Mechanisms of cell competition: Themes and variations

Romain Levayer and Eduardo Moreno

Institut für Zellbiologie, University of Bern, CH-3012 Bern, Switzerland

Cell competition is the short-range elimination of slow-dividing cells through apoptosis when confronted with a faster growing population. It is based on the comparison of relative cell fitness between neighboring cells and is a striking example of tissue adaptability that could play a central role in developmental error correction and cancer progression in both *Drosophila melanogaster* and mammals. Cell competition has led to the discovery of multiple pathways that affect cell fitness and drive cell elimination. The diversity of these pathways could reflect unrelated phenomena, yet recent evidence suggests some common wiring and the existence of a bona fide fitness comparison pathway.

Since the seminal work of Nusslein-Volhard and Wieschaus (1980) on segmentation in *Drosophila melanogaster*, embryogenesis has mostly been described as a top-down process whereby master regulators provided by the mother (the morphogens; Wolpert, 1981) set a cascade of hierarchal events leading to precise pattern formation. Yet, this framework does not allow correction of potential downstream errors and is not sufficient to explain the robustness and the adaptability observed in living organisms. There is indeed an increasing number of studies showing the great adaptability of developing tissues (Braendle and Félix, 2008; Domyan and Sun, 2011), among which cell competition is a striking example.

Cell competition was originally defined by the short-range elimination of slow-dividing cells when confronted with a faster growing population (Morata and Ripoll, 1975; Simpson, 1979; Simpson and Morata, 1981). Outcompeted cell elimination is a complex process involving local fitness comparison between "loser" cells and "winner" cells that eventually drives loser cell apoptosis (Moreno et al., 2002a; de la Cova et al., 2004; Moreno and Basler, 2004; Li and Baker, 2007; Rhiner et al., 2010; Fig. 1 A). Thus, cell competition involves a context-dependent regulation of growth, proliferation, and death that is based on local cell–cell interactions, in contrast to the classical top-down view of embryogenesis.

Correspondence to Eduardo Moreno: eduardo.moreno@izb.unibe.ch

Abbreviation used in this paper: wt, wild type.

Since then, cell competition has led to the discovery of multiple cell selection processes based on local fitness comparison both in *Drosophila* and in mammals (Fig. 1, B and D). These phenomena have repercussions on a large variety of areas from cancer, growth regulation, and cell signaling. In this review, we will describe the different processes driving cell fitness modulation and cell selection while trying to find potential common wiring for all of these phenomena. The analogous competitive interactions described in stem cell niche will not be described here, and a precise description of the phenomenon can be found elsewhere (Johnston, 2009; Zhao and Xi, 2010).

Historical definition of cell competition

Cell competition was originally characterized in *Drosophila* more than 30 years ago through the study of a class of dominant mutations called *Minutes* (Morata and Ripoll, 1975), encoding for ribosomal proteins (Kongsuwan et al., 1985). Heterozygous *Minute*$^{+/-}$ flies showed a general developmental time delay due to a cell-autonomous reduction of growth rate (Morata and Ripoll, 1975), but eventually reached normal body size without profound patterning defects. Interestingly, early induced *Minute*$^{+/-}$ clones in wild type (wt) background were not recovered in adult fly wings, suggesting a context dependent elimination of *Minute*$^{+/-}$ cells. This phenomenon was called cell competition and was subsequently better characterized by P. Simpson and colleagues (Simpson, 1979; Simpson and Morata, 1981). The recovery of *Minute*$^{+/-}$ clones increased when induced late or upon larvae starvation, which suggested that elimination required a differential growth rate. This was later confirmed by combining *Minutes* mutations with variable severity (Simpson and Morata, 1981), as the proportion of recovered clones was proportional to the relative differences in the growth rates of the two confronted cell populations. Interestingly, the final size of the wings and compartments was unaffected by competition, which suggests that wt cells grow at the expense of *Minute*$^{+/-}$ cells (Simpson and Morata, 1981). However, single wt clone expansion was restrained to well-defined and reproducible frontiers, and competition was ineffective across these borders, which outlined the existence of wing disc subdivision in nonmiscible cell populations,

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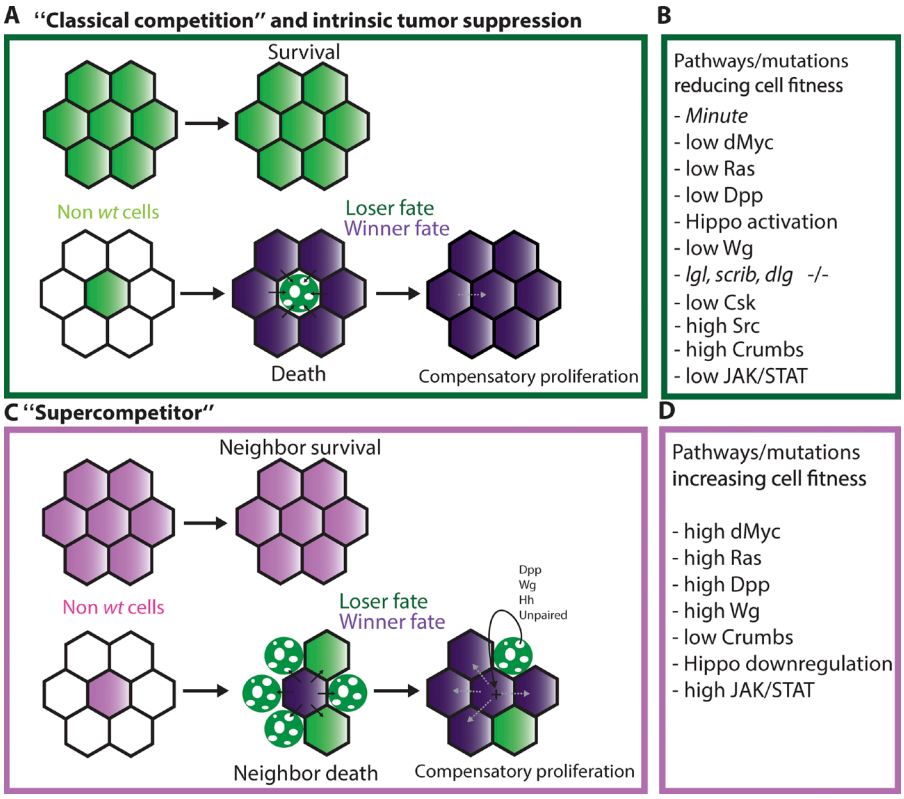

