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Lamellipodia-based migrations of larval epithelial cells are required for normal closure of the adult epidermis of *Drosophila*

Marcus Bischoff *

University of Cambridge, Department of Zoology, Downing Street, CB2 3EJ Cambridge, UK

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ABSTRACT

Cell migrations are an important feature of animal development. They are, furthermore, essential to wound healing and tumour progression. Despite recent progress, it is still mysterious how cell migration is spatially and temporally regulated during morphogenesis and how cell migration is coordinated with other cellular behaviours to shape tissues and organs. The formation of the abdominal epithelium of *Drosophila* during metamorphosis provides an attractive system to study morphogenesis. Here, the diploid adult histoblasts replace the polyploid larval epithelial cells (LECs). Using *in vivo* 4D microscopy, I show that, besides apical constriction and apoptosis, the LECs undergo extensive coordinated migrations. The migrations follow a transition from a stationary (epithelial) to a migratory mode. The migratory behaviour is stimulated by autocrine Dpp signalling. Directed apical lamellipodia-like protrusions propel the cells. Initially, planar cell polarity determines the orientation of LEC migration. While LECs are migrating they also constrict apically, and changes in activity of the small GTPase Rho1 can favour one behaviour over the other. This study shows that the LECs play a more active role in morphogenesis than previously thought, with their migrations contributing to abdominal closure. It furthermore provides insights into how the migratory behaviour of cells is regulated during morphogenesis.

Introduction

Cell migrations are an important aspect of animal development (Montell, 1999). They are crucial to position cells during morphogenesis, where they also have to be coordinated with other cellular behaviours such as shape changes and divisions to form tissues and organs (Bischoff and Cseresnyes, 2009; Butler et al., 2009; Fernandez et al., 2007; Gong et al., 2004). Despite recent progress, it is still mysterious what regulates cell migration to ensure that cells migrate at the right time to the correct position and how the coordination of cell migration with other cell behaviours is achieved.

Moving cells are often of epithelial origin. To become mobile, these cells have to undergo a transition from a stationary to a migratory mode. During such epithelial to mesenchymal transitions (EMTs), cells lose epithelial characteristics, such as cell adhesion, and gain mesenchymal characteristics, such as becoming migratory (Baum et al., 2008; Huber et al., 2005; Thiery et al., 2009). Examples of such processes include *Drosophila* border cell migration (Niewiadomska et al., 1999) and gastrulation (Leptin, 1999), zebrafish lateral line migration (Haas and Gilmour, 2006) and neural crest migration in vertebrates (Matthews et al., 2008). Migration of epithelial cells also contributes to the closure of wounds (Yan et al., 2010) and to tumour progression when cancer cells invade surrounding tissues (Thiery, 2002).

The metamorphosis of the abdominal epithelium of *Drosophila* provides an attractive system to study the migration of epithelial cells. Here, the diploid adult histoblasts replace the polyploid larval epithelial cells (LECs) (Supplementary Movie 1) (Bischoff and Cseresnyes, 2009; Madhavan and Madhavan, 1980; Ninov et al., 2007). While the histoblasts divide and migrate towards the midline, the LECs constrict apically, leave the epithelium (delaminate) and die. This is concurrent with the notion that the larval tissue has to be removed to generate space for the adult tissue (Ninov et al., 2007, 2010). The mechanisms that drive morphogenesis of the adult epidermis are, however, still elusive.

We have previously shown that the LECs relocate dorsally before they die (Bischoff and Cseresnyes, 2009), which suggested that the LECs might be pushed by the histoblasts. Here I analyse the behaviour of the LECs in detail using *in vivo* 4D microscopy (Bischoff and Cseresnyes, 2009; Schnabel et al., 1997). I show that the LECs undergo extensive coordinated migrations, which are propelled by apical lamellipodia-like protrusions. These migrations are well suited to study how migratory behaviour is regulated during different phases of morphogenesis. Prior to migration, the LECs undergo a transition from stationary to migratory behaviour. The migratory behaviour is stimulated by autocrine Decapentaplegic (Dpp) signalling. Initially, the migrations are oriented posteriorly, and this depends on the planar polarity of the epithelium. Eventually, the LECs move dorsally,

* Fax: +44 1223 336676.
E-mail address: mb628@cam.ac.uk.

