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## 淇河鲫*cyp19a1b*基因的克隆表达及芳香化酶抑制剂对其表达的影响

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**摘要:** 为探索脑型芳香化酶基因(*cyp19a1b*)在雌核发育三倍体鱼淇河鲫性别决定与分化过程中的作用, 利用RACE方法克隆淇河鲫*cyp19a1b*基因cDNA全长序列, 采用Real-time PCR分析其不同组织、胚胎及胚后不同发育时期的表达情况, 同时检测芳香化酶抑制剂Letrozole诱导性逆转及腹腔注射人绒毛膜促性腺激素(hCG)后*cyp19a1b*在脑中的表达情况。结果显示, 淇河鲫*cyp19a1b* cDNA全长2 984 bp (GenBank ID: MF926270), 包含132 bp 5'非编码区, 1 319 bp 3'非编码区, 1 533 bp开放读码框, 编码510个氨基酸残基; 氨基酸序列比对及系统进化分析结果显示, 淇河鲫Cyp19a1b与其他鲤科鱼类同源性较高, 与哺乳类、爬行类等脊椎动物同源性较低, 这与其分类地位一致; 组织分布检测结果显示, *cyp19a1b*基因在淇河鲫脑中表达量最高, 在卵巢等其他组织中表达量较低; Real-time PCR结果表明, 在胚胎发育过程中*cyp19a1b*在外源精子刺激后从囊胚期开始上调, 神经胚期达到最高, 随后降低; 出膜后随着发育的进行, 该基因在脑中表达量逐步上调, 在躯体中一直维持在较低水平; 除此之外, 伴随着Letrozole诱导的性逆转, *cyp19a1b*在脑中表达量降低; 腹腔注射hCG可以促进*cyp19a1b*在脑中表达。研究表明, 淇河鲫*cyp19a1b*基因可能通过参与神经系统的形成及神经内分泌活动, 在性别决定与分化过程中起到一定的作用。

**关键词:** 淇河鲫; *cyp19a1b*; 克隆; 表达; 抑制剂

**中图分类号:** Q 786; S 917.4

**文献标志码:** A

芳香化酶(p450arome)是细胞色素P450家族的重要成员, 催化雄激素转化为雌激素, 是雌激素合成过程中的限速酶<sup>[1-3]</sup>。研究表明, 在大多数哺乳动物中, 芳香化酶由单基因编码<sup>[4-9]</sup>。而在多数硬骨鱼类中芳香化酶由两种不同的基因编码, 根据其表达情况分别命名为脑型芳香化酶(Cyp19a1b)和卵巢型芳香化酶(Cyp19a1a)<sup>[10-19]</sup>。鱼类作为较低等的脊椎动物, 其性别决定除了受遗传因素影响, 外界环境特别是激素水平的变化对鱼类的性别决定和分化也有很大影响。在

硬骨鱼类中, 内源性雌激素在卵巢的分化与发育过程中起着决定性作用, 是卵巢分化的天然诱导剂<sup>[20]</sup>。抑制芳香化酶活性可以使遗传性别为雌性的鱼类性逆转为雄性<sup>[21-24]</sup>。目前有关*cyp19a1a*基因在雌激素合成、性别分化及性腺发育中的研究较多, 其功能也得到了证实<sup>[25-26]</sup>, 但有关*cyp19a1b*基因在性别决定与分化过程中的作用机制还不清楚, 需要进一步研究。

在脊椎动物中, 雌核发育是一种单性生殖方式, 通过这种生殖方式可以产生具有相似遗

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传背景的全雌后代。在两性生殖的硬骨鱼类中,脑型芳香化酶基因主要在脑中表达,通过参与脑部雌激素合成,对性别分化等很多生理机能产生影响。然而,脑型芳香化酶基因在单性生殖的硬骨鱼类中是否具有相似的表达模式,并行使类似的功能目前还未见报道。淇河鲫(*Carassius auratus*),鲤形目(Cypriniformes),鲤科(Cyprinidae),鲫属(*Carassius*),是银鲫在长期生态适应过程中形成的地方性变异种群,为天然雌核发育三倍体鱼,是河南省特有的名贵优质鱼类。相关研究表明,在繁殖季节用异源精子刺激淇河鲫卵子,受精卵将通过雌核发育产生全雌的淇河鲫群体<sup>[27]</sup>,但相关的分子机制尚不明确。目前关于淇河鲫性别决定与分化的相关研究很少,本研究从*cyp19a1b*基因入手,克隆了该基因的cDNA全长,分析该基因在淇河鲫不同组织、胚胎及胚后发育不同时期的表达规律,并检测在芳香化酶抑制剂Letrozole诱导的雄鱼中的表达情况,同时检测了人绒毛膜促性腺激素(hCG)对其在脑中表达的影响,为更深入了解该基因在雌核发育(单性生殖)鱼类性别决定与分化中的作用奠定基础。

