

# Mechanisms and mechanics of cell competition in epithelia

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When fast-growing cells are confronted with slow-growing cells in a mosaic tissue, the slow-growing cells are often progressively eliminated by apoptosis through a process known as cell competition. The underlying signalling pathways remain unknown, but recent findings have shown that cell crowding within an epithelium leads to the eviction of cells from the epithelial sheet. This suggests that mechanical forces could contribute to cell elimination during cell competition.

## Genetic mosaics

Tissues comprised of genetically distinct cells. In *Drosophila melanogaster*, these are typically generated by inducing mitotic recombination in a heterozygous background, thus creating twin clones, one being homozygous wild type and the other homozygous mutant at a given locus.

## Blastocysts

Early mammalian embryos composed of about 100 cells. Embryonic stem cells are injected into blastocysts to generate chimaeras before implantation into a surrogate mother.

## Chimaeras

Embryos comprising cells of two distinct genotypes. They are usually generated by cell transplantation.

Living tissues are micro-ecosystems in which cells interact and compete for resources and space. This competition is not easily studied except in genetic mosaics that specifically contain cells with distinct fitness. In such situations, the weaker cells are often eliminated even when they are fully viable in a non-mosaic tissue. Such context-dependent elimination of relatively weaker or slower-growing cells is the hallmark of a process known as cell competition. One example is well known to mouse geneticists, who have observed that the success of embryonic stem (ES) cell transplantation in blastocysts is strongly influenced by the genotypes of the donor and host. For example, an inbred 129Sv mouse (a commonly used laboratory strain) is viable and healthy, and yet when ES cells of this genotype are transplanted into outbred wild-type blastocysts (in which cells are expected to be more vigorous) they are compromised in their ability to contribute to chimaeras<sup>1</sup>. By contrast, 129Sv ES cells give rise to frequent chimaeras when injected into inbred BL6 blastocysts. Therefore, cells seem to assess their relative fitness and eliminate the weaker cells through cell competition.

A common form of cell competition occurs when cells grow at different rates and the faster proliferating cells grow at the expense of slower ones, which are progressively eliminated. Cell competition caused by a growth differential has been best-characterized in *Drosophila melanogaster*, but it has been reported in mammals as well<sup>2–4</sup>. For example, when rapidly proliferating fetal rat liver cells were injected into adult rats after two-thirds of the liver had been resected by hepatectomy, they colonized a disproportionately large part of the regenerating liver and, importantly, they triggered apoptosis among adjacent host cells<sup>3</sup>. Although the significance of cell competition is still under intense debate, it probably contributes to tissue homeostasis. It could even protect against tumorigenesis, as the proliferation of transformed

fibroblasts (for example, by a transfected oncogene) can be restricted by the presence of normal surrounding cells<sup>5–7</sup>. Conversely, however, in certain circumstances cell competition could be subverted by cancer cells enabling them to kill neighbouring normal cells, thus making space for their own growth (reviewed in REFS 8–12). Because of its relevance to tissue homeostasis and tumorigenesis<sup>13,14</sup>, cell competition is attracting considerable interest<sup>10,12,15</sup>.

Although cell competition caused by a growth differential has been observed in various cell types, most studies have been performed in epithelia. This could be because cell competition is more prevalent in such tissues, which are, incidentally, the origin of most tumours (85%; see [CancerStats](#)). Another, non-mutually exclusive possibility is that cell competition may be more readily detected in epithelia because of their planar organization. The discovery of cell competition and most of the subsequent mechanistic insight came from studies of *D. melanogaster* appendages<sup>13,14,16–23</sup> in which it is relatively easy to generate genetic mosaics<sup>24</sup> that confront cells with different features, including growth rates. This can be achieved, for example, by genetically reducing or boosting translational activity in a clone of cells within the tissue<sup>13,14,25,26</sup>. Often this has no impact on organ size and proportion, demonstrating a high degree of flexibility in the allocation of space within an organ<sup>13,16,27</sup>. Interestingly however, such growth differentials lead to the elimination of slow-growing cells by apoptosis, and this increases the ability of faster-growing cells to colonize the tissue<sup>16,17,26,28</sup>. It is the local difference in growth rate that seems to be the determinant of cell death: cells that grow slowly, for example as a result of reduced translational capacity<sup>29</sup>, survive in their own company but are eliminated in the presence of wild-type cells<sup>2,13,14,16,17</sup>. Crucially, the same wild-type cells are themselves eliminated by nearby cells that have been genetically engineered to grow faster<sup>25,26</sup>.

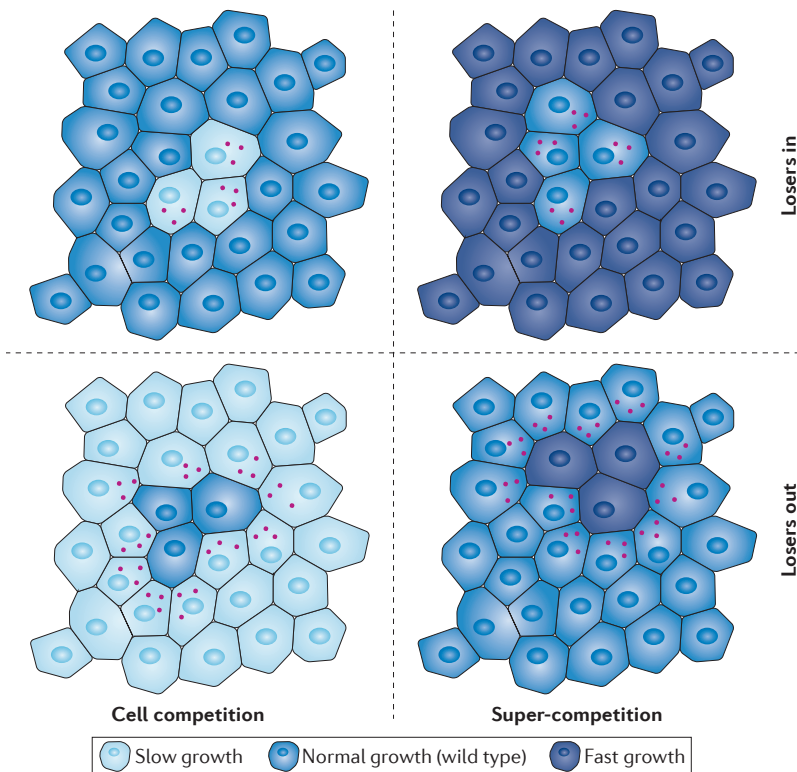
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**Figure 1 | Topologies of cell competition.** Mosaic tissues composed of cells growing at different rates are represented. Red dots indicate loser cells that undergo apoptosis. In each case, the cells that grow more slowly than their neighbours are losers. Loser cells can either be surrounded by faster-growing cells (as shown in the panels at the top) or encircle faster-growing cells (as shown in the bottom panels). Cells that grow faster (and eliminate wild-type cells) are called super-competitors.

The outcompeted cells are often referred to as 'losers', whereas the relatively fast-growing cells are called 'winners'. Abnormally fast-growing cells that have the ability to overgrow and cause the elimination of nearby wild-type cells are called 'super-competitors'<sup>26</sup> (FIG. 1).

