Cell rearrangements, cell divisions and cell death in a migrating epithelial sheet in the abdomen of Drosophila

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During morphogenesis, cell movements, cell divisions and cell death work together to form complex patterns and to shape organs. These events are the outcome of decisions made by many individual cells, but how these decisions are controlled and coordinated is elusive. The adult abdominal epidermis of Drosophila is formed during metamorphosis by divisions and extensive cell migrations of the diploid histoblasts, which replace the polyploid larval cells. Using in vivo 4D microscopy, we have studied the behaviour of the histoblasts and analysed in detail how they reach their final position and to what extent they rearrange during their spreading. Tracking individual cells, we show that the cells migrate in two phases that differ in speed, direction and amount of cellular rearrangement. Cells of the anterior (A) and posterior (P) compartments differ in their behaviour. Cells near the A/P border are more likely to change their neighbours during migration. The mitoses do not show any preferential orientation. After mitosis, the sisters become preferentially aligned with the direction of movement. Thus, in the abdomen, it is the extensive cell migrations that appear to contribute most to morphogenesis. This contrasts with other developing epithelia, such as the wing imaginal disc and the embryonic germband in Drosophila, where oriented mitoses and local cell rearrangements appear to direct morphogenesis. Furthermore, our results suggest that an active force created by the histoblasts contributes to the formation of the adult epidermis. Finally, we show that histoblasts occasionally undergo apoptosis.

KEY WORDS: Morphogenesis, Cell migration, Cell division orientation

INTRODUCTION

How do groups of cells form complex patterns during morphogenesis? Cell movements, cell divisions and cell death work together to place cells in their final positions. Notable examples are provided by cell movements in early nematode development (Schnabel et al., 2006), dorsal closure (Jacinto et al., 2000) and gastrulation movements in Drosophila (McMahon et al., 2008), as well as convergent extension movements (Keller, 2002) and primitive streak formation (Voiculescu et al., 2007) in vertebrate development. Even though the cells move together as groups (Lecaudey and Gilmour, 2006), it is the behaviour of the individual cells that directs morphogenesis. Although new imaging techniques have recently lead to progress in the analysis of patterning (Keller et al., 2008), the mechanisms underlying the final positioning of cells remain elusive.

Oriented mitoses are thought to play some role in Drosophila wing development (Baena-Lopez et al., 2005) and germband extension (da Silva and Vincent, 2007) as well as in zebrafish gastrulation (Gong et al., 2004) and neurulation (Tawk et al., 2007). However, during morphogenesis, cells often divide and migrate at the same time. Thus, it becomes difficult to separate the contributions of mitosis and cell movement to morphogenesis, and for this reason the specific contribution of oriented mitosis to patterning remains unclear (da Silva and Vincent, 2007; Gong et al., 2004).

In some developmental contexts, the removal of cells by apoptosis is important for patterning, e.g. in leg imaginal disc development in Drosophila (Manjon et al., 2007) and limb development in vertebrates (Chen and Zhao, 1998).

In epithelia, adherens junctions ensure the integrity of the tissue and give it some rigidity. Consequently, cell rearrangements during epithelial morphogenesis are limited and depend on the remodelling of cell-cell junctions (Bertet et al., 2004). Examples are the wing imaginal disc and the elongation of the embryonic germband of Drosophila, where oriented mitoses and local cell rearrangements (with only limited changes in neighbourhood relations) drive morphogenesis (Baena-Lopez et al., 2005; da Silva and Vincent, 2007; Blankenship et al., 2006; Classen et al., 2005; Gibson et al., 2006; Irvine and Wieschaus, 1994).

The adult epidermis of the abdomen of Drosophila is newly formed during metamorphosis as the polyploid larval epithelial cells (LECs) are replaced by the descendants of the histoblasts—diploid imaginal cells derived from small lateral nests in the larva. The histoblasts divide and migrate dorsally and ventrally over the abdomen until its whole surface is covered with cells (Madhavan and Madhavan, 1980; Ninov et al., 2007; Roseland and Schneiderman, 1979). During this process, the LECs undergo apoptosis; they constrict apically, are extruded from the epithelium and are subsequently phagocytosed by hemocytes, which patrol underneath the epithelium (Ninov et al., 2007).

