

## Lgl/aPKC and Crb regulate the Salvador/Warts/Hippo pathway

Linda M. Parsons,<sup>1,\*</sup> Nicola A. Grzeschik,<sup>1</sup> Melinda L. Allott<sup>1</sup> and Helena E. Richardson<sup>1-3</sup>

<sup>1</sup>Cell Cycle and Development; Peter MacCallum Cancer Centre; <sup>2</sup>Department of Anatomy and Cell Biology; <sup>3</sup>Department of Biochemistry and Molecular Biology University of Melbourne; Melbourne, Victoria Australia

**A** key goal of developmental biology is to understand the mechanisms that coordinate organ growth. It has long been recognized that the genes that control apico-basal cell polarity also regulate tissue growth. How loss of cell polarity contributes to tissue overgrowth has been the subject of much speculation. Do loss-of-function mutations in cell polarity regulators result in secondary effects that globally deregulate cell proliferation, or do these genes specifically control growth pathways? Three recent papers have shown that the apico-basal polarity determinants Lgl/aPKC and Crb regulate tissue growth independently of their roles in cell polarity and coordinately regulate cell proliferation and cell death via the Salvador/Warts/Hippo (SWH) pathway. Lgl/aPKC are required for the correct localization of Hippo (Hpo)/Ras associated factor (RASSF), while Crb regulates the levels and localization of Expanded (Ex), indicating that cell polarity determinants modify SWH pathway activity by distinct mechanisms. Here, we review the key data that support these conclusions, highlight remaining questions and speculate on the underlying mechanisms by which the cell polarity complexes interact with the SWH pathway. Understanding the interactions between cell polarity regulators and the SWH pathway will improve our knowledge of how epithelial organization and tissue growth are coordinated during development and perturbed in disease states such as cancer.

### Introduction

During development the control of organ size depends on a delicate balance

between cell proliferation, cell death and differentiation. These processes are regulated in response to both global (nutrient, hormones) and local signals (growth factor, cell intrinsic programs). In *Drosophila*, adult organs such as eyes, wings and legs are derived from epithelial precursor tissues known as imaginal discs that mature during larval and pupal development. Initially, spontaneous mutations were isolated that regulated imaginal disc growth but in recent times specific genetic screens have been undertaken to identify genes required for growth control (reviewed in ref. 1 and 2). These screens have identified components of growth regulatory networks, e.g., insulin receptor or TOR that control the rate of cell growth, nutrient use, cell size and body size. Other genes modulate tissue growth by regulating cell proliferation and/or cell death (e.g., *myc*, *p53*, *hpo* and *sav*). Loss-of-function mutations in some of these genes cause tissue overgrowth, consequently they are termed tumor suppressor genes (TSGs). Inactivating mutations in *Drosophila* TSGs broadly fall into two classes, hyperplastic or neoplastic. Hyperplastic TSG mutations cause increased cell numbers but these cells maintain their epithelial characteristics and ultimately differentiate into adult structures. Loss-of-function mutations in the neoplastic TSGs leads to tissue overgrowth, along with a loss of apico-basal cell polarity and differentiation resulting in disrupted tissue architecture. The neoplastic TSGs fall into two classes, *Lgl*, *scrib* and *dlg* function to establish and/or maintain apico-basal cell polarity thereby influencing epithelial structure, the second class regulate endocytosis e.g., *avl* and *rab5*.<sup>1</sup>

**Key words:** *Drosophila*, tumor suppressor gene, cell polarity, Hippo pathway, Crb, Lgl, aPKC

**Abbreviations:** aPKC, atypical protein kinase C; Crb, crumbs; FERM, four-point one, ezrin, radixin, moesin; Hpo, Hippo; Lgl, lethal giant larvae; PDZ, PSD-95, discs large, ZO-1; RASSF, ras associated factor; Sav, Salvador; TSG, tumor suppressor gene; Wts, Warts; Yki, Yorkie

Submitted: 06/28/10

Accepted: 07/21/10

Previously published online:  
www.landesbioscience.com/journals/  
autophagy/article/13116

DOI: 10.4161/fly.4.4.13116

\*Correspondence to: Linda M. Parsons;  
Email: linda.parsons@petermac.org

Addendum to: Grzeschik NA, Parsons LM, Allott ML, Harvey KF, Richardson HE. Lgl, aPKC and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. *Curr Biol* 2010; 20:573–81; PMID: 20362447; DOI: 10.1016/j.cub.2010.01.055.

