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Review

Apical junctions and growth control in *Drosophila*

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ABSTRACT

Recent studies have revealed unexpected links between cell polarity and proliferation, suggesting that the polarized organization of cells is necessary to regulate growth. *Drosophila melanogaster* is a genetically simple model that is especially suited for the study of polarity and growth control, as polarized tissues undergo a well-defined pattern of proliferation and differentiation during the development. In addition, genetic studies have identified a number of tumor suppressor genes, which later studies have shown to be associated with junctions, or in the regulation of junctional proteins. We will explore in this review the links between growth and apical junction proteins in the regulation of growth control in *Drosophila*.

Contents

1. Epithelial organization in *Drosophila*

In *Drosophila* epithelia, the lateral membrane is subdivided into two distinct highly organized cell–cell junctions [51,68]. The Zonula Adherens (ZA) is the first junction to form in the developing epithelium during *Drosophila* embryogenesis. This adhesive complex is localized at the apical apex of the lateral membrane and forms a belt-like structure around the cell. The molecular heart of the ZA is composed of cadherin/catenin complexes that connect apico-lateral actin belts of adjacent cells to each other [1,35]. The vertebrate ZA is structurally and molecularly very similar to the invertebrate ZA. One difference is that while in *Drosophila* the ZA forms the most apical junction, the ZA is situated basal to the Tight Junctions (TJ) in vertebrate epithelial cells. The vertebrate TJ forms a belt of close membrane apposition creating a semi-permeable barrier to the diffusion of molecules through the extra-cellular space. Invertebrate does not have TJs, instead, the barrier function is provided by structurally different junctions named Septate Junctions (SJ). In contrast to the TJ, the *Drosophila* SJ forms regions of close membrane contact that extend over a large part of the lateral membrane, beneath the ZA (Fig. 1).

The formation of the ZA and maintenance of cell polarity depend on two major protein complexes both localized at the apical membrane [68]. These complexes are enriched in a region just apical to the ZA, named the Sub-Apical Region (SAR). The first known complex (named the Crumbs complex) is composed of the trans-membrane protein

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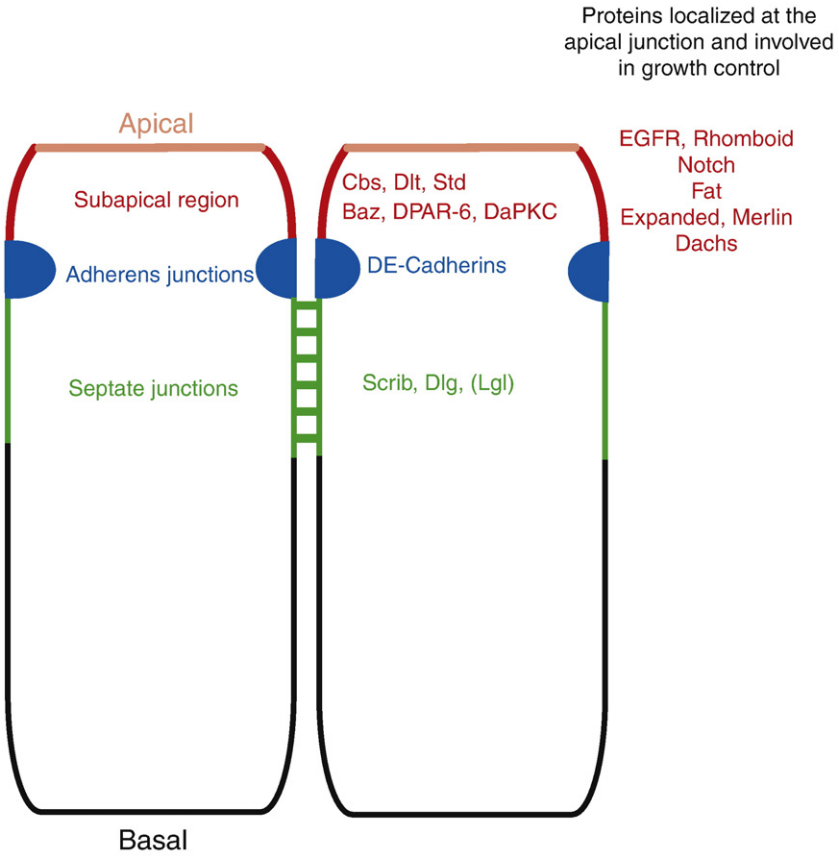

