Abstract

Segments are fundamental units in animal development which are made of distinct cell lineages separated by boundaries. Although boundaries show limited plasticity during their formation for sharpening, cell lineages make compartments that become tightly restricted as development goes on. Here, we characterize a unique case of breaking of the segment boundary in late drosophila embryos. During dorsal closure, specific cells from anterior compartments cross the segment boundary and enter the adjacent posterior compartments. This cell mixing behaviour is driven by an anterior-to-posterior reprogramming mechanism involving de novo expression of the homeodomain protein Engrailed. Mixing is accompanied by stereotyped local cell intercalation, converting the segment boundary into a relaxation compartment important for tension-release during morphogenesis. This process of lineage switching and cell remodelling is controlled by JNK signalling. Our results reveal plasticity of segment boundaries during late morphogenesis and a role for JNK-dependent developmental reprogramming in this process.

Introduction

Patterning of tissue progenitors through specific gene expression precedes tissue morphogenesis. Once cells are committed to a particular lineage, they generally keep to it throughout development. Nonetheless, plasticity of segmental lineages is commonly observed during the stages of boundary sharpening, like for example during Drosophila segmentation [1,2,3,4] and rhombomere formation in the vertebrate hindbrain [5,6,7,8,9,10]. In contrast, during later development, reprogramming of patterned cells is mostly associated with pathological conditions (e.g. regeneration) [11] or experimental procedures (e.g. cloning, grafting, or overexpression of selector genes) [12]. Rare cases of fate switching have nonetheless been reported during somitogenesis and hindbrain segmentation in the chick embryo [5,13,14] and during Caenorhabditis elegans embryogenesis [15]. Still, whether patterning can be re-adjusted during late tissue morphogenesis remains elusive.

Dorsal closure in Drosophila embryos is a powerful model of epithelial morphogenesis and wound-healing [16,17,18]. It proceeds through cell stretching and a zipping mechanism that lead to the convergence and suture of the lateral leading edges (LE) at the dorsal midline (see Video S1). This cell movement is believed to be collective and uniform. By looking at dorsal closure in live Drosophila embryos, we reveal a highly stereotyped pattern of cell reprogramming and intercalation, resulting in the remodelling of segment boundaries during late epithelial morphogenesis.

Results/Discussion

Cell Mixing and Intercalation at the Segment Boundaries during Dorsal Closure

Tracking of the dorsal ectoderm cells using confocal live imaging revealed several unexpected cell rearrangements taking place within the leading edge (Figure 1A–D and Video S2). First, we observed that in abdominal segments, one cell from each anterior compartment mixes with the posterior compartment by the end of dorsal closure. We designate these versatile cells the mixer cells (MCs; yellow in Figure 1B–D). These cells have been noticed recently and have been qualified as an aberration in patterning [19]. Second, we show that two cells from the ventral ectoderm intercalate into the leading edge, posterior to each MC (Figure 1C,D). The two intercalating cells, one from the anterior compartment (anterior intercalating, AI; green in Figure 1B–D) and the other from the posterior compartment (posterior intercalating, PI; red in Figure 1B–D), thus establish new segment boundaries dorsally (Figure 1D and Video S3). This striking
Author Summary

Multicellular organisms are assembled from different cell types, each following a particular fate depending on their history and location. During development, cells are organized into compartments, which are essential for the correct formation of organs. Within the compartments, cells follow two general rules: (i) cells that have acquired a given fate cannot change their differentiation state and (ii) cells from one compartment stay together and never mix with cells from other compartments. In this work, we identified a group of unique cells in Drosophila melanogaster embryos called mixer cells which move from one compartment of the epidermis to another, breaking the compartment boundary rule. Our data show that this unique behaviour depends on the nuclear reprogramming of the mixer cells, which change their fate and acquire the identity of the destination compartment de novo. We show that the shift in identity and compartment mixing are due to the expression of a single gene (Engrailed), under the control of JNK, a signalling pathway that is conserved across species. Interestingly, this process of reprogramming and mixing provides a mechanism for tension relaxation to the tissue during morphogenesis that allows dorsal closure of the Drosophila embryo (an event that resembles wound healing). This work reveals a novel model of cell plasticity that is amenable to genetic study, with potential application in the field of regenerative medicine.

pattern of remodelling is spatially and temporally regulated along the leading edge, with a degree of fluctuation, from embryo-to-embryo, in the timing and number of intercalating cells (Figure 1E).

