Published in final edited form as:
J Genet Genomics. 2011 October 20; 38(10): 483–495. doi:10.1016/j.jgg.2011.09.006.

Cell competition and its implications for development and cancer

Yoichiro Tamori and **Wu-Min Deng***
Department of Biological Science, Florida State University, Tallahassee, FL 32306-4370, USA

Abstract

Cell competition is a struggle for existence between cells in heterogeneous tissues of multicellular organisms. Loser cells, which die during cell competition, are normally viable when grown only with other loser cells, but when mixed with winner cells, they are at a growth disadvantage and undergo apoptosis. Intriguingly, several recent studies have revealed that cells bearing mutant tumor-suppressor genes, which show overgrowth and tumorigenesis in a homotypic situation, are frequently eliminated, through cell competition, from tissues in which they are surrounded by wild-type cells. Here, we focus on the regulation of cellular competitiveness and the mechanism of cell competition as inferred from two different categories of mutant cells: (1) slower-growing cells and (2) structurally defective cells. We also discuss the possible role of cell competition as an intrinsic homeostasis system through which normal cells sense and remove aberrant cells, such as precancerous cells, to maintain the integrity and normal development of tissues and organs.

Keywords

Cell competition; Canalization; Competition-dependent cell death; *Drosophila* models; Cellular proliferation; Cellular growth; Tumor-suppressor genes

1. Introduction

The concept of competition for survival between cells has long been intensively studied by microbiologists—the microbial antagonism induced by bacteriocin of *Escherichia coli* and *Pseudomonas aeruginosa* and the viral killer toxin of yeast and paramecium are representative examples (reviewed by Riley and Wertz, 2002; Schmitt and Breinig, 2006)—but biologists studying multicellular systems had barely considered the concept of competition between cells of the same species in a tissue until Morata and Ripoll (1975) discovered the phenomenon of cell competition in *Drosophila*. They used the developing wing imaginal disc of *Drosophila* to study the behavior of cells bearing a group of dominant mutations (known as the "*Minute*" mutations) that reduced the rate of cell division in a cell-autonomous manner (Morata and Ripoll, 1975). *Minute* heterozygous (*M*/+) flies develop more slowly than wild-type flies because of a defect in their ribosomal proteins but are viable and show normal body size. Morata and Ripoll (1975) generated mitotic clones of wild-type cells in a slower-growing *M*/+ field and found that the wild-type clones occupied most areas of the adult wing, and at the same time, *M*/+ cells were eliminated from the mosaic field. A few years later, Simpson and Morata (1981) showed that this phenomenon depends on a short-range cell–cell interaction. These investigators again generated wild-type

*Corresponding author: Tel: +1 850 645 1501, fax: +1 850 645 8447. wumin@bio.fsu.edu (W.-M. Deng).

clones in an *M*/+ background and studied the growth rates of both clones in greater detail. They found that wild-type cells adjacent to *M*/+ cells tended to divide more often than those positioned in the center of the clone and that *M*/+ cells not in contact with wild-type cells were not eliminated. Because this regulation of balance between proliferation of faster-growing wild-type cells and elimination of *M*/+ cells depended on cell–cell interaction, they named the phenomenon “cell competition.”

Interestingly, several recent studies have revealed that cells bearing mutant tumor-suppressor genes, which show overgrowth and tumorigenesis in a homotypic situation, are frequently eliminated, through cell competition, from epithelial tissues in which they are surrounded by wild-type cells. This suggests that cell competition is an intrinsic tumor-suppression mechanism that assures elimination of precancerous cells and epithelial integration. Cell competition might also be involved, at the cellular level, in canalization, a concept introduced by Waddington (1942) to describe the reduced sensitivity of a phenotype to genetic and environmental perturbations and defined by Wilkins (1986) as the stabilization of developmental pathways by multiple genetic factors within the genome: a form of genetic buffering. A number of studies have suggested that an intrinsic canalization system during development robustly assures the consistent size and shape of organs (Larsen, 2005), and recent studies have shown that cell competition accomplishes just this regulation at the cellular level (de la Cova et al., 2004). Because, in the process of cell competition, elimination of suboptimal loser cells is accompanied by compensatory proliferation of optimal winner cells with the result that final size and shape of organs are finely normalized, adult organ size becomes more variable when cell competition is blocked in developing tissues. The mechanism underlying the phenomenon therefore not only assures sensing and elimination of potentially deleterious (e.g., precancerous) cells during development but is also tightly connected to the organ-size control system.

Tumors are caused by uncontrolled proliferation of transformed mutant cells with activated oncoproteins and/or inactivated tumor-suppressor proteins, and malignant neoplasias arise from tumor cells that have lost the ability to assemble and create tissues of normal form and function (Weinberg, 2007). In other words, cancer can be viewed as a disease that arises when mutant cells fail to obey the intrinsic tissue-integrity and organ-size control system. Because the process of cell competition is deeply involved in maintenance of tissue integrity and regulation of organ size, especially when a mutant cell is spawned in a wild-type tissue, tumorigenesis occurs once the mutant cells break the rules of these systems. Here, we survey different types of genes whose mutant cells have been shown to induce cell competition in *Drosophila* and categorize those mutants into two distinct groups based on their phenotypes in an epithelial tissue. Mutants in the first category (slower-growing cells, SGCs) show slower growth than wild-type cells, and those in the second category (structurally defective cells, SDCs) show structural defects such as epithelial apicobasal polarity defects. Overview and comparison of the processes of cell competition induced by these two groups will help to reveal the mechanisms of cell competition and its relevance to cancer.

2. Hallmarks of cell competition

Since the introduction of accessible genetic-mosaic analysis tools in *Drosophila*, such as FLP–FRT mitotic recombination (Xu and Rubin, 1993) and Flip-out-Gal4-UAS (Ito et al., 1997), phenotypic analysis of a vast array of genes has been performed in later stages of development (late larva and adult). Substantial numbers of these mutant cells experimentally generated in developing wild-type tissues by means of mitotic recombination techniques have shown defects in their proliferation or survival. The first hallmark of cell competition is survival defect of loser cells. In cell competition, outcompeted loser cells undergo apoptosis and are eventually eliminated from tissues. Most of this apoptosis is observed at the

boundary between two clones, winner cells and loser cells, because cell competition is based on direct cell–cell interaction between two different types of clones (Fig. 1A–D). Perhaps more accurate, then, is to say that the first hallmark is not just apoptosis of loser cells but "competition-dependent apoptosis"; loser cells adjacent to winner cells at their clone boundary undergo apoptosis. The second hallmark is that loser cells remain viable in a homotypic field where they come into contact only with the same loser cells. Examination of a group of loser cells mixed with winner cells in a mosaic tissue therefore shows that loser cells in the center of a loser clone do not show apoptosis, whereas those at the periphery do (Fig. 1D).

