Genes and Cancer: Molecular Biology of the Melanoma Oncogene of Xiphophorus

Angelika Schartl and
Manfred Schartl,
Martinsried, FRG

Much of the progress in our understanding of the molecular origins of cancer stems from studies on specific genes, the oncogenes, which appear to be responsible for specifying many of the malignant features of tumor cells. Dominant acting oncogenes arise in large part by an activation process from pre-existing proto-oncogenes, which themselves appear to play essential roles in normal cellular physiology, and are often involved with the regulation of normal cell proliferation and differentiation. Recessive oncogenes contribute to the neoplastic phenotype of a cell due to inactivation of both normal alleles, which in their turn are thought to be critically involved in negative control of cell growth, therefore also designated tumor suppressor genes (see 1). Although the number of known oncogenes increases steadily, and structural and functional analyses have proceeded to extremely high levels, generally the final proof that these genes are causally involved in the process of tumor formation is extremely difficult – if at all – to obtain. The situation is much better in those cases in which genetically defined loci have been found to be responsible for tumor induction. For the human hereditary retinoblastoma and for the Drosophila *lethal* (2) *giant larvae* tumor the causative recessive alleles have been identified and characterized (2, 3, 4, 5). In addition, gene losses of possible tumor suppressor loci have been observed in a variety of human tumors (see 6, for recent compilation and discussion) pointing also to causative recessive oncogenes. For dominant acting oncogenes the melanoma system of the teleost fish Xiphophorus offers a unique experimental situation where – dissimilar to other vertebrate systems – the loci responsible for tumor formation have been clearly defined by Mendelian genetics.

Genetics of melanoma formation in Xiphophorus

In Xiphophorus, some individuals exhibit spot patterns, composed of large, intensily black pigment cells. These cells have been termed macromelanophores while the normal sized black pigment cells that make up the uniform greyish body coloration have been designated micromelanophores (7). Already more than 60 years ago it was discovered that certain hybrids of the macromelanophore pattern carrying platyfish (*Xiphophorus maculatus*) and of the unspotted swordtail (*X. helleri*) develop spontaneously malignant melanoma (7, 8, 9). Shortly thereafter it was recognized that occurence of tumors in hybrids is due to a single locus (the macromelanophore locus) of *X. maculatus* that "interacted" with the *X. helleri* genome (10, 11). This interaction was later on defined as the presence of intensifying genes and/or the absence of repressing genes in the hybrid genome, which act specifically on the macromelanophore locus (12, 13, 14, 15, 16).

In a typical crossing experiment (see Fig. 1) a female *X. maculatus* which carries the X-chromosomal macromelanophore

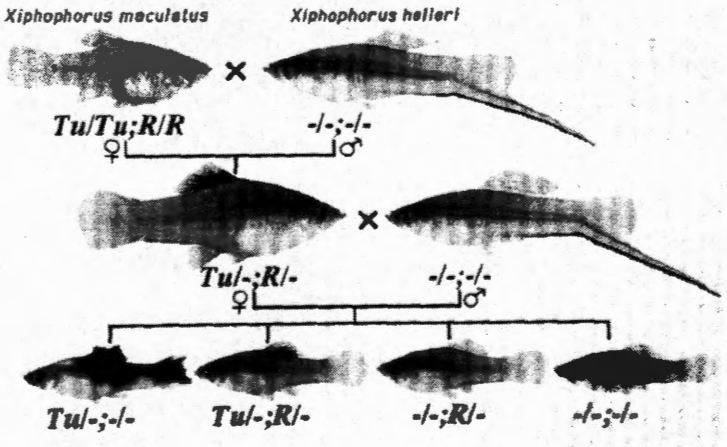

Figure 1: Classical crossing experiment leading to hybrids that develop malignant melanoma. For details see text. *Tu*, tumor gene, *R*, tumor suppressor gene. Note that the parental platyfish (*Xiphophorus maculatus*) carries two pigment spots in the dorsal fin that develop into melanoma in the hybrids.

locus *Sd* (Spotted dorsal, small spots in the dorsal fin) is mated to *X. helleri*, which does not carry the corresponding locus and exhibits the uniform wildtype pigmentation. The F1 hybrid shows enhancement of the *Sd* phenotype. Backcrossing of the F1 hybrid to *X. helleri* results in offspring that segregate into 50% which have not inherited the *Sd*-locus and are phenotypically like the *X. helleri* parental strain and 50% which carry the macromelanophore locus and develop melanoma. The severity of melanoma ranges from very benign in some individuals (phenotype like the F1 hybrids) to highly malignant in others. Highly malignant melanomas of such fish grow invasive and exophytic and are fatal to the individuum. They even grow progressively following transplantation to thymusaplastic (nude) mice (17). Based on a variety of such classical crossing experiments a genetic model has been developed to explain tumor formation in Xiphophorus (18). The macromelanophore locus was formally equated to a melanoma oncogene locus, whose critical constituent was designated "tumor-gene" (*Tu*). Melanoma formation then was attributed to the uncontrolled activity of *Tu*. In non-tumorous fish *Tu* activity was proposed to be negatively controlled by cellular regulatory genes or tumor suppressor genes (*R*-genes, corresponding to the modifying genes mentioned above). For the crossing experiment outlined above this means that *X. maculatus* contains the *Tu-Sd* locus on the X-chromosome and the corresponding major *R* on an autosome, while *X. helleri* is proposed not to contain this particular *Tu*-locus and its corresponding *R*. Backcrossing of the *Tu*-containing hybrids to *X. helleri* results, in effect, in the progressive replacement of *R*-bearing chromosomes from *X. maculatus* by *R*-free chromosomes of *X. helleri*. The stepwise elimination of regulatory genes is thought to allow expression of the *Tu* phenotype, leading to benign melanoma if one functional allele of *R* is still present or malignant melanoma if *R* is absent (for review see 19).

