Xiphophorus as an In Vivo Model for Studies on Oncogenes[1,2]

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ABSTRACT—The capacity of *Xiphophorus* to develop neoplasia can be formally assigned to a "tumor gene" (*Tu*), which appears to be a normal part of the genome of all individuals. The wild fish have evolved population-specific and cell type-specific systems of regulatory genes (*R*) for *Tu* that protect the fish from neoplasia. Hybridization of members of different wild populations in the laboratory followed by treatment of the hybrids with carcinogens led to disintegration of the *R* systems permitting excessive expression of *Tu* and thus resulting in neoplasia. Certain hybrids developed neoplasia even spontaneously. Observations on the genuine phenotypic effect of the derepressed *Tu* in the early embryo indicated an essential normal function of this oncogene in cell differentiation, proliferation and cell-cell communication. *Tu* appeared to be indispensable in the genome but may also be present in accessory copies. Recently, *c-src*, the cellular homolog of the Rous sarcoma virus oncogene *v-src*, was detected in *Xiphophorus*. The protein product of *c-src*, pp60$^{c\text{-}src}$, was identified and then examined by its associated kinase activity. This pp60$^{c\text{-}src}$ was found in all individuals tested, but, depending on the genotype, its kinase activity was different. The genetic characters of *c-src*, such as linkage relations, dosage relations, expression, etc., correspond to those of *Tu*. From a systematic study which showed that pp60$^{c\text{-}src}$ was present in all metazoa tested ranging from mammals down to sponges, we concluded that *c-src* has evolved with the multicellular organization of animals. Neoplasia of animals and humans is a characteristic closely related to this evolution. Our data showed that small aquarium fish, besides being used successfully because they are time-, space-, and money-saving systems for carcinogenicity testing, are also highly suitable for basic studies on neoplasia at the populational, morphological, developmental, cell biological, and molecular levels. — Natl Cancer Inst Monogr 65: 97-109, 1984.

ABBREVIATIONS: MNU = *N*-methyl-*N*-nitrosourea; BC = backcross generation; *Tu* = tumor gene; *R* = pigment cell-specific regulatory gene for *Tu*; *v-src* = transforming gene from Rous sarcoma virus; *c-src* = cellular homolog of *v-src*; pp60$^{v\text{-}src}$ and pp60$^{c\text{-}src}$ = phosphoprotein gene products of *v-src* and *c-src* (mol wt = 60,000).

Neoplasia was found in all taxonomic groups of eumetazoa and was even detected in fossils of prehistoric animals, such as sauria, and mammals including humans (*1-3*).

Neoplasia occurs infrequently in the natural populations of eumetazoa, and induction by carcinogens is difficult (*4, 5*). However, certain nontaxonomically defined groups of animals are highly susceptible to both spontaneously developing and carcinogen-triggered neoplasms [table 1; (*6-31*)]. These groups consist mainly of animals of hybrid origin, such as naturally occurring or experimentally produced interspecific, interracial, and interpopulational hybrids (*5*) as well as laboratory and domesticated animals (*6*) which actually are also hybrids (i.e., homozygous combinations of chromosomes of different population or racial provenance). These animals share their high susceptibility to neoplasia with humans (*4*). The hybrid animals can serve as models for the research on the origin of neoplasia in humans and can serve as tester organisms for the carcinogens in our environment (*32*). The rationale underlying the use of these animals as models is based on the assumption that the biological fundamentals of neoplasia are similar in models and human beings.

Although mice and rats are the classic laboratory animals used in experimental cancer research, several genera of small teleost fish serve increasingly as models in new cancer research programs. Their suitability is beyond question (except for research on breast cancer, lung cancer, etc.), and their efficiency is more economic and time-saving as compared with that of the mammals (*32, 33*). One of these genera is *Xiphophorus*, the animal model that we have used in our laboratories for 25 years (*4, 5, 34-36*).

TAXONOMIC STATUS OF THE WILD *XIPHOPHORUS*

Xiphophorus, including platyfish and swordtails, lives in genetically isolated populations in brooks, rivers, lakes, ponds, and pools in Central America and has evolved into innumerable genotypically and phenotypically distinguishable groups. Based on certain morphological, ecological, and ethological characteristics, 17 of these groups have been listed as species (*37, 38*). All individuals of this genus, however, can be hybridized in the laboratory without difficulty, and all hybrids are fertile. This finding and those results on the conformity of genome organization (*39*), the low degree of enzyme polymorphism (*40*), and the normal chromosome pairing during meiosis in the hybrids (*41*) led to the conclusion that the taxonomic differences between these groups of *Xiphophorus* are not at the species level but at the level of elementary local populations as well as

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TABLE 1.—*Animals that exhibited a high tumor incidence*

Species	Tumor	Reference
Insects		
Drosophila laboratory stocks	Various neoplasms	(7)
Solenobia hybrids	" "	(8)
Teleosts		
Xiphophorus hybrids	" "	(9-11)
Girardinus laboratory stocks	Promoter-induced melanoma	(12)
Ornamental guppy strains	Carcinogen-induced hepatoma	(13)
Orange medaka	Hepatoma	(14)
Domesticated trout	Aflatoxin-induced liver tumors	(15)
Salvelinus hybrids	Fibrosarcoma	(16)
Domestic carp	Neuroepithelioma	(17)
Ornamental hybrid carp	Ovarian neoplasia	(18)
Lake Ontario hybrid carp	Pollution-conditioned gonadal tumors	(19)
Goldfish	Erythrophoroma	(20)
Amphibia		
Bufo calamita and *B. viridis* hybrids	Chordomas	(21)
Birds		
Musk duck and mallard hybrids	Gonadal tumors	(22)
Peacock and guinea fowl hybrids	" "	(23)
Improved breeds of fowl	Leukosis	(6)
Mammals		
Mus musculus and *M. bactrianus* hybrids	Various neoplasms	(24)
Laboratory mice strains	" "	(25)
Hybrids of mice strains	Increased incidence of various neoplasms	(25)
BALB/c and NZB hybrids	Plasma cell tumors (50%)	(26)
Blue ribbon mice	Mammary tumors (100%)	(27)
Sprague-Dawley and Long-Evans hybrids	Increased mammary tumor incidence	(28)
Domestic dogs	Various neoplasms	(6)
Boxers	" "	(29)
Domestic cats	" "	(6)
Sinclair swine	Melanoma	(30)
Lipizzaner horses	"	(31)

ecological and geographical races. The ease of hybridizing animals from the different taxonomic groups of *Xiphophorus* listed as species, therefore, is no curiosity in the animal kingdom but is comparable to the frequently occurring hybridization between animals of different populations or local races from other teleosts as well as amphibia, lizards, birds, mammals (*40*), and humans.

SUSCEPTIBILITY TO NEOPLASIA IN *XIPHOPHORUS* HYBRIDS

About 10,000 specimens of purebred descendants of the wild populations have been treated with powerful carcinogens, such as benzo[*a*]pyrene, MNU, and X-rays, but none developed neoplasia. Contrastingly, of 10,195 hybrids which survived treatment with MNU or X-rays, 805 (8%) of the individuals developed a large variety of neoplasms (table 2). Most of the neoplasms were classified as neurogenic and mesenchymal, with melanoma, neuroblastoma, and fibrosarcoma the predominant types. Epithelial neoplasms were less frequent but comprised those with the largest diversity. Tumors comparable to those of mammals and humans developed in almost all tissues including connective tissue, muscles, and the pigment cell, nervous cell, gastrointestinal, and reticuloendothelial systems.

ASSIGNMENT OF CANCER SUSCEPTIBILITY TO ONCOGENES AND REGULATORY GENES

At present, *Xiphophorus* is the only model, at least to our knowledge, in which susceptibility to carcinogen-triggered neoplasia was assigned to oncogenes and regulatory genes. Information about these genes comes from the analysis of the hereditary trait of certain spontaneously developing melanomas, pterinophoromas, neuroblastomas, thyroid carcinomas, and reticulosarcomas in hybrids. The hybrid animals that develop the neoplasms spontaneously are rare compared with those that require carcinogen induction for tumor development. However, they can be bred at the will of the experimenter and have therefore contributed many facts for development of ideas about how other animals and humans might inherit susceptibility to neoplasia (*34-36, 46, 47*).

Oncogenes and Regulatory Genes in Animals Developing Melanoma Spontaneously

To illustrate the assignment of cancer susceptibility to distinct genes in greater detail, we used melanoma because this type of tumor is easily detectable by its heavy melanin pigmentation. Even singly transformed pigment cells can be distinguished from nontransformed pigment cells by gross

TABLE 2.—*Neoplasia in* Xiphophorus *F_2–F_{24} and BC_n generations*[a]

Type of neoplasm	No. of neoplasms			Percent incidence based on total No. of survivors	
	MNU	X-rays		MNU	X-rays
Neurogenic					
Melanoma (benign)	135	93	491	2.12	2.6
Melanoma (malignant)	138	34		2.09	0.95
Neuroblastoma	84	7		1.27	0.2
Epithelial					
Squamous cell carcinoma	6	0	78	0.09	0
Epithelioma	19	6		0.28	0.17
Carcinoma (low differentiation)	3	4		0.05	0.11
Carcinoma (high differentiation)	2	5		0.03	0.14
Adenocarcinoma (kidney)	8	2		0.12	0.05
Adenocarcinoma (thyroid)	2	3		0.03	0.08
Papilloma	9	0		0.14	0
Hepatoma	5	1		0.07	0.03
Acanthoma	3	0		0.04	0
Mesenchymal					
Fibrosarcoma	190	6	236	2.87	0.17
Rhabdomyosarcoma	33	2		0.5	0.05
Lymphosarcoma	1	0		0.01	0
Reticulosarcoma	4	0		0.06	0
Total	642	163	805		

[a] One year after treatment with MNU (10^{-3} *M* four times for 1 hr in 2-wk intervals) and X-rays (1,000 roentgens three times for 45 min in 6-wk intervals). Survivors: MNU = 6,608; X-rays = 3,587. Data were compiled from (*42–45*).

examination of the living animal (*48*). Furthermore, morphological, ultrastructural, and histochemical markers specific to the successive stages of pigment cell differentiation facilitate the detection of the event of neoplastic transformation. This facilitation and the ease with which crossings in *Xiphophorus* can be accomplished provided the background for the design of the experiments that elucidated the oncogenes and regulatory genes by formal genetics.

