REVIEW

Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors

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Mammalian epithelial tumors lose polarity as they progress toward malignancy, but whether polarity loss might causally contribute to cancer has remained unclear. In *Drosophila*, mutations in the "neoplastic tumor suppressor genes" (nTSGs) *scribble*, *discs-large*, and *lethal giant larvae* disrupt polarity of epithelia and neuroblasts, and simultaneously induce extensive overproliferation of these cells, which exhibit malignant-like characteristics. Herein I review what is known about the role of the fly nTSGs in controlling cell polarity and cell proliferation. Incorporating data from mammalian studies, I consider how polarity and proliferation can be coupled, and how disruption of polarity could promote cancer.

In 1967, Elizabeth Gateff and Howard Schneiderman published a short note in the *American Zoologist* describing a *Drosophila* mutation that caused affected cells to "grow rapidly and invasively and kill their hosts" (Gateff and Schneiderman 1967). The mutant cells "[behaved] like a malignant tumor." This mutation, *lethal giant larvae* (*lgl*), acted in a recessive fashion, and was thus distinct from the transforming oncogenes identified shortly thereafter. The potential existence of "recessive oncogenes" had long been appreciated, dating back to Boveri (Boveri 1929). However, Gateff and Schneiderman's announcement was the first example in vivo of a gene in which loss of function resulted in tumor formation (Gateff and Schneiderman 1967). Thus, prior to Knudson's epidemiological evidence that the retinoblastoma (Rb) locus in human populations acted recessively (Knudson 1971), and contemporary with Harris's somatic cell hybrid experiments that gave rise to the term "tumor suppressor" (Harris et al. 1969), *Drosophila* provided the first example of a tumor-suppressor gene (TSG).

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Subsequent years saw the isolation of additional tumor-causing mutations in *Drosophila*, and the similarities between the *Drosophila* tumors and human tumors, outlined in Gateff's 1978 review in *Science* (Gateff 1978), initially occasioned significant interest. However, as human and fly TSGs were cloned, the lack of overlap in protein sequence, cellular localization, and proposed function led to decreasing attention from cancer biologists. Moreover, as it became appreciated that human cancer results from multiple genetic lesions, the relevance of fly models in which loss of a single gene could lead to dramatic overproliferation aroused skepticism. From an auspicious beginning, *Drosophila* became rather overlooked as a model system for cancer studies.

Recent years have seen a remarkable turnaround. The potential of fly research to address difficult questions in the etiology of cancer (as well as distinctively human pathologies such as Alzheimer's and Parkinson's diseases) is newly appreciated (Bernards and Hariharan 2001). *Drosophila* has come into its own in particular as a system in which to identify new TSGs, and the relevance of fly TSGs to human cancer has become increasingly clear. The past few years have seen reports that TSGs first identified in the fly are mutated in human cancers and can cause tumor susceptibility in mice (e.g., St John et al. 1999; Spruck et al. 2002; Fuja et al. 2004; Rajagopalan et al. 2004). Analyses of fly TSGs have also produced substantial contributions toward understanding the basic cell biology of tumorigenesis, in particular links between cell growth, cell proliferation, and apoptosis.

The unbiased genetic screens possible in *Drosophila* have often provided entry points into studies of phenomena that have been recalcitrant to other approaches. In mammalian tumor biology, one such phenomenon concerns the transition of tumor cells from benign overproliferation to a fully transformed, malignant phenotype. In carcinomas (malignant tumors of epithelial origin), one of the primary diagnostic features of transformation is a pronounced disorganization of cell architecture. The relationship between loss of epithelial organization and progression toward malignancy in mammalian tumors

has long been known, but whether this is merely correlative or whether loss of architecture might have some causative contribution to tumorigenesis remains a compelling and critically important question.

Remarkably, research on the original fly TSG—*lgl*—is now providing insight into this issue. *lgl*, along with a second gene, *discs-large* (*dlg*), controls not only cell proliferation but also epithelial organization. Recently, the identification of a third gene in this class, called *scribble* (*scrib*), and the finding that the three genes act together in a single pathway, has raised interest in the possibility that studies of *lgl*, *dlg*, and *scrib* will reveal a molecular mechanism by which growth control and cellular architecture are linked. This review provides a context to consider what studies of these fly TSGs tell us about links between tumor suppression and cell architecture. I first discuss the functions of *lgl*, *dlg*, and *scrib*, and then consider what aspects of their mutant phenotypes may provide informative parallels to human cancer. Finally, I speculate on the mechanisms by which polarity and proliferation could be coupled in epithelial tissues, and highlight mammalian data that are consistent with this concept.

Drosophila tumor suppressors: one term, many types

Before discussing fly TSGs, it is important to consider what is meant by a fly tumor. A minimal definition of a tumor encompasses a mass of overproliferating tissue whose growth is irreversibly uncoordinated from that of normal cells. Several types of abnormal growths, induced by genetic changes in the fly, meet these criteria. Although the similarity is necessarily inexact, it is reasonable to call these growths fly tumors.

The literature on insect or, indeed, invertebrate tumors in wild populations is quite thin (Harshbarger and Taylor 1968). *Drosophila* tumors have been found serendipitously as well as through genetic screens that seek to identify mutations causing excess cell proliferation. The majority of these mutations act in a recessive rather than dominant manner. They therefore do not act as oncogenes but instead as TSGs. There have been objections to the broadening and varied usage of the TSG designation over the years, and in particular its application to nonmammalian species. However, one current accepted definition is a gene in which loss-of-function mutations give rise to cellular overproliferation (Haber and Harlow 1997), and by this criterion the fly contains many TSGs.

Overproliferation can be assayed in the fly by examining an epithelial organ called the imaginal disc. Imaginal discs are larval tissues that serve as the primordia for most adult structures. They are set aside during embryogenesis as clusters of 20–50 cells, but must proliferate to their final sizes of 20,000–50,000 cells before they undergo metamorphosis in the pupal case. Their well-described development has made imaginal cells, in particular of the eye and the wing discs, a favorite system in which to study cell proliferation control. Moreover, because they proliferate during the larval stages, issues of maternal contribution are avoided, whereas the ease of inducing clones via mitotic recombination makes them amenable to mosaic analysis as well as measures of doubling times. Most screens for proliferation mutants have been performed using imaginal discs, although mutations that affect the proliferation of nonepithelial cells, such as the neuroblasts, germ cells, and hemocytes, have also been identified (Watson et al. 1994).

It is important to distinguish between the several groups of *Drosophila* genes that have been referred to as TSGs. For instance, *Drosophila* contains homologs of most known human tumor suppressors, including p53, Rb, NF1, and APC (Sutcliffe et al. 2003). Mutations in these genes have been generated through reverse genetics, and analyses of their phenotypes in the fly have helped clarify their roles in vertebrate cancer. However, many of these mutations themselves do not cause overproliferation in the fly, and by this criterion are not in fly TSGs.

The "true" fly TSGs can be considered those that, when mutated, cause cellular overproliferation leading to tissue overgrowth (tumors). Fly tumors have traditionally been subdivided into two groups, hyperplastic and neoplastic. In "hyperplastic" tumors, imaginal discs contain increased cell numbers, but, despite often extensive overproliferation, the cells are normally shaped and remain arranged in an epithelial monolayer, ultimately differentiating into adult tissues. Hyperplastic tumors can be caused by inactivating mutations in genes that regulate cell (as opposed to tissue) growth (e.g., *PTEN*, *Tsc1/2*, *salvador*, *warts*, *hippo*; for review, see Pan et al. 2004), in genes that coordinate proliferation and cell death (again *salvador*, *warts*, *hippo*; for review, see Rothenberg and Jan 2003), or genes that function in a pathway apparently limiting total organ size (e.g., *fat*, *ex*; for review, see Johnston and Gallant 2002). In contrast, in "neoplastic" tumors, which are caused by mutations in *lgl*, *dlg*, or *scrib*, the overproliferating cells lose the ability to organize an epithelial monolayer. Neoplastic cells are rounded rather than polygonal, and pile up atop one another as they overproliferate; they are incapable of terminal differentiation. The use of the terms hyperplastic and neoplastic to distinguish between *Drosophila* tumors that retain or lose epithelial organization has caused some confusion among cancer biologists, because it corresponds only loosely to how these terms are used in the context of pathology. In particular, "neoplasm" can connote any abnormal growth, including both well-differentiated benign tumors as well as malignant tumors with aberrant cell architecture. Although the confusion is understandable and regrettable, the terms are sufficiently well-ensconced in the *Drosophila* literature that it is difficult to justify a change of nomenclature.