In this Review, we first briefly speculate on why cell competition might have evolved. Next, we compare various triggers of cell competition, including differences in translational capacity, cell polarity mismatch and steep differences in signalling activity. We then focus on the mechanistic basis of cell competition caused by a growth differential due to differences in translational efficiency. Cell competition inherently requires cell–cell communication, but the molecular mechanisms underlying the detection of growth differences and how this is transduced into apoptosis in the slow-growing territory remains largely elusive. Important components of this communication system have been identified, but no clear signalling pathway has yet emerged. We discuss recent studies demonstrating that cell crowding causes the elimination of cells from epithelia, thus reviving an earlier suggestion that mechanical stress could contribute to cell competition<sup>30</sup>. Finally, we describe how a mechanical model of epithelia that tracks the movement of cell vertices could be used to assess whether inhomogeneous growth could contribute to the elimination of loser cells during cell competition.

#### Apoptosis

One of the main processes leading to cell death. It involves the activation of a proteolytic cascade comprising initiator and executioner caspases.

#### Aneuploid cells

Cells that have lost or gained entire chromosomes or large chromosomal fragments as a consequence of chromosome rearrangements.

### Why does cell competition occur?

Why would multicellular organisms evolve a system to eliminate slow-growing cells? It has been suggested that cell competition could contribute to organ size control<sup>8–11,31</sup>, but so far it has not been possible to directly test this hypothesis. Alternatively, cell competition could constitute a quality control mechanism that ensures the elimination of potentially dangerous or suboptimal cells<sup>31</sup>. Unfortunately, the concept of suboptimal cells has remained frustratingly vague.

It has recently been suggested that cell competition could help detect and eliminate aneuploid cells<sup>32</sup>. It was noted that ribosomes require the stoichiometric association of many proteins and that the genes encoding these proteins are evenly distributed across the genome. Therefore, extensive chromosomal rearrangements that occur in aneuploid cells would lead to an imbalance in ribosomal stoichiometry and hence, in many cases, reduced growth. This is expected to be true for cells in tissues as well as unicellular organisms, which would quickly lose out in the competition for resources. In multicellular organisms, apoptosis might have emerged as an additional feature to ensure the rapid removal of slow-growing cells. This model constitutes an interesting first step towards an assessment of the role of cell competition caused by ribosomal deficiency. However, it does not address how cells carrying supernumerary copies of ribosomal genes would be eliminated. It also does not explain how aneuploid cancer cells can grow at the expense of surrounding tissue. It thus remains unclear whether elimination of aneuploidy is the primary function of cell competition and the significance of this process remains largely unknown. It is nevertheless hoped that functional tests will emerge from the identification of genes specifically required for cell competition.

### Triggers of cell competition

A local difference in protein synthesis capacity, which results in different growth rates, was the first known trigger of cell competition<sup>13,14,16,33</sup>. Additional factors have been implicated in cell competition, including other growth regulators<sup>19</sup>, cell polarity<sup>34–37</sup> and steep differences in signalling activity<sup>14,38,39</sup> (FIG. 2). Although signalling and cell polarity are clearly connected to growth (BOX 1), the extent to which differential growth contributes to all forms of cell competition is unclear. Because of space constraints, here we do not discuss instances when steep differences in the activity of Wingless (which is the fly homologue of WNT), Decapentaplegic (Dpp; which is the fly homologue of transforming growth factor- $\beta$  (TGF $\beta$ )) or JAK (Janus kinase)–STAT (signal transducer and activator of transcription) signalling trigger local apoptosis in a manner akin to cell competition<sup>14,38,39</sup>.

**Growth differential.** Cell competition was first shown in *Minute* (also known as ribosomal protein) mosaics of growing appendages of *D. melanogaster*<sup>13</sup>. *Minute* genes encode protein components of ribosomes<sup>29</sup>. As expected, removal of both alleles of a given *Minute* gene is lethal. However, *Minute* heterozygous larvae are

## Myc

A proto-oncoprotein that controls ribosome biosynthesis, translational activity and other essential cellular activities.

viable<sup>13,17,19,28,29,33</sup>. Because of reduced biosynthetic activity, they grow more slowly than wild-type larvae but give rise to fertile adults with only a minor bristle phenotype. This clearly indicates that *Minute* heterozygous cells are viable and yet, in genetic mosaics that confront them with wild-type cells, *Minute* heterozygous cells are eliminated by apoptosis<sup>14,19</sup>. Somehow the local difference in growth rate triggers apoptosis in the slower-growing cells. Similarly, it was found that mutant mouse ES cells heterozygous at the *Bst* locus, which encodes the RPL24 riboprotein, did not contribute to chimaeras when transplanted into wild-type mouse blastocysts, whereas in reverse transplantation experiments, wild-type cells contributed disproportionately throughout the animal<sup>2</sup>. Although apoptosis was not directly assessed, this study strongly suggests that changes in ribosomal activity lead to cell competition in mice.

The effect of local differences in translational capacity was later confirmed and extended in *D. melanogaster* mosaics that juxtaposed cells with different doses of Myc, a key regulator of cell growth<sup>25,26</sup>. Heterozygous *myc* mutant flies were viable, and yet heterozygous cells were eliminated when in the vicinity of wild-type cells. Importantly, wild-type cells are eliminated if they are surrounded by faster-growing cells carrying three copies of *myc* (3xMyc cells), which are therefore considered super-competitors<sup>26</sup>.

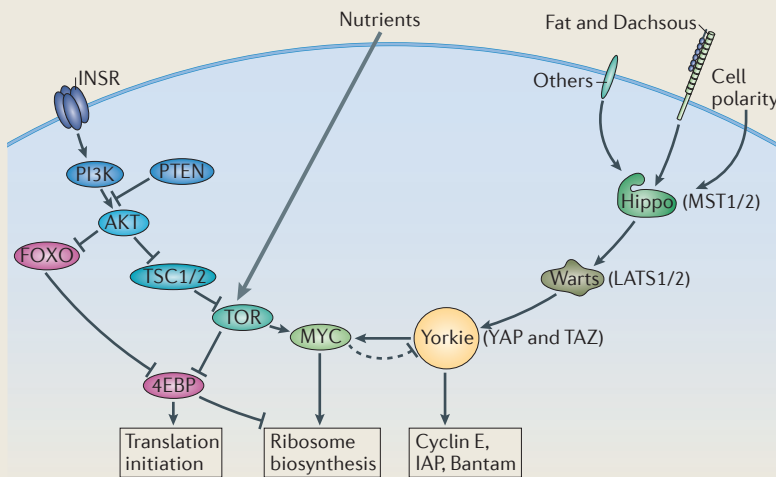
The analysis of *Minute* and *myc* mosaics (FIG. 2) demonstrated that a local difference in translational capacity causes apoptosis in the less-active cells. As translational activity correlates with the ability to proliferate, cell competition is generally thought to occur at the interface between fast- and slow-dividing cells<sup>14,16,19,20</sup>. However, it might constitute a more general response to a growth differential, even in post-mitotic cells<sup>40</sup>.

