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Review

Drosophila Myc: A master regulator of cellular performance ☆

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ABSTRACT

The identification of the *Drosophila* homolog of the human MYC oncogene has fostered a series of studies aimed to address its functions in development and cancer biology. Due to its essential roles in many fundamental biological processes it is hard to imagine a molecular mechanism in which MYC function is not required. For this reason, the easily manipulated *Drosophila* system has greatly helped in the dissection of the genetic and molecular pathways that regulate and are regulated by MYC function. In this review, we focus on studies of MYC in the fruitfly with particular emphasis on metabolism and cell competition, highlighting the contributions of this model system in the last decade to our understanding of MYC's complex biological nature. This article is part of a Special Issue entitled: Myc proteins in cell biology and pathology.

1. Introduction

MYC is one of the most referenced molecules in biology; since its isolation in the chicken genome in early 1980s [1], plenty of literature has covered the many aspects of its function. Despite that, the last decade has witnessed its unanticipated appearance in several cellular mechanisms, demonstrating that we are still far from composing a complete picture of its biological potential. *Drosophila* Myc protein is encoded by the *dm* gene (*diminutive*, from the mutant phenotype) whose molecular characterization [2] was long anticipated by the phenotypic observation. Current technology allows the researcher to manipulate the fly genome at a level of precision that exceeds that of any other multicellular organism, and many studies in the fly aimed at investigating Myc function in different tissues and organs in both physiological and pathological conditions. In this review, we will discuss the most recent advances in MYC biology obtained in studies in *Drosophila*, focusing mainly on its roles in metabolism, tissue growth and cancer.

2. Myc family in flies: a conserved network

The phenotype of Myc mutant flies has been known since 1933 when Eleanor Nichols Skoog, while in Calvin Bridges' lab at California Institute of Technology, observed adult flies with small body size and female sterility and identified a mutation in a gene on the X chromosome that she named *diminutive* [3]. In 1996, 50 years after its phenotypic description, the search for a simple system in which to study the complexity of MYC function was finally satisfied by the discovery that *diminutive* encoded the *Drosophila* homolog of Myc [2,4]. *Drosophila* Myc protein is only 26% identical to its human counterpart, but still contains highly conserved functional domains that allowed its identification in a yeast 2-hybrid screen using human Max as a bait; subsequently, the *max* and *mnt* genes in *Drosophila* were also cloned [2]. As in mammals, the Myc/Max/Mad network also controls fundamental cellular processes in *Drosophila*, including apoptosis, tissue growth and proliferation and consists of single Myc, Max, and Mad/Mxd components [5–7].

Drosophila Myc contains several functional domains, among which is the highly conserved basic-helix-loop-helix leucine zipper domain (bHLH/LZ) present at its C-terminus that mediates Myc:Max heterodimerization [2,4]. Myc:Max heterodimers bind the E-box sequence CACGTG on target genes and activate their transcription. At its N-terminus Myc contains several conserved motifs, including the conserved Myc Boxes I and II [2,8], which are partially required for Myc transcriptional activities [8], the conserved Myc Boxes III and IV, the latter containing the acidic region, whose mutational analysis revealed for these domains a novel, conserved function in controlling

Abbreviations: ACC, acetyl CoA carboxylase; bHLH/LZ, basic helix-loop-helix leucine zipper; Brk, Brinker; CK1α, casein kinase 1α; Desat1, desaturase-1; DILPs, *Drosophila* insulin like peptides; Dlg, discs large; *dm*, *diminutive*; Dpp, decapentaplegic; FAS, fatty acid synthase; Fwe, flower; GSCs, germ stem cells; GSK3, glycogen synthase-3 kinase 3; Hpo, Hippo; InR, insulin receptor; JNK, c-Jun N-terminal kinase; Lgl, lethal giant larvae; Mlx, Max-like; *M*, *minute*; PCP, planar cell polarity; PI3K, phosphoinositide 3 kinase; Rps, ribosomal proteins; Scrib, Scribble; SPARC, secreted protein acidic and rich in cysteine; TOR, target of rapamycin; Upd-2, unpaired-2; Wg, wingless; Wts, warts; TGA, triglyceride acids; YAP, Yes-associated protein; Yki, Yorkie

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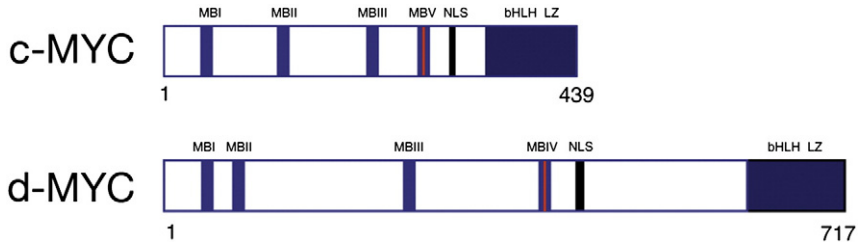

Fig. 1. Myc proteins. Schematic representation of Myc proteins. *Drosophila* Myc is longer than its vertebrate counterpart but the functional domains are conserved. In the draw we have outlined the relative position of the conserved Myc Boxes I–IV, the nuclear localization sequence (NLS), the acidic box (in red) within the sequence of MBIV and at the C-terminus the basic helix-loop-helix leucin zipper (bHLH LZ) DNA-binding domain.

Myc protein stability (Fig. 1) [8,9]. *Drosophila* Mnt is similar to both mammalian Mnt and Mad, and forms heterodimers via its bHLH/LZ domain with the cognate domain of Max [2,10]. Mnt:Max heterodimers repress transcription using the functional "Sin3 binding domain" (SID) at their N-termini to mediate the transcriptional co-repressor activity of Sin3 and histone deacetylase activity (HDACs) [11,12]. Finally, Max is the most conserved member of the *Drosophila* Myc network, being 42% identical to human Max in overall amino acid sequence [2]. Mnt:Max heterodimers bind the same E-boxes as Myc:Max heterodimers *in vitro* and can repress the transcription of Myc:Max targets to antagonize Myc function [10,13]. Interestingly, flies lacking Max show weaker phenotypes than *dm* mutants, suggesting that Myc may have functions independent of Max or Mnt in flies [14]. Experiments where *Drosophila myc* cDNA was used to rescue proliferation defects of mouse embryonic fibroblasts from *c-myc* mutant mice demonstrated that *Drosophila* and vertebrate Myc can functionally substitute for each other [15]. Moreover, *Drosophila myc* cDNA was able to induce transformation of rat embryonic fibroblasts when expressed together with an activated form of Ras (Ras^{V12}) [4]. In addition, developmental defects of *Drosophila* dm^{PG45} hypomorphic mutants were rescued by expression of the human *c-myc* cDNA [16]. These results indicate that many of Myc's functions are conserved from insects to mammals.

An important mammalian Myc-related complex is the Mondo-Max-like (Mlx) protein family, a bHLH/LZ network that works in parallel to the Myc–Max–Mnt node to control glucose and glutamine metabolism. Members of the Mondo-Mlx family bind to carbohydrate response elements (ChoRE) that contain similar E-boxes (CAAGTG) to those of the Myc–Max–Mnt family [17]. In *Drosophila* the product of the *Mio* gene, the single ortholog of the human *Mondo A* and *B* genes, binds to the Bigmax protein, the fly ortholog of human Mlx [17].

Competition between human Mnt monomers and heterodimers of Mnt and Mlx, a member of Mondo-Max-like protein network, to repress the transcription of ChoRE genes [18], suggests the presence of common targets for the two transcriptional networks in regulating metabolic pathways relevant to metabolism and growth (see Section 5).