Fig. 1. Organization of *Drosophila* epithelium. The proteins of the Baz and Cbs complexes are enriched in the Subapical region (red), apical to the Adherens junctions (blue). Scrib, Dlg and Lgl forms a complex just basally to the Adherens junctions (green). Some regulators of growth and proliferation signaling pathways, such as EGFR, Rhomboid, Notch, Fat, Expanded, Merlin and Dachs, localized to the apical junction in the *Drosophila* epithelium (adapted from [68]).

Crumbs (Cbs), the cytoplasmic scaffolding protein Discs lost (Dlt) and the membrane-associated guanylate kinase protein (MAGUK) Stardust (Std) [36,2]. The second complex of the SAR is composed of Bazooka (Baz), the *Drosophila* homologs of *C. elegans* Par-3, *Drosophila* Par-6 (DPar-6), and the homolog of atypical Protein Kinase C (DaPKC) [38,74,56]. Aside from these two apical protein complexes, a lateral protein complex has also been described as a regulator of apical polarization. This third complex is composed of the Myosin II binding protein Lethal Giant Larvae (Lgl) and the multi-PDZ domain proteins Discs Large (Dlg) and Scribble (Scrib) [34,75,76,7,8].

While the relation between these three complexes is not yet exactly understood, it is clear that they cooperate together to control polarity [31]. Genetic analysis in *Drosophila* showed that the lateral Scrib complex acts to oppose Baz initiated apical polarization, whereas the Crb complex, which is recruited to the SAR by the Baz complex, acts to antagonize the Scrib complex [8,66].

2. Potential links between polarity and growth control

Interestingly, the *scrib*/*dlg*/*lgl* genes are described as *Drosophila* neoplastic tumor suppressor (nTGS), as their mutations not only disrupt cell polarity but also simultaneously induce an extensive overproliferation of cells [64,6,7,5]. In fact, zygotic mutations in any of these genes lead to a "giant larva" phenotype, the mutant larvae never pupate but instead continue to grow extensively before dying. In these "giant larvae", the imaginal epithelia is not a flat epithelia composed of columnar cells but rather a mass of rounded, poorly adhesive, misshapen cells that pile aberrantly on top of each over. The similarity of the phenotypes as well as the fact that each of these proteins is required for the proper localization or stability of the others, indicate that Scrib, Dlg and Lgl act together to regulate both polarity and growth [6]. The fact that a mutation in a single gene induces simultaneously a polarity and a growth defect suggests that these two mechanisms are linked. However the nature of this link is still uncertain. One hypothesis is that loss of polarity is directly responsible for growth defects, possibly by mislocalisation of signaling proteins involved in growth control, leading to their inappropriate activation. It has also been suggested that the disruption of junctions could inhibit the poorly understood mechanisms of contact inhibition. An alternative hypothesis is that these proteins may have a direct role in signaling to regulate proliferation. They may interact with different interacting partners to co-ordinate different polarity and growth control pathways. Indeed Scrib and Dlg orthologs bind to EGFR family members, known to regulate cell proliferation/growth [37].

The role of the other junctional complexes in the control of growth is not clear. However the fact that the lateral Scrib complex genetically interacts with the SAR complex suggests that they could also play a role in the control of growth. For example, aPKC has been described to interact with and phosphorylate Lgl, and this phosphorylation could be responsible for the apical inhibition of Lgl by releasing the protein from the apical cortex [4]. Interestingly, some evidence suggests that aPKC may be involved in the regulation of epithelial cells growth, by antagonizing Lgl [59,42].

