Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation
Claire Bertel, Lawrence Sulak & Thomas Lecuit

Laboatoire de Génétique et de Physiologie du Développement (LGPD), Institut de Biologie du Développement de Marseille (IBDM), CNRS-INSERM-Université de la Méditerranée, Campus de Luminy, case 907, 13288 Marseille cedex 9, France

Shaping a developing organ or embryo relies on the spatial regulation of cell division and shape. However, morphogenesis also occurs through changes in cell-neighbourhood relationships produced by intercalation\(^2\). Intercalation poses a special problem in epithelia because of the adherens junctions, which maintain the integrity of the tissue. Here we address the mechanism by which an ordered process of cell intercalation directs polarized epithelial morphogenesis during germ-band elongation, the developmental elongation of the Drosophila embryo. Intercalation processes by which junctions are spatially reorganized in the plane of the epithelium following a patterned process of disassembly and reassembly. The planar remodelling of junctions is not driven by external forces at the tissue boundaries but depends on local forces at cell boundaries. Morphosis is specifically enriched in disassembling junctions, and its planar polarization and activity are required for planar junction remodelling and cell intercalation. This simple cellular mechanism provides a general model for polarized morphogenesis in epithelial organs.

Drosophila germ-band elongation (GBE)\(^3\) leads to an almost doubling in length of the epithelial layer that forms the thorax and abdomen of the embryo (Fig. 1a, b). This extension is associated with a mediolateral convergence of cells along the dorsoventral (D/V) axis. Neither cell divisions nor changes in cell shape contribute to anteroposterior (A/P) axis elongation\(^4\); thus, GBE is solely

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Correspondence and requests for materials should be addressed to B.S. (bsyemour@fil.ion.ucl.ac.uk).
associated with epithelial cell intercalation, which requires the modification of adhesion between neighbouring cells. Cell adhesion at epithelial junctions depends on homotypic binding of E-cadherin between adjacent epithelial cells. Epithelial junctions in Drosophila and other organisms consist of a core E-cadherin/β-catenin/α-catenin complex (the adherens junctions)\(^{4,5}\), whose recruitment at the membrane is stabilized by a crosslinking network of actin filaments\(^{7}\).

To understand the cellular mechanisms of intercalation during GBE, we focused on adherens junctions containing a fully functional fusion protein between E-cadherin and green fluorescent protein (GFP) expressed at endogenous levels in early embryos\(^{6}\). The living embryos were observed by time-lapse microscopy. After 30 min, cells intercalate along the A/P axis in the ventrolateral region of the ectoderm (Fig. 1c). Cells do not delaminate, but instead remain integrated in the epithelium. Moreover, counter to a naive expectation, individual cells do not move relative to a population of more static neighbouring cells. Instead, cell intercalation seems to stem from a global rearrangement of cell junctions in a highly organized spatio-temporal pattern.

At the onset of GBE, epithelial cells form a packed hexagonal array with their boundaries either parallel to the D/V axis (referred as type 1), or at ±60° to it. When GBE begins, groups of four cells form characteristic tetrads around type 1 junctions (Fig. 1d, g, red). In this type 1 configuration, adjacent cells along the A/P axis are in contact but immediately dorsal and ventral cells are not in contact with each other. As GBE proceeds, the ±60° junctions hardly change their orientation or average length (4 μm). In contrast, type 1 junctions specifically shrink (average time 10 min), leading to a configuration in which the four cells of the tetrad now share equal contacts (type 2; Fig. 1e, g). Subsequently, new junctions of type 3 are built perpendicular to the old type 1 junction, resulting in effective intercalation of the cells that were dorsal and ventral (Fig. 1f, g; see also movie in Supplementary Information). This polarized pattern of junction remodelling shows that cells can distinguish between its different cell boundaries and control a specific behaviour in each of them (shrinkage or extension). These observations therefore indicate that intercalating cells are inherently polarized within the plane of the epithelium.

Observation of 314 junctions showed that 100% of them irreversibly underwent the transition type 1 to type 2 to type 3, and never the reverse transition (Fig. 1g). This leads to a relative decrease in the number of type 1 junctions and a corresponding increase in the number of type 3 junctions as intercalation proceeds (Fig. 1h). Junction remodelling occurs only in the intercalating region; that is, in the ventral and lateral ectoderm (Fig. 1a, b, grey). A group of cells that complete the transition from type 1 to type 3 elongates about 50% along the A/P axis, and correspondingly shortens along the D/V axis. Note that the germ band elongates along the A/P axis and shortens along the D/V axis to the same extent in the same time window (about 30 min).