Reintroduction of *R* by crossing malignant melanoma bearing hybrids to parental *X. maculatus* was shown to lead to a reversion of the malignant phenotype resulting in totally tumor-free fish in the succeeding backcross generations using again *X. maculatus* as the recurrent parent (19). This demonstrates that the melanoma oncogene *Tu* itself remains structurally unaltered during the process of activation via hybridization and that the concerted action of *R* and *Tu* results in non-proliferating macromelanophore spots.

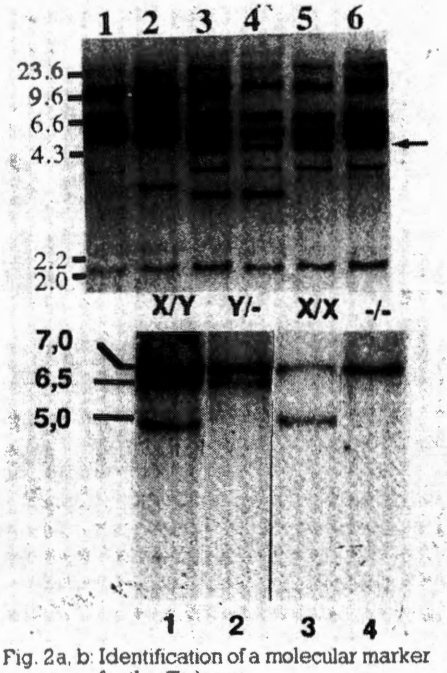

Fig. 2a, b: Identification of a molecular marker sequence for the *Tu*-locus.
a) Southern blot analysis of *Eco*RI digested DNA from fish without *Tu* (lanes 1, 2) or with *Tu* (lane 3–6) hybridized with the viral *erb* B probe under conditions of moderate stringency. A weak hybridizing fragment of 5 kb (arrow) cosegregates with the *Tu*-locus, while all other bands are non-informative (for details see 24).
b) Southern blot analysis using the cloned 5 kb marker sequence revealed three fragments: that one of 5 kb indicative of a X-chromosomal *Tu*, one of 6.5 kb in fish that carry a Y-chromosomal *Tu*, and a 7 kb band that was invariably detected irrespective of the presence or absence of *Tu*.

Reverse genetic approaches towards isolation of the dominant melanoma inducing gene

In order to understand the molecular basis of hereditary melanoma isolation and characterization of the genes involved was attempted. We first concentrated on the dominant acting *Tu* because in the past a large variety of different alleles from natural populations (see 20) and several spontaneous and X-ray induced mutants had been isolated and characterized (21). To that point no candidate gene product of *Tu* had been characterized precluding cloning by conventional recombinant DNA technology. We therefore applied a strategy that has been termed "reverse genetics" (22) to isolate the melanoma inducing gene of *X. maculatus*. This strategy included the following steps: 1.) Determination of the chromosomal location of *Tu*. 2.) Identification and cloning of a molecular marker sequence for the *Tu*-locus, which is apparent due to a restriction fragment length polymorphism (RFLP). 3.) Cloning of the *Tu*-containing region by chromosome walking or jumping. 4.) Identification and isolation of a candidate gene. 5.) Verification that the candidate gene is indeed responsible for the *Tu*-phenotype, namely melanoma induction in the hybrids, thereby proving that the cloned gene is actually the sought *Tu* gene.

After the chromosomal localization of *Tu* had been clearly defined by recombination and mutation analyses as to reside within the distal portion of the sex-chromosomes, the most critical step was to identify a molecular marker sequence. One of several approaches (see 23) was to use heterologous oncogene/proto-oncogene probes for Southern hybridizations under conditions of low stringency.

From all probes tested, the viral *erb* B (v-*erb* B) probe was most informative. It is derived from the B oncogene of avian erythoblastosis virus and represents a truncated and oncogenically activated version of the avian epidermal growth factor receptor (EGFR) gene. The v-*erb* B probe that encompasses most of the highly conserved kinase domain, detects in *EcoRI* digests besides other strongly hybridizing bands two weaker bands that were only detected in the DNA of fish carrying a sex-chromosomal *Tu*-locus, one of 6.5 kb cosegregating with Y-chromosomal *Tu*-loci and one of 5 kb, cosegregating with X-chromosomal *Tu*-loci (24). In linkage analysis employing more than 500 individual fish no recombinant between this RFLP and the *Tu*-locus was found (24, 25, 26, 27) indicating that this sequence is either intimately linked to *Tu* or even an integral part of the locus. The 5 kb band was cloned and found to detect besides the Y-chromosomal 6.5 kb band a third hybridizing sequence of 7 kb which was invariably present in DNA of all fish irrespective of the presence or absence of a *Tu*-locus (28). With the genomic sequence as a probe a corresponding full lenght c-DNA (26) was cloned from melanoma cells and found to encode a typical growth factor receptor protein with an extracellular ligand binding domain, a transmembrane domain and an intracellular catalytic domain, that contains all eleven structural motifs diagnostic for the protein kinase activity of receptor tyrosine kinases (RTK). The gene was designated *Xmrk* for Xiphophorus melanoma receptor kinase. It is closest related to the EGFR of higher vertebrates, but it does not represent the fish homologue of this gene (26). *Xmrk* is a bona fide new member of the RTK gene family, whose physiological ligand is unknown to date.