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while also constricting apically. Altering levels of the small GTPase Rho1 can favour one behaviour over the other — Rho1 activation induces constriction, whereas its down-regulation increases migratory behaviour. Overall my data show that, together with apical constriction, the coordinated migration of LECs is required for normal closure of the adult epithelium. Thus, the LECs play a more active role in morphogenesis than previously thought.

Materials and methods

Fly stocks

FlyBase (Tweedie et al., 2009) entries of the used transgenes are as follows:

en.Gal4: *Scer/Gal4$^{en\text{-}e16E}$*, *hh.Gal4*: *Scer/Gal4$^{hh\text{-}Gal4}$*, *H2AvGFP*: *His2Av$^{T:Avic|GFP\text{-}S65T}$*, *DE-cadherin::GFP*: *shg$^{Ubi\text{-}p63E.T:Avic|GFP\text{-}rs}$*, *UAS.RFP: Disc|RFP$^{Scer|UAS.cWa}$*, *UAS.GFP-actin*: *Act5C$^{Scer|UAS.T:Avic|GFP}$*, *UAS.gma*: *Moe$^{Scer|UAS.T:Avic|GFP\text{-}S65T}$*, *UAS.ectoDs*: *ds$^{ecto.Scer|UAS}$*, *UAS.p35*: *BacA|p35$^{Scer|UAS.cHa}$*, *UAS.DIAP1*: *th$^{Scer|UAS.T:Ivir|HA1}$*, *UAS.DIAP2*: *lap2$^{Scer|UAS.cWa}$*, *UAS.rho^{V14}*: *Rho1$^{V14.Scer|UAS}$*, *UAS.rho^{N19}*: *Rho1$^{N19.Scer/UAS}$*, *UAS.DCR2*: *Dcr-2$^{Scer/UAS.cDa}$*, *UAS.tkv^{DN}*: tkv$^{1\Delta GSK.Scer/UAS}$, *UAS.tkv$^{Q\text{-}D}$*: *tkv$^{QD.Scer|UAS}$*, *UAS.-dad*: *dad$^{Scer/UAS.cTa}$*, *UAS.pio-RNAi*: *pio^{KK112233}*, *UAS.ds-RNAi*: *ds^{GD14350}*, *UAS.rho1-RNAi*: *rho1^{GD4726}*, *UAS.tkv-RNAi*: *tkv^{GD2549}*, *UAS.dpp-RNAi*: *dpp^{JF01371}*, *tub-FRT-CD2-FRT-Gal4*: *Rnor|CD2^{A902}*, *UAS.mCD8-GFP*: *Mmus/Cd8a$^{Scer|UAS.T:Avic/GFP}$*, *UAS.mCD8-RFP*: *Mmus/Cd8a$^{Scer|UAS.T:Disc/RFP\text{-}mRFP}$*, *UAS.myr-RFP*: *Disc/RFP$^{Scer/UAS.T:Myr1}$*, *UAS.FLP*: *FLP1$^{Scer|UAS.cDa}$*.

Expression of UAS-transgenes in LECs

Overexpression of *UAS*-transgenes in the LECs was achieved by using the FLP-out technique (Struhl and Basler, 1993). FLP-out clones were induced by heat-shocking third instar larvae for 6 to 10 min at 35–37 °C. This led to FLP-out events in the polyploid LECs but rarely in the diploid histoblasts (Ninov et al., 2007). Thus, most LECs but only few histoblasts expressed the UAS-transgenes. Additional expression of *UAS.FLP* increased the recombination events in the polyploid cells, thus increasing expression levels. After heat-shock, flies were kept at 23 °C for 3 days or at 29 °C for 2 days before imaging. The *UAS.rho^{V14}* and *UAS.rho^{N19}* pupae were filmed 1 day after heat-shock.

Pupae carrying clones that expressed various *UAS*-transgenes (*UAS.X*) marked with mCD8-GFP, mCD8-RFP or myr-RFP had the following genotypes:

1) *y w hs.FLP; UAS.mCD8-GFP, tub-FRT-CD2-FRT-Gal4, UAS.FLP/+* or *Sp* or *UAS.X; +/+* or *UAS.X*
2) *y w hs.FLP; UAS.mCD8-RFP, tub-FRT-CD2-FRT-Gal4, UAS.FLP/ubi.DE-cadherin::GFP; MKRS/+*
3) *y w hs.FLP; UAS.myrRFP, UAS.myrRFP, tub-FRT-CD2-FRT-Gal4, UAS.FLP/ ubi.DE-cadherin::GFP, UAS.X; +* or *MKRS/+* or *UAS.X*

Transgenes on either chromosome 2 or 3 were used, with the exception of *UAS.tkv^{DN}* where insertions in both chromosomes were used. No differences in phenotype were observed between flies carrying wild-type chromosomes and chromosomes with *Sp* and/or *MRS/MKRS*.

