The Mildred Scheel 1988 Memorial Lecture

A Biologist's View of Human Cancer*

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This biennial lecture reflects the generosity of Dr. Mildred Scheel, whose life was dedicated to the fight against cancer. I met Mildred Scheel personally on the occasion of several conferences on human cancer and remember her with gratitude. It is an honor to have been invited to present the 1988 lecture.

The ultimate purpose of all who study cancer biology falls within the general goal of the efforts of Dr. Scheel: to analyze the biological factors that are involved in tumor development for the purpose of preventing cancer. At times the analytical work of many scientists of Mildred Scheel's generation appeared to meet certain opposition when they have seen printed in large letters "cancer is not inherited" and "genes that determine cancer do not exist." Such statements came from well-meaning people intent on calming the fears of families that have had cancer in their ancestry.

We all are involved in the fight against cancer, the physicians, epidemiologists, biochemists, immunologists, virologists; everybody in his place. I am a zoologist, trained as a geneticist who views human beings as products of nature with all their potentials, limitations, and inadequacies arising from their animal background.

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A. Oncogenes in Phylogeny

Neoplasia is not limited to human beings, or to mammals, but develops in all taxonomic groups of recent Eumetazoa and even in multicellular plants. Neoplasia was also found in Jurassic Sauria and in other fossils including humans. Neoplasia, therefore, was not created by human civilization, but is inherent in the multicellular organization of life [1]. It is, therefore, not surprising that the genes coding for human cancer are distributed throughout the animal kingdom (Fig. 1, [2–10]).

The most venerable oncogene seems to be the *ras* oncogene, which probably has evolved together with the heterotrophic organization of the early **Eucaryotes.** This supposition does not exclude the idea that certain sequences of *ras* (and other oncogenes) might have been evolved before the heterotrophs in the history of life. Actually *ras* is distributed as a normal genomic constituent from yeast [11], where one obviously cannot recognize a cancerous state, through all groups of the animal kingdom studied up to humans and is possibly involved in the development of human tumors such as bladder carcinoma, melanoma, neuroblastoma, fibrosarcoma, lung sarcoma, lung carcinoma, and acute myeloid leukemia (for review see [12, 13]). Its early appearance in the history of life suggests fundamental functions for our life. Its product is a GTP-binding protein which probably activates phospholipase C that generates the internal promoter diazylglycerol for kinase C, thus signalizing cell proliferation [14–16].

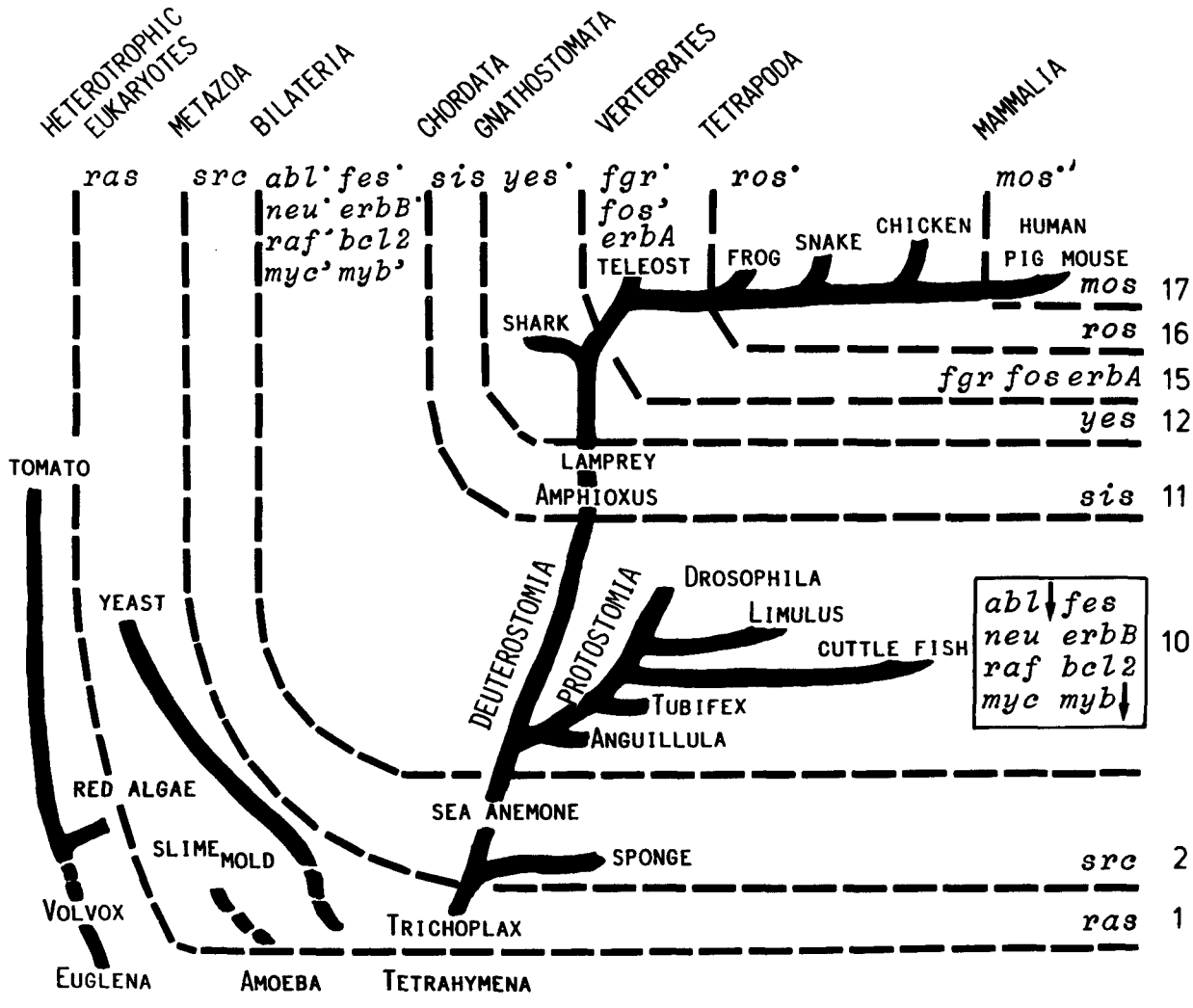


Fig. 1. Attempted outline of the evolution of oncogene systems in the animal kingdom (compiled from [2–10]). See text

As one moves up the evolutionary scale to the multicellular organization of the living beings, i.e., to the **Metazoa,** the *src* oncogene appears in the parazoic sponges and is, thereafter, traceable through the Eumetazoa up to humans [2, 17, 18]. We have not identified cancer in sponges, but *src* was found highly active in the sponges which, because of the autonomy of their cells, can be considered to grow as independently as tumors. In Coelenterata such as sea anemone both *src* activity and abnormal growth comparable to teratomas of higher species have been observed. High activity of *src,* measured as activity of its product, the $pp60^{c\text{-}src}$ kinase, was detected in the nervous cell systems of all groups of animals tested. Its activity is also high in animal and human melanoma [19, 20], the cells of which are probably all derived from the neural crest cell-system. The *src* oncogene is possibly, like *ras,* involved in the transmission of proliferation signals which, on this evolutionary level, possibly include the phosphoinositide phosphoinositol turnover [15]. It serves probably in intercellular communication for coordination of growth and function of the Metazoa, perhaps through gap junctions.

As we go up to the **Bilateria** the Metazoa branch out to the **Protostomia** and **Deuterostomia.** This period must have been evolutionarily very active and successful. A large variety of taxonomic groups containing a large packet of oncogenes has been evolved. In addition to *ras* and *src*, the following have been identified: (a) *abl, fes, neu, erb*B, which

belong to the *src* family and exhibit tyrosine kinase function, (b) *myc* and *myb*, which are assumed to fulfill regulatory functions of gene expression in the nucleus, (d) *raf*, coding for a serine/threonine kinase, and (e) *bcl*2, isolated from human B-cell lymphoma. Since the viral oncogenes which mostly have been used as probes originate from higher vertebrates (i.e., Deuterostomia), one can conclude that the respective cellular genes must have been already present in the last common ancestor of both Protostomia and Deuterostomia. The clear hybridization signals always found with *abl* and *myb* lead to the presumption that they evolved still earlier in the history of life as can be shown by present data (see arrows in Fig. 1). Nothing is known about the tumorigenic function of these oncogenes in the tumors observed in invertebrates. Little is known about these functions in human tumors [12]. *abl, myb, fes, bcl*2 present in *Drosophila, Limulus,* etc., organisms which have no blood in the sense of the blood of mammals, are possibly involved in human hematopoietic malignancies; but no convincing data from human biopsy specimens or fresh cells from a variety of human leukemias und lymphomas are available showing that these early oncogenes are crucial in human neoplasia [12].

The appearance of the *sis* oncogene, which codes for the platelet-derived growth factor (PDGF) in the **Chordata,** represented by *Amphioxus* and lamprey in the outline of the phylogenetic tree, might be critical for the evolution of the closed blood circulation apparatus that exposes the blood to pressure. Up to the teleosts this oncogene is represented by only one copy. Later on, moving from lower Tetrapoda to Mammalia, a second *sis* copy occurs. In humans PDGF is coded by two distinct but related genes, namely the PDGF-A gene and the PDGF-B gene, the latter one being known as human c-*sis,* which is less homologous to the teleost c-*sis* than the PDGF-A gene [6]. Although human c-*sis* is apparently inactive in most human cells, it is supposed that both PDGF A and B (and their receptors) are involved in general regulatory processes, cell proliferation, and tumor formation [12].

