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Bazooka is a permissive factor for the invasive behavior of *discs large* tumor cells in *Drosophila* ovarian follicular epithelia

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SUMMARY

Drosophila **Bazooka and atypical protein kinase C are essential for epithelial polarity and adhesion. We show here that wild-type *bazooka* function is required during cell invasion of epithelial follicle cells mutant for the tumor suppressor *discs large*. Clonal studies indicate that follicle cell Bazooka acts as a permissive factor during cell invasion, possibly by stabilizing adhesion between the invading somatic cells and their substratum, the germline cells. Genetic epistasis experiments demonstrate that *bazooka* acts downstream of *discs large* in tumor cell invasion. In contrast, during the migration of border cells, Bazooka function is dispensable for cell invasion and motility, but rather is required cell-autonomously in mediating cell adhesion within the migrating border cell cluster. Taken together, these studies reveal Bazooka functions distinctly in different types of invasive behaviors of epithelial follicle cells, potentially by regulating adhesion between follicle cells or between follicle cells and their germline substratum.**

Key words: Bazooka, Cell invasion, Border cell migration, Cell polarity, Discs large, *Drosophila melanogaster*, Follicle cell, Cell adhesion

INTRODUCTION

During the development of multicellular organisms, various types of directed cell migration occur that contribute to the development of different tissues. These include the migration of neural crest cells, hematopoietic stem cells and germ cells. Gaining a better understanding of the mechanisms that govern normal cell motility and invasion is crucial for understanding development but may also contribute to understanding forms of aberrant cell invasion and migration of metastatic tumor cells.

Border cell migration during *Drosophila* oogenesis is one well-studied example of invasive and directed migration (Spradling, 1993; Montell, 2001; Rørth, 2002). Border cells are specified within the anterior follicular epithelium that surrounds the germ cells in each egg chamber (Fig. 1). At late egg chamber stage 8, approximately eight border cells delaminate from the monolayer epithelium and, in a highly stereotyped fashion, invade the germ cell cluster within the developing egg chamber (Montell et al., 1992; Niewiadomska et al., 1999). First, they undergo directed cell migration between nurse cells towards the anterior margin of the oocyte and then turn dorsally, coming to rest at the dorsal anterior corner of the egg chamber next to the underlying oocyte nucleus.

Border cell migration displays several features that are reminiscent of metastasis by cancer cells (Thiery, 2002). Initially, border cells are polarized epithelial cells that loose some homophilic cell adhesion, undergo an epithelial-to-mesenchymal transition, acquire adhesion with the substratum, and undergo cell migration. However, not all epithelial characteristics are lost during migration. The migrating cells remain attached to each other and intercellular polarized junctions containing DE-cadherin [also known as Shotgun (Shg)], Armadillo (Arm) and Crumbs are present (Peifer et al., 1993; Niewiadomska et al., 1999). DE-cadherin has been demonstrated to play an essential role in both migrating border cells, and their substratum, the germ cells (Oda et al., 1997; Niewiadomska et al., 1999). These previous studies suggest homophilic interactions between transmembrane receptors, such as DE-cadherin, may provide the necessary adhesion between invasive cells and their substratum.

The *Drosophila* tumor suppressor genes *discs large* (*dlg1* – FlyBase) and *lethal (2) giant larvae* (*lgl*; *l(2)gl* – FlyBase) play essential roles in epithelial cell polarity, adhesion and proliferation (Mechler et al., 1985; Jacob et al., 1987; Woods and Bryant, 1989; Woods and Bryant, 1991; Woods et al., 1996; Woods et al., 1997; De Lorenzo et al., 1999; Bilder et al., 2000). Follicle cell epithelia mutant for *dlg* undergo an epithelial-to-mesenchymal transition driving their invasion between germ cells, in a pattern similar to that observed in migrating border cell clusters (Goode and Perrimon, 1997). Within follicle cells, Dlg localizes to the basolateral membrane and, in germ cells, the protein is present at sites of contact

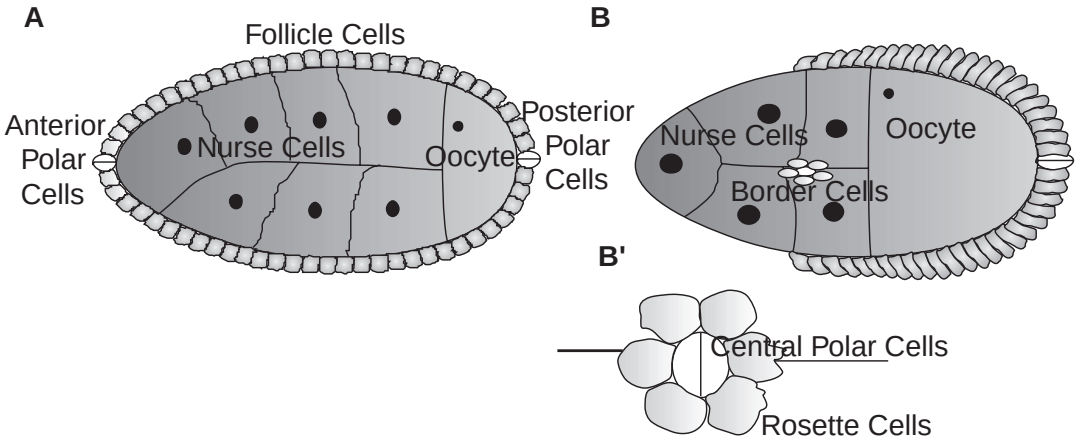

Fig. 1. Schematic summary of border cell migration. (A) Border cells are specified within the anterior follicular epithelium that surrounds the germline. The two anterior polar cells and several neighboring follicle cells undergo an epithelial-to-mesenchymal transition, intrude between the germline nurse cells and (B) migrate towards the oocyte at the posterior of the egg chamber. (B′) Within the migrating border cell cluster, the two central polar cells (previously the anterior polar cells) are carried along by the migratory rosette cells that surround them.

between germ cells. However, the protein is conspicuously absent from sites of contact between follicle and germ cells raising the question as to whether *dlg* affects the follicle and germ cell interactions indirectly via another protein complex.