Figure 1. **Mutations and pathways involved in cell competition and intrinsic tumor suppression have a context-dependent phenotype.** (A) In "classical" cell competition, mutant cells (light green) surrounded by cells with the same genotype survive (top), whereas they are eliminated when surrounded by wt cells (white cells, bottom). Subsequently, wt cells replenish the tissue by compensatory proliferation. Green cells are the mutant cells, purple cells are the winners. (B) Mutation/pathways that induce a reduction of fitness leading to cell competition and intrinsic tumor suppression. (C) Supercompetitor cells (light purple) do not induce apoptosis when surrounded by cells with the same genotype (top), whereas they can grow at the expense of the surrounding wt cells (white) by inducing their death (bottom). Winner cell growth is up-regulated by compensatory proliferation, through the secretion of Dpp, Wg, Hh, and Unpaired from dying cells (dark gray arrow), or the non–cell autonomous down-regulation of Hippo pathway induced by dying cells (not depicted). Purple cells are the winner cells, green cells are the loser cells. (D) Mutation/pathways that induce an increase of fitness, thereby generating supercompetitors.

the so-called compartment boundary (Garcia-Bellido et al., 1973; Simpson and Morata, 1981).

Cell competition became a subject of interest again 20 years later when it was shown that *Minute*$^{+/-}$ clone elimination could also be observed in the wing imaginal disc and was apoptosis dependent. Loser clone elimination required an active induction of *Minute*$^{+/-}$ cell apoptosis by the surrounding wt cells (Abrams, 2002; Milàn, 2002; Moreno et al., 2002a). The elimination of *Minute* clones was driven by a relative deficit of Dpp pathway activation (Decapentaplegic, the fly orthologue of BMP, an extracellular morphogen regulating growth and patterning) leading to ectopic up-regulation of its down-steam inhibited target Brinker (Fig. 2; Moreno et al., 2002a). This subsequently led to JNK (c-Jun N-terminal kinase) pathway activation and apoptosis induction (Moreno et al., 2002a). Based on these results, it was proposed that neighboring cells compete for the uptake of limiting survival factors (here the morphogen Dpp) so that any cell showing a relative fitness deficit could lead to the reduction of Dpp uptake and cell elimination. Thus, cell competition could build a quality control mechanism that maximizes tissue fitness by destroying suboptimal cells. Interestingly, mutation in a ribosomal protein (Rpl 24) also led to competitive interactions in mouse blastocysts (Oliver et al., 2004), which suggests that the same phenomenon occurs in mammals.

Myc and supercompetitors

Cell competition gained further interest when it was related to cancer through the discovery of supercompetitors. Hypothetical supercompetitor mutations should increase cell fitness and lead to the clonal invasion of tissue at the expense of the surrounding wt cells, similarly to the early stage of tumor progression (Abrams, 2002; Fig. 1 B). The proto-oncogene *dmyc* was the first candidate to fit this definition (de la Cova et al., 2004; Moreno and Basler, 2004). Myc is a conserved transcription factor regulating multiple downstream targets involved in cell growth and ribosome biogenesis (Johnston et al., 1999; de la Cova and Johnston, 2006). Clones expressing high levels of *dmyc* overgrew at the expense of the surrounding tissue until they filled the compartment (de la Cova et al., 2004; Moreno and Basler, 2004). Clone expansion required the elimination of the surrounding cells by apoptosis, which was induced through Dpp deprivation, JNK activation (Moreno and Basler, 2004), and induction of the apoptotic activator Hid in the loser cells (de la Cova et al., 2004). On the contrary, down-regulation of Myc in clones led to their elimination, similar to *Minute* mutations (Johnston et al., 1999; de la Cova et al., 2004; Moreno and Basler, 2004). *dmyc* competition could be genetically related to *Minute*, as the *Minute* mutation suppressed the supercompetitor phenotype of *dmyc* overexpression (Moreno and Basler, 2004). Strikingly, cells with two additional copies of *dmyc* alternatively behaved as supercompetitors or losers when confronted, respectively, with wt cells or cells with four copies of *dmyc* (Moreno and Basler, 2004), demonstrating that competition is based on relative and not absolute levels of *dmyc*. Yet, supercompetition is not a general feature induced by any hyperproliferative clones, as overexpression of the phosphoinositide3-kinase (PI3K) Dp110 (an effector of the insulin growth pathway; Böhni et al., 1999) or the cell cycle regulators CyclinD and Cdk4 both generated large clones, but no elimination of the surrounding cells (de la Cova et al., 2004; Moreno and Basler, 2004).