We have tracked individual cells using 4D microscopy (Schnabel et al., 1997) in order to study the morphogenesis of the adult abdominal epidermis of Drosophila in detail. We have investigated which cellular behaviours are important to this process and to what extent cells rearrange during the spreading of the histoblasts. We show here that there are two phases of cell migration. In the anterior (A) and posterior (P) compartments, cells differ in their behaviour. The A/P boundary appears to influence the behaviour of cells near to it. In contrast to in other epithelia (Baena-Lopez et al., 2005; da Silva and Vincent, 2007), we find that cell migrations dominate patterning in the abdominal epidermis; the mitoses do not show any preferential orientation, but following mitosis the two sisters become preferentially aligned with the direction of movement. Our results furthermore suggest that the migrating histoblasts might actively contribute to

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their own movement, thus pushing against the larval cells. Finally, we observe that histoblasts occasionally die and are removed from the epithelium.

MATERIALS AND METHODS
Fly stocks and clonal analysis
FlyBase (Grumbling and Strelets, 2006) entries of the mutations and transgenes are as follows: en.Ga4, Scer/Gal80α-hs.FLP; tub.Gal80, Scer/Gal80α-hs.GFP; tub.Gal4, Scer/Gal80α-hs.GFP; UAS.RedStinger, DiscRFP/RedT4.Scer/UAS.Tub; H2Av:GFP; His2Av::GFP (Clarkson and Saint, 1999); Sagh::GFP, sqh.T::hsb:GFP (Royo et al., 2002); and De-cadherin::GFP, shg.T::hse:GFP; Tav.Gal80α (Oda and Tsukita, 2001).

Pupae carrying ‘wild-type’ clones marked with DsRed had the following genotype; y w hs.FLP; H2Av::GFP/+; tub-Gal80α-Gal4/UAS.RedStinger.

Overexpression clones were induced using the FLP-out technique (Struhl and Basler, 1993). Pupae were induced by heat shocking third instar larvae for 1 hour at 35°C.

4D microscopy
For imaging, pupae were staged according to Baimbridge and Bownes (Baimbridge and Bownes, 1981). A window in the pupal case was made and the pupae were filmed as described (Escudero et al., 2007). All the studied flies developed into pharate adults after imaging and many eclosed.

Histoblasts migrate to their final positions in two phases
Using 4D microscopy (Schnabel et al., 1997), we tracked the movements and divisions of the histoblasts while they spread more than 300 μm in 24 hours (Fig. 1A,B; see Movie 1 in the supplementary material). We tracked the cells at the tip of the moving cell mass, documented their positions, monitored their divisions, traced their descendants and studied cell rearrangements. We can distinguish and follow all the cells of the A and P compartments (Fig. 1C,D; see Movie 2 in the supplementary material).

RESULTS
Histoblasts migrate to their final positions in two phases
To calculate the angle of cell division (θ), we used the 2D coordinates of the sister cells during anaphase (one frame after the metaphase plate is visible). The angle was calculated between the line connecting these points and the dorsoventral (DV) axis of the pupa.

Sister cell rearrangement
To calculate the position of sister cells relative to each other (ρ), we used the last 2D coordinate of the lineage of the two sisters (either before the next mitosis or at the end of the movie) and calculated the angle between the line connecting these points and the DV axis of the pupa. The average time between mitosis and the last coordinate of a cell is 162±90 frames (~7±4 hours).

Cell density
To calculate the density maps, the distance of each cell to its six nearest neighbours was calculated at a given time point and the average distance plotted at the position of the cell using a 16-band look-up table (LUT). This algorithm introduces an error at the borders of the cell mass because these cells have fewer neighbours on one side.

Neighbourhood maps
To calculate the neighbourhood maps, we used the 2D coordinates of all cells at two given time points (the start and the end of the movie). For each cell, the distances to the six closest neighbours at time point 1 were calculated. Then, the distances to the same six cells (regardless of whether they remained closest neighbours or not) were calculated at time point 2. For each of the six neighbours, the difference between these two values was calculated and then the average was plotted at the position of the cell at time point 1 using a banded LUT. Since this calculation is affected by the observed change in cell density over time (cells are more densely packed in some areas at the beginning, see Fig. S2 in the supplementary material), we corrected for this by only displaying differences larger than the average distance between cells (7 μm).

Velocities
To calculate the velocities of cells, we used the distance the cells moved within a 30-minute interval (beginning at the start of the movie). The velocities of each cell were plotted at their positions at the beginning of the time interval using a banded LUT.