Discussions on Myc function generally refer to the activities exerted by components of the Myc/Max/Mnt network. However, in this review we will focus primarily on the function of the most investigated member of the network: Myc, and its role in controlling growth and cell competition.

3. Myc controls growth and size

Overexpression of Myc in cells of the imaginal discs (larval organs, composed of diploid epithelial cells, which give rise to the adult appendages and part of the body wall) induces growth by accelerating mass accumulation and the rate of the G1/S transition of the cell cycle. However, the cells are unable to proliferate faster because their entry into the M-phase is limited by availability of String/CDC25, which is developmentally regulated. This results in larger than normal cells [19]. Conversely, *dm* mutant disc cells slow entry into G1-phase and exhibit slower overall cellular division rates. Animals carrying weak *dm* alleles (dm^1, dm^{P0}) are developmentally delayed, yielding adult flies with small but normally proportioned body size and small bristles [19], while dm^4 null animals die as larvae early in development [20]. Conversely, Myc over-expression results in flies that are about 16% larger than normal [21]. Animals mutant for *mnt*, the fly homolog of the human *mad* gene, are viable and show a growth phenotype similar to Myc over-expression, as cells mutant for *mnt* are larger and *mnt* adults are heavier than wild-type [10]. The *mnt* phenotype was partially rescued by reducing the *dm* gene dosage, indicating that *mnt* and *myc* exert opposite control on animal growth [13]. Surprisingly, analysis of *max* null mutants revealed that development of *max* null animals takes longer than *myc* null or *myc:mnt* double mutant animals, but the flies die as "pharate" (differentiated but non-viable) adults. This suggested the existence of Max-independent Myc activities, perhaps substituted by Mlx, that allow flies to survive longer than dm^4 null animals [14].

Drosophila Myc influences the expression of a large number of genes that are involved in diverse cellular processes; however a preeminent group of Myc-activated targets encodes for proteins involved in ribosome biogenesis, translation, and metabolism [22–24]. Analysis of the target genes demonstrated that the majority of them encode for RNA-binding proteins, rRNA processing and ribosomal proteins, which all contribute to ribosome biogenesis [22,25–27]. Myc function in ribosome biogenesis is conserved in vertebrates, where c-Myc activates the expression of the RNA helicase DDX18 (in flies encoded by the *pitchoune* gene) [28], and the nucleolar proteins Nucleolin and Nol12 (encoded in flies by the *modulo* and *viriato* genes, respectively) [29,30]. Numerous studies in both flies and vertebrates revealed that Myc also contributes to ribosome biogenesis by directly activating RNA polymerases I and III [14,24,31]. A common co-activator for RNA polymerase III is Brf11, which acts also downstream of nutrients and of the TOR (target of rapamycin) pathway to induce organismal growth [32]. Many of Myc target genes, members of the chromatin remodeling complexes, are also repressed by Mnt, consistent with the antagonistic roles for Myc and Mnt in promoting and suppressing cellular growth [13]. Indeed, Myc was shown to be in a complex with the transcriptional cofactors Tip48 (Reptin) and Tip49 (Pontin) [25], components of chromatin remodeling complexes [33]. While most of the functions of mammalian Tip48 and Tip49 are associated with cellular growth and cancer progression [34], in flies, genetic interaction experiments with Myc revealed an unexpected function for Pontin and Reptin to repress gene transcription *in vivo* [25]. This function is conserved also in *Xenopus*, where Myc function is required together with Pontin and Reptin to repress Miz-1 transcriptional activity [35]. Myc regulation of growth is mediated also by direct binding to protein members of complexes that control histone modification or to enzymes that favor transcription. Myc binds to members of the Trithorax-group such as the histonemethyltransferase Ash2 ("Absent, small, or homeotic discs 2"; the homolog of vertebrate ASH2L), the ATPase Brahma (the homolog of human hBrm and Brg1) and finally the histone-demethylase Lid

("Little imaginal discs"; the homolog of vertebrate Rbp-2/JARID1A and PLU-1/JARID1B), which is required to promote growth *in vivo* [36].

4. Myc stability downstream of growth factors

Insulin (InR) and Target of rapamycin (TOR) pathways are key regulators of growth and are highly conserved in *Drosophila* [37]. Animals hypomorphic for *Tor* or *s6k*, components of the TOR signaling pathway [38–40], and mutants for the *Drosophila*-insulin-like peptides (DILPs) or for the adaptor molecule *chico*/*IRS*, are smaller than wild-type [41,42], with a phenotype resembling that of weak *dm* mutants [19,20]. Genomic analysis showed strong correlation of Myc targets with those of the TOR pathway, and to a lesser extent between Myc targets and those of PI3K signaling [43]. *Drosophila* Myc protein stability is regulated by nutrients and by growth factor signaling [44] and activation of InR and TOR signalings both in S2 cells and in the imaginal disc stabilizes Myc protein [45]. These experiments evidenced the presence of few conserved phosphorylation domains that function as substrates for the GSK3β kinase (encoded by *shaggy*/*sgg* in *Drosophila*), acting in combination with CK1α kinases to control Myc protein degradation through the ubiquitin–proteasome pathway [9]. Coordination between phosphorylation and ubiquitination is a conserved feature of mammalian and *Drosophila* Myc, as mutations in the ubiquitin-ligase Archipelago (Ago), the *Drosophila* homolog of the SCF (Skip1-Cullin-F box) ligase Fbw7 (SCF^{Fbw7}), result in elevated Myc protein levels [46]. This proteolytic degradation pathway is facilitated by the presence of the scaffolding protein Axin, which binds GSK3β, Ck1α and Myc (PB, unpublished) to decrease its stability [47,48]. Since GSK3β and CK1α kinases also regulate the stability of the key effector of the Wnt/Wingless pathway beta-catenin (Armadillo in *Drosophila*), which is in a complex with Axin in the absence of Wnt signaling [47], Axin might be the common partner for Myc and Wnt in the mechanisms responsible for early colorectal tumorigenesis [49].

5. Myc controls metabolic pathways that connect nutrition with systemic growth

Growth requires energy as well as protein synthesis and MYC activity plays an important role in controlling metabolic pathways such as glycolysis and glutaminolysis [50,51]. This feature is particularly relevant in cancer, where c-MYC activity couples glucose and glutamine metabolism with growth [52,53]. During *Drosophila* development the fat body, a metabolic organ with physiological functions like the mammalian adipose tissue and liver [54,55], stores sugars and fats so that they can be mobilized systemically to sustain basal cellular energy supplies when nutrients are scarce [56]. Starved animals activate survival pathways to sustain the basal metabolic level and in the fat body *dm* mRNA is induced by transcriptional activity of FOXO upon starvation, while the contrary is true in muscle cells [57]. Studies in vertebrates also report increases in c-*myc* mRNA in adipocytes and liver cells in response to caloric restriction, suggesting that Myc activity is necessary to maintain survival signals during low nutrient conditions [58,59]. In *Drosophila* the fat body also senses the amino acid concentration in the hemolymph (the larval blood), and in response to a protein-rich diet it produces humoral factors that control the release of *Drosophila* DILPs from the brain, allowing the animal to grow [60]. Expression of Myc specifically in the fat body increases animal size [55,61] similar to animals that express Myc ubiquitously [19–21]. However, increasing the size of the fat body cells is not sufficient to alter organismal growth, because expression of Cyclin D + Cdk4 or Rheb, two potent growth inducers, does not affect animal size [55]. Instead, Myc activity induces metabolic changes in the fat body that remotely control the release of unknown factors, which in turn stimulate DILP2 secretion by the brain neurosecretory cells (Fig. 2) [55]. As in vertebrates, excessive Myc activity in the fat body and in imaginal discs favor glucose uptake by inducing expression of a cohort of genes necessary for increased glucose uptake and utilization [62,63]. Studies in vertebrates demonstrated that c-MYC expression in the liver favors glycolysis and lipogenesis [64], with increased blood glucose disposal and resistance to streptozotocian-induced diabetes [65]. This function of Myc in the regulation of carbohydrate metabolism is conserved in flies, and increased Myc activity in fat body cells promotes glucose availability in the hemolymph, suggesting that Myc expression could counteract the increased levels of circulating trehalose (the fly circulating sugar) induced by a high sugar diet [55].