In the initial stage of cell competition, two different types of cells compare their competitive abilities to determine winners and losers. Most previous reports have shown that slowly proliferating cells undergo apoptosis when they are surrounded by rapidly proliferating cells, but activation of cyclin D/Cdk4 or the insulin/insulin-like growth-factor receptor pathway, which accelerates cell division or cellular growth, respectively, does not cause cell competition (de la Cova et al., 2004), suggesting that a difference in cell growth rate alone does not always trigger cell competition. Although all factors conferring competitive ability on cells are not yet known, we know that the competitive ability of *Minute* heterozygous (*M*/+) cells is lower than that of the wild-type cells. When *Drosophila* geneticists confront a phenotype of mutant cells showing a disadvantage in their proliferation or impairment in their survival in a mosaic tissue with wild-type cells, the "*Minute* technique" is commonly used to recover the mutant cells in mosaic tissues. *Minute* causes a marked developmental delay in heterozygotes. Wild-type clones in *M*/+ genotypes have a growth advantage over their *M*/+ neighbors and are often very large. Garcia-Bellido et al. (1973) exploited this phenomenon as a general method for the production of large somatic clones normally showing defects in their proliferation or survival in a wild-type background (Ashburner et al., 2005). Many such mutant cells that are outcompeted by wild-type cells have been shown to survive when they confront *M*/+ cells instead of wild-type cells, in this *Minute* technique. This result could arise from an alleviation of the competitive pressure, but technically this technique reduces proliferation speed of competitors, suggesting that the recovery of mutant clone size could be just a result of a proliferation race. Cell competition is not just a proliferation race but is survival of the fittest based on a cell–cell interaction. Therefore, when the *Minute* technique rescues such cells from elimination and the competition-dependent cell death observed at a boundary with wild-type clones, we can say that the mutant cells fulfill the third hallmark of cell competition (Fig. 1E).

3. Slower-growing cells

3.1. Minute

Cell competition was first experimentally confirmed in *Drosophila* by Morata and Ripoll (1975), who studied the growth parameters of *Minute* (*M*) mutations. *Minute* is lethal to cells when homozygous because the cells lack functional ribosomes and cannot synthesize proteins. Flies heterozygous for *Minute* (*M*/+), on the other hand, are viable and of normal size although they require a few days more than wild-type flies to complete embryonic development. In mosaic imaginal discs containing both *M*/+ and wild-type cells, the *M*/+ cells are disproportionately eliminated from the developing imaginal discs and do not contribute to the adult animal (Morata and Ripoll, 1975). At the same time, growth of the wild-type cells is correspondingly increased, sometimes leading the entire compartment to be constructed from just these cells (Simpson, 1979; Simpson and Morata, 1981). More recently, these findings have been confirmed by Moreno et al. (2002), who observed actual apoptosis of *M*/+ cells surrounded by wild-type cells in a developing wing imaginal disc using modern FLP–FRT mosaic techniques.

3.2. dMyc and Ras

The transcription factor dMyc is the sole *Drosophila* ortholog of the vertebrate c-myc protooncogene and a central regulator of growth and cell-cycle progression during normal development. Using mosaic analysis in *Drosophila* imaginal wing discs, Johnston et al. (1999) showed that loss of dMyc retards cellular growth and reduces cell size, whereas dMyc overexpression increases cellular growth rates and cell size. dMyc-induced growth promotes G1/S progression but fails to accelerate cell division because G2/M progression is independently controlled by Cdc25/String. Although *dMyc* is an essential gene, hypomorphic *dMyc* mutants are viable and show only a modest growth defect. When mitotic clones of the hypomorphic *dMyc* mutant are generated in a developing wild-type imaginal disc, however, they are outcompeted by surrounding wild-type cells and undergo apoptosis. Interestingly, Moreno and Basler (2004) and de la Cova et al. (2004) found that, conversely, when clones overexpressing dMyc are generated in a developing wild-type imaginal disc, wild-type cells are outcompeted and eliminated by the dMyc-overexpressing cells. These findings suggest that the relative expression level of dMyc determines the winner and loser cells in cell competition.

The Ras protooncogene has effects on growth and the cell cycle similar to those of dMyc. In fact, Ras has been shown to regulate expression of dMyc; so Ras may regulate cellular growth through dMyc (Prober and Edgar, 2000). Previously, Diaz-Benjumea and Hafen (1994) showed that cells lacking Ras are eventually eliminated from the developing wing. This survival defect of the *ras* mutant clone is alleviated by inhibition of apoptosis through antiapoptotic p35 expression (Prober and Edgar, 2000). In addition, because the *ras* mutant clones in a *Minute* background had higher survival rates and were larger than *ras* mutant clones in a wild-type background, Prober and Edgar (2000) concluded that Ras promotes growth, leading to cell survival by reducing cell competition.

Regulation of cell growth by dMyc was also confirmed by results of gene-expression profiling analyses indicating that loss of dMyc leads to decreased expression of genes required for ribosome biogenesis and protein synthesis (Pierce et al., 2008). Together, these findings suggest that the reason why the mutant cells defective in Ras become losers in cell competition may be the result of reduced total cellular activity, which is regulated by dMyc and ribosomal proteins.

3.3. The Wingless signaling pathway

The Wingless (Wg)/Wnt signaling pathway has been implicated in growth control in developing *Drosophila* wing imaginal discs. Giraldez and Cohen (2003) examined mutations in two components of the Wg signaling pathway: *arrow*, encoding the *Drosophila* homolog of LRP5/6, an essential Wnt coreceptor, and *pygopus* (*pygo*), encoding a nuclear PHD-finger protein required for Wg signaling. Both of the mutant clones generated in a developing wing disc were eliminated by apoptosis. Suppression of cell death by expression of the viral caspase inhibitor protein p35 only partially rescued clonal growth of both mutant clones, suggesting that reduced Wg signaling not only leads to defects in cell survival but also reduces cell proliferation. Most recently, however, Vincent et al. (2011) showed that Wg signaling is not intrinsically required for cell survival in developing wing imaginal epithelia. Posterior compartments of developing wing discs composed entirely of *wg* or *fz fz2* (double mutant for two *Drosophila* Wg receptors, Frizzled and Frizzled2) mutant cells survive to the end of larval life as long as they are not surrounded by wild-type cells. They also showed that overactivation of Wg signaling cell-autonomously in the mutant clones of either *axin* or *APC* (*Adenomatous Polyposis Coli*), two tumor-suppressor genes encoding negative regulators of Wg signaling, led to the elimination of surrounding wild-type cells; mosaic mutant clones of *axin* or *APC* (double mutant for two *Drosophila* APC, APC1 and

APC2) induced apoptosis in neighboring wild-type cells to overcolonize the imaginal epithelia. Together, these results indicate that relative differences in the levels of Wg signaling activity in neighboring cells lead to cell competition (Vincent et al., 2011).

Wg is required for both the cell cycle and growth arrests in the ZNC (zone of nonproliferating cells in the dorsal–ventral boundary of the wing disc during the third larval instar) (Johnston and Edgar, 1998), because it negatively regulates the expression of dMyc (Johnston et al., 1999). Consistent with these facts, the expression level of dMyc was reduced in *axin* or *APC* mutant cells that outcompeted neighboring wild-type cells (Vincent et al., 2011), suggesting that Wg signaling regulates cellular competitive ability independently of dMyc.

