Dynamics of the basement membrane in invasive epithelial clusters in *Drosophila*

Caroline Medioni and Stéphane Noselli*

Institute of Signaling, Developmental Biology and Cancer, UMR 6543 CNRS, University of Nice Sophia-Antipolis, Parc Valrose, 06108 Nice, cedex 2, France

*Author for correspondence (e-mail: noselli@unice.fr)

Accepted 29 April 2005

Development 132, 3069-3077
Published by The Company of Biologists 2005
doi:10.1242/dev.01886

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 Perlecan. 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 vkg\textsuperscript{G254} (GFP-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 and hypothesis that results are different (ShiK44A), lower (Drab5 S43N) or experiment, we tested the hypothesis that results are equal versus the number of samples, we applied the Bernoulli Rule. In each means of the variables in each experiment. Given the statistically high

### Statistical analysis

Matrix metalloproteinases Dm1-MMP (Llano et al., 2000) + + +

### 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), wild-type and mutant egg chambers were stained and processed at the same time. Thus, each experiment has its own control, which is plotted in the histogram. At least three sets of experiments were performed for each condition.

Images were taken using a Leica TCS-SP1 or a Zeiss LSM 510 Meta confocal microscope and processed using Photoshop 7.0 (Adobe). Three-dimensional reconstruction of anterior egg chambers and apical caps was made using Volocity 2.6 (Improvement).

### 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

### 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>Trafficking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drab5\textsuperscript{WT} (Entchev et al., 2000)</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Drab5\textsuperscript{S43N} (Entchev et al., 2000)</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Shi\textsuperscript{K44A} (strong) (Moline et al., 1999)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Shi\textsuperscript{K44A} (weak) (Moline et al., 1999)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Drab7\textsuperscript{Q67L} (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>
</tr>
<tr>
<td>Dome\textsuperscript{WT} (Brown et al., 2001)</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Dome \textsuperscript{ΔTM} (Brown et al., 2001)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dome \textsuperscript{Δcyt} (Brown et al., 2001)</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Dome \textsuperscript{Dex} (Ghiglione et al., 2002)</td>
<td>–</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Ecdysone, EGF and insulin pathways</td>
<td></td>
<td></td>
<td></td>
</tr>
<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>
</tr>
<tr>
<td>Matrix metalloproteinases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dm1-MMP (Llano et al., 2000)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Dm1-MMP1 (Page-McCaw et al., 2003)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Dm1-MMP2 (Page-McCaw et al., 2003)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Dm2-MMP (Page-McCaw et al., 2003)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Dtip (Page-McCaw et al., 2003)</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Small GTPases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DrhoL\textsuperscript{N25} (Murphy and Montell, 1996)</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>DRac\textsuperscript{C.89} (Luo et al., 1994)</td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>DRac\textsuperscript{C.117} (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DRac\textsuperscript{C.122} (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DCdc2\textsuperscript{Q57} (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DCdc2\textsuperscript{L12} (Luo et al., 1994)</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

The expression of UAS constructs was driven using the *Slbo-GAL4*, GFP-Vkg line at 29°C (except for UAS-Dome\textsuperscript{WT}, 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 Drab5\textsuperscript{S43N} and is described in the legend to Fig. 4.

The 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 Drab5\textsuperscript{S43N} and Shi\textsuperscript{K44A} 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; DrhoL\textsuperscript{N25}, only approximately 30% of the egg chambers show a migration defect.
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.

Cell-type specificity of apical capping
In wild-type egg chambers, the apical cap forms over anterior polar cells only (Fig. 1B). In order to demonstrate the cell-type specificity of apical capping, anterior polar cells were either deleted or made in excess, by manipulating the polar cell fate repressor eyes absent (eya). Overexpression of eya using Upd-GAL4 blocks anterior polar cell differentiation (Bai and Montell, 2002) (Fig. 2A–C). As a consequence, the apical cap is not formed (Fig. 2A,B; compare with Fig. 1B,C). Conversely, when mutant clones for eya are generated in the anterior follicle cells, ectopic polar cells form and are capped (Fig. 2D–I). Consistently, ectopic polar cells made in the posterior region do not show an apical cap (data not shown), indicating a contribution of anteroposterior patterning in the regulation of polar cell capping. Altogether, these results demonstrate that apical capping is a specific feature of the anterior, migrating polar cells.

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.