To investigate the mixing mechanism, we analysed the origin and identity of the MCs during dorsal closure. Originally, the MCs occupy the dorsal-anterior corner of each anterior compartment (Figure 1C,D). They are clearly identifiable as part of a single row of cells, known as the groove cells, which form a morphological furrow that marks each segment border, perpendicularly to the leading edge [20,21]. Like other groove cells, the MCs express higher levels of the actin anti-capping protein Enabled (Ena) (Figure 2A; Figure S1A). The anterior nature of the MCs was confirmed by looking at endogenous Patched (Ptc) expression, which is indeed present throughout the process of cell mixing (Figure 2A; Figure S1B). Thus, both its initial position as well as the expression of Ena, Ptc, and of compartment specific drivers (ptc-gal4 positive and en-lacZ negative; see Figure S1C) show that the MC is the dorsal-most anterior groove cell.

De Novo Expression of Engrailed in the MC Induces Its Shifting to the Posterrior Compartement

The MC behaviour challenges the compartment boundary rule stating that cells from different compartments cannot mix due to different cell affinities that sort them out [2,3,4,10,22,23]. One possible explanation for the violation of this law is that the MCs may be re-programmed to acquire posterior identity. Strikingly, the analysis of endogenous protein levels revealed that the MCs, to promote anterior-to-posterior reprogramming through de novo expression of En, compartment mixing, and segment adjustment.

In order to address the effect of excess JNK activity in the process, we ectopically expressed either a wild type (Hep) or an activated form of DJNKK (Hep+) in the anterior compartment using the ptc-gal4 driver. These gain-of-function conditions induced a dramatic increase in the number of intercalated cells and the formation of ectopic MCs at the segment boundaries (Figure 4B,G; Figures S3C, S5, and Video S5). These ectopic MCs shows that the MCs express both markers (Figure 2A; Figure S1B), Ptc first then both, which supports the idea that the MCs were originally anterior cells that subsequently acquired posterior identity. Consistent with previous work showing that ectopic expression of En in anterior cells is sufficient to determine posterior identity in the posterior compartment [25,26], these results suggest that the MCs undergo anterior-to-posterior reprogramming through de novo expression of the En posterior determinant, thus favouring their mixing into the posterior compartment.

To demonstrate a direct role of En in MC formation, we inhibited its function in the anterior compartment by inducing en RNAi using the ptc-gal4 driver. These embryos showed a decrease of En expression in the MC (Figure 3A). In addition, they exhibited a significant number of segments (40%) with aberrant cell mixing, i.e. with partial or no mixing at all (Figure 3B). These results indicate that de novo expression of En in the MCs is essential for their reprogramming and mixing behaviour.

JNK Signalling Controls En Expression and Cell Mixing

The differentiation of the dorsal leading edge, to which MCs belong, is under the control of the conserved JNK pathway. Embryos lacking the activity of the JNK/Basket (BskDN) was expressed either in the anterior or in the posterior compartment using the ptc-gal4 or en-gal4 driver, respectively. The extinction of JNK activity was assessed by the loss of puc-lacZ expression (Figure 4A). Embryos expressing BskDN in the posterior compartment (en>bskDN) showed no phenotype (Figure 4A). In contrast, expression of BskDN in the anterior compartment (ptc>bskDN) led to the complete absence of MC intercalation, as is observed in fJNKK mutants embryos (Figure 4A,C; Figure S4 and Video S4). The same result was obtained when blocking JNK signalling through overexpression of the JNK phosphatase Puckered (Puc) (Figure 4C; Figure S3B). Absence of JNK activity in ptc>bskDN or ptc>puc embryos (but not with the en-gal4 driver) also led to the abolition of En expression in the MCs (Figure 4A; Figure S3B). Interestingly, although most (87%) ptc>bskDN embryos were able to complete dorsal closure, 92% of them showed a high degree of segment mismatching at the dorsal midline (53% of A1–A6 segments showed defects; Figure 4D). This suggests that MC formation and intercalation play a role in segment adjustment at the time of suture, consistent with a previous hypothesis [19]. In contrast, matching was normal in en>bskDN embryos. Together, these results indicate that JNK signalling is essential in the anterior compartment, most likely in the MCs, to promote anterior-to-posterior reprogramming through de novo expression of En, compartment mixing, and segment adjustment.

