The role of cell polarity in regulating the Salvador/Warts/Hippo tissue size control pathway

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Introduction

Metazoans achieve reproducible sizes by intrinsic genetic controls. It is still largely unknown how this reproducible size control is accomplished and maintained (Leevers *et al*., 2005). It is known however that organ shape relies on managing cell proliferation and the spatial ordering of cells during development (Baena-López *et al*., 2005). The acquisition and maintenance of cell polarity is necessary for this spatial arrangement of cells. Cell polarity is also of great importance in regulating cell proliferation, since loss of cell polarity is a known hallmark of advanced cancers, and accompanies unrestrained proliferation (Hanahan *et* al., 2000, Leevers *et al*., 2005). Currently, it is not known how cell polarity influences tissue size control during development and in the adult. Here we describe how polarity pathways and the Salvador/Warts/Hippo (SWH) pathway interact to control tissue size. We discuss several recent studies, which give important insights into possible molecular mechanisms underlying such an interaction. To set the stage to discuss this recent literature, we will first consider the cellular junctions, the regulators of apicobasal and planar cell polarity, and the components of the SWH pathway.

Cellular junctions

In epithelial monolayers, cells are tightly joined together by junctional complexes that provide mechanical strength, are required for the functioning of epithelia, and are indispensable for establishing and maintaining apical basal polarity. Junctional complexes include tight junctions, adherens junctions, and gap junctions. Tight junctions form a belt-like region of adhesion between adjacent cells and regulate the flow of ions and molecules across the cellular monolayer. Moreover, they stabilize cell polarity by preventing the intermixing of apical and basolateral membrane components (Macara, 2004, Tepass *et al.,*

2001). Additionally, in epithelial cells of *Drosophila* and other arthropods tight junctions are not found. It was first suggested that these junctions were replaced by a more basolateral structure, the septate junction (Noirot-Timothee *et al*., 1980), however more recently it has been indicated that the marginal zone might be the appropriate *Drosophila* homologue for the vertebrate tight junction (Tepass *et al*., 2001). Adherens junctions, which connect to the actin cytoskeleton, form an adhesive band that surrounds the cell just below the apical surface providing adhesion between neighbouring epithelial cells. These junctions are located basal to the tight junctions in mammalian epithelial cells (Wang *et al*., 2007). In *Drosophila* epithelia, the apical and basolateral domains of a cell are separated by the zona adherens, an actin-rich region that forms a belt around the cell and where adherens junctions are located (Genevet *et al*., 2009). An overview of the cellular junctions and the localization of the apicobasal polarity complexes in a *Drosophila* and a vertebrate epidermal cell (Macara et al., 2004) is given in Figure 1.

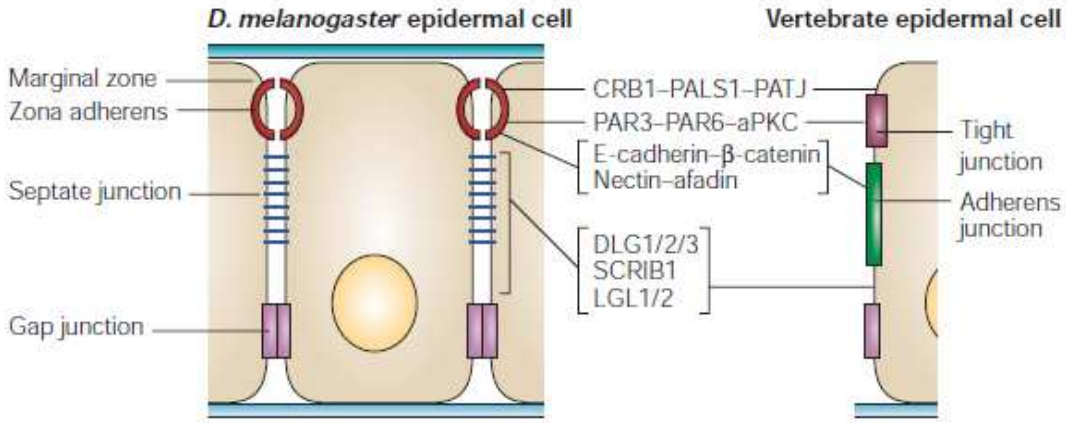

Figure 1: Overview of cellular junctions and the localization of the apicobasal polarity complexes in a *Drosophila* (left) and a vertebrate epidermal cell (right). Adapted from Macara *et al*., 2004. Only mammalian names of polarity proteins are shown. The *Drosophila* homologues of the polarity complexes shown here are: Crb-Sdt-Patj for the mammalian Crb1-Pals1-Patj, Baz-Par-6-aPKC for the mammalian Par3-Par6-aPKC and Scrib-Dlg-Lgl for the mammalian Dlg1/2/3-Scrib1-Lgl1/2 (Bilder *et al*., 2004).

Apicobasal cell polarity

Three conserved protein complexes regulate apicobasal polarity in a variety of settings. A complex consisting of Crumbs (Crb), Patj (Pals associated tight-junction protein), and Stardust (Sdt) is necessary for apical basal polarity (Wang *et al*., 2007). *Crumbs* was the first

Drosophila gene shown to be involved in the basic organization of epithelial composition, when mutations in this gene were found to result in loss of polarity and severe disorganization of epithelia that eventually could lead to cell death (Tepass *et al*., 1990). The *sdt* gene was later suggested to be involved in the same cellular function as *crb* in controlling the organization of epithelia in *Drosophila*, when it was observed that the mutant phenotypes of these genes were very similar (Tepass *et al*., 1993). Years later, Patj, first named Discs Lost, was described to interact with Crumbs. Additionally it was demonstrated that Patj is required to establish and maintain cell polarity, because mutations in *patj* led to abnormal localization of Crumbs and caused loss of epithelial cell polarity (Bhat *et al*., 1999). The Crumbs complex is localized apically in both vertebrate and *Drosophila* epithelial cells (Bulgakova *et al*., 2009, Macara *et al*., 2004, see Figure 1).

A second conserved complex, also located apically, consists of Par6-aPKC-Par3 (Tepass *et al*., 2001). In mammalian epithelial cells this complex is confined to the tight junctions (Macara *et al*., 2004, see Figure 1). In a screen for defects in axis specification in *C. elegans* zygotes six *par* (partioning defective) genes and an atypical protein kinase C (aPKC) were identified (Kemphues *et al*., 1988, Wu *et al*., 1998). Mutations in these genes led to defects in cleavage patterns, timing of cleavages, and localization of cell fate determinants (Kemphues *et al*., 1988). The *par* genes predominantly encode scaffolding proteins and serine-threonine kinases. Par3 and Par6 are two scaffolding proteins that physically interact with aPKC to form the apical PAR complex (Wang *et al*., 2007, Izumi *et al*., 1998).

A third important group of polarity proteins is localized at the lateral membrane (Macara *et al*., 2004, see Figure 1) and consists of Lethal giant larvae (Lgl), Scribble (Scrib) and Disc large (Dlg). These proteins have been shown to depend on one another for correct localization in the cell and are therefore considered part of a functional group (Macara, 2004). Additionally, in *Drosophila*, loss-of-function mutants for the genes encoding these polarity proteins have very similar developmental phenotypes, including loss of apicobasal polarity, impaired cell cycle exit, and tissue overgrowth that results in non-viable larva (Bilder, 2004). Surprisingly, however, no direct physical interaction has been found between any of these proteins (Macara, 2004). Scrib was isolated in a screen for maternal effect mutations in *Drosophila* that disrupted aspects of epithelial morphogenesis such as cell adhesion, shape, and

polarity. Loss of *scrib* function resulted in the misdistribution of apical proteins and adherens junctions to the basolateral cell surface, even though basolateral protein localization remained intact (Bilder *et al*., 2000). The *dlg* and *lgl* genes were originally identified based on their ability to act as a neoplastic tumor suppressor in *Drosophila* imaginal discs (Stewart *et al*., 1972, Gateff *et al*., 1974). Afterwards it became apparent that the Dlg protein is also a critical component of the septate junctions and is required for maintaining apicobasal polarity in *Drosophila* epithelium (Woods *et al*., 1996). Similarly, the Lgl protein was found to play an important role in establishing and maintaining apicobasal polarity in *Drosophila* brains and imaginal discs (Strand *et al*., 1994).

