Journal of Cell Science, Supplement 18, 19-33 (1994)
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Drosophila in cancer research: the first fifty tumor suppressor genes

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SUMMARY

In *Drosophila*, over 50 genes have been identified in which loss-of-function mutations lead to excess cell proliferation in the embryo, in the central nervous system, imaginal discs or hematopoietic organs of the larva, or in the adult gonads. Twenty-two of these genes have been cloned and characterized at the molecular level, and nine of them show clear homology to mammalian genes. Most of these mammalian genes had not been previously implicated in cell proliferation control. Overgrowth in some of the mutants involves conversion to a cell type that, in normal development, shows more cell proliferation than the original cell type. Thus the neurogenic mutants, including *Notch*, show conversion of epidermal cells to neuroblasts, leading to the 'neurogenic' phenotype of excess nervous tissue. The ovarian tumor mutants show conversion of the female germ line to a cell type resembling the male germ line, which undergoes more proliferation than the female germ line. Mutations of the *fat* locus cause hyperplastic overgrowth of imaginal discs, in which the epithelial structure is largely intact. The predicted fat protein product is a giant relative of cadherins, supporting indications from human cancer that cadherins play an important role in tumor suppression. Mutations in the *lethal(2)giant larvae* and *lethal(1)discs large* genes cause neoplastic overgrowth of imaginal discs as well as the larval brain. The *dlg* gene encodes a membrane-associated guanylate kinase homolog that is localized at septate junctions between epithelial cells. This protein is a member of a family of homologs that also includes two proteins found at mammalian tight junctions (ZO-1 and ZO-2) and a protein found at mammalian synaptic junctions (PSD-95/SAP90). Genes in which mutations cause blood cell overproduction include *aberrant immune response-8*, which encodes the RpS6 ribosomal protein and *hopscotch*, which encodes a putative non-receptor protein tyrosine kinase. The gene products identified by ovarian tumor mutants do not show clear amino acid sequence homology to known proteins. *Drosophila* provides an opportunity to rapidly identify and characterize tumor suppressor genes, many of which have mammalian homologs that might also be involved in cell proliferation control and tumor suppression.

Key words: *Drosophila*, tumor suppressor gene, loss-of-function mutation

INTRODUCTION

It is becoming increasingly clear that the deregulated cell growth seen in neoplasia is associated with genetic alterations, which can be multiple and progressive. Proto-oncogenes are activated in a dominant fashion by mutation, chromosome translocation or gene amplification (Bishop, 1991), whereas tumor suppressor genes (TSGs) are inactivated or lost by mutation, chromosome loss, mitotic recombination or gene conversion (Lasko et al., 1991; Fig. 1a,b). The identification of genes in both categories is, of course, important to a full understanding of cancer.

Human TSGs usually have been identified by genetic mapping of tumor susceptibility loci followed by positional cloning, and/or by analyzing the changes in somatic cell DNA that occur during tumor development (Stanbridge, 1990). The protein products of TSGs include cell adhesion proteins, putative signal transduction molecules, transcription factors and molecules involved in cell cycle control. They apparently mediate many different steps in the production and transduction of growth-control signals.

Model experimental systems have not contributed greatly to the discovery of human TSGs, but they have the potential to do so. In this article we show how the genetic procedures available in *Drosophila* are now making it possible to quickly identify and characterize new tumor suppressor genes in this organism, and we discuss the possible functions of their mammalian homologs.

TSG IDENTIFICATION IN *DROSOPHILA*

In *Drosophila*, mutations in over 50 genes cause either hyperplastic or neoplastic overgrowth of various proliferating cell populations in the embryonic, larval and adult stages (Tables 1-5). The mutants vary in the degree to which the overgrowing tissue shows neoplastic characteristics such as loss of differentiated structure and invasiveness. However, for simplicity, since in all cases loss of function leads to overgrowth, we classify these genes as tumor suppressor genes (TSGs). Mutations in most of them are recessive lethals that cause death in pre-adult stages; TSGs that function only in the gonads are

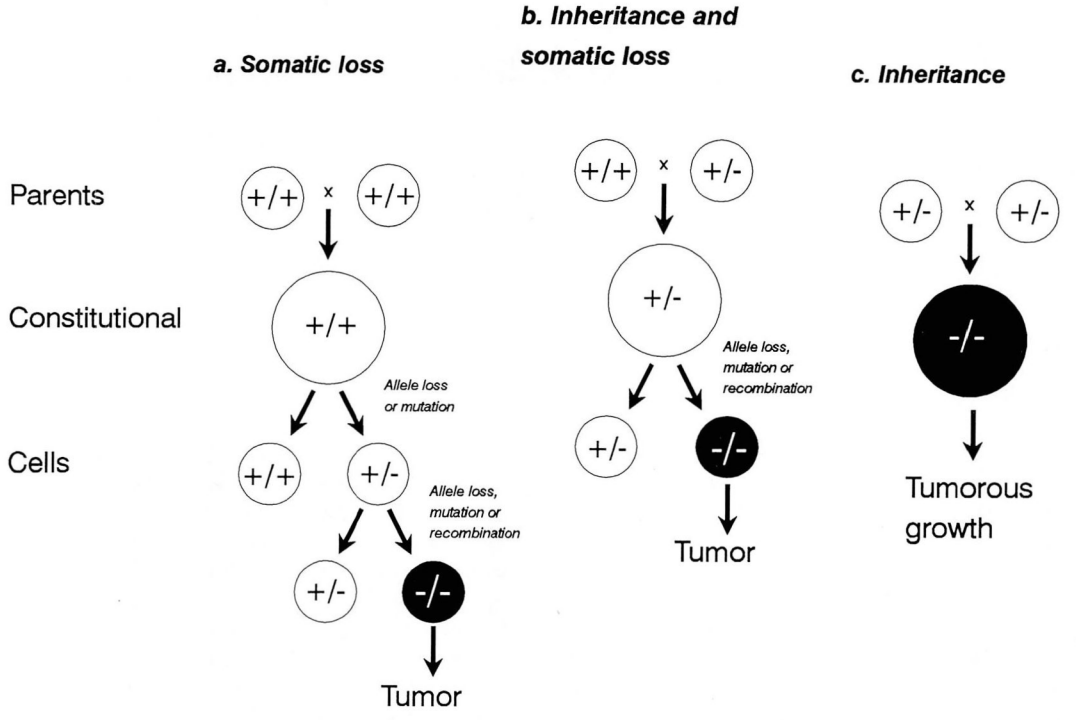

Fig. 1. Three ways in which cells can lose both copies of the normal allele of a TSG. (a) The first allele is lost by mutation, and the second is lost by a second mutation or by mitotic recombination between the two homologous chromosomes. (b) One mutant allele is inherited from a parent, and the second is lost by a second mutation or by mitotic recombination between the two homologous chromosomes. (c) A mutant allele (or a deficiency of the gene) is inherited from each of the two heterozygous parents, giving a zygote that lacks TSG function. Growth abnormalities are expected to arise at the time in development when the normal TSG functions.

exceptional in that they allow survival to adulthood but then cause sterility.

One of the technical advantages of using *Drosophila* as a model system is the availability of balancer chromosomes that make it possible to maintain recessive lethal mutations as stable heterozygous stocks. From such stocks it is simple to produce and study homozygous animals that have lost TSG function in every cell of the body (Fig. 1c). Another technical advantage is that radiation-induced or FLP recombinase-induced mitotic recombination (Golic and Lindquist, 1989) can be used to generate homozygous mutant clones in an otherwise heterozygous animal (Bryant et al., 1993), mimicking the allele loss observed in human tumors (Fig. 1b). Lastly, the ability to alter, reintroduce and manipulate transposable elements in the *Drosophila* genome (Sentry and Kaiser, 1992; Brand and Perrimon, 1993) is providing a series of powerful new tools for the identification and analysis of TSGs and other genes. Several large-scale mutagenesis screens involving mobilization of P elements (Watson et al., 1991; Török et al., 1993) have contributed greatly to our knowledge of *Drosophila* TSGs.

