The Hippo pathway polarizes the actin cytoskeleton during collective migration of *Drosophila* border cells

Eliana P. Lucas, Ichha Khanal, Pedro Gaspar, Georgina C. Fletcher, Cedric Polesello, Nicolas Tapon, and Barry J. Thompson

1 Epithelial Biology Laboratory, and 2 Apoptosis and Cell Proliferation Laboratory, Cancer Research UK, London Research Institute, London WC2A 3LY, England, UK

Collective migration of *Drosophila* border cells depends on a dynamic actin cytoskeleton that is highly polarized such that it concentrates around the outer rim of the migrating cluster of cells. How the actin cytoskeleton becomes polarized in these cells to enable collective movement remains unknown. Here we show that the Hippo signaling pathway links determinants of cell polarity to polarization of the actin cytoskeleton in border cells. Upstream Hippo pathway components localize to contacts between border cells inside the cluster and signal through the Hippo and Warts kinases to polarize actin and promote border cell migration. Phosphorylation of the transcriptional coactivator Yorkie (Yki)/YAP by Warts does not mediate the function of this pathway in promoting border cell migration, but rather provides negative feedback to limit the speed of migration. Instead, Warts phosphorylates and inhibits the actin regulator Ena to activate F-actin Capping protein activity on inner membranes and thereby restricts F-actin polymerization mainly to the outer rim of the migrating cluster.

Introduction

Migration of cells is one of the most dramatic events that underlies the development of animal tissues and the progression of tumors (Condeelis et al., 2005; Sahai, 2005; Montell, 2008). Most of our knowledge of the mechanisms of cell migration comes from the study of single cells migrating in culture (Van Haastert and Devreotes, 2004; Ridley, 2011). However, in vivo, cells often migrate not as individuals but as groups that move collectively (Friedl and Gilmour, 2009; Rørth, 2009; Weijer, 2009).

*Drosophila* border cell migration is a genetically tractable model system for the study of collective cell movement (Starz-Gaiano and Montell, 2004; Rørth, 2009). Border cells arise in the follicular epithelium that surrounds each egg chamber in the *Drosophila* ovary (Fig. 1A). At the anterior pole of the egg chamber, a pair of polar cells recruits a small group (4–8) of neighboring follicle cells into the border cell cluster. At stage 9 of oogenesis, this cluster delaminates from the epithelium and invades the underlying germ line, migrating across the egg chamber between the large nurse cells to reach the oocyte at the posterior pole by stage 10 of oogenesis (Fig. 1, A–C).

A series of important discoveries has revealed many key mechanisms by which border cells are first specified (Montell et al., 1992; Bai et al., 2000; Silver and Montell, 2001; Beccari et al., 2002; Xi et al., 2003; Borghese et al., 2006; Jang et al., 2009), begin their invasive movement (Fulga and Rørth, 2002), detach from the epithelium (McDonald et al., 2008), begin their invasive movement (Fulga and Rørth, 2002), are guided toward the oocyte (Duchek and Rørth, 2001; Duchek et al., 2001; McDonald et al., 2003; Bianco et al., 2007; Poukkula et al., 2011), sense tension (Somogyi and Rørth, 2004), maintain adhesion (Niewiadomska et al., 1999; Pacquelet and Rørth, 2005; Cobreros-Reguera et al., 2010), and organize their polarity (Abdelilah-Seyfried et al., 2003; Pinheiro and Montell, 2004; McDonald et al., 2008). Yet, how border cells control the dynamic organization of the actomyosin cytoskeleton to drive cell locomotion is still not fully understood.

Determinants of cell polarity are required to polarize the border cell cytoskeleton to organize cluster architecture and promote collective migration (Abdelilah-Seyfried et al., 2003; Pinheiro and Montell, 2004; McDonald et al., 2008). Loss of polarity determinants delays migration and can cause the cluster to

© 2013 Lucas et al. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike-No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/).
can be regulated by determinants of cell polarity, such as Crumbs and aPKC, can respond to changes in the actin cytoskeleton, and can influence the level of F-actin in epithelial cells (Chen et al., 2010; Grzeschik et al., 2010; Ling et al., 2010; Fernández et al., 2011; Sansores-Garcia et al., 2011; Wada et al., 2011). However, the physiological roles for the Hippo pathway as a to disintegrate (Abdelilah-Seyfried et al., 2003; Pinheiro and Montell, 2004). The polarity determinants Crumbs, Baz, and the aPKC–Par6 complex localize to membranes where border cells form contacts with one another (Niewiadomska et al., 1999; Abdelilah-Seyfried et al., 2003; Pinheiro and Montell, 2004; McDonald et al., 2008). These determinants do not localize to regions of the membrane where border cells are actively migrating across their nurse cell substrate (Niewiadomska et al., 1999; Abdelilah-Seyfried et al., 2003; Pinheiro and Montell, 2004; McDonald et al., 2008). Thus, by polarizing the cytoskeleton, polarity determinants promote cohesion between border cells and collective migration of the cluster as a whole. Reduced cytoskeletal dynamics at sites of contact between collectively migrating cells is also evident in several other contexts, including invasive human cancer cells, and may be related to the phenomenon of contact inhibition of cell migration in cell culture (Carmona-Fontaine et al., 2008; Hidalgo-Carcedo et al., 2011). However, the molecular mechanisms by which border cell polarity determinants organize cluster architecture to promote migration remain unknown.

