Chinese Journal of Oceanology and Limnology
Vol. 25 No. 2, P. 191-198, 2007
DOI: 10.1007/s00343-007-0191-9

The tyrosinase gene family and albinism in fish*

WANG Jiaqing (王家庆), HOU Lin (侯林)**, ZHANG Ruifeng (张瑞锋), ZHAO Xintao (赵欣涛), JIANG Lijuan (姜丽娟), SUN Wenjing (孙文静), AN Jialu (安家璐), LI Xiaoyan (李晓燕)
(College of Life Science, Liaoning Normal University, Dalian 116029, China)

Received Apr. 18, 2006; revision accepted June 17, 2006

Abstract Tyrosinase exists universally in organisms and is a characteristic enzyme of melanocytes. Tyrosinase family genes in vertebrates consist of 3 related members; tyrosinase (TYR, Tyr), tyrosinase-related protein-1 (TRP-1, Tyrp1), and tyrosinase-related protein-2 (TRP-2, Tyrp2, Dct). These proteins catalyze melanin biosynthesis in pigment cells and play important roles in determining vertebrate coloration. Transcription of the TYR and TRP genes is useful for studying neural crest and optic vesicle cell migration and differentiation during embryogenesis and important in pigment rescue in fish. In this paper, the structure of gene and protein molecular evolution, function and roles of the TYR family in fish were reviewed.

Key words: fish; tyrosinase gene family; molecular evolution; transgene; albinism

1 INTRODUCTION

Tyrosinase, a type of characteristic enzyme of melanocytes, is a copper-containing glycoprotein (Hearing and Jimenez, 1987), and a biochemical/physiological marker of differential maturity of melanocytes. The international enzyme codes of TYR and TRP-2 (Dct, dopachrome tautomerase) are EC1.14.18.1 and EC5.3.2.3, respectively. Tyrosinase catalyzes the rate-limiting conversions of tyrosine to DOPA, and DOPA to DOPA-quinone (DQ), an essential precursor in melanin synthesis (Boonanuntanasarn et al., 2004). In fish and many other vertebrates, mutations and deletions of TYR and TYR-related proteins result in skin coloration abnormalities. Most fish albinisms are related with mutations of the tyrosinase family of genes, where the skin of albinos lack melanin and eye development is affected. The mutations of the zebrafish sdy gene, which encodes tyrosinase, slow down the onset of adaptation to bright light. Retinal pigment epithelium (RPE), which normally expresses tyrosinase, secretes a modulatory factor (possibly L-DOPA), which regulates light adaptation in retinal circuitry (Page-McCaw et al., 2004). The mutation of the mouse tyrosinase gene can arouse albinism and the elevation of intraocular pressure (Savinova et al., 2001). Besides, tyrosinase influences the development of the eye anterior segment in mice (Link et al., 2004). The TYR/L-DOPA pathway may modify human primary congenital glaucoma (Libby et al., 2003). In animals, tyrosinase plays an important role in modifying the pigmentation and regulating weight and feed intake.

2 THE STRUCTURE OF TYR FAMILY GENES

Zebrafish (*Danio rerio*), fugu (*Takifugu rubripes*) and medaka (*Oryzias latipess*), which are members of the TYR family, have been characterized clearly. The amounts and sizes of exons are similar to the mammal TYR family of genes, but the lengths of total coding regions are between 2–6 kb, which are about 1/10th of that of humans and mice. Vertebrate fugu has a compact genome, and the gene amounts are approximately equivalent to that of humans with a lack of introns. Fugu fish has been regarded as one of the best model organism for human genome research. Thus, the TYR family of genes of fugu fish have been

* Supported by NSFC (No. 30271035).
** Corresponding author: houlin01@126.com

researched quite in depth. The fugu TYR gene has a length of 4 966 bp, containing 5 exons; the TRP-1 gene has a length of 4 891 bp, consisting of 7 exons; TRP-2 has a length of 5 755 bp, having 8 exons. The sizes and relative positions of the 3 genes are shown in Fig.1. Genomic DNA blot analysis revealed that the TYR gene is present as a single copy in the fish genome (Inagaki et al., 1994). All 3 genes contain a highly conserved 11 bp regulatory element in their proximal promoter regions, the M-box. The conserved core sequence of the 3 M-boxes, '5-GTCATGTG-3', contains an E-box (CATGTG), which is the recognition site for the microphthalmia transcription factor (Mitf). Since Mitf stimulates transcription of tyrosinase and TRP-1 by binding to the E-box, these M-box elements are believed to be key regulatory elements in pigment cell-specific gene expression (Fang et al., 2002; Vetrini et al., 2002; Camacho-Hubner et al., 2004).