The *yes* oncogene occurs in the animal kingdom together with the appearance of the **Gnathostomata,** which are represented in our studies by sharks. This gene is a member of the *src* family which is highly homologous to *src* itself. This poses the question of gene duplication in evolution. Another example, the single *sis* copy of the teleosts that corresponds to the human PDGF A became duplicated (probably), as mentioned above. One could extend this question asking whether the large *src* family including the already mentioned *abl, fes, neu, erb*B, *yes* and the not yet mentioned *fgr, ros,* and *mos* could have been evolved by gene duplication. The idea that oncogene families might have been evolved by gene duplication contributes to the general concept of evolution by gene duplication proposed by Ohno [21] almost 20 years ago.

At the evolutionary level of **Vertebrates,** *fgr,* a member of the *src* family, *fos,* a nember of the *myc*/*myb* family, and *erb*A, a partial homolog of the receptors of thyroid hormone, estrogen, progesterone, glucocorticoid hormone of humans, and the human X-factor, appear together in the teleosts. Since *erb*A of the fish shows strong homologies to the viral gene, one could assume that it has evolved earlier in the history of life than the present data indicate. It seems not to be involved in neoplastic transformation but in tumor promotion, perhaps supporting *erb*B, which appears to be involved in transformation [22].

It is notable that, based on our earlier genetic and histogenetic experiments, not only have gene patterns favorable to neoplasia been observed in teleost species but also genes which limit the action of these genes to certain cell types [23]. This is an important point to consider in human neoplasia [3]. It appears that nature's way of keeping the oncogenes from

their transforming capacity as soon as they became too dangerous for the increasing complexity of life has been to establish a new category of genes, namely the oncogene-specific regulatory genes [24], today sometimes called anti-oncogenes or oncostatic genes.

Finally, *ros*, a member of the *src* family, possibly involved in cell proliferation and tumor promotion through the internal promoter diazyglycerol [14–16], appears to be specific to the **Tetrapoda,** and *mos*, related to the *src* family and also to *raf*, appears to be specific to **Mammalia** [4, 5, 25]. Nothing is known, at least to my knowledge, about the specificity of these genes to the organization of the Tetrapoda and Mammalia, respectively. *mos* is probably involved in human acute myelogeneous leukemia [12].

In conclusion it appears that, in parallel with the advancement of the animal kingdom, particular oncogenes were subject to their own evolution and that, furthermore, the systems of the oncogenes corresponding to this advancement increased in number, several of them probably by gene duplication. From yeast to mammals we found an increase from 1 to 17 (see Fig. 1, right). This increase might reflect the increase of complexity required for advancement in the animal evolution but might in addition reflect an increase of sensitivity to any endogenous and exogenous impairment of the systems. Therefore, our phylogenetic view might reflect some rough observations on the tumor incidence in the animal kingdom which so far have never been studied seriously. Although both oncogenes and cancer have been observed in all systematic categories of the Eumetazoa, it appears that mammals are more afflicted with cancer than any other group of animals.

B. Low and High Susceptibility to Neoplasia

Neoplasia occurs infrequently in the natural populations of Eumetazoa, and induction of cancer by initiating carcinogens and tumor promoters is difficult to achieve [26]. This phenomenon was studied in detail in the Central American teleost genus *Xiphophorus* [26–29] and in East Asiatic mice [30]. Natural selection in Mendelian populations will not favor one population or race and discriminate against the other but will always work against susceptibility to cancer in all populations and races. However, certain nontaxonomically defined groups of animals are highly susceptible to spontaneously developing, carcinogen-initiated, and promoter-stimulated neoplasms (Table 1). These groups consist mainly of animals of hybrid origin, such as naturally occurring or experimentally produced interspecific, interracial, and interpopulational hybrids as well as laboratory and domesticated animals which actually are also hybrids, i.e., homozygous combinations of chromosomes of different populational or racial provenance. These animals share their high susceptibility to neoplasia with humans [26, 31].

While we do not have data on the relationship between hybridization and cancer in human beings comparable to the data on animals, it is interesting to speculate whether the many facts on tumor incidence in humans that do not agree with the concept of the primacy of environmental factors in carcinogenesis can be explained by interpopulational and interracial matings in our ancestry. Certainly interpopulational and interracial mating may have occurred at any time in any place. Because of the high and increasing mobility of modern humans as compared with other species, one should expect high heterogeneity. Various estimates based on enzyme variation showed that heterogeneity in humans is comparable to that of domestic animals such as cats, but is about six times higher than that of wild macaques, about ten times higher than that observed in the large wild mammals such as elk, moose, polar bear, and elephant seal, and about twice as great as that of most feral rodents studied so far [32–34]. Based on these

Table 1. Animals that exhibit a high tumor incidence (for references see [26, 31])

Species	Tumor
Insects	
Drosophila laboratory stocks	Various neoplasms
Solenobia hybrids	Various neoplasms
Teleosts	
Xiphophorus hybrids	Various neoplasms
Girardinus laboratory stocks	Promoter-induced melanoma
Ornamental guppy strains	Carcinogen-induced hepatoma
Orange medeka	Hepatoma
Domesticated trout	Aflatoxin-induced hepatoma
Salvelinus hybrids	Fibrosarcoma
Domestic carp	Neuroepithelioma
Ornamental hybrid carp	Ovarian neoplasia
Lake Ontario hybrid carp	Pollution-conditioned gonadal tumors
Goldfish	Erythrophoroma
Amphibia	
Bufo calamita and *B. viridis* hybrids	Chordomas
Birds	
Musk duck and mallard hybrids	Gonadal tumors
Peacock and guinea fowl hybrids	Gonadal tumors
Improved breeds of fowl	Leukosis
Mammals	
Mus musculus and *M. bactrianus* hybrids	Various neoplasms
Laboratory mice strains	Various neoplasms
Hybrids of mice strains	Increased incidence of various neoplasms
BALB/c and NZB hybrids	Plasma cell tumors (50%)
Blue ribbon mice	Mammary tumors (100%)
Sprague-Dawley and Long Evans hybrids	Increased mammary tumor incidence
Domestic dogs	Various neoplasms
Boxers	Very high tumor incidence
Domestic cats	Various neoplasms
Sinclair swine	Melanoma
Lipizzaner horses	Melanoma

data and on the assumption that tumor incidence in general is related to interpopulational and interracial matings, one could explain why humans have a high incidence of neoplasia comparable to that of the domestic animals.

Furthermore, there are some data on chromosomal heteromorphisms in human populations that might be useful for estimates of heterogeneity within and among 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 heteromorphism, with blacks having more prominent heteromorphisms than whites [35, 36]. One is tempted to assume that this heteromorphism refects the differences in the degree of heterogeneity among the Japanese and white and black United States populations. In this context it is notable that the ratio of prostatic cancer in Japanese, United States whites, and United States blacks is reported as 1:10:30 and that the black citizens in San Francisco have double the

risk of developing neoplasia as compared with their Japanese fellow citizens [37, 38]. We cannot explain these facts by environmental factors or racial differences. The high susceptibility to neoplasia in domestic or hybrid animals, respectively, could show us how to approach the problem. Of course, it is very difficult to study the heterogeneity of a recent human population of a city or country in terms of biological measures. However, new methods such as the determination of restriction fragment length polymorphisms available today could be helpful in revealing the possible relationship between genetic heterogeneity and tumor incidence in modern human populations.

C. Cancer in *Xiphophorus* as a Model for Cancer in Humans

Human biology is unique, but is not so unique in its fundamentals as to make studies on animal models irrelevant for an explanation of human diseases including cancer. Although mice and rats are the classical laboratory animals used in experimental cancer research, several genera of small teleost fish serve increasingly as models in new cancer research programs [39]. One of these genera is *Xiphophorus* (Fig. 2; for portraits of different phenotypes see [2, 3, 22, 23, 29, 31]), the animal model from Central America that we have used in our laboratories for 30 years [24, 40]. Neoplasia appears to develop only very exceptionally in the wild populations of xiphophorine fish. In spite of the fact that thousands of individuals of many wild populations that are isolated from each other have been collected by several investigators and myself, no tumor has been detected. In the progeny of the wild populations that have been inbred in the laboratory for about 80–100 generations, no tumor has occurred spontaneously and almost

