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瓯江彩鲤酪氨酸酶基因的克隆与序列分析

杨新鑫[1]，王成辉[1]，马玉清[1]，项松平[2]，王　剑[2]

(1. 上海海洋大学 农业部水产种质资源与利用重点开放实验室，上海　201306；2. 浙江龙泉省级瓯江彩鲤良种场，浙江 龙泉　323700)

摘　要：瓯江彩鲤(*Cyprinus carpio* var. *color*)是分布在我国瓯江流域的一种鲤科鱼类，色彩艳丽，体形优美，深受人们喜爱。瓯江彩鲤有5种基本体色："全红"、"大花"、"麻花"、"粉玉"和"粉花"，是研究鱼类体色遗传的良好材料和理想模型。酪氨酸酶(tyrosinase)是影响黑色素合成的关键酶，其转录的提前终止会导致黑色素无法合成。采用RACE技术从瓯江彩鲤皮肤转录本中克隆5种体色酪氨酸酶基因全序列，并对序列进行分析。发现在瓯江彩鲤的5种体色中，酪氨酸酶基因cDNA序列长度存在差异："全红"为2 100 bp，"麻花"为2 107 bp，"大花"为2 073 bp，"粉玉"为1 976 bp，"粉花"为2 111 bp；且每种体色都存在两种类型酪氨酸酶基因(TYR-1，TYR-2)的转录。这两种酪氨酸酶基因mRNA所翻译成的氨基酸序列仅在一二级结构上有所差异，而在结构域和三级结构上不存在差异。但酪氨酸酶基因的这些差异是否与瓯江彩鲤体色相关还有待后续的证明。

研究亮点：在国内外首次克隆了鲤的酪氨酸酶基因，发现不同体色瓯江彩鲤均转录完整的酪氨酸酶基因，且每种体色都存在两种酪氨酸酶的mRNA转录。揭示了5种体色瓯江彩鲤酪氨酸酶基因的序列差异，对进一步研究该基因与体色的关系提供了依据。

关键词：瓯江彩鲤；体色；酪氨酸酶基因
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鲤在全世界广泛养殖，其体色多种多样[1]，是研究体色遗传的极好材料[2]。瓯江彩鲤(*Cyprinus carpio* var. color)是浙江省瓯江流域广泛养殖的一种鲤科鱼类，其肉质鲜美，生长速度快，尤其是色彩绚丽鲜艳、斑纹美观悦目，有重要的观赏价值。瓯江彩鲤主要有5种基本体色："全红"(全身体表为红色)、"粉玉"(全身体表为白色)、"大花"(红色体表分布大块黑色斑块)、"麻花"(红色体表分布黑色小斑点)、"粉花"(粉白色体表分布大块黑色斑块)。瓯江彩鲤如此丰富的体色在我国的淡水鱼类中极为罕见，是我国鲤科鱼类的一大瑰宝，作为食用和观赏鱼类开发具有巨大的发展潜力。国内外对鱼类的体色遗传研究多集中在体色的表型观察与遗传规律研究，而对体色的分子基础研究相对较少[3－6]。酪氨酸酶(tyrosinase)是影响黑色素合成的关键酶，其转录的提前终止会导致黑色素无法合成，造成白化现象。国外对鱼类酪氨酸基因进行了一些研究，如酪氨酸酶基因对体色的决定和影响方面[7－11]，但很少有涉及不同体色间酪氨酸酶基因的差异[12]。本论文通过克隆瓯江彩鲤5种体色的酪氨酸酶基因，探讨酪氨酸酶基因与瓯江彩鲤体色的关系，尤其是与黑色斑纹的关系，试图了解瓯江彩鲤体色变异的分子基础。

1　材料与方法

1.1　实验材料

实验所用材料来自浙江龙泉省级瓯江彩鲤良种场，以"大花"体色为母本，"粉花"体色为父本杂交构建的全同胞家系。

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作者简介：杨新鑫(1987—)，男，硕士研究生，研究方向为水产动物种质资源与苗种工程。E-mail：yxxhnx@126.com
通讯作者：王成辉，E-mail：wangch@shou.edu.cn

