Dynamics of the basement membrane in invasive epithelial clusters in Drosophila

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Summary
The basement membrane (BM) represents a barrier to cell migration, which has to be degraded to promote invasion. However, the role and behaviour of the BM during the development of pre-invasive cells is only poorly understood. Drosophila border cells (BCs) provide an attractive genetic model in which to study the cellular mechanisms underlying the migration of mixed cohorts of epithelial cells. BCs are made of two different epithelial cell types appearing sequentially during oogenesis: the polar cells and the outer BCs. Here, we show that the pre-invasive polar cells undergo an unusual and asymmetrical apical capping with major basement membrane proteins, including the two Drosophila Collagen IV α chains, Laminin A and Perlecian. Capping of polar cells proceeds through a novel, basal-to-apical transcytosis mechanism that involves the small GTPase Drab5. Apical capping is transient and is followed by rapid shedding prior to the initiation of BC migration, suggesting that the apical cap blocks migration. Consistently, non-migratory polar cells remain capped. We further show that JAK/STAT signalling and recruitment of outer BCs are required for correct shedding and migration. The dynamics of the BM represents a marker of migratory BC, revealing a novel developmentally regulated behaviour of BM coupled to epithelial cell invasiveness.

Key words: Basement membrane, Border cells, Migration, JAK/STAT, Transcytosis, Collagen IV

Introduction
The extracellular matrix (ECM), including the basement membrane (BM), plays a major role in the establishment and maintenance of epithelial cell morphogenesis (Sternlicht and Werb, 2001). Its selective synthesis and degradation is developmentally regulated to favor cell rearrangements and migration. In cancer cells, misregulation of ECM break-down strongly promotes cell invasion and metastasis (Egeblad and Werb, 2002). However, the behavior of the ECM in the early stages of migration, such as during migratory cell selection and delamination from an epithelium, remains poorly understood.

Drosophila border cells (BCs) represent an attractive genetic model with which to study the invasiveness of epithelial cell clusters (Montell, 2003; Rorth, 2002). BCs are made of two different cell types that appear sequentially during oogenesis, which form a composite cluster delaminating from the anterior follicular epithelium to migrate posteriorly into the egg chamber (Fig. 1A). In early stages, a pair of polar cells is determined at each pole of the egg chamber. Later, a ring of six outer BCs, surrounding the anterior polar cells, are specified to make a mature cluster, which, collectively with polar cells, is called the BC. We, and others, have recently shown that the formation of the BC cluster depends on signaling from polar cells to the outer BCs (Beccari et al., 2002; Ghiglione et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). In this process, polar cells express the secreted ligand Unpaired, activating the JAK/STAT pathway in neighboring cells. Those cells receiving the highest levels of the Unpaired ligand are committed into the outer BC fate and participate in the formation of a migratory BC cluster.

Recent work has shown that BCs are guided during their migration towards the oocyte through the EGFR and the PDGF/VEGF pathways (Duchek and Rorth, 2001; Duchek et al., 2001; McDonald et al., 2003). Other molecules are also essential for BC migration, including Slbo (Montell et al., 1992), ecdysone (a steroid hormone) (Bai et al., 2000) and myosin VI (Geisbrecht and Montell, 2002), as is the formation of long cellular actin-containing extensions (Fulga and Rorth, 2002). However, the cellular events accompanying the initial phases of BC formation and delamination are poorly understood, and it is particularly unclear what are the role and behavior of the BM in this process.

Here, we show that components of the BM undergo shuttling to the apical surface in anterior polar cells, through transcytosis involving Drab5 (Rab5 – FlyBase). This unusual apical capping is both asymmetrical and transient. Indeed, shortly before invasion starts, JAK/STAT signaling and interaction with outer BCs is required for shedding of the apical cap in polar cells, thus coordinating outer BC recruitment and invasiveness of the cluster. Strikingly, isolated polar cells that are unable to migrate maintain an apical cap.