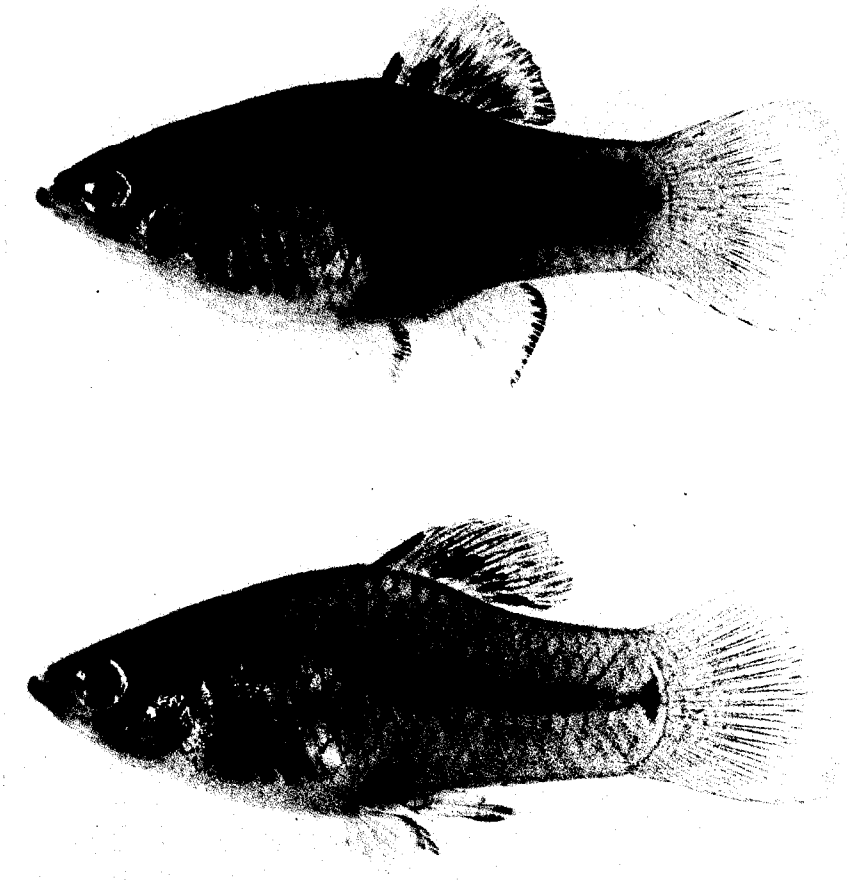


Fig. 2. Female and male of the "spotted dorsal" platyfish, *Xiphophorus maculatus*, from Rio Jamapa (Mexico)

Table 2. Oncogenes in *Xiphophorus*

Oncogene	In *Xiphophorus*	Probe from
*erb*A	+	Avian erythroblastosis virus
*erb*B	+	Avian erythroblastosis virus
sis	+	Simian sarcoma virus
myc	+	Avian myelocytomatosis virus
myc	+	Human
N-*myc*	(+)	Human neuroblastoma
myb	+	Avian myeloblastosis virus
myb	(+)	Human
fos	+	FBJ osteosarcoma virus
fos	?	Human
Ha-*ras*	+	Harvey murine sarcoma virus
Ki-*ras*	+	Kirsten murine sarcoma virus
N-*ras*	(+)	Human promyelotic leukemia
abl	+	Abelson murine leukemia virus
yes	+	Yamaguichi-73 sarcoma virus
fms	+	McDonough feline sarcoma virus
fgr	+	Gardner-Rasheed feline sarcoma virus
src	+	Rous sarcoma virus
raf/mil	+	Murine sarcoma virus
neu	+	Human neuroblastoma
fes/fps	+	Gardner-Arnstein Virus
fes/fps	?	Human
bcl 2	+	Burkitt's lymphoma
Not found		
ros		UR-II sarcoma virus
mos		Moloney murine sarcoma virus
mos		Human

Twenty-six probes were used (gifts from R.C. Gallo, K. Toyoshima, M. Cleary, R. Müller)

no tumor could be induced even with the strongest mutagens-carcinogens such as X-rays and *N*-methyl-*N*-nitrosourea (MNU). This fact requires special clarification since most of the oncogenes that are known to transform the cells and to drive the tumors are present in the fish (Table 2). If, however, interpopulational and interspecific crossings are performed, depending on the genotype, the progeny spontaneously or following treatment with initiating carcinogens (X-rays, MNU, ethylnitrosourea, diethylnitrosamine, 2-amino-3-methylimidazo-(4,5-f)quinoline, etc.) and/or tumor promoters (12-O-tetradecanoylphorbol-13-acetate = TPA, 5-azacytidine, phenobarbital, cyclamate, testosterone, nortestosterone, methyltestosterone, trenbolone, ethinylestradiol, cAMP, biphenyl, butylhydroxytoluene, deoxycholic acid, thioacetamine, bis(2-ethylhexyl)-phthalate, betel nut extract, etc.) develops neoplasia (data in [41]). Neoplasms originate from all neurogenic, epithelial, and mesenchymal tissues (Table 3). The suitability of the model is, except for research on mammalian-specific tumors such as breast cancer, lung cancer, etc., beyond question and its efficiency is more economic and time-saving than that of the laboratory mammals. Agents that induce neoplasia in certain high-risk genotypes of the fish hybrids, might, in principle, also affect certain high-risk human individuals.

Table 3. Neoplasms in xiphophorine hybrid fish induced by physical and chemical agents (i) or spontaneously developed (s)

Neurogenic		Epithelial		Mesenchymal	
Pigment cell system		Surrounding epithelium		Connective tissues	
Benign melanoma	i, s	Epidermal papilloma	i	Intestinal fibroma	i
Malignant melanoma	i, s	Carcinoma	i	Fibrosarcoma	i
Pterinophoroma	i, s	Squamous cell carcinoma	i		
		Epithelioma	i	Muscles	
Nervous cell system				Rhabdomyoma	i
Neurilemmoma	i	Glands	i	Rhabdomyosarcoma	i
Ganglioneuroma	i	Thyroid adenocarcinoma	i, s	Leiomyosarcoma of mesentery	i
Retinoblastoma	i	Pancreatic adenocarcinoma	i		
Neuroblastoma	i, s			Hematopoietic tissues	
		Organs		Reticulosarcoma	i, s
		Liver cell carcinoma	i	Lymphosarcoma	
		Kidney adenocarcinoma	i, s		
		Gallbladder carcinoma	i		

The neoplasms were determined by K. Frese, Institut für Veterinär-Pathologie, Universität Giessen, and by M. Schwab, S. Abdo, G. Kollinger, Genetisches Institut, Universität Giessen, according to Mawdesley-Thomas [42], and were classified essentially according to Weiss [43]

D. Classification of Tumor Etiology in *Xiphophorus* and Humans

The neoplasms of *Xiphophorus* can be classified as:

1. *Mating conditioned:* accessory oncogenes are introduced into, and/or regulatory genes for the oncogenes are eliminated from, the germ line by replacement of chromosomes carrying the respective genes or lacking them, and vice versa.
2. *Mendelian inherited:* regulatory genes for oncogenes are impaired, lost, or dislocated in the germ line by mutation.
3. *Mutagen-carcinogen conditioned:* regulatory genes for oncogenes are impaired, lost, or dislocated in a somatic cell by mutation.
4. *Nutrient and endocrine conditioned:* resting stem cells are pushed to differentiate by tumor promoters (the genetic preconditions according to a, b, and c are fulfilled by earlier events).
5. *Virus conditioned:* accessory oncogenes are introduced (so far not convincingly shown in the fish).

The same classification can be applied to human cancer comprising a small group of (a) "familial"; (b) "hereditary" neoplasms in which genetic factors are supposed to be involved, e.g., retinoblastoma, meningioma, melanoma; (c) a large group of "carcinogen-dependent" neoplasms, e.g., lung cancer; (d) a large group of "endocrine-dependent" and "digestion-related" neoplasms, e.g., breast, prostatic, colon cancer; and, finally, (e) a group of viral-conditioned neoplasms, e.g., leukemia, genital tumors.

In *Xiphophorus* derived from a wild population neoplasia develops in general only if different protocols for the induction of tumors are combined by the experimenter, for instance, (a) the elimination of regulatory genes by selective matings, (b) the induction of germ line mutations, and (c) the induction of somatic mutations, etc. The particular events that alone do not lead to neoplasia, summate, and appear as a multistep process that goes beyond the generations and, finally, reaches the last step that leads to neoplasia in a certain individual. The experimenter must detect the sequence of the

different steps, and it is easy to see that the last step that completes the multistep process determines the etiological type of neoplasia. This was shown for *Xiphophorus* but might be helpful to explain the different types of tumor etiology in humans in which both the ancestry of an individual and the individual itself are involved.

In the following paragraphs we shall try to approach the biological basis of spontaneously developing, carcinogen-mutagen induced, and promoter-dependent neoplasms.

E. Tumors Appearing and Disappearing in the Succeeding Generations

Human tumors such as a certain colon cancer that afflicts individuals 15–20 years sooner than generally may appear "spontaneously" in a family in one generation and may disappear in the succeeding generation. This is demonstrated by means of a cartoon (Fig. 3, upper part) adapted from Lynch and his colleagues [50]. We cannot explain this phenomenon. The *Xiphophorus* model (Fig. 3, lower part) provided the opportunity to study a similar appearance through the fish generations.

Crossings of a spotted platyfish (A) with a nonspotted swordtail (B) result in F_1 hybrids (C) that develop enhanced spot expression and sometimes benign melanoma instead of the spots. Backcrossings of the F_1 hybrids with the swordtail as the recurrent parent result in BC_1 offspring (D, E, F), 50% of which exhibit neither spots nor melanomas (F) while 25% develop benign melanoma (D) and 25% develop malignant melanoma (E). Further backcrossings of the fish (not shown in Fig. 3) carrying benign melanoma with the swordtail result in a BC_2 that exhibits the same segregation as the BC_1.

As opposed to the crossing procedure that gave rise to the melanoma, backcrossings of the melanoma-bearing hybrids (E), with the platyfish as the recurrent parent (A), result in an alleviation of the melanoma in the offspring (C*), which in the following BC generation grow into healthy fish (A*). In conclusion, malignant melanoma of the BC animal (E) originates from the spots of the preceding platyfish generations (A) and is reduced to spots again in succeeding generations (A*).