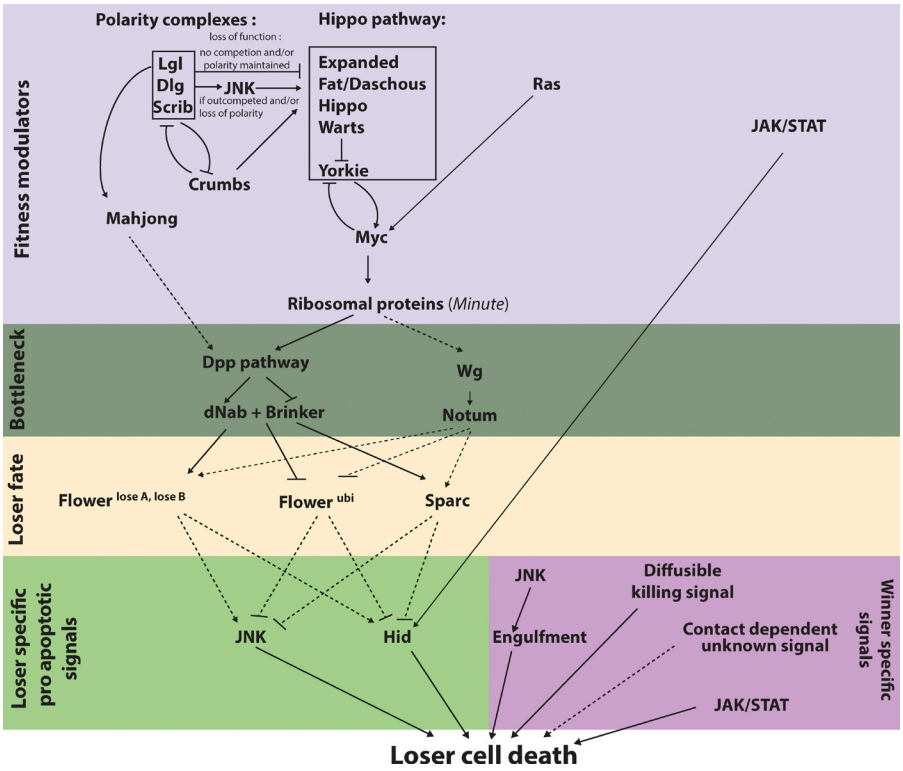

Figure 2. **Cell competition and cell selection are multistep processes.** Schematic of the multiple layers of regulation involved in loser cell elimination. Colored rectangles separate each hypothetical layer of control. Cell selection is initiated by mutations/pathways leading to a gain or a loss of fitness (light purple). The modulation of fitness leads to the deficit/gain of some limiting factors for which cells are competing (bottleneck, dark green). This then activates cell fitness markers (Flower, Sparc; yellow). Eventually, loser cell elimination is induced by different cell autonomous signal (JNK, Hid; light green), and by signals emitted by winner cells (dark purple). Hypothetical epistatic relationships are marked by broken lines.

Other Myc related pathways that induce cell competition

Since the discovery of *Minute*- and Myc-driven competition, the repertoire of pathway-inducing competitive interactions has been constantly growing. This includes the GTPase Ras1 (Prober and Edgar, 2000). Down-regulation of Ras in clones led to their elimination by competition. This phenotype was probably induced by the down-regulation of Myc, as Ras1 can up-regulate Myc concentration by posttranscriptional regulation, and Myc expression is sufficient to rescue Ras1 mutant clones (Prober and Edgar, 2000). The Hippo pathway has also emerged as an important regulator of competition (Tyler et al., 2007; Froldi et al., 2010; Menéndez et al., 2010; Chen et al., 2012). The Hippo pathway is a central regulator of growth, cell death, and tissue homeostasis, and has been involved in a variety of cancers (Zhao et al., 2011; Staley and Irvine, 2012). It is composed of the core kinases Hippo and Warts, and the adaptors Salvador and Mats, which prevent the nuclear accumulation of the transcriptional cofactor Yorkie (Yki; Yap in mammals). Upon Hippo pathway down-regulation, Yki accumulates in the nuclei and induces the transcription of multiple targets promoting cell proliferation, and down-regulates apoptosis through Diap1 (*Drosophila* inhibitor of apoptosis 1) up-regulation (Staley and Irvine, 2012).

The involvement of the Hippo pathway in cell competition was initially identified in a screen for genes preventing *Minute*$^{+/-}$ clone elimination in fly adult eyes (Tyler et al., 2007). Similar to *dmyc* overexpression, mutations in the Hippo pathway components increased Dpp pathway activation and produced supercompetitive clones, which invaded a whole compartment. Hippo-dependent competition could be driven by the transcriptional up-regulation of *dmyc* by Yki (Neto-Silva et al., 2010; Ziosi et al., 2010). Indeed, Myc down-regulation was sufficient to abolish the supercompetitive behavior of activated Yki clones (Neto-Silva et al., 2010; Ziosi et al., 2010). Yet, Myc does not fully recapitulate the features of Hippo-dependent competition, as *dmyc* overexpression was not sufficient to rescue Yki mutant clones (Ziosi et al., 2010), which are probably eliminated through the down-regulation of the apoptosis inhibitor Diap1.

Polarity defects and intrinsic tumor suppression

Elimination of clones mutant for the central regulators of apico-basal polarity Lethal giant Larvae (Lgl), Disc large (Dlg), Scribble, and clones overexpressing the apical determinant Crumbs has also been related to cell competition. Yet, these phenomena differ from the original definition of cell competition, as homozygous mutant organisms were not viable and mutant cells were only eliminated after losing apico-basal polarity and showing profound morphological defects (Wodarz, 2000; Grzeschik et al., 2010b). Moreover, elimination of *lgl* mutant cells did not require differential growth (Menéndez et al., 2010), as apoptosis was still observed in *lgl* mutant clones despite their high proliferation rate induced by Yki or Ras activation. Finally, *lgl* mutant clones were recognized and killed by circulating macrophages through the secretion of the JNK activator TNF/eiger by immune cells (Cordero et al., 2010), whereas *Minute*$^{+/-}$ clone elimination did not require the presence of macrophages, which are only necessary for the removal of apoptotic corpses (Lolo et al., 2012). As such, these selections differ from the original definition of cell competition, and were referred to by others either as intrinsic tumor suppression (Igaki, 2009) or extrinsic tumor suppression (Vidal, 2010; Lolo et al., 2013).

