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Review

Tumor suppressor and overgrowth suppressor genes of *Drosophila melanogaster*: Developmental aspects

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Introduction

Over the past 25 years it has become increasingly evident that cancer is causally related to mutations in specific genes. These genes are instrumental in developmental processes such as cell-cell communication, signal transduction, regulation of gene expression, translation, cytoskeletal organization, protein folding and transport, and differential regulation of cell cycles within developing tissues and organs. In the late sixties a compelling proof for the existence of recessive oncogenes was provided by the work of Harris *et al.* (1969), Gateff and Schneiderman (1967, 1969) and Knudson (1971). Nevertheless, most research during the seventies and early eighties concentrated on the dominant retroviral oncogenes *(v-onc)* and their cellular homologs, the so-called cellular oncogenes *(c-onc)*. The causal relationship between retroviral oncogenes and malignant transformation of cells was unequivocally documented (Bishop, 1982). The causal involvement of their cellular counterparts in cancer, however, is still a matter of debate. Despite numerous studies, no dominant mutations in cellular oncogenes have been shown, either in *Drosophila* or in any other animal, to alone induce cancer as in the case of their viral counterparts.

Attention is therefore shifting to tumor suppressor genes. The designation "tumor suppressor gene" was chosen following the finding that hybrids between tumor and normal cells showed suppression of tumorigenicity (Harris *et al.*,1969). Since the rescue of the *Drosophila* tumor mutant *lethal(2)giant larvae (lgl)* from developing malignant neuroblastomas and imaginal disc tumors, by introducing the wild-type allele into the germ line (Opper *et al.*, 1987), this designation has been applied by analogy to the recessive *Drosophila* tumor genes as well, whose causal involvement in the one-step malignant transformation of a particular cell-type is by now unequivocally proven.

The first sex-linked blood cell-tumor suppressor gene mutation, *lethal(1)7 [l(1)7]*, was found in *Drosophila* by Bridges in 1916. Bridges was also the discoverer of the second, by now legendary, tumor suppressor gene mutation *lgl*. However, the malignant nature of the optic neuroblasts and imaginal discs in this mutant was recognized only 25 years later (Gateff and Schneiderman, 1967, 1969). *lgl* was the first tumor suppressor gene to be cloned (Mechler *et al.*, 1985).

At the time when *lgl* was identified as a tumor mutant, R.C. King *et al.* investigated five benign ovarian tumor mutants (King, 1969, 1970). Thus, already in the very early days of tumor genetics, *Drosophila* was an extremely favorable object of study. Since then, more than 50 tumor suppressor genes and overgrowth suppressor genes have been identified, twenty of which are cloned.

A number of reviews have appeared over the years, discussing the different tumor phenotypes (Gateff, 1978a,b, 1982a), the cloning and functional analysis of some of the tumor suppressor genes (Mechler, 1988, 1990, 1991; Gateff and Mechler, 1989; Mechler *et al.*, 1991), and the putative homologies to vertebrate genes (Bryant, 1993; Watson *et al.*, 1994). This review deals primarily with the developmental aspects of *Drosophila* tumor suppressor and overgrowth suppressor genes, their putative role during cell differentiation and tumorigenesis.

Criteria applied in characterizing tumor suppressor and epithelial overgrowth suppressor genes of *Drosophila*

As mentioned above, the very first gene exhibiting all criteria of tumor suppressor genes was discovered in *Drosophila* as early as 1916 by C.B. Bridges. The mutant allele of this sex-linked gene showed (i) a recessive mode of inheritance, and (ii) caused developmental arrest of late third instar larvae, due to the malignant growth of the blood cells.

Thus, *Drosophila* tumor suppressor genes conform to all criteria defined for tumor suppressor genes in general, i.e. they are recessive-lethal, developmental genes which in the hemi- or homozygously mutated state cause the malignant or benign neoplastic transformation of a specific cell-type. The presence of a wild-type allele suppresses tumorous growth and promotes normal development.

Drosophila malignant and benign neoplasms share all main features with their vertebrate counterparts. Vertebrate cancer is defined as a malignant neoplasm (Cowdry, 1955). "Malignant" denotes the lethal behavior of cells and "neoplasm" a new mode of growth (neos-, new; plasma, a thing formed). Vertebrate malignant neoplastic cells are characterized by autonomous, lethal growth and the loss of the capacity for differentiation. Furthermore, they are invasive, metastatic and show histological and fine structural aberrations (Cowdry, 1955). Malignant neoplasms usually grow in the same autonomous, invasive and lethal fashion after transplantation into nude mice as *in situ*. Benign neoplastic cells, in contrast, grow autonomously in a non-invasive, non-metastatic and non-lethal fashion and resemble histologically more or less closely the tissue of origin (Cowdry, 1955). They usually show some capacity for differentiation and do not grow after being transplanted into nude mice (Cowdry, 1955).

Based on the above characteristics, *Drosophila* neoplasms can also be classified as malignant or benign (Gateff, 1978a). *Drosophila* malignant cells are, like vertebrate malignant cells, differentiation-incompetent and grow autonomously and invasively in a lethal fashion *in situ* as well as after transplantation into a wild-type

host. Since the circulatory system is an open one, metastasis cannot be shown unequivocally in *Drosophila*. Nevertheless, malignant cells injected into the body cavity of a wild-type fly invade many organs by actively traversing their basement membranes.

We refrain from using vertebrate terminology, such as carcinoma or sarcoma, in *Drosophila*, since this would imply evolutionary and ontogenetic homologies between the different *Drosophila* and vertebrate tissues.

In addition to tumor suppressor genes, this review also considers epithelial overgrowth suppressor genes. Epithelial overgrowth differs in one main characteristic from malignant or benign tumors, namely in the capacity of the cells to differentiate. While tumor cells are incapable of differentiation, the cells in overgrown epithelia retain their differentiation capacity.

Methods used to identify malignant or benign tumors and epithelial overgrowth in *Drosophila*

In order to characterize a tissue overgrowth as malignant or benign or simply as hyperplastic, the tissues were first studied *in situ* by anatomical, histological and in some cases by ultrastructural investigations. The capacity of the neoplastic cells for growth and differentiation was tested by the culture and transplantation method of Hadorn (1966).

The differentiation capacities of the mutant cells were investigated by implantation of small tissue pieces into third instar wild-type larvae, allowing the implant to go through metamorphosis with the hosts. Implants capable of differentiation can be recovered from eclosed adults and their differentiation patterns studied, whereas differentiation-incompetent implants cannot be recovered from the body cavity of the adult host, since they are, most probably, eliminated during metamorphosis.

In order to test the mode of growth of the overgrown tissue, small tissue pieces were injected into the body cavity of female adult flies. Malignant cells grow invariably in the same autonomous lethal, invasive fashion as *in situ*. Tissue pieces of benign tumors and epithelial overgrowth, in contrast, grow slower and do not kill their hosts. When tested for their capacity to differentiate, benign neoplastic cells remain differentiation-incompetent, while hyperplastic cells retain their capacity to differentiate when tested by implantation into wild-type larvae. In summary, tissue implants growing autonomously in a lethal fashion in wild-type adult hosts are classified as malignant. Tissue implants showing a non-invasive, non-lethal and differentiation-incompetent growth are considered benign. Implants which retain their capacity to differentiate and grow in a non-lethal fashion are regarded as epithelial overgrowth.

Embryonic tumor suppressor genes

Two lethal mutations are presently known to cause malignant neoplastic transformation of primordial larval cells and tissues in the developing embryo. These are the *Notch (N)* and the *shibire (shi)* mutations (Table 1).

Hemizygous *N* embryos lack ventral and procephalic ectoderm and exhibit instead an overgrown nervous system. Furthermore, mesodermal and endodermal tissues are also affected (Artavanis-Tsakonas *et al.*, 1991; Hartenstein *et al.*, 1992; Fortini and Artavanis-Tsakonas, 1993). Within the neurogenic region the wild-type N^+ gene product determines the epidermal cell fate by serving as a receptor for the transmembrane ligand *Delta*, thus inhibiting the neurogenic pathway by lateral inhibitory signaling (Simpson, 1990). It is assumed that in the mesoderm and endoderm cell fate is established in a similar manner as in the ectoderm (Bate *et al.*, 1993).

Gateff and Schneiderman (1974) tested the possibility that the overgrown *N* nervous system may be tumorous by injecting anterior portions of 24-h-old hemizygous $Df(1)N^8$ (Lindsley and Zimm, 1992) embryos into wild-type, female adult abdomens. After 10-14 days the host flies showed bloated abdomens which were filled, instead of with neuroblasts, with loosely growing blood cell-like cells (Fig. 1), causing host lethality. This lethal, malignant tumor was propagated for 14 transfer generations before the transplantation work was discontinued.

Using the N^{55E11} allele, which is a point mutation that behaves as a null allele, G.E. Gray (personal communication) found, in transplantation experiments with 16-20-h-old embryos, compactly growing tumors that sometimes exhibited melanizations in the central regions. Melanization reactions are typical of the phagocytic blood-cell type, the plasmatocytes and their morphological variant, the lamellocytes (Rizki, 1978; Shrestha and Gateff, 1982a; see later). The above transplantation experiments, using two *N* alleles,

TABLE 1

EMBRYONIC TUMOR SUPPRESSOR GENES

Gene designation	Locus	Affected tissue(s)	Expression	Protein	Homology	Putative functions
(1) *Notch (N)*	3C7	Ventral ectoderm nervous system, mesodermal and endodermal derivatives	10.5 kb transcript; complex expression pattern in many embryonic, larval and adult tissues	2703 aa*, 288 kDa, 36 EGF-like repeats, 3 lin 12/N repeats, 6 ankyrin/cdr 10 repeats, OPA repeat, PEST. In embryonic cell membranes, adherence junctions of imaginal discs	Human TAN-1, 50-70% identity; mouse Motch A,B,C 40-50% identity; Xenopus Xotch 36-70% identity; Zebrafish 55% identity	Lateral inhibition of neuroblast and mesoderm differentiation
(2) *shibire (shi)*	13F-14A1	Embryonic nervous system	Ubiquitous, constitutive	836 aa, GTPase activity, contains SH3 and "pleckstrin homology" domains	Rat and human dynamin, 69% identity	GTP-binding motor vesicular transport in endocytosis; mitosis spindle separation

(1) Gateff and Schneiderman, 1974; Wharton *et al.*, 1985a,b; Coffman *et al.*, 1990; Artavanis-Tsakonas *et al.*, 1991; Ellisen *et al.*, 1991; Del Amo *et al.*, 1992; Bierkamp and Campos-Ortega, 1993; Lardelli and Lehndahl, 1993. (2) MacMorris Swanson and Poodry, 1981; Poodry, 1990; Chen *et al.*, 1991; Van der Bliek and Meyerowitz, 1991. *aa, amino acids.

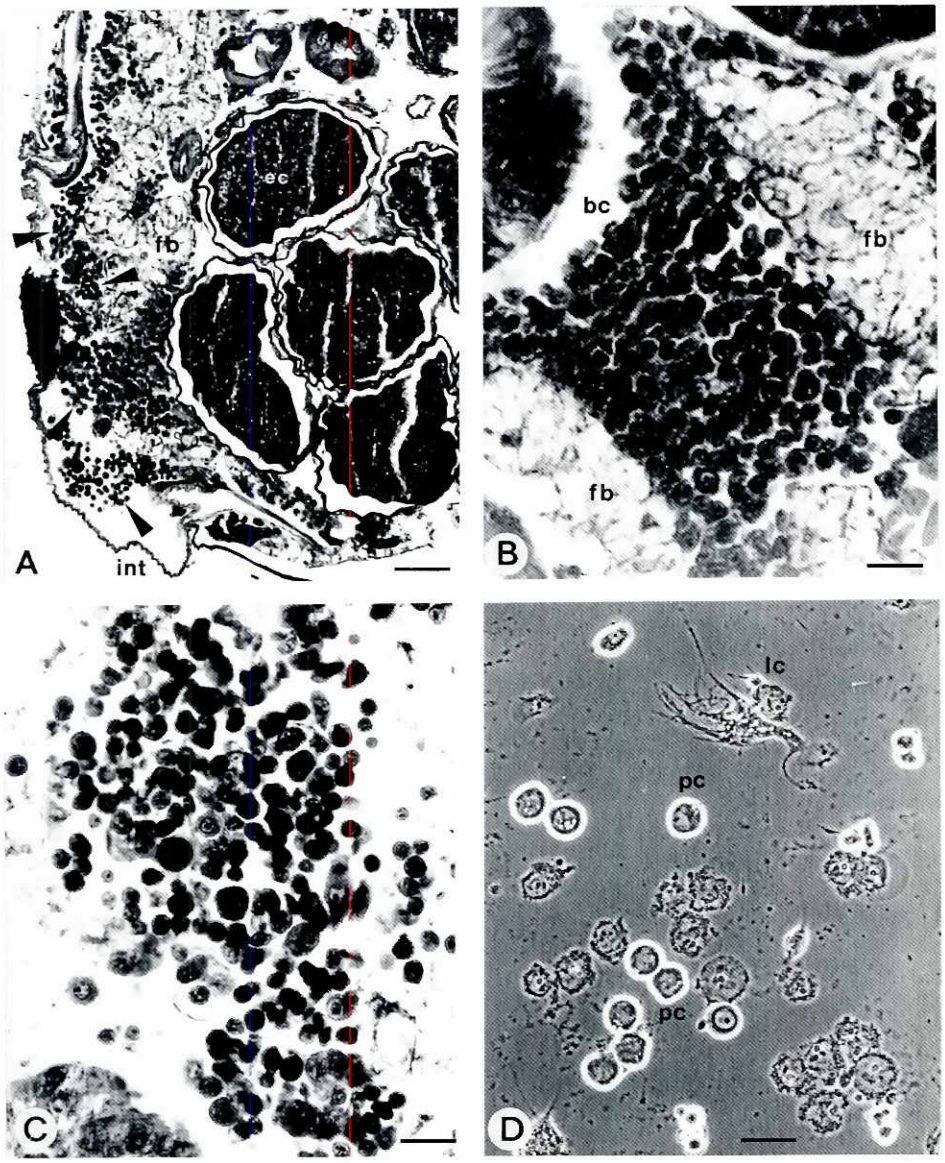


Fig. 1. Histological and phase contrast preparations of *Notch*^{8} transplantable tumor. (A) *Histological cross section through portion of wild-type female abdomen showing Notch*^{8} *tumorous blood cell-like cells (arrows) from the 10th transfer generation, invading the fat body (fb; arrows).* **(B)** *Enlarged histological section through fat body (ft) invaded by tumorous blood cell-like cells (bc).* **(C)** *Histological section through tumorous blood cell-like cells in the body cavity.* **(D)** *Phase contrast of tumorous blood cells from the 10th transfer generation grown in vivo in a female adult abdomen. ec, egg chamber; fb, fat body; int, integument; lc, lamellocyte; pc, podocyte.* **A,B,C**, *hematoxylin and eosin. Bars, A, 1.1 mm; B,C,D, 12 μm.*

indicate strongly that the lack of wild-type *Notch*$^{+}$-gene function causes uncontrolled malignant growth of mesodermal derivatives.

The *N* gene encodes a large transmembrane protein (Table 1) with 36 EGF-like repeats and three cystein-rich *lin12/N* repeats in its extracellular domain (Wharton *et al.*, 1985a; Kidd *et al.*, 1986). The cytoplasmic domain exhibits six *ankyrin/CDC10* repeats (Breeden and Nasmyth, 1987), a *PEST* motif (Rogers *et al.*, 1986) and an *OPA* repeat (Wharton *et al.*, 1985b). *N* functions as a receptor in cell fate decisions (Heitzler and Simpson, 1993).