The recently discovered hyperplastic tumor suppressor pathway, Salvador/Warts/Hippo (SWH), controls both cell proliferation and cell death (reviewed in ref. 3). The SWH pathway comprises a kinase cascade: Hippo (Hpo) binds Salvador (Sav), a WW domain adaptor protein, to phosphorylate Warts (Wts), which in turn phosphorylates the transcriptional coactivator Yorkie (Yki), excluding it from the nucleus. When the SWH pathway is inactivated, Yki becomes dephosphorylated and translocates to the nucleus, resulting in the upregulation of cell proliferation drivers (*cycE* and *E2F1*) and inhibitors of cell death (*Drosophila inhibitor of apoptosis*, (*Diap1*) and *bantam* microRNA). The relationship between the neoplastic TSGs (or regulators of cell polarity) and cell proliferation or survival was unclear. Recently, our studies and two other groups have shown a link between the neoplastic TSG (*lgl*), regulators of apico-basal cell polarity (aPKC and Crb) and the SWH pathway.<sup>4-6</sup>

### Lgl, aPKC and Crb Regulate the SWH Pathway

We have previously shown that *lgl* mutant clones in the developing eye undergo ectopic cell proliferation and increased survival.<sup>7</sup> We investigated the basis of this and observed that *lgl* mutant tissue showed upregulation of SWH pathway target genes (*cycE* and *Diap1*) as well as pathway feedback targets *expanded* (*ex*) and *four-jointed* (*fj*). Consistent with this we also observed that the localization of Yki was more cytoplasmic/nuclear in *lgl* mutant clones and pYki (inactive Yki) levels were reduced in *lgl*-mRNA depleted (*act>lglRNAi*) eye discs. Importantly, when Yki activity was reduced in *lgl* mutant tissue cell proliferation, survival and the activation of SWH targets was also reduced, demonstrating that Yki activity is required for the effect of Lgl depletion on ectopic cell proliferation and survival.

The overexpression of aPKC or Crb mimics *lgl* loss-of-function (see below for details). In a parallel set of experiments, we demonstrated that the overexpression of aPKC or Crb resulted in the upregulation of SWH targets that were also

sensitive to the levels of Yki. Intriguingly, we found that Lgl/aPKC and Crb regulate SWH pathway activity by two distinct mechanisms; Lgl/aPKC activity regulates the localization of Hpo/RASSF (Ras associated factor) while Crb activity regulates the localization of Ex. Below we discuss the possible molecular mechanisms underpinning the interaction between the polarity regulators Lgl/aPKC and Crb, and the SWH pathway.

### Interactions of the Apical-basal Polarity Complexes—Lgl, aPKC and Crb

The establishment and maintenance of cell polarity is coordinated by a conserved network of interacting protein complexes. A detailed understanding of the regulation of these polarity protein complexes has emerged through the sustained efforts of several groups working in both flies and mammalian systems (reviewed in ref. 8). One of these complexes (Lgl, Scrib, Dlg) is localized at the septate junctions (basolateral junctions), which are located basal to the adherens junctions in epithelial cells. This complex antagonizes the activity of the apically localized aPKC and Crb complexes, which act to specify the apical membrane domain. aPKC can interact with several proteins including a scaffolding protein called Par-6. Par-6, via its PDZ (PSD-95 Discs large ZO-1) domain binds to either Lgl or Par-3 bringing them in contact with the kinase domain of aPKC to allow phosphorylation. Once Lgl is phosphorylated it dissociates from the membrane and enters the cytoplasm. Thus, Lgl is excluded from the apical membrane domain, thereby allowing the apical polarity complexes to specify apical identity. In the apical region of the membrane aPKC is found in a complex with Par-3 and Par-6. This complex, via Par-6, interacts with the PDZ binding motif (PBM) located within the C-terminal region of Crb resulting in aPKC-mediated phosphorylation of the intracellular domain of Crb.<sup>9</sup> Thus, aPKC has a dual role in cell polarity; it inactivates the basal polarity complex (Lgl, Scrib, Dlg) while being required for the activity of the apical Crb complex. How then do the Crb and Lgl protein complexes differentially

regulate SWH pathway activity and what is the role of aPKC in this?