Control	Minute	Myc	Hippo	Insulin	Cell polarity	
Wild type	<i>Minute</i> <sup>-/+</sup>	2 × <i>myc</i>	<i>yorkie</i> <sup>-/+</sup>	<i>InsR</i> <sup>-/-</sup>	<i>Igf</i> <sup>-/-</sup>	Loser in
Wild type	<i>Minute</i> <sup>+/+</sup> ≈ wild type	3 × <i>myc</i>	<i>yorkie</i> <sup>-/+</sup> ≈ wild type	<i>InsR</i> <sup>+/-</sup> ≈ wild type	<i>Igf</i> <sup>-/-</sup> ≈ wild type	
Wild type	<i>Minute</i> <sup>+/-</sup>	4 × <i>myc</i>	Yorkie overexpression	<i>Pten</i> <sup>-/-</sup>	<i>Igf</i> <sup>-/-</sup> and <i>Ras</i> <sup>*</sup>	Loser out
	<i>Minute</i> <sup>+/-</sup>	2 × <i>myc</i> = wild type	Wild type	<i>Pten</i> <sup>+/-</sup> ≈ wild type	<i>Igf</i> <sup>-/-</sup> ≈ wild type	
Super-competition demonstrated	No	Yes	Yes		Yes	
Overriding organ size control	No	No	Yes		Yes	
Pro-apoptotic gene involved	<i>hid</i>	<i>hid</i>	Unknown	No cell competition	Unknown	
JNK signalling required	Unclear	Unclear	Unclear		Yes	

**Figure 2 | Triggers of cell competition.** Key findings from several mosaic situations that have been linked to cell competition are shown. In the diagrams, the genotype of the inner cells is shown in light boxes and that of the main tissue is shown underneath. Red dots indicate apoptotic cells (which are characterized by having activated caspase). Only specific examples of genetic mosaics are shown, for example one genetic mosaic to illustrate a specific pathway. The first row shows loser cells in blue, whereas in the second row the blue domains show the behaviour of winner cells created in the corresponding situation with the same pathway. Winner cells overgrow, as indicated by the large size of clones, in comparison to control clones (wild type). *Minute*<sup>-/+</sup> cells (which have reduced ribosomal activity) generate small clones and are eliminated when surrounded by wild-type tissue (*Minute*<sup>+/-</sup>). Conversely, wild-type clones eliminate surrounding heterozygous tissue. The third column shows that relative differences in Myc dose determines cell survival in mosaic tissues, with cells expressing less Myc being eliminated. In both *Minute* and *Myc* competition, the final organ size is normal despite the inhomogeneous growth rates. Clones with overactive Yorkie overgrow particularly dramatically and trigger apoptosis in surrounding wild-type tissue. They themselves undergo increased apoptosis but this is not sufficient to prevent them from overgrowing and deforming overall organ shape. Yorkie-overexpressing clones take on a round appearance and do not seem to mix with surrounding tissue. This is in contrast to clones that are deficient in basolateral determinants (for example, *Lethal giant larvae* (*Igf*) and overexpressed activated Ras (*Ras*<sup>\*</sup>)). Such clones also overgrow dramatically (despite increased apoptosis) and deform overall organ shape. However, in this case, the overgrowing tissue seems to be invasive and takes on a metastatic-like behaviour. Interestingly, at least in wing imaginal discs, steep differences in insulin signalling do not trigger cell competition even though they do cause inhomogeneous growth. The reason for this remains unknown. *Pten*<sup>-/-</sup> cells overactivate insulin signalling as PTEN is a negative regulator of this pathway. The wild-type diploid dose of Myc is represented by 2 × *myc*. Mitotic clones are made in a heterozygous background (for example, *Igf*<sup>-/+</sup> or *myc*<sup>-/+</sup>), which, in most instances, is compatible with normal organismal development (phenotypically wild type). One exception is the *Minute*<sup>-/-</sup> genotype, which causes developmental delay and the thinning of some bristles. *InsR*, insulin receptor; JNK, JUN N-terminal kinase.



# Box 1 | Growth control pathways



Several pathways that interact with each other promote growth (reviewed in REFS 42, 43, 86) (see the figure). Insulin signalling, mediated by insulin receptor (INSR), impinges on key regulators of protein synthesis such as MYC and the translation inhibitor 4EBP (translation initiation factor 4E-binding protein). Activation of the insulin receptor leads to activation of AKT (also known as PKB) via PI3K. This leads to the inhibition of tuberous sclerosis complex 1 (TSC1) and TSC2, which in turn inhibits the central metabolic regulator TOR (target of rapamycin). TOR is also regulated by the amino acid sensing mechanism. TOR modulates ribosomal activity through MYC and translational activity through 4EBP. Another branch of growth control involves Yorkie (the human homologues of which are YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif)), a factor that, with the help of cell type-specific cofactors (not shown), controls transcription of the cell cycle regulator cyclin E, IAP (inhibitor of apoptosis) and Bantam (a microRNA that positively regulates growth). Yorkie itself is regulated by the two protein kinases Hippo (or MST1 (mammalian STE20-like protein kinase 1) and MST2 in humans) and Warts (also known as LATS1 (large tumour suppressor homologue 1) and LATS2 in humans), although further upstream, the pathway is not fully understood. The two protocadherins Fat and Dachshous clearly contribute, but cell polarity and additional receptors could also be involved. The arrows represent genetic interactions but do not imply a direct effect, and only a subset of key regulators is shown.

## Contact inhibition

A phenomenon whereby cells stop growing as their density increases.

## Imaginal discs

Epithelial pouches that grow inside insect larvae and give rise to most adult structures during metamorphosis. They are extensively used to study pattern formation and growth control.

## Apical–basal determinants

Proteins that ensure apical–basal polarity. Apical determinants such as atypical protein kinase A and basolateral determinants such as Scribble or Lethal giant larvae typically oppose each other's activity thus ensuring the partitioning of distinct domains.

In the past decade, the Hippo pathway (BOX 1), named after one of its central kinases, has emerged as a key contributor to growth regulation<sup>41–43</sup>. Repression of Hippo (also known as MST1 (mammalian STE20-like protein kinase 1) and MST2 in humans) or its associated kinase, Warts (also known as LATS1 (large tumour suppressor homologue 1) and LATS2 in humans), leads to the activation of the transcription factor Yorkie (or the human homologues YAP (Yes-associated protein) and TAZ (transcriptional co-activator with PDZ-binding motif)). This in turn promotes the expression of cyclin E and inhibitor of apoptosis proteins (IAPs)<sup>41–43</sup>. Thus, the Hippo pathway represses Yorkie, which otherwise acts as a pro-growth and anti-apoptosis transcription factor. Hence, cells lacking Hippo, or cells overexpressing Yorkie, overgrow<sup>41–43</sup>.

Importantly, these overgrowing cells trigger apoptosis in surrounding wild-type cells, a telltale sign of cell competition<sup>44–47</sup>. Like 3xMyc cells, Yorkie-overexpressing cells act as super-competitors, but there is at least one key difference. Whereas cells with a translational advantage (such as 3xMyc cells) grow within the confines of

normal organ size, Yorkie-overexpressing cells flout such a constraint<sup>41</sup>. Spectacular examples include the massive overgrowth of *D. melanogaster* eyes or mammalian livers harbouring cells overexpressing Yorkie or YAP, respectively<sup>41,48–52</sup>. These and other observations suggest that the Hippo pathway, perhaps activated by a form of contact inhibition, mediates the signal that terminates growth when organs reach target size<sup>53–56</sup>. As Myc is a transcriptional target of Yorkie, it is likely that some of the effects of Yorkie are mediated by Myc<sup>47,57</sup>. Therefore, the underlying mechanisms that trigger apoptosis could be the same in *myc* and *yorkie* mosaics. However, Yorkie has additional effects besides activating Myc, given that Yorkie-overexpressing cells overcome organ size control, whereas Myc-overexpressing cells do not. In addition, Yorkie-overexpressing clones are round and smooth, whereas Myc overexpressing clones are jagged. This difference could be due to Yorkie also having an effect on cell junctions and/or on the extracellular matrix. In such a case, Yorkie would trigger cell competition through two mechanisms: one mediated by Myc, and the other through changes in tissue architecture.

