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EXTRA VIEW

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Regulating mechanical tension at compartment boundaries in *Drosophila*

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ABSTRACT

During animal development, cells with similar function and fate often stay together and sort out from cells with different fates. In *Drosophila* wing imaginal discs, cells of anterior and posterior fates are separated by a straight compartment boundary. Separation of anterior and posterior cells requires the homeodomain-containing protein Engrailed, which is expressed in posterior cells. Engrailed induces the expression of the short-range signaling molecule Hedgehog in posterior cells and confines Hedgehog signal transduction to anterior cells. Transduction of the Hedgehog signal in anterior cells is required for the separation of anterior and posterior cells. Previous work showed that this separation of cells involves a local increase in mechanical tension at cell junctions along the compartment boundary. However, how mechanical tension was locally increased along the compartment boundary remained unknown. A recent paper now shows that the difference in Hedgehog signal transduction between anterior and posterior cells is necessary and sufficient to increase mechanical tension. The local increase in mechanical tension biases junctional rearrangements during cell intercalations to maintain the straight shape of the compartment boundary. These data highlight how developmental signals can generate patterns of mechanical tension important for tissue organization.

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The organization of cells into complex patterns and morphologies during animal development often involves the interplay between cell fate decisions and the spatiotemporal generation of cellular forces. One system in which to study this interplay is the formation of compartment boundaries.[1-3] Compartment boundaries are lineage restrictions that maintain separate groups of cells differing in their fate and gene expression (termed compartments). They were initially discovered by clonal analysis in the wings and abdomen of insects.[4,5] In the developing *Drosophila* wing (wing imaginal disc), a compartment boundary separating anterior and posterior cells (AP boundary) arises during embryogenesis,[4] whereas a second compartment boundary separating dorsal from ventral cells (DV boundary) appears later during larval development.[6,7] The subsequent discovery of compartment boundaries in vertebrate embryos revealed that the formation of compartment boundaries is a developmental strategy common to both insects and vertebrates.[1]

Local signaling across compartment boundaries often induces signaling centers (organizers) along the compartment boundary that play a pivotal role in growth and patterning of the tissue.[8] The separation of cells from neighboring compartments contributes to the stable and straight shape of the organizer and thus its ability to direct precise patterning. These boundaries therefore play an important role as reference lines for growth and patterning within tissues.[9,10]

In *Drosophila*, the *engrailed* gene was early on identified as being important for separating anterior from posterior cells.[11] Adult wings mutant for *engrailed* no longer show a defined lineage restriction between anterior and posterior cells. Moreover, mutant clones for *engrailed* do not respect the lineage restriction and can transgress from the posterior to the anterior region of the developing wing. Engrailed encodes a homeodomain-containing transcription factor whose expression is confined to cells of posterior compartments.[12,13] It was

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proposed that Engrailed defines a 'posterior affinity' that will make the posterior cells separate out from anterior cells (see[14]). However, this 'selector-affinity model' was challenged by experiments indicating that Engrailed may only indirectly contribute to boundary formation by inducing the expression of Hedgehog ('signaling-affinity model').[14,15]

Hedgehog is a short-range signaling molecule that is expressed in cells of the posterior compartment under control of Engrailed (reviewed in[16]). Hedgehog protein secreted by posterior cells moves to a strip approximately 10 cells-wide of anterior cells along the AP boundary, where it sets in motion a signal transduction cascade that ultimately leads to the activation of the transcription factor Cubitus interruptus (Ci). *ci* transcription is repressed in posterior cells by Engrailed. As a consequence, even though the posterior cells express Hedgehog, they cannot activate Hedgehog target genes. Activation of Hedgehog signal transduction and target gene expression is thus confined to the strip of anterior cells. Transduction of the Hedgehog signal in these anterior cells is important for the separation of anterior and posterior cells. Clones of cells mutant for *smoothened*, an essential transducer of the Hedgehog signal, no longer obey the compartment boundary, but instead, when located close to the AP boundary, transgress from anterior to posterior territory.[14,15] These data indicate that Engrailed controls compartment boundary formation and maintenance indirectly by inducing the expression of Hedgehog. Later experiments, however, showed that Engrailed also has a Hedgehog-independent function in separating cells along the AP boundary leading to the proposal that Engrailed acts via 2 pathways to influence boundary formation: A Hedgehog-dependent pathway that leads to the activation of Ci in anterior cells close to the AP boundary and, a poorly understood, Hedgehog-independent pathway that cell autonomously in posterior cells is required for the establishment of the compartment boundary.[17]