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全红 -------ACA TGGGG----- GTGTCTAATT CGTCTCGCTC TCGTCATGTC TCTCCCTCTG CTTCTGTTCT TCATTCAGTT 80
麻花 --------- --------- ---------- -------A. AT.GG..... .......... .......... ..........
大花 -----T··· .....CTTCC .......... .......... .......... .......... .......... ..........
粉玉 -------... .....----- .......... .......... .......... .......... .......... ..........
粉花 ACATGGG.AG C.A.CTGAAG T.C......G .....G.G.. ..A..... .......... .......... ..........
全红 CTTGAGTCCG TCTCTCCAGC AGTTTCCTCG GCCGTGCACC ACGCCGGACG TCCTGCGGAG CAAGCGCTGC TGTCCGGTTT 160
麻花 ..G..... .......... .......... .......... ..A..... ......A... .......... ........C.
大花 .......... .......... .......... .......... .......... ......A... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 GGCCGGGCGA CGGTTCGGTG TGCGGCTCCC TTTCGGGTCG AGGCTTCTGC CAGGACGTCA CGGTCTCCGA TCTTCCCAAC 240
麻花 .A..A..T.. .......... ..T..... .G..... .......... ..A..... .......T.. .......G..
大花 .......... .......... .......... .......... ...G..... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 GGGCCGCAGT ACCCACATTC AGGCCTGGAC GACCGCGAAC AATGGCCTCT GGTGTTTTAC AACCAAACCT GCCAGTGCGC 320
麻花 .......... .......... .........T ........G. GC........ ......C... .......... ........A.
大花 .......... ....G..... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 CGGAAACTAC ATGGGGTTCG ACTGCGGCGA GTGCAAGTTC GGTTACTTCG GCGCCAATTG CGGTGAACGA CGGGAATCTG 400
麻花 .......... .......... .......... .........T ........T. .T....GC.. ...G...... ..........
大花 .......... .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 TGCGCAAAAA CATCTTCCAA TTATCTGTCT CTGAAAGGCA GAGGTTCATC TCGTACCTCA ATCTCGCCAA AACTACAGTC 480
麻花 ......G... .......... .....C..G. ....G..... A......... .......... .C........ ......CA..
大花 .......... .......... .....C.... .......... .......... .......... .......... ..........
粉玉 .......... .......... .....C.... .......... .......... .......... .......... ..........
粉花 .......... .......... .....C.... .......... .......... .......... .......... ..........
全红 AGCCCTGATT ATATGATCGT GACGGGCGTG TACTCACAGA TGAACAACGG CTCGACGCCC ATGTTCACCA ACATCAGTGT 560
麻花 .....C.... ..G..... .......AC. ..TG.G.... .......... .......... .......... ..........
大花 .......... .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 GTATGATCTG TTCGTCTGGA TGCACTATTA CGTGTCCCGT GATGCGCTGC TCGGGGGTCC CGGGAACGTG TGGGCTGACA 640
麻花 ...C..... .......... .......... .......... .......... .......... .......... .....C....
大花 ...C..... .......... .......... .......... .......... .......... .......... .....C....
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 TTGACTTTGC ACACGAATCC GCAGCTTTTC TGCCCTGGCA TCGCGTTTAC CTGCTGTTCT GGGAGCATGA GATCCGAAAG 720
麻花 .......... G........G ..C..G.... .......... .......... .......... .......... ......G...
大花 .......... G........G ..C..G.... .......... .......... .......... .......... ......G...
粉玉 .......... .........G .....G.... .......... .......... .......... .......... ..........
粉花 .......... .........G .....G.... .......... .......... .......... .......... ..........
全红 CTGACCGGTG ACTTTAACTT CACCATCCCG TACTGGGACT GGCGTGACGC TCAGGACTGT CAGGTGTGCA CGGATGAGCT 800
麻花 .......... .......... .......... .......... .......... .......... .......... ..........
大花 .......... .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 GATGGGGGCG CGCAGTCCTC TCAACCCCAA CCTCATCAGC CCGTCCTCGG TGTTCTCCTC CTGGAAGGTG ATCTGTTCAC 880
麻花 .......... .......... .......... .......... .......... .......... .......... ..........