Trajectories of cell movements
To display the direction in which the cells moved we connected the two 2D coordinates of each cell at 30-minute intervals with a straight line. The colour of these lines represents the velocity of the cell (see Velocities).

Because of hemocytes, which patrol underneath the epithelium and thus block viewing of some of the histoblasts, 2% of histoblasts could not be followed all the way to the end of the movie (n=1296 cells). Thus, in some calculations that use two 2D coordinates, only the first coordinate could be determined. In these cases, the affected cells are coloured grey in the plots.

Three wild-type pupae (pupae #1, 2, 3) and one en.Ga4 UAS.RedStinger pupa (pupa #4) were used for these analyses. In total, 747 sister pairs were analysed. Pupa #4 differed from the other three only with respect to an increased number of cell deaths in the P compartment. To allow a classification of cells as either A or P compartment in the three wild-type pupae, the information from six en.Ga4 UAS.RedStinger movies was used.

Statistics
Since only angular data from 0° to 180° were considered, we used linear statistics. Data were tested for normal distribution using the Kolmogorov-Smirnov test. If the test excluded normal distribution, non-parametrical Mann-Whitney tests were performed. Analyses were performed using SPSS 16.0.
The histoblasts moved with an average speed of 15 μm/hour. Their maximum velocity of 46 μm/hour resembles that of fibroblasts extending their leading edge in tissue culture [42 μm/h (Abercrombie et al., 1970)].

The histoblasts moved in two distinct phases. First, they followed a more or less straight line dorsally towards the midline for ~17 hours, starting at ~20 hours after puparium formation (APF) (Fig. 1E). Second, shortly before they reached the midline (at 37 hours APF), when there were about three rows of surviving LECs, the histoblasts turned in an anterior direction (Fig. 1F).

The histoblasts undergo about eight divisions (Madhavan and Madhavan, 1980), of which about two to four divisions occur during dorsal migration. During their spreading, the size of the histoblasts remains constant throughout the divisions (Ninov et al., 2007).

**During the first, dorsal migration phase, the cells rearrange depending on their position along the anteroposterior axis**

During dorsal migration, the histoblasts changed their arrangement relative to each other: the further posterior a cell was positioned, the further dorsal it moved (Fig. 2A; see Movies 3A,B in the supplementary material). This is because cells located more posteriorly moved dorsally towards the midline at a higher speed than those located more anteriorly (Fig. 2B; see Movie 4 in the supplementary material); they also moved for longer and made their anterior turn later (Fig. 2C).

**The second, anterior migration phase completes the formation of the epithelium**

As the histoblasts approached the midline, they slowed and turned to the anterior, now moving at about half their former speed (Fig. 3A). In some pupae, this change coincided with the disappearance of the last row of LECs – that which separates the histoblasts of neighbouring segments (see box in Fig. 3A). In this row, the LECs disappeared consecutively, so that the histoblasts came together like a zipper along the dorsoventral (DV) axis of the pupa (see earlier phases of spreading in Movie 1 in the supplementary material). In other pupae, this row of LECs disappeared earlier, at the time when there were still seven to nine rows of LECs separating the histoblasts from the midline (see Movies 1, 2 in the supplementary material). In this case, the histoblasts turned anteriorly later and only after they had reached the midline.

While moving anteriorly, the histoblasts started to disperse evenly (see Fig. S2 in the supplementary material) and adopted a more uniform shape, being elongated along the DV axis (Fig. 3B; see Movie 5 in the supplementary material). Histoblasts also stopped dividing and ceased to move relative to each other. Eventually, the intersegmental folds formed (see Movie 5 in the supplementary material) as the cells of the P compartment became folded underneath the A compartment, which further contributed to an anterior movement of the histoblasts. Thus, the dispersion of cells into the available space as they form a stable epithelium is directed anteriorly. This packing of cells resembles morphogenesis of the wing imaginal disc, where cells also become more uniformly packed shortly before hair formation (Clasen et al., 2005). Only histoblasts of segments posterior to segment 1 migrated anteriorly, so that all the cells push up against the more stationary segment 1 (Fig. 1F; see Fig. S3 in the supplementary material).

