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Cellular Processes Associated with Germ Band Retraction in *Drosophila*

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Large-scale movements of epithelial sheets are necessary for most embryonic and regenerative morphogenetic events. We have characterized the cellular processes associated with germ band retraction (GBR) in the *Drosophila* embryo. During GBR, the caudal end of the embryo retracts to its final posterior position. We show using time-lapse recordings that, in contrast to germ band extension, cells within the lateral germ band do not intercalate. In addition, the germ band and amnioserosa move as one coherent sheet, and the amnioserosa strongly shortens along its dorsal–ventral axis. Furthermore, during GBR, the amnioserosa adheres to and migrates over the caudal end of the germ band via lamellipodia. Expression of both dominant-negative and constitutively active RhoA in the amnioserosa disrupts GBR. As RhoA acts on both actomyosin contractility and cell–matrix adhesion, it suggests a role for such processes in the amnioserosa during GBR. The results establish the cellular movements and shape changes occurring during GBR and provide the basis for an analysis of the forces acting during GBR.

Key Words: **amnioserosa; epithelial morphogenesis; lamellipodia; migration.**

INTRODUCTION

Epithelial morphogenesis comprises the various processes by which epithelia contribute to organ formation and body shape. Cells employ a number of mechanisms, such as movements, shape changes, proliferation, and death to shape tissues (Fristrom, 1988). Among these processes, cell shape changes and movements play a prominent role, and are used widely and repeatedly during evolution. For example, apical constriction of epithelial cells is necessary for both vertebrate neurulation (Smith and Schoenwolf, 1997) and mesoderm formation during gastrulation in *Drosophila* (Costa *et al.*, 1993). Dorsal closure in the *Drosophila* embryo, in which epithelia move as a coherent sheet, is comparable to ventral enclosure in *Caenorhabditis elegans* (Williams-Masson *et al.*, 1997), as well as some aspects of wound healing in vertebrates (Kiehart *et al.*, 2000). Finally, both *Drosophila* germ band extension (Costa *et al.*, 1993; Irvine and Wieschaus, 1994) and convergent extension during *Xenopus* gastrulation (Wilson and Keller, 1991) involve cell rearrangements within epithelia.

In *Drosophila*, three large-scale epithelial movements, gastrulation, dorsal closure, and germ band extension, have been extensively investigated. Gastrulation starts with the formation of a ventral furrow in a stripe of presumptive mesodermal cells about 10 cells wide (Costa *et al.*, 1993). The first step in ventral furrow formation, apical constriction, is presumably caused by RhoA-mediated contraction of the apical actomyosin cytoskeleton (Barrett *et al.*, 1997; Häcker and Perrimon, 1998). At the end of gastrulation, the three different germ layers have formed from a single epithelium.

Another major morphogenetic event in the embryo is dorsal closure, which is initiated immediately after germ band retraction (GBR). The first step in dorsal closure is the elongation of cells at the leading edge of the dorsal epidermis, which abut the amnioserosa. Elongation of these leading edge cells spreads to more ventrally located cells of the germ band; a process which is concomitant with a dorsal–ventral (DV) contraction of the amnioserosa (Kiehart *et al.*, 2000; Stronach and Perrimon, 1999). These cell shape changes cause the epidermis to stretch and close dorsally over the amnioserosa. Laser ablation studies have demon-

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strated that both amnioserosa and leading edge cells are necessary for dorsal closure (Kiehart *et al.*, 2000).

Germ band extension occurs just before GBR. During germ band extension, cells rearrange with respect to their neighbors, that is, cells along the DV axis intercalate between cells aligned along the anterior–posterior (AP) axis (Costa *et al.*, 1993; Irvine and Wieschaus, 1994). This process takes approximately 1 h and results in elongation of the germ band.

A fourth morphogenetic process of large-scale epithelial movements in the *Drosophila* embryo is GBR, which occurs after germ band extension and prior to dorsal closure. GBR begins at early stage 12 and takes approximately 2 h. During GBR, the thoracic and abdominal segment boundaries form from anterior to posterior, and the caudal end of the embryo comes to lie at its final posterior position with respect to the eggshell (Martinez Arias, 1993). At the end of this process, the amnioserosa, a squamous epithelium, has spread out and covers the yolk sac on the dorsal side of the embryo.

The origin and nature of the mechanical forces necessary for GBR are not known, even though it has been suggested that GBR is associated with cell shape changes rather than intercalation (Martinez Arias, 1993). A number of processes can be ruled out based on mutant phenotypes. For example, cell divisions do not appear to contribute to GBR because the germ band of *string* mutant embryos that do not undergo postblastoderm mitoses retracts normally (Edgar and O'Farrell, 1989). Further, cell death does not appear to play a role because mutant embryos lacking the apoptosis inducers *grim*, *reaper*, and *head involution defective* retract their germ band normally (Lamka and Lipshitz, 1999). Thus, the dramatic movements of GBR are likely to be driven by cell shape changes or movements.

A substantial amount of data has been obtained on the molecular mechanisms that drive gastrulation and dorsal closure, while little is known about GBR. To dissect GBR, we have initiated both a cellular and a genetic analysis of this system. Here, we provide a detailed morphological analysis of GBR employing live imaging of embryos marked with various proteins fused to green fluorescent protein (GFP). This establishes several findings: first, GBR is not mediated by cell intercalation, implying that it is not a reversal of germ band extension (Costa *et al.*, 1993; Irvine and Wieschaus, 1994). Second, the amnioserosa and the germ band move as one coherent sheet during GBR. Third, amnioserosa cells undergo a dramatic shortening of their DV axis during GBR. Fourth, we show that the amnioserosa adheres to the caudal end of the germ band via lamellipodia. The lamellipodia not only adhere to, but migrate over the caudal end of the germ band. Finally, expression of either dominant-negative or constitutively active RhoA in the amnioserosa disrupts GBR. The results establish the cellular movements and shape changes occurring during GBR and provide the basis for an analysis of the forces acting during GBR.