3.4. The Hippo (Salvador/Warts/Hippo) pathway

Recent studies in *Drosophila* have revealed the Salvador/Warts/Hippo (SWH) signaling pathway as a hyperplastic tumor-suppressor pathway. It controls organ size by regulating cell growth, proliferation, and apoptosis through transcriptional activation of downstream target genes including the antiapoptotic gene DIAP1, the cell-cycle regulator Cyclin E, and a *Drosophila*-specific micro-RNA called bantam, which has antiapoptotic and growth-promoting functions. This evolutionarily conserved pathway comprises a phosphorylation cascade regulated by four conserved tumor suppressors: the Ste20-like kinase Hippo (Hpo), its regulatory protein Salvador (Sav), the NDR family kinase Warts (Wts), and its regulatory protein *Mob as tumour suppressor* (Mats) (reviewed by Pan, 2010; Halder and Johnson, 2011). The Hpo–Sav complex phosphorylates and activates the Wts–Mats complex (Wu et al., 2003; Wei et al., 2007), which in turn phosphorylates and inactivates the oncoprotein Yorkie (Yki) (Huang et al., 2005), which normally functions as a coactivator for the TEAD/TEF family transcription factor Scalloped (Sd) (Goulev et al., 2008; Wu et al., 2008; Zhang et al., 2008). Conversely, the Ras-associated-domain-family protein (RASSF) inhibits Hpo activity by preventing the binding of Sav to Hpo (Polesello et al., 2006). Recent studies have also revealed that these core SWH pathway proteins are regulated by inputs from several upstream regulator-gene groups, including the atypical cadherins Fat (Ft) and Dachsous (Ds); the FERM domain proteins Expanded (Ex), Merlin (Mer), and WW-domain-containing protein Kibra; and the apicobasal polarity proteins Crumbs (Crb), atypical Protein Kinase C (aPKC), and Lethal Giant Larvae (Lgl) (reviewed by Grusche et al., 2010). Impairment of this pathway allows nuclear translocation of Yki and its interaction with DNA-binding proteins and transcriptional activation of downstream target genes that regulate apoptosis, cell proliferation, and tissue growth.

Tyler et al. (2007) performed a genetic screen to identify mutant alleles that suppress the elimination of *M*/+ clones normally outcompeted by wild-type clones in developing eyes. They first identified new mutations in *ex* and *fat*, then tested other mutant alleles previously identified in a mosaic screen to show an overgrowth phenotype. Mutations of *salvador* (*sav*), *hippo* (*hpo*), *warts* (*wts*), *mats*, *dacapo*, *argos*, *gap1*, *TSC1*, *TSC2*, *PTEN*, *archipelago*, *karst*, *lethal giant discs* (*lgd*), *scribble* (*scrib*), and *lethal giant larvae* (*lgl*) were tested for their ability to rescue *M*/+ clones from cell competition. Only the mutant alleles of the SWH pathway–component genes *sav*, *hpo*, *wts*, and *mats* rescued *M*/+ clones from elimination, but they did not completely eliminate apoptosis of *M*/+ cells, suggesting that the impairment of the SWH pathway simply confers an acceleration of proliferation on the slow-growing *M*/+ cells.

Recently, Neto-Silva et al. (2010) revealed that dMyc and the SWH pathway are codependent in growth of *Drosophila* imaginal discs. They demonstrated that dMyc is a transcriptional target of Yki and functions as a critical cellular-growth effector of the SWH pathway. At the same time, they showed that dMyc modulates Yki expression and activity

through both transcriptional and posttranscriptional mechanisms to control its own level in a negative feedback loop. In fact, Neto-Silva et al. (2010) and Ziosi et al. (2010) showed that relative differences in the level of SWH pathway activity in neighboring cells lead to cell competition, as is the case with dMyc; *yki* mutant clones were outcompeted by neighboring wild-type cells and that, conversely, Yki overexpressing clones out-competed neighboring wild-type cells. These results suggest that the SWH pathway regulates cellular competitive ability through dMyc.

3.5. mahjong

Mahjong was identified as a novel Lgl-binding protein by Tamori et al. (2010). *lethal giant larvae (lgl)* was originally identified as a tumor-suppressor gene in *Drosophila* (Mechler et al., 1985) and its encoding protein Lgl has the characteristics of a molecular scaffold, with WD40 protein–protein interaction motifs and no known enzymatic activity. Its functional roles are therefore likely to be mediated by protein–-protein interactions. To determine the molecular mechanisms whereby Lgl is involved in cell competition, Tamori et al. (2010) performed immunoprecipitation using MCF-7 human breast epithelial cells expressing GFP-tagged mLgl2, another mammalian Lgl homolog. They identified a novel Lgl-binding protein and named it Mahjong, the name of a table game in which winners and losers are determined through strong competition. In *Drosophila*, one Mahjong homolog protein exists, which was shown to interact with Lgl, indicating that Mahjong is an evolutionally conserved Lgl-binding protein. When Tamori et al. (2010) generated mosaic clones of *mahjong* mutant ($mahj^{-/-}$) cells, they found that $mahj^{-/-}$ cells disappeared from the mosaic wing imaginal epithelium (Fig. 1A–C) and that these $mahj^{-/-}$ clones, when adjacent to wild-type or *mahjong* heterozygous ($mahj^{+/-}$) cells, frequently underwent apoptosis, whereas $mahj^{-/-}$ clones that were not adjacent to wild-type or $mahj^{+/-}$ cells did not (Fig. 1D). The apoptosis of $mahj^{-/-}$ cells was suppressed when they were surrounded by *M*/+ cells (Fig. 1E). Collectively, these results indicate that $mahj^{-/-}$ cells are eliminated from the wild-type disc epithelium through the process of cell competition. To examine the functional role of Mahjong in mammalian cells, they established Madin–Darby canine kidney (MDCK) cell lines that stably express Mahjong shRNA in a tetracycline-inducible manner (MDCK-pTR Mahjong shRNA). Using this cell line, they tested for involvement of Mahjong in cell competition in mammalian cells. MDCK-pTR Mahjong shRNA cells were labeled with fluorescent dye and mixed with normal MDCK cells. The mixture of cells was cultured in the absence of tetracycline until the cells formed a monolayer. Expression of Mahjong shRNA was then induced with tetracycline, and the fate of Mahjong-knockdown cells surrounded by normal cells was observed by time-lapse microscopy. By 24–48 h after tetracycline addition, 50% of Mahjong-knockdown cells had died and been extruded from the apical surface of the monolayer. Addition of a caspase inhibitor significantly suppressed both apical extrusion and cell death, suggesting that the death of the Mahjong-knockdown cells was caused by apoptosis and that apical extrusion resulted from cell death. In contrast, when Mahjong shRNA cells alone were cultured with tetracycline, no cell death was observed, indicating that cell death of Mahjong-knockdown cells occurs only when they are surrounded by normal cells. This was the first demonstration of cell competition in mammalian cultured cells and suggests that at least some cell-competition pathways are evolutionally conserved between flies and mammals and that cell competition might be a general phenomenon in various species.

We still do not know the exact function of Mahjong in regulation of cellular competitive ability, but overexpression of dMyc in the $mahj^{-/-}$ clones did not prevent the elimination of $mahj^{-/-}$ cells, suggesting that dMyc is not involved in the reduced competitive ability of *mahj* mutant cells (Tamori et al., 2010).

Recently, an interaction between Mahjong and the Merlin/NF2 tumor-suppressor gene in mammalian systems was revealed by Li et al. (2010). The tumor-suppressor gene *Merlin*, encoding a member of the Ezrin/Radixin/Moesin (ERM) family of proteins, was originally identified as a causative gene of the familial cancer-predisposition syndrome neurofibromatosis type 2. Li et al. (2010) revealed that wild-type Merlin binds to the E3 ubiquitin ligase $CRL4^{DCAF1}$. They showed that the unphosphorylated form of Merlin, which is presumably stabilized in the closed conformation and able to mediate growth inhibition, translocates into the nucleus and binds to DCAF1, the substrate receptor subunit of $CRL4^{DCAF1}$ and mammalian Mahjong homolog, and inhibits $CRL4^{DCAF1}$-mediated ubiquitylation of target proteins. Interestingly, gene-expression profiling analysis by microarray suggests that $CRL4^{DCAF1}$ regulates a broad program of gene expression, consisting of more than 1000 genes. A survey of the genes coordinately regulated by expression of Merlin or loss of DCAF1/Mahjong revealed that expression of Merlin and inactivation of $CRL4^{DCAF1}$ cause a concordant downregulation of genes promoting cell-cycle progression and upregulation of genes involved in growth arrest and apoptosis, including a fraction of the known SWH-pathway target genes (Li et al., 2010). Collectively, cellular competitive ability regulated by Mahjong can be considered to consist of vast amounts of gene expression involved in cell proliferation and apoptosis.