The apical *Drosophila* Bazooka (Baz), Par-6 (DmPar-6), and atypical protein kinase C (DaPKC/aPKC) protein complex (in this text referred to collectively as the PAR complex) regulates apical-basal polarity in a variety of epithelial and nonepithelial cell types and is functionally conserved among different organisms (Jan and Jan, 1999; Doe, 2001; Knoblich, 2001). Central to the assembly of this complex are the PDZ domain scaffolding proteins Baz and DmPar-6 (Kuchinke et al., 1998; Petronczki and Knoblich, 2001) which together with DaPKC form a subapical membrane protein complex in polarized epithelial cells, where they function to stabilize adhesion complexes at the apical adherens junctions (Wodarz et al., 2000).

In zebrafish, loss of atypical protein kinase Cλ (aPKCλ) causes severe epithelial defects and loss of cell polarity (Horne-Badovinac et al., 2001; Peterson et al., 2001). Furthermore, within the context of an in vitro wound assay, using primary migratory rat astrocytes, and upon integrin-mediated activation of Cdc42, an mPar6/aPKCζ complex is recruited to the wound leading edge where it initiates the establishment of cell polarity (Etienne-Manneville and Hall, 2001).

In this study, we compared the function of *baz* in two distinct types of follicle cell invasion: border cell migration and *dlg* tumor cell invasion. We demonstrate that the tumor suppressor genes *dlg* and *lgl* function cell-autonomously within follicle cells to prevent invasion and that *baz* acts downstream of *dlg* in mediating follicle cell invasion, revealing that wild-type *baz* activity is required for *dlg* mutant follicle cell invasion. Clonal analysis of *dlg baz* double mutant follicle and germ cell clones suggests that *baz* regulates adhesion among migrating follicle cells, as well as between these cells and their substratum. In contrast, neither *baz* nor *DaPKC* appears to be required for border cell invasion and migration per se, since mutant border cells appropriately migrate, reaching the anterior margin of the oocyte at the expected stage. However, border cells mutant for *baz* appear to be deficient in their adhesion to each other and frequently display an altered arrangement of the border cell cluster.

Collectively, these results provide evidence for interactions between components of the basolateral and apical cell-cell junctions in the context of tumorous follicle cell invasion in the ovary, mediated by Dlg and Baz, respectively. Furthermore, our data suggest a role of *baz* in differentially regulating adhesion among distinct types of invasive follicle cells and show that border cell and *dlg* tumor cell invasion can mechanistically be differentiated via the function of *baz*.

MATERIALS AND METHODS

Drosophila strains and genetic clonal analyses

The dlg^{m52} null allele was recombined onto a baz^{EH171} [FRT]$^{9\text{-}2}$ chromosome using the *Dp(1;Y)W73*, y^{31d} B^{1}, f^{+}, B^{S}/*C(1)DX*, y^{1} f^{1}/y^{1} baz^{EH171} stock. The recombination alleles were tested for genetic complementation by crossing candidate dlg^{m52} baz^{EH171} [mini-w^{+}, FRT]$^{9\text{-}2}$/*FM6*, *yw B* single females with *Dp(1;Y)W73*, y^{31d} B^{1}, f^{+}, B^{S}/y^{1} baz^{EH171} males and scoring for the absence of dlg^{m52} baz^{EH171} [mini-w^{+}, FRT]$^{9\text{-}2}$/*Dp(1;Y)W73*, y^{31d} B^{1}, f^{+}, B^{S} males and dlg^{m52} baz^{EH171} [mini-w^{+}, FRT]$^{9\text{-}2}$/y^{1} baz^{EH171} females.

The following strains were used to generate germline clones by the FLP/FRT technique (Xu and Rubin, 1993): *yw* baz^{EH171} P[mini-w^{+}, FRT]$^{9\text{-}2}$/*FM6*, *yw B*; *pr pwn* P[ry^{+}; hsFLP]^{38}/*CyO*, *y* dlg^{m52} P[mini-w^{+}, FRT]^{101}/*FM6*, *yw B*; *pr pwn* P[ry^{+}; hsFLP]^{38}/*CyO*, *yw* P[w^{+}; ubi-nls-GFP] P[mini-w^{+}, FRT]$^{9\text{-}2}$, *yw* P[w^{+}; ubi-nls-GFP] P[mini-w^{+}, FRT]^{101}, *yw*; P[ry^{+}, FRT]^{42D} $DaPKC^{k06403}$/*CyO*, *yw* P[mini-w^{+}, hs-FLP]^{1}; P[ry^{+}, FRT]^{42D} P[mini-w^{+}; ubi-nls-GFP]^{2R}/*CyO*, *yw* P[mini-w^{+}, hs-FLP]^{1}; $l(2)gl^{4}$ P[en.lacZ]^{2L} P[ry^{+}, FRT]^{40A}/*CyO*, *yw* P[mini-w^{+}, hs-FLP]^{1}; P[mini-w^{+}; ubi-nls-GFP]^{2L} P[ry^{+}, FRT]^{40A}/*CyO*, *yw* dlg^{m52} baz^{EH171} P[mini-w^{+}, FRT]$^{9\text{-}2}$/*FM6*, *yw B*, *yw* P[w^{+}; ubi-nls-GFP] P[mini-w^{+}, FRT]$^{9\text{-}2}$, *pr pwn* P[ry^{+}; hsFLP]^{38}/*CyO*, *yw B*, *pr pwn* P[ry^{+}; hsFLP]^{38}/*CyO*. The P[w^{+}; ubi-nls-GFP] P[mini-w^{+}, FRT] chromosomes bear a polyubiquitin promoter that drives ubiquitous nuclear GFP expression. Administering a 1-hour heat shock at 37°C on several consecutive days during late larval and pupal stages induced germline clones. Following immunohistochemistry, germline clones were identified by the absence of nuclear GFP expression.

Immunohistochemistry and imaging

Wild-type and mosaic ovaries were dissected, fixed and stained as described previously (Cox et al., 2001a). The following antisera were used: rabbit anti-Baz (1:1,500; a gift from A. Wodarz, Düsseldorf); rabbit anti-PKCζ C20 (1:1000; Santa Cruz Biotechnology); guinea-pig anti-Dlg (1:1000; a gift from P. Bryant); rabbit anti-Slbo (1:100; a gift from P. Rørth, EMBL Heidelberg); mouse anti-FasIII 7G3 (1:25; Developmental Studies Hybridoma Bank, University of Iowa); rat anti-Dcad2 (1:50; a gift from T. Uemura); mouse anti-Arm (1:100, N2-7A1 Developmental Studies Hybridoma Bank, University of Iowa); rabbit anti-β_H-spectrin [1:25 (Thomas and Kiehart, 1994)].
Cy2-, rhodamine red X- and Cy5-conjugated secondary antibodies (Jackson ImmunoResearch; Molecular Probes) were used at 1:200 to 1:500. Fluorescently labeled samples were counterstained with propidium iodide to visualize DNA. Micrograph images were taken with either BioRad MRC 1024ES confocal laser mounted on a Nikon Eclipse TE300 microscope or a Leica TCS SP2 laser scanning spectral confocal microscope. Images were processed with Adobe Photoshop (Adobe Systems).