In *Drosophila*, homozygous mutants for *lgl*, *dlg*, or *scrib* developed normally until the depletion of the maternal supplied pool. Subsequently, epithelial cells of the imaginal discs lost their polarity and proliferated massively until forming a multilayered and amorphous mass (Wodarz, 2000). As such, these genes were classified as neoplastic tumor suppressors. Similarly, mutant clones of *lgl*, *dlg*, and *scrib* overproliferated in the eye imaginal disc, where apico-basal polarity was maintained (Brumby and Richardson, 2003; Grzeschik et al., 2007, 2010a,b; Doggett et al., 2011). However, early induced *lgl/dlg/scrib* mutant clones failed to proliferate in the wing imaginal disc, where mutant cells surrounded by wt cells were eliminated upon loss of apico-basal polarity through JNK-dependent apoptosis (Brumby and Richardson, 2003; Igaki et al., 2009; Froldi et al., 2010; Grzeschik et al., 2010b; Menéndez et al., 2010; Tamori et al., 2010; Ohsawa et al., 2011; Chen et al., 2012). Similarly, clones overexpressing the apical determinant *crumbs* were eliminated by apoptosis when surrounded by wt cells (Hafezi et al., 2012). Moreover, a disc compartment fully mutant for *lgl* or overexpressing *crumbs* grew indefinitely and was not affected by intrinsic tumor suppression (Menéndez et al., 2010; Hafezi et al., 2012), similar to Myc- and *Minute*-driven competition. On the contrary, *crumbs* mutant clones underwent massive proliferation and induced apoptosis of the wild-type surrounding cells, similar to Myc supercompetitors (Hafezi et al., 2012). Yet, Crumbs "supercompetition" differed from Myc in that it induced death across compartment boundaries. In mammalian epithelial cells, *scrib* mutants also underwent delamination and apoptosis when surrounded by wild-type cells (Norman et al., 2012), and *lgl* mutant cells were also eliminated through JNK activation when surrounded by wt cells (Tamori et al., 2010). Thus, *lgl/dlg/scrib* mutants and *crumbs* overexpression recapitulate some features of cell competition, which suggests the existence of common downstream events required for cell elimination.

Interestingly, neoplastic growth of *scrib* mutant clones could be rescued by the activation of Ras and Notch (Brumby and Richardson, 2003) or by increasing Yki activity (Chen et al., 2012). Similarly, *lgl* mutant clones could be partially rescued by *dmyc* expression (Froldi et al., 2010) and Hippo pathway down-regulation (Menéndez et al., 2010). Accordingly, Myc was down-regulated in *lgl* mutant clones (Froldi et al., 2010). Moreover, the Hippo pathway regulator Expanded was mislocalized in *crumbs* mutant, whereas the Hippo target Diap1 was up-regulated, as previously reported (Chen et al., 2010; Robinson et al., 2010), which suggests that the *crumbs* mutant supercompetitor phenotype is driven by Hippo pathway down-regulation. Thus, the Myc and/or Hippo pathway are core downstream targets required for elimination of polarity-deficient cells and Crumbs supercompetition. Surprisingly, the downstream regulation of Myc and Hippo pathways by polarity proteins is context dependent (Froldi et al., 2010; Grzeschik et al., 2010a,b; Doggett et al., 2011; Chen et al., 2012). *scrib/lgl* mutant clones surrounded by wt cells down-regulated Yki/Myc, whereas the same targets were up-regulated if the clones were surrounded by slow-growing cells (Froldi et al., 2010; Chen et al., 2012). Similarly, Yki was up-regulated in *lgl* mutant clones in the eye imaginal disc where apico-basal polarity was maintained (Grzeschik et al., 2010a), whereas it was down-regulated in *lgl* mutant clones in the wing pouch of the wing imaginal disc, where apico-basal polarity was lost (Froldi et al., 2010; Menéndez et al., 2010). In the absence of intrinsic tumor suppression and strong morphological defects, Yki up-regulation in *scrib* and *lgl* mutants was induced by an increased activity of the apical determinant aPKC (atypical PKC; Leong et al., 2009; Grzeschik et al., 2010a,b; Doggett et al., 2011) or by the mislocalization of the Hippo regulator Expanded in Crumbs overexpressing cells (Grzeschik et al., 2010a). On the contrary, Yki could be down-regulated in polarity-deficient/outcompeted mutant cells through the activation of the JNK pathway (Doggett et al., 2011; Chen et al., 2012). However, other downstream targets of polarity proteins could produce a fitness deficit. For instance, the Lgl interactor Mahjong was a key downstream target of Lgl responsible for *lgl* mutant elimination in both mammalian cells and *Drosophila* imaginal disc (Tamori et al., 2010).

In conclusion, polarity defects are also potent inducers of cell selection and fitness modulation. The close relationship between Hippo pathway and apico-basal polarity cues (for review see Genevet and Tapon, 2011) and the results of the various epistatic experiments suggest that the Hippo pathway is a major inducer of selection acting downstream of polarity defects, which could relate intrinsic tumor suppression to classical cell competition (Fig. 2). However, the partial rescue of mutant clones obtained upon Yki activation (Menéndez et al., 2010) suggest that other downstream targets (such as Mahjong; Tamori et al., 2010) are required for cell selection.

Myc- and *Minute*-independent pathways inducing competition

So far, most of the fitness modulators have been related to differential expression of Myc and/or modification of the Hippo pathway. Yet, several new cases of competition independent of Myc, *Minute*, or Hippo have been reported recently. This includes the differential activation of the Wnt–Wingless pathway and modification of JAK–STAT activation.