4. Structurally defective cells

4.1. scrib, dlg, and lgl

Studies of a group of *Drosophila* tumor-suppressor genes, *lethal giant larvae* (*lgl*), *discs large* (*dlg*), and *scribble* (*scrib*), highlight the critical relationship between loss of epithelial cell polarity and tumor development (Bilder, 2004). These genes play a key role in regulation of apicobasal cell polarity and cell proliferation in epithelial tissues. These proteins interact with other apicobasal polarity-protein complexes, such as the Crumbs complex, composed of transmembrane protein Crumbs (Crb) and the adaptor proteins PATJ and Stardust (PALS), and the aPKC complex, composed of atypical protein kinase C (aPKC) and the adaptor proteins Bazooka/Par3 (Baz) and Par6 (reviewed by St Johnston and Ahringer, 2010). Homozygous mutants of these three genes, *lgl*, *dlg*, and *scrib*, exhibit very similar phenotypes. Larvae develop normally, but as maternal supplies of these proteins are exhausted, cells within the normally monolayered epithelial imaginal discs lose structure and polarity, fail to differentiate, and overproliferate to become multilayered amorphous masses that fuse with adjacent tissues (Bilder, 2004). The larvae are unable to initiate pupal development and eventually die as giant overgrown larvae. The three-dimensional and invasive overgrowth exhibited by the mutant tissue, which fails to differentiate properly and lacks proper morphology, has led to the classification of these three genes as *Drosophila* neoplastic tumor-suppressor genes (nTSGs; Gateff, 1994), but the mosaic mutant clones of these genes made in a heterozygous imaginal disc through mitotic recombination are slower in growth than their wild-type neighbors and are ultimately eliminated by cell competition (Woods and Bryant, 1991; Agrawal et al., 1995; Brumby and Richardson, 2003; Pagliarini and Xu, 2003; Grzeschik et al., 2007; Igaki et al., 2009; Menéndez et al., 2010; Tamori et al., 2010).

Tamori et al. (2010) studied details of *lgl* mutant ($lgl^{-/-}$) cells generated in the developing wing imaginal epithelium. By 96 h after clone induction, a number of $lgl^{-/-}$ cells abutting wild-type cells had become apoptotic and were basally extruded, as previously reported for eye discs by Grzeschik et al. (2007). Some apoptotic cells stayed in the epithelial layer, however, suggesting that the apoptosis was not caused by basal extrusion. At 144 h, most of the $lgl^{-/-}$ clones were basally extruded from the epithelium. In contrast, when they were surrounded by *M*/+ cells, $lgl^{-/-}$ clones survived and showed a tumorigenic overgrowth phenotype. Quantification analyses revealed that the apoptosis was mostly detected in

mutant cells that were at the clone boundary, suggesting that the presence of the abutting or nearby wild-type or $lgl^{+/-}$ cells triggered apoptosis of $lgl^{-/-}$ cells. Tamori et al. (2010) also found that the elimination of $lgl^{-/-}$ clones was suppressed when Mahj was overexpressed in the $lgl^{-/-}$ cells, but over-expression of Mahj did not suppress apoptosis of *scrib* mutant cells in a wild-type wing imaginal disc. Although the mechanism by which Mahj suppresses elimination of $lgl^{-/-}$ cells remains unknown, these data suggest that Mahj acts downstream of Lgl and is involved in Lgl-mediated cell competition but that the effect of Mahj overexpression on apoptosis is specific to $lgl^{-/-}$ cells (Tamori et al., 2010). Froldi et al. (2010) also showed that dMyc expression level is down-regulated in the *lgl* mutant clones generated in a wing-pouch region where high levels of dMyc are endogenously expressed in a wing imaginal disc. Because the slower growth of the nTSGs mutant cells might explain why these mutant clones are outcompeted by surrounding wild-type cells, the downregulation of dMyc level in the *lgl* mutant clones is straightforward.

Grzeschik et al. (2007) demonstrated, however, that *lgl* mutant clones in the developing larval eye disc exhibit ectopic expression of the G1–S regulator Cyclin E and ectopic proliferation. More recently, they also revealed that depletion of Lgl or overexpression of aPKC or Crb results in an upregulation of some target genes of the SWH pathway, such as CycE, DIAP1, *ex*, and *four-jointed*, and also translocation of Yki protein and decrease of phosphorylated (inactive) Yki (Grzeschik et al., 2010). On the other hand, RNAi-mediated knockdown of Scrib or Dlg to levels where epithelial cell polarity is mildly affected does not lead to upregulation of SWH pathway targets, but when apicobasal polarity is lost in *scrib* transheterozygous larval discs, the tumor growth depends on Yki. Furthermore, they demonstrated that Lgl and aPKC act antagonistically to regulate the SWH pathway through localization of Hpo and RASSF (but not Ft or Ex), whereas Crb regulates localization of Ex (but not Hpo, RASSF, or Ft) (Grzeschik et al., 2010). Sun and Irvine (2011) also showed that disruption of apical–basal polarity by depletion of *lgl* or *dlg* activates Yki through the JNK (c-Jun N-terminal kinase) signaling pathway in wing imaginal discs. In wing discs, however, they did not detect the discrete apical localization of Hpo reported by Grzeschik et al. (2010) in their studies of eye discs; so the mechanism of activation of Yki through mis-localization of Hpo and RASSF might be different in the wing discs. Furthermore, the effect of direct JNK activation on Yki was not detected in eye discs. The consistent activation of Yki in *lgl* mutant cells in wing and eye discs seems inconsistent with the outcompetition of *lgl* mutant cells by wild-type cells, but the downregulation of dMyc level in the *lgl* mutant cells (Froldi et al., 2010) and the codependent regulation between Yki and dMyc (Neto-Silva et al., 2010) imply that the level of dMyc is most important for cellular competitive ability.

4.2. vps25 and tsg101

Some recent mosaic genetic screens have identified four other nTSGs in *Drosophila: avalanche* (*avl*), *Rab5*, *erupted/tumor-susceptibility gene-101* (*tsg101*), and *vps25* (Lu and Bilder, 2005; Moberg et al., 2005; Thompson et al., 2005; Vaccari and Bilder, 2005; Herz et al., 2006). Because these genes encode components of the endocytic machinery and are involved in vesicular trafficking of transmembrane proteins, they are also known as “endocytic tumor-suppressor genes.” Each of them is required for different steps in trafficking of proteins from the apical membrane to the lysosome, and mutation of each gene blocks endocytic degradation of certain transmembrane proteins and induces epithelial polarity defect and neoplastic overgrowth in imaginal epithelium. The tumor growth observed when mosaic mutant clones of these genes are generated in developing imaginal epithelium occurs in part through a non-cell-autonomous process (Moberg et al., 2005; Thompson et al., 2005; Vaccari and Bilder, 2005). Endocytic trafficking defects in these mutant cells cause endosomal accumulation of the signaling receptor Notch and increase Notch signaling. Increased Notch activity leads to ectopic production of Unpaired, a

secreted cytokine-like ligand of the JAK–STAT signaling pathway, which induces over-proliferation of the neighboring wild-type cells. On the other hand, the *tsg101* and *vps25* mutant cells undergo apoptosis when they are surrounded by wild-type cells in imaginal epithelium (Moberg et al., 2005; Thompson et al., 2005; Vaccari and Bilder, 2005). Thompson et al. (2005) found that *vps25* mutant clones survive relatively well after arrest of cell proliferation, which occurs in cells behind the morpho-genetic furrow of the eye imaginal disc and in the follicular epithelium of the ovary. The survival defect of *vps25* mutant cells is therefore a consequence of cell proliferation. They also showed that the apoptosis of *vps25* mutant clones can be prevented either by alleviation of competition by means of the *Minute* technique or by expression of the antiapoptotic p35. In both cases, blockade of apoptosis allows the mutant clone to grow to form a tumor mass (Thompson et al., 2005).