Structure and genomic organization of *Xmrk*

In the Xiphophorus genome three different copies of the *Xmrk* gene coexist, which were identified due to their restriction fragment length polymorphism in the region encoding the kinase domain. The three copies represent independent genetic loci (26, 25).

All three copies of *Xmrk* share as far as analysed an identical exon/intron arrangement and show a sequence identity of more than 99% including the noncoding intron sequences. Each locus comprises approximately 25 kb (see fig. 2a, b). *Xmrk* shares an identical exon/intron arrangement and exon sizes with EGFR, HER 2/*neu* and ERB B 3 (29).

All fish, regardless of the presence or absence of the *Tu*-locus, contain one copy of *Xmrk* (recognized by the invariably present 7 kb *Eco*RI fragment, therefore called INV) on each sex-chromosome. It obviously represents a typical proto-oncogene (26, 29, 25). The remaining two copies – named X or Y according to their sex-chromosomal location – are associated with macromelanophore spot pattern loci that can give rise to melanoma in the appropriate crossings (25) and are regarded as oncogenic versions of the INV copy.

All three copies of *Xmrk* show a strong sequence conservation. The kinase domain does not show a single mutation which could lead to an altered protein, such mutations are restricted to the carboxy-terminus of the *Xmrk* receptor tyrosine kinase genes. From 122 sequence differences between the proto-oncogene and the two oncogenes found in a total of 18 kb of genomic sequence (exons and introns), 10 lead to amino acid exchanges, only three of which are nonconservative (29).

It is not clear at present if the observed sequence differences, or possible mutations in the so far not analysed extracellular, transmembrane and juxtamembrane domains in the Y- and X-locus do contribute to the process of neoplastic transformation. Anyway, the mutations in the oncogenic copies of *Xmrk* are not effective as long as the genes are under control of the *R*-locus. Therefore such differences are not primarily responsible for bringing about the appearance of melanoma in the hybrids.