In fact, the term "neoplastic tumor suppressor gene" (nTSG) was originally chosen in part to call tumor biologists' attention to the fact that these mutant fly cells, with their overproliferation, loss of epithelial architecture, and failure to differentiate, share several features with human cancer cells. Although the differences between the developmental abnormalities of an insect and a human pathological condition are great, the evident

parallels suggest that the nTSGs may reveal basic principles by which polarity and proliferation control are linked. The precise mechanisms by which the nTSGs do so remain shrouded, but some themes are becoming clear, and these are discussed below.

nTSGs: an overview

The three currently known nTSGs—*lgl*, *dlg*, and *scrib*—were discovered at different times and in different ways. Although *lgl* alleles were first isolated by Bridges in the 1930s (Bridges and Brehme 1944), it was not until the *lgl4* allele arose spontaneously that its ability to produce neoplastic tumors was appreciated. Shortly after *lgl4* was reported (Gateff and Schneiderman 1967), a genetic screen assaying imaginal disc morphology identified a similar mutation called *discs-large* (*dlg*; Stewart et al. 1972). Twenty-eight years later, a third mutation with the same suite of phenotypes, called *scribble* (*scrib*), was isolated in a screen for regulators of epithelial architecture (Bilder and Perrimon 2000).

The tumorous phenotypes shared by nTSG mutant animals are seen in the larval stage of the fly life cycle. All three genes have large maternal contributions, such that the heterozygous mother deposits sufficient wild-type gene product to allow homozygous mutant embryos (which would otherwise die because of polarity defects; see below) to progress into larval stages. These zygotic mutant animals grow normally until the third larval instar (L3; ~5 d after egg laying). At this stage, imaginal discs in wild-type larvae have reached full size, and proliferation in the discs ceases as hormonal changes induce formation of the pupa. However, L3 nTSG mutant larvae do not pupate, but instead continue to grow for up to 2 wk before their death. During this period, the animals increase in length and become bloated, leading to an easily recognizable "giant larva" phenotype. Dissection of mutant animals reveals that, whereas most nonproliferating larval tissues show fairly normal structure, the proliferating cells of the imaginal epithelia and nervous system are dramatically aberrant. Rather than forming flat epithelial sheets, the mutant imaginal discs are solid spherical masses of cells, much larger than their wild-type counterparts (Fig. 1A). Additionally, the brain is elongated and enlarged because of hyperplasia of the optic lobes.

The strong similarities between the nTSG phenotypes suggested that the three genes might act in a common pathway, regulating a single process. This hypothesis is supported by genetic interactions between the nTSGs, as well as demonstrations that each nTSG protein is required for the proper localization and/or stability of the others (Bilder et al. 2000). Although differences can be discerned among the mutant phenotypes, it is not yet clear if these reveal distinct, independent roles or rather reflect hypomorphic alleles or differential perdurance of gene products. The current evidence is consistent with the view that in most cases the nTSG products together compose a "cassette" in which all three proteins are obligately involved.

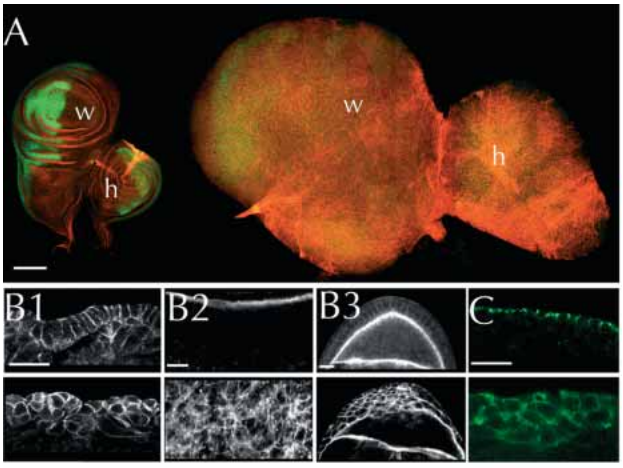

Figure 1. Phenotypes of nTSG mutations. (*A*) Tumorous tissue from nTSG mutant animals. Phalloidin staining of wild-type (WT; *left*) and *scrib* (*right*) wing (w) and haltere (h) discs, shown at identical magnification. *Scrib* discs are disorganized and overproliferative, and tend to fuse with neighboring discs. Note that this confocal section underestimates the size of the spherical, multilayered *scrib* tissue in comparison to the flat monolayered wild-type discs. Bar, 100 µm. (*B*) Defective architecture of nTSG mutant epithelia of the embryo (α-spectrin staining of maternally and zygotically mutant embryonic epidermis; *B1*), larva (phalloidin staining of a zygotic mutant wing imaginal disc; *B2*), and the adult (phalloidin staining of ovarian follicle cells; *B3*). (*Left*) Wild-type tissue. (*Right*) *scrib* mutant tissue. *B1* is reprinted with permission from Bilder and Perrimon (2000; © 2000 Nature http://www.nature.com). Bars, 10 µm. (*C*) Expansion of apical polarity in *scrib* embryonic epithelia, revealed by the apical marker Crumbs (green; apical is oriented up). Bar, 10 µm.

nTSGs encode junction- and cytoskeleton-associated proteins

Given the dramatic effect that loss of the nTSGs has on cell proliferation, it is surprising that all three encode not nuclear factors but, instead, cytoplasmic proteins found at the cell membrane (Fig. 2). *Dlg* encodes a scaffolding protein of the MAGUK family, containing three PDZ domains, an SH3 domain, and a GUK domain (Woods and Bryant 1991). In developing epithelia, Dlg is found throughout the basolateral domain, and becomes enriched at the apex of this domain as the epithelium matures and cell–cell junctions coalesce. These junctions include an adherens junction (AJ) that is identical to the vertebrate AJ in both its molecular composition and its position within the epithelium. However, flies do not have a tight junction (TJ) apical to the AJ. Instead, the paracellular barrier function of epithelia is provided by a distinct junction, the septate junction (SJ), that, although it shares some components with the SJ (Behr et al. 2003; Wu et al. 2004), is located just basal to the AJ (Tepass et al. 2001; Wu et al. 2004). In the mature epithelium, Dlg is found primarily in association with the SJ.

Scrib also encodes a membrane-associated, multi-PDZ scaffolding protein. However, Scrib is a LAP family pro-

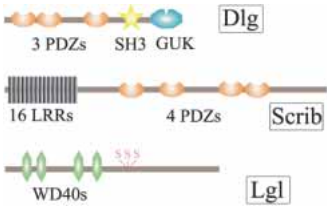

Figure 2. Structure of nTSG proteins. Domain architecture of the *Drosophila* nTSG proteins. This architecture is conserved with nTSG vertebrate homologs. (PDZ) PSD-95, ZO-1, Dlg homology domain; (SH3) Src homology 3 domain; (GUK) guanylate kinase-like domain; (LRR) leucine-rich repeats; (WD40) tryptophan–asparatic acid 40 residue repeat; (S) serine residues that are conserved phosphorylation sites targeted by aPKC.

tein, with 16 N-terminal leucine-rich repeats (LRRs) and four PDZ domains (Bilder and Perrimon 2000). The Scrib LRRs appear to be a protein–protein interaction domain closely resembling those of the Ras-binding proteins yeast Adenylate Cyclase and worm Sur-8. However, neither Ras nor another small GTPase has yet been found to bind to this region (Legouis et al. 2003; D. Bilder, unpubl.). Like Dlg, Scrib is restricted to basolateral epithelial surfaces and SJs, and, indeed, Dlg can be found in Scrib immunoprecipitates, demonstrating that the proteins can associate (Mathew et al. 2002). Consistent with association in a complex, animals mutant for either *scrib* or *dlg* show reduced levels and mislocalization of the other protein (Bilder et al. 2000). Scrib and Dlg are also found together at synaptic junctions and in embryonic neuroblasts, indicating that their association is not limited to epithelial tissues (Mathew et al. 2002; Albertson and Doe 2003).

Unlike Scrib and Dlg, Lgl is not an obvious scaffold, having no compelling structural homologies other than several WD-40 repeats (Jacob et al. 1987). Also unlike the other nTSGs, Lgl localization is not polarized along the apicobasal axis of epithelia (Strand et al. 1994). Yet, like Scrib and Dlg, Lgl is found at cell membranes, and this localization seems to be critical for Lgl function. A thermosensitive form of Lgl associates with the membrane at permissive temperatures, but at restrictive temperatures remains localized in the cytoplasm (Manfruelli et al. 1996). It is not clear through which interactions Lgl is brought to membranes. However, in *scrib* or *dlg* mutant embryos, Lgl plasma membrane association is also lost (Bilder et al. 2000). This finding suggests that a critical role of Scrib and Dlg is to recruit Lgl to the membrane, where Lgl acts to mediate its currently unclear function.

nTSG-induced tumors

The functions of the nTSGs are best illustrated by considering the cellular consequences of their loss. NTSG mutant cells are defective in polarity, proliferation control, and differentiation, and display altered behavior suggestive of invasive capability. Remarkably, these phenotypes are all features of malignant mammalian tumors as well. I discuss below the phenotypes of fly nTSG mutant cells individually, starting with the best understood, polarity.