Herz et al. (2006) also analyzed the apoptosis phenotype of *vps25* mutant clones using the *Minute* technique, but although *vps25* mutant clones surrounded by the *M*/+ cells were larger than the clones surrounded by the wild-type cells, they still showed apoptosis. Furthermore, overexpression of dMyc in the *vps25* mutant clones did not significantly change the apoptosis rate. On the other hand, they found that Ex protein levels were low in *vps25* clones and that the apoptosis was almost completely blocked in *vps25 hippo* double-mutant clones, suggesting that the SWH signaling pathway controls apoptosis in *vps25* mutant cells. The contribution of cell competition to the apoptosis phenotype of *vps25* mutant cells therefore remains to be examined.

5. Other mutant cells

In addition to those listed above as SGCs and SDCs, many mutations whose phenotypes have been studied in *Drosophila* imaginal discs have been described as undergoing cell competition because of their phenotypes in defects of survival or growth, for example, *lgd* (Buratovich and Bryant, 1997), *chico* (Böhni et al., 1999), *peter pan* (Migeon et al., 1999), *dAkt* (Verdú et al., 1999), *dTOR* (Zhang et al., 2000), *cdc42* (Genova et al., 2000), *DRacGAP* (Sotillos and Campuzano, 2000), *Asx* and *E*(*z*) (Be uchle et al., 2001), *TSC1* and *TSC2* (Gao and Pan, 2001), *rheb* (Patel et al., 2003), *slik* (Hipfner and Cohen, 2003), *roughest* (Bao and Cagan, 2005), *pixie* (Coelho et al., 2005), *calderón* (Herranz et al., 2006), *cpa* and *cpb* (Janody and Treisman, 2006), *crooked legs* (Mitchell et al., 2008), *nero* (Patel et al., 2009), *minus* (Szuplewski et al. 2009), *dMCRS2* (Andersen et al., 2010), and *dcr-1* (Herranz et al., 2010). All of these mutant cells showed a growth disadvantage or a survival defect when their mitotic clones were generated in developing wild-type imaginal discs, and the *Minute* technique succeeded in saving them, but if we define cell competition as not just a proliferation race but a survival of the fittest, and if we require exact conformity with all three hallmarks of cell competition described above, then further analyses are still needed to reveal the phenotypes of those mutant cells as a result of cell competition.

6. The mechanisms by which loser cells are eliminated

The puzzle that must be solved is how winner cells kill neighboring loser cells. More than a quarter of a century after the discovery of cell competition, the mechanisms of this phenomenon had not been explained at the molecular level, but in 2002, Moreno et al. described the genetic pathway involved in cell competition induced by *M*/+ cells. They showed that deterioration of uptake of the transforming-growth-factor-β family member Decapentaplegic (Dpp) in the *Minute* or *dMyc* mutant cells that are surrounded by wild-type cells triggers upregulation of its downstream transcription factor *brinker* (*brk*). Ectopic upregulation of *brk* leads to activation of the c-Jun N-terminal kinase (JNK) pathway, thereby triggering the apoptosis pathway and eliminating these mutant cells from the wing

imaginal epithelia (Moreno et al., 2002; Moreno and Basler, 2004). On the other hand, Tyler et al. (2007) recently showed that suppressors of cell competition between *M*/+ and wild-type cells do not require Dpp activity. Paradoxically, some of these suppressors augmented Dpp activity in loser cells and increased their proliferation, but apoptosis was not reduced. Therefore, Dpp-stimulated proliferation has been suggested to outpace their death (Tyler et al., 2007; Johnston, 2009). Moreno and Basler (2004) also found that constitutive activation of the Dpp pathway or over-expression of Rab5, a small GTPase that drives the formation of clathrin-coated vesicles and their subsequent fusion with early endosomes, intended to stimulate endocytosis, and prevented elimination of loser cells (wild-type cells surrounded by dMyc-overexpressing cells). Deterioration of endocytic uptake of Dpp might therefore promote cell competition. On the other hand, Igaki et al. (2009) found that endocytosis is activated in *scrib* mutant clones and that blockade of endocytosis by overexpression of a dominant-negative form of Rab5 suppressed the elimination of *scrib* mutant clones. They therefore concluded that this increase in endocytosis promotes translocation of Eiger, the *Drosophila* tumor-necrosis factor (TNF), to endocytic vesicles and leads to activation of apoptotic JNK signaling in the *scrib* mutant cells. Some mechanisms of elimination of loser cells might also depend on the type of loser cells. In fact, a detailed look at the phenotypes of both SGC and SDC mutant cell types during their cell death induced by cell competition reveals three different modes of elimination: (1) JNK pathway–dependent apoptosis, (2) extrusion from an epithelial layer followed by apoptosis, and (3) engulfment by neighboring winner cells (Fig. 2).

6.1. The puzzle of JNK-dependent apoptosis

Activation of the JNK pathway has been observed in various mutant cells outcompeted by wild-type cells, for example, *Minute* (Moreno et al., 2002), *scrib* (Brumby and Richardson, 2003), *slik* (Hipfner and Cohen, 2003), *dMyc* (Moreno and Basler, 2004), *vps25* (Herz et al., 2006), *dlg* (Igaki et al., 2006), and *lgl* and *mahj* (Tamori et al., 2010). The proapoptotic role of JNK signaling has been further elucidated in mammalian systems (Davis, 2000; Dhanasekaran and Reddy, 2008), and similarly, *Drosophila* genetics and its cell-culture systems have shown that JNK-mediated cell-death machinery also exists in flies (Kuranaga et al., 2002; Kanda and Miura, 2004). Importantly, mosaic clones of both SGC and SDC mutants have been demonstrated to show activation of the JNK pathway, and in addition, blockade of JNK activation by overexpression of Puckered (phosphatase of JNK) or the dominant-negative form of Basket (*Drosophila* JNK) can suppress their apoptosis in cell competition. Furthermore, Tamori et al. (2010) showed that addition of JNK inhibitor significantly suppressed cell death and apical extrusion of MDCK Mahjong shRNA cells that were surrounded by normal MDCK cells, suggesting that the involvement of JNK activation in cell competition is evolutionarily conserved. JNK-dependent apoptosis has therefore been the most plausible and accepted mechanism for explaining the elimination of loser cells. A recent study has suggested that endocytosis-dependent activation of the Eiger–JNK pathway plays an important role in the elimination of *scrib* mutant clones from epithelia, as blocking endocytosis prevents both JNK activation and the elimination of the *scrib* mutant clones (Igaki et al., 2009), but we find a discrepancy in the logic of JNK-dependent apoptosis.