The apical and transient targeting of BM materials in BCs may represent a novel marker of migratory cells during development and cancer, as well as a novel mechanism whereby the transition from pre-migratory to migratory phenotype is controlled.
Materials and methods

Genetics

A description of genetic markers and chromosomes can be found at FlyBase (http://flybase.bio.indiana.edu). Protein-trap lines vkgG454 (GFPG-Vkg) (Morin et al., 2001), and 658, 1700, 1973, 2840, 2867, G6 and G205 (Kelso et al., 2004), were used to mark the basement membrane and the apical cap. GAL4-dependent overexpression in the BC was performed using the slbo-GAL4, vkgG454/CyO line crossed to UAS-X flies (with X representing any reporter gene). The UAS lines used in this study are listed in Table 1. To overexpress eva, UAS-EYAB1 and UAS-EYAB2 were used (see FlyBase) (Bai and Montell, 2002), and crossed to Upd-GAL4 (a gift from D. Montell). Clonal analysis was performed using y w; vkgG454, FRT40A/CyO crossed to either HS-Flip; Hs-c-Myc, FRT40A/CyO or UB-GFP, FRT40A/CyO; T155-GAL4, UAS-Flip/T155-GAL4, UAS-Flip. Myc and nls-GFP were used as clonal markers.

Antibodies and western blotting

Polyclonal antibodies against type IV Collagen α1 chain (Cg25C; 1:1000) were from J. Fessler (Blumberg et al., 1988) and anti-GFP was a gift from R. Arkowitz (1:10,000). Monoclonal antibodies against Crumbs (1:50), Armadillo (1:50) and Fas3 (7G10; 1:50) were from the Developmental Studies Hybridoma Bank; anti-Laminin A (1:3000) was a gift from S. Baumgartner (Gutzeit et al., 1991), anti-β-galactosidase (1:1000) was from Promega and anti-c-Myc was from Calbiochem (1:100). Secondary antibodies were rabbit Alexa 488 (1:400) or Texas Red (1:100), and anti-mouse Texas Red (1:100) or Alexa 488 (1:400), from Molecular Probes.

Protein extracts were prepared from ovaries, loaded on SDS-PAGE gels and blotted onto nitrocellulose filters. Type IV Collagen α1 and α2-GFP chains were detected using anti-α1 and anti-GFP antibodies, respectively.

Confocal microscopy and imaging

From one experiment to another, we found a small variation in the number of egg chambers showing an apical cap in wild type (85±5%), probably because of variations in the fixation conditions. To determine the percentage of egg chambers showing an apical cap (Fig. 4G,H), we chose a confidence interval (α) and applied Student’s t-test to the data. The result of which indicates that the probability that Drab5WT is higher than wild type is 0.99 (99%), the probability that ShiK44A and wild type are not different is 0.99 (99%), and the probability that Drab5WT is higher than wild type is 0.85 (85%).

Results

Apical capping of anterior polar cells with basement membrane material

In order to analyse the dynamics of the BM in developing BCs, we used a specific protein-trap line expressing Drosophila type IV Collagen α2 chain [encoded by the viking (vkg) gene] fused to GFP (Morin et al., 2001). The resulting GFP-Vkg fusion protein is expressed under the control of the endogenous vkg gene.

Table 1. Expression of UAS constructs in BCs and their effect on apical cap formation and shedding