Genetic analysis in *Drosophila* has shown that these cell polarity protein complexes interact with one another in a signalling network. An example of such an interaction; Par3 first localizes to the adherens junction to recruit the Crb complex to the apical surface. There, Crb inhibits the function of the Scrib group by expanding the apical domain and by maintaining the correct localization of the Par3 complex. Scrib, on the other hand, inhibits the function of Par3, to support expansion of the basal domain (Macara, 2004, Suzuki *et al*., 2006, Richardson *et al*., 2010). Eventually, these polarity complexes integrate intracellular and extracellular signals by regulating the restructuring of the cytoskeleton and trafficking of membrane, RNA and protein (Humbert *et al*., 2006). Mechanisms how this is accomplished remain poorly understood.

Planar cell polarity

The majority of epithelial tissues require polarization within the plane of the epithelium in addition to the apicobasal polarity. This conserved polarity is known as planar cell polarity and is important in the orientation of cellular structures such as hairs (Simons *et* al., 2008, Willecke *et al*., 2008). Currently two conserved sets of PCP factors are identified that function together to establish PCP: the Frizzled (Fz)/Flamingo (Fmi) core genes and the Fat (Ft)/Dachsous (Ds) PCP system. The exact relationship between these two sets of factors is currently unresolved, although recent studies suggest that they function in a parallel and independent manner (Simons *et al*., 2008). Alternatively, it has been stated that Ds-Ft heterodimers provide a long-range global directional cue in PCP signalling by orienting the polarization of the Fz system with respect to the tissue axes. Fat is necessary to somehow

orient mitotic spindles and might therefore be involved in organizing the apical microtubule network, by which Fz containing vesicles navigate through the cell during the accumulation of the asymmetric complexes at opposite sides of the cell. This suggests that Ft-Ds heterodimers might establish a subtle asymmetry, which is amplified by the Fz system, which locally coordinates polarization (Axelrod, 2009).

Besides the core PCP signaling proteins Fz and Fmi, components of the Fz/Fmi group include Dishevelled (Dsh), Prickle (Pk), Strabimus/Van Gogh (Stbm/Vang), and Diego (Dgo). Within this group, Fz-Dsh and Stbm/Vang-Pk are known to form complexes. These complexes exclude each other's localization leading to their localization to domains at opposite poles of each cell (Simons *et al*., 2008). An example of how PCP is established in *Drosophila* wing cells is illustrated in Figure 2; Fz-Dsh-Dgo complexes are localized to the distal end of each cell, while Stbm-Pk complexes are enriched proximally. Polarity in this tissue is apparent by the formation of an actin-based hair close to the distal domain of each cell, where Fz-Dsh-Dgo complexes are localized (Simons *et al*., 2008).

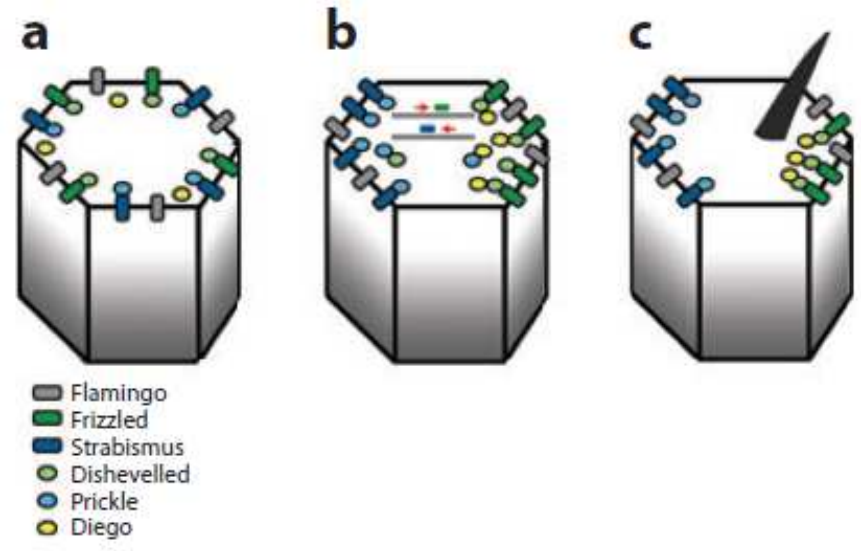

Figure 2: Generation of planar cell polarity in a *Drosophila* wing cell. Adapted from Simons *et al*., 2008. A single cell establishing PCP over time is shown from left to right (a to c). (a) Localization of core PCP components before any asymmetry has been established. (b) Fz-Dsh-Dgo complexes localize at the distal (here right) end of each cell, while Stbm-Pk complexes assemble proximally (here left). It is been suggested that PCP components might travel via microtubule-associated particles, since this has been seen for Fz (Axelrod, 2009). (c) After complexes are distributed asymmetrically, PCP becomes apparent by the formation of an actin-based hair close to the distal domain of each cell, where Fz-Dsh-Dgo complexes are localized (Simons et al., 2008).

The second PCP complex, the Fat/Ds PCP group, is understood in less detail. The main components of this group however are known to be the large proto-cadherins Fat (Ft) and Dachsous (Ds) and the Golgi kinase Four-jointed (Fj). Fat and Ds can interact heterophilically across cell boundaries. In addition, Fj has been suggested to modulate the activity of Ds. In agreement with this, *ds* and *fj* are expressed in opposing gradients in the eye, while *fat* expression is uniform (Simons *et al*., 2008). This led to the "Ds/Fat model" (see Figure 3), which states that the direction of the Ds/Fj gradient determines cell polarity. Ds and Fj concentration gradients cover the organ and interact with uniformly expressed Fat to establish, in one axis, a linear gradient of Ds/Fat heterodimers. In this model Ds and Fat function as ligands and receptors for each other, making it possible for each cell to compare the numbers of Ds/Fat heterodimers on opposing sides, thereby establishing the direction of PCP (Lawrence *et al*., 2008).

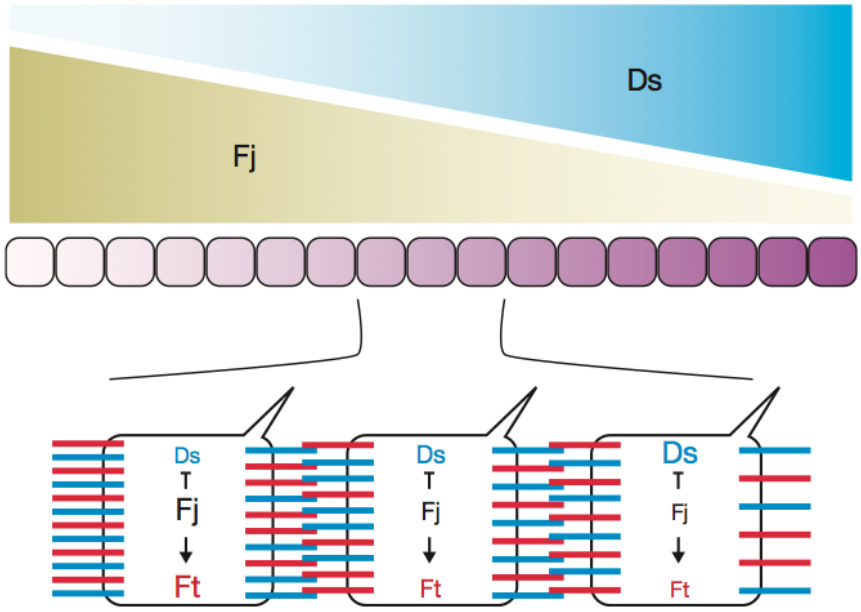

Figure 3: The "Ds/Fat model". Adapted from Lawrence *et al*., 2008. Ds and Fj expressed in opposing gradients, interact with uniformly expressed Fat to establish a linear gradient of Ds/Fat heterodimers. Ds and Fat function as ligands and receptors for each other, making it possible for each cell to compare the numbers of Ds/Fat heterodimers on opposing sides, thereby establishing the direction of PCP (Lawrence *et al*., 2008).

Fj modulates the binding affinity of Ds/Ft

Two groups together recently provided evidence that Fj inhibits the ability of Ds to bind Fat and increases the ability of Fat to bind Ds (Brittle *et al*., 2010, Simon *et al*., 2010). This is nicely illustrated in the *Drosophila* dorsal abdomen, where mutant cells lacking endogenous Fat and Ds but overexpressing an active form of Ds lacking the intracellular domain *(UAS.ectoDs)*, reverse the polarity of hairs behind these cells. Co-expression of *UAS.fj* with *UAS.ectoDs* in these cells however, largely suppressed this effect. Since no Fat was present, it is apparent that Fj blocked the function of Ds independently of Fat. Overexpression of *UAS.ft* in the absence of endogenous Ds and Ft caused a shift in the polarization of the hairs so that they pointed away from the mutant cells and co-expression with *UAS.fj* increased this effect. Since no Ds was present, this suggests that Fj strengthens the function of Ft independently of Ds (Brittle *et al*., 2010).