### What Is the Relationship Between Cell Polarity and Tissue Growth?

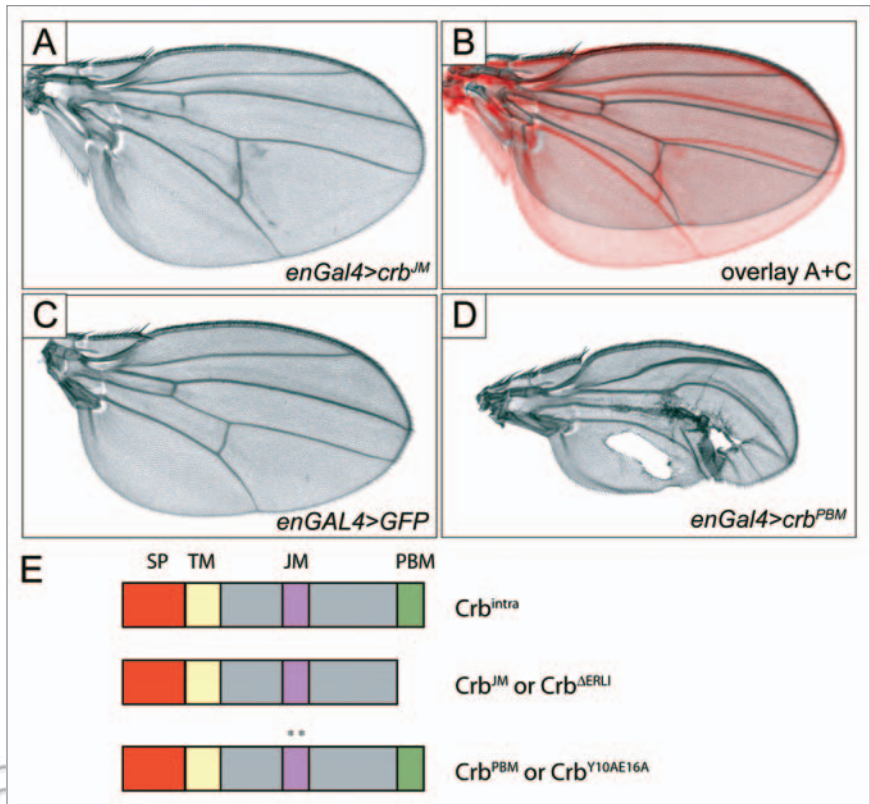
A key finding of both our work and the recent Robinson et al. (Moberg group) paper is that the polarity protein complexes regulate apico-basal cell polarity and tissue growth independently.<sup>5,7</sup> In the developing eye *lgl* mutant tissue undergoes ectopic cell proliferation and shows decreased developmental cell death without loss of apico-basal polarity, indicating that Lgl has separable roles in polarity versus proliferation and survival. The Moberg group also showed that Crb regulates tissue growth and polarity independently by dissecting the function of the Crb intracellular domain (Crb<sup>intra</sup>). Remarkably, a *crb* transgene encoding the transmembrane region and the 37 amino acid (aa) intracellular domain is sufficient to rescue *crb* null mutants,<sup>10</sup> (Crb is 2,189 aa) and when *crb*<sup>intra</sup> was expressed in wing discs (*en>crb*<sup>intra</sup>) it caused tissue overgrowth. Further analysis showed that this overgrowth was due to the downregulation of Ex protein and the concomitant upregulation of SWH targets (*ex*, *diap* and *bantam*).<sup>5</sup> The Crb intracellular region has two functional subdomains conserved from worms to humans.<sup>11</sup> The first 16 aa's are referred to as the juxta-membrane domain (JM) or FERM binding motif (FBM) as it has been shown to recruit the FERM domain containing proteins Yurt and DMOesin (DMoe).<sup>12,13</sup> The second domain is composed of the last four C-terminal residues (ERLI) and encodes the PBM, which binds Patj/Sdt to form the Crb polarity complex. Patj can then recruit the aPKC/Bazooka/Par-6 complex allowing aPKC to phosphorylate Crb on serine and threonine residues within the JM (FBM) domain.<sup>9</sup> Overexpression of a *crb* transgene that lacked the PBM domain *en>crb*<sup>JM</sup> also known as *crb*<sup>ΔERLI</sup>, see Fig. 1E) was sufficient to cause tissue overgrowth and activate the SWH pathway (Fig. 1A and C, see overlay Fig. 1B). This data demonstrated that the apico-basal polarity function of the Crb complex is not required to promote cell proliferation and survival via the SWH

pathway. However, expression of a Crb construct that contained an intact PBM domain but mutations in two amino acids in the JM (FBM) domain (Crb<sup>Y10AE16A</sup> also referred to as Crb<sup>PBM</sup>, see Fig. 1E) failed to induce tissue overgrowth or upregulate Yki transcriptional targets (*en>crb<sup>PBM</sup>*, Fig. 1D), suggesting that the 16 aa JM (FBM) subdomain of Crb mediates SWH signaling. Consistent with the above data, expression of a dominant negative aPKC construct (*aPKC<sup>CAAX-DN</sup>*) does not rescue tissue overgrowth induced by Crb<sup>intra</sup> expression, providing further evidence that aPKC/Bazooka/Par-6 and the Crb polarity complex regulate tissue growth and apico-basal polarity separately. In contrast, expression of *aPKC<sup>CAAX-DN</sup>* rescues tissue overgrowth and Hpo/RASSF mislocalization in *lgl* mutant tissue, further highlighting that Lgl/aPKC and Crb complexes regulate the SWH pathway by distinct mechanisms.

### How Does Lgl/aPKC Regulate the SWH Pathway?

We showed in larval eye discs that Hpo and RASSF were co-mislocalized in *lgl* clones, or when aPKC was overexpressed (*GMR>aPKC<sup>CAAX-WT</sup>*). RASSF competes with Sav for Hpo binding and therefore functions as an inhibitor of the Hpo kinase cascade.<sup>14</sup> We predict that mislocalized Hpo/RASSF forms an inactive SWH signaling complex and Yki fails to be phosphorylated leading to the activation of downstream target genes (Fig. 3A).