The Hippo pathway inhibits cell proliferation in growing epithelial tissues of both Drosophila and mammals (Grusche et al., 2010; Oh and Irvine, 2010; Pan, 2010; Badouel and McNeill, 2011; Halder and Johnson, 2011). Hippo signaling is also activated upon contact inhibition in cell culture, where it contributes to the repression of cell proliferation (Zhao et al., 2007; Kim et al., 2011). Recent work indicates that Hippo signaling can be regulated by determinants of cell polarity, such as Crumbs and aPKC, can respond to changes in the actin cytoskeleton, and can influence the level of F-actin in epithelial cells (Chen et al., 2010; Grzeschik et al., 2010; Ling et al., 2010; Fernández et al., 2011; Sansores-Garcia et al., 2011; Wada et al., 2011). However, the physiological roles for the Hippo pathway as a
The Hippo pathway in collective migration

Lucas et al.

staining—accumulates around the outer rim of the migrating cluster (Fig. 1, A–F). Live imaging of Utrophin-GFP, which labels the actin cytoskeleton, confirms that actin filaments concentrate and are most dynamic around the outer rim of the cluster (Video 1). Unlike F-actin, the key upstream components of the Hippo pathway Kibra (Kib), Expanded (Ex), and Merlin (Mer) (Hamaratoglu et al., 2006; Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010), as well as the recently identified component Zyxin (Rauskolb et al., 2011), localize with the polarity determinants aPKC and Crb to membranes inside the border cell cluster at sites of border cell–border cell contact (Fig. 2, A–G). Note that the bulk Hippo (Hpo) and Warts (Wts) proteins are not localized specifically to any region of the cell, but are well known to be active only in the presence of the upstream components, with which they physically interact.

Results

Polarization of Hippo pathway components and the actin cytoskeleton in migrating border cell clusters

We began by investigating the actin cytoskeleton during border cell migration. We find that F-actin—detected by phalloidin staining—accumulates around the outer rim of the migrating cluster (Fig. 1, A–F). Live imaging of Utrophin-GFP, which labels the actin cytoskeleton, confirms that actin filaments concentrate and are most dynamic around the outer rim of the cluster (Video 1). Unlike F-actin, the key upstream components of the Hippo pathway Kibra (Kib), Expanded (Ex), and Merlin (Mer) (Hamaratoglu et al., 2006; Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010), as well as the recently identified component Zyxin (Rauskolb et al., 2011), localize with the polarity determinants aPKC and Crb to membranes inside the border cell cluster at sites of border cell–border cell contact (Fig. 2, A–G). Note that the bulk Hippo (Hpo) and Warts (Wts) proteins are not localized specifically to any region of the cell, but are well known to be active only in the presence of the upstream components, with which they physically interact;
The Hippo pathway is required to polarize the actin cytoskeleton and promote migration in border cells

We next tested the requirement for Hippo signaling in border cells. In imaginal disc epithelia, the upstream Hippo pathway components Kib, Ex, and Mer are partially redundant in that they each tend to have weaker loss-of-function phenotypes than hpo or wts mutants, whereas ex, kib or ex, mer double mutants cause very strong phenotypes (Hamaratoglu et al., 2006; Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010). Wild-type border cell clusters normally reach the oocyte by stage 10 of oogenesis, whereas inactivation of ex or kib individually delays border cell migration and the double-mutant combination ex, kib causes very strongly delayed migration, with clusters rarely even initiating migration (Fig. 3, A–F). Polarization of F-actin is abnormal in ex mutant clusters and formation of clusters is completely prevented in ex, kib double mutants (Fig. 3, C and D). These results show that upstream Hippo pathway components are essential for organizing the architecture and motility of border cell clusters.

