Requirement for Par-6 and Bazooka in *Drosophila* border cell migration

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Summary

Polarized epithelial cells convert into migratory invasive cells during a number of developmental processes, as well as when tumors metastasize. Much has been learned recently concerning the molecules and mechanisms that are responsible for generating and maintaining epithelial cell polarity. However, less is known about what becomes of epithelial polarity proteins when various cell types become migratory and invasive. Here, we report the localization of several apical epithelial proteins, Par-6, Par-3/Bazooka and aPKC, during border cell migration in the *Drosophila* ovary. All of these proteins remained asymmetrically distributed throughout migration. Moreover, depletion of either Par-6 or Par-3/Bazooka by RNAi resulted in disorganization of the border cell cluster and impaired migration. The distributions of several transmembrane proteins required for migration were abnormal following Par-6 or Par-3/Bazooka downregulation, possibly accounting for the migration defects. Taken together, these results indicate that cells need not lose apical/basal polarity in order to invade neighboring tissues and in some cases even require such polarity for proper motility.

Key words: Par-6, Bazooka, Border cells, Cell migration, *Drosophila*

Introduction

The conversion of epithelial cells to migratory cells plays a central role in development, wound healing and tumor metastasis. Depending on the context, cells may move as integrated sheets, as individual cells or in small groups. During skin repair, large groups of cells migrate collectively as epithelial sheets. On the other hand, neural crest cells and primordial germ cells move in chain-like arrangements. In the latter cases, when migration is initiated, the cells must reduce their adhesion with the epithelial cells that are left behind, while maintaining cohesive contacts with other migrating cells. Changes in expression of specific cell adhesion proteins and intermediate filament proteins have been described that correlate with migration of a variety of epithelial cell types (Ridley et al., 2003). Although some epithelial characteristics are lost when cells become migratory, the fates of the recently characterized epithelial polarity proteins have not been described in migrating cells derived from an epithelium.

The migration of the border cells, a small group of follicle cells in the *Drosophila* ovary, provides a model system to study these events in vivo. The border cells originate from the anterior pole of the follicular epithelium, which surrounds and communicates with an underlying cluster of 16 germline cells to form an egg chamber (Fig. 1A). Migration is initiated when a pair of specialized follicle cells, known as the polar cells, secrete a cytokine signal that activates the Janus Kinase/signal transducer and activator of transcription (JAK/STAT) pathway in the neighboring 6-8 cells, stimulating their motility (Silver and Montell, 2001). Migration is completed once the border cells reach the oocyte.

Initially, the border cells are polarized epithelial cells. These epithelial cells have distinct membrane domains. The apical domain contacts the germline. The basal plasma membrane of follicle cells contacts the basal lamina. The lateral sides touch the plasma membranes of neighboring epithelial cells, connecting neighboring follicle cells through cell junctions. These distinct membrane domains harbor different protein complexes that are important for establishing and/or maintaining the epithelium. The Par-3/Bazooka (Baz), Par-6, atypical protein kinase C (aPKC) complex and Crumbs, Stardust (Sdt), Discs lost (Dlt) complex localize to the apical membrane of follicle cells during oogenesis as well as to subapical adherens junctions. Mutations in these genes cause epithelial discontinuity and multilayering (Abdelilah-Seyfried et al., 2003; Tanentzapf et al., 2000). E-cadherin and Armadillo (Arm), which is the *Drosophila* homolog of β-catenin, localize laterally in follicle cells and are critical for the assembly of adherens junctions. Clones of follicle cells lacking Arm lose adherens junctions, and E-cadherin is no longer detected in the junctional region. In addition, a protein complex composed of Discs large (Dlg), Lethal (2) giant larvae (Lgl), and Scribble (Scrib) localizes to septate junctions in the lateral domain. Mutations in *dlg* disrupt septate junction structure and cell polarity (Woods et al., 1997). Finally, proteins such as integrins localize to the basal domain. Although spatially separated, these protein complexes communicate with each other and in some cases can affect each other's proper localization.

Although much has been learned concerning the molecules and mechanisms that are responsible for generating and maintaining polarity within the follicular epithelium, little is

known of what happens to epithelial polarity when the border cells change from stationary epithelial cells to invasive cells. Earlier studies of the organization of the border cell cluster demonstrated that four to six motile cells are attached to the centrally located pair of anterior polar cells in a rosette arrangement (Niewiadomska et al., 1999). In addition, Crumbs was shown to be localized asymmetrically throughout migration, although the functional significance of this protein was not investigated (Niewiadomska et al., 1999).

It was not clear from the earlier work whether the asymmetric localization of Crumbs was unique to this protein or whether epithelial polarity was more generally maintained during border cell migration. In this study, we focus on Par-6 and Baz, the *Drosophila* homolog of Par-3, which are part of an evolutionarily conserved complex that contributes to the polarization of epithelial cells in a variety of organisms. Par-6 and Baz are localized at the apical surfaces of follicle cells and, interestingly, are maintained asymmetrically in the migrating border cells. Loss-of-function mutations in these genes in somatic follicle cell clones result in epithelial defects as previously described. In addition, we show that disruption of *par-6* and *baz* function in the border cells using RNAi results in delayed migration as well as mislocalization of membrane proteins such as E-cadherin and β_{ps}-integrin. Migration delays and mislocalization of E-cadherin and β_{ps}-integrin are also observed when Par-6 and Baz are overexpressed in border cells, using the Gal4/UAS system. Our observations suggest that border cells retain some degree of epithelial polarity during migration, and that Par-6 and Baz are required for the proper distribution of membrane proteins in the border cell cluster and for successful migration.

Materials and methods

Drosophila stocks and genetics

w^{1118} serves as the wild-type strain. The *slbo* allele used was $slbo^{1}$. Follicle cell clones (Xu and Rubin, 1993) were generated by crossing *yw* baz^{xi106} P[w+,FRT]9-2/*FM6* (Muller and Wieschaus, 1996) or *w*, $par6^{\Delta 226}$ P[w+,FRT]9-2/FM7c ftz *lacZ* (Petronczki and Knoblich, 2001) to *yw*, *ubi-nls*GFP P[w+,FRT]9-2; *e22c*-Gal4 P[w+, UAS-FLP]. One- to five-day-old adult female progeny were incubated at 29°C for 12-14 hours.