Crosses (fig. 1A–H) of a certain *X. maculatus* (platyfish), which is infrequently spotted (A), with a certain *X. helleri* (swordtail), which is never spotted (B), result in F_1 that develop uniformly benign melanoma instead of spots (C). Backcrosses of the first generation hybrids with the swordtail as the recurrent parent (D) result in offspring (BC_1), 50% of which exhibit neither spots nor melanoma (G and H), whereas 25% develop benign melanoma (E), and 25% develop malignant melanoma (F). Further backcrosses (not shown in fig. 1) of the fish carrying benign melanoma with the swordtail result in a BC_2 that exhibits the same segregation as the BC_1. The same applies for further backcrosses of this kind. Backcrossing of the fish carrying malignant melanoma with the swordtail results, however, in a BC_2 in which 50% of the animals do not develop melanoma, whereas the remaining 50% develop malignant melanoma. In contrast, backcrossing of the melanoma-bearing hybrids with the use of the platyfish as the recurrent parent results in a gradual suppression and finally disappearance of neoplasia in the succeeding generations (fig. 2).

The results obtained in these 2 series of crossing experiments revealed several genetic components that are involved in melanoma formation or protection from melanoma (*see* fig. 3):

1) The platyfish genome contains the genetic information for the development of spots which is lacking in the swordtail. Because the spots consist of transformed pigment cells (*48*), this information is considered to be encoded by an oncogene, *Tu*, which is an accessory gene of the platyfish genome. From about 70 structural changes involving crossovers, deletions, duplications, and translocations, we know that *Tu* is normally located at the end of the X-chromosome of *X. maculatus* (*42, 49–51*) and is apparently also responsible for the large variety of the carcinogen-triggered neurogenic, epithelial, and mesenchymal neoplasms (*35, 43*) listed in table 2.

2) The protection of the platyfish from the activity of its own *Tu* is apparently exerted by regulatory genes. After hybridization, *Tu* becomes dysregulated indicating that the swordtail lacks not only the oncogene *Tu* but also the regulatory genes (*34*). Several types of regulatory genes can formally be deduced from the outcome of the crossing experiments: 1) The restriction of crossing-conditioned neoplasia to melanoma indicates the presence of a *Tu*-linked pigment cell-specific regulatory gene which, as we know from mutagenesis studies (*35, 42, 50, 51*) and from comparison of the *Tu*-containing chromosomes shown in the schemes of figs. 3 and 4 (*see* later), is impaired by mutation. Out of the tissue-specific regulatory genes only the impaired pigment cell-specific regulatory gene for *Tu*, i.e., *R′*, is shown in the scheme (fig. 3). 2) The restriction of the crossing-conditioned melanomas to the posterior part of the body and to the dorsal fin indicates the presence of *Tu*-linked regulatory genes that are specific to certain compartments of the body. In additional experiments corresponding to those shown in figs. 1–3, depending on the genotype (mutant) of the platyfish used for the initial crosses, melanoma develops, for instance, in the anterior part of the body, anal fin, tail fin, mouth, eye, peritoneum, or meninx, etc. Thirteen compartments have been identified that correspond to different genes which, in turn, correspond to sites of the body where the melanomas occur (*35*). Out of these compartment-specific regulatory genes only the impaired dorsal fin-specific regulatory gene, i.e., $R_{Df'}$, and the posterior part-specific regulatory gene, i.e., $R_{Pp'}$, are shown in the scheme. 3) The clear-cut 1:1 segregation between the BC-hybrids bearing malignant melanomas and those bearing benign melanomas indicates the existence of a prominent regulatory gene that is not linked to the oncogene *Tu*. Because the benign melanomas consist, in contrast to malignant melanoma, predominantly of terminally differentiated transformed pigment cells (*48, 52*) this regulatory gene is considered to be involved in differentiation of these cells, and was, therefore designated

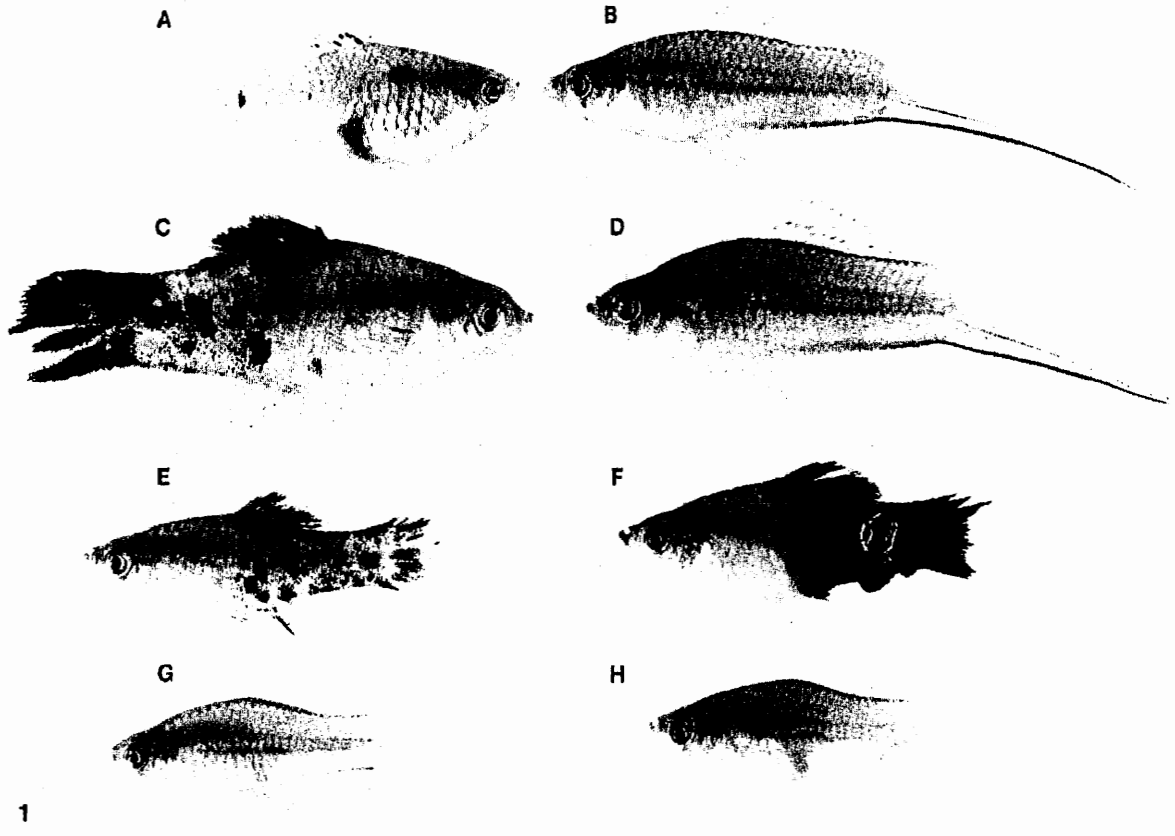

FIGURE 1.—Crossing procedure for the production of melanoma developing hybrids of *Xiphophorus*. A) *X. maculatus* from Rio Jamapa (Mexico); some small spots in the skin of the dorsal fin and the side of the body are visible. Spots consist of terminally differentiated, neoplastically transformed pigment cells. B) *X. helleri* from Rio Lancetilla (Mexico) always lacked spots. C) F_1 developed benign melanoma instead of spots (100% of the F_1). D) *X. helleri* from B) used in the backcross as the recurrent parent. E) Backcross hybrid with benign melanoma (25% of the BC generation). F) Backcross hybrid with malignant melanoma (25% of the BC generation). G), H) Backcross hybrids that do not develop melanoma (50% of the BC generation).

as *Diff*. The *Diff*-containing chromosome can easily be detected by an esterase marker, *Est-1*, which is closely linked to *Diff* (*35; 53-59*). Additional genes involved in melanoma formation have been identified but have not been taken into consideration in this study.

Following crossings and backcrossings with the swordtail as the recurrent parent, the chromosomes of the platyfish are replaced by the homologous chromosomes of the swordtail, resulting in the gradual disintegration of the regulatory gene system for *Tu*. Thus the *Tu* hybrids develop spontaneously benign melanoma if some regulatory genes such as *Diff* are still present in the system and malignant melanoma if the regulatory genes are lacking (*34, 35, 53, 56, 58, 59*). If *Tu* is lacking, no melanomas occur.

