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INSIGHTS FROM MODEL SYSTEMS
Understanding Human Cancer in a Fly?

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> *"As Gregor Samsa awoke one morning from uneasy dreams he found himself transformed in his bed into a gigantic insect . . ."*
>
> [Franz Kafka, *The Metamorphosis*]

As foreign as an insect's body felt to Kafka's Samsa, the homology of flies and humans is astonishing. Approximately 80% of human genes have a genetic homologue in the fruit fly, *Drosophila melanogaster* (Cookson 1997). Although *Drosophila* has only ~10,000 genes in its genome, compared with ~100,000 genes in a mammalian cell, most human genes are duplications and elaborations of their insect equivalents (Lundin 1993; Holland et al. 1994; Miklos and Rubin 1996). In fact, not only individual domains and proteins but entire complexes and multistep pathways are conserved between fly and man (Artavanis-Tsakonas et al. 1995; Wasserman et al. 1995).

Because so many pathways are conserved, a significant number of genes studied in flies have proved to be homologues of human oncogenes and tumor suppressors (Miklos and Rubin 1996). The knowledge that we have gained from studying these *Drosophila* genes and the biological processes in which they participate contributes to our understanding of the mechanisms of action of their human counterparts.

Perhaps it is less widely known that *Drosophila* can develop tumors and that they display the full range of characteristics of human cancers. As we describe below, powerful genetic screens have been designed to identify genes that are responsible for such neoplastic growths in flies. When pursued, human homologues of fly tumor suppressors, and vice versa, have been isolated and have been shown to be functionally conserved. Thus, *Drosophila* is an excellent model for studying the molecular mechanisms of tumorigenesis and directly contributes to our understanding of cancer biology.

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Identification of Tumor Suppressors in *Drosophila*: The Power of Genetic Mosaics

Hereditary tumors were reported in the earliest days of *Drosophila* genetics. In 1916, Bridges discovered a spontaneous lethal mutation that causes homozygous larvae to develop "black granules" in their bodies. Later, these granules were characterized by Stark (1918) as being "melanotic tumors." When human carcinogens were examined in *Drosophila,* they were found to efficiently induce various tumors (Harshbarger and Taylor 1968). Subsequently, the properties of *Drosophila* tumors caused by a number of genetic lesions were further analyzed by Gateff and others (Gateff and Schneiderman 1969; Gateff 1978). Many of these tumor-causing lesions behave as recessive mutations and therefore are defined as tumor suppressors (e.g., see Woods and Bryant 1989; Mahoney et al. 1991). Most of these tumor suppressors were identified because they cause late-larval lethality in homozygous animals. Phenotypic characterization of dead larvae by dissection subsequently revealed occasional overproliferation of internal tissues. Using this information, systematic screens of collections of late-larval lethal mutants identified more tumor suppressors (Török et al. 1993).

Many tumor suppressors regulate cell proliferation and differentiation during early development. Consequently, germ-line mutations in these genes may cause embryonic lethality in homozygous animals. To circumvent this, we have been screening for tumor suppressors in mosaic flies (Xu et al. 1995; also see fig. 1 and sidebar). In such screens, populations of heterozygous embryos are produced from mutagenized parents. A few somatic cells in each developing heterozygous animal are then induced to become homozygous for the newly generated mutation. Mosaic individuals that carry small