Polarity

The loss of epithelial polarity and tissue architecture is a primary diagnostic mark of malignant carcinomas in tissues such as breast, prostate, and colon. Most features of simple epithelia, including regular cell shape, tight adhesivity, monolayered organization, and apicobasal polarity, are well conserved between flies and mammals, allowing epithelial architecture to be profitably studied in *Drosophila*. Three oft-studied fly epithelia are the embryonic ectoderm (the precursor of the larval epidermis), the larval imaginal discs (precursors of the adult epidermis), and the adult ovarian follicle epithelium (a somatic epithelium that surrounds and supports the developing germ line). In these three tissues, cells mutant for nTSGs display disruption of all epithelial features (Fig. 1B). The classic columnar or cuboidal monolayer is replaced by rounded, poorly adhesive, misshapen cells that pile upon each other in a multilayer. Importantly, analysis indicates that polarity loss is the primary defect that leads to other morphological phenotypes. In the absence of an nTSG product, proteins normally restricted to the apical (free) membrane domain are found at ectopic locations in basolateral (contacting) cell surfaces (Fig. 1C; Woods et al. 1997; Bilder and Perrimon 2000). The same is true for proteins of the adherens junctions (AJs), and, indeed, ectopic AJs can be seen ultrastructurally (Woods et al. 1996; D. Bilder, unpubl.). In contrast, basolateral proteins seldom expand into the free apical cell surface, indicating that mutant cells are not completely apolar but, instead, have lost the ability to restrict apically destined proteins. Because this phenotype is seen in all epithelia examined, the nTSGs seem to be critical polarity regulators that are used every time the fly makes a cell with epithelial architecture.

In addition to the three nTSGs, five additional genes that regulate epithelial polarity have been identified through genetic studies (for review, see Knust and Bossinger 2002). The eight genes together encode essentially three protein complexes, each centered around proteins containing the PDZ protein–protein interaction domain. For ease they can be referred to as the Scrib (/Dlg), the Par-3 or Baz (/Par-6/atypical Protein kinase C), and the Crumbs (/Stardust) complexes; Lgl is a special case (see below). Integrin-based matrix adhesion apparently does not play a major role in *Drosophila* apicobasal polarity (Brown 2000). The polarity complexes are highly conserved between invertebrate and vertebrate epithelia, not only in their composition but also (despite the differing positions of the invertebrate SJ and the vertebrate TJ) in their polarized expression along the apicobasal axis of epithelial cells (Fig. 3A). At the resolution of confocal microscopy, the Crumbs complex is the most apical, found just above the ZA; the Par-3 complex overlaps the ZA and the Crumbs-containing region; and the Scrib

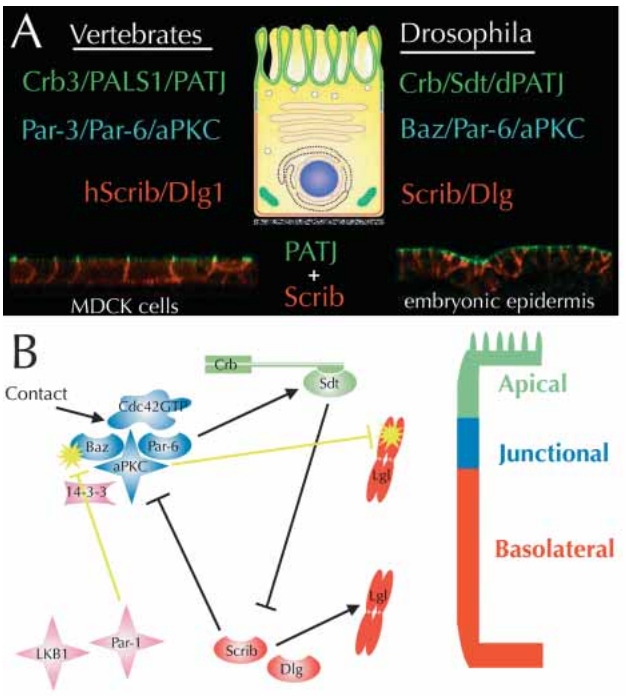

Figure 3. Role of nTSG proteins in epithelial polarity. (*A*) Diagram of subcellular localization of polarity regulators with respect to a generic epithelial cell. NTSG proteins and their vertebrate homologs (*below*, Scrib staining, red) are restricted to lateral surfaces, basal to the zonula adherens and the apical junctional complex (PATJ staining, green). MDCK staining courtesy of J.-P. Borg (Inserm, Marseille, France). (*B*) Speculative model outlining regulatory interactions during *Drosophila* epithelial polarization. Bazooka (Baz) is the fly homolog of Par-3. Yellow stars represent inhibitory phosphorylation events. Note that not all relationships may be active in every tissue.

complex is found just basolateral to the ZA, at the site of the SJ. In the embryo, the three complexes act in a single integrated hierarchy (as opposed to having independent activities) to regulate polarity (Fig. 3B; Bilder et al. 2003; Tanentzapf and Tepass 2003). The Par-3 complex is the initial apical regulator, whereas the Scrib complex distinguishes a basolateral domain by repressing the apicalizing activity of Par-3. Par-1 and 14–3–3 may also contribute to the basolateral exclusion of Par-3 (Benton and St Johnston 2003). To counteract the full repression of the Scrib complex, Par-3 recruits the Crumbs complex apically, and Crumbs indirectly represses Scrib activity. The careful balance of activities between these complexes thus sets the limits of the apical and basolateral membrane domains and positions the ZA appropriately at the interface between the two.

The molecular mechanisms underlying the regulatory hierarchy between the nTSGs and other polarity genes are not fully known. However, a recently described physical interaction between Lgl and apical complex proteins provides a satisfying molecular basis for the genetic data (Betschinger et al. 2003). Although Lgl is clearly in the nTSG class of proteins, sharing phenotypes and showing genetic interactions with Scrib and Dlg, it is found in complex not with these other two nTSGs but rather with Par-6 and aPKC. Lgl is, in fact, an in vivo substrate for aPKC, and phosphorylation of Lgl promotes its dissociation from the plasma membrane (Kalmes et al. 1996; Betschinger et al. 2003). The Par-3-dependent apical localization of aPKC therefore prevents Lgl association with the apicolateral membrane, hence restricting Lgl activity to the basolateral surface. Meanwhile, Scrib and Dlg antagonize Par-3 complex localization, limiting aPKC kinase activity to the apical pole. The net result is a robust partition of apical and basolateral portions of the plasma membrane. Because the membrane localization of Lgl is controlled by the basolateral Scrib complex (Bilder et al. 2000), whereas its activity seems to be negatively regulated by the apical Par-3 complex, Lgl appears to be a critical molecular integrator of apical and basolateral activities.

Although these data are exciting, the cell biological activities through which Lgl regulates polarity remain to be determined. An intriguing hint comes from studies of polarity in yeast. Unlike the other epithelial polarity proteins, Lgl has strong homologs in two *Saccharomyces cerevisiae* proteins: SRO7p and SRO77p (Kagami et al. 1998). Yeast mutant for both proteins are defective in bud formation, a process that, like epithelial polarity, requires polarized trafficking and localization of spatial determinants (Lehman et al. 1999). As with Lgl, the yeast proteins are found at the cell cortex, where they associate with myosin II and the plasma membrane t-SNARE SEC9p. Although a requirement of SNARE and/or myosin binding for SRO7/77 activity has not yet been demonstrated, these interactions have suggested that Lgl family members may function by regulating polarized vesicle trafficking. Indeed, *SRO7/77* mutant yeast show an accumulation of undocked transport vesicles in the bud tip. Because both yeast bud formation and epithelial polarity involve vectorial protein transport, it is appealing to consider that the nTSGs, and perhaps the entire polarity hierarchy, control polarity by regulating polarized vesicle trafficking. Alternative models have also been suggested, such as that the Lgl–myosin II interaction modifies the cortical cytoskeleton to prevent binding of cytoplasmic determinants (Barros et al. 2003). Moreover, a role for the nTSGs in retention of previously localized proteins has not been ruled out. Answers to these questions will demand detailed analyses of protein trafficking in wild-type and mutant fly tissue.

