

# Pigment Genes and Cancer Genes

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## Introduction

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## Glossary

**Gene knockdown** Inhibition of gene expression by the presence of a specific blocking agent such as antisense morpholinos.

**Malignant tumor** Severe tumor type that is able to metastasize.

**Metastasis** Spread of a tumor from the primary place of origin to another part of the body.

**Mutagenesis screen** Forward genetic method to find novel genes of interest by introducing gene mutations into the genome of a model organism.

**Oncogene** Gene driving tumor formation when mutated or expressed at abnormal levels.

**Promoter** The upstream sequence of DNA which regulates the expression of the downstream coding

sequence of the gene. The promoter may sometimes be as big as or bigger than the gene itself. A so-called 'minimal promoter' is the length of the promoter required to achieve expression of the gene, but without additional positive or negative elements.

**Receptor tyrosine kinase** Cell-surface receptor that activates intracellular signaling cascades in response to binding of specific extracellular ligand molecules.

**Transcription factor** DNA-binding protein that influences the expression of genes by binding to their promoter region.

**Tumor modifier** Genetic factor that influences the characteristics of a specific tumor type.

**Tumor suppressor** Gene that prevents tumor formation.

## Introduction

Pigmentation and color patterning of body and fins serve multiple functions in fish, including camouflage, communication, mate choice, or species recognition. Fish pigmentation is highly polymorphic and often sex-specific, particularly during the spawning season. Furthermore, many fish species change their pigmentation during their life cycle. Prominent examples for colorful fishes are coral reef fishes, poeciliids from Middle and South America (guppy, *Poecilia reticulata*; platyfish, *Xiphophorus maculatus*; swordtail, *Xiphophorus hellerii*) or the cichlids of the East African Great Lakes. (see also **Vision**: Color vision and Color Communication in Reef Fish)

Coloration of teleost fishes is based on six different major types of pigment cells. Other fish lineages and tetrapods, in contrast, have a smaller repertoire of such chromatophores. Studying the genetic basis of fish coloration has a long tradition, particularly in East Asia, where color morphs of carp and goldfish have been cultured for more than 1500 years. In the beginning of the last century, poeciliids became popular among geneticists to study the inheritance of color patterns and also of pigment cell-derived tumors.

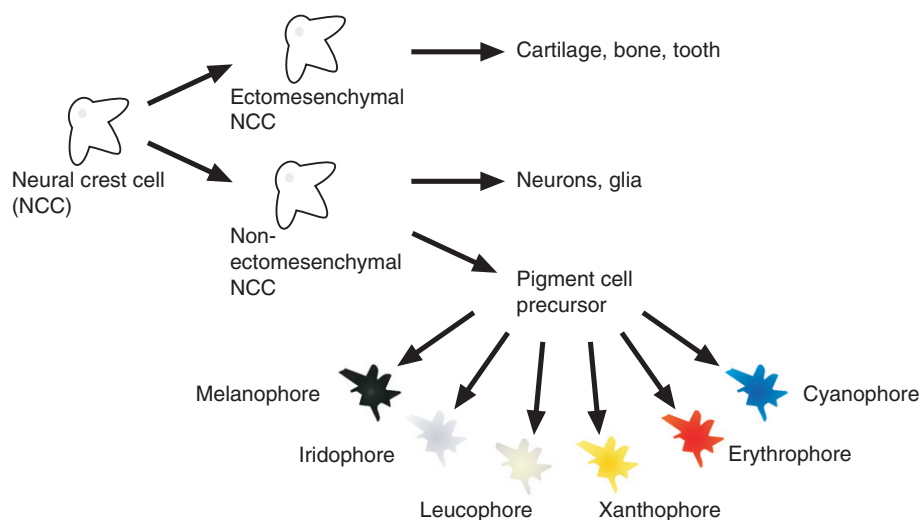
The present article gives an overview of the types of pigment cells found in fish, the genetic control of their development and differentiation, and the genes that underlie color polymorphisms between natural fish populations. Subsequently, we discuss the longstanding interest in fish as model systems for the study of pigment cell-derived types of skin cancer.

## Pigment Cells in Fish

### Development of Pigment Cells

There are two major types of pigmented cells in the vertebrate body. The black cells of the retinal pigment epithelium, the dark cell layer in the eye, are of neuroectodermal origin. In contrast, the pigment cells of the skin (chromatophores), which are the focus of the present article, derive from the neural crest.

The neural crest is a transient cell population during early embryonic development that arises in the dorsal ectoderm between the neuroectoderm and the prospective epidermis. From this layer, neural crest cells migrate along different routes throughout the embryo to their



**Figure 1** Specification of pigment cells from the neural crest.

final destinations. Neural crest cells give rise to around 50 different cell types including cartilage, neurons, and chromatophores. They are all initially multipotent, but they become gradually restricted to a particular cell fate during development. A first distinction can be made between ectomesenchymal and non-ectomesenchymal neural crest cells. The former give rise to cartilage, bone, and tooth tissues, while the latter develop into neurons, glia, and pigment cells. All pigment cell types develop from a common precursor cell (Figure 1).

