The cytoskeletal motor proteins Dynein and MyoV direct apical transport of Crumbs

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ABSTRACT

Crumbs (Crb in Drosophila; CRB1-3 in mammals) is a transmembrane determinant of epithelial cell polarity and a regulator of Hippo signalling. Crb is normally localized to apical cell-cell contacts, just above adherens junctions, but how apical trafficking of Crb is regulated in epithelial cells remains unclear. We use the Drosophila follicular epithelium to demonstrate that polarized trafficking of Crb is mediated by transport along microtubules by the motor protein Dynein and along actin filaments by the motor protein Myosin-V (MyoV). Blocking transport of Crb-containing vesicles by Dynein or MyoV leads to accumulation of Crb within Rab11 endosomes, rather than apical delivery. The final steps of Crb delivery and stabilisation at the plasma membrane requires the exocyst complex and three apical FERM domain proteins – Merlin, Moesin and Expanded – whose simultaneous loss disrupts apical localization of Crb. Accordingly, a knock-in deletion of the Crb FERM-binding motif (FBM) also impairs apical localization. Finally, overexpression of Crb challenges this system, creating a sensitized background to identify components involved in cytoskeletal polarization, apical membrane trafficking and stabilisation of Crb at the apical domain.

1. Introduction

Cell polarity is a fundamental characteristic of living organisms. The molecular determinants of cell polarity have been revealed through pioneering genetic screens in the yeasts S. cerevisae and S. pombe, the worm C. elegans, and the fruit fly Drosophila melanogaster (St Johnston and Ahringer, 2010; Tepass, 2012; Thompson, 2013). In yeast, the small GTPase Cdc42 was discovered to be a fundamental determinant of cell polarity (Adams et al., 1990), localizing to one pole of the cell through a positive feedback loop of self-recruitment (Johnson et al., 2011; Martin, 2015; Slaughter et al., 2009). Two general mechanisms for Cdc42-driven positive feedback were identified in yeast: (1) oligomeric clustering of Cdc42 complexes (Altschuler et al., 2008; Bendeau et al., 2015; Irazoqui et al., 2003) and (2) actin cytoskeleton mediated delivery of Cdc42 containing vesicles by a Myosin motor protein (Lechler et al., 2000; Wedlich-Soldner et al., 2003) in S. cerevisiae or microtubule mediated transport of polarizing factors in S. pombe (Martin and Arkowitz, 2001; Shahab et al., 2015; Tepass et al., 1990). Baz and Crb act in parallel to maintain polarity determinants, is responsible for directing polarity remains a fundamental unsolved problem.

Drosophila epithelial cells exhibit a more complex polarization than oocytes, with the plasma membrane divided into distinct apical and basolateral domains, separated by a ring of adherens junctions (Gibson and Perrimon, 2003; St Johnston and Ahringer, 2010; Tepass, 2012; Thompson, 2013). Like oocytes, epithelial cells express the cortical polarity determinant Par-3/Bazooka (Baz), which is polarized through oligomeric clustering at the plasma membrane (Benton and St Johnston, 2003a; b; Harris, 2017; Krahn et al., 2010; McKinley et al., 2012; Mizuno et al., 2003). In addition, epithelial cells express a second apical polarity determinant, Crumbs (Crb) (Bazellieres et al., 2018; Campbell et al., 2012; Petronczki and Knoblich, 2001; Shahab et al., 2015; Tanentzapf and...
Tepass, 2003). The Cdc42-Par6-aPKC complex promotes Crb polarization, forming a positive feedback loop whose nature is still not fully understood (Fletcher et al., 2012; Harris and Tepass, 2008). The redundancy between Baz and Crb makes it possible to study Crb polarization in isolation, because defects in Crb localization to the apical domain do not disrupt the overall apical-basal polarization of most epithelial cells, and instead disrupts Hippo signalling (Chen et al., 2010; Fletcher et al., 2012, 2015; Ling et al., 2010) – indeed the crucial requirement for Crb in epithelial polarity occurs during embryonic gastrulation (Campbell et al., 2009; Grawe et al., 1996), when Baz is localized to adherens junctions in a planar polarized fashion during germ-band extension (Simoës Sde et al., 2010; Zallen and Wieschaus, 2004).

We previously proposed a model of Crb localization via Cdc42-dependent positive feedback, based on analysis of overexpressed Crb in Drosophila follicle cells (Fletcher et al., 2012). In this model, Crb – a transmembrane protein – is delivered apically from Rab11 endosomes (Blankenship et al., 2007; Li et al., 2007; Roeth et al., 2009) and engages in oligomeric clustering via its extracellular domain, as well as interacting via its cytoplasmic domain with the PDZ domain protein Stardust/PALS1 [Sdt (Bachmann et al., 2001; Hong et al., 2001; Kast et al., 1993; Muller and Wieschaus, 1996; Tepass and Knust, 1993)] and the FERM domain proteins Moein [Moe (Medina et al., 2002; Polesello et al., 2004) and Tiam1 (Cao et al., 2010; Ling et al., 2010)] which both function in directing membrane trafficking of the Crb protein from Rab11 endosomes (Fletcher et al., 2012; Roeth et al., 2009) to the apical domain of the ovarian follicle cell epithelium. Accordingly, disruption of microtubule polarization and/or the actin cytoskeleton impairs Crb localization to the apical domain. We confirm that the Exocyst complex then promotes the final step in delivery of Crb to the plasma membrane, as previously demonstrated in the embryo (Blankenship et al., 2007). Crb must then interact with apical FERM-domain proteins via its FERM-binding motif to be stabilized at the plasma membrane. Finally, we find that overexpression of Crb challenges this system of polarized transport and exocytic delivery, acting as a sensitized background for the testing of molecular mechanisms involved in Crb polarization.

2. Results

We began by re-examining the requirement for the microtubule motor protein Dynein in regulation of Crb in the Drosophila ovarian follicle cell epithelium. Previously, loss of Dynein was reported to cause decreased levels of Crb protein in follicle cells due to failure of crb or sdt mRNA transport (Cao et al., 2017; Horne-Badovinac and Bilder, 2008; Li et al., 2008). Using a Crb-GFP knockin line, we find that, in addition to reduced Crb protein at the apical membrane domain, Crb also accumulates basally in Rab11 endosomes upon silencing of Dynein expression by conditional expression of UAS-dynein-RNAi hairpins with the follicle cell-specific trafficjam.Gal4 (tj.Gal4) driver (Fig. 1A,B; Fig. 1-S1). We find similar results with antibody staining for Crb, which localises basally under clonal expression of actin > flpout > Gal4 UAS-dynein-RNAi hairpins but not in neighbouring wild-type follicle cells (Fig. 1C). Co-staining for Crb and Rab11 in wild-type and dynetin-RNAi expressing follicle cells reveals that Crb protein accumulates within Rab11-positive endosomes (Fig. 1D). These results suggest that upon loss of the microtubule minus-end directed motor protein Dynein, Rab11 endosomes containing Crb protein are transported basally, rather than apically.