The formal parallelism in the occurrence of neoplasia in the human family and in the experimental model is striking. In our search for causes of human cancer there might be some value in realizing the types of factors that can be passed from the fish parents to the fish offspring to influence the occurrence of cancer. The experiment with the model suggests that certain human cancers may be expected to occur in individuals because of a combination of factors from both parents that by themselves did not cause cancer in either parent. More data are required in order to compare more stringently human familiar cancer with mating-conditioned neoplasia in the model.

F. Oncogene Expression in the Tumors

The appearance of tumors in both human and model brings about the question for the oncogenes expressed in human and xiphophorine neoplasms. Data available for melanoma indicate an elevated expression of both the human and the xiphophorine *src*, *erb*B, *sis*, *ras*, and *myc* ([2, 6, 7, 18–20, 40, 44, 45] personal communication, U. Rodeck). Measurements concerning the significance of the xiphophorine *src* oncogene (*x-src*) for the development of melanoma and other kinds of neoplasia in the fish (Table 4) showed that the activity of its product, the $pp60^{x\text{-}src}$ kinase, may be elevated in the tumors up to 50 times over that of the controls [46]. Furthermore, the phosphoinositide phosphoinositol turnover, which is supposed to be linked to the *x-src* activity [14–16], was found up to more than ten times elevated over that of the controls (Table 5). This finding is im-

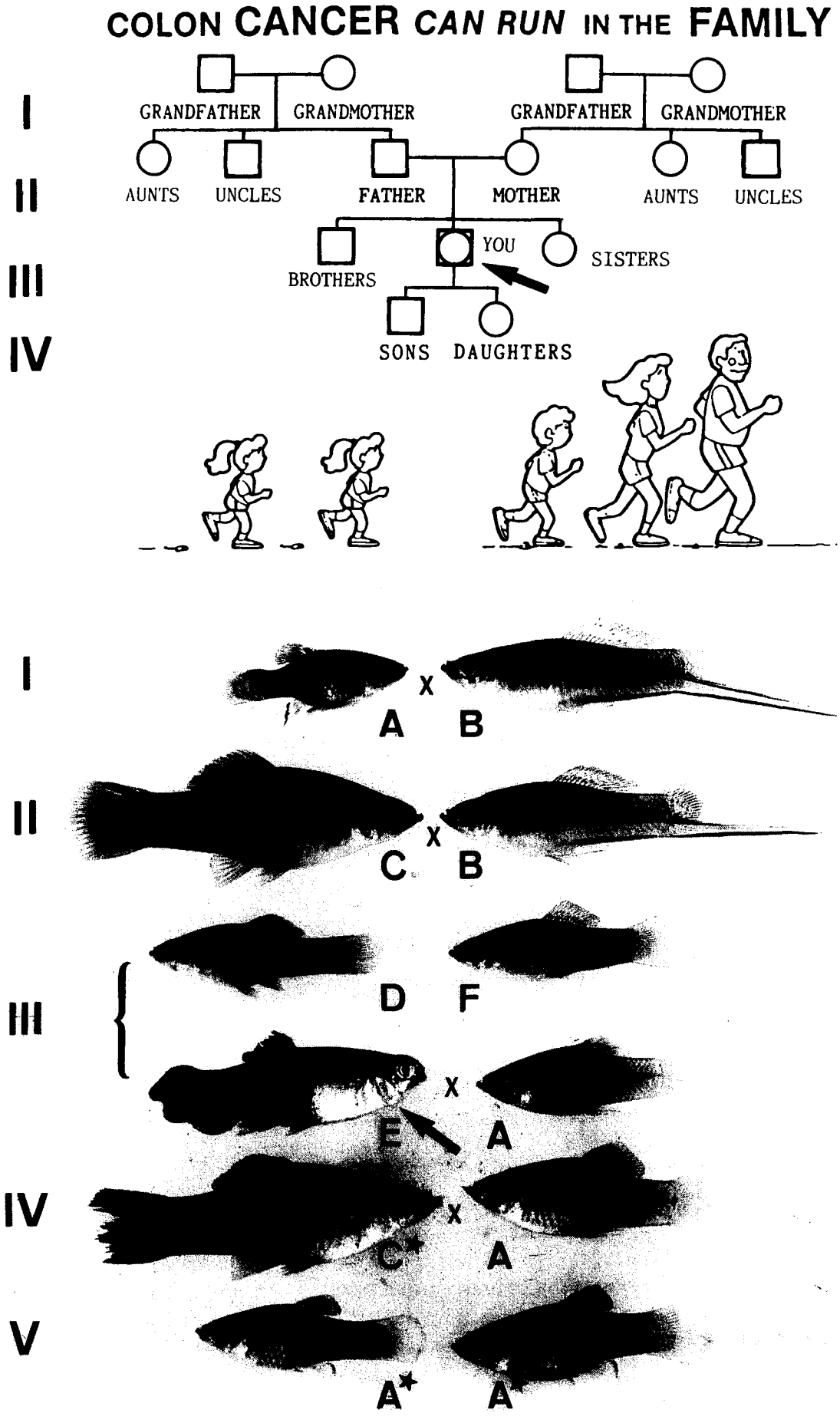


Fig. 3. Appearance and disappearance of neoplasia in succeeding generations (cartoon adapted from [50]). See text

Table 4. Elevation of $pp60^{x\text{-}src}$ kinase activity in tumors and brain of *Xiphophorus* hybrids. (Data from [46])

Tumor	Etiology	Character	Factor of elevation	
			Tumor	Brain
	Carcinogen-induced			
Melanomas	X-ray, adult	Invasive, malignant	5[a]	No
Squamous cell carcinoma	X-ray, adult	Invasive	2[a]	No
Epithelioma	X-ray, adult	Benign	2[a]	No
Fibrosarcoma	ENU, adult	Invasive, malignant	13[c]	No
Fibrosarcoma	MNU, adult	Malignant	10[c]	1.4
Fibrosarcoma	MNU, adult	Invasive, malignant	10[c]	NT
Fibrosarcoma	MNU, adult	Invasive, highly malignant	50[c]	No
Retinoblastoma	MNU, adult	Progressive growth	3[b]	No
Melanoma	MNU, embryo	Invasive	8[a]	No
Melanoma	MNU, embryo	Invasive	10[a]	No
Rhabdomyosarcoma	MNU, embryo		6[c]	NT
Rhabdomyosarcoma	MNU, adult	High malignant, invasive	50[c]	No
	Promoter-induced			
Mesenchymal tumor	MNU + testosterone	Exophytic, slow growing	7[c]	NT
Melanoma, amelanotic	Testosterone	Highly malignant	30[a]	No
	Hereditary			
Melanoma ($n=15$)	Spontaneous	Benign	2–3[a]	1.5–2
Melanoma ($n=28$)	Spontaneous	Malignant	4–8[a]	2, 3
	Unknown			
Rhabdomyosarcoma	Spontaneous	Invasive	20[c]	NT

For comparison nontumorous tissues were used: [a] skin; [b] eye; [c] muscle

Table 5. [^{3}H]-Inositol incorporated into phosphoinositides of xiphophorine melanoma (cpm/10 mg neoplasm). (Data adapted from [47–49])

Melanoma	PtdIns	PtdInsP	$PtdInsP_2$
Benign	9000	1050	800
Malignant, "spontaneous"	29000	1200	2100
Malignant, "induced"	17000	600	2300
Extremely malignant, "spontaneous"	34000	2500	4300
Extremely malignant, inherited	30000	3300	700
Brain (control)	3000	500	300

PtdIns, phosphatidylinositol; PtdInsP, phosphatidylinositol-4-phosphate; $PtdInsP_2$, phosphatidylinositol-4,5-diphosphate

portant because the turnover may serve as a measure for the activation of phospholipase C, which generates the internal promoter diacylglycerol.

A tremendous amount of work on oncogene expression and its possible secondary processes in the tumors and in tumor-derived cell lines of experimental mammals and of humans [12] has been performed in the expectation of finding a particular tumor type-specific initial gene and the initial event of the

formation of a particular neoplasm. While we were never able to identify what one could term a "liver cancer gene" or a "melanoma gene," others have thought they did. Our own studies on the *Xiphophorus* model showed only a relationship of a number of regulatory genes of a number of tissue-specific developmental genes which in total we called "tumor gene-complex" (*Tu* complex); but we interpreted this as an association rather than a true genetic entity, and we assigned the different kinds of neoplasms such as those listed in Tables 3 and 4 to the same *Tu* complex. The nature of the causality of neoplasia remained unclear.

G. An Approach to the Study of the Genetic and Molecular Basis of Neoplasia

The genes underlying neoplasia in *Xiphophorus* were most successfully studied in the generations developing the "spontaneously occurring" mating-conditioned tumors, and it appears to be in the nature of things that those laboratories working presently on the small group of familial and hereditary human tumors approached the fundamentals of neoplasia at least as closely as those working on the large groups of carcinogen- and promoter-dependent tumors.