3 LOCATIONS OF TYR FAMILY GENES

The structures of TYR and TYR-related protein genes of humans and mice have been recognized; the lengths are between 20–70 kb. The encoding region of the human TYR gene comprises 5 exons, locates at chromosome region 11q14–q21; TRP-1 locates at chromosome region 9p23; and TRP-2 locates at chromosome region 13q31–q32. The latter 2 genes consist of 7 and 8 exons, respectively. In medaka fish, TYR gene i locus locates at linkage group 13 (LG13), and TRP-1 gene locates at linkage group 18 (LG18) (Naruse et al., 2000, Fukamachi et al., 2004). In Mexican cavefish (*Astyanax mexicanus*), the TYR gene locates at microsatellite linkage map 15, and TRP-1 gene locates at microsatellite linkage map 13 (Protas et al., 2006). Kelsh et al., (2000) cloned a zebrafish TRP gene that shares all the identified motifs of its mammalian homologues. It maps the lower arm of LG9, which is synteny with human chromosome 13q.

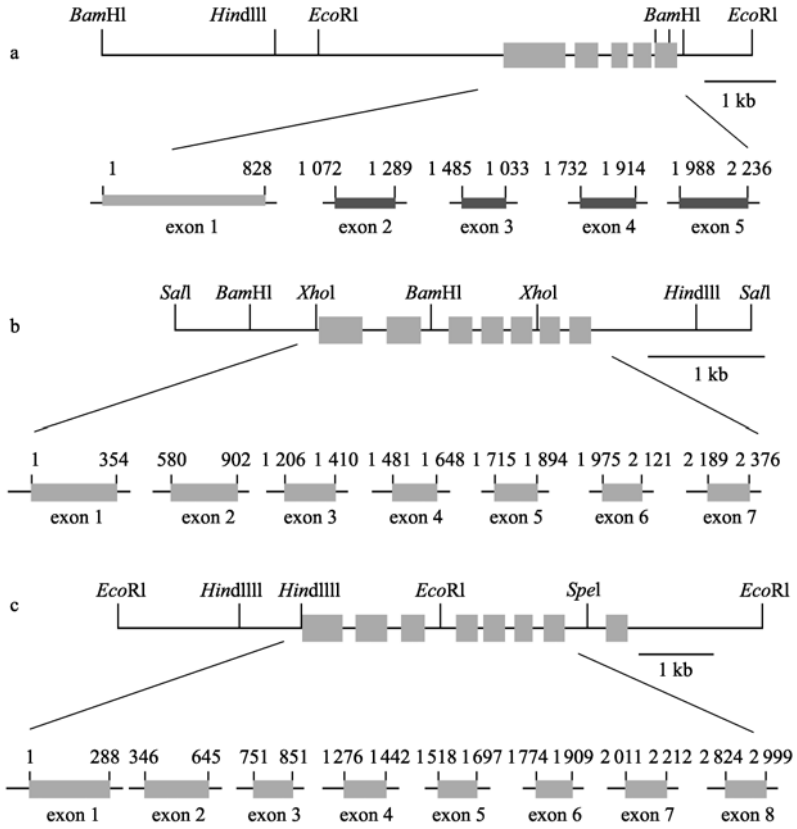

Fig.1 Positions and sizes of TYR gene family exons of fugu (from Camacho-Hubner et al., 2000, 2002)

a. The BamHI-EcoRI fragment of fugu TYR gene, indicating sizes and positions of exons 1–5 (Camacho-Hubner et al., 2000). b. The SalI fragment of fugu Tyrp1 gene, indicating sizes and positions of exons 1–7. c. Two adjacent EcoRI fragments of fugu Dct gene, indicating sizes and positions of exons 1–8 (Camacho-Hubner et al., 2002)