Twenty-two *Drosophila* TSGs have been characterized at the molecular level (Tables 1-5). Nine of them (*Notch*, *shibire*, *fat, twins, expanded, lethal(1)discs large, lethal(2)giant larvae, hopscotch* and *aberrant immune response-8*) show obvious homology to human genes, most of which had not been previously recognized as TSGs. This work is therefore contributing to our understanding of oncogenesis by identifying new candidate TSGs in man. Clearly, the further analysis of this system could lead to the rapid identification of the molecular nature of many more TSG products, and it is likely that these will have functional equivalents in human cells. Many of the known elements controlling cell growth and differentiation (for example, the EGF, FGF and insulin receptors, TGFβ, cyclins, cdc genes, many oncogenes, tyrosine kinases, G proteins, homeobox genes) are highly conserved between human and *Drosophila* (Merriam et al., 1991), making it likely that many tumor-suppressing functions are also conserved.

TSGs FUNCTIONING IN THE EMBRYO

'Neurogenic' genes

A group of *Drosophila* genes with possible roles in tumor suppression is represented by the zygotic 'neurogenic' genes, the prototype being *Notch* (*N*; Artavanis-Tsakonas et al., 1991) (Table 1). The best-known phenotype caused by mutations of the *N* locus is hyperplasia of the embryonic nervous system in which excessive numbers of ectodermal cells adopt the neuroblast fate and delaminate from the ectodermal layer, rather than adopting the alternative epidermal fate (Poulson, 1937). Detailed study of these embryos shows that not only neuroblasts, but also other cell types that delaminate from the ectoderm (sensory neurons, peripheral glial cells and oenocytes) are overproduced (Hartenstein et al., 1992). Although excess neuronal and other cells are produced in these mutant embryos, the phenotype results from the initial determination of excess precursors rather than continuous cell pro-

Table 1. Genes in which mutations cause overgrowth of the nervous system in *Drosophila* embryos (the 'neurogenic' phenotype)

Gene	Symbol	Locus*	Site*	(Predicted) gene product	Reference
Notch	*N*	1-3.0	3B4	Transmembrane protein with EGF repeats	Gateff and Schneiderman, 1974; Wharton et al., 1985a; Artavanis-Tsakonas et al., 1991
lethal(1)discs large	*dlg*	1-34.8	10B8-9	Septate junction-associated guanylate kinase homolog	Woods and Bryant, 1989, 1991
shibire	*shi*	1-52.1	13F-14A1	Dynamin homolog	Poodry, 1990; Chen et al., 1991; Van der Bliek and Meyerowitz, 1991
big brain	*bib*	2-34.7	27D-31E	Transmembrane channel	Rao et al., 1990; Artavanis-Tsakonas et al., 1991
mastermind	*mam*	2-70.3	50C20-23	Nuclear protein rich in homopolymers	Smoller et al., 1990; Artavanis-Tsakonas et al., 1991
neuralized	*neu*	3-50	86C1-D8	Zinc finger protein	Artavanis-Tsakonas et al., 1991; Price et al., 1993
Delta	*Dl*	3-66.2	92A2	Transmembrane protein with EGF repeats	Alton et al., 1989; Artavanis-Tsakonas et al., 1991
Enhancer of split complex	*E(spl)-C*	3-89	96F11-14	G protein (β subunit) plus seven basic helix-loop-helix proteins	Hartley et al., 1988; Delidakis and Artavanis-Tsakonas, 1992; Knust et al., 1992

*Locus and site from Lindsley and Zimm, 1992 or from listed references.

Table 2. Genes in which mutations cause larval brain overgrowth in *Drosophila*

Gene	Symbol	Locus*	Site*	(Predicted) gene product	Reference
lethal(1)discs large	*dlg*	1-34.8	10B8-9	Septate junction-associated guanylate kinase homolog	Woods and Bryant, 1989, 1991
lethal(2)giant larvae	*l(2)gl*	2-0.0	21A	Cell membrane-associated, 78 and 127 kDa	Gateff and Schneiderman, 1974; Mechler et al., 1985; Klämbt and Schmidt, 1986; Jacob et al., 1987
lethal(2)79/18	*l(2)79/18*	2-	22C		Török et al., 1993
lethal(2)giant discs	*lgd*	2-42.7	32A-E		Bryant and Levinson, 1985
lethal(2)37Cf	*brat*	2-53.9	37C5-7	Novel, 99 kDa, pI = 9.25	Wright, 1987; Hankins, 1991
lethal(2)82/25	*l(2)82/25*	2-	35D-E		Török et al., 1993
lethal(2)43/1	*l(2)43/1*	2-	38CD		Török et al., 1993; M. Buratovich and P. J. Bryant, unpublished
lethal(2)90/37	*l(2)90/37*	2-	48		Török et al., 1993
lethal(2)115/12	*l(2)115/12*	2-	50		Török et al., 1993
lethal(3) malignant brain tumor	*l(3)mbt*	3-93	97E8-F11		Loffler et al., 1990; Gateff et al., 1993
lethal(3)1A1	*1A1*	3-	92B		T. Uemura, personal communication; P. J. Bryant, unpublished
prune;Killer of prune	*pn;awd$^{K\text{-}pn}$*	1-0.8; 3-102.9	2E2-3; 100C-D	GAP protein?; nucleoside diphosphate kinase	Teng et al., 1991b; Hackstein, 1992

*Locus and site from Lindsley and Zimm, 1992 or from listed references.

liferation, so the mutant phenotype does not constitute a tumor in the usual sense. However, transplantation studies do show that tissue from embryos lacking *N* function can develop into teratoma-like growths that can be serially cultured (Gateff and Schneiderman, 1974). Unfortunately it is not clear whether this effect is due to loss of *N* function per se or to the loss of a closely linked gene in the deficiency used in these experiments. The *N* gene encodes a 2703 amino acid transmembrane protein with 36 cysteine-rich domains homologous to epidermal growth factor (EGF) and three copies of the cysteine-rich *lin-12/N* repeat in the extracellular portion (Wharton et al., 1985a; Kidd et al., 1986; Fig. 2). In the cytoplasmic domain the protein contains a PEST motif (Rogers et al., 1986) and an OPA repeat (Wharton et al., 1985b), both thought to be associated with high protein turnover rate (Rogers et al., 1986). It also includes six ankyrin repeats, each composed of a 33 amino acid sequence homologous to a repeated sequence in the cell-cycle control genes *cdc10* of *Schizosaccharomyces pombe* and *SW16* of *Saccharomyces cerevisiae* (Breeden and Nasmyth, 1987). Immunolocalization experiments show that the Notch protein is localized at the cell membrane in embryos (Johansen et al., 1989) and in adherens junctions of the imaginal disc epithelium (Artavanis-Tsakonas et al., 1991; Fehon et al., 1991).

The human homolog of Notch, TAN-1, shows the same

Table 3. Genes in which mutations cause imaginal disc overgrowth in *Drosophila*

Gene	Symbol	Locus*	Site*	(Predicted) gene product	Reference
lethal(1)discs large	*dlg*	1-34.8	10B8-9	Septate junction-associated guanylate kinase homolog	Woods and Bryant, 1989, 1991
lethal(2)giant larvae	*l(2)gl*	2-0.0	21A	Cell membrane-associated 78 and 127 kDa	Gateff and Schneiderman, 1974; Mechler et al., 1985; Klämbt and Schmidt, 1986; Jacob et al., 1987
expanded	*ex*	2-0.1	21C1-2	Protein 4.1 family	Boedigheimer and Laughon, 1993; Boedigheimer et al., 1993
lethal(2)79/18	*l(2)79/18*	2-	22C		Török et al., 1993
lethal(2)fat	*fat*	2-12	24D8	Giant cadherin-like molecule	Bryant et al., 1988; Mahoney et al., 1991
lethal(2)giant discs	*lgd*	2-42.7	32A-E		Bryant and Schubiger, 1971; Bryant and Levinson, 1985
lethal(2)161/28	*l(2)161/28*	2-	43B		Török et al., 1993
lethal(2)90/37	*l(2)90/37*	2-	48E		Török et al., 1993
lethal(2)131/7	*l(2)131/7*	2-	48E		Török et al., 1993
lethal(2)115/12	*l(2)115/12*	2-	50E		Török et al., 1993
lethal(2)106/22†	*l(2)106/22*	2-	57		Török et al., 1993
lethal(2) tumorous imaginal discs	*tid*	2-104	59F4-6		Loffler et al., 1990; Kurzik-Dumke et al., 1992
lethal(3)c43	*hyd*	3-49.0	85E	Novel	Martin et al., 1977; Mansfield et al., 1994
twins	*tws*	3-	85F	Protein phosphatase 2A	Uemura et al., 1993
lethal(3)9C3	*l(3)9C3*	3-	90D		T. Uemura, personal communication
lethal(3)1A1	*1A1*	3-	93E1-9		T. Uemura, personal communication; P. J. Bryant, unpublished
lethal(3)malignant brain tumor	*l(3)mbt*	3-93	97E8-F11		Loffler et al., 1990; Gateff et al., 1993
warts‡	*wts*	3-	100A7-B1		Justice and Bryant, 1992
lethal(3)discs overgrown	*dco*	3-	100A7-B7		Jursnich et al., 1990