Apoptosis is suppressed by blockade of JNK activation in loser cells. This result clearly shows that activation of the JNK pathway is necessary to induce apoptosis in loser cells, but in most cases studied previously, the JNK activation is cell-autonomous in loser cells. In the mosaic imaginal epithelia where the mutant clones are induced by mitotic recombination, we can usually detect some groups of mutant cells. Study of mutant clones of *Minute* (Moreno et al., 2002; Li and Baker, 2007), *dMyc* (Moreno and Basler, 2004; de la Cova et al., 2004), *lgl* (Menéndez et al., 2010; Tamori et al., 2010), and *mahj* (Tamori et al., 2010) clearly

showed that loser cells adjacent to winner cells undergo apoptosis, and this “competition-dependent” mode of cell death is one of the most important hallmarks of cell competition. If the cell-autonomous JNK activation observed in loser cells is sufficient to trigger the apoptosis pathway, all mutant cells in a group should undergo apoptosis simultaneously; so JNK-dependent apoptosis cannot explain two paradoxical phenomena: cell-autonomous JNK activation in mutant cells and competition-dependent cell death (cell death that occurs only at the mutant clone boundary).

We therefore examined the JNK-activation patterns in wing imaginal discs dissected from *mahj* or *lgl* homozygous mutant larvae or *Minute* heterozygous mutant larvae with anti-phospho JNK antibody (unpublished data). Intriguingly, JNK was cell-autonomously phosphorylated even in the wing discs of these mutant larvae (Fig. 3B–D), whereas their apoptosis did not differ from that in wild-type wing discs (Fig. 3E–H). This result indicates that the JNK activation is intrinsic to loser cells and also confirms that this intrinsic JNK activation is necessary but not sufficient to induce competition-dependent cell death in loser cells.

Recently, Igaki et al. (2009) showed that *scrib* mutant clones are not eliminated and develop into tumors when they are generated in homozygous *eiger* mutant wing discs. They also found that knockdown of *eiger* expression within *scrib* mutant clones is sufficient to increase animal lethality caused by tumorigenesis significantly, suggesting that Eiger signaling originates within the *scrib* mutant clones and acts in an autocrine fashion. On the basis of these observations, we can hypothesize that two parallel mechanisms can induce competition-dependent apoptosis in cell competition: (1) Eiger promotes JNK activation in an autocrine fashion in loser cells, and (2) a cell-death-promoting factor secreted by abutting wild-type cells cooperates with the JNK pathway to promote apoptosis in loser cells (Fig. 2A). This hypothetical cell-death promoting factor might be soluble molecules secreted from neighboring wild-type cells (Senoo-Matsuda and Johnston, 2007).

6.2. Extrusion from an epithelial layer followed by apoptosis

For the epithelial barrier function to be preserved during cell turnover, dying cells are squeezed out of the epithelium by a process of “apoptotic cell extrusion” (Rosenblatt et al., 2001). The cell destined for apoptosis signals its neighboring epithelial cells to form a ring of actin and myosin II that contracts to squeeze the dying cell out of the epithelial sheet. This contraction not only ejects the dying cell but also closes any gaps that may result from insults to the epithelium (Slattum et al., 2009). In fact, in the loser-cell-elimination process, physical extrusion of loser cells from epithelial layers has been demonstrated in many SGCs and SDCs, but some evidence shows that this extrusion does not depend on the apoptosis of loser cells in cell competition. In this case, the extrusion induced by neighboring winner cells is a positive system for elimination of loser cells and is distinct from “apoptotic cell extrusion.” For example, Vidal et al. (2006) described this apoptosis-independent extrusion of *dCsk* (*Drosophila* C-terminal Src tyrosine kinase) mutant cells in cell competition. C-terminal Src tyrosine kinase (Csk) is a major inhibitor of Src kinase. Rous sarcoma virus Src gene (*v-Src*) was the first identified oncogene (Hunter and Sefton, 1980), and its cellular counterpart *c-Src* is overexpressed and highly activated in various human cancers (Frame, 2002). v-Src and c-Src are nonreceptor tyrosine kinases that phosphorylate multiple proteins on tyrosine residues and thereby regulate the actin cytoskeleton, cell adhesions, cell proliferation, and other cellular processes (Frame et al., 2002; Parsons and Parsons, 2004). The *Drosophila* Csk (*dCsk*) was identified as a factor mediating activity of both the Lats/Warts tumor suppressors (Stewart et al., 2003) and oncogenic isoforms of the Ret receptor tyrosine kinase (Read et al., 2004). When dCsk activity is decreased in the developing eye or wing imaginal epithelia, the result is overproliferation, inhibition of apoptosis, and

decreased cell adhesion, but Vidal et al. (2006) found that the *dCsk* mutant clones generated in a developing wild-type epithelium were outcompeted by neighboring wild-type cells and showed basal extrusion followed by apoptosis.

Tamori et al. (2010) also found that, in the process of elimination of $mahj^{-/-}$ cells, some apoptotic $mahj^{-/-}$ cells remained within the epithelial layer, whereas the majority of apoptotic cells were basally extruded from the disc epithelium. They also found that a number of $lgl^{-/-}$ cells abutting wild-type cells had become apoptotic and were basally extruded, whereas some apoptotic cells stayed in the epithelial layer. Furthermore, even if the apoptosis of these mutant clones was blocked by antiapoptotic p35 expression, some of these mutant cells were basally extruded from the epithelial layer. These results suggest that the apoptosis is not caused by basal extrusion of these mutant cells. This apoptosis-independent extrusion of mutant cells has also been demonstrated in the recent studies of mammalian cell-culture system (Hogan et al., 2009; Kajita et al., 2010), where MDCK cells were used to examine phenomena occurring at the interface between normal and transformed epithelial cells. Both RasV12 expressing MDCK cells (Hogan et al., 2009) and temperature-sensitive mutant of v-Src (ts-Src)-expressing cells (Kajita et al., 2010) are apically extruded from the monolayer of normal cells only when they are surrounded by normal MDCK cells, and this extrusion occurs in an apoptosis-independent manner. These results suggest that the apoptosis-independent extrusion is one of the evolutionarily conserved modes of elimination of loser cells in cell competition (Fig. 2B).

6.3. Engulfment by neighboring winner cells

Cells destined for apoptosis are promptly eliminated from tissues, a process made possible by apoptosis-dependent phagocytosis, in which cells unnecessary, obstructive, or dangerous to organisms are induced to undergo apoptosis and are earmarked for phagocytosis (Nakanishi et al. 2011). In *Drosophila*, apoptotic corpses are engulfed both by macrophage cells and by “nonprofessional” cells, such as imaginal disc cells, expressing the *ced* (*cell death abnormality*) pathways that were first identified in *Caenorhabditis elegans* (Reddien and Horvitz, 2004; Fullard et al., 2009). On the other hand, Li and Baker (2007) found apoptosis-independent engulfment in cell competition. They found that dead *M*/+ cell corpses are engulfed by neighboring wild-type cells rather than extruded from the epithelium and consumed by macrophages and, interestingly, that cells that are mutant for engulfment genes, such as *draper* (*drpr*), *wasp*, *phosphatidylserine receptor* (*psr*), *mbc/doc180*, or *rac1*, do not outcompete *M*/+ cells. These results suggest that the corpse engulfment by winner cells is not simply a passive response to the presence of dying loser (*M*/+) cells but is required to kill and eliminate neighboring loser cells (Li and Baker 2007) (Fig. 2C).

