cytochrome P450 family, participating in the rate limited step of estrogen synthesis by catalyzing the conversion of androgens into estrogens (Ahmed, 1998). Two genes encoding P450 aromatase have been found in teleosts, mainly present in the brain and gonads, but they may have different roles in reproductive physiology (Barney et al., 2008). The brain-specific aromatase is the product of cyp19a1b, while the gonad-specific aromatase is the product of cyp19a1a (Tchoudakova and Callard, 1998). Germ cells have the potential to develop into males or females during sex differentiation. The hormonal balance between estrogens and androgens is crucial for gonad differentiation and development, and it may be partially regulated by P450 aromatase. Thus, the sexual differences in cyp19 gene expression during differentiation and development result in variable synthesis of sex steroids, which in turn regulate fish sex differentiation to some extent.

Previous studies showed that SF-1 can regulate transcription of the cyp19 genes in ovary follicles by binding to their promoters (Watanabe et al., 1999; Yoshiura et al., 2003). However, the SF-1 binding sites were found only in the promoter of cyp19a1a and not that of cyp19a1b in some teleosts, such as zebrafish (Danio rerio), goldfish (Carassius auratus) and gray mullets (Mugil cephalus) (Tchoudakova et al., 2001; Tong and Chung, 2003; Nocilado et al., 2007). Thus, it is still unclear whether there is a cooperative mechanism of regulation of gonad differentiation by SF-1, cyp19a1a and cyp19a1b.

Common carp (Cyprinus carpio) is the oldest cultured and most domesticated fish species in the world with important economic significance. The gonads of carp, a protandrous species, can directly develop into testes or ovaries (Johannes et al., 1992). However, the physiological basis of sex differentiation and development in this species remains unknown, and as mentioned above, no sex-linked gene or sex chromosomes have been found in carp. It is an excellent animal model to study reproduction in teleosts because events such as vitellogenesis, oocyte maturation, and ovulation can be timed accurately. As a first step in clarifying the function of SF-1 and aromatase in the developing gonads of teleosts, we set out to clone the SF-1 gene in carp and to analyze the tissue distribution and developmental patterns of SF-1, cyp19a1a and cyp19a1b.

### 2. Materials and methods

#### 2.1. Animals and gonad histology

Carp at 3, 10, and 23 months of age were obtained from the Zhengzhou Aquatic Research Center and housed at 18–20 °C in circular tanks (1.5 m in diameter; 0.6 m in depth), which are part of a recirculation system, with a photoperiod of 12 h light and 12 h dark. After acclimation for a week, the carp were dissected, frozen in dry ice, and stored at –80 °C until used for RNA extraction. At the same time, the gonads were excised and weighed for calculation of the gonadosomatic index (GSI), which is the gonad weight/body weight × 100, and the samples were fixed in Bouin’s fluid for histology. Total RNA was extracted using the Trizol Plus RNA Purification System (Invitrogen) according to the manufacturer’s instructions. Poly (A) RNA was purified with the PolyATract mRNA Isolation System (Promega).

### Table 1

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5′–3′)</th>
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</tr>
</thead>
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<td><strong>CL-csf1</strong></td>
<td>GCGCCGGGATGCTACAG</td>
<td>Cloning</td>
</tr>
<tr>
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<td>ATGACATTCTCTCCTGCTC</td>
<td>Cloning</td>
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<tr>
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#### 2.2. Isolation of the SF-1 homolog cDNA

For amplification of the SF-1 cDNA fragments, degenerate primers (CL-csf1 and CL-csf2) (Table 1) were designed from highly conserved regions of zebrafish ffr1c (Kuo et al., 2005) and goldfish FTZ-F1 (Choi and Habibi, 2005). The CL-csf1 primer is located at the 5′ side of the start codon ATG, and the CL-csf2 primer is located at the FTZ-F1 box. Total RNA was extracted from the carp pituitary. As a template for RT-PCR, the first-strand cDNA was synthesized from 1 μg pituitary total RNA using ReverTra Ace (Toyobo, Japan) with 9-mer random primers. RT-PCR conditions were 30 °C for 10 min, followed by 42 °C for 1 h. Amplification was performed using LA Taq DNA polymerase (Takara). After an initial 4 min denaturing step at 94 °C, 40 cycles of amplification were performed under the following conditions: denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 50 s. After the last cycle, elongation was extended for a final 5 min at 72 °C. After electrophoresis on a 1% TAE-agarose gel, the DNA fragment was excised and ligated into the pMD-18T vector (Takara) according to the manufacturer’s instructions and confirmed by sequencing.