<table>
<thead>
<tr>
<th>UAS construct (reference)</th>
<th>Apical cap†</th>
<th>Shedding‡</th>
<th>Migration§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trafficlighting†</td>
<td></td>
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<tr>
<td>Drab5WT (Entchev et al., 2000)</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Drab5S45N (Entchev et al., 2000)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ShiK44A (strong) (Moline et al., 1999)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ShiK44A (weak) (Moline et al., 1999)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Drab70E (Entchev et al., 2000)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Deep orange (Dor) (Sevrioukov et al., 1999)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>JAK/STAT pathway‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpaired</td>
<td>+</td>
<td>+</td>
<td>–/–</td>
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<tr>
<td>DomeWT (Brown et al., 2001)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Dome ΔTM (Brown et al., 2001)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dome Δcyt (Brown et al., 2001)</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dome Δext (Ghiglione et al., 2002)</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Ecdysone, EGF and insulin pathways</td>
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<tr>
<td>Ecdysone receptor B1 (Cherbas et al., 2003)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ecdysone receptor B2 (Cherbas et al., 2003)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ecdysone receptor C (Cherbas et al., 2003)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Argos (Michelson et al., 1998)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>EGF receptor (Michelson et al., 1998)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Insulin receptor (Brogiole et al., 2001)</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Matrix metalloproteinases</td>
<td></td>
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<tr>
<td>Dml1-MMP (Llano et al., 2000)</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Dml1-MMPi1 (Page-McCaw et al., 2003)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Dml1-MMPi2 (Page-McCaw et al., 2003)</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Dm2-MMP (Page-McCaw et al., 2003)</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Dtip (Page-McCaw et al., 2003)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Small GTPases</td>
<td></td>
<td></td>
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<tr>
<td>DrholN25 (Murphy and Montell, 1996)</td>
<td>+</td>
<td>+</td>
<td>–/–</td>
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<tr>
<td>DRac1-89 (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DRac1-117 (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DRac1-122 (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DCdc42V12 (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DCdc42V12 (Luo et al., 1994)</td>
<td>+</td>
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The expression of UAS constructs was driven using the Slbo-GAL4, GFP-Vkg line at 29°C (except for UAS-DomeWT, which was done at 25°C). Slbo-GAL4 is expressed in BCs. In each condition, apical cap formation and shedding were analysed. The effect on the anterior-posterior migration of the BC cluster was also analysed.

ND, not determined because of lethality.
†+, like wild type; –, mutant phenotype. A mutant phenotype is observed only with Drab5S45N and is described in the legend to Fig. 4.
‡Same as for Apical Cap defects (in this case a phenotype is observed only with DomeDN forms and is shown in details in Fig. 7).
§+, migration takes place normally; –, migration defect in more than 50-60% of the egg chambers. For most UAS lines tested, a detailed analysis of the phenotype can be found in the original reference. Detailed analysis of the migration phenotype observed in Drab5S45N and ShiK44A egg chambers is described in Fig. 6. +/–, means there is a peculiar phenotype, which is as follows: Unpaired (Upd), ectopic outer border cells form, with the normal cluster migrating normally; DrholN25, only approximately 30% of the egg chambers show a migration defect.
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promoter and thus represents a faithful reporter of Vkg expression. GFP-Vkg strongly accumulates basally in all egg chambers (Fig. 1B; data not shown), consistent with Collagen IV being a major component of the BM (Fessler and Fessler, 1989). Most surprisingly, we found that, in stage 8 egg chambers, GFP-Vkg also accumulates apically into a discrete cap over the anterior polar cells (arrowhead). (C,D) Close up of the framed regions shown in B. Polar cells are marked by Fas2 expression (B-D; red). (E-G) Co-localization of GFP-Vkg and Coll.IV α1 (F,G; red), at the BM and in the apical cap (G). (H) Antibodies are specific to Coll.IV α1, as they do not recognize GFP-Vkg. The lower band (asterisk) is non-specific. w, white. (I-K) Co-localization of GFP-Vkg and Laminin A (red) in the apical cap. (L-N) Apical cap accumulation of BM protein-trap lines G205 (Vkg), 2840 and 2867 (Perlecan; see Fig. S1 in the supplementary material). Anterior is to the left. Scale bars: in B,10 µM; in C, 5 µM for C,D; in E, 5 µM for E-G; in I, 5 µM for I-K; in L, 5 µM for L-M.