4 MUTATIONS OF TYR GENE IN FISH

Tyrosinase gene has many alleles in fish. At present, allele loci that have been researched consist of i1, i4, i5 and i6. In medaka, the genotype i1/i1 exhibits a complete albino phenotype but lacks *in vivo* tyrosinase activity. The genotype i4/i4 shows a quasi-albino phenotype; both skin and eyes have reduced pigmentation. The TYR genes for i1 and i4 were cloned and sequenced, and compared with wild-type tyrosinase. The i1 allele contains a 1.9 kb transposable element *Tol-1* in the first exon, Tyrosinase i1 gene of revertants showed *Tol-1* can excise precisely from the gene, and the i4 allele contains a 4.7 kb transposable element in the fifth exon (Koga et al., 1996), but no i4 revertants were found (Koga et al., 1997). Allele i5 at this i locus (also denoted as ib), causes oculocutaneous albinism in i5/i5 genotype carriers. Its albino phenotype is very weak, characterized mainly by small and various-sized melanophores in juveniles. Cloning and sequencing of the tyrosinase gene for the i5 allele revealed the presence of a 4.7 kb extra DNA fragment in the 5' untranslated region, being *Tol-2*, a DNA-based transposable element of the *hobo Activator Tam3* (*hAT*) family. Its insertion point was 85 bp upstream of the main transcription initiation site and 50 bp downstream of the CATGTA motif, which has been deemed essential to the promoter function of the tyrosinase gene. The transcription level of the tyrosinase gene was decreased in i5/i5 fish, compared with wild-type fish (Iida et al., 2004). Recently, reverse mutation of i5 oculocutaneous albinism to wild-type pigmentation can occur in medaka fish (Iida et al., 2005). Genotype i6/i6 carriers have the same complete albino phenotype with i1/i1. Compared with wild-type tyrosinase gene, we found that i6/i6 carriers were not produced by transposable element, but were due to 3 deletion mutations with the loss of 8, 44 and 245 bp. The largest deletion spans over the last 180 bp of the second intron and the first 65 bp of the third exon. Because of this deletion, the i6 allele lacks the branch point sequence and the acceptor site for the second intron, both being considered necessary for normal RNA splicing. Therefore, the 245 bp deletion is likely to be responsible for the albino phenotype. With a mutant gene of this type, unlike ones bearing transposable element insertion, the possibility of reverse mutation to the wild-type would be negligible. Therefore, fish having the i6/i6 genotype should serve as superior recipients for the tyrosinase gene in rescue experiments (Koga et al., 1999).

5 PROTEIN STRUCTURES OF TYR AND TYR-RELATED PROTEINS

Many conserved regions exist in the peptide structures of the tyrosinase family proteins and may represent functional domains: (1) an N-terminal signal peptide; (2) two histidine-containing domains, binding 2 divalent metal ions (CuA and CuB) are necessary for the catalytic EGF-like motif, which might be implicated in the formation of the multienzyme complex formed by the 3 proteins within the melanosome; (4) several glycosylation sites and cysteine-rich region (Cys-rich); (5) a highly hydrophobic transmembrane domain; (6) leucine- and tyrosine-based sorting motifs in the C-terminus, which appear to mediate the correct targeting of proteins to the melanosomes (Fig.2). All the important structural and functional domains are conserved in fish TYR family protein levels (Camacho-Hubner et al., 2002).

6 EVOLUTION AT GENETIC LEVEL OF TYR GENE FAMILY

The tyrosinase gene family originated from a common ancestor. The family members are conservative in evolution. Ancestral TYR gene produces TYR and TRP-1 gene by duplication and

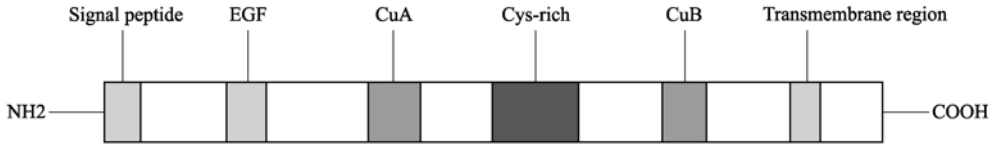

Fig.2 Domain structure of tyrosinase of vertebrate

divergence; subsequently, the latter produces TRP-2 by duplication and divergence. In mice and humans, the exon/intron structure of the tyrosinase gene family differs, with the position of only one intron conserved. The genetic structure of the family suggests a gain of introns with time. Both TRP-1 and TRP-2 are thought to arise from tyrosinase by means of gene duplication and subsequent divergence (Budd, 1995; Camacho-Hubner et al., 2002). The common ancestor of mammals and teleosts, which lived more than 450 million years ago, already had a full set of diversified tyrosinase family of genes. At the genetic level, the intron positions and phases are conserved between teleosts and mammals, suggesting that after the duplication of the ancestral tyrosinase gene during evolution, the TRP gene duplicated to give rise to TRP-1 and TRP-2 before the divergence of the vertebrate lineage (Camacho-Hubner et al., 2002) (Fig.3).