So far, we have implied that growth differentials always trigger cell competition. There is one notable exception to this rule in *D. melanogaster*. Cells unable to transduce the signal from insulin (BOX 1) are reduced in size and grow at a relatively slow rate in imaginal discs. Yet, these cells survive, even when they are surrounded by wild-type cells<sup>58</sup>. Conversely, clonal activation of the insulin pathway causes autonomous overgrowth but no apparent elimination of surrounding cells<sup>59</sup>. Myc and insulin signalling both regulate ribosomal function and hence protein synthesis (BOX 1). Moreover, insulin signalling positively regulates Myc. Why insulin signalling is not associated with cell competition in *D. melanogaster* imaginal discs remains mysterious. Likewise, it is not known why overproliferation induced by overexpression of the cell cycle regulators cyclin D and cyclin-dependent kinase 4 (Cdk4) does not seem to trigger cell competition<sup>25</sup>.

**Cell polarity mismatch.** Apical–basal determinants have been linked to cell competition because cells lacking basolateral determinants such as Scribble, Mahjong and Lethal giant larvae undergo apoptosis if wild-type cells surround them<sup>34,35,37,46,60–63</sup>, but they survive and grow if all of the cells in the animal are mutant cells<sup>61,63–65</sup>. As the names of some of these proteins suggests, the corresponding mutant larvae become enlarged. However, this is not due to enhanced proliferation and faster growth, as larvae lacking these basolateral determinants do not grow at an increased rate<sup>45</sup>. Instead, they fail to metamorphose at the normal developmental stage and thus grow for a longer period than wild type until they die many days later as larvae<sup>45</sup>.

Two recent studies suggest how polarity disruption prevents growing imaginal discs from sensing when they have reached their appropriate size<sup>66,67</sup>. They show that growing imaginal discs that are wounded or lose polarity cues continuously produce Dilp8, an insulin-like peptide that prevents metamorphosis<sup>66,67</sup>. Once disc target size is reached, Dilp8 production stops, allowing the

**Madin–Darby canine kidney cells**  
(MDCK cells). The best-characterized epithelial cell line.

**Neoplastic overgrowth**  
Overgrowth that is accompanied by loss of polarity and general disorganisation of the tissue.

**Hyperplastic overgrowth**  
Overgrowth that maintains cell polarity and tissue integrity. It is often accompanied by tissue folds.

**Caspase**  
A class of proteases that initiate or execute apoptosis.

transition to metamorphosis. Discs lacking basolateral determinants seem insensitive to size control, as they continue to produce Dilp8 and the larvae fail to metamorphose. The underlying size control mechanism is unknown but is likely to have a mechanical component either mediated by cell–cell contact or cell–matrix interactions. Overall, these studies showed that mosaic loss of basolateral determinants leads to apoptosis, whereas removal of basolateral determinants throughout the tissue interferes with organ size sensing without causing much apoptosis.

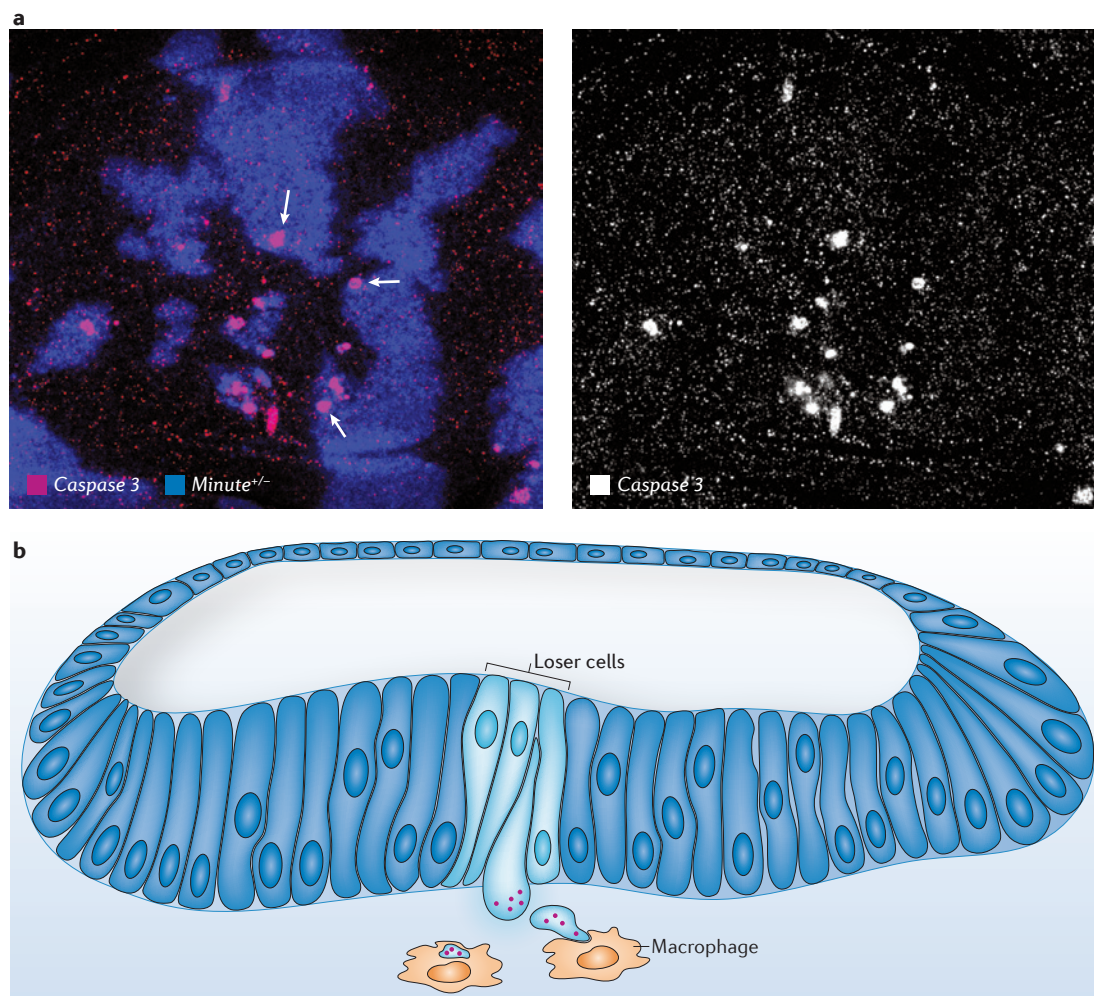
Interestingly, although mutant cells that have lost basolateral determinants behave as loser cells, they adopt features of super-competitors when they overexpress activated Ras (Ras<sup>\*</sup>) or Notch, as they induce apoptosis in surrounding normal tissue (although they also contain an excess of apoptotic cells)<sup>34,35,37</sup>. However, constitutive activation of Ras or Notch on their own is not sufficient to confer this super-competitor phenotype. Crucially, cells lacking basolateral determinants are clearly outcompeted. Two observations suggest that this may not be a consequence of reduced growth. First, basolateral mutant cells, which are eliminated in mosaic imaginal discs, do not show reduced proliferation<sup>45</sup>. The second observation comes from the elegant demonstration that polarity mismatch causes cell elimination in cultured Madin–Darby canine kidney cells (MDCK cells)<sup>68</sup>. Reduction of Scribble activity throughout the culture has no adverse impact on viability or epithelial integrity, whereas clones of cells lacking Scribble are eliminated by extrusion and ultimately apoptosis. These behaviours are akin to cell competition, yet there is no evidence that a growth differential is involved because elimination of Scribble-deficient cells is observed after confluence. Therefore, it seems that at least in this case, polarity mismatch, and not a growth differential, is the primary cause of cell competition. The situation may be different for basolateral mutant cells expressing Ras<sup>\*</sup>, as these cells do overgrow relative to the surrounding tissue and have increased levels of Myc<sup>60,69</sup>. Such overgrowth could explain their super-competitive behaviour although further proof will require specific markers of Myc-induced cell competition. Nevertheless, it is clear that promoters of growth such as Ras<sup>\*</sup>, and perhaps Myc, render basolateral mutant cells overproliferative and invasive<sup>34,37,47,57</sup> in a manner that overrides organ size control. Likewise, in a mammalian model, MYC cooperates with an *Lgl* mutation to trigger invasive tumours in mouse mammary glands<sup>70</sup>. It is worth noting that, in *D. melanogaster*, Ras<sup>\*</sup>-expressing cells that are deficient in basolateral determinants exhibit neoplastic overgrowth, whereas Yorkie-expressing clones show hyperplastic overgrowth. Thus, in Hippo signalling mutants, size control is lost but epithelial organization is maintained, whereas in cell polarity mutants, both the size control mechanism and epithelial organization seem to be disrupted. Accordingly, cell polarity could be an essential component of size sensing by the Hippo signalling pathway. Whether cell polarity is also required for cells to compare their relative fitness remains to be determined.