The essential function of the nTSGs in polarity is not limited to epithelia. In the nervous system, nTSGs are required both for the asymmetric division of neuroblasts and for the organization of the neuromuscular synapse (Budnik et al. 1996; Ohshiro et al. 2000; Peng et al. 2000; Mathew et al. 2002). However, they are by no means required for all events of cell polarization. To give one example, the germ line must be correctly polarized to distinguish an oocyte from supporting nurse cells, as well as to pattern the major body axes within that oocyte; neither event requires nTSG activity. Surprisingly, although all fly epithelial cells require the Scrib complex

for apicobasal polarity, the Par-3 and Crumbs complexes are largely dispensable for disc epithelial architecture (Tepass and Knust 1990; Bellaiche et al. 2001). It is becoming clear that the Scrib, Par-3, and Crumbs complexes are best regarded as molecular cassettes whose activities can be modified and targets altered, from tissue to tissue and from organism to organism. Understanding the fundamental cell biological activity by which the cassettes function, for instance, by promoting targeted vesicle fusion or by retaining proteins at membrane domains, will allow an appreciation of how the diversity of polarized cell architectures seen in animals is generated.

Proliferation

Aberrant proliferation is the most salient characteristic of human tumors. Many tumor cells do not have a dramatically altered cell cycle; instead, overproliferation primarily results from a failure to respond to arrest cues, leading to unchecked cell division. At the level of the single cell, cell cycle control in *Drosophila* is similar to that in humans. In imaginal discs, the primary point of proliferation control is the G1/S regulator cyclin E, which is regulated both transcriptionally and posttranscriptionally. Apoptosis has only a minor function in determining total cell numbers (e.g., de la Cova et al. 2004). Interestingly, imaginal discs have an intrinsic growth control mechanism whereby overall tissue size, rather than cell numbers per se, is regulated (Johnston and Gallant 2002). For instance, an increase in cell size leads to a decrease in total disc cell numbers; moreover, a slowly growing disc will continue to proliferate until it reaches its proper full size. When discs attain their full size, most cell divisions cease and hormonal signals initiate the physiological changes that lead to terminal differentiation, imaginal morphogenesis, and pupa formation. The mechanisms that measure final disc size, and coordinate cell cycle exit and the hormonal changes of metamorphosis, remain poorly understood.

The growth control defects of nTSG mutant animals are most clearly seen in the imaginal discs of zygotic mutant larvae. As the maternal supply of wild-type gene product is exhausted, the nTSG mutant phenotype becomes evident in both the epithelial organization of disc cells and their proliferation. Surprisingly, cell counts reveal that during early larval stages, nTSG mutant discs are much smaller than their wild-type counterparts (Woods and Bryant 1989). The growth rate of mutant discs continues to lag such that, at the normal time of pupation (~5 d after egg laying), their numbers are only about one-third those of wild type. However, nTSG mutant animals never pupate and the disc cells continue to proliferate for up to 10 additional days. During this extended larval phase, disc cell numbers can reach more than five times the wild-type maximum before the animal dies. These data indicate that it is not a more rapid cycle that causes the observed overgrowth; indeed, proliferation in the mutant appears to be slower than wild type. Rather, it is the failure to exit from the proliferative cycle that results in tumor formation.

Because entry into postmitotic arrest correlates with metamorphosis in wild-type animals, one must consider whether the growth control phenotypes of zygotic nTSG mutants might result from a block in pupation of the larva rather than a defect in the disc cells themselves. Several lines of evidence strongly support the argument for a disc-autonomous requirement for the nTSGs in proliferation control. First, failure to pupate does not itself lead to excessive cell proliferation, as mutations that block the ecdysone pulse result in extended larval periods but do not cause large increases in cell numbers (Garen et al. 1977). Second, overproliferation occurs even when mutant discs are transplanted into a wild-type host. Larval discs and brains can be cultured in vivo in the abdomen of the adult (Hadorn 1968), and in this situation, wild-type tissue proliferates only slowly, and ceases as it reaches the proper size. However, when nTSG mutant disc or brain tissue is implanted, it uncontrollably proliferates, increasing in size as much as 200-fold to fill the abdomen before killing the host (Gateff and Schneiderman 1969). Third, when nTSG mutant clones are made in an otherwise wild-type disc through mitotic recombination, the mutant cells show an upregulation of cyclin E (Brumby and Richardson 2003). These cells are slower growing than their wild-type neighbors, and are ultimately eliminated by the process of cell competition (see below). Nevertheless, these data indicate that nTSG mutant cells continue to express critical cell cycle regulators at a time when wild-type cells have permanently exited the cell cycle.

As with polarity, proliferation defects are seen not only in epithelial but also in neuronal tissues. In zygotic mutant larvae, neuroblasts and ganglion mother cells overproliferate, giving rise to characteristically elongated optic lobes (Gateff and Schneiderman 1969; Woods and Bryant 1989). The comparative dynamics of proliferation in wild-type and mutant neural tissue are only now being closely studied (Rolls et al. 2003). However, normal larval neuroblasts are proliferative, polarized, and express nTSG proteins, thus it is reasonable to assume that they may be affected in a manner similar to the neoplastic epithelium. Because these cells are likely to lack some of the features associated with normal epithelial architecture (cell–cell junctions and contact with basement membranes; spatial patterning by diffusible signals), studies on the neuronal tumors may provide an informative comparison with epithelial tumors.

Can all dividing cells be transformed by nTSG mutation? The answer appears to be no. Despite the loss of polarity, embryonic cells lacking all nTSG product do not undergo additional mitoses. Evidence that nTSGs cause overgrowth of nonlarval tissues has been found in the follicle cells, where a mild and spatially restricted increase in cell numbers has been measured (Goode and Perrimon 1997). However, the dynamics of this overproliferation have not been explored sufficiently to reveal potentially general versus imaginal-specific aspects of growth control by the nTSGs.

Differentiation

In most cases, animal cells cease proliferation and permanently exit the cell cycle as they undergo stages of terminal differentiation. However, vertebrate cancer cells often exhibit significant loss of differentiated characteristics, and the lack of differentiation typically correlates with the lethality of the tumor. Similarly, the cells of fly nTSG tumors are incapable of terminal differentiation. For instance, *scrib* mutant cells in the eye neuroepithelium usually do not express markers of neuronal fate, and even when not eliminated by cell competition, the mutant cells do not form adult structures (Brumby and Richardson 2003). Moreover, whereas wild-type discs cultured in a wild-type host are capable of forming well-differentiated adult structures, *lgl* discs cultured in the same manner do not differentiate (Gateff and Schneiderman 1969). Finally, in the brain, affected cells remain as proliferative neuroblasts and ganglion mother cells rather than undergoing normal maturation to become ganglion cells (Gateff and Schneiderman 1969). Increased proliferation alone does not prevent terminal differentiation, as cells mutant for "hyperplastic" TSGs such as *fat* and *l(2)gd* will contribute to adult structures (Watson et al. 1994). Thus, differentiation defects are another aspect of the nTSG phenotype that mimics malignant human tumors.

Invasion and metastasis

Invasion and metastasis, rather than overproliferation per se, are the primary sources of morbidity and mortality in human cancer. Although much has been learned about physiological changes associated with invasion and metastasis, the genetic programs that enable cells to acquire these characteristics are very poorly understood. Understanding invasion and metastasis has been hampered by the absence of a reliable model system in which the advantages of genetic analysis, which have proved so powerful for studying other aspects of tumorigenesis, can be brought to bear. There is therefore great interest in the potential of *Drosophila* to study these phenomena.

Because cells exit the epithelium during the initial stages of invasion, regulators of epithelial polarity are also likely to regulate invasive behavior. Indeed, cells mutant for nTSGs show invasive characteristics in situ. The frequent fusion between mutant imaginal discs (Fig. 1A) could reflect invasive activity, because this is never seen in wild-type discs or comparably overgrowing hyperplastic mutants. A more clear invasion-like phenotype is seen in the adult ovarian follicle epithelium. Unlike the imaginal epithelia, the follicle epithelium is in close contact with a distinct tissue, the germ line, which it encloses in a monolayered sheath. nTSG mutant follicle cells are capable of leaving the epithelium and streaming into the germ-line cyst, where they penetrate between nurse cell membranes (Fig. 4A; Goode and Perrimon 1997). This process resembles border cell migration, a normal developmental event that has been extensively studied as a model of cell invasion (Montell 2001), but nTSG mutant cells do not acquire border cell fates. The invasive follicle cell phenotype is not well understood, and although it differs in several important ways from typical mammalian tumor invasions (e.g., the cells depart from the apical surface rather than degrading the basement membrane and exiting basolaterally), it does indicate acquisition of a novel capability not seen in other polarity or proliferation mutants (Abdelilah-Seyfried et al. 2003).