Notch-homologous genes have been identified on the genomes of man (Ellisen *et al.*, 1991), mouse (Del Amo *et al.*, 1992; Lardelli and Lendahl, 1993; Lardelli *et al.*, 1994), *Xenopus* (Coffman *et al.*, 1990) and zebrafish (Bierkamp and Campos-Ortega, 1993) (Table 1). The homologous genes exhibit similar domain structure and a remarkable conservation of all repeat motifs. The aggregation behavior of cells *in vitro*, which depends on EGF-like repeats 11 and 12, is not altered when the *Drosophila* EGF-like repeats are substituted by the corresponding repeats from *Xenopus* (Rebay *et al.*, 1991). The structural and functional conservation among species in some regions of the protein and the divergence in others, such as the intracellular carboxy terminus, together with the fact that some vertebrates have several homologs (Lardelli and Lendahl, 1993), may indicate species-specific functions for the proteins. *TAN-1*, the human homolog, has been shown to be involved in a t(7;9) chromosomal translocation in three cases of T-lymphoblastic leukemia, suggesting *TAN-1* requirement for normal lymphocyte function (Ellisen *et al.*, 1991). The mesodermal origin of the human tumorous cells corresponds well with the above transplantation results showing malignant growth *in vivo* of mesodermal derivatives. The neurogenic defects may be the result of inductive signals from the mesoderm at gastrulation (Kidd *et al.*, 1989).

N shows a complex pattern of expression in a variety of embryonic (Hartenstein *et al.*, 1992), larval and adult tissues such as the eye (Cagan and Ready, 1989; Fortini *et al.*, 1993), the bristles (Heitzler and Simpson, 1991) and during oogenesis (Xu *et al.*, 1992). Of the many tissues and cells in which *N* is expressed,

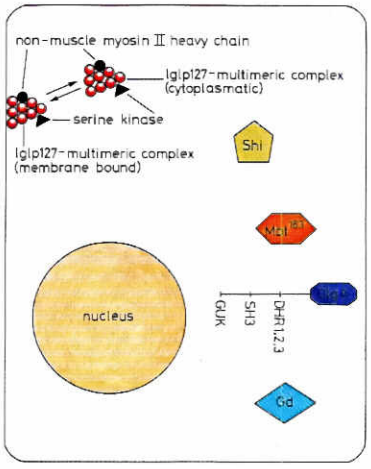


Fig. 2. Schematic drawing of a presumptive adult optic neuroblast. *Shown are the subcellular locations of the lgl127 protein within the cytoplasm and at the inner surface of the cell membrane, where it associates with the cytoplasmic and submembrane cytoskeletons respectively. The septate junction protein DlgA may localize in synaptic junctions (see text). Depicted also is the embryonic tumor suppressor gene product Shi, a dynamin homolog (Table 1). Since the subcellular localization of the remaining three proteins Brat*^{99}*, Mbt*^{163} *and Lgd is unknown, they are placed arbitrarily in the cytoplasm. The drawing is compiled from published and unpublished data cited in Tables 1, 2 and the text. See also Figs. 3 and 5.*

only embryonic mesodermal derivatives seem to become malignantly transformed, which points to functioning in a cell-type-specific manner. Rebay *et al.* (1991) showed that the extracellular domain binds not only the *Delta* but also the *Serrate* extracellular EGF-like repeats, suggesting that *N* may bind a variety of proteins in a functionally and cell-type-specific way. Understanding the specificity of the *N*-ligand binding in mesodermal derivatives is needed to elucidate the events leading to tumorous growth of mesodermal derivatives in hemizygous male *N* embryos.

The second embryonic tumor suppressor gene, *shibire* (*shi*; Table 1), is also a neurogenic gene (Poodry, 1990) showing striking phenotypic similarities to lethal *N* mutations (Shellenbarger and Mohler, 1978). With the help of the temperature-sensitive *shibire*^{ts1} (*shi*^{ts1}) allele, Poodry (1990) determined the temperature-sensitive period responsible for the enormous expansion of the central nervous system at the expense of the epidermis, around the time of neuroblast segregation from the ventral neuroectoderm during gastrulation. Implantation of *shi*$^{ts-1}$ anterior embryonic halves into abdomens of wild-type female flies yielded

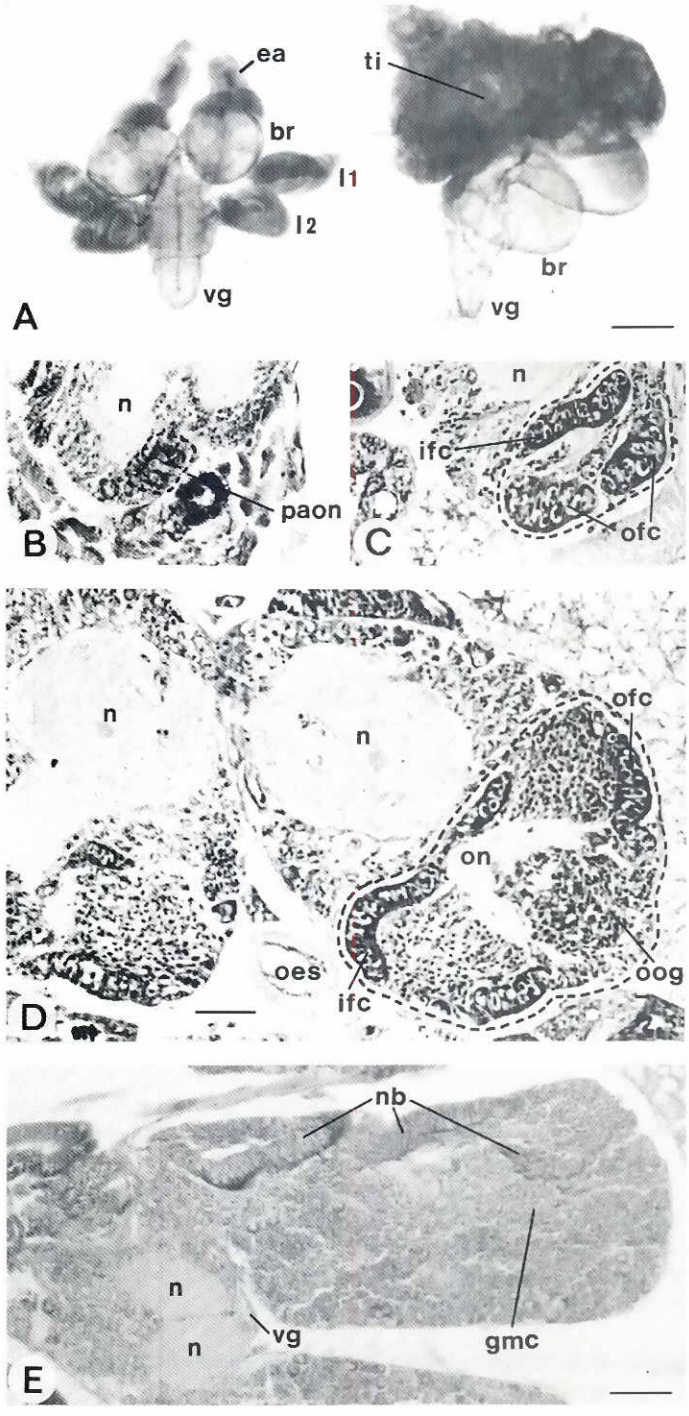


Fig. 3. Comparison of whole-mount and histological preparations of wild type and mutant *lgl* brain-ventral-ganglion complexes and imaginal discs. (A) *Whole-mount preparations of wild-type (left) and lgl mutant (right) brain (br)-ventral ganglion (vg) complexes with attached eye-antennal (ea), first and second leg (l1, l2) imaginal disc pairs. Compare the enlarged mutant brain hemispheres and the fused tumorous imaginal disc mass (ti).* **(B,C)** *Sagittal histological sections through the latero-posterior portion of first* **(B)** *and second* **(C)** *instar wild-type larval brains. The small cluster of larger presumptive adult optic neuroblasts (panb; stippled line) in the first instar larval brain* **(B)** *becomes subdivided in the inner (ifc) and outer (ofc) optic formation centers in the second larval instar brain.* **(D)** *Sagittal histological section through the brain hemisphere of a third instar larva, showing between the inner (if) and outer (of) optic formation centers, consisting of monolayers of large neuroblasts, the differentiating outer optic glomerulus (oog) recognized by the optic neuropile (on) surrounded by optic ganglion-mother cells and optic ganglion cells. The larval neuropile (n), enveloped by the cellular cortex consisting of larval neurons, is also seen.* **(E)** *Sagittal histological section through a lgl brain hemisphere bearing a neuroblastoma consisting of optic neuroblasts (nb) and ganglion-mother cells (gmc). Hematoxylin and eosin. Bars, A, 120 μm; B, C, D, 50 μm; E: 30 μm.*

lethal tumorous growth of neurogenic cells (Poodry, 1990; MacMorris-Swanson and Poodry, 1981).

Kosaka and Ikeda (1983) found in *shi*^{ts1} animals at 29°C blockage of membrane recycling in endocytosis in all investigated cell types, and Masur *et al.* (1990) showed inhibition of neurotransmitter endocytosis during paralysis. The cloning of the gene revealed that *shi* codes for multiple forms of dynamin with a 69% amino acid identity to human and rat dynamin (Chen *et al.*, 1991; van der Bliek and Meyerowitz, 1991). Dynamin is a microtubule-associated GTPase involved in vesicle transport dur-

TABLE 2

BRAIN AND IMAGINAL DISC TUMOR SUPPRESSOR GENES

Gene designation	Locus	Affected cell-type	Expression	Protein	Homology	Putative function
lethal(1)disc large-1 (dlg)	10B8,9	All imaginal discs and brain[1]	Five transcripts, 1.9 - 6.0 kb; expressed in many embryonic and larval epithelia[2]	960 aa*, 102 kDa; septate junction-associated guanylate kinase (GUK); GUK-domain non-functional. DHR**-, SH3+-domain. OPA-repeat, PEST-sequence[2]	MAGUKs++: *Drosophila* p55, rat PSD-95/SAP90, human p55, yeast guanylate kinase, mouse ZO-1. GUK-, DHR-, SH3 domain[2]	May regulate guanine nucleotides acting as messengers in signal transduction by binding GMP and other proteins
lethal(2)giant larvae (lgl)	21A	Adult optic neuroblasts, ganglion-mother cells, imaginal discs[3]	6.5 kb transcript in oogenesis, spermiogenesis, embryogenesis, 3rd larval instar ring gland, salivary gland[4]	1161 aa, 127 kDa; forms oligomers (600-800 kDa); associated with serine kinase, cytoskeletal myosin[5] and up to 10 other proteins	Mouse *(mgl-1)*, human *(hgl-1)*[6], 95% homology to *mgl-1*. *D.pseudoobscura, D.mauritiana, D.hydei, Caliphora erythrocephala*[7]	Signal transduction?
brain tumor (brat) formerly *lethal(2)37Cf*	37C5-7	Adult optic neuroblasts, ganglion-mother cells	4.2 kb, 2.7 kb transcripts, constitutive[8]	99 kDa	—	—
lethal(2)giant discs [l(2)gd]	32A-E	Adult optic neuroblasts, ganglion-mother cells, imaginal discs overgrowth ovary[9]	—	—	—	—
lethal(3)malignant brain tumor [l(3)mbt]	97F3-11	Adult optic neuroblasts, ganglion-mother cells, imaginal discs overgrowth[10]	5.8 kb, 5.0 kb, 4.4 kb transcripts; oogenesis, embryo, larva, pupa, adult[11]	1476 aa, 163 kDa[13]	—	Contains one zinc-finger

(1) Stewart *et al.*, 1972. (2) Woods and Bryant, 1989, 1991, 1993. (3) Gateff and Schneiderman, 1969, 1974. (4) Mechler *et al.*, 1985, 1989; Jacob *et al.*, 1987; Török *et al.*, 1993a. (5) Strand *et al.*, 1994a,b. (6) Tomotsune *et al.*, 1993. (7) Krieg-Schneider, 1993. (8) G.R. Hankins and T.R.F. Wright, personal communication. (9) Bryant and Schubiger, 1971; Bryant and Levinson, 1985; Szabad *et al.*, 1991. (10) Löffler *et al.*, 1990; Gateff *et al.*, 1993. (11) Wismar, 1994. *aa, amino acids; **DHR, Disc Large Homology Region; +SH3, Sarc Homology 3; ++MAGUK, Membrane-associated Guanylate Kinase homolog.

ing endocytosis (Obar *et al.*, 1990). It contains a SH3 domain and a "pleckstrin homology" (PH) domain (Musacchio *et al.*, 1992, 1993), both implicated not only in protein-protein interactions during signal transduction, but also in regulating the interaction with GTP-binding proteins. Recent studies have demonstrated stimulation of dynamin GTPase activity after binding to a subset of 15 recombinant SH3 domains (Gout *et al.*, 1993).

Further studies will be necessary to disclose the neuroblast-specific involvement of the *shi+* gene product in endocytosis and signaling (Fig. 2). Then it will not be difficult to envision the loss of *shi* function to be causally associated with the halt in differentiation and, thus, malignant growth of neuroblasts.

Brain and imaginal disc tumor suppressor genes

The prototype for this group of tumor suppressor genes is the *lethal(2)giant larvae (lgl)* gene which causes, in the mutated state, neuroblastomas in the larval brain (Fig. 3A,E) and tumorous imaginal discs (Fig. 4; Table 2; Gateff and Schneiderman, 1967, 1969, 1974). The neuroblastomas originate from the malignantly transformed adult optic neuroblasts found in the inner and outer formation centers of the presumptive adult optic anlagen, located in the postero-lateral region of each brain hemisphere (Gateff and Schneiderman, 1969, 1974; Fig. 3D).

The adult optic anlage in the wild-type embryonic brain represents about 20 presumptive optic neuroblasts (Campos-Ortega and Hartenstein, 1985; Hofbauer and Campos-Ortega, 1990; Fig. 3B). After the hatching of the larva from the egg the adult optic neuroblasts engage in equal divisions. During the second larval instar, the growing population of optic neuroblasts becomes subdivided into an inner and an outer optic formation center (Fig. 3C). From now on neuroblast divisions are unequal, giving rise to large neuroblasts and small ganglion mother cells which in the late larva and during metamorphosis differentiate into optic neurons.

In all five brain tumor mutants (Table 2), the differentiation of optic ganglion mother cells into optic neurons is interrupted, resulting in an autonomously growing, invasive and lethal tumor *in situ* in the larval brain (Fig. 3E) as well as in wild-type female adult hosts after transplantation of small brain tissue pieces into their body cavity (Gateff and Schneiderman, 1969, 1974; Gateff *et al.*, 1993). Woodhouse *et al.* (1994) showed, on the biochemical and cellular level, by measuring type IV collagenase activity, that the invasive behavior of the malignant *lgl* neuroblasts and ganglion-mother cells is similar to that of some human tumor cells.

lgl encodes a single 6.5 kb transcript expressed in oogenesis, embryogenesis and the third larval instar (Mechler *et al.*, 1985; Table 2). The *lglp127* protein forms multimeric complexes consisting of 6 to 10 *lgl* polypeptide chains (600-1000 kDa), which associate with the cytoplasmic as well as the submembrane cytoskeleton (Fig. 2) in regions of cell-cell contacts (Strand *et al.*, 1994b). In addition, up to 10 different proteins associate with the multimeric *lgl* complex. Two of these proteins have been identified.

One is a serine kinase which phosphorylates the *lgl* protein at specific serine residues (Kalmes, 1993), and the other represents non-muscle myosin II heavy chain (Strand *et al.*, 1994a; Fig. 2) These findings suggest that the *lgl* protein may play a crucial role in the integrity of the cytoskeleton and the polarity of the cells. Furthermore, by associating and interacting with cytoskeletal components it may be involved in intercellular communication and in cell differentiation. The identity of these cytoskeletal proteins is a challenging future task. One such protein may be the putative Tid^{56} protein (see below). This assumption stems from the analysis of the brain and imaginal disc tumor phenotypes in homozygous *lgl/tid* double mutants which show suppression of the brain tumor but not of the imaginal disc tumors (Kurzik-Dumke *et al.*, 1992). Since the *lgl* allele, used for the generation of the double mutant, was a null allele, and no partially functioning or functionally altered gene product can be expected, we hypothesize that the mutant Tid^{56} protein may supplement the *lgl* protein in the brain. In contrast, in the imaginal discs exhibiting the severe *lgl* tumor phenotype, the Tid^{56} protein acts in an *lgl*-independent pathway.