## Seeking mediators of cell competition

Cell competition is clearly a process that requires communication between distinct cell populations. However, the molecular mechanisms underlying this dialogue are still unclear. How do cells assess their relative fitness and how is this transduced into the death of loser cells? The answer to the first question is largely unknown, but some clues to the downstream signalling components are beginning to arise.

**A short-range signal.** One hallmark of cell competition is the elimination of loser cells when they are near winner cells<sup>14,20,26,71</sup>. This is thought to occur through apoptosis induced by a signal emanating from winner cells. Indeed, staining *Minute* mosaic tissues with antibodies against activated caspase has established that apoptosis occurs within the loser cell territory<sup>14,20,26,71</sup> (FIG. 3a). Apoptotic markers provide one way to assess the range of the cell competition signal, although this is complicated by cell dispersal after the onset of apoptosis. Apoptotic cells are rapidly excluded from epithelia to prevent disruption of epithelial integrity<sup>72</sup>. In *D. melanogaster* imaginal discs, dying cells accumulate at the disc basal surface<sup>14,20,21</sup> and are subsequently scavenged by macrophages<sup>21</sup> (FIG. 3b). As a result, cell corpses are not necessarily detected at the site of death, and this can lead to erroneous assessment of the distance reached by the signal that triggers apoptosis. Nevertheless, in *Minute* mosaics, the small number of caspase-positive cells that are still within the epithelium at any one time are predominantly located within one or two cell diameters of the clone edge<sup>14,20,21,71</sup>. This suggests that the pro-apoptotic signal acts at short range (although a longer range has been suggested for *myc* mosaics). The nature of this signal is still unknown. One approach towards its identification has been to take advantage of cell competition in cell culture, where biochemical purification is feasible<sup>23,73</sup>. However, as it is being debated whether the behaviour of cultured cells accurately reflects that of cells within tissues, we do not discuss these studies further.

**Cell competition depends on the membrane protein Flower.** Unbiased screens have been performed to identify specific cellular components that detect growth differentials and lead to the death of loser cells. A screen to identify genes that are upregulated in loser cells brought a dramatic advance as it led to the identification of Flower as a multipass transmembrane protein required for cell competition in *myc* and *Minute* mosaics<sup>22</sup>. The role of Flower has been extensively reviewed and will therefore only be briefly described here. Normal tissue expresses the ubiquitous form, Flower<sup>Ubi</sup>, whereas loser cells express distinct splice isoforms termed Flower<sup>loseA</sup> and Flower<sup>loseB</sup>. Although the regulation of *flower* mRNA splicing is not understood, it is necessary and sufficient to mark cells as loser cells. Importantly, Flower-deficient mice are partially resistant to papillomas, benign skin tumours, adding evidence to the possible link between cell competition and cancer<sup>74</sup>. The identification of Flower provides new insights into the molecular machinery underlying cell competition. Understanding the regulation of *flower*



**Figure 3 | Apoptosis in *Minute*<sup>+/-</sup> cells occurs in the vicinity of wild-type cells in *Drosophila melanogaster* imaginal discs. **a** | Example of a mosaic wing imaginal disc with *Minute*<sup>+/-</sup> tissue labelled in blue, surrounded by unlabelled wild-type tissue. Activated caspase immunoreactivity is marked in red (left panel) or white (right panel). Note that most caspase-positive cells are located in the *Minute*<sup>+/-</sup> tissue where it abuts wild-type cells (see also REFS 14,20,21,71). **b** | This diagram illustrates that loser cells undergoing apoptosis rapidly delaminate and accumulate on the basal surface of the epithelium, where they are removed by circulating macrophages. Images in part **a** courtesy of L. Alberto Baena-Lopez, Medical Research Council (MRC) National Institute for Medical Research, London, UK.**

mRNA splicing could reveal the mechanism that senses a growth differential, whereas identifying molecules that act downstream of Flower could unravel the molecular mechanisms that trigger the apoptotic machinery. Another benefit of the discovery of Flower is that it creates the possibility of designing a cell competition sensor by placing GFP in frame with either Flower<sup>loseA</sup> or Flower<sup>loseB</sup> (as previously described for other splice sensors<sup>75</sup>). This would allow the rapid identification of cell competition events involving Flower and provide an independent measure of the distance that the pro-apoptotic signal covers — two features that are not easily achieved with current splice-specific probes.

**Upstream of the apoptotic machinery.** A pro-apoptotic signalling pathway linked to cell competition is mediated by JUN N-terminal kinase (JNK) (FIG. 4a), although there seem to be controversial reports in the literature. There is no disagreement about the requirement of JNK

signalling in competition caused by polarity mismatch. For example, the elimination of basal polarity mutant cells is strongly reduced if they also lack Basket (which is a JNK kinase) or upstream components such as Eiger (the *D. melanogaster* homologue of tumour necrosis factor (TNF)<sup>36</sup>). However, the requirement of JNK signalling for different forms of cell competition (involving Myc, Yorkie or Minute) is still subject of discussion: evidence for and against a requirement for JNK<sup>25,44</sup> has been published. Nevertheless, there seems to be clear evidence that Eiger is not required for Myc-dependent competition<sup>76</sup>, an indication of the difference between these two cell competition triggers. It is conceivable that JNK signalling contributes to enhancing apoptosis in loser cells or ensuring their subsequent elimination from the epithelium. This view of JNK as an enhancer but not a trigger of cell competition is consistent with increased general (non-localized) phospho-JNK immunoreactivity seen in *Igl*, *mahjong* and *Minute* mutants<sup>15</sup>.