There are other links between growth and junction proteins. The dEGFR is localized along lateral membranes and is present at junctions, and plays an essential role during development by regulating process such as cell fate choice, cell division, migration or cell survival [62]. Its role in growth control has been verified by the observation that activation of the MAPK signaling cascade, which is regulated by the dEGFR, induced a hyperplastic overgrowth of the

imaginal discs [33]. A protease, Rhomboid, is essential for the cleavage and activation of the dEGFR ligand Spitz [21,71,62]. Intruigingly, Rhomboid is localized to the apical junction, suggesting it may act there to modulate EGFR activity [65]. A problem with this model is that Rhomboid promotes the cleavage of Spitz in the Golgi, thus the role of its apical localization is unclear. The receptor Notch is another example of an important regulator of *Drosophila* development that is localized in the apical junction [19]. Notch pathway is involved in the regulation of numerous developmental decisions and also in the regulation of cell proliferation during *Drosophila* development [18].

Another group of tumor suppressor genes has recently been discovered, that together form the Hippo pathway [16,28,53,61]. Mutants in the Hippo pathway are characterized by a massive hyperplastic overgrowth phenotype due to both an increase in growth rate and an inhibition of apoptosis. The upstream regulators of the Hippo pathway (Fat, Expanded and Merlin) are all localized at the Sub-Apical Region, and may be involved in communicating information about cell density and tissue size to the downstream elements of the Hippo pathway.

3. The giant atypical cadherin FAT

Cadherins represent a large family of adhesion protein involved in cell–cell adhesion as well as cell signaling. They are characterized by the presence of specific repeats in the extracellular domain that regulate homophilic and heterophilic interactions (Cadherin repeat) [24]. The classical cadherins appear to modulate cell–cell adhesion through dynamic interaction with the actin cytoskeleton. They form primarily homophilic interactions and are mainly found at the adherens junction. A divergent group of cadherins, containing the tumor suppressor Fat (Ft) and Dachsous (Ds) localizes apical to the adherens junction [10,44,14,43,63]. These atypical cadherins are structurally and functionally different from the classical cadherins. Instead of five extracellular cadherin repeats characteristic of the classical cadherins, Fat and Ds possess respectively 34 and 27 of these repeats. In addition to these 34 cadherin repeats, the giant extracellular domain of Fat possesses five EGF-like repeats and two laminin G repeats. Interestingly, the intracellular domains of these atypical cadherins are divergent from the classical cadherins and do not appear to bind the actin cytoskeleton, suggesting a different cell signaling function. The *Drosophila* atypical cadherins Fat and Ds are both present at sub apical junctions and are involved in planar cell polarity (PCP) signaling, that organizes the cell in the plane of the epithelia. The role of these cadherins in PCP signaling has been extensively studied in *Drosophila* development [60]. Genetic and biochemical data indicate that Ds binds Fat and inhibits Fat activity in PCP signaling [46,47]. In addition to its well-known role in PCP signaling, Fat is also implicated in the regulation of growth. In fact, Fat is known as a *Drosophila* tumor suppressor as mutations in the *fat* gene cause a dramatic hyperplastic overproliferation of imaginal discs during an extended larval phase [10,44,23]. *Fat* mutant discs continue to grow far beyond their normal final size but retain a single-layered epithelial structure as well as the ability to differentiate into adult structures. While Fat's role in growth is long known, we are just beginning to understand the mechanisms involved.

4. Fat and the dEGFR signaling pathway

It was first suggested that Fat specifically cooperates with the *Drosophila* Epidermal Growth factor Receptor (dEGFR) pathway to regulate growth during development [22]. *Fat* mutations and dEGFR activation have a strong synergetic effect on growth. Overgrowth in *fat*