The question remains, what causes Hpo and RASSF to become mislocalized in *lgl* mutant tissue? Based on the mislocalization of Hpo<sup>M1</sup> mutant protein (which is predicted to encode a kinase dead protein), it was suggested that Hpo kinase activity might be required for correct Hpo localization.<sup>15</sup> However, localization of a kinase dead Hpo transgene expressed in eye discs (*GMR>hpo<sup>KD</sup>*) compared with wildtype Hpo (*GMR>hpo<sup>WT</sup>*) appears normal (Fig. 2A and B), indicating that kinase activity is not required for Hpo localization. Therefore, we believe that co-mislocalization of Hpo/RASSF by Lgl/aPKC is not simply due to reduced Hpo kinase activity, but mediated by deregulation of a specific biological process. A remaining issue that



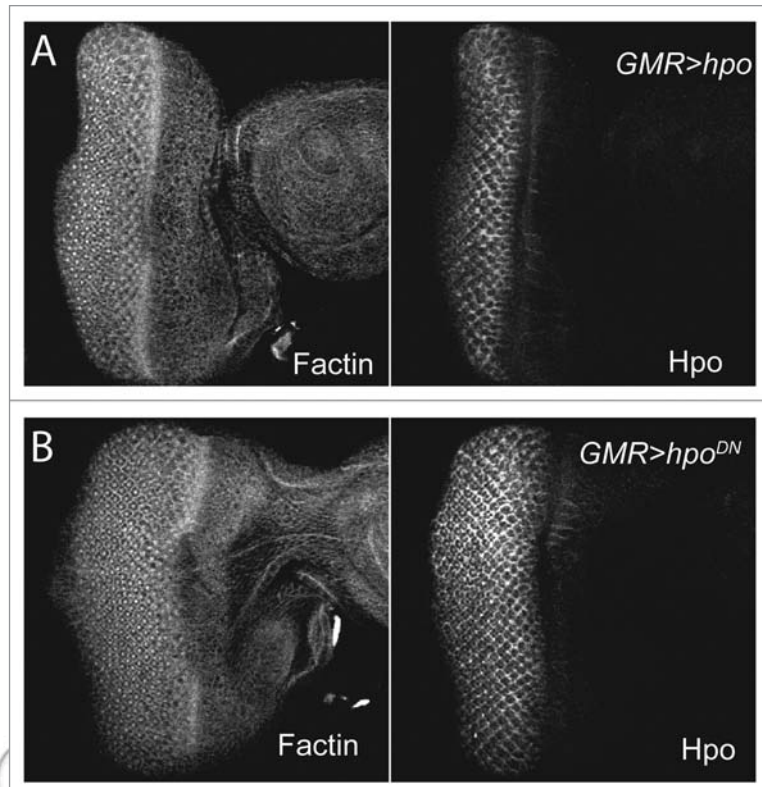
**Figure 1.** The JM domain of Crb is required for SWH pathway signaling. Images and overlay of *en>crb<sup>JM</sup>* and control *en>UASGFP* wings (A–C). Note that *en>crb<sup>JM</sup>* wings are larger than control wings indicating that the PBM is not required for tissue overgrowth. (D) Overexpression of a transgene that encodes a mutated JM domain (*en>crb<sup>PBM</sup>*) does not promote tissue overgrowth but induces polarity defects resulting in lost tissue. (E) Schematic representation of *crb* transgenes. Signal peptide (SP), transmembrane domain (TM), juxtamembrane FERM-binding motif (JM), PDZ-binding motif (PBM), and amino acid substitutions are indicated \*\* (reviewed in ref. 5 and 11).

needs to be addressed is does mislocalized Hpo<sup>M1</sup> alter RASSF localization, and does overexpression or mislocalization of RASSF drive Hpo mislocalization?

We predict that reduced Lgl activity leads to elevated levels of aPKC that result in the co-mislocalization of Hpo and RASSF. Consistent with this we demonstrated that expression of dominant negative aPKC in *lgl* mutant tissue was sufficient to restore Hpo and RASSF localization. It is possible that aPKC phosphorylates a key protein in the SWH signaling pathway. However, aPKC is unlikely to directly phosphorylate Hpo or RASSF as aPKC is not co-localized with RASSF and Hpo in *lgl* mutant tissue. The above data suggests that aPKC plays a fundamental role in the localization of Hpo/RASSF and regulation of the SWH pathway downstream of Lgl. However, it will be necessary to determine if expression

of *lgl* can restore mislocalization of Hpo/RASSF in *GMR>aPKC* eye discs to support the contention that aPKC acts downstream of Lgl.

Across mammalian and fly systems there are several recognized biological functions for aPKC, including regulation of NFκB and JNK activation (p62), cell migration/membrane dynamics (Cdc42/Rac1) and endocytosis (Cdc42/Arp2/3) (reviewed in ref. 16 and 17). It is possible that any of these mechanisms may link aPKC activity to Hpo and RASSF localization but perhaps the most promising link is endocytosis. Georgiou et al. showed that aPKC loss-of-function leads to accumulation of endosomes that contain the apical adherens junction protein E-cadherin, suggesting that aPKC normally plays a role in recycling of E-cadherin to the adherens junction.<sup>18</sup> aPKC appears to be acting via the Rho-family GTPase, Cdc42



**Figure 2.** Localization of Hpo is independent of kinase activity. Planar sections of larval eye discs. Posterior is to the left. *GMRGal4* was crossed to *UAS-hpo* (A), *UAS-hpo<sup>DN</sup>* (B) and stained for F-actin (Phalloidin) and Hpo ( $\alpha$ -Hpo). (A) Control *GMR>hpo* larval disc stained for F-actin and Hpo. Hpo localization is cytoplasmic. (B) *GMR>hpo<sup>DN</sup>* larval disc stained for F-actin and Hpo. Hpo localization is cytoplasmic and similar to control discs (A) suggesting that Hpo kinase activity is not required for localization.