*Locus and site from Lindsley and Zimm, 1992 or from listed references.
†Only labial and antenna discs affected.
‡Phenotype known only from mitotic recombination clones.

domain structure as Notch, with amino acid identities in the range of 50-70% (Fig. 2). It was discovered as a gene spanning the 9q34 breakpoint in a series of translocations found in T lymphoblastic neoplasms (Ellisen et al., 1991). In addition to bearing the translocation chromosome, the tumor cells show frequent loss of the remaining normal chromosome 9. A likely interpretation is that the translocation inactivates one copy of *TAN-1* and that neoplasia is promoted when the second allele is lost from cells already carrying the translocation. Such an association between loss of both functional copies of a gene and the onset of malignancy would be similar to the behavior of other tumor suppressor genes. Thus for both *Drosophila* and human homologs there is preliminary evidence that these genes may function as TSGs.

It has been suggested that Notch function at the cell membrane may be translated into effects on gene expression through a pathway involving transcription factors encoded by other genes in which mutations cause overproduction of neuroblasts in the embryo (the 'neurogenic' class, Table 1; Artavanis-Tsakonas et al., 1991). If so, this effect could be mediated by the ankyrin repeats of Notch. Ankyrin repeats like those in Notch are found in members of the IκB family, proteins that interact with and inhibit the function of members of the rel/NF-κB group of sequence-specific transcription activators by blocking their translocation into the nucleus (Geisler et al., 1992; Kidd, 1992). N and TAN-1 may represent transmembrane relatives of IκB that interact directly with transcription factors, possibly at times when the latter are localized transiently in the cytoplasm.

Another possibly important aspect of Notch action is its interaction with the *Serrate* gene product, another transmembrane protein with EGF-like repeats in its extracellular domain (Fleming et al., 1990). Cells expressing Notch show adhesion to Serrate-expressing cells (Rebay et al., 1991), suggesting a direct interaction between the proteins, and a role for Serrate in cell proliferation control is indicated by the reduced proliferation seen in imaginal discs of *Serrate* mutant larvae, as well as the additional proliferation seen in imaginal discs containing regions that over-express Serrate (Speicher et al., 1994).

Mutations at the *shibire* (*shi*) locus also produce a neuralized phenotype as described for *Notch*, and tissues transplanted from mutant embryos give rise to transplantable tumorous growths (Hummon and Costello, 1988; Poodry, 1990). The predicted amino acid sequence of the *Drosophila shi* gene product shows 68-69% identity of amino acid sequence to that of both rat and human dynamin (Chen et al., 1991; Van der Bliek and Meyerowitz, 1991), a protein that acts as a GTP-binding motor functioning in vesicle trafficking and meiotic spindle separation (Obar et al., 1991). Vesicle trafficking may be required in the intercellular transfer of signals controlling cell fates, as with the products of *wingless* (Van den Heuvel et al., 1989; González et al., 1991) and *boss* (Cagan et al., 1992), which appear to be transported within and between cells in vesicles. Such a requirement has not been demonstrated for signals controlling cell proliferation, but one possibility is suggested by the finding that the Ash/Grb-2 adaptor protein, which is essential for Ras activation and therefore possibly involved in cell proliferation control, co-precipitates with

Table 4. Genes in which mutations cause overgrowth of larval hematopoietic tissues in *Drosophila*

Gene	Symbol	Locus*	Site*	(Predicted) protein product	Reference
lethal(1)air1	*air1*	1-0.5-2.1	1C3-E1;	2B15-18	Watson et al., 1991
lethal(1)air2	*air2*	1-2.8-4.3	1E3-4		Watson et al., 1991
lethal(1)air6	*air6*	1-15.0	5A1-E8		Watson et al., 1991
lethal(1)air7	*air7*	1-15.9	7A6-8		Watson et al., 1991
lethal(1)air8	*air8*	1-22.3	7C4-9	RpS6 ribosomal protein	Watson et al., 1991, 1992; Stewart and Denell, 1993
lethal(1)air9	*air9*	1-20.0	7C9-D10		Watson et al., 1991
lethal(1)air11	*air11*	1-29.0	8A5-9A2; 11A7-13F10		Watson et al., 1991
multi sex combs$^{malignant\ blood\ neoplasm}$	*mxc*^{mbn}		8D3-9		Santamaria and Randsholt, 1994
hopscotch$^{Tumorous\text{-}lethal}$	*hop*$^{Tum\text{-}l}$	1-34.5	10B6-8	Non-receptor protein tyrosine kinase (Jak family)	Hanratty and Ryerse, 1981; Hanratty and Dearolf, 1993; Zinyk et al., 1993; Binari and Perrimon, 1994
lethal(1)air13	*air13*	1-35.4	8A5-9A2; 11A7-13F10		Watson et al., 1991
lethal(1)air15	*air15*	1-55.6	11A7-13F10		Watson et al., 1991
lethal(2)168/14	*l(2)168/14*	2-	23B		Török et al., 1993
lethal(2)144/1	*l(2)144/1*	2-	31A		Török et al., 1993
lethal(2)86/34	*l(2)86/34*	2-	37D		Török et al., 1993
lethal(2)43/1	*l(2)43/1*	2-	38CD		Török et al., 1993; M. Buratovich and P. J. Bryant, unpublished
lethal(2)65/24	*l(2)65/24*	2-	48A		Török et al., 1993
lethal(2)90/37	*l(2)90/37*	2-	48E		Török et al., 1993
lethal(2)131/7†	*l(2)131/7*	2-	48E		Török et al., 1993
lethal(2)154/1†	*l(2)154/1*	2-	48E		Török et al., 1993
lethal(2)211/5	*l(2)211/5*	2-	51		Török et al., 1993
lethal(2)30/7	*l(2)30/7*	2-	55DE		Török et al., 1993
lethal(2)88/10	*l(2)88/10*	2-	56A		Török et al., 1993; M. Buratovich and P. J. Bryant, unpublished
lethal(2) malignant blood neoplasm	*l(2)mbn*	2-			Gateff et al., 1984
lethal(3) malignant blood neoplasm-1	*l(3)mbn-1*	3-13.3	64F4-5	Cytokeratin homolog	Shrestha and Gateff, 1986; Konrad et al., 1994
lethal(3)malign ant blood neoplasm-2	*l(3)mbn-2*	3-	87		Mechler, 1990
prune;Killer of prune	*pn;awdK-pn*	1-0.8; 3-102.9	2E2-3; 100C-D	GAP protein?; nucleoside diphosphate kinase	Teng et al., 1991b; Hackstein, 1992

*Locus and site from Lindsley and Zimm, 1992 or from listed references.
†*l(2)131/7* and *l(2)154/1* are allelic to *l(2)90/37* but are phenotypically distinct (Török et al., 1993).