In contrast, after backcrossings of the melanoma-bearing hybrids with the platyfish as the recurrent parent (fig. 2), the chromosomes carrying regulatory genes are reintroduced into the descendants. This results in a reconstruction of the original regulatory gene system that suppresses the activity of *Tu*.

Oncogenes and Regulatory Genes in Animals Requiring Carcinogenic Inducers for the Development of Melanoma

To relate the genes that are responsible for the spontaneous development of melanoma to the genes that are basically involved in the development of the carcinogen-dependent neoplasms, we replaced the $R'\ R_{Df}'\ R_{Pp}'\ Tu$-chromosome (fig. 3) by the $R\ R_{Df}\ R_{Bs}'\ Tu$-chromosome (fig. 4), the pigment cell-specific R and dorsal fin-specific R_{Df} of which are nonmutated and active, and the compartment-specific R_{Bs} that normally protects the entire side of the body from melanoma is mutated to R_{Bs}'. The R_{Pp} was not taken into consideration in these experiments. Because R is inherited with *Tu*, melanoma does not develop spontaneously in the hybrids. After treatment with carcinogens, those hybrids carrying the $R\ R_{Df}\ R_{Bs}'\ Tu$-chromosome but lacking the nonlinked regulatory genes including *Diff* are highly sensitive to the carcinogens on the body side. Thus development of melanoma requires only impairment or deletion of the pigment cell-specific R gene

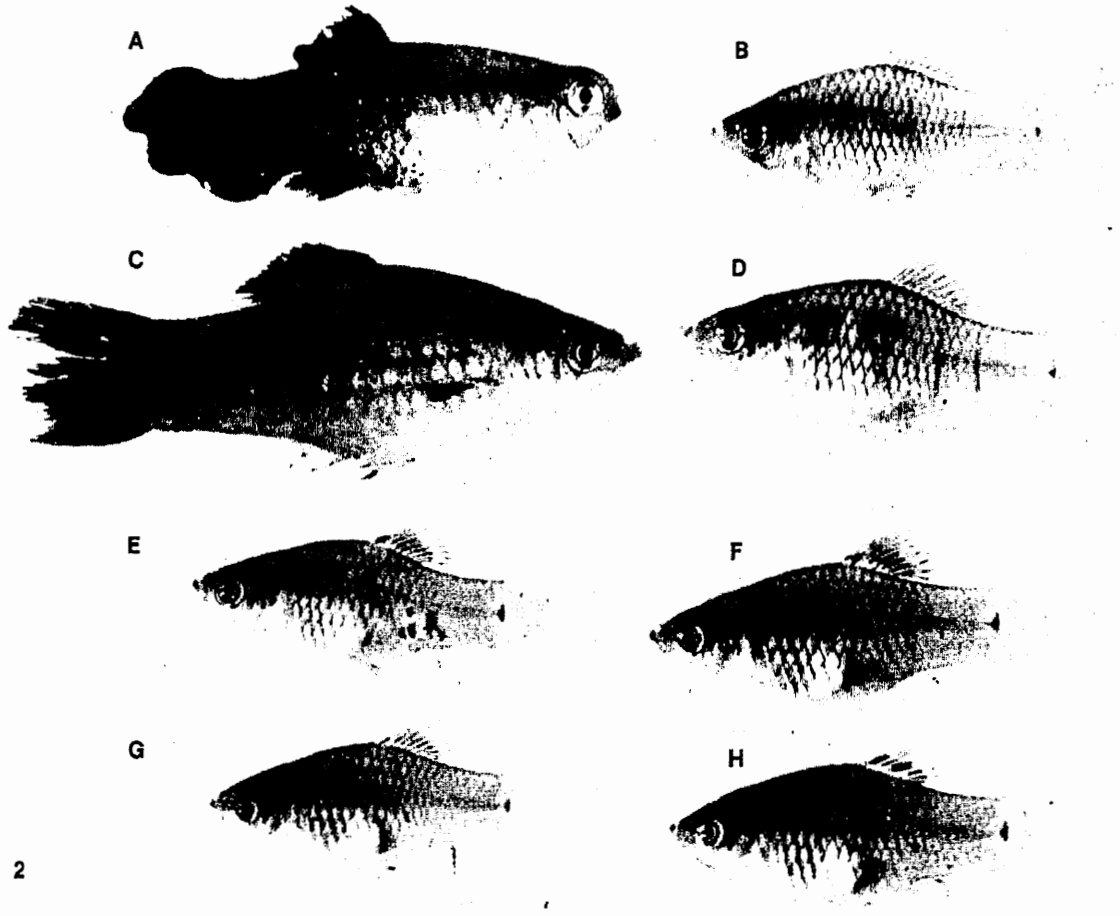

FIGURE 2.—Crossing procedure for the suppression of melanoma in *Xiphophorus*. A) Malignant melanoma bearing backcross hybrid according to figure 1F. B) *X. maculatus* according to figure 1A as the recurrent parent. C) Quasi F_1 exhibiting benign melanoma. D) *X. maculatus* as the recurrent parent. E), F), G), H) Backcross hybrids (quasi *X. maculatus*) exhibiting spots only.

by the carcinogen in a somatic cell at the side of the body (*32, 60, 61*).

Problem of Tissue Specificity of the Oncogene and of the Regulatory Gene System

The hybrid segregants that were highly susceptible to carcinogen-triggered melanoma were also susceptible to the induced neuroblastoma, epithelioma, and fibrosarcoma (*43-45, 60, 61*). Furthermore, many hybrid individuals treated with carcinogens, in addition to the melanoma, may also develop multiple neoplasms, such as neuroblastoma, retinoblastoma, carcinoma, and sarcoma. Therefore, the development of the different carcinogen-induced neoplasms apparently depends on the same *Tu*-containing chromosome like that of melanoma (*43-45, 60, 61*). Additional studies are required before investigators can decide whether 1) *Tu* is tissue-nonspecific although tissue specificity of the tumor comes from *Tu*-linked, tissue-specific *R* genes, or 2) whether *Tu* is an oncogene complex composed of tissue-specific constituents that are under the control of linked *R* genes specific to the *Tu* constituent. Experiments in which susceptibility to neurogenic and epithelial neoplasia could be assigned to a certain chromosome, though susceptibility to neoplasms of mesenchymal origin depended on a different chromosome (*60, 61*), tempt one to assume that *Tu* actually could be composed of several constituents that are tissue specific and could be considered as different oncogenes.

GENUINE ONCOGENIC EFFECT OF *Tu* IN THE PIGMENT CELL SYSTEM OF EARLY EMBRYOS

Information about the genuine oncogenic effect of *Tu* comes from a balanced laboratory stock carrying a lethal *Tu* translocation. The *Tu* copy became translocated from an X-chromosome of *X. maculatus* to an autosome of *X. helleri* and, in its new position, is no longer controlled by its formerly linked regulatory genes that act in *cis*-position only (*42*). Nonlinked regulatory genes are not present in the system except for *Diff*. The *Tu*-carrying progeny of the stock segregates into 50% carrying *Diff* which survive, whereas the corresponding 50% lacking *Diff* are lethal (*42*). As a consequence of the Mendelian inheritance of the