### Pigment Cell Types in Fish

In teleost fishes, six major pigment cell types can be recognized, each displaying characteristic pigments in specialized pigmentary organelles (Figure 2).

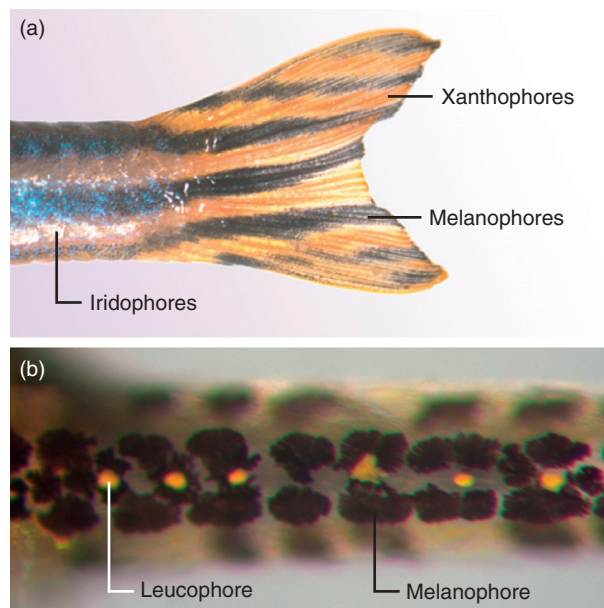
Melanophores synthesize the black to brown eumelanin in their melanosomes (Figure 2). In contrast to mammals and birds, teleosts do not synthesize the lighter pheomelanin.

The metallic-reflective iridophores store crystalline purines, particularly guanine, in so-called reflecting platelets (Figure 2(a)). The creamy-whitish leucophores also contain purines in their leucosomes (Figure 2(b)). Irido- and leucophores are closely related and generate structural colors by the reflection of light.

Yellow to red chromatophores are categorized according to their overall color either as xanthophores (yellow; Figure 2(a)) or as erythrophores (red). Both pigment cell types synthesize yellow to red pteridine pigments in their pigmentary organelles (pterinosomes), which are called xanthosomes or erythrosomes, respectively. In addition, xantho- and erythrophores may store yellow to red carotenoid pigments obtained from the food in carotenoid vesicles.

The blue cyanophores have so far only been found in two species of the family Callionymidae (dragonets), the mandarinfish *Synchiropus splendidus* and the picturesque dragonet *S. picturatus*. The pigmentary organelles of these cells, termed cyanosomes, contain a true blue pigment. In other fishes, blue is usually displayed as a structural color generated by iridophores.

The distribution of chromatophores, often combining stacked layers of different pigment cell types, generates



**Figure 2** Teleost pigment cell types. (a) Tail and tailfin of an adult zebrafish showing the characteristic stripe pattern based on dark melanophores, yellow xanthophores and reflective iridophores. (b) Dorsal view on the trunk of a juvenile medaka. Cream-colored leucophores are surrounded by dark melanophores.

the endless diversity of stripes, spots, and patches of all types of colors in teleosts.

To the best of our knowledge, melanophores and iridophores are the only pigment cell types that have been described so far for agnathans (lamprey and hagfish), cartilaginous fishes (sharks and rays) and lobe-fin fishes (coelacanth and lungfish). (see also **The Skin: Coloration and Chromatophores in Fishes**).

## Genetic Control of Pigment Cell Development

The identification of pigment genes, that is, genes that are involved in pigment cell development, pattern formation, pigment synthesis, or color change, has been spurred by the systematic analysis of pigment mutant collections of zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). An important resource for naturally occurring medaka pigmentation mutants is the Tomita collection. For zebrafish, a large collection of pigmentation mutants has been isolated from different large-scale mutagenesis screens. **Table 1** and **Figure 3** give some examples of pigmentation mutants for which the affected pigment gene has been found. In addition, some pigment genes have been functionally investigated by gene knockdown approaches. Several of the major pigment genes in fish will be introduced in the following.

## Specification of Pigment Cells

The specification of the non-ectomesenchymal neural crest is regulated by the transcription factor Sox10. In the zebrafish *colorless* mutant, which is defective in the *sox10* gene, all types of pigment cells are severely reduced and defects in other non-ectomesenchymal derivatives are also observed.

All chromatophore types develop from a common precursor cell, but their relationships and the progression of fate restrictions to a particular type of chromatophore remain poorly understood. The analysis of zebrafish pigmentation mutants, however, has revealed that during development each type of chromatophore requires the expression of a characteristic transcription factor as well as of a transmembrane receptor from the family of receptor tyrosine kinases.