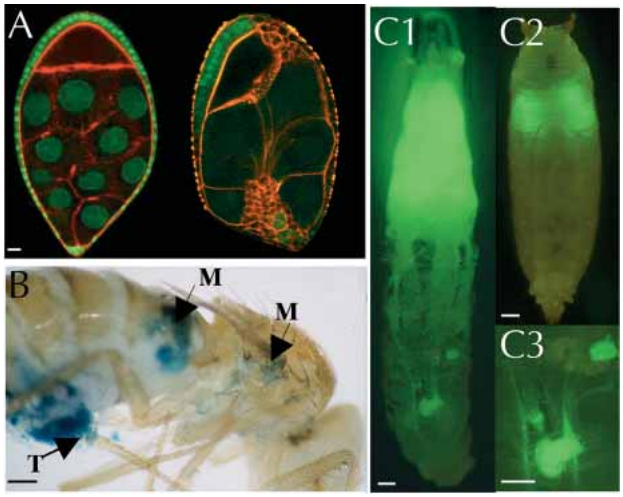

Figure 4. Invasion and metastasis-like phenotypes of nTSG mutant cells. (*A*) Invasive behavior of *lgl* follicle cells. Mutant cells (lacking GFP, compare with wild-type GFP-positive cells) exit the epithelium and move into the germ line. Bar, 10 µm. (*B*) Metastatic behavior of transplanted *lgl* brain cells labeled with *lacZ*. Mutant cells have left the primary tumor (T) and formed secondary metastatic growths (M). Reprinted with permission from Woodhouse et al. (2003). Bar, ~200 µm. (*C*) Metastatic behavior in situ of *scrib* mutant eye disc cells overexpressing activated Ras. These cells (labeled with GFP) invade into the brain and proventriculus, and also leave the primary tumor (T), attaching to other sites of the body (M, higher magnification in *C3*). Wild-type eye cells are shown in *C2*. Images courtesy of Ray Pagliarini (Yale Medical School, New Haven, CT). Bar, ~200 µm.

Invasion-like behavior is also seen in transplant assays. When nTSG mutant tissue labeled with a histological marker is implanted into wild-type adults, small secondary tumors can be seen growing far from the site of the primary tumor, attached to and in some cases invading host tissues such as intestine, muscle, and ovary (Fig. 4B; Woodhouse et al. 1998, 2003). Such dispersal is never seen in transplants of wild-type tissue, and a collagenase activity present in nTSG mutant cells has been described (Woodhouse et al. 1994), providing a potential mechanism for invasion. The broad dispersal of these cells is reminiscent of metastasis in mammals, although evaluation of metastatic phenotypes in the fly is complicated by anatomical differences between the two. *Drosophila* has an open circulatory system, in which the organs are bathed in a cavity containing nutrient-bearing hemolymph, while oxygen distribution is carried out by the

ramifying branches of the tracheal system; thus, intravasion and extravasion as a means of metastatic spread cannot be assayed. Nevertheless, these results suggest that nTSG mutant cells can exhibit behavior similar to cells at early stages of metastasis.

A dramatic demonstration of metastasis-like behavior in situ in *Drosophila* is provided by a recent study evaluating cooperativity between the nTSGs and oncogenic Ras (Pagliarini and Xu 2003). Although eye disc cells expressing activated Ras overproliferate, they are capable of terminal differentiation and remain in the epithelial sheet, without displaying invasive characteristics (Karim and Rubin 1998). However, when activated Ras-expressing eye cells are simultaneously mutant for *scrib*, they degrade the basement membrane and reliably invade into neighboring wild-type tissue. The contiguous larval brain was the most common site of invasion, but secondary metastatic growths can occur as well at distant sites (Fig. 4C). Activated Ras also strongly enhances the metastatic ability of *scrib* mutant tissue in adult transplantation assays. The specific functions that Ras and Scrib contribute to the invasive phenotype remain to be worked out. However, the data suggest that Scrib's contribution comes not from its effects on proliferation (because several hyperplastic tumor suppressors tested do not cooperate with Ras) but, rather, from its effects on polarity, because other polarity-defective mutants can cooperate with Ras, whereas coexpression of E-cadherin suppresses invasion. It appears that even subtle alterations in polarity can synergize with Ras to promote transformation and metastasis.

How closely do the fly tumors resemble human tumors?

The data reviewed above demonstrate that imaginal disc cells mutant for the nTSGs have lost growth control, acquired not only uncontrolled proliferative capacity but also the ability to form lethal tumors in hosts, and show evidence of invasive ability. When considered with respect to the physiological changes seen in malignant epithelial tumors (Hanahan and Weinberg 2000), a striking overlap is evident. It is surprising that mutations in a single fly gene can cause phenotypes reminiscent of so many aspects of human cancer, and it must be considered where such parallels are informative and where merely superficial. Although it is too early to answer this question definitively, a comparative analysis is informative.

Some phenomena seen in human tumors have not been documented in neoplastic fly tumors, although in most cases the data are insufficient to draw a firm conclusion about their absence. For instance, nTSG cells can undergo apoptosis throughout development (Woods and Bryant 1989), and, indeed, in mosaic clones the mutant cells can be actively eliminated by this mechanism (Brumby and Richardson 2003). Resistance to apoptosis does not therefore seem to make a major contribution to overproliferation. Genetic instability, seen in many malignant human tumors, has not been strongly noted in fly tumors (Mishra et al. 1997), nor have cytological differences such as increased nuclear or nucleolar size. A fly analog of angiogenic behavior is the recruitment of new tracheal branches to proliferating cells. Presumably the overgrowing tissue of the fly tumors actively induces tracheal recruitment, but how this happens in the deeply embedded cells of a compact nTSG tumor has not yet been analyzed. Telomere renewal and end protection are telomerase-independent in the fly (Pardue and DeBaryshe 2003) and are unlikely to impose a senescent limit on fly cell proliferation, although control of telomere length in nTSG mutants has not been explored.

Perhaps the most striking difference between mammalian and fly tumors is the number of genetic changes required for cellular transformation. Data from human tumors have long supported a multistep model. In the paradigmatic case of the colonic epithelium, a minimum of four to seven distinct changes are thought to be required for progression into a malignant intestinal neoplasm. In contrast, in the fly, mutation of any one of the three nTSGs alone is sufficient to cause neoplastic growth. This phenotype occurs in every mutant cell after only ~30 cell generations, and intermediate stages of transformation have not been reported. None of this suggests the involvement of additional genetic lesions in the development of fly neoplastic tumors.

What might account for the differences between the organisms, where a single mutation can transform fly tissue but multiple lesions are required to transform mammalian tissue? It is likely that the lengthened life span—months to years for most mammals versus several weeks for flies—has led to the evolution of redundant mechanisms to protect mammals, whereas these mechanisms are absent in the fly. Furthermore, development of fly tissues involves a small number of cell divisions, and in the adult only the gonadal cells along with blood cells and midgut remain proliferative. This fairly limited division of somatic cells makes the risk of spontaneous occurrence of tumorigenic mutations low. Flies may not need the elaborate backup systems and checkpoints that vertebrates do.

A related issue concerns the context in which human tumors and fly nTSG tumors arise. Human tumors originate as genetic mosaics, in which genotypic changes in one or a small number of cells confers upon those cells a proliferative advantage. However, nTSG mutant cells proliferate inherently more slowly than surrounding wild-type cells. In fact, small clones of nTSG mutant cells in otherwise wild-type imaginal discs do not form tumors (Woods and Bryant 1991; Agrawal et al. 1995; Brumby and Richardson 2003). Because discs in which every cell is mutant for an nTSG overgrow to form neoplastic tumors, why don't clones of nTSG mutant cells form tumors? And given that nTSG mosaic discs do not generate tumors, in what sense can the tumors of nTSG mutant animals be compared with human neoplastic tumors?

Answering this question requires an appreciation of the phenomenon of cell competition in *Drosophila*.

Within a given imaginal disc, clones of slow-growing cells are actively eliminated by apoptosis induced by surrounding cells. The molecular mechanisms of this cell competition are under investigation; current research has implicated levels of the proto-oncogene Myc as well as transforming growth factor β and Jun N-terminal kinase (JNK) signaling in this process (Adachi-Yamada and O'Connor 2002; Moreno et al. 2002; de la Cova et al. 2004; Moreno and Basler 2004). Brumby and Richardson (2003) found that when *scrib* clones in mosaic eye discs are protected from competition by blocking JNK signaling, the mutant cells, indeed, overgrow and kill the animal. Moreover, elimination of the wild-type disc cells via genetic ablation also enables *scrib* clones to overgrow and kill the animal. These results suggest that the failure of nTSG mutant clones to form tumors, in the manner that entirely mutant discs do, may be a consequence of their comparatively slow growth rate, which makes them fall prey to cell competition.

