XIPHOPHORUS AS AN *IN VIVO* MODEL FOR STUDIES ON NORMAL AND DEFECTIVE CONTROL OF ONCOGENES[1]

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I. Introduction and Historical Background

Oncogenes are genes that code for neoplastic transformation and possibly for the maintenance of the neoplastic state of a cell. Although they have been

[1]Dedicated to Peter Karlson on the occasion of his 65th birthday.

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deduced from the inheritance of certain tumors many decades ago by geneticists (see Strong, 1958; Lynch, 1967; Bauer, 1968; Heston, 1974), their general significance for tumor development remained widely unnoticed until they became identified as constituents of the genomes of tumor viruses and cells in recent years by virologists (see Bishop, 1982a,b).

The history of the discovery of the oncogenes considered in this article can be traced back to the year 1928, when Kurt Kosswig in Muenster, Georg Haeussler in Heidelberg, and Myron Gordon in New York found that certain hybrids between the ornamental platyfish (*Xiphophorus maculatus*) and the ornamental swordtail (*Xiphophorus helleri*) are capable of developing melanoma spontaneously. Subsequently these authors showed that the melanomas of the hybrids originated from black spots that were inherited from the platyfish following Mendelian laws. Both the spots and the melanomas were formally assigned to Mendelian genes which were designated as "color genes" (because of the black coloration of the spots and of the melanomas), or "macromelanophore genes" (because of the giant pigment cells found in the spots and in the melanomas). The enhancement of the spots to melanomas was initially believed to be the result of enhanced color gene expressivity in the pigment cells exerted by "intensifier genes" that were assumed to be contributed to the hybrid genome by the swordtail. Thus, positive control of the color genes was believed to be the major cause for melanoma formation in *Xiphophorus* (Kosswig, 1929, 1937; Gordon, 1931, 1958). Sporadically occurring hybridization-conditioned tumors involving tissues other than the pigment cell system, e.g., ocular tumors (Gordon, 1947b), thyroid tumors (Aronowitz *et al.*, 1951; Berg *et al.*, 1953), and kidney tumors (Baker *et al.*, 1953), were difficult to interpret in terms of formal genetics.

Our group started its research on cancer in the platyfish–swordtail system in 1957 when Gordon and Kosswig provided us with some of their fish stocks. During the following 10 years it became clear that development of melanoma and some other types of neoplasia in the fish is due to a certain gene that is endowed with the capacity to mediate neoplastic transformation (F. Anders, 1967). This gene is an oncogene by definition (A. Anders *et al.*, 1973a,b; see Heston, 1974), and was designated as "tumor gene" (*Tu*) (F. Anders *et al.*, 1974). In contrast to the initial concept of positive control of "color genes" mentioned above it was shown that *Tu* is normally under negative control by certain linked and nonlinked regulatory genes (*R* genes). Following hybridization, chromosomes carrying nonlinked *R* genes may be replaced by chromosomes lacking them: the oncogene *Tu*, then may become derepressed to a certain degree and may mediate neoplasia. Thus, spontaneous tumor formation in platyfish–swordtail hybrids was finally recognized as a problem of negative regulation of an oncogene (F. Anders, 1967). Some positive control on tumor growth detected in the derepressed *Tu* system could be related to metabolites such as amino acids which may serve as nutrient factors in the transformed cells (F. Anders *et al.*, 1962b, 1963, 1969; F. Anders and Klinke, 1965; M. Sieger *et al.*, 1968; F. Sieger *et al.*, 1969), and to hormonal influences (Siciliano and Perlmutter, 1972; A. Anders *et al.*, 1973a; A. Schartl *et al.*, 1982).

During the second decade of our studies we found that *Tu* is not only responsible for the spontaneous development of neoplasms in hybrids but also for germ line mutation-conditioned tumors as well as for the large variety of neurogenic, epithelial, and mesenchymal neoplasms (Abdo, 1979) that can be triggered in somatic cells by mutagenic (A. Anders *et al.*, 1973a; Schwab *et al.*, 1978a,b, 1979; Schwab and A. Anders, 1981) and chromatin-damaging agents (F. Anders *et al.*, 1981a), and by tumor promoters (A. Schartl *et al.*, 1982).

While genetically conditioned and environmentally triggered neoplasia in *Xiphophorus* became rather well understood in terms of formal genetics, population genetics, cytogenetics, and developmental genetics (A. Anders and F. Anders, 1978; F. Anders, 1981; F. Anders *et al.*, 1981b; Prescott and Flexer, 1982), its molecular basis remained extremely resistant to any elucidation.

New directions for our research arose when we related the concept of the oncogene of endogenous viruses freshly developed by Bentvelzen (1972) to our earlier concept of the tumor gene in the fish genome (see the discussions in A. Anders *et al.*, 1973a; Heston, 1974; Kollinger *et al.*, 1979). The appropriate experiments were undertaken once tumor virologists realized that the oncogenes of certain tumor-mediating retroviruses such as the *src* oncogene from Rous sarcoma virus (RSV) of chicken are also present in the noninfected cells of the host organisms which are not taxonomically related to the host (see Bishop, 1982a,b). In *Xiphophorus* the cellular counterparts (*c-onc* genes) of 10 different avian and mammalian retroviral oncogenes (*v-onc* genes) were detected in a cooperative work of the laboratories of the Genetics Institute (Giessen) with the laboratories of H. Bauer (Giessen) and R. Gallo (Bethesda), by M. Schartl, A. P. Czernilofsky, and G. Franchini (see F. Anders, 1982; M. Schartl and Barnekow, 1982). One of these *onc*-genes, namely *c-src* (the cellular counterpart of the oncogene of Rous sarcoma virus, *v-src*), was studied in more detail and its expression could be correlated with that of the oncogene *Tu* (Barnekow *et al.*, 1982; M. Schartl *et al.*, 1982; Bauer *et al.*, 1982; F. Anders, 1982, 1983; F. Anders *et al.*, 1983).

The present article aims to unify our populational, morphological, developmental, and cell biological findings obtained during research on the biology of the oncogene *Tu* of *Xiphophorus*. Furthermore, it aims to show that neoplasia of multicellular animals including humans results from elimination, deletion, impairment, or insufficiency of regulatory genes that nor-

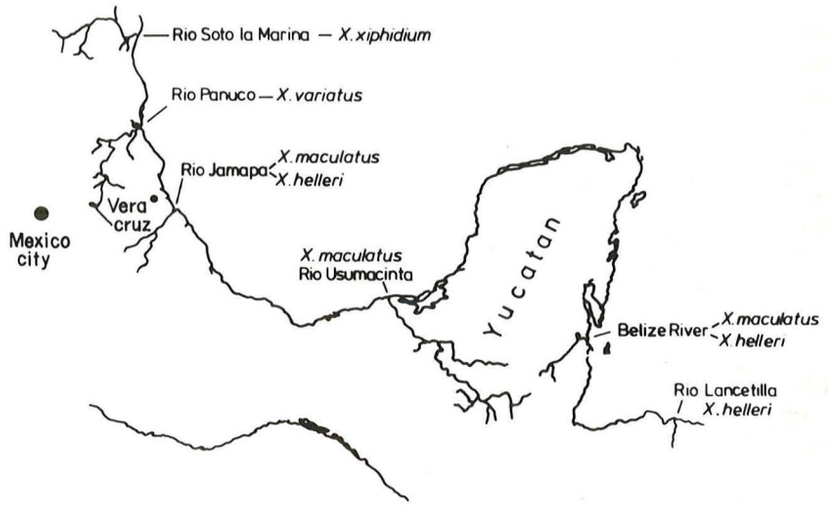


FIG. 1. Map of Mexico and adjacent parts of Central America showing the distribution of these species of *Xiphophorus* which were mainly used in this study. For details see Kallman (1975).

mally control the oncogene, or from the introduction of uncontrolled accessory oncogenes into the genome.

Many earlier and recent findings cited in this article were to date unpublished.

II. Ubiquity of Oncogenes in Purebred Animals Derived from Wild Populations

We shall first report some basic facts on *Xiphophorus* (see Rosen and Bailey, 1963; Kallman, 1975) that are important for an understanding of the model character of our system for studies on neoplasia in general.

A. Biology and Taxonomy of *Xiphophorus*

Xiphophorus is a viviparous genus of topminnows inhabiting ditches, rivers, lakes, brooks, ponds, and pools in the Atlantic coastal drainage systems from northern Mexico southeast to northern Honduras (Fig. 1). The peninsula of Yucatan is not inhabited by these animals indicating that the ancestors of the recent genus *Xiphophorus* were already present in the pliocenic Central America before Yucatan emerged from the sea.

Xiphophorus reproduces in closed populations and has evolved into innu-

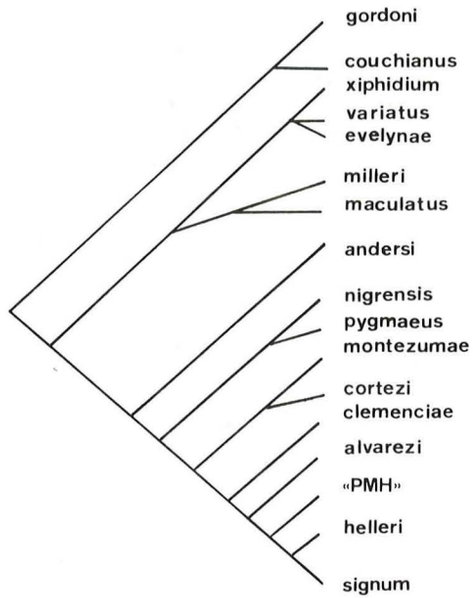


FIG. 2. Cladogenetic divergence of the presently described species of the genus *Xiphophorus*. From Radda (1980), modified.

merable, phenotypically distinguishable groups which are isolated geographically or ecologically (see Atz, 1962; Zander, 1967). Seventeen of these groups which differ clearly from each other by their phenotype have been classified as species (Rosen, 1979; Radda, 1980) (Fig. 2). They can be arranged in species groups, as follows: the *maculatus* species group (the platyfish), e.g., *X. maculatus* (Fig. 3A), *X. variatus*, and *X. couchianus;* the *helleri* species group (the swordtails), e.g., *X. helleri* (Fig. 3B), *X. clemenciae*, and *X. signum;* and the *montezumae* species group (the montezuma swordtails), e.g., *X. cortezi*, *X. montezumae*, and *X. pygmaeus*. Some of these species are endemic to only an extremely restricted area, occupying at best a few spring pools or lakes; others inhabit a single river system; and still others, such as *X. maculatus* and *X. helleri* which are the main species of this research, can be found from the Rio Jamapa (Mexico, near Veracruz; northwest from Yucatan), eastward to the Belize River (British Honduras; southeast from Yucatan), which represents a distance of over 3500 km (Fig. 1). Some species, e.g., *X. maculatus* and *X. helleri*, are sympatric, for instance in the Belize River and in the Rio Jamapa; but interspecific hybrids have never been found in the natural habitat (see Kallman and Atz, 1967; Zander, 1967).

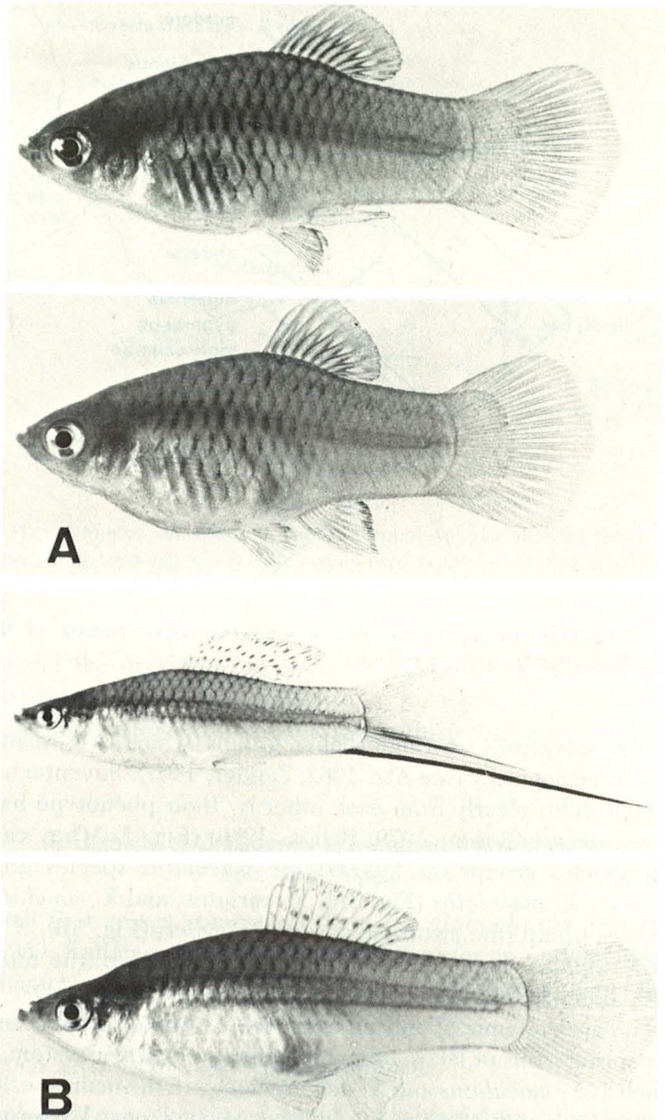


FIG. 3. Male (top) and female (bottom) of (A) *Xiphophorus maculatus* (from a population of the Rio Usumacinta) and (B) *Xiphophorus helleri* (from Rio Lancetilla) (up to about 8 cm in length).

The animals of all taxonomic groups of *Xiphophorus* can be hybridized in the laboratory, and all hybrids are fertile. This, together with the findings that the degree of enzyme polymorphism is low (A. Scholl, 1973; A. Scholl and F. Anders, 1973a; E. Scholl, 1977; Morizot and Siciliano, 1982), that pairing of the chromosomes in the hybrids during meiosis is normal (Siegmund, 1982; Kollinger and Siegmund, 1981), and that genome organization shows conformity in all cases tested (Schwab, 1982b; Herbert, 1983), led us to the conclusion that the relationship between these taxonomic groups known as species in the literature, actually is at the level of geographical and ecological populations and races comparable to the populations and races of most of the wild animal species and the human species (F. Anders *et al.*, 1981b). Even the large morphological differences between *X. helleri* and *X. maculatus* (compare Fig. 3A with 3B) that culminate in the development or lack, respectively, of a "sword" (an elongation of the lower part of the tail fin in the adult males) is of minor taxonomic value: following treatment of newborn *X. maculatus* with methyl-testosterone a sword develops indicating that even the sword-lacking fish carry the genetic information for the development of the sword like their sword-carrying taxonomic counterparts (Dzwillo, 1964; A. Schartl, 1981).

B. Insusceptibility of Animals from Wild Populations to Development of Neoplasia

Tens of thousands of individuals from wild populations of the different taxonomic groups of *Xiphophorus* have been collected by several authors (Gordon, Kallman, Borowsky, Siciliano, Zander, and ourselves), but no tumors were detected. Furthermore, almost all species listed in Figs. 1 and 2 have been inbred or bred in closed stocks in our laboratories (e.g., *X. helleri* from Rio Lancetilla and *X. maculatus* from Rio Jamapa, collected by Gordon in 1939; inbred for about 90 and 130 generations, respectively), but no tumors developed.

The pure-bred descendants of the wild populations also proved to be highly insensitive to carcinogens such as X rays (A. Anders *et al.*, 1971, 1973a,b; Pursglove *et al.*, 1971; Pursglove, 1972; Haas, 1981), Benzo(*a*)pyrene (Maas, 1967), *N*-methyl-*N*-nitrosourea (MNU) (Schwab *et al.*, 1978a,b; C. R. Schmidt, 1983), *N*-ethyl-*N*-nitrosourea (ENU), diethylnitrosamine (DENA), dimethyl sulfoxide (DMSO) (C. R. Schmidt, 1983; Herbert, 1983), and to tumor promoters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Schwab, 1982a; C. R. Schmidt, 1983; Herbert, 1983), 17-methyltestosterone, and other steroids (A. Schartl, 1981; A. Schartl *et al.*, 1982) as well as to potential tumor promoters such as saccharine, cyclamate and diazepam (C. R. Schmidt, 1983; Herbert, 1983). The first part of Table I

TABLE I

NEOPLASIA IN *Xiphophorus* 1 YEAR AFTER TREATMENT WITH MNU[a] AND X RAYS[b]

	Number of survivors		Number of neoplasms	
	MNU	X Rays	MNU	X Rays
Pure-bred				
X. maculatus (Rio Jamapa)	410	3405	0	0
X. variatus (Rio Panuco)	~100	~500	0	0
X. xiphidium (Rio Soto la Marina)	~100	~100	0	0
X. helleri (Rio Lancetilla)	415	~2000	0	0
X. cortezi (Rio Axtla)	~100	~100	0	0
		~7200	0	
Hybrids				
F_1	470	~1000	18(4%)	0
F_2–F_n; BC_1–BC_{24}	8258	3587	826(10%)	163(5%)
		~13,500	1007 (7.5%)	

[a] 10^{-3} *M*; four times for 1 hr in 2-week intervals.

[b] 1000 R; three times for 45 min in 6-week intervals.

summarizes material, methods, and results of a recent broad-scale treatment experiment of this kind: about 7200 pure-bred individuals survived treatment with X rays and MNU, but none developed neoplasia (for germ line mutation-conditioned neoplasms that develop in the offspring of the treated wild animals see Section IV,A,2).

C. Occurrence of Neoplastically Transformed Cells in Animals from Wild Populations

While neoplasms have never been observed in wild *Xiphophorus* there are strong indications that neoplastic transformation of a certain number of cells is a common process in these animals (F. Anders *et al.*, 1980). Up to the present, however, this process could only be determined in pigment cells of the skin which, because of their natural pigmentation, can easily be observed *in vivo*.

Evidence for transformation of pigment cells in the nontumorous wild fish comes from studies on certain population-specific and species-specific patterns of black spots (Fig. 4). Gross inspection of the spots showed that they are composed of abnormal melanophores which differ from the regular melanophores of the skin by their enormous size and their heavy black pigmentation (Figs. 5 and 6). Gordon (1958) has considered them as an additional type of melanophore in the pigment cell system, namely the "macromelanophore," which develops following a process of "macromelanophore differentiation." Out of 9000 adults of *X. maculatus* collected by him, 1879 had these macromelanophore spots.

Morphological, ultrastructural, biochemical, and developmental studies revealed similarities and differences between the regular and the abnormal melanophores which led to the disclosure of the true nature of the abnormal melanophores and of the spots.

The *regular melanophores* of the skin (Fig. 5) can easily be recognized by their lobulated or dendritic shape and by their content of melanin (Becker-Carus, 1965; Lueken *et al.*, 1973; E. R. Schmidt, 1978). They contain completely melanized melanosomes but lack other cytoplasmic structures such as the endoplasmatic reticulum and the Golgi complexes (Weissenfels *et al.*, 1970; U. Vielkind, 1972). Tyrosinase activity has not been found in the melanophores. They do not proliferate. These features suggest that the melanophores have reached the final stage of pigment cell differentiation. After having reached a certain age, they are removed by macrophages and become replaced by younger melanophores (see Fig. 8).

It is important to note that the melanophores are spaced singly and keep a certain distance between each other (Lueken and Kaeser, 1972; Lueken *et al.*, 1973; E. R. Schmidt, 1978). One can assume, therefore, that the dis-

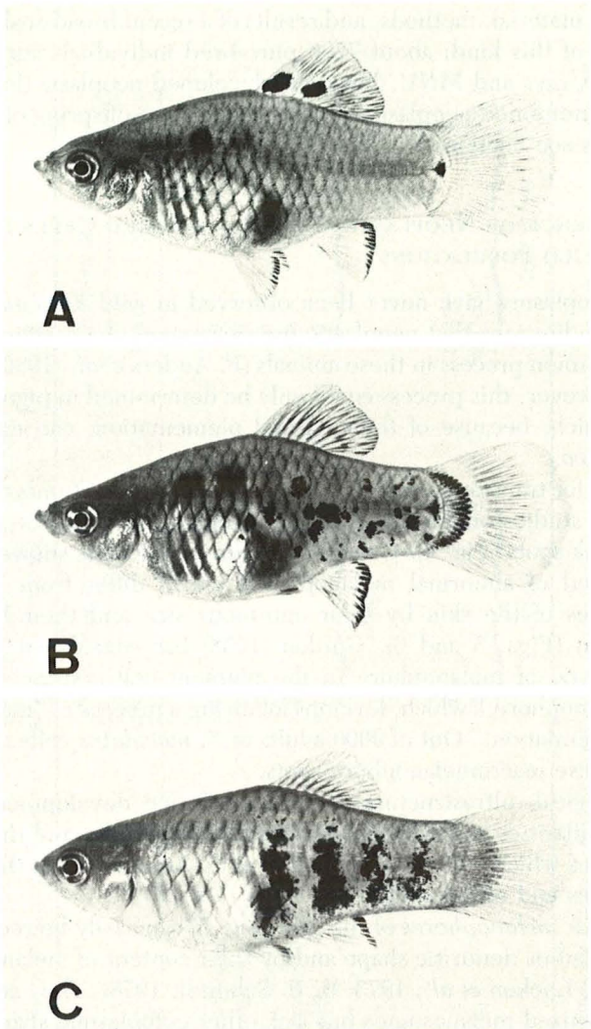


FIG. 4. Patterns of spots in *Xiphophorus* consisting of terminally differentiated neoplastically transformed pigment cells. (A) "Spotted dorsal" of *X. maculatus* from Rio Jamapa; (B) "Spotted" pattern of the same population; (C) "Nigra" of *X. maculatus* from Belize River; (D) "Lineatus" and "Punctatus" patterns of *X. variatus* from Rio Panuco; (E) "Flecked" of *X. xiphidium* from Rio Soto La Marina; (F) "Dabbed" of *X. helleri* from Belize River. See Figs. 1 and 2.

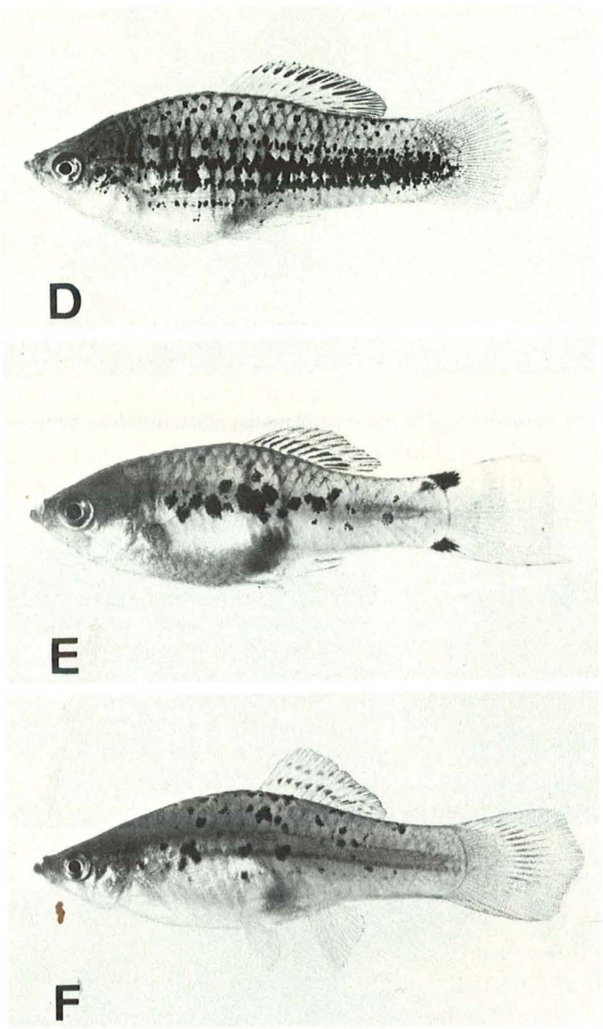


FIG. 4D–F.

tances between the particular melanophores are controlled by a mechanism like density-dependent regulation in the sense proposed by Holley (1975) for control of cell growth in cultures.

The *abnormal melanophores* of the spots of the skin (Fig. 6A and B), also lobulated and heavily pigmented, can easily be recognized by their enor-

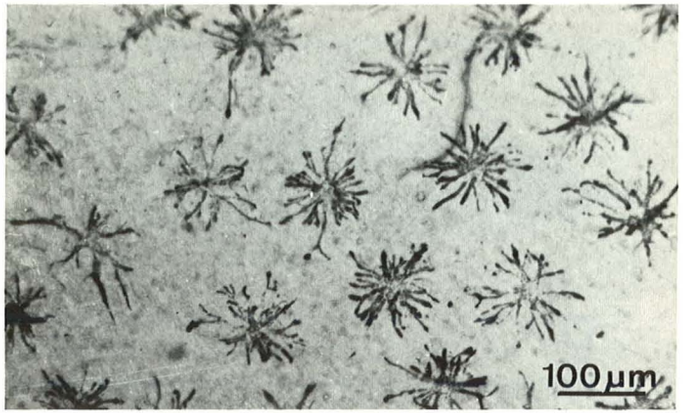


FIG. 5. Regular melanophores in the skin. Note the equal distances between the cells.

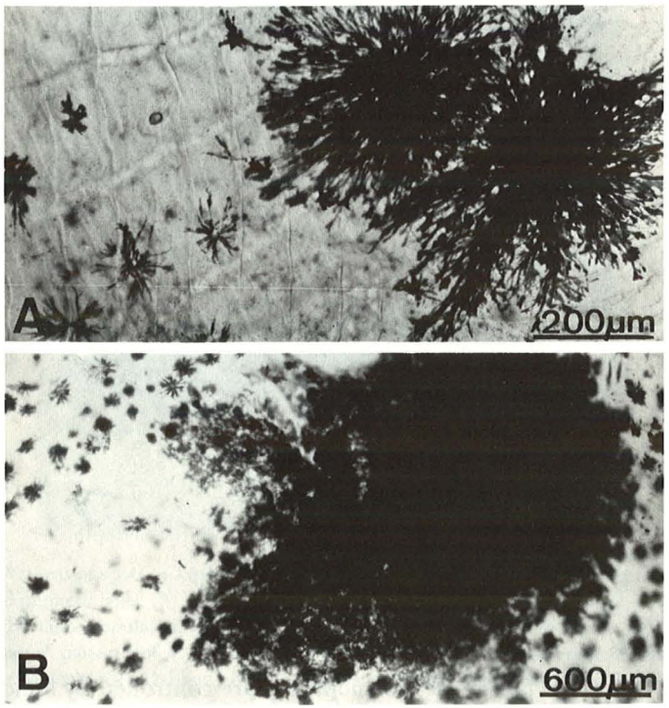


FIG. 6. The accumulations of neoplastically transformed melanophores (Tr melanophores) in the skin. (A) Two interlacing Tr melanophores in the early development of a spot; note the surrounding distance-regulated regular melanophores. (B) Spot, composed of many Tr melanophores.

mous size as compared to that of the normal melanophores (Breider and Seeliger, 1938; F. Anders *et al.*, 1980). They are multinucleated and highly endopolyploid, and can enlarge up to 0.5 mm in diameter. Like the regular melanophores and abnormal melanophores are not able to divide, show no tyrosinase activity (Schlage, 1978; U. Vielkind *et al.*, 1977), contain only mature melanosomes, and lack the other cytoplasmatic structures (U. Vielkind, 1972, 1976) indicating that they have also reached their final stage of cell differentiation. After they have reached a certain age, they are also removed by macrophages and replaced by younger abnormal melanophores (Lueken *et al.*, 1973; U. Vielkind, 1976; E. R. Schmidt, 1978; Diehl, 1982) (see Fig. 8, Tr cells). The radically different feature of the abnormal melanophores as compared to the regular melanophores is, however, that they are not subjected to distance-dependent regulation. Their lobules and dendrites interlace (Fig. 6A) and the cells grow onto each other, thus forming compact three-dimensional accumulations of some hundred cells each, which appear as the heavily pigmented black spots in the skin (Fig. 6B).

As generally accepted by tumor cell biologists, normal cells after having reached a certain density become terminally differentiated and stop dividing, whereas tumor cells are not regulated by density dependency; they remain poorly differentiated and continue to proliferate (see Pierce and Wallace, 1971; Holley, 1975; Prescott, 1976; Prescott and Flexer, 1982). Dysfunction of density-dependent regulation is a fundamental process underlying the change of a cell from the normal to a neoplastic state. The abnormal pigment cells of *Xiphophorus*, however, although not regulated by density dependency or distance dependency, respectively, are completely differentiated and are incapable of dividing further. These facts suggest that the early pigment cells, after being neoplastically transformed, become restrained from proliferation by genes that constrain them to differentiate to the final stage of abnormal melanophores. Dysfunction of distance-dependent regulation of the abnormal pigment cells, therefore, appears reminiscent of an early transformation event that was posttransformationally "neutralized" by cell differentiation. We will show later that differentiation of the transformed cells is exerted by a certain "differentiation gene" (see Section IV,D).