Fig. 1. Apical capping of anterior polar cells with basement membrane material. (A) Formation and migration of the BC cluster. (a) A stage 8 egg chamber made of a monolayer epithelium (follicle cells, light gray) surrounding the germline (blue, nurse cells; yellow, oocyte). (b) Anterior polar cells (orange) express the Upd ligand activating the JAK/STAT pathway in follicle cells (arrows). (c) Cells receiving the highest levels of Upd are committed into the outer BC fate (beige). (d) A mature cluster delaminates and starts migration. (B) GFP-Vkg (green) accumulates in the BM. At stage 8, an apical cap forms over anterior polar cells (arrowhead). (C,D) Close up of the framed regions shown in B. Polar cells are marked by Fas2 expression (B-D; red). (E-G) Co-localization of GFP-Vkg and Coll.IV α1 (F,G; red), at the BM and in the apical cap (G). (H) Antibodies are specific to Coll.IV α1, as they do not recognize GFP-Vkg. The lower band (asterisk) is non-specific. w, white. (I-K) Co-localization of GFP-Vkg and Laminin A (red) in the apical cap. (L-N) Apical cap accumulation of BM protein-trap lines G205 (Vkg), 2840 and 2867 (Perlecan; see Fig. S1 in the supplementary material). Anterior is to the left. Scale bars: in B,10 µM; in C, 5 µM for C,D; in E, 5 µM for E-G; in I, 5 µM for I-K; in L, 5 µM for L-M.

Apical capping is asymmetrical and dynamic
Three-dimensional imaging of anterior stage 8 egg chambers shows that the apical cap is rod-shaped and runs along the apical surface (Fig. 3A-F; see Movies 1 and 2 in the supplementary material). Interestingly, the apical cap preferentially associates with one of the two polar cells (see below), thus revealing a previously unknown intrinsic asymmetry within the pair of anterior polar cells.
Careful staging of egg chambers indicates that apical capping is highly dynamic and proceeds through four discrete steps. (1) From stages 1-8 of oogenesis, GFP-Vkg and other BM component localization is restricted to the basal surface of epithelial cells (Fig. 3G and data not shown). (2) During stage 8, the formation of a discrete apical cap above each of the two polar cells is observed, suggesting that, initially, each polar cell makes its own apical cap (Fig. 3H, arrowheads; Fig. 2D,F). Subsequently, when the apical surface of polar cells constricts, one cap develops (large arrowhead) while the other remains rudimentary (small arrowhead), thus leading to asymmetrical capping (Fig. 3I-K). (3) The apical cap is kept in place at stages when polar cells undergo rounding and detach from the BM (Fig. 3J). In rare cases, it is possible to observe an intermediate stage showing two opposite caps, one at the basal and one at the apical surface (Fig. 3K). (4) Finally, when the BC cluster starts to migrate, the apical cap is no longer observed and only the basal cap remains (Fig. 3L). Thus, apical capping is transient, appearing at stage 8 and being shed at stage 9. The timing and cell-type specificity of apical capping indicate that BM cap dynamics are tightly coordinated with the formation of migratory border cells.

Subsequently, when the apical surface of polar cells constricts, one cap develops (large arrowhead) while the other remains rudimentary (small arrowhead), thus leading to asymmetrical capping (Fig. 3I-K). (3) The apical cap is kept in place at stages when polar cells undergo rounding and detach from the BM (Fig. 3J). In rare cases, it is possible to observe an intermediate stage showing two opposite caps, one at the basal and one at the apical surface (Fig. 3K). (4) Finally, when the BC cluster starts to migrate, the apical cap is no longer observed and only the basal cap remains (Fig. 3L). Thus, apical capping is transient, appearing at stage 8 and being shed at stage 9. The timing and cell-type specificity of apical capping indicate that BM cap dynamics are tightly coordinated with the formation of migratory border cells.
Polar cells, but not border cells, maintain a basement membrane during migration