In epithelia, Kib, Ex, and Mer are known to function by activating the Hpo and Wts kinases at the apical membrane (Hamaratoglu et al., 2006; Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010), but can also act independently of Hpo and Wts to help polarize apical determinants (Fletcher et al., 2012). To identify the specific role of signaling through Hpo and Wts in border cell migration, we examined hpo and wts mutant clusters. Approximately 60% of hpo and wts mutant border cell clusters are delayed at stage 10 of oogenesis (Fig. 3, G–K). Unlike control clusters, F-actin fails to polarize to the outer rim of hpo and wts mutant clusters and instead tends to accumulate throughout the cluster (Fig. 3, A–L). Similar results were obtained for phosphorylated myosin II (Fig. 3 L). Live imaging reveals that wts mutant clusters, or clusters expressing RNAi against the Wts cofactor Mats, tend to tumble rather than move directionally and sometimes disintegrate (Fig. S1; Videos 2–5). These results show that signaling through Hpo and Wts is essential to polarize the actin cytoskeleton and promote collective migration in border cells.

To rule out an indirect effect of Wts on border cell migration via misregulation of border cell specification, we tested the effect of wts mutants on markers of border cell fate. We find that expression of slbo.lacZ and upd.lacZ is not affected in wts mutants (Fig. 4, A–D). These results show that border cell specification was not affected by inactivation of Hpo or Wts and support the notion that the Hippo pathway acts directly at the cell cortex to control cluster architecture and motility.

We also sought to rule out the possibility that Wts might regulate polarization of polarity determinants or adherens...
The Hippo pathway in collective migration • Lucas et al.

The Hippo pathway regulates the activity of the Ena/Capping protein system to organize cluster architecture and motility

To explore how the Hippo pathway regulates the actomyosin cytoskeleton, we considered the role of the actin regulator Enabled (Ena; VASP in mammals). At the leading edge of migrating cells in culture, Ena/VASP proteins are known to drive actin polymerization and cortical protrusions by inhibiting the activity of F-actin Capping proteins, which normally limit actin polymerization (Bear and Gertler, 2009). Several lines of evidence suggest potential links between the Hippo pathway and Ena. First, at focal adhesions in cultured cells, Zyxin binds to Ena/VASP proteins and modulates their activity to produce a stable cortex. Second, loss of Capping proteins Cpa and Cpb (Cpa and Cpb) has been shown to induce Hippo signaling in the context of growth control (Fernández et al., 2011; Sansores-Garcia et al., 2011). Third, Ena and Capping proteins have been implicated as regulators of border cell migration (Gates et al., 2009). Fourth, we identify a conserved Wts consensus phosphorylation site in Ena that is highly similar to the site in Yki whose phosphorylation inhibits Yki (Fig. 6A). We find that this site in Ena can be directly phosphorylated by Wts in vitro, similar to the site in Yki (Fig. 6B). These results suggest that Hippo signaling may act by phosphorylating and inactivating Ena to polarize the actin cytoskeleton.

If Hippo signaling acts by inhibiting Ena, then the phenotype of hpo mutant border cell clusters should be caused by excessive Ena activity and rescued in hpo ena double mutants. Accordingly, we find that hpo ena double-mutant clusters migrate normally and exhibit a normally polarized actin cytoskeleton (Fig. 6, C and D). Furthermore, overexpression of Ena is sufficient to mimic a mild Hippo pathway loss-of-function phenotype, with F-actin accumulating throughout the Ena-expressing clusters and delayed migration during stage 9 (Fig. 6E). However, Ena-expressing clusters recover and are not delayed by stage 10 (Fig. 6F). Expression of phospho-mutant EnaS187A has a stronger effect, with clusters delayed at both stage 9 and 10, whereas expression of an Ena S187D phosphomimetic mutant does not delay migration (Fig. 6, G–K). Live imaging of Ena-expressing

junctions. We find that the polarity determinant aPKC is normally localized in wts mutant border cell clusters, as is the adherens junctions protein Armadillo/β-catenin (Fig. 4, E–H).

Phosphorylation of Yki by Wts does not mediate the function of Wts but rather provides negative feedback.

In many tissues, Hippo signal transduction proceeds by the Wts kinase phosphorylating and inhibiting the transcriptional coactivator Yorkie (Yki; YAP/TAZ in mammals; Huang et al., 2005; Dong et al., 2007). In Drosophila, most known phenotypes of hpo and wts mutants can be phenocopied by ectopic expression of Yki (Huang et al., 2005; Shaw et al., 2010; Staley and Irvine, 2010). We therefore expected ectopic expression of Yki to inhibit border cell migration. In contrast, we find that expression of wild-type Yki or a constitutively active form of Yki lacking the major Wts phosphorylation site (YkiS168A) does not inhibit border cell migration and instead accelerates it (Fig. 5, A–J). This surprising result indicates that Hpo and Wts act directly to promote border cell migration, rather than by signaling through Yki to the nucleus, and that repression of Yki by the Hippo pathway provides negative feedback to limit migration.
These results indicate that Hippo signaling promotes border cell migration by inhibiting Ena and thus promoting Cpb activity inside the cluster to help restrict F-actin to the outer rim of migrating clusters.

