Published in final edited form as:

Nat Rev Genet. 2020 November ; 21(11): 683–697. doi:10.1038/s41576-020-0262-8.

Emerging mechanisms of cell competition

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Abstract

The growth and survival of cells within tissues can be affected by 'cell competition' between different cell clones. This phenomenon was initially recognized between wild type cells and cells with mutations in ribosomal protein (Rp) genes in *Drosophila melanogaster*. However, competition also affects *D. melanogaster* cells with mutations in epithelial polarity genes, and wild type cells exposed to 'super-competitor' cells with mutation in the Salvador–Warts–Hippo tumour suppressor pathway or expressing elevated levels of Myc. More recently, cell competition and super-competition were recognized in mammalian development, organ homeostasis and cancer. Genetic and cell biological studies have revealed that mechanisms underlying cell competition include the molecular recognition of 'different' cells, signaling imbalances between distinct cell populations, and mechanical consequences of differential growth rates; these mechanisms may also involve innate immune proteins, p53, and changes in translation.

Graphical Abstract

The growth and survival of cells within tissues can be influenced by competition between different cell clones. Genetic and cell biological studies suggest that cell competition may occur through the molecular recognition of 'different' cells, signalling imbalances between cell populations or the mechanical consequences of differential growth rates.

Introduction

As multicellular life relies on cell–cell interactions it is unsurprising that individual somatic cells might compete with one another. For example, in the developing nervous system, many neurons compete for appropriate targets to survive[1]. The phrase 'cell competition' acquired a more specific meaning through the study of genetic mosaics of *Drosophila melanogaster* that first revealed unexpected consequences of differences between cells, namely the non-autonomous elimination of a class of mutant cells only from genetic mosaics[2]. The recessively-lethal mutations in these genetic mosaics, which are now known to be in genes encoding Ribosomal Proteins (*Rp*), allowed heterozygous flies to survive, albeit with a slower developmental rate than wild type flies. In contrast to this non-mosaic survival, in mosaic flies $Rp^{+/-}$ cells are progressively eliminated from rapidly growing tissues in which wild type cells are also present[2]. Over several days, even a single $Rp^{+/+}$ cell can colonize

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Competing interests

The author declares no competing interests.

virtually entire developmental compartments as $Rp^{+/-}$ cells disappear[2] (Figure 1A). Further studies suggested that the growth of $Rp^{+/+}$ cells was enhanced in the presence of $Rp^{+/-}$ cells, and that these reciprocal changes in growth occurred at short range[3]. We now know that $Rp^{+/-}$ cells are eliminated by caspase-dependent apoptosis that is induced by nearby $Rp^{+/+}$ cells, indicating that a specific process affects cell survival at the boundary between these genetically different cells[4–6](Figure 1B). This disappearance of intrinsically viable cells, as a consequence of differing from their neighbors, was called ‘cell competition’. Cell competition seems to be a process that sometimes occurs where cells differ, genetically or otherwise, and does not simply reflect changes in clone proportions that occur over time as a passive consequence of cell-autonomous growth differences.

The existence of an active cell competition process was made particularly clear by the discovery of ‘super-competitor’ genotypes; super-competitors are genotypes that can eliminate nearby wild type cells (Figure 1C). For example, *D. melanogaster* imaginal disc cells expressing elevated levels of *Myc* (the mammalian homologue of which is an oncogene), owing either to an extra copy of the genomic locus or to a transgene modestly over-expressing *Myc* from the tubulin promoter, eliminate wild type cells[7,8]. The failure of wild type cells to survive cannot reflect an intrinsic defect in the eliminated cells, which are genetically normal. Other super-competitor genotypes in *D. melanogaster* include cells harboring mutations in tumor suppressor genes of the Salvador–Warts–Hippo (SWH) pathway[9], or cells overexpressing the YAP/TAZ homologue *Yki* and thus Yki, the activity of which is restrained by the SWH pathway[9,10]. A positive feedback relationship between Yki and Myc over-expression may underlie the shared ability of elevated *Yki* or *Myc* to result in a super-competitor genotype[10,11].

Cell competition and super-competition have now been described for multiple cell genotypes in *D. melanogaster* (Table 1)[12]. These examples are all thought to represent the active elimination of cells that differ in some way from the rest of the tissue, rather than passive clonal expansion or contraction.

Similar phenomena are now also recognized in mammals (Table 1). A very clear example is provided by the moderate overexpression of mouse *Myc* under the control of the Rosa26 promoter, which creates a super-competitor genotype leading to the elimination of control cells in the mouse epiblast[13]. In the absence of the super-competitors, the control cells exhibit no abnormalities in development. The over-representation of super-competitor cells with elevated *Myc* is abolished by expression of the anti-apoptotic protein baculovirus p35, indicating that wild type cells are eliminated by a specific mechanism in the presence of super-competitors and not as a result of cell-autonomous differences in growth rate[13]. Interestingly, $Mycn^{+/-}$ cells are eliminated by apoptosis when they encounter $Mycn^{+/+}$ cells in embryonic mouse skin mosaics, and they can themselves eliminate $Mycn^{-/-}$ cells in mosaics, showing that reduced *Mycn* function can also result in cell competition[14]. These observations further emphasize the comparative nature of cell competition, whereby $Mycn^{+/+}$ and $Mycn^{+/-}$ can each be either the ‘winner’ or the ‘loser’, depending on the genotype of the competitor[13,14].

How prevalent cell competition processes are, their molecular mechanism or mechanisms, and the purposes they serve continues to be studied. One possibility raised by the plethora of oncogene homologues among super-competitor genes in *D. melanogaster* and mammals is that mammalian tumors may be super-competitors in comparison to nearby normal cells, such that cell competition contributes to tumor expansion; however, cell competition is also thought to have tumor suppressive roles in addition (Table 1; Box 1).

In this Review, before discussing the essential features and mechanisms of cell competition, a brief historical survey of mammalian cell competition is given to exemplify the potential scope of cell competition, and to provide more material for discussion. The Review will then address some molecular mechanisms of cell interaction that have been recently described in cell competition. Perhaps surprisingly, multiple mechanisms of cell competition have been discovered, including mechanisms that involve: the specific molecular recognition of differences between cells, imbalances in general signaling processes that occur where distinct cell populations meet, and the mechanical consequences of differences between cells. In mammals, p53 signaling is implicated in multiple examples of cell competition, and is part of a body of evidence suggesting that cell competition occurs in cancer (Box 1).

A variety of competitive processes

Cell competition in the liver

Some of the first descriptions of cell competition-like phenomena in mammals concerned liver repopulation[15,16]. Partial hepatectomy stimulates fetal stem cells and progenitor cells introduced into rat liver to overtake the liver; this colonization is accompanied by the apoptosis of surrounding hepatocytes as donor tissue replaces host tissue, which is indicative of an active process that would not occur without the transplanted cells[15]. Liver repopulation is faster and more extensive in older hosts, for example, it is 5x more extensive after transplants into 14-month old rats than into 2-month old rats, suggesting that the speed of repopulation is related to the age difference between the host and donor cells[17]. Cell competition was also seen when wild type donor hepatocytes were engrafted into mice expressing a mutant human α1-anti-trypsin gene[16]. Apoptosis of hepatocytes expressing the mutant gene increased in the presence of transplanted wild type cells and the donor hepatocytes repopulated the liver at the expense of the mutant host hepatocytes, without changing the overall size of the liver, indicating that donor hepatocytes had a competitive advantage (Figure 2A)[16].