Expression of Myc in fat body cells regulates lipid metabolism and increases the storage of Triglyceride acids (TGA). At the molecular level, Myc increases the transcription of genes encoding for *fatty acid synthase* (*FAS*), *acetyl CoA carboxylase* (*ACC*), both targets of ChREBP, and of the *stearoyl-CoA desaturaseSCD1* (whose *Drosophila* homolog is called *Desat1*) (Fig. 2) [55]. The expression of these enzymes is increased in cancer cells and might promote *de novo* synthesis of lipids to provide tumor cells with a growth advantage [66–68]. Desat1 is the rate-limiting enzyme for the biosynthesis of monosaturated fatty acids into TGA and phospholipids [69]. Interestingly, in the mouse, mutations in Desat1/SCD1 result in a dramatic reduction of whole body fat and in reduced animal size [70,71]. In *Drosophila*, Desat1 is necessary for Myc's ability to accumulate TGA and to control body size [55]. Desat1 is not a direct target of Myc, but its expression is necessary in fat body cells to promote autophagy in starved conditions [72], identifying this enzyme as a novel component in Myc-mediated control of lipid metabolism and systemic growth. In the vertebrate liver and adipocytes FAS and ACC are transcriptionally regulated in response to glucose concentration by the Mondo–Mlx network [73]; Mondo:Mlx heterodimers reduce glucose uptake [18,74] and their activity is susceptible to high glutamine concentration [75]. It is not still clear whether this mechanism is conserved in *Drosophila*; however a possible genetic interaction between *Drosophila* Mondo and Myc has been suggested [18], raising the possibility that high levels of Myc change the stoichiometry of Mondo:Mlx heterodimers to favor lipid metabolism in the fat body by promoting *FAS* and *ACC* transcription.

Myc activity regulates glutamine flux and reprograms glutamine metabolism, a condition that favors tumor proliferation [53]. As in vertebrates, *Drosophila* Myc expression increases the levels of *glutaminase* and *glutamine synthetase* (*GS1*) mRNAs, key regulators of glutamine metabolism (Fig. 2) [55,62]. Notably, Myc expression also increases the level of a family of amino acid transporters homolog to SLC1A5 (encoded by *minidiscs* in *Drosophila* [76]) and SLC3811 [55,77]. SLC1A5 was shown to control the efflux of L-glutamine to import essential amino acids and to induce activation of mTOR signaling [78]. A similar mechanism may occur in the cells of the fat body , where Myc activity might permit entry of essential amino acids to maintain a basal level of activated TOR signaling (PB, unpublished). Recent data in mammals also identified a novel function for GS1 in activation of FOXO-induced autophagy and reduced mTOR signaling in autophagosomes [79]. How and if these pathways are also conserved in flies, and if they depend on Myc is not clear yet, but we speculate that the FOXO-Myc-GS1 axis may also exist in the fat body to regulate glutamine-dependent pathways that promote animal survival.

A limited number of other factors also contribute to systemic growth and have been linked to TOR signaling pathway, including the transcription factor DREF, which activates several genes involved in ribosome biogenesis [80,81], and Brf1, a component necessary for the activation of RNA polymerase III. Both DREF and Brf1 have also been linked to Myc activity [32,82]. In addition, expression of Unpaired-2 (Upd2), an adipokine homolog to human class I cytokines that acts as a ligand for the JAK/STAT signaling pathway, in fat body cells promotes DILP2 release from neurosecretory cells of the brain during feeding conditions [83]. Release of Upd2 from the gut of adult animals was shown to be cell-autonomous and Myc-dependent [84,85], suggesting that in the fat body Upd2 may also be downstream of Myc in its control of systemic growth. The fat body responds to the steroid hormone ecdysone in the control of developmental timing, and in fat body cells ecdysone

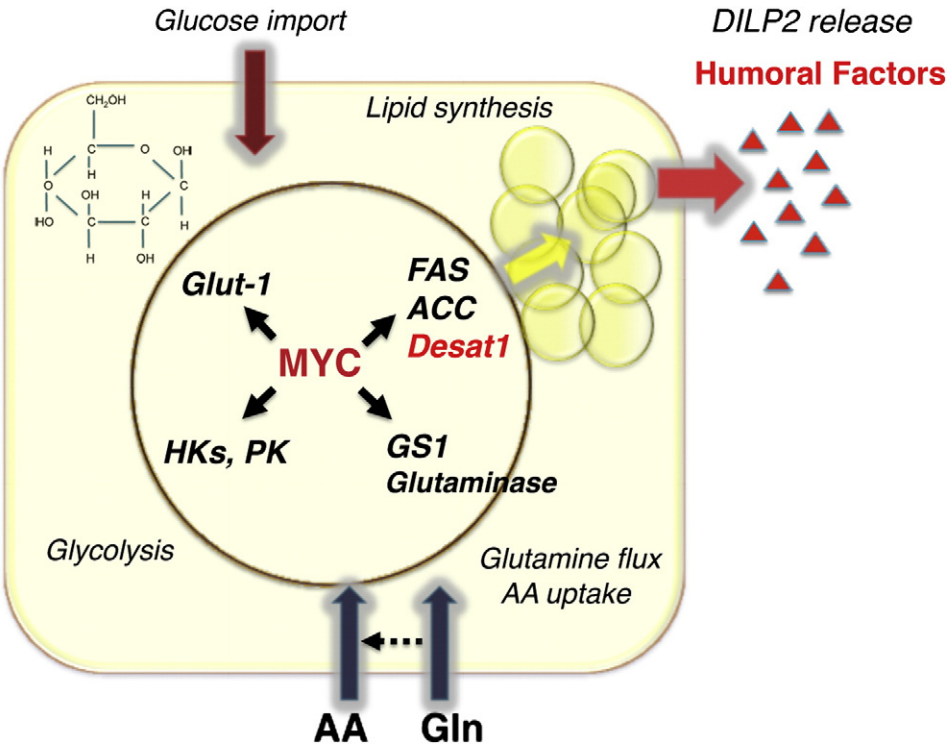

Fig. 2. Major pathways induced by Myc in the larval fat body. Expression of Myc increases the activity of fatty acid synthase (FAS), acetyl CoA (ACC) and Desat1, responsible for the synthesis and modification of fatty acids. Hexokinases (HK-A and C), piruvate kinases (PK), glycolytic enzymes and glucose transporter-1 (Glut-1) are also induced by Myc expression, together with glutamate synthase (GS1) and glutaminase, key enzymes of glutamine synthesis and glutaminolysis. Evidence also indicates that Myc increases the expression of SLC38-11 and SLC1A5 aminoacid/glutamine transporters, suggesting a role in controlling glutamine flux in the fat body. At the systemic level, the metabolic changes induced by Myc in the fat body cause the release of unknown factors which in turn trigger DILP2 secretion by the brain neurosecretory cells. From Parisi et al., *Dev Biol* 2013, 379(1):64–75.

represses Myc transcriptional activity to control systemic growth [61]. Ecdysone also suppresses the ability of fat cells to accumulate lipids [86], corroborating the relevance of Myc in controlling lipid metabolism.