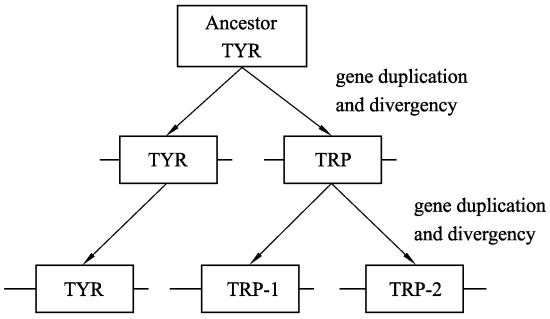

Fig. 3 Ancestor TYR gene duplicated and subsequently diverged to TYR family: TYR, TRP-1 and TRP-2

7 EVOLUTION AT THE PROTEIN LEVEL OF TYR GENE FAMILY

According to the accession numbers of the GenBank, the amino acid level homologies are as follows: the degree of identity of fugu TRP-1 and TRP-2 is higher than fugu TYR and TRP-1 homology (43%), and TYR and TRP-2 homology (45%). The homology of zebrafish TRP-1 and TRP-2 (50%) is higher than fugu TYR and TRP-1 homology (43%), and TYR and TRP-2 homology (46%). The results also show that during the evolution of fish, TYR gene appeared early, and subsequently, TRP-1 and TRP-2 appeared by means of gene duplication and subsequent divergence. The homology of the 3 orthologous genes between fugu and zebrafish are TYR (73%), TRP-1 (75%), and TRP-2 (79%) higher than the homology of the paralogous genes between 2 fishes (the percentages of homology are not shown here). Using CLUSTALX program, the protein sequence alignments were finished (partial alignment results and the GenBank accession numbers are shown in Fig.4). The tree was constructed using neighbor-joining (NJ) method, and the analysis results are shown by TREEVIEW software (Fig.5) (this topology structure agrees with the result of Mega 3.0 program bootstrap 1 000 times, not shown here). As shown in Fig.5, the fish TYR family clearly congregates into 3 groups, which demonstrates that homology of the fish TYR family orthologues is higher than that of its paralogues.

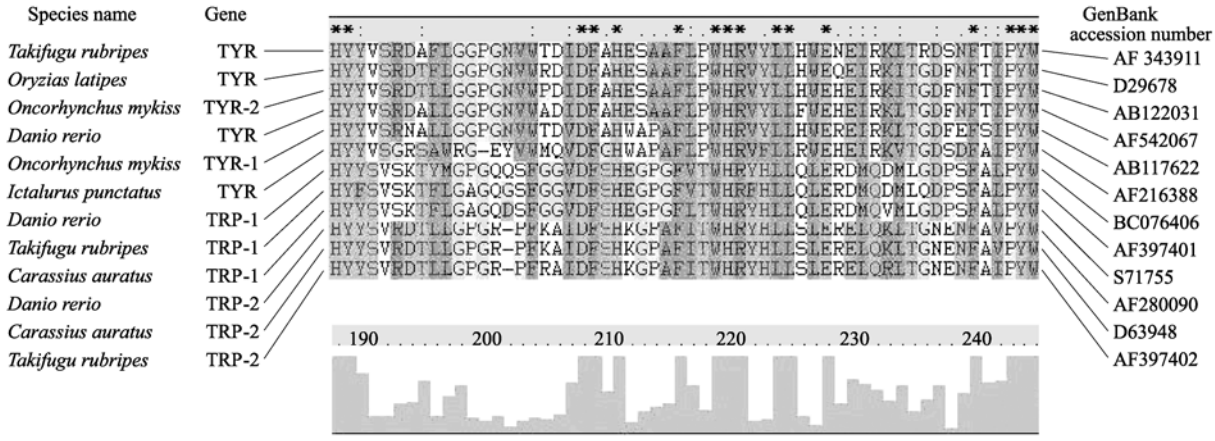

Fig.4 The multiple sequence alignments of TYR gene family members in fish

The multiple sequence alignments produced by the newest CLUSTALX 1.81 (only partial alignments were shown). Species and gene names were on the left, the corresponding GenBank accession numbers on the right.