JNK Controls Segment Boundary Remodelling

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In order to address the effect of excess JNK activity in the process, we ectopically expressed either a wild type (Hep) or an activated form of DJNKK (Hep+) in the anterior compartment using the ptc-gal4 driver. These gain-of-function conditions induced a dramatic increase in the number of intercalated cells and the formation of ectopic MCs at the segment boundaries (Figure 4B,G; Figures S3C, S5, and Video S5). These ectopic MCs
express Ptc, Ena, and En like normal MCs. These results show that more lateral groove cells are competent for reprogramming, but they are restricted by the field of JNK activity in the leading edge.

Wingless Inhibits Groove and MC Formation

Each MC has a mirror-image counterpart at the LE parasegment (PS) boundary (MC* in Figure 5A) that never develops into a MC. Interestingly, the asymmetry of the MC pattern correlates with Wingless (Wg) activity across the segment [28] and the presence of the groove at the segment boundary (Figure 5A) [20,21]. In addition, in JNK gain-of-function embryos, extra MCs only appear along the segment boundary (Figure 4B), suggesting that only groove cells can differentiate into MCs. To test this hypothesis, we made use of specific wg mutant embryos in which an ectopic groove is formed at the PS boundary [20]. In this context, MCs* were transformed into ectopic MCs at the PS boundary (Figure 5B). Like genuine MCs, transformed MCs* express Ptc, Ena, and most importantly En, which suggests that Wg suppresses the MC pathway at the PS boundary. To test whether Wg itself can repress MC formation, Wg was expressed ectopically in the MCs (ptc>wg) where it is not normally active [28]. This blocks MC reprogramming and cell remodelling (Figure 5C; Video S6). Consistently, En expression is no longer detected in MCs. These results indicate that Wg has a non-permissive function at the PS boundary through the blocking of groove cell differentiation, thus restricting the MC pathway to the segment boundary (Figure 5D). Therefore, only dorsal groove cells are competent for MC formation (Figure 5D).

Local Tissue Tension Modifies the Dynamics of Cell Intercalation

Dorsal closure is characterised by dramatic cell elongation (3-fold in the DV axis) accompanied by the formation of a LE supracellular actin cable and amnioserosa contraction, all of which contribute to tissue tension (see Video S1) [29–33]. To test the effect of tension on the intercalation process, we applied laser ablation to live embryos expressing βCatenin-GFP. The tension in tested segments was assessed in three conditions (control, amnioserosa ablation, and cable ablation) by measuring the ectoderm recoil after a single cell ablation at the leading edge (Figure 6B). Increase in LE tension was induced by ablation of the pulling amnioserosa, while its release was induced through a double ablation of the actin cable on each side of a test segment (Figure 6A). We next compared the dynamics of LE insertion in the controls and in embryos mechanically challenged by laser. In control embryos, the PI cell (red in Figure 6C) takes, on average,
14 min to complete insertion in the leading edge. This time increases dramatically when tension is reduced in the cable (cable ablation condition; 60 min, Figure 6C middle panel, 6D), while it is shortened (4 min) in conditions of higher tension generated by amnioserosa ablation (Figure 6C bottom panel, 5D). These data show that the dynamics of intercalation depends on local tissue tension and suggest a role of intercalation in tension modulation. Improper tension release along the leading edge, in the absence of cell intercalation, could therefore explain the reduced ability of segments to match with their counterparts, as observed in JNK mutant conditions (Figure 4D). To perturb tension genetically, we analyzed the pattern of intercalation in *zipper* (*zip*, encoding MyoII) mutants, in which a reduced tissue tension has been reported [34]. Interestingly, these embryos show a reduced level of cell intercalation, supporting our model of a link between tissue tension and the rate of intercalation (Figure 4C).