MATERIALS AND METHODS

Fly Strains

pnr-Gal4 (Calleja *et al.*, 1996) was obtained from the Bloomington Stock Center, *Kr-Gal4 10°* and *Kr-Gal4 40°* (Castelli-Gair *et al.*, 1994) were obtained from J. P. Gergen, *UASrho*^{N19} (Strutt *et al.*, 1997) from M. Mlodzik, *UASrho1*^{V12} (Rangarajan *et al.*, 1999) from U. Gaul, *UASmEGFP* from E. Spana, *c381-Gal4* (Manseau *et al.*, 1997) from L. Kockel, *arm-Gal4 UASα-cateninGFP* (Oda and Tsukita, 1999), *UASactin5CGFP* (Verkhusha *et al.*, 1999), and *ubiDE-cadherinGFP* (Oda and Tsukita, 2001) from H. Oda.

Time-Lapse Recording

Embryos were dechorionated in 50% Clorox for 3 min, dried shortly on Whatman paper, and transferred to Petriperm 50 (Unisyn Technologies) plates, which have a gas-permeable bottom. The embryos were slightly flattened with a coverslip after covering them with halocarbon 27 oil. Micrographs were taken on a Leica TCS-NT laser confocal microscope and further processed with Adobe Photoshop 5.0. Morphometric measurements were performed with NIH Image 1.62. For each picture shown, two to four z-sections were averaged. For each experiment, at least three different embryos were analyzed.

To analyze embryos, we used *UASα-cateninGFP* driven by the ubiquitously expressed *arm-Gal4* (Oda and Tsukita, 1999) and *ubiDE-cadherinGFP* (Oda and Tsukita, 2001). Both α-cateninGFP and DE-cadherinGFP localize to the adherens junction. We also used *UASactin5CGFP* (Verkhusha *et al.*, 1999) and *UASmEGFP*, driven by *Kr-Gal4* or *c381-Gal4*. mEGFP localizes to both cytoplasm and plasma membrane due to the Src myristilation anchor attached to EGFP. Actin5CGFP is incorporated into filamentous actin (Verkhusha *et al.*, 1999).

Cuticle Preparations and Histochemistry

Cuticle preparations were performed as previously described (van der Meer, 1977). For actin staining, dechorionated embryos were fixed for 45 min in formaldehyde-saturated heptane, then hand-devitellinized and stained for 20 min with Texas Red-phalloidin (Molecular Probes; 1:100).

RESULTS

Germ Band Cells Do Not Intercalate

We used the Gal4/UAS system (Brand and Perrimon, 1993) to produce time-lapse recordings of embryos expressing a UAS*α-cateninGFP* transgene driven by the ubiquitously expressed *arm-Gal4* (see Materials and Methods). α-CateninGFP labels adherens junctions allowing dynamic visualization of cell boundaries (Oda and Tsukita, 1999). Figure 1A displays the course of germ band retraction in a typical wild type embryo (see also Movie 1). The DV axis of most amnioserosa cells is oriented along the long axis of the embryo at the beginning of GBR, but coincides with the embryonic DV axis after retraction (Fig. 1A).

While retracting, the height of the germ band (its DV axis) approximately doubles, and the width of the germ band (its

AP axis) decreases by 50% (Martinez Arias, 1993). We therefore asked whether these changes come about by cell intercalation as they do during germ band extension (Costa *et al.,* 1993; Irvine and Wieschaus, 1994). Individual cells along the AP and DV axes on the lateral side of the germ band were identified and monitored at 2-min intervals (Figs. 1B and 2A). Along the AP axis, cells do not stack up (Fig. 1B). Conversely, cells along the DV axis are not separated, i.e., no cells intercalate into the column of marked cells (Fig. 2A). Germ band cells are only separated along the DV axis by mitoses (see numbered cells in Fig. 2A; also see Movie 2). Cell divisions occur throughout GBR, but with decreasing frequency. The orientation of the plane of cell division is random, but most daughter cells appear to align along the DV axis (see cells 2a and 2b in Fig. 1B and cells 2a, 2b, 4a, and 4b in Fig. 2A). We found only minimal rearrangements during GBR (e.g., follow the position of cells 6 and 7 in Fig. 2A).

To determine whether amnioserosa and germ band cells are stably attached to each other via adherens junctions during GBR, we identified and followed cells in the amnioserosa and in the adjacent leading edge cells of the germ band. Cells of both epithelia remain conjoined during GBR and move as one coherent sheet (Fig. 2B; also see Movie 2).

Shape Changes in the Amnioserosa and Germ Band Are Reciprocal

After demonstrating that local cell rearrangements do not contribute to GBR, we examined the cell shape changes in amnioserosa and germ band cells. We analyzed individual cell shape changes by following cells marked with ubiquitously expressed α-cateninGFP or DE-cadherinGFP (Oda and Tsukita, 1999, 2001). Along the AP axis, two types of cell shape changes are visible in the germ band: a slight shortening of each cell (from 3.3 ± 1.1 to 2.1 ± 0.3 μm, 50 cells measured), and the formation of a furrow at each segment boundary (Fig. 1). In addition, DV elongation occurs throughout the germ band but is much more pronounced in about 8 cell rows next to the amnioserosa (Fig. 2A). In those cell rows, germ band cells elongate along their DV axis from 3.3 ± 0.7 to 8.8 ± 1.9 μm (50 cells measured). In contrast, amnioserosa cells undergo shape changes that are opposite to those seen in epidermal cells of the lateral germ band. Amnioserosa cells strongly shorten along their DV axis (from 78 ± 19 to 28 ± 6.5 μm, 30 cells measured) (Fig. 3A).

In both germ band and amnioserosa cells, the actin cytoskeleton is localized cortically (data not shown), indicating that forces within these cell sheets are expected to occur at cell boundaries. We therefore investigated the border between amnioserosa and germ band more closely, which revealed that the row of leading edge cells is pulled in wherever the amnioserosa plasma membranes perpendicular to the leading edge contact the germ band (arrows in Fig. 3B). This suggests that the amnioserosa exerts a force on the germ band at the boundary of the two epithelia (see Discussion).

Thus, in contrast to cell intercalation that characterizes germ band extension, we observe major cell shape changes within the amnioserosa and the germ band during GBR.

The Amnioserosa Adheres to the Caudal End of the Germ Band

Histological sections of embryos at the beginning of stage 12 revealed that the amnioserosa overlaps the caudal end of the germ band (Campos-Ortega and Hartenstein, 1997). To investigate the nature of this overlap in more detail, we generated time-lapse recordings of sagittal sections of retracting wild type embryos labeled with *UASmEGFP* expressed in the amnioserosa with the driver line *c381-Gal4* (see Materials and Methods). *UASmEGFP* localizes to both cytoplasm and plasma membrane due to the Src myristilation anchor attached to EGFP. At the beginning of embryonic stage 12, much of the amnioserosa overlaps the germ band (Fig. 4, panel 1). Time-lapse recordings show that this overlap gradually decreases. At the same time, the area of contact between amnioserosa and yolk sac increases (Fig. 4).