There are also indications for neoplastic transformation of pigment cells in nonspotted animals, for instance in *X. maculatus* from Rio Usumacinto. Studies on late embryos and neonates of these animals have shown that during the time in which the immune system matures, single heavily pigmented cells that occur in the skin are rejected as pigmented aggregates of dead cell masses from the surface of the skin (Fig. 7) (M. Schartl, 1979). This process is interpreted at present as an autoimmune rejection of transformed pigment cells. It can be suppressed by the immune-suppressive steroid

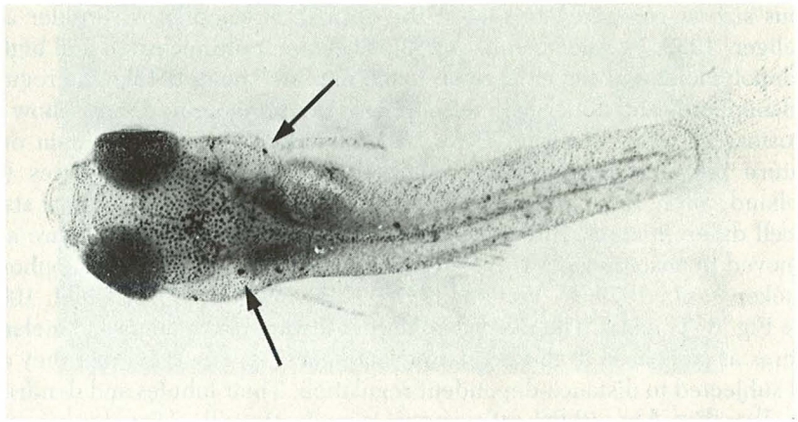


FIG. 7. Rejected aggregates of dead pigment cell masses (arrows) presumably originating from transformed cells. The fish is a neonate of *X. maculatus* from Rio Usumacinto. Adults are shown in Fig. 3A.

prednisolon. Treatment with testosterone (A. Schartl, 1981) or BUdR (Haas-Andela, 1978) leads to an acceleration of the rejection of the melanin aggregates.

D. Competence for Neoplastic Transformation

The occurrence of neoplastically transformed melanophores (Tr melanophores; all transformed cells are designated Tr cells in the following sections) in the fish raises the question of the stage of differentiation in which the pigment cells are capable of changing from the normal state to the neoplastic state. This stage of cell differentiation is considered the competent stage for neoplastic transformation. To detect this stage we studied first the normal differentiation of the pigment cells. According to Fitzpatrick and Lerner (1953) and Bagnara and Hadley (1973), we denote the very early common precursors of all types of pigment cells as *chromatoblasts*, the embryonic cells potentially capable of producing melanine as *melanoblasts*, the mature but not yet terminally differentiated melanin-producing cells as *melanocytes*, and the terminally differentiated melanin-containing cells as *melanophores* (for these latter cell-types see Section II,C).

The pigment cells of vertebrates originate from neural crest cells (DuShane, 1938; Weston, 1970). In *Xiphophorus* (see Fig. 8) (Humm and Young, 1956; F. Anders *et al.*, 1979a; Diehl, 1982) at the fourth day of embryonic life the neural crest cells start migrating. Those entering their

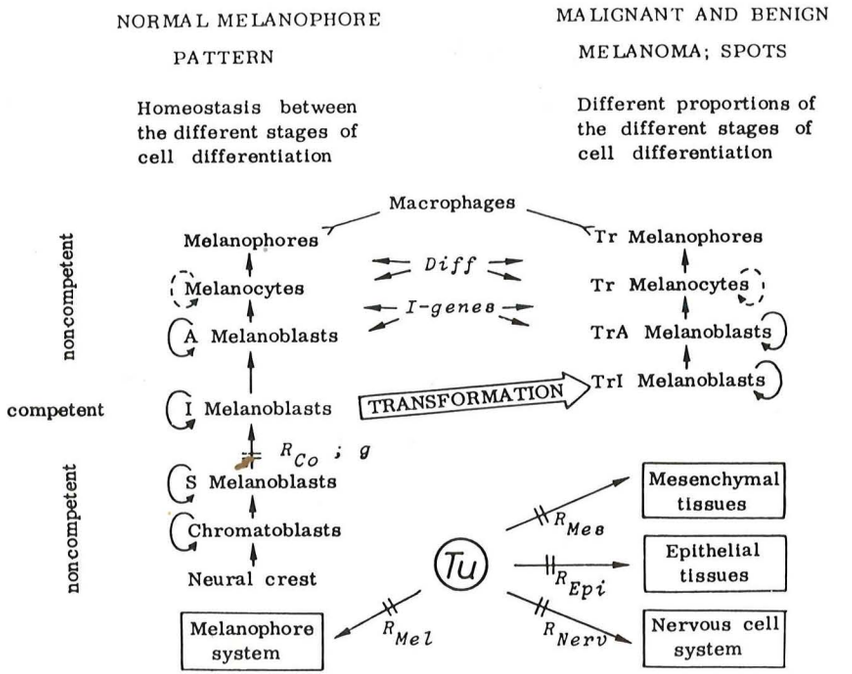


FIG. 8. Schematic presentation of the differentiation of normal and neoplastically transformed pigment cells. S, I, and A melanoblasts are stem, intermediate, and advanced melanoblasts, respectively. The Tr cells represent the transformed cells. Only I melanoblasts are competent for neoplastic transformation. *Tu*, tumor gene (oncogene) (see Section IV,A); R_{Mel}, regulatory gene for control of *Tu* in the melanophore system (see Section IV,B); R_{Nerv}, R_{Epi}, R_{Mes}, regulatory genes controlling *Tu* in the nervous cell system, the epithelial tissues and the mesenchymal tissues, respectively; R_{Co}, compartment-specific regulatory genes (see Section IV,C); *g*, "golden" gene that blocks pigment cell differentiation; *Diff*, differentiation gene (see Section IV,D); I genes, intensifier genes, which support proliferation of poorly differentiated transformed pigment cells. Macrophages attack melanophores and Tr melanophores. From A. Anders and F. Anders (1978), modified.

final locations, including the corium of the skin and the extracutaneous connective tissues of the peritoneum, pericardium, dura mater, fascia abdominalis, chorioidea, and vascular structures (Peter, 1982, 1983), become determined to differentiate to pigment cells. These cells are considered to be *chromatoblasts*, which are the common precursors of all pigment cells (*chromatophores*) including pterinophores, purinophores, and melanophores. Those chromatoblasts committed to differentiate to melanophores give rise to stem cells for melanophore differentiation (*S melanoblasts*). These may reproduce throughout the life of the fish, but may also differenti-

ate to an intermediate stage of melanoblasts (*I melanoblasts*). All cells that have reached this stage continue differentiation to the most advanced stage of melanoblasts (A melanoblasts). These cells can be distinguished from their precursors by their reaction to dopa. The first A melanoblasts occur in the skin of the 5-day-old embryos. They may differentiate within about 15 hr to *melanocytes*. The melanocytes differentiate to *melanophores*, the first of which occur in the 11-day-old embryo. In contrast to their precursors, they do not divide and are removed by macrophages after they have reached a certain age. A homeostasis exists between the different stages of melanophore differentiation, which is controlled by distance-dependent regulation (see Section II,C).

Evidence for the restriction of competence for neoplastic transformation to a certain stage of pigment cell differentiation comes from two lines of observations (F. Anders *et al.*, 1972). (1) A certain mutant (golden *gg*) (A. Anders *et al.*, 1973a), in which the melanophore differentiation is almost completely blocked at the stage of the S melanoblasts (see *g* in Fig. 8), fails to differentiate Tr cells, indicating that S melanoblasts as well as earlier stages (chromatoblasts, neural crest cells) are noncompetent for neoplastic transformation. (2) A melanoblasts, melanocytes, and melanophores, which can easily be recognized, have never been found to undergo neoplastic transformation. These cells appear to be too advanced in the normal process of differentiation, and therefore have lost the competence for transformation.

The cells competent for neoplastic transformation, therefore, are the I melanoblasts.

The I melanoblasts, after being transformed to TrI melanoblasts, differentiate to the easily recognizable, proliferating TrA melanoblasts. These Tr cells differentiate to the heavily pigmented, endopolyploid Tr melanocytes which proceed to the terminal stage of differentiation of transformed pigment cells, represented by the abnormal melanophores (see Section II,C), i.e., the Tr melanophores.

As will be shown later, spots and melanomas differ in the different proportions of Tr cells found in the different stages of pigment cell differentiation: Tr melanophores represent the predominant cells of the spots and the benign melanomas while TrA melanoblasts and Tr melanocytes represent the majority of cells of the malignant melanoma.

We assume that competence to neoplastic transformation is, in general, restricted in all organisms to a certain stage of cell differentiation in any one of the organisms systems, and that Tr cells in any case may differentiate to a terminal stage, in which they, although still neoplastically transformed, are no longer deleterious to the organism; this assumption is supported by many observations from under systems (see Pierce and Wallace, 1971; Sachs, 1978, 1982).

E. Cellular Homologs of Retroviral Oncogenes in Animals from Wild Populations.

Our interest in the cellular homologs of retroviral oncogenes in *Xiphophorus* came from several loosely connected lines of observations, and from the literature: (1) the ubiquity of transformed cells in all individuals of the fish (Section II,C), (2) the inheritance of genetic information for neoplastic transformation (Sections III,C,D), and (3) the identification of *c-src*, i.e., the cellular homolog of the Rous sarcoma virus transforming gene, in the genome of various vertebrates such as uninfected chicken (Stehelin *et al.*, 1976), mouse, calf, humans, and salmons (Spector *et al.*, 1978). These facts raised the question whether cellular oncogenes (*c-onc* genes), equivalent or even homologous to retroviral oncogenes (*v-onc* genes), are ubiquitously present in *Xiphophorus*, and whether they play any role in neoplasia.

The cellular counterparts of all 10 *v-onc* genes tested by molecular hybridization have been detected in DNA from *X. helleri* and *X. maculatus* with *v-onc* probes (Fig. 9) carried out by M. Schartl and Czernilofsky (1982) and M. Schartl and Franchini (1982) (see F. Anders, 1982; M. Schartl and Barnekow, 1982). From one of these *c-onc* genes, namely the *c-src*, the RNA transcript was identified (M. Schartl and Franchini, 1984), and a phosphoprotein was detected that, as shown by its antigenic, biophysical, and enzymatic properties is closely related to the transforming protein of the RSV *v-src* oncogene ($pp60^{v\text{-}src}$) (Fig. 10): it was immunoprecipitated with antiserum against $pp60^{v\text{-}src}$, has an estimated molecular weight of 60,000, and is associated with a kinase activity which phosphorylates the tyrosine residue in the heavy chain of anti-$pp60^{src}$ IgG. This phosphoprotein is, therefore, assumed to be the product of *c-src* of the fish, i.e., the $pp60^{c\text{-}src}$ (Barnekow *et al.*, 1982).

As measured by means of the $pp60^{c\text{-}src}$ kinase assay according to Collet and Erikson (1978) (see Collet *et al.*, 1980) (Fig. 11), *c-src* is active in all individuals of all wild populations of the different taxonomic groups of *Xiphophorus* tested (Table II). Brain tissue shows always a considerably higher kinase activity as compared to that of skin, liver, spleen, and testes. Muscle tissue showed no or a barely detectable kinase activity (Fig. 12).

The fact that the oncogenes *c-src* is active in the nonneoplastic cells of the pure-bred animals indicates that neither the oncogene itself nor its product is necessarily correlated with tumor development (Barnekow *et al.*, 1982; M. Schartl *et al.*, 1982).

F. The *c-src* Oncogene in the Animal Kingdom

The identification of *c-src* in the genome of various vertebrates such as chicken (Stehelin *et al.*, 1976), mouse, calf, humans, salmon (Spector *et al.*,

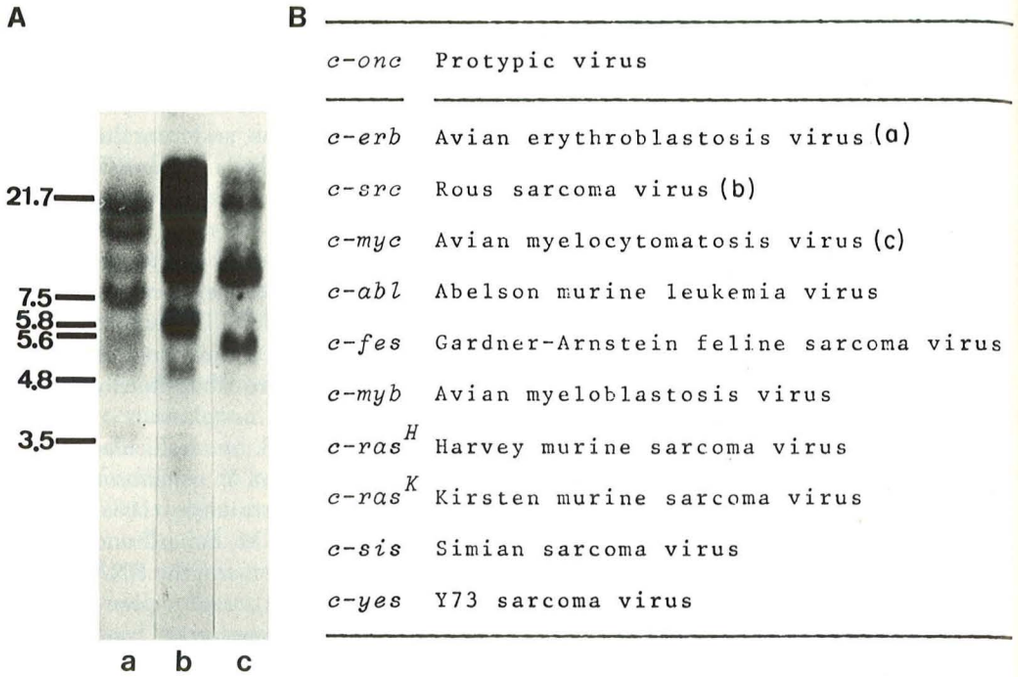


FIG. 9. Cellular homologs of viral oncogenes detected in *Xiphophorus*. (A) Southern blots of *Xiphophorus* total genomic DNA digested with *Eco*RI, hybridized to nick-translated viral *onc* probes (a, *v-erb;* b, *v-src;* c, *v-myc*). (B) *c-onc* genes so far found in *Xiphophorus*. Viral *onc* probes were gifts from R. C. Gallo and K. Toyoshima, in collaboration with G. Franchini.

1978), and four different species of *Xiphophorus* from eight different localities (Barnekow *et al.*, 1982; M. Schartl *et al.*, 1982) (Table II) led us to a more systematic search for this oncogene in additional taxonomic groups of the animal kingdom, and in the plant kingdom. First, different fish genera of the family of *Poeciliidae* that are more or less taxonomically related to *Xiphophorus* were investigated. All fish tested showed a pp60$^{c\text{-}src}$ kinase activity indicating that *c-src* is present in their genome (Barnekow *et al.*, 1982). Second, *c-src* has been detected partly by molecular hybridization, partly by serological identification of pp60$^{c\text{-}src}$, and partly by the tyrosine kinase activity of pp60$^{c\text{-}src}$ in a large variety of other metazoa ranging from mammals, coelenterates (Fig. 13), down to the sponges (Schartl and Barnekow, 1982). Our results together with those of Spector *et al.* (1978) obtained with humans, calf, and salmon and those of Shilo and Weinberg (1981) obtained with *Drosophila* are listed in Table III.

All unicellular eukaryotes tested, including the protists *Euglena, Cryptomonas, Chlorogonium, Paramecium, Tetrahymena* as well as the colony forming *Volvox*, were negative for the tyrosine phosphorylating kinase activity. The same is true for *Trichoplax adhaerens*, which is regarded to represent an intermediate form between the protozoan and metazoan organization. Algae and higher plants also showed no kinase activity.

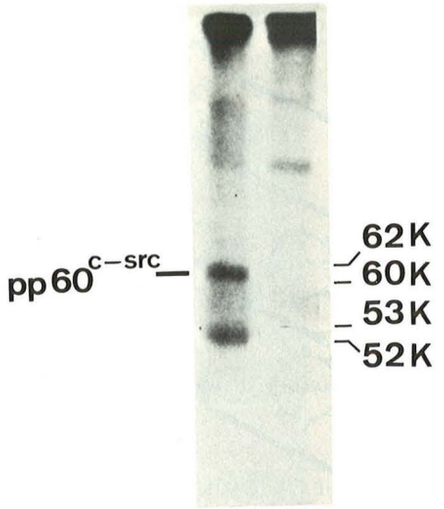


FIG. 10. Demonstration of ^{32}P-labeled pp60$^{c\text{-}src}$ in fish brain extracts. Left track: Immunoprecipitation of fish brain extracts, labeled before with [^{32}P]P_i *in vitro*, with antisera from RSV tumor-bearing rabbits (TBR serum). The M_r 50,000 protein is presumably a degradation product of pp60$^{c\text{-}src}$. Right track: Immunoprecipitation of the same extracts with normal rabbit serum. Both 60K and 52K proteins could not be detected. From Barnekow *et al.* (1982).

The ubiquity of *c-src* in metazoa raises the idea that this homolog of the viral oncogene *v-src* might have still unknown basic functions closely related to the evolution of the multicellular organization of the animals, and that neoplasia might be a character that is closely related to this evolution. As the sponges are known to have evolved in the proterozoicum, the origin of the *c-src* oncogene has to be estimated at over 1.5×10^9 years ago.

At present several groups are trying to determine whether a cellular oncogene, such as *c-src*, is capable of mediating neoplastic transformation like its viral counterpart (Oskarsson *et al.*, 1980; Pulciani *et al.*, 1980; Reddy *et al.*, 1982; de Feo *et al.*, 1981; Takeya and Hanafusa, 1982; de Klein *et al.*, 1982; Tabin *et al.*, 1982). If this should be proven we suggest that all individuals of all metazoa are endowed with the capacity to develop neoplasia. Support for this idea comes from the fact that neoplasia is distributed—although sporadically—in all groups of multicellular animals (Huxley, 1958;

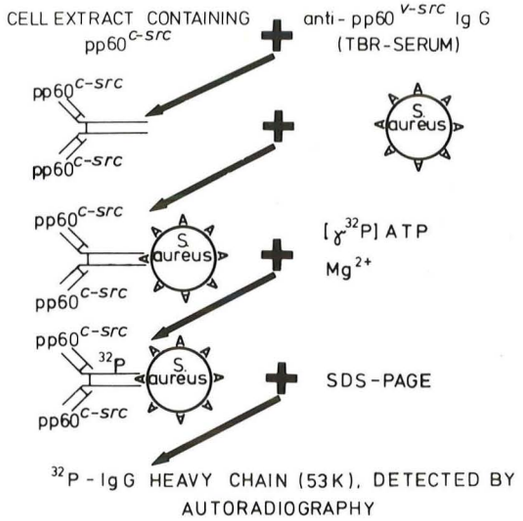


FIG. 11. Assay for pp60$^{c\text{-}src}$ kinase activity according to Collet and Erikson (1978). See Barnekow *et al.* (1982).

Dawe and Harshbarger, 1969, 1975; Krieg, 1973; Kraybill *et al.*, 1977; Dawe *et al.*, 1981; Kaiser, 1981). Ubiquity of oncogenes in the wild animals on the one hand and the infrequent occurrence of neoplasia in these animals on the other hand raises the question of the mechanisms that protect the majority of the individuals of all metazoans from the action of their own oncogenes (F. Anders, 1981; F. Anders *et al.*, 1979a,b, 1981b). The *Xiphophorus* model provides an opportunity to contribute to the study of this problem. This

TABLE II
EXPRESSION OF pp60$^{c\text{-}src}$-ASSOCIATED KINASE IN BRAIN EXTRACTS OF *Xiphophorus* OF DIFFERENT PROVENANCE

Species	Population	Kinase activity[a]
X. helleri	Belize River	+++
X. helleri	Rio Lancetilla	+++
X. maculatus	Belize River	+++
X. maculatus	Rio Jamapa	++
X. maculatus	Rio Usumacinta	++
X. cortezi	Rio Axtla	+++
X. variatus	Rio Coy	++
X. variatus	Rio Panuco	++

[a] ++, 100–200 cpm/mg protein; +++, 200–400 cpm/mg protein.

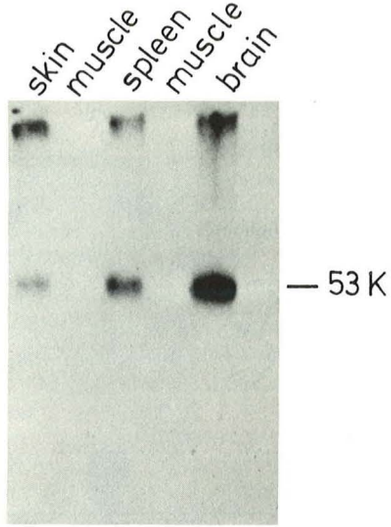


FIG. 12. Demonstration of pp60$^{c\text{-}src}$ associated protein kinase activity in extracts of different fish organs. 53K = ^{32}P-labeled IgG heavy chain, detected by autoradiography (see Fig. 11). Autoradiograms show equal amounts of protein for each sample. Note that brain shows the highest kinase activity. See Fig. 28.

contribution comes from studies on defective control of the oncogenes which has been observed in certain hybrids (see Section III).

III. Defective Control of Oncogenes in Hybrids

In contrast to the purebred descendents of wild populations of *Xiphophorus* that are highly insusceptible to neoplasia (see Section II,B), certain interpopulational, interracial, and interspecific hybrids are susceptible.

A. Susceptibility of Hybrids to Development of Neoplasia

Following treatment with carcinogens, depending on the wild populations used for hybridization, about 1 to 4% of the first hybrid generation (F_1) develop neoplasia. Tumor incidence increases in the second hybrid generation (F_2) and the first backcross generation (BC_1) up to about 8% and remains stable in the succeeding generations, which in the case of the backcrosses have been tested up to BC_{24} (Table I, second part).

As compiled from the results of several investigators in our laboratories (A. Anders *et al.*, 1973a, 1983; Schwab *et al.*, 1978a,b, 1979; Kollinger *et al.*,

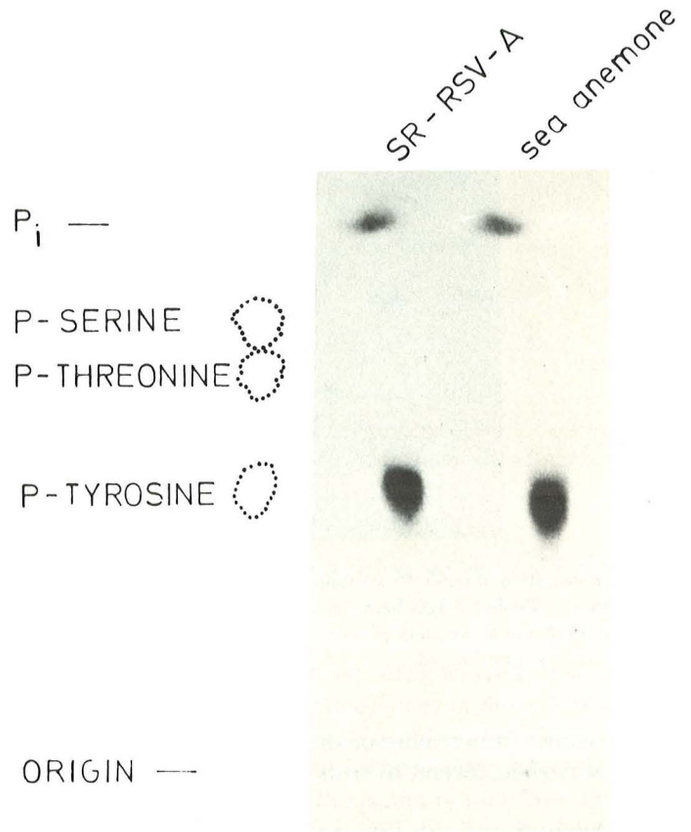


FIG. 13. Identification of phospho-amino acids in ^{32}P-labeled IgG heavy chain of TBR serum precipitated sea anemone extracts and subsequent performance of the kinase assay. Note that the phosphorylation site is exclusively tyrosine. See legends Figs. 10–12, and Barnekow *et al.* (1982).

1979; Haas, 1981; C. R. Schmidt, 1983), 805 of 10,195 (8%) hybrids which survived treatment with MNU and X rays developed a large variety of different neoplasms (Table IV; Fig. 14). Most of the neoplasms were classified as neurogenic and mesenchymal, with melanoma, neuroblastoma, and fibrosarcoma being the predominating types (Schwab *et al.*, 1978a,b; Schwab and A. Anders, 1981; Abdo, 1979). Epithelial neoplasms were less frequent but comprised those with the largest diversity. Some individuals developed several tumors of different types, for instance melanoma, neuroblastoma, and rhabdomyosarcoma. Almost all tissues including the respiratory, endocrine, exocrine, excretory, reproductive, gastrointestinal, re-

TABLE III
c-src IN EUKARYOTES

Mammals	Bony fish	Cartilaginous fish	Molluçs
Humans	Poeciliidae	Shark	Cuttle fish (?)
Calf	*Xiphophorus*	Jawless fish	Coelenterates
Rat	*Girardinus*	Lamprey	Sea anemone
Mouse	*Poecilia*	Acrania	Sponges
Birds	*Belonesox*	*Amphioxus*	Marine sponge
Chicken	*Heterandria*	Insects	Freshwater sponge
Quail	*Xenotoca*	Cockroach	
Frogs	Flat fish	*Drosophila*	
Xenopus	Sea robin		
	Mackerel		
	Roach		
	Gudgeon		
	Salmon		
	Codfish		
	Cichlid		

ticuloendothelial, hepatobiliary, skeletal, muscular, nervous cell, and pigment cell system developed tumors. The tumors show some fish-specific features, but their specific structure and growth are essentially identical to that of the corresponding tumors of other vertebrates (see Schlumberger and Lucké, 1948; Schlumberger, 1957; Scarpelli, 1969; Abdo, 1979) including humans (Sobel *et al.*, 1975; Riehl *et al.*, 1984).

B. Hybridization as a Step toward Neoplasia

The occurrence of individuals susceptible to neoplasia among hybrids brings about the question whether this phenomenon is unique for *Xiphophorus* or represents a more or less general phenomenon in the animal kingdom. A survey of the literature (F. Anders, 1968, 1981; F. Anders *et al.*, 1979b, 1981b,c) and the experience of pathologists (see Weiss, 1972) illustrates the general rule that (1) the incidence of spontaneously developing neoplasms is low in pure-bred animals from wild populations, and neoplasia is difficult to induce in these same animals, while (2) the incidence of spontaneously developing neoplasms is high in animals of hybrid origin, and neoplasia is easily inducible in these animals (e.g., naturally occurring or experimentally produced interspecific and interpopulational hybrids, domestic, laboratory, and ornamental animals, pets) (see Stünzi, 1972; Hayes, 1978). Many examples of neoplasia in hybrids and improved breeds of hybrid origin in the animal kingdom have been cited in the literature: (1) *insects:* experimental hybrids and laboratory stocks, e.g., *Drosophila* (Gateff, 1978,

TABLE IV
NEOPLASIA IN *Xiphophorus* HYBRIDS (F_2–F_n; BC_1–BC_{24}) 1 YEAR AFTER TREATMENT WITH MNU AND X RAYS[a,b,c,d]

Type of neoplasm	Number of animals which developed neoplasms[e]			Percentage incidence based on total number 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 carinoma	6	0	78	0.09	0
Epithelioma	19	6		0.28	0.17
Carcinoma (low-differentiated)	3	4		0.05	0.11
Carcinoma (high-differentiated)	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] Classification according to Mawdesley-Thomas (1975).
[b] Total number of survivors: 10,195 (MNU, 6608; X rays, 3587).
[c] MNU, 10^{-3} *M*; four times for 1 hr in 2-week intervals (Schwab *et al.*, 1979). X Rays, 1000 R; three times for 45 min in 6-week intervals (Pursglove *et al.*, 1971).
[d] Out of 10,195, 805 (7.9%) hybrids developed neoplasia.
[e] Several animals developed several different kinds of neoplasms.

1982), Solenobia (Seiler *et al.*, 1958); (2) *fish:* naturally occurring hybrids, e.g., Lake Ontario hybrid carp (Leatherland and Sonstegard, 1978), ornamental fish, e.g., red swordtail (*Xiphophorus*), guppy (Poecilia reticulata) (Sato *et al.*, 1973), *Girardinus* (A. Schartl *et al.*, 1982), orange medaka (*Oryzias*) (Takayama and Ishikawa, 1977), goldfish (Ishikawa *et al.*, 1978a), ornamental carp (Ishikawa and Takayama, 1977), domestic fish bred for economic reasons, e.g., domestic carp (Ishikawa *et al.*, 1978b), domestic trout (Halver and Mitchel, 1967; Sinnhuber *et al.*, 1977); (3) *birds:* ornamental

hybrids, e.g., musk duck × mallard duck (Crew and Koller, 1936), peacock × guinea fowl (Poll, 1920), improved breeds of domestic chicken (Weiss, 1972); and (4) *mammals:* interspecific hybrid mice, e.g., *Mus musculus* × *M. bactrianus* (Little, 1947), laboratory mice strains, e.g., blue ribbon mice (Heston and Vlahakis, 1968), hybrids between laboratory rat strains, e.g., BALB/c × NZB (Warner *et al.*, 1974), hybrids between laboratory rat strains, e.g., Sprague–Dawley × Long–Evans (Gross and Dreyfuss, 1979), domestic dogs, e.g., boxer dog (Stünzi, 1972; Weiss, 1972; Denlinger *et al.*, 1978), cats. cattle, swine, e.g., Sinclair swine (Hook *et al.*, 1979), horses, e.g., Lippizaner (Gebhard and Niebauer, 1979), etc.