The basal transport of endosomes is normally mediated by the plus-end directed microtubule motor protein Kinesin (Mellman and Nelson, 2008; Mostov et al., 2006; Nelson, 1991, 2003; Rodriguez-Boulan and Macara, 2014; Weiss and Rodriguez-Boulan, 2009). We therefore sought to confirm that, in the absence of Dynein, Kinesin is responsible for basal transport of Crb, using the Gal4-UAS system. We find that the basal transport of Crb-GFP in dynein-RNAi expressing follicle cells is disrupted by simultaneous expression of kinesin-RNAi, leading to an abnormal depolarised localization of Crb (Fig. 2A–D). Note that co-expression of UAS-driven dynetin-RNAi with a UAS-driven GFP does not alter the dynein-RNAi phenotype, which rules out a dilution effect of the GAL4 transcription factor. These results confirm that both Dynein and Kinesin are capable of transporting Crb containing Rab11-positive endosomes,
Fig. 1. Dynein is required to traffic Crumbs protein apically in the Drosophila follicle cell epithelium
A) Endogenously tagged knockin Crb-GFP localises apically in the follicle cell epithelium (stage 7/8 egg chamber; scale bar approximately 10 μm).
B) Expression of Dynein hairpin RNAi throughout the follicle cell epithelium with tj.Gal4 causes relocalisation of Crb-GFP to the basal surface of follicle cells. Anti-GFP antibody staining is shown (stage 7/8 egg chamber; scale bar approximately 10 μm).
C) Clonal expression of Dynein hairpin RNAi, marked by expression of nls-GFP, which causes cell-autonomous relocalisation of Crb to the basal surface of follicle cells within the clone (stage 7/8 egg chamber; scale bar approximately 10 μm).
D) Co-immunostaining for Crb and Rab11 upon clonal expression of Dynein hairpin RNAi, marked by expression of nls-GFP, reveals co-localization within basal endosomes (stage 7/8 egg chamber; scale bar approximately 10 μm). Asterisks indicate a clone of cells expressing UAS.Dynein-RNAi.
but that under normal conditions the Dynein-dependent transport is more efficient, leading to apical localization of Rab11 endosomes and delivery of the Crb protein to the apical plasma membrane domain.

In addition to transport along microtubules by the Dynein motor, it is conceivable that Crb may also be transported along actin filaments by motors such as Myosin V (MyoV), which has been previously shown to form a complex with Crb and to promote apical secretion from Rab11 endosomes in the Drosophila photoreceptor (Li et al., 2007; Pocha et al., 2011). Class V Myosins were originally discovered in the yeast S. cerevisiae to promote polarized transport along F-actin filaments (Johnston et al., 1991; Pruyne et al., 1998, 2004), alongside Class I Myosins (Anderson et al., 1998; Goodson et al., 1996; Lechler et al., 2000, 2001; Wedlich-Soldner et al., 2003), suggesting possible redundancy between Class I and Class V Myosins. We find that ectopically expressed MyoV-GFP primarily localises apically in the follicle cell epithelium, while a dominant-negative form of MyoV named MyoV-GT-GFP, which retains cargo-binding activity but cannot transport it (Krauss et al., 2009), fails to localise apically and instead localises on endosomes (Fig. 3A,B). Use of the dominant-negative MyoV-GT-GFP thus circumvents any possible redundancy between Drosophila Myosins. Accordingly, ectopic expression of MyoV-GT-GFP prevents delivery of Crb to the apical membrane, such that the Crb protein is trapped inside

![Fig. 2. Dynnein loss is partially rescued by Kinesin-RNAi.](image-url)

A) Endogenously tagged knockin Crb-GFP localises apically in the follicle cell epithelium (stage 7 egg chamber; scale bar approximately 10 μm).

B) Expression of Dynnein hairpin RNAi throughout the follicle cell epithelium with $\hat{g}.Gal4$ causes relocalisation of Crb-GFP to the basal surface of follicle cells (stage 7 egg chamber; scale bar approximately 10 μm).

C) Expression of Kinesin hairpin RNAi throughout the follicle cell epithelium with $\hat{g}.Gal4$ does not affect localization of Crb-GFP in follicle cells (stage 7 egg chamber; scale bar approximately 10 μm).

D) Expression of both Dynnein and Kinesin hairpin RNAi throughout the follicle cell epithelium with $\hat{g}.Gal4$ does partially rescue the effect of Dynnein RNAi on localization of Crb-GFP in follicle cells. Anti-GFP antibody staining is shown (stage 7 egg chamber; scale bar approximately 10 μm).
large endosomes that reside just underneath the apical domain (Fig. 3C–E). The apical localization of these enlarged Crb-positive endosomes is completely disrupted upon co-expression of UAS.dynein-RNAi, which leads to basal localization in cells that are still polarized, or to random distribution in those cells that have been extruded (Fig. 3F–H), or by depolymerization of microtubules with Colchicine (Fig. S1B). Co-staining of Crb and Rab11 confirms that these Crb-containing endosomes are indeed Rab11-positive (Fig. 3F,G). These results confirm a dual requirement for transport of the Crb protein by F-actin and microtubule motor proteins.

Given our findings with MyoV, we sought to confirm that the F-actin cytoskeleton is also necessary for apical localization of Crb. As expected, disruption of F-actin by acute treatment with Latrunculin A (Lat A) caused a strong loss of MyoV-GFP and Crb-GFP from the apical domain, with Crb-GFP accumulating in endosomes (Fig. 4A–F). Treatment with another F-actin cytoskeleton disrupting compound, Cytochalasin D (Cyto D), had a similar effect on Crb-GFP localization (Fig. 4G). These results confirm the essential requirement for the F-actin cytoskeleton in polarization of Crb.