Based on these results, we propose that the MC pathway provides an adaptive response to tissue tension by allowing an increase of cell number in the leading edge. Indeed, one major consequence of boundary remodelling is the addition of intercalating cells (AI and PI), which increases the cellular number of the leading edge by approximately 10% (Figure 1E). The adaptive nature of cell intercalation is reflected by the flexibility in the number (from 0 to 3) of intercalating cells (Figure 1E), which contrasts with the robustness of MC reprogramming assessed by de novo expression of En. In our model, MC formation would weaken the segment boundary (i.e., through a change in cell affinity), making it a preferred site competent for tension-dependent intercalation. Our data and work published by Peralta et al. [35] indicate that the width remains constant on average with only slight oscillations during dorsal closure. Therefore, for constant width the increase in the number of cells implies that each cell is less stretched, thus inducing tension relaxation in individual cells. MC formation and associated local cell intercalation thus provide each segment with a tuneable relaxation compartment, important for tension release during morphogenesis (Figure 6E).

In this study we unravel the mechanism of a unique case of breaching of the segment boundary during late morphogenesis, i.e. post-patterning and post-boundary sharpening. This process is shown to be highly stereotyped and developmentally regulated.
through JNK signalling. Our data indicate that it takes place through a two-step mechanism, involving first MC formation, which is then followed by cell intercalation. Indeed, de novo expression of En in the dorsal groove cell always precedes intercalation (Figure 2B). Furthermore, we can observe MC formation and mixing without intercalation like in the thoracic segments (Figure 1E), but intercalation was never observed in the absence of MC formation: for example, when MC reprogramming is blocked in JNK loss-of-function conditions, no intercalation occurs (Figure 4A). Cell mixing thus takes place through a novel morphogenetic mechanism involving plasticity of the segment boundary and compartment relaxation via patterned intercalation. It would be interesting to see if plasticity of boundaries can be a general mechanism for fine tuning late morphogenesis. Intriguingly, late expression of En in anterior cells has been reported at the anterior-posterior boundary in the wing imaginal disc. But contrary to the MC process, the so-called “S. Blair cells” do not mix with the posterior En-expressing cells [36], and their function remains elusive. It would be interesting to reinvestigate their late behaviour using time lapse approaches [37].

Interestingly, the JNK pathway has been shown to be involved in transdetermination of injured imaginal discs [38], reminiscent of

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**Figure 4. JNK signalling controls En expression in mixer cells.** (A, B) Pattern of cell mixing and intercalation in JNK loss and gain of function embryos. A summary of the terminal phenotypes is shown on the right panel. Ena, white; Ptc, green; DAPI, turquoise; β-Galactosidase (puc-lacZ), purple; En, red. Still images from live embryos show terminal phenotypes using the colour code as in Figure 1D: mixer (M) cell, yellow; anterior and posterior intercalating cells, green and red, respectively; unidentified intercalating cells, gray. (A) Cell mixing, cell intercalation, and En expression in the mixer cell (yellow dotted circle) are blocked in hep mutants and when JNK signalling is selectively down-regulated in the anterior compartment (ptc->bskDN). Quantification of En expression in hep mutants is shown in Figure 2B. Mixing, intercalation, and En expression are normal when JNK signalling is down-regulated in the posterior compartment (en->bskDN). (B) Up-regulation of the JNK pathway in the anterior compartment (ptc->hep or ptc->hep^αCatGFP) induces ectopic mixer cells expressing En in the groove. (C) Total number of intercalating cells per leading edge from wild type (n=6), ptc->hep (n=8), ptc->bskDN (n=6), ptc->puc (n=10), and zipper mutant (n=5) backgrounds. Data are means ± s.d. (*p = 0.0015, **p = 0.0026, ***p < 0.001; ****p = 0.06). (D) Segment mismatching in ptc->αCatGFP, bskDN embryos. Percentages of defects are given for segments A1 to A6 (n=144 segments of 24 embryos). Scale bars: 5 μm.

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the MC reprogramming described here. Hence, JNK signalling represents a fundamental morphogenetic and cell reprogramming pathway essential for developmental and regenerative sealing.

Materials and Methods

Genetics

The following fly lines were used: βcatenin-GFP (8556), UAS-h-actin-CFP (7064), UAS-myr-RFP (7119), UAS-Dxcatenin-GFP, UAS-hep\textsuperscript{ tet } (9306), UAS-bsh\textsuperscript{DN} (6409), zip\textsuperscript{I} (4199), UAS-lamGFP (7377) (all from the Bloomington stock center), UAS-en-RNAi (VDRC #35697), ptc-gal4 (gift from N. Perrimon), en-gal4 (gift from A. Brand; see Figure S6 for en-gal4 expression pattern in MCs), puc\textsuperscript{LacZ} [39], UAS-puc\textsubscript{2a} [40], UAS-wg [41], hep\textsuperscript{I}, hep\textsuperscript{II} and UAS-hep4E\textsuperscript{E} [27], wg\textsuperscript{cx4}, en-gal4, and wg\textsuperscript{cx4}, arm\textsuperscript{sch} [20].