6. Myc controls apoptosis and autophagy

A conserved feature of Myc activation is its ability to trigger cell death [6,16]. High levels of Myc cause cell-autonomous apoptosis by inducing expression of the pro-apoptotic genes *hid*, *grim*, *reaper* and *sickle* [21,87]. Moreover, Myc was shown to induce apoptosis non-cell-autonomously in imaginal disc cells, in a process called cell competition (see Section 7). The role of Myc in autonomous cell death puzzled scientists for many years and contradicted Myc's pro-survival role in tumor formation. However recent data identify a new function for Myc in macro-autophagy, a process used by cells to re-utilize macromolecules (fat, sugars, organelles) to promote survival when nutrients are scarce (PB, unpublished) and during cellular stress [88]. Recent work in flies and in vertebrates showed that Myc may act on the signals that induce autophagy in response to the unfolded protein response (UPR) with the activation of PERK- (ER stress dependent kinase), while its expression correlates to the ability of c-Jun N-terminal kinase 1 (JNK1) to induce autophagy [89,90]. Both JNK and Myc are key components of the pathways that regulate autophagy, but what are the signals triggered by Myc that activate a "detrimental" or a "survival" pathway? Is Myc responsible for a switch between these two pathways? Very recently Myc-nick, a short, cytoplasmic form of Myc generated by calpain-dependent proteolysis, was shown to promote cell survival in response to apoptotic stress [91]. Myc-nick levels are increased in tumor cell lines and tumor samples, while in tissue culture Myc-nick-expressing cells show increased autophagy and reduced cell death, suggesting a tumorigenic mechanism induced by inhibition of the apoptotic pathway [92]. Calpain is an ubiquitous enzyme whose function is conserved in *Drosophila*, but its role in promoting Myc cleavage in flies has not been addressed yet.

7. Myc and cell competition

Arguably, one of the most novel contributions of *Drosophila* to the field of Myc biology is the discovery of its role in regulation of competitive interactions between cells during tissue growth. First evidence that genetically different cells growing within a tissue contribute unequally to the adult organ was obtained about 40 years ago in *Drosophila*, but it took a long time before the leading role of Myc in cell competition was unveiled. In the last decade, plenty of literature has shown how cell competition regulates tissue growth both in *Drosophila* and mammals, emphasizing its relevance in development and cancer biology.

7.1. The beginning: Minute mutations

Cell competition is one of the most intriguing mechanisms in the field of developmental biology. It results from bidirectional interactions between cells that are genetically different within a growing epithelium [93]. In the 1970s, pioneering work demonstrated that in a growing tissue, wild-type cells and cells carrying a *Minute* (*M*) mutant allele, a group of dominant, homozygous-lethal mutations in genes encoding various ribosomal proteins (Rp) [94], compete for occupancy of the adult tissue [93]. The authors utilized genetic mosaic analysis to study the behavior of wild-type cells in *M*/+ individuals (for a comprehensive review of clonal analysis see [95]). They found that when the growing tissue or organ was composed of cells of the same genotype the *M*/+ cells were viable, although showed a delay in the rate of cell proliferation due to ribosome impairment. However, when juxtaposed to wild-type cells, *M*/+ mutant cells were out-competed, and much of the resulting adult organ was composed of wild-type cells [93] (Fig. 3A). The tissue in which this analysis was carried out was the epithelium of the wing imaginal disc (the larval primordium of the adult wing and thorax), an organ whose tissue grows much like the somatic tissues in

mammals and in which physiological homeostasis has been extensively studied [96,97]. The wing imaginal disc consists of a highly-proliferating, pseudostratified sheet of columnar epithelial cells organized into different developmental compartments, which are inter-dependent but also function as autonomous units of growth [98]. It was next demonstrated that cell competition only occurs among cells growing in the same compartment [99] (Fig. 3A,B).

Mutations in Rp genes also occur in mammals, but it was not until 2004 that the *M*/+ phenotype was assigned to an Rp gene. The *Belly spot and tail* (*Bst*) mutation is a deletion within the mouse *RpL24* riboprotein gene, and it was demonstrated that *Bst*/+ mutant cells were out-competed by wild-type cells in developing chimeric blastocysts [100]. This was the first indication that cell competition was conserved and suggested that it had evolved as a mechanism to ensure that viable but unfit cells do not enter the final pool of cells committed to form the whole organism.

In the same years, a molecule emerged in *Drosophila* as a factor associated with cell fitness: the Decapentaplegic (Dpp)/TGFβ protein, long known and documented as a key morphogen in *Drosophila* and vertebrate development [101–103]. In 2002 it was observed that *brinker* (*brk*), a gene repressed by Dpp, was upregulated in *M*+ cells when compared to wild-type neighbors in the growing wing disc; *brk* upregulation triggered JNK (c-*J*un *N*-terminal *k*inase) activation and apoptosis, whose inhibition abolished cell competition; indeed, the expression of the anti-apoptotic baculovirus protein p35 not only rescued *M*/+ cells from death but also reduced the rate of proliferation of the wild-type counterparts [104,105]. Since Dpp functions as a growth-promoting factor in this tissue, the authors proposed the existence of a rate-limiting molecule involved in capturing Dpp which production might be hampered in *M*/+ cells [106]. However, this hypothesis is still under debate since no differences were found in the Dpp/Brk gradient between *M*/+ and wild-type tissue in a later study [107].

7.2. Myc enters competition

More recently, studies identified Myc as a key regulator of cell competition. Clones of cells bearing hypomorphic *dm* mutations, although viable in a homotypic environment, died when generated in a wild-type imaginal disc [19]. In 2004, two papers clearly demonstrated that this phenomenon was triggered by the difference in Myc expression levels between the two adjacent cell populations [21,108]. Both groups took advantage of *Drosophila*'s genetic propensity for mitotic recombination and engineered different assays to modulate Myc levels in clones in which each daughter cell and its clonal progeny were marked. In one paper, the recombination event generated clones that over-expressed Myc, while the relative sister clones were composed of wild-type cells. As expected, the clones expressing Myc grew to a significantly larger size than control wild-type clones induced in parallel. However, the sister clones were at a significant disadvantage and were smaller than control wild-type clones induced in parallel (Fig. 3A). This was shown to depend on an increase in apoptosis in the clones of wild-type cells (called "losers") that were growing adjacent to the Myc-expressing clones (called "winners"). Moreover, these wild-type "loser" cells appeared to be eliminated only if within eight cell diameters from the Myc-expressing cells [21]; this was an important observation as it was later suggested that cell competition is mediated by as yet unknown soluble factors [109]. Similar results were obtained with a tandem duplication model, in which sibling clones resulted composed of cells expressing four copies (4× *dm*) and two copies (2× *dm*) of the *dm* gene. In these experiments, not only 4× *dm* clones were significantly larger than their 2× *dm* siblings, but they were also larger than 4× *dm* clones induced in a homotypic background. Likewise, clones carrying two copies of *dm* were not only smaller than their 4× *dm* siblings, but also smaller than 2× *dm* clones growing in a homotypic background. Altogether, these studies reinforced the