Similarly to Dpp, the morphogen Wingless (Wg; *Drosophila* orthologue of Wnt) is also involved in cell competition (Vincent et al., 2011). Wg is also a central regulator of growth, patterning, and cell survival (Clevers, 2006), which forms a gradient along the dorso-ventral axis in the wing imaginal disc (Baena-Lopez et al., 2012). Like Myc, local down-regulation of Wg signaling led to clone elimination, whereas local hyperactivation generated supercompetitors and apoptosis of neighboring wt cells. Elimination of Wg-deprived cells also involved both JNK activation (Giraldez and Cohen, 2003) and Hid (Johnston and Sanders, 2003). Yet competitive interactions induced by Wg were independent of Myc, as Myc was unexpectedly down-regulated in Wg supercompetitor clones, and supercompetition was not abolished by down-regulation of Myc throughout the disc or up-regulation of Myc in the surrounding wt cells. Alternatively, Wg dependent supercompetition required the secretion of notum, an inhibitor of Wg signaling that was activated downstream of Wg and secreted from Wg hyperactivated clones (Vincent et al., 2011).

Modulation of JAK–STAT pathway activity also led to competitive interactions in the wing and eye imaginal discs of *Drosophila* (Rodrigues et al., 2012). The JAK–STAT pathway has been involved previously in the regulation of growth and tissue size (Arbouzova and Zeidler, 2006; Li, 2008). Similarly to Myc, down-regulation of JAK–STAT in a subset of cells led to their elimination by apoptosis. On the contrary, STAT hyperactivation produced supercompetitors that grow at the expense of the surrounding cells. Yet, the competitive interactions induced by JAK–STAT modulation were independent of Myc, Hippo pathway, Wg, and ribosome biogenesis. Moreover, JNK was not activated in *StatE92* mutant clones, whereas elimination of wt cells by STAT hyperactivated cells required the apoptotic gene *hid*.

Finally, other pathways have been related to cell competition, notably through their context-dependent effect on epithelial cell extrusion. Yet, these phenomena differ from classical cell competition, as they do not require induction of apoptosis. This includes activation of Ras (Hogan et al., 2009) and Src in MDCK cells (Kajita et al., 2010), and *Drosophila* clones mutants for C-terminal Src kinase (Csk; Vidal et al., 2006).

What are the cells competing for?

The similar characteristics shared by the selection processes described so far suggest that they share some common ground. Part of this could be the downstream limiting factors for which cells are competing (Fig. 2, Bottleneck). This hypothesis was suggested early on, when *Minute*- and Myc-deficient clones were shown to compete for the uptake of the Dpp morphogen (Moreno et al., 2002a; Moreno and Basler, 2004). The competition observed upon Wg perturbation (Vincent et al., 2011) suggests that the same phenomena occur for multiple morphogens and growth factor. Indeed, elimination of Wg-deficient cells still occurred when surrounded by slow-dividing cells (Giraldez and Cohen, 2003), which suggests that Wg also acts downstream of the metabolism deficit (Fig. 2). In this framework, any mutation leading to a relative fitness deficit will eventually lead to reduced survival/growth factor uptake, thereby inducing cell death. Death could then be induced by the inconformity of the morphogen readout in the loser cells compared with their neighbors, as previously characterized for Dpp (Adachi-Yamada et al., 1999; Milán et al., 2001; Adachi-Yamada and O'Connor, 2002; Gibson and Perrimon, 2005; Shen and Dahmann, 2005). Dpp inconformity could be detected by the abnormal colocalization of Brinker and its interactor dNab, which are normally expressed in exclusive complementary patterns (Ziv et al., 2009).

Alternatively, cell competition could occur because of limited space and mechanical constrains. It was suggested previously that *Minute*- and Myc-driven competitions were related to size control, as the final size of the wings and the compartments undergoing competition was unaffected and similar to control discs (Simpson, 1979; Simpson and Morata, 1981; de la Cova et al., 2004). This result suggests that wing final size is a fixed parameter, which is not affected by the autonomous growth rate of clones. Growth in a limited space could be a key parameter required to drive cell competition. It was previously suggested that differential growth between neighboring cells could induce mechanical stress and feedback on the rate of cell division and apoptosis (Shraiman, 2005). In this model, the compression experienced by slow-dividing cells neighboring fast-growing cells would drive apoptosis. More recently, random cell delamination due to cell crowding was described in the pupal notum of *Drosophila* (Marinari et al., 2012). However, only a minor proportion of delamination was apoptosis dependent, which suggests a process different from cell competition. Yet, elimination of loser clones could also be partially induced by the mechanical constraints imposed by the vicinity of fast growing clones and cell crowding. This could explain the insulating role of compartment boundaries on cell competition, which through the local up-regulation of cortical tension (Landsberg et al., 2009) could mitigate the communication of mechanical stress from one compartment to the other.

Induction of a loser fate and the Flower code

Cell competition has shed new light on multiple phenomena driving elimination of cells showing reduced fitness compared with their neighbors. Since then, identifying the potential molecular effectors allowing fitness comparison between cells has become a central question in the field. The transmembrane protein Flower could be this so-called fitness marker (Rhiner et al., 2010). The *flower* gene was identified in a microarray for genes induced early in loser cells during Myc-dependent competition. The Flower locus encodes three different isoforms mostly differing in their extracellular domain. One isoform ($flower^{ubi}$, "ubi" stands for ubiquitous) is normally expressed throughout the wing disc but down-regulated in loser cells during competition, whereas the two other isoforms ($flower^{loseA}$ and $flower^{loseB}$) are only detected in loser cells during cell competition. Interestingly, this pattern is observed downstream of multiple fitness modulators, including Myc, *Minute*, *scrib* mutant, and Dpp pathway. $flower^{lose}$ expression is required for loser elimination, as RNAi targeting the two *lose* isoforms was sufficient to rescue loser clone elimination. On the contrary, $flower^{lose}$ expression in clones or in S2 cells was sufficient to drive their elimination when contacting wt cells or *Minute* mutants. Thus, a relative increase of lose isoforms expression is necessary and sufficient to induce loser fate and cell elimination. Similarly, $flower^{ubi}$ knockdown in a subset of cells also drove their elimination. Moreover, Flower differential expression was induced upstream of the JNK pathway and apoptosis induction, and was specific to cell competition. Thus, the "Flower code" is a central and common downstream regulator of cell selection, which would inform neighboring cells on their relative fitness status and subsequently activates the elimination of loser cells.