Figure 5. Wg inhibits groove and Mixer cell formation at the parasegment boundaries. (A) Leading edge expression of En and Wg in stage 13 embryos; mixer cell (M), yellow dotted circles; mixer mirror cell (M*), white dotted circles; En, red; Wg, turquoise; β-galactosidase (puc-lacZ), purple. (B) Specific loss of Wg signalling induces ectopic mixer cell formation at the parasegment boundary as seen by expression of En (white, top) and En (red, top and bottom); Ptc (green, bottom); DAPI is turquoise. (C) Overexpression of Wg in the mixer cell (ptc\textgreater wg, top panel) inhibits anterior-to-posterior reprogramming as seen by the absence of En in the mixer cell, leading to the absence of the mixing (see Video S6). Overexpression of Wg in the posterior compartment (en\textgreater wg, bottom panel) has no effect on reprogramming and mixing. The histogram shows the total number of intercalating cells for control (n = 8), ptc\textgreater wg (n = 8), and en\textgreater wg (n = 6) embryos. Data are means ± s.d. (*p = 0.06, **p = 0.0012, ***p = 0.0005). β-galactosidase (puc-lacZ), purple; Ptc, green; DAPI, turquoise; En, red. (A–C) (right panels) Scheme of the phenotype and expression patterns of Wg and En; PS is for parasegment boundary and S for segment boundary. (D) Model of JNK induced reprogramming at the segment boundary and Wg inhibition at the parasegment boundary. Scale bars: 5 \textmu m.

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Figure 6. Local tissue tension modifies the dynamics of cell intercalation. (A) Still confocal images showing cable recoil following laser ablation (from stage 14 live embryos expressing βCatenin-GFP, in gray). Single cell ablations are done in the mixer cell of a central segment (red arrowheads). The upper panel shows ablation in a control embryo cut once. The middle panel shows the recoil following a double ablation targeting adjacent segments (yellow sparkles in scheme on the left side). The bottom panel shows the recoil following the ablation of half of the amnioserosa (see scheme on the left side). (B) Indirect measurements of local tension at the segment boundary (recoil speeds) in control embryos, embryos with cable ablation, and embryos with amnioserosa ablation (n = 6, 3, and 6, respectively). Data are means ± s.d. (*p = 0.167, **p = 0.048). (C) Still confocal images showing the timing of insertion in the leading edge of a control embryo (upper panel), and embryos with continuous cable ablation (middle panel) or with amnioserosa ablation (bottom panel). Mixer cell, yellow; posterior intercalating cell, red. (D) Timing of the final phase of intercalation
(leading edge insertion) in all three conditions (control, n = 22; cable ablation, n = 2; amnioserosa ablation, n = 11). Data are means ± s.d. (*p = 0.032, **p = 0.005). (E) Scheme showing the relaxation (blue arrow) at the segment boundary as a result of cell rearrangements (left panel) and the pattern and variability of relaxation from segment to segment (blue circles of variable sizes, right panel). Scale bars: 10 μm in (A) and (C).

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UAS-lacZ; (6) w*/UAS-bap2DK57; βcatenin-GFP, ptc-gal4; (7) w*; βcatenin-GFP, ptc-gal4; UAS-puc2x; (8) w*; βcatenin-GFP, en-gal4/UAS-lacZ; (9) w*; βcatenin-GFP, en-gal4; UAS-ug (10) w*; βcatenin-GFP, ptc-gal4/UAS-ug and (11) w*/UAS-bap2DK57, βcatenin-GFP, ptc-gal4; UAS-puc-lacZ. Removal of late wg function was obtained using wg 