A mouse *lgl* homolog, designated as *mgl-1*, was recently found by Tomotsune *et al.* (1993). With the help of the *mgl-1* probe a homologous human cDNA was isolated exhibiting 95% homology to the *mgl-1* gene (D. Strand and B.M. Mechler, personal communication; Table 2). Figure 4 compares wild type and tumorous *lgl* imaginal discs. For discussion of imaginal disc tumors see next section.

Another gene, developing malignant brain and imaginal disc tumors, is the *lethal(1)disc large-1 (dlg)* gene (Fig. 5B; Table 2). The gene is constitutively expressed in various epithelial cells, the nervous system and the hematopoietic organs (Woods and Bryant, 1989, 1991). The predicted *DlgA* protein, localized in septate junctions of imaginal discs (Woods and Bryant, 1991; Fig. 6), includes a number of domains which are also present in a family of related mammalian proteins designated as *MAGUKS* and a *Drosophila* protein Dp55 (Bryant *et al.*, 1993; Woods and Bryant, 1993). Within the *DlgA* protein three tandemly arranged *D*isc-large *H*omologous *R*egions (*DHR*1,2,3), each 80 amino acids long, an *OPA* trinucleotid repeat (Wharton *et al.*, 1985b), a *SH3* (src-homology region 3; Musacchio *et al.*, 1992), a *PEST* domain (Rogers *et al.*, 1986; Kidd *et al.*, 1989) and a guanylate kinase-like *(GUK)* domain (Berger *et al.*, 1989) were identified. A number of mammalian proteins and at least one other *Drosophila* gene product show considerable homology to the above *DHR*, *SH3* and *GUK*-like domains. The *OPA* and *PEST* domains have not been identified in other, presently known, *MAGUKS*. Studies to localize the *DlgA* protein in neuroblasts of the adult optic centers have not yet been done. There are no septate junctions between the cells in the central nervous system; nevertheless, *DlgA* may associate with synaptic junctions characteristic for nervous tissue (see below).

DlgA shows 67% identity to a predicted rat protein *PSD-95/SAP90*, which is a component of brain synaptic junctions (Cho *et al.*, 1992; Kistner *et al.*, 1993). Unlike *DlgA* it is expressed only in the nervous tissue. However, similarly to *DlgA* it localizes to specialized junctions involved in cell-cell communication. Furthermore, the putative kinase ATP-binding site shows the same deficiency as the corresponding putative kinase ATP-binding site in *DlgA*. A homolog protein was also identified in human brain (Adams *et al.*, 1992). A second human *MAGUK*, the *p55* protein, shows 60% identity to the recently cloned *Drosophila Dp55* homolog (Bryant and Woods, 1992). The *MAGUK* family of proteins co-purify with spectrin, fasciclin III, the cytoskeletal Band 4.1 protein, actin and tubulin (Bryant *et al.*, 1993). These proteins are components of the cytoskeletal network and signal transducing pathways.

The putative *DlgA* associations with membrane cytoskeleton links point to its possible role in signal transduction. However, *DlgA* may also contribute to the structural integrity of the septate desmosomes, which are considered the invertebrate equivalents of the vertebrate tight junctions and which are responsible for the establishment and maintenance of cell polarity (Citi, 1993).

A mouse *DlgA* homolog was cloned recently (Y. Nakano, personal communication). The putative *MDlg* protein shows highly conserved GUK-, DER- and SH3-domains, and is expressed in the

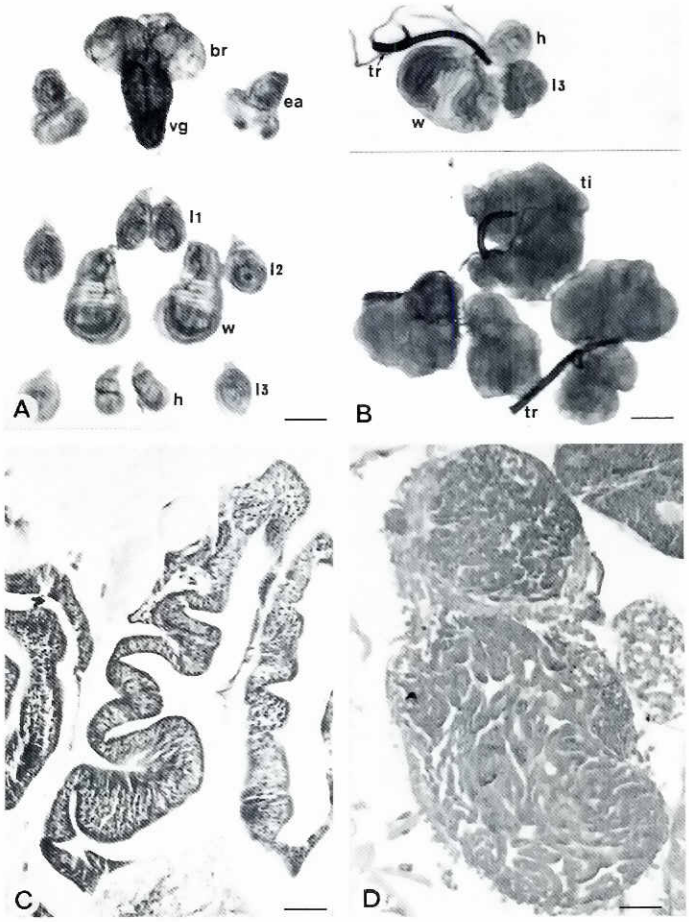


Fig. 4. Whole-mounts and histological preparations of wild-type and tumorous *lgl* imaginal discs. (A) *Whole-mount preparation of wild-type imaginal discs and brain-ventral-ganglion complex (br, vg).* **(B)** *Whole-mount preparation of tumorous wing, third leg and haltere imaginal discs (ti), associated with the trachea (tr) as compared to their wild-type counterparts (above). In contrast to the wild type, the identity of the individual, tumorous imaginal discs (ti) cannot be determined with certainty.* **(C,D)** *Longitudinal histological section through wild-type and lgl tumorous wing imaginal disc. Note the folded monolayer arrangement of the wild-type wing imaginal disc cells and the clumped configuration of the tumorous imaginal disc cells. br, brain; ea, eye-antennal imaginal disc; h, haltere imaginal disc; l1,2,3, leg 1,2,3 imaginal disc; ti, tumorous imaginal disc; tr, trachea; vg, ventral ganglion; w, wing imaginal disc.* **C,D**, *hematoxylin and eosin. Bars, A,B, 30 μm; C, 100 μm; D 130 μm.*

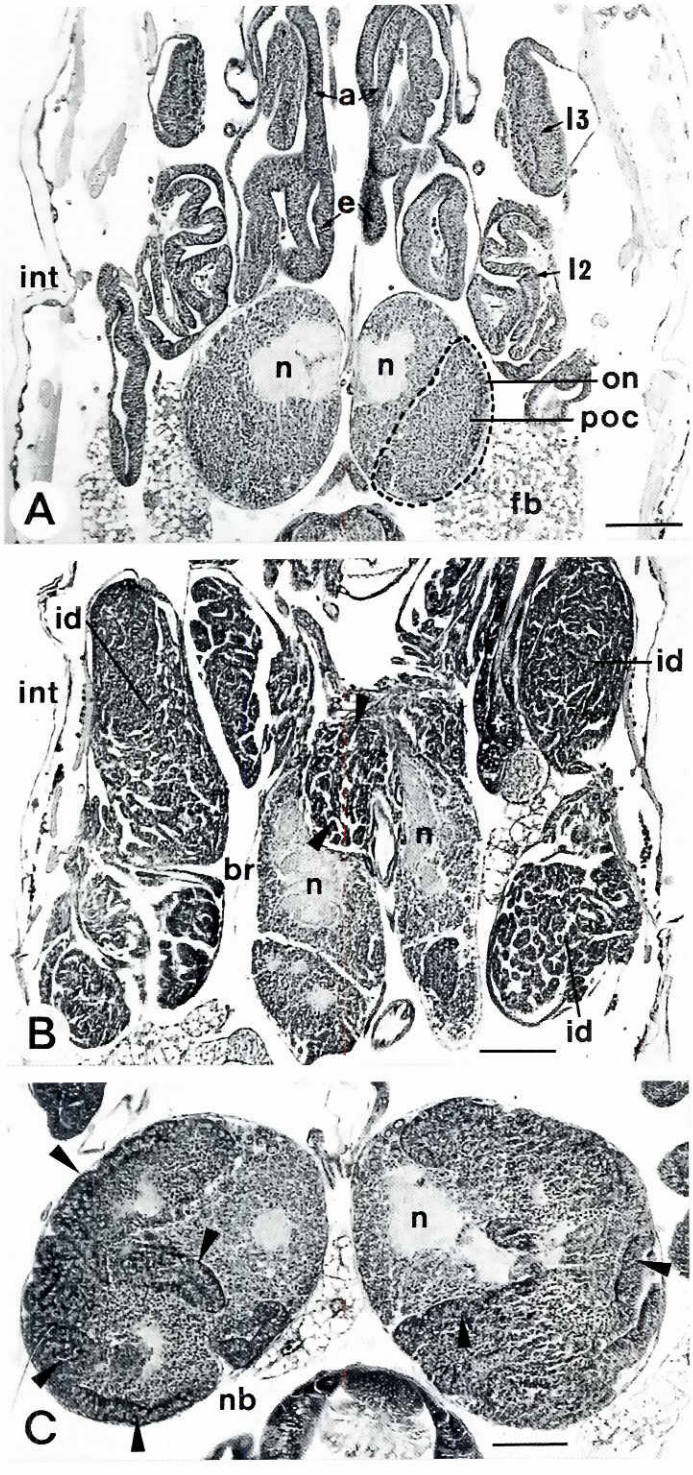


Fig. 5. Sagittal histological preparations of third larval instar wild-type and mutant brain and imaginal discs. (A) *Wild-type eye (e), antennal (a), first and second leg (l2, l3) and wing (w) imaginal discs surrounding the two brain (br) hemispheres. Note the folded imaginal disc epithelia representing cellular monolayers, and in the brain the neuropile (n) surrounded by the cellular cortex including the presumptive adult optic center (poc; stippled line). The large optic neuroblasts (nb) of the outer optic formation center are seen.* **(B)** *dlg tumorous imaginal discs (id) surrounding the two tumorous brain hemispheres (br). Compare the epithelial structure of the tumorous imaginal discs and the brain with the corresponding wild-type organs in* ***A****. Note the imaginal disc invasion (arrows) into the left brain hemisphere. The exact designation of the tumorous imaginal discs is not possible, since they are often fused and spatially dislocated.* **(C)** *l(3)mbt brain tumor showing a highly disorganized cellular cortex with supernumerary, spatially dislocated optic formation centers (arrows) consisting of monolayers of adult optic neuroblasts (nb) surrounded by numerous differentiation-incompetent and invasive ganglion-mother cells. fb, fat body; int, integument. Hematoxylin and eosin. Bars, A, 120 μm; B, 200 μm; C, 65 μm.*

mouse central nervous system only. In parallel to the *DlgA GUK* domain, the *MDlg GUK*-domain also seems to be deficient and thus may have acquired a different function.

The remaining three tumor suppressor genes *brat*, *l(3)mbt* and *l(2)gd* develop exclusively brain tumors (Table 2). The *brat* imaginal discs are absent, while *l(3)mbt* and *l(2)gd* imaginal discs show the epithelial overgrowth phenotype (see later). The medium size *l(3)mbt*$^{+}$ gene extends over 6.0 kb of genomic DNA. Despite extensive mutagenesis experiments only two alleles could be isolated, a P-element induced deletion and a temperature-sensitive allele *l(3)mbt*^{ts1}. Temperature "shift" experiments performed with the *l(3)mbt*^{ts1} allele determined the temperature-sensitive period for brain tumor development and imaginal disc overgrowth between 0 and 6 h of embryonic life (Gateff *et al.*, 1993). All of the embryos shifted during this time to the restrictive temperature developed brain tumors which consist of disorganized sheaths of optic neuroblasts surrounded by differentiation-incompetent, invasive ganglion-mother cells (Fig. 5C). The *l(3)mbt*$^{+}$ gene encodes three constitutively expressed transcripts of 4.4 kb, 5.0 kb and 5.8 kb respectively. *In situ* expression studies show in preblastoderm embryos a strong maternal component. During gastrulation and thereafter *l(3)mbt*$^{+}$ is expressed ubiquitously in the germ band. At the end of embryonic life *l(3)mbt*$^{+}$ expression is restricted to the central nervous system (Wismar, 1994). In the larval imaginal discs, the presumptive optic centers in the brain and few other organs express *l(3)mbt*$^{+}$. Constitutive *l(3)mbt*$^{+}$ expression was detected throughout oogenesis. The putative *Mbt*^{163} protein possesses a single zinc-finger, but otherwise shows no homology to known proteins (Table 2). Since no further protein data are available, *Mbt*^{163} is arbitrarily placed in the cytoplasm in Figs. 2 and 6.

brat gene mutations induce in the larval brain an extreme expansion of optic neuroblasts and ganglion-mother cells, which behave autonomously in the transplantation test. The *brat* gene was cloned by G.R. Hankins (personal communication). The putative 99 kDa *Brat*^{99} protein exhibits no significant homology to other known proteins (Table 2). Double mutants of *brat/lgl* and *brat/fat* yielded embryonic lethality, which possibly demonstrates that these three genes are active in different developmental pathways (Kurzik-Dumke *et al.*, 1992).

l(2)gd was discovered by Bryant and Schubiger (1971). The *l(2)gd* gene mutation is responsible for a transplantable, malignant brain tumor (Table 2) and overgrown imaginal discs (Bryant and Levinson, 1985; see later Table 4). The *l(2)gd* gene is not yet cloned. The cellular location of the putative *Brat*^{99} and *l(2)gd* proteins are unknown and they are also placed arbitrarily in the cytoplasm (Fig. 2).

Imaginal disc tumor suppressor genes

In the early embryo small groups of 20-50 determined cells become the primordia for the various parts of the imaginal integu-

TABLE 3

IMAGINAL DISC TUMOR SUPPRESSOR GENES

Gene designation	Locus	Affected imaginal discs	Expression	Protein	Homology	Putative function(s)
lethal(2)tumorous imaginal discs [l(2)tid]	59F5	All imaginal discs[1]	2.0 kb transcript constitutive; 1.4 kb, 0.8 kb transcripts, larval-specific[2]	518 aa*, 56 kDa; 90 aa DNAJ-domain; cytoplasmic location[2]	Up to 60% identity to DNAJ-domain of bacteria, yeast and man[2]	Cooperating factor in protein folding and transport in Hsp 70 chaperonin machinery ?
lethal(2)tumorous antennal, labial, clypeo-labral imaginal discs [l(2)talc], formerly l(2)106/22	57	Antennal-, labial-, clypeo-labbral imaginal discs[3]	–	–	–	–

(1) Kurzik-Dumke *et al.*, 1992. (2) Kurzik-Dumke *et al.*, 1994. (3) Török *et al.*, 1993b. *aa, amino acids.

ment (Bryant, 1978; Younossi-Hartenstein *et al.*, 1993). During larval life the adult integumental anlagen grow by cell division into imaginal discs consisting of folded monolayer epithelia (Fig. 4A,C) in which the cells are polarized in basal-apical direction and connected laterally to each other by adherence and septate desmosomes (Poodry, 1980; Fig. 6). Under the influence of the hormones at metamorphosis they evaginate and secrete their prospective cuticular bristle patterns. In comparison, tumorous imaginal disc cells have lost their apical-basal polarity and most of their junctional complexes (Abbott and Natzler, 1992), which interrupts their monolayer epithelial organization (Fig. 4B,D) and their capacity to differentiate.