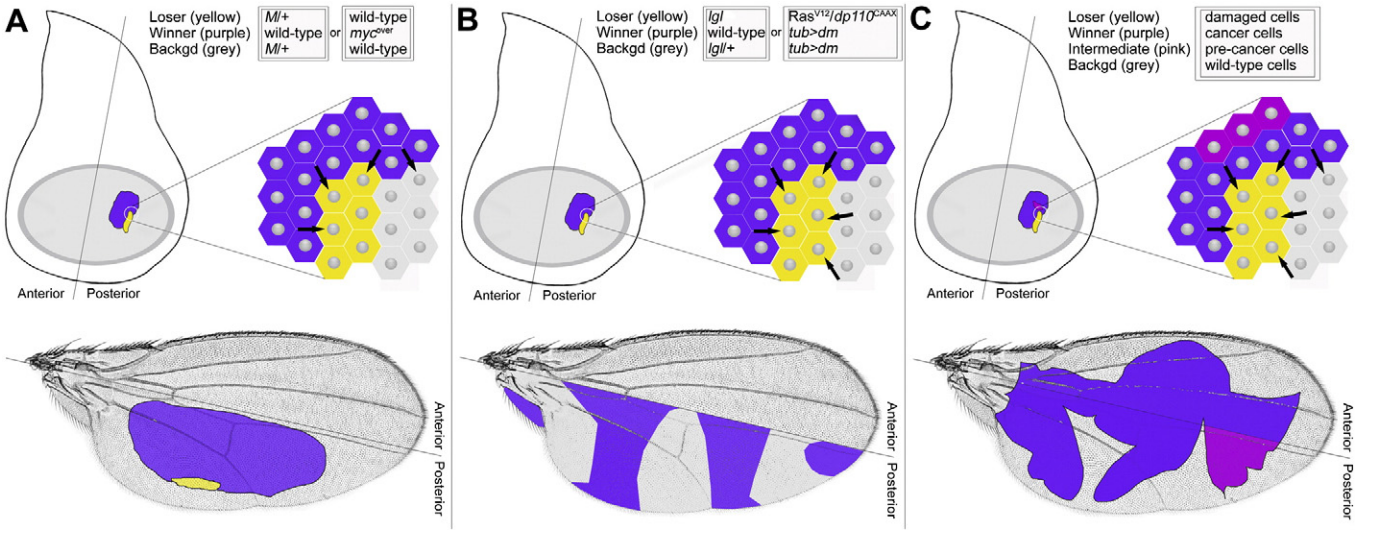

Fig. 3. Possible outcomes of cell competition. In each panel, the imaginal wing disc is shown with the wing-pouch region (gray) and the anterior/posterior border outlined, a hypothetical clone originated in the posterior compartment, a magnification of the encircled clonal region and the resulting adult wing. Arrows point towards the outcompeted cells. The boxed genotypes represent the different examples described in the main text. A. *Winner cells outcompete losers and background*. Wild-type clones induced in a *M*/+ background [98] or *myc*-overexpressing (*myc*^{over}) clones induced in a wild-type background [65] (purple) outcompete both loser cells (yellow) and the cells in the background (gray). At the end of development, the major part of the posterior compartment of the adult wing is composed of winner cells (purple). B. *Winner cells and background outcompete losers*. *lgl* mutant clones induced in an *lgl*/+ background [149] or *Ras*^{V12}/*dp110*^{CAAX} clones induced in a *tub* > *dm* background [113] (yellow) are outcompeted by both winners (purple) and cells from the background (gray), which fill the posterior compartment of the adult wing (purple/gray). C. *Several cell populations compete for organ colonization*. In *lgl*; *Ras*^{V12} or *scrib*; *Ras*^{V12} models of oncogenic cooperation [149,162–164,171–174], such super-competitive cells (purple) eliminate cells from the background (gray) and grow unrestrainedly. In the same field, damaged cells are possibly generated during clonal growth (yellow) and pre-malignant cells carrying intermediate genotypes may also appear in the tumor mass (pink). If these animals would not die during development, clone competition would result in an adult wing in which the anterior/posterior border, usually constrained, would be overridden and cancer cells would colonize the most part of the organ (purple). In this hypothetical example, pink cells are still present at the end of development but do not cross anterior/posterior border, displaying a non-invasive behavior. *M* = *Minute*; *myc*^{over} = *myc*-overexpression; *lgl* = *lethal giant larvae*; *Ras*^{V12} and *dp110*^{CAAX} encode activated forms of the proteins; *tub* > *dm* = a construct expressing a *dm* transgene under the control of the *α1-tubulin* promoter.

idea that cellular behavior is not dictated just by cell genotype, since it varies in different contexts, and introduced Myc as a key player in modulating the behavior of cells growing in diverse environments [108]. These results also defined an essential trait of cell competition: "winner" cells over-proliferate to fill the space left by the "losers".

Given Myc's role in regulating ribosome function and Rps (see Section 3), it has been hypothesized that Myc-mediated cell competition may require its ability to influence ribosomal activity. Consistent with this idea, Myc-expressing clones bearing one copy of *M(2)60E* (encoding for the Myc transcriptional target *RpL19*) were no longer able to out-compete surrounding cells [108]. However, regulation of protein synthesis "*per se*" seems to be insufficient to trigger Myc-induced cell competition, since the expression of another regulator of protein synthesis such as PI3K was shown to be unable to induce cell competition [21] and to protect cells from being out-competed [108]. As for *Minute*-mediated cell competition, Moreno and Basler found a reduction of Dpp signaling in the out-competed cells; according to the "ligand capture" model, cells compete with different success for limiting amounts of extracellular survival and growth factors, such as Dpp, by actively internalizing them. "Winner" cells, due to their optimal growth status, would then be able to capture more survival factors than "loser" cells that instead would die by JNK-mediated apoptosis [108]. To confirm this hypothesis and to test the rescue properties of some genes implicated in cell growth, the authors carried out a cell competition assay [21] inducing mutant clones of the chosen genes in a Myc-overexpressing background; mutations that provided cells with a growth advantage in this competitive context were defined as "super-competitors" [108]. From these experiments the authors concluded that the overexpression of the early endosome marker Rab5, involved in Dpp trafficking and signaling [110], of the activated Dpp receptor Thickveins (Tkv) and of Dpp itself, was able to confer to the cells the capacity to resist Myc-mediated cell death, while expression of activated forms of Ras and PI3K, two major effectors of cell growth in *Drosophila* and mammals, was not able to rescue mutant cells from death [108] (Fig. 3B). From these studies emerged that a difference in Dpp signaling between "loser" and "winner" cells is relevant for Myc-induced cell competition; however, in a parallel study it was observed that cells undergoing Myc-induced competition carried sufficient levels of signaling from Dpp [21]. It was later demonstrated that expression of Brinker and its partner Nab, both repressed by Dpp signaling, was necessary and sufficient in unfit cells with reduced Dpp uptake to trigger Myc-induced cell competition [111]. Therefore, the relevance of Dpp signaling in Myc-mediated cell competition is still an open question. The role of Dpp in Myc-mediated cell competition was also investigated in the fly ovary germline stem cell niche [112]. Niche cells produce high levels of Dpp [113] and, while the proximal germ stem cell (GSC) shows high levels of pMad, the downstream effector of Dpp signaling, the distal cell expresses the differentiation factor Bag of marbles (Bam), normally repressed by pMad, and differentiates into a cystoblast [114]. In contrast to a previous study which found no role for *dm* in *bam*-mediated GSC competition [115], the authors found that Myc was expressed at high levels in cells transducing Dpp signaling and much less in differentiating cells. Manipulation of Myc levels in the GSCs demonstrated that Myc-deficient cells move out from the germ niche and differentiate, while Myc-overexpressing GSCs are able to outcompete and substitute the wild-type counterparts [112].