## 1 材料与方法

### 1.1 实验材料

淇河鲫取自河南师范大学水产养殖基地,个体大小均一(约500 g左右)。收集健康成鱼脑、肌肉、肠、心脏、卵巢、肾脏、肝脏、脾脏等组织。在繁殖季节将雌雄亲鱼按8:1的比例放入亲鱼池,人工注射催产素,随后放入养殖系统进行流水刺激,效应时间结束后采用干法受精获得受精卵,用羽毛搅拌后布于网片上,在静水中进行孵化。孵化过程中定期取样观察胚胎发育情况。并取不同发育时期(未受精、受精、囊胚期、原肠胚期、神经胚期、尾芽期、心跳期、出膜期)胚胎和胚后10、20、25、30、40、60 dah(孵化后天数)等时期的脑及去除脑、卵黄和内脏的躯体材料,每个组织或不同时期各取3个平行样品,置于液氮中保存。按TaKaRa RNAiso Plus (TaKaRa, 大连)试剂盒说明书提取总RNA。使用ND-2000核酸蛋白仪测定RNA浓度与OD值,选取A260/A280在1.8~2.0,且琼脂糖凝胶电泳条带完整的RNA样品用于后续实验。

### 1.2 淇河鲫*cyp19a1b*基因cDNA全长的克隆

根据GenBank上已报道的硬骨鱼类*cyp19a1b*保守序列设计淇河鲫*cyp19a1b*正、反向引物(表1),以淇河鲫成鱼脑组织总RNA为模板,按照Prime Script II 1st Strand cDNA Synthesis Kit (含gDNA Eraser)(TaKaRa, 大连)说明书合成第一链cDNA。以第一链cDNA为模板进行PCR扩增:94 °C预变性3 min; 94 °C 30 s, 59 °C 30 s, 72 °C 1 min 30 s, 共35个循环; 72 °C延伸10 min。PCR扩增产物经1.5%琼脂糖凝胶电泳检测,纯化后连接、转化,将阳性菌落送北京英俊公司进行测序。在已获得序列的基础上设计3'-RACE和5'-RACE的特异性引物(表1),进行3'和5'末端扩增。

表1 所用引物序列

引物 primer	序列(5'→3') sequence	用途 usage
<i>cyp19a1b-F</i>	AGGTGTGTCTTCA GATGATG	中间片段克隆 partial sequence PCR
<i>cyp19a1b-R</i>	CATGCGTCCGATG TTCAGGA	
<i>cyp19a1b-3' out</i>	TGTCGTGGAGGAG CAGATCG	3'端扩增 3'-RACE PCR
<i>cyp19a1b-3' in</i>	CATTCCACGAGCT CAGAATA	
<i>cyp19a1b-5' out</i>	CAACCCGGACCTG GGACAC	5'端扩增 5'-RACE PCR
<i>cyp19a1b-5' in</i>	CTGTGCAGGAGGA TCAGCAG	
UPM-L	CTAATACGACTCA CTATAGGGC AAGCAGTGGTATC AACGCAGAGT	通用引物 universal primer
UPM-S	CTAATACGACTCA CTATAGGGC	
<i>cyp19a1b-real-F</i>	GGTCTCAAGGGC ACGGTAA	定量PCR real-time PCR
<i>cyp19a1b-real-R</i>	CAACCCGGACCTG GGACAC	
<i>β-actinF</i>	ACCATCTACCCCG GTATTGC	内参基因 housekeeping gene
<i>β-actinR</i>	TGGAAGGTGGACA GGGAAGC	

### 1.3 淇河鲫*cyp19a1b*基因cDNA序列及系统进化分析

将测序所得*cyp19a1b*基因cDNA序列用DNA-star软件进行拼接,利用NCBI进行Blastn同源性比对(<http://blast.ncbi.nlm.nih.gov/>), ExPASy程序(<http://www.expasy.ch/tools/>)推算其开放阅读框和编码氨基酸序列情况,用SMART (<http://smart.emblheidelberg.de/>)进行信号序列和蛋白结构域分

析。利用MEGA 6.0软件邻位相连法Neighbor-Joining (bootstrap values为1 000)构建分子系统进化树。

#### 1.4 淇河鲫*cyp19a1b*组织分布

为探索*cyp19a1b*基因在淇河鲫不同组织中的表达模式,取健康成鱼脑、肌肉、肠、心脏、卵巢、肾脏、肝脏、脾脏等组织,按照“实验材料”中的方法提取RNA,以总RNA为模板,按照Prime Script RT reagent Kit with gDNA Eraser (TaKaRa, 大连)操作说明进行逆转录合成cDNA,将cDNA稀释10倍作为Real-time PCR模板。利用克隆得到的*cyp19a1b*基因开放读码框设计定量PCR引物,并以包含相应基因的质粒10倍梯度稀释来检测相应引物的扩增效率。所用的Real-time PCR反应体系(20  $\mu$ L): 2 $\times$ SYBR Premix Ex Taq (TaKaRa, 大连)10  $\mu$ L, 上、下游引物各0.5  $\mu$ L, cDNA模板2  $\mu$ L, ddH<sub>2</sub>O 7  $\mu$ L; 反应程序采用两步法: 95  $^{\circ}$ C预变性30 s; 95  $^{\circ}$ C 5 s, 60  $^{\circ}$ C 20 s, 共40个循环。为了保证Real-time PCR的可靠性,每个组织分别取自3条鱼,即3个平行样品,以 $\beta$ -actin为内参,依据 $2^{-\Delta\Delta C_T}$ 法计算*cyp19a1b*相对表达量,以mean $\pm$ SE表示。使用SPSS软件进行方差分析和多重比较,显著性水平设为 $P<0.05$ 。