Cell competition in the immune system

Competition also occurs between stressed cells and unstressed cells in the immune system. Lethal irradiation ablates hematopoietic stem cells, leading to immunodeficiency[18,19]. However, milder irradiation that permits cell survival and the maintenance of immune function stresses hematopoietic stem cells, putting them at a competitive disadvantage when they are in chimeras with unirradiated cells or with irradiated cells deficient in the tumor suppressor gene *Tp53*[18,19]. The tumorigenic effects of radiation might reflect competition between cells differing in p53 activity, which can contribute to a selective advantage for lineages expressing mutant *Tp53*[18,19]. Cell competition occurs later than the radiation-

induced DNA damage response, and predominantly affects hematopoietic stem cells rather than lymphocytes[18]. The stem cells undergo p16-dependent senescence only in the presence of competitor stem cells that express lower levels of p53 activity than themselves[18].

More recently it has been proposed that an endogenous cell competition occurs between resident and new T lymphocyte precursors in the thymus, and is important to reduce the risk of leukemogenesis from the resident cells[20,21].

Examples in mouse embryogenesis

p53 activity also influences cell competition in mouse embryogenesis. A screen for knock-down genotypes with a potential advantage during embryonic development was conducted in induced pluripotent stem (iPS) cells, for which cell growth rate, embryoid body differentiation and the reacquisition of pluripotency were selected successively[22]. *Tp53* and the gene encoding Topoisomerase 1 were found to have an advantage in this embryogenesis-mimicking regime, and the subsequent knock down of either gene in mouse embryonic stem (ES) cells enabled these cells to displace wild type ES cells when co-injected into blastocysts[22]. Thus, during mouse embryonic development, *Tp53* loss seems to favor cells early in differentiation[22].

Changes in p53 activity may also mediate the effects of other pathways on survival of cells in mosaics. For example, tetraploid (4n) cells in mouse embryos in chimeras with diploid (2n) cells are removed by apoptosis following gastrulation, although they survive in extra-embryonic tissues[23,24]. The loss of 4n cells from embryonic tissues is p53-dependent[25]. p53 levels are increased in 4n cells compared with 2n cells and knockdown *Tp53* reduces the disadvantage of these cells in co-culture with 2n cells[26]. In another example, cells harboring mutant *Bmpr1a* (the gene encoding bone morphogenetic protein receptor type-1A) are also eliminated from embryos and co-cultures by competition with non-mutant cells in a manner dependent on p53 activity[27]. Unsurprisingly, mutations affecting the MDM2 family of ubiquitin ligases that target p53 for degradation are also disadvantageous to cells during mouse embryogenesis[28].

These examples indicate that cell competition might occur at multiple stages of mouse embryogenesis. Much of the endogenous apoptosis that characterizes the mouse epiblast around embryonic day 6 (E6) is proposed to reflect cell competition between cells that express different levels of *Myc*[13]. This competition might reflect a selection for fit cells, or it has been suggested that, since *Myc* promotes pluripotency in embryonic cells, eliminating cells with lower *Myc* may guard against premature differentiation and maintain a pluripotent cell population[29]. Recently, competition has been reported even earlier in mouse embryogenesis, in the pre-implantation epiblast, where competition of cells with lower activities of the transcription factor TEAD1 and its coactivator YAP maintains pluripotency through Myc and other factors[30]. The elimination of $Bmpr1a^{-/-}$ cells, meanwhile, occurs after E6 when differentiation is beginning, even though this elimination might also involve Myc[26]. Cells with less *Mycn* are eliminated from mouse epidermis by various mechanisms between E12.5 – E17.5, and such competition is hypothesized to be required endogenously for optimal epithelial barrier function[14]. The examples of cell competition in liver and the immune system above occur post-embryonically, as does cell competition in the mouse heart

whereby cells expressing elevated levels of *Myc* can progressively supplant normal cardiomyocytes within hearts that retain normal function during this replacement[31].

Cell competition in tissue culture

Cell competition phenomena have also been modelled in tissue culture, particularly using the Madin-Darby canine kidney (MDCK) cell line that can form an epithelial layer. Cells expressing RAS^{V12}, an oncogenic variant of RAS that is associated with enhanced proliferation and cell survival, maintain epithelial organization and growth; however, when RAS^{V12} expression is induced in sporadic cells within an epithelial layer of MDCK cells in culture, they are frequently lost by extrusion **[G]**[32]. Similar observations have been made for MDCK cells expressing v-SRC[33], ERBB2[34], or constitutively active YAP[35], or for MDCK cells in which the tumor-suppressor gene *SCRIB*[36](encoding the cell polarity determinant Scribble)(Figure 2B) or *MAHJ*[37](encoding the E3 ubiquitin ligase Mahjong) have been knocked down. Thus, epithelia may recognize and eliminate potentially-transformed single cells in a process termed epithelial defense against cancer (EDAC)[38].

Some of these observations made in cell culture have been corroborated in vivo, where single cells expressing oncogenes are influenced by the normal cells surrounding them. Indeed, RAS^{V12}-expressing cells are extruded from intestinal organoids as they are from cultured epithelia[39]. In other examples, whereas expression of activated β-catenin or H-RAS^{V12} causes hypertrophy in mouse hair follicles or skin, hypertrophy regresses in chimeras where cells expressing these genes compete with wild type cells[40].

Spontaneous genetic mosaicism

One feature common to many of the aforementioned examples of cell competition is that they are experimental. That is, they result from artificial genetic mosaicism induced by the researcher (endogenous competition in the mouse epiblast is one exception)[13,29]. Historically, this fact has led some scientists to question the physiological relevance of cell competition. It is important, therefore, to recognize the extent of spontaneous genetic mosaicism in vivo, as a consequence both of chromosome abnormalities resulting from errors in mitosis and of somatic mutation.

Genetic mosaicism is in fact common in early human development, where the majority of embryos contain some aneuploid cells **[G]**[41,42]. This fact seems surprising, given that aneuploidy is a major cause of miscarriage and birth defects, but aneuploid cells are usually eliminated from mosaic embryos before birth[43]. This elimination has been modeled in mice by transiently inhibiting the spindle assembly checkpoint **[G]** to generate aneuploid blastomeres; when introduced into embryos, the chimeras generally eliminated the aneuploid cells before implantation, and embryos initially comprising up to 50% aneuploid blastomeres frequently were born as live mice with little or no evidence of the abnormal cells[44]. Aneuploid cells might also be eliminated from embryos later in development; for example, embryonic mouse cortex is ~30% aneuploid cells at E13.5 but only ~1% aneuploid cells six months after birth[45].

A second endogenous process leading to genetic mosaicism is somatic mutation. Somatic mutations accumulate substantially with age and can lead to clonal selection and competition-like population swings.[46]. Clonal selection is very apparent in the immune system, which in older age can become dominated by a single hematopoietic clone, apparently selected on the basis of mutations commonly found in leukaemias and lymphomas that still support normal blood function and do not yet cause transformation[47]. Similarly, normal human skin and oesophagus frequently contain expanded lineages of mutant cells, as revealed by deep-sequencing studies[48,49]. For example, in the oesophagus, mutations in the cell surface receptor-encoding gene *NOTCH1*, which were previously considered to be cancer drivers, were actually more frequent in normal tissue than in tumors, suggesting their role precedes, rather than promotes, transformation[49]. Finally, mining of mRNA-sequence data also indicates that many mutant clones that arise expand in functionally normal tissues over time[50].