RESULTS

baz and *DaPKC* regulate cell polarity and zonula adherens maintenance in follicular epithelial cells

To test whether *DaPKC* and *baz* function in the regulation of cell polarity within the follicular epithelium surrounding the germ cells, we generated follicle cell clones mutant for the null alleles $DaPKC^{k06403}$ (Wodarz et al., 2000) and baz^{EH171} using the FLP/FRT technique in which the wild-type chromosome was marked with green fluorescent protein (GFP) (Xu and Rubin, 1993). Mutant follicle cell clones identified by the absence of nuclear GFP expression were characterized by immunohistochemistry to assess follicle cell polarity and zonula adherens (ZA) integrity. Baz and DaPKC colocalize to the apical membrane of follicle cells during oogenesis (Huynh et al., 2001; Cox et al., 2001b). We first tested whether both proteins depend on each other for their correct apical localization. Indeed, Baz failed to localize to the apical membrane in $DaPKC^{k06403}$ follicle cell clones (Fig. 2A) and DaPKC mislocalized in baz^{EH171} mutant follicle cells (Fig. 2B) consistent with a role for each protein in stabilizing the PAR complex. Next, mutant $DaPKC^{k06403}$ and baz^{EH171} follicle cell clones were labeled with anti-Dlg, anti-DE-cadherin, anti-Arm, and anti-β_H-spectrin antibodies to visualize the integrity of apical and basolateral membrane compartments and of the ZA. Dlg normally localizes to the lateral membrane between follicle cell surfaces but, in $DaPKC^{k06403}$ (Fig. 2C) and baz^{EH171} mutant follicle cells (Fig. 2D), it was dispersed throughout the entire membrane. Arm and DE-cadherin strongly localize to the apicolateral ZA and, at lower levels, to the lateral membrane between follicle cell surfaces (Peifer et al., 1993; Niewiadomska et al., 1999; Cox et al., 2001b). However, within mutant clones, Arm (Fig. 2E) and DE-cadherin (Fig. 2F) were found in ectopic foci along the entire membrane. Moreover, we observed that those polarity defects were generally more severe at the poles of the egg chambers whereas, in lateral positions, the polarity of $DaPKC^{k06403}$ and baz^{EH171} mutant follicular epithelial cells was frequently almost normal (Fig. 2F, left arrow). This is possibly because of their direct contact with the germline as opposed to mutant follicle cell clones at the poles that are often multi-layered thus maintaining no direct contact with the germline cells. This phenotype was indicative of some non cell-autonomous rescue. Indeed, $DaPKC^{k06403}$ and baz^{EH171}mutant egg chambers in both the germline and the follicle cell epithelium have more severe polarity defects in lateral positions, indicative of some stabilizing role of the germline in polarizing the follicle cell epithelium (compare Fig. 2F, right and left arrows). In contrast to the distinctive apical localization pattern in wild-type follicle cells (Thomas and Williams, 1999; Tanentzapf et al., 2000), β_H-spectrin protein was mislocalized to ectopic sites in both $DaPKC^{k06403}$ and baz^{EH171} mutant follicle cells (data not shown). Taken together, the defects in Baz, DaPKC, Dlg, Arm, DE-cadherin, and β_H-spectrin localization indicate a loss of cell polarity and a breakdown of ZA in $DaPKC^{k06403}$ and baz^{EH171} mutant follicle cells. This conclusion is further supported by the rounded mesenchymal-like morphologies displayed by mutant

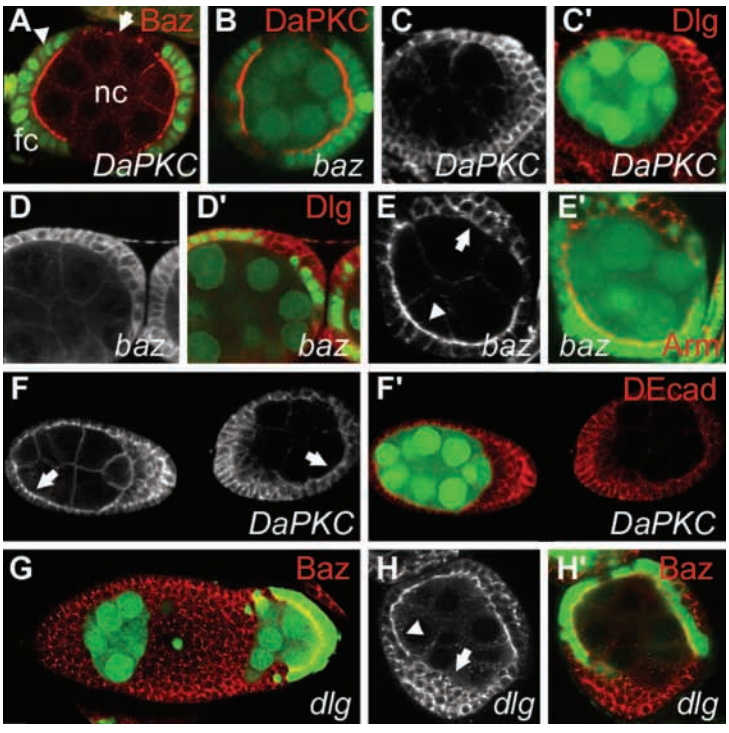

Fig. 2. Loss of cell polarity in $DaPKC^{k06403}$ and baz^{EH171} mutant follicle cells. (A) $DaPKC^{k06403}$ mutant follicle cells stained for Baz (red) which is mislocalized. (B) Within baz^{EH171} mutant follicle cells, DaPKC (red) is mislocalized. (C) $DaPKC^{k06403}$ mutant follicle cells stained for Dlg, which is mislocalized throughout the entire membrane. (D,E) baz^{EH171} mutant follicle cells stained for Dlg, which is mislocalized throughout the entire membrane (D) and Arm, which is mislocalized to ectopic foci (E). (F) $DaPKC^{k06403}$ mutant follicle cells stained for DE-cadherin, which is present in ectopic foci (F). In lateral positions, the polarity defects are stronger in the absence of germline DaPKC (left arrow: egg chamber with wild-type germline; right arrow: egg chamber with $DaPKC^{k06403}$ mutant germline). (G,H) dlg^{m52} mutant follicle cells stained for Baz display ectopic foci of Baz. Arrowhead indicates wild-type GFP-positive follicle cells; arrows show mutant GFP-negative follicle cells. fc, follicle cells; nc, nurse cells.

follicle cells. This loss of cell polarity is reminiscent of follicle cell clones mutant for the tumor suppressor genes *lgl* or *dlg*. Within follicle cells mutant for the severe tumor suppressor alleles dlg^{m52} and lgl^{4}, including those cells that behave like invasive tumor cells, Baz and DaPKC were localized to the cell membrane but dispersed to ectopic foci (Fig. 2G,H; data not shown).