#### 1.5 淇河鲫*cyp19a1b*基因在不同时期的表达模式

取不同发育时期(未受精、受精、囊胚期、原肠胚期、神经胚期、尾芽期、心跳期、出膜期)全胚和胚后(10、20、25、30、40、60 dah)各时期的脑及去除脑、卵黄和内脏的躯体材料,不同时期各取3个平行样品,提取RNA,并逆转录为cDNA,检测*cyp19a1b*表达量。

#### 1.6 Letrozole处理

按Letrozole 200 mg/kg量将药物溶于无水乙醇后混入相应重量的饵料,混匀、37  $^{\circ}$ C烘干备用。用Letrozole处理孵化后5 d的淇河鲫(200尾),持续处理60 d,然后正常饲养至210 dah,同时用只混有酒精的正常饲料饲养的淇河鲫群体作为对照组。分别取对照组和处理组淇河鲫性腺,波恩氏液固定,石蜡包埋、切片。H.E染色,观察处理组和对照组淇河鲫性腺表型。使用RT-PCR检测*cyp19a1b*在处理组和对照组脑中表达差异。RNA提取、逆转录参照“实验材料”和“淇河鲫*cyp19a1b*基因cDNA全长的克隆”。PCR条

件: 94  $^{\circ}$ C 3 min; 94  $^{\circ}$ C 30 s, 59  $^{\circ}$ C 30 s, 72  $^{\circ}$ C 20 s, 共28个循环; 72  $^{\circ}$ C延伸10 min。PCR产物进行琼脂糖凝胶电泳,分析处理组和对照组条带亮度。

#### 1.7 腹腔注射hCG对淇河鲫脑中*cyp19a1b*表达的影响

取同一时期、健康、个体大小均一的淇河鲫,分成两组,处理组腹腔注射hCG (1 000 IU/kg),对照组注射生理盐水(0.65%)<sup>[28]</sup>。在注射后0、8、12、24 h取脑组织,提取RNA,检测脑中*cyp19a1b*的表达变化。RNA提取、逆转录、Real-time PCR方法同“实验材料”和“淇河鲫*cyp19a1b*组织分布”。

## 2 结果

### 2.1 淇河鲫脑型芳香化酶基因cDNA全长和氨基酸序列分析

将扩增所得序列经测序、拼接,得到淇河鲫*cyp19a1b*基因cDNA序列,全长2 984 bp (MF926270)。其中5'非编码区132 bp, 3'非编码区1 319 bp (包括典型的AATAA转录终止子加A信号),开放读码框1 533 bp,编码510个氨基酸残基(图1),预测相对分子质量58.11 ku,蛋白等电点7.57。序列分析发现,淇河鲫Cyp19a1b含有细胞色素p450家族5个典型的结构域:跨膜区、I-螺旋区、特异的芳香化区、Ozol's区以及血红素结合区(图2)。

### 2.2 淇河鲫Cyp19a1b系统进化树构建及蛋白质同源性比较

使用MegAlign软件进行序列比对,结果显示淇河鲫Cyp19a1b与金鱼同源性高达99%,与鲤(Cyprinus carpio)、斑马鱼(Danio rerio)等鲤科鱼类相似性为87%~95%,与尼罗罗非鱼(Oreochromis niloticus)、青鳉(Oryzias latipes)等非鲤科鱼类相似性为50%~63%,与哺乳类、爬行类等高等脊椎动物的相似性为51%~52%(表2)。利用MEGA 6.0构建系统进化树,进化树分为两支,鱼类聚为一支,哺乳类、鸟类、爬行类和两栖类聚为一支。淇河鲫Cyp19a1b位于鱼类的一支,并且与鲤科鱼类聚为一小支,与青鳉、尼罗罗非鱼等非鲤科鱼类聚为一大支,这与淇河鲫的分类地位相一致(图3)。