More recently, Ohsawa et al. (2011) revealed that Eiger–JNK signaling in surrounding normal cells promotes elimination of nTSGs mutant cells. They found that non-cell-autonomous JNK activation occurred in wild-type cells surrounding *scrib*, *dlg*, or *vps25* mutant clones and that this activation induced upregulation of PVR, the *Drosophila* PDGF/VEGF receptor, which activates the genes involved in the phagocytic pathway, such as *ELMO* (engulfment and cell motility, a Ced-12 homolog) and *Mbc* (myoblast city, a Ced-5/DOCK180 homolog), and causes them to engulf these nTSGs mutant cells. They also showed, however, that *M*/+ cells were still eliminated in an *eiger*-null mutant background and that JNK was activated neither in the SWH pathway mutant clones nor in their surrounding wild-type cells. They therefore concluded that this Eiger–JNK signaling–mediated elimination mechanism may have evolved specifically to eliminate highly malignant neoplastic cells from the tissue.

7. Relevance to cancer

Recently, cell competition has come to be considered deeply relevant to initial stages of carcinogenesis (Baker and Li, 2008; Moreno, 2008; Johnston, 2009; Hogan et al., 2010). During these initial stages, transformation events involving mutations in oncogenes and/or tumor-suppressor genes occur in a single cell within an epithelial monolayer. Although events at the interface between normal and transformed epithelial cells during this process remain unclear, cell competition is the most plausible candidate for the phenomenon exerting a decisive influence on the future of the transformed protumor cells in this stage. Considering the phenotypes of SGC and SDC during cell competition, we can postulate two different significances of the phenomenon in the early stage of tumor progression: elimination of protumor cells and "field cancerization." The concept of field cancerization was first introduced by Slaughter et al. (1953) as a result of their studies of the presence of histologically abnormal tissue surrounding oral squamous-cell carcinoma. The concept was proposed to explain the development of multiple primary tumors and locally recurrent cancer. According to one model of field cancerization, one cell sustains an initiating mutation. After extensive proliferation, some of its clonal descendants may subsequently acquire a second mutant allele, and the doubly mutated "initiated" cells may then proliferate and eventually occupy a large patch of epithelium in which chances of development of a malignant neoplasm are higher (Weinberg, 2007).

The mutant phenotypes of nTSGs, like *scrib*, *dlg*, and *lgl*, are clearly different from those of hyperplastic tumor-suppressor genes, like the SWH pathway components or the gain-of-function mutant of protooncogenes like *dMyc*. nTSGs mutant cells show defects in epithelial apicobasal cell polarity and induce disorganization in epithelial integration; so they cannot undergo terminal differentiation and never stop dividing, producing massive tumor growth and metastasis. Interestingly, however, the mosaic mutant clones of nTSGs generated in a heterozygous imaginal disc through mitotic recombination grow more slowly than their wild-type neighbors and are eliminated by cell competition. On the other hand, mutant cells of the SWH pathway component genes or cells overexpressing protooncogene *dMyc* do not show epithelial disintegration but outcompete neighboring wild-type cells and proliferate to form a field as supercompetitors. Therefore, if the transformed cell spawned in an epithelium is a mutant cell of nTSG, these cells should be outcompeted by surrounding normal cells and eventually eliminated from the epithelium. In this case, cell competition functions as an intrinsic tumor-suppression mechanism to maintain homeostasis of epithelia, but if the transformed cell is a gain-of-function mutant cell of a protooncogene, such as *Myc*, or a loss-of-function mutant cell of a hyperplastic tumor-suppressor gene, such as the SWH pathway, these cells will outcompete neighboring wild-type cells and form a field in which possibilities of development of a malignant neoplasm are higher. In this case, cell competition as an intrinsic tumor-suppression mechanism is hijacked by the protumor cells.

8. Concluding remarks

A survey of various types of mutant cells that induce cell competition with wild-type cells reveals two distinct categories based on their phenotypes in an epithelial tissue: SGCs and SDCs. Because most of the mutant cells, including SDCs, that have been shown to be eliminated in cell competition are slower-proliferating cells, proliferation (or growth) speed is generally interpreted as a primary factor determining winners and losers. In population genetics, fitness is a measure of survival and reproduction of the different genotypes, and natural selection will occur if individuals with different genotypes differ in fitness (Smith, 1998). If we consider cell competition as natural selection at the cellular level, proliferation rate could be considered a measure of cellular fitness.

Some types of loser cells cannot, however, be rescued by an increase in growth rate alone. For example, dMyc over-expression cannot alleviate the elimination of *mahj* mutant clones (Tamori et al., 2010). Furthermore, increase of proliferation rate by activation of cyclin D/Cdk4 or the insulin/insulin-like growth-factor receptor pathway does not cause cell competition (de la Cova et al., 2004). Menéndez et al. (2010) also showed that even large patches of tumorigenic *lgl* mutant clones overexpressing oncogenic RasV12 or Yki undergo apoptosis at the clone boundary with wild-type cells and that the majority of small clones of those tumorigenic mutant cells are eliminated despite their high proliferation rate. These results suggest that proliferation (or growth) speed is not the sole factor determining winners and losers in cell competition. Multiple factors may define cellular fitness in cell competition, although cells' ability to sense and measure the multiple factors properly is surprising. Cell competition might be a heterogeneous phenomenon induced by multiple causal phenotypes and might lead to elimination of loser cells by three different modes.

Recently, microarray gene expression analysis during cell competition between dMyc-overexpressing cells and wild-type cells led to the identification of two important genes: *flower* (Rhiner et al., 2010), encoding a cell-membrane protein that is conserved in multicellular animals and proposed to be a Ca^{2+} channel in neurons, and *SPARC* (Portela et al., 2010), the *Drosophila* homolog of the SPARC/Osteonectin protein family, which encodes a multifunctional secreted glycoprotein. In cell competition, one of the splicing isoforms of *flower* (fwe^{Lose}) is specifically upregulated in loser cells and functions as an extracellular code that is required and sufficient to label cells as losers. *SPARC* is also upregulated in loser cells and provides a transient protection by inhibiting Caspase activation in outcompeted cells. Importantly, the authors found that the two genes function in different types of loser cells: fwe^{Lose} is expressed in *M*/+ or *scrib* mutant cells, and *SPARC* is expressed in *M*/+ or *lgl* mutant cells. This pattern suggests not only common roles for these two genes in cell competition but also presence of a common pathway in cell competition.

The molecular mechanism of cell competition is a newly emerging challenge in the fields of developmental biology and cancer biology; so many questions still remain unanswered. Further studies designed to identify key molecules that play a pivotal role in cell competition and to elucidate each mechanism in different types of mutant cells or in different types of tissues will eventually reveal the whole picture of the phenomenon and show us a new concept in the field.

Acknowledgments

This work was supported by National Science Foundation grant (IOS-1052333) and National Institutes of Health grant (R01GM072562) to W.-M. D. We thank Anne B. Thistle, John S. Poulton, Nicholas Leake, and Gengqiang Xie for critical reading and helpful input with the manuscript.