To express genes ectopically as well as to express RNAi trangenes, the Gal4/UAS system was utilized (Brand and Perrimon, 1993). The Gal4 strains used were *Act5c* (Ito et al., 1997), *e22c* (Lawrence et al., 1995), *c306* (Manseau et al., 1997), *slbo* (Rorth, 1998), *upd* (Doug Harrison) and *tubulin* (Lee and Luo, 1999). UAS lines used were UAS-Baz (Kuchinke et al., 1998), UAS-Par-6 (J. A. Knoblich, unpublished) and UAS-*dome*ΔCYT (Brown et al., 2001).

RNAi constructs

The RNAi-*par-6* construct contains the 1.5 kilobase coding region of *par-6* from the expressed sequence tag LD08317 cloned into the *Sym-pUAST* vector (Giordano et al., 2002). The RNAi-*baz* construct was obtained by cloning a two-kilobase PCR-generated *baz* fragment (from nucleotides 28506 to 30554 of a genomic clone, GenBank Accession number: AE003504) into the *Sym-pUAST* vector. The PCR-amplified genomic fragment was obtained using the following primer pair: *Eco*RI-5′ (5′-CGGAATCGGAGGACGACGATCCC-AGTCATC) and *Eco*RI-3′ (3′-CTGAATTCGAGGAGCAAATGC-CAC). Each construct was verified by restriction mapping and DNA sequencing. A control RNAi-*white* transgenic fly line was obtained (Giordano et al., 2002).

P element-mediated germline transformation

Plasmid DNA to be injected was prepared and purified by using the Qiagen plasmid kit. DNA was dissolved in injection buffer (0.1 mM phosphate buffer pH 6.8, 5 mM KCl) at a concentration of 500 μg/ml for the transforming plasmid and 160 μg/ml for the helper plasmid pπ25.7 (wings-clipped). The RNAi *par-6* construct and RNAi *baz* construct were injected into w^{1118} embryos as described (Spradling, 1986). Eighteen independent insertion lines were obtained for RNAi *par-6*, and were combined to create RNAi *par-6* lines with two copies of the transgene. One insertion on the second chromosome was obtained for RNAi *baz*. Additional insertions were obtained by mobilizing the original RNAi *baz* transgene.

Immunofluorescence and antibody production

Rabbit anti-PAR-6 antibodies were generated against the peptide HHQQAASNASTIMASDVKDGVLHL, affinity purified, (Proteintech Group) and used at 1:500 dilution. Ovary dissection and fixation, and antibody staining were performed essentially as described (Bai et al., 2000; Montell, 1999). For immunostaining, the following primary antibodies were used: mouse anti-Armadillo monoclonal [N27A1, 1:75; Developmental Studies Hybridoma Bank (DSHB)]; rat anti-DE-cadherin monoclonal (DCAD2, 1:10; (Uemura et al., 1996); mouse anti-Fascilin III monoclonal (7G10, 1:50; DSHB); rabbit anti-GFP serum (1:4000; Molecular Probes); mouse monoclonal anti-β_{ps}-integrin (CF.6G11, 1:10; DSHB); mouse anti-Singed monoclonal (7C, 1:25; DSHB); mouse anti-α-tubulin (1:500; Sigma). For rabbit polyclonal DmPAR-6 [1:500 (Petronczki and Knoblich, 2001)]; rabbit anti-Bazooka N-term (1:250) and rat anti-Bazooka N-term monoclonal [1:250 (Wodarz et al., 1999)] and rabbit anti-nPKCζ polyclonal (C20, 1:250; Santa Cruz Biotechnology), ovaries were first washed with PBS and blocked with 1% BSA in PBS, then incubated with the primary antibody at 4°C overnight. Secondary antibodies conjugated to Alexa-488 and Alexa-568 (Molecular Probes) were used at a dilution of 1:400. F-actin filaments were detected with rhodamine-phalloidin (Molecular Probes), at a dilution of 1:400. Images were captured with the Ultraview confocal microscope or with a digital camera on a Zeiss Axioplan fluorescent microscope. Three-dimensional reconstructions of confocal image stacks were generated using Volocity software.

Results

Asymmetric distribution of Par-6 and Baz in migrating border cells

Par-6 and Baz are PDZ domain proteins that associate with each other and with aPKC in apical epithelial domains, where they are required for apical/basal polarity. In single confocal sections of wild-type egg chambers, Par-6 and Baz were clearly localized apically in the follicle cells, including those cells that delaminate to form the border cell cluster (Fig. 1B-C′), as previously reported (Abdelilah-Seyfried et al., 2003). This asymmetric distribution of Par-6 and Baz was also observed in the border cells as they initiated their migration at early stage 9. At this stage, Par-6 and Baz localized predominantly to the leading edge of the cluster (Fig. 1E-F′). However, subsequently Par-6 and Baz accumulated at the junctions between adjacent border cells and were not found at high levels at the leading edge (Fig. 1H-I′). At stage 10, when the border cell cluster reaches the oocyte, Par-6 and Baz were found at high levels at the surface of the border cell cluster facing the oocyte (Fig. 1K-L′).

To get a more complete view of the distributions of Par-6, Baz and aPKC during migration, we examined the expression of these proteins in three-dimensional reconstructions of

migrating border cell clusters. Par-6, Baz and aPKC were found at high levels on one side of the cluster, which was roughly orthogonal to the direction of migration, whereas E-cadherin could be detected throughout the lateral surfaces of these cells (Fig. 1N-P′) (see Movie 1 in the supplementary material). Just as in epithelial cells, a small region of overlap between Par-6, Baz, aPKC and E-cadherin was detected. These findings are consistent with the previous observation that another apical epithelial protein, Crumbs, is also found on one side of the border cell cluster during migration, perpendicular to the direction of migration (Niewiadomska et al., 1999). These findings suggest that early in migration the entire cluster rotates so that the leading edge is roughly orthogonal to the apical domain.