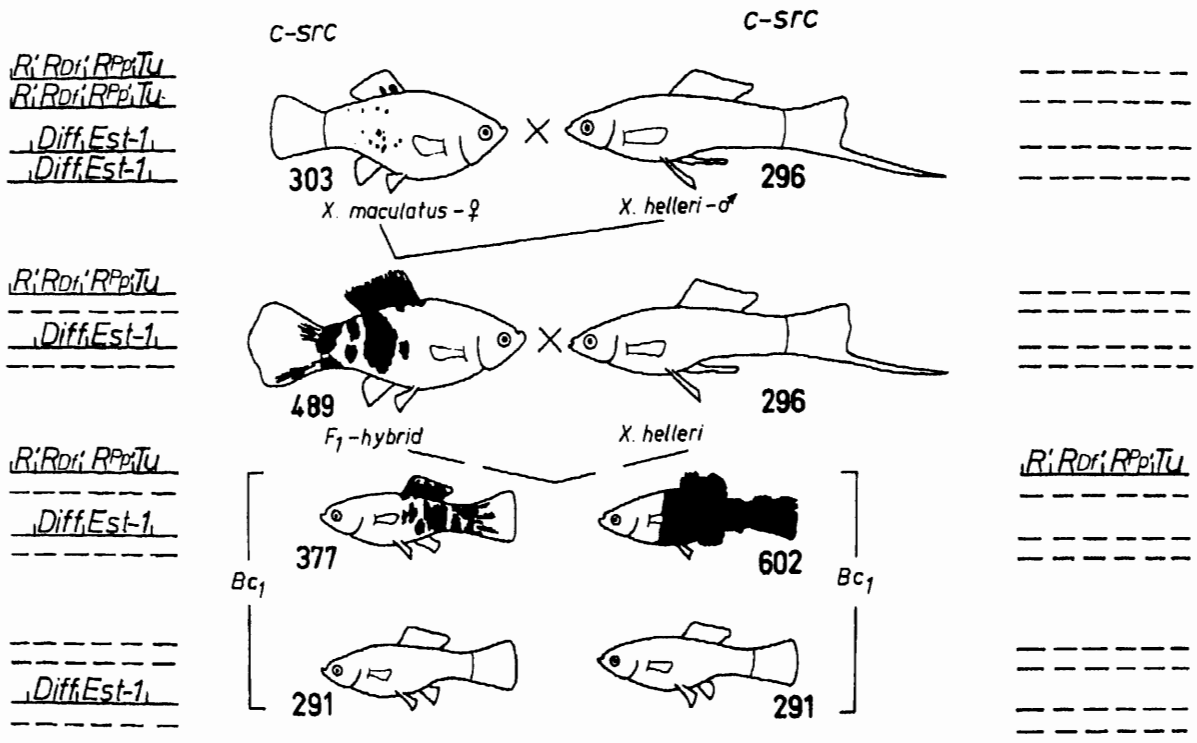

FIGURE 3.—Crossing scheme according to figure 1, which displays the genetic conditions for the "spontaneous" development of spots, benign melanoma, and malignant melanoma. ——— = chromosomes of *X. maculatus;* - - - - = chromosomes of *X. helleri; Tu* = tumor gene; $R_{Pp'}$ and $R_{Df'}$ = impaired regulatory genes controlling *Tu* in the compartments of the posterior part of the body (Pp) and the dorsal fin (Df); *R'* = impaired regulatory gene specific to pigment cells but nonspecific to the compartments; *Diff* = regulatory gene controlling differentiation of neoplastically transformed cells; *Est-1* = locus for esterase-1 of *X. maculatus. c-src* = $pp60^{c\text{-}src}$ kinase activity expressed as counts per minute/milligram protein); *note* basic and excessive activity and correlation between *c-src* expression and *Tu* expression. Data from (*4, 5, 32, 75*) were combined.

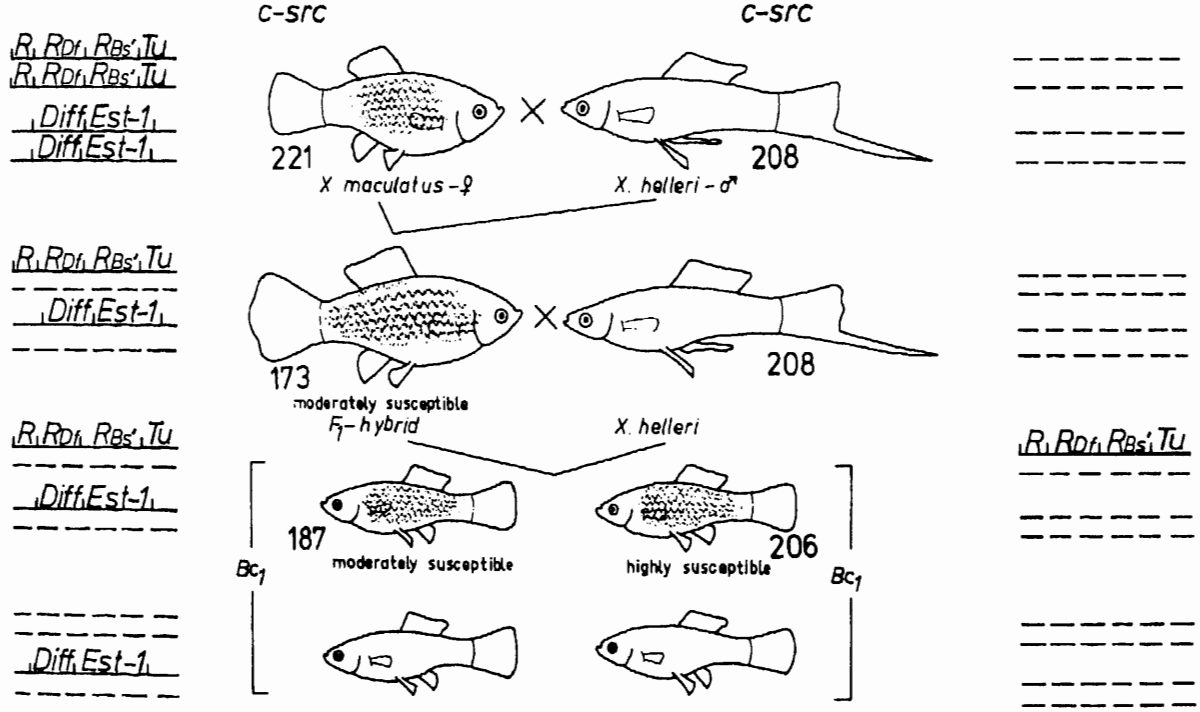

FIGURE 4.—Crossing scheme displaying the genetic conditions for susceptibility to carcinogen-dependent neoplasia. The highly susceptible genotype is extremely sensitive to the carcinogenic (mutagenic) trigger. *See* legend to figure 3 for explanation of abbreviations. $R_{Bs'}$ = impaired regulatory gene controlling *Tu* in the compartment of the entire side of the body (Bs). Data from (*4, 5, 32, 75*) were combined.

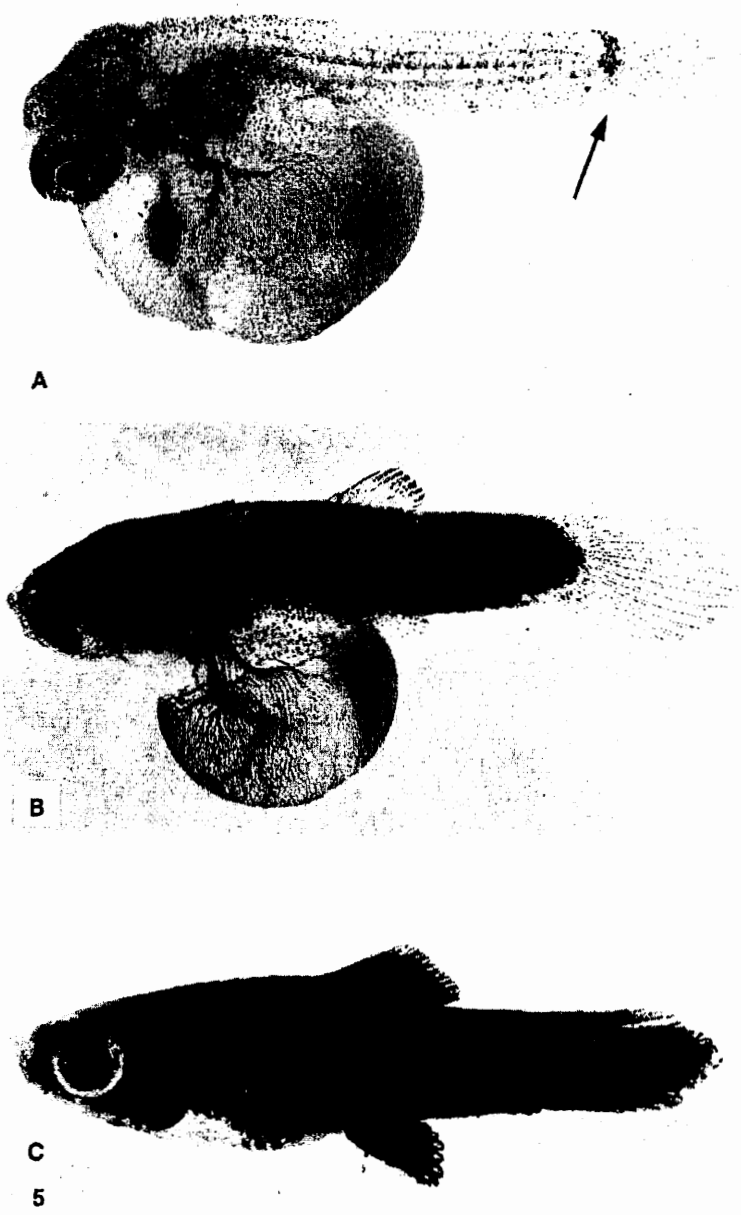

FIGURE 5.—Genuine oncogenic effect of the tumor gene *Tu*. A) Ten-day-old embryo (3 mm long) exhibiting some T-melanocytes at the peduncle of the tail fin (*see arrow*). B) Same fish, 5 days later (4 mm long). C) Neonate of the same genotype (6 mm long).

unrestrained *Tu* through the germ line, the pigment cell precursors become transformed as soon as they become competent for neoplastic transformation by cell differentiation in the embryo (*62*). During the first days of embryonic life, differentiation of pigment cells still undergoes the normal course. After the embryo is 5 days old, some single cells become transformed, and at a later time about 10–20 dividing transformed melanoblasts appear in the peduncle of the tail fin. These melanoblasts differentiate within approximately 15 hours to transformed melanocytes (fig. 5A), which represent the predominant cells of the growing melanoma. During further development of the embryo, neoplastic transformation continues in all areas where pigment cell precursors become competent (fig. 5B), and the melanoma grows by both transformation and proliferation, thus developing into a "whole body melanoma" (fig. 5C), which will kill the fish before or after birth. The development of melanoma in the early embryo reflects the genuine oncogenic effect of the completely derepressed *Tu* on the pigment cell system (*35, 42, 62*).

CONCEIVABLE NONONCOGENIC FUNCTIONS OF *Tu* IN THE EMBRYO

The observations on the genuine oncogenic effect of *Tu* in the embryo tempt us to assume that *Tu* exerts important normal functions in cytodifferentiation and proliferation in the early embryo that are related to the neural crest where the pigment cell precursors originate. Moreover, we assume that in normal embryogenesis these functions become switched off or choked by the regulatory genes before the fifth day of embryonic life. If, however, the regulatory

genes (i.e., the entire switch in the lethal *Tu* translocation) are lacking, *Tu* continues to exert its early embryo-specific functions which, as an extension of the cellular development in the early embryo, appear as transformation of the competent cells to the neoplastic state.