Three identified conserved serine residues in Ds have been shown to be involved in the modulation of Ft-Ds binding affinity by Fj in *Drosophila* S2 cells. Mutating these serines in *ds-EGFP* to alanine to prevent phosphorylation at these sites (*ds$^{S>Ax3}$-EGFP)* made these cells unresponsive to coexpression with *GNT-Fj* (a Golgi associated form of the protein which is more active *in vivo*) in a cell aggregation assay, unlike control *ds-EGFP*-expressing cells. Conversely, mutating the three serines to aspartates (*ds$^{S>Dx3}$-EGFP)* to mimic phosphorylation by adding a negative charge, demonstrated that these *ds$^{S>Dx3}$-EGFP* had a significantly decreased level of binding to Ft expressing cells when compared to wild-type *ds-EGFP*-or *ds$^{S>Ax3}$-EGFP*-expressing cells (Brittle *et al*., 2010). These results demonstrate that phosphorylation of Ds by Fj diminishes the binding affinity of Ds for Ft, suggesting that phosphorylation of Ds is important in the regulation of Ft/Ds heterodimerization and its subsequent effects on PCP and growth (Brittle *et al*., 2010).

The second group affinity purified Fat1-10:FLAG (the first ten Fat cadherin domains labelled with a C-terminal FLAG epitope tag) using anti-FLAG beads from conditioned medium of cells without exogenous Fj expression. Binding activity of Ds1-10:AP (the first ten Ds cadherin domains labelled with an alkaline phosphatase tag) to *in vitro* phophorylated Fat1-10:FLAG (with affinity-purified Fj, ATP and buffer) was higher than to non-phosphorylated Fat1-10:Flag. Conversely, *in vitro* removal of phosphates from Fat1-10:FLAG revealed a decrease

in its ability to bind Ds1-10:AP. These results show that phosphorylation by Fj is enhances the binding activity of Fat to Ds. A phosphorylation site was mapped to the Fat cadherin domain 3, specifically to Ser273, which when mutated reduces, but not abolishes, the ability of Fj to promote Fat:Ds binding. This suggests that the binding interactions of full-length Fat and Ds are elaborate, with important roles for multiple Fj phosphorylation sites (Simon *et al*., 2010).

These findings of these two groups led to a model (Figure 4) that accounts for how Fat activity is polarised in response to an Fj gradient. In this model, Fj is expressed in a linear gradient in which (Figure 4A) Fj-mediated phosphorylation simultaneously decreases the Fat-binding activity of Ds (block arrows) and increases the Ds-binding activity of Fat (pointed arrows). In a particular cell at a higher point in the Fj gradient, Fj-mediated phosphorylation will have a relatively greater effect in both enhancing the ability of Fat to bind to Ds and inhibiting the ability of Ds to bind to Fat. Each cell in this gradient with a relatively high Fj level is therefore better at receiving a Fat signal (large red arrows) and worse at sending a Fat signal (small red arrows) than an adjacent cell with lower Fj expression (Figure 4B). This mechanism results in polarized Fat activity (asterisks) within each cell (Figure 4C). The direction of this Fat polarization mirrors the vector of the Fj gradient and the magnitude of Fat polarization reflects its slope (Simon *et al*., 2010).

Critical to this model is the dual and opposite action of Fj on Fat and Ds. If Fj would only modulate Fat, Fat activity would differ across a tissue, but would not be polarized within the individual cells of that tissue (Figure 4D). Conversely, if Fj would only affect Ds, individual cells would have to make a distinction between relatively high levels of Fat activity and between relatively low levels of Fat activity in order to polarize (Figure 4E) (Simon *et al*., 2010). In this model it is assumed that Ds is uniformly expressed (for simplicity), which is not the case in reality, where it generally is expressed in a gradient opposing the Fj gradient (Figure 3). The opposing Ds gradient would strengthen the Fj-driven polarization of Fat activity shown in Figure 4 (Simon *et al*., 2010).

The Salvador/Warts/Hippo (SWH) pathway

The SWH pathway provides crucial control of organ size in both *Drosophila* and mammals (Milton *et al*., 2009). It limits cell proliferation by controlling various regulators of proliferation. For example, this pathway controls the abundance of cyclin E, a protein essential for driving progression from G_1 to S phase; additionally it promotes apoptosis by down-regulating the *Drosophila* inhibitor of apoptosis protein, DIAP1 (Bennett *et al*., 2006).

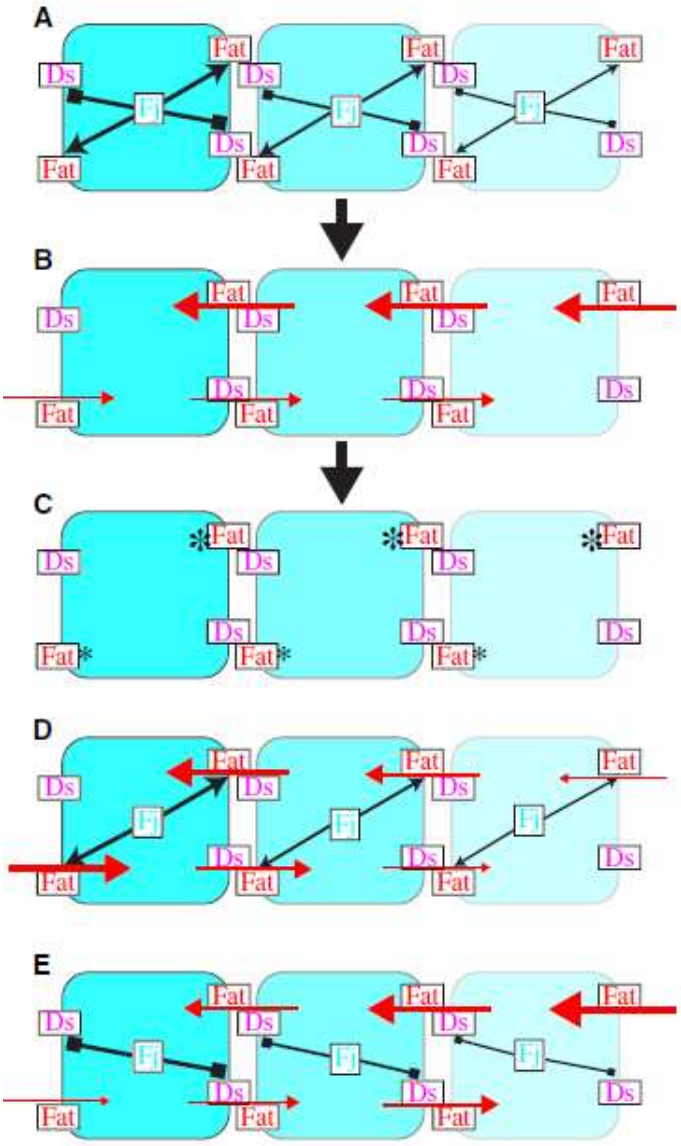

Figure 4: Model for polarization of Fat activity in response to an Fj gradient. Adapted from Simon *et* al., 2010. Squares represent cells in a gradient of Fj shown by the levels of shading. (A) Fj simultaneously decreases the Fat-binding activity of Ds (block arrows) and increases the Ds-binding activity of Fat (pointed arrows). (B) Therefore, each cell with a relatively high Fj level is better at receiving a Fat signal (large red arrows) and worse at sending a Fat signal (small red arrows) than an adjacent cell with a lower Fj expression. (C) This leads to polarized Fat activity (asterisks) in each cell. (D) If Fj would only modulate Fat, Fat activity would differ across a tissue, but would not be polarized within the individual cells of that tissue. (E) If Fj would only affect Ds, individual cells would have to make a distinction between relatively similar (high or low) levels of Fat activity in order to polarize (Simon *et* al., 2010).

Nearly all of the components of the SWH pathway were isolated in genetic screens as genes that, when inactivated, allowed excessive tissue growth in developing *Drosophila* wings or eyes (Saucedo *et al*., 2007). An elegant example of such a genetic screen method is to generate clones of homozygous mutant cells and sister clones of wild-type cells in the eyes of otherwise heterozygous *Drosophila* animals. Mutant and wild-type tissue are then

identified by the presence of eye colour markers, a white colour indicating mutant tissue and a red colour indicating wild-type tissue. Their relative contribution to the adult eye is eventually assessed (Harvey *et al*., 2003); that is, mutations with more white than red are analyzed.