大花 .......... .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 AACCCGAAGA CTACAACCAG CGTGAGGTTT TGTGTGACGG GTCTCCAGAG GGACCGTTAC TGCGTAATCC AGGAAACCAC 960
麻花 .......... .......... .......... .......... .......... .......... .......... ..........
大花 .......... .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... ......T... .....T.... .......... ..........
粉花 .......... .......... .......... .......... .......... .......... .......... ..........
全红 GACCCAAACC GTGTCCCGCG GCTGCCCACC TCCGCAGACG TGGAGTCAGT GCTGAGCCTA ACAGAGTACG AGACGGGTCT 1040
麻花 ....G...G. .......... .......... .......... .......... .......... .......... ..........
大花 ....G...G. .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 ....G...G. .......... .......... .......... .......... .......... .......... ..........
全红 GATGGACAGA AGCGCCAACA TGAGCTTCAG GAACGCTCTG GAAGGTTTTG CGAGTCCTGA GACGGGGCTG GCAGTAACGG 1120
麻花 .......... C......... .......... .......... .....A.... .......... .......... ..........
大花 .......... C......... .......... .......... .....A.... .......... .......... ..........
粉玉 .......... C......... .......... .......... .G........ .......... .......... ..........
粉花 .......... C......... .......... .......... .....A.... .......... .......... ..........
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全红 GGCAGAGCTT GATGCACAAC TCCTTACACG TCTTCATGAA CGGATCCATG TCTTCAGTGC AGGGATCCGC CAACGACCCC 1200
麻花 .......... .......... ..T....... C.C....... .......... .......... .......... ......T...
大花 .......... .......... ..T....... C.......... .......... .......... .......... ......T...
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .T........ .......... ..T....... C.C....... .......... .......... .......... ......T...
全红 ATCTTCCTTC TACATCATGC CTTCATCGAC AGTATCTTTG AGCAATGGCT GAGGAGACAC CAGCCCCCCC -GCACACACT 1280
麻花 .......... .......... ...TG..... ..C....... .......... .......... .....T.... -.........
大花 .......... .......... ...TG..... ..C....... .......... .......... .....T.... -.........
粉玉 .......... .......... .......... ..C....... .......... .......... .......... C.........
粉花 .......... .......... ...TG..... ..C....... .........A .......... .....T.... -.........
全红 ACCCGACAGC CAACGCCCCG ATCGGACACA ACGACGGCTT TTACATGGTC CCCTTCATCC CTCTGTACAG AAACGGAGAT 1360
麻花 .......C.. .......... .......... .........A .......... .......... ....C..... ..........
大花 .......C.. .......... .......... .........A .......... .......... ....C..... ..........
粉玉 .......... .........A .......... .......... .......... .......... .......... ..........
粉花 .......C.. .......... .......... .........A .......... .......... ....C..... ..........
全红 TATTTCCTCT CGACTAAAGC TCTGGGATAT GAATATGCAT ATTTACAGGA CCCAAGTCAG CGGTTTGTGC AGGAGTTTCT 1440
麻花 .......... .......... ......T... .......... .......... .......... .......... ..........
大花 .......... .......... ......T... .......... .......... .......... .......... ..........
粉玉 .......... .......... .......... .......... .......... .......... .......... ..........
粉花 .......... .......... ......T... .......... .......... .......... .......... ..........
全红 GACGCCGTAT CTAGAGCAAG CTCAGCAGAT CTGGCGCTGG CTGCTGGCCG CGGGGCTCCT CGGCGCGGCT GTGGCGGGAA 1520
麻花 .......... ..G....... ...G...... .......... .......... .....A.... ...G.....C ....T.....
大花 .......... ..G....... ...G...... .......... .......... .....A.... ...G.....C ....T.....
粉玉 .......... .......... .......... ...C...... .......... .......... .......... ..........
粉花 .......... ..G....... ...G...... .......... .......... .....A.... ...G.....C ....T.....
全红 TTATTGCAAC TATTATCACC GTGGCGTGTC GCAGACGCCT GAAGAGGCGA AAGTCATCAG CGTACGGGGA GAGACAGCCA 1600