Table 5. Genes in which mutations cause ovarian or gonial cell tumors in *Drosophila*

Gene	Symbol	Locus*	Site*	(Predicted) gene product	Reference
ovo	*ovo*	1-10.2	4E2	Zinc finger protein	Mével-Ninio et al., 1991; Mohler, 1977
female sterile(1)1621, sans-fille, liz	*fs(1)1621, snf*	1-11.7	4F1-5A1		Gollin and King, 1981; Salz, 1992
Sex-lethal	*Sxl*	1-19.2	6F4-7B3	RNA-binding protein	Bell et al., 1988; McKearin and Spradling, 1990
ovarian tumors	*otu*	1-23.2	7F1	Novel, proline-rich	Steinhauer et al., 1989; Geyer et al., 1993; Sass et al., 1993
fused	*fu*	1-59.5	17C3-D2	Ser/thr protein kinase	Therond et al., 1993; King et al., 1957
female sterile(1)231	*fs(1)231*	1-			King et al., 1957
female sterile(2)of Bridges	*fs(2)B*	2-5			King et al., 1957
narrow	*nw*	2-83?	54A-55A		King et al., 1957
benign(2)gonial cell neoplasm	*b(2)gcn*	2-106.7	60A3-7		Loffler et al., 1990
bag-of-marbles	*bam*	3-85	96C	Novel	McKearin and Spradling, 1990

*Locus and site from Lindsley and Zimm, 1992 or from listed references.

dynamin from PC12 cells (Miki et al., 1994). Interaction between these proteins would provide a link between signal transduction pathways important in cell proliferation control, and endocytic processes.

Mutations in several genes other than *Notch* and *shi* give rise to the neuralized phenotype (Artavanis-Tsakonas et al., 1991; Hartenstein et al., 1992; Table 1); these include *neuralized* (*neu*), *mastermind* (*mam*), *Delta* (*Dl*), *big brain* (*bib*) and the

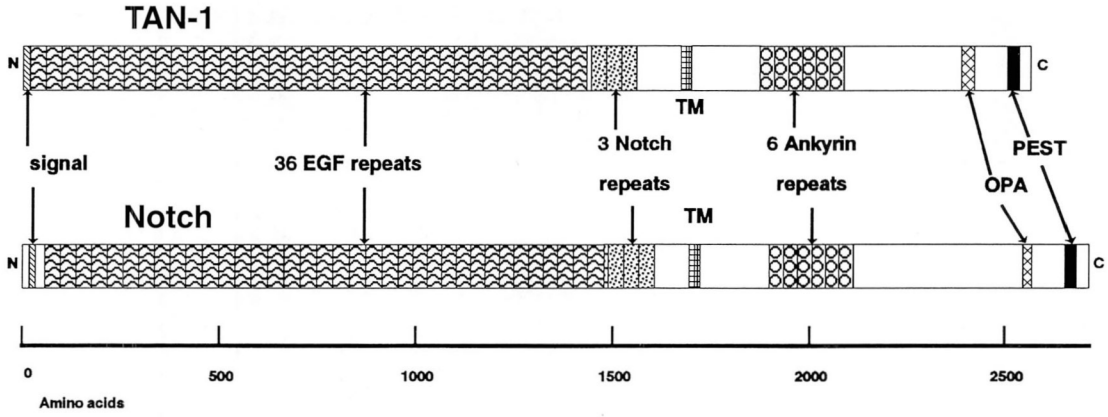

Fig. 2. Domain comparison between *Drosophila* Notch and human TAN-1 (after Ellisen et al., 1991). Both proteins contain a signal sequence, 36 tandemly repeated epidermal growth factor (EGF) domains, three tandemly-repeated Notch domains, a transmembrane domain (TM), six ankyrin domains, an OPA domain and a PEST domain. C, carboxy terminus; N, amino terminus.

Enhancer of split complex (*E(spl)-C*). Unfortunately, the appropriate transplantation tests to identify neoplastic growth like that seen with *N* and *shi* have not been reported for these genes. Therefore, whether or not they should be considered true TSGs must await further analysis.

TSGs FUNCTIONING IN BRAIN DEVELOPMENT

Several genes have been found to be necessary to control proliferation in the developing central nervous system of the larva. In wild-type larvae, the central nervous system is composed of two brain hemispheres and a ventral ganglion. The brain hemispheres are surrounded by a single layer of glial cells (the perineurium) together with an extracellular matrix, the neurilemma. The outer cortical region of the brain hemispheres contains several cell types (Gateff et al., 1984): neuroblasts, functioning as stem cells; ganglion mother cells, which are derived from the neuroblasts; and differentiated cells including neurons, neurosecretory cells and glial cells. The mass of axons making up the neuropile is in the center of the hemispheres. The lateral part of each hemisphere is occupied by the inner and outer proliferation centers - layers of neuroblasts that give rise to the optic lobes during metamorphosis. Between the two proliferation centers are several layers of ganglion mother cells and neurons.

Mutations in brain TSGs cause dramatic enlargement (to 2-5× the normal volume) of the two brain hemispheres, usually with no noticeable effect on the ventral ganglion. In the three cases that have been investigated in detail (*lethal(2)giant larvae (lgl)*, Gateff and Schneiderman, 1974; *brain tumor (brat)*, Hankins, 1991; and *lethal(3)malignant brain tumor (l(3)mbt)*, Gateff et al., 1993) the mutant brain hemispheres contain large numbers of undifferentiated cells, interpreted as small undifferentiated neuroblasts (*brat)*, or as neuroblasts and ganglion mother cells (*lgl* and *l(3)mbt)*. The predominance of neuroblasts leads to the suggestion that these growths are neuroblastomas (Gateff and Schneiderman, 1974; Hankins, 1991; Gateff et al., 1993). The *lgl* brain hemispheres contain all of the cell types found in wild-type animals, but they are intermingled rather than separated into well-defined layers and proliferation centers. In *l(3)mbt* mutants, the brain is disorganized and the excess growth seems to obliterate at least part of the neuropile and to cause fragmentation of the remaining neuropile. In all three mutants, pieces of the mutant larval brain continue to grow by cell proliferation when transplanted into the abdomens of wild-type adults, and the body cavity becomes filled with cells interpreted as neuroblasts (*brat*) or neuroblasts and ganglion mother cells (*lgl* and *l(3)mbt*). Tumor cell types include neurons in the case of *brat*, and 'preganglion cells' and polyploid giant cells in the case of *lgl*. In all three mutants tumor cells invade host tissues such as ovaries, fat body, gut, and thoracic muscles, and in *lgl* the invasiveness has been confirmed by the use of genetic markers to distinguish the transplanted cells from the host (Timmons et al., 1993). For *lgl* and *l(3)mbt* the tumors are lethal to the host, supporting the idea that they should be considered malignant neuroblastomas (Gateff and Schneiderman, 1974).

lethal(2)giant larvae (lgl)

Although the *lgl* gene was the first TSG in any organism to be identified, cloned and sequenced (Klämbt et al., 1989), an understanding of how its product functions has been elusive. cDNA sequencing predicts the existence of two protein products, one of which is predicted to be 78 kDa and the other 127 kDa due to the addition of a C-terminal tail (Jacob et al., 1987). Neither sequence contains a transmembrane domain or other recognizable functional domain. The 78 kDa product is sufficient for tumor suppression, as judged by transformation rescue experiments (Jacob et al., 1987). Immunocytochemical studies show that the lgl protein is localized mainly in the cytoplasm, but about 20% is membrane-associated during early embryonic stages. At late embryonic stages the protein becomes restricted to the lateral surfaces of midgut and salivary gland epithelial cells, and to the neuropile of the central nervous system (Klämbt and Schmidt, 1986; Klämbt et al., 1989; Mechler and Strand, 1990; Strand et al., 1991). This pattern of embryonic expression is somewhat puzzling since the midgut and salivary gland are not abnormal in mutant animals, and since homozygous mutant *lgl* embryos can successfully develop and reach late third instar before they show the characteristic brain and imaginal disc tumors. These findings could be explained if the mutant embryos are rescued by maternally derived gene products (Schmidt, 1989). The lgl