A decade later its discover in *Drosophila*, Myc-mediated cell competition was also identified in mammalian development. Clavería and colleagues induced functional genetic mosaics in the mouse and observed that imbalanced Myc levels in the epiblast were sufficient to induce the expansion of Myc-overexpressing cells at the expense of the wild-type cells, which were eliminated by apoptosis [116]. Moreover, the authors found that wild-type cells composing the early embryo were heterogeneous in Myc content during development and a physiological mechanism akin cell competition refined the final epiblast selecting for cells with higher Myc levels [116]. In a parallel study, Sancho and colleagues observed that defective embryonic stem cells underwent apoptosis during embryo development through a mechanism that depended on the establishment of differential Myc levels throughout the embryo [117]. This was convincing evidence that Myc-mediated cell competition is an evolutionarily conserved phenomenon that defines cell chances in terms of ground occupancy during animal development.

Very recently, some new mechanisms of cell competition have been found in *Drosophila* that seem to be independent of Myc. Vincent and colleagues tested the role of Wingless (Wg) signaling, the fly ortholog of the vertebrate morphogenetic protein Wnt, in clonal growth in the wing disc [118]. They induced *axin* mutant clones, which have high Wg signaling activity, and found that those cells were able to overgrow and to induce apoptosis in the surrounding wild-type cells. Modulation of Myc activity did not modify *axin* clonal behavior, and interactions with *M*/+ mutations were additive with respect to *axin*-induced competition, suggesting for those clones a parallel pathway of competition [118]. Similarly, variations in the levels of the JAK-STAT signaling cascade, another central regulator of growth in *Drosophila* [119], modulated the cellular competitive behavior in the imaginal wing disc but in a Myc- and *Minute*-independent manner [120]. Cells mutant for STAT were out-competed by wild-type neighboring cells, and cells with hyperactivation of STAT killed wild-type cells through a mechanism independent of Myc, or Wingless signaling, or ribosome biogenesis [120]. These last data suggest that Wingless, STAT and Myc use parallel pathways for the regulation of tissue homeostasis through cell competition in *Drosophila*.

7.3. Factors involved in Myc-mediated cell competition

The evidence that cell death induced by competition was detectable in a maximum range of 8–10 cells away from the source of competitive induction [21] led to the search of possible secreted mediators responsible for this phenomenon. First evidence that Myc-induced cell competition was mediated by soluble factors came from a study carried out on *Drosophila* S2 cells, where cells overexpressing Myc and control, GFP-positive cells, were co-cultured. The two populations were separated by porous filters that were permissive only to the transit of soluble molecules. Over a short period of time, the control GFP-positive cells were induced to die, while death of the Myc-expressing cells did not increase above background, demonstrating that cell competition did not require a cell–cell contact and was mediated by soluble factors [109]. In addition, these studies showed that both populations seem to contribute to the active process because conditioned medium from the competing co-cultured populations was able to induce death of control wild-type cells, while Myc-expressing cells were resistant. In addition, medium from single cultures was not sufficient to trigger the apoptotic signals [109]. Unfortunately, no such sharp-cut evidence has so far been obtained in mammalian cells, however it was demonstrated that murine embryonic stem cells (ESCs) overexpressing c-MYC eliminate wild-type ESCs in a co-culture assay [117], demonstrating that the difference in Myc levels is "*per se*" sufficient to trigger a competitive behavior also in mammalian cells.

Cell competition can also be mediated by membrane proteins, as some years later Flower (Fwe), an evolutionarily conserved membrane channel protein, was shown to participate in Myc-induced cell competition [121]. Different Few isoforms were observed to tag "winner" and "loser" cells in *Drosophila* imaginal epithelia. Fwe is present in three isoforms with different C-terminal extracellular domains; the *Ubi* form is constitutively expressed by the epithelial cells of the discs, while the *Lose-A* and *Lose-B* forms were only observed in the loser cells. The confrontation between the *Ubi* and the *Lose*(*s*) forms was postulated to be the cause for loser cells to die, since knockdown of the *fwe* gene resulted in a reduced expansion of clones overexpressing Myc [121]. The isoform *Lose-B* was also associated with neuronal culling in the *Drosophila* retina; in this case, *Lose-B* specifically marked the neurons at

the periphery of the retina that must be eliminated for a fully functional eye [122]. This is an important discovery because it demonstrates that cell competition is at work also in post-mitotic tissues, in the absence of external insults or genetic alterations [122]. Cell competition in post-mitotic tissues was already observed in the follicular cells of the *Drosophila* egg chamber. When *M*/+ clones are induced in the follicle cells, after they have transitioned from mitotic cycles to endoreplicative cycles, they are eliminated by apoptotic death via interactions with surrounding wild-type cells; remarkably, the space left by the loser cells is filled by hypertrophic cells rather than over-proliferating cells [123]. In this case, apoptosis is Myc-independent and JNK-independent, and the cellular hypertrophy is due to additional endocycles triggered by the insulin/IGF-like signaling pathway [123]. Regulation of organ integrity and size may therefore be achieved through cell plasticity even under the constraints of a tissue-specific differentiation program [123]. Another factor that has been shown to mark loser cells in *Drosophila* is SPARC (*s*ecreted *p*rotein *a*cidic and *r*ich in *c*ysteine) [124]. This molecule, a secreted matrix glycoprotein homolog to the SPARC/Osteonectin protein family [125], has an unexpected role in loser cells, since its transient expression inhibits Caspase 3 activation protecting losers from cell death. Stressed but still useful cells may be rescued by untimely elimination. SPARC upregulation in loser cells is induced by different genetic alterations, such *M*/+ mutations, or in wild-type twins of Myc- or Brk-overexpressing clones, demonstrating a general role for this protein in survival of loser cells [124]. Like Fwe, SPARC seems to be engaged specifically in cell competition since its expression does not rescue apoptosis induced in non-competitive contexts [124]. Concerning specific factors expressed by the winner cells, a very recent study characterizes a novel role for p53 in promoting growth of Myc-expressing cells in heterotypic contexts [62]. Myc-expressing cells undergo an enhanced glycolytic flux that is buffered by a p53-mediated boost of oxidative respiration; the metabolism of winner cell is thus optimized. Indeed, winner cells deprived of p53 function are no longer able to kill adjacent wild-type cells, and do not undergo clonal expansion because they are subject to massive death. p53 is necessary in Myc-overexpressing cells for competitiveness, survival and genomic stability, and this role is partially conserved in other winner cells such as wild-type siblings of *M*/+ or *dm* mutant clones [21].

7.4. Principles and phases of cell competition

Cell competition occurs in distinct, inter-dependent phases, whose order and significance is under continuous modification due to the complex nature of the phenomenon. Here we make an attempt to organize in few major points the information that has emerged from literature about cell competition in the imaginal wing disc.

A *A cell is weakened by a priming event that reduces its overall fitness*. Mutations that modify cell proliferation or growth rate [21,108], metabolic rate or protein synthesis [93,106] can trigger competitive interactions. In general, these mutations do not lead to cell-autonomous death.

B *Survival signals are reduced in the primed cell*. It has been suggested that loser cells from both *Minute* and Myc-mediated competition suffer from deprivation of morphogens and survival molecules. Some authors found an unbalance in Dpp/TGFβ signaling [106,108] that has led to the "ligand capture" hypothesis [108] and it has been shown that Wg-deficient cells are eliminated by Myc-independent cell competition only when growing in a heterotypic background [118], but demonstration of an active role in cell competition for this class of molecules is still pending.