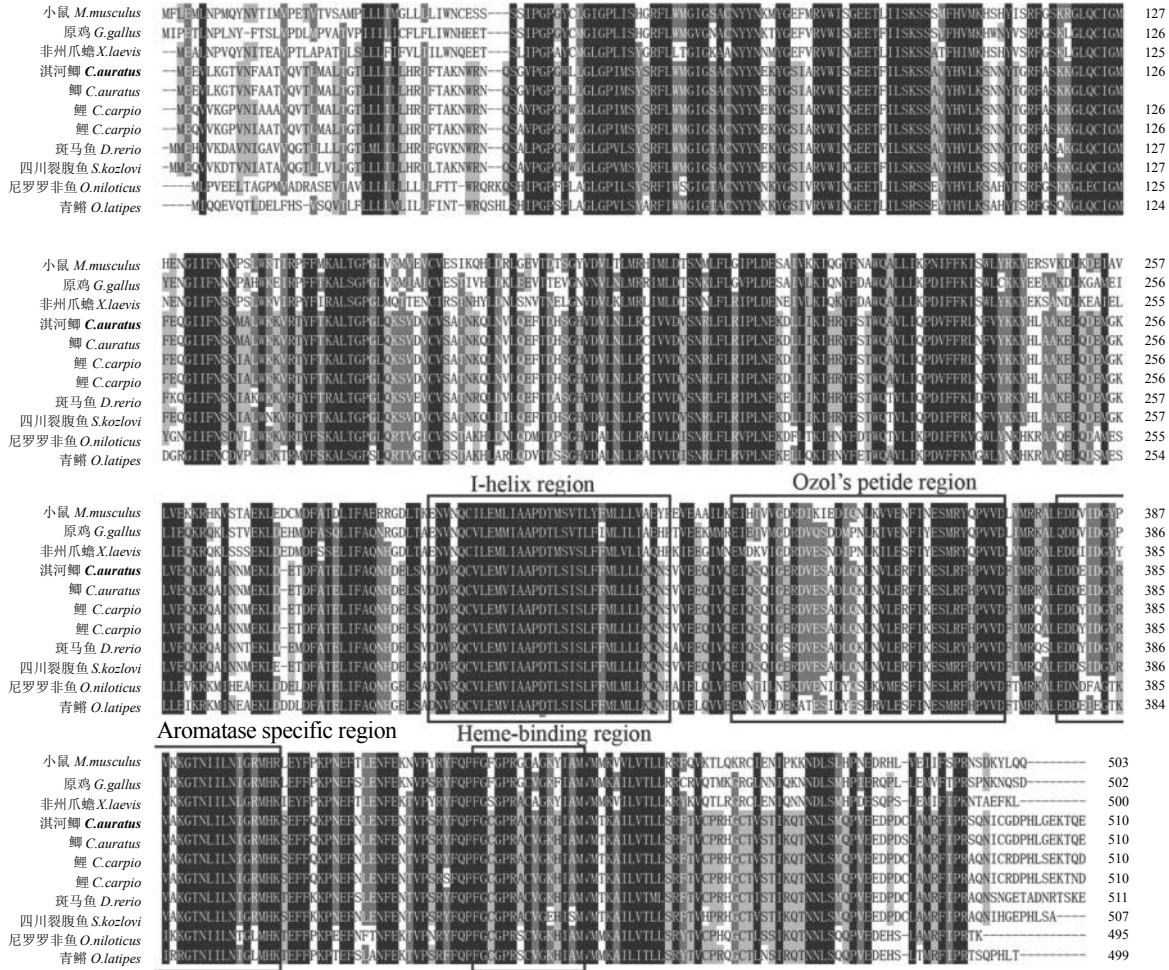


图 2 淇河鲫 *Cyp19a1b* 与其他物种 *Cyp19a1b* 氨基酸序列比对

使用 ClustalX 进行多重序列比对, 黑色方框表示保守结构域: I-螺旋区、Ozol's 区、特有的芳香化区以及血红素结合区

Fig. 2 Alignment of *Cyp19a1b* in *C. auratus* with those of other vertebrates

The program ClustalX is used to align the *Cyp19a1b* sequences. The conserved domains (I-helix region, aromatase specific region Ozol's petide region, and Heme-binding region) are boxed

淇河鲫 60 d, 并正常饲养至 210 d, 组织学结果显示, Letrozole 处理可以使原本将发育为卵巢的淇河鲫性腺发育为精巢(图 7)。半定量 PCR 结果显示, 与对照组相比, *cyp19a1b* 在处理组脑中表达量显著降低 ( $P < 0.05$ ) (图 8)。

2.6 腹腔注射 hCG 对脑中 *cyp19a1b* 表达的影响

同时检测了腹腔注射 hCG 对脑中 *cyp19a1b* 表达的影响, 结果显示, hCG 处理能够显著促进 *cyp19a1b* 在脑中表达, 并持续至 24 h ( $P < 0.05$ ) (图 9)。

3 讨论

本研究克隆得到淇河鲫 *cyp19a1b* 基因 cDNA 全长序列, 其中开放阅读框长 1 533 bp, 编码 510 个

氨基酸, 淇河鲫 *cyp19a1b* 基因与金鱼、鲤等鲤科鱼类亲缘关系最近, 相似性最高; *Cyp19a1b* 氨基酸序列具有跨膜区、I-螺旋区、特有的芳香化区、Ozol's 区, 同时具有能体现细胞色素 p450 家族特征的血红素结合保守基序 F-X-X-G-X-R-X-C-X-G (淇河鲫中为 F-G-C-G-P-R-A-C-V-G, 与亚铁血红素发生作用的半胱氨酸残基表现出绝对的保守性), 以上结果证明本研究中克隆得到的序列是淇河鲫 *cyp19a1b* 基因。