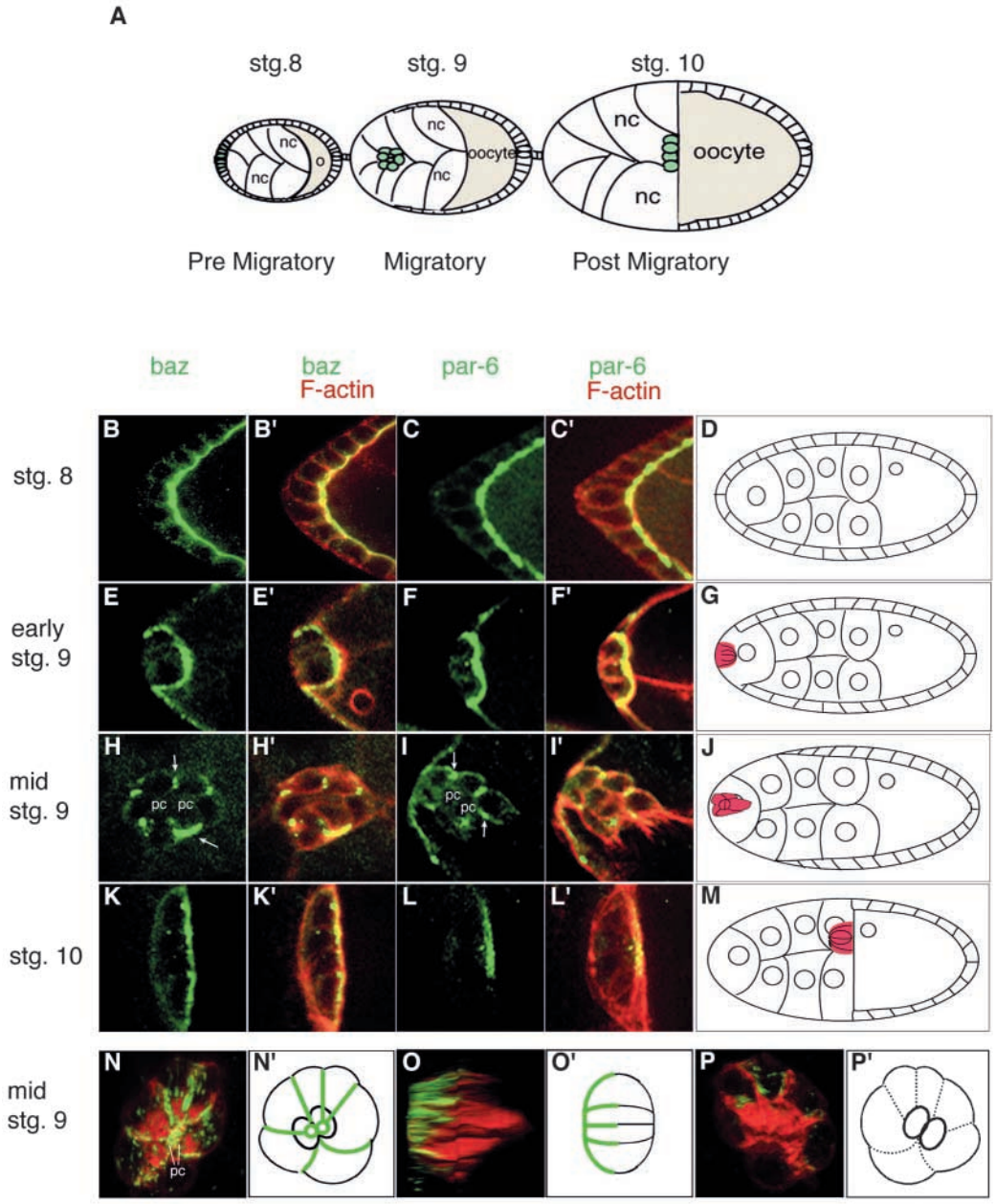

Fig. 1. Par-6 and Baz expression in the ovary. (A) Schematic of egg chambers at stages 8-10. Nurse cells (nc), and oocyte (o) are indicated. Border cells (green) are shown prior to, during and after migration. (B-M) Single confocal sections of wild-type anterior follicle cells (B-C′), and border cell clusters at the initiation of migration (E-F′), during migration (H-I′), and at the completion of migration (K-L′). Schematics of wild type stage 8 (D), early stage 9 (G), mid stage 9 (J) and stage 10 (M) egg chambers depict the position of the border cells (red) in the adjacent confocal images. Baz staining (green) is shown in panels (B,E,H,K) and double labeling with rhodamine phalloidin (red) to mark F-actin in panels (B′,E′,H′,K′). Co-localization appears yellow. Par-6 staining (green) is shown in panels (C,F,I,L) and double labeling with phalloidin (red) in panels (C′,F′,I′,L′). Polar cells (pc) are indicated in migrating clusters. High levels of Baz and Par-6 expression at border cell/border cell boundaries are indicated by arrows. (N-P′) Three-dimensional confocal reconstruction and schematic representation of a border cell cluster in mid migration. The orientation of the border cell cluster in panel N is identical to that of the border cell cluster shown in panel H. Par-6 (green) and E-cadherin (red) are shown. Par-6 expression at the apices of the central polar cells (pc) is indicated by two straight lines.

Par-6, Baz and aPKC were not detected at the surfaces of the border cells in contact with the polar cells. The polar cells occupy a central position in the border cell cluster during migration, and high levels of Par-6, Baz and aPKC were observed in a ring-like pattern at the polar cell apices (Fig. 1N). The asymmetric distributions of Par-6, Baz, aPKC and Crumbs (Niewiadomska et al., 1999) in the migrating border cells indicated that some apical/basal polarity was maintained.