We next sought to examine the role of the apically-localized FERM domain proteins – Merlin (Mer), Expanded (Ex) and Moesin (Moe). FERM domains link the actin cytoskeleton to the plasma membrane (Chishti et al., 1998) and bind directly to spectrins (Baines et al., 2014), which are required to polarize microtubules and regulate the Hippo pathway in Drosophila (Fletcher et al., 2015; Khanal et al., 2016). Mer and Ex are redundantly required to regulate the Hippo signaling pathway in Drosophila, a parallel function that may arise from the different subcellular localizations of Mer, which is found across the apical surface, and Ex, which localises to the sub-apical junction through direct interaction with the Crb intracellular domain (Fletcher et al., 2015; Hamaratoglu et al., 2006; Su et al., 2017). Moe has important roles in linking cortical F-actin to the plasma membrane, particularly during mitotic cell rounding and microvilli formation (Carreno et al., 2008; Fehon et al., 2010; Kunda et al., 2008; Sauvanet et al., 2015). We find that mutation of moe does not affect Crb localization in follicle cells, similar to mer, ex double mutants (Fig. 5A,B). Double mutants of mer, moe also have no effect on Crb (Fig. 5C). However, triple mutant clones of mer, ex, moe do lead to a strong disruption of Crb localization (Fig. 5D). Accordingly, knock-in deletion of the Crb FERM-binding motif (FBM) (Huang et al., 2009) also reduces the apical localization of Crb in follicle cells (Fig. 5E,F). These findings demonstrate that the apical FERM domain proteins are collectively required to promote Crb polarization, a role that is consistent with their molecular functions in polarising the cytoskeleton in epithelia and with their ability to directly bind the Crb intracellular domain.

Once Crb has been successfully trafficked to the apical membrane of the cell, it must be delivered to the plasma membrane through a process of regulated exocytosis. The exocyst complex was discovered to mediate regulated exocytosis in the yeast S. cerevisiae (He and Guo, 2009; Novick et al., 1980; TerBush et al., 1996). The exocyst subunit Sec15 directly interacts with the Cdc42-mediated polarity establishment complex in yeast (Franz et al., 2006). In mammalian epithelial cells in culture, the exocyst associates with adherens junctions, tight junctions (Yeaman et al., 2004), and Par3 (Ahmed and Macara, 2017). In Drosophila epithelia, the exocyst component Exo84 is required for apical trafficking of Crb from Rab11 endosomes to the plasma membrane in embryos (Blankenship et al., 2007); Sec6 is required for apical exocytosis in Drosophila photoreceptors (Beronja et al., 2005); and Sec5 is required for...
efficient delivery of E-cadherin to adherens junctions in the pupal notum (Langevin et al., 2005) but is not required for cytoskeletal polarization in follicle cell epithelium (Murthy and Schwarz, 2004). We therefore tested whether Sec15 and Sec5 were required for apical localization of Crb in the follicle cell epithelium. We find that mutants clones for sec15 or sec5 strongly disrupt the apical localization of Crb (Fig. 5G–J). These findings confirm an essential requirement for the exocyst in delivery of Crb to the apical membrane in the follicular epithelium.

Finally, we sought to challenge the system of polarized Crb exocytosis by overexpressing full-length Crb protein in the follicle cell epithelium. We find that this results in ectopic localization of Crb (Fig. 5G–J). These findings confirm an essential requirement for the exocyst in delivery of Crb to the apical membrane in the follicular epithelium.

Fig. 4. The F-actin cytoskeleton, which concentrates apically, is required for apical localization of both MyoV and Crb.
A) Control (DMSO-treated) egg chamber stained for F-actin with phalloidin (n > 9 stage 7/8 egg chambers; scale bar approximately 10 μm).
B) Latrunculin A treated egg chamber stained for F-actin with phalloidin (n > 10 stage 7/8 egg chambers; scale bar approximately 10 μm).
C) Control (DMSO-treated) egg chamber expressing MyoV-GFP with tj.Gal4 (n > 10 stage 7/8 egg chambers; scale bar approximately 10 μm). C’ shows zoom image.
D) Latrunculin A treated egg chamber expressing MyoV-GFP with tj.Gal4 shows loss of MyoV-GFP apical localization (n > 12 stage 7/8 egg chambers; scale bar approximately 10 μm). D’ shows zoom image.
E) Control (DMSO-treated) Crb-GFP egg chamber showing normal apical localization of Crb in follicle cells (n > 7 stage 7/8 egg chambers; scale bar approximately 10 μm). E’ shows zoom image.
F) Latrunculin A treated Crb-GFP egg chamber showing loss of apical Crb localization and localization to endosomal punctae (n > 5 stage 7/8 egg chambers; scale bar approximately 10 μm). F’ shows zoom image.
G) Cytochalasin D treated Crb-GFP egg chamber showing loss of apical Crb localization and localization to endosomal punctae (n > 7 stage 7/8 egg chambers; scale bar approximately 10 μm). G’ shows zoom image.

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wild-type background, such as patronin or shot. The assay further highlights the key role of adherens junctions, whose disruption with alpha-catenin-RNAi strongly affects the F-actin cytoskeleton and causes a striking endosomal accumulation of overexpressed Crb (Fig. 6B). The assay similarly reveals a key role for the Crb extracellular domain, whose loss leads to strong accumulation of the overexpressed Crb transmembrane and intracellular domain (CrbICD) in endosomes, even though endogenously-expressed CrbICD exhibits only a moderate reduction in apical localization (Fig. 6C–F). Thus, Crb overexpression in follicle cells is a highly sensitized assay for detecting components of the Crb trafficking and polarization machinery, even where such components exhibit redundancy or degeneracy with others or have pleiotropic phenotypes. Novel genes identified in this assay should then be examined for their effect on endogenous Crb in double and triple mutant combinations with other redundantly required genes. A summary of the key components of cytoskeletal polarization and Crb trafficking is shown schematically in the final diagram (Fig. 6F).
3. Discussion