Our approach in the model is described by means of Fig. 4, which refers to the same fish as indicated in Fig. 3 by the same capital letters (for the mutants see later). Based on breakpoint data the genes responsible for melanoma inheritance are located terminally in one Giemsa band of the X chromosome [51] and represent a complex consisting of (a) the pterinophore locus (*Ptr*) which is responsible for pterinophore differentiation, (b) the compartment-specific dorsal fin locus (*Df*, impaired to *Df'*) which restricts both pterinophore and macromelanophore differentiation to the dorsal part of the body, (c) the region in which a viral *erb*B-related oncogene (*erb*B*, an oncogene related to the receptor of the human epidermal growth factor, EGF, x-*egfr*) is located, (d) the melanophore locus (*Mel*), which appears to be under control of *Df* and *erb*B*, and (e) the arbitrarily symbolized "tumor gene" (*Tu*), which appears as a Mendelian factor but might possibly be composed of both *erb*B* and *Mel* [22, 52]. Oncogenes in addition to the xiphophorine *erb*B* (x-*erb*B*) could not be detected in the X chromosome. Based on our present knowledge, the respective region of the X chromosome of the platyfish, the "*Tu* complex," can be roughly mapped as follows (commas represent breaking points observed):

X . . ., Ptr, Df, erbB, Mel-Tu*

At least about 20 linked genes are involved in the regulation of the *Tu* complex, but there are also several nonlinked regulatory genes, e.g., the *Diff* gene, which, if present in the homozygous state, restrains the transformed pigment cells from proliferation by terminal differentiation [53].

The swordtail (B) has neither evolved a comparable *Tu* complex nor the linked and nonlinked regulatory genes.

Since platyfish and swordtails have a rather high number of chromosomes ($n=48$) and since clear-cut chromosomal conditions concerning their origin were required, the experimental animals, besides the purebreds (A, B), were taken from the F_1 (C), which contains one platyfish and one swordtail genome, and from high backcross generations comprising BC_8 up to BC_{22} (F, E), the genome of which virtually consists of swordtail chromosomes except for the *Tu* complex containing X chromosome selected from the platyfish by the crossings. The phenotypic overexpression of the *Tu* complex thus depends mainly on the crossing-conditioned replacement of platyfish autosomes carrying regulatory genes such as the differentiation gene *Diff*, by swordtail autosomes lacking such genes.

More information about the *Tu* complex comes from studies on the restriction length polymorphism of the onco-

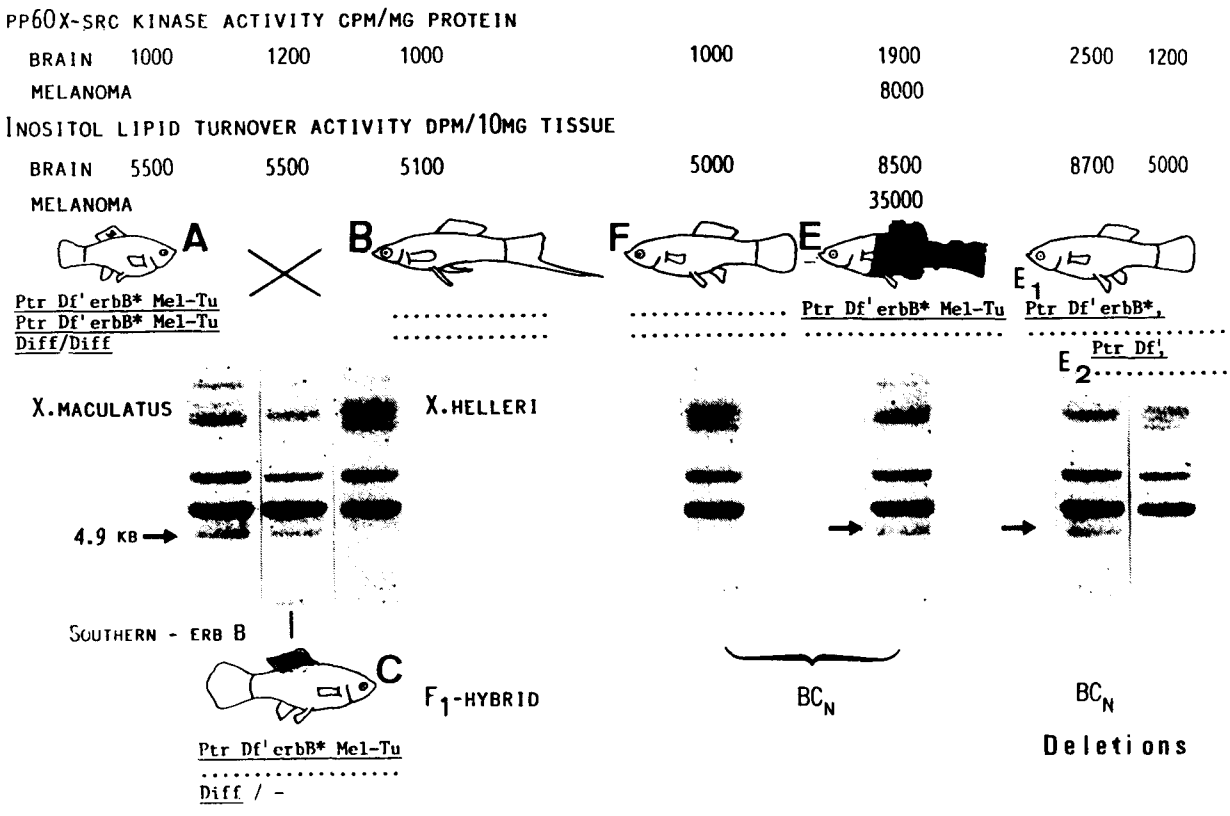


Fig. 4. Appearance of mating-conditioned development of melanoma after crossings of *X. maculatus* × *X. helleri* (platyfish × swordtail; A × B) and backcrossings of the F_1 hybrid (C) with *X. helleri*. F and E represent the backcross generation (BC_n). E_1 and E_2 represent deletions. The fish indicated by the *capital letters* correspond to those indicated in Fig. 3 by the same letters. Note that the 4.9-kb *Eco*R1 Southern fragment is inherited along with the tumor gene-complex. *Ptr*, pterinophore locus; *Df'*, impaired dorsal fin-specific regulatory gene; *erb*B*, xiphophorine copy of an oncogene related to the viral *erb*B; *Mel-Tu*, melanophore locus containing the potential for tumor formation. *Diff*, a nonlinked differentiation gene; ——, chromosomes of *X. maculatus*; . . ., chromosomes of *X. helleri*. See text

genes derived from platyfish and from swordtail. Some of the xiphophorine oncogenes (x-*onc*s) listed in Table 2 show restriction fragment length polymorphism (RFLP) the patterns of which have been differently evolved in the wild fish of different provenance [6–8, 22, 40]. For instance, the patterns of the lengths of the restriction fragments of x-*sis* are specific to each of the different species, but show no RFLP within each of the species; actually these species show a monomorphism of the restriction fragment lengths of x-*sis*. In contrast, the patterns of the lengths of restriction fragments of x-*erb*A and x-*erb*B are species nonspecific, but are specific to the different races and populations of the species. The lengths of certain fragments of x-*erb*B are even different in females and males of the same population.

We used the RFLP phenomenon as an indicator for the Mendelian inheritance of the x-*onc*s through the purebred and hybrid generations. If a certain oncogene fragment is independently inherited from the inheritance of spot or melanoma formation, then one can conclude that the respective oncogene is not "critical" for the first step of melanoma formation. This is not to say that such an oncogene is not involved in melanoma formation at all; as already mentioned, x-*src*, x-*sis*, x-*ras*, x-*myc* are expressed in the mela-

nomas and are certainly involved in tumor growth or tumor progression, but they are not involved in the first step leading to melanoma because they are contributed by the swordtail to the hybrid genome whereas the appearance of the spots and the melanomas is contributed by the X chromosome of the platyfish. Furthermore, since 47 chromosomes of the malignant melanoma bearing backcross hybrids are contributed by the swordtail and only 1, namely the *Tu* complex carrying X chromosome, is contributed by the platyfish, one can assume that most of the oncogenes in the genome of the tumorous backcross animals are contributed by the swordtail genome. Actually, the only x-*onc* detected so far on the platyfish chromosome carrying the *Tu* complex is the x-*erb*B*. This oncogene is represented in Fig. 4 by a 4.9-kb *Eco*R1 Southern restriction fragment which is inherited along with spot and/or melanoma development (A, C, E) and is lacking in the melanoma-free swordtail (B) and the melanoma-free BC hybrid (F). The other *Eco*R1 fragments that also indicate *erb*B sequences could not be assigned to the X-chromosomal locus where the inheritance of the melanomas comes from.

Additional information about the correlation between the inheritance of melanoma formation and the inheritance of the x-*erb*B*-representing 4.9-kb Southern fragment comes from two mutants of the type E BC hybrids. Both types (Fig. 4, E_1 and E_2) have lost the locus *Mel-Tu*, i.e., the capability to develop melanoma, but only one type (E_2) has also lost x-*erb*B* as is shown by the lack of the 4.9-kb fragment. This result indicates that (a) x-*erb*B* is located between *Df* and *Mel-Tu* and (b) information crucial for melanoma formation depends on *Mel-Tu*, which codes for the differentiation of certain pigment cells. This is, however, not to say that there are no links in the chain of events leading to the very beginning of melanoma formation that precede the function of *Mel-Tu*.