RNA interference

RNAi lines were obtained from the VDRC (Dietzl et al., 2007) and the BDSC. Various observations made here and by others suggested that the RNAi phenotypes are specific to a knock-down of the targeted gene and also allowed an assessment of the strength of the knock-down:

- *UAS.rho-RNAi*: *UAS.rho^{N19}* pupae have a similar phenotype. *UAS.rho-RNAi* has been shown to generate abdominal closure defects when using another driver than in this study (Sekyrova et al., 2010).
- *UAS.ds-RNAi*: The adult cuticle in *UAS.ds-RNAi pupae* shows PCP defects similar to *ds* mutants (data not shown). *UAS.ectoDs* pupae have a similar phenotype.
- *UAS.tkv-RNAi*, *UAS.dpp-RNAi*: *UAS.tkv^{DN}* and *UAS.dad* have similar phenotypes. *UAS.dpp-RNAi* was shown to interfere with histoblast development and Dpp is expressed in LECs (Ninov et al., 2010).

4D microscopy

For imaging, pupae were staged according to (Bainbridge and Bownes, 1981). A window in the pupal case was made and the pupae were filmed as described in Escudero et al. (2007). The analysis focused on the dorsal side (tergite) of the abdomen. All imaged flies developed into pharate adults and many hatched. *Z*-stacks of around 40 μm with a step size of 2.5 or 3.0 μm were recorded every 150 or 180 s using a Leica SP5 confocal microscope at 23 ± 2 °C. Each genotype was recorded at least three times (Supplementary Table 1). All images and movies shown are projections of *z*-stacks. Figures and movies were made using Adobe Illustrator, Adobe Image Ready, Adobe Photoshop, ImageJ (NIH, Bethesda, USA), Volocity (Improvision), and Quicktime Pro (Apple Inc.).

Analysis of 4D movies

To analyse cell behaviour in detail, the LECs were tracked manually using the software SIMI Biocell (Schnabel et al., 1997). The 3D coordinates of the nuclei (for H2AvGFP and mCD8GFP) or the centres of the cells (for DE-cadherin::GFP) were saved at least every 30 min.

Trajectory plots were calculated using a programme written in C# using Microsoft Visual Studio 2005 with the Microsoft .NET 2.0 framework (Bischoff and Cseresnyes, 2009). All calculations were performed in two dimensions due to the planar character of the epithelial sheet.

1) Trajectory plots: To display the trajectories of the cells, the coordinates of a cell at a certain time and the coordinates of the same cell 30 min later were connected with a straight 'beeline'. The colour of these lines represents the velocity of the cell using a banded look-up table. If nuclei were tracked, the trajectories of the cells not only represent the movement of the cell *per se* but also the movement of the nucleus within the cell — for the general direction of the movement, however, this is negligible. In most figures, two migration phases are shown separately — 'posterior' and 'dorsal'. These two phases represent the time intervals in which cells mainly move posteriorly (up to approx. 28 h after puparium formation (APF)) and dorsally (from approx. 28 h APF onwards), respectively.
2) Measurement of the speed of abdominal closure: The average speed of the dorsally expanding histoblast mass was measured from the beginning of dorsal LEC migration to its halt when there were about five rows of LECs left.
3) The relative fluorescence of DE-cadherin::GFP was measured along a line in anterio-posterior (a–p) direction using LAS AF Lite (Leica Microsystems, Mannheim, Germany).
4) The length and width of LECs were measured manually using ImageJ (NIH, Bethesda, USA), at the time when LECs have undergone cell shape changes and started to move (around 26 h APF). The longest line fitting in the apical area of the LECs of segment A2 along their a–p and dorso-ventral (d–v) axes, respectively, was measured.

Results

LECs undergo a transition from stationary to migratory behaviour

The LECs are large cells (up to 70 μm in apical diameter), which makes them well suited to study cell behaviour *in vivo*. I analysed