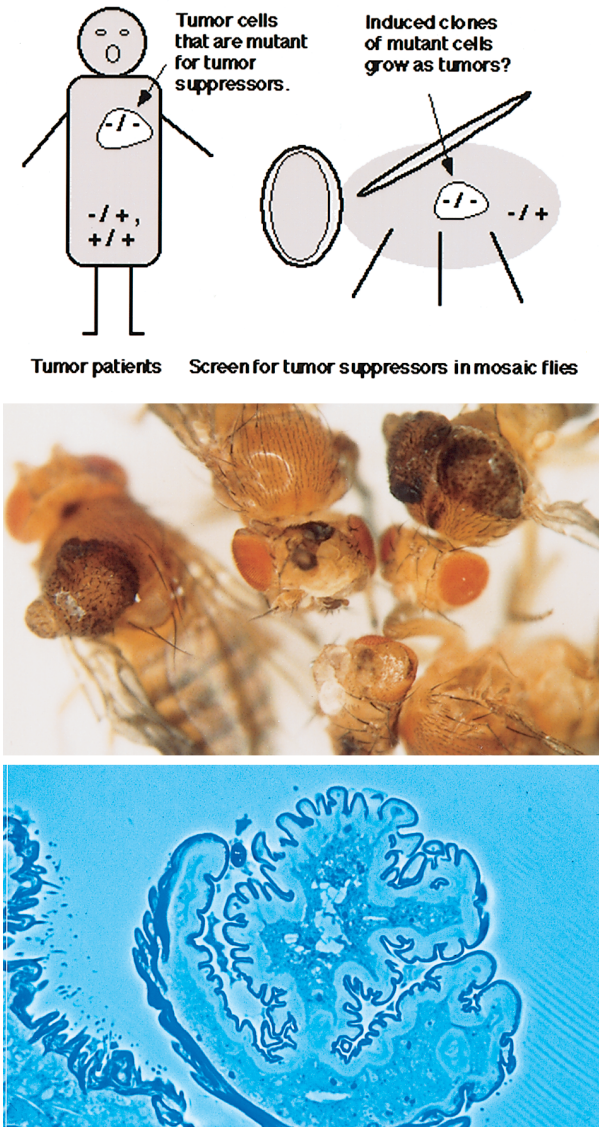


Figure 1 Identification of tumor suppressors in mosaic flies. Although animals that are homozygous for a lethal mutation could die at an early developmental stage, mosaic flies carrying clones of cells that are homozygous for the same mutation can live. One can identify potential tumor suppressors by generating and examining clones of overproliferated mutant cells in mosaic animals. The genetic constitution of these mosaic flies is similar to the mosaicism of cancer patients. The middle panel shows that mosaic flies carrying clones of cells mutant for the *lats* tumor suppressor develop large tumors in various tissues; the bottom panel shows the irregular growth and lumen-containing structures in a *lats* tumor section. (The top panel of this figure is reprinted from the article by Xu et al. [1995, p. 1055] and is reprinted here with permission from the journal *Development* and Company of Biologists Ltd.)

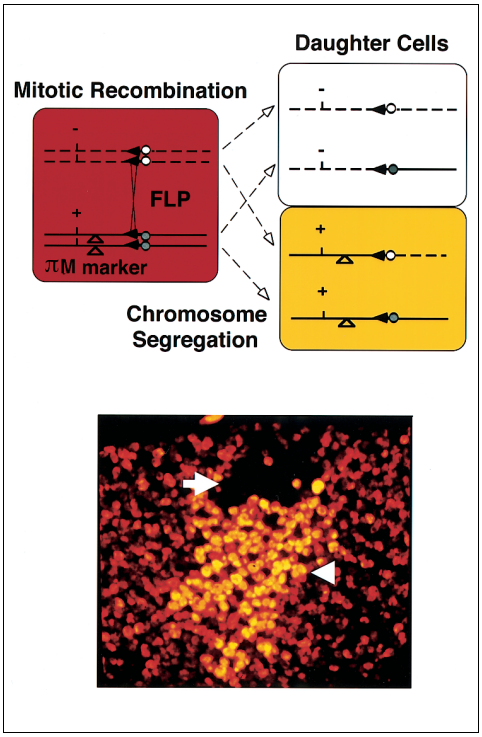
patches of cells mutated for a tumor suppressor are viable, yet the overproliferation mutant phenotype is readily detectable. Once a tumor-carrying fly is identified, the mutation of interest can be recovered from its heterozygous germ-line cells by mating (Xu and Harrison 1994; also see sidebar). The mosaic nature of these flies mimics the genetic constitution of human cancer patients who are chimeric individuals carrying a small number of cells that are mutant for tumor suppressors or proto-oncogenes (fig. 1). Such mosaic screens were achieved because of the construction of special *Drosophila* chromosomes (Xu and Rubin 1993). The FRT (*F*LP *r*ecombinase *t*arget) sequence, which is the recombination target site of the yeast FLP enzyme, has been inserted near the centromere of each chromosome arm. When FLP is induced in developing flies, high frequencies of mitotic recombination occur between FRT sites on homologous arms (Golic 1991). Thus, mutant clones can be efficiently induced for most of the fly genome. Various genetic and cell markers have also been introduced into these FRT-carrying chromosomes (Xu and Rubin 1993; Xu et al. 1995). Cosegregation of these markers and the mutation of interest allows the identification of the clones of mutant cells in both adult and developing tissues (see sidebar).