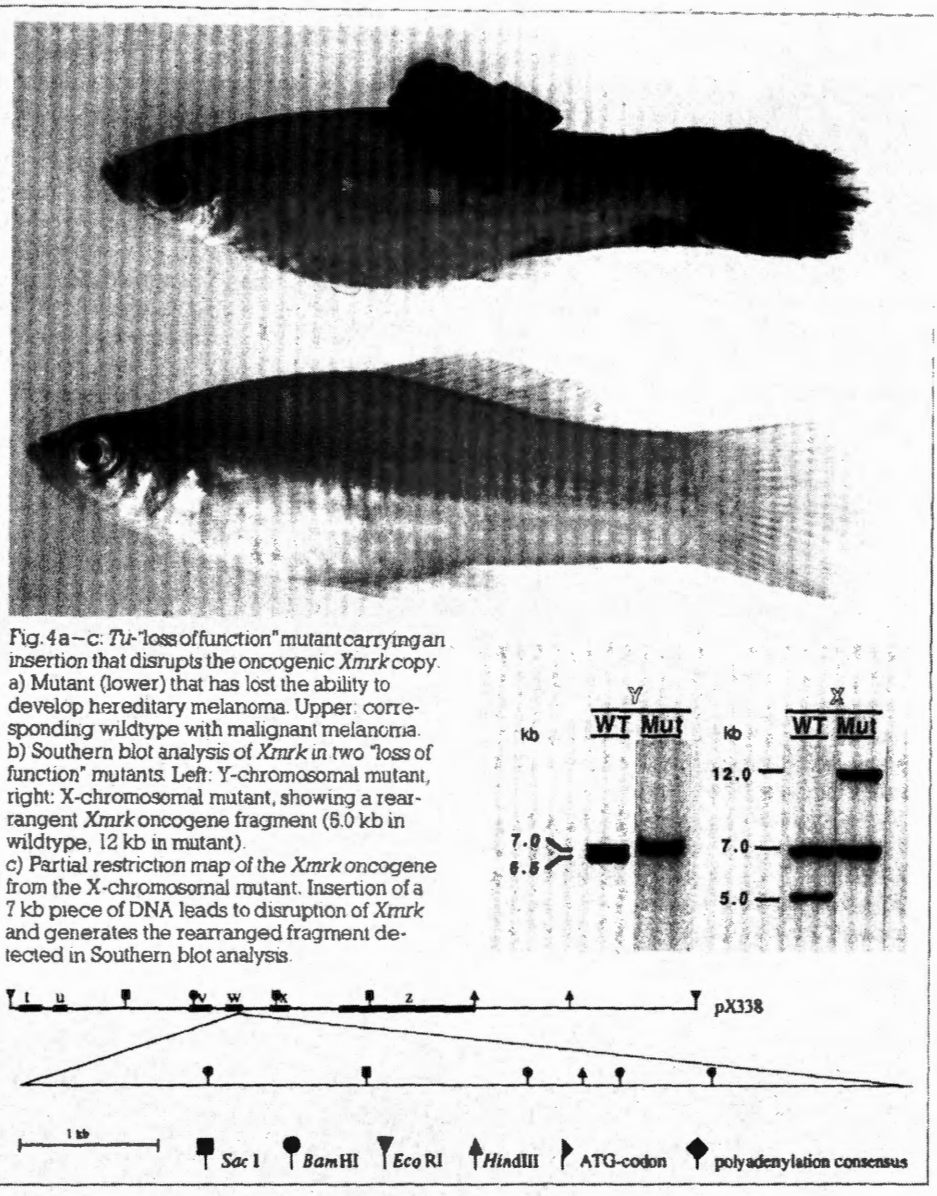

Fig. 4a–c: *Tu*-"loss of function" mutant carrying an insertion that disrupts the oncogenic *Xmrk* copy. a) Mutant (lower) that has lost the ability to develop hereditary melanoma. Upper: corresponding wildtype with malignant melanoma. b) Southern blot analysis of *Xmrk* in two "loss of function" mutants. Left: Y-chromosomal mutant, right: X-chromosomal mutant, showing a rearrangent *Xmrk* oncogene fragment (6.0 kb in wildtype, 12 kb in mutant). c) Partial restriction map of the *Xmrk* oncogene from the X-chromosomal mutant. Insertion of a 7 kb piece of DNA leads to disruption of *Xmrk* and generates the rearranged fragment detected in Southern blot analysis.

Evaluation of the sequence differences found in all three *Xmrk* loci with respect to phylogeny strongly supports the idea of a gene duplication event which created a new copy of the INV gene. This copy was translocated 2 cM apart on the Y-chromosome during this duplication process and at a later stage transferred also to the X-chromosome by homologous recombination (29; see fig. 3).

Verification that the additional X- or Y-chromosomal copies of *Xmrk* are actually the critical, i.e. melanoma inducing constituent of the *Tu*-locus, came from analyses of "loss of function" mutants of *Tu*. Such mutants arise spontaneously with a very low frequency ($< 10^{-5}$) in broods of melanoma-bearing hybrids and are characterized by a loss of the ability to develop spontaneous melanoma. One such mutant was found to carry an insertion within one exon of the additional X-chromosomal *Xmrk* locus (26), resulting in the inability to develop hereditary melanoma (see fig. 4a–c). This showed that the *Xmrk* oncogene is necessary for tumorigenesis and therefore is the critical constituent of the *Tu*-locus.

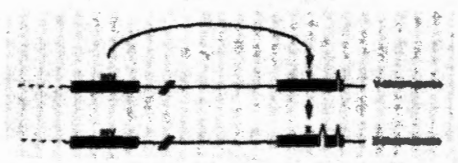

Figure 3: Model for the generation of the oncogenic *Xmrk* copies (X, Y) by gene duplication of the proto-oncogenic INV *Xmrk* gene and sex chromosomal crossing-over (indicated by arrows). Large deletions are indicated by gaps.

Transcriptional activation of the *Xmrk* oncogene

The *Xmrk* genes give rise to two transcripts of different size, one of 5.8 kb and one of 4.7 kb. The shorter transcripts are product of the oncogenic X- and Y-

chromosomal *Xmrk* copies while the 5.8 kb mRNA is transcribed from the proto-oncogenic INV copy (26; 29).

The proto-oncogene transcript is highly abundant as maternal RNA in unfertilized eggs and is differentially expressed during organogenesis. In adult non-tumorous fish, expression of the INV gene is restricted to low levels of transcripts in skin, fins and gills. Low levels of the 5.8 kb transcript are also found in melanoma (29). This expression is not influenced by presence or absence of the *R*-locus, as the 5.8 kb transcript is found at a similar level in melanomas of differing malignancy.

In contrast, expression of the X- and Y-copy of *Xmrk* is depending on the absence of the *R*-locus. Expression of the 4.7 kb transcript is limited to melanoma, it cannot be detected in any other tissue. The degree of *Xmrk* oncogene expression and the malignancy of melanoma are definitely correlated: the amount of the 4.7 kb transcript is low in benign and very high in malignant melanomas (26; 29). Overexpression of the oncogenic *Xmrk* copies seems to be a prerequisite for tumor formation after loss of the *R*-locus.

With respect to the size difference of 1.1 kb of the INV transcript on the one, and the X- and Y-transcript on the other side, differences in the 5′ region were found to account for this phenomenon. Both oncogenic copies of *Xmrk* use a transcription start site located about 1.1 kb 3′ to that used by the proto-oncogene. This is due to the presence of two different promoters in the different *Xmrk* genes (29). The promoter of the oncogenic *Xmrk* loci is obviously only active in the melanoma cells of the hybrid fish but appears inactive in the purebred parental fish.

Based on this results, it is conceivable to argue that the *R*-locus is somehow involved in transcriptional control of the promoter of the oncogenic *Xmrk* loci (X, Y). Loss of the *R*-locus then would lead to uncontrolled expression of X and Y resulting in melanoma formation.

Cooperation of multiple oncogenes?

It is generally accepted that cancer is a multistep process. This is reflected on the molecular level by the fact that in a variety of experimental situations activation of a single oncogene is not sufficient to induce and maintain the neoplastic phenotype of a cell. Moreover a second or even more activated oncogenes are required (for review see 30). On the first sight it might appear that the situation is more simple in cases where activation of a single genetic locus, like *Tu*, is the only event needed for tumor formation. Similar to the retroviral oncogenes expression of the X- or Y-copy of *Xmrk* should be the single event necessary and sufficient for melanoma induction. However, overexpression of *Xmrk* in the pigment cell lineage leads to a very complex system of alterations, not all of which may be simply explained as physiological consequences of an enhanced receptor tyrosine kinase activity, but may be regarded as secondary activation steps that are the prerequisite to obtain the full neoplastic phenotype of the melanoma.