How do the Hedgehog-dependent and independent pathways of Engrailed control cell separation? A long-standing hypothesis poses that differences in the adhesiveness of cells underlies cell sorting.[18,19] It has therefore initially been proposed that Hedgehog signal transduction and Engrailed control the expression of one or more cell adhesion molecules.[17] However, seminal findings by Major and Irvine indicated that not differences in the expression of cell adhesion molecules, but rather differences in the organization of the cells' cytoskeleton may be responsible for setting up compartment boundaries.[20,21] These authors observed that filamentous (F-) actin and non-muscle Myosin II (Myosin II) levels were enriched along the DV boundary in wing imaginal discs.[20,21] Similar increases were later seen at the AP boundary of wing imaginal discs and at compartment boundaries in the embryonic epidermis.[22,23] The enrichment of actomyosin along compartment boundaries resembles supracellular actomyosin cables and it has been proposed that such cables act as fences preventing cells from either side of the compartment boundary to intermingle.[20,21,23,24]

Actomyosin associated with adherens junctions can generate mechanical tension along cell junctions (referred to as cell bond tension). Experiments ablating adherens junctions by laser light and quantifying the resulting tissue relaxation demonstrated that cell bond tension is similar in the anterior and posterior compartments. Mechanical tension, however, is locally increased at cell bonds along the AP boundary.[22] Similar findings were later made for the DV boundary and for compartment boundaries in the abdomen of the fly.[25,26] Reducing Myosin II activity, either throughout the tissue or locally in cells along the compartment boundary disturbs the straight shape of compartment boundaries in wing imaginal discs and the embryonic epidermis.[20-23] Taken together, these results are consistent with a model in which a local increase in mechanical tension at cell bonds along a compartment boundary is important for separating cells from neighboring compartments.

Does this local increase in cell bond tension depend on the Hedgehog-dependent or Hedgehog-independent function of Engrailed, or both? A recent paper addressed this question by combining measurements of cell bond tension with several genetic conditions in which Hedgehog signal transduction was altered.[27] In one scenario, Hedgehog signal transduction was approximately equalized between anterior and posterior cells, either by reducing Hedgehog activity or by activating Hedgehog signal transduction in posterior cells. Importantly, Engrailed expression was unaffected and still confined to posterior cells in these experiments. In this scenario, a local increase in cell bond tension along the AP boundary relative to the bulk of the tissue was no longer observed. These data allow 2 conclusions: First, a difference in Hedgehog

signal transduction between anterior and posterior cells is required for increased cell bond tension along the AP boundary. Second, the difference in Engrailed expression between anterior and posterior cells is not sufficient to increase cell bond tension in the absence of a difference in Hedgehog signal transduction between these 2 cell populations.

In a second scenario, Hedgehog signal transduction was increased in posterior cells, but reduced in anterior cells, thus reversing the normal situation. Strikingly, cell bond tension was again locally increased along the AP boundary.[27] This experiment revealed that the difference in Hedgehog signal transduction is not only necessary, but also sufficient to increase cell bond tension along the AP boundary. These findings further stress that Hedgehog signal transductions per se does not influence cell bond tension. Rather, it is the difference in Hedgehog signal transduction between anterior and posterior cells that elicits a local increase in cell bond tension along the AP boundary. Moreover, since in the absence of Hedgehog differences in Engrailed expression between anterior and posterior cells do not lead to a local increase in cell bond tension, these results furthermore indicate that the Hedgehog-dependent pathway, but not the Hedgehog-independent pathway of Engrailed results in the local increase in cell bond tension along the AP boundary. Since both pathways are required for the straight shape of the AP boundary, this suggests that both cell bond tension-dependent and -independent mechanisms contribute to the shaping of this compartment boundary. Thus, the Hedgehog-dependent and Hedgehog-independent pathways of Engrailed appear to impinge on different boundary-forming mechanisms. Using two independent mechanisms might increase the fidelity and robustness of boundary formation and maintenance. It may also explain the scarcity of molecules that have been identified to date in genetic screens aimed at revealing novel molecules important for AP boundary formation.[28,29]

How does a difference in Hedgehog signal transduction between anterior and posterior cells induce a local increase in cell bond tension along the AP boundary? The observation that actomyosin is elevated along compartment boundaries in a supracellular cable-like structure indicated that the local increase in cell bond tension is a collective property of cell bonds along the AP boundary. Indeed, experiments in the *Drosophila* embryo showed that mechanical tension is higher on cell bonds that are aligned and form part of an actomyosin cable compared to 'isolated' cell bonds.[30] However, inducing Hedgehog signal transduction in a single cell surrounded by non-Hedgehog transducing cells is sufficient to increase cell bond tension to the same level as compared to the normal AP boundary.[27] These data indicate that a difference in Hedgehog signal transduction can locally increase cell bond tension cell bond by cell bond, not requiring the collective activity of neighboring cell bonds. Furthermore, experiments in which the integrity of the actomyosin cable was compromised by laser ablation showed that the integrity of the actomyosin cable is not required to increase cell bond tension.[27] These findings do not support the idea that cell bonds along the AP boundary are part of a 'conventional' elastic actomyosin cable. Rather, these data argue in favor of a model in which the difference in Hedgehog signal transduction between 2 cell populations causes a local increase in cell bond tension, cell bond by cell bond, at the interface.