BCs are of epithelial origin and their detachment from the follicle epithelium requires remodelling of cellular junctions (Niewiadomska et al., 1999). Apical and adherens junction markers are reorganized during the transition from an epithelial to a migratory phenotype, leading to an extension of the apical and junctional domains and the adoption of a rosette-like shape (Fig. 3M) (Niewiadomska et al., 1999). After migration, when BCs reach the oocyte and re-epithelialize, the apical domain returns to its original organization (Fig. 3O). Interestingly, we found that polar cells keep a BM throughout the process, in a position opposite to the apical side as shown by double staining with the apical marker Crumbs (Fig. 3M,O). This is in sharp contrast with outer BCs, whose BM is detached or degraded when they delaminate from the follicle epithelium. This result indicates that, contrary to outer BCs, polar cells maintain a fully polarized phenotype with a normal BM during migration.

Apical capping is controlled by Drab5-dependent transcytosis

Two simple mechanisms could lead to the formation of an apical cap over polar cells. In a first scenario, this cap could originate from apical targeting of neo-synthesized BM proteins by polar cells. In a second mechanism, the apical cap could form by transcytosis of BM material from the basal to the apical surface. In order to discriminate between these two mechanisms, we first tested whether inhibition of GFP-Vkg expression in polar cells and/or BCs could affect apical capping. Clonal analysis was used to make non-expressing GFP-Vkg cells in a GFP-Vkg background. In such mosaic egg chambers, polar cells that do not express GFP-Vkg still show an apical cap (Fig. 4A), indicating that it is not originating from de novo synthesis in the polar cells. This conclusion is consistent with the fact that polar cells, unlike other follicle cells, do not express GFP-Vkg around stage 8, as shown by the absence of GFP-Vkg in the cytoplasm (Fig. 4 and data not shown). Apical capping does not depend on GFP-Vkg expression in outer BC either, as clones of outer BCs that do not express GFP-Vkg do not affect the assembly of an apical cap (Fig. 4B,C). Thus, BM proteins present in the apical cap have a non-autonomous origin. We found that the normal BM, which is present in all stages throughout oogenesis (Fig. 5A), is also made in a non-autonomous manner (Fig. 5B-G), indicating that it is not possible to directly test the effect of genetically removing components of the BM. Indeed, the BM was normal whatever the developmental stage or the size of the clones; consistently, we found that vkg mutations do not show any defect in BC migration (data not shown).

The above results suggest that the apical cap originates from outside the polar cells. Transcytosis is a mechanism allowing proteins from one membrane sub-domain to be targeted to another domain after internalization (Tuma and Hubbard, 2003). To test whether internalization could be a mechanism by which the apical cap forms, we blocked Drab5 and Shibire (a Drosophila dynamin homolog) function in the BCs. These proteins are required in intracellular trafficking in Drosophila and other organisms (Seto et al., 2002), and we show here that both proteins are essential for BC migration (Table 1; Fig. 6).

Interestingly, the expression of a Drab5 dominant-negative (DN) form (Drab5S43N) (Wucherpfennig et al., 2003) results in the absence of the apical cap in stage 8 egg chambers, as compared with wild type (Fig. 4D,E,G). Moreover, among clusters still showing a cap in Drab5S43N egg chambers, 47% show a ‘micro-cap’ (Fig. 4F,H), which is twice as frequent as in wild-type egg chambers (20%) (Fig. 4H). Conversely, the expression of Drab5S43N induces a 5% increase in the number of egg chambers with an apical cap (Fig. 4G). In this case, microcaps are less frequent (15%) than in wild type (25%) (Fig. 4H).

The expression of a dominant-negative form of Shibire (ShiS44A) (Moline et al., 1999) did not block the formation of the apical cap (Fig. 4G). Thus, contrary to clathrin-dependent endocytosis, Drab5 and Shi have distinct roles in the formation of an apical cap over anterior polar cells.