The assumption of normal (nononcogenic) functions of *Tu* in early embryogenesis raises the question regarding the genes that might exert these functions in the animals lacking *Tu*, such as swordtails, used in the experiments described earlier. This question leads to the problem of indispensable and accessory copies of the oncogenes that will be discussed later.

PROBLEM OF INDISPENSABLE AND ACCESSORY COPIES OF *Tu*

About 30 deletions of *Tu* have been genetically characterized, and some of the major deletions involving both *Tu* and its linked regulatory genes were, in addition to the genetic results, observed cytologically (*50*). Furthermore, deletions of *Tu* copies have been observed in all of our *Tu*-containing stocks of the genus *Xiphophorus*, including *X. maculatus, X. variatus, X. xiphidium, X. helleri* and *X. montezumae* (*42*). All deletions, even the loss of one *Tu*-containing Giemsa band in the homozygous condition in the female or in the heterozygous state in the male (observed in the X-chromosome of *X. maculatus*) have no detectable effect on the viability of the fish (*50*). This observation and the fact that wild populations may lack *Tu* in total led us to the conclusion that the *Tu* considered so far is accessory for the fish. We do not imply that the accessory *Tu* is lacking normal functioning. One could, for instance, assume that certain indispensable copies of *Tu* are present in the genome and may compensate the loss of the accessory *Tu* loci according to a gene dosage compensation mechanism, which warrants normal functions. Support for this assumption comes from the following experiment. Platyfish carrying the X-chromosome deletion of the Giemsa band that involves the accessory *Tu* were crossed with the swordtail according to the procedure outlined in figure 3. As was expected, no X-chromosome inherited tumors developed spontaneously in the hybrids, but after treatment with MNU, the backcross hybrids developed melanomas that could be assigned to an autosome of *X. maculatus*. Thus it appears that the platyfish, besides the easily detectable accessory *Tu* copies, also contains indispensable ones that require more intricate experiments for their detection.

So far we could not detect any individual of the genus *Xiphophorus* that lacks the genetic information for neoplastic transformation. Even individuals having lost the easily detectable *Tu* copies still contain oncogenes for the development of neoplasia. These oncogenes can always be noted after the appropriate crosses and with the appropriate carcinogenic treatment that trigger the neoplasms that are always chromosome specific. Therefore, oncogenes seem to be indispensable for the fish. Accessory copies of the oncogenes may be present but are not essential. If they are present, special regulatory gene systems are required for their control. We introduced up to 10 authentic copies of *Tu* (4 copies in homozygous duplications in both X-chromosomes of the female, 6 copies in 3 nonhomologous pairs of autosomes) and their linked regulatory genes into the genome containing nonlinked regulatory genes, but no tumors developed spontaneously nor could any effect on viability be observed. We assume that the *Tu* copies present in a certain genome are not strongly limited in number if their control is warranted by regulatory genes.

ONCOGENE DOSAGE EFFECT AND COMPENSATION

The following experiments illustrate the conditions leading to oncogene dosage effect and compensation. Both the X-chromosome of *X. maculatus* containing the accessory *Tu* (the X-chromosome according to fig. 3) and the X-chromosome of *X. maculatus* having lost the Giemsa band carrying the accessory *Tu* were introduced into the genome of *X. helleri* lacking both the accessory *Tu* and the regulatory gene system. The $X^{Tu}X^{Del} \times X^{Tu}$ Y-matings were accomplished. The segregating offspring having none, 1, or 2, respectively, accessory *Tu* copies showed a clear-cut gene dosage effect (fig. 6). The animals lacking *Tu* show no neoplastically transformed pigment cell (A), the littermates containing 1 *Tu* copy exhibit a limited number of transformed cells (B), and the segregants having inherited the double dosage of *Tu* develop malignant melanoma (C). However, if the experiment was modified by the use of animals as recipients for the X^{Tu}, X^{Del}- and Y-chromosomes that have retained the nonlinked regulatory genes, we observed gene dosage compensation (*63*). Dosage effect and compensation of the accessory oncogene *Tu*, therefore, depend on the absence or presence, respectively, of the nonlinked regulatory genes in these experiments. Oncogene dosage effect and compensation have been observed in many experiments of this kind (*63*).

CELLULAR COUNTERPARTS OF RETROVIRAL ONCOGENES IN THE *Tu* MELANOMA SYSTEM

Although neoplasia in *Xiphophorus* is well understood with regard to formal genetics, thus far its molecular basis has been extremely resistant to any elucidation.

Ten years ago, *Tu* of *Xiphophorus* was considered formally equated with oncogenes of endogenous tumor viruses (*42, 64*) that were interpreted in the sense proposed by Bentvelzen (*65*), Todaro and Huebner (*66*), and others (*67*). Our (*68*) experimental examination of this hypothesis, however, showed that B- and C-type particles present in melanoma and neuroblastoma following induction with 5-bromodeoxyuridine apparently are not related to neoplasia of the fish, and efforts to detect reverse transcriptase proved fruitless. This absence of prospects changed immediately when cancer virology progressed to the elucidation of cellular oncogenes.

Oncogene *v-src* from Rous sarcoma virus has a counterpart, *c-src*, in noninfected cells of the chicken (*69*). This *c-src* was also found in the human being, mouse, and calf (*70*). Commercial DNA derived from salmon obviously contains the same gene (*70*). However, no convincing evidence exists that relates the cellular *src* or its gene product, a 60,000-dalton phosphoprotein ($pp60^{c\text{-}src}$), with a

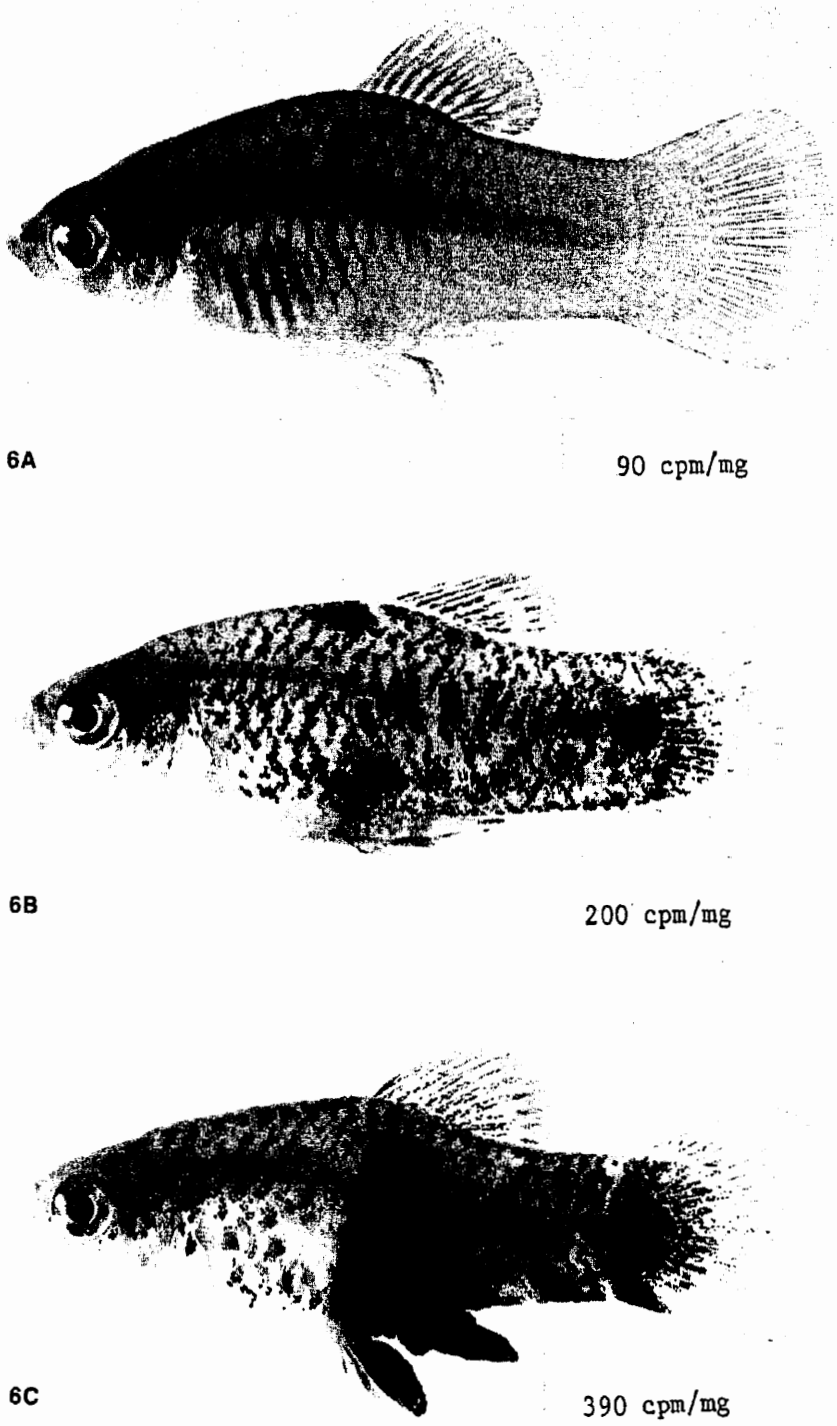

FIGURE 6.—Correlation between gene dosage effect of *Tu* (specified as the phenotype of the tumor) and $pp60^{c-src}$ kinase activity (expressed as counts per minute/milligram protein) in littermates containing A) no accessory *Tu*, B) single dose of *Tu*, and C) double dose of *Tu*. Genetic background of the fish is identical. A) *Tu* is deleted in the germ line. B), C) The pigment cell-specific *R* linked to *Tu* is impaired by germ-line mutation.

tyrosine phosphorylating kinase activity to neoplasia that depends on conditions other than virus infections (*71, 72*). On the other hand, evidence had been presented that $pp60^{c-src}$ is expressed in a differentiation-dependent and tissue-specific manner (*71*). This was the state of knowledge about cellular oncogenes when we started our search for *c-src* and its product $pp60^{c-src}$ in *Xiphophorus*.