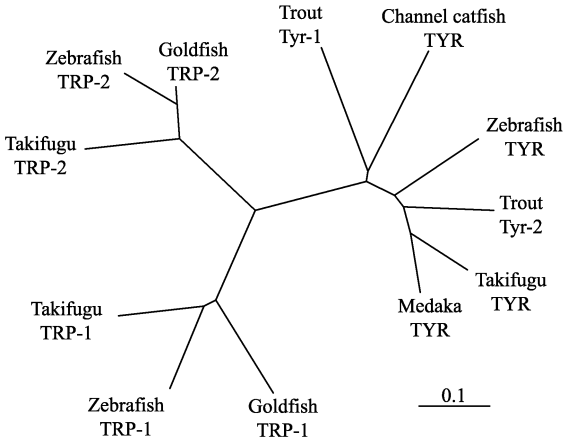

Fig.5 Phylogenetic tree of tyrosinase and tyrosinase-related proteins from several fish species

8 TISSUE-SPECIFICITY OF TYR FAMILY IN NORMAL AND ALBINO FISH

Using cDNA probe, Inagaki et al. (2000) carried out RNA blot analysis in medaka. In wild type medaka, the 2.2 kb TYR mRNA was expressed in the eyes and skin of the fish but not in liver corresponding to tissue-specific melanin formation. Guo et al. (2003), using substrate L-3, 4-dihydroxyphenylalanine (L-DOPA) staining, found that: (1) tyrosinase activity existed in all the skin extracts tested, including pigmented and non-pigmented skins from the ocular or blind side of normal and albino turbot, and the tyrosinase activity of the ocular skin extracts was significantly higher than that of the blind skin extracts from a single individual for both normal and albino turbot. Unexpectedly, the tyrosinase activity in the extracts from albino skin of the ocular side of albino turbot (termed as AOA skin) was about 56% higher than that from pigmented skin of the ocular side of normal turbot (termed as PON skin); (2) histochemical staining showed that tyrosinase activity was present only in the PON skin but not in the AOA skin, and the white skin of the blind side of albino (termed as WBA skin) and normal turbot (termed as WBN skin). A large amount of positive black granules was formed in the epidermal cells of PON skin but no black granules were formed in the skins of AOA, WBA and WBN; (3) temperature and salinity have similar effects on the tyrosinase activity of ocular pigmented and of albino skin extracts, and the optimal temperature was 55–60ºC and optimal salinity 26. However, different pH values had different effects on these tyrosinase activities, and the optimal pH value of ocular pigmented skin extracts was 7.0 and ocular albino skin extracts 8.0; all the 3 activators tested (SDS, trypsin and zymosan) can increase the activity of tyrosinase in both ocular pigmented and albino skin extracts of turbot. But the level of activation on the ocular albino skin extracts of albino turbot was significantly higher than that on ocular pigmented skin extracts of normal turbot. Boonanuntanasarn et al. (2004) cloned tyrosinase-1 (Tyr-1) and tyrosinase-2 (Tyr-2) of trout; Tyr-1 transcript was first detected in embryos at 5 d post-fertilization (dpf) and Tyr-2 transcript at 15 dpf. 3, 4-dihydroxyphenylalanine assays significantly revealed the reduced tyrosinase activities in dominant and recessive albino mutants compared with wild-type embryos. However, RT-PCR showed no difference in amount or length of the coding regions of Tyr-1 and Tyr-2 transcripts between wild-type embryos and albino mutants. The expression of TRP-2 precedes visible melanin formulation in melanophores and retinal pigment epithelium (RPE). In wild-type zebrafish melanocytes, the expression of TRP-2 can be detected at least 5 dpf. The expression of TRP-2 is the early marker of melanocyte differentiation (Kelsh et al., 2000).

9 TRANSGENE AND GENE KNOCKDOWN OF TYR GENE FAMILY IN FISH

Transgenic fish is more and more considered as an excellent system for molecular genetics. In the most vertebrates, redundant intragenic sequences make it quite difficult to study the whole gene and its modulatory region. The compact genomes of fish, especially fugu fish, bring convenience to transgenic research. Camacho-Hubner et al. (2000) transferred TYR gene into mouse pigment cells, and started up the expressions of the report genes in melanocytes and RPE, respectively. In medaka, a genomic clone from wild-type medaka containing the 5 kb tyrosinase gene with its 5 exons, 10 kb of upstream sequences and 2 kb downstream sequences, were introduced into fertilized eggs from a tyrosinase-negative albino strain. The results show that the genomic clone predominantly conferred mosaic expression ending before the hatching stage. The partial adulthood transgenic fishes can mate to