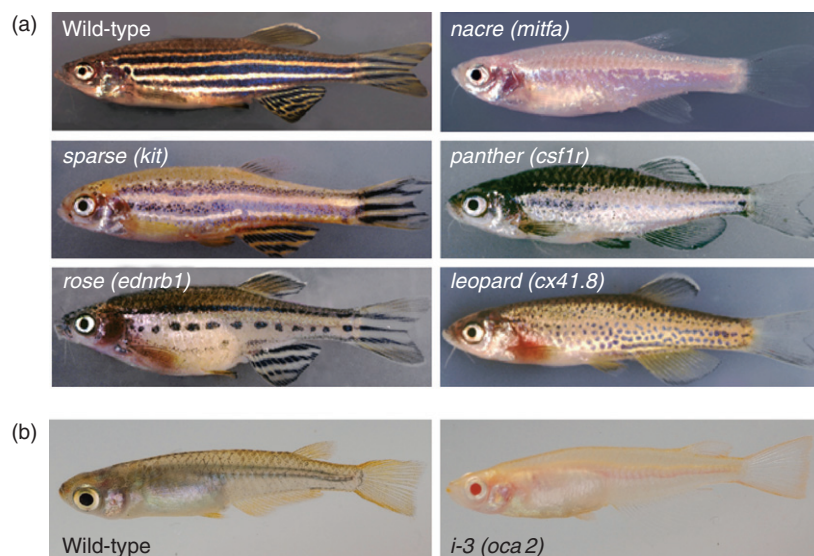
## Melanophores

The zebrafish mutant *nacre* lacks almost all melanophores (**Figure 3(a)**). These fish are mutated in the *mitfa* gene. The Mitf transcription factor is the master regulator of melanophore development in vertebrates, and expression of *mitfa* is sufficient to specify precursor cells for the melanophore fate. Mitf binds to the promoters of melanin synthesis enzyme genes and upregulates their expression. The expression of *mitfa* itself is regulated by Sox10. Because of its specific activity, DNA constructs

**Table 1** Examples of teleost pigment genes

Developmental/cellular function	Gene	Teleost mutant <sup>a</sup>	Molecular function
Neural crest specification	<i>sox10</i>	<i>colorless</i> (Dre)	Transcription factor
Melanophore specification	<i>mitfa</i>	<i>nacre</i> (Dre)	Transcription factor
Melanophore migration/survival	<i>kita</i>	<i>sparse</i> (Dre)	Receptor tyrosine kinase
Melanophore development	<i>tfap2a</i>	<i>lockjaw</i> (Dre)	Transcription factor
	<i>hdac1</i>	<i>colgate</i> (Dre)	Histone deacetylase
	<i>trpm7</i>	<i>touchtone</i> (Dre)	Cation channel
	<i>erbb3b</i>	<i>picasso</i> (Dre)	Receptor tyrosine kinase
Xanthophore specification	<i>pax3a</i>	KD (Dre)	Transcription factor
Xanthophore migration/survival	<i>csf1ra</i>	<i>panther</i> (Dre)	Receptor tyrosine kinase
Iridophore specification	<i>foxd3</i>	<i>mother superior</i> (Dre)	Transcription factor
Iridophore migration/survival	<i>ltk</i>	<i>shady</i> (Dre)	Receptor tyrosine kinase
Pattern formation	<i>cx41.8</i>	<i>leopard</i> (Dre)	Gap junction protien
	<i>kcnj13</i>	<i>obelix/jagular</i> (Dre)	Potassium channel
	<i>fbxw4</i>	<i>hagoromo</i> (Dre)	Protein degradation
Melanosome dispersal	<i>mc1r</i>	KD (Dre)	Melanocortin receptor
	<i>mlpha</i>	<i>j120</i> (Dre)	Protein transport
Melanin synthesis	<i>tyra</i>	<i>sandy</i> (Dre), <i>i</i> (Ola)	Enzyme
	<i>tyrp1a/tyrp1b</i>	KD (Dre)	Enzyme
	<i>silva</i>	<i>fading vision</i> (Dre)	Structural protien/enzyme
	<i>oca2</i>	<i>i-3</i> (Ola)	Melanosomal transporter
	<i>slc45a2</i>	<i>b</i> (Ola)	Melanosomal transporter
	<i>slc24a5</i>	<i>golden</i> (Dre)	Melanosomal transporter
Pteridine synthesis regulation	<i>mycbp2</i>	<i>esrom</i> (Dre)	?

<sup>a</sup>Names of mutant lines from zebrafish (Dre) or medaka (Ola). Some genes have been studied by gene knockdown (KD).