**JUN N-terminal kinase (JNK).** The key mediator of a kinase cascade that is often activated by stress. Depending on the context, it can trigger cell migration or apoptosis.



**hid**

A gene encoding one of the five pro-apoptotic proteins in *Drosophila melanogaster*. It inhibits the activity of DIAP1 (*D. melanogaster* inhibitor of apoptosis 1), which itself inhibits caspases.

**Epithelial-to-mesenchymal transition**

(EMT). A process whereby epithelial cells lose their epithelial characteristics (for example, polarity), detach from the epithelium and become migratory.

One possible approach to uncovering the signal transduction pathway that leads to loser cell death is to unravel the pathway backwards. Identifying the pro-apoptotic gene involved could provide an experimental foothold. In *D. melanogaster*, almost all apoptotic events are triggered by the transcriptional activation of one or several so-called pro-apoptotic genes, *reaper*, *hid* (also known as *Wrinkled*), *sickle* and *grim*. One of these genes, *hid*, is specifically activated in loser cells within *myc* mosaics<sup>25</sup> (FIG. 4a). Moreover, a mutation in *hid* strongly reduces apoptosis, suggesting that the remaining pro-apoptotic genes are less relevant<sup>25</sup>. Therefore, identifying the upstream regulators of *hid* in loser cells could uncover the signal transduction pathway that kills them. This is easier said than done because *hid* lies in a complex genetic locus (see [FlyBase](#)). Moreover, the absence of good quality antibodies or reporter genes is an impediment to high-throughput screens. Until these become available, an unbiased identification of the pathway upstream of *hid* will remain problematic.

**Engulfment of live loser cells**

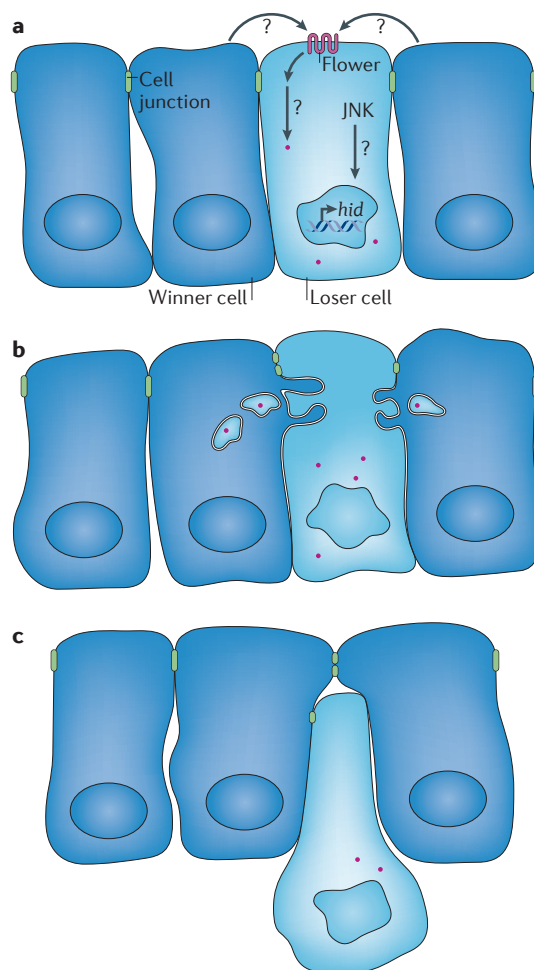
Apoptosis at discontinuities is the defining feature of all forms of cell competition. This suggests that a specific signal emanating from winner cells triggers apoptosis (FIG. 4a). However, recent work indicates that winner cells may not produce a specific pro-apoptotic ligand. Instead, it has been suggested that winner cells could be engulfing bits of loser cells, and this in turn would lead to apoptosis (FIG. 4b). Indeed, caspase immunoreactivity was observed in live winner cells, indicating that winner cells engulf losers and that such engulfment could be the primary trigger for caspase activity<sup>20</sup> (FIG. 4b). Supporting evidence came from the finding that various 'engulfment genes' were required for cell competition in *Minute* mosaics<sup>20</sup>, as well as at zones of polarity mismatch<sup>76</sup>. However, as many of these genes encode cytoskeletal regulators, their specificity to cell competition is debatable. Moreover, the primary observation that these genes are required in winner cells for competition to occur has been challenged<sup>21</sup>. Even though the role of engulfment remains controversial, these studies have raised the possibility that apoptosis at mosaic boundaries may not involve a classic ligand–receptor signalling pathway.

**Eviction from epithelia by crowding**

Another process that is indirectly associated with apoptosis in epithelia is the eviction of cells from the epithelial sheet (FIG. 4c). This can occur either towards the apical surface (a process known as extrusion) or towards the basal surface (a process known as delamination). Delamination only disrupts cell–cell contacts, whereas extrusion causes detachment from the extracellular matrix, as well as loss of cell–cell contacts. In both cases, unless it is part of a programmed process such as epithelial-to-mesenchymal transition (EMT), exit from the epithelium leads to the loss of essential survival signals and thus apoptosis. Two recent studies have shown that cell crowding within an epithelium triggers delamination and extrusion<sup>77,78</sup>. This has prompted the investigation of whether mechanical strain could be an important contributor to cell competition.

**Stretch receptors could mediate the response to crowding.**

It was shown that an abnormally high cell density (crowding) leads to the stochastic exit of cells, which then die because of lack of cell adhesion<sup>77,78</sup>. In one case<sup>78</sup>, crowding was induced by culturing epithelial



**Figure 4 | Direct and indirect triggers of apoptosis.** Three possible routes leading to apoptosis during cell competition. The outcome is always the activation of apoptosis (red dots) in loser cells. **a** | Signalling between winners and losers leads to the production of a specific splicing variant of the multipass transmembrane protein Flower (represented by a wiggle within the membrane). This is expected to cause the transcriptional activation of the pro-apoptotic gene *hid* via an unknown signalling pathway. JUN N-terminal kinase (JNK) signalling could contribute to *hid* expression and/or apoptosis through an uncharacterized pathway. **b** | Winner cells have been shown to engulf loser cells. How engulfing activity is induced is not known, but it has been suggested that, as winner cells phagocytose fragments of loser cells, they trigger apoptosis in the loser cells. **c** | Epithelial crowding causes extrusion or delamination (delamination is shown here). As the loser cell delaminates, it loses survival signals from adherens junctions, and this leads to apoptosis. It is noteworthy that these scenarios are not mutually exclusive. For example, the distinct splice isoforms of Flower expressed in loser cells could contribute to the activation of apoptosis in delaminating cells.

cells (MDCK cells) on a stretched latex substrate and then allowing the surface to relax, which caused an acute 1.3-fold increase in cell density. Within a few hours, normal cell density was restored, as cells randomly exit the epithelium by extrusion. As shown in this study, these cells were alive while extruding and showed signs of apoptosis only after they exited the epithelium. This and other findings demonstrate that apoptosis is a consequence of extrusion and not vice versa. Interestingly, however, inhibitors of sphingosine 1-phosphate (S1P) signalling or RHO kinase, which were previously shown to prevent extrusion of dying cells<sup>79</sup>, also interfered with live-cell extrusion. It is therefore likely that live cells and dying cells use the same mechanism for extrusion. However, the authors suggest that the upstream trigger is different, as gadolinium, a pharmacological inhibitor of stretch-activated ion channels, prevents crowding-induced extrusion but not the extrusion of cells killed by ultraviolet (UV) light irradiation. Similarly, gadolinium prevents extrusion in the zebrafish fin<sup>78</sup>, as do morpholino oligonucleotides against the zebrafish gene encoding Piezo, a mechanically activated channel<sup>80</sup>. This *in vivo* validation is tantalizing despite the difficulties in assessing the specificity of the phenotype. Mosaic analysis will be needed to determine whether the relevant membrane deformation occurs in the prospective extruding cells, as would be expected, or in surrounding cells. Conversely, it is thought that activation of RHO kinase occurs in surrounding cells to allow them to 'squeeze out' the doomed cells. This too will require direct experimental tests in mosaic embryos. Another question, to which we will return below, is how a mechanically activated channel could be activated by crowding.