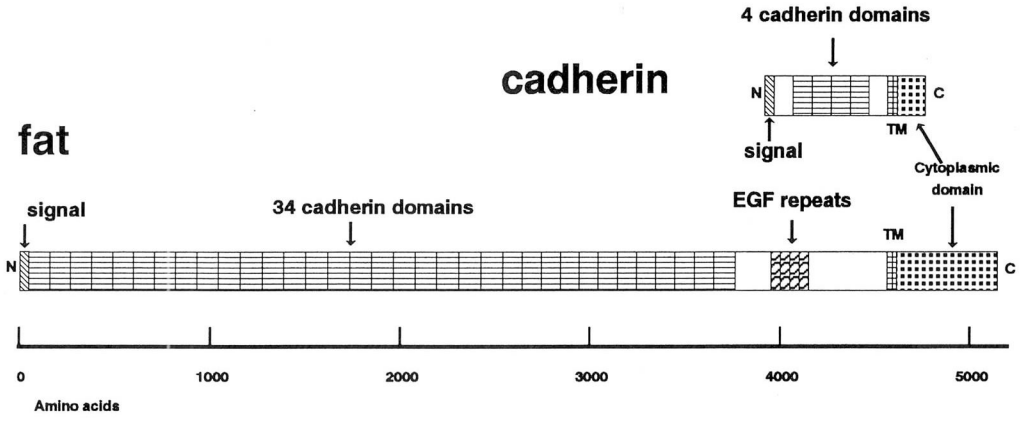

Fig. 3. Domain comparisons between the *Drosophila* fat protein and a typical vertebrate cadherin (Mahoney et al., 1991). The predicted fat product contains 34 extracellular domains with sequence similarity to the four extracellular domains of typical cadherins, and contains four epidermal growth factor (EGF) repeats that are not found in cadherins. In the predicted fat protein the first cadherin domain occurs immediately after the signal sequence, whereas in typical cadherins the signal sequence is followed by approximately 100 amino acids that are cleaved off to produce the mature protein. There is no significant homology between fat and cadherin in the cytoplasmic domain. C, carboxy terminus; N, amino terminus; TM, transmembrane domain.

protein is also expressed in the late third instar, in imaginal discs and in the neuroblasts of the larval brain (Klämbt and Schmidt, 1986), which are the tissues that show overgrowth in the mutant. In general, the protein seems to be present in tissues that are near or at the end of their proliferative growth phase, consistent with the conclusion from phenotypic studies that lgl plays a role in arresting cell proliferation. A mouse homolog of lgl has been identified as a gene under the control of the Hox-C8 homeobox gene, by immunopurification of DNA sequences that bind to Hox-C8 in native chromatin (Tomotsune et al., 1993). The predicted amino acid sequence shows about 41% identity to that of *Drosophila* lgl, and the gene is expressed at high levels in the nervous system.

brat has also been cloned and cDNAs sequenced (Hankins, 1991), but the sequence shows no clear homology to any other sequence and reveals no clues about the protein's function.

prune-killer-of-prune (*pn;awd*$^{K\text{-}pn}$) and neurofibromatosis

An unusual genetic interaction occurs in *Drosophila* between recessive mutations at the *prune* (*pn*) locus and the dominant *Killer-of-prune* allele of the *abnormal wing disc* gene (*awd*$^{K\text{-}pn}$; Teng et al., 1991a), and this genetic combination gives rise to an unusual tumorous phenotype. Animals carrying both of these mutations exhibit hypertrophy of the brain perineurium, the neuroglia and the lymph glands (Hackstein, 1992), and they die as late larvae. This is the only reported example of a TSG functioning in glial cells in *Drosophila*. The *awd* gene encodes a nucleoside diphosphate (NDP) kinase that is 78% identical to the mammalian Nm23 protein, which was identified by its low expression in cell lines with high metastatic potential (Rosengard et al., 1989). It has been proposed that NDP kinases regulate developmental processes and metastasis by increasing the availability of GTP, which activates GTP-binding proteins (Liotta et al., 1991). It has also been proposed that the *pn* gene product may be a GTPase activating protein (GAP), based on its slight similarity to bovine GAP (Teng et al., 1991b; but see Barnes and Burglin, 1992; Venkatesh and Teng, 1992, for a discussion of this putative similarity). These ideas and some phenotypic similarities have led to the formulation of a detailed model relating the *Drosophila* double mutant *pn;awd*$^{K\text{-}pn}$ to human neurofibromatosis (Hackstein, 1992).

TSGs FUNCTIONING IN IMAGINAL DISCS

Mutations in some genes cause hyperplastic overgrowth of imaginal discs, in which the disc tissue retains most of its epithelial structure and is able to secrete cuticle during metamorphosis after transplantation to a larval host. In contrast in another group of mutants, the mutant imaginal discs lose their epithelial structure to varying extents, and lose their ability to differentiate after transplantation into a larval host. The latter are called neoplastic overgrowth mutants.

Hyperplastic overgrowth mutants

fat: a TSG encoding a putative cell adhesion molecule

Lethal mutations at the *fat* locus cause overgrowth of all of the imaginal discs, and the overgrowth is classified as hyperplasia because the tissues retain their single-layered epithelial structure and their ability to differentiate (Bryant et al., 1988; Mahoney et al., 1991). The gene is transcribed in the embryonic ectoderm and larval imaginal discs (Mahoney et al., 1991). Sequencing of overlapping partial cDNAs indicates that the gene product is a transmembrane protein with strong sequence homology in its extracellular domain to calcium-dependent cell adhesion molecules (cadherins; Fig. 3). However, the predicted product is much larger than typical cadherins, with 34 extracellular domains corresponding to the four domains of typical cadherins, as well as four EGF-like repeats and other cysteine-rich regions in the extracellular domain. The cytoplasmic domain shows no significant homology to that of typical cadherins. Some of the excess tissue from the overgrown imaginal discs in *fat* mutants is shed as vesicles during the pupal stage, supporting the idea that the gene product is required for epithelial cell adhesion as well as proliferation control.

In human patients, loss or reduction of E-cadherin expression has been reported in breast, head-and-neck, gastric, colorectal, bladder, prostate and liver cancer (Schipper et al., 1991; Shimoyama and Hirohashi, 1991; Field, 1992; Bringuier et al., 1993; Mayer et al., 1993; Morton et al., 1993; Nigam et al., 1993; Oka et al., 1993; Oda et al., 1994), and E-cadherin loss is correlated with invasiveness of carcinoma and epithelial cell lines (Behrens et al., 1989; Frixen et al., 1991; Doki et al., 1993). Treatment with anti-E-cadherin antibodies can cause

non-invasive carcinoma and epithelial cell lines to become invasive (Behrens et al., 1989; Frixen et al., 1991), and invasiveness of carcinoma cell lines can be prevented by transfection with E-cadherin cDNA (Frixen et al., 1991). These correlative findings are highly suggestive of an important role of E-cadherin in preventing the transition to tumorous and/or invasive growth. Even more intriguing is the possibility that the human E-cadherin gene located at 16q22.1 (Natt et al., 1989) may correspond to a TSG identified by loss of heterozygosity in this genetic region in hepatocellular, breast, and prostate carcinomas (Carter et al., 1990; Sato et al., 1990; Tsuda et al., 1990; Zhang et al., 1990). If E-cadherin plays an important role in tumor suppression, the *fat* locus may provide a useful model system for investigating the mechanism of this effect. Both E-cadherin and the fat protein may play important direct roles in proliferation control, perhaps by initiating a signal transduction process at the cell membrane. Alternatively, their roles may be simply to support cell adhesion as a crucial prerequisite for the cell signalling events that control proliferation and for the mechanical attachments that prevent metastasis.

Other hyperplastic overgrowth mutants

Three more genes in which mutations cause hyperplastic imaginal disc overgrowth have been cloned. The *hyperplastic discs (hyd)* gene (*=l(3)c43*; Martin et al., 1977) encodes a novel protein with a region homologous to a part of poly(A)-binding proteins (Mansfield et al., 1994). Alterations at the *twins* locus cause an overgrowth phenotype in which the posterior part of the wing disc is duplicated with mirror-image symmetry (Uemura et al., 1993). cDNAs representing the *twins* gene encode a product with 75-78% amino acid identity to the regulatory B subunits of human protein phosphatase 2A (Uemura et al., 1993). The *expanded* locus, in which mutations result in overgrowth of the posterior part of the wing disc and parts of the leg and antenna discs (Boedigheimer and Laughon, 1993), encodes a member of the protein 4.1 family of membrane-cytoskeletal linker molecules that includes the NF2 (neurofibromatosis type 2) tumor suppressor (Boedigheimer et al., 1993).