(which directly binds aPKC) and the F-actin regulator WASP, to regulate E-cadherin protein localization by endocytosis.<sup>18–21</sup> While aPKC has been linked to E-cadherin trafficking, it is yet to be determined whether aPKC can affect the trafficking of other proteins, such as RASSF or Hpo. It is likely that SWH pathway regulation requires the correct localization and turnover of signaling complexes and we believe that active Hpo/Sav or inactive Hpo/RASSF complexes maybe regulated by endocytosis.

### How does Crb Regulate the SWH Pathway?

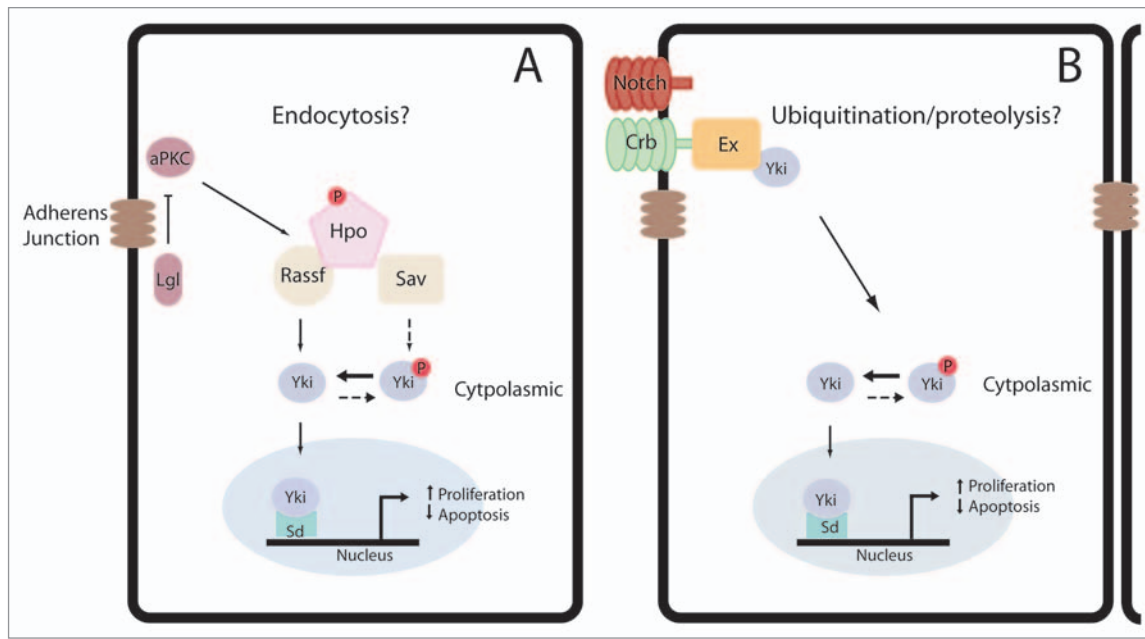
Robinson et al. showed that overexpression of *crb<sup>inttra</sup>* (*en>crb<sup>inttra</sup>*) resulted in reduced levels of Ex protein.<sup>5</sup> This data has recently been confirmed and extended by Duoia Pan's group, who also demonstrated that Ex directly binds the Crb<sup>JM</sup> (FBM) sequence via its FERM domain.<sup>6</sup>

The McNeill group has previously shown that the C-terminal region of Ex directly binds Yki. This interaction is proposed to sequester Yki in the cytoplasm, thereby preventing it from entering the nucleus and activating SWH pathway target genes.<sup>22</sup> As shown in **Figure 3B**, it is tempting to speculate that Crb/Ex/Yki form a tripartite complex and that reduced levels of Ex/Yki complexes increase the available pool of Yki that can enter the nucleus and upregulate the expression of SWH pathway targets.