Among the first core members of the SWH pathway to be described was warts (*wts*). Wts, a NDR family protein kinase, was identified by its overproliferation mutant phenotype in *Drosophila* (Justice *et al*. 1995). Loss of the *wts* gene not only results in overproliferation but also in apical hypertrophy of epithelial cells, leading to abnormal deposition of extracellular matrix (cuticle) during adult development (Justice *et al*., 1995), which resembles warts. Salvador (*sav*), a gene that promotes both cell cycle exit and cell death, was identified in a genetic screen in the *Drosophila* eye for mutations that increase the relative representation of mutant tissue compared to wild-type tissue. In this screen *sav* mutant cells were found to have elevated cyclin E and DIAP1 levels, resulting in delayed cell cycle exit and impaired apoptosis (Tapon *et al*., 2002). Moreover, Salvador was found to contain two WW domains and to bind to the Warts protein kinase (Tapon *et al*., 2002). Hippo (*Hpo*), a gene that regulates cell growth, cell cycle exit, and cell death was identified simultaneously by two different groups by genetic screens in the *Drosophila* eye (Harvey *et al*., 2003, Wu *et al*., 2003). *Hpo* encodes a Ste-20 family protein kinase that together with *Sav* and *Wts* forms a signalling pathway that affects both cell cycle exit and apoptosis (Wu *et al*., 2003, Harvey *et al*., 2003).

Yki, a gene encoding the *Drosophila* ortholog of yes-associated protein (YAP), which is a transcriptional coactivator in mammalian cells, was found in a study that aimed to extend the SWH pathway further downstream than the Hpo, Sav and Wts components. To accomplish this, a yeast two-hybrid screen for Wts binding proteins was done, since Wts was placed as the most downstream component of this pathway at that time. This screen discovered Yki as the missing connection between Wts and transcriptional regulation. Overexpression of Yki led to similar effects as a loss-of-function mutation of *hpo* and *wts*, including elevated transcription of *cyclinE* and *DIAP1*, increased proliferation, defective apoptosis, and tissue overgrowth (Huang *et al*., 2005). The activity of the SWH pathway results in phosphorylation of Yki, which prevents Yki from entering the nucleus and

transcribing its target genes. In 2009, Milton *et al*. classified the components of this pathway that have distinct roles in controlling cell growth, proliferation and apoptosis (such as *sav*, *wts*, *hpo* and *yki*) as core pathway members.

Upstream regulators of SWH pathway

The molecular mechanisms regulating the core kinase cascade of the SWH pathway are now established (Figure 5); the current issue is to decipher how SWH signalling is regulated by upstream components of the SWH pathway or by other regulators. Mer, Ex, and Fat are all *Drosophila* tumor suppressors proposed to act as upstream components of the SWH pathway (Genevet *et al*., 2009) based on the finding that double mutant cells for *ex* and *mer* have phenotypes comparable to that of other components of the SWH pathway (Feng *et al*., 2007). Moreover, Ex and Mer were found to modulate phosphorylation of Hpo and Wts and thereby the transcriptional activity of Yki in cultured cells (Hamaratoglu *et* al., 2006). Fat, in addition, controls the same downstream genes as are regulated by the SWH pathway and *ft* mutant tissue also displays similar phenotypic features as tissue lacking other SWH pathway activity promoting genes (Bennet *et al*., 2006).

Merlin (Mer) and Expanded (Ex) are members of the FERM-domain superfamily, which consists of membrane-associated cytoplasmic proteins that interact with transmembrane proteins and therefore may function as transmitters of signals from the membrane to protein complexes and/or the cytoskeleton (Saucedo *et al*., 2007, Maitra *et al*., 2006). In addition, Mer and Ex are known to physically interact and co-localize in the apical junctional region of epithelial cells (Miatra *et al*., 2006). The molecular mechanisms through which these proteins regulate SWH signalling were long unknown. A few years ago however, a yeast two-hybrid system detected weak interactions between Ex and Hpo and between Mer and Sav (Formstecher *et al*., 2005). This suggests that these upstream regulators induce Hpo activity, since Hpo needs to interact with Sav to phosphorylate Warts, which is facilitated by Mats to phosphorylate Yki, thereby excluding it from the nucleus (Grzeschik *et al*. 2010, Robinson et al., 2010). Additionally it was found that Ex binds Yki, localizing it to the cell cortex (Badouel *et al*., 2009). This suggests that Ex restricts Yki to the cytoplasm, blocking translocation of Yki to the nucleus and its subsequent activity there (Saucedo *et al*., 2007). Therefore, these two upstream components of the SWH pathway seem to have a balanced

mode of action in that loss of Ex will not have a large consequence on cell proliferation and apoptosis, since the activity of the kinase cascade is also regulated via Mer which brings Sav into close proximity to Hpo. On the other hand, as long as Ex is functional, extreme pathway activity brought about by loss of Mer can be effectively diminished by the binding of Yki by Ex (Badouel *et al*., 2009). Several feedback loops present in the SWH pathway further ensure a coordinated SWH signalling activity. For example, Yki regulates the expression of both *mer* and *ex* (Miatra *et al*., 2006, Badouel *et al*., 2009).

The other suggested upstream component of the SWH signalling pathway, Fat, is different from Ex, Mer, and core components of the hippo kinase cascade, in that Fat also regulates PCP (Yu *et al*., 2010). It has been suggested that Fat acts through Ex, since Fat also localizes to apical junctions (Saucedo *et al*., 2007) and mutation of *fat* causes a decrease in the levels of Ex at the apical membrane without affecting the levels and localization of Hpo, Sav, and Mer (Feng *et al*., 2007, Saucedo *et al*., 2007). Combined with the interactions between Ex and Hpo and between Mer and Sav described above, these results indicate that Fat strengthens SWH signalling by bringing Hpo into close proximity to Sav through Ex (Saucedo *et al*., 2007). However, there are also indications that Ex and Fat act in separate parallel pathways that regulate growth. In *fat* mutant cells, Ex is relocalized basally rather than lost. However, overexpressing Ex within *fat* mutant cells resulted in the detection of high levels of Ex staining and its normal subapical location. Fat can therefore modulate Ex membrane localization, but is not necessary for it. Additionally, the mutation of *fat* was found to considerably increase the growth of cells overexpressing Ex, while Ex staining remained strong at the subapical membrane. Fat signalling can thus occur independent of an effect of Ex levels or localization. These findings obviously dispute models in which Fat signals mostly through modulation of Ex levels or localization. By contrast, it is in agreement with the hypothesis that *fat* and *ex* act in parallel to regulate growth (Feng *et al*., 2007).

Fat has also been suggested to act through the unconventional myosin Dachs to regulate the levels of Wts and thereby the activity of Yki. Cells deficient for *fat* showed reduced levels of *wts* and this effect was dependent on the presence of Dachs. Fat might therefore stimulate SWH signalling by stabilizing Wts via the restriction of the function of Dachs (Feng *et al*., 2007, Cho *et al*., 2006, Saucedo *et al*., 2007). In summary, at present several models for

modulation of SWH signalling through Fat exist and further experiments are required to determine the exact mode of action of Fat on the SWH pathway.

One of the latest upstream component of the SWH signalling pathway to be identified was Kibra, a cytoplasmic protein. Loss of *kibra* results in imaginal disc overgrowth and abnormal gene expression distinctive of deregulated SWH signalling. Kibra seems to function together with Mer and Ex in an apical protein complex in epithelial cells, which regulates the Hippo kinase cascade via direct binding to Hpo and Sav, collaborating to bring about Wts phosphorylation and thereby modulation of Yki phosphorylation (Yu *et al*., 2010).