The *c-src* was detected in *Xiphophorus* by molecular hybridization of *src*-specific probe from cloned *v-src* with DNA from fish (Czernilofsky AP, Schartl M: Unpublished observations). To identify $pp60^{c-src}$, they labeled brains of the fish with [^{32}P]orthophosphate; the cell extracts were immunoprecipitated with antisera from Rous sarcoma virus tumor-bearing rabbits followed by polyacrylamide gel electrophoresis. The 60,000-dalton protein detected in the gel has a tyrosine-specific kinase activity and represents the $pp60^{c-src}$ (*73*). The kinase activity was measured according to Collett and associates (*74*). For a detailed description of

the experimental procedures, *see* the reports of Barnekow et al. (*73*), Schartl et al. (*75*), and Schartl and Barnekow (*76*). The experiments are based on the assumption that the activity of the pp60^{c-src}-associated phosphokinase monitors the activity of the endogenous *c-src* oncogene in *Xiphophorus*.

The kinase activity was determined in several tissues including skin, liver, spleen, testes, brain, and melanoma (*73*). The latter two always had the highest kinase activity, and genotype-specific differences in kinase activity were similar in both (*73*). Hence we could determine pp60^{c-src}-associated protein kinase activity mainly in brain extracts and relate the activity observed to the expression of *Tu* ascertained by the development of melanoma (*75, 77*). The possibility that the differences in kinase activity measured in the fish of different *Tu* genotypes are due to epiphenomena of the melanoma appears unlikely (*75*) because our results reflect the actual genetic activity of the *c-src* oncogene in the nontumorous brain tissue of the tumorous and nontumorous fish.

To study the possible relation between *Tu*-conditioned neoplasia and *c-src* expression, we took advantage of the 3 genetic experiments outlined in figures 3, 4, and 6.

In the experiment recorded in figure 3, the purebred *X. maculatus* carrying 2 repressed copies of the accessory *Tu*, as well as the purebred *X. helleri* and the BC-hybrids lacking the accessory *Tu*, display the same activity of *c-src* kinase (about 300 cpm/mg protein). This activity appears to be the basic expression of *c-src*. In contrast, the melanoma-bearing hybrids which contain the derepressed *Tu* show an increase in *c-src* activity, with the malignant melanoma bearing BC-hybrids displaying the highest activities (about 600 cpm/mg protein).

In the experiment illustrated in figure 4, all purebred and hybrid animals, irrespective of the lack and the dosage of the accessory *Tu*, but dependent on the nontumorous state exerted either by several regulatory genes or by a linked *R* alone (*see* the highly susceptible genotype), display a uniform *c-src* activity (about 200 cpm/mg protein) which seems to represent the basic *c-src* expression like that in purebred animals and *Tu*-lacking hybrids in the previous experiment (fig. 3).

In littermates (fig. 6) genetically identical except for the lack of the accessory *Tu* and the presence of 1 or 2 partially derepressed accessory *Tu* copies, *c-src* displays a kinase activity that increases stepwise and parallel to the lack and the dosage of *Tu* (100, 200, 400 cpm/mg protein), which, in turn, determine whether the animals will develop no tumors or slow- or rapid-growing tumors. Table 3 shows other experiments of the same kind that yielded similar results.

The main results of these experiments are that the nontumorous fish display a basic expression of *c-src*, which, in the tumorous fish, may increase stepwise under 2 conditions, i.e., the stepwise 1) derepression of an accessory *Tu* and 2) introduction of additional copies of a derepressed accessory *Tu*. These findings indicate several possibilities for an interpretation about how *Tu* is related to *c-src*: 1) It is independent from *c-src*, and the correspondence between both *Tu* and *c-src* is due to linkage relationships. 2) The *c-src* represents a regulatory gene for *Tu* or *vice versa*. 3) It might consist of different oncogenes responsible for different kinds of neoplasia, and *c-src* is one of these genes. 4) Also, *Tu* is identical to *c-src*, and this oncogene can code for a wide variety of neoplasia. At present, we cannot make a firm interpretation; additional data are required.

TABLE 3.—*The pp60^{c-src}-associated kinase activity in brain extracts specified by counts per milligram soluble protein in F_2 segregants*[a]

Tu gene complex[b]	Dosage of *Tu*, cpm/mg[c]		
	None	Single	Double
Striped[d]	90	200	390
Dabbed[e]	170	NT	390
Dabbed[f]	200	260	1,240

[a] Three to 8 brains per measurement were used.
[b] Different gels each were measured.
[c] One gel each was measured. NT = not tested.
[d] The *Tu* copy of *striped* originates from the X- and Y-chromosomes of *X. maculatus* from Rio Jamapa, Mexico; the linked *R* and R_{Bs} are impaired (*see* fig. 4), which resulted in the phenotype shown in figure 6.
[e] The *Tu* copy of *dabbed* originates from an autosome of *X. helleri* from Belize River, British Honduras; *Tu*-control is partly impaired in the compartment of the side of the body.
[f] Phenotype and genotype are similar to those of *e*, but *X. helleri* originates from Rio Lancetilla, Mexico.

DISTRIBUTION OF *c-src* IN THE ANIMAL KINGDOM

The presence of *c-src* in the genome of taxonomically different animals, such as chicken, salmon, and *Xiphophorus*, led us to a more systematic search for this oncogene in additional taxonomic groups of animals. First, different groups of *Xiphophorus* and different fish genera taxonomically related to *Xiphophorus* were investigated. All fish tested (table 4) show a pp60^{c-src} kinase activity indicating that *c-src* must be present (*73*). In addition, *c-src* was evidenced by its kinase activity in a large variety of fish

TABLE 4.—*Expression of pp60^{c-src} kinase in brain extracts of different fish species*[a]

Species	Source
X. helleri	Belize River, British Honduras
X. helleri	Rio Lancetilla, Mexico
X. maculatus	Belize River, British Honduras
"	Rio Jamapa, Mexico
"	Rio Usumacinta, Mexico; British Honduras
X. cortezi	Rio Axtla/Panuco System, Mexico
X. variatus	Rio Coy, Mexico
X. variatus	Rio Panuco, Mexico
Girardinus falcatus	Cuba
G. metallicus	Cuba
Poecilia sphenops	Puerto Barrios, Guatemala
Belonesox belizanus	Chetumal, Mexico
Heterandria bimaculata	Lake Catemaco, Mexico
Xenotoca eiseni	Highlands of Mexico

[a] *See* (*73*).

TABLE 5.—*The* c-src *in eukaryotes*[a]

Mammals	Cartilaginous fish
Humans (*70*)	Shark
Calf (*70*)	
Rat	
Mouse	Jawless fish
Birds	Lamprey
Chicken	
Quail	Acrania
Bony fish	*Amphioxus*
Flat fish	
Sea robin	Insects
Mackerel	Cockroach
Roach	*Drosophila*
Gudgeon	*melanogaster* (*78*)
Xiphophorus	
Salmon (*70*)	Sponges
Codfish	Marine sponge
Cichlid	Freshwater sponge

[a] *See* (*76*).

and metazoa (other than fish) ranging from mammals to sponges (*76*), which, with the results of other laboratories, are listed in table 5. Schartl and Barnekow (*76*) found no *c-src* in protozoa, algae, and higher plants.

The ubiquity of *c-src* in metazoa suggested that this homolog of the viral oncogene *v-src* still has unknown basic functions of life closely related to the evolution of the multicellular organization of the animals and that neoplasia is a characteristic closely related to this evolution. As the sponges are known to have evolved in the Proterozoic era, the origin of the *c-src* oncogene has to be estimated as having occurred over 1.5×10^9 years ago (*76*).

CONCLUSIONS

We studied neoplasia of *Xiphophorus* at different levels of the biological organization, including species, races, populations, generations, littermates, embryos, adults, tissues, cells, genomes, chromosomes, and genes and gene products. In doing so, we traced neoplastic transformation to the activity of 1 copy or several copies of the oncogene *Tu* that shows a relation to the cellular counterpart of the transforming oncogene of Rous sarcoma virus, the *c-src*.

Although we cannot decide now what the basis of the relationship between the Mendelian *Tu* and the molecular *c-src* in *Xiphophorus* might be (linkage, identity, etc.), and, although we still do not have data on the distribution of *Tu* in the animal kingdom comparable to that of *c-src*, our experiments support the assumption that the biological fundamentals of neoplasia are, in principle, the same in all groups of metazoa including humans (*77*). Genes that are apparently essential for the differentiation and proliferation of cells to form tissues in the early embryo and to maintain the differentiated state of the tissue by cell-cell communication in development and during adulthood obviously can relapse into their early function, which, beyond the early embryonic state, appears as the transforming function of the oncogene.