Antibodies, Immunostaining, Imaging

Embryos were dechorionated in 1.6% bleach, fixed for 15 min in heptane and 4% paraformaldehyde diluted in PBS (50:50 mix), devitellinised in heptane and methanol (or chilled 70% ethanol when presence of GFP) (50:50 mix) for 2 min using a vortex (or incubated at −20 °C for 7 min before vortexing when GFP), rinsed 3 times in methanol, then 3 times in ethanol, rehydrated sequentially in ethanol/PBS 0.1% triton solutions (70/30, 50, 30/70, 0/100) for 5 min each time, then blocked in PBS 0.1% triton 1% BSA for a minimum of 2 h at room temperature before applying primary antibodies for overnight incubation at 4 °C. Primary antibodies were washed 6×10 min with the blocking solution at room temperature before adding secondary antibodies for a minimum of 2 h at room temperature. Finally, embryos were treated with DAPI (10 μg/ml, Biochemika) for 5 min at room temperature. 6×10 min washing in PBS 0.1% triton preceded mounting in Mowiol® 4-88 Reagent (Calbiochem). Antibodies used: mouse anti-Ena 5G2 (1/500), mouse anti-Ptc apa I (1/50), rabbit anti-En (1/200; Santa Cruz), chicken anti-β-Galactosidase (1/1000; Genetex), anti-mouse H488 (1/400; Molecular Probe), rabbit anti-En (1/200; Santa Cruz), chicken anti-β-Galactosidase (1/1000; Genetex), anti-mouse H488 (1/400; Molecular Probe), anti-rabbit cy3 (1/100), and anti-chicken cy3 (1/400) both from Jackson.

Images were taken with a Zeiss LSM 510 Meta confocal microscope using ×40 1.3 NA or ×63 oil immersion objectives.

Live Imaging, Laser Ablation, and Image Treatment

Embryos were dechorionated in bleach, then staged and placed dorsal side down on a coverslip. Embryos were then coated with halocarbon oil and covered with a hermetic chamber containing a piece of damp paper for hydration. This mounting system ensures normal development of 95% of embryos. Videos last from 2 to 5 h with stacks of 25 images (thickness from 30 to 40 μm) taken every 5 min.

Image and video assembly was done using ImageJ. Stacks are projected using either a maximal intensity or an average projection. Cell intercalations were analysed by tracking manually each cell with ImageJ. Graphs were made using Microsoft Excel. Video S3 was made using Microsoft PowerPoint and Alcoosoft PPT2Video video converter.

Ablations were performed using a two-photon pulsed Spectraphys’s Tsunami laser combined with a Zeiss LSM 510 Meta confocal microscope for imaging. The power was calibrated in each experiment using a test embryo and ablations were performed with the ZEiss “bleach” macro to control the size and timing of each cut. For MC ablation, the actin cable on the dorsal side was targeted, while for amnioserosa ablation, the laser beam was focused on the apical area to destroy adherens junctions and the cytoskeleton in a region of interest of 10–30 micrometers long, parallel to the AP axis. To determine cable tension, we used the classical definition of tension in a pulse string as the magnitude of the pulling force exerted by the string [29]. The application of Newton’s second law under the conditions of low Reynolds number (viscous fluid) shows that the initial recoil speed of a cable after the cut is proportional to the contribution of the suppressed force, i.e. tension [32,42].

Protein Level Quantification in MCs

ImageJ was used to quantify En and β-Galactosidase levels on projections of non-saturated stacks of images. For a given segment, the absolute intensity of En in the MCs was normalised to the average absolute intensities of the bona fide En-expressing cells of the leading edge. An average of these relative intensities was calculated for stages of intercalation as shown in Figure 2. For each embryo, only segments A2, A3, and A4 were considered as they are most representative of mixing and intercalation. Relative intensity in the MCs is the ratio of absolute MC intensity/average of absolute intensities in bona fide En cells.

Statistical Analysis

All analyses were performed using the Mann-Whitney non-parametric test, which does not assume any condition on the distribution and is adapted to independent experiments and small sample sizes. p values were computed using the statistics toolbox from the Matlab software.