The phenomenon of introducing susceptibility to neoplasia by means of hybridization is not limited to the animal kingdom. Susceptibility to neoplasia has also been observed in a large variety of plant hybrids, especially in cultivated plants that are mainly bred by hybridization. Hybrids of cabbage, lilies, tobacco, tomatoes, calanchoe, thorn-apples, poplar, etc., are well-known examples (see Ahuja, 1965; F. Anders, 1968; F. Anders *et al.*, 1981b,c; Beiderbeck, 1977; Braun, 1978).

While there are no data on the relation between hybridization and cancer in human beings comparable to those in animals and plants, it is interesting to speculate whether the many facts on the high tumor incidence in humans that do not agree with the concept of the primacy of environmental factors and life style in carcinogenesis (Burch, 1976; Higginson, 1969; Maugh, 1979; Oeser and Koeppe, 1979; Koeppe, 1980) may be explained by civilization-conditioned interpopulational and interracial hybridization in our preceding generations. Such speculations are probably of little value in the fight against cancer. They might, however, help to find those factors that make a particular individual susceptible or insusceptible to cancer, or sensitive or insensitive to carcinogens (Heston, 1974).

Interpopulational and interracial human mating (we use the term population and race in the sense of "Mendelian population" and "Mendelian race" proposed by Dobzhansky, 1937) may have occurred at any time and place. Because of the high mobility of humans as compared to other mammals one should expect high values of genetic heterogeneity. Various estimates based on enzyme variation showed that heterogeneity in humans (Schull, 1979) is comparable to that of domestic mammals, such as cats (O'Brien, 1980), but is about 6 times higher than that of wild macaques, about 10 times higher than that observed in big wild mammals such as elk, moose, polar bear, black bear, and elephant seal, and about twice as high as that of most feral rodents studied so far (Fuerst *et al.*, 1977). On the basis of these data one could assume that the high tumor incidence in humans might also be related to hybridization like that in domestic animals.

There are also some data on chromosomal heteromorphisms in human

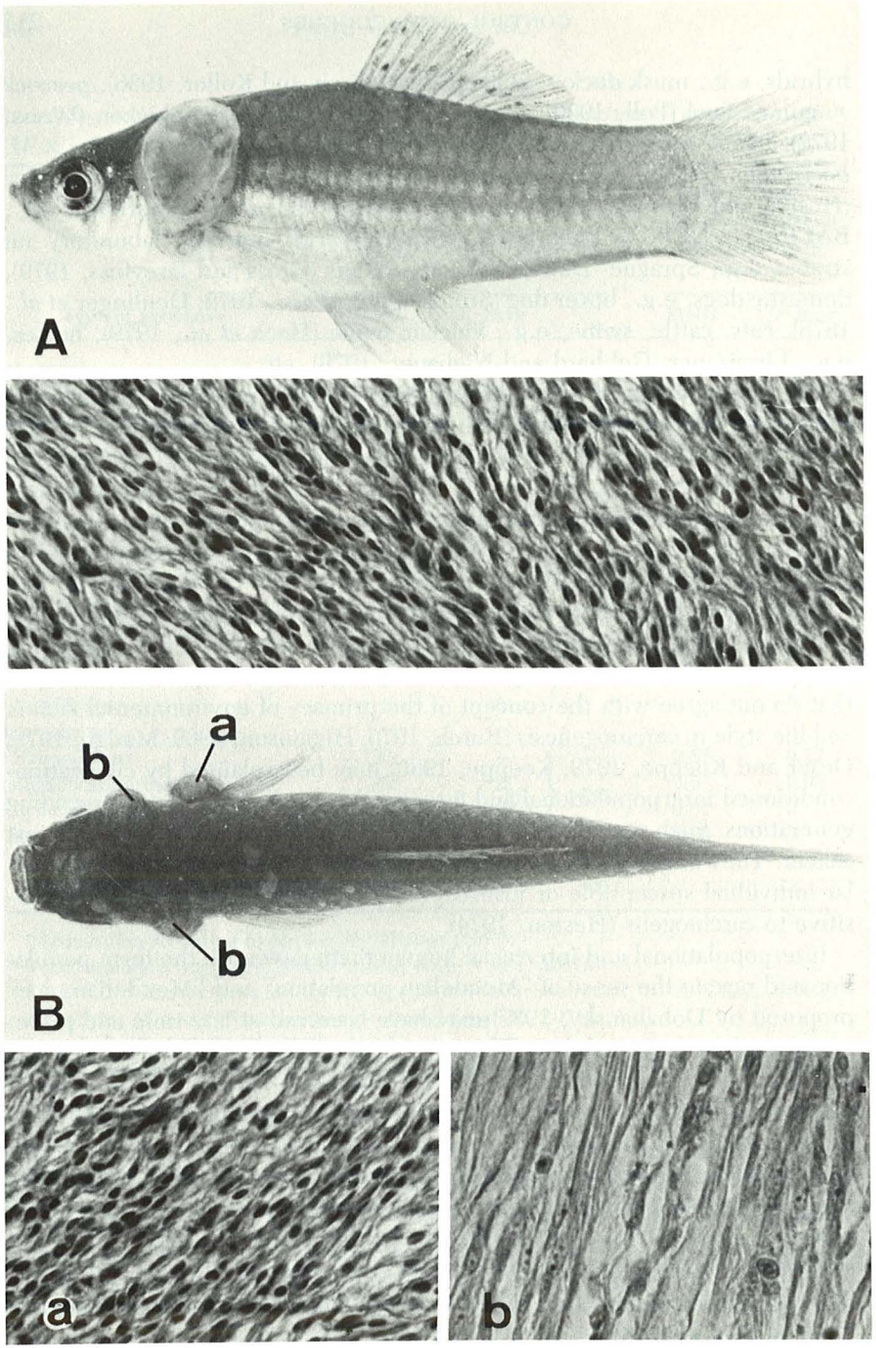
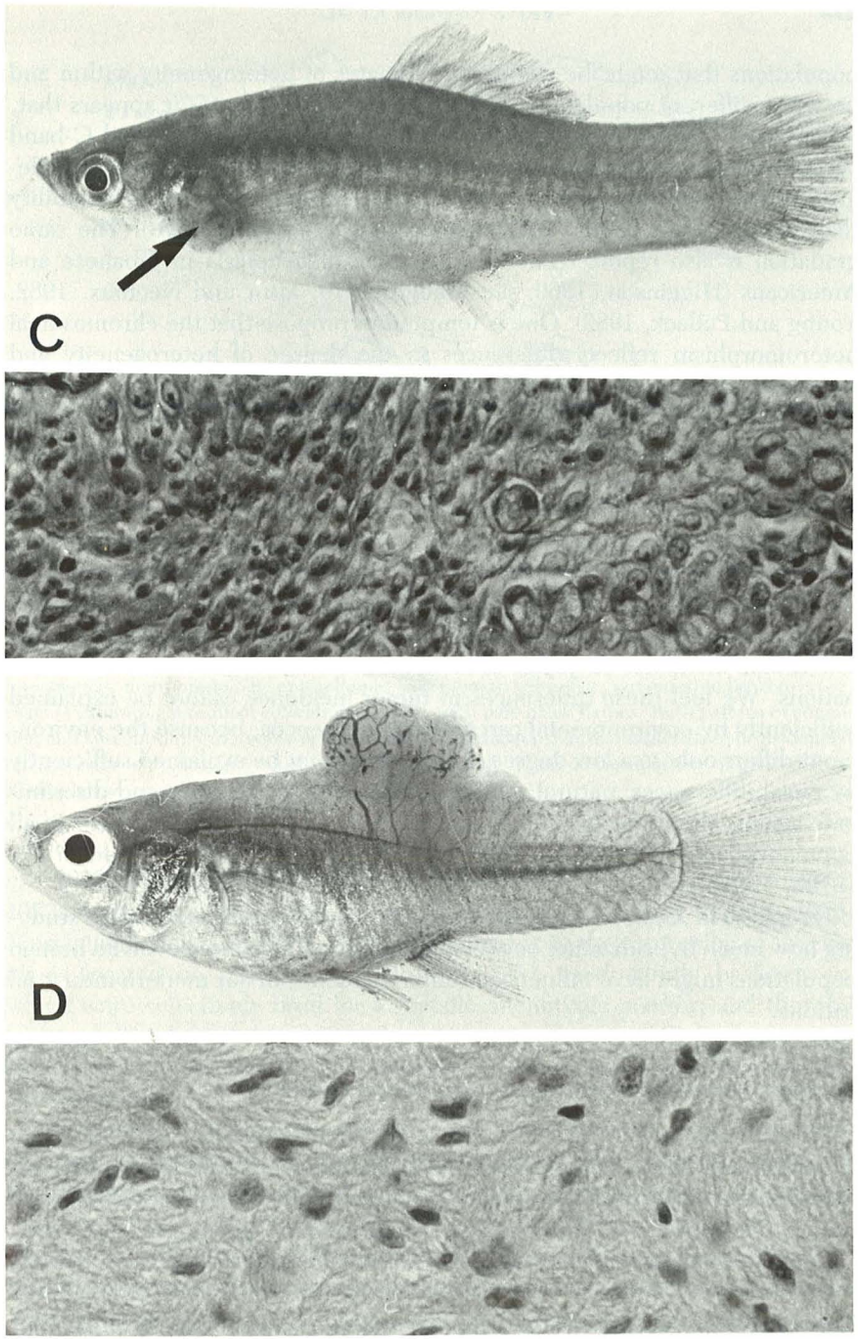


FIG. 14. *Xiphophorus maculatus-X. helleri* hybrids exhibiting A: MNU-triggered fibrosarcoma; B: different MNU-triggered fibrosarcoma (a) and rhabdomyosarcoma (b); C: MNU-trig-



gered signet ring cell carcinoma; D: spontaneous amelanotic melanoma on an albino (note vascularization). From C. R. Schmidt (1983).

populations that might be useful for estimates of heterogeneity within and between different populations. According to such estimates it appears that, for instance, Japanese populations exhibit a low degree of Q- and C-band chromosome heteromorphisms, whereas Americans have a much higher degree of this heteromorphisms, with blacks showing even more variability than whites (Lubs *et al.*, 1977; Yamada and Hasegawa, 1978). The same gradation is also reported for the incidence of neoplasia in Japanese and Americans (Higginson, 1969; see Maugh, 1979; Muir and Nectoux, 1982; Young and Pollack, 1982). One is tempted to propose that the chromosomal heteromorphism reflects differences in the degree of heterogeneity and therefore differences in tumor incidence between the Japanese and the white and black United States populations. We suggest that these differences in tumor incidence are due to different degrees of interpopulational and interracial matings in nations thereby affecting genetic heterogeneity as it does in *Xiphophorus*. On the other hand, the extremely low tumor incidence of active Mormons and Seventh-Day Adventists, as compared to total United States whites (see Cairns *et al.*, 1980; Gardner, 1980) might be due to the biological homogeneity of their populations (which favors insusceptibility to cancer) rather than to environmental factors. The same could apply to the low tumor incidence in Japan as compared to that of the other industrial nations. We feel these differences in tumor incidence cannot be explained sufficiently by environmental carcinogenic influences, because the environment differs only to a low degree. They also cannot be explained sufficiently by racial differences: natural selection will not favor one race and discriminate against the other but it will work against susceptibility to cancer in all populations and all races (for details see F. Anders, 1981; F. Anders *et al.*, 1979b, 1981b,c).

Neoplasia in *Xiphophorus* may serve as a model system suitable for studying how much hybridization between members of differently evolved human populations might have influenced tumor incidence in our modern industrial nations.

C. Assignment of Neoplasia to Chromosomes

To study the crucial differences between the fish that are insusceptible to neoplasia and those that develop neoplasia following treatment with carcinogens, we attempted to assign the susceptibility to specific tumors to specific chromosomes. For this purpose 65 defined genotypes of *X. maculatus, X. xiphidium, X. variatus, X. cortezi, X. helleri*, and their hybrids were employed (Schwab, 1980; Schwab *et al.*, 1978a,b, 1979; Schwab and Anders, 1981). These genotypes exhibit, or lack, specific color patterns (Gordon, 1947a; Atz, 1962; Kallman, 1968; Kallman and Atz, 1967; A. Anders *et al.*, 1973a,b) or enzyme markers (A. Scholl, 1973; A. Scholl and Anders, 1973a,b; Ahuja *et al.*, 1980; E. Scholl, 1979; Siciliano and Wright, 1976; Morizot and Siciliano, 1979, 1982) which are due to the expression of specific genes located on different chromosomes. We used mainly backcrosses, which were selectively bred for a specific phenotypic marker, and thereby for a specific chromosome. Such backcross generations (BC) segregate into 50% animals carrying the chromosome marker, and 50% lacking this chromosome.

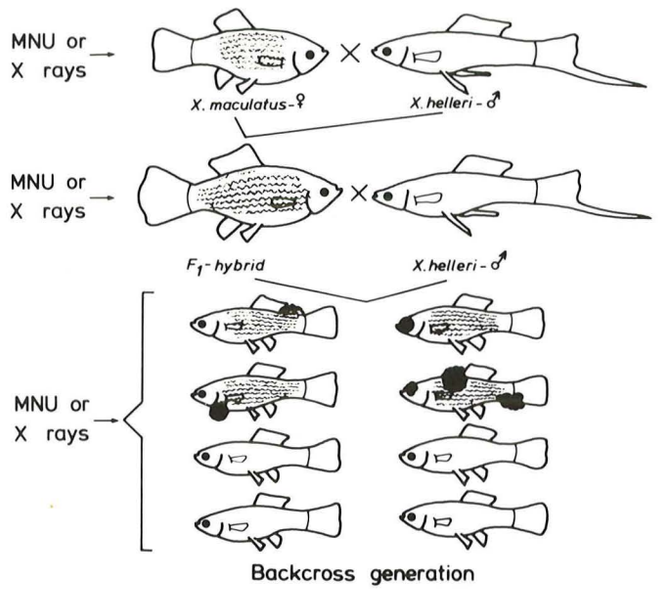


FIG. 15. Crossing scheme showing the assignment of carcinogen-triggered neoplasms (melanomas, neuroblastomas, epitheliomas, carcinomas, fribromas, sarcomas) to backcross segregants exhibiting a reddish coloration together with pale black stripes. Both pattern of coloration and susceptibility to neoplasia (sensitivity to carcinogens) in the backcross hybrids depend on the same chromosome which, in this experiment, is an X chromosome inherited from *Xiphophorus macultatus*.

Depending on the wild populations used in the crossings, neoplasia could be assigned specifically to defined chromosomes. The crossing scheme of Fig. 15 shows an example: in the cross of the insusceptible *X. maculatus* exhibiting a pale reddish coloration and some longitudinal dark stripes, with the also insusceptible *X. helleri* exhibiting a homogeneous gray coloration, almost exclusively the BC segregants exhibiting the reddish coloration and the stripes were sensitive to the carcinogens while the homogeneous gray segregants were, with few exceptions, insensitive. The susceptible fish de-

veloped those kinds of neoplasms which are listed in Table IV; some of these hybrid individuals developed several tumors of different tissue types, for instance fibrosarcoma and rhabdomyosarcoma (Fig. 14B), or melanoma, neuroblastoma, and carcinoma. This experiment clearly shows that the loci coding for the pattern of coloration and for susceptibility to develop different neoplasms are linked to the same chromosome. This is not to say that all BC segregants exhibiting the reddish coloration and the stripes develop neoplasia; but the fish in which the neoplasms developed belong almost exclusively to that group of BC segregants that exhibits the reddish coloration and the stripes. In the present experiment both characters, susceptibility to neoplasia and the color pattern, belong to linkage group I (the sex chromosomes); they also contain loci coding for the enzymes adenosine deaminase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase (Morizot *et al.*, 1977). Analysis of sex determination of the fish used in this experiment revealed that the sex chromosome to which the different kinds of neoplasms could be assigned is the X chromosome of *X. maculatus* (A. Anders and F. Anders, 1963; Schwab, 1980). Other crossing protocols using different pure-bred strains of *Xiphophorus* have shown that the chromosome on which susceptibility to develop neoplasia depends may be a Y chromosome, or an autosome. Other examples, again, have shown that susceptibility to neurogenic and epithelial neoplasms depends on a certain chromosome (e.g., and X chromosome) while susceptibility to neoplasms of mesenchymal origin depends on another chromosome (e.g., a Y chromosome or an autosome) (Schwab *et al.*, 1978b; Schwab and Anders, 1981).

D. Assignment of Cancer Susceptibility to Oncogenes and Regulatory Genes

The assignment of carcinogen-triggered neoplasia to specific chromosomes raised the question whether the neoplasms could be assigned to specific genes. The pursuit of this problem proved to be extremely difficult because the development of carcinogen-triggered neoplasia is a rather rare event (see Table I) and, although assignable to chromosomes, would require immense efforts for the production of sufficient experimental material for a finer genetic analysis. The rationale for the design of the experiments that raised the opportunity to assign the carcinogen-triggered neoplasms to genes is based on studies on the occurrence of hereditary neoplasms such as melanomas, pterinophoromas (pigment cell tumors that consist predominantly of drosopterine-containing cells; Henze *et al.*, 1977; Rempeters *et al.*, 1981), neuroblastomas, thyroid carcinomas, kidney adenocarcinomas, and reticulosarcomas, in certain hybrids.

1. *Oncogenes and Regulatory Genes in Animals Developing Neoplasia Spontaneously*

The individuals of laboratory hybrid populations of *Xiphophorus* (wild hybrid populations do not exist; Kallman and Atz, 1967; Zander, 1967; see Section II,A) that develop neoplasia spontaneously are extremely rare. They can, however, be propagated in large numbers at the will of the experimenter, because they and their descendants develop neoplasia exactly following Mendelian predictions (Kosswig, 1937; Gordon, 1947a, 1958; F. Anders, 1967; Kallman, 1975). Hundreds of thousands of individuals that develop neoplasia following Mendelian laws have been studied.

To illustrate the assignment of cancer susceptibility to distinct genes we used melanoma because development of this type of tumor can easily be observed. Even a singly transformed pigment cell can be distinguished from a regular pigment cell by gross examination of the living animal (see Section II,C,D). Furthermore, morphological, ultrastructural, and histochemical markers specific to the successive stages of pigment cell differentiation facilitate the detection of the first events involved in neoplastic transformation (see Section II,D). These facts, and the ease with which crossings in *Xiphophorus* can be accomplished, provided the background for the design of the two series of crossing experiments that elucidated the existence of oncogenes and regulatory genes by formal genetics (F. Anders, 1967) (Figs. 16 and 17).

(1) The animals used for the initial crosses were females of a mutant stock of *X. maculatus* (platyfish) derived from the wild population of Rio Jamapa (Fig. 16A), and males of *X. helleri* (swordtail) from Rio Lancetilla (Fig. 16B; see Section II,A). The platyfish infrequently exhibit spots consisting of transformed pigment cells that are terminally differentiated (Tr melanophores) whereas the swordtails are never spotted (see Section II,C). The development of the spots is restricted to the dorsal fin and to the skin of the posterior part of the body of the platyfish, and is X chromosome linked.

Crosses of the platyfish (A) with the swordtail (B) result in F_1 hybrids (C) that develop uniformly in all individuals melanomas consisting mainly of Tr melanophores that are similar to those of the spots of the parental platyfish. These melanomas are benign. They occur only in those compartments of the body where the platyfish parent infrequently exhibits the spots. In older F_1 animals, the melanoma of the dorsal fin and that of the posterior part of the body combine to form a large superficially spreading benign melanoma. Backcrosses of the F_1 hybrids with the swordtails as the recurrent parent (D) result in offspring (BC_1) exhibiting three types of segregants: 25% of the BC_1 (E) develop benign melanoma like that of the F_1, 25% (F) develop malignant melanoma consisting mainly of incompletely differentiated Tr cells which invade other tissues (except for brain, gonads, intestine) and kill the fish,

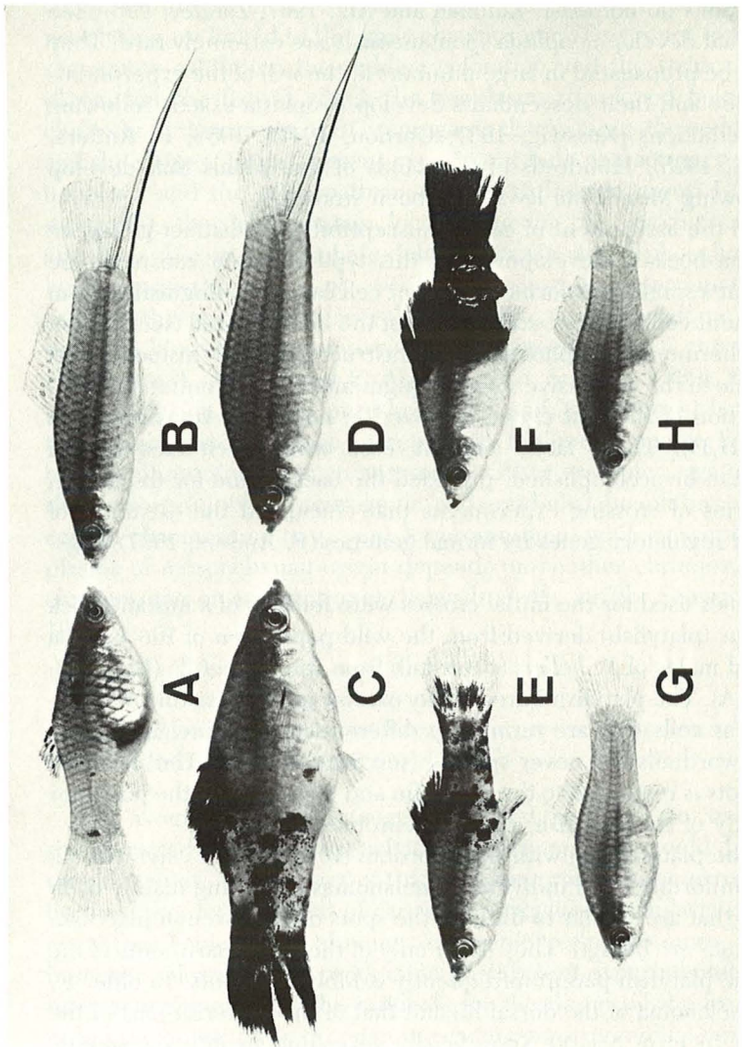


FIG. 16. Crossing procedure for the production of melanoma developing hybrids of *Xiphophorus*. (A) *X. maculatus* from Rio Jamapa; some small spots in the skin of the dorsal fin and the side of the body are visible. Spots consist of terminally differentiated neoplastically transformed pigment cells. (B) *X. helleri* from Rio Lancetilla, always lacking spots. (C) F_1 hybrid 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 developing benign melanoma (25% of the BC generation). (F) Backcross hybrid with developing malignant melanoma (25% of the BC generation. (G, H) Backcross hybrids that do not develop melanoma (50% of the BC generation). From F. Anders *et al.* (1984), modified.

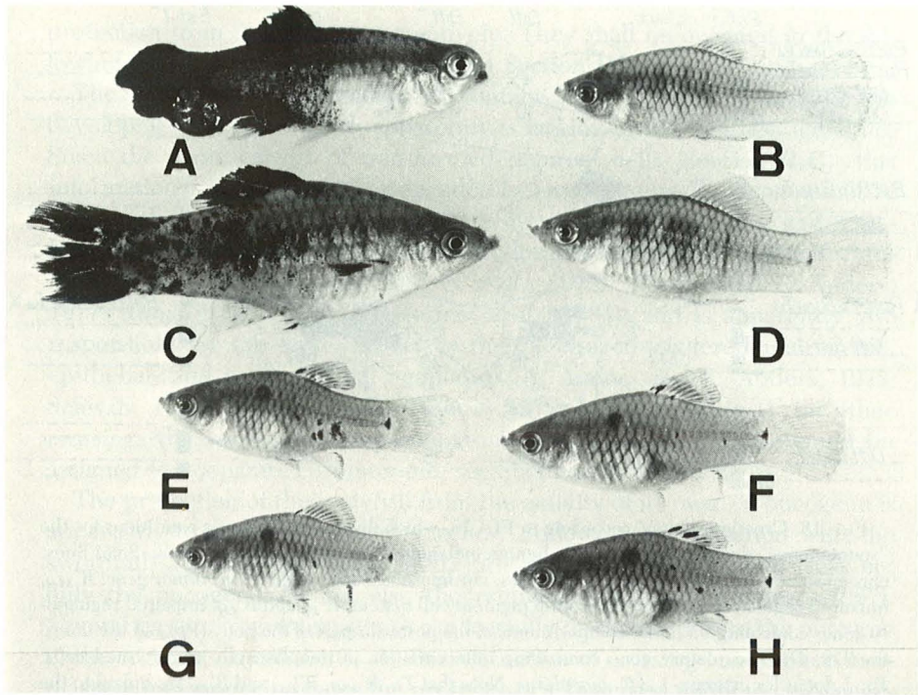


FIG. 17. Crossing procedure for the suppression of melanoma in *Xiphophorus*. (A) Malignant melanoma bearing backcross hybrid according to Fig. 16F. (B) *X. maculatus* according to Fig. 16A as the recurrent parent. (C) Quasi-F_1 exhibiting benign melanoma. (D) *X. maculatus* as the recurrent parent. (E, F, G, H) Backcross hybrids (quasi-*X. maculatus*) exhibiting spots only. From F. Anders *et al.* (1984), modified.

whereas 50% (G and H) develop neither spots nor melanomas. Further backcrosses (not shown in Fig. 16) of the fish carrying benign melanoma, with the swordtail, result in a BC_2 that exhibits the same segregation pattern as the BC_1. The same applies for further backcrosses of this kind. Backcrosses of the fish carrying the malignant melanoma with the swordtail show a different result: 50% of the BC segregants develop malignant melanoma, whereas the remaining 50% are melanoma free; benign melanomas do not occur. Whenever melanomas occur in these crossing experiments, they develop in both the compartment of the dorsal fin and the compartment of the posterior part of the body.

(2) In contrast to the above results (Fig. 17A–H), backcrosses of malignant melanoma bearing hybrids (a) with platyfish (B) (like those of Fig. 16A, but lacking the spot factor by deletion of a Giemsa band; see Section IV,A,3) result in a quasi-F_1 that segregates in 50% animals displaying benign melanoma (C) and 50% exhibiting neither melanomas nor spots (not shown in the

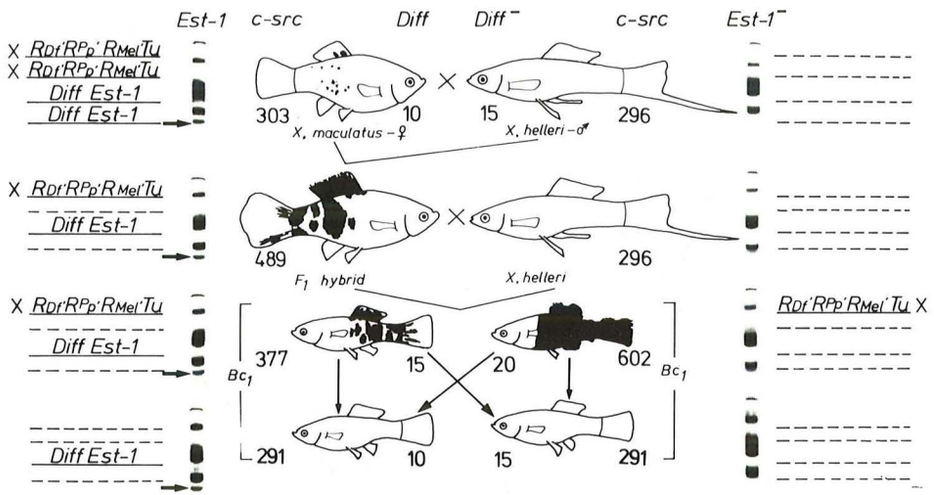


FIG. 18. Crossing scheme according to Fig. 16, which displays the genetic conditions for the "spontaneous" development of spots, benign melanoma, and malignant melanoma. Solid lines, chromosomes of *X. maculatus;* dashed lines, chromosomes of *X. helleri; Tu, tumor gene;* R'_{Mel}, impaired regulatory gene specific to the pigment cell system; R'_{Pp} and R'_{Df}, impaired regulatory genes controling *Tu* in the compartments of the posterior part of the body (Pp) and the dorsal fin (Df); *Diff*, regulatory gene controlling differentiation of neoplastically transformed cells; *Est-1*, locus for esterase-1 of *X. maculatus*. Note that *Tu*, R'_{Mel}, R'_{Pp}, and R'_{Df} are linked to the X chromosome, whereas *Diff* and *Est-1* are linked to an autosome. *Est*, esterase profiles (polyacrylamide gels stained using α-naphthyl-acetate as substrate). Note Est-1 isozyme in the *X. maculatus*, the F_1 hybrid, and half of the backcross segregants, which include fish with benign melanoma and others without melanoma (see arrows pointing to the gels). Data from Ahuja *et al.* (1980). *c-src* (cellular counterpart of the viral src oncogene), $pp60^{c\text{-}src}$ 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 Barnekow *et al.* (1982); M. Schartl *et al.* (1982); see Section IV,A,5. *Diff*, activity of *Diff* expressed as the replacement of guanine in position 34 (first position of the anticodon) of the tRNAs for Asp, Asn, His, and Tyr by labeled guanine, pmol Gua incorporated/A_{260} tRNA. Note that high values indicate high contents of guanosine (G), whereas low values indicate high values of queuosine (Q) in position 34. Data from Dess (1983); Kersten *et al.* (1983); see Section IV,D,4. Arrows between the backcross segregants, design of transplantation experiments described in Section IV,D,3.

scheme). Further backcrosses of the benign melanoma bearing quasi-F_1 hybrids with the platyfish (D) result in spotted and nonspotted fish that are similar to the purebred platyfish (E, F, G, H). Genetically they segregate into those that inherit the capability to develop melanoma after crossings with swordtails and those that do not inherit the capability to develop melanoma (not shown in the scheme). These two genotypes are hard to distinguish phenotypically.