In certain mutants that affect A/P patterning in the embryo, the

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**Figure 1** Polarized junction remodelling underlies planar intercalation. All the panels have the same orientation: dorsal (D) at the top, anterior (A) to the left, a, b, Drosophila embryo at the onset (a) of GBE and 30 min later (b). The germ band is posterior to the cephalic furrow (CF) and folds back dorsally (arrow) from its posterior end (arrowhead) after intercalation in the ventral–lateral region (grey). c, An E-cadherin–GFP fusion protein outlines the cell junctions in the boxed regions shown in a and b at corresponding time points. d–g, Cell contacts in the type 1 configuration (red dashed line in d and g) progress towards the type 2 (yellow dashed line in e and g) and to the type 3 configuration (green dashed line in f and g). h, Percentage of type 1, type 2 and type 3 configurations at successive stages of GBE. i, GBE defects in a Kr(RNAi) mutant embryo. j, Distribution of type 1–type 3 configurations in Kr(RNAi) mutants. k, Junction remodelling is reduced and bi-directional in a Kr(RNAi) mutant. Pink arrows point to ‘backward’ transitions; orange arrows point to type 1 junctions that fail to remodel in 20 min. l, Schematic representation of junction remodelling with type 1 junctions (red) and type 3 junctions (green). Scale bar, 100 μm in a, b, i; 5 μm elsewhere.
germ band does not elongate and intercalation is affected. If planar junction remodelling is required for axis elongation, then it should also be disrupted under these mutant conditions. The gap gene Krüppel (Kr) is required to specify distinct cell fates along the A/P axis in the central region of the germ bandP10. Elimination of the activity of Kr by RNA-mediated interference (RNAi) causes an incomplete elongation of the germ band (Fig. 1i), a fully penetrant defect similar to that of Kr mutant embryos. In Kr(RNAi) mutant embryos, cell intercalation is strongly reduced in the central region of the epithelium. Correspondingly, type 1, type 2 and type 3 junction configurations are still observed but no change in the quantitative distribution is observed with time (Fig. 1j). This defect could occur either because cells are 'frozen' in a quasi-immobile state or because junctions are remodelled, but with no specific spatial order. Detailed analysis shows that cell intercalation is strongly reduced (junction remodelling occasionally stalls for as long as 20 min; Fig. 1k). In addition, when junction remodelling occurs, the transitions between type 1, type 2 and type 3 configurations are no longer unidirectional but tend to be more random (Fig. 1k, l). In some cases, complete ‘backward’ transitions are observed (Fig. 1k, pink arrows). This suggests that, in a Kr mutant, GBE is reduced because junction remodelling is strongly reduced and no longer polarized in the plane of the epithelium and that, as a result, intercalation is slower and no longer oriented.

Unidirectional junction remodelling could be driven by an external force at the posterior end of the embryo, for example, associated with posterior-midgut invagination (PMI) (see Supplementary Fig. S1a), thus pulling the tissue along its axis of elongation. Alternatively, junction remodelling could depend on intrinsic forces acting locally at cell boundaries (Supplementary Fig. S1a). In the folded-gastrulation (fog) mutant, PMI and meso-derm invagination (MI) are inhibitedP11, removing possible contributions of morphogenetic processes at the boundaries. In fog(RNAi) mutant embryos, PMI (Supplementary Fig. S1b) and MI (not shown) are blocked as in null mutant embryos (not shown), but we find that intercalation and junction remodelling occur normally (Supplementary Fig. S1c, e). To accommodate the space required for intercalation in the fog mutant, the intercalating tissue can no longer extend dorsally, because this requires PMI. Instead, intercalation causes a folding of the tissue (Supplementary Fig. S1a, c, d). This argues against the possibility that intercalation is driven by a posterior pulling force, indicating instead that it might be controlled by local mechanisms at cell junctions. The data also indicate that intercalation might be so robust that it can overcome the mechanical resistance generated during tissue compression.

The cellular effectors of these mechanisms have to fulfil several requirements. The proteins should be localized at junctions and have a localization polarized in the plane of the epithelium. Moreover, this localization should be under the control of the embryonic A/P patterning system. Finally, the proteins should be functionally required for axis elongation.