As Crb overexpression results in activation of SWH target genes it would have been expected that *crb* loss-of-function would lead to reduced levels of SWH target gene expression. Surprisingly, Robinson et al. found that *crb* mutant tissue also resulted in overgrowth of the wing and a growth advantage of *crb* clones in the developing eye, along with increased expression of the SWH target gene, *Diap1*. This is consistent with other

studies showing that *crb* loss-of-function provides a proliferative advantage in eye disc clones without loss of cell polarity.<sup>23,24</sup> Robinson et al. also found that Ex protein was affected in *crb* mutant tissue. Although Ex protein levels were increased, Ex was mislocalized baso—laterally, suggesting that although Ex accumulates in *crb* mutants, since *crb* mutants is mislocalized it may be unable to effectively repress Yki activity. Furthermore, since a mutant allele of *crb* that lacks the PBM binding domain only shows mild defects relative to a *crb*-null mutant, Robinson et al. argue that the JM (FBM) domain may also be responsible for this effect of Crb on Ex accumulation and function. Similar to eye tissue, Duoia Pan's group showed that Crb was required to localize Ex apically in wing discs.<sup>6</sup> Ling et al. also used genomic engineering to generate *crb* alleles that contained mutations within the JM (FBM) domain known to disrupt FERM domain interactions (*crb<sup>V10API2AE16A</sup>* analogous to *crb<sup>PBM</sup>*) and an allele that lacked the PBM domain (*crb<sup>ΔERLI</sup>* equivalent to *crb<sup>JM</sup>*). As expected, Ex was localized normally in *crb<sup>ΔERLI</sup>* mutant tissue but Ex levels were increased and mislocalized basolaterally in *crb<sup>V10API2AE16A</sup>* clones. Thus both *crb* loss-of-function and *crb* overexpression deregulate SWH pathway signaling through different effects on Ex.

How can the different effects of *crb* loss or gain-of-function mutations on Ex protein levels and localization be explained? Robinson et al. showed that in *en>crb<sup>inttra</sup>* wing discs genetic reduction of proteasome activity with a dominant-negative allele of the proteasomal subunit *Pros2 $\beta$*  partially restored Ex:GFP levels. When *crb<sup>inttra</sup>* was expressed in S2 cells the presence of a proteasome inhibitor MG132 could partially restore Ex levels. In contrast, Ex levels remained low in S2 cells expressing Crb<sup>inttra</sup> in the presence of the lysosomal inhibitor chloroquine. These data hint that ubiquitin mediated proteasome proteolysis but not endocytosis may play a role in the regulation of Ex levels. However, it should be noted that although ubiquitin is best recognized for its role in protein degradation, it is now well established that it can play nonproteolytic roles within the cell, such as membrane trafficking, protein oligomerization and protein



**Figure 3.** (A) Lgl/aPKC regulates Yki activity by promoting the formation of Hpo/RASSF complexes. Lgl/aPKC regulate the SWH pathway by favoring the formation of inactive Hpo/Rassf over active Hpo/Sav protein complexes. Hpo/Rassf are unable to phosphorylate Yki. Consequently, pYki levels are reduced and dephosphorylated Yki enters the nucleus to form a complex with Scalloped (Sd) and activate SWH pathway targets. Perhaps the mislocalization and formation of Hpo/Rassf complexes is due to disruptions in endocytosis. (B). Crb regulates Yki activity by reducing the levels of Ex/Yki complexes. Crb/Ex/Yki form a tripartite complex and Yki is sequestered in the cytoplasm and prevented from entering the nucleus by binding to Expanded (Ex). The overexpression of Crb reduces Ex/Yki complexes, freeing Yki to enter the nucleus, interact with Scalloped (Sd) and activate SWH target genes. It is possible that Ex/Yki complexes are regulated by ubiquitination and/or proteolysis. *crb* mutant tissue also shows overgrowth due to deregulation of Notch endocytosis but the influence of Notch signaling on SWH pathway targets remains to be determined.

kinase activation (reviewed in ref. 25). The precise mechanism of Crb function in Ex localization/stability remains to be elucidated and it is possible that Ex may be regulated by multiple mechanisms including ubiquitin, protein degradation and membrane trafficking. It is important to clarify these issues as the *crb* mutant data suggests *crb* acts as a tumor suppressor while the overexpression data supports *crb* behaving as an oncogene.

Previous studies have shown that the Crb<sup>JM</sup> (FBM) domain indirectly interacts with another FERM domain protein DMOESIN via  $\beta$ H-spectrin.<sup>13</sup> Thus, linking Crb to the actin-spectrin cytoskeleton and raising the possibility that Crb may also recruit Ex to the cortical actin cytoskeleton via a similar mechanism. The interaction of Crb with Moe is intriguing and Robinson et al. provided evidence that Moe may also be linked to the SWH pathway, since depletion of Moe reduced Yki target gene expression and the overgrowth phenotype of Crb<sup>intra</sup> (*en>crb<sup>intra</sup>; moe-RNAi*). Thus, downregulation of

Moe promotes SWH activity and prevents SWH target gene expression, whereas reduced levels of Ex inactivate the SWH pathway and upregulate target gene expression. Robinson et al. also showed that Ex levels are still reduced in wings discs overexpressing Crb and depleted of Moe mRNA (*en>crb<sup>intra</sup>; moe-RNAi*), suggesting that Moe may act downstream of Crb/Ex activity to modulate SWH pathway target gene expression. Clearly, regulation of the SWH pathway via Crb<sup>JM</sup> (FBM) domain is more complicated than initially envisaged and whether or not Moe is downstream of Ex or in parallel and how this influences Yki activity will require further investigation.