The results obtained in these two series of crossing experiments revealed several genetic components that are involved in melanoma formation, or

protection from melanoma, respectively. They shall be outlined in the following, and discussed in more detail in Section IV.

The platyfish genome (see Fig. 18) contains the genetic information for the development of spots which apparently is lacking in the swordtail genome. Since the spots consist of transformed pigment cells (Section II,C), this information is considered to be encoded by an oncogene, *Tu*. 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* (A. Anders *et al.*, 1973b; Förster and F. Anders, 1977; Ahuja *et al.*, 1979; Chatterjee *et al.*, 1981), and is apparently also responsible for the large variety of the carcinogen-triggered neurogenic, epithelial, and mesenchymal neoplasms (A. Anders and F. Anders, 1978; Schwab, 1980; Schwab *et al.*, 1978a,b, 1979) listed in Table IV (in other crossings the susceptibility to neoplasms of mesenchymal origin could be assigned to a separate chromosome; see Sections III,C and E).

The protection of the platyfish from the activity of its own *Tu* oncogene is apparently exerted by regulatory genes. Following hybridization with the swordtail, *Tu* becomes deregulated, indicating that the swordtail lacks not only the oncogene *Tu* but also the regulatory genes (F. Anders, 1967). 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 known from mutagenesis studies (A. Anders *et al.*, 1973a; A. Anders and F. Anders, 1978; Ahuja, 1979), is impaired by mutation. Out of the tissue-specific regulatory genes only the impaired pigment cell-specific regulatory gene for *Tu*, i.e., R'_{Mel}, is shown in the scheme (Fig. 18). (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. 16–18, depending on the genotype (mutant) of the platyfish used for the initial crosses, melanoma develops, for instance, in the anterior part of the body, the anal fin, tail fin, the mouth, the eye, the peritoneum, the 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 (A. Anders and F. Anders, 1978). Of these compartment-specific regulatory genes which have been designated in total as R_{Co} (Fig. 8) only the impaired dorsal fin-specific regulatory gene, i.e., R'_{Df}, and the impaired posterior part-specific regulatory gene, i.e., R'_{Pp}, are shown in the scheme. (3) The clearcut 1:1 segregation between the BC hybrids bearing malignant melanomas and those bearing benign melanomas indicates the existance of a prominent regulatory gene derived from *X. maculatus* that is nonlinked to the oncogene *Tu*. Since the benign melanomas consist, in contrast to the

malignant melanoma, predominantly of terminally differentiated transformed pigment cells (U. Vielkind, 1972, 1976; Diehl, 1982; F. Anders *et al.*, 1980) this regulatory gene is considered to be involved in differentiation of these cells, and was, therefore, designated as *Diff*. The *Diff*-containing chromosome can easily be detected by an esterase marker, *Est-1* which is closely linked to *Diff* (Siciliano and Wright, 1976; E. Scholl, 1977; Ahuja *et al.*, 1980; Morizot and Siciliano, 1982). Additional genes of minor importance involved in melanoma formation have been identified but are not taken into consideration in this study.

Following crossings and backcrossings according to Fig. 18, the chromosomes of the platyfish (continuous lines) are replaced by the homologous chromosomes of the swordtail (broken lines), 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. If *Tu* is lacking, no melanomas occur.

In contrast, after backcrossings of the melanoma-bearing hybrids with the platyfish as the recurrent parent (see Fig. 17) the chromosomes carrying regulatory genes for *Tu* are reintroduced into the descendants. This results in a reconstruction of the original regulatory gene system that suppresses the activity of *Tu*.

2. *Oncogenes and Regulatory Genes in Animals Requiring Carcinogenic Triggers for the Development of Melanoma*

To relate the genes that are responsible for the development of spontaneous melanoma to the genes that are involved in the development of the carcinogen-dependent neoplasms, we replaced the $R'_{Df}\,R'_{Pp}\,R'_{Mel}\,Tu$ chromosome by another one the crucial difference of which is that R_{Mel} is nonmutated and active ($R_{Df}\,R'_{Bs}\,R_{Mel}\,TU$) (Fig. 19). Because R_{Mel} is inherited along with *Tu*, melanoma does not develop spontaneously in the hybrids. Following treatment with carcinogens, those hybrids carrying the $R_{Df}\,R'_{Bs}\,R_{Mel}\,Tu$ chromosome but lacking the nonlinked regulatory genes including *Diff* are highly sensitive to the carcinogens because development of melanoma requires only impairment or deletion of the crucial pigment cell-specific R_{Mel} gene in a melanophore precursor at the side of the body.

Crosses between two BC hybrids of this genotype were the basis for the establishment of a strain homozygous for the $R_{Df}\,R'_{Bs}\,R_{Mel}\,Tu$ chromosome (Fig. 20). Because of the fact that each of the two copies of the *Tu* oncogene is repressed by its own linked R_{Mel} which acts in cis position only (see Section IV,B) the incidence of animals developing melanoma following treatment with carcinogens potentially doubles. These animals are highly suitable as test animals for mutagenic carcinogens in the water (F. Anders *et al.*, 1981d, 1983; A. Anders *et al.*, 1983; Schmidt, 1983).

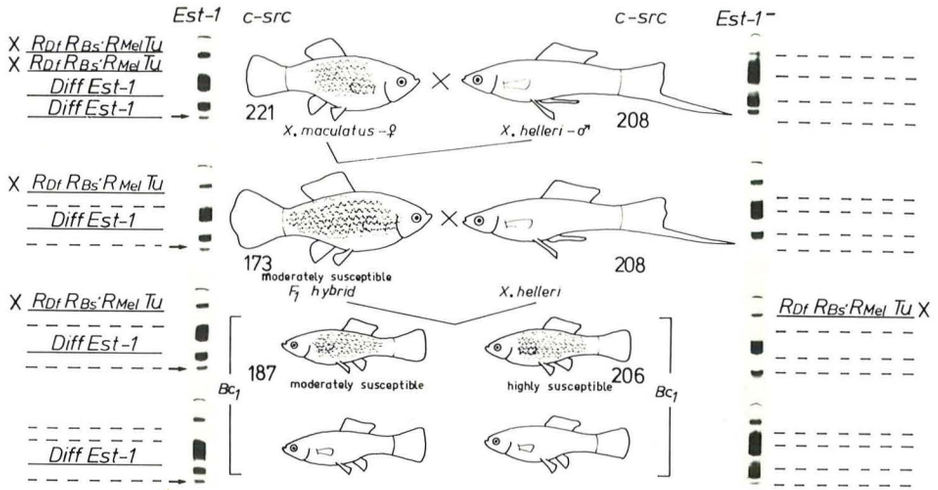


FIG. 19. Crossing scheme displaying the genetic conditions of susceptibility to carcinogen-dependent neoplasia. The highly susceptible genotype is extremely sensitive to the carcinogenic (mutagenic) inducer. See legend to Fig. 18 for explanation of abbreviations. R'_{Bs}, impaired regulatory gene controlling *Tu* in the compartment of the entire side of the body (Bs).

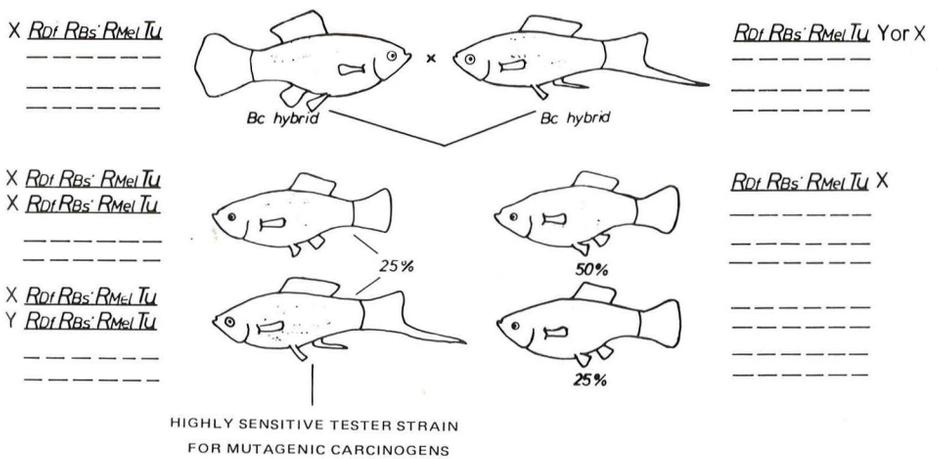


FIG. 20. Breeding procedure of an established tester strain which is very highly sensitive to mutagenic carcinogens. Both X and Y chromosome contain *Tu* and R_{Mel}. This strain was derived from the highly susceptible segregants shown in Fig. 19. See legends to Figs. 18 and 19 for explanation of abbreviations.

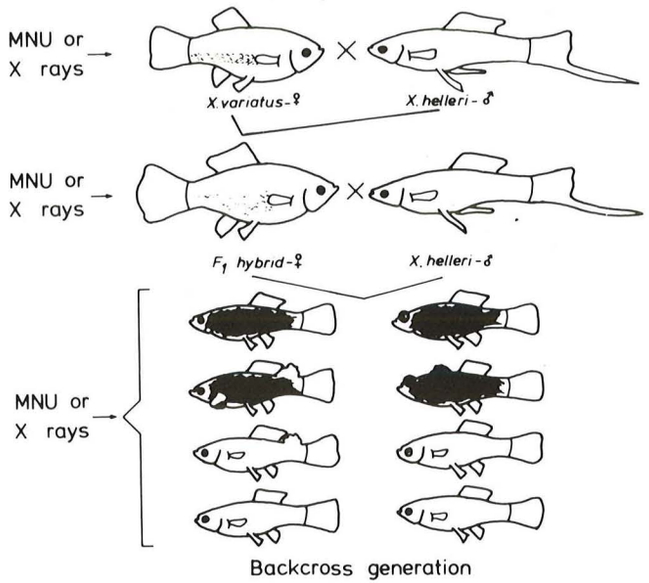


FIG. 21. Schematic presentation of crossings and backcrossings between *X. variatus* (Fig. 4D) and *X. helleri* (Fig. 3B) with *X. helleri* as the recurrent parent that have shown spontaneous development of benign melanoma in 50% of the BC generation, and a high rate of carcinogen-triggered malignant melanoma, neuroblastoma and different kinds of benign and malignant epithelial tumors in the same segregants. Carcinogen-triggered neoplasms of mesenchymal origin occur in both the melanomatous and the nonmelanomatous segregants indicating that they are genetically independent from neurogenic and epithelial neoplasms.

E. Tissue Specificity or Tissue Nonspecificity of Oncogenes and Regulatory Genes

The hybrid segregants that were highly susceptible for carcinogen-triggered melanoma (see Fig. 19) were also susceptible to many kinds of carcinogen-triggered neurogenic, epithelial, and mesenthymal neoplasm (see Fig. 15; Sections III,A and D). Furthermore, many hybrid individuals treated with carcinogens, in addition to the melanoma, developed multiple neoplasms such as neuroblastoma, retinoblastoma, carcinoma, and sarcoma (Abdo, 1979; Kollinger, 1980; Schmid, 1983). Therefore, the development of the different carcinogen-triggered neoplasms apparently depends on the same *Tu* (Schwab *et al.*, 1978a,b).

Additional information about the assignment of the different kinds of neoplasms to the oncogene *Tu* came from crosses and backcrosses between *X. variatus* (Fig. 4D) and *X. helleri* (Fig. 3B) (Fig. 21) that correspond to those between *X. maculatus* and *X. helleri* (see Fig. 19). In this experiment 50% of

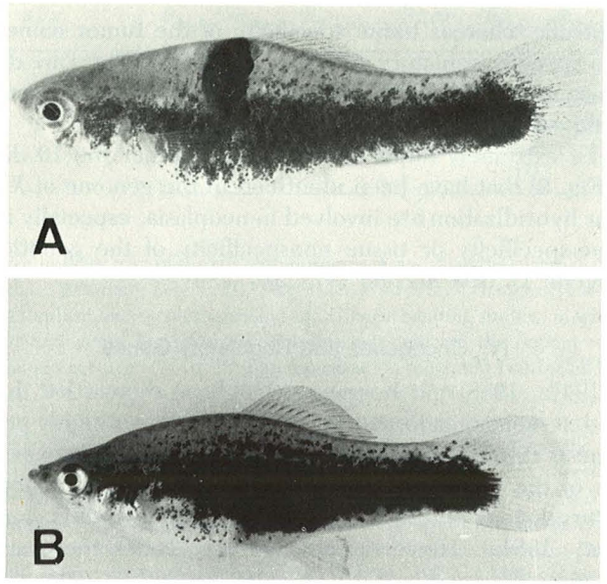


FIG. 22. Backcross hybrids between *X. variatus* and *X. helleri* according to Fig. 21. (A) Fish exhibiting crossing-conditioned (germ line hereditary) superficial spread benign melanoma, and MNU-triggered invasive malignant melanoma. (B) Fish exhibiting the crossing-conditioned benign melanoma only.

the animals of the BC generation develop superficial spreading benign melanoma but maintain their normal vitality and fertility; the melanoma is not invasive in these animals (Altmaier and F. Anders, 1968). The remaining 50% of the animals of the BC generation are melanoma free. After treatment with X rays and MNU the melanomatous segregants may develop foci of malignant melanoma on the skin and even on the superficial spreading benign melanoma (Fig. 22). Furthermore, these animals may develop different kinds of neurogenic (mostly neuroblastoma) and epithelial neoplasms. The crossing-conditioned benign melanoma as well as the carcinogen-triggered malignant melanoma, neurogenic, and epithelial neoplasms can be assigned to the same chromosome. Neoplasms of mesenchymal origin, however, develop in both the melanomatous and the nonmelanomatous segregants. Further crossing analysis of the potential of the fish to develop neoplasia following the carcinogenic trigger has shown that the mesenchymal tumors are mediated by oncogenes other than those that mediate the neurogenic (including melanoma) and epithelial neoplasms (Schwab *et al.*, 1979; Schwab and A. Anders, 1981).

Additional studies are required before we can decide whether (1) *Tu* is

tissue-nonspecific whereas tissue specificity of the tumor comes from *Tu*-linked tissue-specific regulatory genes, or (2) whether there are different *onc* genes that are under the control of linked regulatory genes specific to the tissue-specific *onc* genes.

It would be extremely important to know whether the 10 different *onc* genes (see Fig. 9) that have been identified in the genome of *Xiphophorus* by molecular hybridization are involved in neoplasia, especially in the problem of tissue-specificity or tissue nonspecificity of the genetically determined oncogene *Tu* (see Section IV,A,5).

IV. Oncogenes and Regulatory Genes

Gordon (1947a, 1958) and Kosswig (1937) have shown that the gene system involved in melanoma formation in *Xiphophorus* is highly polygenic. X-ray mutagenesis studies have confirmed these results, and revealed that the constituents of the polygenic system are distributed throughout all chromosomes (Pursglove *et al.*, 1971; Pursglove, 1972; A. Anders *et al.*, 1971; F. Anders *et al.*, 1981a). However, among these genes there are only few prominent genes that predominatly determine whether melanoma develops or not (Section III,D). These genes are *Tu*, the oncogene; R_{Mel}, the pigment cell-specific regulatory gene; R_{Co} (e.g., R_{Df}, R_{Pp}), the series of compartment-specific regulatory genes (Df = dorsal fin; Pp = posterior part of the body); and *Diff*, the differentiation gene.

Studies on 48 structural changes of sex chromosomes of *X. maculatus*, *X. variatus*, and *X. xiphidium* (19 deletions, 14 duplications, 4 translocations, and 11 X–Y crossovers) indicate that *Tu*, at least as far as the sex chromosomes are concerned, is located terminally, and that R_{Mel} and the R_{Co} series are closely linked to *Tu* (Fig. 23) (A. Anders *et al.*, 1973a,b; and unpublished data). Some of the major chromosome aberrations involving loss and translocation of both *Tu* and its linked regulatory genes controlling *Tu* in the melanophore system (the region including R_{Co}, R_{Mel}, and *Tu*), were, in addition to their genetic identification, also observed cytologically (Ahuja, 1979; Ahuja *et al.*, 1979). *Diff* is not linked to *Tu* and is located together with the loci for esterases-1 and -4, and malatedehydrogenase-2 on an autosome corresponding to linkage group V according to Morizot and Siciliano (1983).

A. The Oncogene

1. General Features of Tu

All deletions of *Tu* are nonlethal in both the heterozygous and the homozygous state, indicating that this sex chromosome-linked oncogene is not

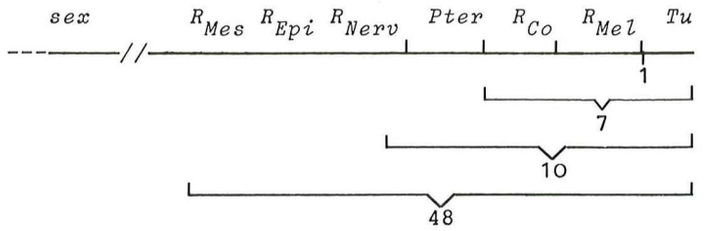


FIG. 23. Preliminary map of the sex chromosomes (X and Y) of the platyfish (*X. maculatus*, *X. variatus*, and *X. xiphidium;* Section II,A) based on 48 structural changes (11 X–Y crossovers, 19 deletions, 14 duplications, 4 translocations) and Giemsa banding studies, in which oncogene *Tu* and its regulatory genes are involved. The brackets indicate the regions within which the structural changes occurred (7, 10, 48); one translocation separated *Tu* from all linked regulatory genes (Section IV,A,2, Fig. 24). sex, sex determining region; R_{Mes}, R_{Epi}, R_{Nerv}, sets of regulatory genes controlling *Tu* in mesenchymal, epithelial tissues, and in the nervous cell system. Pter, pterinophore locus; R_{Co}, region containing at least 13 compartment-specific regulatory genes such as R_{Df} (dorsal fin-specific), R_{Pp} (posterior part-specific) etc. (Section IV,C); R_{Mel}, melanophore-specific regulatory gene. The unit including R_{Co}, R_{Mel}, and *Tu* corresponds to the specific "color genes" known as *Sd* (spotted dorsal), *Sp* (spotted), etc. in the literature. The unit including Pter corresponds to the so-called "erythrophore genes" known as *Dr* (dorsal red), *Ar* (anal red), *Or* (orange), etc. in the literature (see Kallman, 1975). Additional regulatory genes for *Tu* are distributed throughout other chromosomes, e.g., *Diff* (nonlinked to *Tu*). From A. Anders and F. Anders (1978), modified.

essential for the fish. One could, for instance, assume that additional copies of *Tu* present in the autosomes may compensate for the loss of the sex chromosome-linked *Tu* locus according to a gene dosage compensation mechanism, which permits normal cellular functions to continue. Such a function might possibly be the normal outburst and control of cell reproduction in different tissues at different periods during different stages of embryonic and postembryonic development as well as in regeneration processes. Impairment or loss of control of this function might give rise to the initial phase of tumor formation. Nevertheless, the only function of *Tu* known so far is that it mediates neoplastic transformation.

Up to the present we have not found any mutation of *Tu* itself although one may expect this to be possible based on the findings of the laboratories of Weinberg (Tabin *et al.*, 1982) and Barbacid (Reddy *et al.*, 1982) that mutation converts a silent *onc* gene to the transforming state. *Tu* of *Xiphophorus* displays its transforming activity as a normal nonmutated gene which, however, is deregulated following elimination, deletion, or impairment of regulatory genes (see Section IV,B,3).

Tu might be related to virus particles found in the crossing-conditioned and in the MNU-triggered neuroblastomas and melanomas (Kollinger *et al.*, 1979). Normally these particles are not present in the neoplasms but occur

following the treatment of BUdR. Up to the present there is no indication that these particles may trigger neoplastic transformation or may stimulate tumor growth. In most neoplasms the virus particles resemble small virions of the DNA tumor virus SV40 or polyoma virus. In addition to these particles, B-type- and C-type-like particles were also found. One could speculate that *Tu* normally governs normal functions in the cell as mentioned above, but may also generate virus particles, if the cell is treated with an inducing chemical. According to Gillespie and Gallo (1975), who refer to the B-type and C-type particles, such a gene would be a "class I gene" that can create a "class I virus." Normal tissues of nontumorous and tumorous fish as well as the tumors showed RNA-dependent DNA polymerase activity. This activity, however, could not be assigned to the supposed endogenous virus reverse transcriptase activity (Lueke, 1984; Lueke and F. Anders, 1983). Regardless of any future findings, that might relate *Tu* to an endogenous oncogenic virus, *Tu* acts, is regulated, and is inherited as a chromosomal gene of the natural gene pool of the Xiphophorine fish (Section III,D).

Tu was transferred by purified DNA (Haas-Andela, 1978; J. Vielkind *et al.*, 1982): donor DNA of various fish strains carrying copies of deregulated *Tu* was injected into recipient embryos lacking these *Tu* copies. Up to 8% of the treated embryos and the resulting young fish developed colonies of neoplastically transformed cells. If embryos were treated with DNA from strains lacking these *Tu* copies, no such colonies were observed (see Section IV,B).

In accordance with Breider (1939) we found that *Tu* may mediate melanoma formation in albinos (Fig. 14D). The developing amelanotic melanomas revealed defective premelanosomes and a lack of melanosomes and melanin (U. Vielkind, 1972, 1976; J. Vielkind *et al.*, 1971). The occurrence of the albino melanomas indicates that *Tu* acts independently from melanin synthesis, and that the black pigmentation of the melanotic melanoma is an epiphenomenon of melanoma development.

2. *The Completely Deregulated Oncogenic Effect of Tu in the Pigment Cell System*

Information about the genuine oncogenic effect of *Tu* comes from a balanced laboratory stock carrying a lethal *Tu* translocation (Fig. 24). The *Tu* gene, minus all linked regulatory genes, 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 (A. Anders *et al.*, 1973a,b). Nonlinked regulatory genes are not present in the system except for *Diff*. The *Tu*-carrying progeny of the stocks segregate into 50% animals carrying *Diff* which survive, whereas the corresponding 50% lacking *Diff* are lethal. As a consequence of the

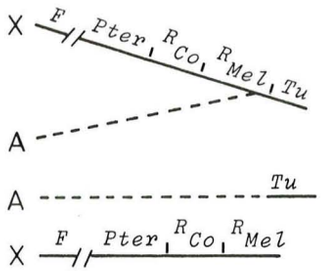


FIG. 24. Translocation of *Tu* from the X chromosome (X) of *X. maculatus* to an autosome (A) of *X. helleri*. Note separation of *Tu* from its linked regulatory genes (see Fig. 23). F, female determining region of the X chromosome. See legend to Fig. 23 for further abbreviations.

transmission of the unrestrained *Tu* through the germ line, the pigment cell precursors become transformed in the embryo as soon as they become competent for neoplastic transformation by cell differentiation (F. Anders *et al.*, 1979a). During the first days of embryogenesis, 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 differentiate within about 15 hr to transformed melanocytes (Fig. 25A), which represent the predominant cells of the growing melanoma. During the further development of the embryo, neoplastic transformation contineus in all areas where pigment cell precursors become competent (Fig. 25B) and the melanoma grows by both transformation and proliferation, thus developing into a "whole body melanoma" (Fig. 25C), which will kill the fish before or shortly 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. These observations suggest to us 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, appears 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 crossing experiments (Section III,D) or the deletion animals mentioned above. This

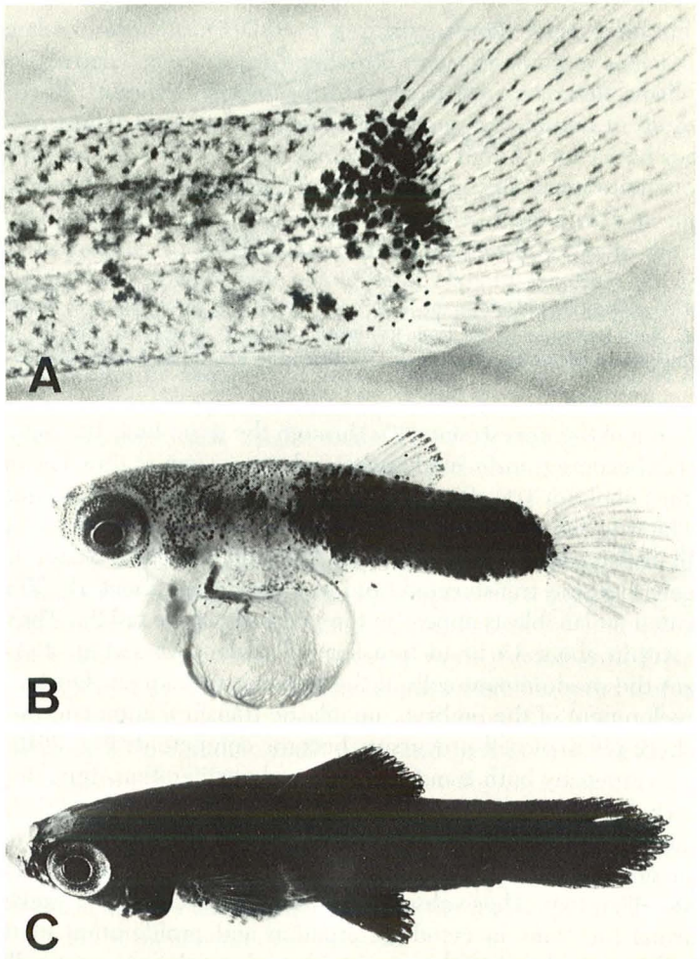


FIG. 25. Completely deregulated oncogenic effect of the oncogene *Tu* in the melanophore system following *Tu* translocation shown in Fig. 24. (A) Ten-day-old embryo (3 mm long) exhibiting some Tr melanocytes at the peduncle of the tail fin. (B) Same fish, 5 days later (4 mm long). (C) Neonate of the same genotype (6 mm long).

question leads to the problem of indispensable and accessory copies of the oncogenes, and to the problem of oncogene dosage.

3. *Indispensable and Accessory Copies of Tu*

In total, about 30 deletions of *Tu* copies have been observed in our *Tu*-containing stocks of *X. maculatus*, *X. variatus*, *X. xiphidium*, *X. helleri*, and *X. montezumae*. All deletions, even the loss of a *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 (Ahuja, 1979; Ahuja *et al.*, 1979). This observation and the fact that wild populations may lack *Tu* in total led us to the conclusion that the *Tu* oncogene considered so far is accessory for the fish. This does not imply that the accessory *Tu* is lacking normal functioning. One could, for instance, assume that certain copies of *Tu* are present in the genome which are indispensable and may compensate the loss of the accessory *Tu* loci. On the other hand, one could also expect that indispensable copies of *Tu* might mediate neoplastic transformation after disturbance of their regulation. Support for the assumption of the existence of indispensable *Tu* copies comes from the following experiment. Platyfish carrying the X chromosomal deletion of the Giemsa band that includes the accessory *Tu* (Ahuja, 1979) were crossed with the swordtail according to the procedure outlined in Figs. 16 and 18. As was expected no X chromosomal inherited tumors developed spontaneously in the hybrid offspring, but after treatment with MNU in rare cases, the backcross hybrids developed different kinds of neoplasms (including melanoma) that could be assigned partly to the deleted X chromosome of *X. maculatus* (42/408) and partly to autosomes of unknown origin (20/470). 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 (Schmidt, 1983).

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 known potentially tumorigenic copies of *Tu* (four copies in homozygous duplications in both X chromosomes of the female, six copies in three nonhomologous pairs of autosomes) together with their linked regulatory genes into a 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 maintained by regulatory genes.

4. *Oncogene Dosage*

More information about control of the *Tu* oncogene comes from studies on oncogene dosage compensation and oncogene dosage effect. We shall refer first to dosage compensation: An X chromosome showing the *Tu* deletion according to Fig. 24 (X^{Del}; the chromosome originates from *X. maculatus* but occurred in an *X. maculatus*–*X. helleri* hybrid) was introduced by introgressive breeding into a stock of *X. maculatus*, the X chromosome of which contains the accessory *Tu* (X^{Tu}) which, because of the impairment of its

linked regulatory genes R_{Mel} and R_{Df}, exhibits the "spotted dorsal" phenotype (see Fig. 18). Both X chromosomes are identical except for the *Tu* deletion. The autosomal regulatory system for *Tu* is complete. The resulting animals contain none, one, or two copies of the accessory *Tu* (Fig. 26A–C). The animals lacking the *Tu* oncogene (A) do not show any neoplastically transformed cell, whereas the littermates containing one (B) or two (C) *Tu* copies, due to the impairment of R_{Mel} and R_{Df}, exhibit spots in the dorsal fin that are phenotypically identical. If the experiment was modified by using autosomes of *X. helleri* that lack the nonlinked regulatory genes for *Tu* as the genetic background (Fig. 27A–C), the animals lacking the *Tu* oncogene, as expected, do also not show any neoplastically transformed cell (A), whereas the littermates containing one *Tu* copy exhibit malignant melanoma (B) while those having inherited the double dosage of *Tu* develop extreme malignant melanoma (C). Oncogene dosage compensation (Fig. 26B and C) and oncogene dosage effect (Fig. 27B and C) have been observed in many experiments of this kind (F. Anders and Klinke, 1966).