The prevalence of genetic mosaicism in normal development and in ageing, arising through multiple processes, means that cell competition could occur as a consequence of somatic genetic differences in normal individuals as well as after experimental manipulation.

Defining features of cell competition

The diverse competition-like behaviors described above have common and unique features, raising the question of how cell competition should best be defined. Although neither the interest of the respective molecular mechanisms nor the physiological significance of any competition-like process will be affected by the terminology used to describe it, it is useful if the term ‘cell competition’ carries an accepted meaning. Currently it seems useful to consider that ‘cell competition’ describes processes that eliminate cells only when they differ from their neighbors, either genetically or by having distinct levels of gene expression, regardless of their growth rates. The mechanism of elimination (that is, elimination by apoptosis or by another mechanism), or some of the other features that are only clearly associated with particular examples of cell competition, are less important to consider. The reasons for this view are outlined below. It is possible that there are multiple cell competition processes that may acquire more specific names as… their molecular bases are uncovered.

It is unavoidable that ‘cell competition’ must remain applicable to the original circumstance in which the term was coined, namely the competition of $Rp^{+/-}$ cells with wild type cells in *D. melanogaster* imaginal disc mosaics[2]. In this case competition is between cells of different genotypes and different intrinsic growth rates. As genetic mosaicism is common in nature, cell competition between genotypes will not only be restricted to experimental situations … In the normal mouse blastocyst, however, cells may well be genetically identical and gene expression differences epigenetically determined or even merely transient[29]. If the process that eliminates cells in the mouse epiblast with fewer copies of *Myc* or *Tead* is the same as the process that eliminates wild-type cells expressing lower levels of *Myc* or other pluripotency factors through non-genetic differences, it makes sense to call the phenomenon cell competition in both cases, and not to restrict the term only to cells with genetic differences[13,30]. This example also illustrates a practical issue, which is

that the genetic status of endogenous cells is not necessarily certain, unless single cell genotyping is to be a requirement of any study that refers to cell competition in an endogenous context. One, usually unstated, reason that cell competition studies focus on genetic mosaics is that cell competition usually refers to competition between cells of the same cell type. In this case, genetic mosaicism provides a clear distinction between competing cells. It is generally accepted that replacement of one cell type by a different cell type during development would not be a good example of cell competition.

Intrinsic differences in growth rate, often reflected by changes in overall translation rate, are a shared feature of many examples of cell competition, including competition between $Rp^{+/-}$ and wild type cells in *D. melanogaster* and competition of wild type cells with Myc expressing super-competitors in *D. melanogaster* and in mice[2,3,7,8,13], amongst others. As discussed below, differential growth may contribute directly to cell competition mechanisms through mechanical effects. However, increasing the intrinsic growth rate is not sufficient to make a cell super-competitive, as seen in the example of cells over-expressing PI3K or CyclinD/Cdk4 in *D. melanogaster*, which are not super-competitors[51]. In addition, differential growth has not been shown for all examples of cell competition. Indeed, *D. melanogatser* cells harboring *scrib* mutations are eliminated by competition with normal cells, but whether the *scrib* mutant has a cell-autonomous growth effect is unclear in *D. melanogaster*[52,53]. Thus, differential growth may contribute to, but may not be an obligate feature of, cell competition.

$Rp^{+/-}$ cells are eliminated from *D. melanogaster* imaginal discs by apoptosis[4], and Myc super-competitors eliminate wild type cells through apoptosis in both *D. melanogaster* imaginal discs and in mouse epiblasts[7,8,13]. Apoptosis induced by super-competitors in *D. melanogaster* occurs over a range of ~10 cell diameters, and an apoptotic signal is transferable from media conditioned by *D. melanogaster* cells expressing different Myc levels[54]. By contrast, dying $Rp^{+/-}$ cells are usually adjacent to wild type cells; although, it is neither proven that they all are nor that the mechanism triggering apoptosis is only juxtacrine[5,55]. Apoptosis also seems to be restricted to cells that are directly exposed to those cells with more Myc than themselves in mouse ES cell cultures[29].

Cell competition can also eliminate cells by mechanisms other than apoptosis. In a mouse model, cardiomyocytes expressing less MYC than their overexpressing neighbors undergo autophagy[31], while stratified epithelia cells with less MYC[14] or more p53[56] activity than their counterparts are eliminated from the stem cell compartment by oriented cell divisions that direct them towards terminal differentiation and sloughing as part of normal epithelia turnover. Since all of these processes facilitate the elimination and replacement of competed cells, there seems no reason to consider apoptosis a defining feature of cell competition, to the exclusion of other elimination mechanisms. Furthermore, irradiated immune cells are forced into a senescent state[18] and, although it could be argued that such cells have not been eliminated, they are prevented from contributing to the functional immune system.

Another necessary feature of cell competition is the replacement of the competed cells by the more successful population. In some cases, such as the case of Myc super-competitors in *D. melanogaster*, cell competition measurably enhances the growth of the cells that take

over[8]. This has recently been attributed to transfer of lactate from the competed wild type cells to nearby Myc super-competitors[57] with corresponding effects on metabolism in both populations[51,57]. Interestingly, metabolic changes are also implicated in EDAC[58]. As it has become controversial as to whether the growth of wild type cells growing near $Rp^{+/-}$ cells is similarly stimulated during their competition[3,59], however, it is uncertain whether reciprocal growth regulation is a universal feature of cell competition.

The core feature common to all of the examples of cell competition discussed here is the induction of a mechanism of specific cell elimination by competitive interactions within a tissue. In cell competition, therefore, cells acquire new survival properties as a consequence of differences with other cells. This acquisition is distinct from changes in clonal cell populations that occur over time as a passive consequence of cellular differences that are cell-autonomous and unaffected by other cells. A change in survival that is contingent on differences between cells is the distinctive feature of cell competition, regardless of the underlying molecular mechanisms. In many experimental situations the competing cells are genetically distinct, but differences with non-genetic origins may also trigger these processes.

Even with a seemingly simple criterion, cell competition will sometimes be suspected but not confirmed; mammalian *Rp* mutants provide a case in point (Figure 3). $Rp^{+/-}$ mosaic mice have been investigated using *Belly Spot and Tail* (*Bst*), a hypomorphic mutation that reduces the splicing and expression level of RPL24 (the mutation name derives from the effects on pigmentation)[60]. Genetically marked, wild type ES cells injected into $Rpl24^{Bst/+}$ blastocysts made an ~10 times greater contribution to coat color than when the same ES cells were injected into control blastocysts, which would be consistent with cell competition but might also be explained by a passive process (Figure 3A)[60]. Mutations in several other *Rp* loci can cause the autosomal dominant disease Diamond-Blackfan Anemia **[G]** (DBA) in humans[61,62]. Some cases of DBA remission reflect molecular reversion events, where newly-arising $Rp^{+/+}$ cells substantially colonize the bone marrow, suggesting that descendants of $Rp^{+/+}$ cells might replace $Rp^{+/-}$ cells[63–65]. The notion that $Rp^{+/-}$ cells are removed by active cell competition in mammals as they are in *D. melanogaster* is plausible given that the *Rp* mutations cause chronic p53 activity in both mouse and human cells by triggering nucleolar stress[66,67], and as noted already, differential p53 activity is a known cause of competition between mammalian cells (Figure 3B)[18,19,22]. To date, however, it is still not formally demonstrated if mammalian $Rp^{+/-}$ cells are actively eliminated or if they are just disadvantaged because they have an intrinsically slower growth rate than wild type cells cells (for example, $Rpl24^{Bst/+}$ mouse fibroblasts exhibit ~ 20% longer cell doubling time than $Rpl24^{+/+}$ cells)[60].