A further characteristic of tumorous imaginal discs is their autonomous, lethal growth after transplantation into wild-type adult hosts (Gateff and Schneiderman, 1969, 1974; Gateff *et al.*, 1974; Gateff, 1978a). Transplanted wild-type imaginal discs, in contrast, show regulated, non-lethal growth (Gateff and Schneiderman, 1969).

Recessive mutations in four tumor suppressor genes cause malignant neoplastic transformation of imaginal discs (Tables 2 and 3). The homozygous, tumor-bearing larvae die around the time of puparium formation. In this section two mutations, transforming exclusively the imaginal discs, will be discussed (Table 3).

One of the two imaginal disc tumor suppressor genes is the *lethal(2) tumorous imaginal discs* [*l(2)tid*] gene (Kurzik-Dumke *et al.*, 1992, 1994; Table 3). Mutations in the *l(2)tid* gene cause in homozygotes malignant neoplasms of all imaginal discs characterized by differentiation incompetence and autonomous lethal growth *in situ* and after transplantation into wild-type female hosts (Kurzik-Dumke *et al.*, 1992).

The molecular organization of the *l(2)tid*$^{+}$ locus revealed the *l(2)tid*$^{+}$ gene localized in the intron of the cotranscribed gene *lethal(2)neighbor of tid [l(2)not]* (Kurzik- Dumke *et al.*, 1994). Both genetic and molecular data suggest a functional relationship between the *l(2)tid* and *l(2)not* genes (M. Kaymer and U. Kurzik-Dumke, personal communication).

The *l(2)tid*$^{+}$ gene, spanning a total of 2,539 nucleotides, encodes a putative *Tid*^{56} protein of 518 amino acids with a predicted molecular weight of 56 kDa (Table 3). The predicted amino acid sequence shows significant homology to eleven *DnaJ* proteins from bacteria, yeast and man. The highest identity scores, up to 60%, were found in the 90 amino acid *DnaJ*-domain (Kurzik-Dumke *et al.*, 1994), characteristic for all *DnaJ* homologous proteins (Silver and Way, 1993). The *l(2)tid*$^{+}$ gene is expressed in the cytoplasm of all cells throughout development (A. Debes and U. Kurzik-Dumke, personal communication; Fig. 6). The fact that in *l(2)tid* mutants the imaginal discs grow in a malignant fashion implies that the *Tid*^{56} protein must be involved in the differentiation of these epithelia.

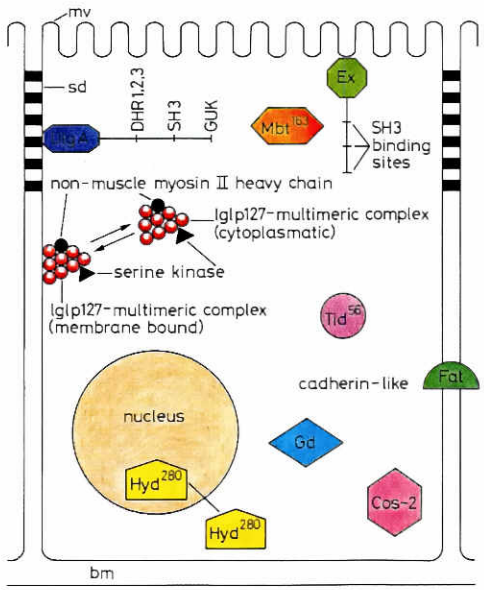
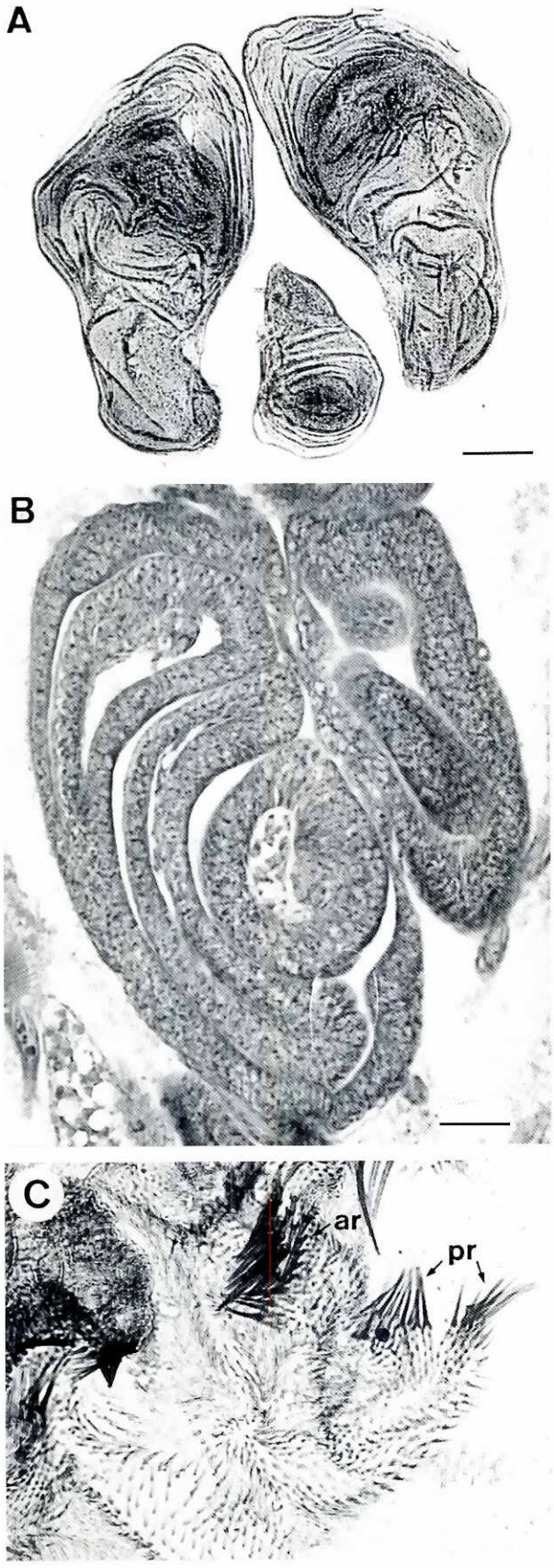


Fig. 6. Schematic drawing of an imaginal disc cell depicting the apical microvilli *(mv)*, the basement membrane *(bm)* and laterally septate desmosomes *(sd)*. *The known cellular localization of tumor suppressor and overgrowth suppressor proteins such as DlgA, Iglp127, Tid*^{56}*, Fat, Ex and Hyd*^{280} *are shown. Arbitrarily placed in the cytoplasm are the Mbt*^{163} *and Cos-2 proteins. GUK, GUanylate Kinase; SH3, Sarc Homology region 3; DHR, Disc-large Homologous Regions. The data are based on publications cited in Tables 3,4 and the text.*

A second, recently isolated tumor suppressor gene *lethal(2)talc* (*talc*), shows in contrast to *l(2)tid* mutant animals tumorous growth only of the antennal, labial and clypeo-labral imaginal discs, while all remaining imaginal discs are normal (Török *et al.*, 1993b; Table 3). The gene is presently being cloned.



Imaginal disc epithelial overgrowth suppressor genes

Bryant (1987) reviewed a number of recessive-lethal mutations which he named "epithelial overgrowth mutations". The mutant imaginal discs are several times larger than their corresponding wild-type counterparts (Fig. 7A). However, contrary to the tumorous imaginal disc cells, which have lost their polarity and consequently their monolayer, folded epithelial structure (Abbot and Natzler, 1992; compare Figs.4 and 5) and the capacity to differentiate, the overgrown imaginal discs maintain their monolayer epithelial arrangement (Fig. 7B) and differentiation capacity (Fig. 7C). Thus, mutations in epithelial overgrowth suppressor genes cause additional cell divisions without affecting the capacity of the imaginal disc cells to differentiate. This indicates that differentiation and regulation of cell division are independent processes. In contrast, tumor suppressor mutations seem to interrupt the differentiation process. Table 4 shows twelve epithelial overgrowth mutations, four of which (*fat*, *cos-2*$^{161/21}$, *l(3)mbt* and *ex*) are cloned (see also Fig. 6).

The predicted *Fat* amino acid sequence shows an enormous transmembrane protein, whose extracellular domain exhibits a strong homology to extracellular domains of vertebrate calcium-dependent cadherins involved in cell adhesion (Mahoney *et al.*, 1991). However, this domain with its 34 cadherin-like repeats is much larger when compared to the four repeats of vertebrate cadherins. Furthermore, it contains four EGF-like repeats and cysteine-rich regions which vertebrate cadherins do not possess. The cytoplasmic domain shows no homology to vertebrate cadherins. The single, large transcript is expressed in embryonic ectoderm and imaginal disc cells. A function as a calcium-dependent adhesion molecule has not yet been shown for the putative *Fat* protein. Nevertheless, based on the cell-autonomous behavior of mutant *fat* clones induced by mitotic recombination and the mutant phenotype, Mahoney *et al.* (1991) concluded that *the Fat* protein may be required for cell-cell communication controlling cell proliferation and morphogenesis of imaginal discs. In various human cancers E-cadherin has been found to be deleted or non-functional (for review Bryant *et al.*, 1993).

Initiation, maintenance and termination of cell proliferation is postulated as the hypothetical function of the *hyperplastic discs (hyd)* gene (Mansfield *et al.*, 1994; Table 4). The 280 kDa *Hyd* protein, found in the cytoplasm as well as in the nucleus (Fig. 6), is present at all developmental stages. It shows 36% sequence identity and 51% sequence similarity over 982 amino acids to a rat protein of unknown function designated *100 kDa Protein* (Müller *et al.*, 1992). Fifty-five amino acids in the *Hyd* and rat *100 kDa Protein* show sequence similarity to the C-terminal domain of a poly(A)-binding protein. About two thirds of the *Hyd* amino acid sequence, however, does not show any homology, implying that the nuclear and cytoplasmic function of this protein in cell proliferation control will have to be elucidated in the future.

Fig. 7. Whole-mount preparations and histology of *fat* imaginal discs and a metamorphosed explant. (A) *Whole-mount preparation of two overgrown fat wing imaginal discs compared to a wild-type counterpart (center).* **(B)** *Longitudinal histological section through a fat wing imaginal disc showing excessively folded, cellular monolayers (compare with wild-type wing imaginal disc in Fig. 5C).* **(C)** *Metamorphosed fat wing imaginal disc explant, exhibiting hair and bristle patterns typical of wing blade with anterior (ar) and posterior (pr) bristle rows.* **B**, *hematoxylin and eosin. Bars, A, 50 μm; B, 30 μm.*

TABLE 4

IMAGINAL DISC EPITHELIAL OVERGROWTH SUPPRESSOR GENES

Gene designation	Locus	Affected imaginal discs	Expression	Protein	Homology	Putative function
(1) *lethal(2)fat, [l(2)ft]*	24D5	All imaginal discs	15-20 kb transcript in embryonic ectoderm and imaginal discs	5147 aa*, transmembrane protein	Vertebrate cadherin S, extracellular domain	Cell-cell adhesion, controlling cell proliferation and morphogenesis
(2) *lethal(2)giant discs (lgd)*	32A-E	All imaginal discs, ovary, brain[2]	—	—	—	—
(3) *costal-2$^{161/28}$, (cos-2)*; formerly *lethal(1)161/28*	43B2-C3	All imaginal discs[3]	—	—	—	—
(4) *lethal(2)43/1 [l(2)43/1]*	38C,D	All imaginal discs[4]	—	—	—	—
(5) *lethal(2)79/18 [l(2)43/1]*	22C	All imaginal discs	—	—	—	—
(6) *lethal(2)overgrown hematopoietic organs 31 [l(2)oho31]*, formerly *l(2)144/1*	31A	All imaginal discs, especially genital imaginal disc; plasmatocytes	0.9 kb transcript: maternal, embryo, imaginal discs	522 aa, 60 kDa	Yeast SRPI**, 41% identity: human APC***	?
(7) *lethal(2)88/10 [l(2)88/10]*	56A	All imaginal discs, 65% melanotic pseudotumors in hematopoietic organs	—	—	—	—
(8) *hyperplastic discs (hyd)*, formerly *l(3)c43*	85E1-10	All imaginal discs, germ cells	9.5 kb transcript; constitutive	2897 aa; 280 kDa cytoplasm and nucleus	Poly-A binding protein. Rat 100 kDa protein	Initiation, maintenance and termination of cell proliferation
(9) *lethal(3)disc overgrown-1 (dco-1)*	100A1-Ba	All imaginal discs	—	—	—	—
(10) *warts (wts)*	100A2-7	All imaginal discs	—	—	—	
(11) *lethal(3)malignant brain tumor [l(3)mbt]*	97F3-11	All imaginal discs	5.8 kb, 5.0 kb, 4.4 kb transcripts; oogenesis, embryo, larva, pupa, adult	1476 aa, 163 kDa	—	Contains one zinc-finger
(12) *expanded (ex)*	21C3	Proximal antenna, wing, leg	6.9 kb transcript; antenna, wing, leg	1429 aa, three SH3 binding sites. Apical surface of imaginal disc cells	20 - 50% identity to 7 blocks present in members of Band 4.1 and NF2 gene super-families	Interaction with SH3 containing proteins in signal transduction

(1) Bryant *et al.*, 1988; Mahoney *et al.*, 1991. (2) Bryant and Schubiger, 1971; Bryant and Levinson, 1985; Szabad *et al.*, 1991. (3) Grau and Simpson, 1987; Török *et al.*, 1993b. (4-7) Yano *et al.*, 1992; Török *et al.*, 1993b; Peifer *et al.*, 1994. (8) Martin *et al.*, 1977; Mansfield *et al.*, 1994. (9) Jursnich *et al.*, 1990. (10) Justice and Bryant, 1992. (11) Löffler *et al.*, 1990; Gateff *et al.*, 1993; Wismar, 1994. (12) Algrain *et al.*, 1993a; Boedigheimer and Laughon, 1993; Boedigheimer *et al.*, 1993, Trofalter *et al.*, 1993. *aa, amino acids; **SRPI, Suppressor of PolI - A190 subunit; ***APC, tumor suppressor adenomatous poliposis coli.

The *expanded* mutation causes overgrowth of proximal antennal, wing and leg discs (Boedigheimer and Laughon, 1993; Table 4). It encodes a novel 1429 amino acid protein localized at the apical surface of imaginal disc cells (Fig. 6). The protein contains three potential SH3-binding sites and thus may interact with other SH3-containing proteins such as *DlgA*, *Drosophila Abl (Dabl)* and *Src (Dsrc)* in signal transduction (Boedigheimer *et al.*, 1993; Table 4). It also contains seven blocks of considerable identity to members of *Band4.1* and *NF2* protein super-families associated with the cell membrane and cytoskeleton (Algrain *et al.*, 1993b) and thus may also be involved in shaping the cytoskeletal architecture.

Mutations in the tumor suppressor gene *lethal(3)malignant brain tumor [l(3)mbt*; Gateff *et al.*, 1993; Wismar, 1994; Table 4] and *lethal(2)giant disc [l(2)gd*; Bryant and Schubiger, 1971; Bryant and Levinson, 1985] induce, in addition to imaginal disc overgrowth, malignant brain tumors (Table 2). Török *et al.* (1993b) isolated five new imaginal disc overgrowth mutations (Table 4), one of which, the *l(2)161/21* mutation, proved to be an allele of the segment polarity gene *costal-2* (Whittle, 1976; Grau and Simpson, 1987), which has been cloned and is functionally analyzed by M. Scott (personal communication).