The 3′ RACE technique was used to obtain sequences downstream of the PCR product. First-strand cDNA synthesis was initiated at the poly (A) RNA using the oligo (dT) anchor (Table 1). The 3′ RACE PCR product was amplified by PCR using the gene specific primer 3′RA-csf1, 3′ RACE adaptor 1 and 3′ RACE adaptor 2 (Table 1) under the following conditions: 0.2 μg cDNA as template; 3′RA-csf1, 3′ RACE adaptor 1 and 3′ RACE adaptor 2 each at 10 μM; 10 mM of each dNTP; and LA Taq DNA polymerase (5 U μl−1, Takara) in 50 μl buffer. After electrophoresis on a 1% TAE-agarose gel, the DNA fragment was excised. The gene specific primer 3′RA-csf1 and 3′ RACE adaptor 2 were used for nested PCR with 35 cycles of 94 °C for 30 s for denaturing, 60 °C for 30 s for primer annealing, and 72 °C for 2 min for extension, followed by one last extension cycle of 5 min at 72 °C. The final PCR product was amplified and T-A cloned into the pMD-18T vector (Takara) and sequenced.

#### 2.3. Sequence analysis

Sequence analysis and comparisons of homology between SF-1 sequences were performed using the ClustalX program version
1.81. Phylogenetic analysis was performed by multiple alignments of deduced amino acid sequences using the neighbor-joining method (Saitou and Nei, 1987) within the ClustalX program. Evolutionary trees were constructed using MEGA 3.1.

2.4. Tissue specific expression analysis of carp SF-1, cyp19a1a and cyp19a1b

Gene-specific primers were designed for RT-PCR of SF-1, cyp19a1a (accession number EU375455) and cyp19a1b (accession number EU375456) (Table 1), and tested for specificity and efficiency. RT-PCR was carried out on a 9600 PCR thermocycler (Perkin Elmer) with β -actin as an endogenous control. The first-strand cDNA was synthesized using 1 μg total RNA from brain, pituitary, testis, ovary, liver, kidney and spleen of 10-month-old carp. RT reactions were performed with ReverTra Ace and 9-mer random primers. The PCR reaction mixture was activated at 94 °C for 4 min and 30 reaction cycles were conducted as follows: 30 s denaturing at 94 °C, 30 s annealing at 60 °C, and 30 s extension at 72 °C, followed by 1 cycle of 5 min extension at 72 °C. Ten microliters of each PCR product was electrophoresed on a 1.5% TAE agarose gel with a DL2000 DNA ladder (Dong sheng) used as a reference to estimate the molecular weights of the amplified fragments. A high resolution scanner was used for quantification of PCR amplified fragments, and the band densities were estimated using NIH Image software (NIH, Bethesda, MD). The densitometry measurements of anthodium bromide stained gels were optimized for linearity as described in a previous study (Kermouni et al., 1998). As a control for loading, carp β -actin was amplified from each sample.

2.5. Northern blot analysis

Poly(A) + enriched RNA from the testes (10 μg), oocytes (10 μg) and brains (10 μg) of 4-month-old male and female carp was electrophoresed on a 1.2% formaldehyde agarose gel with a high range RNA Marker (MBI Fermentas) and transferred to a nylon membrane (Hybond-N+, Amersham) using a Trans-Blot SD semi-dry electrophoretic transfer cell (Bio-Rad). The membrane was fixed with a UV-crosslinker at 120 mJ/cm2. The hybridization probe was a 52 bp RNA fragment for P450aromB mRNA (nt 1095–1108) (accession number EU375456) (Table 1), and tested for specificity and efficiency. Phylogenetic analysis was performed by multiple alignments using MEGA 3.1. Phylogenetic trees were constructed using MEGA 3.1. Evolutionary trees were constructed using the ClustalX program. Evolutionary trees were constructed using the ClustalX program. Evolutionary trees were constructed using the ClustalX program.

3. Results

3.1. Cloning of carp SF-1 cDNA

Using primers designed as described in Section 2 for SF-1, one major PCR fragment of 346 bp was amplified from the carp pituitary and separated by electrophoresis. The carp SF-1 UTR sequence acquired by the 3' RACE method was subsequently combined to generate a full-length coding sequence. The 1749 bp cDNA has an open reading frame (ORF) of 1509 bp that begins with the start codon ATG at the 9th bp and ends with a TGA stop codon at bp 1518 (accession number EU022463). The carp SF-1 ORF is 1509 bp, encoding a polypeptide of 503 amino acid residues with a predicted size of 57 kDa.