**Figure 3** Examples of teleost pigmentation mutants. (a) Zebrafish mutants. Name and affected gene (in brackets) are given. (b) Wild-type medaka compared to the albino mutant *i-3*. (a) Adapted from figure 5 in Parichy DM (2006) Evolution of danio pigment pattern development. *Heredity* 92: 200–210.

containing the *mitfa* promoter can be used to artificially drive the expression of other genes into melanophores of transgenic fish. Such *mitfa* promoter constructs are essential tools for pigment cell and skin cancer research in fish (see below).

In teleosts, a second *mitf* gene, *mitfb*, regulates the expression of melanin synthesis enzymes in retinal pigment epithelium cells. The two *mitf* duplicates were generated in a teleost-specific whole genome duplication (see also Cellular, Molecular, Genomics, and Biomedical Approaches: Evolution of Fish Genomes). Many pigment genes are present in two copies in teleosts due to this event (recognizable by the suffix ‘a’ or ‘b’ after the gene name).

The receptor tyrosine kinase Kita is required for the migration and survival of melanophores. The *sparse* mutant lacks Kita function and is characterized by loss of embryonic as well as early stripe melanophores. Late stripe melanophores, in contrast, are present in *sparse* mutants (Figure 3(a)). The Kita receptor is activated and sends intracellular signals upon binding to Kit ligand a (*Kitla*). Knockdown of *kitla* in zebrafish decreases and *kitla* overexpression increases the number of melanophores.

### Xanthophores

The specification of xanthophores in zebrafish requires the function of the Pax3 transcription factor. Pax3 is known to be important for melanocyte specification in mammals. Knockdown of *pax3a* genes in zebrafish, however, leads to a decrease of xanthophores, while melanophores increase in number. Pax3 presumably regulates

the expression of genes required for xanthophore differentiation like the pteridine synthesis enzymes.

Very similar to the role of Kita for melanophores, the related receptor tyrosine kinase *Csf1ra* is required for xanthophore migration and survival. The corresponding *panther* mutant is devoid of most xanthophores and this absence of xanthophores seems to be responsible for the loss of the adult melanophore stripe pattern (Figure 3(a)).

### Iridophores and Leucophores

The presence of the Foxd3 transcription factor is essential for the differentiation of iridophores. Its function appears to be the downregulation of *mitfa* expression in those precursor cells that will give rise to iridophores.

Iridophores also need a specific receptor tyrosine kinase for their development. The *shady* mutant, which shows reduced numbers of iridophores, was shown to be defective in the *leucocyte tyrosine kinase* (*ltk*) gene.

Although there are leucophore mutants in medaka (e.g., *leucophore free*), so far no genes has been identified that is involved in leucophore development.

### Color Pattern Formation

Studies on color pattern formation have been focused mostly on the adult stripe pattern in zebrafish (Figure 1(a)). Mathematical models predict a reaction–diffusion mechanism generating this pattern and developmental studies have shown that interactions among melanophores and xanthophores are essential for stripe formation.



In the *leopard* mutant (Figure 3(a)), the adult stripe pattern is disrupted into a series of spots. It is mutated in the gap junction gene *connexin 41.8* (*cx41.8*). Gap junctions mediate tight cell–cell contacts, which is in line with the cell–cell interaction theory of stripe pattern formation.

Several genes are involved in the development of more than one type of pigment cell. One example is the *ednrb1a* gene, which is expressed in melano-, irido-, and xanthophores. Loss of this gene leads to defects in stripe patterning in the zebrafish *rose* mutant (Figure 3(a)). The loss of the growth hormone somatolactin in the medaka mutant *color interfere* results in an increase in leucophores and a simultaneous decrease in xanthophores. Many more genes involved in chromatophore development have been identified (some further examples are given in Table 1), but their discussion is beyond the scope of this article.

## Pigment Synthesis Genes

Many teleost pigmentation mutants are characterized by lighter pigmentation (hypopigmentation) of chromatophores because of defects in pigment synthesis. Some of the responsible genes have been identified.

### Melanin Synthesis

In melanophores and the retinal pigment epithelium, black eumelanin is synthesized from tyrosine by enzymes of the tyrosinase family: tyrosinase (Tyr), dopachrome