Neoplastic overgrowth mutants

discs-large: a TSG encoding a junctional protein

Mutations in the *lethal(1)discs large (dlg)* gene cause neoplastic imaginal disc overgrowth; the mutant tissues, which are normally single-layered, become disorganized masses and lose the ability to develop into adult parts after transplantation into normal hosts. This is similar to the effect on imaginal discs produced by mutations in the *lgl* gene (Gateff and Schneiderman, 1974). The *dlg* gene encodes several products including a 960 amino acid protein DlgA (Fig. 4) that is expressed in most epithelial tissues as well as other tissues throughout development (Woods and Bryant, 1989, 1991). The carboxy-terminal 179 amino acids of DlgA show strong homology (35.5% identity) to yeast guanylate kinase (GUK; Berger et al., 1989), an enzyme that transfers a phosphate group from ATP to GMP, converting it to GDP. Although the GMP-binding features of yeast GUK are highly conserved in DlgA, the putative ATP-binding site has a 3 amino acid deficiency (Koonin et al., 1992) suggesting that the protein may simply bind GMP or catalyze a reaction other than phosphorylation of GMP. DlgA also contains the 59 amino acid SH3 domain (Musacchio et al., 1992), which is found in many membrane-associated signal transduction proteins and mediates binding to other proteins, including GTPase-activating proteins (Ren et al., 1993). It also has an OPA domain and a PEST domain, as mentioned above for the Notch protein. The N-terminal half of the molecule contains three copies of a newly identified 91 amino acid motif called DHR (previously GLGF; Cho et al., 1992), of unknown function (Fig. 4).

In epithelial cells the Dlg protein is localized in an apical belt of the lateral cell membrane at the position of a specialized structure, called the septate junction, which connects cell neighbors (Woods and Bryant, 1991). The role of these junctions is unknown, although they have often been considered the invertebrate equivalent of vertebrate tight junctions (Noirot-Timothee and Noirot, 1980). The Dlg protein is required for septate junction structure as shown by the absence or reduction of septate junctions in *dlg* mutant larvae (D. F. Woods and P. J. Bryant, unpublished). The analysis of this gene suggests that cell interactions important for growth control occur at septate junctions, and that the interactions may regulate the production of guanine nucleotides that act as messenger molecules within the cell.

Four human homologs of *dlg* have been identified, and none was previously suspected to be involved in tumor suppression (Fig. 4; Woods and Bryant, 1993). We refer to this growing family of gene products as MAGUKs (membrane-associated guanylate kinase homologs). Three of the mammalian MAGUKs are associated with cell junctions: ZO-1 and ZO-2 are two well-known components of epithelial tight junctions, while PSD95/SAP-90 is a component of synaptic junctions in the brain.

ZO-1 is a major protein component of epithelial and endothelial tight junctions (Anderson et al., 1988; Itoh et al., 1993; Willott et al., 1993). It shows clear homology to DlgA in the DHR, SH3, and GUK domains, but it differs from it in the presence of a large proline-rich C-terminal extension (Fig. 4). The ZO-1 GUK domain shows 29% identity of amino-acid sequence with the DlgA GUK domain and 24% with pig GUK, and it has a well conserved Mg^{2+}-binding site (the B motif) including the Asp residue implicated in direct interaction with the Mg^{2+} cofactor (Yan and Tsai, 1991). However, ZO-1 has a 32 amino acid deficiency within the GUK domain as compared to DlgA and pig GUK, and this deficiency removes four of the ten residues expected to interact with GMP. The A-motif of ZO-1 has the same three amino acid deficiency as in DlgA, and is also missing an otherwise highly conserved lysine residue. These features would appear to make it unlikely that ZO-1 functions as a guanylate kinase and suggest that it might not even interact with GMP. The ZO-1 amino acid sequence shows 14.6-44.8% identity with DlgA in the other domains (Fig. 4).

The second mammalian MAGUK is ZO-2 (Fig. 4; Jesaitis and Goodenough, 1994), another protein component of epithelial tight junctions that has been shown to bind to ZO-1 (Gumbiner et al., 1991). The available predicted amino acid sequence shows the presence of DHR3, SH3 and GUK domains, together with a proline-rich C-terminal domain that is considerably shorter than that of ZO-1. The sequence is incomplete at the N-terminal end, so it is still possible that DHR1 and DHR2 domains are present. The sequence is more similar to that of ZO-1 than to the other known members of the MAGUK family.

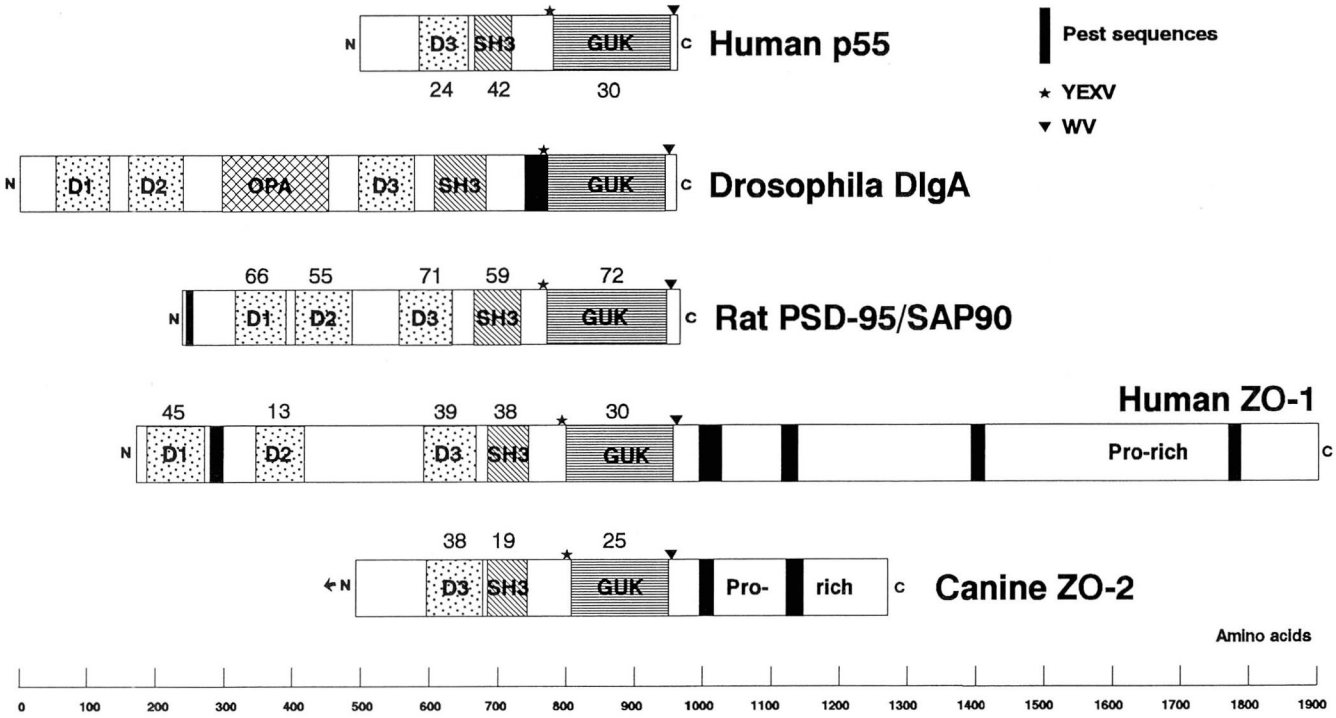

Fig. 4. Comparison of the modular structures of human p55 (Ruff et al., 1991), *Drosophila* DlgA (Woods and Bryant, 1991), rat PSD-95/SAP90 (Cho et al., 1992; Kistner et al., 1993), human ZO-1 (Willott et al., 1993) and canine ZO-2 (Jesaitis and Goodenough, 1994). D1, D2, D3, discs-large homologous regions (DHRs), previously designated GLGF (Cho et al., 1992); SH3, src-homology region 3 (Musacchio et al., 1992); GUK, guanylate kinase-homologous region; OPA, repetitive sequence (Wharton et al., 1985b). Domain boundaries and sequence identities were determined using the BLAST algorithm (Altschul et al., 1990) on line at the National Center for Biotechnology Information, and the multiple alignment program MACAW (Schuler et al., 1991). Numbers outside the boxes indicate percentage amino acid identity compared with the corresponding domain of DlgA. Regions shown in black are PEST sequences, scoring >6.0 on the PESTFIND program (Rogers et al., 1986). Arrow indicates incomplete sequence.