produce offspring, some of which show complete rescue of pigmentation. The resulting transgenic line harbors a single copy of the wild-type tyrosinase gene and all fish are wild-type with respect to pigmentation. These experiments suggest that the TYR gene can be used in fish to routinely detect transgenic line (Fu et al., 1994). Also in medaka, transgenic experiments were carried out by transferring the genomic tyrosinase gene with the 10 kb 5' upstream region and the other tyrosinase cDNA with the 3 kb 5' upstream region into fertilized eggs of the albino i1 mutant; in both cases, transgenic eggs and a mosaic pattern of pigmentation were obtained in fish after hatching (Inagaki et al 1998). However, the exogenous gene introduced in fish genome may alter the specific ecological environments and environment realities. Also, the technique of fish transgene is not yet mature, thus reports related to fish transgene are rare. In some studies, antisense morpholino oligonucleotides (AMOs) designed to knockdown tyrosinase gene expression in wild-type embryos led to reduced pigmentation in the retina and skin of embryos (Boonanuntanasarn et al., 2004; Pickart et al., 2004). This inspires us to think that the AMOs method can be applied in the treatment of hyperpigmentation in fish and other animals.

10 INFLUENCE FACTORS OF EXPRESSION OF TYR GENE

Many factors can influence the synthesis and activity of the TYR gene. For example, Mitf can regulate the expression of TYR, TRP-1 and TRP-2 in melanocytes (Carreira et al., 2000; Goding, 2000; Bejar et al., 2003). Activation of mitogen-activated protein (MAP) kinase can phosphorylate the target serine, and this phosphorylation can upregulate Mitf transactivation of the tyrosinase pigmentation gene promoter (Hemesath et al., 1998). Pink-eyed dilution protein (P protein) can affect the activities of the TYR proteins in teleost melanocyte morphogenesis (Fukamachi et al., 2004). pH values can affect TYR mature and catalysis activity, which influences melanin biosynthesis (Smith et al., 2004). Fatty acids can regulate the degradation of tyrosinase (Ando et al., 2004).

11 PROSPECT OF THE STUDY ON TYR GENE FAMILY IN FISH

The TYR family can affect melanin synthesis in melanocytes, and TYR expression directly reflects the color of fish body. Therefore, TYR family genes can be taken as genotype marker genes in albino fish or body transparent fish, such as medaka fish (Wakamatsu et al., 2001), and zebrafish embryo (Camp et al., 2001). In zebrafish melanophores, TRP-2 is used as early marker gene (Kelsh et al., 2000). Both TYR and TPR-2 can be used as melanocyte marker genes (Ziegler, 2003; Quigley et al., 2004). In the *Xenopus laevis* family genes, TRP-2 is expressed earlier than TYR and TRP-1, which suggests that TRP-2 is the most suitable marker gene for melanin-producing cells among them (Kumasaka et al., 2003). Fish is the most primitive and quantitatively dominant group, and is traditionally considered as the main protein source for humans. The activity of TYR and TYR-related protein closely relate to fish coloration. The body color of abnormal fish cannot meet consumers' demands, which puts a negative influence on the protein sources of humans and economic efficiency of aquaculture. Solving the skin coloration abnormality in fish has important economic significance, and can also provide a theoretical basis for cases in humans and other vertebrates.

References

Ando, H., H. Watabe, J. C. Valencia, K. Yasumoto, M. Furumura, Y. Funasaka, M. Oka, M. Ichihashi and V. J. Hearing, 2004. Fatty acids regulate pigmentation via proteasomal degradation of tyrosinase: a new aspect of ubiquitin-proteasome function. *J. Biol. Chem.* **279**: 15 427-15 433.

Budd, P. S. and I. J. Jackson, 1995. Structure of the mouse tyrosinase-related protein-2/dopachrome tautomerase (Tyrp2/Dct) gene and sequence of two novel slaty alleles. *Genomics* **29**: 35-43.

Bejar, J., Y. Hong and M. Schartl, 2003. Mitf expression is sufficient to direct differentiation of medaka blastula derived stem cells to melanocytes. *Development* **130** (26): 6 545-6 553.

Boonanuntanasarn, S., G. Yoshizaki, K. Iwai and T. Takeushi, 2004. Molecular cloning, gene expression in albino mutants and gene knockdown studies of tyrosinase mRNA in rainbow trout. *Pigment Cell Res.* **17**: 413-421.