All three members of the gene family of *src*-related cytoplasmic tyrosine kinases of Xiphophorus, namely X*src*, X*yes* and X*fyn*, that have been cloned and analysed so far are highly expressed in melanoma (31, 32, 33, 34). The transcript levels of X*yes* and X*fyn* are even higher as in embryonal cells and in adult brain, the latter being the organ of preferential expression of these genes in non-tumorous fish. The situation is even more intruiging with X*src*. Its expression in melanoma cells is specific for the transformed state because non-transformed pigment cells were found not to contain detectable amounts of X*src* transcripts (31). The enzymatic activity of the X*src* encoded protein, $pp60^{X}$*src*, is strongly enhanced in tumors as compared to any other normal organ, and correlates positively with the degree of malignancy of the melanoma (35, 36). The X*src* gene is not associated structurally with the *Tu*-locus and consequently with *Xmrk* (37, 24), therefore it obviously represents a "secondarily" activated oncogene. If this holds also true for X*yes* and X*fyn* remains to be clarified. It will be also of interest to know why a certain subset of the *src*-family genes which are either suspected or shown to exert a normal function that is totally uncoupled from proliferation in neural tissue (38) is activated in the highly proliferative melanoma cells, which according to their embryological origin are also derived from the neuroectoderm.

Other observations that possibly will turn out to mark important steps in the generation of the full neoplastic phenotype are: 1.) Increased phospholipid turnover (39), that might be indicative of an active second messenger system involved in signal transduction of the proliferation response from a receptor tyrosine kinase activation. This has been proposed for the EGFR and might be conceivable also for *Xmrk*. 2.) Growth of melanoma cells in low serum, accompanied by an accumulation of *Xmrk* transcripts (34), possibly related to an autocrine stimulatory loop in proliferation control of Xiphophorus melanoma cells.

Modulation of the neoplastic phenotype

Besides a genetic control by the action of regulatory genes like *R* malignancy of melanoma can also be influenced by a variety of epigentic factors some of which are androgens. In certain genotypes melanoma formation starts earlier in males and leads to a higher malignancy (40) and in other genotypes only mature males develop melanoma (19). Treatment of Xiphophorus with testosterone has dramatic effects on melanoma formation depending on the genotype and the developmental stage of the tumor of the fish. All the various effects can be explained by a promotion of pigment cell differentiation by androgens.

The degree of malignancy of melanoma in Xiphophorus is as in other neoplasia determined by the stage of differentiation of the melanoma cell which itself is regarded as an incompletely differentiated pigment cell (41). Benign melanoma consist mainly of cells in advanced stages of differentiation which have lost their capacity to divide whereas malignant tumors mainly consist of poorly differentiated pigment cells which are still capable to devide (42, 43, 44). Based on this the effect of androgens are detailled as follows:

1.) Induction of melanoma occurs in such genotypes that are predisposed to tumor development according to the deregulation of *Tu* but usually do not develop melanoma due to a delay or a total inhibition of pigment cell differentiation prior to the stage where neoplastic transformation can occur. In this case a promotion of pigment cell differentiation results in supply of stem cells which can be neoplastically transformed. They give rise to fast growing malignant melanoma (see fig. 5).

2.) Enhancement of tumor growth occurs in such genotypes that bear malignant melanoma consisting predominantly of poorly differentiated precursor cells. A promotion of cell differentiation via testosterone leads to an increased number of neoplastically transformed pigment cells which are still capable to divide.

3.) Suppression of melanoma growth and tumor regression was induced by treating such genotypes that bear relatively benign melanoma consisting predominantly of highly differentiated pigment cells. In this

case a promotion of cell differentiation decreases the number of cells capable to divide. That results in a suppression of tumor growth. Removal of terminally differentiated macromelanophores by macrophages then leads to regression of the melanoma.

Treatment with other sexual steroids, derivatives and analogues indicated that the observed modulations of the melanoma phenotype are specific for androgens (45).

Another substance that was found to modulate the malignancy of melanoma in Xiphophorus is dinitrochlorphenol (DNCB). Application of this compound led to tumor regression (46). DNCB is known to stimulate the immune system and thus may enhance an immune response against the melanoma cells. The same mechanism may explain the observation that raising tumor-bearing backcross hybrids under hyperthermic conditions also suppresses melanoma formation (47). The high temperature in the environment most likely induces some kind of "artificial fever" in the poikilothermic animals.

Besides steroids and the immune system many more factors may exist that modulate the neoplastic phenotype. Due to the fact that the genetic factors determining malignancy are defined and lead to tumors of high pathophysiological uniformity, melanoma formation in Xiphophorus provides a unique system for studies on epigenetic modulatory factors and their mechanism of action. This hopefully will also offer new perspectives for therapeutic approaches.

Implications and perspectives

The classical model to explain spontaneous melanoma formation in platyfish/swordtail hybrids employing the sex-chromosomal dominant acting oncogene locus *Tu*, which we have shown to encode a copy of the *Xmrk* gene, and the autosomal tumor suppressor locus *R*, has been extended to explain also a variety of other phenomena and experimental observations such as the formation of carcinogen- and X-ray-induced tumors of all histiotypes in Xiphophorus hybrids, and the occurence of macromelanophore spot patterns in several other Xiphophorus species besides *X. maculatus*, some of which predispose to spontaneous melanoma formation in hybrids while others do not (48). With the availability of the *Xmrk* gene these problems can be approached experimentally.