麻花 ........G. .........T .......... A......GAC ..G....A.. .......—— ——.T..... .........G
大花 ........G. .........T .......... A......GAC ..G....A.. .......—— ——.T..... .........G
粉玉 .......G.. .......... .......... .T........ ..G....... .....G..G. .......... ..........
粉花 ........G. .........T .......... A......GAC ..G....A.. .......—— ——.T..... .........G
全红 CTGCTGAACA GCAGTGAAGA GGAGGGATCG ACTTCATATC AGACTACACT GTGA—————— ————-CCC TACAGACACG 1680
麻花 ..T....... ....C..... .........C .......... .......... ....———— ————-... ..........
大花 ..T....... ....C..... .......... .......... .......... ....———— ————-... ..........
粉玉 .......... .......... .......... .......... .......... ....ATCAGA GCGTAAG..T -.........
粉花 ..T....... ....C..... .........C .......... .......... ....———— ————-... ..........
全红 TGTCAGCGAG GACAGATACA CCGTACAGAT A————————— CGCCAGTTAA ACCAGGTCAT GTGACAAATG AGACAAGGTC 1760
麻花 .......... .......... .....G... .————————— .......... .......... ........CA ..........
大花 .......... .......... .......... .————————— .......... .......... .......... ..........
粉玉 ..C....... .........G ..AA..T..C .GAAAAAAGA .A.A.CA... C......... .......T.. .......C..
粉花 .......... .......... ......G... .————————— .......... .......... ........CA ..........
全红 TCGCCTCTAA ACCAGGGTCA TGCAACAAGG GCTGCGTTCT GAATCCTGCT ATAGTATTCA TAATGCGCAC TACATACACT 1840
麻花 ......G... .......... .......... .......... .......... .......... ......A... .....T....
大花 .......... .......... .......... .......... .......... .......... .......... ..........
粉玉 .......... ..TG.A.... .......... .........A .....T.... .......G.. .....T.... ........G.
粉花 ......G... .......... .......... .......... .......... .......... ......A... .....T....
全红 ACTGTTCAAA TGTTTGGGGT CAGTAAGAGT TTACTCAGCA AGGATGCATT AAATGAATCA AAAGTGAC— ———————TTT 1920
麻花 ......T... .......... ....C..... .......... .......... .......... ........AG TAAAGAC...
大花 .......... .......... .......... .......... .......... .......... ........—— ————————...
粉玉 C.....T... A....A.... .........T. ...T...... C......... ....TG.... ........AG TAAAGACA..
粉花 ......T... .......... ....C..... .......... .......... .......... ........AG TAAAGAC...
全红 TTTAACATCT TTCTATTTCA AATAAATGCT GCTCATTTGA ACTTGATATT GAGCAGCACA ACTGTTTTCA ACATTGATAA 2000
麻花 .G...T.... .......... .T........ .......... ....TC.... C.T..———. .GAA....G. .G..AA.-.T
大花 .....T.... .......... .......... .......... .......... .......... .......... ..........
粉玉 .G...TG... .......... .......T.. .......... .AAAA.A.AA A.AA.A—. .AAAAAAAA. .A.———————
粉花 .G...T.... .......... .T........ .......... ....TC.... C.T..———. .GAA....G. .G..AA.-.T
全红 TAATCAGAAA TGTTTCTTGA GCAGCAAATT AGCATATTAG AATGATTTCT GAAG-ATCAT GTGATACTGA AGTGATGAGT 2080
麻花 CTG...C... A———.A.... .........C .......... .......... ....G...C. ..A....... ..........
大花 .......... .......... .......... .......... .......... ....-..... .......... ..........
粉玉 ———————— ———————— ———————— ———————— ———————— ———————— ———————— ————————
粉花 CTG...C... A———.A.... .........C .......... .......... ....G...C. ..A....... ..........
全红 GATTAACTGT TGTGCTGTAC AGAATACAAA AGAAATAAAT ATTTAAAAAG AATGAAAAAA AAAAA-                2145
麻花 .......... .......... .......... .T........ .......... ———-..... .....A
大花 .......... .......... .......... .......... .......... .......... .....A
粉玉 ———————— ———————— ———————— ———————— ———————— ———————— ———————
粉花 .......... .......... .......... .T........ .......... ———-..... .....A
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图 1　5 种体色瓯江彩鲤酪氨酸酶基因的 cDNA 序列