If nTSG mutations are sufficient to induce tumors when all cells in the disc are mutant, are there factors that will allow nTSG mosaic clones to do the same? Brumby and Richardson (2003) and Pagliarini and Xu (2003) have recently described a strong cooperative effect between *scrib* and a prototypical human oncogene, activated Ras, in proliferation control as well as metastasis. Coexpression of activated Ras in *scrib* mutant cells enables these cells, rather than being eliminated by surrounding cells, instead to outcompete their wild-type counterparts and form a large neoplastic tumor. Activated Ras alone does not impart this strong competitive advantage; it appears only in the context of polarity disruption, and cannot be solely attributed to Ras's ability to promote cell cycle progression or block cell death. These findings suggest that nTSG activities intersect with distinct pathways that are also involved in human tumorigenesis. It may be that other examples of cooperativity between fly TSGs and fly homologs of mammalian TSGs and oncogenes await discovery.

The requirement for additional genetic changes to induce tumor formation in nTSG mosaics is reminiscent of the multistage transformation of mammalian cancer cells. This analogy raises several intriguing questions. First, to what extent does manipulating JNK or Ras signaling protect *scrib* cells from cell competition alone, and to what extent does it endow *scrib* cells with additional tumorous properties? Are *scrib* cells eliminated only because of their slow growth, or do polarity defects or additional phenotypes also trigger their elimination by surrounding cells? Second, what is the relevance of the cell-competition-dependent elimination of nTSG mutant cells in flies to the formation of human tumors? Mammalian tumor cells can be held in check by surrounding tissues (Bissell and Radisky 2001; Liotta and Kohn 2001; Rubin 2003), and the epithelial organization of cells can influence their susceptibility to apoptosis (Jacks and Weinberg 2002), but whether these processes have functional or mechanistic similarities to *Drosophila* cell competition is not known. It will be fascinating to learn whether cell competition or an analogous form of "tissue surveillance" exists in mammalian epithelia.

Do vertebrate nTSG homologs play a role in cancer?

The role of Scrib, Dlg, and Lgl in fly tumor suppression has, of course, aroused interest in whether related human proteins play a role in cancer. Close vertebrate homologs of each fly nTSG exist. Dlg has four homologs among human proteins; these proteins have been renamed Dlg1–4 but are perhaps more familiar by previous names such as hDlg, Chapsyn-110, NE-Dlg, and PSD-95 (Fujita and Kurachi 2000). In contrast, Scrib has only one human homolog, hScrib, although related proteins include ERBIN, Lano, and Densin-180 (Santoni et al. 2002). Lgl has two human homologs, Llgl1 and Llgl2, and a somewhat related protein called Tomosyn is also present (Tomotsune et al. 1993; Strand et al. 1995; Fujita et al. 1998).

Like the fly nTSG proteins, their human homologs are broadly expressed in epithelial tissues, where they are localized to the basolateral domain (Wu et al. 1998; Legouis et al. 2003; Nakagawa et al. 2004). Also like their fly homologs, many of these proteins or their relatives are found at synaptic junctions (Sheng 2001). Synaptic partners of Dlg family members are conserved between human and fly, and complexes between LAP family proteins and MAGUKs have been reported in both species (Huang et al. 2001; Saito et al. 2001; Ohtakara et al. 2002). Binding between Lgl and Par-6 also occurs in vertebrates, as does phosphorylation of Lgl by aPKC (Plant et al. 2003; Yamanaka et al. 2003). Moreover, transgenic rescue experiments have shown that vertebrate Dlg and Scrib homologs can rescue their cognate mutants in *Drosophila* (Thomas et al. 1997; Dow et al. 2003). Together, these data indicate that many aspects of nTSG function have been evolutionarily conserved.

Is a role in tumor suppression one of the functions that has been conserved? Evidence to date has been suggestive but indirect. Large-scale sequencing of nTSG homologs from mammalian tumors has not yet been performed, but a small-scale study has reported Dlg1 mutations in breast cancers (Fuja et al. 2004), and microarray experiments have detected significant changes of nTSG homolog expression in several human cancers (Liu et al. 2002; Boussioutas et al. 2003). Immunohistochemistry has revealed a reduction of hScrib in invasive cervical cancers and Dlg1 in mammary ductal carcinoma, as well as reduction of Dlg4 in a fully defined model of mouse ovarian cancer (Huang et al. 2003; Nakagawa et al. 2004). Binding partners of nTSG homologs are also suggestive of a role in tumor progression. The vertebrate APC tumor-suppressor protein contains a C-terminal PDZ-binding motif that has been found to interact with hDlg1 (Matsumine et al. 1996). HScrib partners have yet to be identified, but the related LAP protein ERBIN was isolated via its interaction with ErbB2, a mammalian receptor tyrosine kinase that is frequently overexpressed in epithelial cancers (Borg et al. 2000). However, in neither

of these cases is the effect of the interaction on influencing tumor progression clear.

Currently, the most direct evidence for nTSG homolog activity in regulating mammalian tumor progression involves the activities of certain viral oncoproteins. Viral oncoproteins often transform mammalian cells by binding to and inactivating or degrading tumor-suppressor proteins such as p53. However, p53 inactivation is not sufficient for full transformation, suggesting that additional oncoprotein targets are involved. One class of targets is PDZ proteins. HPV E6, HTLV-1 TAX, and AdE4 9ORF1 are unrelated oncoproteins that share the ability to bind via a C-terminal sequence to various PDZ domains (Kiyono et al. 1997; Lee et al. 1997). In the most clear case, the presence of a PDZ-binding C-terminal tail in HPV E6 distinguishes between low-risk subtypes that cause mild cervical overproliferation and high-risk subtypes that can lead to carcinoma in situ (Mantovani and Banks 2001). When expressed transgenically in the mouse lens epithelium, high-risk E6 increases cell proliferation, inhibits differentiation, and induces architectural defects reminiscent of the fly neoplastic tumors (Nguyen et al. 2003). However, expression of high-risk E6 lacking the PDZ-binding tail is largely inactive. Binding through the E6 C-tail can lead to proteasome-mediated degradation of both hDlg and hScrib (Gardiol et al. 1999; Nakagawa and Huibregtse 2000), and, indeed, hScrib is absent in HPV-positive (but not HPV-negative) cervical carcinomas (Nakagawa et al. 2004). Other, degradation-independent mechanisms for viral oncoprotein interference with Scrib and Dlg function have also been proposed. Although it has not yet been demonstrated that viral transformation, in fact, requires inactivation of Scrib or Dlg inactivation, the correlation between PDZ-binding ability and the ability to induce malignant tumors is strong.

The recent identification of mice mutant for Scrib and Dlg1, the Dlg homolog most broadly expressed in epithelia, should allow a direct genetic analysis of cancer predisposition in heterozygotes (Caruana and Bernstein 2001; Murdoch et al. 2003). However, this is limited by the possibility that the currently available alleles (a point mutation truncating mScrib after the second PDZ domain, and a transposon insertion producing a Dlg1 protein lacking the SH3 and GUK domains) may not be functionally null. The homozygous mutant phenotypes (including cleft palate and neural tube closure defects, respectively) suggest limited failures of morphogenesis rather than widespread defects in apicobasal polarity and proliferation control. Conserved functions could be masked in these animals by hypomorphic gene activity or by coexpression of related, functionally redundant molecules. Alternatively, the essential role of Scrib and Dlg in epithelial architecture and tumor suppression may not, in fact, have been retained in mammals. A definitive answer will await analysis of true knockout animals and their tissues.

Indeed, a recently published knockout of mouse *Lgl1* documents disruption of apicobasal polarity in embryonic neuroepithelia that is associated with lack of differentiation and overproliferation (Klezovitch et al. 2004). These defects are quite reminiscent of those seen in *lgl* mutant brains (Rolls et al. 2003). Among the many proteins mislocalized in *Lgl1* mutant neuroepithelial cells is Numb, whose asymmetric inheritance in dividing cells influences a Notch signal-dependent pathway through which neural progenitors become differentiated neurons. The similarities between *Lgl1* and *Numb* mutant brains (Li et al. 2003) lead the authors to propose that overproliferation and failure of differentiation is caused by a cell fate transformation, leading to an increased presence of neural progenitors. Although limited to neural (rather than ectodermal) epithelial tissues, the dysplastic phenotype of *lgl1* is consistent with causal involvement of mammalian nTSG homologs in regulating both cell polarity and cell proliferation. *Lgl1* maps to a chromosomal region altered in many human medulloblastomas (Koyama et al. 1996); further studies are sure to examine the role of *Lgl1* in susceptibility to, and progression of, adult tumors.