硬骨鱼类成鱼的脑以高芳香化酶活性著称 (主要由于脑型芳香化酶基因高表达) [29-32]。相关研究表明, 芳香化酶在鱼类脑中的活性比其他脊椎动物中的高出 100~1 000 倍, 这主要与脑中产生的雌二醇和许多生理及性行为有关。在斑

表2 Cyp19a1b氨基酸序列的同源性  
Tab.2 GenBank accession numbers and the identity of Cyp19a1b

物种(登录号) species (accession no.)	同源性/% identity
小鼠 <i>Mus musculus</i> (NP_031836.1)	51
原鸡 <i>Gallus gallus</i> (NP_001001761.2)	53
绿安乐蜥 <i>Anolis carolinensis</i> (XP_016852003.1)	54
非洲爪蟾 <i>Xenopus laevis</i> (NP_001079122.1)	52
刺鲃 <i>Dasyatis sabina</i> (AAF04617.1)	50
青鳉 <i>Oryzias latipes</i> (AAP83449.1)	63
尼罗罗非鱼 <i>Oreochromis niloticus</i> (AAG49480.2)	63
斑马鱼 <i>Danio rerio</i> (AAG12245.1)	87
四川裂腹鱼 <i>Schizothorax kozlovi</i> (ANW82316.1)	91
鲤1 <i>Cyprinus carpio</i> (ACB13198.1)	95
鲤2 <i>Cyprinus carpio</i> (ACC95443.1)	95
金鱼 <i>Carassius auratus</i> (BAA23757.1)	99

马鱼、金鱼、青鳉、尼罗罗非鱼、稀有鮎鲫(*Gobiocypris rarus*)、鲤中, *cyp19a1b*主要在脑中表

达, 在性腺中表达量很低或不表达<sup>[11, 34-38]</sup>。本研究中, 淇河鲫*cyp19a1b*也主要在脑组织中表达, 在性腺等组织中表达量很低, 这与其他硬骨鱼类中的研究结果一致, 表明在进化过程中*cyp19a1b*可能具有参与脑内雌激素合成, 进而影响脑的发育与性别分化的保守功能, 这也暗示*cyp19a1b*在进行单性生殖的硬骨鱼类中很可能具有与进行两性生殖的硬骨鱼类中相同的作用。在青鳉、尼罗罗非鱼、稀有鮎鲫中*cyp19a1b*在雌性个体脑中表达量高于雄性, 而在斑马鱼中却相反<sup>[11, 34, 36-37]</sup>, 在本研究中, 经Letrozole处理导致的性逆转过程中, *cyp19a1b*在雄性个体脑中表达量较雌性低。所以, 不同硬骨鱼类*cyp19a1b*基因在雌雄个体脑中表达模式可能不同。

在稀有鮎鲫中, *cyp19a1b*从囊胚期开始表达, 至神经胚期达较高水平, 随后逐渐降低; 而在青鳉中, 从受精卵至4~6体节期都没有*cyp19a1b*的表达, 直到出膜前才检测到其表达, 暗示*cyp19a1b*在不同硬骨鱼类胚胎发育过程中的表达具有物种特异性<sup>[37, 39]</sup>。外源精子刺激后*cyp19a1b*基因在淇河鲫胚胎内表达逐步上调, 在神经胚期达到最高, 然后降低, 表明该基因可能参与