We next asked how the overlap between amnioserosa and germ band can be maintained, even though the germ band retracts rapidly. To this end, we followed dorsal views of wild type embryos marked with *UASmEGFP* expressed with *Kr-Gal4* in the amnioserosa. Strikingly, the overlap of the amnioserosa over the caudal end of the germ band is maintained by adhesion of the posteriormost amnioserosa cells (Fig. 5; also see Movie 3). The amnioserosa adheres by extending protrusions. They emanate mostly from posterior amnioserosa cells, and project toward the posterior (Fig. 5A). During GBR, on average 90% of protrusions extend toward the posterior (7 embryos analyzed). The protrusions become longer during GBR and collapse as soon as GBR is completed (Fig. 5B), and no new lamellipodia are observed (data not shown).

In tissue culture cells, structures called lamellipodia arise and protrude by localized actin polymerization (Geiger *et al.,* 2001). We therefore tested for the presence of actin in these protrusions by using *c381-Gal4* to express *UAS-actin5CGFP* in the amnioserosa (Verkhusha *et al.,* 1999). Indeed, we observe the presence of actinGFP at the leading edge of protrusions and in foci within the protrusions (Fig. 6; also see Movie 4). Furthermore, actinGFP dynamically changes its location with progressive protrusion of these structures, which presumably corresponds to actin polymerization. Therefore, these protrusions resemble lamellipodia in all respects described in motile tissue culture cells, and are referred to as lamellipodia from now on (Geiger *et al.,* 2001).

We next analyzed whether lamellipodia migrate over the germ band or stably adhere to it and are then dragged by the retracting germ band. We therefore labeled embryos simultaneously with ubiquitously expressed *ubiDE-cadherinGFP* and with *UAS actin5CGFP* expressed in the amnioserosa

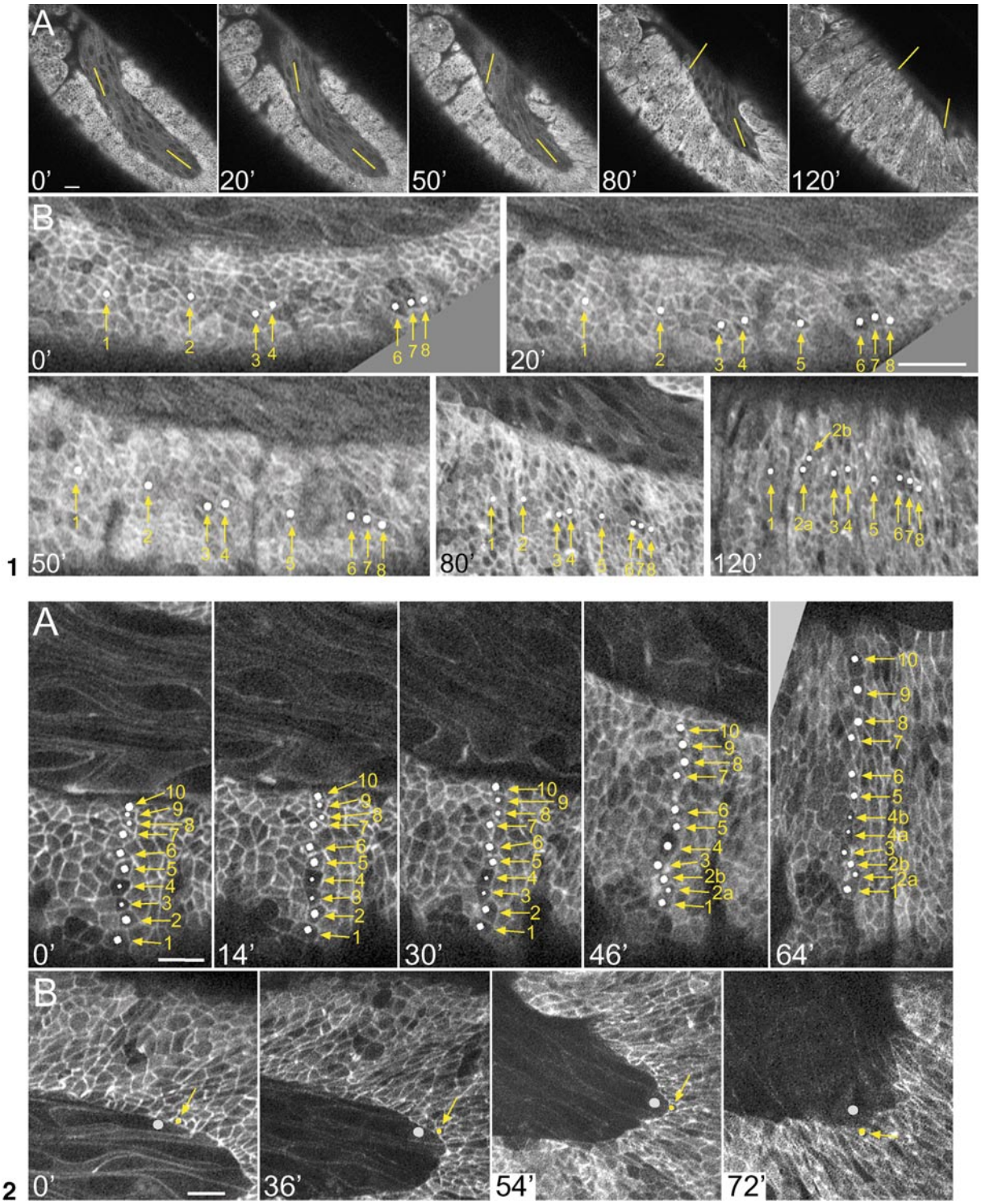


FIG. 1. Time-lapse recording of GBR in a wild type embryo visualized with *arm-Gal4 UASα-cateninGFP*. α-CateninGFP localizes to adherens junctions outlining the shape of epidermal cells (Oda and Tsukita, 1999). Frames were taken every 2 min by using a laser confocal microscope at room temperature. Selected frames with the time scale in minutes are shown. In all panels, anterior is left or in the upper left corner and dorsal is up or in the upper right corner. (A) Overview of the time course of GBR. The DV axis of amnioserosa cells is oriented along the long axis of the embryo at the beginning of GBR, but coincides with the embryonic DV axis at the end of GBR (yellow lines). (B) Enlarged view of lateral aspect of germ band. Cells along the AP axis of the germ band were identified and followed every 2 min, by marking them with white dots to follow the movement of individual cells. Cells are numbered to indicate dividing cells. More cells were followed than shown. For each experiment, at least three different embryos were analyzed. Bars, 20 μm.