Fig. 2. Apical capping is specific to anterior polar cells. (A) Overexpression of the polar cell fate repressor eya. The polar cell marker Fas2 is shown in red. (B,C) Enlarged views of the framed regions shown in A. Note the absence of anterior polar cells and an apical cap (compare with Fig. 1B-D). Egg chambers show Laminin A (green) and nuclei (blue). (D-I) Mosaic egg chambers containing eya mutant follicle cells making ectopic polar cells (Fas2, in red). (D-F) Anterior stage 8 egg chamber with two normal polar cells and their apical cap (D, arrowheads 1, 2). (E) A ectopic polar cell close by shows a nascent apical cap (arrowhead 3). (F) Merged image of D and E. (G-I) A group of five to six ectopic polar cells have assembled two separate and large apical caps (arrowheads 1, 2). (I) Merged image of G and H. Scale bars: in A, 10 µM; in B, 10 µM for B,C; in D, 5 µM for D-F.

Fig. 3. The apical cap is asymmetric and dynamic. (A-F) Three-dimensional imaging and reconstruction of the anterior region of stage 8 egg chambers, showing GFP-Vkg (green), Fas2 (red) and Hoeschst (blue, in A). (A) The original stack is a projection of 20 z sections (total depth is 7.6 µm). (B) Intermediate processing showing BM (green), polar cells (white) and apical cap (blue). (C) The resulting 3D-reconstructed polar cells with the apical cap. (D-F) Three other examples of reconstructed polar cells with their apical cap. Inset in C-F show the apical side of polar cells. The red line is the boundary between the two polar cells. (G-O) GFP-Vkg (green; G-O) egg chambers showing Fas2 (G-L), Crumbs (M,O) or Fas3 (N) in red. The process of apical capping is transient and can be described in four discrete phases (1-4; see text for details). After shedding (L), the BC delaminate and invade the nurse cell compartment (M). During migration (phase 5), a BM containing GFP-Vkg is present in polar cells specifically (M-O), opposite to the apical side (marked with Crumbs in M and O). The polar cell BM is maintained when BCs reach the oocyte (O). Anterior is to the left. Scale bars: in A, 5 µM for A-F; in G, 5 µM for G-O.
Polar cells, but not border cells, maintain a basement membrane during migration

BCs are of epithelial origin and their detachment from the follicular 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 follicular 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 de novo 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 in BCs 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’ phenotype. Frequent caps (G) and microcaps (H) of stage 8 egg chambers in wild-type (n=625) flies, or flies expressing Drab5S43N (n=298), Drab5WT (n=201) or ShiS44A (strong; n=183). Data are presented as mean±s.e.m. (see Materials and methods). Anterior is to the left. Scale bar: in A, 5 µM for A-F.

Fig. 4. Drab5-dependent basal to apical transcytosis of basement membrane. (A-C) GFP-Vkg non-expressing cells in a GFP-Vkg/+ background. The Myc clonal marker is in red; GFP-Vkg non-expressing cells are outlined (dashed lines). When polar cells only (A), or polar and outer BCs (B,C), do not express GFP-Vkg, the apical cap forms normally. (D) Wild type; (E,F) polar cells expressing Drab5S43N (E) do not form an apical cap or (F) show a ‘microcap’ phenotype. Frequency of caps (G) and microcaps (H) of stage 8 egg chambers with an apical cap (Fig. 4G). In this case, micro caps are less frequent (15%) than in wild type (25%) (Fig. 4H). Conversely, the expression of Drab5WT induces a 5% increase in the number of egg chambers with an apical cap (Fig. 4G). In this case, micro caps are less frequent (15%) than in wild type (25%) (Fig. 4H).
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) (Dome\(^\Delta\)Ext and Dome\(^\Delta\)Cyt), 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 (Dome\(^\Delta\)Ext and Dome\(^\Delta\)Cyt, 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 Dome\(^\Delta\)Cyt 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 (Drac\(^{N17}\)) (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...
Basement membrane dynamics in cell migration

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 DomeCyto (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 DomeN cyt 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 collagen 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.

We thank R. Arkowitz, S. Baumgartner, L. Bianchini, N. Brown, L. Cooley, J. Fessler, M. Gonzalez-Gaitan, P. Léopolis, C. Lopez-Otin, D. Montell, X. Morin, A. Page McCaw, P. Rorth, P. Théron, the Bloomington Stock Center and DSHB for providing us with fly stocks, materials and/or for critical reading; O. Devergne for Fig. 6; P. Spéder for statistical analysis and D. Cerezo for technical help. Work in S.N.’s Laboratory is supported by grants from CNRS P. Spéder for statistical analysis and D. Cerezo for technical help.

Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/132/13/3069/DC1

References