Camacho-Hubner, A., C. Richard and F. Beermann, 2002. Genomic structure and evolutionary conservation of the tyrosinase gene family from Fugu. *Gene* **285**: 59-68.

Camacho-Hubner, A., A. Rossier and F. Beermann, 2000. The Fugu rubripes tyrosinase gene promoter targets transgene expression to pigment cells in the mouse. *Genesis* **28**: 99-105.

Carreira, S., B. Liu and C. R. Goding, 2000. The gene encoding the Tbox factor Tbx-2 is a target for the microphtalmia-associated transcription factor in melanocytes. *J. Biol. Chem.* **275**: 21 920-21 927.

Camp, E. and M. Lardelli, 2001. Tyrosinase gene expression in zebrafish embryos. *Dev. Genes Evol.* **211:** 150-153.

Fang, D., Y. Tsuji and V. Setaluri, 2002. Selective down-regulation of tyrosinase family gene TYRP1 by inhibition of the activity of melanocyte transcription factor, MITF. *Nucleic Acids Res.* **30**(14): 3 096-3 106.

Fukamachi, S., M. Sugimoto, H. Mitani and A. Shima, 2004. Somatolactin selectively regulates proliferation and morphogenesis of neural-crest derived pigment cells in medaka. *Proc. Natl. Acad. Sci. USA* **101**: 10 661-10 666.

Fu, L. J., M. Mambrini, E. Perrot and D. Chourrout, 2000. Stable and full rescue of the pigmentation in a medaka albino mutant by transfer of a 17 kb genomic clone containing the medaka tyrosinase gene. *Gene* **241**: 205-211.

Fukamachi, S., .S Asakawa, Y. Wakamatsu, N. Shimizu, H. Mitani and A. Shima, 2004. Conserved Function of Medaka pink-eyed dilution in Melanin Synthesis and Its Divergent Transcriptional Regulation in Gonads Among Vertebrates. *Genetics* **168:** 1 519-1 527.

Guo, H., B. Huang, S. Zhang and F. Qi, 2003. Biochemical and histochemical activities of tyrosinase in the skins of normal and albino turbot *scophthalmus maximus*. *Fish Physiology and Biochemistry* **29**: 67-76.

Goding, C. R., 2000. Mitf from neural crest to melanoma: signal transduction and transcription in the melanocyte lineage. *Genes Dev.* **14**: 1 712-1 728.

Hearing, V. J. and M. Jimenez, 1987. Mammalian tyrosinase-the critical regulatory control point in melanocyte pigmentation. *J. Biochem.* **19**(12): 1 141-1 147.

Hemesath, T. J., E. R. Price, C. Takemoto, T. Badalian and D. E. Fisher, 1998. MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. *Nature* **391**: 298-301.

Inagaki, H., Y. Bessho, A. Koga and H. Hori, 1994. Expression of the tyrosinase gene in a colorless melanophore mutant of the medaka fish, Oryzias latipes. *Gene* **150**: 319-324.

Iida, A., H. Inagaki, M. Suzuki, Y. Wakamatsu, H. Hori and A. Koga, 2004. The tyrosinase gene of the i(b) albino mutant of the medaka fish carries a transposable element insertion in the promoter region. *Pigment Cell Res.* **17**: 158-164.

Iida, A., N. Takamatsu, H. Hori, Y. Wakamatsu, A. Shimada, A. Shima and A. Koga, 2005. Reversion mutation of ib oculocutaneous albinism to wild-type pigmentation in medaka fish. *Pigment Cell Research* **18**: 5 382-5 384.

Inagaki, H., A. Koga, Y. Bessho and H. Hori, 1998. The tyrosinase gene from medakafish: Transgenic expression rescues albino mutation. *Pigment Cell Research* **11**: 283-290.

Kumasaka, M., S. Sato, I. Yajima and H. Yamamoto, 2003. Isolation and Developmental Expression of Tyrosinase Family Genes in Xenopus laevis. *Pigment Cell Research* **16**(5): 455-462.

Kelsh, R. N., B. Schmid and J. S. Eisen, 2000. Genetic analysis of melanophore development in zebrafish embryos. *Dev. Biol.* **225**: 277-293.

Koga, A., M. Suzuki, H. Inagaki, Y. Bessho and H. Hori, 1996. Transposable element in fish. *Nature* **383**: 30-30.