Hematopoietic tumor suppressor and overgrowth suppressor genes

The two larval blood cell types, the plasmatocytes and the crystal cells (Fig. 8A), originate in the hematopoietic organs from proplasmatocytes and procrystal cells (Rizki, 1978; Shresta and Gateff, 1982a). Plasmatocytes and their morphological variants, the podo- and lamellocytes (Fig. 8B), defend the animal against smaller intruders by phagocytosis and remove larger invaders by encapsulation and melanization. Thus, plasmatocytes resemble in

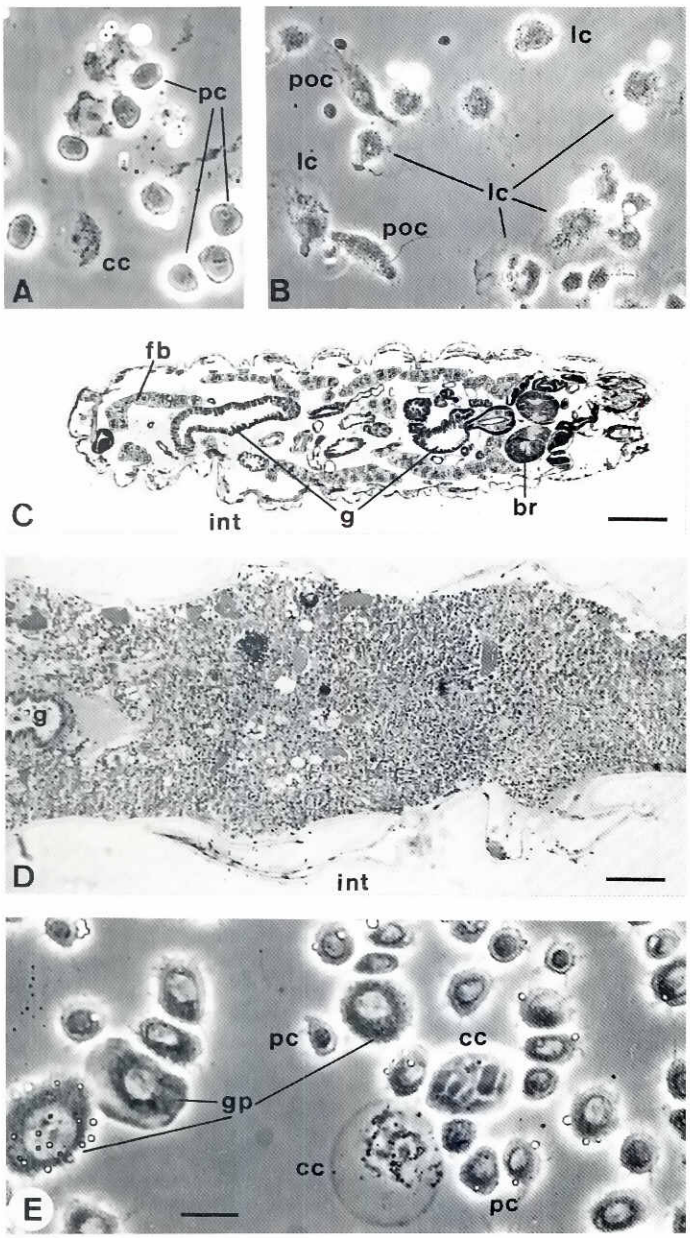


Fig. 8. Phase contrast representations of wild-type and mutant blood cells and sagittal histological sections through wild-type and *l(3)mbn-1* mutant larvae. (A,B) *Phase contrast of wild-type larval blood cells showing plasmatocytes (pc), crystal cells (cc), podocytes (poc) and lamellocytes (lc).* **(C)** *Sagittal histological sections through third instar wild-type larva and* **(D)** *third instar l(3)mbn-1 moribund larva. Note the almost complete absence of larval tissues and the numerous plasmatocytes within the body cavity in the mutant as compared to the wild-type in* **C**. **(E)** *Phase contrast of l(3)mbn-1 blood cells consisting of malignant plasmatocytes (pc), giant plasmatocytes (gp) and non-tumorous crystal cells (cc). br, brain; g, gut; fb, fat body; int, integument.* **C,D**, *hematoxylin and eosin. Bars, A,B,E, 10 μm; C, 0.3 mm; D, 0.2 mm.*

their capacity to phagocytose and encapsulate vertebrate macrophages. The second blood cell type, the non-phagocytic crystal cells, seem to play a role in hemolymph coagulation and melanization as a reaction to external and internal wounding.

Gene mutations causing larval hematopoietic disorders fall into three main categories; (i) mutations preventing blood cell proliferation (Gateff, 1994), (ii) mutations causing plasmatocyte overgrowth and their extensive differentiation into lamellocytes associated with the formation of melanotic pseudotumors (Sparrow, 1978; Watson *et al.*, 1991; Török *et al.*, 1993b), and (iii) mutations inducing malignant neoplastic transformation of larval plasmatocytes (Gateff and Mechler, 1989; Bryant *et al.*, 1993). The following text will deal with eight blood cell tumor suppressor mutations (Table 5).

The early work of Stark (1918, 1919, 1935, 1937) on the blood tumor mutant *lethal(1)7* [*l(1)*7; Bridges, 1916] showed, for the first time, the existence of recessive-lethal gene mutations responsible for the malignant growth of blood cells. Many years later Gateff (1974; 1978a) described a new, X-linked blood tumor suppressor gene mutation *lethal(1)malignant blood neoplasm [l(1)mbn]* (Table 5), which will serve as a prototype example of a malignant blood cell tumor.

The mature, *l(1)mbn* hemizygous male, mutant larvae exhibit extremely enlarged hematopoietic organs, which often take up half of the body cavity. In contrast to the wild-type hematopoietic organs which harbor only blood cell precursors, such as the proplasmatocytes and procrystal cells (Shresta and Gateff, 1982a), the mutant hematopoietic organs contain, in addition to precursor blood cells, large numbers of plasmatocytes, podocytes and lamellocytes (Shresta and Gateff, 1982b), which normally are found exclusively in the hemolymph. Thus, the site of blood cell maturation in the mutant is shifted from the hemolymph to the hematopoietic organs. The transformed plasmatocytes engage in uncontrolled proliferation in the hematopoietic organs as well as after their release into the hemolymph, where a 3-7 fold increased blood cell count can be registered. The numerous free plasmatocytes, unable to recognize "self" from "non-self", invade all larval tissues causing their destruction by phagocytosis, and consequently the death of the larvae around the time of puparium formation. The invasive and lethal plasmatocyte growth is also observed after transplantation of small tissue pieces of mutant hematopoietic organs into wild-type larvae (Gateff, 1994) and adult female hosts (Shresta and Gateff, 1982b), where they grow in an autonomous malignant fashion. The commitment of plasmatocytes for tumorous growth takes place as early as the beginning of larval life (G. Schuler and E. Gateff, unpublished result).

A further characteristic of the hemizygous, tumor-bearing larvae is the constitutive expression of the antibacterial peptide diptericin (Gateff, 1994) which, in the wild-type and all other blood tumor mutants, is strictly inducible upon bacterial inoculation. This intriguing result indicates that the diptericin gene is uncoupled from the coordinated control of the remaining antibacterial peptide genes expressed in the fat body after bacterial stimulation (Bowman *et al.*, 1972; Robertson and Postlethwait, 1986; Wicker *et al.*, 1990). The question of whether the constitutive diptericin gene expression is caused by the *l(1)mbn* mutation remains to be investigated.

In recent mutagenesis experiments aiming to characterize the complementation groups in the polytene chromosome region *8D*, where *l(1)mbn*$^{+}$ is located, Santamaria and Randholt (1994) isolated four mutant alleles of the *multi sex combs (mxc)* gene, which is a member of the *Polycomb* group *(Pc-G)* gene family. A complementation analysis of the *mxc* alleles with *l(1)mbn* showed it to be a *lethal mxc* allele (Santamaria and Randholt, 1994). Consequently, the original designation of the *lethal(1)malignant blood neoplasm* gene was changed to *multi sex combs*$^{malignant\ blood\ neoplasm}$ (*mxc*^{mbn}), and the three of the seven *mxc* alleles developing blood tumors were renamed to *mxc*^{mbn1}, *mxc*^{mbnS0} and *mxc*^{mbnG43}

(Table 5). The finding that *l(1)mbn* is a lethal *mxc* allele and, thus, a new member of the *Pc-G* gene family is of uppermost importance in elucidating its role in the suppression of malignant plasmatocyte growth.

Genetic, cytogenetic and molecular work on different members of the *Pc-G* genes showed that they not only control, in a synergistic way, the homeotic genes clustered in the *Antennapedia*- and *Bithorax*-complexes, but also a great number of other genes (Paro, 1990, 1993). This is further substantiated by the fact that *Pc-G* proteins can be detected in 80 to 100 specific target sites along polytene chromosomes (Franke *et al.*, 1992). The mechanism by which these proteins exert their specificity in controlling developmental genes is not yet clear. However, evidence is accumulating that they play a crucial role in maintaining the spatial pattern of determined cells over many cell generations along the anterior-posterior body axis (Jürgens, 1985; McKeon and Brock, 1991; Simon *et al.*, 1992). Viable *mxc* mutations, for instance, cause homeotic transformations that mimic the ectopic gain of function of *BX-C* and *ANT-C* gene mutations. The *Pc-G* phenotype indicates that the wild-type alleles of these genes must be involved in the silencing of the homeotic and other developmental genes in specific cell-types. Furthermore, their constitutive expression allows the heritable maintenance of the suppressed state, over many cell generations.

Molecular data have shed some light on the possible mechanism by which gene silencing may be achieved. Three *Pc-G* proteins have been shown to contain DNA-binding domains (Singh *et al.*, 1991; DeCamillis *et al.*, 1992), and to associate with 10-15 different proteins forming large multimeric complexes (Franke *et al.*, 1992). The members of the *Pc-G* genes are functionally related and part of an integrated control system which specifically induces stable changes in the higher order chromatin and which is transmitted from one cell generation to the next (Paro, 1993). *Pc-G* mutations derepress target selector genes by altering the chromatin structure (McKeon and Brock, 1991; Simon *et al.*, 1992).

How can we envision the causal involvement of some *mxc* alleles, such as mxc^{mbn1}, mxc^{mbnS0} and mxc^{G43} (Table 5) in the malignant transformation of plasmatocytes? The phenotypes of the different *mxc* alleles ranging from viable homeotic transformation and sterility, malignant plasmatocyte growth and first larval instar lethality, indicate differential gene functions in the various cell types. If the putative *mxc*-protein has DNA-binding capacity as well as the ability to participate in the formation of multimeric protein complexes involved in the silencing of cell-type-specific sets of

TABLE 5

HEMATOPOIETIC TUMOR SUPPRESSOR GENES

Gene designation	Locus	Affected cell-type	Expression	Protein	Homology	Putative function
(1) *lethal(1)7* [*l(1)7*], also *deep orange lethal-1* (*dor1*)	left of eosin	Plasmatocytes	–	–	–	–
(2) *multi sex combs*^{mbn}; alleles: mxc^{mbn1}, mxc^{mbnS0}, mxc^{G43}; formerly *lethal(1)malignant blood neoplasm*	8D3-8,9	Plasmatocytes	–	–	–	Member of Pc-G-gene family. May regulate developmental genes via chromatin condensation
(3) *ribosomal protein S6* (*rpS6*), formerly *air8, hen1/hen2*	7C4-9	Plasmatocytes	1.1 kb constitutive	248 aa*, 70 kDa	Human and rat ribosomal protein S6; 94,8% homology; 75,4% identity	Regulation of plasmatocyte cell division rate
(4) *lethal(2)overgrown hematopoietic organs 31* [*l(2)oho31*], formerly *l(2)144/1*	31A	Plasmatocytes, all imaginal discs, especially genital imaginal disc	0.9 kb transcript; maternal,embryo, imaginal discs	522 aa, 60 kDa	Yeast SRPI**, 41% identity; human APC***	?
(5) *lethal(2)malignant blood neoplasm* [*l(2)mbn*]		Plasmatocytes	–	–	–	–
(6) *lethal(3)malignant blood neoplasm-1* [*l(3)mbn-1*]	65A1	Plasmatocytes	2.6 kb transcript embryo, larva; 1.5 kb, 1.8 kb transcripts pupa, adult	796 aa, 83 kDa	G/S-rich tail repeats of acidic cytokeratin KI and K10 of man 40% homology	?
(7) *lethal(3)malignant blood neoplasm-2* [*l(3)mbn-2*]	n.d.	Plasmatocytes	–	–	–	–
(8) *hopscotch*$^{Tumorous\text{-}lethal\text{-}1}$ ($hop^{Tum\text{-}l}$), formerly Tum^{l}	10B6-8	Plasmatocytes	5.4 kb, 5.1 kb, 1.8kb, 0.9kb transcripts, constitutive	1177 aa, non-receptor tyrosine kinase, cytoplasmic	Janus family of non-receptor tyrosine kinases (JaK)	Signal transduction

(1) Bridges, 1916. (2) Shresta and Gateff, 1982b; Santamaria and RAndholt, 1994. (3) Watson *et al.*, 1992; Stewart and Denell, 1993. (4) Yano *et al.*, 1992; Török *et al.*, 1993b; I. Török and B.M. Mechler, personal communication; Peifer *et al.*, 1994. (5) Gateff *et al.*, 1980; Gateff, 1994; available as permanent *in vitro* cell-line designated *mbn-2*. (6) Konrad *et al.*, 1994. (7) E. Gateff, unpublished. (8) Hanratty and Ryerse, 1981; Hanratty and Dearolf, 1993; Binary and Perrimon, 1994; the only dominant oncogene. *aa, amino acids; **SRPI, Suppressor of PolI - A190 subunit; ***APC, tumor suppressor adenomatous poliposis coli.

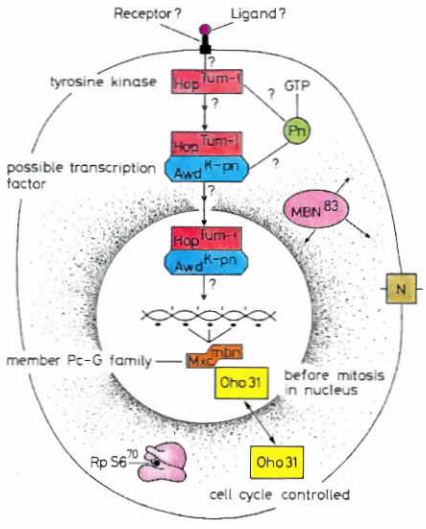


Fig. 9. Schematic representation of a plasmatocyte, showing the four putative blood tumor suppressor gene products *RpS6, MBN^{83}, Oho31* and *Mxc^{mbn}* (Table 5) and the gene product of the only dominant oncogene *hop$^{Tum\text{-}l}$* in their established or predicted positions within the cell. *Shown are also possible interactions between Hop$^{Tum\text{-}l}$ tyrosine kinase and the gene product of the Awd$^{K\text{-}pn}$ and Pn proteins, both of which are suppressors of the Hop$^{Tum\text{-}l}$ blood cell tumor. A highly speculative signaling pathway, starting with a hypothetical membrane receptor and involving Hop$^{Tum\text{-}l}$ and AwdK-pn is shown. The MBN^{83} cytoplasmic distribution is indicated by stippling (arrows). For details see text. The information in this figure stems from published and unpublished data cited in Table 5 and the text. The unpublished, cell cycle-dependent positioning of Oho31 to the premitotic nucleus and the postmitotic cytoplasm was kindly provided by I. Török and B.M. Mechler. Table 5 shows the gene designations.*

selector genes in plasmatocytes, epidermal cells and germ cells, then we may assume that in each cell type the Mxc-protein combines with different proteins which induce specific chromatin changes and by this suppresses cell-specific sets of genes (Fig. 9).