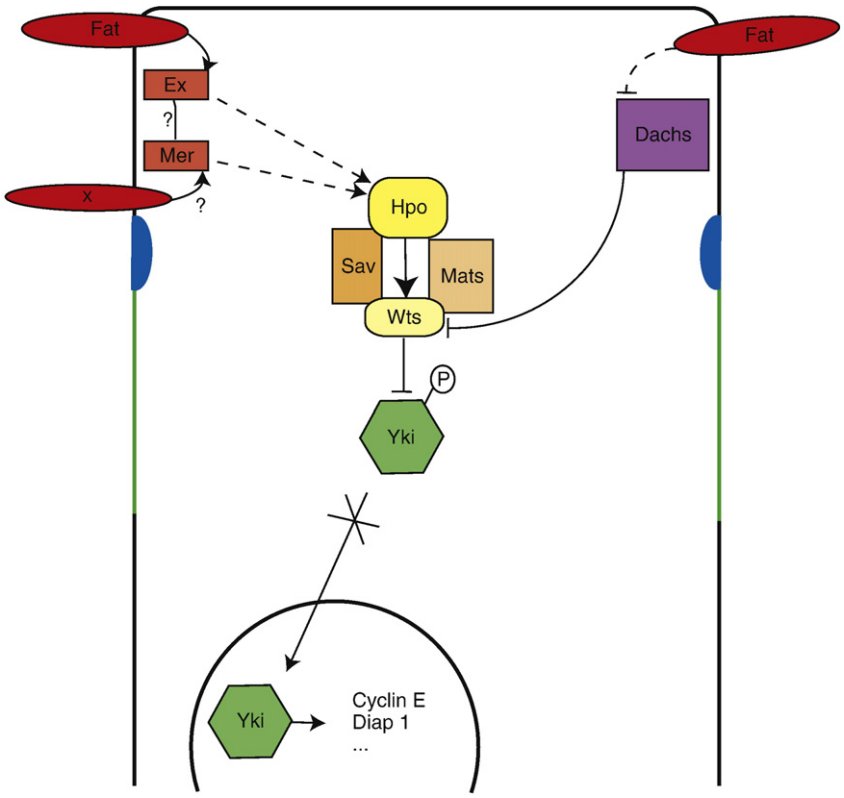

Fig. 2. The Hippo pathway cascade in a *Drosophila* epithelial cell. Hippo (Hpo) phosphorylates and activates the kinase Warts (Wts), which in turn phosphorylates and inactivates the co-transcriptional activator Yorkie (Yki), by restricting it in the cytoplasm These reactions appear to be facilitated by two scaffold proteins, Salvador (Sav) and Mats that bind Hpo and Wts. In its activated form, Yki activates growth and inhibits apoptosis by controlling the expression of genes such as the cell cycle regulator cyclin E and the anti-apoptotic protein Diap1. Upstream of the Hippo pathway are the atypical cadherin Fat, the FERM domain proteins Expanded (Ex) and Merlin (Mer), and the unconventional myosin Dachs, that all localized at apical junctions.

mutant tissues is enhanced when dEGFR signaling is activated, whereas the overproliferation is reduced by a decrease in the dEGFR signaling [22]. This suggests that Fat might regulate growth control partly via the dEGFR signaling. The molecular link between Fat and the dEGFR pathway is still unclear.

5. Fat and the Hippo pathway

Very recently, a growing number of publications revealed that Fat regulates growth via the Hippo kinase pathway [3,13,63,73,69]. This recently described signaling pathway appears to be a crucial regulator of epithelial growth control during *Drosophila* development [16,28,53,61]. Mutation in any member of the Hippo pathway leads to dramatic hyperplastic overgrowth phenotype similar to *fat* mutants. Whereas it was at first studied only in *Drosophila*, there is now growing evidence that the Hippo pathway is well conserved in mammals where it is also involved in the restriction of tissue growth and organ size [12,11,15,80,26]. The heart of this pathway is composed of a cascade of phosphorylation: the sterile-20-like kinase Hippo (Hpo) phosphorylates and activates the Dbf-2-related-type kinase Warts (Wts) [32,17,29,70,77], which in turn phosphorylates and inactivates the co-transcriptional activator Yorkie (Yki), by restricting it in the cytoplasm [30,15,80,52]. These reactions appear to be facilitated by two scaffold proteins, Salvador (Sav) and Mats that bind Hpo and Wts [67,54,39,72]. The downstream transcriptional co-activator Yki activates growth/proliferation and inhibits apoptosis by controlling the expression of genes such as the cell cycle regulator cyclin E and the anti-apoptotic protein Diap1 (Fig. 2) [30,78,79].