Molecular mechanisms of competition

The molecular mechanisms of cell competition and the changes that occur in competing cells are of much interest, not least because alterations in proto-oncogenes and tumor suppressors often underscore cell competition, suggesting a relationship to cancer (Box 1). If active cell competition is a feature of cancer, its underlying mechanisms could be manipulated to help prevent and treat this disease. Cell competition might also be exploited

to maximize the potential of tissue replacement in regenerative medicine. The events presumed to occur at the interface between competing populations of cells are of particular interest, although this is not the only level at which cell competition might be manipulated.

Three general mechanisms through which differences between cells could lead to cell competition can be envisaged (Figure 4). Most obviously, a specific molecular recognition event might identify differences between cells, leading to local activation of the cell competition process (Figure 4A). Alternatively, differences between cells may disrupt the normal balance of cell death and cell survival signals, leading to a locally inhospitable environment for one cell population, and thus cell competition, without the need for any specific molecular recognition (Figure 4B). Finally, mechanical stresses generated by the differential growth of cell populations may affect cell survival, cell proliferation, and cell mobility to cause cell competition (Figure 4C)[68,69].

Molecular recognition mechanisms

In *D. melanogaster, scrib* encodes a PDZ protein that helps define the apical–basal axis **[G]** of epithelial cells. The general mutation or knock down of *scrib* causes imaginal disc epithelia to overgrow into disorganized cell masses; by contrast, *scrib* mutant clones in wild type tissues are eliminated[52,70]. Owing to the neoplasia caused by general *scrib* mutants, competition that removes cells harboring *scrib* mutations or mutations in functionally related epithelial polarity genes is considered tumor suppressive. Genetic screens designed to elucidate the basis of tumor-suppressive cell competition identified signaling events that are unique to the interface between *scrib* mutant cells and wild type cells (Figure 5A). In *scrib* mutant cells at this interface, the tyrosine phosphatase PTP10D is relocalized from the apical to the basolateral cell membrane where it can interact with its transmembrane ligand Stranded at second protein (Sas)[71]. This relocalization may be related to alterations in cell polarity, since it is also observed during cell competition in cells harboring defectives in other polarity genes[71]. PTP10D-dependent signaling downregulates Ras activity in the *scrib* mutant cells that appose wild type cells. This downregulation dampens a signal that otherwise counteracts Jnk signaling, which is elevated in all *scrib* mutant cells due to autocrine signals from Eiger (Egr), the homologue of mammalian tumour necrosis factor (TNF)[71]. Away from the interface with wild type cells, Jnk and Ras activities synergize to promote proliferation and neoplasia in *scrib* mutant cells (Figure 5Aa). At the boundary, where Ras activity is suppressed, Jnk promotes the basal extrusion of *scrib* mutant cells, which is associated with the downregulation of E-cadherin levels through autocrine Slit–Robo signaling and the activity of Enabled (encoded by *Ena*) (Figure 5Ab)[72]. Neighboring wild type cells also help to remove *scrib* mutant cells by engulfment, stimulated by upregulation of the platelet derived growth factor receptor homologue Pvr in surrounding wild type cells (Figure 5Ac)[73].

In summary, this example of tumor-suppressive cell competition depends on direct molecular interactions, particularly the interaction of PTP10D with Sas that only occurs between the competing cell populations (Figure 5Ab)[71–73].

Although it is not known how *scrib* depletion could affect protein synthesis, a recent study reports that in *D. melanogaster*, imaginal disc cells depleted for *scrib* are sensitive to

hyperinsulinemia and the enhanced translation that results specifically in *scrib* depleted cells is sufficient to rescue them from cell competition[74]. The authors suggest that this mechanism contributes to the elevated risk of human cancer in individuals with hyperinsulinemia and, although it is not clear that human *Scrib* is a tumor-suppressor, loss of epithelial polarity is a common feature of tumor cells[74].

Mechanisms without molecular recognition

The elimination of wild type cells by cells with a Myc super-competitor genotype depends on genes that are also associated with innate immune signaling and, in particular, on one or more Toll-like receptors (TLRs) and NF-κβ family transcription factors[75]. In *D. melanogaster* imaginal disc cells overexpressing Myc, the serine proteases modular serine protease (modSP) and spaetzle-processing enzyme (SPE) are expressed at higher levels than in wild type cells and locally cleave more of the secreted protein Spaetzle (Spz) to its active form[76]. Active Spz then impairs the survival of nearby wild type cells by binding to their TLRs to activate NF-κβ signaling to toxic levels[76]. Interestingly, as cells overexpressing Myc express TLRs Toll-2, Toll-8 and Toll-9, at reduced levels, a working model for super competition is that wild type cells adjacent to cells expressing high Myc levels experience toxic levels of the NF-κβ signaling because only they express normal levels of these TLRs near to elevated levels of active Spz (Figure 5B)[76]. Although these studies are interpreted as local functions of molecules that are involved in innate immunity elsewhere, it has also been reported that the role of innate immune genes in Myc super-competition is reduced flies raised in a sterile environment[77]. Accordingly, the interplay between local and systemic functions of proteins in the innate immune pathway remains to be fully resolved. Nevertheless, the model shows how wild type cells can be compromised close to cells expressing higher levels of Myc without the need for molecular the recognition of the wild type cells by the super-competitor cells (Figure 5B).

TLRs, NF-κβ homologues, and other innate immune factors from *D. melanogaster* are also implicated in the competition of $Rp^{+/-}$ cells by wild type cells, although it is not known whether a similar mechanism to that seen in Myc super-competition applies[75]. It will also be interesting to determine whether innate immune factors could contribute to metabolic differences between Myc-competing cells in *D. melanogaster*[51,57], or to competition between mouse cells that differ in terms of the level of *Myc* expression, which occurs both in epithelia and in other tissues that lack epithelial structure[13,14,31].

Mechanical cell competition

Cell competition often occurs between cells with different growth rates, and compensation for variation in growth is one possible adaptive function of cell competition[3,7]. Differential cell growth can have mechanical consequences, especially within epithelia where cell rearrangements are constrained by adhesion junctions **[G]**[68]. For example, a clone of hyperplastic cells constrained within a generally less-proliferative epithelium is expected to experience compression, and to exert a reactive force on the surrounding cells[78]. As compression can promote the elimination of cells from epithelia, super-competition by hyperplastic cells could result from the compression-induced apoptosis of surrounding cells, without any other signaling between cells, especially if the less proliferative cells have a

heightened sensitivity to compression and thus elimination (Figure 4C).[79–81] Compressed cells surrounding a hyperplastic clone may simultaneously be stretched in the perpendicular direction, which is another way in which they could potentially differ from compressed cells within the hyperplastic clone[69].

