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Compartment boundaries

Sorting cells with tension

Daiki Umetsu and Christian Dahmann*
Max Planck Institute of Molecular Cell Biology and Genetics; Dresden, Germany

The subdivision of proliferating tissues into groups of non-intermingling sets of cells, termed compartments, is a common process of animal development. Signaling between adjacent compartments induces the local expression of morphogens that pattern the surrounding tissue. Sharp and straight boundaries between compartments stabilize the source of such morphogens during tissue growth and, thus, are of crucial importance for pattern formation. Signaling pathways required to maintain compartment boundaries have been identified, yet the physical mechanisms that maintain compartment boundaries remained elusive. Recent data now show that a local increase in actomyosin-based mechanical tension on cell bonds is vital for maintaining compartment boundaries in Drosophila.

Key words: Drosophila, wing imaginal disc, compartment boundary, cell sorting, mechanical tension

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*Correspondence to: Christian Dahmann; Email: dahmann@mpi-cbg.de

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Compartments were first identified in the wings and abdomen of insects by clonal analysis.[1,2] When single cells were genetically marked during early development, the descendant cells ('clone') grew up in the adult structure to a boundary line (the compartment boundary), and frequently ran along it, but never extended to the other side. These experiments revealed that, in Drosophila, the developing wing is subdivided during embryogenesis into anterior (A) and posterior (P) compartments (**Fig. 1A**) and later, during larval development, into dorsal (D) and ventral (V) compartments. Compartments were subsequently identified in different parts of the fly, including the leg, haltere, head and abdomen.[3-7] More recently, lineage tracing also revealed compartments in vertebrate embryos,[8-16] indicating that the formation of compartments is a common strategy during both insect and vertebrate development.

Meinhardt's theoretical work on pattern formation proposed that boundaries between compartments act as reference lines for positional information during tissue development, and that they serve as sources of morphogen synthesis.[17,18] Indeed, many compartment boundaries, both in insects and vertebrates, have by now been shown experimentally to be associated with signaling centers that produce morphogens (reviewed in refs. 19 and 20). The defined position and shape of signaling centers is important for the establishment of precise morphogen gradients and patterning.[21,22] In growing tissues, however, the position and shape of signaling centers is challenged by cell rearrangements that take place during cell division.[23,24] By inducing signaling centers along stable and straight compartment boundaries, precise morphogen gradients can be maintained in proliferating tissues.[25] Compartment boundaries therefore play vital roles during the patterning of proliferating tissues.

How are straight and sharp compartment boundaries maintained despite cell re-arrangements caused by cell division? The maintenance of compartment boundaries often requires local signaling between cells from the two adjacent compartments. In the developing hindbrain, for example, signaling by Eph receptors and ephrins is required to maintain the boundaries between adjacent rhombomeres.[26,27] In the developing wing of the fly, signaling downstream of