Fig. 1　The cDNA nucleotide and its variation of tyrosinase gene in five color patterns of Oujiang color common carp

相同的核苷酸以点表示,缺失的核苷酸以横线表示。

TYR-1	MSLPLLLFFI	QFLSPSLQQF	PRPCTTPDVL	RSKRCCPVWP	GDGSVCGSLS	GRGFCQDVTV	60
TYR-2	....	....	....	Q.........	....	....	
TYR-1	SDLPNGPQYP	HSGLDDREQW	PLVFYNQTCQ	CAGNYMGFDC	GECKFGYFGA	NCGERRESVR	120
TYR-2	....D.....	........R.	....	....	....	S.........	
TYR-1	KNIFQLSVSE	RQRFISYLNL	AKTTVSPDYM	IVTGVYSQMN	NGSTPMFTNI	SVYDLFVWMH	180
TYR-2	R.........	....	....I....V	....T.A...	....	....	
TYR-1	YYVSRDALLG	GPGNVWADID	FAHESAAFLP	WHRVYLLFWE	HEIRKLTGDF	NFTIPYWDWR	240
TYR-2	....	....	....	....	....	....	
TYR-1	DAQDCQVCTD	ELMGARSPLN	PNLISPSSVF	SSWKVICSQP	EDYNQREVLC	DGSPEGPLLR	300
TYR-2	....	....	....	....	....	....	
TYR-1	NPGNHDPNRV	PRLPTSADVE	SVLSLTEYET	GLMDRSANMS	FRNALEGFAS	PETGLAVTGQ	360
TYR-2	......RK..	....	....	.....R....	....	....	
TYR-1	SLMHNSLHVF	MNGSMSSVQG	SANDPIFLLH	HAFIDSIFEQ	WLRRHQPPRT	HYPTANAPIG	420
TYR-2	........A.	....	....	....	....	....	
TYR-1	HNDGFYMVPF	IPLYRNGDYF	LSTKALGYEY	AYLQDPSQRF	VQEFLTPYLE	QAQQIWRWLL	480
TYR-2	....	....	....	....	....	..R.......	
TYR-1	AAGILGAAVV	GIIAAIITVA	CRRRLKRRKS	SAYGERQPLL	NSSEEEGSTS	YQTTL	535
TYR-2	....	....	....	....	....	.....	

图 2 瓯江彩鲤两种类型酪氨酸酶基因的氨基酸序列

Fig. 2 The amino acid sequence and its variation of two types of tyrosinase genes in Oujiang color common carp

相同的氨基酸以点表示。

2.3 蛋白质结构预测

蛋白质二级结构预测表明,瓯江彩鲤酪氨酸酶氨基酸分子主要由 α 螺旋、β 折叠组成,兼有少量无规则卷曲。三级结构预测显示:瓯江彩鲤酪氨酸酶基因的两种蛋白质序列三级结构的空间布局非常相似,主要的差异区段全部为无规则卷曲结构(图 3)。

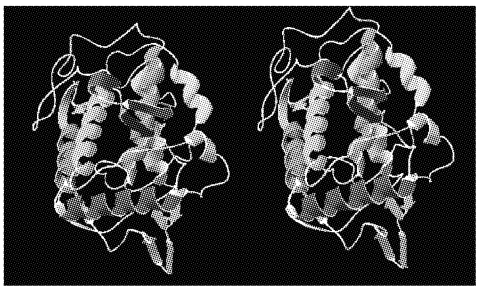

图 3 瓯江彩鲤酪氨酸酶基因 TYR-1(左) TYR-2(右)的蛋白质三级结构预测

Fig. 3 The tertiary structure of TYR-1 (left) and TYR-2 (right) from tyrosinase gene in Oujiang color common carp