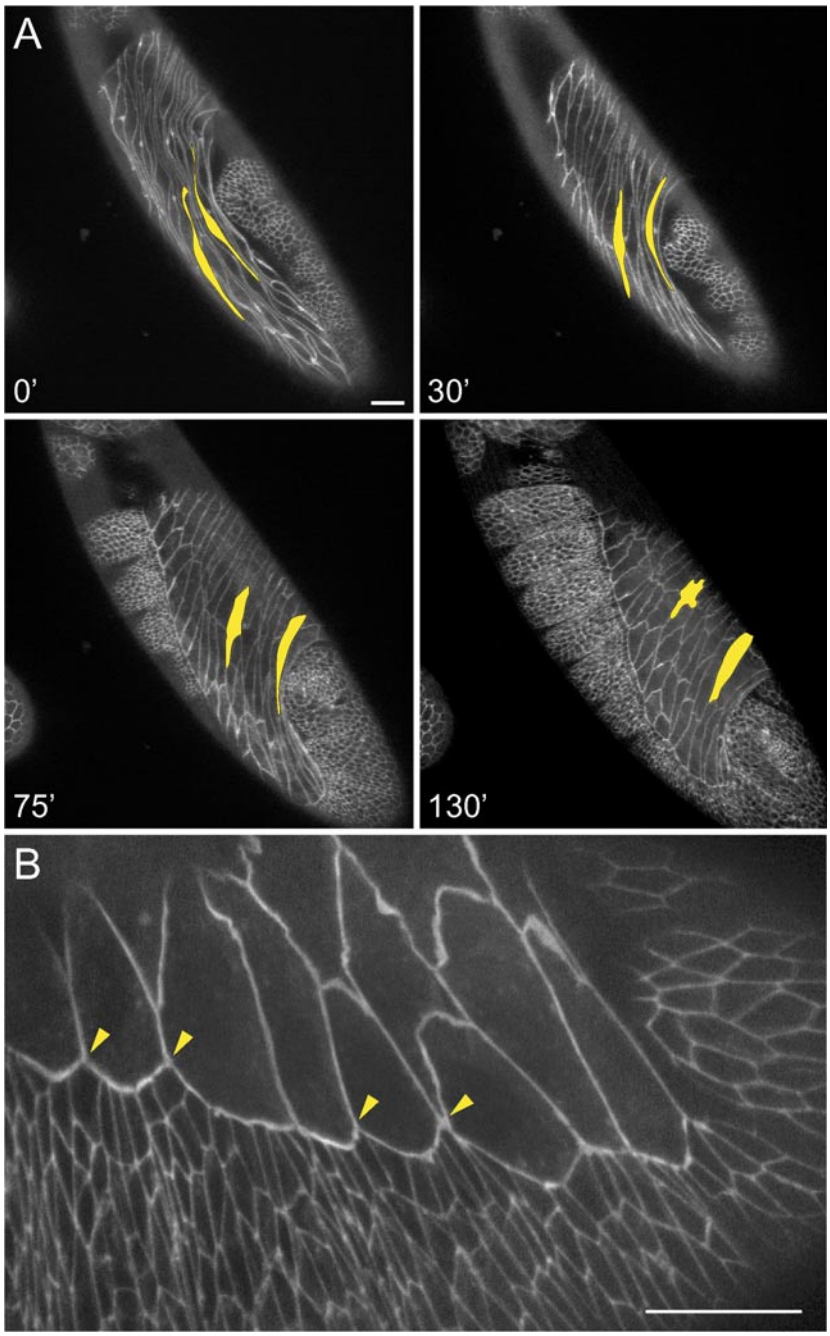


FIG. 3. Cell shape changes in amnioserosa cells. (A) Time-lapse recording of a wild type embryo marked with *ubiDE-cadherinGFP* taken at 5-min intervals. Two individual amnioserosa cells were followed through most of GBR and are shown in yellow. They shorten along their DV axis from 92 to 31 μm and 91 to 48 μm, respectively. (B) Enlarged view of the boundary between amnioserosa and germ band. Arrowheads indicate sites where amnioserosa cell membranes perpendicular to the leading edge of the germ band touch the leading edge. Note that the germ band appears to be pulled in at these sites. Bars, 20 μm.

FIG. 2. Germ band cells are not separated along the DV axis, and amnioserosa and germ band are stably attached to each other via adherens junctions. Time-lapse recording of wild type embryo marked with *arm-Gal4 UASα-cateninGFP* taken at 2-min intervals. (A) A column of cells along the DV axis was monitored by marking cells with white dots. Cells are not separated by intercalating cells. Note that cells close to the amnioserosa show a stronger DV elongation than more lateral cells. (B) One amnioserosa cell and an epidermal cell directly bordering each other were followed through GBR. The amnioserosa cell is marked with a gray dot. The germ band cell is marked with a yellow dot and an arrow. Each amnioserosa cell attaches to about five germ band cells and is generally wider at the site of attachment than more dorsally. Bars, 10 μm.