Discussion

Our results show that the Hippo pathway provides a mechanism linking determinants of cell polarity with polarization of the actin cytoskeleton—a mechanism that is responsible for organizing the architecture and motility of collectively migrating border cell clusters. Collective migration depends on actomyosin polymerizing and contracting around the outer rim of the cluster, where border cells migrate over their nurse cell substrates, but not in the center of the cluster, where polarity determinants

clusters revealed a tumbling motion highly reminiscent of wts mutant clusters (compare Video 5 with Videos 2–4). These results indicate that Ena is a key target of Hippo pathway in polarizing the actin cytoskeleton during border cell migration.

Ena is thought to antagonize the action of Capping proteins, which compete with Ena for binding to F-actin barbed ends (Bear and Gertler, 2009). Ena promotes F-actin polymerization, whereas Capping proteins inhibit polymerization. In border cells, mutation of cpb caused clusters to accumulate F-actin inside the cluster and to exhibit delayed migration at stage 9 and 10 (Fig. 7, A–D). Around 10% of cpb mutant clusters disintegrated, highly similar to wts or hpo mutants (Fig. S1; Video 3 and Video 6). Finally, overexpression of Cpb was able to fully rescue the migration defect and F-actin polarization defects of wts mutant border cell clusters (Fig. 7, E–G; Video 7). These results indicate that Hippo signaling promotes border cell migration by inhibiting Ena and thus promoting Cpb activity inside the cluster to help restrict F-actin to the outer rim of migrating clusters.
localize to sites of contact between border cells. Our results show that upstream components of the Hippo pathway—Kib, Ex, and Mer—are recruited to border cell contacts and signal through Hpo and Wts to polarize the actin cytoskeleton. Double mutants for ex, kib have an even stronger phenotype than loss of hpo or wts, demonstrating that these upstream components have an additional role aside from activating Hippo signaling that is likely to involve directly assisting polarization of polarity determinants (Fletcher et al., 2012). Nevertheless, the upstream components also signal via Hpo and Wts to polarize the actin cytoskeleton and promote migration.

Our results indicate that Wts acts by regulating the Ena/Capping protein system, which is one system that cells use to control polymerization of actin (see model in Fig. 8). Loss of Wts results in excessive F-actin polymerization inside the cluster. Loss of Capping protein has the same effect, as does overexpression of the Capping protein inhibitor Ena. Ena contains a conserved Wts phosphorylation site located at the start of the proline-rich region (PRR) domain, which mediates binding to Profilin, so phosphorylation might disturb this binding interaction and thus inhibit Ena function. Our results support the notion that Ena is inactivated upon Hippo signaling, so that Capping protein can be active and thereby repress actin polymerization on inner membranes. Hence, in hpo or wts mutants, ectopic Ena activation inhibits Capping protein activity and leads to ectopic F-actin polymerization inside the cluster. In support of this view, hpo or wts mutants can be rescued by loss of Ena or overexpression of Capping protein, respectively. Notably, the rescued clusters show normal polarization of F-actin and can migrate normally, indicating that mechanisms other than polarization of Ena activity must also exist to help polarize the actin cytoskeleton in border cells, consistent with the fact that aside from Ena there are many other regulators of F-actin polymerization. Nevertheless, Hpo–Wts signaling is clearly one important mechanism of F-actin polarization for border cells because its disruption leads to the majority of border cell clusters migrating slowly in a tumbling fashion or even disintegrating.

Our results show that the role of the Hippo pathway in restraining F-actin polymerization at inner membranes is a direct one that is not mediated by the nuclear signaling effector Yki. Instead, our results indicate that repression of Yki by Wts functions solely as a negative feedback loop that is important to limit the speed of migration. Previous work has shown that excessive F-actin levels can cause a loss of Hippo pathway activity, which activates Yki, inducing expression of several key upstream components of the Hippo pathway to bolster pathway activity at the cortex (Fernández et al., 2011; Sansores-Garcia et al., 2011). In the context of border cell migration, such a negative feedback loop mechanism may be important for homeostatic control of F-actin polymerization. Excessive F-actin levels might therefore be expected to feedback to restrain F-actin polymerization via the Yki-mediated negative feedback loop. This phenomenon may explain the unusual behavior of Ena-overexpressing clusters, which strongly up-regulate F-actin and delay migration at stage 9.
but always recover to reach the oocyte by stage 10. In contrast, border cells never recover from loss of Capping protein, which, unlike overexpressed Ena, cannot be ameliorated by Wts phosphorylation. Hence, our results provide a physiological context for understanding the role of Yki as a negative feedback regulator of Hippo signaling.