5. *Tu and c-src*

After the cellular homologs of retroviral oncogenes, particularly *c-src* (Section II,E), and the Mendelian oncogene *Tu* (Section III,D) were identified in all individuals of all groups of *Xiphophorus* tested, we combined the molecular studies on *c-src* with the genetic studies on *Tu*.

Based on the assumption that the activity of the pp60$^{c\text{-}src}$ associated phosphokinase monitors the activity of the *c-src* oncogene, we carried out comparisons between kinase activity (Section II,E), i.e., *c-src* expression, and tumor development, i.e., *Tu* expression (Section III). In somatic mutation-conditioned neoplasms (melanomas and others) triggered by MNU and X rays (Section III,A) we found elevated levels of kinase activity as compared to the corresponding nontumorous tissues (M. Schartl *et al.*, 1983). The results are compatible with those obtained by other authors in other systems (see Jacobs and Rübsamen, 1983), but still left it unclear to determine whether the kinase activity in tumors is causally related to neoplasia or is only one of the numerous epiphenomena associated with neoplasia (see Section IV,D).

More informative data come from studies on hybrids developing melanoma spontaneously according to Mendelian laws (Section III,D,1; Figs. 16 and 18). These hybrids show tissue-specificity in kinase activity like the nontumorous fish (see Fig. 12). This activity is high in melanoma and in brain, and varies genotype specifically in both melanoma and brain in the same direction (Fig. 28) (Barnekow *et al.*, 1982; M. Schartl *et al.*, 1982). The genotype-specific similarity of kinase activity in brain and melanoma was not found in the animals developing the somatic mutation-conditioned neo-

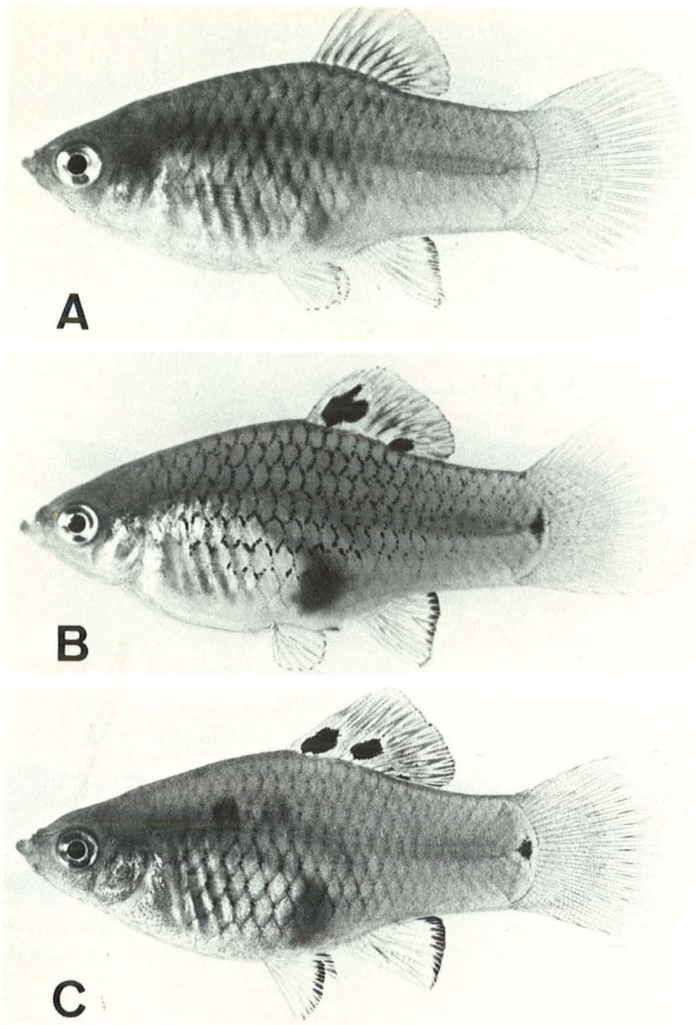


FIG. 26. Gene dosage compensation in the "spotted dorsal" phenotype (*Tu* expression in the dorsal fin) in littermates that are genetically identical except for the dosage of the accessory *Tu*. *Tu* is controlled by the complete nonlinked regulatory gene system. (A) No *Tu*, no spots. (B) One copy of *Tu*, spots in the dorsal fin. (C) Two copies of *Tu*, spots do not differ from those of B.

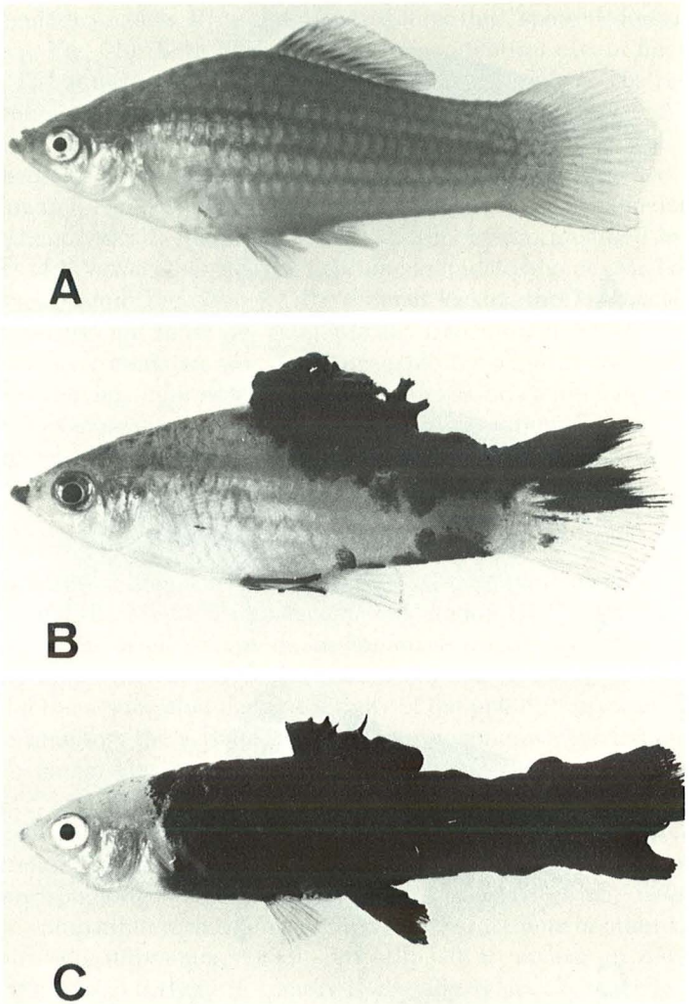


FIG. 27. Gene dosage effect of the same *Tu* as used in the experiments shows in Fig. 26 except that the nonlinked regulatory gene system is lacking. (A) No *Tu*, no neoplastically transformed cell. (B) One copy of *Tu*, malignant melanoma originates at the dorsal fin. (C) Two copies of *Tu*, extreme malignant melanoma develops.

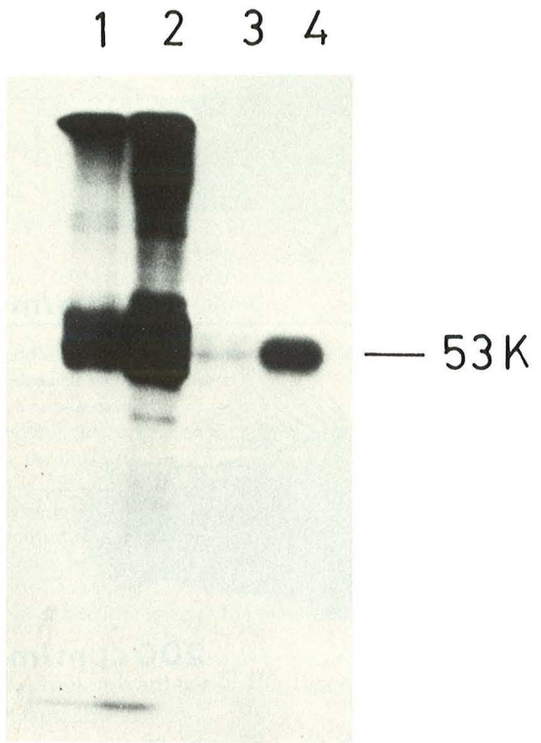


FIG. 28. Demonstration of pp60$^{c\text{-}src}$-associated protein kinase activity in extracts of brain and melanoma. Track 1: brain from benign melanoma bearing BC hybrids. Track 2: brain from malignant melanoma bearing BC hybrids. Track 3: benign melanoma. Track 4: malignant melanoma. 53K indicates the heavy chain of immunoglobulin G of the pp60$^{c\text{-}src}$ immunocomplex. Note that kinase activity in both brain and melanoma changes in the same direction. BC hybrids according to Figs. 16, 18, and 36. See legends to Figs. 11 and 12 for kinase assay.

plasms indicating that there is no secondary interdependence between kinase activity in brain and melanoma (M. Schartl *et al.*, 1983). Hence, we could determine pp60$^{c\text{-}src}$ associated protein kinase activity mainly in brain extracts and relate the activity observed to the expression of *Tu* ascertained by the development of melanoma (M. Schartl *et al.*, 1982). 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. Therefore, the results reflect the actual genetic activity of the *c-src* oncogene in the nontumorous brain tissue of the tumorous and nontumorous fish.

To study possible relations between *Tu*-conditioned neoplasia and *c-src*

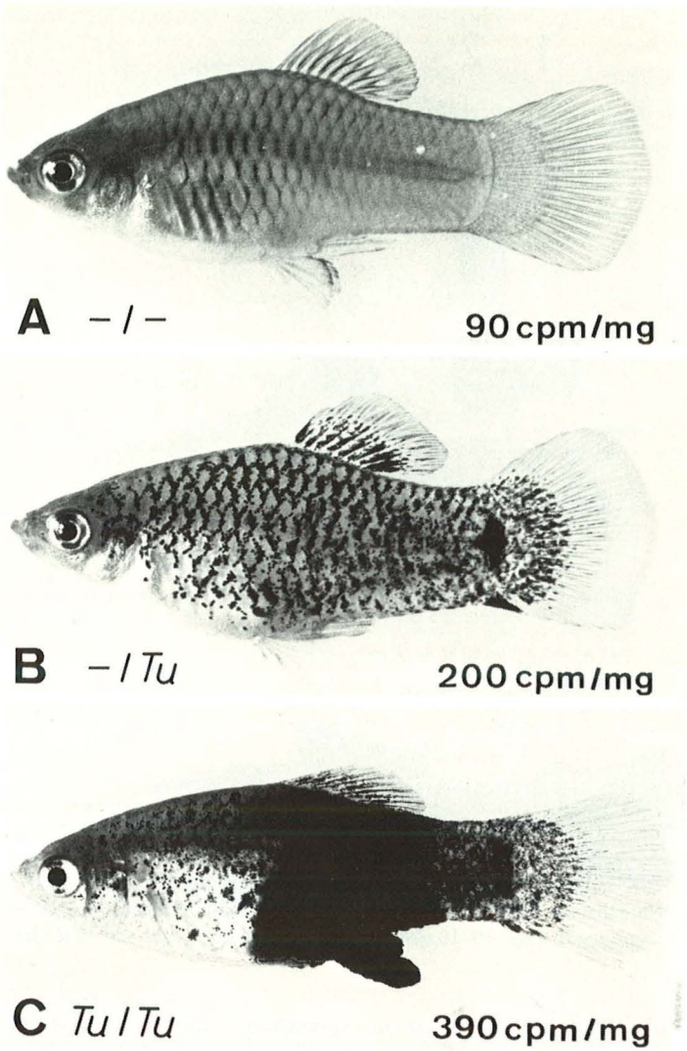


FIG. 29. Correlation between gene dosage effect of *Tu* (specified as the phenotype of the tumor) and gene dosage effect of *c-src* (pp60$^{c\text{-}src}$ kinase activity expressed as counts per minute/milligram protein) in littermates containing (A) no accessory *Tu*, (B) single dose of *Tu*, (C) double dose of *Tu*. The genetic background of the fish is identical; the pigment cell-specific R_{Mel} which is linked to *Tu* is impaired by germ line mutation. From F. Anders *et al.* (1984), modified.

TABLE V

pp60$^{c\text{-}src}$ ASSOCIATED KINASE ACTIVITY IN BRAIN EXTRACTS SPECIFIED BY cpm/mg SOLUBLE PROTEIN[a] IN F_2 SEGREGANTS HAVING NONE, ONE, OR TWO, RESPECTIVELY, ACCESSORY COPIES OF *Tu*

Tu gene complex corresponding to phentype[b]	Dosage of the accessory *Tu*[c]		
	No *Tu* (cpm/mg)	One dosage (cpm/mg)	Double dosage (cpm/mg)
striped[d]	90 (18)[g]	200 (4)	390 (8)
dabbed[e]	170 (11)	190 (10)	390 (51)
dabbed[f]	200 (5)	260 (3)	1240 (4)

[a] Three to eight brains per measurement were used.

[b] Different gels each were measured.

[c] One gel each was measured.

[d] The *Tu* copy of *striped* originates from the X and Y chromosomes of *X. maculatus* from Rio Jamapa, Mexico; the linked R_{Mel} and R_{Bs} are impaired resulting in the phenotype shown in Fig. 29B and C (this is a mutant of *X. maculatus* shown in Fig. 19).

[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.

[g] Total number of brains is indicated in parentheses.

expression we took advantage of the three genetic experiments outlined in Figs. 18, 19, and 29.

In the experiment outlined in Fig. 18, the pure-bred *X. maculatus* carrying two repressed copies of the accessory *Tu*, as well as the pure-bred *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 we interpret to be the basic expression of *c-src*. In contrast, the melanoma-bearing hybrids which contain the derepressed *Tu* show an increase of *c-src* activity, with the malignant melanoma-bearing BC hybrids displaying the highest activities (about 600 cpm/mg protein).

In the experiment outlined in Fig. 19, all pure-bred and hybrid animals, irrespective of the dosage of the accessory *Tu*, but dependent on their nontumorous state maintained by several nonlinked regulatory genes or by a single linked regulatory gene (see the highly susceptible genotype), display a similar uniform base-level *c-src* activity.

In littermates (Fig. 29 A–C), however, genetically identical except for the absence (A) or presence of one (B) or two (C) partially derepressed accessory *Tu* copies, pp60$^{c\text{-}src}$ displays a kinase activity that increases stepwise and in parallel with the dosage of *Tu*. This *Tu* gene dosage, in turn, determines whether the animals will develop no tumors or slow or rapidly growing

tumors. Table V shows additional 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 two conditions, i.e., one, the stepwise derepression of an accessory *Tu*, and two, the stepwise introduction of additional copies of a derepressed accessory *Tu*. These findings suggest several possible interpretations of the relationship between *Tu* and *c-src:* (1) *Tu* might be independent from *c-src*, and the correspondence between both *Tu* and *c-src* is due to linkage relationships. (2) the *c-src* might represent a regulatory gene for *Tu* or vice versa. (3) *Tu* might be identical to *c-src*, and this oncogene can code for a large variety of neoplasms. (4) *Tu* might consist of different oncogenes responsible for different kinds of neoplasia, and *c-src* is one of these genes. At present, we cannot make a firm interpretation; additional data are required. Two points favor the idea that the accessory *Tu* oncogene is composed of several *c-onc* genes homologous to the retroviral *v-onc* genes. These are (1) the fact that all types of neoplasia including epithelial, neurogenic, and mesenchymal neoplasms could be assigned, for instance, to an accessory *Tu* located on a particular Giemsa band of an X chromosome, and (2) the assumption that each of the different *c-onc* genes identified by molecular hybridization codes for a different type of neoplasia. Those crossing experiments, in which the backcross hybrids segregated into one group of animals susceptible to epithelial and neurogenic neoplasms, and into the other group susceptible to mesenchymal tumors (see Section III,E) could be explained by the assumption that part of the tissue-specific *onc* genes that normally might compose *Tu* were translocated to a nonhomologues chromosome.

B. The Pigment Cell-Specific Regulatory Gene

1. *Significance of Regulatory Genes in Neoplasia*

Emphasis is being placed at present in cancer research on the molecular characterization, amplification, rearrangement, mutation, and overexpression of oncogenes, and on their normal and abnormal functions. We know from the *Xiphophorus* model, on the other hand, that the most important process involved in neoplasia in these animals is probably loss, deletion, impairment, or any other dysfunction of the regulatory gene system permitting the abnormal expression of the ubiquitously present oncogenes. Deregulation of an oncogene caused by spontaneous or environmentally induced molecular or structural changes in the regulatory gene system is the biological basis for the development of neoplasia in *Xiphophorus*. It is these regulatory genes and not the *Tu* oncogene itself that the carcinogens act on

when they trigger neoplasia in *Xiphophorus* (A Anders *et al.*, 1973a,b; A. Anders and F. Anders, 1978).

Based on this insight our group has considered the possibility (Ahuja and F. Anders, 1976, 1977) that the specific chromosome abnormalities which have been found to be associated with chemically (Mitelman and Levan, 1972; Levan *et al.*, 1974) and virally (Mitelman *et al.*, 1972) induced neoplasms of mammals, and with certain neoplasms of humans (Rowley, 1973, 1974, 1977; Zech *et al.*, 1976; Mark, 1977; Sandberg, 1980), might arise as a result of molecular change, chromosome translocation, or deletion of tissue-specific regulatory genes which control oncogene expression in those tissues. Thus, impairment or deletion of regulatory genes, on the one hand, presumably leads to neoplastic development, whereas loss or impairment of oncogenes, on the other hand, would lead to a decrease of the tumor-mediating potential in a specific tissue. Recent results on chromosomal location of oncogenes and on specific chromosome aberrations associated with certain tumors (Dalla-Favera *et al.*, 1982a,b; de Klein *et al.*, 1982; Neel *et al.*, 1982; Rowley, 1982, 1983; Taub *et al.*, 1982) may also be interpreted by the assumption that the development of many human neoplasms results from the release of oncogenes from their regulatory genes caused by chromosome aberration and impairments.

2. *General Features of R_{Mel}*

The pigment cell-specific regulatory gene R_{Mel} was first detected by Kallman (1970) when he observed an unequal crossover that separated a *Tu* oncogene-containing chromosome segment from its linked R_{Mel}. Mutagenesis studies (A. Anders *et al.*, 1973a,b) confirmed that R_{Mel} is closely linked to *Tu* (Fig. 23). R_{Mel} controls *Tu* in the cis position only, i.e., the R_{Mel} gene controlling the closely linked *Tu* of a specific chromosome does not influence the *Tu* located on the homologous chromosome or any other chromosome. Based on these findings we developed an extensive breeding program to produce donor and recipient fish for gene transfer (transfection) experiments. These were expected to furnish new data on the functional interrelationship between R_{Mel} and *Tu*.

3. *Cotransfer of R_{Mel} and Tu by DNA Injection*

Total genomic DNA extracted from nontumorous male gonads of laboratory stocks of *X. maculatus* carrying accessory copies of *Tu* and normal, impaired, or deleted R_{Mel} genes was injected into the neural crest region of early embryos (0.05 to 0.9 μg DNA per embryo) of *X. helleri* which apparently lack both the accessory *Tu* copies and the R_{Mel} genes (Fig. 30) (Haas-Andela, 1978; J. Vielkind, 1979; J. Vielkind *et al.*, 1982). In some of the experiments DNA from animals that contained six additional but rigidly

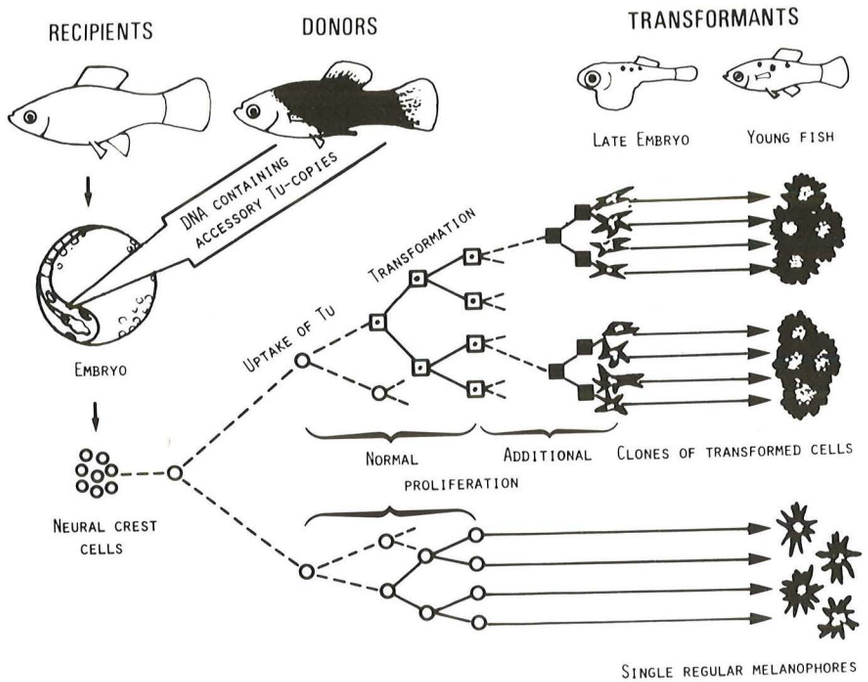


FIG. 30. Outline of the transfection experiments. See text. From Haas-Andela (1978) and J. Vielkind *et al.* (1982), modified.

repressed accessory *Tu* copies was also used. The injected DNA maintains its high molecular weight for about 2 hr (Schwab, 1974; Schwab *et al.*, 1976b) and thereafter becomes degraded to pieces which are too small to contain genetic information (J. Vielkind, 1971; J. Vielkind *et al.*, 1973a; Schwab *et al.*, 1976b). Therefore, the timing of DNA injection with the appropriate developmental state of the embryo, i.e., the stage just prior to the migration of neural crest cells and their descendants to their final location in the skin and the extracutaneous tissues (Sections II,D and IV,C) is very important for the outcome of the experiments. A neural crest cell or its early descendant still close to the neural crest region may take up the donor DNA, and may divide and differentiate to I melanoblasts, the only pigment cell precursors that are competent to the transforming *Tu* activity (Section II,D; Fig. 8). These cells eventually may become transformed to TrI melanoblasts. Additional proliferation of the Tr cells may amplify the transfected *Tu* in the pigment cell population, and the expected result should become visible after differentiation of the still nonpigmented TrI melanoblasts to the heavily

pigmented and large Tr melanocytes and Tr melanophores that form black cell colonies on the greenish-gray normal skin. Similar colonies consisting of transformed pigment cells were also expected to occur in extracutaneous tissues that normally are also populated by regular melanophores (see Section IV,C).

Most of the transformants (embryos and young fish) developed single Tr cells or small colonies of Tr cells (Fig. 31A) that were indistinguishable from those of the first stages of inherited melanoma in embryos and young fish (Fig. 31C) (Haas-Andela, 1978; J. Vielkind *et al.*, 1982), and from those developing from transplanted stem-melanoblasts containing an active *Tu* (Fig. 31B) (M. Schartl, 1979). It is important to note that most of the Tr cell colonies observed had about the same size indicating that they had undergone the same number of cell divisions. Several transformants exhibited large amounts of melanoma cells that grew along the meninx primitiva of the spinal cord (Fig. 32) and killed the embryos.

The transforming activity of the DNA of different donor breeds (Fig. 33A–E) lacking the accessory *Tu* and R_{Mel} (A), or exhibiting phenotypically different degrees of control of *Tu* by R_{Mel} (B–D) was compared. If a donor was used which lacks R_{Mel} *Tu* (A), no recipient exhibited transformed pigment cells. If a donor strain was used that carries a *Tu* slightly derepressed in the pigment cell system by a "weak" mutation of its linked R_{Mel}, i.e., R'_{Mel} *Tu* (B), then 0.4% of the recipients developed colonies of neoplastically transformed cells. If the DNA originated from a strain that carries a *Tu* derepressed to a greater degree, due to a "stronger" mutation of R_{Mel}, i.e., R''_{Mel} (C), the incidence of recipients exhibiting transformed pigment cells increased to 2.6%. If, finally, a donor was used in which the *Tu* lacks the R_{Mel} (due to a chromosomal translocation) (D) this incidence increased to 6.3%. DNase degradation prior to injection eliminates the transforming activity of the DNA (E). DNA from animals carrying additional but rigidly repressed accessory *Tu* copies (not shown in the figure) did not influence the incidence of transformants.

These results obtained by the gene transfer experiments in *Xiphophorus* may be helpful in our efforts to analyze the processes leading to neoplasia.

Besides the fact that the information for neoplastic transformation, presumably *Tu* itself, was transfered via total genomic DNA injection, it is important to note that the transforming donor DNA did not originate from tumor cells but from the nonneoplastic testes indicating that ongocenes must not necessarily be changed or amplified in order to acquire the transforming potential. The many oncogene transfection experiments accomplished during the last years by several authors with other systems (Der *et al.*, 1982; Goldfarb *et al.*, 1982; Krontiris *et al.*, 1981; Perucho *et al.*, 1981; Reddy *et al.*, 1982; Tabin *et al.*, 1982) in which DNA extracts from tumors were used

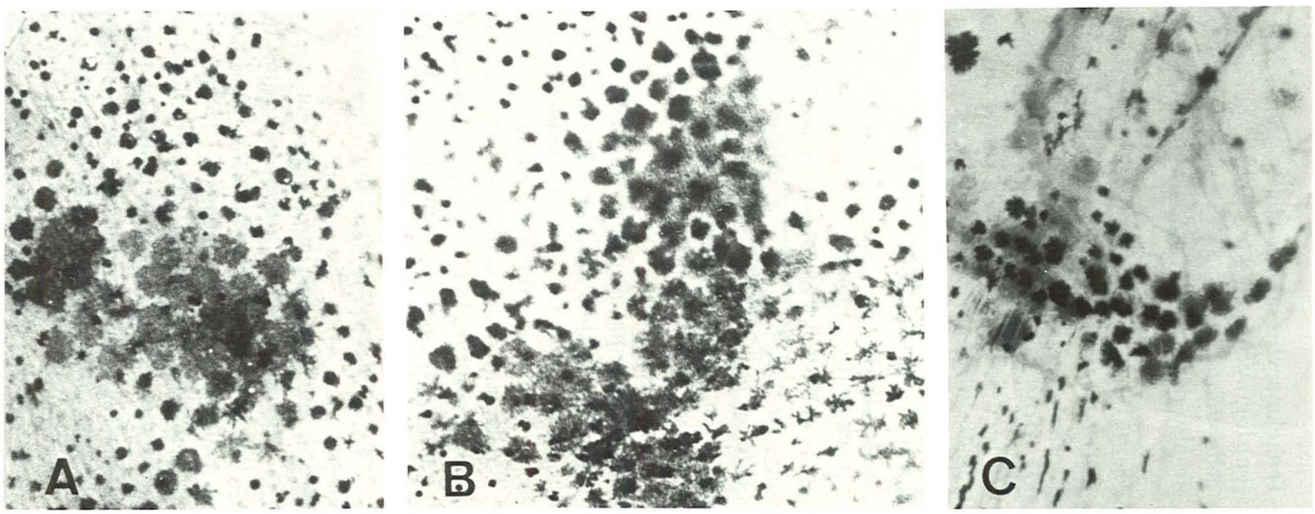


FIG. 31. Colonies of neoplastically transformed pigment cells in embryos and fry normally not capable of developing transformed cells (A and B). (A) Tr melanocytes induced by injection of *Tu* DNA into an early embryo. From Haas-Andela (1978). (B) Control experiment by M. Schartl (1979): Tr melanocytes differentiating from stem cells of melanoma transplanted into an embryo. (C) Unmanipulated control. Tr melanocytes normally differentiating in *Tu*-containing genotypes capable of developing transformed cells. Note that the Tr cells are all alike. For details see J. Vielkind *et al.* (1982). See also Fig. 38.