3.2. Homology comparisons of the deduced SF-1 amino acid sequence of carp with those of other species

Carp SF-1 contains a P-Box, D-Box, FTZ-F1 box, zinc finger modules I–II, Regions II–III and activation function-2 (AF-2) hexamer (Fig. 1). Multiple sequence alignments showed that the deduced carp SF-1 amino acid sequences of functional domains are highly conserved with those of other species. Among those functional domains, the P-box determines DNA binding specificity, and the FTZ-F1 box is a characteristic feature of the FTZ-F1 family. The P-box and FTZ-F1 box are completely identical in all investigated species of this study. A proline-rich domain found in SF-1 of mammals was not detected in that of teleosts, amphibians, birds and reptiles.

3.3. Phylogenetic analysis

To investigate the relationship between the putative carp SF-1 and other members of the nuclear receptor 5A (NR5A) subgroup, phylogenetic analysis was performed by the neighbor-joining method with zebrafish NR5A5 forming an outgroup. We found that the carp SF-1 belongs to the NR5A, or Ftz-F1 subfamily, which is comprised of four identified members (NR5A1–NR5A4; Fig. 2). The previously established representatives of NR5A1 (e.g., human SF-1, horse SF-1 and rat SF-1) and NR5A2 (e.g., human LRH-1, horse LRH-1 and rat LRH-1) were included in the analysis. The teleost medaka, yellow catfish LRH-1 and zebrafish ftla also grouped with the NR5A2 clade. Phylogenetically, carp SF-1 was most closely related to goldfish, catfish (Ictalurus Punctatus), tilapia (Oreochromis niloticus) SF-1 and zebrafish ft1b and ft1d (Fig. 2) and grouped with the NR5A4 clade. The zebrafish ft1b grouped with zebrafish ft1d in NR5A4, whereas ft1c did not align clearly within any of the previously defined NR5A subgroups. The results indicate that the putative carp SF-1 does not belong to NR5A1, but rather to the NR5A4 family.

2.7. Statistical analysis

Carp GSI % at different developmental stages are indicated as mean ± standard error of the mean (SEM). Data generated by qPCR were compiled and collected using SDS 1.1 software (Applied Biosystems). Acceptability for each set of triplicate reactions was set at 15% of the SEM. The mean of samples in 3-month-old females was set as 1. Relative gene expression was analyzed using the 2-ΔΔCt method (Livak and Schmittgen, 2001). Data from biological replicates were averaged and shown as normalized gene expression ± SEM. Student’s t test was used to compare expression levels of SF-1, cyp19a1a and cyp19a1b mRNA where the significance was set at P < 0.05.
3.4. GSI and histological analysis

To characterize the developmental stage, we analyzed the GSI and gonads by histology in carp at different stages of development. The results show that GSI in 3-month-old males was 0.08 ± 0.03% (n = 6). At an early spermatogenic stage, the immature testes in 3-month-old male carp were characterized by spermatogonias, spermatids and a few spermatozoas in some lobules (Fig. 3A). The GSI in 10-month-old males was 1.70 ± 0.37% (n = 5) and in 23-month-old males was 2.69 ± 0.68% (n = 6). Ten-month-old males were at a functional maturation stage (Fig. 3B), and 23-month-old males were at a functional maturation stage for the second time (Fig. 3C). Both testes of 10- and 23-month-old males showed abundant spermatids and spermatozoa in all lobules.

The GSI in 3-month-old females was 0.20 ± 0.01% (n = 6). At an early developmental stage, the ovaries of 3-month-old juvenile females contained primary oocytes in a early growth stage (Fig. 3D). The GSI of 10-month-old females was 3.72 ± 0.85% (n = 5). At the middle developmental stage, ovaries of 10-month-old females were characterized by the presence of growing oocytes at an early vitellogenic stage, and yolk globules were rarely observed in cortical alveoli (Fig. 3E). The GSI in 23-month-old females was 7.94 ± 0.92% (n = 5). At a functionally mature stage, ovaries of 23-month-old females were characterized by oocytes at the post-vitellogenic stage; the cortical alveoli were pushed to the periphery of the oocytes, and most of the cytoplasm was filled with yolk globules (Fig. 3F).