The identity of the *Xmrk* gene product as a putative novel growth factor receptor with a tyrosine kinase activity implicates further questions the answers to which should help to understand how overexpression of the gene mediates the initiation and maintenance of the neoplastic phenotype of pigment cells. It will be important to find those cellular substrates for the *Xmrk* kinase that transduce the mitogenic signal exerted by the *Xmrk* ligand to the nucleus as well as to identify the genes that are activated following *Xmrk* stimulation. Isolation and characterization of the ligand will not only help to elucidate the normal, physiological function of *Xmrk* but also help to investigate if the melanoma cells constitute an autocrine growth stimulatory loop sensu Todaro and/or if the oncogenic *Xmrk* loci encode an "activated" mutant protein, that is constitutively active.

To obtain information how the oncogenic activity of the X- and Y-copies of *Xmrk* is suppressed in the parental purebred fish isolation and characterization of the *R* encoded gene(s) is required. The finding that transcriptional control may be the mechanism through which *R* regulates *Xmrk* emphasises the importance of identifying the factors that control transcription of the oncogenic *Xmrk* copies. An alternative approach will be to use also for cloning of *R* encoded gene(s) the methodology of reverse genetics.

Melanomas in feral Xiphophorus populations are extremely rare (49, 50), however, their existance gives additional significance to the reasoning that the duplicated *Xmrk* genes that reside closely to the macromelanophore locus, are potentially injurious. It will be important to exploit also from an evolutionary and social behavioural genetic point of view how such a potential deleterious gene has been maintained in the natural populations of most species of Xiphophorus.

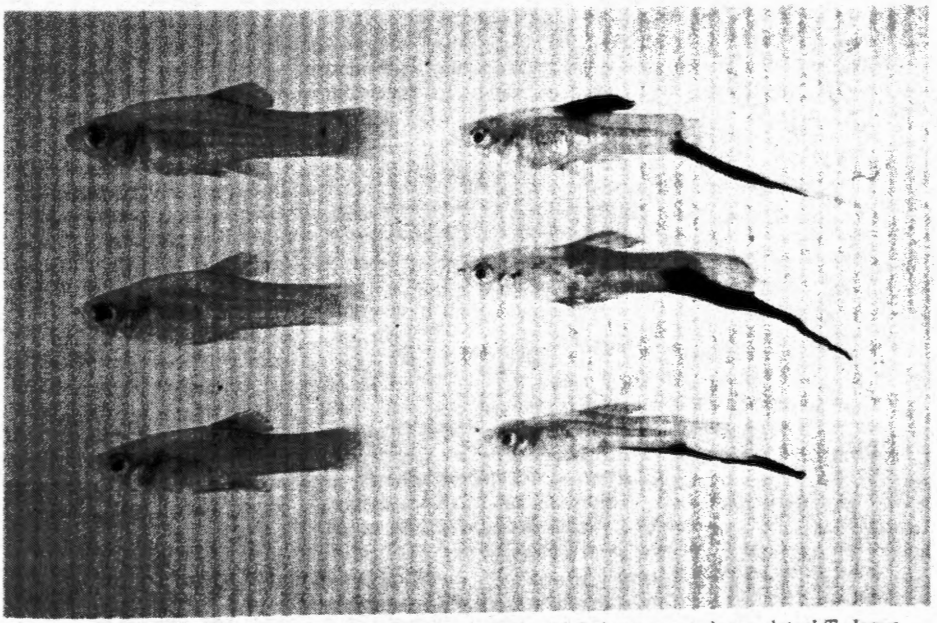

Figure 5: Melanoma induction by testosterone. Treatment of fish that carry a deregulated *Tu*-locus but do not develop melanoma due to a genetic arrest of pigment cell differentiation leads to melanoma formation. Testosterone promotes pigment cell differentiation thus pushing precursor cells to a stage where they are competent for transformation by *Tu* Left, control; right, fish treated with 10^{-6} M methyltestosterone. As a side effect, treated animals develop a sword-like extension of the tail fin as male secondary sex character.

Acknowledgements

We thank A. Geishauser for breeding of the fish, and S. Romanow for typing the manuscript. This work was supported by the Bundesministerium für Forschung und Technologie through Schwerpunkt "Grundlagen und Anwendungen der Gentechnologie" (Grant No 26), the Deutsche Forschungsgemeinschaft through Schwerpunkt "Molekulare Tumorzytogenetik" (Scha 406/2–3) and SFB 190, the Stiftung Volkswagenwerk "Wettbewerb Biowissenschaften", and the Max-Planck-Gesellschaft.

References

(1) Friend, S.H., Dryja, T.P., Weinberg, R.A. (1988). Oncogenes and tumor-suppressing genes. *The New England Journal of Medicine* 318: 618–622.