The hypothetical, cell-type-specific combinatorial interactions of the *mxc* protein with different sets of *Pc-G* proteins may be responsible for the various developmental defects observed in the different cells and tissues of the *mxc* allelic strains. The constitutively expressed antibacterial peptide gene diptericin in the fat body of *mxc^{mbn}* mutant animals (see above) strongly suggests that *mxc* may be involved in its suppression. This new aspect of a *Drosophila* tumor suppressor gene being involved in the shaping of heritable chromatin changes and by this regulating the expression of cell-specific genes responsible for the maintenance and stability of the differentiated state is challenging, and all the more so, since the mouse *Bim-1* protein, found to cause leukemias, shows high sequence identity to the *Posterior Sex Combs* protein, a *Pc-G* member (Brunk *et al.*, 1991; van Lohuizen *et al.*, 1991; van der Lugt *et al.*, 1994). The *mxc^{mbn}* gene is presently being cloned in the laboratory of P. Santamaria.

Table 5 lists seven further blood tumor suppressor genes, all of which cause in the mutated state malignant transformation of plasmatocytes. Four of these genes, *rpS6* (Watson *et al.*, 1992; Stewart and Denell, 1993), *Hop$^{Tum\text{-}l}$* (Hanratty and Dearolf, 1993; Binary and Perrimon, 1994), *l(3)mbn-1* (Konrad *et al.*, 1994), and *l(2)oho31* (I. Török and B. Mechler, personal communication) are cloned.

The finding that mutations in the *rpS6* gene, coding for ribosomal protein *S6*, cause malignant transformation of plasmatocytes is unexpected and breaks the conventional thinking about housekeeping genes, usually considered not to be involved in cell division control and differentiation. Ribosomal protein S6 is the major phosphoprotein in the 40S ribosomal subunit. In response to mitogenic stimulation, a number of serine residues at the carboxy terminus become phosphorylated (Traugh and Pendergast, 1986; Sturgill and Wu, 1991), which induces massive protein synthesis.

The *Drosophila RpS6* protein exhibits a 75.4% amino acid identity to human and rat S6 ribosomal proteins (Table 5). As the gene is expressed ubiquitously future studies will have to show the mechanism by which the lack of S6 protein function causes the malignant growth specifically of plasmatocytes. The specificity of the malignant transformation process precludes the possibility that in plasmatocytes the non-functioning of *the RpS6* protein prevents the synthesis of specific proteins controlling plasmatocyte replication in the hematopoietic organs and the hemolymph. A hint how this is possibly achieved may come from recent studies on S6-phosphorylation in humans (Ming *et al.*, 1994). In this system S6 activation takes place via a novel S6-specific kinase represented by a cytoplasmic (p70^{S6k}) and a nuclear (p85^{S6k}) splice product, and which functions in a *p21^{ras}*-independent signaling pathway. Assuming that *Drosophila* also has S6-specific kinases, it can be hypothesized that in blood cell precursors the mutant *RpS6* protein cannot be phosphorylated, which renders it non-functional.

A somewhat different blood tumor phenotype is found in the homozygous *l(3)mbn-1* mutant larvae. Numerous, immature, dividing plasmatocytes fill the enlarged hematopoietic organs (Shresta and Gateff, 1986). After their release into the hemolymph, they continue to divide, causing an immense blood cell count of 1×10^6 blood cells per larva as compared to 5×10^4 blood cells per wild-type larva (compare Fig. 8C,D). Mutant larvae show their entire body cavity filled with numerous blood cells, which actively invade and destroy larval tissues and organs. The vigorously dividing malignant blood cell type is the plasmatocyte (Fig. 8E). Only 2-3% of the plasmatocytes differentiate into giant podo- and giant lamellocyte-like cells (Fig. 8E). The neoplastic growth is, however, non-autonomous since pieces of hematopoietic organs implanted into wild-type adult hosts do not grow (Shresta and Gateff, 1986).

The *l(3)mbn-1^{+}* gene was recently cloned and sequenced (Konrad *et al.*, 1994). Except for the C-terminal domain, the deduced amino acid sequence of the N-terminal and central domain revealed no significant homology to any known protein. The C-terminal domain, characterized by seven *Gly/Ser*-rich repeats, shows a 40% identity to the tail domains of human cytokeratins K1 and K10. The putative *MBN^{83}* protein is, however, not a cytokeratin, as shown by *in situ* hybridization of anti *MBN^{83}* antibodies. It can be detected concentrated around the nucleus and radiating toward the cell periphery (S. Dreschers, A. Hotz-Wagenblatt, E. Gateff, unpublished results). Figure 9 shows schematically its cytoplasmic distribution.

A similar blood tumor phenotype is also caused by mutations in the blood tumor suppressor gene on chromosome 2, designated as *l(2)mbn* (Table 5). The mutant strain was lost. There is, however, a cell line, designated *mbn-2*, derived from the tumorous *l(2)mbn* plasmatocytes (Gateff *et al.*, 1980) which has proven useful in studying the expression and regulation of a variety of antibacterial peptides (Samakovlis *et al.*, 1992; Kappler *et al.*, 1993).

The *l(2)oho31* blood and imaginal disc tumor suppressor gene has only recently been cloned. By the kind permission of I. Török

and B.M. Mechler, preliminary unpublished data are included in Tables 4 and 5, and in Fig. 9. The considerable amino acid identity over the entire protein to a group of proteins with diverse functions, all of which share 7 to 12 *Armadillo* repeats (Yano *et al.*, 1992; Peifer *et al.*, 1994) may help to unravel the function of this protein in normal and tumor development.

The only dominant *Drosophila* oncogene is the X-linked, temperature-sensitive $Hop^{Tum\text{-}l}$ gene (Table 5), originally designated by Corwin and Hanratty (1976) as *Tumorous-lethal (Tum-l)*. The renaming of the above allele was necessary after it became obvious that *Tum-l* is a dominant mutation of the *hopscotch (hop)* locus (Hanratty and Dearolf, 1993). The recessive *hop* mutations cause smaller imaginal discs, larval/pupal lethality, and in homozygous embryos lacking maternal *hop* activity, segmentation defects (Perrimon and Mahowald, 1986). The hemi- and homozygous $hop^{Tum\text{-}1}$ animals show extremely enlarged hematopoietic organs and an increased blood cell count at 29°C. As in the case of the mxc^{mbn} blood tumor mutant (see above), plasmatocytes divide excessively and differentiate prematurely in large numbers into podo- and lamellocytes already in the hematopoietic organs (Silvers and Hanratty, 1984). Released into the hemolymph, the numerous lamellocytes form large melanotic masses (Hanratty and Ryerse, 1981).

hop has recently been cloned and shown to encode a non-receptor tyrosine kinase of the *JaK*-type (Binari and Perrimon, 1994). Mammalian members of this family are instrumental in signal transduction via the interferon α-, erythropoietin- and growth hormone-receptors (Velazquez *et al.*, 1992; Witthuhn *et al.*, 1993; Algetsinger *et al.*, 1993). They may define new pathways which link membrane receptors directly to the activation of nuclear genes via latent cytoplasmic transcription factors known as STATs (**S**ignal **T**ransducers and **A**ctivators of **T**ranscription). Thus, hypothetically the $Hop^{Tum\text{-}l}$ tyrosine kinase may be a link between a not yet identified membrane receptor lacking kinase activity, and $Awd^{K\text{-}pn}$ a homolog of the metastasis suppressor gene *nm23* (Zinyk *et al.*, 1993; Fig. 9), which may be a transcription factor (Ch. Dearolf, personal communication). $Awd^{K\text{-}pn}$ has been shown to be a suppressor of $hop^{Tum\text{-}1}$ plasmatocyte growth and lethality (Zinyk *et al.*, 1993). The isolation of five further dominant second site suppressor genes of $hop^{Tum\text{-}1}$ (Ch. Dearolf, personal communication) points to additional interactions along this hypothetical signaling pathway. However, it has to be shown that $hop^{Tum\text{-}1}$ is indeed capable of phosphorylating one or more components along this hypothetical signaling pathway (Fig. 9).

Table 6 shows eleven further hematopoietic mutations which have been classified as non-tumorous, but which show a slight blood cell hyperplasia. All have recently been isolated and, for that reason, none has been studied extensively (Török *et al.*, 1993b).

TABLE 6

HEMATOPOIETIC OVERGROWTH SUPPRESSOR GENES

	Gene designation	Cytogenetic locus	Affected cell-type	Melanotic masses
(1)	*lethal(2)overgrown hematopoietic organs 48E (oho48E)*; previously *l(2)131/17*	48E	Plasmatocytes	yes
(2)	*lethal(2)overgrown hematopoietic organs 23B (oho23B)*; previously *l(2)168/14*	23B	Plasmatocytes	yes
(3)	*lethal(2)overgrown hematopoietic organs 48A (oho48A)*; previously *l(2)65/24*	48A	Plasmatocytes	yes
(4)	*lethal(2)overgrown hematopoietic organs 51 (oho51)*; previously *l(2)211/15*	51	Plasmatocytes	no
(5)	*lethal(2)overgrown hematopoietic organs 55D,E (oho55D,E)*; previously *l(2)30/7*	55D,E	Plasmatocytes	no
(6)	*lethal(1)air1 (air1)*	1C3,4-D3-E; 1B15-2B17,18	Plasmatocytes	yes
(7)	*lethal(1)air2 (air2)*	1E3,4	Plasmatocytes	yes
(8)	*lethal(1)air6 (air6)*	5A1,2-E8	Plasmatocytes	yes
(9)	*lethal(1)air7 (air7)*	7A6,8	Plasmatocytes	yes
(10)	*lethal(1)air9 (air9)*	7C9-D1; 7D5-10	Plasmatocytes	yes
(11)	*lethal(1)air11 (air11)*	8A5-9A2	Plasmatocytes	yes
(12)	*lethal(1)air15 (air15)*	11A7-13F10	Plasmatocytes	yes

(1-5) Török *et al.*, 1993b. The designations of the genes have been changed. Therefore the previous designations, as used in the publication Török *et al.*, 1993b, are also indicated. (6-12) Watson *et al.*, 1992.

Germ line tumor suppressor genes

The primordial germ cells, the so-called pole cells, are the first to be individualized at the posterior pole of the preblastoderm embryo. During gastrulation they migrate through the posterior mid-gut rudiment into the prospective mesodermal gonads. The male and female embryonic gonads are indistinguishable from each other, but shortly after the larva hatches from the egg the male gonad becomes larger. This sexual dimorphism persists throughout larval life and is caused by the higher division rate of the male gonial cells. The first steps of male and female germ cell differentiation are very similar. Each gonial stem cell division gives rise to a new gonial stem cell and a cyst progenitor cell. The female cyst progenitor cell is designated as cystoblast (King, 1970), and the male as primary gonial cell (Lindsley and Takuyasu, 1980). Each cyst progenitor cell gives rise to a syncytium of sixteen interconnected cystocytes or primary spermatocytes respectively. In the male two further synchronous meiotic cell divisions give rise to a syncytium of 64 spermatids, which in a complex order of events differentiate into mature sperm. In the female, the cluster of sixteen interconnected cystocytes becomes enveloped by follicle cells to form the egg chamber. Within the egg chamber one of the sixteen cystocytes differentiates into the oocyte, while the remaining fifteen cystocytes take on the nurse cell fate. The metabolically active nurse cells transfer into the oocyte, in a temporal and spatial order of events, a multitude of molecules involved in the shaping of the oocyte and the establishment of the future body plan (Lasco, 1992).

Reciprocal pole cell transplantations demonstrate the autonomy of the male germ cells in a female environment and the non-autonomy of the female germ cells in a male surrounding. Thus, in a male gonad female germ cells attain the appearance of primary spermatocytes (Nöthiger *et al.*, 1989; Steinemann-Zwicky *et al.*, 1989; Steinemann-Zwicky, 1992). These observations indicate that sexual differentiation of the female germ line requires signals from the female soma which the male soma cannot provide. The absence of this signal(s) causes a partial female-to-male germ line transformation (see later).

TABLE 7

FEMALE AND MALE GERM LINE TUMOR SUPPRESSOR GENES

Gene designation	Locus	Affected cell-type	Expression	Protein	Homology	Putative function
(1) *bag-of-marbles (bam)*	96C	Cystoblasts, cystocytes, primary spermatocytes	2.0 kb transcript in cystoblasts, germarium region 1; egg chamber stage 10, primary spermatocytes; 0-4 h embryo (maternal)	442 aa**, 55 kDa; 2 forms: form 1 in cytoplasm of cystoblasts and 2-, 4-, 8- cell cysts; form 2 fusome-associated	20% identity to *otu*	Incomplete cytokinesis of cystocytes and primary spermatocytes
(2) *benign(2)gonial cell neoplasm [b(2)gcn]*	60A3	Cystocytes, primary spermatocytes	6 overlapping transcripts (0.5 - 4.2 kb); germarium region 2; egg chamber stages S2-S5, S8-S13; primary spermatocytes not limited to female and male germ lines	C-terminal region; 245 aa open reading frame	contains highly charged domain as found in: U1 snRNP 70 kb, U2 snRNP, splice factors SC35, ASF, U2AF; transformer, *suppressor of white apricot*	RNA binding? RNA splicing?
(3) *female sterile(2) of Bridges [fs(2)B]*	2-5 cM*	Cystocytes	–	–	–	–
(4) *female lethal(2)d [fl(2)d]*	50A2-FA1	Cystocytes	constitutive	–	–	Female-specific splicing of *Sxl* (genetic data)
(5) *fused (fu)*	17C3-D2	Cystocytes, segment polarity	3.2 kb transcript; higher expression in females than in males; maternally in posterior segment region	Serine/threonine kinase; in cytoplasm	–	Signal transduction; not required in germ line cells
(6) *narrow (nw)*	54A-55A	Cystocytes	–	–	–	–
(7) *ovarian tumor (otu)* formerly *[fs(1)231]*	7F1	Cystocytes	3.2 kb, 4.0 kb ovary-specific transcripts; 1.1 kb, 3.5 kb testes-specific transcripts	Two isoforms 98 kDa, 104 kDa; in cytoplasm	20% identity to *bam*	Sex determination, female germ cell differentiation
(8) *ovo*	4E1,2	Cystocytes	Stage 8-14 nurse cells, early embryogenesis	1209 aa**, four Zn-fingers; in nucleus	*Krüppel* Zn-fingers	Sex determination, transcription factor?
(9) *sans fille (snf)* formerly *fs(1)1621* or *liz*	4F1A-5A	Cystocytes	1.0 kb transcript; constitutive, in soma and germ line	28 kDa, in nucleus	Mammalian U1A-, U2B-snRNP, 70% - 72% identity	Female specific splicing of *Sxl* in soma and germ cells
(10) *Sex-lethal (Sxl)*	6F5-7A1	Cystocytes	10 transcripts (1.7 - 4.4 kb), 3 female-, 3 male-, 2 germ line-specific	Multiple forms of proteins in different tissues and developmental stages: in nucleus	–	Autoregulatory control of female- specific splicing of its own pre-mRNAs and that of transformer
(11) *male sterile(3)only diploid stages [ms(3)ods]*	–	Primary spermatocytes	–	–	–	–

(1) McKearin and Spradling, 1990. (2) Gateff, 1982b; Kaiser, 1994; Protin, 1994. (3) King, 1970. (4) Granadino *et al.*, 1990, 1992. (5) King, 1970; Busson *et al.*, 1988; Préat, 1992; Bopp *et al.*, 1993. (6) King, 1970. (7) King *et al.*, 1986; Storto and King, 1987, 1988; Champe and Laird, 1989; King and Storto, 1988; Mulligan *et al.*, 1988; Steinhauer and Kalfayan, 1992; Tirronen *et al.*, 1992; Bopp *et al.*, 1993; Pauli *et al.*, 1993. (8) Oliver *et al.*, 1987, 1990; Mével-Ninio *et al.*, 1991. (9) Gollin and King, 1981; Oliver *et al.*, 1988, 1993; Steinemann-Zwicky, 1988; Salz, 1992; Albrecht and Salz, 1993; Bopp *et al.*, 1993; Flickinger and Salz, 1994. (10) Schüpbach, 1985; Steinemann-Zwicky *et al.*, 1989; Bopp *et al.*, 1993; Oliver *et al.*, 1993. (11) U. Schäfer, unpublished. *cM, centi Morgans; **aa, amino acids.