Requirement for Par-6 and Baz expression in the border cells for proper migration and adhesion within the cluster

The reason for maintaining apical/basal polarity during migration is not understood. It has been postulated that this would have the advantage that epithelial polarity does not need to be established de novo when the border cells reach the oocyte (Niewiadomska et al., 1999). If this were the only purpose for maintaining polarity during migration, then the functions of apical proteins would not be required for migration. Alternatively, or in addition, Par-6 and Baz may play a specific role in migration, in which case disrupting their expression would impair migration. To distinguish between these hypotheses, *par-6* and *baz* homozygous mutant follicle cell clones were generated using the FLP/FRT system, since null alleles of *par-6* and *baz* are embryonic lethal.

Follicle cell clones, mutant for the baz^{xi106} or $par6^{\Delta 226}$ null allele, exhibited epithelial defects including discontinuity and multilayering of the epithelium, as previously reported (Abdelilah-Seyfried et al., 2003; Huynh et al., 2001). Although mutant border cell clusters were rare, we observed some examples of border cell clusters that failed to migrate to the oocyte (Fig. 2B,D). In these examples, the majority of cells in the cluster were homozygous mutant. Clusters with smaller numbers of mutant cells migrated normally (data not shown), as previously reported (Abdelilah-Seyfried et al., 2003), and mutant cells could be located in any position within the cluster. The morphology of the mutant clusters was frequently abnormal (Fig. 2C). In the case of baz^{xi106}, mosaic clusters were frequently elongated. In some cases, trailing cells were observed in $par\text{-}6^{\Delta 226}$ and baz^{xi106} mutant clusters. This suggests that Par-6 and Baz are important for maintaining adhesion between cells of the migrating cluster.

The general epithelial defects observed in $par\text{-}6^{\Delta 226}$ and baz^{xi106} follicle cell clones, and the infrequency of mutant border cell clusters, made it difficult to distinguish whether the observed migration defects were secondary to the epithelial defects. To circumvent the pleiotropic effects, we restricted the disruption of *par-6* and *baz* function to the border cells by using double-stranded RNA interference (RNAi). The UAS-Gal4 system was utilized to drive the expression of symmetrically transcribed RNAi *par-6* and RNAi *baz* transgenes in the border cells. When these RNAi lines were crossed to a Gal4 line under the control of the strong, ubiquitous *tubulin (tub)* or *Actin5C (Act5C)* promoter, lethality occurred in the F1 progeny (see Table S1 in the supplementary material). Control *tub-GAL4*/UAS-lacZ flies and control *Act5C*-Gal4 flies, which carry a Gal4-defective transposon, showed normal development. To test the function of Par-6 and Baz in border cell migration, the RNAi transgenes were activated using *slbo*-GAL4 which is expressed in the border cells, but not in the polar cells (Fig. 2E), long after the epithelium forms. Upon activation of the RNAi *par-6* transgene, Par-6 protein was reduced appreciably in 100% of stage 9 and 10 border cell clusters (n=85) compared to wild type (Fig. 2F,G). Similarly, a reduction of Baz protein levels was observed in 100% of stage 9 and 10 RNAi *baz*-expressing clusters (n=118) compared to wild type (Fig. 2H,I), indicating that the RNAi effect is completely penetrant.

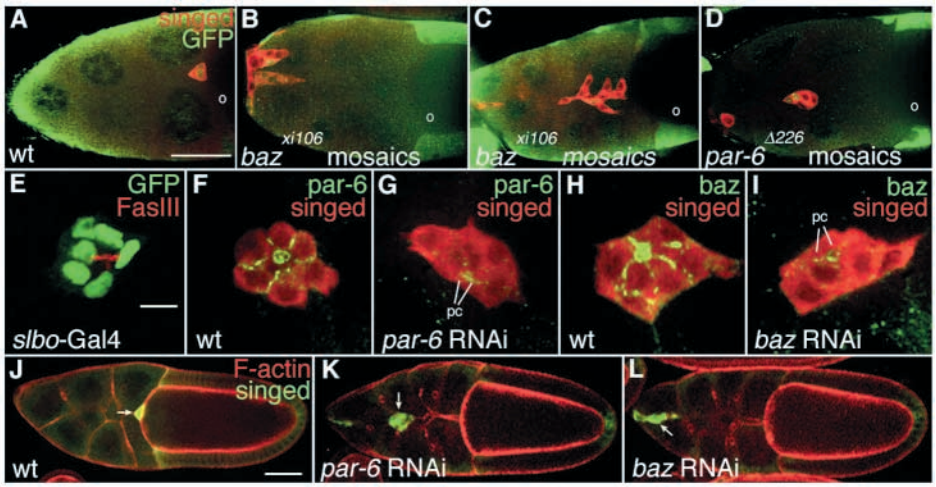

Fig. 2. Loss of Par-6 and Baz impedes border cell migration. (A-D) Single confocal section of stage 10 egg chambers stained with anti-Singed antibody (red) or GFP (green) that are wild type (wt) (A) or contain mosaic clones for a null mutation in the *baz* (B-C) or the *par-6* (D) locus. Homozygous mutant cells are labeled by the absence of GFP. o, Oocyte. Scale bar: 50 μm. (E) *slbo*-Gal4 driver, expressed in the border cells (UAS-GFP) (green), but not in the polar cells, Fascilin III (red). (F-L) Confocal images of border cell clusters that are wild type (F,H,J) or that express either two copies of RNAi *par-6* in a *par-6* heterozygous null background (G,K) or two copies of RNAi *baz* in a *baz* heterozygous null background (I,L). RNAi transgenes were activated using *slbo*-Gal4. (F-I) Merged confocal z sections of border cell clusters stained with anti-Singed antibody (red) (F-I) showing either Par-6 (green) (F,G) or Baz (green) (H,I). The position of the central polar cells (pc) is indicated by two straight lines. Scale bar: 5 μm. (J-L) Single confocal sections of stage 10 egg chambers showing Singed (green) and F-actin (red). Scale bar: 50 μm. Arrows indicate border cell cluster.