C *Both loser and winner cells participate in the competitive process*. First evidence of biochemical modifications in cells under competition was the finding that the production of soluble factors by both competing populations was necessary for death of the loser cells [109]. Later, two molecules were found expressed in loser cells which balance determines if losers can be rescued to life or definitely committed to die: these molecules are the membrane channel Fwe^{Lose} [121] and the extracellular matrix component SPARC [124], whose role in competition was described in the previous section. On the other hand, in the winner cells a metabolic change occurs which, following an increase in glucose uptake and utilization, biases ATP production to that produced from aerobic glycolysis, known as the Warburg effect [126]. In the absence of p53 function, this metabolic shift leads Myc-overexpressing cells to genomic instability and death, while if p53 function is retained, it boosts oxidative respiration optimizing cell metabolism and allowing the "winners" to put into play their super-competitive properties [62].

D *Apoptosis is activated in loser cells and phagocytes participate in apoptotic cell clearance*. Activation of apoptosis leads to activation of Caspase 3 in the loser cells during cell competition. As they begin to die, the cells shift basally and the apoptotic corpses are extruded from the epithelium [127]. Cell clearance was first found to be carried out by winner cells that acquired phagocytic properties [105]; it was later shown that recruited phagocytes carry out the most part of debris removal from the basal side of the epithelium [127].

E *Apoptosis-dependent proliferation of winner cells*. Cell competition is a process that assures that organs reach proper size and are composed of perfectly functional cells; unfit cells are promptly removed from growing tissues and substituted by fitter cells, avoiding unwanted cell heterogeneity during development. Upon death and removal of unfit cells, winner cells are stimulated to repopulate the developing organ undergoing additional proliferation, that has been named "apoptosis-dependent proliferation" because it does not occur if apoptosis is blocked in loser cells [105,108].

8. Cell competition in cancer

As described in the previous section, the biological nature of cell competition involves the active participation of at least two cell populations with different growth properties in sustaining their own clonal expansion at the expense of the others. This is also a basic trait of cancer, where clonal growth is boosted not only by an increased proliferative ability but also by active selection that results from confrontation with neighbor cells [128,129].

Cell competition has been proposed as a possible explanation for "field cancerization" [108,112,130,131], a clinical phenomenon originally inferred from the observation that oral cancers were frequently surrounded by patches of abnormal tissue that, in time, led to tumors [132]. The clinical observations provided evidence that early clones with mutations in common with the ultimate detectable tumor expanded slowly until they accumulated further genetic lesions enabling them to grow as cancers [133,134]. In fact, many years are often required for cells that carry priming mutations to develop into cancers, and this is mainly due to suppressive forces exerted by the tumor microenvironment. On the other hand, molecular and structural miscues from the microenvironment can destabilize tissue homeostasis and, even in the absence of any genetic susceptibility, initiate normal cells to malignancy [135]. Investigations about the nature of the interactions occurring between cancer and its environment are just beginning to be undertaken in mammals and, given the frequent upregulation of Myc in human cancers [136], an increased focus on the role of cell competition in cancer biology is predicted. Initial evidence of functional competitive interactions in cancer was reported in a mouse colon cancer model. In intestinal crypts, loss of *APC* (*adenomatous polyposis coli*) leads to unrestricted proliferation; however simultaneous loss of APC and *c-myc* provoked rapid competition against the mutant cells by surrounding wild-type stem cells, leading to a complete rescue of pathological changes [137]. Although the authors did not analyze cell competition in their system, this study indicates the existence of mechanisms, dependent on the proximity of cells with different Myc levels, able to restrain cancer growth. In addition, in a mouse model of chemical carcinogenesis,

Fwe abundance was found to be high in normal cells at the tumor boundary in a chemically-induced papilloma mouse model, and Fwe knock-out mice are much less prone to carcinogenesis than wild-type mice [138], suggesting that cancer cells may take advantage of Fwe expression in normal cells to out-compete them. Prior to its identification as a factor transiently expressed in loser cells in *Drosophila*, SPARC expression was studied in a variety of experimental models and human cancers, but its role in tumorigenesis seems to be controversial, showing both anti-cancer and pro-metastatic properties [138,139]. Petrova and colleagues suggest that Myc-overexpressing cancers could behave as super-competitors and loss of SPARC expression in normal cells would accelerate their elimination [138].

8.1. Cell polarity and cell competition

Epithelial cells show a strong polarization along the apical–basal axis and most part of them are also polarized along the plane of the epithelium, a polarization called "planar cell polarity" (PCP) [140]. Apical–basal polarity and PCP are interconnected because of the juxtaposition of their determinants, and both have been investigated for involvement in cell competition. Li and Baker investigated the role of PCP in the orientation of cell division in the wing disc epithelium upon competitive cell death induced by *M*/+ mutations [141]. They found that competitive cell death re-oriented mitosis of winner cells at the clonal border, and this function required the activity of the PCP proteins Dachsous (Ds) and Fat (Ft). As a control, they induced *pineapple eye* (*pie*) mutant clones which suffer from autonomous, non-competitive cell death and found that mitoses occurring near the mutant clones were also re-oriented in a PCP-dependent manner, concluding that involvement of PCP proteins in the re-orientation of apoptotic-dependent cell division is not specific for cell competition [141]. PCP is involved in multiple pathways of cell migration, morphogenesis and epithelial repair from *Drosophila* to vertebrates and several PCP proteins are implicated in human cancer; the strict regulatory interactions with apical–basal polarity complexes and downstream signaling pathways further contribute to carcinogenesis [142].

Defects in apical–basal cell polarity are intimately associated with carcinogenesis; the majority of human cancers derive from epithelial tissues, show loss of polarity and, as a consequence, tissue disorganization [143]. Apical–basal cell polarity is maintained in *Drosophila* epithelia through the organization of different conserved complexes which include the "Crumbs(Crb)/Stardust(Sdt)/PATJ" apical module, the "Bazooka(Baz)/Par6/aPKC" sub-apical module, and the "Scribble(Scrib)/Discs large(Dlg)/Lethal giant larvae(Lgl)" baso-lateral module. Clonal loss of members from each these complexes has been shown to trigger competitive interactions [144–147]. In particular, members of the baso-lateral module represent a class of evolutionarily conserved proteins that has been extensively investigated because of the malignant and metastatic properties of the epithelial and nervous tissues of the mutant animals [148–151]. *lgl* mutant cells are eliminated in the wing disc by Myc-dependent cell competition [147] (Fig. 3B). When surrounded by wild-type tissue, *lgl* mutant cells undergo apoptotic death mediated by the activation of JNK signaling and, as expected when competitive interactions take place, wild-type sister clones of $lgl^{-/-}$ clones overgrow to fill the space left by the $lgl^{-/-}$ losers [147]. $lgl^{-/-}$ clones express low levels of Myc compared to the background, and this difference in Myc expression triggers elimination of the mutant cells. In fact, overexpression of Myc in the $lgl^{-/-}$ clones not only rescues their viability but promotes malignant growth and induces death in surrounding wild-type cells, turning *lgl*-deficient cells from losers into super-competitors [147]. Tamori and colleagues identified the VprBP homolog Mahjong (Mahj) as a novel binding partner for Lgl [152]. $mahj^{-/-}$ cells are viable in a homotypic background but die by JNK-mediated apoptosis when surrounded by wild-type cells, both in *Drosophila* and in the Madin–Darby Canine Kidney (MDCK) epithelial cell line, and its ectopic expression in *lgl* mutant cells in *Drosophila* rescued their competitive elimination [152]. Similar results were obtained by Norman and colleagues upon *scrib* knockdown in the same cell line: while $scrib^{KD}$ cells were viable when surrounded by cells of the same kind, they died and were apically extruded in the presence of wild-type MDCK cells [153].