To further analyze the role of *baz* and *DaPKC* in epithelial organization, we counterstained mutant epithelial clones with propidium iodide to examine the position of follicle cell nuclei. Within the ovarian follicular epithelium, proliferation generates a monolayer ranging between 650 and 1000 follicle cells (depending on the study) before egg chamber stage 6 when mitoses cease (Spradling, 1993) (Fig. 3A). Our analysis revealed that follicular epithelia in which all cells were mutant for $DaPKC^{k06403}$ were multi-layered, most strikingly at the anterior and posterior poles (Fig. 3B); another phenotype reminiscent of follicle cell clones mutant for the severe tumor suppressor alleles lgl^{4} (Fig. 3C) or dlg^{m52} (Goode and Perrimon, 1997). In $DaPKC^{k06403}$ mutant follicle cell clones, the density of nuclei in lateral positions was significantly decreased (compare Fig. 3E and F). Therefore, multi-layering at the poles probably results from a combination of mispositioning of cells and, possibly, mild over-proliferation. Similarly, follicle cell clones mutant for the baz^{EH171} null allele displayed a multi-layering phenotype similar to $DaPKC^{k06403}$ mutant follicular epithelia (Fig. 3D, arrow). In summary, we conclude from these data that signaling via the PAR complex and the basolateral tumor suppressors regulates follicle cell polarity and ZA maintenance and that loss of these protein complexes appears to cause epithelial-to-mesenchymal-like transformations of the follicular layer.

baz is essential for invasion of *dlg* tumor cells

Follicle cell clones mutant for the dlg^{m52} null allele have been shown to acquire invasive properties (Goode and Perrimon, 1997). In mutant follicle cell clones, the edges between soma and germline were rough (Fig. 4A, arrow), indicating that follicle cells generate some force upon the germ cell cluster and, during invasion, clusters of interconnected cells migrate between the germline nurse cells (Fig. 4B,C). In contrast, follicle cell clones mutant for baz^{EH171} and $DaPKC^{k06403}$ did not show any invasion of the germline proper and the edges at the boundary between the mutant follicular epithelium and the germline had a smooth appearance (Fig. 3B; $n>150$ for each). Therefore, we next quantified and compared the occurrence of heterogeneous cell mixing and invasion in dlg^{m52}, lgl^{4}, baz^{EH171} and $DaPKC^{k06403}$ follicle cell clones. Cells were counterstained with propidium iodide to identify the position and ploidy of follicle and germ cell nuclei. Follicle cells were counted as being invasive when they had forced their way between germline cells, thereby separating germ cell boundaries (Fig. 4D, arrow). Those cases in which follicle cells only deformed the germ cell cluster were not counted as being invasive (Fig. 4A,E; arrow). Our analysis revealed that follicle cell clones mutant for dlg^{m52} became invasive in 51% (85/167) of cases recorded. Streams of invasive follicle cells were found to break out of the follicular epithelium at random points (Fig. 4B,C) and to cross the germ cell proper, predominantly along the anterior-posterior axis of the egg chamber, but also in diverging directions relative to the position of the oocyte, identified by its ploidy state. Furthermore, follicle cell invasion occurred in 69% of egg chambers in which the entire germline was wild-type and 13% when at least some wild-type germ cells were present. We conclude, therefore, that *dlg* functions primarily in a follicle cell-autonomous fashion to inhibit cell mixing. In some cases, wild-type follicle cells were recruited into the streams of invading cells (Fig. 4C, white bracket). This non cell-autonomous effect may be caused by the adherence of wild-type and *dlg* mutant follicular cells to each other during invasion. Similar to dlg^{m52}, lgl^{4} mutant follicle cells were able to invade wild-type germ cell clusters (7.7%; 8/104) (Fig. 4D). Therefore, inhibiting follicle cell invasion appears to be an essential function of the tumor suppressor genes *dlg* and *lgl*.

In contrast to *dlg* and *lgl*, the two PAR complex components *baz* and *DaPKC* appeared to regulate cell polarity and to prevent multi-layering but not to block follicle cell invasion. To further investigate the failure of baz^{EH171} mutant follicle cells to invade the germline, we performed a genetic epistasis experiment examining follicle cell clones that were double mutant for dlg^{m52} and baz^{EH171}. We predicted that if wild-type *baz* is essential during follicle cell invasion, dlg^{m52} baz^{EH171} double mutants should lack invasive properties. Indeed, dlg^{m52} baz^{EH171} double mutant follicle cell epithelia were never invasive (Fig. 4E,F), except when the germline was dlg^{m52} baz^{EH171} double mutant. In this latter genetic combination, dlg^{m52} baz^{EH171} germline mutant egg chambers failed to differentiate an oocyte, resulting in a 16-nurse cell phenotype as revealed by the presence of 16 polyploid nurse cells and lack of an oocyte nucleus (data not shown). The 16-nurse cell phenotype, characteristic of baz^{EH171} germline mutant egg chambers (Huynh et al., 2001; Cox et al., 2001b), demonstrates that follicle cell invasion can occur in the absence of an oocyte. In total, only 8% (5/63) of egg chambers displayed an invasive phenotype and in those cases, follicle cell invasion always correlated with a dlg^{m52} baz^{EH171} double mutant germline. In