In conclusion, our findings establish a novel role for Hippo signaling in collective migration and provide a novel mechanism for polarization of the actin cytoskeleton. Our results suggest that examination of the role of the Hippo pathway in human cancer should consider not only its potential to regulate cell proliferation and survival, but also its potential to regulate cell polarity, the actomyosin cytoskeleton, and collective cell invasion.

Materials and methods

Drosophila stocks and genetics

Flies were raised and crossed at 25°C according to standard procedures. w or yw flies were used as the wild-type stock. The FLP/FRT site-specific recombination system was used to generate mutant clones with a heat-shock promoter (Xu and Rubin, 1993; Lee and Luo, 1999). Flies of the following genotypes were generated: yw hsFLP UAS-nucGFPmyc; FRT42D hpo42-47/FRT42D tubGal80; tubGal4/+ (Wu et al., 2003); yw hsFLP UAS-nucGFPmyc; FRT42D hpo42-47/FRT42D tubGal80; tubGal4/+ (Jia et al., 2003); yw hsFLP, tubGal4, UAS-nucGFPmyc; FRT82B wtsX1/FRT82B tubGal80 (Xu et al., 1995); yw hsFLP, tubGal4, UAS-nucGFPmyc; FRT82B kib32/FRT82B tubGal80 (Genevet et al., 2010); yw hsFLP; exAP50FRT40A/FRT40A ubi-GFP (Hamaratoglu et al., 2006); yw hsFLP; exAP50FRT40A/FRT40A ubi-GFP; FRT82B kib32/FRT82B ubi-GFP; yw hsFLP, tubGal4, UAS-nucGFPmyc; FRT82B cpbM143/FRT82B tubGal80 (Fernández et al., 2011); yw hsFLP, tubGal4, UAS-nucGFPmyc; UAS cpb+/; FRT82B wts11/FRT82B tubGal80; yw hsFLP UAS-nucGFPmyc; FRT42D hpo42-47, ena210 contractility

Figure 8. Model. The role of the Hippo pathway in apico-basal polarization of border cells is shown schematically. At sites of contact between border cells inside the cluster (green), the Hippo pathway acts to suppress actin polymerization via regulation of the Ena/Capping protein system. Consequently, actin polymerization and motility occur primarily at the “basal” outer rim of the border cell cluster.
and FBS. Individual egg chambers were carefully removed and transferred to poly-lysine–coated imaging chambers containing Schneider’s media, insulin, FBS, trehalose, adenosine deaminase, methoprene, ecysone, and M4-64 dye (Bianco et al., 2007). Videos were acquired on an inverted confocal microscope (LSM780; Carl Zeiss) using 40× water immersion objectives; 15 sections were taken 1.6 µm apart with a 3-min interval period between stacks. 3–5 egg chambers were simultaneously imaged using multi-position imaging. Sections covering the migrating cluster were projected for each time point using LSM Image Examiner software (Carl Zeiss) and the videos were processed into a montage using MetaMorph software (Molecular Devices).

In vitro kinase assay

Peptides used in this study [RII peptide synthesis were: Yki S168 HSRLAH-HSRRASPSLQQNY (molecular weight 2,516.8 D); Yki S168A HSRLAH-SRRASPSLQQNY (molecular weight 2,500.8 D); Ena S187 SPPTQGGHRTSSAPPPQGQQQ (molecular weight 2,431.6 D); Ena S187A SPPTQGGHRTSSAPPPQQQQ (molecular weight 2,415.6 D). HPLC purified peptide substrates were diluted with deionised water to working dilutions (1 mg/ml) and stored at −20°C. The activity of recombinant Lats1 kinase (SignalChem) was measured in a kinase assay with 8,000 ng of peptide [Yki S168, Yki S168A, Ena S187, Ena S187A] and 350 ng of Lats1 kinase diluted in kinase dilution buffer III (SignalChem). The kinase reaction mixture consisted of 2 µl of 5x kinase assay buffer I [SignalChem], 10 µl of ATP cocktail (9.4 µl of kinase dilution buffer III, 10 µM cold ATP, and 3 µCi of γ-[32P]ATP [PerkinElmer]). The kinase assay was incubated for 30 min at 30°C. After 30 min on ice, samples were loaded on 8% SDS-PAGE and the phosphorylated bands were visualized with a phosphoimager (Molecular Dynamics). The relative cpm was determined by dividing the absolute cpm by the cpm in the control sample lacking substrate.

Online supplemental material

Fig. S1 shows quantification of disintegration defects in wts mutant clusters. Video 1. Shows polarization of F-actin visualized with Utrophin-GFP in border cells. Video 2 shows tumbling migration of wts or mats-R clusters versus a control. Video 3 shows disintegration of wts or mats-R clusters versus a control. Video 4 shows failure of detachment of wts or mats-R clusters versus a control. Video 5 shows tumbling migration of an UAS-enclusters versus a control. Video 6 shows disintegration of a cbp60D clusters versus a control. Video 7 shows rescue of wts mutant migration by expression of Cbp. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201210073/D1. Additional data are available in the JCB DataViewer at http://dx.doi.org/10.1083/jcb.201210073.dv.