8.2. The Hippo pathway and cell competition

Understanding the link between disruption of cell polarity and uncontrolled cell proliferation would represent a significant advance in our comprehension of the molecular basis of epithelial cancers. In order to grow as frank malignancies, cells mutant for polarity genes must resist cell competition by neighbors, and then transform into super-competitors. Cells from human epithelial cancers exhibit an absence or mislocalization of polarity proteins [154] and Myc upregulation, but what are the mechanisms that link these two players? In *Drosophila*, *lgl*, *scrib* and *dlg* were identified in a screen for dominant suppressors of a hypomorphic mutation of *cyclin E* [155], indicating a role in regulation of cell cycle; indeed $lgl^{-/-}$ clones show ectopic Cyclin E expression [144]. A mechanistic link between polarity genes and cell proliferation was established with the demonstration that the proliferative defects above described were due to deregulation of the Hippo (Hpo) pathway [156], a signaling network that plays a key role in organ growth from *Drosophila* to humans [157–159]. Briefly, the downstream effector of the Hpo pathway is the transcriptional co-activator Yorkie (Yki), the *Drosophila* homolog of the YAP (Yes-associated-protein) oncogene, which is directly regulated by the core kinases Hpo and Warts (Wts), whose functions are modulated by a myriad of upstream cellular factors. When the pathway is active, Wts phosphorylates Yki, sequestering it in the cytoplasm; upon genetic or mechanical stresses that inactivate the pathway, unphosphorylated Yki enters nucleus and, binding to tissue-specific partners, activates the transcription of several target genes involved in cell growth and resistance to apoptosis such as *cyc E*, *dIAP1* (*Drosophila Inhibitor of APoptosis 1*) and the miRNA *bantam* [159]. Thus Yki promotes growth, whereas the upstream components act as tumor suppressors. Initially, mutations in several genes of the Hpo pathway were found to protect *M*/+ cells from being eliminated during cell competition with wild-type cells, providing cells with super-competitive capabilities [160]. Regulation of Yki by polarity genes was recently shown in the wing imaginal disc, where tumors generated by knocking down *lgl* or *dlg* transcripts in the posterior compartment of the wing disc led to overgrowth and loss of cell polarity [161]. In cells depleted of *lgl* or *dlg* Yki localized to the nucleus, and knockdown of Yki or overexpression of Wts in this context rescued the tissue overgrowth and induced high levels of cell death, suggesting that Yki activity is required for growth and survival of *lgl*- or *dlg*-depleted cells. Interestingly, Yki activation was found dependent on JNK signaling [161]. The same result was obtained in clonal experiments in the wing imaginal disc where *lgl* and *scrib* mutations were coupled with Yki overexpression; in this case, the mutant cells upregulating Myc resisted cell competition, overgrew and became super-competitors with the ability to eliminate the surrounding loser cells and repopulate the tissue [162,163]. In 2010, two studies demonstrated that Myc is a direct target of Yki in the *Drosophila* epithelia [164,165], supporting a possible link between loss of cell polarity and Myc overexpression in these cells. In contexts in which *lgl*, *dlg* or *scrib* mutations promote growth, the Hpo pathway becomes compromised, thereby activating Yki; Yki then activates transcription of a series of genes that provide cells with growth and survival properties, allowing them to turn from losers into super-competitors and overwhelm the adjacent normal tissue (Fig. 3C). In summary, mutations in core components of the Hpo pathway give rise to hyperplastic growth, and a functional cooperation between loss of apical–basal cell polarity and hyperplasia seems to be necessary and sufficient for pushing the cells towards a malignant phenotype.

Myc has been identified as a downstream effector of the Hpo pathway also in mammals; YAP promotes transcription, among others, of *c-myc* and *survivin* in a mice model of hepatocellular carcinoma [158] and both YAP and c-MYC are necessary for liver carcinogenesis in a nude mouse model [166]. YAP is amplified in several human tumors and high nuclear expression is observed in many types of cancers [167], partly accounting for the high MYC expression in human tumors. In an attempt to delineate a signature of cell competition in human epithelial cancers, samples from a variety of primary carcinomas and respective metastases in different organs were investigated, and a precise pattern of Lgl cytoplasmic localization, YAP nuclear accumulation and Myc upregulation was found frequently within the tumor, while caspase 3 activation was observed in stromal cells at the outer border of the cancer mass, or trapped within infiltrating malignant cells (DG, unpublished). Cytoplasmic release of Lgl was previously demonstrated in ovarian cancers [168] and represents a phosphorylated, inactive pool of Lgl [169]. This retrospective study provides evidence for a possible causative link in human cancers between disruption of apical–basal cell polarity, Hpo pathway deregulation, Myc upregulation and outcompetition of stromal cells at the invasive front.

Deregulation of the Hpo pathway also contributes to the overgrowth that characterizes the oncogenic cooperation between activated Ras/Raf and polarity gene loss [162,170,171] (Fig. 3C). In 2003, two studies showed that constitutive activation of Ras is able to rescue *scrib*, *lgl* or *dlg* mutant cell viability and trigger massive overgrowth and invasiveness [172,173]. Dogget and colleagues found that Yki target genes were upregulated in $scrib^{-/-}$; Ras^{V12} tumors and inhibition of Yki activity significantly reduced tumor growth [170]. In another study, analysis of fast-proliferating $lgl^{-/-}$; Ras^{V12} clones in the wing disc revealed nuclear Yki staining and, accordingly, a strong upregulation of its target genes, including Myc [162]. Similarly, $scrib^{-/-}$; Ras^{V12} clones in the wing disc show upregulation of Yki target genes and Yki overexpression is able to promote overgrowth of *scrib* mutant cells [171].

Successively, it has been shown that isolated clones of *lgl*; Ras^{V12} cells, despite their well-known proliferative advantage, may undergo cell death [162]; on the other hand, loser cells embedded in a homotypic environment escape cell competition; as an example, Rab5-deficient cells turn from "losers" into "super-competitors" when a substantial number of mutant cells are simultaneously present in the tissue [174]. These two studies highlighted the relevance of the homotypic environment in cell competition: super-competitive but sporadic cells can encounter death also in a context formed by disadvantaged neighbors and losers can bypass competition if a certain mass of mutant cells is present in the field: "*united we stand, divided we fall*".

9. Conclusions

In the last decades work in the fruitfly has greatly helped elucidate complex issues about Myc function, from tissue homeostasis during development to cancer biology. Significant progresses have been made in understanding Myc's role in many signaling pathways, thanks to the availability of tissue-specific, sophisticated tools, which allowed to dissect intricate genetic interactions. One of the most important aspects of Myc biology, that is currently undergoing intense investigation, is its direct implication in the non-autonomous control of cellular growth, a phenomenon called cell competition. During development, the control of the mechanisms responsible for correct cell-to-cell communications is essential to the establishment and maintenance of organ homeostasis. The finding that in *Drosophila* the Myc protein acts in this context to trigger death of unfit cells during organ development has proven fascinating and, as it was expected, this mechanism is conserved also in mammals during development. Since the unfit cells subject to Myc-induced cell competition comprehend damaged and pre-neoplastic cells, future research on this topic will be of great advantage to open new possibilities in cancer diagnosis and treatment.

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