Epithelial polarity and proliferation control in vertebrate tissues

Whether or not nTSG homologs act as mammalian tumor suppressors, studies of fly nTSGs may reveal a general theme in which a stable epithelial architecture acts to limit cellular proliferation. This model proposes that disruption of epithelial architecture has a causative contribution to tumor progression. Such a notion is supported by data from many areas of mammalian biology, ranging from work on cultured cells to studies of mouse knockouts and classical human TSGs.

Early investigations into architectural regulation of tissue growth explored the phenomenon of density-dependent inhibition of growth, often called contact inhibition, in cell culture. When cultured, most normal cells slow their growth as they touch and become confluent. However, the proliferation of cells derived from malignant tumors or transformed in other ways is unchecked by confluence, and the cells pile atop one another as they continue to divide. The analogy with simple epithelial tissues in vivo, where monolayered and controlled proliferation of normal tissue contrasts with multilayered, invasive growth of transformed cells, is appealing. Yet contact inhibition appears to be a complex phenomenon involving the ability of cells to spread on substrate and access to growth factors as well as cell-contact-dependent signals (Cavallaro and Christofori 2001). Although several molecules have been implicated in contact inhibition, few general conclusions have been available on the molecular mechanisms underlying this process, and its relationship, if any, to in vivo proliferation control remains unclear.

The desire to mimic living tissues more closely has led researchers to culture cells in 3D matrices containing components of basement membrane. These culture systems, which better recapitulate the cell–cell and cell–matrix contacts formed in vivo, have demonstrated the remarkable impact of cell architecture on a variety of

cancer-related phenomena. Studies contrasting 2D and 3D cultures of cells derived from mammary epithelia reveal that these parameters can influence tumor growth, migration, susceptibility to apoptosis, and differentiation (Bissell et al. 2003). For example, in 3D cultures, normal mammary epithelial cells arrest after forming polarized, monolayered structures that resemble the alveoli seen in vivo. In contrast, cells derived from malignant breast tumors overproliferate in a disorganized, multilayered fashion. If, however, integrin or EGFR signaling pathways are inactivated in malignant cells, polarity is restored and the malignant growth phenotype is also reverted (Weaver et al. 1997; Wang et al. 1998). These data suggest that a cell's loss of contextual information, including disruption of contacts with neighboring cells and the ECM, plays a role in promoting malignancy. Moreover, studies indicate that the tissue microenvironment, in particular, the stroma that surrounds epithelial tissues, can impose control on the growth of even genotypically malignant cells (Kenny and Bissell 2003). Such findings highlight the intimate and reciprocal relationship between cell architecture and proliferation control signals in mammalian tissue.

Outside of cell culture, data linking cell architecture and proliferation control have been found using vertebrate genetics. An obvious candidate for such links is E-cadherin, the transmembrane component of AJs that is a primary regulator of epithelial organization. Indeed, heterozygosity for *E-Cadherin* mutations predispose families to diffuse gastric cancer, somatic *E-cadherin* mutations have been found in other epithelial cancers, and E-cadherin is down-regulated in many human tumors (Cavallaro and Christofori 2004). Cultured cell lines lacking E-cadherin are also more invasive and proliferative, phenotypes that can be ameliorated by E-cadherin reintroduction. Although mice containing *E-cadherin* mutant skin or mammary epithelia do not form tumors (Tinkle et al. 2004), interepretation of these data is somewhat confounded by the ability of cells to upregulate alternative classical cadherins in response to E-cadherin loss.

The mechanisms by which E-cadherin exerts tumor-suppressive activity are controversial. E-Cadherin loss could promote malignancy through a disruption of cell–cell adhesion, freeing cells to invade as well as inducing architectural defects with adverse consequences on cell–cell signaling. Significant evidence supports the view that the tumor-promoting effects of E-cadherin loss may, in fact, relate to freeing of its binding partner β-catenin into the cytosol, leading to increases in oncogenic Wnt signaling activity (Wong and Gumbiner 2003). However, analysis of α-*catenin* mosaic mice supports a β-catenin-independent role of AJs in regulating cell proliferation (Vasioukhin et al. 2001). α-Catenin ablation in skin does not disrupt the assembly of E-cadherin–β-catenin complexes, which are still found at the cell membrane, but prevents the organization of AJs at the ultrastructural level. The mutant skin shows not only disruptions in epithelial polarity and architecture and acquires invasive characteristics, but also displays inappropriate mitoses that reflect cellular overproliferation. Although these phenotypes are not seen in all α-*catenin* mutant epithelia (Nemade et al. 2004), they suggest that in certain contexts disruption of junctional proteins can have significant consequences beyond loss of adhesion.

The familial cancer syndrome Neurofibromatosis type 2 (NF2) has provided one example suggesting loss of contact-dependent proliferation control in human tumors. In NF2 patients, inheritance of heterozygosity for the *NF2* locus is followed by somatic loss of the wild-type allele in developing tumors, principally benign slow-growing CNS tumors called schwannomas. Mice heterozygous for *NF2* are also cancer-prone, albeit to a different spectrum of tumors (McClatchey and Cichowski 2001). The *NF2* gene product, called Merlin, encodes a plasma-membrane-associated protein related to the ezrin–radixin–moesin (ERM) family of proteins. Although the basic cellular activity of Merlin remains a mystery, the protein is enriched at AJs, and fibroblasts derived from *NF2* mutant mice are defective in AJ formation (Lallemand et al. 2003). In these cells, cadherin–catenin complexes are assembled and transported to the membrane properly, but remain diffusely localized around the cell rather than focused at sites of cell–cell contact. In vitro, *NF2* mutant cells lack contact inhibition. Interestingly, Merlin plays a role in signaling through the p21-activated protein kinase Pak (Kissil et al. 2003), which has itself been implicated in contact inhibition (Zegers et al. 2003). Merlin associates not only with cadherin–catenin complexes, but also with the transmembrane glycoprotein CD44, which serves as a receptor for hyaluronic acid (Morrison et al. 2001). These interactions highlight Merlin as a candidate cytoplasmic transducer of cell–cell and/or cell–matrix cues to the proliferation control machinery.

The analyses of *NF2* mutant fibroblasts and α-*catenin* mutant skin described above demonstrate that loss of a single protein associated with cell junctions can, in fact, endow mammalian cells with characteristics of transformed tumors. A phenotype common to both situations is mislocalization, but not total loss, of cadherin–catenin complexes. This is strikingly reminiscent of the situation in the fly, in which junctional mislocalization resulting from nTSG mutations, but not junctional loss caused by, for instance, mutations in β-*catenin*, are correlated with overproliferation. The human tumor suppressor LKB1, a kinase mutated in Peutz-Jeghers syndrome, has recently been linked to regulation of epithelial polarity (Baas et al. 2004); and, indeed, *LKB1* mutations in fly epithelia cause ectopic apical polarity in a manner resembling the nTSG mutations (Martin and St Johnston 2003). Although LKB1 has been linked to several pathways that influence cell proliferation (Yoo et al. 2002; Carling 2004), it will be interesting to see whether tumorigenesis in *LKB1* mutant mammals is, indeed, influenced by polarity defects.

Polarity and proliferation control: models

Although the fly nTSGs demonstrate a clear link between cellular architecture and proliferation control, it

remains to be determined exactly how these two functions are coupled, and how polarity defects result in overgrowth. In one group of models, overgrowth is an indirect consequence of epithelial polarity loss, owing to spatial disorganization of signaling pathways that control cell proliferation (Fig. 5). There are several ways in which this could happen. First, mispolarization could disrupt hypothetical signals, mediated by cell junctions, that convey environmental information to restrain cell growth. β-Catenin has been proposed to act as such a signal, and is the best known example of a junctional protein that can also regulate proliferation, but an increasing number of proteins have been found to transit between cell junctions and the nucleus and could influence the cell cycle (Balda and Matter 2003). Second, mispolarization could alter the distribution and turnover of diffusible signals that regulate proliferation or the receptors that bind to them. Either signaling loss, caused by insufficient local concentrations of activated receptors, or ectopic signaling, caused by receptor activation at inappropriate cellular locations or excessive levels of activated receptor, could result. A third, related model derives from the observation that, in some epithelial sheets, basolaterally polarized growth factor receptors are compartmentally separated from their apically secreted ligands (Vermeer et al. 2003). When the epithelial barrier is breached by, for instance, wounding, ligand diffusion can stimulate proliferation until the epithelial barrier is restored. In this context, polarity defects would not only lead to activation of mitogenic pathways through loss of the epithelial partition, but would be further exacerbated by the inability to reform a tight epithelial seal. Fourth, polarity loss could cause disorganization of internal rather than external aspects of signaling, by skewing the subcellular localization of polarized signal transduction components. Intracellular disorganization could also disrupt subcellular locales, such as cell–cell or cell–matrix junctions, that are involved in integrating and coordinating multiple signaling pathways. Fifth, as suggested by *lgl1* mice, mislocalization of cell fate determinants could alter fate-regulating signaling pathways, leading to expansion of a proliferative "stem cell"-like population. Clearly, the above models are not mutually exclusive, as polarity defects are likely to disrupt many aspects of cell–cell signaling. In contrast, in an alternative second group of models, polarity and proliferation are not inherently coupled but, rather, linked by separable, independent functions of proteins such as those encoded by the nTSGs.