The same microarray data identified Sparc, another general marker of loser cells (Portela et al., 2010). Sparc is a secreted protein involved in extracellular matrix remodeling and has been related to cancer progression (Bradshaw, 2012). Sparc is also up-regulated in loser clones downstream of multiple selection phenomena. Yet, contrary to Flower, Sparc down-regulation accelerated loser elimination, whereas its overexpression delayed it. This protective effect is specific to cell competition and

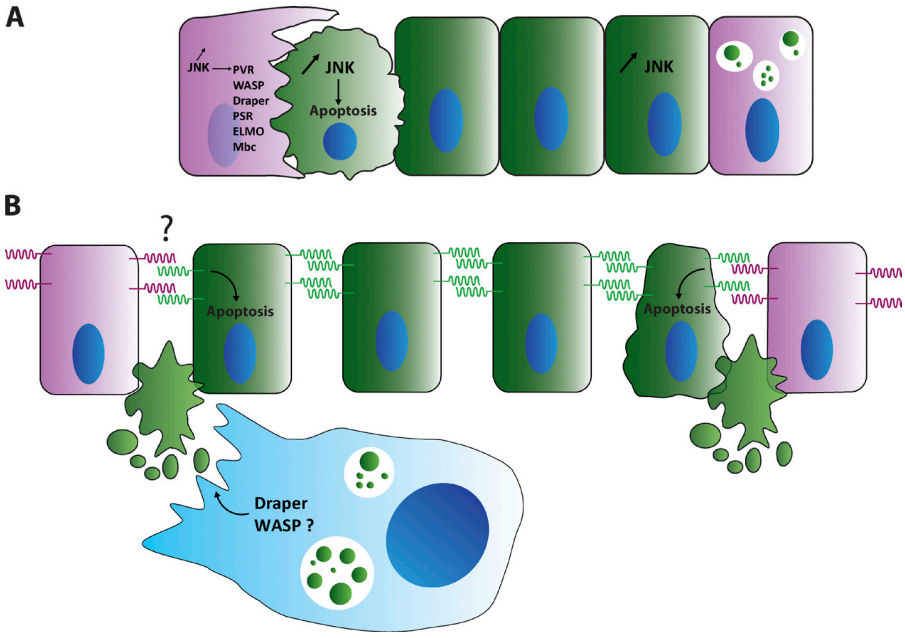

Figure 3. **Different scenarios regarding the requirement of loser cell engulfment.** (A) Engulfment of loser cells (green) by winner cells (purple) is required for their elimination. Engulfment is induced by JNK activation in winner cells, which in turn activates the engulfment-specific gene PVR. Engulfment also requires the presence of WASP, Draper, PSR, ELMO, and Mbc. Winner cell lysosomes contain fragments of loser cells (green particles). (B) Alternatively, engulfment is not required for loser cell elimination, but only for the clearance of already delaminated apoptotic corpses. This is achieved by macrophages (blue) through Draper activation and, potentially, WASP.

intrinsic tumor suppression, as Sparc overexpression did not prevent developmentally regulated apoptosis. Moreover, epistatic experiments showed that Sparc was activated in parallel with Flower. Thus, Sparc is a self-protective signal induced in loser cells, which could prevent inappropriate elimination of cells experiencing a transient fitness deficit.

These results suggest that loser cell elimination is a very tightly regulated cell decision event that relies on the balance between pro-loser signals (e.g., *flower*^{lose}) and protective signals (e.g., Sparc). Moreover, the characterization of Flower and Sparc suggests the existence of a bona fide cell fitness comparison pathway acting downstream of several fitness modulators. Yet, Flower and Sparc expression patterns are not sufficient to fully comprehend loser cell elimination, as apoptosis occurs mostly at the periphery of loser clones, while they are both expressed homogenously throughout the clone. These observations reveal the existence of downstream events required for loser cell elimination that are based on winner/loser interactions.

Downstream events leading to loser elimination

In the early descriptions of *Minute*-induced competition, investigators noticed that competition occurred only in cells directly contacting *Minute*$^{+/+}$ cells (Simpson and Morata, 1981). Later on, it was confirmed that apoptosis was mostly observed at the periphery of loser clones (Li and Baker, 2007; Ohsawa et al., 2011). Similarly, apoptosis was only observed at the periphery of *flower*^{lose}-expressing cells (Rhiner et al., 2010). These observations suggest the existence a winner/loser contact–dependent induction of death that acts downstream of loser fate induction (here marked by *flower*^{lose}). One explanation could be the requirement of loser cell engulfment by winner cells (Li and Baker, 2007; Ohsawa et al., 2011; Fig. 3 A). This is supported by the requirement of engulfment-specific genes (the actin regulators *WASP* and *draper*, phosphatidylserine receptor [*PSR*], and the phagocytosis genes engulfment and cell motility [*ELMO*], and myoblast city [*mbc*]) in winner cells for the elimination of *Minute* and *scrib* mutant clones, and the presence of loser cell fragments in winner cell lysosomes. Interestingly, *scrib* mutant engulfment was induced by the activation of the JNK pathway in winner cells (Ohsawa et al., 2011), which suggests a different outcome of JNK activation depending on the cell fitness status. JNK activation was induced by the upstream secreted activator TNF/Eiger (Igaki et al., 2002; Moreno et al., 2002b; Igaki et al., 2009) in both winner and loser, and led to induction of PVR (the fly orthologue of the PDGF/VEGF receptor, an engulfment factor) in the winner cells (Ohsawa et al., 2011). Yet, loser cell engulfment is probably not sufficient to explain the distribution of apoptotic cells at the periphery of clones. Indeed, blocking apoptosis by overexpressing the baculovirus protein p35 was sufficient to block engulfment, suggesting that it acts downstream of apoptosis (Li and Baker, 2007). Moreover, a recent study failed to reproduce the key experiments demonstrating the requirement of engulfment genes in winner cells (Lolo et al., 2012). Lolo and colleagues showed instead that engulfment was only required for the elimination of already delaminated cells and was mostly performed by macrophages, therefore reopening the question of which mechanism drives loser death at clone boundaries (Lolo et al., 2012, 2013; Fig. 3 B). This controversy could simply point to the existence of multiple mechanisms responsible for loser cell elimination. For instance, JAK–STAT signaling was also required in wt cells to eliminate *scrib* mutant clones independently of Hippo signaling (Schroeder et al., 2012). Alternatively, polarity-deficient cells could also be eliminated by the secretion of Eiger from circulating hemocytes (Cordero et al., 2010).