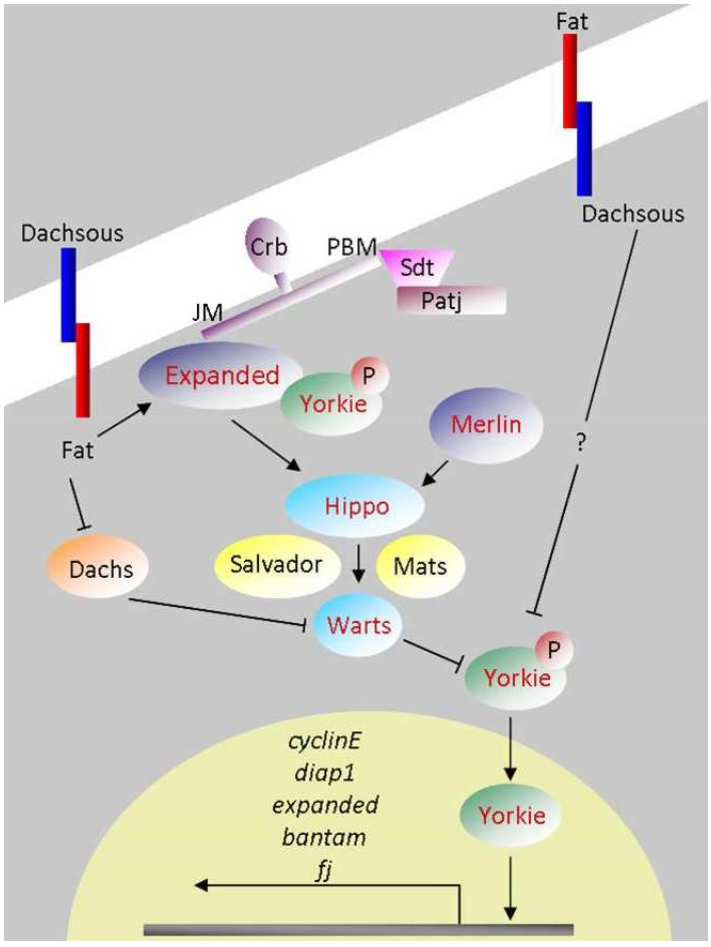

Figure 5:The evolutionarily conserved SWH kinase cascade. Adapted and modified from Lawrence *et* al., 2008. Hippo binds Salvador to phosphorylate Warts, which is facilitated by Mats, a Mob family protein, to phosphorylate Yorkie, thereby excluding it from the nucleus. When the SWH pathway is inactivated, Yki becomes dephosphorylated and translocates to the nucleus, where it binds the Scalloped (Sd) transcription factor, leading to the upregulation of various genes promoting cell proliferation and survival (Grzeschik *et al*. 2010, Robinson et al., 2010). SWH signalling suppresses cell proliferation by inhibiting the activity of Yki through phosphorylation thereby suppressing the expression of its target genes (Hamaratoglu *et al*., 2009). Recently it has been found that Expanded binds Yki, relocating it to the cell cortex (Badouel *et al*., 2009), and that Crumbs binds Expanded (Ling *et al*., 2010, Grzeschik *et al*. 2010, Robinson *et al*., 2010). See text for details.

Boundaries of PCP regulators modulate SWH signalling to control growth

PCP regulators have been found to affect the SWH pathway. One study found that Ds overexpression in *Drosophila* epithelial cells causes an up-regulation of known

transcriptional targets of the SWH pathway; *ex*, *diap*, and *fj* (Willecke *et al*., 2008). Surprisingly these SWH target genes were not up-regulated in all cells overexpressing Ds, but were up-regulated merely along both sides of the borders of Ds overexpressing cells. Additionally, loss-of-function *ds* mutant cells caused an up-regulation of *fj* expression on the outside of the border where cells with different levels of Ds activity are confronted. This expression was only found on the outside of the border since *fj* was only up-regulated in wild-type cells and not in *ds* mutant cells. This is an indication that Ds is necessary for cells to react to Ds boundaries. Moreover, loss and gain of Fj caused an up-regulation of SWH target genes on both sides of the boundary between cells expressing different levels of Fj activity. Taken together, these results suggest that differences of Ds and Fj activity between cells instead of the absolute amounts of Ds and Fj presented to a cell modulate the SWH pathway (Willecke *et al*., 2008). The up-regulation of SWH target genes could also occur through another pathway than the SWH pathway; the Fat signalling pathway, which is involved in the establishment of PCP and is linked to the SWH pathway to regulate a common set of downstream target genes (Rogulja *et al*., 2008). Interestingly Fj is known to phosphorylate Fat and Ds during the establishment of PCP (Simon *et* al., 2010, Brittle *et* al., 2010) and is a SWH target (Willecke *et al*., 2008), implying a feedback loop that aims to maintain an equilibrium of Fat signalling and PCP with SWH signalling.

Ds is identified to interact with Fat on neighbouring cells (Lawrence *et al*., 2008), this is an indication that Fat indeed is required for the modulation of the SWH pathway by Ds/Fj boundaries. To examine this, Ds expressing cells in *fat* mutant discs were made. These showed no additional up-regulation of *ex* and *diap1* on top of the up-regulation already seen in *fat* mutant discs. This suggests that Fat is necessary for the Ds boundary effect. Additionally, loss of Dachs, acting downstream of Fat, suppressed the up-regulation of SWH target genes at Ds boundaries. This finding suggests that Ds boundaries restrain the activity of the Fat and the SWH pathway to modulate gene expression (Willecke *et al*., 2008). Moreover a decrease of Ex protein was found at the plasma membrane of cells located on both sides at the boundaries of Ds activity. This reduction of Ex levels required Fat and Dachs, since no reduction of Ex at the plasma membrane was found at Ds boundaries in *fat* and *dachs* mutant discs. These results therefore indicate that Ds boundaries might suppress the activity of the SWH pathway, to a degree at least, through a Fat- and Dachs-dependent

mechanism that modulates the localization and stability of Ex (Willecke *et al*., 2008). Based on these findings a model has been suggested for regulation of Fat signalling by a Ds gradient, in which the inhibitory effect of Fat on Dachs function is central (Figure 6), instead of its effects on Ex localization or stability (Rogulja *et al*., 2008). This model, see figure 6, explains how graded Ds expression might lead to the regulation of SWH gene transcription through Fat signalling and how affecting this gradient changes the expression of SWH genes.

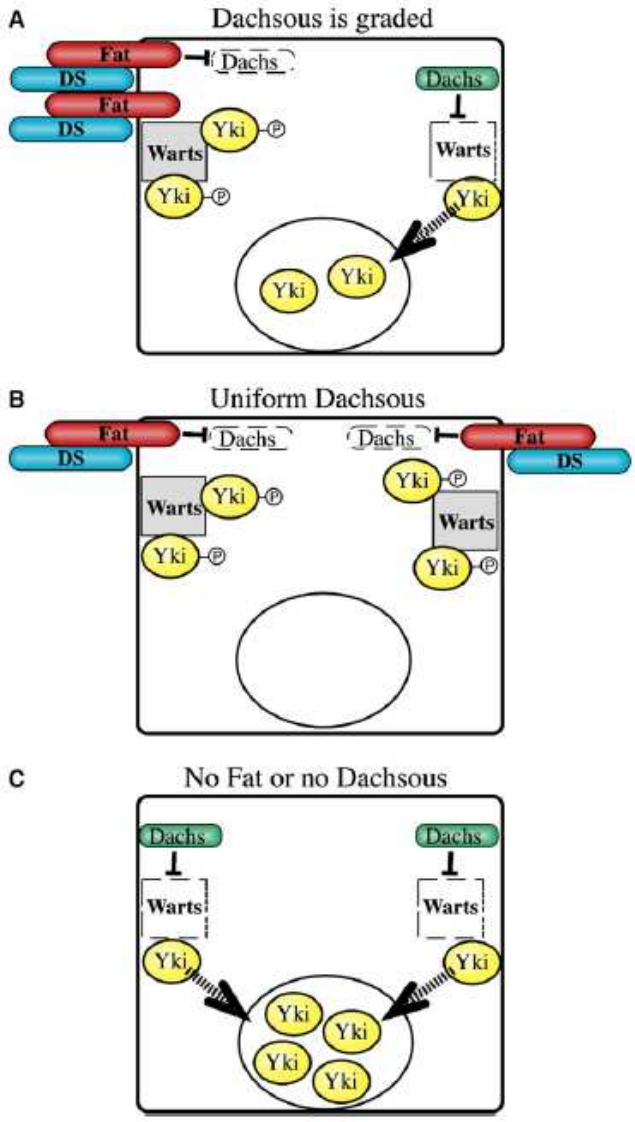

Figure 6:Model for modulation of Fat signalling and regulation of SWH genes by a Ds gradient. Adapted from Rogulja *et* al., 2008. (A) If Ds is expressed in a gradient and is present only on one side of a cell, Fat will also show graded accumulation to interact with Ds. Fat will therefore inhibit the function of Dachs only on one side of the cell, leading to active Dachs on the opposite side of that cell where it inhibits Yki phosphorylation by Wts, thereby allowing intermediate levels of Yki-mediated gene transcription in the nucleus. (B) If Ds is expressed uniformly on both sides of a cell, Fat is also uniformly localized on both sides, leading to inhibition of Dachs thereby de-repressing Yki phosphorylation by Wts on both sides. (C) When no Fat or Ds would be present; Dachs would not be inhibited by Fat and would block Yki phosphorylation by Wts on both sides of that cell thereby resulting in high levels of Yki-mediated gene transcription in the nucleus.