Using this mosaic-screen approach, we have identified several novel genes that, when inactivated, cause dramatic overproliferation phenotypes (Xu et al. 1995). These mutations cause embryonic lethality without obvious morphological defects and thus were not identified in previous genetic screens that examined homozygous mutants. The identified overproliferation mutations can be classified into two different groups. Mutations in the first group cause mutant cells to overproliferate and to form unpatterned tumorous tissues, whereas the second type of mutations induce patterned outgrowth structures in addition to tumorous tissues. Our genetic and molecular characterization suggests that the first type of mutations affect components that are involved in the regulation of the cell cycle, whereas the second type of mutations perturb developmental signals regulating pattern formation (W. Tao, M. A. R. St. John, S. Zhang, R. A. Stewart, and T. Xu, unpublished data).

The *Drosophila large tumor suppressor* gene (*lats* [which is also known as *warts*]) is an example of the first group of mutations and has been extensively characterized (Justice et al. 1995; Xu et al. 1995). Molecular characterization of *lats* predicts its gene product to be a novel Ser/Thr protein kinase with a putative SH3-binding site. Somatic cells that are mutant for *lats* undergo extensive proliferation and form large tumors in many tissues of mosaic animals (fig. 1). Animals that are homozygous for the various *lats* alleles display a wide range of developmental defects, including embryonic lethality, overproliferation of both neural and epidermal tissues, rough eyes, and sterility.

FLPing out tumor-suppressor genes

Working with *Drosophila* permits us to generate and analyze chimeric animals with distinct and defined genotypes in different tissues. The FLP/FRT site-specific recombination system from yeast has been introduced into the fly genome to efficiently generate genetic mosaics. The FRT sequence is integrated into chromosome arms proximal to the centromeres (Xu and Rubin 1993). Expression of FLP recombinase is under the control of the inducible-heat-shock promoter (Golic and Lindquist 1989). By inducing heat shock, we can activate mitotic recombination at any point during fly development. In a heterozygous cell, FLP induces mitotic recombination between FRT sites on homologous chromosome arms. Segregation of recombinant chromosomes at mitosis can produce a cell homozygous for the part of the chromosome distal to the FRT sites, where a mutant tumor-suppressor gene, such as *lats,* may reside. The cosegregation of π-MYC (πM), a cell marker, on the same arm allows the identification of the mutant cells and its descendants (white cells). The complementary recombination product is homozygous wild type and carries two copies of the πM marker.



This micrograph shows a section of a mosaic larval eye disk in which recombination was induced. The disk was stained to allow visualization of the πM cell marker. A clone of nonfluorescent cells that lack the πM marker (*arrow*) is accompanied by a brightly stained twin-spot clone (*arrowhead*), which expresses the πM protein at a higher level than the heterozygous background cells. Clones lacking the marker are homozygous for a mutation in a tumor-suppressor gene and, as such, are destined to overproliferate to form large tumors. Mosaic animals are instrumental for the identification of tumor-suppressor genes such as *lats* (Xu et al. 1995), whose functions are conserved from flies to humans. (The bottom of this figure is from the article by Xu and Rubin [1993, p. 1226] and is reprinted here with permission from the journal *Development* and Company of Biologists Ltd.)