We thank K. Irvine, D.J. Pan, G. Halder, T. Lecuit, H. Jiang, A. Laughon, Developmental Studies Hybridoma Bank (DSHB), and Bloomington Stock Center for antibodies and fly stocks. Special thanks to the IR Light Microscopy Unit for help with microscopy and image analysis. This work was funded by Cancer Research UK.

Accepted: 15 October 2012

References

Abdelilah-Seyfried, S., D.N. Cox, and Y.N. Jan. 2003. Bazooka is a permissive protein related to AIB1, a steroid receptor coactivator that regulates gene expression in Drosophila ovarian follicular epithelia. Development. 130:1927–1935. http://dx.doi.org/10.1242/dev.00420

Badeau, C., and H. McNeill. 2011. Snapshot: The hippo signaling pathway. Cell. 145:484–484; e1. http://dx.doi.org/10.1016/j.cell.2011.04.009

Bai, J., Y. Uehara, and D.J. Montell. 2000. Regulation of invasive cell behavior by the Cdc42 small GTPase. J. Cell Sci. 113:4843–4854. http://dx.doi.org/10.1242/jcs.113.4843

Bajer, J.E., and F.B. Gertler. 2009. Ena/V ASP: towards resolving a pointed conundrum. J. Cell Sci. 122:1947–1953. http://dx.doi.org/10.1242/jcs.038125

Beccari, S., L. Teixeira, and P. Roeth. 2002. The JAK/STAT pathway is required for border cell migration during Drosophila oogenesis. Mech. Dev. 111:115–123. http://dx.doi.org/10.1016/S0925-4773(01)00615-3

http://dx.doi.org/10.1016/S0092-8674(00)00208-3

http://dx.doi.org/10.1242/dev.00420

http://dx.doi.org/10.1016/j.cell.2011.04.009

http://dx.doi.org/10.1083/jcb.201210073.dv.
Bianco, A., M. Poukkula, A. Cliffe, J. Mathieu, C.M. Luque, T.A. Fulga, and P. Roth. 2007. Two distinct modes of guidance signalling during collective migration of border cells. Nature. 448:362–365. http://dx.doi.org/10.1038/nature05965

Boggiano, J.C., and R.G. Feohan. 2012. Growth control by intercellular junctions, cell polarity, and the cytoskeleton regulate Hippo signaling. Dev. Cell. 22:695–702. http://dx.doi.org/10.1016/j.devcel.2012.03.013

Borghese, L., G. Fletcher, J. Mathieu, A. Atzberger, W.C. Eades, R.L. Cagan, and P. Roth. 2006. Systematic analysis of the transcriptional switch inducing migration of border cells. Dev. Cell. 10:497–508. http://dx.doi.org/10.1016/j.devcel.2006.02.004

Carmona-Fontaine, C., H.K. Matthews, S. Kurtycz, M. Moreno, G.A. Dunn, M. Parsons, C.D. Stern, and R. Mayor. 2008. Contact inhibition of locomotion in vivo controls neural crest directional migration. Nature. 456:957–961. http://dx.doi.org/10.1038/nature07441

Chen, C.L., K.M. Gajewski, F. Hamafraglou, W. Bossuyt, L. Sansores-García, C. Tao, and G. Halder. 2010. The apical-basal cell polarity determinant Crumbs regulates Hippo signaling in Drosophila. Proc. Natl. Acad. Sci. USA. 107:15810–15815. http://dx.doi.org/10.1073/pnas.1004060107

Cobreros-Reguera, L., A. Fernández-Míchán, H. Fernández-Espartero, H. López-Schier, A. González-Reyes, and M.D. Martín-Bermudo. 2009. Enabled signaling in oogenesis. Curr. Biol. 19:27–36. http://dx.doi.org/10.1016/j.cub.2008.12.013

Deng, Y., Y. Matsui, Y. Zhang, and Z.C. Lai. 2013. Hippo activation and feedback and mutual antagonism combine to polarise Crumbs in epithelial cells. Nat. Cell Biol. 15:87–96. http://dx.doi.org/10.1038/ncb2233

Dong, J., G. Feldmann, J. Huang, S. Wu, N. Zhang, S.A. Comerford, M.F. Gayld, R.A. Anders, A. Maitra, and D. Pan. 2007. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell. 130:1120–1133. http://dx.doi.org/10.1016/j.cell.2007.07.019