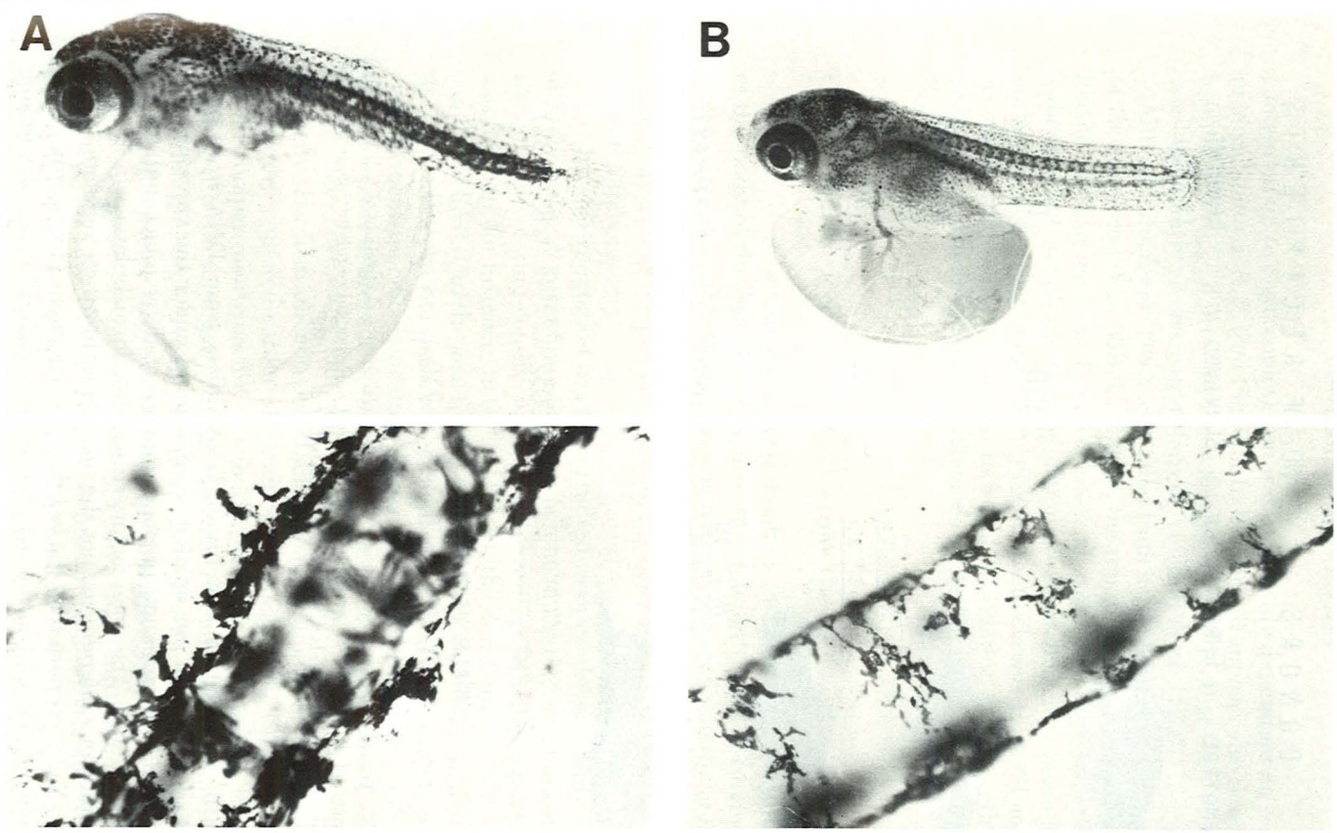


FIG. 32. Twelve-day-old embryo (3 mm long) raised *in vitro* with pigment cells of the embryos populating the dura mater of the spinal cord. (A) Transformant of a transfection experiment outlined in Fig. 30. Note the neoplastically transformed cells. (B) Control with regular pigment cells. From Haas-Andela (1978).

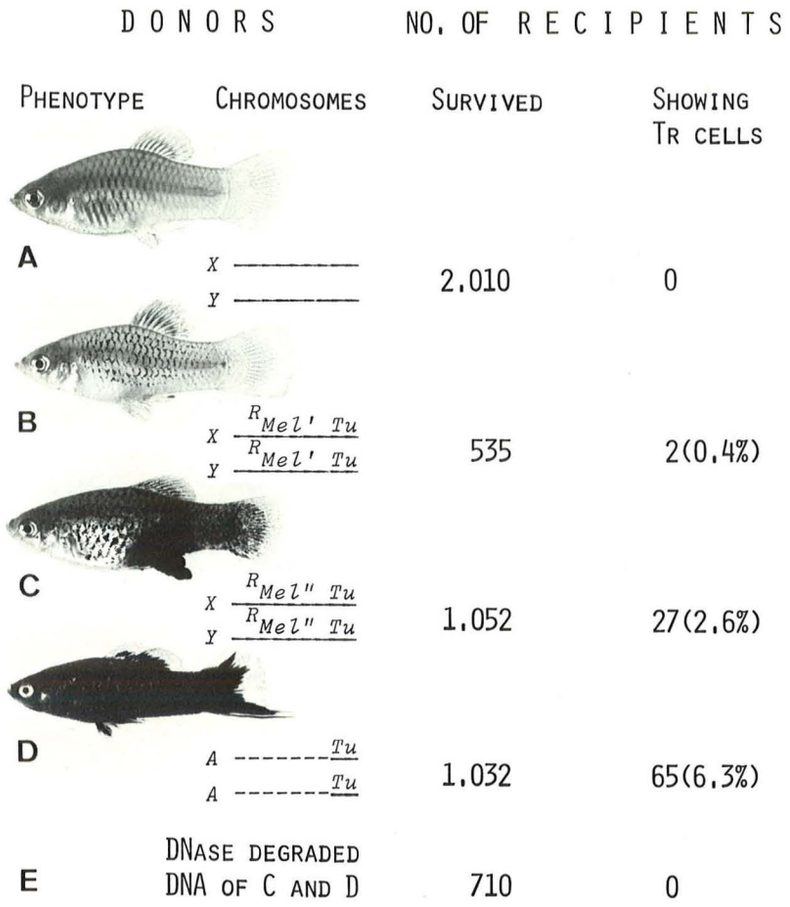


FIG. 33. Transfection activity of donor DNA extracted from male gonads of fish differing in gene dosage of *Tu*, and in *Tu* control by the pigment cell-specific regulatory gene R_{Mel}. See text. Data from Haas-Andela (1978) and J. Vielkind *et al.*, (1982).

under the expectation that tumor DNA differs from DNA from normal tissues should be, in our opinion, reconsidered under the viewpoint of repression and derepression of oncogenes exerted by intact and defective regulatory genes. The main factors responsible for neoplasia are, in view of our results on *Xiphophorus*, not the *onc* genes, but their regulatory genes.

This view is supported by the fact that the incidence of transformants, i.e., the incidence of the transformation events mediated by *Tu*, was independent from the number of *Tu* copies in the donor DNA (we tested DNA

containing up to eight copies), but was exclusively dependent on the degree of impairment of R_{Mel} (see Fig. 33). In this view it appears also reasonable to assume that R_{Mel}, if present, is so closely linked to *Tu* that both *Tu* and R_{Mel} were apparently always cotransferred (Vielkind *et al.*, 1982).

Although the donor DNA originated from fish exhibiting different degrees of *Tu* expression (Fig. 33B, C, and D), the transformed cells of the recipients looked all alike, and the Tr cell colonies all were about the same size. This indicates that neoplastic transformation of a certain cell is more an all-or-nothing process than a multistep process that releases this cell and its descendants from negative control by regulatory genes. On the other hand, growth of the tumor is neither influenced by *Tu* nor by the intact or impaired R_{Mel}.

C. Compartment-Specific Regulatory Genes

Most of the neoplasms are preferentially located in certain compartments of the body of the fish. For instance, the different kinds of carcinomas, as well as sarcomas, and melanomas originate preferentially in the area around the peduncle of the dorsal fin and tail fin; epitheliomas develop frequently in the region around the posterior part of the operculum (see Figs. 15–18, 21, 26, and 27).

The compartmentation has been studied in more detail mainly by means of X-ray-induced germ line mutations (a total of 28) that affect one or several sites of the crossing-conditioned melanomas (Fig. 34A–H). These melanomas develop, for instance, in the tail fin (A), the dorsal fin (B), in both tail fin and dorsal fin (C), in the anal fin (D), in the tail fin, dorsal fin, anal fin, mouth tip, and the posterior part of the side of the body (E; mutations of five compartment-specific regulatory genes are involved), in the anterior and posterior parts of the side of the body (F), in all compartments except for the mouth, belly, eye, dorsal fin, and tail fin (G), or even in all compartments of the body (H). The phenotypes of additional combinations of impaired R_{Co} genes were depicted earlier (A. Anders and F. Anders, 1978).

The compartment-specific distribution of these melanomas is inherited according to the segregation of the parental *Tu*-carrying chromosome, indicating that the respective R_{Co} genes are linked to *Tu*, and structural changes of the chromosome have verified that this linkage is very close (see Fig. 23). At least 14 genes corresponding to 14 different compartments have been identified (Fig. 35). They represent regulatory genes that were designated R_{Co} in total and R_{Ap} (anterior part), R_{Pp} (posterior part), R_{Df} (dorsal fin), R_{Tf} (tail fin), etc. in reference to each specific body compartment. Intact R_{Co} genes repress *Tu*, and impaired R_{Co} genes permit *Tu* activity. They act,

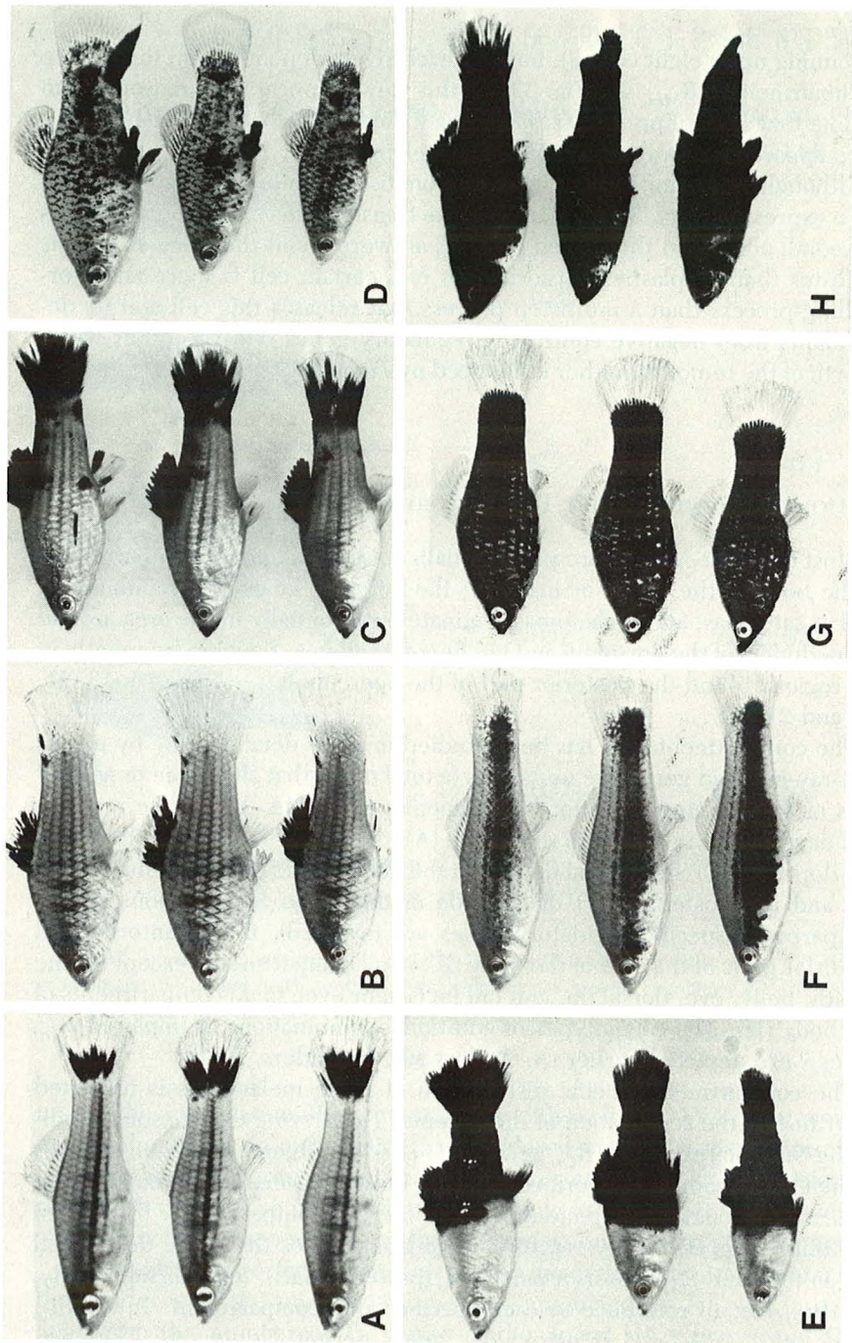


FIG. 34. Examples of compartmentation depending on compartment-specific regulatory genes (R_{Co} genes) which, in case of impairment, permit compartment-specific melanoma formation. See Fig. 35 and text.

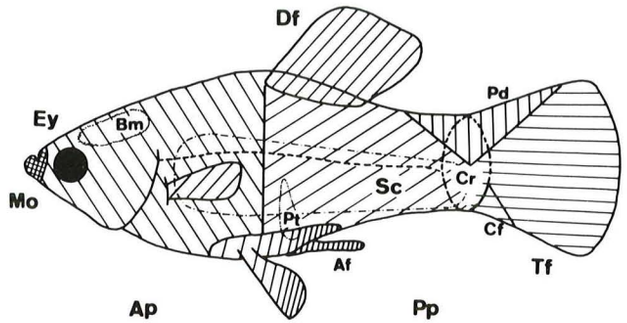


FIG. 35. Compartments for the development of melanomas in the fish (see Fig. 34) that are due to compartment-specific regulatory genes (R_{Co} genes). Mo, mouth; Ey, eye; Bm, brain membrane; Ap, anterior part; Df, dorsal fin; Pt, peritoneum; Af, anal fin; Sc, spinal cord; Pp, posterior part; Cr, crescent region; Pd, peduncle of the tail fin; Cf, caudal fin stripe; Tf, tail fin. From A. Anders and F. Anders (1978).

however, in the cis position only, indicating that the compartment-specific regulation of *Tu* exerted by the R_{Co} genes acts at the DNA level.

In the active state the R_{Co} genes appear to delay the differentiation of pigment cells in the stem cell stage (S melanoblasts; see R_{Co} in Fig. 8; Diehl, 1982). Additional mechanisms that are not understood provide the fish with differentiating pigment cells that mostly escape neoplastic transformation, but very exceptionally may be transformed. If, however, one or several R_{Co} genes are impaired by mutation, compartment-specific melanomas develop in the hybrids and are inherited according to Mendelian prediction.

There are many observations cited in the literature indicating that human melanomas are also preferentially located in certain areas of the body (Olsen, 1966). *Xiphophorus*, therefore, may provide a model to study this phenomenon.

D. The Differentiation Gene

1. General Features

As shown in Section III,D and Figs. 16 and 18 benignancy and malignancy in the hybridization-conditioned melanomas depend upon the presence or absence, respectively, of the chromosome carrying *Diff*. Linkage relationship studies using biochemical markers for this chromosome, i.e., the esterase *Est-1* and the isozyme A of the glyceraldehyde-3-phosphate dehydrogenase, have confirmed that the *Diff*-carrying chromosome, like the accessory *Tu*, is derived from the platyfish. Animals from those wild popula-

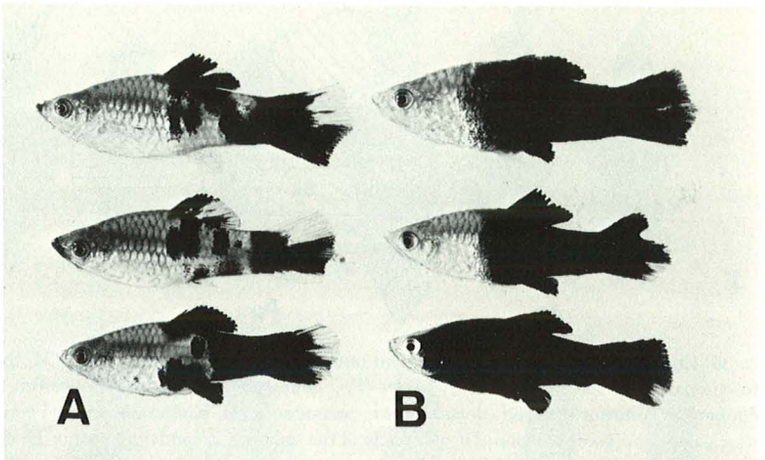


FIG. 36. Melanoma bearing backcross segregants according to the schematic drawings of Fig. 18. (A) Benign melanoma bearing fish containing one dose of the differentiation gene *Diff*. (B) Malignant melanoma bearing littermates that lack the *Diff* gene.

tions that apparently lack the accessory *Tu* oncogene also apparently lack the *Diff* gene (E. Scholl, 1977; Ahuja *et al.*, 1980).

Diff may be present in double dosage, single dosage, or be lacking, and the *Tu*-containing animals carrying two, one, or no *Diff* can easily be distinguished by gross examination of their phenotype. If *Diff* is present in double dosage (*Diff*/*Diff*) the oncogene *Tu* phenotypically may express at the most some occasional spots which are considered to be extreme benign melanomas (Section II,C); if present in single dosage (*Diff*/−) the *Tu* oncogene may express benign melanoma. If, however, *Diff* is lacking (−/−) the oncogene *Tu* expresses almost always malignant melanoma.

2. *Diff-Dependent Characters*

To study the basic difference between the benign and the malignant state of the melanoma, thousands of melanoma bearing BC segregants have been produced according to the crossing scheme shown in Fig. 18, and all showed a clearcut 1:1 segregation into animals developing spontaneously benign or malignant melanomas (Fig. 36; Table VI). BC hybrids that require the carcinogenic trigger for the development of carcinoma (see Fig. 19) show also a clearcut *Diff* effect: The *Diff*-carrying segregants (identified by esterase 1) that are moderately susceptible to neoplasia develop benign melanoma, whereas the *Diff*-lacking segregants that are extremely susceptible develop malignant melanoma (Fig. 37).

TABLE VI
SEGREGATION OF FISH CARRYING BENIGN AND MALIGNANT MELANOMA IN BC GENERATIONS

Stock from which *Tu* and *Diff* originated		BC generation	Melanoma: Benign *Diff*/−	Melanoma: Malignant −/−
Spotted	*Sp*	1–4	173	207
Spotted dorsal	*Sd*	1–12	1.358	1.344
Spotted dorsal mutant	*Sd′*	1–4	1.270	1.235
Spotted dorsal mutant	Sd^{rec}	1–5	1.831	1.806
Stripe sided mutant	*Sr′*	1	263	251
Lineatus mutant	*Li′*	1–2	232	228
		Total	5.118	5.071

The cytological, fine structural, biochemical, and biological data obtained by the comparison of benign (with *Diff*) and malignant (without *Diff*) melanoma are listed in Table VII. The data show that the cells of the benign melanoma are well differentiated whereas those of the malignant melanoma are poorly differentiated, and that differentiation of the Tr cells is controlled by *Diff*. This, together with the findings on pigment cell differentiation (Section II,D; Fig. 8) led us to the following conclusions: (1) if *Diff* is lacking, the majority of the melanoma cells persist in the stage of the poorly differentiated, continuously dividing TrA melanoblasts and Tr melanocytes, and only a few cells differentiate to Tr melanophores which are incapable of dividing and, at a certain age, are removed by macrophages; (2) if, however, the *Diff* is present, the majority of the melanoma cells become terminally differentiated to Tr melanophores, whereas only a few cells remain in the stage of TrA melanoblasts and Tr melanocytes. Terminal differentiation and removal of the melanoma cells are antagonistic to the permanent supply of melanoma cells from S melanoblasts by transformation, and the melanoma renders benign. This observation is in accordance with the outcome of experiments in which newborn BC segregants were treated with methyltestosterone which strongly promotes pigment cell differentiation from the precompetent to the competent state for neoplastic transformation (A. Schartl, 1981): both, *Diff*-carrying and *Diff*-lacking newborn segregants, developed malignant melanomas that killed them. A benign period of the melanoma in the *Diff*-carrying fish was not observed. Consequently, the level of *Diff*-controlled benignancy is not at the premalignant stage but at the malignant stage of the tumor. On the other hand, if one considers the stepwise disintegration of the regulatory gene system for *Tu*, which is re-

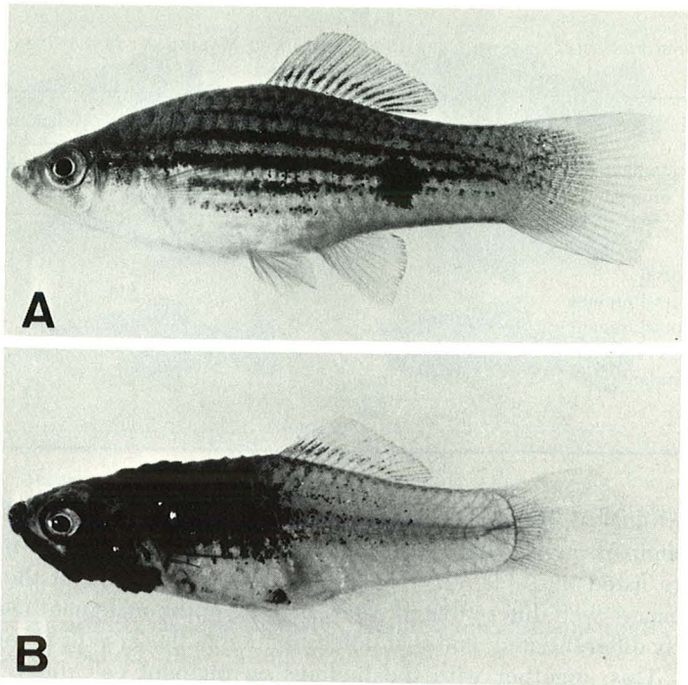


FIG. 37. Backcross hybrids according to Fig. 19 after MNU treatment. (A) *Diff*-carrying segregant (identified by esterase-1) developing benign melanoma. (B) *Diff*-lacking segregant (identified by lack of esterase-1) developing malignant melanoma.

quired for neoplasia, and considers elimination, deletion, or impairment, respectively, of the *Diff* as the last step of disintegration, benignancy might appear as the premalignant state of a malignant melanoma. The existence of animals carrying malignant melanoma on the surface of a benign melanoma like that shown in Fig. 22 might support this concept; actually, however, such a malignant melanoma is an additional, independently derived malignant melanoma that initiates following a mutation of *Diff* in an early pigment cell precursor. The concept of the change of a tumor from the benign to the malignant state which is generally accepted in cancer research could not be verified in *Xiphophorus*, because such a change has never been observed in *Xiphophorus*, and is even unlikely to exist. Malignant melanoma (and the malignant state of other neoplasms) in *Xiphophorus* is malignant from the very beginning, even in its initial stage consisting of one or only several cells (Section II,D).

TABLE VII
THE GENE *Diff* IN *Xiphophorus*

Diff/−	−/−	Reference
Benign melanoma	Malignant melanoma	
Well differentiated	Poorly differentiated	U. Vielkind (1976)
Slow growing	Fast growing	A. Anders *et al.* (1973a,b)
Noninvasive	Invasive	
Nonlethal	Lethal	
Difficult to transplant	Easily transplantable	
No vascularization	Vascularization	
Difficult to trigger by promoters	Sensitive to promoters (testosterone, cAMP, corticotropin, BUdR, TPA, cyclamate, saccharin, phenobarbital, etc.)	M. Schartl *et al.* (1981); C. R. Schmidt (1983); Herbert (1983)
Regression following testosterone treatment, etc.	No regression	A. Schartl *et al.* (1982)
Weak effect of external factors on growth rate	Drastic effect of external factors on growth rate (temperature, salinity, UV, etc.)	F. Anders *et al.* (1962b); F. Sieger *et al.* (1969)
No effect of nutrient factors	Drastic effect of nutrient factors (amino acids)	M. Sieger *et al.* (1968); F. Anders *et al.* (1969); F. Sieger *et al.* (1969)
Many macrophages	Few macrophages	Diehl (1982)
Tr melanophores are prevailing	TrA melanoblasts and Tr melanocytes are prevailing	U. Vielkind (1976)
Endopolyploid and multinucleated	Diploid and uninucleated	
Mature melanosomes	Immature melanosomes	
Lack of ER and Golgi complexes	Well-developed ER and Golgi complexes	
Low enzyme activities	High enzyme activities (tyrosinase, LDH B^4, MDH etc.)	Schwab *et al.* (1976a); E. Scholl (1977); U. Vielkind *et al.* (1977)
Less complex glycosphingolipids	More complex glycosphingolipids	Felding-Habermann *et al.* (1983)
Low rate of thymidine incorporation	High rate of thymidine incorporation	M. Sieger *et al.* (1968); J. Vielkind *et al.* (1973b)
Tumorous and nontumorous tissues	Tumorous and nontumorous tissues	
Low *c-src* activity	High *c-src* activity	M. Schartl *et al.* (1982)

TABLE VII (*Continued*)

Diff/–	–/–	Reference
Dispersed chromatin	Condensed chromatin	F. Anders *et al.* (1981a); Heil (1983)
High rate of DNA nuclease digestion	Low rate of DNA nuclease digestion	Heil (1983)
Low pteridine contents	High pteridine contents	Henze *et al.* (1977)
First position of the anticodon of $tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{Tyr}$, $tRNA^{His}$ contains predominantly Queuosine	First position of the anticodon of $tRNA^{Asp}$, $tRNA^{Asn}$, $tRNA^{Tyr}$, $tRNA^{His}$ contains predominantly guanosine	Dess (1982); Kersten *et al.* (1983)
Diff product is diffusible	No product	M. Schartl (1979)

3. *A Differentiation-Promoting Diff-Dependent Product*

The most convincing data supporting *Diff*-dependent control of pigment cell differentiation come from transplantation experiments which were designed according to the arrows drawn in Fig. 18 between the BC hybrids (M. Schartl, 1979). The crucial transplantation experiments, including the composition of secondary chimeras by fusion of parts of early embryos, have shown that pigment cell precursors present in the transplant taken from fish carrying *Tu* but lacking *Diff* (material of still tumor-free early embryos of the malignant melanoma developing genotype) become transformed and remain incompletely differentiated Tr cells if transplanted into embryos lacking *Tu* and *Diff*: the resulting animals develop malignant melanoma. If, however, the pigment cell precursors of the same genotype were transplanted into *Tu*-lacking embryos that contain the *Diff*, the cells of the developing melanoma become terminally differentiated and regain their distance regulation (Fig. 38): these resulting animals develop extreme benign melanomas which regress and eventually may become removed by macrophages. Thus the effect of *Diff* on the differentiation of the neoplastically transformed pigment cells can be traced to a diffusible substance. The nature of this substance is unknown at present.

4. *Modified tRNAs in Diff-Dependent Differentiation*

There is considerable evidence for the involvement of modified nucleosides containing tRNAs in the process of cell differentiation in eubacteria, slime molds, and normal neoplastic tissues of vertebrates (Nishimura and Kuchino, 1979; Kersten, 1982a, 1983; Katze *et al.*, 1983; see Nass,

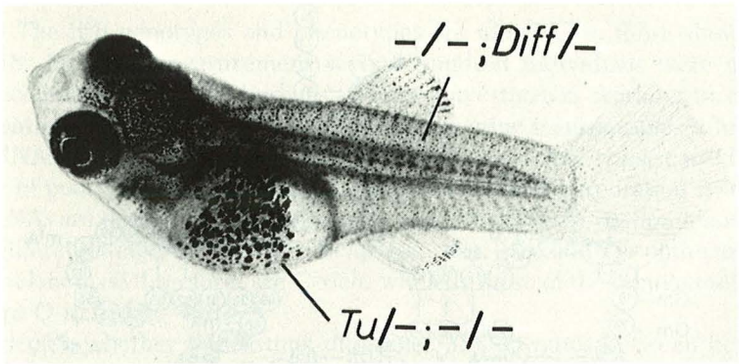
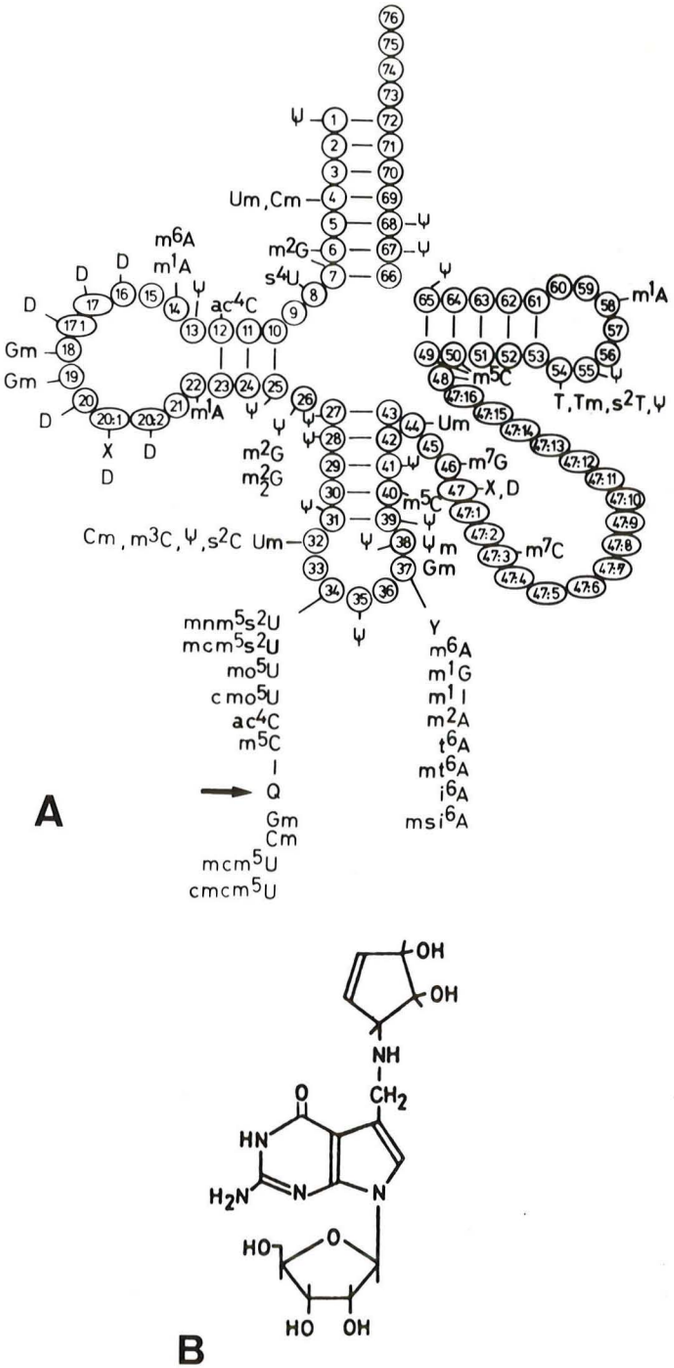


FIG. 38. Secondary chimera composed by transplantation of tissues containing precursor cells of malignant melanoma that originated from a young backcross hybrid containing *Tu* but lacking *Diff* (malignant melanoma developing BC segregant according to Fig. 18), to an embryo lacking *Tu* but containing *Diff* (nontumorous BC segregant in Fig. 18 at bottom left). Note terminal differentiation and distance (density) regulation of the transformed cells of the transplant according to the *Diff* genotype of the host. From M. Schartl (1979).