Two cortical proteins, Expanded (Ex) and Merlin (Mer), have been described to act upstream of the Hippo pathway. Ex and Mer are two FERM domains containing proteins that localize at apical junctions [40,9,48,63]. The fact that Ex and Merlin have similar phenotypes, and that far-Western experiments showed that the protein physically interact, suggested that Ex and Mer work together to regulate growth [48]. Mutation of both genes recapitulates the effects of loss of *hpo* or *wts*. Genetic experiments suggest that Hpo and Wts are necessary for Ex/Mer regulation of growth, placing Ex/Mer upstream of the Hpo pathway [25]. Interestingly, *wts* phenotype is somewhat stronger than *hpo* or *ex*/*mer* phenotype, suggesting several layers of regulation exist.

It is unknown how Ex and Mer activate the growth regulator pathway. Interestingly, interactions between Mer and Sav as well as between Ex and Hpo have been detected in a large scale yeast two hybrid screen, but have not been confirmed yet [20]. Also open is the question of how Ex and Mer co-operate in the regulation of growth. The fact that mutations in both genes induce a more severe overgrowth phenotype than mutation in *ex* or *mer* separately, suggests that they can act in parallel to regulate growth. In fact, recent studies highlighted the fact that they have distinct effect on cell cycle and apoptosis regulation, suggesting that even if Ex and Mer have some overlapping function, they also have some specific roles and that they might be able to differentially modulate the Hippo pathway signaling [55].

Both biochemical and genetic experiments indicate that Fat is upstream of the Hippo pathway, identifying for the first time a potential link between the extracellular environment and Hippo pathway activation [3,13,27,63,73]. While the *fat* hyperplastic overgrowth phenotype is very similar to *hpo* or *wts* mutants, it is less dramatic, particularly regarding the mild anti-apoptotic effect. The *fat* phenotype is actually closer to *ex* phenotype and current models suggest that Fat acts upstream of Ex but not Mer to activate the Hippo pathway [3,63,73,69]. Interestingly Ex co-localizes with Fat at sub-apical junctions and loss of *fat* appears to perturb this localization. These results suggest that Ex localization at junctions is required to regulate the Hippo pathway. Fat can also regulate the Hippo pathway independently of Expanded and Hippo, by regulating the stability of Wts [13]. Wts levels decrease in *fat* mutants, independently of Hippo but dependant on inhibition of the unconventional myosin Dachs [45]. Dachs also localizes to apical junctions in *Drosophila* imaginal discs.

wts was recently identified in a screen for genes that enhance the phenotype of *dlg* in ovaries [81]. This suggests that there may be cross-talk between the Scrib/Lgl/Dlg signaling pathway and the Hippo pathway. Interestingly, *fat* and *ex* mutants did not enhance the *dlg* phenotype, indicating a separation of signaling inputs to Wts in this system. Other studies have shown a separation between Fat/Ex and the Hippo pathway in the ovaries, suggesting that there may be tissue dependant mechanism to regulate Wts activity [50,57].

6. Summary and future directions

Taken together, these studies highlight the fact that junctions play an important role, not only in the organization of epithelia but also in the regulation of tissue growth. Thus the apical junctions might provide a privileged platform of communication between cells, possibly integrating polarity and density signals from neighboring cells to regulate the proliferation rate.

However, many questions remain as to how junctions regulate tissue growth. We still do not understand how Fat, Ex and Merlin regulate the activity of the Hippo pathway. In addition, it is unknown what extracellular signals regulate Fat to control Hippo activity, or the as yet unidentified cell-surface receptor that controls Merlin activity and or localization. Finally, how cells use the Hippo pathway to regulate organ size, and how they sense when a tissue has reached the correct size is a mystery. Some of the answers may lie in understanding how global patterning mechanisms provide input and cross talk with the growth-regulatory apparatus [58]. There are also hints from recent literature that the regulation of tissue growth via the Hippo pathway may underlie the long-known but poorly understood phenomena of contact-inhibition of growth [41,49,80]. While there are still many unanswered questions to the mechanisms of communication from the junctions to the nucleus, these studies focus our attention on apical junctions as a crucial nexus point for the regulation of growth and polarity.

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