Interestingly, the anterior movements of the histoblasts help match cells on either side of the midline. During the dorsal migration, the cells of the two facing hemisegments (left and right) do not head precisely towards each other but, as they turn anteriorly, they migrate to different extents so as to settle in precise registration across the midline (Fig. 1F). A similar matching of cells occurs during dorsal closure in *Drosophila* embryos (Millard and Martin, 2008).
Fig. 2. The velocity and duration of the dorsal migration of cells determine their position. 
(A) Topology of the cell mass. The left and right panels show the same 3D representations of pupa #4 differently colour-coded to illustrate the change in cell positioning from 25 to 41 hours APF (n=273 and 443 cells, respectively). Spheres indicate anterior (A) compartment cells, and ovals indicate posterior (P) compartment cells. At the left, the cells are colour-coded arbitrarily in stripes with respect to the DV axis at the beginning of the recording; at the right, colour-coding is with respect to the AP axis at the end of the recording. The more posteriorly that cells are positioned the more dorsally they will move. Furthermore, cells do not change their positions along the AP axis. Cells in the P compartment appear to rearrange more extensively than those in A. Cells close to the A/P boundary move the furthest. See also Movies 3A,B in the supplementary material. (B) The velocity of each cell is plotted at its position at three consecutive time points (pupa #1). The number of hours after the start of the recording is indicated in the top right corner. The velocities of the cells change over time. Histoblasts which are more posterior in the segment tend to move faster. Cells are coloured grey if their velocity cannot be calculated because one of the coordinate-pair is missing. See also Movie 4 in the supplementary material. (C) The trajectories of all cells are plotted by connecting the coordinates of a cell in 30-minute intervals with a line (pupa #1). The colour of the line represents the velocity of the cells. More anteriorly positioned cells turn anteriorly earlier. The cell that turns first is marked with an arrow. More posteriorly positioned cells tend to move faster and turn anteriorly later. In all images, anterior is to the left.

Overall changes in neighbourhood relations are relatively small
Next, we analysed the behaviour of individual cells in the moving cell mass, particularly the dorsal migration, which is accompanied by cell divisions.

We first asked to what extent cells change their neighbours during their movements and found that most cells maintain their nearest neighbours (Fig. 4A); for example, only ~7% of sister cells lose contact with each other during their migration (Fig. 4B). Such stability of neighbour relations during morphogenesis is found in other developing epithelia, such as the pupal wing imaginal disc (Classen et al., 2005; Gibson et al., 2006). Even in the extending germband of the Drosophila embryo, where cells intercalate, the changes in neighbourhood relations are moderate (Bertet et al., 2004; Blankenship et al., 2006; Irvine and Wieschaus, 1994). Any changes in the relative positions of cells involve dynamic remodelling of cell-cell junctions (Bertet et al., 2004) (see Movies 5-7 in the supplementary material).

Changes in neighbourhood relations are most extensive at the A/P boundary
In certain regions, we observed histoblasts changing their neighbours. Those changes took place in the centre of the moving cell mass and especially near the boundary between the A and P compartments (Fig. 4A). In the centre of the cell mass, cells are more columnar and more densely packed and thus appeared to influence each other more strongly, which could lead to the separation of cells by the division or movement of neighbours that push in between them (Fig. 4C; see Movie 8 in the supplementary material). In most cases, this separated cells by no more than one cell diameter.

Those cells situated near the A/P boundary differed in their behaviour from other cells in the segment in that they moved furthest dorsally before they turned anteriorly (Fig. 2A; see Movies 3A,B in the supplementary material). Furthermore, they changed their neighbours more often (Fig. 4A); some sister cells even became separated by several cell diameters (see Movie 9 in the supplementary material). This unusual behaviour near the A/P border might be due to the unusual adhesive properties of the A/P boundary; it has been shown that cells of the two compartments do not mix because of the action of engrailed in the P compartment (Morata and Lawrence, 1975). Thus, the A/P boundary might be a region where cells are able to move more freely against each other, as they adhere to each other less tightly.

The divisions of histoblasts are not oriented
Calculating the division angles relative to the direction of movement of the cell mass showed that the histoblast divisions are not preferentially oriented (Fig. 5A,B; see Table S1 in the supplementary material). Furthermore, divisions deviated from the direction of movement equally in the anterior and posterior directions. Indeed, there was no part of the moving cell mass where the orientation of cell division differed noticeably from the random (see Fig. S4 and Table S1 in the supplementary material) and thus it is the pattern of migrations that mostly shapes the adult segment.