It is also interesting to note that Pichaud et al. show that Crb activity regulates the size of the developing head and eye tissue.<sup>24</sup> Under circumstances where *crb* mutant clones are generated in a background where the wild-type tissue dies (as a result of a *Minute* mutation or *GMR-hid*, *cell lethal* background), *crb* mutant tissue overgrows so that the adult

head is approximately 1.3X larger than wild-type. They showed that *crb* mutant tissue overgrowth was not due to reduced apoptosis but increased cell proliferation. In stark contrast to the Moberg group, these authors were able to fully rescue the *crb* head overgrowth phenotype with a *crb* transgene encoding the extracellular domain and transmembrane domain, but lacking the intracellular domain, indicating that in hyper-proliferating tissue the Crb<sup>extra</sup> domain is required to restrict tissue growth. Whether this represents a Crb/Crb interaction, or involves unidentified proteins on the cell surface of neighboring cells remains to be determined. However, these authors provided evidence that Crb acts to limit the endocytosis of Notch, thus restricting growth by reducing Notch activity. This is consistent with previous studies showing that Crb suppresses  $\gamma$ -secretase activity, which is required for the activation of Notch.<sup>26</sup> Importantly, this study provides evidence that Crb may have a role in modulating endocytosis at the apical membrane. Unfortunately,

these authors did not examine SWH targets, but it is tempting to speculate that in addition to regulating Notch signaling, Crb mediated regulation of apical endocytosis may also have a role in modulating Ex levels and localization. Interestingly, Crb is not required for the accumulation of Fat or Epidermal Growth Factor receptors observed in *wts* mutant tissue, suggesting that Crb is not a general regulator of apical endocytosis but may have a specific role in trafficking particular membrane proteins.<sup>15,27</sup> How Crb regulates Ex localization and/or levels remains to be elucidated and will require understanding how Crb specifically regulates proteolysis or endocytosis in different biological contexts.

Taken together the Moberg, Pan and Pichaud studies reveal that Crb regulates tissue growth during development via multiple mechanisms. In rapidly dividing tissues or regenerative contexts the Crb extracellular domain may interact with key cell/cell proteins to coordinate proliferation and epithelial integrity, while the intracellular domain may be required to regulate developmental or homeostatic growth via the SWH pathway. Further studies are necessary to determine how the regulation of tissue growth by Crb extracellular and intracellular domains is integrated, and whether Notch signaling can also be linked to SWH pathway regulation.

### Concluding Remarks

The link between neoplastic tumor suppressor mutants and tissue growth has remained elusive. We and two other groups have shown that Lgl/aPKC and Crb regulate tissue growth via the SWH pathway independently of their roles in cell polarity. Upstream regulation of the core components of the SWH pathway is the focus of much enquiry. Our results linking apico-basal polarity regulators to the SWH pathway adds to the complexity of SWH regulation by upstream components such as the atypical cadherins *Ft* and *Ds*, which also play roles in planar cell polarity, and by Dpp signaling (reviewed in ref. 28). In addition, further cross-talk within the SWH pathways occurs since the heparan sulfate proteoglycans, Dally and Dally-like, which modulate Dpp,

Hh and Wg morphogen signaling, were recently found to be targets of Yki.<sup>29</sup> How Lgl/aPKC and Crb are linked to these and to the recently discovered Kibra protein, a WW domain protein that complexes with both Mer and Ex to recruit the core SWH pathway proteins to the apical membrane for activation remains to be determined (reviewed in ref. 28). The next few years should begin to reveal the molecular mechanisms underlying interactions between the SWH pathway and the apical-basal cell polarity regulators in developing tissues undergoing normal proliferation, as well as within the context of wound healing and tumor growth.

### Acknowledgements

We would like to thank Nathalie Martinek for comments on the manuscript. Eli Knust for *UAS-crb<sup>ΔDERL1</sup>* (also referred to as *UAS-crb<sup>JM</sup>* FBal0103869) and *UAS-crb<sup>Y10AE16A</sup>* (also referred to as *UAS-crb<sup>PBM</sup>* FBal0103862) Georg Halder for *UAS-hpo<sup>WT</sup>* (FBal0191267) and *UAS-hpo<sup>K71R</sup>* hereafter referred to as *UAS-hpo<sup>DN</sup>* (FBal0190115) stocks. Nic Tapon for Hpo66 antibody.