Another alternative hypothesis could lie in a short-range diffusible signal secreted by winner cells and required to induce loser cell elimination (Fig. 4 A, diffusible killing signal). In Myc and JAK–STAT supercompetition assays, wt cell elimination was observed several cell diameters away from winner clones (de la Cova et al., 2004; Rodrigues et al., 2012). This could be

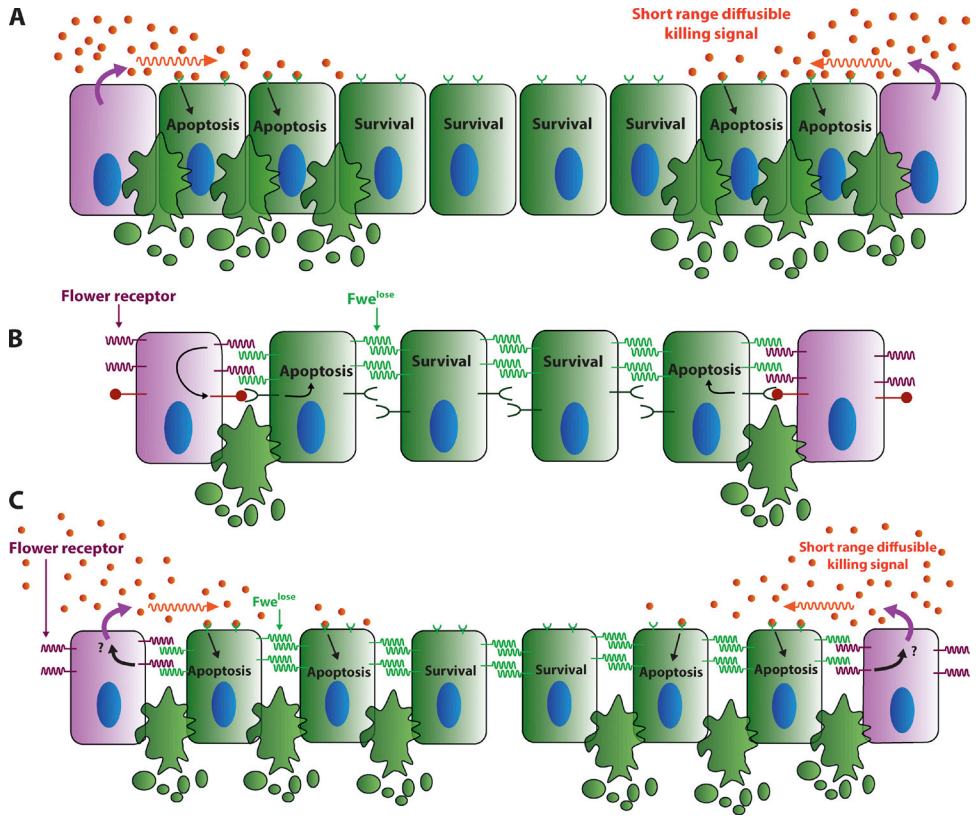

Figure 4. **The diffusible killing signal and the transmembrane receptor–dependent hypothesis.** (A) Loser cell death is induced by an unknown short-range diffusing signal produced by winner cells. The killing signal (orange dots) diffuses on short range and activates apoptosis two to three cell diameters away from winner cells, whereas cells located farther survive ("survival"). Only cells with a fitness deficit respond to the signal (here, depicted by the presence of a green receptor on loser cells). The winners are in purple and the losers in green. (B) Loser cell elimination is induced by a direct contact with winner cells. An uncharacterized receptor in the winner cells (purple transmembrane protein) recognizes Flower^{lose} (Fwe^{lose}, green transmembrane protein) or the absence of Flower^{ubi} and activates a contact-dependent killing signal (interaction between brown ligand and dark green receptor). The winners are in purple and the losers in green. (C) Combined model where winner/loser contact is required for fitness comparison (through Flower code), which then induces the production of a diffusible killing signal in the contacting winners. Only cells with a fitness deficit (green receptor) are sensitive to the killing signal. The winners are in purple and the losers in green.

explained by the existence of a diffusible killing signal produced by winner cells (Senoo-Matsuda and Johnston, 2007). This hypothesis is supported by experiments performed in S2 cells. Cells overexpressing *dmyc* induced wt cell elimination across a porous barrier, preventing cell contact but allowing protein diffusion (Senoo-Matsuda and Johnston, 2007). Moreover, a culture medium where cells had previously undergone competition was sufficient to induce death on a homogenous population of control cells, whereas it increased proliferation of *dmyc*-expressing cells. Thus, some diffusible factors inform cells about their fitness status and can induce loser cell elimination. Yet the diffusible killing signal hypothesis can hardly explain some early observations, including the insulation effect of compartment boundaries, which do not prevent protein diffusion (Zhou et al., 2012), and the strict requirement of contact for *Minute*$^{-/+}$ and *flower*^{lose} clone elimination (Simpson and Morata, 1981; Li and Baker, 2007; Rhiner et al., 2010; Ohsawa et al., 2011). The killing signal must diffuse on very short distances and its diffusion must be blocked at compartment boundaries by some unknown mechanisms in order to fit with these observations.