Polarization of the cytoskeleton is a universal feature of columnar epithelial cells that determines polarized membrane trafficking of many cargo proteins and is known to depend on fundamental determinants of epithelial polarity (Mellman and Nelson, 2008; Mostov et al., 2000; Nelson, 1991, 2003; Rodriguez-Boulan and Macara, 2014; Weisz and Rodriguez-Boulan, 2009). Whether the localization of apical-basal polarity determinants themselves requires cytoskeletal polarization has been uncertain, particularly because apical-basal polarization of the Baz/Par-3 system can arise without cytoskeletal polarization or membrane trafficking in asymmetrically dividing Drosophila neuroblasts or C. elegans zygotes (Halbgut et al., 2011; St Johnstone and Ahiringer, 2010; Tepass, 2012; Thompson, 2013). Furthermore, disruption of microtubules or preventing polarization of microtubules does not strongly interfere with polarization of the plasma membrane in S. cerevisiae (Martin and Arkowitz, 2014), in the Drosophila follicle cell epithelium (Khanal et al., 2016) or in cultured mammalian epithelial cells (Noordstra et al., 2016; Toya et al., 2016). In the case of Crb, an apical polarity determinant and transmembrane protein that moves through the secretory pathway via Rab11 endosomes and the exocyst to reach the apical domain (Blankenship et al., 2007; Fletcher et al., 2012; Li et al., 2007; Roeth et al., 2009) (Fig. S1), it was previously unclear whether polarized cytoskeletal transport was in fact required for apical membrane trafficking and localization of the Crb protein, rather than Crb or sdt mRNA (Cao et al., 2017; Horne-Badovinac and Bilder, 2008; Li et al., 2008).

Our results demonstrate that cytoskeletal polarization and directed motor transport of Crb protein are necessary for its localization to the apical domain of the follicular epithelium. Disruption of either Dynein-mediated microtubule transport or MyoV-mediated actin transport leads to trapping of Crb in endosomes, such that it is unable to reach the apical domain (Figs. 1–3), without obviously affecting overall polarization of aPKC (Fig. S1). Microtubules are polarized along the apical-basal axis of epithelial cells, with minus ends apical, such that loss of either of these microtubules, but appear unable to traverse the thick cortical F-actin at the apical surface to reach the plasma membrane (Figs. 3 and 4). Our findings confirm and extend previous work demonstrating that mutation of exocyst complex components also prevents apical delivery of Crb (Fig. 5). Thus, membrane trafficking of Crb occurs by directed motor-driven transport along polarized microtubules and F-actin filaments, Roeth et al., 2009) (Fig. S1), it was previously unclear whether polarized cytoskeletal transport was in fact required for apical membrane trafficking and localization of the Crb protein, rather than Crb or sdt mRNA (Cao et al., 2017; Horne-Badovinac and Bilder, 2008; Li et al., 2008).

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followed by exocyst-mediated delivery to the plasma membrane, and is crucial for the apical localization of this key polarity determinant.

Our findings also shed light on the mechanisms of cytoskeletal polarization. We have identified a key role for the apical FERM domain proteins, which link the PIP2-rich plasma membrane with F-actin, apical spectrins and the microtubule minus-end binding proteins Shot and Patronin (Chishti et al., 1998; Fehon et al., 2010; Khanal et al., 2016). This apical cortical meshwork (equivalent to the terminal web in mammalian epithelia) is then responsible for polarizing microtubules and thus ensuring directed apical trafficking of Crb by Dynein. Further work is necessary to understand precisely how the three apical FERM domain proteins become localized, and how the apical meshwork is organized, particularly as Moe and Mer are normally found across the apical surface (with Shot) while Ex is found at the sub-apical junctions (with a βH-Spectrin-Patronin complex) (Su et al., 2017). Importantly, cytoskeletal polarization ultimately depends on the core apical-basal polarity determinants, including Crb itself, which acts redundantly with Baz to organize epithelial polarity (Tepass, 2012; Thompson et al., 2013). Thus, there is a positive feedback loop between apical polarity determinants and cytoskeletal polarization, which then directs further delivery of Crb to the same location on the plasma membrane to reinforce apical identity and maintain a polarized cytoskeleton.

The above mechanism of cytoskeletal polarization is required for localization of Crb, a transmembrane protein, but is not required for polarization of Baz, a cytoplasmic protein that associates with the plasma membrane. Thus, during asymmetric division of Drosophila neuroblasts, which are polarized along the apical-basal axis by Baz, there is no role for either Crb or membrane trafficking (Halbsgut et al., 2011; Hong et al., 2001). Furthermore, during the early establishment of epithelial polarity in the Drosophila embryo, polarity is initiated by Baz before Crb becomes expressed (Blankenship et al., 2007; Harris and Peifer, 2004). It is conceivable that the Baz system is able to polarize more rapidly than the
Crb system, which may be an advantage in asymmetric cell division and in early establishment of epithelial polarity, but that the Crb system is advantageous in mature epithelial cells, where stable polarization of the cytoskeleton is fundamental to both cellular structure and function. Importantly, redundancy between Baz and Crb-Sdt in recruiting the Cdc42-Par6-aPKC complex enables one system to maintain apical-basal polarity while the other is deployed in a planar polarized fashion during various episodes of morphogenetic change during development (Campbell et al., 2009; Grawe et al., 1996; Thompson et al., 2013).

Our findings also indicate that overexpression of Crb can saturate this system of polarized transport, such that Crb accumulates abnormally along lateral membranes (Fig. 6A,B). Overexpressed Crb can also accumulate dramatically within endosomes when individual components of the polarized transport machinery are compromised (Fig. 6A,B). The sensitized background caused by Crb overexpression explains the discrepancy between deletion of the conserved extracellular domain in overexpressed Crb, which has dramatic consequences (Das and Knust, 2018; Fletcher et al., 2012; Letizia et al., 2013; Roper, 2012; Thompson et al., 2013), versus the same experiment in endogenous Crb, which only modestly affects Crb localization (Das and Knust, 2018) likely due to the continued operation of polarized cytoskeletal transport (Fig. 6C–E). Similarly, mutation of the FERM-binding domain of Crb has strong effects upon overexpressed Crb (Fletcher et al., 2012; Letizia et al., 2011) but only causes a moderate reduction of endogenously expressed Crb (Cao et al., 2017; Klose et al., 2013; Sherrard and Feohon, 2015a) (Fig. 5E,F). Thus, important mechanisms of Crb polarization that are obscured through genetic redundancy can be revealed through the study of overexpressed Crb to provide a unifying model of polarization (Fig. 6F).