Up-regulation (de-repression) of SWH pathway target genes at the Ds/Fj boundaries induces imaginal disc growth and thereby contributes to the regulation of organ size. Flattening the gradients of Fj and Ds by uniformly expressing Ds and Fj in ds, *fj* double mutant or otherwise

wild-type *Drosophila* flies, using the ubiquitous *tub-Gal* driver, resulted in animals with significant smaller bodies and relative smaller wings and legs (Willecke *et al*., 2008, Rogulja *et al*., 2008). Overall, this study suggests that the effect of Ds/Fj boundaries on SWH gene regulation is scalar (levels of target gene expression) whereas the effect of Ds/Fj boundaries on PCP is vectorial (direction of polarity). The direction of the Ds/Fj gradients therefore determines the direction of cell polarity, whereas the difference in Ds/Fj activity (steepness of the gradients) affects SWH signalling (Willecke *et al*., 2008). To nuance this conclusion however, though *Drosophila* flies with a uniform Ds/Fj expression have significant growth defects, growth was not abolished altogether. This indicates that the Ds/Fj gradient accounts for some but not all growth control (Willecke *et al*., 2008, Rogulja *et al*., 2008). Nevertheless these results seem to be in agreement with the steepness hypothesis, which states that the steepness of a linear gradient, in this case the Ds/Fj gradient can serve to regulate the activity of a particular process. Here the Ds/Fj gradient drives proliferation of a tissue in one dimension, by means of the SWH pathway, thereby decreasing the steepness of this linear gradient until the slope of the gradient becomes too low to drive growth (Lawrence *et al*., 2008).

Apicobasal polarity regulators affect localization of SWH members

Besides the apparent loss of apicobasal cell polarity, homozygous *Drosophila* mutants for Scrib, Dlg, and Lgl show an increased proliferation in the brain and epithelial imaginal tissues, which also fail to differentiate. Overexpression of other known polarity regulators, such as aPKC and Crb, also leads to tissue overgrowth. How these polarity regulators modulate the cell cycle and apoptosis machinery is not known in detail (Brumby *et al*., 2005).

Apicobasal polarity regulators however have recently been implicated in the regulation of the SWH pathway. In wild-type tissue, Hpo localizes apicolaterally, overlapping with aPKC, and Dlg, here used as an apical and lateral marker respectively. In *lgl*$^{-}$ mutant cells of the *Drosophila* larval eye disc, however, Hpo expanded from its apicobasal localization towards a more basolateral localization (Grezschik *et al*. 2010). RASSF, a Ras-associated domain family protein and a known tumor suppressor (Avruch *et al*., 2008) normally localizing in the apical cortex, also overlaps with aPKC and Dlg. In *lgl*$^{-}$ tissue RASSF was also mislocalized and colocalized with Hpo (Grezschik *et al*. 2010). Ex and Fat levels and localization at the apical

cortex were not changed in *lgl*$^{-}$ larval eye disc tissue, indicating that the defect is specific for Hpo and RASSF localization. In addition, the SWH pathway targets CycE, DIAP1, *fj*, and *ex*, were upregulated in an Yki dependent manner after Lgl depletion, suggesting that the mislocalization of Hpo and RASSF affects Hpo kinase activity. Upregulation of aPKC in *Drosophila* eye discs had a similar effect on Hpo and RASSF, in that their normal localization at the apical cortex was changed towards a more basolateral localization, though Ex and Fat localization remained unchanged. Additionally, Crb overexpression in the *Drosophila* eye disc resulted in mislocalization of Ex from the apical cortex towards a more diffused lateral localization, while leaving the localization of Hpo, RASSF, and Fat unaffected. Moreover, overexpression of *aPKC* or *crb* in the *Drosophila* larval eye disc resulted in an Yki dependent up-regulation of DIAP1 and *ex*. Taken together this study shows that apicobasal polarity regulators can modulate the SWH pathway via two distinct mechanisms: Lgl and aPKC can act by opposing each other in their regulation of the SWH pathway through localization of Hpo and RASSF (but not Fat or Ex), whereas Crb can affect the localization of Ex (but not Hpo, RASSF, or Ft) (Grezschik *et al*. 2010).

Crb: a dual regulator of polarity and growth

The finding that Crb overexpression results in Ex mislocalization was also recently observed by another group, who additionally showed that Ex levels are reduced in this situation (Robinson *et al*., 2010). To investigate the molecular mechanisms behind the regulation of Crb on Ex levels and localization, this study focussed on the Crb intracellular tail. This tail contains two functional motifs that are conserved across Crb proteins in many species: (1) the juxtamembrane FERM-binding motif (JM) which links Crb to the actin/spectrin cytoskeleton via FERM-domain proteins, and (2) the C-terminal PDZ binding motif (PBM) which establishes interactions with Sdt and Patj to form the Crb polarity complex. Overexpressing a *crb* transgene containing the JM motif, but lacking the PBM motif, in the *Drosophila* wing disc increased adult wing size, depleted apical Ex and increased Yki activity. By contrast, a *crb* transgene containing the PBM motif, but lacking a functional JM motif, did not increase wing size and had no effect on Ex levels or localization. Expression of *crb-PBM* however resulted in a disrupted organization of the disc epithelium and wing morphology, which was not seen when expressing *crb-JM*. These results suggest that Crb might act as a cross point between apicobasal polarity signals and proliferation through its dual regulation

of these processes by means of its two tail domains (Grzeschik *et al*. 2010, Robinson *et al*., 2010).

Only recently it has been proved that Crb binds Ex directly via its juxtamembrane FERM-binding motif (JM). Direct binding of Ex by Crb was suspected after examination of $Crb^{Myc-Intra}$, a construct of truncated Crb encoding the membrane-bound cytoplasmic domain of Crb activity in S2R+ cells, to investigate Crb's relation to SWH signalling. $Crb^{Myc-Intra}$ alone, did not promote Wts phosphorylation, however it did increase Ex-mediated Wts phosphorylation. Crb therefore seems to positively regulate SWH signaling via Ex. Moreover, $Crb^{Myc-Intra}$ seemed to promote Ex phosphorylation together with a decrease of Ex levels, which could be caused by a feed-back loop since *ex* is a target gene of SWH signaling. Mutation of the JM motif, but not of the C-terminal PDZ binding motif (PBM) which establishes interactions with Sdt and Patj, decreased Crb-induced Ex phosphorylation. By localizing at the cell membrane, Ex might be phophorylated by unknown kinases, and indeed targeting Ex to the cell membrane by attaching a myristylation signal to its N terminus also induces Ex phosphorylation. Crb might therefore function by localizing Ex to the cell membrane by directly binding to it. This was examined by GST pull-down assays between GST-Crb-intra (with and without JM or PBM) and cell lysates expressing epitope-tagged Ex or bacterially purified Ex proteins. In both assays, GST-Crb-intra bound Ex in a JM, but PBM independent, manner. Crb therefore seems to bind Ex through its JM domain. Moreover, it was found that loss of Crb or mutation of its JM domain results in mislocalizaton of Ex stainings to the basolateral domain in *Drosophila* imaginal discs. Localization of merlin, another FERM domain protein, however was not affected in *crb* mutant cells. This indicates that Crb's JM domain binds to Ex's FERM domain and that this is specifically required in localizing Ex to the apical membranes of epithelial cells, where Ex can be activated by phosphorylation by unknown kinases (Ling *et al*., 2010). This study implicates Crb, a transmembrane protein that binds directly to an apical component of the SWH pathway, as a potential cell surface receptor for SWH signalling (Ling *et al*., 2010).

One question is to what extent these findings represent direct regulation of the SWH pathway by apical polarity regulators, rather than an indirect effect caused by loss of proper apical-basal polarity. For example, *crb* overexpression causes a general expansion of the

apical domain with a concurrent reduction of the basolateral domain size. This may account for the observed mislocalization of Ex. Two findings argue that there is direct signalling from crb to the SWH pathway. First, Hpo, RASSF, and Ft are not mislocalized in *crb* overexpressing cells. Second, separate domains of the Crumbs intracellular tail mediate its effects on Ex and on apical-basal polarity.

Mammalian Crumbs involved in tumorigenesis

Crumbs is also specifically interesting, because the expression of a mammalian orthologue of this *Drosophila* polarity regulator, identified as *crb3*, was found to be repressed in a screening for tumorigenicity in immortal baby mouse kidney epithelial (iBMK) cells. This suggests that Crb3 plays an important role in maintaining the epithelial phenotype, and downregulation or loss of function of Crb3 contributes to tumor progression (Karp *et al*., 2008), which is in agreement with what is known from *Drosophila* studies. Tumorigenesis is associated with loss of epithelial features through initiation of an epithelial to mesenchymal transition (EMT). EMT is the process by which cells are transformed from a polarized, epithelial phenotype to a migratory mesenchymal phenotype. EMT is characterized by distinct morphological and functional modifications in cells such as disruption of epithelial tight junctions, reorganization of the actin cytoskeleton, and loss of apicobasal polarity. It was found that cells that undergo tumorigenesis *in vivo*, also undergo EMT. Moreover, loss of *Crb3* expression was found to be, at least partly, responsible for the impaired junction formation during tumorigenesis (Karp *et al*., 2008).