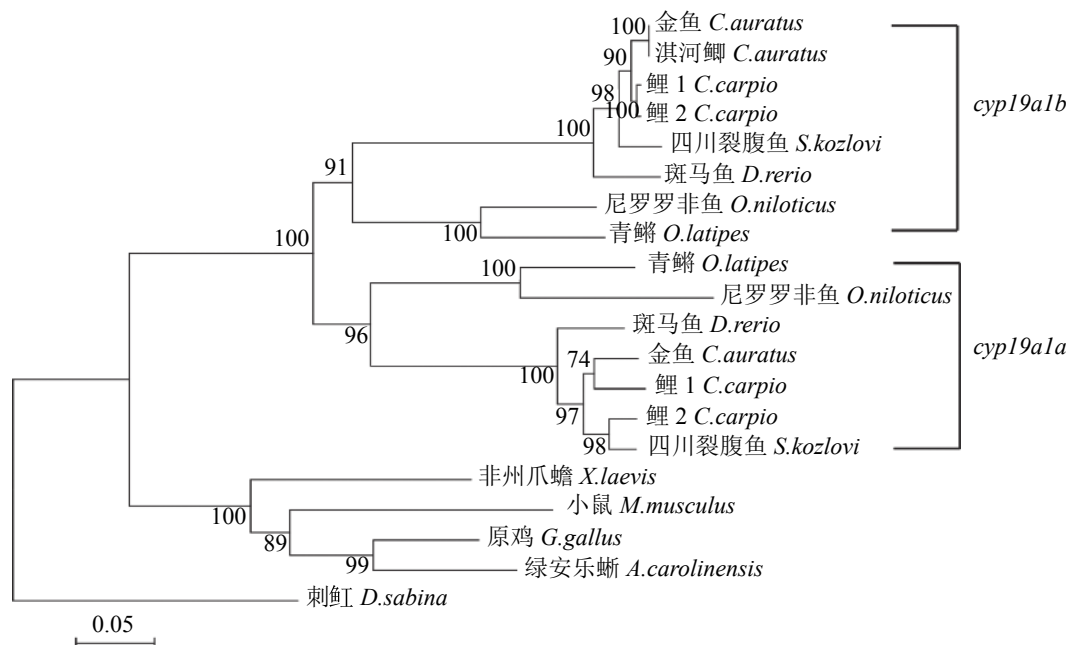


图3 Cyp19a1系统进化树

Cyp19a1a所用序列登录号: 青鳉(NP\_001265808.1)、尼罗罗非鱼(NP\_001266515.1)、斑马鱼(NP\_571229.3)、金鱼(AEX97168.1)、鲤(ACB13197.1/ABF82249.1)、四川裂腹鱼(ANW08806.1); Cyp19a1b及其他脊椎动物序列号见表2

Fig.3 Phylogenetic tree of Cyp19a1 from vertebrates

Accession number of Cyp19a1a: *O. latipes* (NP\_001265808.1), *O. niloticus* (NP\_001266515.1), *D. rerio* (NP\_571229.3), *C. auratus* (AEX97168.1), *C. carpio* (ACB13197.1/ABF82249.1), *S. kozlovi* (ANW08806.1); accession numbers of Cyp19a1b are listed in table 2

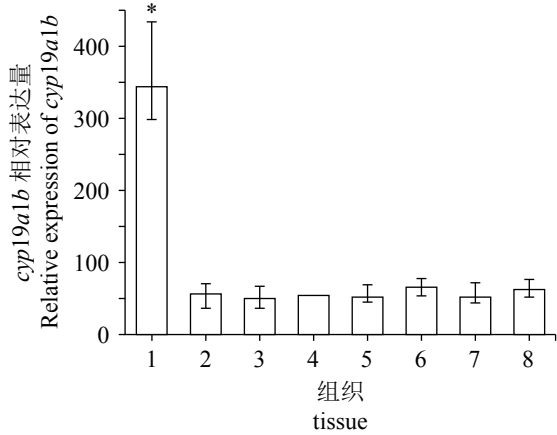


图 4 *cyp19a1b* 组织分布

\*表示差异显著 ( $P < 0.05$ )。1. 脑; 2. 心脏; 3. 脾脏; 4. 肝脏; 5. 肠; 6. 卵巢; 7. 肾脏; 8. 肌肉

Fig. 4 Tissue distribution of *C. auratus cyp19a1b* by Real-time PCR

Asterisk indicates significant difference ( $P < 0.05$ ). 1. brain; 2. heart; 3. spleen; 4. liver; 5. intestines; 6. ovary; 7. kidney; 8. muscle

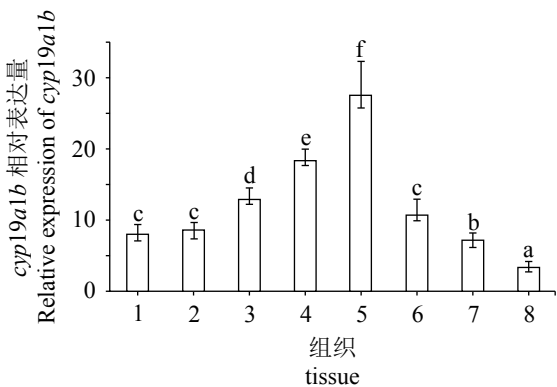


图 5 *Cyp19a1b* 基因在淇河鲫胚胎期的相对表达量

1. 未受精卵; 2. 受精卵; 3. 囊胚期; 4. 原肠胚期; 5. 神经胚期; 6. 尾芽期; 7. 心跳期; 8. 出膜期。不同字母表示差异显著 ( $P < 0.05$ ), 下同

Fig. 5 Expression of *cyp19a1b* in different embryonic development stages of *C. auratus*

1. unfertilized eggs; 2. fertilized egg; 3. blastula stage; 4. gastrula stage; 5. neurula stage; 6. tail-bud; 7. heart-beating; 8. hatching stage. Different lowercases indicate significant difference ( $P < 0.05$ ), the same below

淇河鲫神经系统的发育。在个体发育过程中, 较早发育和分化的是脑神经系统, 而神经胚期就是中枢神经发育的重要时期。尽管在青鳉胚胎发育初期并没有检测到该基因的表达, 但在性别分化关键时期检测到该基因的表达, 之后一直维持在较高水平<sup>[39]</sup>。除此之外, 在尼罗罗非鱼以及草鱼(*Ctenopharyngodon idella*)性别分化之