Our results explain the striking genetic interactions we have observed between upstream components of the Hippo signaling pathway, whose disruption leads to increased Crb expression (Fletcher et al., 2018; Genevet et al., 2009; Hamaratoglu et al., 2009; Zhu et al., 2015), and components of the Crb trafficking machinery such as exocyst components (Fletcher et al., 2012, 2015). Specifically, RNAi knockdown of spectrin cytoskeleton components in sec15 mutants or kib mutants, or alternatively double mutants of ex kib, all cause strong Crb accumulation in endosomes in the follicle cell epithelium (Fletcher et al., 2012, 2015) (Fig. S2). Since Crb itself functions with the spectrin cytoskeleton, the FERM domain proteins Merlin and Expanded, and the exocyst-binding partner Kibra to directly regulate Hippo signaling, follicle cells make use of this pathway to sense mechanical stretching of the apical domain to promote increased expression of crb, ex, kib and other target genes to help maintain the apical domain and accommodate mechanical perturbation (Fletcher et al., 2018). Thus, by virtue of being a transmembrane protein, an apical polarity determinant, as well as an upstream component of the Hippo pathway, Crb helps orchestrate cytoskeletal polarization, but is also transported apically, so can act as a sensor of the successful maintenance of cytoskeletal polarity in the face of significant mechanical stress or strain exerted upon the cytoskeleton of epithelial cells during development.

Finally, the model we propose in Drosophila may be conserved in humans, as many of the components have human orthologs, including CRB3, which localises to sub-apical tight junctions with PALS1 and PAR6, while MYO8A/B, MERLIN, KIBRA, CAMSAPs, spectrins and exocyst components localise across the entire apical surface (www.proteinatlas.org). Notably, CRB3 lacks the homophilic extracellular domain of CRB1 and CRB2, but is still able to localise to tight junctions, suggesting that other tight junction proteins such as JAMs, Occludins or Claudins may mediate extracellular domain clustering, with the entire complex clustered by intracellular multi-PDZ domain proteins such as ZO-1 and MUPP1/PATJ (Bazellieres et al., 2018; Ebnet et al., 2004; Michel et al., 2005). In addition, our findings suggest that polarization of the cytoskeleton in human columnar epithelial cells may also contribute to apically-directed membrane trafficking of CRB1-3 and other transmembrane tight junction components. In future, it will be of interest to test whether the mechanisms of epithelial polarization uncovered in Drosophila are conserved in epithelial organoids, an experimentally tractable model system which forms highly columnar cells similar to those observed in human epithelial tissues.

4. Materials and methods

Drosophila stocks were obtained from the Bloomington Drosophila Stock Centre and are described in FlyBase. Mitotic clones were generated using the FLP/FRT system and were either marked positively (presence of GFP; MARCM) or negatively (absence of GFP). Third instar larvae were heat-shocked once at 37 °C for 1 h and dissected 3 days after eclosion. Expression of UAS-driven transgenic lines was achieved with traffic jam.Gal4 (tg.Gal4) driver, the actin ‘flip-out’ and MARCM systems. The tg.Gal4 line is weakly expressed from the beginning of follicular development and strongly from stage 7 onward. For ‘flip-out’ clones, third instar larvae were heat-shocked at 37 °C for 20 min, and dissected 3 days after eclosion. Fly crosses were kept at a temperature of 25 °C.

4.1. Immunohistochemistry

Ovaries were dissected in PBS, fixed for 20 min in 4% paraformaldehyde in PBS, washed for 30 min in PBS/0.1% Triton X-100 (PBT) and blocked for 15 min in 5% normal goat serum/PBT (PBT/NGS). Primary antibodies were diluted in PBT/NGS and samples were incubated overnight at 4 °C. Secondary antibodies were used for 2 h at room temperature and then mounted on slides in Vectashield (Vector Labs). Images were taken with a Leica SP5 confocal using 40x oil immersion objective and processed with Adobe Photoshop and ImageJ.

Primary antibodies used were: rat anti-Crums (1:200, E. Knust), mouse anti-Crums (Cq4) (1:10, DSHB), rat anti-Crb intra (1:500 M.Bhat), rabbit anti-Lgl (1:50, Santa Cruz), mouse anti-Dlg (1:250, DSHB) and FITC-conjugated anti-GFP (1:400, Abcam).

Secondary antibodies used were goat Alexa fluor 488, 546 or 647 (1:500, Invitrogen), Phalloidin (2.5:250, Life Technologies) to stain F-actin and DAPI (1 μg/ml, Life Technologies) to visualize nuclei.

4.2. Inhibitor treatments

Treatment of ovaries expressing Crb:GFP was performed by isolating egg chambers and culturing them as described (Aguilar-Aragon et al., 2018) with Colchicine (0.2 mg/ml), Latrunculin A (0.05 mM), Cytochalasin D (0.05 mM), Jasplakinolide (0.05 mM), Ethanol or DMSO control (all of them from Sigma) for 2 h. After treatment, samples were fixed and processed normally for imaging.

4.3. Statistical analysis

Experiments were performed with at least three biological replicates. Prism software was used to plot the mean of the experimental data and error bars represent the standard deviation. T-test for all conditions tested in the paper was found to be p < 0.01.
Atwood, S.X., Chabu, C., Penkett, R.R., Doe, C.Q., Prehoda, K.E., 2007. CDC42 acts downstream of BAZooka to regulate neuronal plasticity through Par-6 aPKC. J. Cell Sci. 120, 3200–3206.

Bachmann, A., Schneider, M., Theilenberg, E., Grawe, F., Knust, E., 2001. Drosophila Stardust is a partner of Crumbs in the control of epithelial cell polarity. Nature 414, 432–434.

Baines, A.J., Lu, H.C., Bennett, P.M., 2014. The Protein 4.1 family: hub proteins in animals for organizing membrane proteins. Biochem. Biophys. Acta 1838, 605–619.

Bazellieres, E., Aksenova, V., Barthelmy-Requin, M., Mansey-Harroche, D., Le Bivic, A., 2018. Role of the Crumbs proteins in clionogenesis, cell migration and actin organization. Semin. Cell Dev. Biol. 81, 13–20.

Benton, R., St Johnston, D., 2003a. A conserved oligomerization domain in drosophila BAZooka/PAR-3 is important for apical localization and epithelial polarity. Curr. Biol. 13, 1330–1334.

Benton, R., St Johnston, D., 2003b. Drosophila PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. Cell 115, 691–704.

Berenjena, S., Laprise, P., Papoulos, O., Pellikka, M., Sisson, J., Tepass, U., 2005. Essential function of Drosophila Sec6 in apical exocytosis of epithelial photoreceptor cells. J. Cell Biol. 169, 635–646.

Bilder, D., Li, M., Perrimon, N., 2000. Cooperative regulation of cell polarity and growth by Drosophila tumor suppressors. Science 289, 115–116.