### References

- Hariharan IK, Bilder D. Regulation of imaginal disc growth by tumor-suppressor genes in *Drosophila*. *Annu Rev Genet* 2006; 40:335-61.
- Wodarz A, Näthke I. Cell polarity in development and cancer. *Nat Cell Biol* 2007; 9:1016-24.
- Badouel C, Garg A, McNeill H. Herding Hippos: regulating growth in flies and man. *Current Opinion in Cell Biology* 2009; 21:837-43.
- Grzeschik NA, Parsons L, Allott M, Harvey KF, Richardson HE. Lgl, aPKC and Crumbs regulate the Salvador/Warts/Hippo pathway through two distinct mechanisms. *Curr Biol* 2010; 20:573-81.
- Robinson B, Huang J, Hong Y, Moberg K. Crumbs regulates Salvador/Warts/Hippo signaling in *Drosophila* via the FERM-domain protein expanded. *Curr Biol* 2010; 20:582-90.
- Ling C, Zheng Y, Yin F, Yu J, Huang J, Hong Y, et al. The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. *Proc Natl Acad Sci USA* 2010; 1-6.
- Grzeschik NA, Amin N, Secombe J, Brumby AM, Richardson HE. Abnormalities in cell proliferation and apico-basal cell polarity are separable in *Drosophila* *lgl* mutant clones in the developing eye. *Dev Biol* 2007; 311:106-23.
- Assémat E, Bazellières E, Palesi-Pocachard E, Le Bivic A, Massey-Harroche D. Polarity complex proteins. *Biochim Biophys Acta* 2008; 1778:614-30.
- Sotillos S, Díaz-Meco MT, Caminero E, Moscat J, Campuzano S. DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in *Drosophila*. *J Cell Biol* 2004; 166:549-57.
- Wodarz A, Hinz U, Engelbert M, Knust E. Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* 1995; 82:67-76.

- Izaddoust S, Nam SC, Bhat MA, Choi HJBK. *Drosophila* Crumbs is a positional cue in photoreceptor adherens junctions and rhabdomeres. *Nature* 2002; 416:178-82.
- Laprise P, Beronja S, Silva-Gagliardi N, Pellikka M, Jensen A, Mcglade C, et al. The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size. *Dev Cell* 2006; 11:363-74.
- Médina E, Williams J, Klipfell E, Zarnescu D, Thomas G, Le Bivic A. Crumbs interacts with moesin and beta(Heavy)-spectrin in the apical membrane skeleton of *Drosophila*. *J Cell Biol* 2002; 158:941-51.
- Polesello C, Huelsmann S, Brown NH, Tapon N. The *Drosophila* RASSF homolog antagonizes the hippo pathway. *Curr Biol* 2006; 16:2459-65.
- Genevet A, Polesello C, Blight K, Robertson F, Collinson LM, Pichaud F, et al. The Hippo pathway regulates apical-domain size independently of its growth-control function. *J Cell Sci* 2009; 122:2360-70.
- Moscat J, Diaz-Meco MT, Albert A, Campuzano S. Cell signaling and function organized by PBI domain interactions. *Mol Cell* 2006; 23:631-40.
- Atsushi Suzuki KAASO. Protein Kinase Ci (PKCi): A PKC isotype essential for the development of multicellular organisms. *J Biochem* 2003; 133:9-16.
- Georgiou M, Marinari E, Burden J, Baum B. Cdc42, Par6 and aPKC regulate Arp2/3-mediated endocytosis to control local adherens junction stability. *Curr Biol* 2008; 18:1631-8.
- Joberty G, Petersen C, Gao L, Macara IG. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat Cell Biol* 2000; 2:531-9.
- Leibfried A, Fricke R, Morgan MJ, Bogdan S, Bellaiche Y. *Drosophila* Cip4 and WASp define a branch of the Cdc42-Par6-aPKC pathway regulating E-cadherin endocytosis. *Curr Biol* 2008; 18:1639-48.
- Harris KR, Tepass U. Cdc42 and Par proteins stabilize dynamic adherens junctions in the *Drosophila* neuroectoderm through regulation of apical endocytosis. *J Cell Biol* 2008; 183:1129-43.
- Caroline Badouel LG, Nancy Amin, Ankush Garg, Robyn Rosenfeld, Thierry Le Bihan, Helen McNeill. The FERM-domain protein expanded regulates Hippo pathway activity via direct interactions with the transcriptional activator Yorkie. *Developmental Cell* 2009; 16:411-20.
- Pellikka M, Tanentzapf G, Pinto M, Smith C, McGlade CJ, Ready DF, et al. Crumbs, the *Drosophila* homologue of human CRB1/RP12, is essential for photoreceptor morphogenesis. *Nature* 2002; 416:143-9.
- Richardson EP, Pichaud F. Crumbs is required to achieve proper organ size control during *Drosophila* head development. *Development* 2010; 137:641-50.
- Chen ZJ, Sun LJ. Nonproteolytic functions of ubiquitin in cell signaling. *Mol Cell* 2009; 33:275-86.
- Herranz H, Stamatakis E, Feiguin F, Milán M. Self-refinement of Notch activity through the transmembrane protein Crumbs: modulation of gamma-secretase activity. *EMBO Rep* 2006; 7:297-302.
- Hamaratoglu F, Gajewski K, Sansores-Garcia L, Morrison C, Tao C, Halder G. The Hippo tumor-suppressor pathway regulates apical-domain size in parallel to tissue growth. *J Cell Sci* 2009; 122:2351-9.
- Grusche FA, Richardson HE, Harvey KF. Upstream regulation of the Hippo size control pathway. *Current Biology* 2010; 20:R574-82.
- Baena-Lopez LA, Iguez IR, Baonza A. The tumor suppressor genes dachsous and fat modulate different signalling pathways by regulating dally and dally-like. *Proc Natl Acad Sci USA* 2008; 105:9645-50.