As was already mentioned, $pp60^{x\text{-}src}$ kinase activity and inositol lipid turnover activity was found enormously elevated in the melanomas. This is true for all kinds of tumors so far studied and for all types of tumor etiology (Tables 4, 5). Unexpectedly, these activities were also found elevated in the healthy tissues of the fish carrying mating-conditioned and Mendelian-inherited melanomas. Figure 4 (upper part) shows the rounded data measured in the brain of the melanomatous BC hybrids type E in comparison to those of types A, B, C, F. The results suggest that the genes controlling $pp60^{x\text{-}src}$ and the inositol lipid turnover are expressed not only in the melanoma tissues but also in the healthy tissues of the tumorous individuals, independently of whether they are involved in neoplasia or not [46, 49]. Possibly this phenomenon corresponds to the often-occurring multiple tumors in combinations such as melanoma, neuroblastoma, rhabdomyosarcoma, and retinoblastoma in the BC segregants, sometimes even in a particular animal.

Multiple tumors and cancer family syndromes have been reported also in humans [54]. The working group of Lampert [55], for instance, studied a family which, despite a healthy ancestry, developed neuroblastoma, ganglioneuroma, and other neurogenic tumors running through two generations. Lynch and his colleagues [56] reported the pedigree of a family afflicted with cancer on breast, urinary bladder, brain, colon, cervix, endometrium, pancreas, prostate, skin, stomach, and uterus. We cannot explain this phenomenon, but the model shows us the possibility of an approach to the study of some of its molecular and biochemical fundamentals.

It appears that the measurements of $pp60^{x\text{-}src}$ kinase activity and inositol incorporation into phosphoinositides in the brain of the deletion mutants of the fish which are incapable of developing melanoma (Fig. 4, right) open new possibilities for intervention in key signals critical to the endogenous induction of

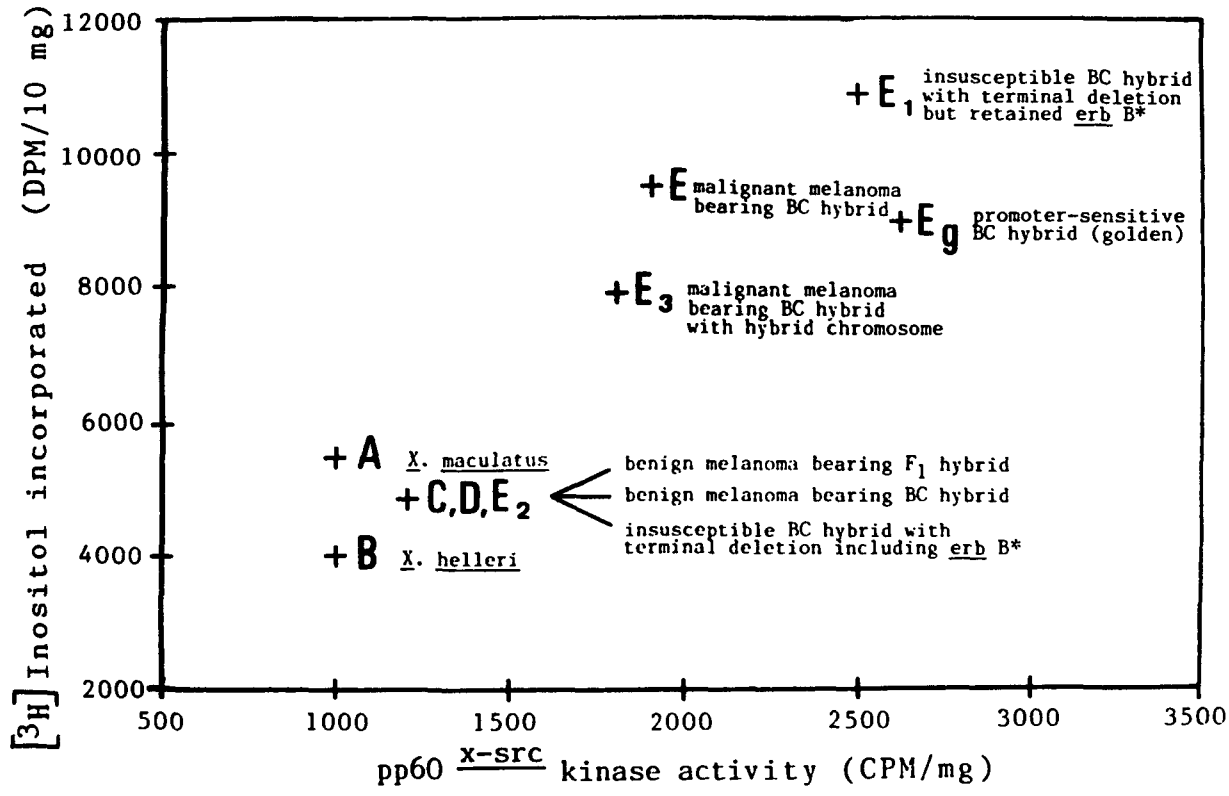


Fig. 5. Incorporation of [^{3}H] inositol in phosphatidylinositol in brain extracts, plotted against activity of pp60$^{x\text{-}src}$. *Capital letters* correspond to the fish indicated by the same letters in Figs. 3 and 4; E_3 ist not shown. E_g corresponds to the promoter-sensitive fish shown in Fig. 9 (*on right*). Note the high correlation of both parameters. Data from [49]. See text

neoplastic transformation in the animal model and possibly in humans. Both pp60$^{x\text{-}src}$ kinase activity and inositol lipid turnover activity are highly elevated in the brain of those insusceptible deletion animals that have lost the *Mel-Tu* locus but have retained the x-*erb*B* oncogene (Fig. 4, E_1). In contrast, the deletion animals having lost the x-*erb*B* together with the *Mel-Tu* locus (E_2) exhibit no elevation. This result suggests that the molecular and biochemical machinery supposedly involved in melanoma formation may be running for genetic reasons, without forming melanoma. Our results, moreover, suggest that there may be a particular type of activation of x-*src* and the inositol phospholipid system that is a marker for predisposition to cancer and could be used for the determination of pro-neoplasia conditions in cancer risk studies. Support for this suggestion comes from the excellent correlation existing between pp60$^{x\text{-}src}$ kinase activity and the [^{3}H]inositol incorporation into phosphatidylinositol (Fig. 5).

One more suggestion arises if one compares the different results obtained with the E_1 and E_2 BC hybrids. Because of the backcross procedure applied to the animals most of the genes involved in melanoma formation are contributed to the hybrids by the swordtail genome. In the deletion hybrid E_2 lacking x-*erb*B* they appear to rest in low activity, indicating that, in order to become involved in melanoma formation, they require a signal for the change from a resting to activated state. The results obtained with the deletion hybrid E_1 show that this signal is transmitted from that region of the *Tu* complex containing platyfish chromosome where x-*erb*B* is located and where the inheritance of the melanoma is determined.

In conclusion, based on the possibility of distinguishing between genes originating from platyfish and swordtail in the

genome of certain hybrids, we found that development and growth of melanoma is mainly run by a set of genes that requires a signal for its activation which, due to the onset of the crossing experiments with the mutants, is transmitted from an x-*erb*B*-containing chromosome locus. This locus, however, is probably deregulated by the crossing-conditioned replacement of platyfish chromosomes carrying regulatory genes for the *Tu* complex (i.e., probably x-*erb*B*) by swordtail chromosomes lacking them.

The 4.9-kb *Eco*R1 restriction fragment was cloned, subcloned, and sequenced. It contains exon c and d of the kinase domain and shows high homology to the respective sequences of the human epidermal growth factor receptor (H-EGFR) gene and to the viral *erb*B (for complete data see [22, 40]). Hybridization of this xiphophorine fragment against genomic xiphophorine DNA revealed the presence of highly homologous sequences located on the Y-chromosome (6.7 kb; see later), on the Z-chromosome, and on an autosome present in all individuals. Another species, *Xiphophorus variatus,* which was studied for comparison, also exhibited an homologous fragment which is inherited along with tumor susceptibility. Each of the x-*erb*B* copies corresponding to these homologous fragments from different chromosomes is also part of a *Tu* complex [40]. Hybrids carrying these *Tu* complexes, however, require treatment with carcinogens as a precondition for melanoma development.

H. Carcinogen-Dependent Neoplasia

The remainder of my review of human cancer is devoted to the large groups of mutagen-carcinogen conditioned (somatic mutation conditioned) and nutrient and endocrine conditioned (promoter conditioned) neoplasms. Both types of etiology comprise probably more than 90% of all tumors. A large body of consistent and contradictory observations on their causation are available.

Lung tumors of humans probably offer the most convincing observations on the involvement of exogeneously induced somatic mutations in the initiation of the tumor. They appear not to be influenced by many environmental factors, and there is no evidence that hormonal or nutritional factors are involved in their causation. The simple interpretation of the induction of a somatic mutation by a physical or chemical carcinogen, however, does not explain the different susceptibility of the different individuals that are exposed to the carcinogen. There must exist hereditary factors that enable most of the individuals to escape lung cancer while others become victims. We cannot explain this observation.

Recently Newman and her colleagues [57] reported on breast cancer in an extended family (Fig. 6). A complex segregation analysis indicated that susceptibility to breast cancer in the family can be explained by autosomal inheritance of a defective regulatory gene while the appearance of the tumor requires a somatic mutation in a target cell. This example shows that steps toward breast cancer had already occurred unnoticed in the preceding generation; the somatic mutation represents only the last step that completes the chain of events leading to cancer.