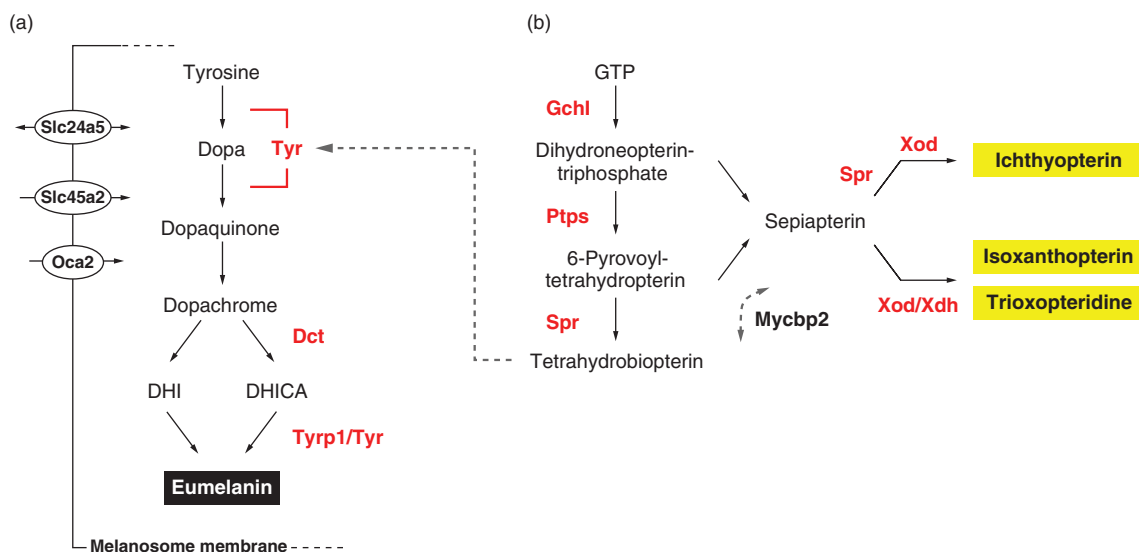
tautomerase (Dct), and tyrosinase-related protein 1 (Typr1) (Figure 4 (a)). Loss of eumelanin formation leads to different types of albinism. Mutant lines of the *i* (medaka) and *sandy* (zebrafish) type are complete albinos devoid of melanin pigments. Both mutants have a defective *tyra* gene. Knockdown of *tyrp1* genes in the zebrafish leads to a brownish type of albinism.

Several transporter proteins that reside in the melanosome membrane (Figure 4(a)) also have an influence on melanin synthesis, although their exact roles for melanin synthesis remain unclear. The albinistic medaka mutants *i-3* (Figure 3(b)) and *b* have defects in the *oca2* and *slc45a2* (also known as *aim1*) gene, respectively. The zebrafish *golden* mutant possesses hypopigmented melanophores. It was found to be affected in the *slc24a5* transporter gene.

### Pteridine Synthesis

The synthesis of yellow to red pteridine pigments is highly complex and less well understood than melanin synthesis. The pteridine synthesis pathway has different branches, one of them leading to the formation of sepiapterin as a precursor for different types of pteridine pigments. Another branch leads to the formation of tetrahydrobiopterin (Figure 4(b)). Tetrahydrobiopterin is an important cofactor of the tyrosinase enzyme in melanophores, which directly links melanin and pteridine synthesis.

Although several mutants with altered pteridine pigments have been found in zebrafish, only one of them has been cloned so far. A mutation in the *mycbp2* gene in the



**Figure 4** Pigment synthesis pathways. (a) Black eumelanin is synthesized from tyrosine by enzymes of the tyrosinase family. Tyr, tyrosinase; Dct, dopachrome tautomerase; Typr1, tyrosinase-related protein. Three melanosomal transporters (Slc24a5, Slc45a2, and Oca2) also play essential roles for melanin synthesis. (b) The pteridine synthesis pathway has two major branches: *de novo* synthesis of tetrahydrobiopterin (top to bottom) and formation of pteridine pigments via sepiapterin in xanthosomes (left to right). The switch between pathways is regulated by Mycbp2. In melanophores, Tetrahydrobiopterin is a cofactor of Tyr. Gchl, GTP cyclohydrolase I; Ptps, 6-pyruvoyl tetrahydropterin synthase; Spr, sepiapterin reductase; Xod/Xdh, xanthine oxidase/dehydrogenase. Note that some intermediate pathway steps are not shown.

*esrom* mutant results in the reduction of yellow pigments. *Mycbp2* may regulate the switching between different branches of pteridine synthesis (Figure 4(b)).

## Color Change

Teleosts are famous for their ability to change colors (see also **Sensory Systems, Perception, and Learning: Communication Behavior: Visual Signals**). Morphological color changes are based on slow changes of pigment amounts or the distribution of chromatophores. Physiological color changes, in contrast, are accomplished by rapid movements of pigmentary organelles within chromatophores. In response to the endocrine and nervous systems, pigmentary organelles are moved along microtubules to the pigment cell center (lightening) or the periphery (darkening) (see also **The Skin: Coloration and Chromatophores in Fishes**).

A large body of literature exists on the regulatory role of hormones such as the melanophore-stimulating hormone (MSH) and the melanin-concentrating hormone (MCH) for physiological color change. Knockdown of the melanocortin-1-receptor (*Mcl1r*), the receptor of MSH, impairs the dispersal of melanosomes. Melanosome transport mutants have been isolated in zebrafish. One of them has a defect in the *melanophilin a (mlpha)* gene, which encodes a structural protein coordinating the movement of melanosomes along microtubules.