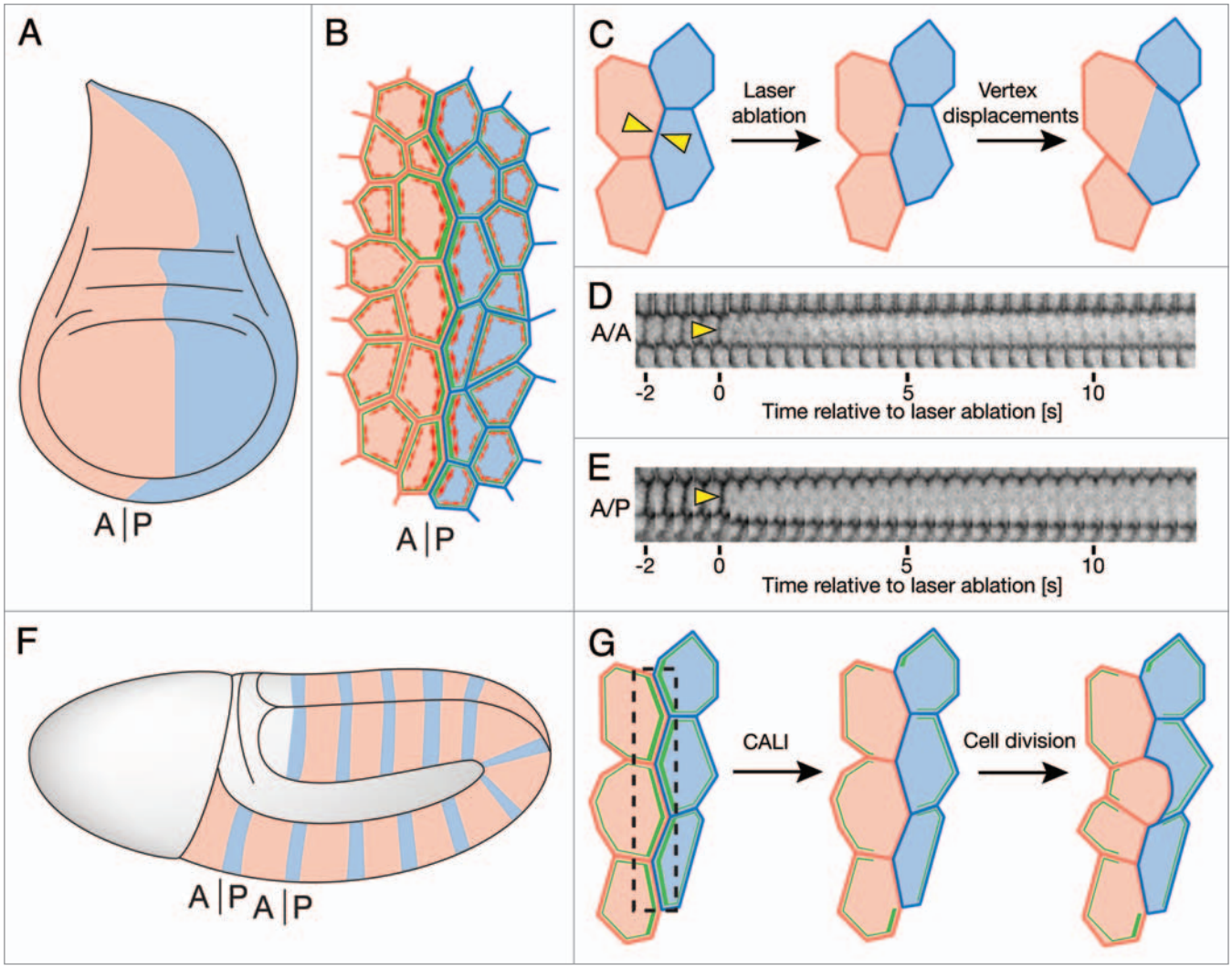

Figure 1. Increased cell bond tension at compartment boundaries in Drosophila. (A) The Drosophila wing imaginal disc is subdivided into anterior (A) and posterior (P) compartments. (B) Myosin II and F-actin (green lines) are enriched at the cell bonds between anterior cells and posterior cells compared to cell bonds elsewhere in the tissue. Mechanical tension (arrows) on cell bonds along the A/P boundary is increased. (C) Measurement of cell bond tension by laser ablation. Arrowheads depict the site of ablation. The two vertices at the ends of the ablated cell bond are displaced. (D and E) Sequential images of an E-cadherin-GFP-labelled cell bond within the anterior compartment (D) or at the A/P boundary (E) before and after laser ablation in wing imaginal discs. (F) Each parasegment of the Drosophila embryo is subdivided into anterior and posterior compartments. (G) Chromophore-assisted laser inactivation (CALI) to locally reduce Myosin II (green lines) in cells along the parasegment boundary (boxed area). As a consequence, dividing cells at the parasegment boundary intermingle.

Hedgehog and Dpp is required to maintain the A/P boundary,[28-31] and Notch signaling is required to maintain the D/V boundary.[32,33] The physical mechanisms maintaining compartment boundaries, however, remained elusive for a long time. Two recent papers, by Landsberg et al. and Monier et al. now provide evidence that actomyosin-dependent tension on cell bonds is an important mechanism to maintain straight and sharp compartment boundaries.[34,35]