Bilder, D., Perrimon, N., 2000. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature 405, 676–680.

Bilder, D., Schober, M., Perrimon, N., 2003. Integrated activity of PDZ protein complexes regulates epithelial polarity. Nat. Cell Biol. 5, 53–58.

Blankemeyer, J.T., Fuller, M.T., Zallen, J.A., 2007. The Drosophila homolog of the Ectodex escutent promot apical epidermal identity. J. Cell Sci. 120, 3099–3110.

Campbell, K., Knust, E., Skager, H., 2009. Crumbs stabilises epithelial polarity during tissue remodelling. J. Cell Sci. 122, 2604–2612.

Cao, H., Xu, R., Shi, Q., Zhang, D., Huang, J., Hong, Y., 2017. FERM domain phosphorylation and endogenous 3’UTR are not essential for regulating the function and subcellular localization of polarity protein Crumbs. J Genet Genomics 44, 409–412.

Carreno, S., Kouranti, I., Glusman, E.S., Fuller, M.T., Echard, A., Payne, F., 2008. Moesin and its activating kinase Slik are required for cortical stability and microtubule organization in mitotic cells. J. Cell Biol. 180, 779–794.

Chen, C.L., Gajewski, K.M., Haramatoglu, F., Bosuut, W., Sanes-Rosera, L., Tao, C., Halder, G., 2010. The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. Proc. Natl. Acad. Sci. U. S. A 107, 15810–15815.

Chishiti, A.H., Kim, A.C., Marfatia, S.M., Lutchman, M., Hanspal, M., Jindal, H., Liu, S.C., Law, P.S., Rouleau, G.A., Mohandas, N., et al., 1998. The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane. Trends Biochem. Sci. 23, 282–286.

Conrider, R., Yu, H., Zahedi, B., Harden, N., 2007. The serine/threonine kinase dPak1 is required for polarized assembly of F-actin bundles and apical-basal polarity in the Drosophila follicle epithelium. Dev. Biol. 305, 470–482.

Cowen, R.T., Kirkpatrick, C., Peifer, M., 1996. Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during Drosophila embryogenesis. J. Cell Biol. 134, 133–148.

Das, S., Knust, E., 2018. A dual role of the extracellular domain of Drosophila Crumbs for morphogenesis of the embryonic neuroectoderm. Biology open 7.

Dickinson, D.J., Schwager, F., Pintard, L., Gotta, M., Goldstein, B., 2017. A single-cell biochemistry approach reveals PAR complex dynamics during cell polarization. Dev. Cell 42, 416–434 e411.

Eaton, S., Auvine, P., Luo, L., Jan, Y.N., Simmons, K., 1995. CDC42 and Rac1 control different actin-dependent processes in the Drosophila wing disc epithelium. J. Cell Biol. 131, 151–164.

Ebert, K., Suzuki, A., Ohno, S., Vestweber, D., 2004. Junctional adhesion molecules (JAMs): more molecules with dual functions? J. Cell Sci. 117, 19–29.

Fehon, R.G., McClatchey, A.I., Bretscher, A., 2010. Organizing the cell cortex: the role of ERM proteins. Nat. Rev. Mol. Cell Biol. 11, 276–287.

Fletcher, G.C., Diaz-de-la-Lozas, M.D., Borreguero-Munoz, N., Holder, M., Aguilar-Aragon, M., Thompson, B.J., 2018. Mechanical Strain Regulates the Hippo Pathway in Drosophila, vol. 145. Development.
Georgiou, M., Marinari, E., Burden, J., Baum, B., 2008. Cdc42, Par6, and aPKC regulate Arp2/3-mediated endocytosis to control local adherence junction stability. Curr. Biol. : CB 18, 1631–1636.

Gibson, M.C., Perriman, N., 2003. Apicobasal polarization: epithelial form and function. Curr. Opin. Cell Biol. 15, 747–752.

Goehring, N.W., Trong, P.K., Bois, J.S., Chowdhury, D., Nicola, E.M., Hyman, A.A., Geiger, B., 2011, 1005–1011. Regulation of PAR proteins by advection of a patterning-forming system. Science 334, 1137–1141.

Goodson, H.V., Anderson, B.L., Warrick, H.M., Pon, L.A., Spudich, J.A., 1996. Synthetic lethality screen identifies a novel yeast myosin I gene (MYOS): myosin I proteins are required for polarization of the actin cytoskeleton. J. Cell. Biol. 133, 1277–1291.

Gotta, M., Abraham, M.C., Abringer, J., 2001. CDC42 controls early cell polarity and spindle orientation in C. elegans. Curr. Biol. : CB 11, 482–488.

Grawe, F., Wodarz, A., Lee, B., Knust, E., Skarr, H., 1996. The Drosophila crumbs and stardust are involved in the biogenesis of adherens junctions. Development 122, 951–959.

Halbgut, N., Lin nemmannstons, K., Zimmermann, L.I., Wodarz, A., 2011. Apical-basal polarity in Drosophila neuroblasts is independent of vesicular trafficking. Mol. Biol. Cell 22, 4373–4379.

Hamaratoglu, F., Gajewski, K., Sansores-Garcia, L., Morrison, C., Tao, C., Halder, G., 2006. Regulated lysosomes are required for the development of epithelial cell polarity. Dev. Suppl. 261.

Kunda, P., Pelling, A.E., Liu, T., Baum, B., 2008. Moesin controls cortical rigidity, cell polarization, and apical membrane size. Cell 133, 669–680.

Johnson, J.M., Jin, M., Lew, D.J., 2011. Symmetry breaking and the establishment of cell polarity in epithelial cells. Curr. Biol. 21, 454–464.

Knust, E., Tepass, U., Wodarz, A., 1993. Crumbs and stardust, two genes of Drosophila melanogaster that control cell polarity. Nature 362, 180–183.

Khanal, I., Elbediwy, A., Diaz de la Loza Mdel, C., Fletcher, G.C., Thompson, B.J., 2016. FCH1 and WASp define a branch of the Cdc42-Pak-APK pathway regulating E-cadherin endocytosis. Curr. Biol. : CB 18, 1639–1648.

Kleidal, A., Ricard, S., Mousain, B., Martin, N., Limlaris, M., 2013. A functional role of the extracellular domain of Crumbs in cell architecture and apicobasal polarity. J. Cell. Sci. 126, 2157–2163.

Letizia, A., Sottolis, S., Campuzano, S., Limlaris, M., 2011. Regulated Clch accumulation controls apical constriction and invagination in Drosophila tracheal cells. J. Cell Sci. 124, 240–251.