In contrast to the abdomen, in both wing imaginal discs and the extending germband of Drosophila, oriented mitoses have been reported to contribute to morphogenesis (Baena-Lopez et al., 2005; da Silva and Vincent, 2007). This is perhaps because there it is the displacement of cells by mitoses and not cell rearrangements that affect the shape of the developing organ (Gibson et al., 2006).
Sister cells change their positions relative to each other in the direction of movement

After cell division, the two daughter cells change their position relative to each other. We measured the angle between the line connecting the two sisters and the DV axis, either at the last time point before their next division or at the end of the recording if there was no further division (Fig. 5A,C). We found that sister pairs became preferentially aligned with the DV rather than the anteroposterior (AP) axis (Fig. 5B; see Table S1 in the supplementary material). P compartment cells rearranged more extensively than A compartment cells (see Table S1 and Movies 3A,B in the supplementary material; see also Fig. 2A).

We then asked whether the rearrangement of sister cells is correlated with the direction of division of their mother. We found that sisters arising from divisions more or less along the DV axis were likely to remain in this orientation (Fig. 5D). More than 50% of sisters arising from divisions more or less orthogonal to the DV axis rearranged (Fig. 5D). The more the division of the mother cell deviated from the DV axis, the less likely was a rearrangement of the sister cells (Fig. 5E), suggesting that the rearrangement might not be an active process but largely due to the movement of the cell mass per se. This is supported by the observation that sister pairs in the P compartment, where cells move faster and for a longer period of time (Fig. 2B,C), rearranged more extensively (see Table S1 in the supplementary material).

Most sisters rearranged within the first 24 minutes after division (Fig. 5D), perhaps because rearrangement occurs when cell-cell contacts are still weak following mitosis.

The histoblasts appear to contribute actively to their own movement

In summary, we find that the morphogenesis of the abdominal epithelium is dominated by extensive cell movements that replace the LECs and deliver the histoblasts to their final positions. What drives these movements? One possibility is that the histoblasts actively contribute to their own displacement and move towards the...
disappearing LECs. As the histoblasts approached the midline, we occasionally observed that the LECs retreated more slowly, and the histoblasts slowed down and started ‘whirling’ (Fig. 6A; see Movie 11 in the supplementary material), suggesting that the histoblasts ‘ran into’ the LECs. When the larval cells eventually disappeared, the histoblasts showed a surge of movement. These observations suggested to us that the histoblasts might move actively towards the LECs, pushing against them if they had not retreated in time. Furthermore, in many pupae, we observed a fold in the epithelial sheet, a few cell rows behind the most dorsal row of histoblasts, which developed as the histoblasts approached the midline (Fig. 6B). This could also be a consequence of a pushing force exerted by the histoblasts. This force could be generated by the extensive divisions in the centre of the moving cell mass and/or by the active crawling of the cells. The previously observed active planar intercalation of the histoblasts into the larval epithelium (Ninov et al., 2007) also supports the idea that the histoblasts actively contribute to the forces that drive the process.
However, the observation that the histoblasts are hampered in their movement in those cases in which the LECs do not retreat in time, argues that the progression of histoblasts and the retreat of larval cells work together. Indeed, both the death of LECs and the division of histoblasts have been shown to be necessary for proper development (Ninov et al., 2007).

It might thus be possible that the dying larval cells also contribute to the movement of the histoblasts by pulling the moving cell mass forward as they constrict apically. Such ‘apoptotic forces’ have been suggested for the amnioserosa cells in dorsal closure (Toyama et al., 2008). We therefore studied how LECs disappear from the epithelium. Most dying LECs make contact with the histoblasts, but LECs that do not touch the histoblasts also die (Ninov et al., 2007): 18% of the LECs that disappear are located more than one larval cell diameter away from the histoblasts. Many LECs were observed to drift far dorsally, moving together with the histoblasts, before they were extruded from the epithelium (Fig. 6C; see Movies 10, 1 and 5 in the supplementary material). The LECs changed shape dynamically before they constricted apically (see Movie 5 in the supplementary material). Whether these dynamic movements and cell shape changes are able to generate a coordinated pulling force remains unclear.