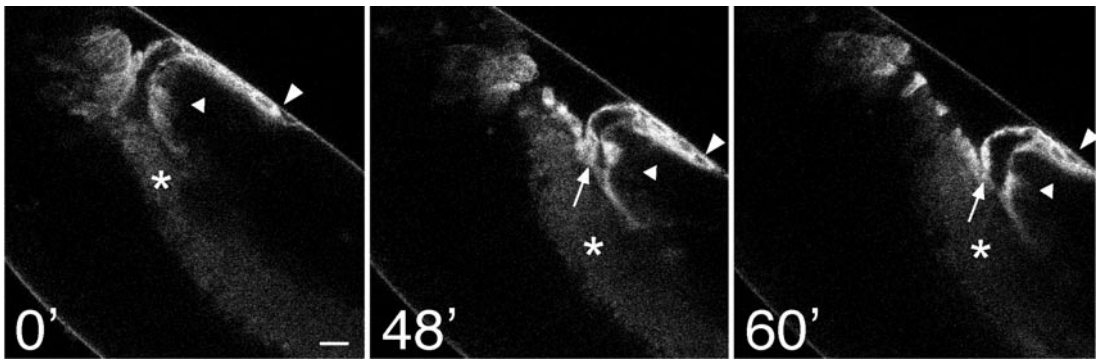


FIG. 4. Sagittal time-lapse recording of amnioserosa during GBR. (A) Time-lapse recording of a parasagittal section of germ band-retracting embryo expressing *UASmEGFP* under the control of the amnioserosa driver line *c381-Gal4*. Frames were taken every 4 min at room temperature. Two regions in the amnioserosa can be distinguished: one overlapping the caudal end of the germband, and the other closely apposed to the underlying yolk sac. The arrowhead marks the tip of the overlapping part of the amnioserosa, while a triangle marks the caudal end of the germband. The arrow indicates the border between the overlapping and the nonoverlapping part of the amnioserosa. The star indicates the autofluorescence of the yolk sac. The overlap is gradually diminishing during GBR. Bar, 20 μm.

with *c381-Gal4*. This visualizes lamellipodia through the expression of actinGFP, while germ band cells are outlined due to the expression of cadherinGFP in the adherens junction. This allowed us to monitor individual cells in the caudal germ band and individual lamellipodia by marking them in each frame of the time-lapse sequence (Fig. 7A). The experiment demonstrates a net migration of lamellipodia over the germ band independent of the retraction of the germ band itself.

We finally asked on which cells the lamellipodia migrate. They adhere to cells of the anal pads, the most caudal structure of the germ band (Fig. 7B).

Constitutively Active and Dominant-Negative RhoA Cause GBR Defects

To address whether the amnioserosa contributes to GBR, we expressed the dominant-negative and constitutively active GTPase RhoA either in the amnioserosa or along the leading edge (Fig. 8). RhoA is an important cytoskeletal regulator affecting adhesion and contractility (Hall, 1998) and interacts genetically with nonmuscle myosin in *Drosophila* (Halsell *et al.*, 2001). In particular, constitutively active RhoA has been shown to induce actomyosin contractility and is a strong inhibitor of cell migration in a wound closure assay, whereas dominant-negative RhoA is disrupting actomyosin contractility as well as inhibiting cell migration (Chrzanowska-Wodnicka and Burridge, 1996; Nobes and Hall, 1999). Expression of both *rhoA* mutants in the amnioserosa results in severe GBR defects using two different Gal4 driver lines (Figs. 8D, 8E, 8G, and 8H). In contrast, expression of these RhoA mutant proteins along the leading edge affects dorsal closure, but does not disrupt germ band extension (not shown) or retraction (Figs. 8F and 8I). This suggests that the amnioserosa contributes to GBR.

DISCUSSION

In this paper, we have analyzed the process of GBR during *Drosophila* embryogenesis. We demonstrate that the amnioserosa plays an active role during GBR by adhering to and migrating over the caudal end of the germ band. In addition, amnioserosa cells strongly shorten along their DV axis. The amnioserosa, therefore, is a novel system to study cell shape changes and cell–matrix adhesion.

GBR is completed during embryonic stage 12. This is a time of exceptional morphogenetic activity, as the midgut fuses and encloses the yolk sac laterally, the tracheal pit extensions fuse to form the tracheal tree, and the segmental furrows form from anterior to posterior (Campos-Ortega and Hartenstein, 1997; Martinez Arias, 1993). At stage 12, the embryo consists of two major epithelia, the squamous extraembryonic amnioserosa and the ectodermal germ band epithelium, as well as a mesenchymal mass of mesodermal and central nervous system precursor cells. Also found in the embryo are the epithelia of the foregut, hindgut, and salivary and tracheal pit invaginations, which are of ectodermal origin, and the midgut epithelium, which forms at that stage by mesenchymal to epithelial transition (Campos-Ortega and Hartenstein, 1997). The syncytial yolk sac, which is enclosed by a yolk sac membrane, sits in the middle of the embryo at the beginning of GBR, but moves more dorsally, directly beneath the amnioserosa, by the end of GBR (Campos-Ortega and Hartenstein, 1997; Turner and Mahowald, 1977). We focused our analysis on the amnioserosa and the germ band, because they appear to be the most likely candidates of the above tissues to participate in GBR.

We first showed that, as suggested earlier, cells in the germ band do not intercalate (Figs. 1 and 2) (Martinez Arias, 1993). GBR is therefore not a reversal of germ band extension (Costa *et al.*, 1993; Irvine and Wieschaus, 1994).