The *Xiphophorus* model provided more details for the study of the complex situation in the somatic mutation-dependent tumors. In mutagenesis studies [52] we detected nontumorous hybrid genotypes which, following treatment with directly acting carcinogens (X-rays, MNU), develop after a latent period of 8–12 months foci of transformed pigment cells that grow out to compact melanomas (Fig. 7). The smallest cell clones to which these melanomas could be traced consisted of eight cells indicating that there were three cell divisions between a somatic mutation event and the occurrence of the transformed pigment cells [23]. The incidence of these tumors depends on the dosage of the treatment, and may reach up to 100%.

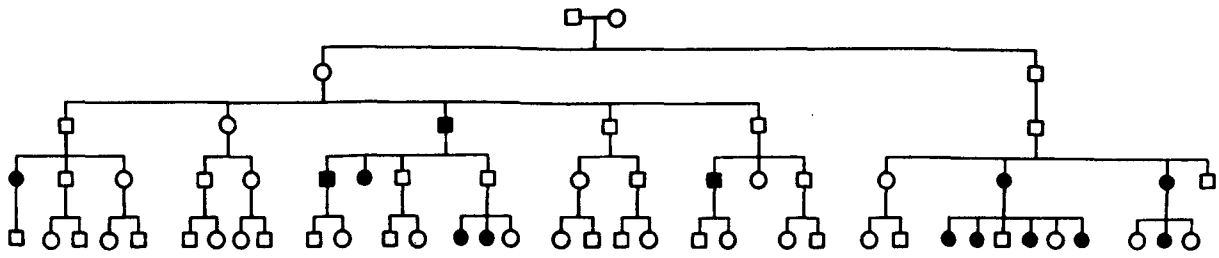


Fig. 6. Pedigree of a family at high risk of breast cancer, adapted from [57]. See text

Fig. 7. Mutagen-carcinogen-sensitive fish developing MNU-induced melanoma. Note the closely circumscribed growth reminiscent of the somatic mutation-conditioned unicellular origin of the tumor

These observations led to the assumption that the *Tu* complex of the treated hybrids is under control of only one regulatory gene which, following treatment, is impaired in a particular pigment cell. Assuming the total of the pigment cell precursors that are competent for neoplastic transformation is 10^6 (this is the average number in the pigment cell system of young fish), and the induced mutation rate is 10^{-6}, then the tumor incidence is 1 (on average 100% of the treated animals will develop one tumor). If, however, the *Tu* complex is under the control of two regulatory genes, the rate of simultaneous mutations of both of these regulatory genes in 1 cell is 10^{-12}, and the tumor incidence is 10^{-6}. This calculation shows that it is difficult to succeed in inducing somatic mutation-conditioned neoplasms if the *Tu* complex is controlled by more than one regulatory gene. This calculation also suggests that the insusceptibility of the animals of the purebred wild populations is based on a polygenic system of regulatory genes directed against cancer.

Support for the assumption that the *Tu* complex of these animals is controlled by only one regulatory gene comes from germinal mutation-conditioned melanoma which occurred in the same genotype. As a consequence of the inheritance of the mutation through the germ line, the *Tu* complex becomes active in the developing progeny as soon as the pigment cell precursors become competent for neoplastic transformation. This process starts in the embryo and continues in all areas of the developing fish where the pigment cell precursors become competent, thus building a lethal "whole body melanoma," which reflects the genuine effect of the *Tu* complex on the pigment cell system. It should be emphasized that the tumorous growths that appear on germinal inherited melanoma (and other hereditary neoplasms), i.e., both the mating-conditioned and the germ line mutation-conditioned melanomas, are not due to the occurrence of somatic mutations during development, because, in contrast to the somatic mutation-conditioned tumors, the transformed cells always occur simultaneously in large areas of the body and show permanent transformation and relapse after complete removal.

To study the molecular and biochemical background of the somatic mutation-conditioned melanomas we modified the experiment that led to mating-conditioned spontaneously occurring melanomas (see Fig. 8 and compare with Fig. 4). The *Tu* complex containing platyfish chromosome was replaced by another which, instead of the mutated dorsal fin-

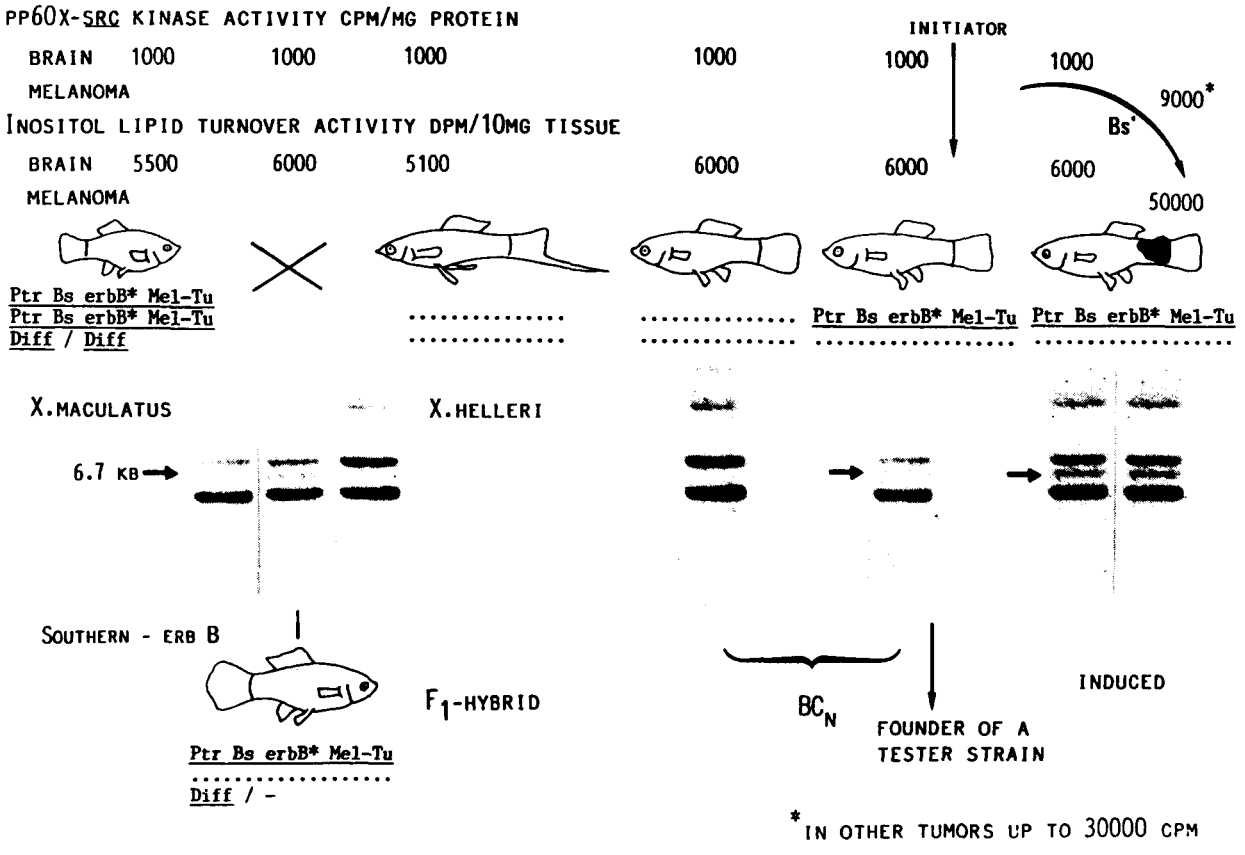


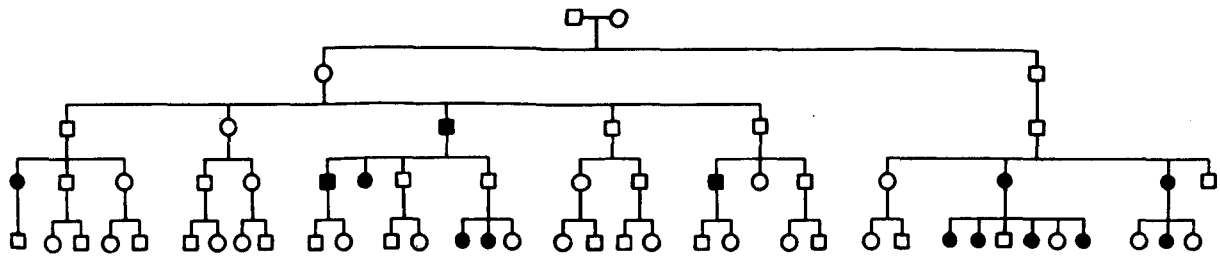
Fig. 8. Crossing procedure for the production of mutagen-carcinogen-sensitive backcross hybrids. Differences to the scheme shown in Fig. 3 are the replacement of the mutated *Df'* by the nonmutated body side-specific regulatory gene *Bs* that suppresses melanoma formation, and the replacement of the 4.9-kb *Eco*R1 fragment indicating *x-erb*B* by a 6.7-kb fragment indicating the same *x-erb*B* gene. See text

specific regulatory gene *Df'*, contains the nonmutated body side-specific regulatory gene *Bs;* in addition, the x-*erb*B* oncogene represented by the 4.9-kb fragment was replaced by a translocated Y-chromosomal copy that is represented by a 6.7-kb fragment. The other genetic conditions are the same as those described in Fig. 4. Melanoma development is suppressed by *Bs* in all purebred and hybrid animals carrying the *Tu* complex. All BC hybrids carrying the *Tu* complex including x-*erb*B* (they can be recognized by their pterinophore-specific reddish coloration coded by *Ptr*) are susceptible to melanoma (and other neoplasms) and may develop melanoma after treatment with physical or chemical carcinogens. Susceptibility to neoplasia or sensitivity to carcinogens, respectively, is inherited in a Mendelian fashion, but the tumors are, as a consequence of a somatic mutation of *Bs* to *Bs'*, nonhereditary and show no relapse after complete removal.