## Pigment Polymorphisms in the Wild

Geneticists, ecologists, and evolutionary biologists have extensively studied color differences among fish populations and species in the wild. For example, classic genetic studies have identified numerous pigmentation loci responsible for color differences among guppy and platyfish populations, many of them residing on the sex chromosomes. There are, however, only few studies in which the particular gene underlying a naturally occurring color polymorphism in fish has been identified.

### Blind Cavefish

The Mexican tetra (*Astyanax mexicanus*) is a dramatic example for naturally occurring pigmentation divergence. While surface populations have a silvery-grayish appearance, blind cave populations independently lost the ability for proper melanin synthesis. It was shown that some of these albino forms of *A. mexicanus* are caused by loss-of-function mutation in the *oca2* gene. Brownish cave populations, in contrast, show changes in the melanocortin 1 receptor (*Mcl1r*) compared to the surface populations. Modulation of *Mcl1r* function is well known

for being responsible for pigmentation polymorphisms and melanism in mammals, birds, and reptiles (see also **Bony Fishes: Blind Cavefish**).

### Danio Species

Parichy and colleagues used an elegant method to pinpoint the developmental pathways responsible for stripe pattern differences between zebrafish and other species of the genus *Danio*. Several *Danio* species were crossed with pigmentation mutants of the zebrafish. These studies showed that stripe pattern differences between zebrafish and the stripe-less pearl danio (*D. albolineatus*) are due to modulations of the aforementioned *Csf1r* and *Kit* receptor tyrosine kinase signaling pathways.

### Stickleback

The three-spined stickleback (*Gasterosteus aculeatus*) is well known for its pigmentary diversity around the globe. A recent study determined the gene responsible for differences in melanophore distribution between freshwater populations with light gills and bellies and ancestral marine populations with dark gills and bellies. These differences in melanophore patterning were traced back to differences in the expression of the *kit ligand a (kitla)* gene causing changes in melanophore distribution.

## Pigment Cell Tumors

Under abnormal conditions, the broad spectrum of pigment cell types in fish can give rise to different types of skin tumors. Teleosts are therefore suitable models for the study of skin cancers, such as malignant melanoma, which is one of the most aggressive cancers with high rates of metastasis formation in humans. Naturally occurring spontaneous tumor growth from pigment cells in fish has been studied since the 1920s. Scientific reports on abnormalities in the skin of fish include erythrophoroma, melanoma, and iridophoroma, but only the first two types have been studied in more detail.

### Goldfish Erythrophoroma

Large malformations in the skin of the goldfish (*Carassius auratus*) that are caused by uncontrolled growth of red pigment cells have been described. Further studies identified these lesions as erythrophoroma, that is, tumors arising from erythrophores. In subsequent studies, erythrophoroma cells were cultured and the erythrophoroma cell lines have since then been used for investigations of general pigment cell and tumor properties. However, the genetic alterations that may have caused the erythrophoroma formation in goldfish remain elusive.

## *Xiphophorus* Melanoma

An extensively studied example of fish skin tumors is the appearance of black tumor lesions (melanoma) in hybrid species of the genus *Xiphophorus*. By crossing, for example, platyfish (*X. maculatus*) and swordtail (*X. hellerii*), hybrids can be generated that have spots with high numbers of melanophores (hyperpigmentation) and also invasive melanoma in the following generations.