A longstanding hypothesis posed that the sorting of cells at compartment boundaries is due to differences in the affinities between cells of adjacent compartments.[36] Earlier theoretical work by Malcom Steinberg had indeed proposed that differences in the adhesiveness of cells lead to cell sorting.[37] Steinberg's hypothesis was based on the important insight that cell sorting closely resembles the separation of immiscible liquids and that quantitative differences in cell properties suffice to explain cell sorting. Cadherins are a class of Ca^{2+}-dependent cell adhesion molecules that can confer differential cell adhesion in vitro and in vivo.[38-40] Circumstantial evidence indicates that cadherins may play a role in maintaining compartment boundaries. In the telencephalon of mouse embryos, for example, the interface between cells expressing R-cadherin and cells expressing cadherin-6 coincide with the cortico-striatal compartment boundary.[11] Interestingly, cortical cells ectopically expressing cadherin-6 sort into the striatal compartment, and the reverse is observed for striatal cells engineered to express R-cadherin. In addition to cadherins, further cell adhesion proteins have been

implicated in maintaining compartment boundaries. In the Drosophila wing imaginal disc, an epithelium that gives rise to the adult wing, the two leucine-rich-repeat domain proteins Capricious and Tartan are expressed specifically in cells of the dorsal compartment.[41] Strikingly, forced expression of either of these proteins in the dorsal compartment can restore a normal straight and sharp D/V boundary in mutants for *apterous*, the selector gene required to establish this boundary.[41]

More recent hypotheses to explain the sorting of cells in animal development are based on differential surface contraction[42] or differential interfacial tension.[43] These hypotheses do not treat cells as liquid molecules, as Steinberg's differential adhesion hypothesis does, but emphasize that cells can generate mechanical tension that allows them to contract the surface to neighboring cells. Minimizing cell surfaces at interfaces between different cell populations could contribute to cell sorting.

Mechanical tension in cells can be generated by tensile elements located at the cellular cortex underlying the plasma membrane, including contractile actomyosin filaments (reviewed in ref. 44). Irvine and colleagues made the important observation that, in Drosophila wing imaginal discs, Filamentous (F)-actin and the motor protein non-muscle Myosin II (Myosin II) were enriched at adherens junctions along the D/V boundary,[45,46] indicating a distinct mechanical property of bonds between cells along this compartment boundary. Moreover, these authors found that in mutants for *zipper*, which encodes myosin heavy chain, the D/V boundary was irregular,[46] showing a requirement for Myosin II in maintaining this boundary.

Landsberg et al. show that F-actin and Myosin II were also enriched on cell bonds along the A/P boundary in Drosophila wing imaginal discs, and that also the A/P boundary was irregular in *zipper* mutants.[34] Moreover, they now provide direct evidence that mechanical tension at cell bonds along the A/P boundary is increased (**Fig. 1B**). Differences in mechanical tension on cell bonds have been proposed to result in differences in the shape of cells and the angles between bonds of cells.[24,47] Landsberg et al. demonstrate that the two rows of cells along the A/P boundary display a unique shape and that angles between cell bonds along the A/P boundary are widened, providing evidence that mechanical tension is elevated along these cell bonds.[34] Distinct shapes have also been previously reported for cells along compartment boundaries in Oncopeltus,[48] indicating that they are commonly associated with compartment boundaries.

Ablation of cell bonds generates displacements of the corners (vertices) of the ablated bonds, providing direct evidence for tension on cell bonds.[49] Landsberg et al. ablated individual cell bonds in wing imaginal discs using an UV laser beam, and quantified the displacements of the two vertices of the ablated cell bonds (**Fig. 1C–E**). The relative initial velocities with which these vertices are separated in response to laser ablation is a relative measure of cell bond tension.[50] Ablation of cell bonds within the anterior compartment and the posterior compartment resulted in similar initial velocities.[34] However, when cell bonds along the A/P boundary were ablated, the initial velocity of vertex separation was approximately 2.5-fold higher.[34] Displacements of cell vertices after laser ablation were strongly reduced in the presence of Y-27632, a drug that specifically inhibits Rho-kinase,[51] which is a major activator of Myosin II.[52] These results suggest that actomyosin-based cell bond tension along the A/P boundary is increased 2.5-fold compared to the tension on cell bonds located elsewhere.