Among other constituents of apical junctions, myosin II heavy chain (encoded by the zipper (zip) geneP12), and myosin II regulatory light chain (encoded by the spaghetti squash (sqh) geneP13) co-localize with the β-catenin/E-cadherin complex during GBE. Sqh and Zip form a very stable heterotetrameric complex that associates with F-actin and is subsequently activated by Sqh phosphorylationP14 (Fig. 2a). We find that Zip localizes at apical junctions (Fig. 2d) only in intercalating cells, that is, in the ventrolateral ectoderm, and not in the non-intercalating dorsal ectoderm (Fig. 2b, c). Zip is not detected along the basal–lateral surface (Fig. 2d). Similar observations were made with a fully functional Sqh–GFP fusion protein (not shown). However, unlike β-catenin and E-cadherin, Zip and Sqh–GFP are not uniformly localized at junctions and show a polarized localization in the plane of the epithelium (Fig. 2e–g). Time-lapse analysis revealed thatSqh–GFP is present at all junctions but that it is enriched along shrinking type 1 junctions (Fig. 2g; t = 0, 85% of type 1 junctions, n = 46). Sqh–GFP is still strongly enriched at the vertex of type 2 junctions (Fig. 2g; t = 20 min, 87.5%, n = 16) but is only present at low levels along the expanding type 3 junctions (Fig. 2g; t = 33 min, 90%, n = 32). Thus, Sqh–GFP is enriched in shrinking cell junctions and reduced as they expand. F-actin localization revealed by phalloidin staining shows no planar polarized distribution at junctions (data not shown). Previous studies showed that Slam, a protein that co-localizes with myosin II and is required for membrane invagination during cellularizationP15,16, localizes in a planar polarized fashion at junctions when ectopically expressed during GBE. In this ectopic situation, Slam serves as a cell marker of planar cell intercalation. Like myosin II, Slam’s planar polarized localization reflects a specific enrichment at type 1 junctions (Fig. 2i); ectopic Slam is absent from the new type 3 junctions (Fig. 2j). The planar polarized localization of myosin II is strongly affected in Kr(RNAi) mutants in which junction remodelling is strongly reduced and no longer polarized. Instead of being enriched at type 1 junctions, Sqh–GFP is globally detected at lower levels and present in dispersed speckles at tricellular junctions (Fig. 2h; 9% of enriched Sqh–GFP at type 1 junctions, n = 56, compared with 85% in the wild type, n = 46).

**Figure 2** Myosin II-polarized localization. a, Myosin II regulatory light chain (Myo-II-RLC)/Sqh and myosin II heavy chain (Myo-II-HC)/Zip hetero-tetramer. b–e, Double staining for Zip (green) and Nrt (red) during GBE shown from the surface (b) and in cross-section (c). Zip is present at cell junctions in the ventral (V) lateral intercalating ectoderm (b, c, arrow, d) and cytoplasmic in the non-intercalating dorsal (D) ectoderm (b, c, arrowheads). Zip is specifically enriched in type-1 junctions (e, f). Enrichment of Sqh–GFP at type 1 junctions (arrowheads). g, Time-lapse sequence of Sqh–GFP (times in minutes), showing the enrichment at type 1 junctions (g, t = 0, pink and orange arrowheads), in type 2 junctions (+20 min, pink arrowheads) and the reduced localization at type 3 junctions (+33 min, pink and orange arrowheads). h, In a Kr(RNAi) mutant embryo, Sqh–GFP is present at lower levels with severe planar polarity defects (9% of type 1 junctions enriched for Sqh–GFP instead of 85% in the wild type). i–k, Ectopic Slam localization at junctions (green) also concentrates in type 1 junctions (arrowheads), but is absent from type-3 junctions (arrowheads). Nrt (red) marks the cell outline. k, In a Kr(RNAi)/mutant, ectopic Slam is also localized more uniformly. Scale bar, 5 μm.
This loss of polarity within the plane of the epithelium is also seen with ectopic Slam at junctions (Fig. 2k).