Ling, C., Cheng, Y., Yin, F., Yu, J., Huang, J., Hong, Y., Wu, S., Pan, D., 2010. The apical membrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. Proc. Natl. Acad. Sci. U. S. A 107, 10532–10537.

Luo, L., Lee, T., Tsai, L., Tang, C., Jan, L.Y., Jan, N.N., 1997. Gercheg Khan (Gek) as a putative effector for Drosophila Cdc42 and regulator of actin polymerization. Proc. Natl. Acad. Sci. U. S. A 94, 12963–12968.

Lin, Y.H., Cremian, H., Pocha, S.M., Rothnie, A., Wassmer, T., Knust, E., 2015. AP-2 complex-mediated endocytosis of Drosophila Crumbs regulates polarity by antagonizing Stardust. J. Cell Sci. 128, 4538–4549.

Ling, C., Zheng, Y., Yin, F., Yu, J., Huang, J., Hong, Y., Wu, S., Pan, D., 2010. The apical membrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. Proc. Natl. Acad. Sci. U. S. A 107, 10532–10537.

Lin, Y.H., Currinn, H., Pocha, S.M., Rothnie, A., Wassmer, T., Knust, E., 2015. AP-2 complex-mediated endocytosis of Drosophila Crumbs regulates polarity by antagonizing Stardust. J. Cell Sci. 128, 4538–4549.

Luo, L., Lee, T., Tsai, L., Tang, C., Jan, L.Y., Jan, N.N., 1997. Gercheg Khan (Gek) as a putative effector for Drosophila Cdc42 and regulator of actin polymerization. Proc. Natl. Acad. Sci. U. S. A 94, 12963–12968.

Ma, C., Benick, H.A., Cheng, D., Montplaisir, V., Wang, L., Xi, Y., Zheng, P.P., Bement, W.M., Liu, X.J., 2006. Cdc42 activation couples spindle positioning to first polar body formation in mouse oocytes. J. Biol. Chem. : CB 281, 31240–31250.

McKinley, R.F., Yu, C.G., Harris, T.J., 2012. Assembly of Bazooka polarity landmarks requires formin for3p in the establishment of cell polarity. Dev. Cell 8, 945–954.

Mata, J., Nurse, P., 1997. tea1 and the microtubular cytoskeleton are important for breaks cellular symmetry. Bioessays 37, 1193–1197.

McKinley, R.F., Yu, C.G., Harris, T.J., 2012. Assembly of Bazooka polarity landmarks requires formin for3p in the establishment of cell polarity. Dev. Cell 8, 945–954.

Medina, E., Williams, J., Klipfell, E., Zarnescu, D., Thomas, G., Le Bivic, A., 2002. Crumbs interacts with moesin and beta(Heav)y-spectrin in the apical membrane skeleton of Drosophila. J. Cell. Biol. 158, 941–951.

Mellman, I., Nelson, W.J., 2008. Cell polarity: protein sorting, targeting and distribution in polarized cells. Nat. Rev. Mol. Cell Biol. 9, 833–845.

Michel, D., Arpando, J.A., Massey-Harroche, D., Beclin, C., Wijhijlts, J., Le Bivic, A., 2005. PATJ connects and stabilizes apical and lateral components of tight junctions in human intestinal cells. J. Cell Biol. 168, 4049–4057.

Minc, N., Martin, M.S., Baas, R., Chang, F., 2009. Establishing new sites of polarization by microtubules. Curr. Biol. : CB 19, 85–94.

Mizuno, K., Suzuki, A., Hirose, T., Kitamura, K., Kotsuzawa, K., Futaki, M., Amano, Y., Ohe, S., 2003. Self-association of PAR-3-mediated by the conserved b-terminal domain contributes to the development of epithelial tight junctions. J. Biol. Chem. 278, 31240–31250.

Mottair, E., Verges, M., Autscheler, Y., 2000. Membrane traffic in polarized epithelial cells. Curr. Opin. Cell Biol. 12, 483–490.

Motegi, F., Zonies, S., Hao, Y., Cuenca, A.A., Griffin, E., Seydoux, G., 2011. Microtubules induce self-organization of polarized PAR domains in Caenorhabditis elegans zygotes. Nat. Cell. Biol. 13, 1361–1367.
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Muller, H.A., Wieschaus, E., 1996. armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithium in Drosophila. J. Cell Biol. 134, 149–163.

Murthy, M., Schwarz, T.L., 2004. The exocyst component Sec3 is required for membrane traffic and polarity in the Drosophila ovary. Development 131, 377–388.

Nelson, W.J., 1991. Cytoskeleton functions in membrane traffic in polarized epithelial cells. Semin. Cell Biol. 2, 395–385.

Nelson, W.J., 2003. Adaptation of core mechanisms to generate cell polarity. Nature 422, 766–774.

Noordstra, I., Liu, Q., Nijenhuis, W., Hua, S., Jiang, K., Baars, M., Remmerswaal, S., Martin, M., Kapiton, L.C., Akhmanova, A., 2016. Control of apico-basal epithelial polarity by the microtubule minus-end-binding protein CAMSAP3 and spectraplakin ACF7. J. Cell Sci. 129, 4278–4288.

Novick, P., Field, C., Schekman, R., 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 21, 205–215.

Peifer, M., Omurlic, S., Sweeten, D., Wieschaus, E., 1993. A role for the Drosophila segment polarity gene armadillo in cell adhesion and cytoskeletal integrity during oogenesis. Development 118, 1191–1207.

Peterson, F.C., Penkert, R.R., Volkman, B.F., Prehoda, K.E., 2004. Cdc42 regulates the Par-6 PDZ domain through an allosteric Crb-PDZ transition. Mol. Cell. 13, 665–676.

Petronczki, M., Knoblich, J.A., 2001. DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in Drosophila. Nat. Cell Biol. 3, 43–49.

Pocha, S.M., Shevchenko, A., Knust, E., 2011. Crumbs regulates rhodopsin transport by interacting with and stabilizing myosin V. J. Cell Biol. 195, 827–838.

Polesello, C., Delon, I., Valenti, P., Ferrer, P., Payre, F., 2002. Dmoesin controls actin-based cell shape and polarity during Drosophila melanogaster oogenesis. Nat. Cell Biol. 4, 782–789.