Abbreviations

nTSGs	neoplastic tumor-suppressor genes
SGCs	slower-growing cells
SDCs	structurally defective cells
JNK	c-Jun N-terminal kinase
***M*/+**	*Minute* heterozygous
dMyc	*Drosophila* Myc

SWH	Salvador/Warts/Hippo
mahj	*mahjong*
lgl	*lethal giant larvae*
MDCK	Madin–Darby canine kidney

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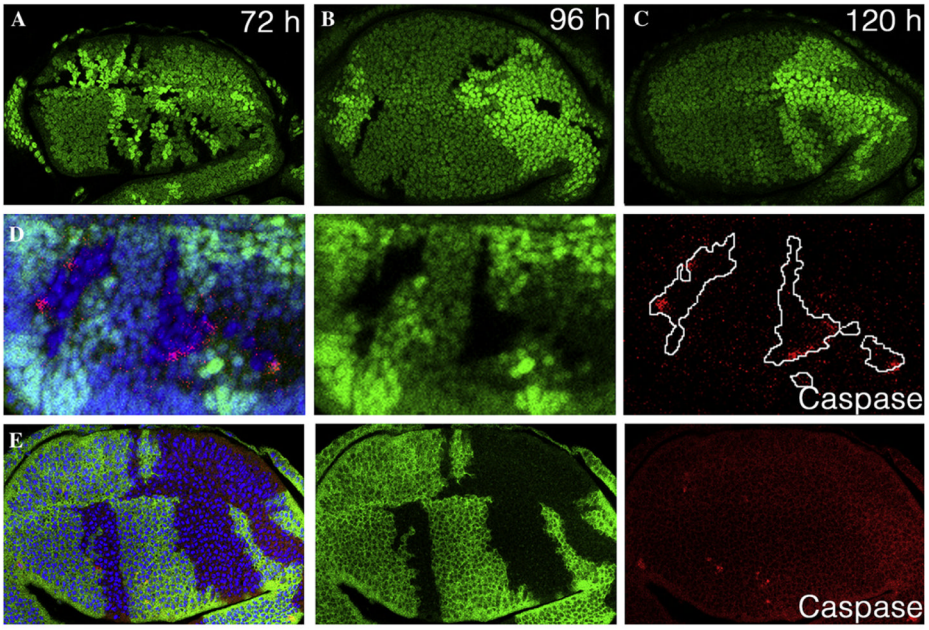

Fig. 1.

Hallmarks of cell competition. **A–C:** elimination of loser cells. Wing imaginal discs with *mahj*$^{-/-}$ (lacking GFP), wild-type (expressing GFP strongly), and *mahj*$^{+/-}$ clones (expressing GFP moderately) at 72 h (**A**), 96 h (**B**), and 120 h (**C**) after clone induction. **D:** competition-dependent apoptosis. Magnified images of *mahj*$^{-/-}$ clones 96 h after clone induction. Note that the apoptotic *mahj*$^{-/-}$ cells are on the clone boundary. **E:** rescue of loser cells by the *Minute* technique. A wing imaginal disc in which *mahj*$^{-/-}$ clones are surrounded by *Minute*/+ heterozygous cells (expressing β-gal, green) at 144 h after clone induction. Nuclei were stained with DAPI (blue). **D** and **E:** apoptotic cells were labeled with anti-cleaved Caspase-3 antibody (red).

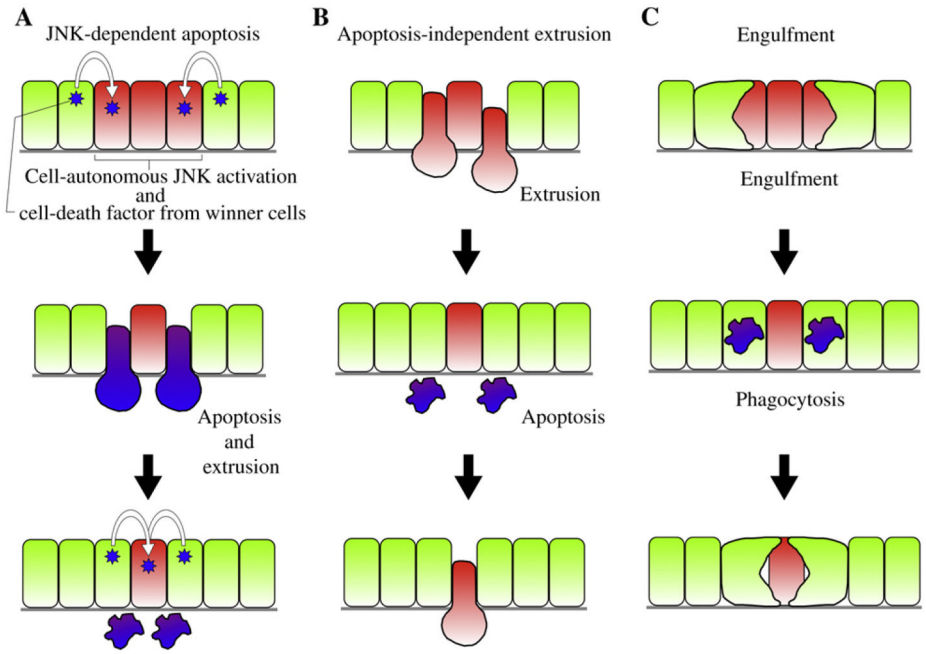

Fig. 2.

Three modes of loser-cell elimination: JNK pathway–dependent apoptosis (**A**), apoptosis-independent extrusion (**B**), and engulfment by neighboring winner cells (**C**). **A:** in loser cells (red), JNK is intrinsically activated in a cell-autonomous manner. A cell-death-promoting factor (blue stars) secreted by abutting wild-type cells (green) cooperates with the JNK pathway to promote apoptosis in loser cells adjacent to wild-type cells. **B:** loser cells (red) are physically extruded by abutting wild-type cells (green). The extrusion process is followed by apoptosis. **C:** abutting wild-type cells (green) engulf and therefore kill loser cells (red).

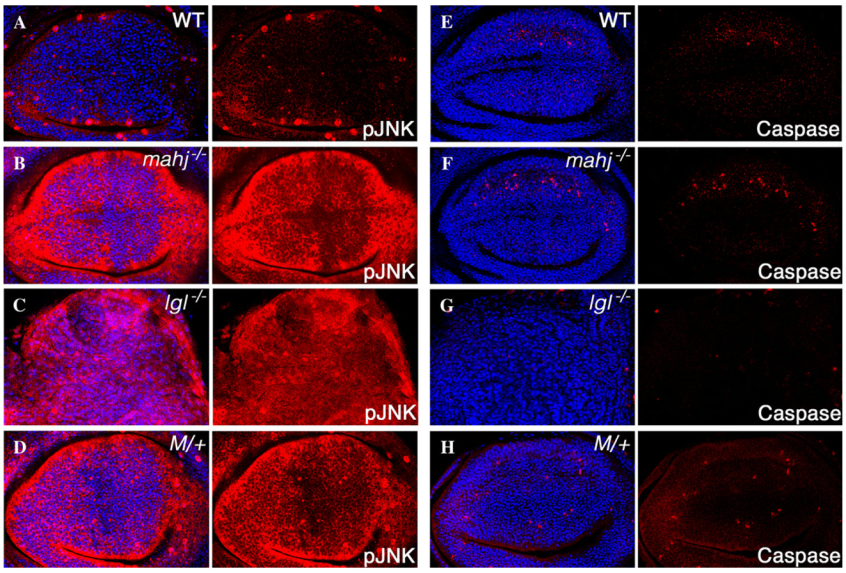

Fig. 3.

Cell-autonomous JNK activation in loser cells. **A**–**H:** wing imaginal discs dissected from wild-type (**A** and **E**), *mahj* homozygous (**B** and **F**), *lgl* homozygous (**C** and **G**), and *M*/+ (**D** and **H**) third-instar larvae. Note that JNK was cell-autonomously activated in the wing discs of these mutant larvae (**B**–**D**), whereas their apoptosis did not differ from that in wild-type wing discs (**F**–**H**). Nuclei were stained with DAPI (blue). **A**–**D:** JNK activation was labeled with anti-phospho JNK antibody (red). **E**–**H:** apoptotic cells were labeled with anti-cleaved Caspase-3 antibody (red).