The third mammalian DlgA homolog is a protein localized in synaptic junctions and named either PSD-95 (Cho et al., 1992) or SAP90 (Kistner et al., 1993) (Fig. 4). The deduced amino acid sequence of this protein shows strong homology (about 67% identity) to DlgA throughout its length, and includes three DHRs, an SH3 and a modified GUK. The PSD-95/SAP90 sequence shows strong conservation of the GMP-binding site and has the same deficiency as DlgA in the putative ATP-binding site, suggesting that its function is similar to that of the *Drosophila* protein (Koonin et al., 1992).

The fourth mammalian MAGUK is the heavily palmitoylated phosphoprotein p55, which was purified from red blood cell membrane ghosts and copurifies with the cytoskeletal actin-bundling protein dematin, protein 4.1, and actin (Ruff et al., 1991). p55 shows striking similarity to the DlgA protein in the DHR3, SH3, and GUK domains (Fig. 4; Ruff et al., 1991; Bryant and Woods, 1992). The ATP-binding site of p55 does not show a deficiency like those seen in the junction-associated MAGUKs, but it does show a K to R substitution that might affect enzyme activity. The p55 gene is located near the tip of the long arm of the X chromosome, between the genes encoding the blood-clotting protein, factor VIII, and the enzyme glucose-6-phosphate dehydrogenase (Metzenberg and Gitschier, 1992).

p55 binds with high affinity to protein 4.1, which is thought to link the cytoskeleton to the cell membrane, and to the transmembrane protein glycophorin C (Alloisio et al., 1993; Marfatia et al., 1994). Protein 4.1 and glycophorin C also bind to each other, suggesting the existence of a ternary complex at the erythrocyte plasma membrane. Genetic loss of either protein 4.1 or glycophorin C leads to loss of p55 and to abnormal erythrocyte shape in the hereditary elliptocytoses, indicating that the complex plays a critical role in stabilizing the erythrocyte plasma membrane. Isoforms of p55, protein 4.1 and glycophorin C are present in many cell types in addition to blood cells (Metzenberg and Gitschier, 1992; Marfatia et al., 1994), so similar membrane-associated complexes may exist in these other cell types. Also, the strong similarity between p55 and other MAGUKs raises the possibility that the MAGUKs are components of similar membrane-associated molecular complexes that might be important structural components of cell junctions.

TSGs FUNCTIONING IN BLOOD CELL DEVELOPMENT

In *Drosophila*, mutations at more than 25 loci lead to a characteristic larval phenotype including hyperplastic hematopoietic organs, excess blood cells in circulation, and melanizing aggregates formed by the mutant blood cells (Table 4). The hematopoietic organs are the lymph glands, which line the dorsal vessel at its anterior end and give rise to two distinct

populations of hemocytes (blood cells): the plasmatocytes and the crystal cells (Rizki, 1978; Gateff et al., 1984). Three of these genes (*aberrant immune response-8, lethal(3)malignant blood neoplasm-1* and *hopscotch*) have been cloned and characterized at the molecular level.

aberrant immune response-8: a TSG encoding a ribosomal protein

The *aberrant immune response-8* (*air8*) locus was the first TSG affecting the functioning of the *Drosophila* hematopoietic system that was characterized at the molecular level. Animals mutant for the *air8* locus show a generalized slowing of larval growth, but they also exhibit gross hypertrophy, hyperplasia and melanization of the larval lymph glands (Watson et al., 1991). Blood cells are overproduced and their differentiation is altered such that lamellocytes, not normally produced until the late larval or pupal stage, are found both in the lymph glands and in circulation. The normal role of lamellocytes is to aggregate and form melanized capsules around foreign objects during an immune response, but in *air8* animals the lamellocytes aggregate and form large melanotic tumors in the absence of a foreign challenge. The mutant animals die during the late larval stage. Transplantation of *air8* lymph glands into permissive hosts results in the formation of melanotic tumors and an increased rate of host death when compared to wild-type lymph gland transplants (Watson, 1993).

The product of the *air8* gene, predicted from cDNA sequencing, shows 75% identity and 95% similarity to the human ribosomal protein S6 (Watson et al., 1992; Fig. 5). On northern blots, the 1 kb RpS6 transcript is barely detectable in hemizygous *air8* larvae but is very abundant in *air8* revertant animals and in wild-type animals throughout development. Thus a loss or major reduction in RpS6 expression appears to result in tumor formation in the *Drosophila* hematopoietic system. Two other RpS6 gene mutations (*RpS6^{WG1288}* and *RpS6^{hen}*) have been identified (Watson et al., 1992; Stewart and Denell, 1993) and shown to result in reduced RpS6 transcription although the hematopoietic tissues in these mutants have not been analyzed for neoplastic properties. Expression of a single wild-type RpS6 gene in germ-line transformants is sufficient to rescue the lethality associated with the *RpS6^{WG1288}* and *RpS6^{hen}* mutations, and to partially rescue the phenotype of the *RpS6^{air8}* mutation (K. Watson, unpublished).

In mammals, RpS6 is the major phosphoprotein of the 40 S ribosomal subunit, occupying the mRNA-binding site (Ballou et al., 1988). Its phosphorylation, on a cluster of serine residues at the C terminus (Fig. 5), represents the end-point of a mitogen-stimulated serine/threonine phosphorylation cascade triggered by growth factor binding at the cell surface (Pelech and Sanghera, 1992; Posada and Cooper, 1992). RpS6 phosphorylation is developmentally regulated, and can be stimulated in quiescent cells by treatments that increase protein synthesis and cell proliferation such as serum growth factors, insulin, tumor promoting agents, transforming viruses, mitogens and chemical carcinogens (see Traugh and Pendergast, 1986, for review). The mitogenic stimulation of RpS6 phosphorylation is reversible, and rapid dephosphorylation occurs when cells are deprived of serum or exposed to other treatments that arrest growth (Glover, 1982; Traugh and Pendergast, 1986). Similarly, cultured blood cells derived from

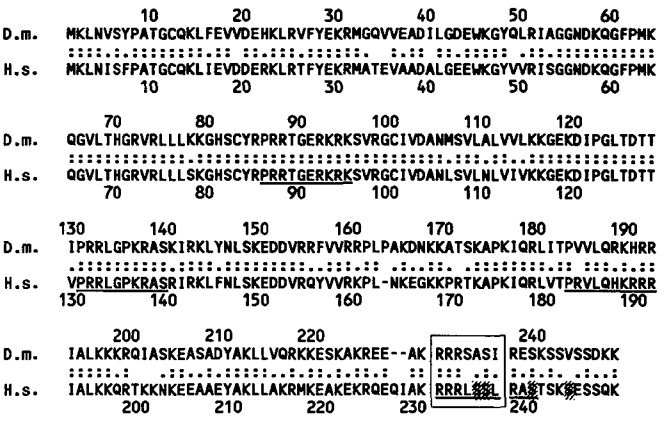

Fig. 5. Sequence comparison between *Drosophila* (D.m.; Watson et al., 1992) and human (H.s.; Pata et al., 1992) RpS6 ribosomal proteins (from Watson et al., 1992). The *Drosophila* RpS6 protein is encoded by the *air8* gene. Both sequences include four copies of a nuclear localization signal (underlined), and a cluster of serine residues at the C terminus. Some of these serines in the human protein (shaded) are sites of phosphorylation by a variety of kinases and an S6-kinase (Ferrari et al., 1991). The kinase recognition sequence is boxed. Alignments were made using the FASTA program (Pearson and Lipman, 1990).

air8 mutant animals become quiescent when deprived of insulin and can be stimulated to proliferate when insulin is added back to the medium (K. L. Watson, unpublished). RpS6 and other ribosomal phosphoprotein mRNAs are overexpressed in human colon carcinomas and liver metastases (Barnard et al., 1992).