Data from *Drosophila* inform a consideration of the above models. With respect to the causal relationship of polarity and proliferation control, it is clear that ectopic proliferation alone does not lead to polarity defects, as the overgrown epithelia found in hyperplastic TSG mutants (like hyperplastic tissues in vertebrates) have normal architecture. In contrast, significant data indicate that the fundamental defect of nTSG mutant cells is altered polarity. Dramatic and consistent polarity loss is seen in mutant epithelial and neuronal tissue at all stages of development. Yet proliferation defects have been well documented primarily in larval stages, raising the possibility that only in specific developmental contexts does polarity loss contribute to overproliferation.

If the nTSGs, indeed, regulate proliferation solely through controlling polarity, one might expect that independent methods of disrupting polarity should also induce overproliferation, at least in the context of imaginal discs. This does not appear to be the case. Imaginal cells with mutations in *Moesin* lose polarity and adhesion and exit the epithelium, but do not display proliferation phenotypes resembling those of the nTSGs (Speck et al. 2003). Cells lacking the AJ component Armadillo/β-catenin do not survive in discs (Peifer et al. 1991), whereas cells mutant for *E-cadherin* show mild polarity defects with no apparent effects on proliferation (Le Borgne et al. 2002). *Rap1* mutations disrupt AJs in imaginal discs, inducing ectopic cell dispersal, but do not show proliferation defects (Knox and Brown 2002). Simi-

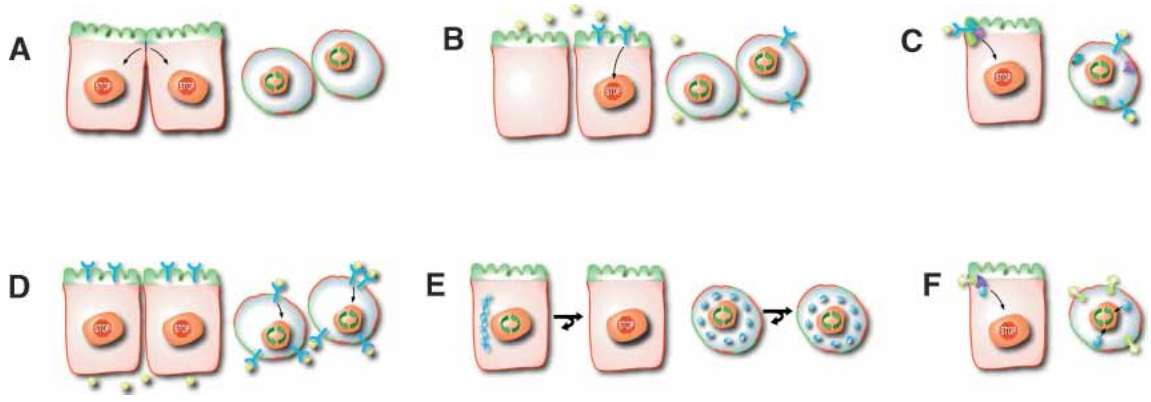

Figure 5. Models linking cell architecture with proliferation control in epithelial tissues. Mispolarization of epithelia could induce overproliferation by disrupting contact-mediated arrest signaling (*A*), polarized distribution of mitogenic or mitostatic diffusible signals and their receptors (*B*), polarized distribution of intracellular signaling components for such pathways (*C*), compartmental segregation of proliferative signaling components (*D*), asymmetric inheritance of fate determinants distinguishing proliferative progenitors from differentiated progeny (*E*), and separable polarity and proliferation control activities of regulatory proteins (*F*). See text for details.

larly, imaginal disc cells lacking SJs do not overproliferate (Ward et al. 1998; D. Bilder, unpubl.). It is important to recall, however, that nTSGs mutations cause a distinctive polarity phenotype that contrasts with the mutations mentioned above. In nTSG mutant cells, apical polarity and AJs are not lost, but rather mislocalized to basolateral sites; so far, all *Drosophila* mutants that show this phenotype also display imaginal disc overgrowth. The possibility that ectopically localized apical or junctional components specifically promote proliferation defects is worth exploring.

Many of the models outlined above invoke misregulation of cell–cell signaling to account for overproliferation. In *Drosophila*, the availability of tools to block or hyperactivate conserved signaling pathways allows this hypothesis to be tested. Interestingly, the manipulations performed to date, involving the Notch, TGF-β, Wnt, Hh, and EGFR signaling pathways, can stimulate some excess proliferation but not to the extent of nTSG mutations. Although inactivation or constitutive activity of a single signaling pathway has not yet induced overgrowth to the extent of the neoplastic tumors, the severe polarity defects seen in nTSG mutant tissue have the potential to alter not just one but multiple, and perhaps all, of the above signaling pathways. It may be that the simultaneous misregulation of several or many pathways, like the mislocalization of polarized fate determinants, leads the cell to revert to a relatively undifferentiated and therefore continuously proliferative fate.

Although models in which global alterations of epithelial polarity disrupt proliferation control systems have an intuitive appeal, it cannot be ruled out that the nTSGs themselves have a more direct role in signaling to regulate proliferation. Interestingly, structure–function analyses of nTSG proteins suggest that polarity and proliferation control are to some extent separable, with distinct regions of the multidomain Dlg and Scrib proteins contributing to the two functions (Hough et al. 1997; J. Zeitler, C. Hsu, and D. Bilder, unpubl.). Although they do not exclude effects on specific signaling systems, these data are also consistent with separate interacting partner proteins that work through independent polarity and proliferation pathways, whereas the nTSG scaffolds act to coordinate the two pathways. PDZ domains are well-known binding partners of growth factor receptors (Simske et al. 1996; Shelly et al. 2003), and, indeed, Scrib and Dlg orthologs bind to EGFR family members (Kolch 2003). Alternatively, Scrib and Dlg could themselves interact with cytoplasmic and nuclear factors that regulate the cell cycle. The genetic interaction seen between *scrib* and *cyclin E* suggests a fairly close link between the nTSGs and the cell cycle (Brumby and Richardson 2003), whereas human Dlg1 overexpression slows G1- to S-phase progression in cultured cells (Ishidate et al. 2000). Identification and characterization of nTSG-binding effectors will help in resolving this conundrum.

When the data from *Drosophila* and vertebrates are considered together, it seems likely that both groups of models are true: the pathways that control cell proliferation and cell polarity are independent in some contexts while influencing each other in different contexts. For instance, in mammalian epithelial cells, oncogenic activation of signaling pathways by Ras and Src cannot only increase cell proliferation but also alter cell shape and organization (Frame 2002; Campbell and Der 2004). Moreover, a recent study has demonstrated that ectopic phosphatidylinositol-3 kinase activity in cultured mammary epithelia disrupts both polarity and proliferation control, but does so by distinct and separable downstream pathways (Liu et al. 2004). In the integrated environment of a cell in vivo, reciprocity between the pathways that regulate proliferation and polarity, as well as cell survival, is likely to occur at several levels, with defects in one pathway having profound consequences for the others. Untangling these threads will be a major challenge to a complete understanding of malignant transformation.

Coupling cell architecture and proliferation control

The evidence discussed in this review suggests that proper epithelial architecture is intimately involved in a cell's ability to control its growth. Cells need to respond to environmental signals to proliferate during development in a coordinated fashion, and to cease proliferation when proper tissue size is reached. Disruption of contacts, for instance, during wounding, can also induce cell division, whereas reestablishment of contacts may help arrest it. Vertebrate cell culture and mouse genetics are now converging with fly studies on the mechanisms by which architecture-dependent proliferation arrest cues are conveyed. Evidence from mammals suggests that there are not just a few but many pathways through which cell architecture can influence the aberrant proliferation, survival, and migratory abilities that result in the appearance of a malignant tumor. In *Drosophila*, the nTSGs seem to be a molecular "node" that coordinates withdrawal from the cell cycle with the maintenance of proper tissue architecture. Such nodes, which integrate information from multiple regulatory inputs, are particularly vulnerable to dysregulation in disease. The relative simplicity of the fly system may give us insight into the general themes by which polarity loss can promote tumor progression, and perhaps even allow us to identify conserved central players. Through studies of the fly nTSGs and their role in organizing polarized cells, a fresh perspective on the mechanisms that contribute to malignancy in human cancer may be attained.

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