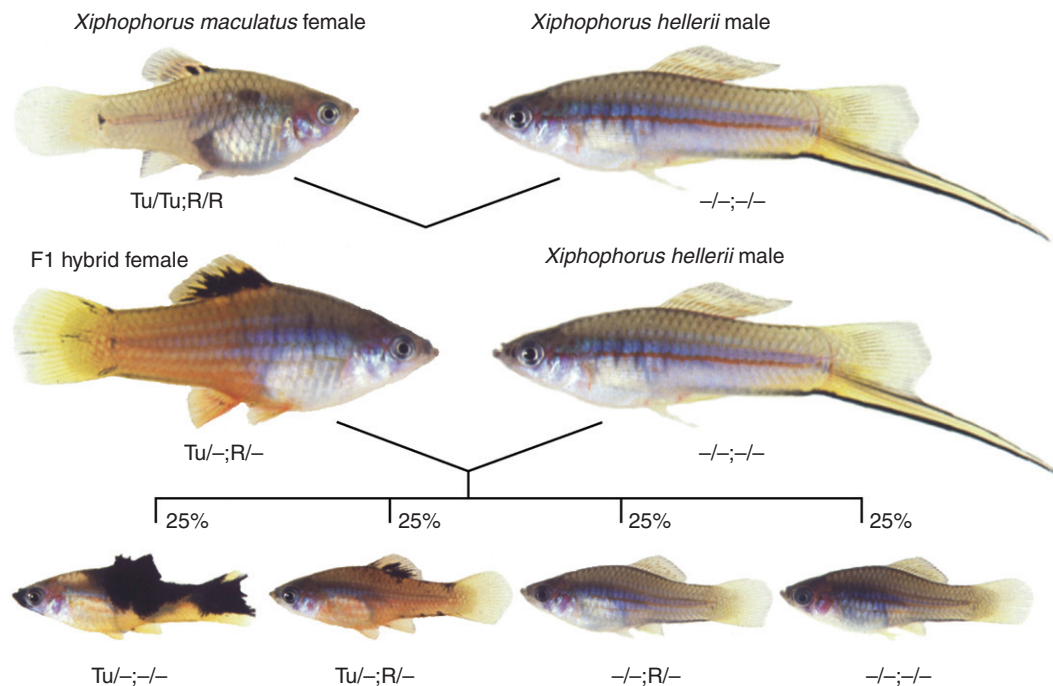
The spontaneous tumor formation in *Xiphophorus* hybrids can be explained by the interplay of a tumor locus (*Tu*) that is under the control of a repressor locus (*R*). Different chromosomal locations of *Tu* and *R* enable the stepwise separation of these two loci by repeated crosses to swordtail.

As shown in the crossing scheme in **Figure 5**, the platyfish genome contains, as well as the *Tu* locus, also the *R* locus, which represses the development of melanoma. After crossing a platyfish to a swordtail, which possesses neither *Tu* nor *R*, hybrids are born which have one *Tu*- and one *R*-bearing chromosome. This first hybrid generation is characterized by hyperpigmentation. A second crossing of these hybrids to swordtail results in four different genotypes. Half of the individuals from this generation will not show a pigmentary phenotype, because their genome is free of the *Tu* locus. One-quarter of fish will be strongly hyperpigmented due to the presence of one *Tu* and one

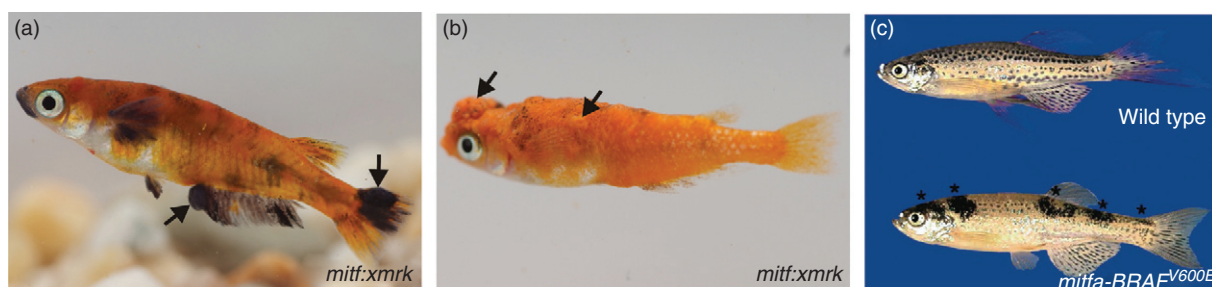
*R* locus allele. One-quarter of individuals will develop malignant melanoma due to the presence of the *Tu* locus and the absence of an *R* locus in their genome.

Positional cloning of the *Tu* locus identified the oncogene *xmrk* (*Xiphophorus* melanoma receptor kinase) as the genetic factor driving the transformation of a melanophore into a malignant melanoma cell. *Xmrk* is an epidermal growth factor receptor (Egfr) that belongs to the group of receptor tyrosine kinases. The *xmrk* gene originated by a local gene duplication of the *egfrb* gene, which is located like *xmrk* on the platyfish sex chromosome.

The *xmrk* gene is highly expressed in melanoma tissues. Two mutations keep the *Xmrk* receptor in a constantly active form, thereby allowing ligand-independent signaling. *Xmrk* turns on different signaling pathways such as the phosphatidylinositol 3-kinase, the Ras–Raf–Mapk, or the Fyn/focal adhesion kinase pathways. These downstream signaling cascades increase cell proliferation, cell survival, and cell motility of the transformed pigment cell. Such changes in cell characteristics as well as activation of the same signaling pathways are also observed in human melanoma. For example, aberrant signaling through the Raf–Ras–Mapk pathway is well described for human melanomas, and mutated receptor tyrosine kinases are known to be involved in human cancer formation and progression.