Cell–cell interactions may be influenced by the geometry of the interface between cell populations, which is controlled by the orientation of cell division and by cell rearrangements, both of which are influenced by mechanical stress[82]. Interestingly, boundaries between competing $Rp^{+/+}$ and $Rp^{+/-}$ cells[83,84], and between wild type cells and cells with a super-competitor Myc phenotype, do become unusually irregular[85]. The more irregular the interface is between cell populations, the greater the exposure of individual cells is to the opposing cell population; slow growing cells surrounded by hyperplastic cells, for example, experience greater compression. This fact may explain why the rate of competitive apoptosis is higher in $Rp^{+/-}$ cells when they are more surrounded by wild type cells[5,69]. Conversely, smooth boundaries between populations are thought to diminish cell competition by distributing force more evenly. This fact could explain why the boundaries between developmental compartments in the *D. melanogaster* wing, which are relatively straight and prevent cell intermingling through higher junctional tension, are barriers to cell competition[85].

The converse of compression-induced cell loss is an increase in cell proliferation due to low epithelial density or stretching[86,87]. Accordingly, each epithelium maintains a homeostatic cell density within the range defined by proliferative (stretched) and apoptotic (compressed) regimes[69]. If two cell populations in a mixture have different homeostatic densities, then the cells with the higher homeostatic density should expand at the expense of the other cell population, because the mixture is likely to enter a regime that is proliferative for one population but promotes the loss of the other[69].

More information about how mechanical forces are distributed in tissues and the pathways that respond to them are required to fully evaluate the contributions of mechanical stress to cell competition. In live cells extruded in response to compression in zebrafish epithelium, the stretch-activated channel Piezo1 is required[80]. However, as Piezo1 is also required for stretch-activated proliferation, the factors that distinguish cell compression from cell stretching remain uncertain[88]. In *D. melanogaster* pupa, in which compaction eliminates surplus cells by extrusion at the future dorsal midline, diminished Egfr–Mapk survival signaling contributes to the mechanism[89], although how cell mechanics influence Egfr signaling is unknown. It will also be interesting to explore further how mechanical forces might impact cell competition in non-epithelial tissues.

Distinct mechanically-induced biochemical changes have been identified in mammalian cells depleted of *SCRIB* (Figure 5C). The inducible knockdown of *SCRIB* did not intrinsically affect the viability of MDCK cells, but its knockdown in sporadic MDCK cells led to their elimination[90]. Specifically, when grown separately, *SCRIB* depleted cells were flatter and grew at lower density than their wild type counterparts; however, in mixed cultures, *SCRIB* depleted cells became compressed and vertically elongated. Importantly, compression of *SCRIB* depleted cells was sufficient to trigger their apoptosis even in the

absence of normal cells. The *SCRIB* depleted cells had chronically elevated levels of p53 that were exacerbated by competition and by compression-induced, Rho-associated protein kinase (ROCK)-dependent p38 kinase activity. Mild p53 activation alone was sufficient to induce competition between otherwise wild type MDCK cells, suggesting that p53 is the ultimate effector of compression in this system (Figure 5C)[90].

Mechanisms awaiting more definition

Notable progress has been made in our understanding of other examples of cell competition. However, in these examples the nature of the cell–cell interactions between populations remain uncertain so it is not yet clear which class of mechanism shown in Figure 4 applies to them.

Elimination of Rp mutant cells in D. melanogaster.—Although the competition of $Rp^{+/-}$ cells in *D. melanogaster* is thought to depend on innate immune genes[75], roles for other distinct pathways have also been elucidated. A combination of Jnk activity and autophagy is reported to cause the death of eliminated $Rp^{+/-}$ cells[55]. Jnk activity is elevated in $Rp^{+/-}$ genotypes, but not as a consequence of signaling by Egr as is the case in *scrib* mutant cells, and its elevation does not lead to Ptp10D activity[4,71,72,91]. Instead, JnK activity is controlled by the bZip domain transcription factor Xrp1, which is expressed in a manner dependent on the cytoplasmic 40S ribosomal protein RpS12 in response to defective ribosome assembly[92,93]. Whereas Jnk is active in all $Rp^{+/-}$ cells, autophagy is induced only in $Rp^{+/-}$ cells that are close to wild type cells, and this synergy is proposed to account for the localized cell death. In mammalian cells and in zebrafish, mutations in *Rp* genes can trigger autophagy cell-autonomously[94], so the observation of localized autophagy in *D. melanogaster* is intriguing. It has been suggested that the onset of autophagy might be related to different rates of protein synthesis between cells, because protein synthesis is lower in $Rp^{+/-}$ cells than in wild type cells[55]. Interestingly, the overall reduction in protein synthesis in $Rp^{+/-}$ cells occurs indirectly, downstream of Xrp1, the same transcription factor that upregulates JnK signaling[92,93]. Perhaps owing to these roles, mutations in *Xrp1* prevent the elimination of $Rp^{+/-}$ cells[93,95]. Xrp1 is also required for an oxidative stress response in *D. melanogaster* $Rp^{+/-}$ cells[96], and differences in the oxidative stress response between these cells and wild type cells have also been implicated in cell competition[91]. Elimination of *D. melanogaster* $Rp^{+/-}$ cells has also been attributed to reduced Dpp signaling, but it is not known how this might be related to autophagy or the function of Xrp1[4].

$Rp^{+/-}$ genotypes activate signaling pathways in mammalian cells also. In particular, RPL5 and RPL11, which are components of the 5S ribonucleoprotein particle that becomes part of the large ribosomal subunit, are permitted by defects in ribosome assembly to interact with MDM2; this interaction results in chronic p53 activation (Figure 3C)[97,98]. Although p53 activation is implicated in multiple cases of mammalian cell competition, it is not yet known whether $Rp^{+/-}$ cells undergo p53-dependent competition in mammals, nor how differences in p53 activity between cells are sensed. p53 activity does not eliminate $Rp^{+/-}$ cells in *D. melanogaster*[6], but Xrp1 is a transcriptional target of p53 and might substitute for the mammalian role of p53 during cell competition in *D. melanogaster*[99].

In short, because it remains unclear how differences in translation rate, the oxidative stress response or other consequences of Xrp1 activity are detected between cells, it is not yet clear which general class of cell competition mechanism best describes competition of $Rp^{+/-}$ cells.

Epithelial defence against cancer.—The elimination of mammalian cells transformed by the expression of activated RAS, or YAP, or by the overexpression of SRC or EEBB2, has been described as EDAC, because these genotypes are potentially oncogenic[58,100]. During EDAC, the transformed cells are initially extruded from the apical epithelial surface before becoming prone to apoptosis owing to a loss of survival signaling (Figure 6)[58]. Apical extrusion is considered tumor suppressive for epithelia, because basal extrusion could promote metastasis if the extruded cells survived.

EDAC involves active changes in neighboring wild type cells, which accumulate filamin; filamin recruits vimentin filaments that are thought to extrude the transformed cell[101]. Transformed cells accumulate the actin-binding protein EPLIN, which is believed to promote their extrusion through stimulating the activity of protein kinase A (PKA) and myosin II[102]. Filamin accumulation in neighboring wild type cells is thought to depend on the activation of Sphingosine-1-phosphate receptor-2-induced Rho, which might be triggered by a higher level of Ephrin-A2 expression in RAS-transformed cells than in wild type cells[58,101]. Thus, EDAC appears to involve cellular processes specific to the interface between the normal and pre-neoplastic cells. How cells recognize these interfaces is not yet clear; that is, it is unknown whether a specific receptor–ligand interaction, such as the Ptp10D–Sas interaction that is observed at the boundaries between *scrib* mutant cells and wild type cells in *D. melanogaster*, occurs (Figure 6)[58]. Elimination of *SCRIB*-depleted cells from MDCK cell epithelia depends on mechanical differences between cells,[90] but it is not known whether this is the case for other examples of EDAC.