Some LECs appear to canalise the dorsal migration of the histoblasts

The segmental groups of dorsally migrating histoblasts are typically separated by a row of LECs that persists almost until the histoblasts meet at the midline (Madhavan and Madhavan, 1980) (see box in Fig. 3A). These LECs, which lie at the future segment boundary, exhibited higher expression of Spaghetti squash (Sqh, Myosin II regulatory light chain) than the other larval cells (Fig. 6D). Similar cells expressing higher levels of Sqh have been found at the DV compartment boundary of wing discs and are thought to act as a fence between dorsal and ventral cells (Major and Irvine, 2006). The LECs at the segmental borders in the abdomen might also act as a fence, canalising the moving cell mass and restraining its lateral expansion. This behaviour would constitute one aspect of how the larval segmental ‘blueprint’ prefigures the adult pattern (Madhavan and Madhavan, 1980).

Histoblasts are removed by apoptosis

During the migration, 3% of the histoblasts disappeared (n=923); sometimes we observed the nucleus fragmenting (see Movie 12 in the supplementary material) and the cells constricting apically (see Movie 13 in the supplementary material). Hemocytes could be seen patrolling underneath these cells. Thus, it seems that histoblasts undergo apoptosis, as do the LECs (Ninov et al., 2007); for a morphological definition of apoptosis, see Galluzzi et al. (Galluzzi et al., 2007).

P compartment cells were much more likely to die than cells of the A compartment (10% in P versus 1% in A). Furthermore, most of the cell death (81%) occurred at the end of the dorsal migration, close to the midline or close to the segment boundaries. A possible explanation for this is that there is increased remodelling of cell-cell contacts at these regions owing to rearrangements of cells and/or the meeting of histoblasts of neighbouring segments. This might lead to more imbalances in the junctional network, which has been suggested as a reason for cell elimination (Farhadifar et al., 2007). Another possibility for the observed cell death is that the removed cells are less ‘fit’ than the remaining cells (Abrams, 2002).
DISCUSSION

We find that the formation of the abdominal epidermis differs from that of other epithelia, such as in the wing imaginal disc (Classen et al., 2005; Gibson et al., 2006) and during germband extension in the Drosophila embryo (Blankenship et al., 2006; Irvine and Wieschaus, 1994). In contrast to these more static epithelia, the formation of the abdominal epidermis is driven by extensive cell migrations. The final positioning of cells appears not to depend on the orientation of cell divisions, but particularly on cell movements, the speed and extent of which vary with the position along the AP axis (Fig. 2A-C). These movements also appear to lead to a rearrangement of sister cells along the direction of movement (Fig. 5B). During migration, cells only occasionally change their neighbours; the most extensive changes occur near the A/P boundary (Fig. 4A). These results explain why fluorescently marked wild-type clones tend to be elongated along the DV axis and do not split (see Fig. 5S in the supplementary material).

One explanation for the differential movements of cells within the AP axis could be a gradient of cell affinities (Lawrence et al., 1999). This gradient might be manifest in a differential stickiness of cells along the AP axis, with posterior cells adhering less to each other, allowing their more extensive rearrangement (see Fig. S6 in the supplementary material).

Interestingly, the behaviour of the cells in the moving epithelial sheet appears to be influenced by the presence of the A/P boundary (see Fig. S6 in the supplementary material). The A/P boundary, with its differential adhesive properties, seems to act like an expansion field (Crick and Lawrence, 1975), and highlight the differences between the A and P compartments (Zecca et al., 1995), but also appears to influence the positioning of the histoblasts.

In addition, we find that the A and the P compartment cells behave differently, with cells of the P compartment rearranging more extensively (Fig. 2A; see Table S1 in the supplementary material) and also being more likely to undergo cell death. These findings highlight the differences between the A and P compartments, which may act as two independent fields (Crick and Lawrence, 1975), and provide insights into differences in cellular behaviours.

In many developmental contexts, cells need to coordinate their behaviour; for example, in order to move as a group (Lecaudy and Gilmour, 2006). Our work complements the study of Ninov et al. (Ninov et al., 2007), who focussed on the interactions of histoblasts and LECs, and highlights the behaviour of individual cells, the sum of which is responsible for morphogenesis. A combination of cell tracking using 4D microscopy and clonal analysis should help us tackle questions such as what mechanisms guide the cells to their final position and what positions them relative to each other. Addressing these questions is important in order to understand the morphogenesis of all epithelia, including in gastrulation and neurulation (Keller, 2002).

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Author contributions

M.B. planned and performed the experiments and wrote the manuscript. Z.C. wrote the software to analyse the SIMI BioCell data and commented on the manuscript.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/136/14/2403/DC1

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