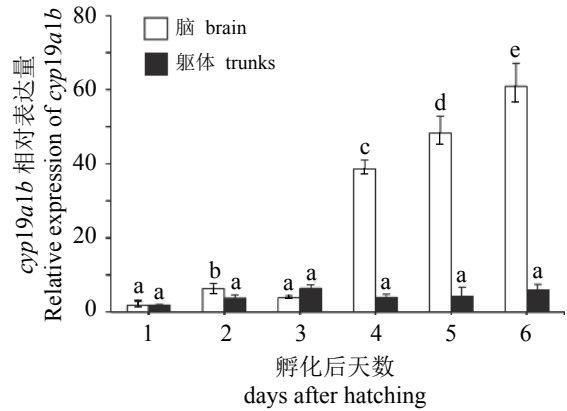


图 6 *Cyp19a1b* 基因在淇河鲫胚后发育过程中脑部和躯体的相对表达量

1. 10 dah; 2. 20 dah; 3. 25 dah; 4. 30 dah; 5. 40 dah; 6. 60 dah

Fig. 6 Expression of *cyp19a1b* in the brain and trunks of *C. auratus* at different larval developmental stages by Real-time PCR

1. 10 dah; 2. 20 dah; 3. 25 dah; 4. 30 dah; 5. 40 dah; 6. 60 dah

前, 脑中不表达脑型芳香化酶基因, 但在性别分化关键时期却高表达, 在脑部以外的躯体中表达量一直很低<sup>[40-41]</sup>。本研究中, 淇河鲫 *cyp19a1b* 在脑中的表达量也是从孵化后 30 d (淇河鲫性别决定与分化关键时期) 开始上调, 但在脑部以外的躯体部分表达量一直很低。以上研究均暗示该基因对鱼类的性别分化也起到一定作用, 即鱼类脑组织开始分泌雌激素和促性腺激素, 通过脑—垂体—性腺轴对未分化的性腺产生影响, 进而影响脑和性腺的性别分化。尽管在胚胎期 *cyp19a1b* 表达模式具有物种特异性, 在硬骨鱼类性别决定与分化关键时期其表达模式却具有共性, 但这也需要进一步验证。鱼类发生了基因组复制导致芳香化酶存在两种不同的类型, 并呈现不同表达模式, 但它们可能在某种程度上存在合作, 即脑型芳香化酶在淇河鲫的性腺发育中也存在一定的作用, 这需要进一步研究。

激素水平的变化对鱼类脑型芳香化酶基因的表达有较大影响。研究发现, 在 *cyp19a1b* 的 5' 侧翼区域存在雌激素的敏感元件<sup>[12-13, 16, 34]</sup>。在斑马鱼中, 睾酮的处理会导致 *cyp19a1b* 基因表达的减少<sup>[42]</sup>; 相反, 雌激素处理使 *cyp19a1b* 基因表达升高; 在斑马鱼、胡子鲶 (*Clarias fuscus*) 中, 芳香化酶抑制剂 Letrozole 处理使脑芳香化酶基因的表达降低<sup>[43-44]</sup>; 黑鲷 (*Sparus macrocephalus*) 短期注射芳香化酶抑制剂 fadrozole 会显著抑制脑中芳香

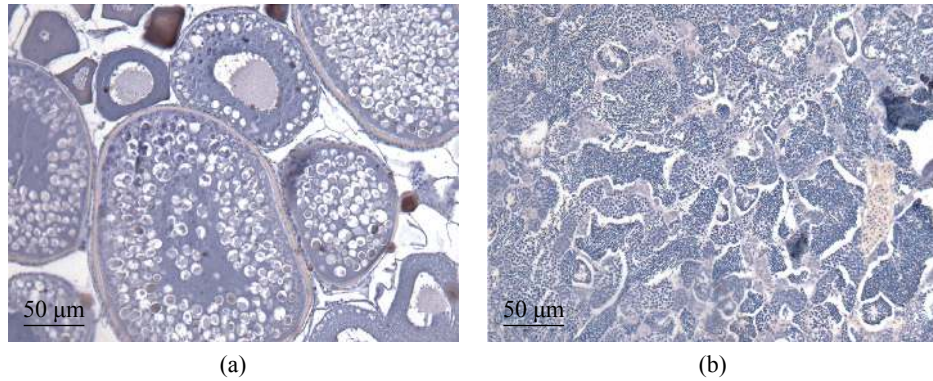


图 7 Letrozole从孵化后5 d处理60 d对雌性个体性腺分化的影响

(a)雌性对照, 卵巢, 性腺中充满不同时相的卵母细胞; (b)Letrozole处理雌性个体, 无卵母细胞, 可见不同时期精母细胞

Fig. 7 Morphological observation of gonad in female fish treated with Letrozole for 60 days

(a) XX control, ovary with oocytes at different phases; (b) Letrozole treated fish, no oocytes, the gonads are filled with spermatocytes

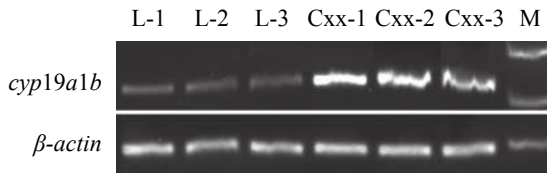


图 8 RT-PCR检测Letrozole处理后cyp19a1b在脑中的表达变化

对照组: Cxx-1、Cxx-2、Cxx-3; Letrozole处理组: L-1、L-2、L-3; M表示 DL 2000 DNA Marker

Fig. 8 RT-PCR analysis of the expression of cyp19a1b in brains of the control and Letrozole-treated female C. auratus