In view of the correlations between RpS6 phosphorylation and growth, the loss of RpS6 expression accompanied by tumor formation in the *air8* mutant appears paradoxical. But one way that RpS6 may play a role in controlling cell growth is through the selective translation of particular mRNAs due to different rates of mRNA initiation or translation (Ballou et al., 1988; Erikson, 1991). If the regulated genes included some that are directly involved in tissue-specific cell proliferation control, the loss of RpS6 expression could slow growth in some tissues while accelerating it in others.

The human RpS6 shares many features with *Drosophila* RpS6 including a polypyrimidine tract at the transcription start site, short untranslated regions, the first intron beginning after the second codon and the absence of consensus TATA or CAAT sequence motifs in the 5′ regulatory region (Antoine and Fried, 1992). The chromosome location of the human RpS6 gene is 9p21, a chromosome region at which genetic alterations are associated with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and pleomorphic adenoma of the salivary gland (Antoine and Fried, 1992). This raises the possibility of RpS6 mutations being involved in human tumorigenesis.

lethal(3)malignant blood neoplasm-1 (*l(3)mbn-1*)

Mutations in the *l(3)mbn-1* gene cause hyperplasia of the lymph glands, abnormal differentiation of plasmatocytes and lamellocytes, invasion of the larval tissues by blood cells and lethality in the late larval stage (Konrad et al., 1994). The gene produces a 2.6 kb transcript during embryonic and larval stages of development and two transcripts of 1.4 and 1.8 kb in pupae

and adults. The predicted gene product contains 796 residues bearing some similarity in the C-terminal domain to the G-S repeats found in human cytokeratins K1 and K10 (Konrad et al., 1994). It is unclear what role this protein performs in blood cells or how loss of the function leads to overproliferation of blood cells.

hopscotch$^{Tumorous\ lethal}$ (*hop*$^{Tum\text{-}l}$): a TSG encoding a tyrosine kinase

Loss-of-function mutations in the *hopscotch (hop)* gene result in small imaginal organs, and mutant animals die at the larval/pupal stage (Binari and Perrimon, 1994). Wild-type *hop* activity is required maternally for the proper segmentation of embryos (Binari and Perrimon, 1994). Conceptual translation of *hop* cDNAs yields a 1,177 amino acid protein proposed to be a member of the Janus family of non-receptor protein tyrosine kinases (JAKs) (Binari and Perrimon, 1994). Although most lesions in the *hop* gene lead to undersized imaginal tissues, one unusual allele (*hop*$^{Tum\text{-}l}$) results in the dominant overproduction of lamellocytes (Hanratty and Dearolf, 1993; Zinyk et al., 1993). The *hop*$^{Tum\text{-}l}$ mutation is complex in that the dominant transformation of hemocytes is temperature-insensitive whereas the recessive lethality and dominant enhancement of melanotic tumors is temperature-sensitive (Hanratty and Ryerse, 1981; Silvers and Hanratty, 1984; Nappi and Carton, 1986). The alteration in the *hop* gene that causes this complex phenotype, and its effect on kinase activity, is not known.

The double mutant *pn;awd*$^{K\text{-}pn}$ (see above) suppresses the hematopoietic defect in *hop*$^{Tum\text{-}l}$ mutant animals (Zinyk et al., 1993). This interaction has led to the suggestion that the wild-type products of the *awd, pn,* and *hop* genes may function in a common hematopoietic regulatory pathway (Zinyk et al., 1993).

TSGs FUNCTIONING IN GONAD DEVELOPMENT

Ovarian tumor mutants

These mutants are characterized by the production of large numbers of poorly differentiated germ cells in the adult ovary, causing female sterility. In the normal ovary each egg chamber contains one oocyte and fifteen nurse cells, which together represent the progeny from four successive divisions of a single cystoblast. In the ovarian tumor mutants (Table 5) the germ cells overproliferate to produce a mass of much smaller cells that show some cytological features characteristic of spermatocytes (Pauli et al., 1993). In *Sxl, snf* and *otu* these cells also show expression of male-specific enhancer traps (Wei et al., 1994). Since in the normal male the spermatocytes show much more proliferation than oogonial cells, it has been suggested that the ovarian tumor phenotype is a result of sex transformation in the germ line (Pauli and Mahowald, 1990; Steinmann-Zwicky, 1992). Indeed, mutations in some genes known to function in somatic cell sex determination (*Sex-lethal* and *sans-fille*) also give rise to ovarian tumors. The expression of some male-specific enhancer traps is restricted to the testis apex in normal males, but is much more widespread in the ovarian tumors. Furthermore, some cells in the tumors show cytological features of nurse cells (female germ-line cells) while expressing male-specific genes (Wei et al., 1994). These results suggest that the ovarian tumor phenotype is not due simply to sex transformation but that it also involves the over-proliferation of cells that are in an early stage of germ-cell development and/or show incomplete sex transformation.

ovarian tumor (otu)

Mutations in this gene yield a variety of phenotypes including excess cell proliferation and differentiation defects during oogenesis (Geyer et al., 1993; Sass et al., 1993). The most severe mutants lack germ cell proliferation; less severe mutants develop tumorous ovarian chambers, and the least severe ones exhibit some oocyte/nurse cell differentiation but fail to complete oogenesis (Geyer et al., 1993; Sass et al., 1993). In *otu* null mutants, ovaries contain tumorous egg chambers indicating that *otu* function is required for the control of normal germ cell proliferation (Geyer et al., 1993). This gene produces two 3.2 kb alternatively spliced, ovary-specific transcripts that differ in their temporal expression (Geyer et al., 1993; Sass et al., 1993). P-element insertions in the *otu* promoter and noncoding regions affect the levels of *otu* expression and this variable expression is correlated with the severity of the mutant phenotype even though transcript initiation occurs at the major start site (Sass et al., 1993). The predicted product is proline-rich and hydrophilic (Steinhauer et al., 1989), but the amino acid sequence is otherwise unrevealing.

Gonial cell tumors

Mutations in two genes give rise to tumorous adult gonads in both sexes. Null mutations in the *bag of marbles* (*bam*) gene result in the formation of abnormal germ cell clusters with increased cell numbers in both ovaries and testes (McKearin and Spradling, 1990). The *bam* gene gives rise to a 2.2 kb transcript, expressed in both male and female gonads, that encodes a protein with some similarity to the central portion of the otu protein (see above; McKearin and Spradling, 1990). Unfortunately, the protein sequence does not immediately reveal a putative functional role for this protein other than the presence of a PEST sequence, which is usually correlated with protein instability or rapid turnover (Rogers et al., 1986). The sequence and phenotypic similarity between *otu* and *bam* suggests that they may have related functions. Mutations in *benign(2)gonial cell neoplasm* also cause tumorous growth of the germ line in both males and females. The ring canals that join nurse cells to the oocyte in the wild type are missing in the ovaries of this mutant, suggesting that the differentiation of the female germ line does not progress beyond the gonial stage; the male germ line also fails to progress beyond the gonial stage (Gateff and Mechler, 1989).

FUTURE DIRECTIONS

In this article, we have shown the utility of *Drosophila* as a model system for identifying and analyzing TSG functions. At least nine cloned *Drosophila* TSGs (*Notch, shibire, fat, twins, expanded, lethal(1)discs large, lethal(2)giant larvae, hopscotch* and *aberrant immune response-8*) have human homologs that may have important roles in growth control and differentiation. Many more TSGs have been identified in *Drosophila* but not yet cloned, and these may direct attention

to additional mammalian homologs. Furthermore, not all chromosomes have been exhaustively screened for such mutations and it is therefore very likely that many more TSGs remain to be discovered. Little effort has been devoted to finding mutations that cause overgrowth in embryonic stages or in mesodermal tissues, so that even more genes could probably be found by searching for mutations with such phenotypes. In addition to its utility for rapidly discovering new TSGs, *Drosophila* provides a variety of genetic methods that will be useful in analyzing the interactions and functions of these genes at the molecular, cellular and developmental levels.

The authors' research is supported by grants from the National Institutes of Health and the National Science Foundation.

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