In short, it remains unclear how differences in cytoskeletal organization are detected between cells and thus which general class of cell competition mechanism best describes EDAC.

The Flower proteins in cell competition.—Initial studies comparing differential gene expression in *D. melanogaster* identified an isoform switch in expression of the *flower (fwe)* gene, encoding a Calcium channel protein, in wild type cells competing with Myc super-competitors[103]. Apposition of cells expressing different isoforms of Fwe is proposed to kill cells by inducing expression of the EF-hand protein Ahuizotl (encoded by *Azot*)[104]. The Fwe proteins were also proposed to function during elimination of *scrib* and $Rp^{+/-}$ cells, in the latter case also via *Azot*[103,104]. It is not yet understood how genetic differences between cells lead to changes in the expression of different Fwe isoforms nor how cells expressing these isoforms interact, and it's unclear how the proposed Fwe mechanism is related to the more-recently described mechanisms outlined in the above sections. Importantly, however, there is also evidence for an important role of the human Fwe homologue in multiple cancers[105], described in more detail in Box 1.

Conclusions and perspectives

This Review summarizes studies of cell competition, with a focus on the underlying molecular mechanisms. Cell competition today refers to the elimination of a cell population due to changes in survival properties in response to differences between cells in the tissue (Figure 1). Cell competition studies were pioneered in *D. melanogaster* although many examples of cell competition in mammals are coming to light. In addition to the history of genetic studies in *D. melanogaster*, experimental mosaic methods have contributed to the discovery of cell competition phenomena. In *D. melanogaster* the genetically encoded, site-specific recombination system FLP–*FRT* is widely used to generate genetic mosaics using stochastic mitotic recombination to generate marked clones within tissues[106]. In mammals the more common approach to mosaic studies has been to eliminate gene function conditionally from the entire tissue of interest using the similar Cre–*loxP* system[107,108]. Complete elimination is necessary when recombined cells are not directly marked. Thus, an increase in mouse studies where mosaic tissues of mixed cells with directly marked genotypes may further highlight examples of cell competition and of other cell-nonautonomous phenomena in mammals.

The recognition of cell competition would be greatly facilitated if a specific marker for the process was discovered. There was initial optimism that a common molecular pathway involving Fwe and Ahuizotl might underlie multiple examples of cell competition in *D. melanogaster*[103,104,109]. It now looks as though cell competition encompasses processes with multiple distinct molecular mechanisms. Thus, confirming examples of cell competition still relies on demonstrating the differential growth and survival of cells in mosaic versus non-mosaic conditions, which is sometimes challenging.

Cell recognition mechanisms in *D. melanogaster* have generally been identified by forward genetic screens, and the mapping of these cell competition mutants has been facilitated by constant advances in genome sequencing and mapping methods[110], and by the availability of large strain collections containing genetic mutations[111], insertions[112], deletions[113,114], and knock-down reagents[115,116] maintained by stock centres[117,118]. Mutant screens remain an important approach for directly assessing processes in vivo. It remains to be seen whether similar approaches can be applied in mammals. It is possible that deep sequencing of human samples to identify dominant genotypes and potential suppressor mutations that protect disadvantageous genotypes may be more convenient than performing experimental screens in a mammalian model[48–50]. Cell competition mechanisms have also been uncovered using mammalian tissue culture models, which remains useful, especially when conclusions can be confirmed in vivo[26,27,58].

The cell competition mechanisms described so far differ in their molecular components. The events occurring at competing boundaries fall into three general classes (Figure 4). These classes include cell–cell recognition through receptors and ligands previously known to play roles in the development of the nervous system (Figure 5A). Cell–cell recognition, however, might not be necessary for the elimination of wild type cells by *D. melanogaster* cells expressing high levels of Myc (Figure 5B). Finally, the mechanical effects of mixing different cells seem paramount in the elimination of MDCK cells depleted of *SCRIB* (Figure

5C). In other cases, such as in EDAC and in the original example of *Rp* mutations in *D. melanogaster*, many aspects of the process have been revealed but these do not yet include the molecular mechanisms whereby differences between cells become important (Figures 3,6).

Remarkably, no two examples of cell competition have yet been shown to exhibit the same molecular mechanism and even the complex mechanisms by which *scrib* mutant cells are eliminated from fly tissues and *SCRIB* knock-down cells are eliminated from mammalian epithelia seem distinct (Figure 3)[71,72,90]. This need not be disheartening, as the deepening knowledge we have gained can only be beneficial and common features of cell competition mechanisms may yet appear. A number of cell competition scenarios in *D. melanogaster* and possibly mammals are associated with differences in the level of overall protein synthesis, which is affected by the expression level of Myc[119] and by mutations in *mahj*[55], as well as by *Rp* mutations, and which can interfere with elimination of *scrib* mutant cells[74].

Notably, many examples of cell competition in mammals involve p53[99]. One important question is whether cell competition contributes to the role of p53 as a tumor suppressor. The idea that p53 acts as a tumor suppressor through its roles in the acute DNA-damage response has been increasingly challenged[120]. Temporal studies show that p53 suppresses tumorigenesis not during the acute DNA damage response but after it has occurred, and that it does not require the main genes that are necessary for cell cycle arrest and apoptosis in response to DNA damage to do so[121–126]. Whether the roles of p53 in cell competition involve other targets that represent the tumor-suppressive aspect of p53 function remains to be elucidated.

In addition to cell competition with tumor-suppressive properties, such as EDAC in mammals or the elimination of *scrib* mutant cells in *D. melanogaster*, there are examples of cell competition promoting tumor growth by eliminating surrounding normal cells, and even examples in which cell competition is simultaneously tumor-suppressive and tumor-promoting (see Box 1). It is also possible that, when genotypes such as Ras^{V12} escape tumor-suppressive competition through further mutation, they may then out-compete normal cells in the host (see Box 1).

As more is learned about the mechanisms of cell competition, and more approaches to detect and manipulate cell competition can be developed, the contributions of cell competition to cancer development and suppression, as well as its potential roles in growth regulation, ageing, pluripotency, and in the suppression of developmental defects, will become more defined[7,14,29,104]. In addition to the connection of cell competition with cancer, cell competition might also promote the phenotypically silent expansion of a single clone throughout a tissue, as has been seen in rodent liver and heart[15,16,31]. In principle this could facilitate the replacement of existing tissues by genetically modified or repaired cells, if this could be applied in humans.

Acknowledgements

I thank H. Buelow, S. Emmons, C. Khan, M. Kiparaki and anonymous reviewers for comments on the manuscript and C.-H. Lee for Figure 1B. Research into cell competition mechanisms in the author's laboratory is supported by NIH grants GM104213 and GM120451 and by the Albert Einstein College Human Genetics Program.

Glossary

Diamond-Blackfan Anemia
A dominant Mendelian disease often characterized by childhood anemia caused in the majority of cases by heterozygous mutations or deletions affecting one of more than 20 Ribosomal protein (Rp) genes.

Aneuploid cells
aneuploidy refers to abnormal chromosome complements due to missing or additional copies of individual chromosomes, is found in most cancers and is a cause of birth defects and miscarriage.

Spindle Assembly Checkpoint
Process that can arrest the cell cycle to ensure proper coupling of chromosomes to the mitotic spindle, without which chromosome segregation errors often result.

Apical–basal axis
the distinction in epithelial cells between the apical surface that faces the exterior or lumen and the basal surface that faces the interior.