Additionally, in a wound-healing essay, tumor derived epithelial cells with decreased *Crb3* expression showed random, uncoordinated cell movements and failed to efficiently migrate and fill the empty space between the epithelial cells. Moreover tight junctions were not properly formed in these tumor derived cells and cells eventually proliferated on top of each other. Conversely, tumor derived epithelial cells expressing *crb3* displayed an obvious improvement in coordinated migration, junction formation and contact inhibited growth. Restoring Crb3 expression in tumor derived cells also showed to suppress cell migration through a porous membrane, while disrupting Crb3 function in non-tumorigenic parental cells repressed junction formation and stimulated migration through a porous membrane. Moreover, injecting nude mice with tumor derived cells, with decreased *Crb3* expression, in

the tail vein caused these animals to develope multiple tumors colonizing the kidney, and bone of the leg and spinal cord, demonstrating the metastatic growth of these tumor derived cells. However mice injected with tumor derived cells expressing *Crb3* remained tumor free. Taken together, these results demonstrate that Crb3 is required for tight junction formation, establishment of polarity, contact inhibited growth, and suppression of migration, which restrains metastasis. Thus, in mammalian epithelial tumors, similar mechanisms as in *Drosophila* may control tumor growth by interacting with polarity determinants (Karp *et al*., 2008).

SWH regulation of polarity determinants

So far we have discussed studies that focus on the effect of polarity regulators on the SWH pathway. However the opposite effect has also been observed. We will now consider two studies that examine the effect of SWH signalling on polarity determinants.

Adult *Drosophila* epithelial cells mutant for the SWH pathway kinases Hpo and Wts demonstrate hypertrophic apical areas. The molecular features of this apical hypertrophy have not yet been completely characterized. Two independent studies investigated how SWH pathway deregulation leads to cell polarity defects (Hamaratoglu *et al*., 2009, Genevet *et al*., 2009). Stainings in *Drosophila* wing imaginal discs for aPKC and Crb showed that, when *hpo* is mutated, there is an increased staining for these apical polarity proteins. Additionally, adherens junctions in *hpo* mutant cells show increased protein localization at the membrane, reflected by staining for E-cadherin (DE-cad) and Armadillo (central components of the *Drosophila* zona adherens). In contrast, the basolateral complexes were not changed by loss of Hpo signalling, as demonstrated by Dlg (lateral marker) and Dystroglycan (basal marker) stainings. Even though aPKC and DE-cad domains were found to be broader, these domains remained predominantly non-overlapping in *hpo* mutant cells, comparable to wild-type cells. Additionally, in cells mutant for *mer;ex* (double mutant), *ft,* or *wts* and in *yki*-overexpressing cells, staining for apical complex members was brighter and broader, whereas a normal distribution of basolateral markers was found (Genevet *et al*., 2009). Another study found similar results in that the membrane levels of the apical polarity proteins; Patj, Crb, and aPKC were significantly increased in *ft*, *mer;ex*, *hpo* and *wts* mutant cells, and in cells overexpressing Yki. Moreover, these results were observed in many

Drosophila tissues including the wing, antennae, and eye imaginal discs (Hamaratoglu *et al*., 2009). Taken together, these results suggest that broader and more intense apical stainings observed in cells after deregulation of the SWH pathway at different levels, are not caused by a mixing of apical subdomains, but rather by an apical enlargement caused by the presence of additional Crb, aPKC, or another apicobasal polarity protein (Hamaratoglu *et al*., 2009, Genevet *et al*., 2009). This appeared to be verified by transmission electron microscopy analysis, which showed a significantly larger apical domain in *yki*-overexpressing cells, compared to control cells. Since Crb overexpression increases the size of the apical domain, it was hypothesized that removing Crb might compensate for the apical membrane hypertrophy induced by deregulating the SWH pathway (Lu *et al*., 2005). Indeed, *crb*/*wts* clones showed wild-type levels of aPKC staining, demonstrating that loss of *crb* rescued the apical polarity protein build up seen in *wts* mutant tissue (Genevet *et al*., 2009). Finally both groups demonstrate that the growth benefits and apoptosis rates of *wts* and *crb*/*wts* clones are very similar, suggesting that the apical hypertrophy, caused by an upregulation of the apical polarity proteins, is not required for the overgrowth phenotype elicited by disruption of the SWH pathway. Therefore, both studies conclude that SWH signalling regulates both cell polarity complexes and proliferation, through separate and independent mechanisms (Hamaratoglu *et al*., 2009, Genevet *et al*., 2009).

Conclusions

From the literature discussed above it can be concluded that determinants of both apical-basal polarity and tissue polarity interact with the SWH pathway at several levels. The apical-basal polarity regulator Crumbs seems to play an important role in regulating both polarity and the SWH pathway through two different and independent domains in its intracellular tail (Grzeschik *et al*. 2010, Robinson *et al*., 2010, Ling *et al*., 2010). One of these Crumbs domains ensures a proper localization of Ex by directly binding this SWH component, thereby allowing its function within this signalling cascade. Disruption of Crumbs therefore indirectly affects SWH signalling (Ling *et al*., 2010). The interaction between PCP determinants and SWH signalling provides an attractive mechanism to maintain tissues at a particular size. Ds/Fj boundaries have an important effect on SWH signalling by forming a gradient that has a scalar effect on SWH gene transcription, possibly through the polarization of Fat signalling which is an upstream component of SWH signalling (Simon *et* al., 2010, Willecke *et al*., 2008,

Rogulja *et* al., 2008). Tissues would continue to grow until the Ds/Fj gradient is sufficiently shallow, and no longer activates SWH signalling.

Overall, tissue polarity and tissue proliferation seem to be regulated by independent and parallel mechanisms which are connected indirectly at several points, possibly in the form of proteins such as Crumbs and Fat, that integrate information about a tissue's 3D-architecture with its ability to proliferate by separable independent functions of these polarity proteins (Bilder *et al*., 2004). These independent mechanisms of polarity and proliferation also become apparent after examination of the effect of SWH signalling on polarity determinants. Apical hypertrophy, seen after upregulation of the apical polarity proteins, is not required for the overgrowth phenotype elicited by disruption of the SWH pathway (Hamaratoglu *et al*., 2009, Genevet *et al*., 2009). However, when we combine the finding that apicobasal polarity regulators can indirectly modulate the SWH pathway by affecting the localization of SWH components (Grezschik *et al*. 2010) with the finding that apicobasal regulators are increased after SWH pathway deregulation (Hamaratoglu *et al*., 2009, Genevet *et al*., 2009), we are led to the possibility of a positive feedback loop between the SWH pathway and the apical polarity proteins. This positive feedback loop might explain in part the continuing loss of polarity accompanied with the massive overproliferation seen during neoplasm formation (Grezschik *et al*. 2010).

In conclusion, we agree with a group of models that state that overgrowth of a tissue is an indirect result of epithelial polarity loss, due to spatial disorganization of signalling pathways (such as the SWH pathway) that control cell proliferation. Specifically, this could happen in several ways, for example by distorting the subcellular localization of polarized signal transduction components (Bilder *et al*., 2004), which is in agreement with what we found in the discussed literature. In the *in vivo* environment of a cell, multiple reciprocal links, between pathways that regulate proliferation, cell survival and polarity, are expected to occur at several stages in order to maintain a tissue's 3D architecture and size homeostasis (Robinson *et al*., 2010). Defects in one pathway might thereby have intense effects on the others pathways (Bilder *et al*., 2004). Unravelling these links remains a major challenge to a full comprehension of malignant transformation

References

Do the protocadherins Fat and Dachsaus link up to determine both planar cell polarity and dimensions of organs? P.A. Lawrence, G. Struhl, J. Casal. Nature Cell Biol 10 (12). Dec. 2008

Crumbs regulates Salvador/Warts/ Hippo signaling in Drosophila via the FERM-domain protein Expanded. B.S. Robinson, J. Huang, Y. Hong, K.H. Moberg. Current Biol 20. Apr. 2010

Lgl, aPKC and Crumbs regulate the Salvador/Warts/ Hippo pathway through two distinct mechanisms. N.A. Grzeschik, L.M. Parsons, M.L. Allott, K.F. Harvey, H.E. Richardson. Current Biol 20. Apr. 2010

Delineation of a Fat tumor suppressor pathway. E. Cho, Y. Feng, C. Rauskolb, S. Maitra, R. Fehon, K.D. Irvine. Nature Gen 38 (10). Oct. 2006