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Drosophila neoplasms display a range of characteristics. The assessments used to define neoplasia in *Drosophila* are quite similar to those used by clinical tumor pathologists: (1) in situ cell overproliferation, (2) altered cell morphology, (3) loss or decrease of differentiation capacity, (4) in situ invasiveness, and (5) transplantability (Mechler and Strand 1990). The two last characteristics distinguish malignant from benign neoplasms. After transplantation, malignant neoplasms grow aggressively and kill the host, whereas benign neoplasms are unable to proliferate. As in human cancer, benign neoplasms remain confined to the tissue of origin.

Tumors that result from inactivation of *lats* display many features of human neoplasms. The *lats* mutant cells grow aggressively, and a single mutant cell can develop into a tumor that is one-fifth the size of the animal. These tumors are highly irregular in shape and size and are often poorly differentiated. Sections of these tumors reveal that they harbor lumen-containing structures, which is reminiscent of aggressive angiogenesis in human tumors (Xu et al. 1995; also see fig. 1).

Malignant growth has been well documented in fly tumors derived from *lgl* mutants (Woodhouse et al. 1994). The flies with the *lgl* mutation do not survive beyond the larval stage, but, amazingly, when the larval brain tumors are transplanted into a normal adult fly, the tumors invade and spread to distant organs. Interestingly, examination of specific proteins (e.g., type IV collagenase) that are differentially expressed in mammalian metastatic tumors have revealed that homologous proteins are also expressed in *lgl* tumors (Woodhouse et al. 1994). These data strongly suggest that metastasis of *Drosophila* tumor cells is similar to that of human tumors.

Moreover, evidence has started to emerge that some human homologues of fly tumor suppressors may play roles similar to those in their fly counterparts. In the case of *lats,* its human homologue has been isolated. The human *lats* gene is able to suppress tumorigenesis and to rescue all developmental defects in *lats* mutants (W. Tao, M. A. R. St. John, S. Zhang, R. A. Stewart, and T. Xu, unpublished data). Such data provide strong evidence that *lats* is conserved from fly to human, indicating that we can use the fly to understand many aspects of human cancer.

Tip of the Iceberg

In addition to recessive overproliferation lesions, gain-of-function mutations that lead to tumor formation have also been identified in *Drosophila.* For example, *Tumorous-lethal* (*hoptum-l*), a dominant mutation in the *Drosophila Hopscotch*/Janus kinase gene, causes hematopoietic overproliferation (Harrison et al. 1995; Luo

et al. 1995). Similar to their human counterparts, activation mutations of the fly *ras* and *Notch* genes cause overproliferation defects (G. Rubin and S. Artavanis-Tsakonas, personal communication). In each case, the entire signal-transduction pathway in which the gene participates is conserved from fly to human (Artavanis-Tsakonas et al. 1995; Wasserman et al. 1995).

Because the molecular pathways that have been studied are well conserved, other essential pathways for regulation of cell proliferation in humans should also be present in flies. The wealth of knowledge about *Drosophila* developmental biology, as well as experimental tools such as genetic modifier screens for assembling genes into pathways, make the fly one of the best systems for dissection of functions for human cancer genes. The characterization of *RBF,* the *Drosophila* homologue of the retinoblastoma-susceptibility gene, is one such example (Du et al. 1996). Once again, the molecules of the *Rb* pathway and their biochemical properties are conserved in flies. *RBF* regulates cell proliferation by modulation of gene expression, through binding of the *Drosophila E2F/DP* complex (Dynlacht et al. 1994; Du et al. 1996). However, it is clear that the fly *RBF* pathway is much simpler than the human one. Although one *RBF* and two *E2F* have been identified in *Drosophila,* three *Rb*-family molecules (Rb, p107, and p130) and at least five *E2F* members have been found in mammals (Weinberg 1995). Characterization of the *Enhancer* and *Suppressor* modifier genes of *RBF* and *dE2F* will soon provide new details about the *Rb* pathway.

The mosaic screens used to identify tumor suppressors in *Drosophila* are by no means saturated. Future genetic screens will identify more and perhaps most of the negative regulators of cell proliferation. On the other hand, as the human- and fly-genome projects progress, many more fly homologues of human cancer-causing genes will be revealed. Given the extraordinary conservation of the molecular pathways that exists between flies and humans, *Drosophila* is destined to become one of the hottest models for cancer research.

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