**Crowding and planar cell elongation induce delamination.** A recent study<sup>77</sup> provided support, *in vivo*, for a model whereby crowding forces cells out of epithelia. The fly notum, which is the dorsal side of the thorax, develops from an epithelium. The live epithelium can be visualized using imaging during pupal stages. At these stages, cells divide but the overall size of the tissue remains relatively constant<sup>81</sup>, and thus tissue pressure is expected to rise. During this time, many cells at the prospective dorsal midline undergo delamination. Artificially boosting growth by activating the insulin signalling pathway within the dorsal midline (for example, through RNAi-mediated knockdown of PTEN) leads to an increase in the frequency of delaminating events. By contrast, reduction of insulin signalling (by overexpressing tuberous sclerosis complex 1 (TSC1) or TSC2 or RNAi against PI3K) causes the opposite phenotype<sup>77</sup>. These findings suggest that delamination is, at least in part, a consequence of tissue crowding. In a tissue under increased compression, delamination is expected to occur randomly throughout, as seen among compressed MDCK cells on the latex substrate<sup>78</sup>. This is not the case in the fly notum; delamination occurs in the midline, which suggests that these cells are prone to delaminating, perhaps because they are genetically programmed to be mechanically weaker as a result of active JNK signalling. One shortcoming of this study therefore is that all the genetic manipulations

to modulate growth are targeted to a tissue that is pre-disposed to delaminate. In the midline, two paths to delamination can be recognized. One pathway, which is relatively rare, involves synchronous junction shortening (apical constriction), a behaviour that is greatly enhanced by an apoptotic stimulus. A more common path in the untreated notum involves the progressive loss of individual junctions. Through a combination of modelling and experimental observations, the authors suggest that junction length fluctuates so that shorter junctions, which are prevalent at the tips of cells that are elongated within the plane of the epithelium, are more readily lost. The authors therefore propose that the primary trigger of delamination is the loss of junctions, and they do not consider the possible role of membrane stretching, which could easily be tested in *piezo* mutant pupae. Overall, analysis of the *D. melanogaster* notum suggests that the two main contributors to delamination are planar cell elongation and crowding pressure.

## A mechanical view of cell competition

As tissue compression affects the ability of cells to remain within an epithelium and hence their survival, and as inhomogeneous growth probably create stresses and strains within an epithelium, it is worth considering cell competition from a mechanical point of view.

**Stress and strains caused by differential growth.** Is there a specific type of stress or strain caused by a growth differential that correlates with delamination and hence the death of loser cells? Mathematical modelling can be used to examine the mechanical implications of heterogeneous growth in epithelial tissues<sup>30</sup>. As adherens junctions are the nexus of cell contacts, a good representation of epithelial mechanics can be obtained by modelling the array of polygons that their adherens junctions form in two dimensions. In a particularly successful model, the key features governing the movement of junctional vertices (and hence the polygons) are the strength of cell–cell adhesion and the contractility of actomyosin at the cell periphery, along with a requirement that the surface of each polygon approaches an optimal set value<sup>82</sup> (BOX 2). We have used this model to predict stresses and strains within and around a clone of cells that grow faster than surrounding cells (as would occur around super-competitors or in a 'winners in' situation). In our simulations, all cells were given identical mechanical parameters, and cell divisions were induced in a random pattern throughout the tissue, although at a higher frequency in the fast-growing territory. As expected intuitively, this model predicts that pressure (radial stress) builds up within the clone (FIG. 5). This suggests that tissue pressure is unlikely to be the trigger of cell elimination during cell competition. However, further preliminary analysis of the simulations (J.-P.V., A.G.F. and A.B.-L., unpublished observations) suggests that the first row of cells surrounding a fast-growing clone could become elongated in a tangential direction (FIG. 5). Therefore, a growth differential could specifically cause planar elongation in the slower-growing cells that are located closest to the faster-growing cells. Although further work is needed to confirm this suggestion and

### PTEN

A negative regulator of insulin signalling. Loss of PTEN is associated with numerous cancers.

### Tuberous sclerosis complex 1

(TSC1). A tumour suppressor that negatively regulates insulin signalling.

### PI3K

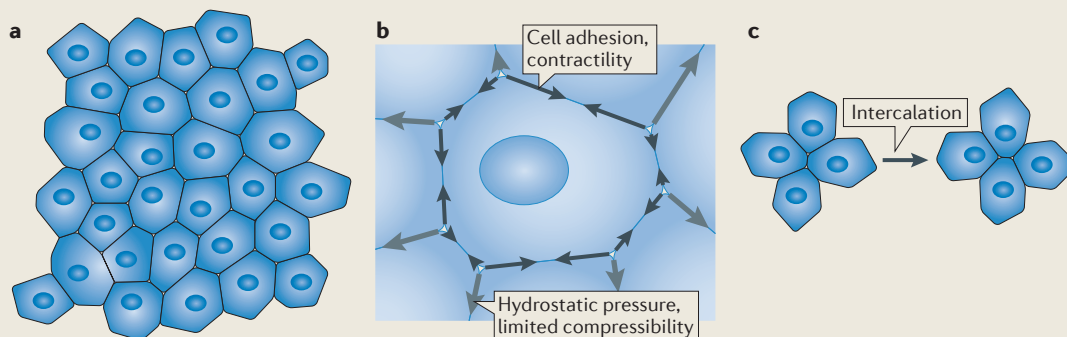
A key mediator of insulin signalling. Upon activation of the insulin receptor, it converts phosphatidylinositol-4,5-bisphosphate to phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P<sub>3</sub>), an activity that is antagonized by PTEN. PtdIns(3,4,5)P<sub>3</sub> activates AKT, which in turn triggers further downstream events.

### Adherens junctions

Molecular complex that mediates cell–cell adhesion in epithelia. They are typically organized in belts that surround every cell near the apical side.