(2) Friend, S.H., Bernards, R., Rogelej, S., Weinberg, R.A., Rapaport, J.M., Albert, D.M., Dryja, T.P. (1986). A human DNA segment with properties of the gene that predisposes to

retinoblastoma and osteosarcoma. *Nature* 323: 643–646.
(3) Huang, H.J.S., Yee, J.-K., Shew, J.-Y., Chen, P.-L., Bookstein, R., Friedmann, T., Lee, E.Y.-H.P., Lee, W.-H. (1988). Suppression of the neoplastic phenotype by replacement of the RB gene in human cancer cells. *Science* 242: 1563–1566.
(4) Mechler, B.M., McGinnis, W., Gehring W.J. (1985). Molecular cloning of *lethal(2)giant larvae*, a recessive oncogene of Drosophila melanogaster. Embo J. 4: 1551–1557.
(5) Jacob, L., Opper, M., Metzroth, B., Phannavong, B., Mechler, B.M. (1987). Structure of the *l(2)gl* gene of Drosophila and delimitation of its tumor suppressor domain. *Cell* 50: 215–225.
(6) Ponder, B. (1988). Gene losses in human tumours. *Nature* 335: 400–402.
(7) Gordon, M. (1927). The genetics of a viviparous top-minnow *Platypoecilus*: The inheritance of two kinds of melanophores. *Genetics* 12: 253–283.
(8) Häussler, G. (1928). Über Melanombildungen bei Bastarden von *Xiphophorus maculatus* var. rubra. *Klin. Wochenschr.* 7: 1561–1562.
(9) Kosswig, C. (1928). Über Kreuzungen zwischen den Teleostiern *Xiphophorus helleri* und *Platypoecilus maculatus*. *Z. Indukt. Abstammungs.-Vererbungsl.* 47: 150–158.
(10) Gordon, M. (1931). Hereditary basis of melanosis in hybrid fishes. *Am. J. Cancer* 15: 1495–1519.
(11) Kosswig, C. (1929). Das Gen in fremder Erbmasse. *Züchter* 1: 152–157.
(12) Gordon, M. (1958). A genetic concept for the origin of melanomas. *Ann. N.Y. Acad. Sci.* 71: 1213–1222.
(13) Atz, J.W. (1962). Effects of hybridization on pigmentation in fishes of the genus *Xiphophorus*. *Zoologica* 47: 163–181.
(14) Kosswig, C. (1965). Genetische Grundlagen des Polymorphismus. *Zool. Anz.* 176: 21–50.
(15) Zander, C.D. (1969). Über die Entstehung und Veränderung von Farbmustern in der Gattung Xiphophorus (Pisces). *Mitt. Hamburg Zool. Mus. Inst.* 66: 241–271.
(16) Kallman, K.D. (1970). Different genetic basis of identical pigment patterns in two populations of platyfish, *Xiphophorus maculatus*. *Copeia* 3: 472–487.
(17) Schartl, M., Peter, R.U. (1988). Progressive growth of fish tumors after transplantation into thymus-aplastic (nu/nu) mice. *Cancer Res.* 48: 741–744.
(18) Ahuja, M.R., Anders, F. (1976). A genetic concept of the origin of cancer, based in part upon studies of neoplasms in fishes. *Prog. Exp. Tumor Res.* 20: 380–397.
(19) Anders, F., Schartl, M., Barnekow, A., Anders, A. (1984). Xiphophorus as an in vivo model for studies on normal and defective control of oncogenes. *Adv. Cancer Res.* 42: 191–276.
(20) Kallman, K.D. (1975). The platyfish, *Xiphophorus maculatus*. In: King, R.C. (ed.): *Handbook of Genetics* 4: pp. 8–132, Plenum Press, New York.
(21) Anders, A., Anders, F., Klinke, K., (1973). Regulation of gene expression in the Gordon-Kosswig melanoma system I, II. In: Schröder, J.H. (ed.): *Genetics and Mutagenesis of Fish*, pp. 33–63, Springer-Verlag, New York.
(22) Orkin, S.J. (1986). Reverse genetics and human disease. *Cell* 47: 845–850.
(23) Schartl, M., Wittbrodt, J., Mäueler, W., Raulf, F., Adam, D., Hannig, G., Telling, A., Storch, F., Andexinger, S., Robertson, S.M. (1990). Oncogenes and melanoma formation in *Xiphophorus* (Teleostei: Poeciliidae) In: Schöder, J.H., Schartl, M. (eds.): New Trends in Ichthyology. Parey, Hamburg.
(24) Schartl, M. (1988). A sex chromosomal restriction-fragment-length marker linked to melanoma-determining Tu loci in Xiphophorus. Genetics 119: 679–685.
(25) Schartl, M. (1990). Homology of melanoma-inducing loci in the genus Xiphophorus. *Genetics* 126: in press.
(26) Wittbrodt, J., Adam, D., Malitschek, B., Mäueler, W., Raulf, F., Telling, A., Robertson, S. M., Schartl, M. (1989). Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in Xiphophorus. *Nature* 341: 415–421.
(27) Zechel, C., Schleenbecker, U., Anders, A., Anders, F. (1988). v-*erbB* related sequences in Xiphophorus that map to melanoma determining Mendelian loci and overexpress in a melanoma cell line. *Oncogene* 3: 605–617.
(28) Adam, D., Wittbrodt, J., Telling, A. und Schartl, M. (1988). RFLP for an EGF-receptor related gene associated with the melanoma oncogene locus of *Xiphophorus maculatus*. *Nucl. Acids Res.* 16: 7212.
(29) Adam, D., Mäueler, W. und Schartl, M. (1990). Transcriptional activation of the melanoma inducing *Xmrk* oncogene in Xiphophorus. *Oncogene*: in press.