Of the acutely transforming retroviruses thus far investigated, all have oncogenes related to cellular genes that are also present in the normal genome of various vertebrate species. We need to know whether they are also present in *Xiphophorus* and, if so, whether their function is also related to *Tu*. No other animal model would be as suitable for this research as the *Xiphophorus* fish model.

Normally, the oncogenes are under the control of tissue-specific systems of regulatory genes that have evolved population specifically. Interpopulational and interracial hybridization in the preceding generation is the main event contributing to the disintegration of the regulatory gene systems for the oncogene. Germ-line mutations that may also disturb the regulatory gene systems are probably less important than hybridization because they are always rare or may become repaired. Somatic mutations, which are among the major factors that trigger carcinogenicity, may complete this disintegration. This implies that somatic mutation is the primary end of a sequence of events that leads to initiation of neoplasia (*4, 5, 35, 77*).

Although we do not have data on interpopulational hybridization in human beings comparable to those of *Xiphophorus* or to the domestic and the classical laboratory animals, it is interesting for one to speculate how much effect hybridization might have contributed to the sequence of events that led to the high tumor incidence observable in most of our highly developed nations. Such speculations are probably of little value for our fight against cancer in humans but they could help to identify the factors that make an individual insensitive or sensitive to the carcinogens of our environment. They could further help in our decisions whether we should direct our attention more to the promoters in our environment or to the initiators because elimination of initiators would have less effect in our fight against cancer if individuals would have already undergone important events of initiation.

REFERENCES

(*1*) KAISER HE: Phylogeny and paleopathology of animal and human neoplasms. *In* Neoplasms — Comparative Pathology of Growth in Animals, Plants, and Man (Kaiser HE, ed). Baltimore, London: Williams & Wilkins, 1981, pp 725-728

(*2*) ORTNER DJ: Bone tumors in archaeological human skeletons. *In* Neoplasms—Comparative Pathology of Growth in Animals, Plants, and Man (Kaiser HE, ed). Baltimore, London: Williams & Wilkins, 1981, pp 733-738

(*3*) KAISER HE: Neoplasms — A systematic review. *In* Neoplasms — Comparative Pathology of Growth in Animals, Plants, and Man (Kaiser HE, ed). Baltimore, London: Williams & Wilkins, 1981, pp 747-821

(*4*) ANDERS F: Erb- und Umweltfaktoren im Ursachengefüge des neoplastischen Wachstums nach Studien an *Xiphophorus*. Klin Wochenschr 59:943-956, 1981

(*5*) ANDERS F, SCHARTL M, SCHOLL E: Evaluation of environmental and hereditary factors in carcinogenesis, based on studies in *Xiphophorus*. *In* Phyletic Approaches to Cancer (Dawe CJ, Harshbarger JC, Kondo S, et al, eds). Tokyo: Jpn Sci Soc Press, 1981, pp 289-309

(*6*) WEISS E: Geschwülste. *In* Allgemeine Pathologie (Frei A, ed). Berlin: Verlag Paul Parey, 1972, pp 295-347

(*7*) GATEFF E: Malignant neoplasms of genetic origin in *Drosophila melanogaster*. Science 200:1448-1459, 1978

(*8*) SEILER J, PUCHTA O, BRUNOLD E, et al: Die Entwicklung des Genitalapparates bei triploiden Intersexen von *Solen-*

obia triquetrella F. R. (Lepid. Psychidae). Deutung des Intersexualitätsphänomens. Archiv Entwickl-Mech 150: 199-372, 1958

(*9*) KOSSWIG C: Über Bastarde der Teleostier *Platypoecilus* und *Xiphophorus*. Z. Indukt Abstamm Vererbungsl 44:253, 1927

(*10*) HÄUSSLER G: Über Melanombildung bei Bastarden von *Xiphophorus helleri* und *Platypoecilus maculatus* var. *Rubra*. Klin Wochenschr 7:1561-1562, 1928

(*11*) GORDON M: The genetics of a viviparous topminnow *Platypoecilus* — The inheritance of two kinds of melanophores. Genetics 12:253-283, 1927

(*12*) SCHARTL A, SCHARTL M, ANDERS F: Promotion and regression of neoplasia by testosterone promoted cell differentiation in *Xiphophorus* and *Girardinus*. *In* Carcinogenesis (Hecker E, ed). New York: Raven Press, 1982, pp 427-434

(*13*) SATO S, MATSUSHIMA T, TANAKA N, et al: Hepatic tumors in the guppy (*Lebistes reticulatus*) induced by aflatoxin B_1, dimethylnitrosamine, and 2-acetylaminofluorene. J Natl Cancer Inst 50:767-778, 1973

(*14*) TAKAYAMA S, ISHIKAWA T: Comparability of histological alteration during carcinogenesis in animals and man in special reference to hepatocarcinogenesis in fish. IARC Sci Publ No. 16. Lyon: IARC, 1977, pp 271-286

(*15*) HALVER JE, MITCHEL IA: Trout hepatoma research conference papers. U.S. Fish Wildl Serv Res Rep 70:1-99, 1967

(*16*) TAKASHIMA F, HIBIYA T: Fibrosarcoma in pond-cultured hybrids of *Salvelinus*. Jpn J Ichthyol 19:97-101, 1972

(*17*) ISHIKAWA T, PRINCE MASAHITO, TAKAYAMA S: Olfactory neuroepithelioma in a domestic carp (*Cyprinus carpio*). Cancer Res 38:3954-3959, 1978

(*18*) ISHIKAWA T, TAKAYAMA S: Ovarian neoplasia in ornamental hybrid carp (Nishikigoi) in Japan. Ann NY Acad Sci 198:330-341, 1977

(*19*) LEATHERLAND JR, SONSTEGARD RA: Structure of normal testis and testicular tumors in cyprinids from Lake Ontario. Cancer Res 38:3164-3173, 1978

(*20*) ISHIKAWA T, PRINCE MASAHITO, MATSUMOTO J, et al: Morphologic and biochemical characterization of erythrophoromas in goldfish (*Carassius auratus*). J Natl Cancer Inst 61:1461-1470, 1978

(*21*) FLINDT R, HEMMER H, SCHIPP R: Zur Morphogenesis von Miβbildungen bei Bastardlarven von *Bufo calamita* ♀ und *Bufo viridis* ♂: Störungen in der Ausbildung des Axialskeletts. Zool Jb Anat 85:51-71, 1968

(*22*) CREW FA, KOLLER P: Genetical and cytological studies on the intergeneric hybrid of *Cairina moschata* and *Anas platyrhynchos*. Proc R Soc (Edinburgh) 56:210, 1936

(*23*) POLL H: Zwischenzellengeschwulst des Hodens bei Vogelmischlingen. Beitr Pathol Anat 67:40, 1920

(*24*) LITTLE CC: The genetics of cancer in mice. Biol Rev 22:315, 1947

(*25*) WOOLLEY GW, DICKIE MM, LITTLE CC: Adrenal tumors and other pathological changes in reciprocal crosses in mice. I. Strain DBA × strain CE and the reciprocal. II. An introduction to results of four reciprocal crosses. Cancer Res 12:142-230, 1952; 13:231-240, 1953

(*26*) WARNER NL, POTTER M, METCALF D, eds: Multiple myeloma and related immunoglobulin-producing neoplasms. UICC Tech Rep Ser No. 13. Geneva: UICC, 1974, pp 41-42

(*27*) HESTON WE, VLAHAKIS G: C3H-A^{vy} — A high hepatoma and high mammary tumor strain of mice. J Natl Cancer Inst 40:1161-1166, 1968

(*28*) GROSS L, DREYFUSS Y: Spontaneous tumors in Sprague-Dawley and Long-Evans rats and their F_1 hybrids: Carcinogenic effect of total body X-irradiation. Proc Natl Acad Sci USA 76:5910-5913, 1979

(*29*) DENLINGER RH, KOESTNER A, SWENBER JA: Neoplasms in purebred boxer dogs following long-term administration of *N*-methyl-*N*-nitrosourea. Cancer Res 38:1711-1717, 1978

(*30*) HOOK RR, AULTMAN MD, ADELSTEIN EH, et al: Influence of selective breeding on the incidence of melanomas in Sinclair miniature swine. Int J Cancer 24:668-672, 1979

(*31*) GEBHARD K, NIEBAUER F: Presented at the Second European Workshop on Mammalian Melanin Pigmentation, London, 1979

(*32*) ANDERS F, SCHWAB M, SCHOLL E: Strategy for breeding test animals of high susceptibility to carcinogens. *In* Short-Term Tests for Chemical Carcinogens (Stich HF, San RH, eds). New York, Berlin: Springer-Verlag, 1981, pp 399-407

(*33*) MATSUSHIMA T, SUGIMURA T: Experimental carcinogenesis in small aquarium fishes. *In* Tumors in Aquatic Animals (Dawe CJ, Scarpelli DG, Wellings SR, eds), vol 20. Basel: S Karger, 1976, pp 367-379

(*34*) ANDERS F: Tumor formation in platyfish-swordtail hybrids as a problem of gene regulation. Experientia 23:1-10, 1967

(*35*) ANDERS A, ANDERS F: Etiology of cancer as studied in the platyfish-swordtail system. Biochim Biophys Acta 516: 61-95, 1978