Koga, A. and H. Hori, 1997. Albinism due to transposable element insertion in fish. *Pigment Cell Res.* **10**(6): 377-381.

Koga, A., Y Wakamatsu, J. Kurosawa and H. Hori, 1999. Oculocutaneous albinism in the i6 mutant of the medaka fish is associated with a deletion in the tyrosinase gene. *Pigment Cell Res*. **12**: 252-258.

Link, B. A., M. P. Gray, R. S. Smith and S. W. John, 2004. Intraocular Pressure in Zebrafish: Comparison of Inbred Strains and Identification of a Reduced Melanin Mutant with Raised IOP. *Invest Ophthalmol. Vis. Sci.* **45**: 4 415-4 422.

Libby, R. T., R. S. Smith, O. V. Savinova, A. Zabaleta, J. E. Martin, F. J. Gonzalez and SWM John. 2003. Modification of ocular defects in mouse developmental glaucoma models by tyrosinase. *Science* **299**: 1 578-1 581.

Lister, J. A., 2002. Development of pigment cells in the zebrafish embryo. *Microsc. Res. Tech*. **58**: 435-441.

Naruse, K., S. Fukamachi, H. Mitani and M. Kondo, 2000. A detailed linkage map of Medaka, Oryzias latipes: comparative genomics and genome evolution. *Genetics* **154**: 1 773-1 784.

Page-McCaw, P. S., S. C. Chung, A. Muto, T. Roeser, W. Staub, K. C. Finger-Baier, J. I. Korenbrot and H. Baier, 2004. Retinal network adaptation to bright light requires tyrosinase. *Nat. Neurosci***. 7**(12): 1 329-1 336.

Protas, M. E., C. Hersey, D. Kochanek, Y. Zhou, H. Wilkens, W. Jeffery, LI Zon, R. Borowsky and C. J. Tabin, 2006. Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nature Genetics* **38**: 107-111.

Pickart, M. A., S. Sivasubbu, A. L. Nielsen, S. Shriram, R. A. King and S. C. Ekker, 2004. Functional Genomics Tools

for the Analysis of Zebrafish Pigment. *Pigment Cell Research* **17**(5): 461-470.

Quigley, I. K., J M. Turner, R. J. Nuckels, J. L. Manuel, E. H. Budi, E. L. MacDonald, D. M. Parichy, 2004. Pigment pattern evolution by differential deployment of neural crest and post-embryonic melanophore lineages in Danio fishes. *Development* **131**: 6 053-6 069.

Savinova, O. V., F. Sugiyama, J. E. Martin, S. I. Tomarev, B. J. Paigen and R. S. Smith, 2001. Intraocular pressure in genetically distinct mice: an update and strain survey. *BMC Genet* 2: 12.

Smith D. R., D. T. Spaulding, H. M. Glenn, B. B. Fuller, 2004. The relationship between Na^+/H^+ exchanger expression and tyrosinase activity in human melanocytes. *Exp. Cell Res.* **298**: 521-534.

Tsutsumi, M., S. Imai, Y. Kyono-Hamaguchi, S. Hamaguchi, A.Koga and H. Hori, 2006. Color reversion of the albino medaka fish associated with spontaneous somatic excision of the Tol-1 transposable element from the tyrosinase gene. *Pigment Cell Research* **19**(3): 245-247.

Toyodaa, R., A. Kasaia, S. Satob, S. Wadac, H. Saigac, K. Ikeod, T. Gojoborid, T.Numakunaie and H. Yamamotoa, 2004. Pigment cell lineage-specific expression activity of the ascidian tyrosinase-related gene. *Gene* **332**: 61-69.

Vetrini, F., A. Auricchio, J. Du, B. Angeletti, D. E. Fisher, A. Ballabio and V. Marigo, 2004. The Microphthalmia Transcription Factor (Mitf) Controls Expression of the Ocular Albinism Type 1 Gene: Link between Melanin Synthesis and Melanosome Biogenesis. *Molecular and CellularBbiology* **24**(15): 6 550-6 559.

Wakamatsu, Y., S. Pristyazhnyuk, M. Kinoshita, M. Tanaka and K. Ozato, 2001. The see-through medaka: A fish model that is transparent throughout life. *PNAS.* **98**(18): 10 046-10 050.

Ziegler, I., 2003. The pteridine pathway in zebrafish: regulation and specification during the determination of neural crest cell-fate. *Pigment Cell Res.* **16**: 172-182.