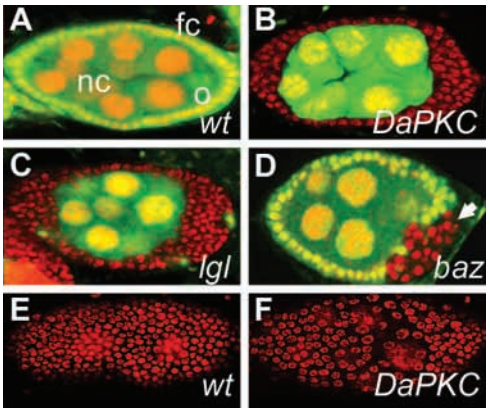

Fig. 3. $DaPKC^{k06403}$ and baz^{EH171} mutant follicle cells are multi-layered. (A) Wild-type follicle cell epithelium is mono-layered. (B) $DaPKC^{k06403}$ and (C) lgl^{4} mutant follicle cells are multi-layered. (D) baz^{EH171} mutant follicle cell clone is multi-layered (arrow). (E,F) Lateral views of the wild-type and $DaPKC^{k06403}$ follicle cell epithelium. Density of nuclei is higher in the wild-type epithelium (E) indicating that mispositioning of cells occurs in the lateral $DaPKC^{k06403}$ mutant follicle cell epithelium (F). Nuclear staining with propidium iodide and GFP clonal marker for wild-type cells (absence of GFP expression indicates mutant follicle cells). fc, follicle cells; nc, nurse cells; o, oocyte.

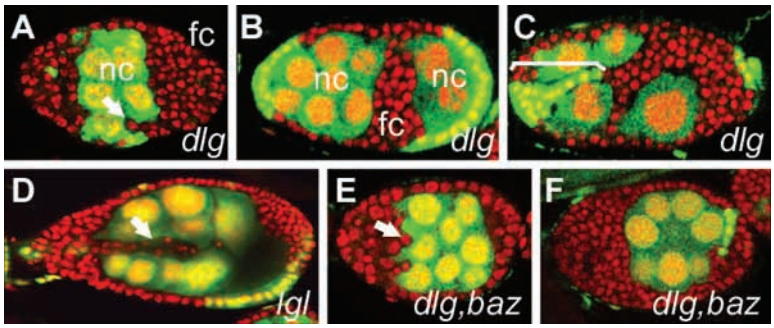

Fig. 4. *dlg* mutant follicle cell invasion requires Baz. (A-C) dlg^{m52} mutant follicle cells cause a rough germline boundary (A, arrow), or are invasive (B,C). Occasionally, wild-type cells are recruited into the streams of invading follicle cells (C, white bracket). (D) lgl^{4} mutant follicle cells can be invasive (arrow). (E,F) dlg^{m52} baz^{EH171} mutant follicle cells are not invasive but can cause a rough germline boundary (E, arrow). Nuclear staining with propidium iodide and GFP clonal marker for wild-type cells (absence of GFP expression indicates mutant follicle cells). fc, follicle cells; nc, nurse cells.

dlg^{m52} baz^{EH171} double mutant follicular epithelia, the boundary between follicle cells and germline frequently appeared rough (Fig. 4E), similar to the phenotypes observed with dlg^{m52}. Collectively, these results reveal that *baz* is epistatic over *dlg* in the control of follicle cell invasion. We suggest that Baz functions as a permissive factor required during dlg^{m52} tumor cell invasion perhaps by stabilizing adhesion between the invading cells and germline cells. Moreover, *baz*-dependent adhesion among germ cells may provide the resistance to block dlg^{m52} baz^{EH171} mutant follicle cells from invasion.

Baz and DaPKC protein localization within the border cell cluster

To further investigate the roles of *baz* and *DaPKC* during invasive cell migration, we characterized their function during border cell migration, a process that bears many similarities to tumor cell invasion (Montell, 2001). The border cell cluster comprises the two polar or central cells in the anterior follicular epithelium and several surrounding rosette cells that carry the central polar cells along as they migrate (Niewiadomska et al., 1999). The two central polar cells can specifically be labeled with anti-Fasciclin III antibodies (Brower et al., 1980; Patel et al., 1987) and the whole cluster of migrating cells can be marked with anti-Slow border cells (Slbo), the *Drosophila* homolog of C/EBP, a transcriptional activator that is essential for border cell migration (Montell et al., 1992). We first analyzed the distribution of Baz and DaPKC proteins within the border cell cluster. At late stage 8, when border cells segregate from the follicular epithelium, both Baz and DaPKC proteins display a tight apical localization to the junctional zone and reduced uniform levels of expression along all surface membranes (Fig. 5A).