Because the localization of myosin II remarkably mirrors the planar polarized remodelling of junctions in the epithelium, myosin II is a very good candidate for driving this process during GBE. Given the known role of the myosin II motor in other cellular contexts, a myosin-dependent polarized contractile force might direct cell intercalation by forcing E-cadherin contact remodelling through the underlying actin–myosin cytoskeleton. Myosin II is maternally provided in the early embryo. However, removing the maternal contribution is not possible because the oocyte does not develop properly when it is homozygous mutant for zip or sqh.

In contrast, removing the zygotic contribution of zip and sqh has been reported to cause late developmental defects. However, using time-lapse analysis of a large number of embryos mutant for different alleles of zip and sqh, we also found a clear requirement for myosin II during GBE, with GBE defects in up to 20% of embryos from heterozygous zipper or sqh parents (Fig. 3a–c). We then looked at cell intercalation by using Ecad–GFP in a zip2 mutant and found that indeed intercalation is severely disrupted, leading to a quasi-‘frozen’ tissue (Fig. 3d, e). Junction remodelling did not occur, and type 1 junctions could remain unchanged for at least 40 min (Fig. 3f–h). In spite of this, the epithelium remained intact and in particular E-cadherin localization was normal at the junctions. Rho kinase (Rok) activates the Zip/Sqh complex by the phosphorylation of Sqh. We used a specific inhibitor of Rok (Y-27632) to further address the role of myosin II during planar junction remodelling. We injected Y-27632 during cellularization and found that Sqh–GFP recruitment at the junctions was strongly reduced (Fig. 4a, b), although cell junctions were normal (Fig. 4d–h). GBE was severely affected in a dose-dependent fashion (Fig. 4c) and this phenotype was rescued when the injection was performed in a strain heterozygous for a transgene that expresses low levels of a phospho-mimetic sqh mutant (sqhe20E21) immune to Rok inhibition (Fig. 4c), which by itself did not cause any phenotype (not shown). Both cell intercalation (Fig. 4d, e) and planar junction remodelling (Fig. 4f–h) were severely affected after Y-27632 injection, as in the zip mutant embryos (Fig. 3h).

The data presented here identify a particularly simple and novel cellular mechanism for planar intercalation within an epithelium that undergoes elongation (Fig. 4i, j). Planar epithelial intercalation

Figure 3 Myosin II is required for junction remodelling. a, b, GBE defect in an embryo mutant for zip2 in comparison with a wild-type embryo (a). The germ band is shaded in red and its posterior end is marked with a white arrowhead. c, Statistics of GBE defects in various zipper and sqh mutant conditions. All embryos were revealed by time-lapse microscopy and GBE defects are similar to or stronger than the embryo shown in b. A few embryos had some defects at the cortex of the embryos before cellularization (white bars). We scored only embryos with GBE defects that had cellularized normally (grey bars).

d, e, Cells expressing E-cadherin–GFP in a zip2 mutant embryo are marked with a coloured dot and tracked from the onset of GBE (d) until 40 min later (e). Cell intercalation is blocked in this embryo. f, g, Junction remodelling is ‘frozen’ from the onset of GBE (f) until 40 min later (g). h, The percentage of type-1, type-2 and type-3 junctions is unchanged at 40-min intervals.