1983). The modified nucleosides occur at well-defined positions in specific tRNAs (Fig. 39A) (see Kersten, 1982b; Spritzel and Gauss, 1982). Many studies were focused on a family of tRNAs including $tRNA^{Asn}$, $tRNA^{Asp}$, $tRNA^{His}$, and $tRNA^{Tyr}$ which may contain queuosine (Q) instead of guanosine (G) in the first position of the anticodon (position 34; see arrow in Fig. 39A). The Q nucleoside (7-{[(4,5-*cis*-dihydroxy-2-cyclopenten-1-yl)-amino]methyl}-7-deazaguanosine) is unique in that its purine skeleton is modified to a 7-deaza structure (Fig. 39B). Eubacteria synthesize the base queuine *de novo* (see Nishimura, 1983) whereas vertebrates are supplied with queuine by nutrition or the intestinal flora (Reyniers *et al.*, 1981), and queuine itself is inserted into the nucleotide chain of tRNA by exchange with guanine by tRNA-guanine transglycosylases. The more the differentiation progresses, the more G is replaced by Q in position 34 (see Nass, 1983).

The method to estimate the G:Q ratio in a given population of the tRNA family consisted of following the replacement of guanine in position 34 by a labeled guanine exerted by a guanine transglycosylase (insertase) of *E. coli* (Okada and Nishimura, 1979; Dess, 1982, 1983; Kersten *et al.*, 1983).

The results obtained in *Xiphophorus* by measurement of [^{3}H]guanine incorporation in the tRNAs for Asn, Asp, His, and Tyr, differing in the ratio of G:Q in position 34 are summarized in Fig. 40. The graphs show the kinetics of the exchange of G 34 of the tRNA family by [^{3}H]Gua, which is the reaction used to evaluate the amount of (Q)-tRNA (Okada and Nishimura,



1979). The fish genotypes and phenotypes are identical to those shown in Fig. 18. For each measurement several hundred individuals were used.

In accordance with the findings of many investigators working in other differentiation systems (see Nass, 1983) [^{3}H]guanine incorporation is high if the tRNAs are prepared from malignant melanomas that consist predominantly of poorly differentiated cells. In contrast, the incorporation is low if the tRNAs are derived from benign melanomas that consist predominantly of well-differentiated cells. The tRNAs for Asn, Asp, His, and Tyr of the malignant melanomas, therefore, are G-rich, whereas those of the benign melanomas are Q-rich (Fig. 40B).

To decide whether the distinct difference in G:Q ratios between benign and malignant melanoma is *Diff* dependent or represents an epiphenomenon of benignancy and malignancy, the skin of nontumorous littermates that segregate into animals carrying *Diff* and lacking *Diff* like the tumorous fish in a 1:1 ratio (see Fig. 18) was used for the measurement (Fig. 40C). The *Diff*-lacking segregants (specified by the lack of Est-1) had always higher amounts of Q-lacking tRNA than the *Diff*-carrying ones. The skin of corresponding segregants derived from crossings between stocks other than that used in Fig. 40 showed the same differences in [^{3}H]guanine incorporation (Dess, 1982; Kersten, 1982a; F. Anders, 1982). The skin of the parent animals used for the initial crosses showed the same differences: *X. helleri* that lacks the *Diff* has a high [^{3}H]Gua incorporation (i.e., is G-rich) whereas *X. maculatus* that contains the *Diff* has a lower [^{3}h]Gua incorporation (i.e., is Q-rich). Similar differences, although not as pronounced as in the skin and tumors, were also found in the nontumorous liver. From these results we conclude that the differences of G:Q ratios between benign and malignant melanoma are no epiphenomena of benignancy and malignancy, but are very closely related to the primary effect of *Diff* that in tumorous fish converts the malignant to the benign state.

The differences in the functional properties of Q-containing and Q-lacking tRNAs requires further elucidation. The (Q)tRNAs are suggested to prefer condons NAU over NAC, whereas the Q-lacking tRNAs read NAC and NAU equally well (Nishimura, 1983). This can be an important mechanism in the regulation of translation. For eukaryotic tRNA^{Tyr} it has been shown that the Q-lacking species reads a terminator codon, probably UAG (Bienz and Kubli, 1981). Therefore the Q-lacking and Q-containing tRNAs of vertebrates might select mRNAs for translation by a regulatory mechanism, a

FIG. 39. (A) General cloverleaf structure of tRNA and positions of modified nucleosides. Positions and abbreviations of modifications in Sprinzel and Gauss (1982). (B) Structure of queuosine. From Kersten *et al.* (1983).

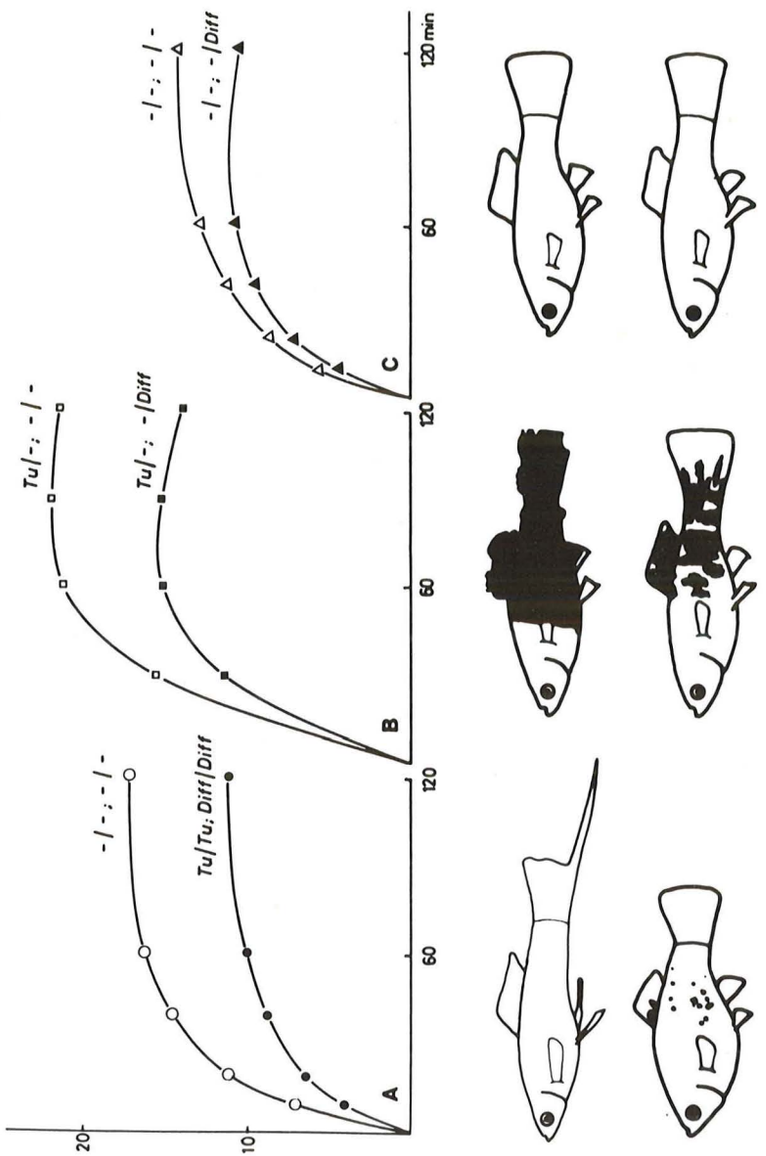


FIG. 40. Incorporation of [^{3}H]guanine in position 34 of tRNA for Asp, Asn, His, and Tyr of *Xiphophorus* catalyzed by tRNA-guanine-transglycosylase (insertase) of *E. coli*. The graphs show the kinetics of the exchange of G34 of tRNA by [^{3}H]Gua, a reaction used to evaluate the amount of (Q−) tRNA (Okada and Nishimura, 1979). A,B,C, according to the fish shown below the curves. These fish correspond to those shown in Fig. 18. High incorporation of [^{3}H]Gua in the *Diff*-lacking animals corresponds to low content of Q, whereas low incorporation of [^{3}H]Gua in *Diff*-containing animals corresponds to high content of Q. (A) Skin of pure-bred *Xiphophorus*: (○) *X. helleri*; (●) *X. maculatus*. (B) Melanoma of BC segregants: (□) malignant; (■) benign. (C) Skin of nonmelanomatous BC segregants: (△) lacking *Diff*; (▲) containing *Diff* (identified by esterase-1). Note that comparable *Diff*-containing animals always have a lower G content and higher Q content than *Diff*-lacking ones. See Fig. 18: The pairs of *Diff* and *Diff*$^-$ values (10 and 15; 15 and 20; 10 and 15) are based on these and additional data of this kind. Data from Kersten *et al.* (1983).

process already demonstrated for bacteria and designated as termination transcription control (Yanofsky, 1976; for details see Kersten *et al.*, 1983).

V. Theoretical Considerations on a General Concept of Neoplasia

A number of theories have been put forth concerning the origin of neoplasia; some have suggested that tumors are caused by viruses (Huebner and Todaro, 1969), while others have proposed that altered control mechanisms of cell differentiation are the main cause (Markert, 1968; Pitot, 1968), often brought about by promoters (Cairns, 1982; Weinstein *et al.*, 1982). Further, it has been suggested that somatic mutations induced by physical or chemical agents might have relevance to the origin of tumors (Boveri, 1929; Bauer, 1928), but there are also genetically conditioned tumors that arise apparently independent of any external influence (Knudson, 1973, 1982). Evaluating these theories, it appears that, regardless of what causes neoplasia, three basic events leading to neoplasia are always the same, namely (1) neoplastic transformation, followed by (2) restrained cell differentiation, and (3) continued cell proliferation. This implies that the principle underlying neoplasia must be also always the same (Holley, 1975; Ahuja and F. Anders, 1976).

A. The Common Basis of Neoplasia in Metazoa

There is a considerable accumulation of evidence that the basic prerequisite of tumor formation is a genetic factor which, under normally regulated conditions probably exerts essential but unknown normal functions in the multicellular organization of apparently all metazoa (Sections II,F and III,B); if deregulated, however, it appears as an oncogene or an accumulation of oncogenes, respectively (Section IV,A). Whether the oncogene is primarily a cellular gene or is contributed as a viral oncogene which is anyway derived from a cellular gene, is of minor importance in this context.

B. The Common Basis of Neoplasia of Different Tissues

While the development of the large variety of mesenchymal, epithelial, and neurogenic neoplasms observed in vertebrates is mostly considered as completely independent, the *Xiphophorus* model provides some new perspectives for a unified consideration of this diversity. The different kinds of neoplasia are possibly mediated by a common *Tu* oncogene, or set of oncogenes that compose *Tu*, which is probably present in all tissues (Fig. 8). Tissue specificity of the activity of *Tu*, however, comes apparently from

tissue-specific sets of regulatory genes (R_{Mes}; R_{Epi}; R_{Nerv}; R_{Mel} in Fig. 8) which, if impaired or deleted, permit further events leading to the neoplasms in the respective tissue(s). In addition, there also seems to exist a genetic mechanism that controls *Tu* independently from the respective tissue. This is derived from the observation that, following treatment with a carcinogen, fish like the BC hybrids shown in Figs. 15 and 21, may develop multiple tumors such as melanoma, neuroblastoma, rhabdomyosarcoma, and epithelioma. Our results, therefore, may unify the origin of the different kinds of neoplasia in *Xiphophorus* to a common principle. It would be worthwhile to examine whether the unity of neoplasia suggested in the *Xiphophorus* model may be also valid for neoplasia in other vertebrates including humans.

C. The Common Basis of Tumor Etiology

Since there are many factors that regulate the oncogene, many events are required for the disturbance of this regulation: the process leading to neoplasia is, necessarily, a multistep process. This is not to say that the final event, i.e., neoplastic transformation of a cell itself, is a multistep process. The *Xiphophorus* model has rather shown that neoplastic transformation of a particular cell is an all-or-nothing-process which represents the last step of a long chain of events that dismantle the regulatory gene system for *Tu*. This dismantlement may start with a series of hybridization, may accumulate through many generations, and, finally, may complete it in a somatic cell during the period from youth to senescence of a particular individual. The sequence of events leading to the last step, however, may be specific to the particular individual, and to the nature of the carcinogenic trigger, thus providing the basis for the singularity of a particular tumor.

The laboratory fish stocks derived from wild populations require a large variety of events to dismantle the regulatory gene system for the *Tu* oncogene. They develop neoplasia only if the many steps leading to neoplasia are completed by a combination of different carcinogenic influences, such as (1) elimination of regulatory genes from the germ line by selective mating, (2) impairment or deletion of regulatory genes in the germ line by mutagens, (3) impairment or deletion of the last active regulatory gene in a somatic cell by mutagens, and (4) promotion of noncompetent cells to competence for neoplastic transformation. It is easy to see that the specific cancer etiology that finally comes to our notice after the animals are treated is determined by the next to last step completing the multistage process permitting that last step, i.e., neoplastic transformation that initiates tumor formation (A. Anders and F. Anders, 1978).

D. A Unified View of Tumor Etiology

So far we have recognized six main types of tumor etiology all of which are based on deregulation of the *Tu* oncogene. These types are neoplasia conditioned by (1) carcinogen-dependent somatic mutation, (2) carcinogen-dependent germ line mutation, (3) crossing-conditioned gene elimination, (4) promoter-dependent cell differentiation, (5) carcinogen-dependent chromatin condensation, and (6) increase of ongocene dosage in somatic cells or germ line. This will be demonstrated in genotypes showing a high susceptibility to melanoma based on the presence of one copy of an accessory *Tu* that is repressed by only one regulatory gene (Fig. 41; for genotype see Fig. 19).

1. Neoplasia Conditioned by Carcinogen-Dependent Somatic Mutation

If the only regulatory gene present in the system remains unchanged (Fig. 41, left), or becomes impaired or deleted in a postcompetent cell like an A melanoblast (Aa), no melanoma will develop. Melanoma will, however, develop if the mutation of the regulatory gene occurs in an I melanoblast (Ab). This cell is competent and becomes neoplastically transformed. Following the processes of cell division and cell differentiation, the TrI melanoblast gives rise to an easily detectable Tr cell clone. These Tr cells may continue to divide, but finally differentiate to Tr melanophores; these, after having reached a certain age, are removed by macrophages. Because less differentiated Tr cells are not present, the development of the melanoma stops. Eventually the melanoma regresses. As is demonstrated in the scheme, the origin of such a somatic mutation-conditioned melanoma is unicellular and its growth proceeds only by proliferation. Following complete removal, we observe no relapse. This was expected because the system lacks supply with cells competent for transformation.

The mutation of the regulatory gene may also occur in an S melanoblast (Ac), or an earlier precompetent pigment cell. This cell remains nontransformed and may multiply over a long period ("latent period") as a normal stem cell. Those descendants reaching the stage of competence by differentiation are transformed simultaneously. After some cell divisions, paralleled by cell differentiation, they become visible as a large cell clone consisting of hundreds or thousands of dividing TrA melanoblasts and Tr melanocytes, which give rise to the melanoma. Those melanoma cells, that complete differentiation to Tr melanophores, are attacked and removed by macrophages. Those S melanoblasts, however, which do not further differentiate may reproduce identically throughout the further life of the fish and may serve as a permanent source of I melanoblasts, which then becomes neoplastically transformed. As explained by means of the scheme, the origin of such a melanoma is multicellular, although it can be traced back to a single

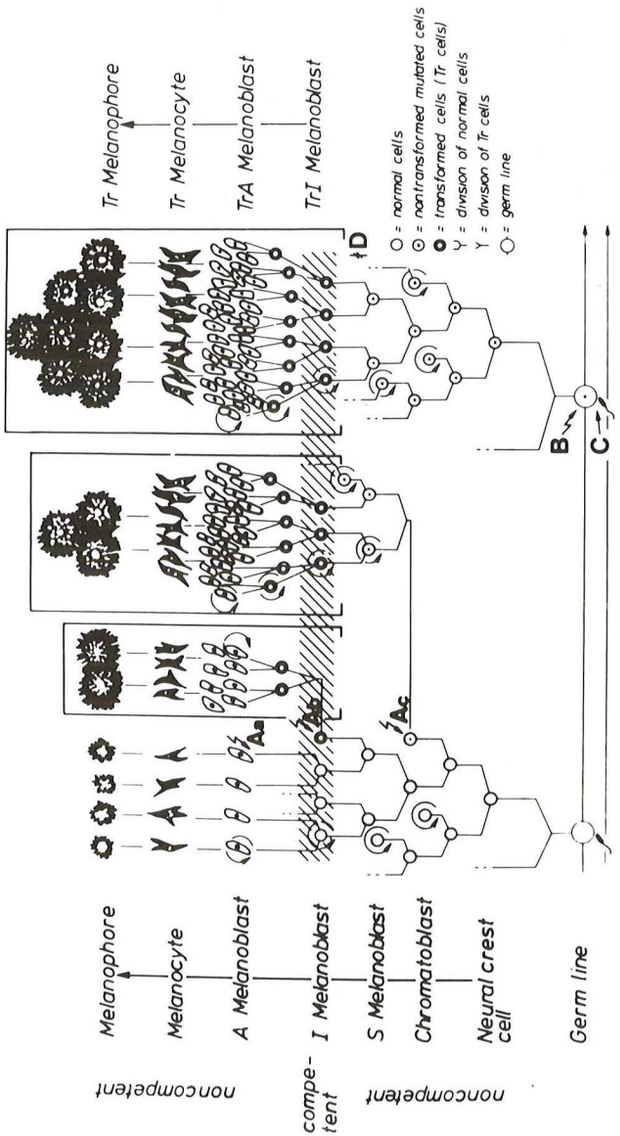


FIG. 41. Differentiation of normal and neoplastically transformed pigment cells in different etiological types of neoplasia. See text. From F. Anders *et al.* (1981b).

mutational event in a somatic cell. It grows by both permanent transformation and proliferation of the descendants of the mutated cell. After complete removal of the melanoma there may or may not be a relapse. We assume that relapse occurs if some mutated stem cells in the surroundings of the melanoma escape removal and differentiate to the competent I melanoblasts, which become neoplastically transformed. No relapse, however, has ever been observed after extirpation of entire fins or large parts of the skin that carry the localized clonal melanomas.

2. *Neoplasia Conditioned by Carcinogen-Dependent Germ Line Mutation*

The same genotype that develops the somatic mutation-conditioned melanomas may also produce germ line mutation-conditioned neoplasms (Fig. 41B) (A. Anders *et al.*, 1981; Chatterjee *et al.*, 1981). In this case melanoma develops following carcinogen-induced impairment or deletion of the only regulatory gene in a germ line cell, and is inherited according to Mendelian rules. As a consequence of the inheritance of the mutation through the germ line, the *Tu* oncogene becomes active in the developing progeny as soon as the melanophore precursor cells differentiate to the competent I melanoblasts, and melanoma develops "spontaneously" in embryos and young individuals. This type of neoplasm is of multicellular origin and grows by both transformation and proliferation. In the event that it is restricted to a certain compartment of the body (see Section IV,C) removal is possible. The melanoma shown, however, relapse, because stem cells still remain available for differentiation to the competent cells, which become transformed.

Although the somatic mutation-conditioned and the germ line mutation-conditioned melanomas are apparently of completely different etiology (they are hereditary or nonhereditary tumors, respectively) their cells must be genetically identical if one considers that they originate from the mutation of the same regulatory gene and the activity of the same *Tu* oncogene. Morphological, pathological, and cytological studies, however, unexpectedly revealed many differences between these two types of melanomas. For instance, germ line mutation-conditioned melanomas lack chromosome aberrations whereas such aberrations are typical for somatic mutation-conditioned melanoma (Chatterjee *et al.*, 1981). These differences, we believe, represent epiphenomena of tumor development and may be useful for diagnosis and prognosis of tumors of unknown etiology in the fish.

3. *Neoplasia Conditioned by Crossings*

Although the etiology of this type of tumor (Fig. 41C) is quite different from that of the germ line mutation-conditioned melanoma, both these types of melanoma are, however, closely related (Fig. 41, compare B and C). The solitary regulatory gene present in the system, if nonlinked to *Tu*, can be

eliminated by crossings with animals that do not carry this gene (Figs. 16 and 18). Neoplasms of this type are always passed on to the offspring if the crucial regulatory gene remains lacking, and are not passed on (and therefore appear nonhereditary) if chromosomes lacking the regulatory gene are resubstituted by those carrying them. Like the germ line mutation-conditioned melanomas, the crossing-conditioned ones occur as the cells become competent for neoplastic transformation. The resulting tumor is of multicellular origin and grows and invades the nontumorous tissues by both transformation and proliferation. Following complete removal, which is possible in the case of compartment-specific tumors, they show relapse because stem cells differentiate to the competent stage and become transformed. In our laboratory we harvest tissues of melanoma of this type several times during the life of the fish. Harvest of the melanoma results in a postponement of death caused by neoplasia.

4. *Neoplasia Conditioned by Promoter-Dependent Cell Differentiation*

Neoplasia of this type can be induced in fish of certain genotypes the *Tu* of which is pigment cell-specifically derepressed by impairment or deletion of the only regulatory gene, but cannot become active because melanophore differentiation is delayed in the stage of the noncompetent S melanoblasts (see g and R_{Co} in Fig. 8, and D in Fig. 41) (A. Anders *et al.*, 1983). Chemical agents and drugs, such as 27-methyltestosterone and other steroids (A Schartl, 1981; A. Schartl *et al.*, 1982; M. Schartl *et al.*, 1981), cyclic AMP, corticotropin, BUdR, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), saccharine, cyclamate, diazepam, and others (Herbert, 1983; Schmidt, 1983), as well as general environmental changes, such as the decrease of the temperature and the increase of the salinity of the water in the tank, promote almost simultaneously the differentiation of large amounts of the noncompetent cells to competent onces, which subsequently become neoplastically transformed. X Rays and *N*-methyl-*N*-nitrosourea (MNU), which are powerful mutagenic carcinogens, may also trigger neoplasms of this type. It appears, however, that in this case neither of these agents acts as a mutagen, but as a differentiation-promoting agent, such as methyltestosterone, cyclic AMP, low temperature, etc., which certainly are not mutagens.

The melanoma of this type of etiology may develop simultaneously within the whole skin and inside the body wherever the differentiation of the pigment cell precursors is promoted from the noncompetent to the competent stage. They are of multicellular origin and grow permanently by both transformation of normal cells and proliferation of Tr cells. They are nonhereditary. We use the fish developing neoplasia of this kind of etiology as tester fish for tumor promoters (A. Anders *et al.*, 1983; Schmidt, 1983).

5. *Neoplasia Conditioned by Carcinogen-Dependent Chromatin Condensation*

The principle underlying this etiological type of neoplasia was first recognized when pure-bred spotted *X. maculatus* from Rio Jamapa were treated as embryos with X rays. The developing animals exhibited an enhancement of spots to benign melanoma (Pursglove, 1972; Pursglove *et al.*, 1971) and have inherited this change of phenotype for about 45 generations. Genetic analysis showed that all chromosomes were involved in the phenotypic change (A. Anders *et al.*, 1971), and phenogenetic, cytological (Lueken and Knoll, 1968), electron microscopic, and biochemical studies revealed a correlation between the hereditary enhancement of oncogene expression and a hereditary change in interphase chromatin appearance from a dispersed to a condensed state (F. Anders *et al.*, 1981a; Heil, 1983).

Similar experiments were done by treatment of the hybrid fish carrying only one regulatory gene for its *Tu* oncogene with several carcinogens. Almost all treated fish developed hereditary melanomas indicating that the regulatory gene is affected rather by a general hereditary change of the chromatin than by mutation (Schmidt, 1983).

Neoplasia based on a general change of the genome following treatment with X rays and chemicals has also apparently been observed in mice (Nomura, 1982). Additional studies are required for an understanding of this phenomenon.

6. *Neoplasia Conditioned by Additional Introduction of Uncontrolled Oncogenes*

Many genotypes have been produced from the *Tu* oncogenes which are derepressed either by mutation-conditioned impairment or hybridization-conditioned elimination of the regulatory gene, but these animals only developed some neoplastically transformed cells instead of malignant melanoma. Following inbreeding, the offspring carrying the double dosage of *Tu* (the fish homozygous for *Tu*) developed malignant melanoma (F. Anders and Klinke, 1966). The genetic situation is similar to that of the oncogene dosage effect shown in Figs. 27 and 29. It is easy to see that the etiology of hybridization-conditioned and that of inbreeding-conditioned neoplasms is closely related. The crucial event in the inbreeding-conditioned neoplasms is, however, the introduction of an additional oncogene into the system.

Introduction of additional oncogenes that are derepressed is also the basis of experimental oncogene transfer by DNA injection of embryos that led to the induction of neoplastically transformed cells (Section IV,A,3).

Additional types of tumor etiology are conceivable, for instance neoplasia

following the deficiency of repair of damage to DNA or following the deficiency of immune surveillance. These and other deficiencies, however, are involved in control of tumor growth rather than in the actual process of neoplastic transformation.

VI. Conclusions

The *Xiphophorus* tumor system has provided the opportunity to reduce the enormous complexity of cancer etiology to a few biological elements basically involved in neoplasia. The development of a tumor requires an oncogene which, after impairment, deletion, or elimination of its regulatory genes is permitted to mediate neoplastic transformation. Emphasis is being placed today in cancer research on the actual oncogenes themselves, but, in our opinion, the most important genes involved in neoplasia are these regulatory genes. However, although detected by classical genetics in the *Xiphophorus* system, these genes are not at present open to a more finely detailed molecular biological analysis. Their actual mode of action is therefore still far from being understood.

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References

Abdo, S. (1979). Thesis, University of Giessen.
Ahuja, M. R. (1965). *Q. Rev. Biol.* **4,** 329–340.
Ahuja, M. R. (1979). *In* "Antiviral Mechanisms in the Control of Neoplasia" (P. Chandra, ed.), pp. 17–37. Plenum, New York.
Ahuja, M. R., and Anders, F. (1976). *Prog. Exp. Tumor Res.* **20,** 380–397.
Ahuja, M. R., and Anders, F. (1977). *Recent Adv. Cancer Res. Cell Biol. Tumor Virol.* **1,** 103–117.
Ahuja, M. R., Lepper, K., and Anders, F. (1979). *Experimentia* **35,** 28–29.
Ahuja, M. R., Schwab, M., and Anders, F. (1980). *J. Hered.* **71,** 403–407.
Altmaier, A., and Anders, F. (1968). *Verh. Dtsch. Zool. Ges. Zool. Anz.* **32** (Suppl.), 225–238.
Anders, A., and Anders, F. (1963). *Z. Vererbrungslehre* **94,** 1–18.
Anders, A., and Anders, F. (1978). *Biochim. Biophys. Acta* **516,** 61–95.
Anders, A., Anders, F., and Pursglove, D. L. (1971). *Experientia* **27,** 931–932.
Anders, A., Anders, F., and Klinke, K. (1973a). *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 53–63. Springer-Verlag, Berlin and New York.
Anders, A., Anders, F., and Klinke, K. (1973b). *In* "Genetics and Mutagenesis in Fish" (J. H. Schröder, ed.), pp. 33–52. Springer-Verlag, Berlin and New York.