We also tested the effect of other trafficking molecules and of signaling pathways known to be important for BC migration (see Table 1). None of these induced a defect in apical cap formation, indicating that Drab5 plays a specific role in this process.
Apical cap shedding requires JAK/STAT signaling and outer BCs

Once BC delaminate and start migration, the apical cap is no longer observed, indicating that an active process is responsible for removing the BM apically. It is thus important to determine the mechanism by which apical shedding and migration are coordinated. One likely possibility is that outer BCs themselves could control the status of the apical BM in polar cells. In order to test this possibility, we blocked the formation of outer BCs by expressing dominant-negative forms of Domeless (Dome) (DomeΔExt and DomeΔCyt), the receptor of the JAK/STAT pathway. In wild-type egg chambers, polar cells secrete the Unpaired ligand, which activates the JAK/STAT pathway in neighboring cells and recruits them as outer BCs (Fig. 1A) (Beccari et al., 2002; Ghiglione et al., 2002; McGregor et al., 2002; Silver and Montell, 2001). Those cells receiving the highest levels of Unpaired are committed into the outer BC fate and participate in the formation of a migratory BC cluster. Expression of dominant-negative forms of Dome lacking either the extracellular or the intracellular receptor domain (DomeΔExt and DomeΔCyt, respectively), induces most egg chambers to develop without outer BCs (Ghiglione et al., 2002). Consequently, the two polar cells remain at the anterior tip and do not migrate (Fig. 7B). Strikingly, the apical cap forms normally in DomeΔCyt stage 9 egg chambers but is not shed in stage 10, or later, egg chambers (Fig. 7A,B), suggesting that outer BCs are required for removing of the apical cap. In order to confirm this result, and to rule out the possibility that the absence of migration itself could block shedding, migration of a fully formed BC cluster was inhibited by expression of either a dominant-negative form of Drac (DracN17) (Murphy and Montell, 1996) (Fig. 7C,D) or a wild-type form of Dome (Ghiglione et al., 2002) (Table 1). In both cases, and despite a lack of migration, the apical cap forms normally and is shed like in wild-type clusters, confirming that outer BCs are essential for shedding before the cluster starts migration, probably by interacting back with the polar cells. Note that, in the Drac1 condition, detachment from the BM appears normal, as indicated by the presence of a basal cap (Fig. 7D), and the migratory phenotype originates from actin cytoskeleton defects, as shown previously (Geisbrecht and Montell, 2004; Murphy and Montell, 1996).

The Drosophila genome encodes only two matrix metalloproteinases (MMPs), which are natural regulators of the extracellular matrix. Interestingly, Drosophila Dm1-MMP (Mmp1 – FlyBase) and Dm2-MMP (Mmp2 – FlyBase) have been shown to degrade Collagen IV in vitro (Llano et al., 2002; Llano et al., 2000). However, we could not see any defect in apical cap dynamics upon expression of Dm1-MMP (native or activated form) or the Drosophila MMP inhibitor DTIMP (Timp – FlyBase; Table 1), which suggests that a different protease or mechanism is responsible for apical cap degradation. It is also important to mention that neither Dynamin-dependent endocytosis induces...
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(ShiK44A) nor lysosomal degradation (Drab7Q67L and Dor) seem to be involved in cap formation or shedding (see Table 1).