Control:Cxx-1, Cxx-2, Cxx-3; Letrozole treatment: L-1, L-2, L-3; M. DL 2000 DNA Marker

化酶的活性, 但不抑制性腺中的芳香化酶的活性<sup>[45]</sup>。伴随着Letrozole诱导的淇河鲫性逆转, cyp19a1b基因在脑中的表达量降低, 说明cyp19a1b在淇河鲫脑中表达具有两性差异, 由于芳香化酶是唯一不可逆的催化雄激素转化为雌激素的酶, cyp19a1b在鱼类脑中的性别差异表达可能与雌雄个体对雌激素需求量不同有关。淇河鲫成鱼腹腔注射hCG导致cyp19a1b表达量升高, 并具有时间依赖性, 这与印度囊鳃鲇(*Heteropneustes fossilis*)中的研究结果一致, 暗示鱼体内源激素水平的变化对鱼类脑型芳香化酶的影响也较大, hCG促进芳香化酶的表达与促性腺激素可以上调芳香化酶活性和雌激素分泌水平具有相关性<sup>[46-48]</sup>。

本研究成功克隆到淇河鲫cyp19a1b基因的cDNA全长序列, 进行了系统进化分析, 并对其在不同组织、不同发育时期的表达量进行了检

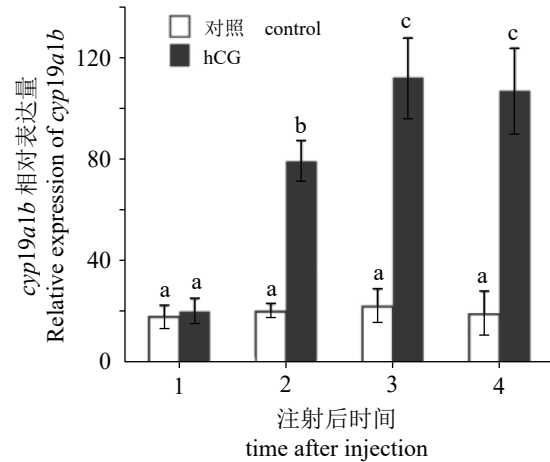


图 9 腹腔注射人绒毛膜促性腺激素对脑中cyp19a1b表达的影响

1.注射后0 h; 2.注射后8 h; 3.注射后12 h; 4.注射后24 h

Fig. 9 Expression profile of cyp19a1b in the brain after hCG injection by Real-time PCR

1. 0 h after injection; 2. 8 h after injection; 3. 12 h after injection; 4. 24 h after injection

测, 同时检测Letrozole处理导致性逆转过程以及腹腔注射hCG对脑中cyp19a1b的表达变化, 为进一步探讨cyp19a1b基因在雌核发育鱼类性别决定与分化中的作用机制奠定基础。

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## Molecular cloning and characterization of *cyp19a1b* gene and the effect of Letrozole on its expression in *Carassius auratus*

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**Abstract:** In the present study, brain aromatase gene (*cyp19a1b*) was cloned by using reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA 5' and 3' ends (RACE) in Qihe crucian carp (*Carassius auratus*). We also examined the expression level of *cyp19a1b* in different development stages and different tissues by real-time PCR, and at the same time, expression profile of *cyp19a1b* after hCG injection and sex reversal induced by Letrozole treatment was also examined. The results showed that the full-length cDNA of *cyp19a1b* was 2 894 bp, including a 132 bp 5'- untranslated region (UTR), a 1 319 bp 3'UTR, and a 1 533 bp open reading frame (ORF) encoding a deduced protein with 510 amino acids. Sequence analysis revealed that Cyp19a1b from *C. auratus* showed the highest amino acid similarity with Cyprinidae fish, while has low similarity with other vertebrates include mammalian and reptiles. Tissue distribution by real-time PCR revealed that *cyp19a1b* was abundantly expressed in the brain, while barely detectable in other tissues. The expression of *cyp19a1b* was gradually upregulated from blastula stage, and peaked at neurula stage, then declined during the embryonic development stage. Ontogenic analysis demonstrated that *cyp19a1b* was expressed abundantly from 30 days after hatching (dah) in the brain, but kept a low level in the trunks. However, *cyp19a1b* decreased in the brain of sex-reversed males by Letrozole treatment. Intraperitoneal injection of human chorionic gonadotropin experiments showed that expression of *cyp19a1b* was significantly elevated at 8 h and persisted until 24 h after injection in the brain. These results suggested that *cyp19a1b* might be involved in the course of nervous system formation, gonad development and sex determination in *C. auratus*.

**Key words:** Qihe crucian carp (*Carassius auratus*); *cyp19a1b*; cloning; expression; inhibitor

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