(*36*) KALLMAN K: The platyfish, *Xiphophorus maculatus*. *In* Handbook of Genetics (King RC, ed), vol 4. New York: Plenum Press, 1973, pp 81-132

(*37*) ROSEN D: Fishes from uplands and inter-mountain basins of Guatemala: Revisionary studies and comparative geography. Bull Am Nat Hist 162:267-376, 1979

(*38*) RADDA AC: Synopsis der Gattung *Xiphophorus* Heckel. Aquaria 27:39-44, 1980

(*39*) SCHWAB M: Genome organization in the tumor model of *Xiphophorus*. Verh Dtsch Zool Ges 1980:285, 1980

(*40*) SCHOLL A, ANDERS F: Electrophoretic variation of enzyme proteins in platyfish and swordtails. Arch Genet 46: 121-129, 1973

(*41*) KOLLINGER G, SIEGMUND E: Meiosis of species and interspecific hybrids of *Xiphophorus*. Verh Dtsch Zool Ges 1981:206, 1981

(*42*) ANDERS A, ANDERS F, KLINKE K: Regulation of gene expression in the Gordon-Kosswig melanoma system. *In* Genetics and Mutagenesis of Fish (Schröder HJ, ed). New York, Berlin: Springer-Verlag, 1973, pp 33-63

(*43*) SCHWAB M, HAAS J, ABDO S, et al: Genetic basis of susceptibility for development of neoplasms following treatment with *N*-methyl-*N*-nitrosourea (MNU) or X-rays in the platyfish/swordtail system. Experientia 34:780-782, 1978

(*44*) HAAS J: Nachweis somatischer Mutationen im Melanomsystem der lebendgebärenden Zahnkarpfen (Poeciliidae). Thesis, Univ Giessen, Fed Republic of Germany, 1981

(*45*) SCHMIDT CR: Untersuchungen über MNU-induzierte Neoplasmen bei *Xiphophorus*. Abhängigkeit der Induzierbarkeit von Genotyp und Entwicklungsstadium. Thesis, Univ Giessen, Fed Republic of Germany, 1984 (Cand)

(*46*) GORDON M: A genetic concept for the origin of melanomas. Ann NY Acad Sci 71:1213-1222, 1958

(*47*) PRESCOTT DM, FLEXER AS: Cancer — The Misguided Cell. New York: Scribner, 1982

(*48*) ANDERS F, DIEHL H, SCHOLL E: Differentiation of normal melanophores and neoplastically transformed melano-

phores in the skin of fishes of genus *Xiphophorus*. *In* The Skin of Vertebrates (Spearman RI, Riley PA, eds). New York, London: Academic Press, 1980, pp 211-224

(*49*) FÖRSTER W, ANDERS F: Chromosome complements from different subspecies and species of *Xiphophorus*. Zool Anz 19:167-177, 1977 (in German)

(*50*) AHUJA MR, LEPPER K, ANDERS F: Sex chromosome aberrations involving loss and translocation of tumor-inducing loci in *Xiphophorus*. Experientia 35:28-29, 1979

(*51*) CHATTERJEE K, KOLLINGER G, SCHMIDT CR, et al: Cytogenetics of neoplasia of *Xiphophorus*. Cancer Genet Cytogenet 3:195-209, 1981

(*52*) VIELKIND U: Genetic control of cell differentiation in platyfish-swordtail melanomas. J Exp Zool 196:197-204, 1976

(*53*) SICILIANO MJ, WRIGHT DA: Biochemical genetics of the platyfish-swordtail melanoma system. Prog Exp Tumor Res 20:398-411, 1976

(*54*) SCHOLL E: Untersuchungen am Melanomsystem von *Xiphophorus:* Kopplung eines Esterase-Gens (*Est-1*) mit einem Differenzierungsgen (*Diff*). Thesis, Univ Giessen, Fed Republic of Germany, 1980

(*55*) AHUJA MR, SCHWAB M, ANDERS F: Linkage between a regulatory locus for melanoma cell differentiation and an esterase locus in *Xiphophorus*. J Hered 71:403-407, 1980

(*56*) SCHARTL M, SCHARTL A, ANDERS F: Phenotypic conversion of malignant melanoma to benign melanoma and vice versa in *Xiphophorus*. *In* Phenotypic Expression of Pigment Cells (Seiji M, ed). Tokyo: Univ Tokyo Press, 1981, pp 517-524

(*57*) MORIZOT DC, SICILIANO MJ: Linkage of two enzyme loci in fishes of the genus *Xiphophorus*. J Hered 73:163-167, 1982

(*58*) DESS G: Das übermodifizierte Nukleosid Queuosin in Transfer-Ribonucleinsäuren aus Melanomen von *Xiphophorus*. Thesis, Univ Giessen, Fed Republic of Germany, 1982

(*59*) KERSTEN H: Involvement of tRNA and the modified nucleoside queuosine in cell development and differentiation. *In* The Biochemistry of Differentiation and Morphogenesis (Jaenicke L, ed). New York, Berlin: Springer-Verlag, 1982, pp 116-119

(*60*) SCHWAB M, ABDO S, AHUJA MR, et al: Genetics of susceptibility in the platyfish/swordtail tumor system to develop fibrosarcoma and rhabdomyosarcoma following treatment with *N*-methyl-*N*-nitrosourea (MNU). Z Krebsforsch 91:301-315, 1978

(*61*) SCHWAB M, ANDERS A: Carcinogenesis in *Xiphophorus* and the role of the genotype in tumor susceptibility. *In* Neoplasms — Comparative Pathology of Growth in Animals, Plants, and Man (Kaiser HE, ed). Baltimore, London: Williams & Wilkins, 1981, pp 451-459

(*62*) ANDERS F, DIEHL H, SCHWAB M, et al: Contributions to an understanding of the cellular origin of melanoma in the Gordon-Kosswig *Xiphophorus* fish tumor system. *In* Pigment Cell, vol 4. Basel: S Karger, 1979, pp 142-149

(*63*) ANDERS F, KLINKE K: Über Gen-Dosis, Gen-Dosiseffekt und Gen-Dosiskompensation. Verh Dtsch Zool Ges 1966: 391-401, 1966

(*64*) HESTON WE: Genetics of cancer. J Hered 65:262-272, 1974

(*65*) BENTVELZEN P: Genetical Control of the Vertical Transmission of the Mühlbock Mammary Tumor Virus in the GR Mouse Strain. Amsterdam: Hollandia, 1968

(*66*) TODARO GJ, HUEBNER RC: The viral oncogene hypothesis: New evidence. Proc Natl Acad Sci USA 69:1009-1015, 1972

(*67*) EMMELOT P, BENTVELZEN P (eds): RNA Viruses and Host Genome in Oncogenesis. Amsterdam, London: North-Holland, 1972

(*68*) KOLLINGER G, SCHWAB M, ANDERS F: Virus-like particles induced by bromodeoxyuridine in melanoma and neuroblastoma of *Xiphophorus*. J Cancer Res Clin Oncol 95:239-246, 1979

(*69*) STEHELIN D, VARMUS HE, BISHOP JM: DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal DNA. Nature 260:170-173, 1976

(*70*) SPECTOR DH, VARMUS HE, BISHOP JM: Nucleotide sequences related to the transforming gene of avian sarcoma virus are present in DNA of uninfected vertebrates. Proc Natl Acad Sci USA 75:4102-4106, 1978

(*71*) BAUER H, BARNEKOW A, ROSE G: The transforming protein of Rous sarcoma virus, pp60^{src}: Growth and cell proliferation inducing properties. *In* Hormones and Cell Regulation (Dumont JE, Nunez J, Schultz G, eds), vol 6. Amsterdam: Elsevier Biomedical Press, 1982, pp 187-205

(*72*) BAUER H, BARNEKOW A, BOSCHEK CB, et al: The dualism of expression of *onc*-genes: Transformation versus differentiation. *In* The Biochemistry of Differentiation and Morphogenesis (Jaenicke L, ed). New York, Berlin: Springer-Verlag, 1982, pp 74-90

(*73*) BARNEKOW A, SCHARTL M, ANDERS F, et al: Identification of a fish protein associated with kinase activity and related to the Rous sarcoma virus transforming protein. Cancer Res 42:2429-2433, 1982

(*74*) COLLETT MS, PURCHIO AF, ERIKSON RL: Avian sarcoma virus transforming protein, pp60^{src}, shows protein kinase activity specific for tyrosine. Nature 285:167-169, 1980

(*75*) SCHARTL M, BARNEKOW A, BAUER H, et al: Correlations of inheritance and expression between a tumor gene and the cellular homolog of the Rous sarcoma virus-transforming gene in *Xiphophorus*. Cancer Res 42:4222-4227, 1982

(*76*) SCHARTL M, BARNEKOW A: *c-src* Oncogene expression in eukaryotes. Differentiation 23:109-114, 1982

(*77*) ANDERS F: The biology of an oncogene based upon studies on neoplasia in *Xiphophorus*. *In* The Biochemistry of Differentiation and Morphogenesis (Jaenicke L, ed). New York, Berlin: Springer-Verlag, 1982, pp 91-115

(*78*) SHILO BZ, WEINBERG RA: DNA sequences homologous to vertebrate oncogenes are conserved in *Drosophila melanogaster*. Proc Natl Acad Sci USA 78:6789-6792, 1981