Is a local increase in cell bond tension sufficient to maintain straight interfaces between proliferating groups of cells? To test this, Landsberg et al. simulated the growth of a tissue based on a vertex model.[24] In this model, the network of adherens junctions in a tissue is described by polygons characterized by the position of vertices. Stable configurations of this network are local minima of an energy function that describes the area elasticity of cells, cell bond tension, and the elasticity of cell perimeters. In these simulations, two adjacent cell populations, anterior and posterior compartments, separated by a straight and sharp interface, are introduced into this network. Tissue growth is simulated by randomly selecting a cell, increasing its area two-fold, and dividing the cell at a random angle. The energy in the whole network is then minimized and the procedure is repeated. Simulation of tissue growth renders the initially straight and sharp interface between the two compartments rough and irregular.[34] However, by increasing locally cell bond tension at the interface between the two simulated compartments, the interface remains straight.[34] These computer simulations provide evidence that a local increase in cell bond tension is sufficient to maintain straight boundaries between compartments in proliferating tissues.

Monier et al. analyzed boundaries in the Drosophila embryo.[35] The embryonic epidermis is subdivided into parasegments, and cells from adjacent parasegments do not intermingle[53] (**Fig. 1F**). Similar to the D/V and A/P boundaries of larval wing imaginal discs, the authors found that the parasegment boundaries also display elevated levels of F-actin and Myosin II.[35] Injection of the Rho-kinase inhibitor Y-27632 into embryos, or expression of a dominant-negative form of *zipper*, resulted in cell sorting defects at the parasegment boundaries. Live imaging of embryos furthermore showed that mitotic cells locally deform the parasegment boundaries, but that the boundaries straighten out at the onset of cytokinesis. When Myosin II activity was locally reduced by chromophore-assisted laser inactivation (CALI), the parasegment boundaries failed to straighten out after cells had divided, and anterior and posterior cells partially intermingled[35] (**Fig. 1G**). These results demonstrate an important role for Myosin II in separating anterior and posterior cells at parasegment boundaries.

Cell sorting is a general phenomenon of developing animals not restricted to compartment boundaries. A well-studied example is the sorting out of cells from the different germ layers during gastrulation. Interestingly, during zebrafish gastrulation, differential actomyosin-dependent cell-cortex tension has recently been implicated in the sorting out of cells from different germ layers.[54] A differential mechanical tension might, therefore, be a general mechanism to prevent the mixing of cells in developing animals.

Does differential cell adhesion play a role in regulating mechanical tension? At least two contributions can be envisioned. First, cell bond tension depends on both contractile forces along cell bonds as well as the strength of adhesion between neighboring cells.[24,43] Elevating contractile forces can increase cell bond tension, whereas increasing adhesive contacts between cells can release tension. Differences in the adhesion between neighboring cells along compartment boundaries, compared to the remaining cells within the compartments, could therefore contribute to the maintenance of compartment boundaries. Second, differential expression of some cell adhesion molecules results in a local increase of F-actin and Myosin II. For example, interfaces between cells expressing the cell adhesion molecule Echinoid and cells lacking Echinoid display elevated levels of F-actin and Myosin II in Drosophila wing imaginal discs.[55] Therefore, it seems plausible that, at least in some cases, the increase of F-actin and Myosin II at compartment boundaries could be the consequence of the differential expression of adhesion molecules. In this model, differential cell adhesion would play an indirect role in maintaining compartment boundaries by resulting in local enrichment of F-actin and Myosin II, which in turn could lead to an elevated mechanical tension.

The local enrichment of F-actin and Myosin II at distinct sites within cells, and a presumed modulation of tensile stresses, is not restricted to compartment boundaries, but appears to be common to diverse developmental processes. In gastrulating Drosophila embryos, for example, tissue elongation is driven by cell intercalation that depends on the enrichment of Myosin II on shrinking cell bonds.[56,57] Similarly, during mesoderm invagination of Drosophila embryos, F-actin and Myosin II accumulate in a central web-like structure at the apical side of cells resulting in apical cell constriction.[58] Recruitment of F-actin and Myosin II to this medial web can be induced by expression of an activated form of Wasp, a known regulator of actin polymerization, providing a mechanism for the local enrichment of actomyosin within cells.[59] In addition to biochemical mechanisms, mechanical signals have also been shown to help localize Myosin II to specific sites within cells. During germband elongation in the Drosophila embryo, for example, cell bonds that are under high tension have elevated levels of Myosin II, and the experimental application of mechanical force is sufficient to recruit Myosin II to the cell cortex.[60] Increased tension at cell bonds along compartment boundaries might, therefore, be also a consequence of both biochemical and mechanical mechanisms. It will be interesting to investigate the nature of these mechanisms, and how they are linked to the developmental signals that control the formation of compartment boundaries.

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