Discussion

The migration of cohorts of cells is an alternative to single-cell migration, which is used by normal and cancer cells to invade tissues. One advantage for mixed clusters is to transport tumorigenic (for example, apoptotic resistant) cells with no migratory abilities to a distant destination that they could not reach on their own (Friedl and Wolf, 2003). In this case, migration is executed by migratory capable cells within the cluster. Clusters illustrate how separate functions (tumorigenesis and migration) can be merged through collaboration between two cell populations. It is thus important to understand how migrating cell clusters are assembled and organized. The BC cluster is made of two distinct populations of cells, i.e. the polar cells and the outer BCs, making it a good model with which to determine the cellular mechanisms underlying the recruitment and migration of mixed cohorts of cells. Here, we identify three novel steps in the formation of BCs (Fig. 7E). First, we show that a developmentally regulated basal to apical transport of BM material takes place in the polar cells, the first population of cells to form in the cluster. The apical cap is the earliest known marker of anterior polar cells. Second, the asymmetrical positioning of the apical cap suggests that despite an apparent identity, the two polar cells are different and might play distinct roles. Third, our data indicate that a two-way interaction takes place between the two differentiated subpopulations of invasive cells before they

Fig. 6. BC migration defects of Drab5S43N and ShiK44A egg chambers. Expression of Drab5S43N, Drab5WT and ShiK44A in egg chambers was driven by slbo-GAL4 at 29°C. To measure the extent of migration, the nurse cell compartment was divided into four equivalent regions (1 to 4; clusters in region 1 have almost completely migrated whereas clusters found in region 4 have not migrated at all; see inset) and the position of the BC relative to this coordinate system monitored. Drab5S43N and ShiK44A led to similar and strong migration defects.

Fig. 7. Apical cap shedding requires JAK/STAT signaling and the recruitment of outer BCs. GFP-Vkg (green) egg chambers expressing DomeΔCyt (A,B) or Drac1N17 (C,D). Follicle cells are marked with phalloidin (A,C,D; red). In B, polar cells are marked with slbo-lacZ (red). (A) Blocking outer BC recruitment does not affect the formation of the apical cap at stage 9. However, shedding does not take place and a cap is still present in stage 10 DomeΔCyt egg chambers (B; phenotype schematized in the upper left panel). Expression of Drac1N17 blocks migration (D) but has no effect on outer BC recruitment and apical cap formation (C) (phenotype schematized in the lower left panel). (E) A four-step model for BC formation and migration: (1) formation of an asymmetrical apical cap (green; containing Collagen IV α1 and α2 chains, Laminin A, Perlecan) over the anterior polar cells (orange) through Drab5-dependent transcytosis; (2) polar cells send a JAK/STAT signal to recruit outer BCs (beige) from the surrounding follicle cells (light gray); (3) shedding of the apical cap is dependent on the presence of outer BCs; (4) migration of the mixed BC cluster starts, with polar cells maintaining a BM. Anterior is to the left. Scale bar: in A, 5 µM for A-D.
migrate. A first signal, activating the JAK/STAT pathway is sent by the polar cells to recruit the outer BCs. In a second step, the outer BCs are essential for shedding the apical cap of polar cells (Fig. 7E).

Outer BCs are not required for apical cap formation (Fig. 7A,B). Similarly, outer BCs form normally in the absence of a cap (Fig. 4; data not shown), indicating that apical capping is not a pre-requisite for outer BCs to be recruited and the cluster to be assembled. Interestingly, we found that immotile polar cells remained capped (Fig. 7A,B). Thus, a possible role for apical capping is to block the migration of immature clusters, a finding that could explain the long standing observation that isolated polar cells cannot migrate on their own. Indeed, the coordination between apical cap degradation and the recruitment of outer BCs indicates that degradation of the apical cap could serve as a check point or quality control ensuring that only finalized clusters can start migration. It is important to note that degradation of the ECM at the leading edge of migrating clusters is essential for tumour progression, and examples of cancer cells showing a reduction or absence of some basement membrane markers, including Collagen IV, have been reported. In particular, human α3(IV) type IV Collagen is found at the apical surface in normal colon tissue, but is absent in colorectal neoplastic cells (Hiki et al., 2002), making the differential distribution of type IV collagens potential diagnostic markers for the invasiveness of cancer cells. The BC model will be central for future studies aimed at understanding BM dynamics and function in invasive clusters.

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Supplementary material
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References