3 讨论

酪氨酸酶广泛存在于植物、动物、细菌,是一个跨膜的转运蛋白,主要存在于黑色素小体中(melanosomes)[23],仅有一小部分存在于细胞质中。不同物种的酪氨酸酶基因结构也各不相同。本研究克隆出 5 种体色瓯江彩鲤的酪氨酸酶 cDNA 全序列,发现无论是纯色的瓯江彩鲤(如“全红”、“粉玉”),还是带有黑色斑点的瓯江彩鲤(如“麻花”),或是黑色斑块的瓯江彩鲤(如“大花”、“麻花”),都转录产生完整的 mRNA,并且在每种体色中都有两种类型 mRNA(TYR-1, TYR-2),开放阅读框长度一致,序列基本相同。

通过氨基酸的二三级结构分析,发现仅在二级结构上有些差异,但这些差异可信度并不是很高。两种氨基酸序列的三级结构完全相同。因此,我们推测,这两种氨基酸序列功能可能是重叠的。

先前一些报道,白化现象的产生是由于酪氨酸基因开放阅读框内突变的产生,使转录无法顺

利进行,如在猕猴中,由于酪氨酸酶编码区域的点突变造成了白化[9]。通过我们的克隆分析发现,彩鲤酪氨酸酶基因的两种序列虽然有差异,但是这些差异位点也都没有形成终止子,使酪氨酸酶翻译提前终止。可见这两种 mRNA 其蛋白质的翻译都不会提前终止,而是翻译成完整的酪氨酸酶,未出现其它物种白化病例中所表现出的转录提前终止现象。但是这些个别位点上的差异是否与瓯江彩鲤 5 种体色的不同有所关联,还需要进一步的实验验证。

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Cloning and sequence analysis of tyrosinase gene in Oujiang color common carp

YANG Xin-xin[1], WANG Cheng-hui[1], MA Yu-qing[1], XIANG Song-ping[2], WANG Jian[2]

(1. *Key Laboratory of Aquatic Genetic Resources and Utilization, Certificated by Ministry of Aquaculture, Shanghai Ocean University, Shanghai* 201306, *China*; 2. *Oujiang Color Common Carp Provincial Farm, Longquan* 323700, *Zhejiang, China*)

Abstract: Oujiang color common carp (*Cyprinus carpio* var. *color*) is a regional freshwater species distributed in the Oujiang River basin in China. Due to its rich color patterns and graceful body shape, Oujiang color common carp is a very popular fish to local inhabitants of the Oujiang River drainage. The fish has five kinds of body color patterns, namely "Whole red", "Whole red with big black patch", "Whole red with scattered black spots", "Whole white" and "Whole white with big black patch". This fish can be a very good model or material for studying body color inheritance in fish. Tyrosinase is a key enzyme involved in the formation of melanin compounds. The melanin can not be synthesized if premature transcription termination occurs in this gene. The cDNA sequences of tyrosinase gene were obtained from the skin tissue of five body color patterns of Oujiang color common carp using the technique of rapid amplification of cDNA ends (RACE). The results showed that the lengths of tyrosinase gene cDNA sequence are different in different color patterns of Oujiang color common carp. For instance, the length of "Whole red" is 2 100 bp, "Whole red with scattered spots" is 2 107 bp, "Whole red with big black patch" is 2 073 bp, "Whole white" is 1 976 bp and "Whole white with big black patch" is 2 111 bp. There are two types of mRNA transcripts in each kind of body color patterns. Analyzing the amino acid sequence from two types mRNA, the first and second structures of predicted protein are different, whereas the third structure and typical structural domain are same in tyrosinase gene. It would be left behind to further research whether the sequence difference of tyrosinase gene would be a direct factor or not in determining different color patterns of Oujiang color common carp.

Key words: *Cyprinus carpio* var. *color*; body color; tyrosinase gene