**Figure 5** Crossing scheme of spontaneous melanoma formation in *Xiphophorus* (Gordon–Kosswig–Anders cross). Mating of a *X. maculatus* female (carrying *Tu* and the repressor *R*) to a *X. hellerii* male (lacking *Tu* and *R*) gives rise to a hyperpigmented hybrid generation (heterozygous for *Tu* and *R*). Further crossing of these hybrids to a *X. hellerii* male results in (from right to left) 50% fish with normal pigmentation (lacking *Tu*), 25% hyperpigmented fish (one copy of *Tu* and *R*), and 25% fish that develop malignant melanoma (carrying *Tu* but lacking *R*). Reproduced from **figure 1** in Meierjohann S and Schartl M (2006) From Mendelian to molecular genetics: The *Xiphophorus* melanoma model. *Trends in Genetics* 22: 654–661.



**Figure 6** Different skin tumor phenotypes in transgenic medaka and zebrafish. Transgenic Medaka expressing *xmrk* in pigment cells under the control of the *mitfa* promoter develop (a) black melanoma and (b) red xantho-/erythrophoroma (arrows). (c) Zebrafish of the *leopard* strain expressing human *BRAF*<sup>V600E</sup> in pigment cells (bottom) show growth of nevi (asterisks). Reproduced from figure 1B in Patton EE, Widlund HR, Kutok JL, et al. (2005) BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Current Biology* 15: 249–254.

The *Xiphoborus* crossings indicate that besides the presence of *Tu*, the absence of the tumor suppressor *R* is necessary for tumor formation (Figure 5). In the platyfish, *R* counteracts *xmrk* and impedes the formation of tumors. The corresponding gene has not been identified yet, but based on genetic studies, one major candidate gene has been identified, *cyclin-dependent kinase inhibitor 2 (cdkn2)*, which maps to the chromosomal region of the *R* locus. *Cdkn2* proteins act as cell-cycle and cell-proliferation regulators and have been intensively studied in human melanoma. *CDKN2* genes are often mutated in human melanomas. Conclusive evidence that *cdkn2* in *Xiphoborus* is indeed the *R* locus-encoded gene, however, is still elusive.

### Transgenic Skin Tumor Models

Besides the appearance of natural spontaneous pigment cell tumors, several new approaches have been developed to study melanoma formation in fish. Recently, progress in molecular biology opened up the possibility to design genetic models of skin tumors in laboratory fish species such as zebrafish and medaka (see also Cellular, Molecular, Genomics, and Biomedical Approaches: Fish as Model Organisms for Medical Research). These skin tumor models show melanoma formation after chemical treatment, radiation, or the expression of an oncogene under a pigment cell-specific promoter.

One example for such a transgenic tumor model is the *mitf:xmrk* medaka, in which the *xmrk* gene from *Xiphoborus* is artificially expressed in medaka pigment cells under the control of the *mitfa* promoter. The transgenic fish develop, after a relatively short time, red xantho-/erythrophoroma and black melanoma (Figure 6). Both tumor cell types show specific characteristics of their own, such as different gene expression profiles, different tumor growth properties, and differences in invasiveness. Interestingly, appearance of the different tumor types depends on the genetic background of the fish, indicating the presence of different tumor modifiers genes.

Another example of newly established melanoma models is the *BRAF*<sup>V600E</sup> zebrafish. *Braf* is a protein kinase of the Ras–Raf–Mapk pathway. In humans, mutations in this gene, for example, the common *BRAF*<sup>V600E</sup> mutation, are associated with various cancers including malignant melanoma. In the transgenic zebrafish, the expression of the human *BRAF*<sup>V600E</sup> gene under the control of the *mitfa* promoter drives the appearance of nevi (Figure 6(c)). Nevi, commonly called moles, are nonmalignant proliferations of melanophores. Further depletion of the tumor suppressor gene *p53* is necessary for the *BRAF*<sup>V600E</sup> zebrafish to develop malignant melanoma. In a second zebrafish model, artificial expression of oncogenic human *RAS* in melanophores is sufficient to induce the formation of malignant melanoma.

Besides the transgenic models described here, other known melanoma oncogenes remain largely uninvestigated in fish. However, there are many promising candidate genes from human melanoma research including some of the pigment genes discussed in the previous sections (e.g., *mitf* and *kit*). Currently, fish models for other known oncogenes or other types of cancer are generated and make fish a valuable model system for cancer research.

See also: **Bony Fishes: Blind Cavefish. Cellular, Molecular, Genomics, and Biomedical Approaches: Evolution of Fish Genomes; Fish as Model Organisms for Medical Research. Sensory Systems, Perception, and Learning: Communication Behavior: Visual Signals. The Skin: Coloration and Chromatophores in Fishes.**

### Further Reading

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## Relevant Websites

- <http://www.espcr.org> – European Society for Pigment Cell Research (ESPCR): Color Genes.
- <http://www.shigen.nig.ac.jp/medaka> – Shared Information on GENetic Resources (SHIGEN): NBRP Medaka.
- <http://zfin.org> – The Zebrafish Model Organism Database (ZFIN).