Supporting Information

Figure S1 Expression of cellular markers in the mixer cells. (A, B) Pattern of Ena (A), Ptc (B), puc-lacZ and En expression at three different stages of cell mixing: before anterior-to-posterior mixer cell mixing (top panels); after the onset of mixer cell mixing and cell intercalation (middle panels); end of cell mixing and intercalation (bottom panels). En, white; Ptc, green; DAPI, turquoise; β-Galactosidase, purple; En, red. (C) Pattern of endogenous En expression in an embryo expressing the β-Galactosidase under the control of the enhancer (en–lacZ) and αCateninGFP under the control of the ptc-gal4 driver (ptc>Gal4). Note the expression of En in the most anterior ptc-gal4 expressing cell (i.e. the mixer cell). αCateninGFP, green; β-Galactosidase, turquoise; En, red. Yellow lines in (A–C) outline the segment boundary. The right panel is a scheme of the intercalation stages (mixer cell, yellow; PI, red; AI, green; posterior, orange; anterior, light green). Scale bars: 2 μm. Found at: doi:10.1371/journal.pbio.1000390.s001 (3.08 MB TIF)

Figure S2 Time-course of Engrailed expression in the mixer cells. Examples of En stainings used for the quantification of the relative amounts of En in the mixer cells compared to neighbouring bona fide En cells. The bottom panel shows a scheme of the intercalation stages (mixer cell, yellow; PI, red; AI, green; posterior, orange; anterior, light green). En, white; DAPI, turquoise; β-Galactosidase, purple; En, red. Yellow lines outline the segment boundary. Scale bars: 100 μm in 1st row; others 5 μm. Found at: doi:10.1371/journal.pbio.1000390.s002 (1.78 MB TIF)

Figure S3 JNK activity controls mixer cell formation and En expression. (A) Expression of endogenous Ptc and En proteins in a hepr75/ hepr mutant embryo. Note that in these mutant embryos the mixer cell expresses Ptc but not En in contrast to wild type embryos. Ptc, green; DAPI, turquoise; En, red. Scale bars:
Figure 1B. Scale bars: 10 μm.

Figure S4 JNK down-regulation in the anterior compartment inhibits cell intercalation. Confocal still images from Video S4 showing an embryo expressing BskΔN in the anterior compartment. Genotype: ptc-gal4, UAS-bskΔN, UAS-RFP; βcatenin-GFP, βCatenin-GFP, white; RFP, green. Colour code as in Figure 1B. Scale bars: 10 μm.

Found at: doi:10.1371/journal.pbio.1000390.s003 (1.34 MB TIF)

Video S3 Dynamics of cell mixing and intercalations at the segment boundaries. Summary cartoon showing the spatial and temporal dynamics of mixer cell shifting and cell intercalations at the segment boundaries.

Found at: doi:10.1371/journal.pbio.1000390.s010 (2.73 MB MOV)

Video S4 Absence of intercalation in an embryo defective for JNK signalling in the anterior compartment. Confocal time-lapse imaging of an embryo expressing a dominant negative form of Bsk in the anterior compartment. Genotype: ptc-gal4, UAS-bskΔN; βcatenin-GFP, βCatenin-GFP, green. Colour code as in Figure 4A. Scale bars: 10 μm.

Found at: doi:10.1371/journal.pbio.1000390.s011 (0.82 MB MOV)

Video S5 Excessive intercalations in an embryo overexpressing JNKK in the anterior compartment. Confocal time-lapse imaging of an embryo overexpressing the JNKK Hep in the anterior compartment from Video S5. Genotype: ptc-gal4, UAS-hepact; puc-lacZ, white; DAPI, purple; En, red. Genotype: ptc-gal4, UAS-bskΔN; βcatenin-GFP, βCatenin-GFP, green. Colour code as in Figure 4A. Scale bars: 10 μm.

Found at: doi:10.1371/journal.pbio.1000390.s012 (0.56 MB MOV)

Video S6 Absence of intercalation in an embryo overexpressing Wg in the anterior compartment. Confocal time-lapse imaging of an embryo overexpressing Wg in the anterior compartment. Putative non anterior cells of segments A2 to A6 are coloured in green to show that no intercalation occurs in the entire bracketed region. Genotype: ptc-gal4, UAS-wg; βcatenin-GFP, βCatenin-GFP, white. Scale bars: 10 μm.

Found at: doi:10.1371/journal.pbio.1000390.s013 (1.00 MB MOV)

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MG and FS have contributed equally to this work and their name is listed in alphabetical order. We wish to thank C. Alexandre, Y. Bellaiche, R. Delanoue, A. Gallet, T. Lecuit, P. Léopold, and members of the SN laboratory for sharing materials and for critical reading of the manuscript.

Author Contributions

The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: MG FS SN. Performed the experiments: MG FS RR. Analyzed the data: MG FS LA SN. Contributed reagents/materials/analysis tools: RR PB LA. Wrote the paper: SN.

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