Expression of the RNAi *par-6* transgene or the RNAi *baz* transgene in the border cells, using *slbo*-Gal4, inhibited their migration (Fig. 2J-L). We observed incomplete border cell migration in 13% (n=685; P=0.016, Student's t-test) of stage 10 egg chambers from females carrying two copies of the RNAi *par-6* transgene (Fig. 3). Activation of two copies of the RNAi *par-6* transgene in flies heterozygous for the $par\text{-}6^{\Delta 226}$ null allele increased the severity of this defect to 34% (n=256; P=0.006, Student's t-test). Similarly, activation of two copies of the RNAi *baz* transgene in a baz^{xi106} heterozygous null background showed a stronger phenotype (33% migration delay; n=493; P=0.0001, Student's t-test) than activation of two copies of the transgene in a wild-type background (14% migration delay; n=685; P=0.013) (Fig. 3). A control RNAi *white* transgene (Giordano et al., 2002) did not cause a migration defect (Fig. 3); nor did it cause a reduction of Par-6 or Baz protein expression. These data demonstrate that Par-6 and Baz are required in migratory border cells for normal migration.

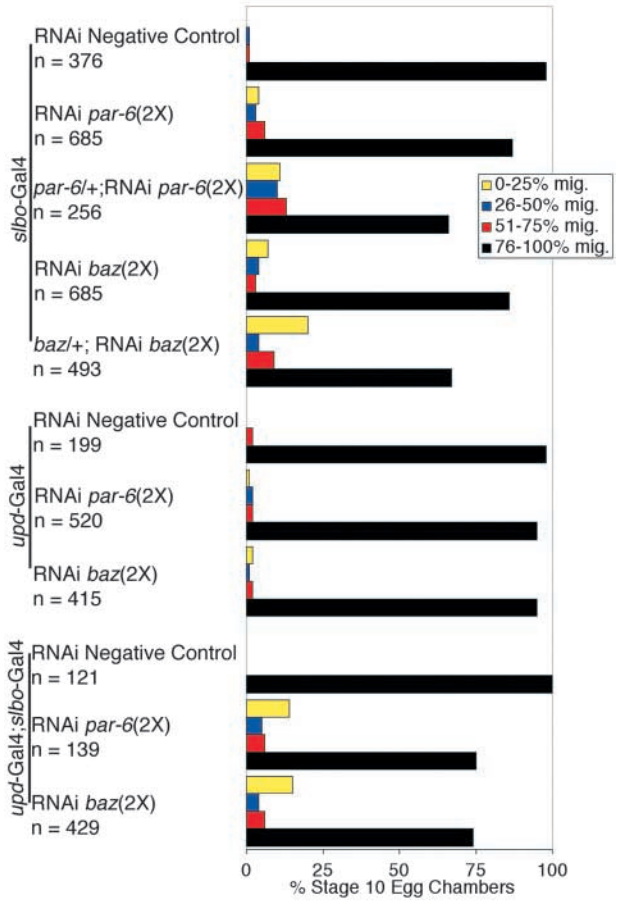

Fig. 3. Quantification of the delay in border cell migration caused by the depletion of Baz or Par-6 protein. RNAi lines and control line crossed to *slbo*-Gal4, *upd*-Gal4 or *upd*-Gal4; *slbo*-Gal4, driving expression of sense and antisense RNA in the border cells, polar cells or both. Two copies of the RNAi *par-6* and two copies of RNAi *baz* transgenes were activated respectively. Quantification of border cell migration shown as the proportion of egg chambers in which the border cells migrated: 0-25% (yellow), 26-50% (blue), 51-75% (red) and 76-100% (black) of the normal distance. *n*, number of egg chambers examined.

Activation of the RNAi *par-6* and RNAi *baz* transgenes in the central non-migratory polar cells alone, using *upd*-Gal4, did not cause a significant defect in border cell migration (Fig. 3). However, activation of the RNAi *par-6* transgene in the border cells and polar cells increased the migration delay to 25% (n=139; P=0.03, Student's t-test) (Fig. 3) from 13% when activated in the border cells alone. Similary, activation of the RNAi *baz* trangene in the border cells and polar cells vs. the border cells alone increased the migration defect from 14% to 25% (n=429; P=0.0002, Student's t-test) (Fig. 3).

Depletion of either Par-6 or Baz caused dramatic abnormalities in the organization of border cell clusters and the morphology of the cells, whereas wild-type border cells are typically firmly attached to one another and fairly regular in shape (Fig. 4A). When protrusions are observed in wild type, they are typically seen only in the leading and/or trailing cell of the cluster (Fig. 4B). In cells lacking Par-6 or Baz, border cells frequently appeared to adhere less well to one another and protrusions were observed in many cells and in more of the clusters than in wild type (Fig. 4C,D). Sometimes the protrusions were very long with one end remaining attached to a distant cell (Fig. 4E). These effects were even more obvious and frequent in clusters in which Par-6 or Baz were depleted from both polar cells and border cells than they were in clusters lacking Par-6 or Baz in border cells alone. Taken together these observations suggest that Par proteins are required for adhesion of border cells to each other and to the polar cells.

To gain further insight into how Par-6 and Baz affect proper border cell migration, we examined the localization of a variety of proteins, in cells depleted of Par-6 or Baz. E-cadherin, a homophilic cell adhesion molecule, is required for border cell migration. In wild-type egg chambers, E-cadherin is found at highest levels between border cells and polar cells and between adjacent border cells (Niewiadomska et al., 1999) (Fig. 4F). Substantially lower levels are observed at the interface between border cells and nurse cells (Fig. 4F). This is proposed to be a consequence of the necessarily dynamic nature of E-cadherin-mediated adhesion at the interface between border cells and nurse cells during migration (Bai et al., 2000; Niewiadomska et al., 1999). In contrast, a uniform distribution of E-cadherin at the membrane was observed in border cells depleted of Par-6 as well as an intracellular accumulation (Fig. 4G). We also observed a misdistribution of cell surface receptors, such as integrin (Fig. 4I) and PVR (data not shown). Normally, β_{ps}-integrin is expressed at high levels at the membrane between border cells (Fig. 4H). In border cell clusters depleted of Par-6 protein, β_{ps}-integrin appeared to accumulate in the cytoplasm (Fig. 4I). PVR aggregates unevenly on the surface of cells depleted of Par-6 compared to wild type.