In contrast to the mating-conditioned spontaneous melanoma developing BC hybrids the carcinogen-sensitive BC hybrids show no elevation of $pp60^{x\text{-}src}$ activity as well as no elevation of inositol lipid turnover in the brain. Elevations of these functions are only detected in the neoplasm.

I. Nutrient- and Endocrine-Conditioned Neoplasia

Evidence for nutritional as well as endogenous and exogenous hormonal influences on human cancer has been accumulating over the past 20 years [58]. The agents exerting these influences, of-



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ten called "promoters" or "cocarcinogens," are by no means mutagenic carcinogens, i.e., "initiators," but appear as agents affecting the course of differentation and the rate of proliferation of cells that have already undergone the genetic key event underlying neoplasia irrespective of whether they are tumor precursor cells or definite tumor cells; the changes in cell differentiation and cell proliferation appear as the last step in the chain of events resulting in cancer.

Many data on this subject come from epidemiological studies [59, 60]. It has been found that breast and colon cancer, which represent a high percentage of total neoplasias in humans, are highly correlated to animal fat intake in a large number of countries, and it has been proposed that low animal fat intake is responsible for a low incidence of these neoplasms, while high animal fat intake is responsible for a high incidence. The order of countries begins (low fat intake, low tumor rate) with Thailand, the Philippines, Japan, Taiwan, continues to Czechoslovakia, Austria, France, Switzerland, Poland, the Netherlands, and Finland, and ends with the United States, Canada, Denmark, and New Zealand (high fat intake, high tumor rate). A more critical view, however, indicates that the tumor incidence of the Dutch is twice as high as that of the Finns, though both have the same fat intake. The same is true, if we compare the Swiss (high tumor incidence) with the Poles (low tumor incidence, but same fat intake). The Danes have an extremely high animal fat intake and an extremely high incidence of breast cancer. If one compares, however, the population of Copenhagen with that of the rural Denmark one finds that fat intake in Copenhagen is much lower than in rural Denmark while urban Danes have a higher tumor incidence than rural Danes.

This is not to say that fat intake will have no influence on the incidence of breast and colon cancer; however, our critical view of the data makes clear that fat intake alone cannot explain the differences in tumor incidence in different countries. There could be genetic factors involved in such a way that countries showing a high tumor incidence not only have a high fat intake but also contain a high percentage of individuals that are highly sensitive to the tumor-promoting effect of the fat. These genetic factors may also be related to an effect on normal body growth as has been reported in mouse studies [61, 62]. Thus, these genes might interact with a multitude of other nutritional factors, such as simple caloric intake, quantity and quality of protein ingested, as well as drugs that influence the general condition of an individual.

Our own studies concentrated first on the construction of strains of *Xiphophorus* that are highly sensitive to tumor promoters. Figure 9 shows the development of such a strain based upon the same genotypes and crossing procedures as were used for the production of BC hybrids that develop melanoma spontaneously (see Fig. 4). The only difference is that the genome of the animals contains a homozygous autosomal gene, "golden" (g/g), by which pigment cell differentiation is delayed in the stage of stem melanoblasts. Thus, the BC hybrids corresponding to those developing malignant melanoma spontaneously are incapable of developing a neoplasm. Chemical agents, such as cyclic AMP, corticotropin, a large variety of steroid hormones including testosterone, trenbolone [41], as well as general environmental changes, such as decrease in temperature and increase in salinity of the water in the tank, promote after a latent period of only 4 weeks (latent period of the carcinogen-dependent melanomas is 8–12 months; see preceding paragraph) almost simultaneously the differentiation of large amounts of the noncompetent cells to the competent ones, which subsequently give rise to the melanoma exactly at that place at the body of the fish where they are expected to grow according to the basic crossing experiment (compare Figs. 4, 9).

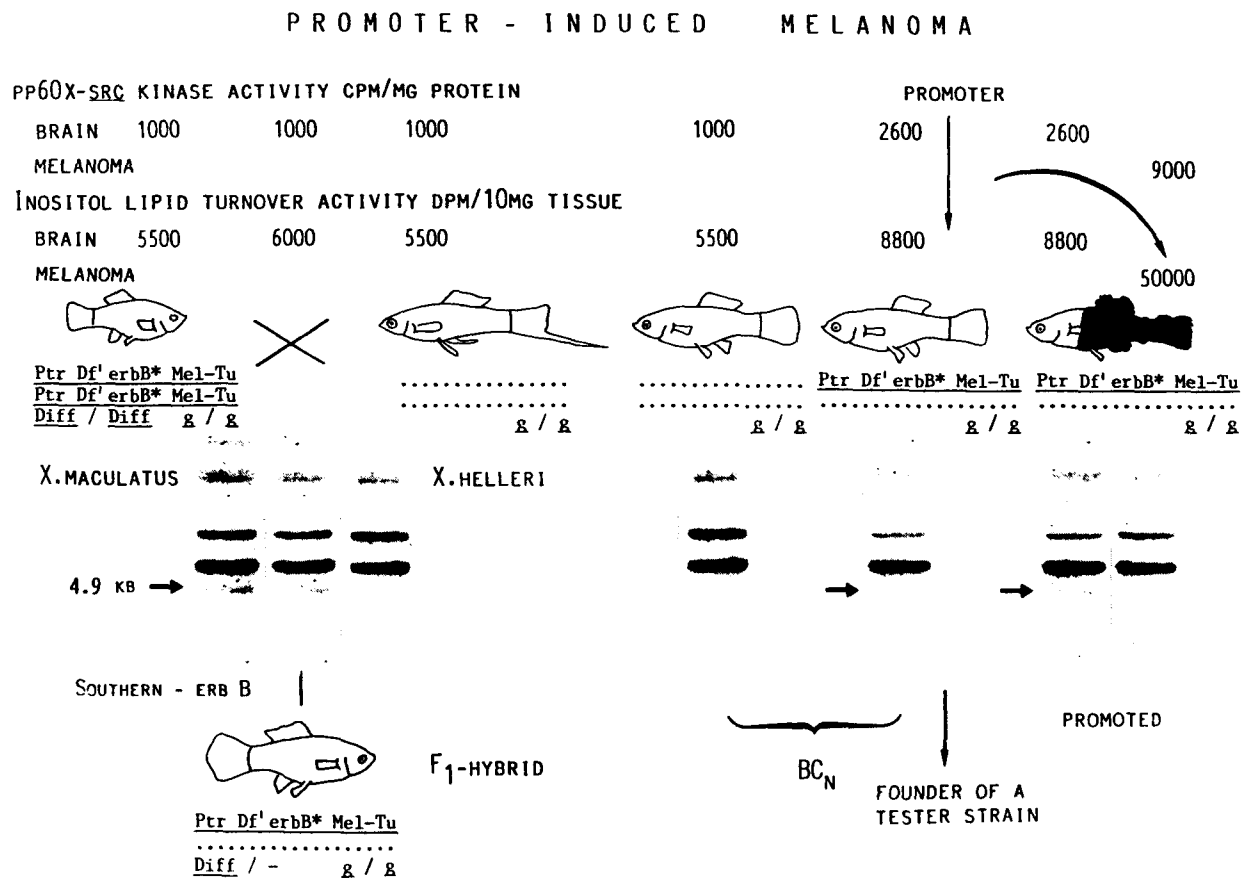

Fig. 9. Crossing procedure for the production of promoter-sensitive backcross hybrids. The only difference to the scheme shown in Fig. 3 is the presence of the homozygous gene "golden", *g/g*, by which pigment cell differentiation is delayed. See text

The very short latent period and the very fast growth of the occurring melanomas as compared with that of the carcinogen-induced tumors is remarkable, but is in line with the enhanced $pp60^{x\text{-}src}$ kinase activity and the enhanced phosphoinositide turnover found in the healthy tissues. It appears that, corresponding to the deletion mutant E_1 (see Fig. 4), the molecular and biochemical machinery leading to neoplasia is running in the susceptible but still tumor-free fish and becomes immediately effective as the competent cells become available for promotion of cell differentiation.

The latter results, again, indicate that both the enhanced activity of the xiphophorine *src* oncogene and the enhanced phosphoinositide turnover are intimately linked with the inheritance of x-*erb*B*, which is presumably involved in the key signal preceding melanoma formation in *Xiphophorus*. They furthermore show again that it should be possible to screen for sensitivity and insensitivity to tumor promoters.

J. Future Goals

In this lecture I have tried to explain some observations on human cancer from the view of a biologist working with a fish model. Of course, what I have presented is not altogether new. Nevertheless, what can we learn from the fish? First of all we should make informed decisions to control the chemical and physical carcinogens and promoters we receive today from our polluted environment. However, we should keep in mind that cancer not only depends on the agents but also on the genes that have been part of our evolution since life began. These genes have experienced mutation, duplication, selection, and genetic drift, and are controlled by oncostatic genes that keep a tight rein on them. To

learn how these regulatory genes keep the oncogenes in check should be an challenging but fulfilling task in the future of cancer research.

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