Pruyne, D., Legesse-Miller, A., Gao, L., Dong, Y., Bretscher, A., 2004. Mechanisms of polarized growth and organelle segregation in yeast. Annu. Rev. Cell Dev. Biol. 20, 559–591.

Pruyne, D.W., Schott, D.H., Bretscher, A., 1998. Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. J. Cell Biol. 143, 1931–1945.

Rodriguez, J., Peglion, F., Martin, J., Hubatch, L., Reich, J., Hirani, N., Gubidi, A.G., Rolley, J., Fernandes, A.R., St Johnston, D., et al., 2017. apPKC cycles between functionally distinct PAR protein assemblies to drive cell polarity. Dev. Cell. 42, 400–415 e409.

Rodriguez-Boulan, E., Macara, I.G., 2014. Organization and execution of the epithelial polarity programme. Nat. Rev. Mol. Cell Biol. 15, 225–242.

Roeth, J.F., Sawyer, J.K., Wilner, D.A., Peifer, M., 2009. Rab11 helps maintain apical crumbs and adherens junctions in the Drosophila embryonic ectoderm. PloS One 4, e7634.

Roper, K., 2012. Anisotropy of Crumbs and aPKC drives myosin cable assembly during polarized epithelial cell movement. Curr. Biol. 22, 1235–1239.

Saueret, C., Wayt, J., Pelaseyed, T., Bretscher, A., 2015. Structure, regulation, and function of the Exocyst complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. 34, 6483–6494.

Thompson, B.J., 2013. Cell polarity: models and mechanisms from yeast, worms and flies. Development 140, 13–21.

TerBush, D.R., Maurice, T., Roth, D., Novick, P., 1996. The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. 15, 6483–6494.

Zihni, C., Vlassaks, E., Terry, S., Carlton, J., Leung, T.K.C., Olson, M., Pichaud, F., 2016. Pol4 is required during epithelial polarity remodeling through regulating AJ stability and bazooka retention at the ZA. Cell Rep. 15, 45–53.

Zallen, J.A., Wieschaus, E., 2004. Patterned gene expression directs bipolar planar polarization in epithelial cells. J. Cell Sci. 117, 559–570.

St Johnston, D., Ahringer, J., 2010. Cell polarity in eggs and epithelia: parallels and diversity. Cell 141, 757–774.

Su, T., Ludwig, M.Z., Xu, J., Feigon, R.G., 2017. Kibra and Merlin activate the Hippo pathway spatially distinct from and independent of expanded. Dev. Cell 40, 478–490 e473.

Tanentzapf, G., Smith, C., McGlade, J., Tepas, U., 2000. Apical, lateral, and basal polarization cues contribute to the development of the follicular epithelium during Drosophila oogenesis. J. Cell Biol. 151, 891–904.

Tanentzapf, G., Tepas, U., 2003. Interactions between the crumbs, lethal giant larva and bazooka pathways in epithelial polarization. Nat. Cell Biol. 5, 46–52.

Tepas, U., 2012. The apical polarity protein network in Drosophila epithelial cells: regulation of polarity, junctions, morphogenesis, cell growth, and survival. Annu. Rev. Cell Dev. Biol. 28, 655–685.

Tepas, U., Knust, E., 1993. Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in Drosophila melanogaster. Dev. Biol. 159, 311–326.

Tepas, U., Therres, C., Knust, E., 1990. Crumbs encodes an EGF-like protein expressed on apical membranes of Drosophila epithelial cells and required for organization of epithelia. Cell 61, 787–799.

TerBush, D.R., Maurice, T., Roth, D., Novick, P., 1996. The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. 15, 6483–6494.

Thompson, B.J., 2013. Cell polarity: models and mechanisms from yeast, worms and flies. Development 140, 13–21.

Thompson, B.J., Pichaud, F., Roper, K., 2013. Sticking together the Crumbs - an unexpected function for an old friend. Nat. Rev. Mol. Cell Biol. 14, 307–314.

Toya, M., Kobayashi, S., Kawasaki, M., Shioi, G., Kaneke, M., Ishiuchi, T., Misaki, K., Mork, W., Takeichi, M., 2016. CAMSAP3 orients the apical-to-basal polarity of microtubule arrays in epithelial cells. Proc. Natl. Acad. Sci. U. S. A. 113, 332–337.

Walther, R.F., Nunes de Almeida, F., Vlassaks, E., Burden, J.D., Pichaud, F., 2016. Pol4 is required during epithelial polarity remodeling through regulating AJ stability and bazooka retention at the ZA. Cell Rep. 15, 45–53.

Zallen, J.A., Wieschaus, E., 2004. Patterned gene expression directs bipolar planar polarity in Drosophila. Cell 117, 528–541.

Zhang, X., Li, C., Xiong, J., Yamada, K., Deng, M., Slaughter, B.D., Rubinstein, B., Li, R., 2013. Sequential actin-based pushing forces drive meiosis I chromosome migration and symmetry breaking in oocytes. J. Cell Biol. 200, 567–576.

Zhang, X., Li, C., Xiong, J., Deng, M., Slaughter, B.D., Rubinstein, B., Li, R., 2011. Dynamic maintenance of asymmetric meiotic spindle position through Par2/3-complex-driven cytoplasmic streaming in mouse oocytes. Nat. Cell Biol. 13, 1252–1258.

Zallen, J.A., Wieschaus, E., 2004. Patterned gene expression directs bipolar planar polarity in Drosophila. Dev. Cell 6, 343–355.

Zhang, X., Ma, C., Miller, A.L., Kishi, H.A., Bement, W.M., Liu, X.J., 2008. Polar body emission requires a rhoA contractile ring and Cdc42-mediated membrane protrusion. Dev. Cell 15, 386–400.

Zhu, Y., Li, D., Wang, Y., Pei, C., Liu, S., Zhang, L., Yuan, Z., Zhang, P., 2015. Brhama regulates the Hippo pathway activity through forming complex with yki-Sd and regulating the transcription of Crumbs. Cell. Signal. 27, 606–613.

Zilhão, C., Vlassaks, E., Terry, S., Carlton, J., Leung, T.K.C., Olson, M., Pichaud, F., Balda, M.S., Matter, K., 2017. An apical MRCK-driven morphogenetic pathway controls epithelial polarity. Nat. Cell Biol. 19, 1049–1060.

Zonies, S., Motegi, F., Hao, Y., Seydoux, G., 2010. Symmetry breaking and polarization of the C. elegans zygote by the polarity protein PAR-2. Development 137, 1669–1677.