This pattern changes dynamically during border

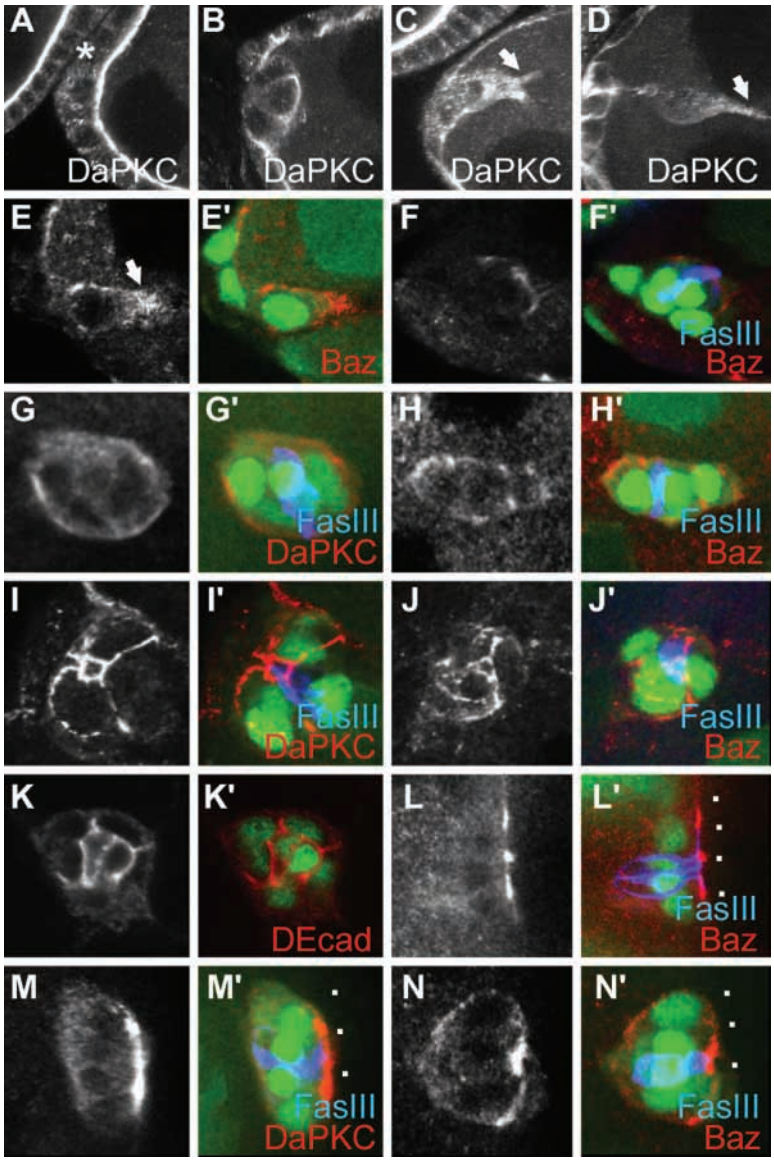

Fig. 5. Baz and DaPKC protein localization within the border cell cluster. (A-F) Late stage 8 and early stage 9 egg chambers stained for DaPKC (A-D) and Baz (E,F). Asterisk indicates central follicle cell prior to invasion (A). Both proteins localize to leading extensions of rosette cells during migration (arrows C-E). (G,H) Within the migrating border cell cluster, DaPKC and Baz strongly localize to the outer surfaces of the rosette cells, facing germline cells. (I-K) Confocal sections through the apical ring between the two central cells, I and J are apical sections, K is a more basal section. Prior to the rosette cells reaching the oocyte, DaPKC, Baz and DE-cadherin overlap on the membranes between rosette cells and in an apical ring between the two central cells. (L) When the cells reach the oocyte (dotted line) Baz localizes to the apical attachment side. (M,N) DaPKC and Baz localize to the apical attachment side between border cells and oocyte (dotted lines) as border cells migrate dorsally. In all panels posterior is to the right.

cell migration. At stage 9, a single rosette cell that invades neighboring germline cells initiates border cell migration. Within this leading cell, Baz and DaPKC strongly localize to the leading extensions (Fig. 5C-F) as well as to those membranes that face the germline. Once the entire border cell cluster has invaded the germline and started to migrate towards the oocyte, Baz and DaPKC strongly localize to the outer surfaces of the rosette cells, facing the germline cells (Fig. 5G,H). BAZ is also present at lower levels, on all germ cell membranes in contact with the migrating border cells. The two proteins also localize to the contact sites between rosette cells and with the central polar cells (Fig. 5I,J) in pattern similar to DE-cadherin (Fig. 5K). As the border cell cluster approaches the oocyte (Fig. 5L) and finally turns dorsally to join up with the inwardly migrating centripetal cells (Fig. 5M,N), Baz and DaPKC are strongly localized to the apical attachment site between oocyte and border cells. Taken together, the dynamic localization patterns of Baz and DaPKC proteins during border cell migration are suggestive of a role of these proteins in this invasive cell behavior.

baz affects adherence within the border cell cluster

We next tested whether *baz* and *DaPKC* function during border cell migration. In baz^{EH171} and $DaPKC^{k06403}$ mutant follicle cell epithelia, specification of central polar border cells was mostly normal as indicated by the presence of FasIII positive cells (Fig. 6A,B; and not shown). In addition, baz^{EH171} or $DaPKC^{k06403}$ mutant rosette cells (Slbo-positive) were present (Fig. 6D-H,J). Therefore, neither *baz* nor *DaPKC* is required for border cell specification.

We observed different requirements for *baz* and *DaPKC* during border cell migration. In the case of baz^{EH171}, we characterized migrating border cell clusters that were mixed with mutant and wild-type cells. Whereas border cell migration of these mosaic clones occurred at the correct stages, there were frequently abnormal arrangements of rosette and central polar cells. Most strikingly, border cell clusters were often elongated with individual cells trailing each other rather than migrating as a group of closely attached cells (Fig. 6F,G,I). Those cells that trailed behind often had elongated extensions which contacted anterior cells. Occasionally, long FasIII-positive extensions between central polar cells were observed (Fig. 6F, arrow). However, whereas DE-cadherin mutant border cells always lag behind wild-type cells (Niewiadomska et al., 1999), baz^{EH171}mutant border cells were found both as leading (Fig. 6D,E) and lagging cells (Fig. 6F,G,I). Therefore, baz^{EH171}mutant cells appear to migrate in a manner essentially indistinguishable from that of wild type. To test whether germline Baz is required for border cell migration, we characterized a rare class of egg chambers in which nurse cells, but not the oocyte, were baz^{EH171} mutant, since *baz* is required for oocyte differentiation (Huynh et al., 2001; Cox et al., 2001b) and lack of the oocyte may impair border cell migration. We documented only a few egg chambers with this genetic mosaicism. In three cases, in which most nurse cells were mutant, border cell migration occurred normally (data not shown). This result suggests that germline Baz, at least, is not strictly required for border cell migration.

In the case of DaPKC, no defects in border cell migration were observed. Border cell mosaic clones in which all rosette cells were mutant for $DaPKC^{k06403}$, migrated properly to the anterior margin of the oocyte. Moreover, adherence of mutant cells within the border cell clusters was unaffected (Fig. 6J). Based on these results, we conclude that *baz* is dispensable for both border cell invasion and motility, but appears to play a role in the adherence among border cells within the cluster, however, a similar requirement for *DaPKC* was not detected in this process.