Duchek, P., and K. Somogyi. 2001. Guidance of cell migration by EGF receptor signaling during Drosophila oogenesis. Science. 291:131–133. http://dx.doi.org/10.1126/science.291.5501.131

Duchek, P., K. Somogyi, G. Jékely, S. Beccari, and P. Rørth. 2006. Systematic analysis of the transcriptional switch inducing migration of border cells. Dev. Cell. 13:49–58. http://dx.doi.org/10.1016/j.devcel.2005.06.007

Jang, A.C., Y.C. Chang, J. Bai, and D. Montell. 2009. Border-cell migration requires integration of spatial and temporal signals by the RTB protein AbluPt. Nat. Cell Biol. 11:569–579. http://dx.doi.org/10.1038/ncl21836

Jia, J., W. Zhang, B. Wang, R. Trinko, and J. Jiang. 2003. The Drosophila Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Genes Dev. 17:2514–2519. http://dx.doi.org/10.1101/gad.113403

Kim, N.G., E. Koh, X. Chen, and B.M. Gumbiner. 2011. E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. Proc. Natl. Acad. Sci. USA. 108:11930–11935. http://dx.doi.org/10.1073/pnas.1103345108

Lee, T., and L. Luo. 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neural morphogenesis. Neuron. 22:451–461. http://dx.doi.org/10.1016/S0896-6273(00)80701-1

Ling, C., Y. Zheng, F. Yin, J. Yu, J. Huang, Y. Hong, S. Wu, and D. Pan. 2010. The apical transmembrane protein Crumbs functions as a tumor suppressor that regulates Hippo signaling by binding to Expanded. Proc. Natl. Acad. Sci. USA. 107:10532–10537. http://dx.doi.org/10.1073/pnas.1004279107

McDonald, J.A., E.M. Pinheiro, and D.J. Montell. 2003. PVF1, a PDGFr/VEGF homolog, is sufficient to guide border cells and interacts genetically with Taiman. Development. 130:3469–3478. http://dx.doi.org/10.1242/dev.00574

McDonald, J.A., A. Khodyakova, G. Aranjuiez, C. Dudley, and D.J. Montell. 2008. PAR-1 kinase regulates epithelial detachment and directional protrusion of migrating border cells. Curr. Biol. 18:1659–1667. http://dx.doi.org/10.1016/j.cub.2008.09.041

Montell, D.J. 2008. Morphogenetic cell movements: diversity from modular mechanical properties. Science. 322:1502–1505. http://dx.doi.org/10.1126/science.1159073

Montell, D.J., P. Rorth, and A.C. Spradling. 1992. slow border cells, a locus required for a developmentally regulated cell migration during oogenesis, encodes Drosophila C/EBP. Cell. 71:51–62. http://dx.doi.org/10.1016/0092-8674(92)90265-E

Niewiadomska, P., D. Gott, and U. Tepass. 1999. DE-Cadherin is required for intercellular motility during Drosophila oogenesis. J. Cell Biol. 144:533–547. http://dx.doi.org/10.1083/jcb.144.3.533

Oh, H., and K.D. Irvine. 2008. In vivo regulation of Yorkshire phosphorylation and localization. Development. 135:1081–1088. http://dx.doi.org/10.1242/dev.015255

Oh, H., and K.D. Irvine. 2009. In vivo analysis of Yorkshire phosphorylation status and localization. Oncogene. 28:1916–1927. http://dx.doi.org/10.1038/onc.2009.4

Oh, H., and K.D. Irvine. 2010. Yorkshire phosphorylation and Hippo signaling. Trends Cell Biol. 20:410–417. http://dx.doi.org/10.1016/j.tcb.2010.04.005

Paqueseut, A., and P. Roth. 2005. Regulatory mechanisms required for DE-cadherin function in cell migration and other types of adhesion. J. Cell Biol. 170:803–812. http://dx.doi.org/10.1083/jcb.200506131

Pan, D. 2010. The hippo signaling pathway in development and cancer. Dev. Cell. 19:491–505. http://dx.doi.org/10.1016/j.devcel.2010.09.011

Pignoni, F., and S.L. Zipursky. 1997. Induction of Drosophila eye development by decapentaplegic. Development. 124:271–278.