Anders, A., Kollinger, G., and Chatterjee, K. (1981). *In* "Pigment Cell 1981" (M. Seiji, ed.), pp. 501–506. Univ. of Tokyo Press.
Anders, A., Schmidt, C.-R., Herbert, A., and Anders, F. (1983). *In* "Prüfung von Chemikalien auf Kanzerogenität, Mutagenität und Teratogenität," pp. 253–268. Gesellschaft für Strahlen und Umweltforschung, München.
Anders, F. (1967). *Experientia* **23,** 1–10.
Anders, F. (1968). *Zentralbl. Vet. Med. B* **15,** 29–46.
Anders, F. (1981). *Klin. Wochenschr.* **59,** 943–956.
Anders, F. (1982). *In* "Biochemistry of Differentiation and Morphogenesis" (L. Jaenicke, ed.), pp. 91–115. Springer-Verlag, Berlin and New York.
Anders, F. (1983). *In* "Modern Trends in Human Leukemia V" (R. Neth, R. C. Gallo, M. F. Greaves, M. A. S. Moore, and K. Winkler, eds.), pp. 186–206. Springer-Verlag, Berlin and New York.
Anders, F., and Klinke, K. (1965). *Z. Vererbungslehre* **96,** 49–65.
Anders, F., and Klinke, K. (1966). *Verh. Dtsch. Zool. Ges. Zool. Anz.* **30** (Suppl.), 391–401.
Anders, F., Anders, A., and Klinke, K. (1962a). *Verh. Dtsch. Zool. Ges. Zool. Anz.* **26** (Suppl.), 97–118.
Anders, F., Vester, F., Klinke, K., and Schuhmacher, H. (1962b). *Biol. Zentralbl.* **81,** 45–65.
Anders, F., Drawert, F., Klinke, K., and Reuther, K. H. (1963). *Experientia* **19,** 219–224.
Anders, F., Sieger, M., and Klinke, K. (1969). *Experientia* **25,** 871–814.
Anders, F., Klinke, K., and Vielkind, U. (1972). *Biol. Zeit* **2,** 35–45.
Anders, F., Anders, A., and Vielkind, U. (1974). *Int. Cancer Congr., 11th, Florence* **3,** 305 (Abstr.).
Anders, F., Diehl, H., Schwab, M., and Anders, A. (1979a). "Pigment Cell" (S. N. Klaus, ed.), Vol. 4, pp. 142–149. Karger, Basel.
Anders, F., Scholl, E., and Schartl, M. (1979b). *In* "Neue Perspektiven in der Medizin" (H. Porcher and K. Theurer, eds.), pp. 38–100. Enke, Stuttgart.
Anders, F., Diehl, H., and Scholl, E. (1980). *Linnean Soc. Symp. Ser.* **9,** 211–224.
Anders, F., Chatterjee, K., Schwab, M., Scholl, E., and Anders, A. (1981a). *Am. Zool.* **21,** 535–548.
Anders, F., Schartl, M., and Scholl, E. (1981b). *In* "Phyletic approaches to Cancer" (C. J. Dawe, ed.), pp. 289–309. Japan Scientific Societies Press, Tokyo.
Anders, F., Scholl, E., and Schartl, M. (1981c). *In* "Pigment Cell 1981" (M. Seiji, ed.), pp. 491–499. Univ. of Tokyo Press.
Anders, F., Schwab, M., and Scholl, E. (1981d). *In* "Short-term Tests for Chemical Carcinogens" (H. F. Stich and R. H. C. San, eds.), pp. 399–407. Springer-Verlag, Berlin and New York.
Anders, F., Schartl, M., and Barnekow, A. (1984). *J. Natl. Cancer Inst.*, in press.
Aronowitz, O., Nigrelli, R. F., and Gordon, M. (1951). *Zoologica* **36,** 239–242.
Atz, J. W. (1962). *Zoologica* **47,** 153–181.
Bagnara, J. T., and Hadley, M. E. (1973). "Chromatophores and Color Change." Prentice-Hall, New York.
Baker, K. F., Berg, O., Gorbman, R. F., Nigrelli, R. F., and Gordon, M. (1953). *Genetics* **38,** 655.
Barnekow, A., Schartl, M., Anders, F., and Bauer, H. (1982). *Cancer Res.* **42,** 2429–2433.
Bauer, H., Barnekow, A., Boschek, C. B., Friis, R. R., and Ziemiecki, A. (1982). *In* "Biochemistry of Differentiation and Morphogenesis" (L. Jaenicke, ed.), pp. 74–90. Springer-Verlag, Berlin and New York.
Bauer, K. H. (1928). "Mutationstheorie der Geschwulstentstehung." Springer-Verlag, Berlin and New York.

Bauer, K. H. (1968). *In* "Aktuelle Probleme aus dem Gebiet der Cancerologie II" (H. Lettrè and G. Wagner, eds.), pp. 63–84. Springer-Verlag, Berlin and New York.
Becker-Carus, C. (1965). *Z. Wiss. Zool. A* **172,** 37–103.
Beiderbeck, R. (1977). "Pflanzentumoren." Ulmer, Stuttgart.
Bentvelzen, P. (1972). *In* "RNA Viruses and Host Genome in Oncogenesis" (P. Emmelot and P. Bentvelzen, eds.), pp. 309–337. North-Holland Publ., Amsterdam.
Berg, O., Edgar, M., and Gordon, M. (1953). *Cancer Res.* **13,** 1–9.
Bienz, M., and Kubli, E. (1981). *Nature (London)* **294,** 188–190.
Bishop, J. M. (1982a). *Sci. Am.* **246,** 69–78.
Bishop, J. M. (1982b). *Adv. Cancer Res.* **37,** 1–32.
Boveri, T. (1929). "The Origin of Malignant Tumors." Williams & Wilkins, Baltimore, Maryland.
Braun, A. C. (1978). *Biochim. Biophys. Acta* **516,** 167–191.
Breider, H. (1939). *Z. Wiss. Zool.* **152,** 107–128.
Breider, H., and Seeliger, R. (1938). *Z. Wiss. Zool. A* **151,** 243–285.
Burch, P. R. J. (1976). "The Biology of Cancer: A New Approach." MTP, Lancaster.
Cairns, J. (1982). *In* "Carcinogenesis and Biological Effects of Tumor Promoters" (E. Hecker, N. E. Fusenig, W. Kunz, F. Marks, and H. W. Thielmann, eds.), pp. 647–651. Raven, New York.
Cairns, J., Lyon, J. L., and Skolnick, M. (1980). "Cancer Incidence in Defined Populations." Cold Spring Harbor Press, Cold Spring Harbor, New York.
Chatterjee, K., Kollinger, G., Schmidt, C. R., Anders, A., and Anders, F. (1981). *Cancer Genet. Cytogenet.* **3,** 195–209.
Collet, M. S., and Erikson, R. L. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75,** 2021–2024.
Collet, M. S., Purchio, A. F., and Erikson, R. L. (1980). *Nature (London)* **285,** 167–169.
Crew, F. A., and Koller, P. (1936). *Proc. R. Soc. (Edinburgh)* **56,** 210.
Dalla-Favera, R., Bergni, M., Erikson, J., Patterson, D., Gallo, R. C., and Croce, C. M. (1982a). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 7824.
Dalla-Favera, R., Gallo, R. C., Giallongo, A., and Croce, C. (1982b). *Science* **218,** 686.
Dawe, C. D., and Harshbarger, J. C. (1969). *Natl. Cancer Inst. Monogr.* **31.**
Dawe, C. J., and Harshbarger, J. C. (1975). *In* "The Pathology of Fishes" (W. E. Ribelin and G. Migaki,eds.), pp. 871–894. Univ. of Wisconsin Press, Madison, Wisconsin.
Dawe, C. J., Harshbarger, J. C., Kondo, S., Sugimura, T., and Takayama, S. (1981). "Phyletic Approaches to Cancer." Japan Scientific Societies Press, Tokyo.
DeFeo, D., Gonda, M. A., Young, H. A., Chang, E. H., Lowt, D. R., Scolnick, E. M., and Ellis, R. W. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 3328–3332.
de Klein, A., van Kessel, A. G., Grosveld, G., Bartram, C. R., Hagemeijer, A., Bootsma, D., Spurr, N. K., Heisterkamp, N., Groffen, J., and Stephenson, J. R. (1982). *Nature (London)* **300,** 765–767.
Denlinger, R. H., Koestner, A., and Swenber, J. A. (1978). *Cancer Res.* **38,** 1711–1717.
Der, C. J., Krontiris, T. G., and Cooper, G. M. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** pp. 3637–3640.
Dess, G. (1982). Thesis, Univ. Giessen.
Dess, G. (1983). Thesis, Univ. Erlangen.
Diehl, H. (1982). Thesis, Univ. Giessen.
Dobzhansky, T. (1937). "Genetics and the Origin of Species." Columbia Univ. Press, New York.
Dushane, G. P. (1938). *J. Exp. Zool.* **78,** 485–503.
Dzwillo, M. (1964). *Mitt. Hamb. Zool. Mus.* **62,** 15–22.

Felding-Habermann, B., Anders, A., and Wiegandt, H. (1983). *Int. Symp. Glycoconjugates, 7th,* Lund (Abstr.).
Fitzpatrick, T. B., and Lerner, A. B. (1953). *Science* **117,** 640–642.
Förster, W., and Anders, F. (1977). *Zool. Anz.* **198,** 167–177.
Fuerst, P. A., Chakraborty, R., and Nei, M. (1977). *Genetics* **86,** 455–483.
Gardner, E. J. (1980). *In* "Prevention and Cure of Hereditary Cancers" (J. Cairns, J. L. Lyon, and M. Skolnick, eds.), pp. 365–377. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
Gateff, E. (1978). *Science* **200,** 1448–1459.
Gateff, E. (1982). *Adv. Cancer Res.* **37,** 33–74.
Gebhard, K., and Niebauer, F. (1979). *Eur. Workshop Melanin Pigment., 4th London* (Abstr.).
Gillespie, D., and Gallo, R. C. (1975). *Science* **188,** 802–811.
Goldfarb, M., Schimizu, K., Perucho, M., and Wigler, M. (1982). *Nature (London)* **296,** 404–409.
Gordon, M. (1928). *J. Hered.* **19,** 551–556.
Gordon, M. (1931). *Am. J. Cancer* **15,** 1495–1523.
Gordon, M. (1947a). *Adv. Genet.* **1,** 95–132.
Gordon, M. (1947b). *J. Natl. Cancer Inst.* **7,** 87–92.
Gordon, M. (1958). *Ann. N.Y. Acad. Sci.* **71,** 1213–1222.
Gross, L., and Dreyfuss, Y. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76,** 5910–5913.
Haas, J. (1981). Thesis, Univ. Giessen.
Haas-Andela, H. (1978). Thesis, Univ. Giessen.
Halver, J. E., and Mitchel, I. A. (1967). *Res. Rep. U.S. Fish Wildlife Serv.* **70,** 1–99.
Häussler, G. (1928). *Klin. Wochenschr.* **7,** 1561–1562.
Hayes, H. M. (1978). *Eur. J. Cancer* **14,** 1299–1308.
Heil, M. (1983). Thesis, Univ. Giessen.
Henze, M., Rempeters, G., and Anders, F. (1977). *Comp. Biochem. Physiol.* **56B,** 33–64.
Herbert, A. (1983). Thesis, Univ. Giessen.
Heston, W. E. (1974). *J. Hered.* **65,** 262–272.
Heston, W. E., and Vlahakis, G. (1968). *J. Natl. Cancer Inst.* **40.** 1161–1166.
Higginson, J. (1969). *Proc. Can. Cancer Conf., 8th,* pp. 40–75.
Holley, R. W. (1975). *Nature (London)* **258,** 487–490.
Hook, R. R., Aultman, M. D., Adelstein, E. H., Oxenhandler, R. W., Milliken, L. E., and Middleton, C. C. (1979). *Int. J. Cancer* **24,** 668–672.
Huebner, R. J., and Todaro, G. J. (1969). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 1087–1094.
Humm, D. G., and Young, R. S. (1956). *Zoologica* **41,** 1–10.
Huxley, J. (1958). "Biological Aspects of Cancer." Allen & Unwin, London.
Ishikawa, T., and Takayama, S. (1977). *Ann. N.Y. Acad. Sci.* **298,** 330–341.
Ishikawa, T., Prince Masahito, Matsumoto, J., and Takayama, S. (1978a). *J. Natl. Cancer Inst.* **61,** 1461–1470.
Ishikawa, T., Prince Masahito, and Takayama, S. (1978b). *Cancer Res.* **38,** 3954–3959.
Jacobs, C., and Rübsamen, H. (1983). *Cancer Res.* **43,** 1696–1702.
Kaiser, H. E. (1981). "Neoplasms-Comparative Pathology of Growth in Animals, Plants and Man." Williams & Wilkins, Baltimore, Maryland.
Kallman, K. D. (1968). "Enjoy your Platys and Swordtail." Pet Library, New York.
Kallman, K. D. (1970). *Genetics* **64,** 32.
Kallman, K. D. (1975). *Handb. Genet.* **4,** 81–132.
Kallman, K. D., and Atz, J. W. (1967). *Zoologica* **51,** 107–135.
Katze, J. R., Beck, W. T., Cheng, C. S., and McCloskey, J. A. (1983). *In* "Recent Results in

Cancer Research: Modified Nucleosides and Cancer" (G. Nass, ed.), pp. 146–159. Springer-Verlag, Berlin and New York.
Kersten, H. (1982a). *In* "Biochemistry of Differentiation and Morphogenesis" (L. Jaenicke, ed.), pp. 116–119. Springer-Verlag, Berlin and New York.
Kersten, H. (1982b). *In* "Biochemistry of S-adenosylmethionine and Related Compounds" (E. Usdein, R. T. Borchardt, and R. C. Creveling, eds.), pp. 357–369. Macmillan, New York.
Kersten, H. (1983). *In* "Recent Results in Cancer Research: Modified Nucleosides and Cancer" (G. Nass, ed.), pp. 255–263. Springer-Verlag, Berlin and New York.
Kersten, H., Schachner, E., Dess, G., Anders, A., Nishimura, S., and Shindo-Okada, N. (1983). *In* "Biochemical and Clinical Aspects of Pteridines" (Wachter, Curtius, and Pfleiderer, eds.), pp. 367–382. De Gruyter, Berlin.
Knudson, A. G. (1973). *Adv. Cancer Res.* **17,** 317–352.
Knudson, A. G. (1982). *Proc. Int. Cancer Congr., 13th, Seattle* (Abstr.).
Koeppe, P. (1980). *Biol. Med.* **9,** 99–110.
Kollinger, G. (1980). Thesis, Univ. Giessen.
Kollinger, G., and Siegmund, E. (1981). *Verh. Dtsch. Zool. Ges.* **74,** 206.
Kollinger, G., Schwab, M., and Anders, F. (1979). *J. Cancer Res. Clin. Oncol.* **95,** 239–246.
Kosswig, C. (1928). *Z. Indukt. Abstamm. Vererb. Lehre* **47,** 150–158.
Kosswig, C. (1929). *Der Züchter* **1,** 152–157.
Kosswig, C. (1937). *Biol. Gen.* **13,** 276–293.
Kraybill, H. F., Dawe, C. J., Harshbarger, J. C., and Tardiff, R. G. (1977). *Ann. N.Y. Acad. Sci.* **298.**
Krieg, K. (1973). *Biol. Zentralbl.* **92,** 617–623.
Krontiris, T. G., and Cooper, G. M. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 1181–1184.
Leatherland, J. F., and Sonstegard, R. A. (1978). *Cancer Res.* **38,** 3164–3173.
Levan, G., Ahlstrom, U., and Mittelman, F. (1974). *Hereditas* **77,** 263–280.
Little, C. C. (1947). *Biol. Rev.* **22,** 315.
Lubs, H. A., Kimberling, W. J., Hecht, F., Patil, S. R., Brown, J., Gerald, P., and Summit, R. L. (1977). *Nature (London)* **268,** 631–632.
Lueke, W. (1984). Thesis, Univ. Giessen.
Lueke, W., and Anders, F. (1983). *Int. Pigment Cell Conf., 12th* **131,** (Abstr.).
Lueken, W., and Knoll, C. (1968). *Experientia* **24,** 595–596.
Lueken, W., and Kaeser, U. (1972). *Experientia* **28,** 1340–1341.
Lueken, W., Schmidt, E. R., and Lepper, K. (1973). *In* "Genetics and Mutagenesis in Fish" (J. H. Schröder, ed.), pp. 139–160. Springer-Verlag, Berlin and New York.
Lynch, H. T. (1967). *Recent Results Cancer Res.* **12.**
Maas, G. (1967). Thesis, Univ. Saarbrücken.
Mark, J. (1977). *Adv. Cancer Res.* **24,** 165–222.
Markert, C. L. (1968). *Cancer Res.* **28,** 1908–1914.
Maugh, T. H. (1979). *Science* **205,** 1363–1366.
Maugh, T. H. (1980). *J. Hered.* **71,** 2–8.
Mawdesley-Thomas, L. E. (1975). *In* "The Pathology of Fishes" (W. E. Ribelin and G. Migaki, eds.), pp. 805–870. Univ. of Wisconsin Press, Madison, Wisconsin.
Mittelman, F., and Levan, G. (1972). *Hereditas,* **71,** 325–334.
Mittelman, F., Mark, J., Levan, G., and Levan, A. (1972). *Science* **176,** 1340–1341.
Morizot, D. C., and Siciliano, M. J. (1979). *Genetics* **93,** 947–960.
Morizot, D. C., and Sicliano, M. J. (1982). *Genetics* **102,** 539–566.
Morizot, D. C., and Siciliano, M. J. (1983). *J. Natl. Cancer Inst.* **71,** 809–813.
Morizot, D. C., Wright, D. A., and Siciliano, M. J. (1977). *Genetics* **86,** 645–656.
Muir, C. S., and Nectoux, J. (1982). *In* "Cancer Epidemiology and Prevention" (D. Schottenfeld and J. F. Fraumeni, eds.), pp. 119–137. Saunders Company, Philadelphia, Pennsylvania.
Nass, G. (1983). "Recent Results in Cancer Research. Modified Nucleosides and Cancer." Springer-Verlag, Berlin and New York.
Neel, G. B., Jhanwar, S. C., Chaganti, R. S. K., and Hayward, W. S. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 7842–7846.
Nishimura, S. (1983). *Prog. Nucleic Acid Res. Mol. Biol.*, in press.
Nishimura, S., and Kuchino, Y. (1979). *Gann* **24,** 245–262.
Nomura, T. (1982). *Nature (London)* **296,** 575–577.
O'Brien, S. J. (1980). *J. Hered.* **71,** 2–8.
Oeser, H., and Koeppe, P. (1979). "Krebs: Schicksal oder Verschulden?" Thieme, Stuttgart.
Okada, N., and Nishimura, S. (1979). *J. Biol. Chem.* **254,** 3062–3066.
Olsen, G. (1966). *Acta Chir. Scand.* **60,** 149–162.
Oskarson, M., McClements, W. L., Blair, D. G., V. Maizel, J., and Vande Woude, G. F. (1980). *Science* **207,** 1222–1224.
Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J., and Wigler, M. (1981). *Cell* **27,** 467–476.
Peter, R. U. (1982). *Eur. Workshop Melanin Pigment., 4th, Edinburgh* (Abstr.).
Peter, R. U. (1983). Thesis, Univ. Giessen.
Pierce, G. B., and Wallace, C. (1971). *Cancer Res.* **31,** 127–134.
Pitot, H. C. (1968). *Cancer Res.* **28,** 1880–1887.
Poll, H. (1920). *Beitr. Pathol. Anat.* **67,** 40.
Prescott, D. M. (1976). *Adv. Genet.* **18,** 99–177.
Prescott, D. M., and Flexer, A. S. (1982). "Cancer: The Misguided Cell." Sinauer, Sunderland, Massachusetts.
Pulciani, S., Santos, E., Lanver, A. V., Long, L. K., Aronson, S. A., and Barbacid, M. (1980). *Nature (London)* **300,** 539–541.
Pursglove, D. L. (1972). Thesis, Univ. Giessen.
Pursglove, D. L., Anders, A., Döll, G., and Anders, F. (1971). *Experientia* **27,** 695–697.
Radda, A. C. (1980). *Aquearia* **27,** 39–44.
Reddy, E. P., Reynolds, R. K., Santos, E., and Barbacid, M. (1982). *Nature (London)* **300,** 149–152.
Rempeters, G., Henze, M., and Anders, F. (1981). *Comp. Biochem. Physiol.* **69B,** 91–98.
Reyniers, J. P., Pleasants, J. R., Wostman, B. S., Katze, J. R., and Farkas, J. R. (1981). *J. Biol. Chem.* **256,** 11591–11594.
Riehl, R., Schartl, M., and Kollinger, G. (1984). *J. Cancer Res. Clin. Oncol.* **107,** 21–31.
Rosen, D. E. (1979). *Bull. Am. Mus. Natl. Hist.* **162,** 267–376.
Rosen, D. E., and Bailey, R. M. (1963). *Bull. Am. Mus. Natl. Hist.* **126,** 1–176.
Rowley, J. D. (1973). *Nature (London)* **243,** 290–293.
Rowley, J. D. (1974). *J. Natl. Cancer Inst.* **52,** 315–320.
Rowley, J. D. (1977). *In* "Genetics of Human Cancer" (J. J. Mulvihill, R. W. Miller, and J. F. Fraumeni, eds.), pp. 125–136. Raven, New York.
Rowley, J. D. (1982). *Science* **216,** 749–751.
Rowley, J. D. (1983). *Nature (London)* **301,** 290–291.
Sachs, L. (1978). *Nature (London)* **274,** 535–539.
Sachs, L. (1982). *In* "Biochemistry of Differentiation and Morphogenesis" (L. Jaenicke, ed.), pp. 3–15. Springer-Verlag, Berlin and New York.
Sandberg, A. A. (1980). "The Chromosome in Human Cancer and Leukemia." Elsevier, Amsterdam.

Sato, S., Matsushiam, T., Tanaka, N., Sugimura, T., and Takashima, F. (1973). *J. Natl. Cancer Inst.* **50,** 767–776.
Scarpelli, D. G. (1969). *In* "Fish in Research" (O. W. Neuhaus and J. E. Nalver, eds.), pp. 45–48. Academic Press, New York.
Schartl, A. (1981). Thesis, Univ. Giessen.
Schartl, A., Schartl, M., and Anders, F. (1982). *In* "Carcinogenesis and Biological Effects of Tumor Promoters" (E. Hecker, N. E. Fusenig, W. Kunz, F. Marks, and H. W. Thielmann, eds.), pp. 427–434. Raven, New York.
Schartl, M. (1979). *Thesis, Univ. Giessen.*
Schartl, M., and Barnekow, A. (1982). Differentiation **23,** 109–114.
Schartl, M., and Franchini, G. (1982). Unpublished (see Anders *et al.*, 1983).
Schartl, M., and Franchini (1984). In preparation.
Schartl, M., and Czernilofsky, A. P. (1982). Unpublished (see Anders, F. 1982a).
Schartl, M., Schartl, A., and Anders, F. (1981). *In* "Pigment Cell 1981" (M. Seiji, ed.), pp. 517–524. University of Tokyo Press.
Schartl, M., Barnekow, A., Bauer, H., and Anders, F. (1982). *Cancer Res.* **42,** 4222–4227.
Schartl, M., Barnekow, A., Schmidt, C. R., Bauer, H., and Anders, A. (1983). In preparation.
Schlage, W. (1978). Thesis, Univ. Giessen.
Schlumberger, H. G. (1957). *Cancer Res.* **17,** 823–832.
Schlumberger, H. G., and Luckè, B. (1948). *Cancer Res.* **8,** 657–712.
Schmidt, C. R. (1983). Thesis, Univ. Giessen.
Schmidt, E. R. (1978). *Roux Arch. Entwicklungsmech.* **184,** 115–134.
Scholl, A. (1973). *In* "Genetics and Mutagenesis in Fish" (J. H. Schröder, ed.), pp. 277–299. Springer-Verlag, Berlin and New York.
Scholl, A., and Anders, F. (1973a). *In* "Genetics and Mutagenesis of Fish" (J. H. Schröder, ed.), pp. 301–313. Springer-Verlag, Berlin and New York.
Scholl, A., and Anders, F. (1973b). *Arch. Genet.* **46,** 121–129.
Scholl, E. (1977). Thesis, Univ. Giessen.
Scholl, E. (1979). *Mitt. Hamb. Zool. Mus. Inst.* **76,** 489–490.
Schull, W. J. (1979). *J. Toxicol. Environ. Health* **5,** 17–25.
Schwab, M. (1974). Thesis, Univ. Giessen.
Schwab, M. (1980). Thesis for Habilitation, Univ. Giessen.
Schwab, M. (1982a). *In* "Carcinogenesis and Biological Effects of Tumor Promoters" (E. Hecker, N. E. Fusenig, W. Kunz, F. Marks, and H. W. Thielmann, eds.), pp. 417–426.
Schwab, M. (1982b). *Mol. Gen. Genet.* **188,** 410–417.
Schwab, M., and Anders, A. (1981). *In* "Neoplasms-Comparative Pathology of Growth in Animals, Plants, and Man" (H. E. Kaiser, ed.), pp. 451–459. Williams & Wilkins, Baltimore, Maryland.
Schwab, M., Ahuja, M. R., and Anders, F. (1976a). *Comp. Biochem. Physiol.* **54B,** 197–199.
Schwab, M., Vielkind, J., and Anders, F. (1976b). *Mol. Gen. Genet.* **144,** 151–158.
Schwab, M., Haas, J., Abdo, S., Ahuja, M. R., Kollinger, G., Anders, A., and Anders, F. (1978a). *Experientia* **34,** 780–782.
Schwab, M., Abdo, S., Ahuja, M. R., Kollinger, G., Anders, A., and Anders, F. (1978b). *Z. Krebsforsch.* **91,** 301–315.
Schwab, M., Kollinger, G., Haas, J., Ahuja, M. R., Abdo, S., Anders, A., and Anders, F. (1979). *Cancer Res.* **39,** 519–526.
Seiler, J., Puchta, O., Brunold, E., and Rainer, M. (1958). *Roux Arch. Entwicklungsmech.* **150,** 199–372.
Shilo, B. Z., and Weinberg, R. A. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 6789–6792.
Siciliano, M. J., and Perlmutter, A. (1972). *J. Natl. Cancer Inst.* **49,** 415–421.

Siciliano, M. J., and Wright, D. A. (1976). *Prog. Exp. Tumor Res.* **20,** 398–411.
Sieger, F., Sieger, M., Prüssing, R., and Anders, F. (1969). *Experientia* **25,** 778–779.
Sieger, M., Sieger, F., and Anders, F. (1968). *Verh. Dtsch. Zool. Ges. Zool. Anz.* **32** (Suppl.), 210–225.
Siegmund, E. (1982). Thesis, Univ. Giessen.
Sinnhuber, R. O., Hendricks, J. D., Wales, J. H., and Putman, G. B. (1977). *Ann. N.Y. Acad. Sci.* **298,** 389–408.
Sobel, H. J. Marquet, E., Kallman, K. D., and Corley, G. J. (1975). *In* "The Pathology of Fishes" (W. E. Ribelin and G. Migaki, eds.), pp. 945–981. Univ. of Wisconsin Press, Madison, Wisconsin.
Spector, D. H., Varmus, H. E., and Bishop, J. M. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75,** 4102–4106.
Sprinzl, M., and Gauss, D. H. (1982). *Nucleic Acids Res.* **10,** r1–r55.
Stehelein, D., Varmus, H. E., and Bishop, J. M. (1976). *Nature (London)* **260,** 170–173.
Strong, L. C. (1958). *Ann. N.Y. Acad. Sci.* **71,** 807–1241.
Stünzi, H. (1972). *Kleintierpraxis* **17,** 66–70.
Tabin, C. J., Bradley, S. M., Bargmann, C. I., Weinberg, R. A., Papageorge, A. G., Scolnick, E. M., Dhar, R., Lowy, D. R., and Chang, E. H. (1982). *Nature (London)* **300,** 143–149.
Takayama, S., and Ishikawa, T. (1977). *IARC Sci. Publ.* **16,** 271–286.
Takeya, T., and Hanafusa, H. (1982). *J. Virol.* **44,** 12–18.
Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S., and Leder, P. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 7837–7841.
Vielkind, J. (1971). Thesis, Univ. Giessen.
Vielkind, J. (1979). Thesis for Habilitation, Univ. Giessen.
Vielkind, J., Vielkind, U., and Anders, F. (1971). *Cancer Res.* **31,** 868–875.
Vielkind, J., Vielkind, U., and Anders, F. (1973a). *Z. Krebsforsch.* **80,** 103–112.
Vielkind, J., Schwab, M., and Anders, F. (1973b). *In* "Genetics and Mutagenesis in Fish" (J. H. Schröder, ed.), pp. 123–137. Springer-Verlag, Berlin and New York.
Vielkind, J., Haas-Andela, H., Vielkind, U., and Anders, F. (1982). *Mol. Gen. Genet.* **185,** 379–389.
Vielkind, U. (1972). Thesis, Univ. Giessen.
Vielkind, U. (1976). *J. Exp. Zool.* **196,** 197–204.
Vielkind, U., Schlage, W., and Anders, F. (1977). *Z. Krebsforsch.* **90,** 285–299.
Warner, N. L., Potter, M., and Metcalf, D. (1974). *UICC Tech. Rep. Ser. Genf.* **13.**
Weinstein, I. B., Horowitz, A. D., Mufson, R. A., Fisher, P. B., Ivanovic, V., and Greenbaum, E. (1982). *In* "Carcinogenesis and Biological Effects of Tumor Promoters" (E. Hecker, N. E. Fusenig, W. Kunz, F. Marks, and H. W. Thielmann, eds.), pp. 599–613. Raven, New York.
Weiss, E. (1972). *In* "Allgemeine Pathologie" (A. Frei, ed.), pp. 295–347. Paray, Berlin.
Weissenfels, N., Schäfer, D., and Bretthauer, R. (1970). *Virchows Arch. Abt. B* **5,** 144–158.
Weston, J. A. (1970). *Adv. Morphog.* **8,** 41–114.
Yamada, K., and Hasegawa, T. (1978). *Hum. Genet.* **44,** 89–98.
Yanofsky, C. (1976). *In* "Molecular Mechanisms in the Control of Gene Expression" (D. P. Nierlich, W. J. Rutter, and C. J. Fox, eds.), pp. 75–86. Academic Press, New York.
Young, J. L., and Pollack, E. S. (1982). *In* "Cancer Epidemiology and Prevention" (D. Schottenfeld and J. F. Fraumeni, eds.), pp. 138–165. Saunders, Philadelphia, Pennsylvania.
Zander, C. D. (1967). *Mitt. Hamb. Zool. Mus. Inst.* **64,** 87–125.
Zech, L., Haglund, V., Nilsson, K., and Klein, G. (1976). *Int. J. Cancer* **17,** 47–56.