Adhesion junctions
where epithelial cells bind one another through cadherin adhesion molecules expressed on each cell surface.

Extrusion
progressive ejection of a single cell from an epithelium. Extrusion usually maintains the epithelial barrier as surrounding cells close in and eventually contact one another to maintain a seal.

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Box1:

Pro-cancer cell competition

Many examples of cell competition, such as competition seen in epithelial defense against cancer (EDAC) or in the elimination of *scrib* mutant cells from *Drosophila melanogaster,* remove potentially neoplastic cells and thus may be tumor suppressive. By contrast, super-competition that removes wild type cells could drive the expansion of tumors expressing oncogenes such as *Myc* and *Yki*, at the expense of normal cells[137,138]. This idea has been substantiated in *D. melanogaster* in which, similarly to the situation in mammals, cells harbouring mutations in homologues of adenomatous polyposis coli protein (APC) develop into intestinal adenomas[139]. The expanding tumors eliminate normal tissue through super-competition; blocking apoptosis prevents cell competition, and consequently tumor expansion. Thus, in this example, tumor development relies on cell competition to eliminate surrounding healthy cells[139].

Calcium channel flower homologs, also known as Human Flower (hFwe) proteins, contribute to tumor growth in mammals[105]. The gene encoding these proteins was named after a homologous gene in *D. melanogaster* that is also implicated in cell competition[103]. In *D. melanogaster Fwe* encodes a potential Ca^{2+} channel originally found to affect synaptic vesicle recycling[140]. *hFwe* encodes four isoforms of putative transmembrane proteins, of which hFWE1 and hFWE3 are pro-apoptotic only when neighboring cells express hFWE2 or hFWE4. This configuration, of hFWE2 and/or hFWE4 in tumor cells and hFWE1 and/or hFWE3 in surrounding stromal cells, occurs in samples from human breast and colon cancer[105]. Recreating this expression pattern in mouse models using MCF7 breast tumor cells enhanced tumor volume, whereas silencing all FWE isoforms in colon and prostate tumor xenografts reduced tumour growth and metastasis, and synergized strongly with conventional chemotherapies such as fluorouracil (also known as 5-FU) or cisplatin with docetaxel[105]. These data argue that FWE-mediated cell competition contributes to the expansion of solid tumors, although how these tumor-promoting expression patterns of hFWE arise, and how they lead to a competitive interaction, is unknown. Individual hFWE proteins do not have much effect on the proliferation rate or survival of normal or transformed human cells[105].

An important role for the transcriptional coactivators YAP and TAZ, homologous of Yki, the expression of which converts *D. melanogaster* cells into super-competitors, was recently identified in intrahepatic cholangiocarcinoma (CCA) in mice[141]. Intriguingly, in mouse liver YAP and TAZ expression were also elevated in peritumoral hepatocytes (that is, in cells surrounding the tumor). Not only was CCA expansion dependent on YAP and TAZ in the tumor cells, but it was opposed by the expression of YAP and TAZ in cells surrounding the tumor; the growth rate of the tumor was dependent on competition between these groups of cells, which was determined by their relative levels of YAP and TAZ expression[141].

The ability of competitive processes, including EDAC, to eliminate cells carrying oncogenic mutations such as RAS^{V12} suggests that neoplastic cells might have to escape competition, perhaps by acquiring additional mutations. In *D. melanogaster*, cells

expressing both *Ras*^{V12} and mutant *scrib* are neoplastic, although cells carrying either genotype alone are eliminated[52,142]. It has also been suggested that, above a threshold size, competitive cell death might paradoxically stimulate tumor growth if apoptosis-dependent proliferation outweighs competitive cell loss restricted to clone boundaries[130].

Figure 1: The basics of cell competition.

A. Cell competition reflects the specific elimination of a particular cell population by competitive interactions within a tissue. In the classic example of cell competition, $Rp^{+/-}$ cells progressively undergo apoptosis when close to $Rp^{+/+}$ cells in *Drosophila melanogaster* imaginal discs[2,4,5]. This apoptosis results in $Rp^{+/+}$ territories (blue cells) expanding at the expense of $Rp^{+/-}$ cells (black cells). Since $Rp^{+/-}$ cells can generate an almost normal fly by themselves, but competitive apoptosis is induced where $Rp^{+/+}$ cells and $Rp^{+/-}$ cells meet, cell competition is specifically induced by the differences between cells, which are genetic in this case. Cell competition does not necessarily require apoptosis: competed cells can also be removed by senescence, autophagy, epithelial extrusion, or they can be eliminated from a stem cell layer by oriented cell divisions. Adapted with permission from REF. 12.

B. An example of cell competition in a wing imaginal disc from *D. melanogaster* is shown. Activated caspases mark dying cells, which are predominantly $Rp^{+/-}$ cells near to $Rp^{+/+}$ cells. By contrast, $Rp^{+/-}$ cells are generally viable away from the border. Image courtesy of Dr. C-H Lee.

C. Super-competition occurs when a particular cell population (grey cells) eliminates normal wild type cells (blue cells). The elimination of wild type cells by super-competition illustrates how cell properties are changed by differences between cells. Adapted with permission from REF. 12

NOTE: for Figure 1 please refer to Figure 1 of the paper at *Nature Reviews Genetics* **volume 21**, pages 683–697(2020)

Figure 2: Examples of cell competition.

A. Competitive repopulation of mutant human α1-anti-trypsin transgenic mouse liver by transplanted, LacZ-expressing wild-type progenitor cells (labeled blue). Initially, transplanted wild type cells constitute only a small fraction of the liver, but over time they replace the transgenic cells; transgenic cells undergo an elevated rate of apoptosis in the presence of the transplanted cells. No such population replacements would be seen in non-chimeric animals. Red stain corresponds to globules of the non-functional human protein. Scale bars: 100 μm. Reproduced with permission from REF. 16.

B. Competitive elimination of individual Madin-Darby canine kidney (MDCK) cells expressing a *Scribble* shRNA from an epithelium of normal MDCK cells. *Scribble* knock-down cells (labeled with red dye) round up and are apically extruded from mixed epithelia 37–48 h after induction of shRNA and are soon detected as dying cells by Sytox. Scale bar: 10 μm. Reproduced with permission from REF. 34.

NOTE: for Figure 2 please refer to Figure 2 of the paper at *Nature Reviews Genetics* **volume 21**, pages 683–697(2020)

Figure 3. Cell competition of *Rp*$^{+/-}$ cells in mammals.

A. Chimeric mice were obtained by injecting injecting control (Rosa26) embryonic stem (ES) cells into blastocysts from a cross between C57BLKS *Bst*$^{+/-}$ and *Bst*$^{+/+}$ strains. The injected blastocysts were transferred into wild type females. When the host blastocyst was *Bst*$^{+/+}$, most of the adults are derived from the host cells, as indicated by the fact that only a few patches of pale fur, which is phenotypic of agouti Rosa26 cells, are present. When the host blastocyst was *Bst*$^{+/-}$ the contribution of Rosa26 cells was markedly greater, as indicated by the fact that adults predominantly have pale fur. This fact could represent active competition of *Bst*$^{+/-}$ cells or a passive consequence of the reduced growth rate and slower cell cycle of *Bst*$^{+/-}$ cells compared to wild type ES cells.