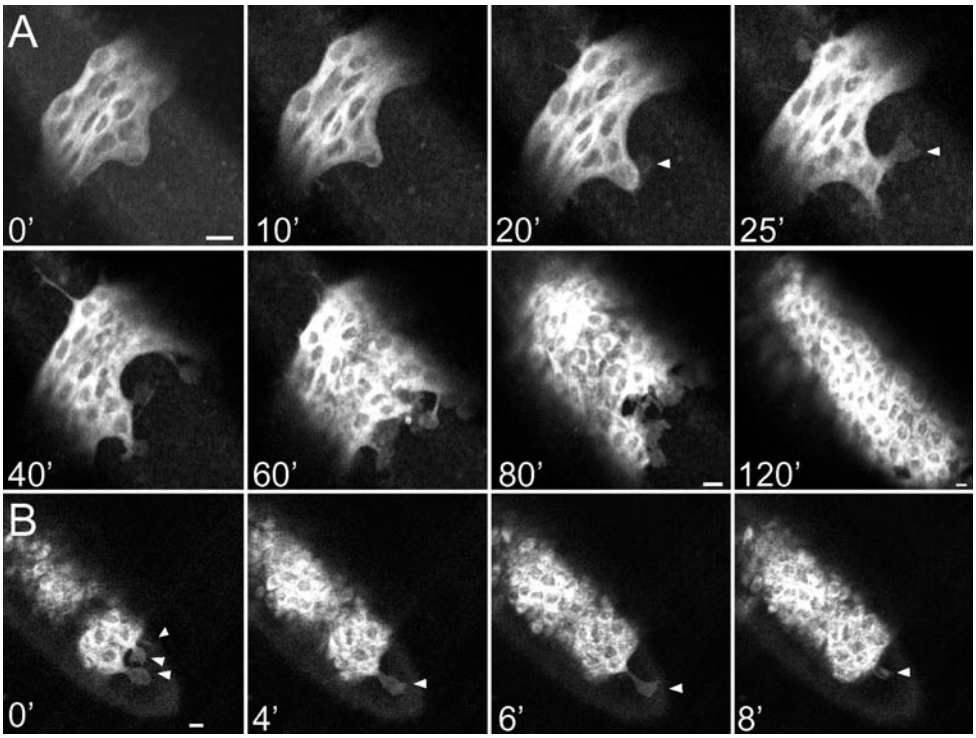


FIG. 5. The amnioserosa adheres to the germ band during GBR. (A) Time-lapse recording of a dorsal view of a wild type embryo marked with *Kr-Gal UASmEGFP* taken at 5-min intervals. During GBR on average 90% of lamellipodia arise on the posterior side of the amnioserosa and project toward the posterior (counted in 7 embryos). Arrowheads mark the first posterior lamellipodium. (B) Time-lapse recording of a dorsal view of a wild type embryo marked with *Kr-Gal4 UASmEGFP* at the end of GBR taken at 2-min intervals. Lamellipodia become longer until they collapse and disappear at the end of GBR. No new lamellipodia form. All lamellipodia are marked by arrowheads. Bars, 10 μm.

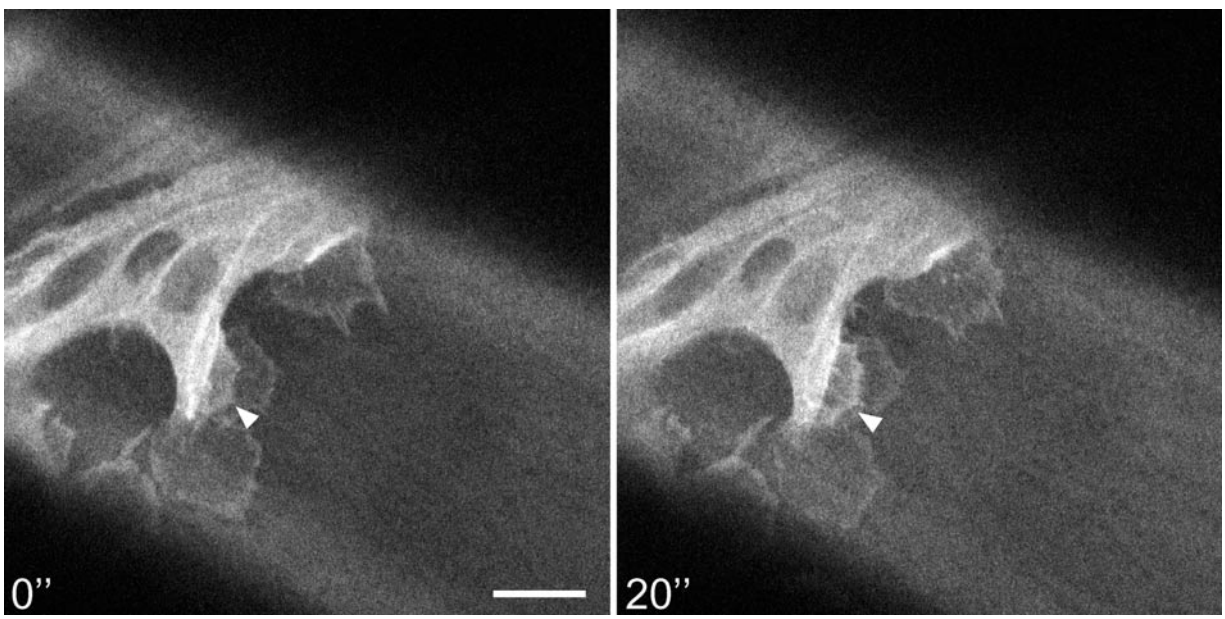


FIG. 6. Lamellipodia formation. Time-lapse recording of a dorsal view of a *c381-Gal4; UASactin5CGFP* embryo during GBR taken at 20-s intervals. Arrowheads point to a newly arising lamellipodium, visualized by the increased presence of actinGFP presumably due to actin polymerization. Note actin polymerization along the edge of the lamellipodia and in foci within the lamellipodia. Bar, 10 μm.