Alternatively, loser elimination could also be controlled by direct winner/loser interaction through unknown transmembrane receptors (Fig. 4 B). A contact-dependent killing response could be activated in the winner cells by the recognition of Flower^{lose} and/or the absence of Flower^{ubi} in the neighboring loser cells (Fig. 4 B). In this framework, the probability of eliminating loser cells would be set by the surface of contact shared with winner cells. Interestingly, this hypothesis could nicely explain the effect of compartment boundaries, which through increased cell tension (Landsberg et al., 2009) mitigate the surface of contact shared with the cells across the boundary, and could reduce the probability of inducing apoptosis. In that respect, the abnormal shape of loser clones (high number of involuting cells) and the high frequency of clone fragmentation (Simpson, 1979) could accelerate loser clone elimination. The high degree of cell mixing could be explained by the reorientation of winner cell division toward dying cells (Li et al., 2009). Alternatively, winner/loser interactions could induce an active cell mixing process based on adhesion regulation or subcellular regulation of tension machinery (Lecuit and Lenne, 2007). A better assessment of clone shape and the relationship between cell topology and apoptosis could shed new light on the process of loser clone elimination.

Finally, loser elimination could involve a combination of these two models. Winner/loser contact could be required first to read the fitness status of the neighboring cell (through Flower),

which would then induce the production of the killing signal in the winner cells (Fig. 4 C). In the two last models, the requirement of winner/loser interactions at several steps would ensure a tight regulation of apoptosis induction (Fig. 2 and Fig. 4).

Compensatory proliferation

Despite the elimination of loser cells by apoptosis during cell competition (Moreno et al., 2002a), the final tissue/compartment size was not reduced (Garcia-Bellido et al., 1973; Morata and Ripoll, 1975). This suggests that winner cells can compensate for the loss of eliminated loser cells by increasing their proliferation rate (Fig. 1). Non–cell autonomous induction of proliferation by apoptotic cells has been widely described under the term of compensatory proliferation (Fan and Bergmann, 2008a; Martín et al., 2009; Bergmann and Steller, 2010; Morata et al., 2011; Ryoo and Bergmann, 2012). Dying cells secreted a variety of pro-mitotic factors, which increased the proliferation of the neighboring cells, including the JNK-dependent secretion of the morphogens Dpp and Wg (Huh et al., 2004; Pérez-Garijo et al., 2004, 2009; Ryoo et al., 2004) and the JNK-dependent secretion of the ligand of JAK–STAT unpaired (Wu et al., 2010) and Hedgehog (Hh; Fan and Bergmann, 2008b). Compensatory proliferation was also reinforced by the non–cell autonomous down-regulation of the Hippo pathway (Grusche et al., 2011; Sun and Irvine, 2011; Graves et al., 2012; Kagey et al., 2012), which was mediated by the activation of Notch (Graves et al., 2012), JNK (Sun and Irvine, 2011), and/or Hh up-regulation (Christiansen et al., 2012; Kagey et al., 2012) in the dying cells. Loser cells might be less sensitive to these pro-mitotic signals due the low efficiency of their uptake machinery (Moreno et al., 2002a). Thus, in addition to its role in tissue replenishment, compensatory proliferation could also accelerate competition by increasing the relative differences of growth between losers and winners and by increasing the survival of winner cells.

Perspectives: Cell competition, cell selection, cancer, and evolution

The strong similarities between clonal expansion observed during cell competition and tumor growth was probably the main source of growing interest for the field (Moreno, 2008; Rhiner and Moreno, 2009). The majority of genes modulating cell fitness are conserved and have known tumor suppressor functions. Cell competition has also been characterized in mammalian tissue and cell culture (for review see Hogan et al., 2011). More recently, the tumor suppressor p53 has also been involved in the competitive interaction in hematopoietic stem cells, whereby after exposition to environmental stress cells with higher p53 levels are eliminated from the population (Bondar and Medzhitov, 2010). Thus, the features of cell competition outlined in *Drosophila* might apply to a large extent to mammals and strongly suggest an involvement of cell competition in cancer progression.

On theoretical ground, cell selection could have two opposite functions in the process of tumor expansion. On the one hand, the elimination of *lgl/dlg/scrib* mutant cells in *Drosophila* tissue and cell culture argues for a tumor suppressor function. Elimination of abnormal cells that would otherwise overproliferate could mitigate the appearance of the fast-growing clonal population and the expansion of a precancerous field. On the other hand, supercompetitive interactions could be exploited by the early tumor in order to kill neighboring cells and accelerate its expansion. This is supported by the pattern of expression of the loser-specific marker Sparc in the wt tissue neighboring human tumor (Petrova et al., 2011). Moreover, Flower knockout mice had a lower probability of developing skin papilloma (Petrova et al., 2012). These results suggest that the pro-tumor growth effect of cell competition is dominant, raising the question of the conservation of a detrimental signaling pathway. Yet, this late detrimental effect could be balanced by an early beneficial role of cell competition during development, which would help to build proper body plan by eliminating suboptimal cells through a fine-tuned error control system. Characterization of fitness comparison pathways in a larger set of organism would open exciting evolutionary prospective studies related to cell–cell cooperation and the onset of multicellularity.

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