Figure 4 Role of Rok during intercalation. a, b, Sqh–GFP localization at cell junctions in a control embryo (a) and an embryo injected with the Rok inhibitor Y-27632 (b). c, GBE defects scored after Y-27632 injection in control (wild-type; WT) embryos (left and middle) and embryos expressing Sqhe20E21 (right). Embryos with the most severe GBE defects (less than 35%) are shown in orange, intermediate elongation defects (35–70%) in yellow and near wild-type GBE (more than 70%) in pale yellow. d, e, Cells expressing E-cadherin–GFP in an embryo injected with Y-27632 are marked with a colour dot and tracked from the onset of GBE (d) until 45 min later (e). Note that cell intercalation is blocked. This embryo elongates its germ band less than 35%. f, g, Junction remodelling is also ‘frozen’ from the onset of GBE (f) until 45 min later (g). h, The percentage of type-1, type-2 and type-3 junctions is unchanged at 45-min intervals. i, j, Model: planar cell intercalation depends on the polarized remodelling of junctions along the A/P axis (i). This irreversible process is controlled by the polarized recruitment of myosin II (Myo-II) in a subset of cell junctions (j), thereby forcing polarized junction remodelling.
depends on the polarized remodelling of cell junctions. Junctions are remodelled through the polarized recruitment of myosin II within the plane of the epithelium. The contractile activity of myosin II might create a local tension that orients the disassembly of E-cadherin junctions. Alternatively, myosin II might interact with and control the activity of regulators of E-cadherin stabilization. Myosin II is required to progress from the type 1 to the type 2 configuration, counteracting the effect of junction formation and stabilization present at all cell junctions in epithelial cells before and during GBE. In principle, when cell–cell contacts are in the type 2 configuration, new contacts would form either by reverting to the type 1 configuration or by progressing to the type 3 configuration. However, we propose that the polarized localization of myosin II counteracts attempts to form new type 1 junctions, thus allowing the formation of only type 3 junctions. In other words, the polarized localization of myosin II determines the irreversible transformation from type 1 to type 3. This basic cellular mechanism could be involved in a variety of biological contexts in different organisms. Epithelial tubes, for instance, elongate during organogenesis from Drosophila to vertebrates\textsuperscript{24,27,28}. Nevertheless, the mechanisms that orient the polarized recruitment of myosin might vary. During GBE, local signalling between adjacent cells along the A/P axis might suffice to recruit myosin II along A/P junctions specifically. In other contexts, long-range signalling by the Fruzzled/Dishevelled planar cell polarity pathway might be involved\textsuperscript{19,20}. The work presented focuses on the cellular effectors of such signalling pathways and shows how they might underlie a variety of morphogenetic processes.

Methods

Imaging techniques

Confocal time-lapse images were collected using a spinning-disc confocal head (Perkin Elmer) run by Metamorph software on an inverted microscope (Zeiss). Phase-contrast time-lapse images were collected on an inverted microscope (Zeiss) and a programmable motorized stage to record different positions over time (Mark&Find module from Zeiss). The system was run with Axiovision software (Zeiss) and allowed the acquisition of large time-lapse data sets in mutant or injected embryos.

RNA interference

db RNA probes to Kri\textsuperscript{1}pnel (between positions 494 and 1268 of the coding sequence) and \textit{fog} (860 bases, \textquoteright 24 from 5\textsuperscript{\prime} of the ATG to position 837 3\textsuperscript{\prime} of the ATG) were synthesized \textit{in vitro} and injected in early embryos (less than 1 h old) at 0.5 μg μl\textsuperscript{-1}.

Injections

Y27632 was prepared as a 50 mM stock in water and injected at 600 μM at the onset of cellularization. The dilution factor of Y-27632 in the syncytial embryo was estimated to be 1:62.

Genetics

The following stocks were used. \textit{ubi}-E-cad-GFP homozygous flies (chromosome II) express a functional E-cadherin–GFP fusion\textsuperscript{4}. The functional Sph–GFP fusion was expressed with a genomic rescue transgene in a \textit{sph}\textsuperscript{AX3}-null mutant background\textsuperscript{26}. The following stocks were used to assess the role of myosin II in Fig. 5: \textit{cn bw sp. zip}\textsuperscript{7}Cyo, \textit{c wr zip}\textsuperscript{1}Cyo, \textit{Ecad}\textsuperscript{flk} \textit{sp. zip} \textit{C}yo and \textit{y sph}\textsuperscript{AS}/\textit{F}M7. The ectopic expression of a \textit{UAS-Slam\textsuperscript{II}A} transgene during GBE was monitored in embryos laid by matutub\textsuperscript{Gal4}V16 mothers crossed to \textit{UAS-Slam\textsuperscript{II}A} males. Received 27 February; accepted 23 April 2005.

Structure of a complex between a voltage-gated calcium channel β-subunit and an α-subunit domain

Filip Van Petegem, Kimberly A. Clark, Franck C. Chatelain & Daniel L. Minor Jr

Cardiovascular Research Institute, Departments of Biochemistry and Biophysics, and Cellular and Molecular Pharmacology, University of California San Francisco, 513 Parnassus Avenue, Box 0130, San Francisco, California 94143, USA

Voltage-gated calcium channels (Cav\textsubscript{s}) govern muscle contraction, hormone and neurotransmitter release, neuronal migration, activation of calcium-dependent signalling cascades, and synaptic input integration\textsuperscript{1}. An essential Cav\textsubscript{s} intracellular protein, the β-subunit (Cav\textsubscript{β})\textsuperscript{2,9}, binds a conserved domain (the α-interaction domain, AID) between transmembrane domains I

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