Par-6 and Baz overexpression in the border cells disrupts membrane protein distribution and delays border cell migration

In *Drosophila* embryos, overexpression of Baz disrupts epithelial polarity (Petronczki and Knoblich, 2001). To carry out overexpression studies in the ovary, we used *slbo*-Gal4 (Rorth et al., 1998). We found that overexpressing Par-6 and Baz in the centripetal cells and posterior cells in the ovary, using *slbo*-Gal4, led to an expansion of the domain of expression of aPKC, an apical marker, into the lateral

membrane (Fig. 5A-D) of these cells. This suggests that overexpression of Baz and Par-6 disrupts the distinction between apical and lateral membrane domains. We examined whether overexpression of Par-6 and Baz in the border cells could alter the localization of aPKC, a serine threonine kinase that forms a physical complex with Par-6 and Bazooka. Three-dimensional reconstructions of confocal micrographs of border cell clusters, under these conditions, showed a disruption in the localization of aPKC and accumulation of aPKC at ectopic sites (Fig. 5I-L) (see Movie 2 in the supplementary material) compared to wild type (Fig. 5E-H) (see Movie 1 in the supplementary material). In addition, overexpression of Baz resulted in its accumulation at ectopic sites in border cells, and Par-6 colocalized to these sites (see Fig. S1 in the supplementary material). However, Par-6 overexpression did not result in the accumulation of Baz at ectopic sites.

We next determined whether overexpression of Baz and Par-6 affected the proper distribution of other proteins in the border cells. Membrane-associated proteins that localize to the apical, basal or lateral domain of the anterior follicle cells were analyzed. We found that ectopic expression of Baz and Par-6 disrupted the localization of E-cadherin (Fig. 5M,Q), β_{ps}-integrin (Fig. 5O,S) and Sdt (Fig. 5N,R). In addition the membrane-associated proteins, Dlt, Dlg, and Arm were also misdistributed (data not shown). However, the localization of cytoplasmic proteins, such as α-tubulin (Fig. 5P,T), Singed, FAK, and Myosin VI were not detectably affected (data not shown).

Overexpressing Par-6 in the border cells caused a delay in migration in 15% (n=119) of stage 10 egg chambers (Fig. 6). Overexpressing Baz in the border cells caused a delay in 32% (n=223) of stage 10 egg chambers (Fig. 6). Overexpression of both Baz and Par-6 enhanced the severity of the migration defect to 83% (n=124) (Fig. 6).

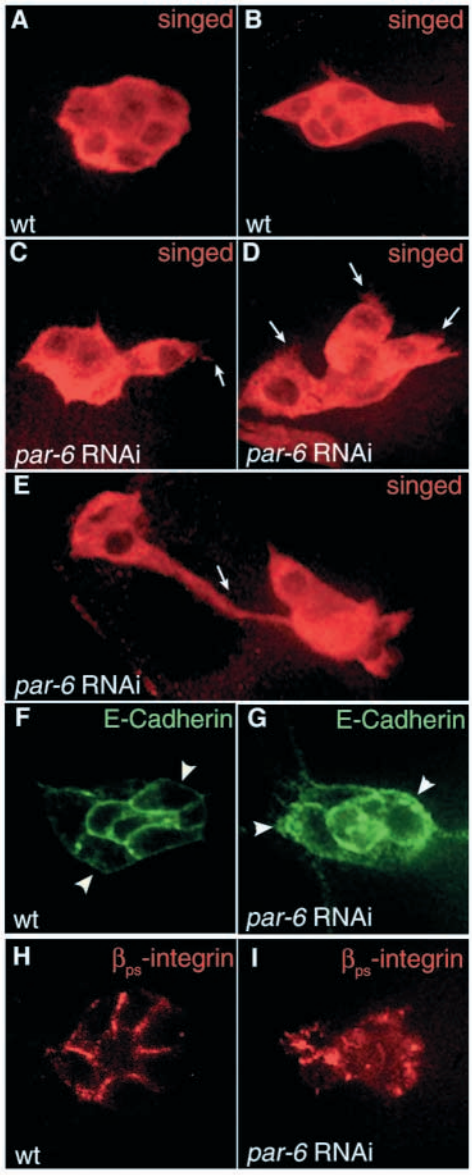

Fig. 4. Loss of Par-6 results in disorganized border cell clusters and a misdistribution of E-cadherin and β_{ps}-integrin. (A-B) Confocal sections of wild-type migrating border cell clusters stained with anti-Singed antibody. (C-E) Morphology changes due to activation of two copies of the RNAi *par-6* transgene in the border cells and the two central polar cells, using *slbo*-Gal4, expressed in the border cells, and *upd*-Gal4, expressed in the polar cells. Arrows show lamellipodia and long processes in cells depleted of Par-6. (F) E-cadherin protein expression in a wild-type border cell cluster. Border cell/border cell and border cell/polar cell boundaries express higher levels of E-cadherin than border cell/nurse cell junctions which are indicated by arrowheads. (G) Border cells depleted of Par-6. E-cadherin levels are high throughout the cluster. β_{ps}-integrin expression is disrupted in Par-6 depleted (I) versus wild-type cells (H).

Par-6 and Bazooka localization are altered in *slbo*, *domeless* and *myosin VI* mutants

A number of genes have been identified that exhibit defects in border cell migration including *slow border cells* (*slbo*), which encodes a basic region/leucine zipper transcription factor of the C/EBP family (Montell et al., 1992). To study the relationship between *par-6*, *baz* and *slbo*, we analyzed the localization of these polarity proteins in *slbo* mutant border cells. Par-6 (Fig. 7B) and Baz were concentrated at the polar cell/border cell junction in *slbo* mutants (Fig. 7B). This is different from wild type, in which Par-6 and Baz are absent from the lateral surfaces of border cells that are in contact with the polar cells (Fig. 7A). Expression of *slbo* requires the activity of the JAK/STAT pathway (Silver and Montell, 2001), and we found a similar disruption in Par-6 and Baz localization in border cell clusters expressing a dominant negative form of the receptor for the JAK/STAT pathway (Fig. 7C,D). A different pattern of mislocalization of Par-6 and Baz was also observed in border cell clusters depleted of Myosin VI (Fig. 7E,F). Instead of the normal accumulation at apical cell-cell junctions, puncta were observed randomly throughout the border cells. Since the JAK/STAT pathway and SLBO both affect transcription, the mislocalization of Par-6 and Baz in these mutants is probably due to an indirect effect on expression of one or more transcriptional targets. In contrast, MyoVI is unlikely to affect transcription and may have a more direct effect on protein trafficking. Nevertheless, it appears that mislocalization of Par-6 and Baz proteins is a common feature of several mutants that affect border cell migration.