3.5. Tissue distribution pattern of carp SF-1, cyp19a1a and cyp19a1b mRNA

Expression of SF-1, cyp19a1a and cyp19a1b mRNA in different tissues of 10-month-old carp were examined by semi-quantitative RT-PCR. At the age of 10 months, male carp were already sexually mature, and female carp were in mid-puberty.
mature, while females were still at a middle developmental stage. SF-1 expression was detected in the hypothalamus, pituitary, gonad, and spleen of both males and females, but liver expression was only detectable in females (Fig. 4). Compared to females, male carp SF-1 expression was much higher in the gonad but lower in spleen; similar expression levels were present in male and female hypothalamus and pituitary. Cyp19a1a expression was detected in the telencephalon, midbrain, cerebellum, hypothalamus, gonad, kidney and spleen (male only). Cyp19a1b expression was detected in the telencephalon, midbrain, cerebellum, medulla oblongata, hypothalamus, pituitary, gonad and kidney (female only). Telecephalon, midbrain, cerebellum, hypothalamus and pituitary were the predominant cyp19a1b expressing tissues (Fig. 4). Cyp19a1b was predominantly expressed in ovaries where cyp19a1b was expressed only at basal levels (Fig. 4). The size and abundance of carp cyp19a1b transcripts were also examined by Northern blot analysis. Using the carp cyp19a1b cRNA probe, a transcript of about 2.8 kb was detected in the poly (A) + enriched testes RNA sample and brain RNA samples of both sexes, whereas the transcript abundance was presumably too low to be seen in the ovary sample (Fig. 5).

3.6. Expression levels of SF-1, cyp19a1a and cyp19a1b mRNA in carp gonads at different developmental stages

To compare the expression levels of SF-1, cyp19a1a and cyp19a1b mRNA in carp of different developmental stages, ovaries and testes were analyzed by real-time PCR. The expression levels of SF-1 in testes from 3-month, 10-month and 23-month-old carp were 4.97-, 4.27- and 0.89-fold of those in female ovaries, respectively (Fig. 6). SF-1 showed similar expression levels in testes of 3- and 10-month-old, but was present at much lower levels at 23 months. SF-1 in ovaries also showed similar expression levels in 3-, 10- and 23-month-old. The expression levels of cyp19a1a from ovaries of 3-, 10- and 23-month-old female carp were 99.29, 233.16 and 56.94-fold higher, respectively, than those in male testes (Fig. 6). Cyp19a1a in testes showed similar expression levels in 3- and 10-month-old males, but was present at much higher levels at 23 months. Cyp19a1a expression in ovaries was very low in 3-month-old, higher in 10-month-old and highest in 23-month old. The expression levels of cyp19a1b in testes of 3-, 10- and 23-month-old male carp were 63.41-, 0.56- and 1.24-fold of those in female ovaries, respectively (Fig. 6). Meanwhile, cy-
p19a1b in testes showed similar expression levels throughout development. Cyp19a1b expression in ovaries was very low in 3-month-old, but higher in 10- and 23-month-old.

4. Discussion

SF-1, first identified from mouse kidney as a steroidogenic tissue-specific transcription factor (Lala et al., 1992), is a mammalian homolog of Drosophila fushi-tarazu factor-1; hence, in some species, it is also known as FTZ-F1 (Ito et al., 1998; Yoshiura et al., 2003). In mammals, the FTZ-F1 genes can be divided into two subgroups, SF-1/NR5A1 and LRH-1 (liver receptor homolog-1)/NR5A2 (Fayard et al., 2004). The carp SF-1 cDNA contains an open reading frame of 1509 nucleotides encoding a protein of 503 amino acids. Multiple amino acid sequence alignments indicated that the carp SF-1 protein has typical features of nuclear receptors. Conserved regions in the carp SF-1 protein that are essential for DNA and ligand-binding domains show high homology with other vertebrates (Fig. 1). Only one or two FTZ-F1 genes have been identified in mammals, but four FTZ-F1 genes, ff1a, ff1b, ff1c and ff1d were isolated in zebrafish (Kuo et al., 2005). Our phylogenetic analysis suggests that carp SF-1 may belong to the NR5A4 subgroup together with goldfish SF-1, catfish SF-1, tilapia SF-1 and zebrafish ff1b and ff1d (Fig. 2). By the results of the multiple alignments and phylogenetic analysis, we can conclude that carp SF-1 is a member of the vertebrate NR5A4 family.