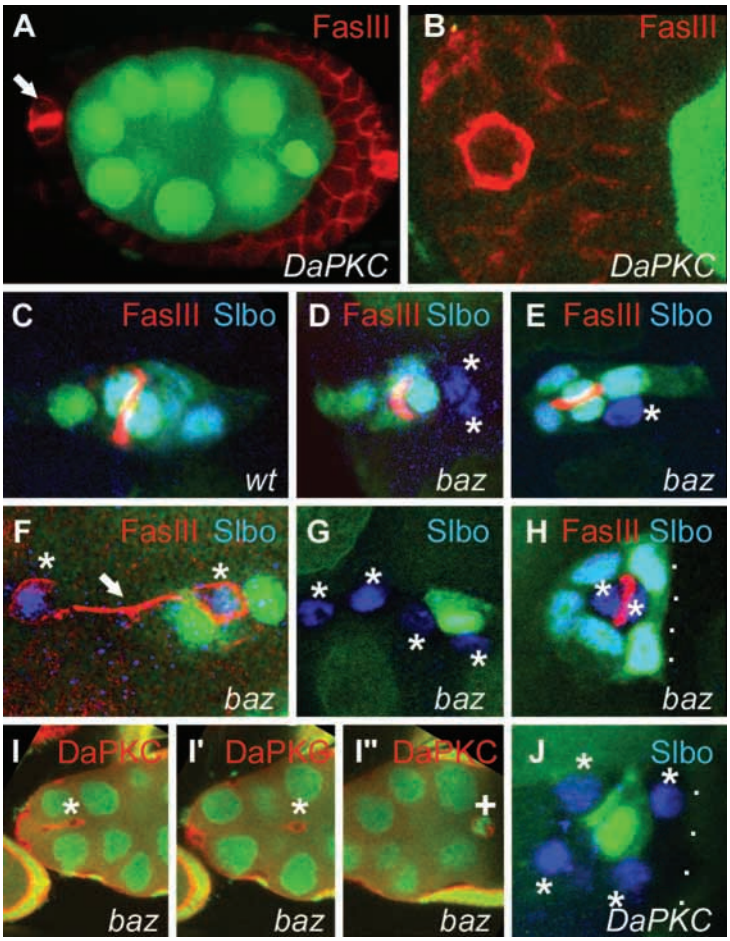

Fig. 6. Role of *baz* and *DaPKC* in border cell migration. (A,B) Egg chambers stained with FasIII. Polar cell induction is not dependent on *DaPKC* (arrow). (C-F,H) Migrating border cell clusters stained for Slbo (blue) and FasIII (red). (G,J) Border cell cluster stained for Slbo (blue). (I) Border cell cluster stained for DaPKC (sequence of several confocal z-sections). (D-I) baz^{EH171} mutant border cells (asterisks) are found in leading positions (D,E), trailing behind wild-type cells within the migrating cluster (F,G,I), or in central positions (H). Long FasIII-positive extension can be seen between baz^{EH171} mutant central polar cells (F, arrow). (I′′) Wild-type cells within the cluster are marked with a plus. (J) $DaPKC^{k06403}$ mutant rosette cells (asterisks) migrate properly to the oocyte (dotted line) and adhesion between border cells is not affected. GFP clonal marker for wild-type cells (absence of GFP expression indicates mutant follicle or border cells). In all panels posterior is to the right.

DISCUSSION

Our results suggest that *baz* is an essential component of *dlg* mutant follicle cell invasion into the germline. During border cell migration, which is another type of follicle cell invasion, *baz* is dispensable for invasion and motility but appears to be required for correct cell adhesion within the migrating cluster. We show that *baz* acts downstream of *dlg* in controlling follicle cell invasion. Taken together, these results suggest that loss of *dlg* initiates epithelial-to-mesenchymal transition and results in increased follicle cell motility. One role of wild-type *baz* may be to ensure the proper adherence between invading cells and their substratum.

baz differentiates tumor cell from border cell invasion

Two lines of evidence suggest mechanistic differences between tumor cell and border cell invasion. First, while both *dlg* tumor cell and border cell invasion undergo a series of similar morphogenetic behaviors, the molecular mechanisms regulating each cellular repertoire appear, at least in part, to be distinct. Whereas tumor cell invasion is dependent on *baz*, border cell invasion and motility are not. Therefore, *baz* genetically discriminates between these processes. Conversely, border cell migration requires *slbo* function, whereas *dlg* mutant follicle cell invasion can occur with much lower levels of Slbo and FasIII proteins and therefore *dlg* mutant cells appear not to adopt a border cell fate (Goode and Perrimon, 1997). A second line of evidence for mechanistic differences is that the patterns of cell invasion are distinct. The timing, direction and cohesion of border cells during their migration is highly stereotyped. In contrast, *dlg* tumor cells can invade at any stage in egg chamber development and in any orientation relative to the oocyte, possibly due to the position where follicle cell over-accumulation and multi-layering occur. Moreover, invasion also occurs in the absence of an oocyte, for example when the germline is dlg^{m52} baz^{EH171} double mutant.

Our data suggest that wild-type Baz is a permissive factor required for follicle cell invasion but that *baz* gene function is dispensable for border cell specification and invasion. Therefore, in the absence of *baz*, the specification of Slbo-positive cells and activation of the appropriate downstream targets that are required for the orchestration of border cell migration is normal. The activation of *slbo* and its target genes may largely mask the permissive role of *baz* in follicle cell migration, a requirement that is uncovered in the context of the *slbo*-independent type of follicle cell invasion caused by the loss of *dlg*. Moreover, in contrast to *DE-cadherin*, another gene with an essential function during border cell migration, Baz and DaPKC levels are not increased in border cells prior to and during border cell migration. The defects observed in *baz* mutant border cell migration are best explained by the lack of adhesion within the border cell cluster rather than by migratory defects.

This interpretation is consistent with the observation that, during border cell migration, *baz* function is clearly distinct from that of *DE-cadherin*, which, though essential for cell motility, does not affect border cell adhesion. The requirement of DE-cadherin for border cell motility appears to be independent of its role as a structural component of the ZA since another essential ZA component, the cytoskeletal linker protein Armadillo, is not required for border cell migration (Peifer et al., 1993). In mosaic border cell clones with *DE-cadherin* mutant cells, the mutant cells consistently lag behind wild-type cells, indicating that the mutant cells have a compromised migratory ability and that they are being dragged along by their wild-type neighbor cells (Niewiadomska et al., 1999). Conversely, in mosaic border cell clones, baz^{EH171} mutant cells were found in every position within the migrating border cell clusters, including the leading position, indicating normal motility. Indeed, cohesion between the migrating cells appears to be defective as mosaic cell clones were frequently dispersed and misarranged. Therefore, *baz* and *DE-cadherin* appear to have different functions during border cell migration.