## Box 2 | A mechanical model of epithelia



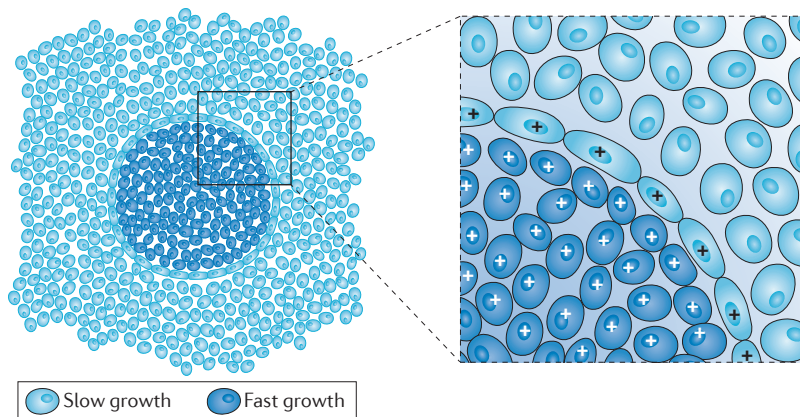
Two approaches can be used to simulate the balance of forces within an epithelial sheet. In one approach, the epithelium is treated as a continuous sheet<sup>30,87</sup>, whereas the other approach tracks the movement of discrete elements. Examples of the second approach are vertex models that consider cells as individual objects<sup>82</sup>. They are increasingly used to study cellular processes within epithelia, including cell motility, cell–cell adhesion, mitosis, delamination and apoptosis<sup>77,84,88,89</sup>. As currently implemented, these models share the key assumption that epithelial mechanics are dominated by forces acting within the plane of cellular junctions (adherens junctions). Cells are considered as two-dimensional polygons that represent cellular interfaces, with vertices forming where three or more polygons meet (see the figure, part a). Every vertex moves in response to forces due to growth, interfacial tension and hydrostatic pressure within each cell. The precise nature of the forces considered and the method of implementation vary across models. For example, the model described by Farahdifar and colleagues<sup>82</sup> considers two opposing forces: an outward force due to limited cell compressibility and an opposing line tension resulting from the combined effect of myosin-dependent cortical contractility and cell–cell adhesion (see the figure, part b). These forces are incorporated into an ‘energy function’ that is calculated and used to update the position of each vertex over time. Within this framework, the contributions of cell growth, mitosis, apoptosis and cell intercalation (see the figure, panel c) are incorporated to predict the evolution of a tissue towards a stable mechanical equilibrium. It is important to note that this model ignores the mechanical contributions of cell–matrix adhesion<sup>90</sup> and centripetal cytoplasmic contractile activity<sup>91</sup>. Another limitation of most current vertex models is that they are restricted to two dimensions, although recent work suggests that three-dimensional modelling is possible<sup>91</sup>. Despite these simplifications, vertex models have successfully represented biologically relevant processes<sup>77,84,88,89,92</sup>. Nevertheless, there is a need for validation with experimental measurements of forces within tissues. The most successful approach so far involves ablating individual junctions with an infrared laser and inferring the tension from the initial speed of vertex recoil<sup>81,84</sup>. Because this method is invasive and samples only a small number of junctions, alternative approaches are needed. Two avenues are being pursued. In one, computational methods are used to infer forces, up to a scaling factor, from observed tissue deformations<sup>93</sup>. Another approach that is currently being developed introduces genetically encoded fluorescence resonance energy transfer (FRET)-based force sensors within the tissue<sup>94</sup>.

to determine whether this would also be true for clones of slow-growing cells located within a field of relatively faster-growing cells (‘losers in’), it is conceivable that planar cell elongation could be an important consequence of a growth differential.

#### *Mechanical features that could cause cell competition.*

The possibility that a growth differential could lead to planar cell elongation at mosaic boundaries is intriguing in light of the suggestion that planar elongation contributes to delamination from the epithelium<sup>77</sup>. It was suggested that planar elongation causes a reduction in the lifetime of cell junctions, specifically the smaller ones, thus accelerating delamination. Planar elongation was not assessed<sup>78</sup>, but it is conceivable that crowded MDCK cells could become elongated within the plane of their junctions, thus increasing their surface-to-volume ratio which leads to the induction of a mechanically activated channel like Piezo. Another possibility that was put forward<sup>78</sup> is that crowding of MDCK cells could cause random buckling of stochastically weaker cells. In this case, buckling would entail loss of the ability to withstand compression within the junctional area. Junctional

buckling is expected to cause compensatory expansion of the cell surface elsewhere and hence activation of Piezo. Alternatively, cell compression could lead to other types of membrane deformation that could activate Piezo. This view suggests that compression and stochastic variations in the mechanical resilience of a cell are key to extrusion. As most compression probably occurs in fast-growing cells (FIG. 5), this view is unlikely to apply directly to cell competition, although a rigorous assessment will require three-dimensional modelling. Another feature that needs to be considered is the ability of cells to change neighbours, which can be described as tissue fluidity. This could relieve the stresses that build up at cellular interfaces. In addition, this might explain why, in imaginal discs, certain situations of inhomogeneous growth (for example, caused by changes in insulin signalling) do not trigger cell competition. Perhaps, differences in tissue fluidity could also explain why activation of insulin signalling is sufficient to trigger delamination in the notum but not in imaginal discs. However, to assess the influence of tissue fluidity, which cannot solely be inferred from clone shape<sup>83</sup>, will require new experimental approaches.



**Figure 5 | Predicted stresses and strains in and around a fast-growing clone of cells.** A representation of expected stresses and strains in and around a clone of cells that grow faster than the surrounding tissue. Radial pressure is expected to increase within the inner cells as indicated by the white crosses within the fast-growing territory. At the same time, slow-growing cells surrounding the fast-growing cells are expected to become elongated in the tangential direction (black crosses). Therefore, cell elongation could contribute to delamination and hence apoptosis of loser cells.

As we have argued above, mechanical features could contribute to cell competition caused by a growth differential. This could explain why the influence of cell competition does not cross the boundary that separates the anterior and posterior compartment in *D. melanogaster* imaginal discs. As this boundary is characterized by increased junctional tension<sup>84</sup>, it could act as a mechanical insulator, thus preventing transmission of the strains caused by inhomogeneous growth. Mechanical influences could also explain how cells, such as cancer cells, that acquire extra DNA or chromosomes could become super-competitors, as these cells tend to increase in size as a result of hyperploidy<sup>85</sup>. The importance of spatial constraints has also been recently highlighted in a recent report showing that post-mitotic epithelial cells increase in size to fill the void caused by tissue

injury (that is, compensatory cellular hypertrophy)<sup>40</sup>. Nevertheless, despite its likely relevance, mechanics alone cannot account for cell competition. In particular, signalling through Flower<sup>22</sup> is likely to be equally, if not more important. Moreover, other signalling pathways, especially JNK signalling, could contribute important positive feedback, ensuring the decisive elimination of loser cells.

### Conclusion and perspective

The ability of cells to compare their relative fitness is a remarkable feat that could have profound relevance to tissue homeostasis and early tumour development. Yet, the underlying mechanism is still poorly understood. The identification of one essential feature in *D. melanogaster*, the alternative splicing of Flower, is an important advance, although there is a need to test whether it is a general feature of all forms of cell competition. How Flower transduces local differences in protein synthesis capacity to the death of loser cells is also an outstanding question. We suggest that mechanical deformation (for example, cell elongation) caused by inhomogeneous growth could be important for cell competition and hence could be part of the upstream mechanism that modulates alternative splicing of the *flower* mRNA. Testing the role of mechanical deformation will require extensive morphometric analysis and the development of methods to further characterize stresses and strains in developing epithelia. Uncovering the sequence of events that transduce differential growth into apoptosis is an exciting multidisciplinary challenge.

### Note added in proof

While the proofs of this Review were being finalized, an article showing that relative differences in MYC levels trigger cell competition in early mouse embryos has been published. This paper also suggests that intrinsic variation in endogenous MYC levels could contribute to selection of the fittest cells<sup>95</sup>.

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# Competing interests statement

The authors declare no competing financial interests.

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