The determination and differentiation of the male and female germ lines are under the control of numerous genes (King and Mohler, 1975; Romrell, 1975; Schüpbach and Wieschaus, 1991; Gönczy *et al.*, 1992; Castrillon *et al.* 1993). A small number (i.e. twelve) of these genes cause germ line tumors (Table 7).

Germ line tumor mutations fall into two main classes. Class one mutations induce tumorous gonial cell growth only in the male or the female. The majority (i.e. nine) of the mutations belonging to this class transform the female germ line. Only one case of a gonial cell tumor in the male has recently been found (*ms(3)ods*; U. Schäfer, personal communication; Table 7). Class-two mutations cause germ line tumors in both sexes (*bam, b(2)gcn*; Table 7; Fig. 10).

In all germ cell tumor mutants differentiation is arrested at a premeiotic stage, i.e. the cystoblast stages in the female (Fig. 10D) and the primary spermatocyte stage in the male (Fig. 10F). The

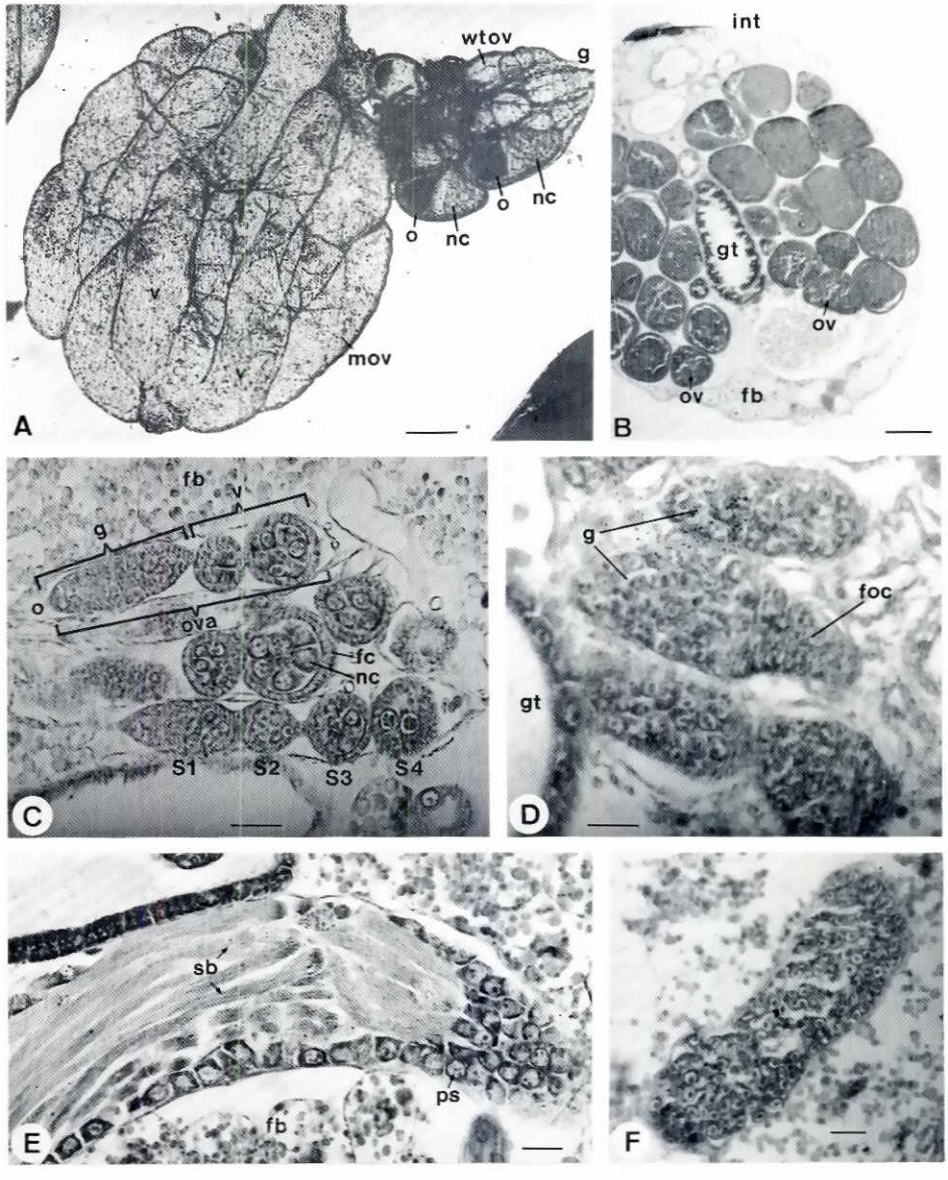


Fig. 10. Whole-mount and histological preparations of wild-type and *b(2)gcn* mutant ovaries and testes. (A) *Whole-mount of 7-day-old b(2)gcn mutant ovary (mov) compared to a wild-type ovary (wtov) from a young female. Note in the wild-type ovary the apical germaria (g) and young follicles (f) harboring the oocyte (o) and nurse cells (nc). In the b(2)gcn vitellaria (v) no follicles can be detected.* **(B)** *Histological cross section through an adult abdomen showing the 15 vitellaria (v) of each of the two 7-day-old mutant ovaries lacking follicles. The left ovary is only partially represented.* **(C,D)** *Sagittal histological sections through young wild-type* (**C**) *and b(2)gcn mutant ovary* (**D**). *The wild-type ovarioles (ova) consist of the germarium (g) and the vitellarium (v), the latter harboring consecutive stages of follicles (S1, S2, S3, S4). Each follicle contains 15 nurse cells (nc) and an oocyte (oc) surrounded by follicle cells (fc). The young mutant ovary* (**D**) *consists of germaria in which no follicles form and which are filled with cystocyte-like cells (cc).* **(E,F)** *Longitudinal histological section through wild-type and b(2)gcn testes. The mutant testis* (**F**) *is filled with primary spermatocyte-like cells, and no postmeiotic spermatid bundles can be detected as in the wild-type testis* (**E**), *where primary spermatocytes (ps) are seen in the apex and laterally, and spermatid bundles (sb) in the center region. fb, fat body. Hematoxylin and eosin. Bars, A, 150 μm; B, 200 μm; C, 10 μm; D, 15 μm; E, F, 20 μm.*

differentiation-incompetent tumorous germ cells divide autonomously and populate the gonads in large numbers (Fig. 10A,B), which leads to sterility. The gonial cell tumors are not lethal to their hosts and thus can be classified as benign.

The first ovarian tumor mutation, *fused (fu)*, was discovered by T. Morgan and C.B. Bridges in 1916 (Lynch, 1919). C.B. Bridges subsequently isolated two further ovarian tumor mutations, named *female sterile(2)of Bridges* [*fs(2)B*] and *narrow* (*nw*; Table 7). King and his colleagues investigated these mutants and recognized the benign nature of the autonomously growing germ cells (King and Bodenstein, 1965; for review , see King, 1969, 1970). Subsequently more such mutants were isolated (Table 7).

In searching for a possible function of these genes, it turned out that some alleles of *Sex lethal (Sxl)*, known to play a central role in female somatic and germ line sex determination (Schüpbach, 1985; Steinemann-Zwicky *et al.*, 1989; Steinemann-Zwicky, 1994), develop similar ovarian tumors as *fu*, *fs(2)B*, *otu*, *snf*, *fl(2)d*, *nw* and *ovo* (Table 7). Thus, the question arose as to whether these genes could also be involved in sex determination of the female germ line. Recent studies have indeed confirmed this assumption for the *fu*$^+$, *otu*$^+$, *ovo*$^+$ *fl(2)d*$^+$ and *snf*$^+$ genes (Bopp *et al.*, 1993; Oliver *et al.*, 1993; Pauli *et al.*, 1993). A large proportion of the genotypically XX:AA female tumorous cells in the above mutant ovaries resemble phenotypically primary spermatocytes, and thus seem to be sexually transformed. The partial sexual transformation of the tumor cells could be shown by the expression of male germ line-specific genes (Wei *et al.*, 1994). Furthermore, Bopp *et al.* (1993) demonstrated in tumorous *otu* ovaries, male-specific *Sxl* mRNA splicing. Finally, introducing into *otu* strains two enhancer trap lines, expressing the ß-galactosidase reporter gene only in the male germ line, Pauli *et al.* (1993) demonstrated the male nature of some of the *otu* ovarian tumor cells. In a different approach Granadino *et al.* (1990) analyzed the *Sxl* transcript pattern in *fl(2)d* female and male larvae and found in female larvae the male *Sxl* transcript pattern.

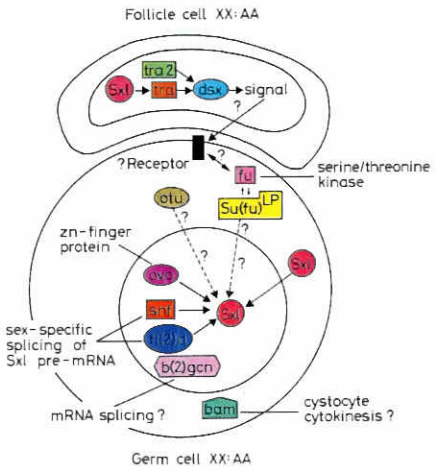


Fig. 11. Schematic representation of a female (XX:AA) follicle cell contacting a female germ cell. *The well-established regulatory gene hierarchy, leading to somatic female sex determination, is seen in the follicle cell. The signal, of unknown nature, sent by the follicle cell, may be received in the germ cell, via a putative receptor. A hypothetical signaling pathway is depicted involving fu serine/threonine kinase and the Su(fu)^{LP} gene product which possibly turns on indirectly the following five germ line sex determining genes: otu, ovo, snf, fl(2)d and Sxl. Mutations in any one of the five genes and the fu gene induce ovarian tumors. Not involved in sex determination are the genes bam and b(2)gcn, which in the mutated state cause gonial cell tumors in both sexes. For details see text. The data in this scheme are compiled from publications cited in Table 7 and in the text. The gene designations are seen in Table 7.*

Genetic studies revealed further that the constitutive expression of *Sxl*$^+$ suppresses the *ovo, otu, fl(2)d* and *snf* tumor phenotype, implying that these genes are instrumental in germ cell sex determination prior to *Sxl*$^+$ function (Steinemann-Zwicky, 1988, 1994; Oliver *et al.*, 1990, 1993; Granadino *et al.*, 1992; Salz, 1992; Pauli *et al.*, 1993; Fig. 11). Thus, germ line sex determination contrasts with the hierarchical female somatic sex determination pathway, where *Sxl* initiates a cascade of autoregulatory female-specific splicing of its own pre-mRNA and that of *transformer* (*tra*). *tra* and *tra2* induce female-specific expression of *doublesex (dsx)* and further the activation of female-specific germ line differentiation genes via a not yet identified signal-receptor system (Baker, 1989; Steinemann-Zwicky *et al.*, 1990; Belote, 1992; Cline, 1993; Fig. 11). These studies provided strong support for the idea that ovarian tumor genes function in female germ line sex determination (Oliver *et al.*, 1987, 1988; Steinemann-Zwicky, 1988).

In summary: female germ line sex determination and differentiation involves the genes *fu, otu, fl(2)d, ovo, snf* and *Sxl* (Table 7; Fig. 11). Specific mutations in these genes cause a partial sexual transformation of cystoblasts into primary spermatocytes, thus preventing egg differentiation. The differentiation-incompetent but division-proficient germ cells engage, consequently, in autonomous benign tumorous growth. In humans, the development of gonadoblastomas is also considered to be associated with aberrant sex determination and differentiation (Scully, 1970; Page, 1987).

The male identity of the ovarian tumor cells was recently questioned. Bae *et al.* (1994) showed that female-specific *orb* and *otu* transcripts were present in *Sxl* alleles developing tumorous egg chambers. Likewise *otu* transcripts accumulated in *snf* ovarian tumors. These findings contradict the studies discussed above, which show male-specific gene expressions in ovarian tumors. The final resolution of these controversial results will require further investigations into the function and possible interactions of the ovarian tumor suppressor genes.

The five ovarian tumor genes listed above are cloned and sequenced. Table 7 and Fig. 11 show the present status of our knowledge concerning their putative function(s) and developmental hierarchy.

The ovarian tumor and segment polarity gene *fu*$^+$ encodes a 3.2 kb transcript, which is expressed in females at a higher level than in males. The gene product, a putative serine/threonine kinase (Préat *et al.*, 1990), points to a possible involvement in signal transduction. It is tempting to postulate a hypothetical interaction of the *fu* kinase with the not yet identified receptor for the female-specific somatic sex determining signal (Fig. 11; Table 7).

The *otu* gene specifies two protein isoforms which are restricted to the germ cell cytoplasm (Steinhauer and Kalfayan, 1992; Table 7). The larger 104 kDa isoform seems to regulate *Sxl*, which in turn is required for the differentiation of nurse cells and oocytes (Bae *et al.*, 1994). The lack of function of this isoform causes ovarian tumor development. The smaller 98 kDa isoform is not involved in tumor formation. Its absence inhibits gonial stem cells and cystoblast proliferation resulting in agamic ovaries. The mode of action of these polypeptide isoforms is, however, unknown. Genetic and molecular investigations have revealed that *otu* acts upstream of *Sxl*, and that most probably *otu* and *ovo* function in the same pathway (Pauli *et al.*, 1993; Fig. 11). Further studies are, however, needed in order to establish unequivocally the functional correlation of *otu* to *snf, fu* and *fl(2)d*. It will certainly be necessary to identify and characterize other gene products which may interact with the ovarian tumor genes along the germ line differentiation pathway(s). For instance, double mutants between *otu* and *quit*, a female sterile mutation, show that *otu* is epistatic over *quit* (Tirronen *et al.*, 1992).

Genetic and molecular studies revealed that *Sxl* plays a crucial role in somatic sex differentiation, germ line development and X-chromosome dosage compensation (Cline, 1993; Cronmiller and Salz, 1994). In the soma, *Sxl* autoregulates its own expression by the well-known "binary switch" mechanism, namely "on" in females and "off" in males. This is achieved via the binding of *Sxl* protein to its own pre-mRNA, thus preventing the splicing of the male-specific exon which, when removed, inactivates the gene product (Horabin and Schedl, 1993). *Sxl* pre-mRNA splicing is controlled additionally by two ovarian tumor suppressor genes *snf* (Oliver *et al.*, 1988, 1993; Steinemann-Zwicky, 1988; Salz, 1992; Albrecht and Salz, 1993; Bopp *et al.*, 1993), and *ft(2)d* (Granadino *et al.*, 1990, 1991, 1992).

snf shows sequence homology to the mammalian *U1A* and *U2B* snRNP proteins and is thus an integral component of the splice site recognition machinery (Flickinger and Salz, 1994; Table 7). Wild-type *Snf*-protein is found in the nucleus of all cells and developmental stages. The apparent germ line-specificity of *Sxl* pre-mRNA 5' splice site recognition by the *Snf* U1A-snRNP-protein complex is difficult to reconcile with its ubiquitous distribution at all developmental stages. *fl(2)d*, the second ovarian tumor gene involved in the female-specific splicing of *Sxl* (Granadino *et al.*, 1990; Table 7, Fig. 11), has recently been cloned, but no further data are yet available (B. Granadino and L. Sanchez, personal communication).

A gene involved in the maintenance of the female germ line is *ovo* (Oliver *et al.*, 1987). *ovo* encodes a zinc-finger protein, pointing to a possible function as transcription factor (Mével-Ninio *et al.*, 1991; Table 7, Fig. 11).