Pinheiro, E.M., and D.J. Montell. 2004. Requirement for Par-6 and Bazooka in Drosophila border cell migration. Development. 131:5243–5251. http://dx.doi.org/10.1242/dev.01412

Poukkula, M., A. Cliffe, R. Changede, and P. Rorth. 2011. Cell behaviors regulated by guidance cues in collective migration of border cells. J. Cell Biol. 192:513–524. http://dx.doi.org/10.1083/jcb.201010003

Rauskolb, C., G. Pan, B.V. Reddy, H. Oh, and K.D. Irvine. 2011. Zyxin links fat signaling to the hippo pathway. PLoS Biol. 9:e1000624. http://dx.doi.org/10.1371/journal.pbio.1000624

Ridley, A.J. 2011. Life at the leading edge. Cell. 145:1012–1022. http://dx.doi.org/10.1016/j.cell.2011.06.010

Roth, P. 2009. Collective cell migration. Annu. Rev. Cell Dev. Biol. 25:407–429. http://dx.doi.org/10.1146/annurev.cellbio.092808.132311

Roth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm, G.M. Rubin, K. Weigmann, M. Milán, V. Benes, W. Ansorge, and S.M. Cohen. 1998. Systematic gain-of-function genetics in Drosophila. Development. 125:1049–1057.

Sahai, E. 2005. Mechanisms of cancer cell invasion. Curr. Opin. Genet. Dev. 15:87–96. http://dx.doi.org/10.1016/j.gde.2004.12.002
Sansores-Garcia, L., W. Bossuyt, K. Wada, S. Yonemura, C. Tao, H. Sasaki, and G. Halder. 2011. Modulating F-actin organization induces organ growth by affecting the Hippo pathway. *EMBO J.* 30:2325–2335. http://dx.doi.org/10.1038/emboj.2011.157

Shaw, R.L., A. Kohlmaier, C. Polesello, C. Veelken, B.A. Edgar, and N. Tapon. 2010. The Hippo pathway regulates intestinal stem cell proliferation during *Drosophila* adult midgut regeneration. *Development.* 137:4147–4158. http://dx.doi.org/10.1242/dev.052506

Silver, D.L., and D.J. Montell. 2001. Paracrine signaling through the JAK/STAT pathway activates invasive behavior of ovarian epithelial cells in *Drosophila*. Cell. 107:831–841. http://dx.doi.org/10.1016/S0092-8674(01)00607-9

Somogyi, K., and P. Rørth. 2004. Evidence for tension-based regulation of *Drosophila* MAL and SRF during invasive cell migration. *Dev. Cell.* 7:85–93. http://dx.doi.org/10.1016/j.devcel.2004.05.020

Staley, B.K., and K.D. Irvine. 2010. Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. *Curr. Biol.* 20:1580–1587. http://dx.doi.org/10.1016/j.cub.2010.07.041

Starz-Gaiano, M., and D.J. Montell. 2004. Genes that drive invasion and migration in *Drosophila*. *Curr. Opin. Genet. Dev.* 14:86–91. http://dx.doi.org/10.1016/j.gde.2003.12.001

Sudol, M., and K.F. Harvey. 2010. Modularity in the Hippo signaling pathway. *Trends Biochem. Sci.* 35:627–633. http://dx.doi.org/10.1016/j.tibs.2010.05.010

Tepass, 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. http://dx.doi.org/10.1146/annurev-cellbio-092910-154033

Udan, R.S., M. Kango-Singh, R. Nolo, C. Tao, and G. Halder. 2003. Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. *Nat. Cell Biol.* 5:914–920. http://dx.doi.org/10.1038/ncb1050

Van Haastert, P.J., and P.N. Devreotes. 2004. Chemotaxis: signalling the way forward. *Nat. Rev. Mol. Cell Biol.* 5:626–634. http://dx.doi.org/10.1038/nrm1435

Wada, K., K. Ito, T. Okano, S. Yonemura, and H. Sasaki. 2011. Hippo pathway regulation by cell morphology and stress fibers. *Development.* 138:3907–3914. http://dx.doi.org/10.1242/dev.070987

Weijer, C.J. 2009. Collective cell migration in development. *J. Cell Sci.* 122:3215–3223. http://dx.doi.org/10.1242/jcs.036517

Wu, S., J. Huang, J. Dong, and D. Pan. 2003. *hippo* encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with *salvador* and *warts*. *Cell.* 114:445–456. http://dx.doi.org/10.1016/S0092-8674(03)00549-X

Xu, T., and G.M. Rubin. 1993. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development.* 117:1223–1237.

Xu, T., W. Wang, S. Zhang, R.A. Stewart, and W. Yu. 1995. Identifying tumor suppressors in genetic mosaics: the *Drosophila* lats gene encodes a putative protein kinase. *Development.* 121:1053–1063.

Yu, J., Y. Zheng, J. Dong, S. Klusza, W.M. Deng, and D. Pan. 2010. Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. *Dev. Cell.* 18:298–299. http://dx.doi.org/10.1016/j.devcel.2009.12.012

Zhao, B., X. Wei, W. Li, R.S. Udan, Q. Yang, J. Kim, J. Xie, T. Ikenoue, J. Yu, L. Li, et al. 2007. Inactivation of YAP oncprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev.* 21:2747–2761. http://dx.doi.org/10.1101/gad.1602907