(30) Weinberg, R.A. (1989). Oncogenes, antioncogenes, and the molecular basis of multistep carcinogenesis. *Cancer Research* 49: 3713–3721.
(31) Raulf, F., Mäueler, W., Robertson, S.M., Schartl, M. (1989). Localization of cellular *src* mRNA during development and in the differentiated bipolar neurons of the adult neural retina in Xiphophorus. *Oncogene Res.* 5: 39–47.
(32) Hannig, G., Ottilie, S., Schartl, M. (1990). Conservation of structure and expression of the *c-yes* and *fyn* genes in lower vertebrates. *Oncogene*, in press.
(33) Mäueler, W., Raulf, F., Schartl, M. (1988a). Expression of proto-oncogenes in embryonic, adult, and transformed tissue of Xiphophorus (Teleostei: Poeciliidae). *Oncogene* 2: 421–430.
(34) Mäueler, W., Barnekow, A., Eigenbrodt, E., Raulf, F., Falk, H.F., Telling, A., Schartl, M. (1988b). Different regulation of oncogene expression in tumor and embryonal cells of Xiphophorus. *Oncogene* 3: 113–122.
(35) Schartl, A., Schartl, M. Anders, F. (1982). Promotion and regression of neoplasia by testosterone promoted cell differentiation in Xiphophorus and Girardinus. *Carcinogensis* 7: 427–434.
(36) Schartl, M., Schmidt, C.-R., Anders, A., Barnekow, A. (1985). Elevated expression of the cellular *src* gene in tumors of differing etiologies in Xiphophorus. *Int. J. Cancer* 36: 199–207.
(37) Robertson, S.M. (1989). Isolation and characterization of *scr*-related sequences from the platyfish *Xiphophorus maculatus* (Poeciliidae: Teleostei) and an evolutionary analyses of the *src* gene-family. Dissertation thesis, LMU München.
(38) Barnekow A., Jahn, R., Schartl, M. (1990). Synaptophysin: a substrate for the protein tyrosine kinase pp60$^{c\text{-}src}$ in intact synaptic vesicles. *Oncogene* 5: 1019–1024.
(39) Smith, A.D., Gronau, T., Pöfrock, A., Zechel, C., Bird, J.M., Lane, P.A., Barnekow, A., Anders A., Anders, F. (1989). EGF receptor gene, inositol lipid turnover and *c-src* activity in key processes preceding melanoma in Xiphophorus. In: Lynch HT, Fusaro RM (eds.): Hereditary Malignant Melanoma. CRC, Boca Raton (in press).
(40) Siciliano, M. J., Perlmutter, A., Clark, E. (1971). Effects of sex on the development of melanoma in hybrid fish of the genus Xiphophorus. *Cancer Res.* 31: 725–729.
(41) Gordon, M. (1939). The melanoma cell as an incompletely differentiated pigment cell. In: Gordon, M. (ed.): *Pigment Cell Biology*, pp. 215–239. Academic Press, New York.
(42) Anders, F., Diehl, H., Scholl, E. (1980). Differentiation of normal melanophores in the skin of the genus Xiphophorus. In: Spearman, R. I. C., Riley, P. A. (eds): The Skin of Vertebrates. *Linnean Society Symposium Series* 9: pp. 211–218.
(43) Vielkind, J., Vielkind, U. (1982). Melanoma formation in fish of the genus Xiphophorus: a genetically based disorder in the determination and differentiation of a specific pigment cell. *Canadian Journal of Genetics and Cytology* 24: 133–149.
(44) Anders, F., Diehl, H., Schwab, M., Anders, A. (1979). Contributions to an understanding of the cellular origin of melanoma in the Gordon-Kosswig Xiphophorine fish tumor system. In: Klaus, S.N. (ed.): *Pigment Cell* 4: pp. 142–149, Karger, Basel.
(45) Schartl, A., Schartl, M. (1988). Modulation of pigment cell differentiation by steroids in melanoma of *Xiphophorus*. *Acta Endocrinologica* 117 (Suppl. 287): 170.
(46) Scholz, A. (1977). Untersuchungen zur Dinitrochlorbenzol (DNCB)-induzierten Regression kreuzungsbedingter Melanome der lebendgebärenden Zahnkarpfen (Poeciliidae). Staatsexamensarbeit, Universität Gießen.
(47) Perlmutter, A., Potter, H. 1988. Hyperthermic suppression of a genetically programmed melanoma in hybrids of fishes: genus Xiphophorus. *J. Cancer Res. Clin. Oncol.* 114: 339–362.
(48) Anders, F. (1990). A biologist's view of human cancer. In: Neth, R. et al. (eds.): *Modern Trends in Human Leukemia* 8: pp. XXIII–XIV, Springer Verlag, Berlin.
(49) Borowsky, R.L. (1973). Melanomas in *Xiphophorus variatus* (Pisces, Poeciliidae) in the absence of hybridization. *Experientia* 29: 1431–1433.
(50) Kallman, K.D. (1971). Inheritance of melanophore patterns and sex determination in the montezuma swordtail, *Xiphophorus montezumae cortezi* Rosen. *Zoologica (N.Y.)* 56: 77–94.

Drs. Angelika und Manfred Schartl
Genzentrum/Max-Planck-Institut für Biochemie, Am Klopferspitz 18 a,
D-8033 Martinsried, Fed. Rep. Germany

Received: October 30, 1990
Accepted: November 7, 1990