In contrast to the comparatively numerous ovarian tumor mutations, testes tumor mutants were until recently unknown. Table 7 shows the *ms(3)ods* mutation (U. Schäfer, personal communication), which is, for the time being, the only of its kind. While the mutant females are fertile, in the mutant males cysts of sixteen primary spermatocytes form, but meiosis and consequently spermiogenesis do not take place. Instead, the primary spermatocytes engage in continuous and autonomous tumorous growth. Since this gene was recently discovered no further information is yet available.

The second class of germ line tumor suppressor genes causes tumorous gonial cell growth in both sexes. Only two such genes are known, namely *bag-of-marbles* (*bam*; McKearin and Spradling, 1990; McKearin and Christerson, 1994) and *benign(2)gonial cell neoplasm (b(2)gcn*; Gateff, 1982b; Kaiser, 1994; Protin, 1994; Table 7). In both mutants and both sexes germ line differentiation is blocked at an early premeiotic stage. In the female germ line, bam^+ is first expressed in cystoblasts and early cystocytes in germarial region 1, while $b(2)gcn^+$ transcripts are first seen in the cystocytes of germarial region 2. In the vitellarium bam^+ transcripts are encountered only in the nurse cells of stage 10 egg chambers, while $b(2)gcn^+$ is found in the nurse cells of egg chamber stages S2 to S5, S8 and S13. After gastrulation, bam^+ mRNA cannot be detected in any subsequent developmental stage. $b(2)gcn^+$, in contrast, is expressed throughout embryonic life, in the pupa and the adult. *bam* represents a relatively short gene coding for a 2.0 kb transcript. The *b(2)gcn* gene extends over 13 kb of genomic DNA and encodes six transcripts ranging from 0.5 kb to 4.2 kb in size (Kaiser, 1994; Protin, 1994; Table 7).

The 20% similarity of the putative *Bam* protein to *Otu* protein is circumstantial and cannot serve as a basis for possible functional implications, especially since *otu* is not expressed in the testes (Steinhauer *et al.*, 1989). Within the 3' *b(2)gcn* sequence two regions of homology were encountered. The first region shows homology to the so-called *paired* repeats found in genes such as *bicoid, daughterless* and *forked*, and the second region represents a 65 amino acids Arg-Ser rich stretch, which is present in proteins involved in RNA-binding and splicing (Table 7). The complex transcript pattern may indeed preclude $b(2)gcn^+$ coding for different splicing factors or RNA-binding proteins. This may be congruent with the finding that $b(2)gcn^+$ is not only expressed in the germ line but also in various embryonic, larval and adult tissues *in situ* and thus may have different functions during different developmental stages in different tissues and cells (Kaiser, 1994; Protin, 1994).

The causal relationship between the malfunctioning of genes involved in germ line sex determination, and germ line tumor induction and development, as shown above for the ovarian tumor genes, prompted the question whether bam^+ and $b(2)gcn^+$ may also be involved in germ line sex determination. The following findings characterize *bam* as a true ovarian and testes tumor gene, functioning independently of germ line sex determination. bam^+ expression is not required for female-specific Sxl^+ pre-mRNA splicing (Bopp *et al.*, 1993). Females derived from a *bam* null allelic strain express *Sxl* protein in large amounts (D.M. McKearin, personal communication). Furthermore, the allele Sxl^{M1}, which expresses constitutively *Sxl* protein, rescues partially the *snf* and *otu* ovarian tumors but not *bam* germ line tumors (D.M. McKearin, personal communication). These experiments allow the conclusion that *bam* germ cells are correctly determined sexually. Nevertheless, the issue seems not completely settled, since Bopp *et al.* (1993) found *Sxl* transcript splicing in *bam* ovaries almost normal but the subcellular localization of the splice product severely affected.

Since the *b(2)gcn* tumor phenotype closely resembles that of *bam* we assume that it also does not function primarily in sex determination. This assumption was confirmed by crossing *b(2)gcn* with the *P-LacZ* enhancer trap line 606 (Gönczy *et al.*, 1992), which expresses *LacZ* only in male germ line stem cells and mitotically dividing spermatogonial cells. The ovaries of animals from this cross expressed *LacZ* at an extremely low level, while the tumorous spermatogonial-like cells within the testes were intensely stained (Protin, 1994). This result demonstrates that the tumorous cells in the *b(2)gcn* ovaries possess the proper sex. From comparisons of the expression patterns of the two genes in the germarium, namely bam^+ in region 1 and $b(2)gcn^+$ in region 2, we tentatively conclude that $b(2)gcn^+$ functions downstream of bam^+.

In conclusion: from the data presented here, we can conclude that tumorous development of the germ line is causally related to mutations in specific genes involved in differentiation processes, such as female germ line sex determination *[snf, fl(2)d, Sxl]* and early stages of cystocyte and primary spermatocyte cytokinesis *[bam, b(2)gcn]*. Furthermore, not only must the soma and germ line know their sex, but they also have to communicate with each other. *fu* and *ovo* may be candidates acting in signal transduction.

Suppressors and enhancers of tumor suppressor genes

Understanding the function of genes in general and of tumor suppressor genes in particular precludes the identification of the gene products with which they interact during development. The isolation of gene mutations, either suppressing or enhancing a tumor phenotype, is a powerful approach for encountering and studying such interactions. Suppressors of tumor suppressor genes have recently been isolated for the ovarian tumor and segment polarity gene *fused (fu*; Préat, 1992; Table 7), the dominant blood tumor mutant $hop^{Tum\text{-}l}$ (Zinyk *et al.*, 1993; Table 5), and for *lethal(2)giant larvae* (M.Sémériva, personal communication; Table 4).

The semidominant *Suppressor of fused*^{LP} *[Su (fu)*^{LP}*]*, a complete loss of function mutation, is homozygously viable without an obvious mutant phenotype. It completely suppresses the *fu* ovarian tumors as well as the embryonic segment polarity defects. Enhancement of the suppressed phenotype can be achieved via a wild-type $Su(fu)^+$ gene duplication. Based on these results Préat (1992) suggested that the $Su(fu)^+$ gene product is involved together with the Fu^{46} kinase in the same developmental pathway (Fig. 11). $Su(fu)^{LP}$ codes for a 46 kDa protein without homology to any known protein.

The second case represents the suppression of the lethal blood tumor developing in hemizygous male $hopscotch^{Tum\text{-}l}$ ($hop^{Tum\text{-}l}$) larvae (Table 5), by dominant and recessive alleles of the *abnormal wing discs* (*awd*) and the *prune* (*pn*) mutations (Zinyk *et al.*, 1993). The *Awd* protein exhibits about 78% amino acid identity to the human *Non-metastatic 23-H1 (Nm23-H1)* and *Nm23-H2* proteins (Rosengard *et al.*, 1988; Evans, 1991; Liotta *et al.*, 1991; Stahl *et al.*, 1991). The *awd* mutation causes abnormal imaginal discs, brains and ovaries (Dearolf *et al.*, 1988a). A 0.8 kb transcript, expressed in the imaginal discs, the brain and the ovaries (Dearolf

et al., 1988b), is translated into a 17 kDa polypeptide subunit belonging to a 100 kDa nucleotide diphosphate kinase (NDP kinase; Biggs *et al.*, 1988, 1990; Lascu *et al.*, 1992). Two classes of *awd* alleles exist: (i) recessive loss-of-function mutations showing larval-pupal lethality due to the above mentioned abnormalities, and (ii) the dominant $awd^{Killer\ of\ prune}$ ($awd^{K\text{-}pn}$) allele (Sturtevant, 1956; Biggs *et al.*, 1988), which alone exhibits wild-type phenotype, but which in combination with the eye color mutation *prune (pn)* causes lethality. Sequence analysis of the mutant $Awd^{K\text{-}pn}$ protein revealed only one amino-acid substitution (A. Shearn, personal communication).

The molecular events leading to $hop^{Tum\text{-}l}$ blood tumor suppression by $Awd^{K\text{-}pn}$ and *pn* mutant alleles are not known. Since the products of both genes have been identified, and since in *awd* mutants NDP kinase activity is unchanged, Zinyk *et al.* (1993) suggested two hypothetical possibilities which may be responsible for the suppression of the $hop^{Tum\text{-}l}$ blood tumor: (i) The mutant $Awd^{K\text{-}pn}$ protein may have attained different biochemical properties, unrelated to the normal NPD kinase function, which in combination with the altered *Pn*-protein is responsible for the suppression of the *Tum-l* mutant phenotype. (ii) Based on the facts that in *pn* mutants the concentration of pteridines (Hackstein, 1992) and *GTP*-cyclohydrolase is reduced (Evans and Howells, 1978; MacKey and O'Donnell, 1983), and that the pn^{+} gene product is required for proper *GTP* metabolism, it is assumed that the altered $Awd^{K\text{-}pn}$ - *GDP/GTP* complex and the changed *pn* regulation may result in an NDP kinase using a different substrate or the same substrate but with slightly different kinetics. Thus, the above authors propose $Hop^{Tum\text{-}l}/Awd^{K\text{-}pn}$ and *Pn* to participate in a common regulatory pathway. Figure 9 suggests hypothetically a direct interaction of the two proteins which will, however, have to be proven experimentally. Five additional, autosomal dominant, second site suppressor genes of the $Hop^{Tum\text{-}l}$ phenotype have recently been isolated (C. Dearolf, personal communication). One of these proved to be a much stronger suppressor than the others or $Awd^{K\text{-}pn}$.

hopscotch represents a putative protein tyrosine kinase belonging to the *JaK family* of nonreceptor tyrosine kinases (Binari and Perrimon, 1994; Table 5). A second site enhancer has been identified in the original $Hop^{Tum\text{-}l}$ mutant stock which shows a strong tumor phenotype. Crossing out the one or two dominant autosomal genes tentatively named *Enhancer of Tum-l [E(Tum-1)]*, present in the original $hop^{Tum\text{-}l}$ stock, resulted in a moderately weaker hemizygous male blood cell tumor (Zinyk *et al.*, 1993).

Four different recessive genes were found to suppress the lethality of $l(2)gl^{ts3}/Df(2L)net62$ larvae at 29°C (M. Sémériva, personal communication). The $l(2)gl^{ts3}/Df(2L)net62$; *su(lgl)/su(lgl)* flies showed good viability and fertility. Since these suppressor mutations have been isolated only recently, further information will have to be awaited.

Additional cases of suppression of the ovarian tumors in germ line mutants like *snf, ovo*, and *otu* by the loss-of-function alleles Sxl^{M1} and Sxl^{M2} (Steinemann-Zwicky, 1988; Salz, 1992) were mentioned in the previous section. An important task for the future should be the identification of suppressors and enhancers for all above tumor suppressor genes.

In addition, double mutants between tumor suppressor genes should be extremely helpful in elucidating whether two genes are part of the same or a different pathway (Kurzik-Dumke *et al.*, 1992).

Perspectives

Genetic analysis of twenty-seven *Drosophila* tumor suppressor genes demonstrates unequivocally their causal and cell-specific involvement in early developmental events, setting the stage for a particular differentiation pathway. In contrast to mammalian cancer, where up to six independent mutations are required for the genesis of a tumor (Yokata and Sugimura, 1993), in *Drosophila* only one recessive gene mutation is sufficient. The fact that none of the dominant cellular oncogenes, such as *c-src, c-ras* etc., cause tumors in *Drosophila*, indicates that, in contrast to mammalian tumors, dominant oncogenes are not involved in the genesis of *Drosophila* malignant growth. Moreover, the genetic analysis revealed that not all mutational events within a particular tumor suppressor gene cause malignant transformation. For instance, of the seven *mxc* alleles only three develop malignant blood tumors. Three of the four remaining alleles are viable and one allele exhibits first instar larval lethality (Santamaria and Randholt, 1994). This holds true for all other tumor suppressor mutations. Further studies, aiming to establish the identity of a specific mutational event and its implication for the gene product and mutant phenotype, are needed in order to correlate specific mutations within a gene to malignant transformation, or else to non-tumorous developmental changes.

In situ expression studies of eleven cloned tumor suppressor genes showed that most are active in more than one tissue, but only one or, at the most, two cell-types become malignantly transformed by a particular mutation (see Tables). Taking these results into account, an important future task would be to compare the cell-type-specific "wiring" of the protein-protein interactions in the wild-type with that in the tumorous cells in order to identify the initial and causal molecular event leading to malignant growth in a particular cell-type.

The gene products of the eleven tumor-suppressor genes localize to various cellular compartments (Figs. 2,6,9,11). Their putative functions were deduced by homology to well-known vertebrate and yeast gene products (see Tables). Most of these gene products localize in the cytoplasm, where they seem to function in conjunction with the submembrane and/or cytoplasmic cytoskeleton.

The extensive homologies found for some of the *Drosophila* tumor suppressor genes to corresponding genes in *Xenopus*, mouse and man, are a promising prospect to elaborate their common functions. On the other hand, during their long, independent evolution the homologous proteins may have acquired different functions (Murzin, 1993). Furthermore, we have to take into account that differentiation is a species-specific process and thus homologous proteins may engage in different cells and species in different molecular pathways. The differentiation of optic neuroblast in the *Drosophila* brain, as compared to that of an optic neuroblast in the optic tectum of man, will certainly need species-specific information.

The tumor suppressor genes demonstrate the antagonism between growth and differentiation, which is apparent during normal development, where in most cases differentiation takes place only after the cells have excited the cell-cycle. None of the cloned tumor suppressor genes of *Drosophila* seem to be directly involved in cell cycle control, but rather encode proteins which seem to be instrumental in key events establishing and maintaining the differentiated state. Cell differentiation can thus be inter-

rupted by specific gene mutations at key checkpoints, leaving the cells differentiation-incompetent but capable of growth. The suggested putative functions of the different gene products (see Tables) show that tumorous growth can result from the loss of cell-cell contact and communication, signal transduction, chromatin structure, protein synthesis, protein folding and transport, splicing, sex determination, cytokinesis. The sets of genes instrumental in cell cycle control and the ones involved in differentiation, which in normal development are in fine tune with each other, become mutually exclusive in malignant growth.

The overgrowth suppressor genes, on the other hand, do not show the phenomenon of mutual exclusion of cell growth and differentiation. These genes must be involved in the fine tuning of the differential cell divisions within a developing organ tissue. Studies in this direction will be especially illuminating, since they will help to understand the events which control cell division rates within a morphogenetic field of a developing organ.

A further important field of study should consider the activity of tumor suppressor gene products in conjunction with the cytoplasmic and/or nuclear skeleton.

Finally, the one-to-one relationship of a recessive developmental gene mutation to a well-defined malignant tumor phenotype in *Drosophila* is beyond doubt the system of choice, providing unique opportunities to unravel the causal event inducing malignant neoplastic transformation of specific cell types.

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Summary

In *Drosophila* about 27 developmental genes have been identified which suppress tumorous growth and about as many genes are known to suppress overgrowth. Recessive lethal mutations in tumor suppressor genes block in one step the differentiation of specific target cells, leaving unaffected their capacity to divide in an autonomous, malignant and lethal fashion. The structural analysis of eight tumor suppressor genes predicts putative functions in differentiation events, such as cell-cell communication, protein transport and protein synthesis, signal transduction, sex determination splicing and cytokinesis. Their predicted products function as effectors of the differentiated state being vital components of cell junctions, the cytoskeleton, the protein synthetic apparatus, the splicing machinery and signal transduction. In contrast to the tumor suppressor genes, which are instrumental in the establishment and maintenance of the differentiated state, overgrowth suppressor genes seem to control cell-specific division rates while leaving unaffected the capacity of the cells to differentiate. The *Drosophila* tumor suppressor and overgrowth suppressor genes show clearly the mutual exclusion of the genetic programs controlling cell division and cell differentiation. Some of the genes exhibit homologies to mammalian genes. Their functional homology, however, is still an open question.

KEY WORDS: *Drosophila, tumor suppressor genes, differentiation*

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