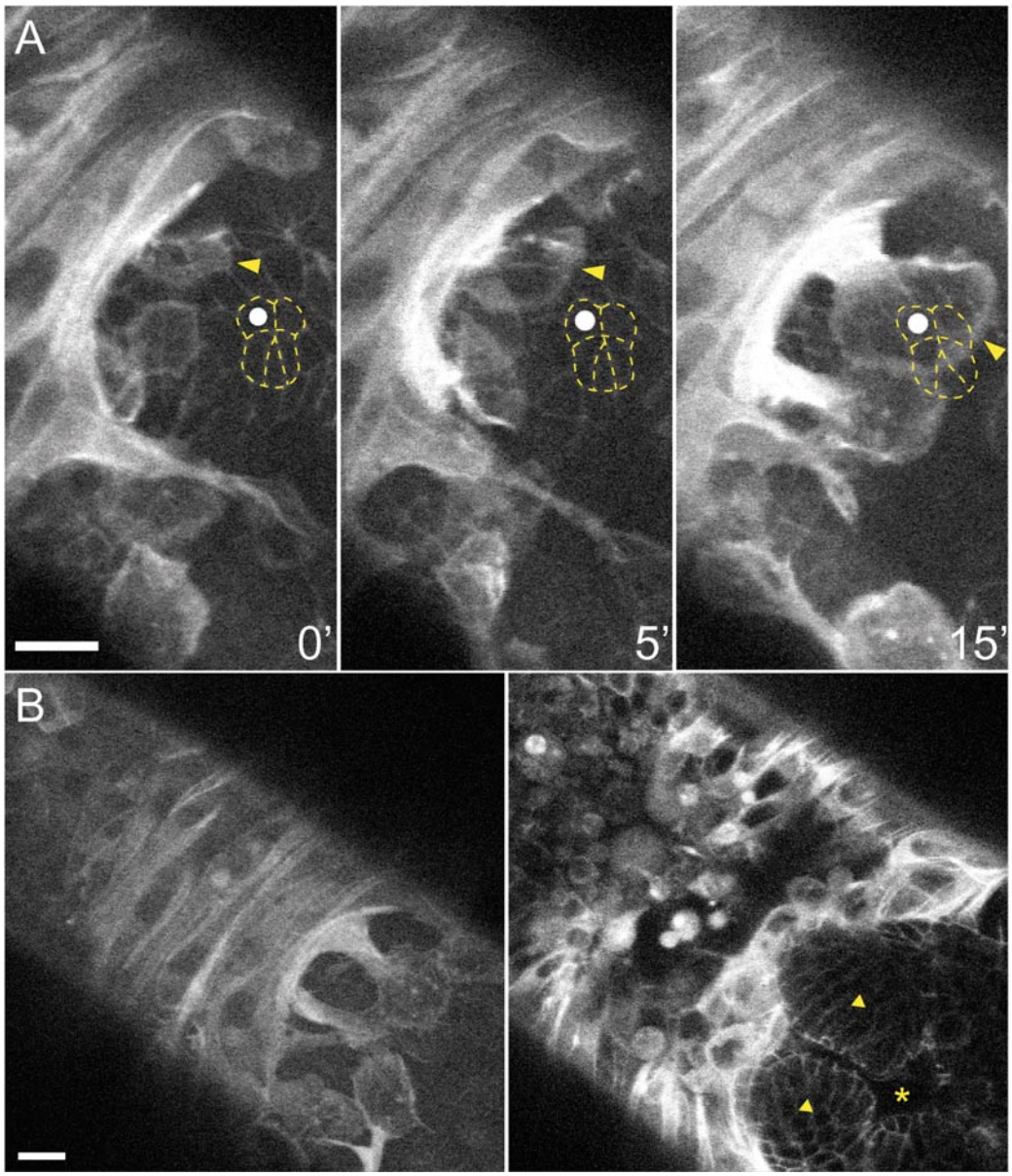
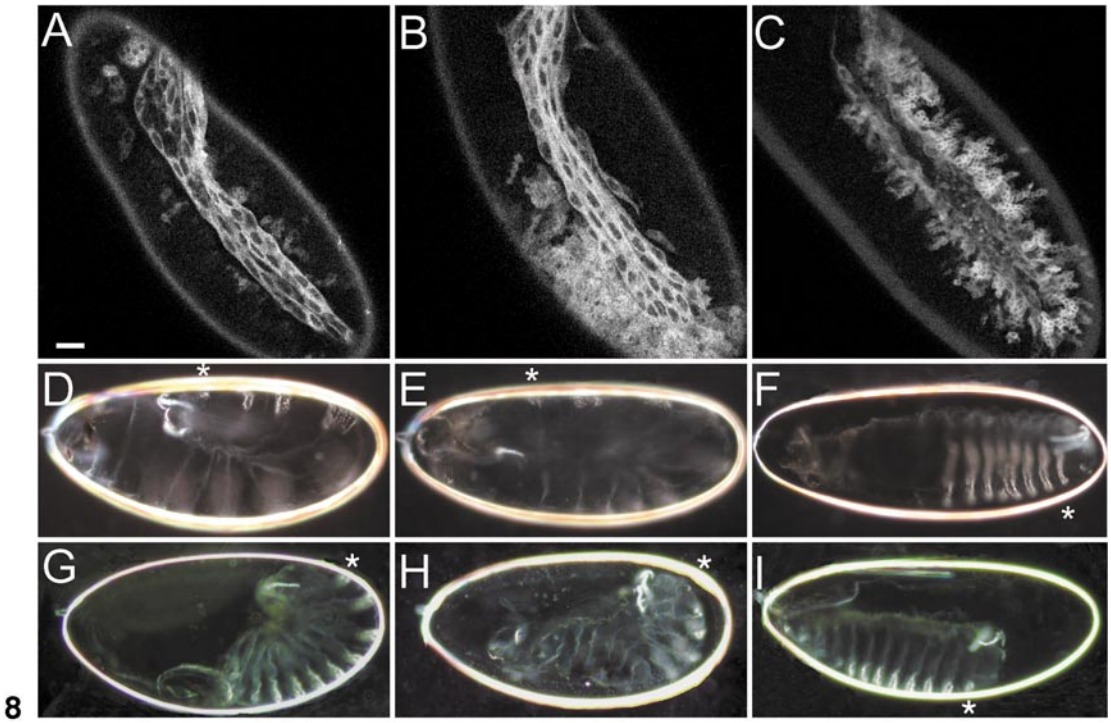


FIG. 7. Lamellipodia migration. (A) Time-lapse recording of a dorsal view of a wild type embryo marked with *c381-Gal4; UASactin5CGFP* and *ubiDE-cadherinGFP* taken at 5-min intervals. UASactin5CGFP is expressed only in the amnioserosa and labels filamentous actin in the cytoplasm (Verkhusha *et al.*, 1999). ubiDE-cadherinGFP is additionally expressed in the germ band cells and outlines them by labeling the adherens junction (Oda and Tsukita, 2001). A yellow arrowhead marks the edge of a lamellipodium, a white dot labels an individual germ band cell. For better visibility of germ band cells, a cluster of five cells is additionally outlined with yellow dotted lines. Within 15 min, the indicated lamellipodium migrates over the cluster of germ band cells marked by yellow dotted lines. (B) Two different z-sections of a retracting wild type embryo illustrating that the lamellipodia adhere to the anal pads. They represent the most caudal structure of the germ band during retraction (Jürgens and Hartenstein, 1993). Triangles mark the anal pads, and the star marks the anus. Bars, 10 μm.

Rather, we find that reciprocal cell shape changes within the amnioserosa and the germ band are associated with the changes in embryo morphology at this stage. That is, the amnioserosa shortens along the DV axis, while the germ band elongates along the DV axis (Fig. 3). This is possible because the amnioserosa and germ band are tightly attached to each other via adherens junctions, i.e., both epithelia move as one coherent sheet (Fig. 2B).