Differential requirements of Salvador-Warts-Hippo pathway members for organ size control in Drosophila melanogaster. C.C. Milton, X. Zhang, N.O. Albanese, K.F. Harvey. Development 137. Dec. 2009

Boundaries of Dachsous cadherin activity modulate the Hippo signaling pathway to induce cell proliferation. M. Willecke, F. Hamaratoglu, L. Sansores-Garcia, C. Tao, G. Halder. PNAS 105 (39). Sept. 2008

Fat cadherin modulates organ size in *Drosophila* via the Salvador/Warts/Hippo signaling pathway. F.C. Bennett, K.F. Harvey. Curr Biol 16. Nov. 2006

The orientation of cell divisions determines the shape of *Drosophila* organs. L.A. Baena-López, A. Baonza, A. García-Bellido. Curr Biol 15. Sept. 2005

Crumbs is required to achieve proper organ size control during *Drosophila* head development. E.C.N. Richardson, F. Picaud. Development 137. Dec. 2009

Controlling the size of organs and organisms. S.J. Leevers, H. McNeill. Curr Opin Cell Biol 17. Oct. 2005

Parsing the polarity code. I.G. Macara. Nat Rev Mol Cell Biol 5. Mar. 2004

The hallmarks of cancer. D. Hanahan, R.A. Weinberg. Cell 100. Jan. 2000

Hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with Salvador and warts. S. Wu, J. Huang, J. Dong, D. Pan. Cell 114. Aug. 2003

The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis. K.F. Harvey, S.M. Pfleger, I.K. Hariharan. Cell 114. Aug. 2003

The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. R.W. Justice, O. Zilian, D.F. Woods, M. Noll, P.J. Bryant. Gen Dev 9. Jan. 1995

Salvador promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines. N. Tapon, K.F. Harvey, D.W. Bell, D.C.R. Wahrer, T.A. Schiripo, D.A. Haber, I.K. Hariharan. Cell 110. Aug. 2002

The Scribble and Par complexes in polarity and migration: friends or foes? P.O. Humbert, L.E. Dow, S.M. Russell. Trends Cell Biol 16(12). Oct. 2006

Filling out the Hippo pathway. L.J. Saucedo, B.A. Edgar. Nat Rev Mol Cell Biol 8. Aug. 2007

The Par-aPKC system: lessons in polarity. A. Suzuki, S. Ohno. J Cell Sci 119. Jan. 2006

Planar cell polarity signaling: from fly development to human disease. M. Simons, M. Mlodzik. Annu Rev Genet 42. 2008

Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. D. Bilder, N. Perrimon. Nature 403. Feb. 2000

Identification of genes required for cytoplasmic localization in early C. elegans embryos. K.J. Kemphues, J.R. Priess, D.G. Morton, N. Cheng. Cell 52. Feb. 1988

Septate and scalariform junctions in arthropods. C. Noirot-Timothee, C. Noirot. Int Rev Cytol 63. 1980

The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila homolog of YAP. J. Huang, S. Wu, J. Barrera, K. Matthews, D. Pan. Cell 122. Aug. 2005

Epithelial cell polarity and cell junctions in Drosophila. U. Tepass, G. Tanentzapf, R. Ward, R. Fehon. Annu Rev Genet 35. 2001

Using Drosophila melanogaster to map human cancer pathways. A.M. Brumby, H.E. Richardson. Nature Rev Can 5. Aug. 2005

Apical junctional complexes and cell polarity. Q. Wang, B. Margolis. Kidney Int 72. Oct. 2007

Role of the polarity determinant Crumbs in suppressing mammalian epithelial tumor progression. C.M. Karp, T.T. Tan, R. Mathew, D. Nelson, C. Mukherjee, K. Degenhardt, V. Karantza-Wadsworth, E. White. Cancer Res 68 (11). June 2008

Four-jointed modulates growth and planar polarity by reducing the affinity of Dachsous for Fat. A.L. Brittle, A. Repiso, J. Casal, P.A. Lawrence, D. Strutt. Curr Biol 20. May 2010

Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. J. Yu, Y. Zheng, J. Dong, S. Klusza, W. Deng, D. Pan. Dev Cell 18. Feb. 2010

The FERM-domain protein Expanded regulates Hippo pathway activity via direct interactions with the transcriptional activator Yorkie. C. Badouel, L. Gardano, N. Amin, A. Garg, R. Rosenfeld, T. Le Bihan, H. McNeill. Dev Cell 16. Mar. 2009

The tumor suppressors Merlin and Expanded function cooperatively to modulate receptor endocytosis and signaling. S. Maitra, R.M. Kulikauskas, H. Gavilan, R.G. Fehon. Curr Biol 16. Apr. 2006

Fat and Expanded act in parallel to regulate growth through Warts. Y. Feng, K.D. Irvine. PNAS 104(51). Dec. 2007

Crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. U. Tepass, C. Theres, E. Knust. Cell 61. June 1990

Dlg protein is required for junction structure, cell polarity, and proliferation control in *Drosophila* epithelia. D.F. Woods, C. Hough, D. Peel, G. Callaini, P.J. Bryant. J Cell Biol 134 (6). Sept. 1996

Epithelial polarity and proliferation control: links from the *Drosophila* neoplastic tumor suppressors. D. Bilder. Genes Dev 18. 2004

Structure, expression, and properties of an atypical protein kinase C (PKC3) from *Caenorhabditis elegans*. S. Wu, J. Staudinger, E.N. Olson, C.S. Rubin. J. Biol Chem 273 (2). Jan 1998

Rassf family of tumor suppressor polypeptides. J. Avruch, R. Xavier, N. Bardeesy, X. Zhang, M. Praskova, D. Zhou, F Xia. J. Biol Chem 284 (17). Apr. 2009

Crumbs and *stardust* act in a genetic pathway that controls the organization of epithelia in *Drosophila melanogaster*. U. Tepass, E. Knust. Dev Biol 159. April. 1993

Discs lost, a novel multi-PDZ domain protein, establishes and maintains epithelial polarity. M.A. Bhat, S. Izaddoost, Y. Lu, K.O. Cho, K.W. Choi, H.J. Bellen. Cell 96. Mar. 1999

The Crums complex: from epithelial-cell polarity to retinal degenaration. N.A. Bulgakova, E. Knust. J Cell Science 122 (15). 2009

An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. Y. Izumi, T. Hirose, Y. Tamai, S. Hirai, Y. Nagashima, T. Fujimoto, Y. Tabuse, K.J. Kemphues, S. Ohno. J Cell Biol 43. Oct. 1998

The recovery and preliminary characterization of X-chromosome mutants affecting imaginal discs in *Drosophila melanogaster.* M. Stewart, C. Murphy, J. Fristrom. Dev Biol 27. 1972

Developmental capacities of benign and malignant neoplasms of *Drosophila*. E.A. Gateff, H.A. Schneiderman. Roux's Arch Dev Biol 176. 1974

The Drosophila lethal(2) giant larvae tumor suppressor protein is a component of the cytoskeleton. D. Strand, I. Raska, B.M. Mechler. J Cell Biol 127 (5). Dec. 1994

Endocytic control of epithelial polarity and proliferation in *Drosophila*. H. Lu, D. Bilder. Nature Cell Biol 7 (12). Dec. 2005

The tumour-suppressor genes *NF2/Merlin* and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. F. Hamaratoglu, M. Willecke, M. Kango-Singh, R. Nolo, E. Hyun, C. Tao, H. Jafar-Nejad, G. Halder. Nature Cell Biol 8 (1). Jan. 2006

Protein interaction mapping: a *Drosophila* case study. E. Formstecher, S. Aresta, V. Collura, A. Hamburger, A. Meil, A. Trehin, C. Reverdy, V. Betin, S. Maire, C. Brun, B. Jacq, M. Arpin *et al.* Genome Res 15 (3). Mar. 2005

Modulation of Fat: Dachsous binding by the cadherin domain kinase four-jointed. M.A. Simon, A. Xu, H.O. Ishikawa, K.D. Irvine. Curr Biol 20. May 2010

Four-jointed modulates growth and planar polarity by reducing the affinity of Dachsous for Fat. A.L. Brittle, A. Repiso, J. Casal, P.A. Lawrence, D. Strutt. Curr Biol 20. May 2010

Morphogen control of wing growth through the Fat signaling pathway. D. Rogulja, C. Rauskolb, K.D. Irvine. Dev Cell 15. Aug. 2008

The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. C. Ling, Y. Zheng, F. Yin, J. Yu, J. Huang, Y. Hong, S. Wu, D. Pan. PNAS. Mar. 2010