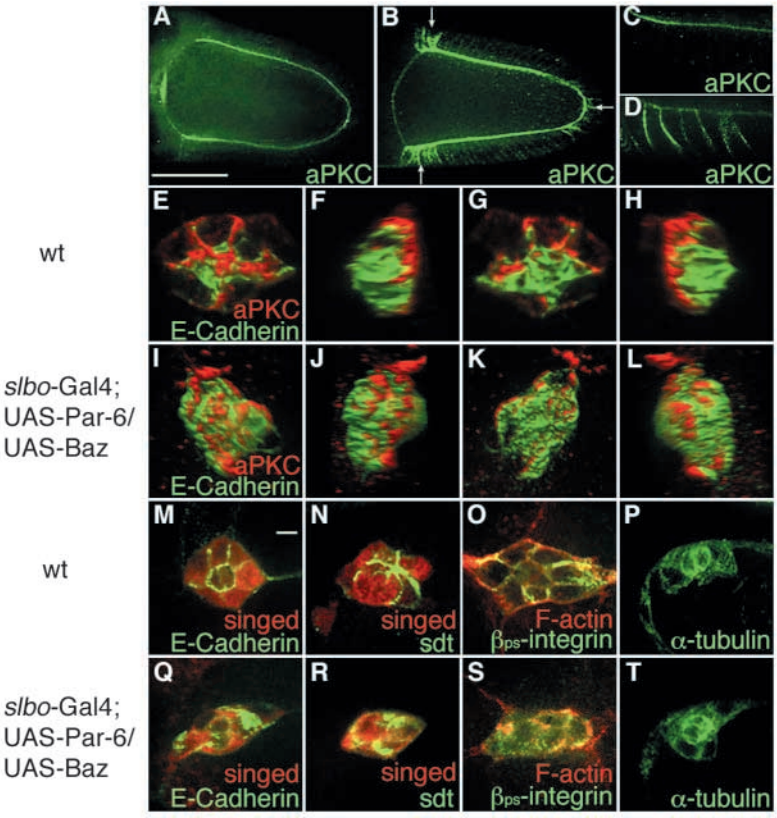

Fig. 5. Overexpression of Par-6 and Baz in the border cells disrupts polarity. (A-D) Single confocal section of stage 10 egg chambers stained with anti-aPKC. Wild-type egg chambers are shown in A and C. Egg chambers in which Par-6 and Baz are overexpressed in centripetal and posterior follicle cells, which are indicated by the arrows, are shown in B and D. C and D show higher magnification of the cells in A and B. Scale bar: 50 μm. (E-T) Confocal images of border cell clusters, stained for different polarity markers that are wild type (E-H,M-P) or overexpress Par-6 and Baz (I-L,Q-T) using *slbo*-Gal4. Scale bar: 5 μm. (E-L) Three-dimensional confocal reconstruction of migrating border cells, stained for aPKC (red) and E-cadherin (green). Each panel represents a different view of the same cluster. (M-T) Confocal *z* sections of border cell clusters of the indicated genotypes: E-cadherin (green) and Singed (red) (M,Q); Sdt (green) and Singed (red) (N,R); β_{ps}-integrin (green) and rhodamine phalloidine (red) (O,S); and α-tubulin (green) (P,T).

Discussion

Par-6 and Baz play a role in border cell migration

The conversion of epithelial cells to migratory cells occurs in a number of developmental processes, including border cell migration in the *Drosophila* ovary.

The border cells derive from a polarized follicular epithelium, however little is known of how their epithelial characteristics are modified as they begin to migrate. In this study, we investigated what happens to the distributions and functions of epithelial polarity proteins in these migratory cells. We found that border cells retain an asymmetric distribution of the apical epithelial proteins Baz, Par-6 and aPKC throughout their migration, raising the question as to why. One possibility could be that these proteins contribute to the cells' direction-sensing mechanism. However, neither Par-6 nor Baz localized asymmetrically with respect to the direction of migration, making this possibility seem less likely. In premigratory border cells, the apical domain is oriented towards the nurse cells and the direction of migration. However, once the cells separate from the epithelium, the side of the cluster with the highest levels of Baz, Par-6 and aPKC was found to be roughly orthogonal to the direction of migration. These findings are consistent with previous observations regarding the distribution of Crumbs, another apical marker (Niewiadomska et al., 1999), and suggest that early in migration the entire cluster rotates so that the leading edge is roughly perpendicular to the apical domain.

A second possibility is that maintaining some aspects of epithelial polarity during migration eliminates the need to re-establish polarity de novo when the border cells reach the oocyte (Niewiadomska et al., 1999). While possible, this hypothesis is difficult to test and cannot be the only function for Par-6 and Baz in border cells, since these proteins were also required during migration.

A third possibility is that cellular asymmetry is retained during border cell migration in order to achieve the proper asymmetries in the distributions of other proteins. Consistent

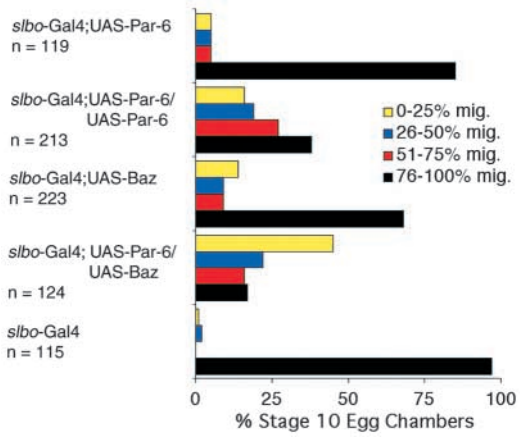

Fig. 6. Overexpression of Par-6 and Baz in the border cells delays border cell migration. Quantification of border cell migration defects caused by overexpression of Par-6 and Baz. Quantification of border cell migration shown as the proportion of egg chambers in which the border cells migrated: 0-25% (yellow), 26-50% (blue), 51-75% (red) and 76-100% (black) of the normal distance. *n*, number of egg chambers examined.