Previous research indicates that SF-1 play an important role in mammalian ventromedial nucleus development (Roselli et al., 1997; Fleming et al., 1999; Dellovade et al., 2000) and regulation of GTH expression in pituitary (Ingraham et al., 1994; Ahmed, 1998; Bakke et al., 2001). We analyzed five regions of the carp brain including telecephalon, midbrain, cerebellum, and medulla oblongata, and found that SF-1 was expressed in the hypothalamus of both sexes (Fig. 4). SF-1 mRNA was also detected in carp pituitary and gonads (Fig. 4). We conclude that SF-1 may play an essential role in reproductive regulation of the hypothalamus–pituitary–gonadal axis as in mammals. The spleen is associated with steroidogenesis (Forneris et al., 1999), and we found that basal SF-1 mRNA expression was high in the carp spleen (Fig. 4). This finding is consistent with previous reports in humans and rats (Ramayya et al., 1997; Morohashi et al., 1999). In carp, SF-1 mRNA is only expressed abundantly in the liver of females, but not in males (Fig. 4). The sex differences of SF-1 expression in the liver may be associated with regulation of bile acid homeostasis. SF-1 is a well-characterized competence factor, directing the expression of steroid hormone metabolism in the gonads and adrenal glands (Parker, 1998). Cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the classic pathway of bile acid biosynthesis, is also regulated by SF-1 (Lee and Moore, 2002), when the bile acid pool size grows too large in the male liver, and the transcription of CYP7A1 is presumably repressed by the inhibition of SF-1 expression.
The present study clearly showed the differential expression of the two forms of P450 aromatase transcripts in carp tissues. Cyp19a1a expression was preferentially expressed in carp ovaries, but also was present at much lower levels in testes and brains of both males and females. This expression pattern was also found in other species including zebrafish (Trant et al., 2001), sea bass (Dicentrarchus labrax) (Blazquez and Piferrer, 2004) and wrasse (Halichoeres tenuispinis) (Choi et al., 2005). These studies support the idea that high cyp19a1a levels are essential for estradiol biosynthesis and ovary development in fish (Masaru et al., 1998).

On the other hand, cyp19a1b was preferentially expressed in brains of both male and female carp, while present at much lower levels in testes and ovaries. Others have also found this expression pattern of cyp19a1b in carp (Barney et al., 2008) as well as other species including zebrafish (Trant et al., 2001), sea bass (Blazquez and Piferrer, 2004) and wrasse (Choi et al., 2005). RT-PCR analysis of different regions of the brain also revealed that cyp19a1b was expressed significantly higher than cyp19a1a in the telecephalon, midbrain, medulla oblongata, hypothalamus and pituitary. The expression of cyp19a1b in the various parts of the brain and pituitary indicates its involvement in the brain–pituitary–gonadal axis as previously described in the channel catfish (Kazeto et al., 2003) and pejerrey fish (Odontesthes bonariensis) (Strobl-Mazzulla et al., 2005). High aromatase activity levels in the brain of adult teleosts have been suggested to play a role in sexual plasticity (Forlano et al., 2001). In our study, both cyp19a1a and cyp19a1b could be detected in carp kidney, consistent with previous reports in pejerrey fish (Strobl-Mazzulla et al., 2005) and nile tilapia (Chang et al., 2005). Interestingly, cyp19a1b was not detectable in kidney of male carp. Additionally, cyp19a1b was expressed in the spleen of male but not of female carp; meanwhile, cyp19a1a could not be detected in the spleen of either sex. These results suggest that the kidney and spleen are capable of producing estrogen from precursors. However, the potential functions of locally-produced steroids in these organs remain unknown. A 2.8 kb transcript was detected by Northern blot analysis (Fig. 5). However, we also detected another 3.4 kb transcript, which is larger than the size of the cloned full-length cyp19a1b cDNA, suggesting the possible presence of different forms of the cyp19a1b transcript in carp.

To further understand the coordinated regulation of SF-1, cyp19a1a and cyp19a1b in gonadal development, we analyzed their gene expressions in carp gonads at different developmental stages (Fig. 6). Testes from 3- and 10-month-old carp showed higher SF-1 expression levels than those of female ovaries. Sex differences of SF-1 mRNA expression were found at the early and middle stages of gonadal development, similar to the results found in rat fetuses (Hatano et al., 1994) and mature frogs (Mayer et al., 2002). This indicates that SF-1 may participate in the development of carp testes.
Expressions of both P450 aromatase genes were also found to be sexually dimorphic in the gonads. Similar to zebrafish (Sawyer et al., 2006), the male carp in our study had higher cyp19a1b expression, and the females had higher cyp19a1a expression at 3 months of age; however, these expression patterns changed in the older carp. As found in zebrafish, tilapia and sea bass (Dicentrarchus labrax), females had higher cyp19a1a expression at 23 months of age; however, these expression patterns changed in the older carp. As found in zebrafish, tilapia and sea bass (Dicentrarchus labrax), females had higher cyp19a1a expression at 23 months of age; however, these expression patterns changed in the older carp. As found in zebrafish, tilapia and sea bass (Dicentrarchus labrax), females had higher cyp19a1a expression at 23 months of age; however, these expression patterns changed in the older carp.