B. Model for potential cell competition of *Rp*$^{+/-}$ cells in mammals. Heterozygous *Rp* mutations in mouse models, or in human patients with Diamond-Blackfan anemia (DBA), activate p53 by causing nucleolar stress. Specifically, under nucleolar stress, excess RPL5 and RPL11 inhibit MDM2, the ubiquitin ligase for p53, leading to chronic activation of p53 (the precise location of this interaction, shown in the nucleoplasm, is uncertain). Elimination of cells with higher p53 activities by competition with cells of lower p53 activities has been found in several contexts. Although each step of this model has been verified independently in different experiments, overall it has still not been directly demonstrated that *Rp*$^{+/-}$ cells are actively competed in mammalian chimeras, as indicated by the question mark…….

NOTE: for Figure 3 please refer to Figure 3 of the paper at *Nature Reviews Genetics* **volume 21**, pages 683–697(2020)

Figure 4. Interactions between cell populations leading to cell competition.

Cell competition could be mediated by several general mechanisms of cell interaction. A) Molecular recognition of distinct surface properties might occur at the interface between cell populations, triggering a response to eliminate one class of cell. Here a receptor is shown on the competed cells, recognizing the presence of cells that will eliminate them, but the reverse is also plausible. B) Non-autonomous toxic and protective signals that are balanced within homogenous populations may become unbalanced at the boundary between non-homogenous populations, leading to a local toxic signal. C) Where winners and losers grow at different rates, mechanical stress may result and have different consequences for each cell population[68,69].

NOTE: for Figure 4 please refer to Figure 4 of the paper at *Nature Reviews Genetics* **volume 21**, pages 683–697(2020)

Figure 5: Mechanisms of cell competition.

A) Summary of pathways contributing to the elimination of *scrib* mutant cells from mosaic imaginal discs in *D. melanogaster*[71–73]. **Aa|** Jnk activity is a response to autocrine TNF (also known as protein Eiger in *D. melanogaster*) signaling in all *scrib* mutant cells, perhaps as a consequence of altered cell polarity. Jnk activity is not apoptotic, but promotes the proliferation of *scrib* cells in conjunction with signaling via the epidermal growth factor receptor (Egfr)–Ras pathway. **Ab|** However, in *scrib* mutant cells at the interface with wild type cells, ligation of the receptor tyrosine phosphatase Ptp10D by its ligand Stranded at second protein (Sas) antagonizes Egfr signaling and, in this context, Jnk potentiates Slit–Robo2-Ena signaling to down-regulate the expression of E-cadherins and promote the basal extrusion and apoptosis of *scrib* mutant cells. **Ac|** In addition, Jnk signaling in wild type cells neighboring *Scrib* mutant cells contributes to the basal extrusion and apoptosis of *scrib* mutant cells, by promoting the expression of the PDGF receptor homolog Pvr. Pvr promotes the Rac-dependent engulfment and clearance of apoptotic *scrib* mutant cells. Reproduced with permission from REF. 12.

B) A model for the elimination of wild type cells by toxic signaling by Toll-like receptors (TLRs) in *D. melanogaster* imaginal discs[76]. The secreted protein Spz is produced as an inactive precursor by wild type cells. However, in cells with elevated levels of Myc the transcription of Spz-processing enzymes is increased while the expression of multiple genes encoding TLRs is downregulated. As both Spz and its processing enzymes are extracellular, wild type cells near to tub>myc cells that express elevated levels of Myc under control of Tubulin-Gal4 are also exposed to elevated active Spz; as wild type cells express TLR at normal levels they acquire high levels of toxic NF-κβ signaling. C) Summary of pathways eliminating *scrib* knockdown cells from Madin-Darby canine kidney cell epithelia in culture[90]. When cultured alone, *scrib* knockdown cells exhibit higher levels of baseline p53 activity and a flattened, lower-density growth habit than wild type cells (1). In mixed cultures, however, *scrib* knockdown cells are compressed by wild type neighbors, promoting Rho-associated protein kinase (ROCK) activity and further p53 activation via p38 (2). Chronic p53 hyperactivation leads to extrusion and apoptosis of compressed *scrib* mutant cells (3).

NOTE: for Figure 5 please refer to Figure 5 of the paper at *Nature Reviews Genetics* **volume 21**, pages 683–697(2020)

Figure 6. Current mechanistic understanding of epithelial defense against cancer.

Multiple cellular changes have been observed in mixtures of mammalian cells bearing transforming mutations such as the RAS^{V12} in a concept termed epithelial defence against cancer (EDAC). The cytoskeletal proteins filamin, and then vimentin, accumulate in wild type cells apposing the transformed cells, whereas Eplin accumulates in the cell expressing RAS^{V12}. These cytoskeletal changes help implement a shift towards glycolytic metabolism in RAS^{V12} cells and to the apical extrusion of these cells from the epithelial monolayer (reviewed in[58]).

NOTE: for Figure 6 please refer to Figure 6 of the paper at *Nature Reviews Genetics* **volume 21**, pages 683–697(2020)

TABLE 1|

Genotypes resulting in cell competition or super-competition.

Genotype	Outcompeting genotype	Reference
Drosophila melanogaster		
Rp$^{+/-}$	Wild type	2
scrib	Wild type	52
lethal giant larvae (l(2)gl)	Wild type	37,127
Discs large (dlg1)	Wild type	53
Vps25	Wild type	128
Tumor susceptibility gene 101 (Tsg101)	Wild type	129
fwe	Wild type	103
mahj (the *D. melanogaster* homologue of VPRBP, aka DCAF1)	Wild type	37
Dominant negative Rab5	Wild type	130
frizzled frizzled2 (fzfz2) double mutant	Wild type	131
Sccro	Wild type	132
Troponin I (also known as *wupA*)	Wild type	133
Upf1 or upf2	Wild type	134
Hel25E	Wild type	55
NMDA Receptor 2 (nmdar2)	Wild type	57
Wild type	Elevated *myc*	7,8
Wild type	*Ex, sav, wts, hpo,* or mats	9
Wild type	Elevated *yki*	10
Wild type	Elevated *Stat*	135
Wild type	*Apc* or *axn*	131
Wild type	*Crumbs (crb)* mutant	136
Mammals (Species)		
Wild type (Rat: after regenerative stimulus)	Liver progenitor cells	15,17
PiZ, transgenic for human al anti-trypsin allele (mouse)	Wild type	16
Wild type (mouse)	*Rosa26>Myc*	13,31
Mycn$^{+/-}$ (mouse)	Wild type	14
Mycn$^{-/-}$ (mouse)	*Mycn*$^{+/-}$	14
Wild type (mouse)	*p53*$^{-/-}$	22
Wild type (Mouse, after irradiation)	*p53*$^{-/-}$	18,19,56
Wild type (mouse)	*Top1*$^{-/-}$	22
Tetrapoid (4n) cells (mouse)	Diploid (2n) cells	27
Bmpr1a$^{-/-}$ (mouse)	Wild type	26
Ras^{V12} (MDCK cells)	Wild type	32
v-Src (MDCK cells)	Wild type	33

Genotype	Outcompeting genotype	Reference
Yap^{act} (that is, constitutively active *Yap*) (MDCK cells)	Wild type	35
Scrib knocdown (MDCK cells)	Wild type	36,90
VPRBP (also known as *DCAF1*, and the homologue of *mahj* from *D. melanogaster*) knockdown (MDCK cells)	Wild type	37
Aneuploid cells (mouse)	Wild type	44

MDCK, Madin-Darby Canine Kidney cells;