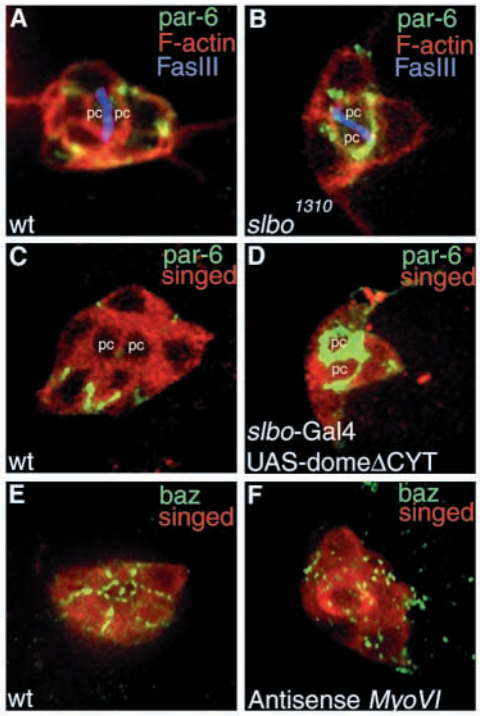

Fig. 7. Par-6 and Baz localization are altered in *slbo*, *domeless*, and *myosin VI* mutants. Single confocal image section of a (A) wild type (wt) and (B) $slbo^{1310}/slbo^{1310}$ female sterile mutant stained for Par-6 (green), rhodamine phalloidin (red), and Fascilin III (blue). (C) Wild type and (D) dominant-negative *domeless* (UAS-*dome*ΔCYT)-expressing border cells stained for Par-6 (green) and Singed (red). Polar cells are indicated (pc). Confocal *z* section of border cells from (E) wild type and (F) MyosinVI-depleted cells (Antisense MyoVI) that have been stained for Baz (green) and Singed (red).

with this proposal, the normally asymmetric accumulations of E-cadherin and β_{ps}-integrin within border cells were dramatically altered in cells depleted of Baz or Par-6. Previous studies indicate that loss of E-cadherin from border cells inhibits migration and that misdistribution of E-cadherin at border cell/nurse cell boundaries correlates with a migration defect (Bai et al., 2000; McDonald et al., 2003; Niewiadomska et al., 1999). The defects in the distributions of E-cadherin and other membrane-associated proteins in border cells depleted of, or overexpressing, Par-6 and Baz may collectively lead to the observed migration defect.

We examined a large number of mosaic egg chambers containing clones mutant for *par-6* or *baz* and observed delays in border cell migration as well as defects in cohesion within the cluster. It was previously reported that mosaic clones of *baz* showed a lack of adhesion within the border cell cluster but no migratory defects (Abdelilah-Seyfried et al., 2003). It is likely that this difference is due to clone size and/or protein perdurance, since only large clones in which the majority of the border cells were mutant, showed defects in border cell migration. Consistent with this, RNAi-mediated reduction of Par-6 and Baz in the border cells resulted in delayed migration, suggesting that the strongest migration defects are observed only when all the border cells lack Par-6 or Baz. This is not unusual. Mutations in *slbo*, *jing*, *stat92E* and *shotgun*, which encode E-cadherin, exhibit similar behavior such that clusters containing some wild-type cells can migrate. These findings seem to indicate that wild-type cells can 'drag' a few mutant cells, but when the number of migration-defective cells exceeds the number of migration-competent cells, migration slows or stops.

RNAi-mediated reduction of Par-6 and Baz in the polar cells, in addition to the outer border cells, exacerbates the defects caused by expression in the migratory cells alone. This suggests that polar cells contribute to organizing the cluster. Cohesion of the cluster may be necessary in order for the migratory cells to receive continuous activation of the JAK/STAT pathway during migration. Consistent with this, in those clusters that split, those cells that remain attached to the polar cells migrate further than the cells that become detached. Polar cells require the migratory cells to reach the oocyte because they are not motile themselves (Han et al., 2000), but the migratory cells also appear to need the polar cells in order to sustain their motility. This mutual requirement may serve to ensure that the migratory cells do not run off without the polar cells, since the polar cells are required at the oocyte surface to form the pore in the micropyle through which a sperm enters at fertilization.

Migrating border cells possess both epithelial and mesenchymal characteristics

The observations presented here demonstrate that Par-6 and Par-3/Baz are distributed asymmetrically in migrating border cells, suggesting that not all epithelial polarity is lost when these epithelial cells become motile. In spite of this, the morphology of the border cells, particularly at the border cell/nurse cell interface, can appear fibroblast-like. This interface must support protrusive behavior and dynamic adhesion, so that the cells can move along the nurse cells, while they simultaneously remain firmly attached to each other and to the polar cells. Therefore, migrating border cells possess both epithelial and mesenchymal characteristics.

We propose that the Par-3/Par-6/aPKC complex functions in these cells, as it does in an epithelium or in asymmetrically dividing neuroblasts, to maintain distinct protein distributions and functional domains in different parts of the cell. In the case of the border cells, three important domains are the interfaces between border cells and nurse cells, between border cells and polar cells and between adjacent border cells. Such distinct domains may be present in other types of cells that maintain contacts with an intact epithelium while they migrate, such as motile keratinocytes at a wound edge or leading endothelial cells during angiogenesis. Tumor cells that metastasize in groups or 'nests' may also possess both epithelial and mesenchymal characteristics. Thus the Par-3/Par-6/aPKC complex may contribute to the invasiveness of other cell populations as well.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/131/21/5243/DC1

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