We also investigated the boundary between amnioserosa and germ band at high magnification to obtain an idea of whether the shape changes observed are of an active or a passive nature. We assume that contractile forces would be



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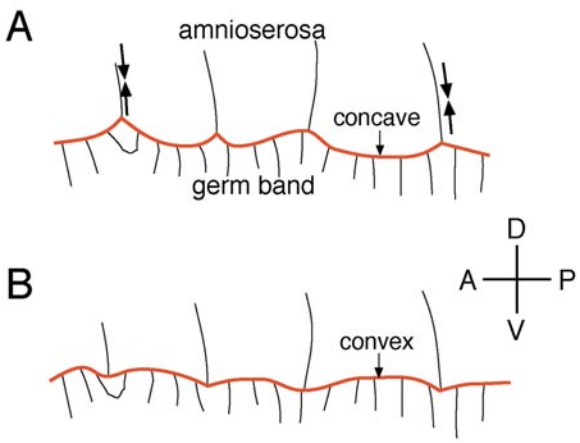

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FIG. 8. Constitutively active and dominant-negative RhoA cause GBR defects when expressed in the amnioserosa. (A–C) The expression of the Gal4 driver lines as detected using *UASmEGFP*. *c381-Gal4* is predominantly expressed in the amnioserosa, and only in small clusters of cells along the leading edge (A); *Kr-Gal4* is expressed in the amnioserosa but also in a central epithelial domain that reflects the earlier expression of *Kr* as a gap gene (B); and *pnr-Gal4* is mainly expressed along the leading edge (C). (D–F) Cuticle phenotypes of embryos that express a constitutively active form of *rhoA* ($rho1^{V12}$) in the amnioserosa (D, E) or along the leading edge (F). (G–I) Cuticle phenotypes associated with the expression of dominant-negative *rhoA* ($rho1^{N17}$) in the amnioserosa (G, H) or along the leading edge (I). Expression of *rhoA* mutants along the leading edge does not affect germ band extension (not shown). Stars indicate the position of abdominal segment eight. Bar, 20 μm.

FIG. 9. Diagrams of the amnioserosa–germ band boundary. (A) Amnioserosa–germ band boundary as observed (see Fig. 3B), suggesting that the amnioserosa exerts a force on the germ band. Double arrows illustrate putative contraction of two amnioserosa cells along their DV axis. (B) Amnioserosa–germ band boundary as envisioned if the germ band actively extends along its DV axis, e.g., by contracting along its AP axis, while the amnioserosa remains passive. The amnioserosa–germ band boundary is indicated in orange. A, anterior; D, dorsal; P, posterior; V, ventral.

exerted along the plasma membranes, because the actin cytoskeleton is localized cortically in both germ band and amnioserosa cells. The row of leading edge germ band cells is pulled in where the amnioserosa membranes perpendicular to the leading edge are attached (Fig. 3B). This suggests that amnioserosa cells contract along their DV axis (Fig.

9A). Cells of the germ band would be expected to push into the larger amnioserosa cells in the case of an active DV extension of the germ band, thus resulting in a convex shape of the amnioserosa–germ band boundary (see Fig. 9B).

We further demonstrate the presence of protrusions, which are formed predominantly at the posterior edge of the amnioserosa projecting toward the posterior (Fig. 5). These protrusions exhibit high levels of dynamic actinGFP at the migration front, indicating actin polymerization (Fig. 6). We classify these protrusions as lamellipodia, because their appearance, behavior, and dynamic actin content are identical to lamellipodia in other motile cells (Geiger *et al.*, 2001). We show that these lamellipodia migrate over the germ band instead of being passively dragged by the retracting germ band (Fig. 7A). The lamellipodia may migrate on an apical extracellular matrix secreted by germ band cells as a precursor to the larval cuticle (Tepass and Hartenstein, 1994). Our observations indicate that the overlap of the amnioserosa over the caudal end of the germ band during GBR is maintained by lamellipodia-mediated migration. Furthermore, both constitutively active and dominant-negative RhoA disrupt GBR, when expressed in the amnioserosa (Fig. 8). This suggests that actomyosin contractility or cell migration within the amnioserosa contribute to GBR, as these processes are affected by expression of *rhoA* mutants in tissue culture (Chrzanowska-Wodnicka and Burridge, 1996; Nobes and Hall, 1999).

It has been shown that the amnioserosa is required for GBR because embryos that lack this tissue fail to undergo GBR (Frank and Rushlow, 1996). It was recently proposed that this requirement for the amnioserosa may involve signaling from the amnioserosa to the germ band (Lamka and Lipshitz, 1999). The cell shape changes and motility observed within the amnioserosa and the overexpression experiments suggest that the amnioserosa additionally contributes to GBR in other ways than signaling.

The processes observed in this study allow several mechanisms, which are not mutually exclusive, to participate in GBR. Segment furrow formation within the germ band may facilitate GBR by causing AP shortening of the germ band. Second, active DV shortening of the amnioserosa may contribute to GBR by pulling in the lateral sides of the anterior germ band, thereby resulting in retraction of the germ band behind the bend of the U-shaped germ band-extended embryo. Third, the pulling force that appears to be exerted by DV shortening of the amnioserosa may be assisted by active DV extension of the germ band cells. Finally, the overlap of the amnioserosa over the germ band may allow proper deployment of forces occurring within the amnioserosa.

In conclusion, we described the cellular processes occurring during GBR. Due to the large size of amnioserosa cells and their location at the surface of the embryo, the amnioserosa appears to be an excellent model system to dissect the molecular and cellular basis of cell shape changes and lamellipodia migration at real-time resolution *in vivo*. Moreover, further genetic analysis of this system, in particular of mutants associated with GBR defects, should allow the identification of molecules that drive the cellular changes occurring during GBR.

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Online Supplemental Material

Movie 1. *arm-Gal4 UASαcateninGFP*-marked wild type embryo, lateral view. A 120-min time-lapse recording was produced by taking a stack of z-sections every 2 min at 512 × 512 pixels resolution using a Leica laser-scanning confocal microscope. Three z-sections were averaged for each frame.

Movie 2. *arm-Gal4 UASαcateninGFP*-marked wild type embryo, lateral view. A 114-min time-lapse recording was produced by taking a stack of z-sections every 2 min at 1024 × 1024 pixels resolution. Three z-sections were averaged for each frame. Individual cells were marked as long as they could be faithfully followed. Note the rapid DV extension of lateral germ band cells close to the amnioserosa.

Movie 3. *Kr-Gal4 UASmEGFP*-marked wild type embryo, dorsal view. A 125-min time-lapse recording was produced by taking a stack of z-sections every 5 min at 1024 × 1024 pixels resolution. Three z-sections were averaged for each frame.

Movie 4. *c381-Gal4; UASactin5CGFP*-marked wild type embryo, dorsal view. A 6-min 40-s time-lapse recording was produced by taking a stack of z-sections every 20 s at 512 × 512 pixels resolution. Two z-sections were averaged for each frame.

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