A model for *baz* function during tumor cell invasion in the *Drosophila* ovary

This study provides an example of a genetic interaction between the apical PAR complex and basolateral tumor suppressor genes. This interaction was assessed based on tumor cell invasion. We have demonstrated that *baz* is epistatic over *dlg* in regulating this process. One possible explanation for the mechanism by which Dlg, a basolateral protein absent from the sites of contact between follicle and germ cells, regulates motility is that it acts via another protein complex. In this study, we have presented evidence that the apical PAR complex may serve such a function. We suggest a model in which follicle cell invasion is a two-step process: first, the loss of *dlg* releases a repression of motility and, second, the apical PAR complex protein Baz serves as a permissive factor for invasion (see model, Fig. 7). Based on our mosaic analysis, we propose a model in which invasion might be mediated by two separate *baz*-dependent interactions between follicle and germline cells. During invasion of *dlg* mutant follicle cells, Baz functions as a permissive factor to promote follicle cell invasive behavior (Fig. 7B). This invasive behavior is blocked in the absence of follicle cell Baz (Fig. 7C), as dlg^{m52} baz^{EH171} or baz^{EH171} mutant follicle cells lack invasive properties. Within the germline, Baz functions as both a permissive factor during invasion of dlg^{m52} mutant follicle cells that express Baz, possibly by stabilizing adhesion between the invading somatic cells and the germline cells (Fig. 7B) and, in the absence of follicle cell Baz, as a repressor of follicle cell invasion, possibly by regulating germ cell adhesion and preventing invasion of Baz-deficient follicle cells (Fig. 7C). The repression of Baz-deficient follicle cell invasion is neutralized in dlg^{m52} baz^{EH171} mutant germ cell clones possibly by a reduction of germ cell adhesion that may increase the ease with which dlg^{m52} baz^{EH171} mutant follicle cells can invade (Fig. 7D). These observations raise the question as to the molecular machinery and the adhesion molecules that mediate *baz*-dependent invasion and to the mechanisms that are in place in dlg^{m52} baz^{EH171} mutant follicle and germ cells in which invasion occurs. An alternative explanation to the loss of motility is that the removal of a second cell polarity system from follicle cells may cause such severe disturbances as to prevent cell invasion. However, dlg^{m52} baz^{EH171} double mutant follicle cells retain their capability to invade into dlg^{m52} baz^{EH171} double mutant germline proper, contradicting this explanation.

The data presented in this study raise the possibility that DaPKC serves similar, essential functions during *dlg* tumor cell invasion. However, this hypothesis was not tested since it

was genetically not possible to generate *dlg DaPKC* double mutant follicle and germline clones. During border cell migration, there is a different requirement for Baz and DaPKC.

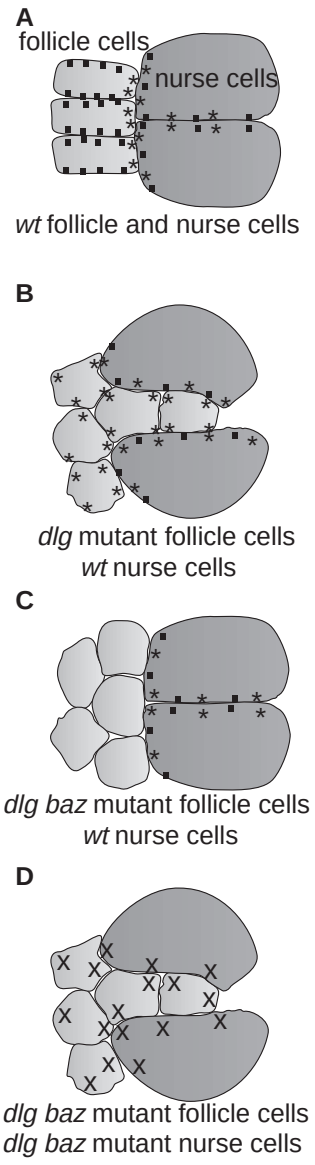

Fig. 7. Model of *baz*-dependent interactions between follicle and germline cells. (A) In wild-type, Baz (asterisks) localizes to the apical follicle cell membranes that are in contact with the germline nurse cell membranes and to the entire nurse cell membranes. Within the nurse cell membranes, Baz and Dlg have an overlapping localization pattern. In contrast, follicle cell Dlg localizes only to the basolateral membranes (rectangles). (B) Within invasive *dlg* mutant follicle cells, Baz functions as a permissive factor that is present along ectopic membrane sites and stabilizes, via *baz*-dependent interactions, the contacts with nurse cells. (C) *dlg baz* double mutant follicle cells lack invasive behavior. In the absence of follicle cell Baz, nurse cell Baz enhances germ cell adhesion thereby repressing follicle cell invasion. (D) The repression of *baz*-deficient follicle cell invasion is released in the absence of nurse cell Baz, possibly due to a reduction of nurse cell adhesion. The cellular machinery underlying this type of follicle cell invasion remains to be identified (X, unknown molecular player mediating cell invasion).

Whereas Baz appears to affect adhesion within the migratory border cell cluster, *DaPKC* function is dispensable for normal border cell invasion, migration, and adherence.

In contrast to previous findings reported by Goode and Perrimon (Goode and Perrimon, 1997), our results indicate *dlg* predominantly functions cell-autonomously to prevent invasion of follicle cells. This finding is consistent with our data on *lgl*, which also functions cell-autonomously within the follicle cell layer to prevent heterogeneous cell mixing and invasion. Indeed, Goode and Perrimon documented cases of cell-autonomous invasions of follicle cells into the germline, which support the notion that, despite quantitative differences between the studies, *dlg* functions cell-autonomously within the follicle cell layer. The FLP/FRT technique combined with GFP imaging used in our study allows for the unambiguous identification of mosaic tissues, clarifying issues of cell-autonomous gene function.

The multiple PDZ domain protein Baz and its vertebrate homolog ASIP is a membrane scaffolding factor required for assembly and sub-membrane attachment of the apical PAR complex (Joberty et al., 2000; Lin et al., 2000; Ebnet et al., 2001). The effects of the PAR complex on *dlg* mutant follicle cell invasion may be exerted via a separate but *baz*-dependent transmembrane adhesion complex, the nature of which is currently unknown (Fig. 7D). In contrast to its function during border cell migration, in humans, loss of E-cadherin correlates with and appears to promote the occurrence of invasive tumor formation (Birchmeier, 1995). It has been suggested, therefore, that E-cadherins serve distinct functions in different cell types, either by promoting or inhibiting cell motility (Montell, 2001). Further studies are required to test whether the homologous proteins of Baz (ASIP) and DaPKC (atypical PKCs iota and zeta) serve conserved functions in mammalian cells and, in contrast to E-cadherin function, whether their loss prevents tumor cell invasion. Moreover, it is unclear whether *baz* function is restricted to the behavior of *dlg* mutant follicle cells or is essential in other forms of tumor cell invasions.

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