How could such a local increase in cell bond tension contribute to the straight shape of compartment boundaries? Previous work has shown that cell intercalations can lead to cell mixing and can thus be detrimental to maintaining the compartmental organization of epithelia.[26] Cell intercalations involve the sequential shrinkage and extension of cell junctions, thereby leading to the exchange of cell neighbors. A local increase in cell bond tension biases these junctional rearrangements in a way that the straight shape of the AP boundary is maintained and the mixing of anterior and posterior cells is prevented.[26] Live imaging of cell intercalations in the vicinity of the AP boundary in wing imaginal discs now showed that this bias in junctional rearrangements requires a difference in Hedgehog signal transduction.[27] These data are consistent with the view that the local increase in cell bond tension at the AP boundary contributes to the straight shape of the compartment boundary by biasing junctional rearrangements during cell intercalations.

The following model of how the AP boundary is established and maintained emerges (Fig. 1): The activity of the selector gene *engrailed* in posterior cells of wing imaginal discs has 2 functions in shaping the AP boundary: One function is independent of Hedgehog. The mechanism by which this function shapes the AP boundary is currently unknown. It apparently does not involve a local modulation of cell

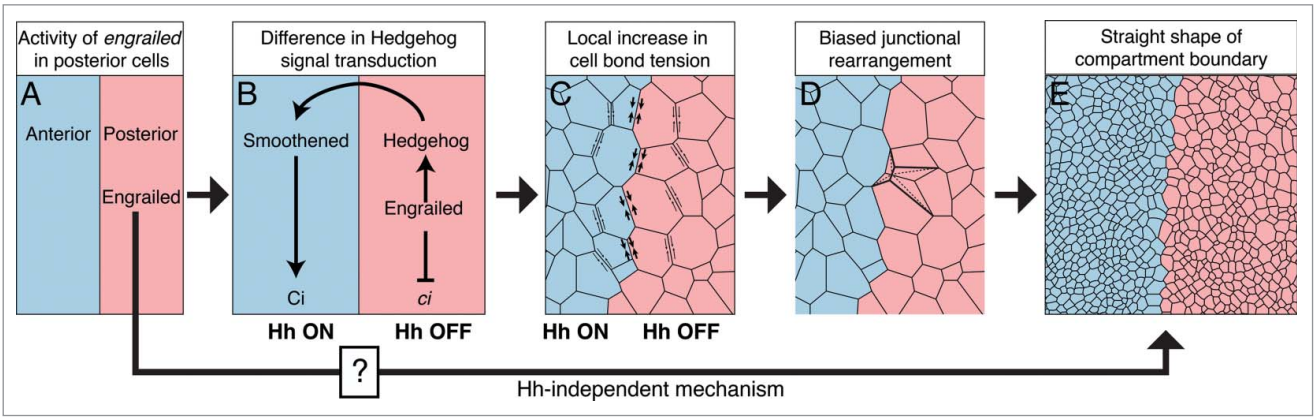

Figure 1. Mechanisms by which Engrailed and Hedgehog contribute to the shaping of the AP boundary. (A) The selector gene *engrailed* is expressed in all cells of the posterior compartment and specifies posterior cell identity. (B) Engrailed induces the expression of the short-range signaling molecule Hedgehog (Hh). Hedgehog protein spreads to cells of the anterior compartment. Transduction of the Hedgehog signal requires the transmembrane protein Smoothened and leads to the activation of the transcription factor Ci, which induces the expression of Hedgehog target genes. Engrailed represses *ci* transcription in posterior cells. Thus, Engrailed results in a difference in Hedgehog signal transduction between anterior (ON) and posterior (OFF) cells. (C) The difference in Hedgehog signal transduction between anterior and posterior cells results in a local increase in mechanical tension (arrows) at cell bonds along the AP boundary. (D) The local increase in cell bond tension biases junctional rearrangements during cell intercalations along the AP boundary. (E) The bias in junctional rearrangements during cell intercalations contributes to the characteristic straight shape of the AP boundary. Engrailed, via a Hedgehog independent pathway, further contributes to the straight shape of the compartment boundary by an unknown mechanism.

bond tension. The second function of Engrailed is to generate a difference in Hedgehog signal transduction activity between anterior (ON) and posterior (OFF) cells. This difference in Hedgehog signal transduction between anterior and posterior cells results in a local increase in cell bond tension along the AP boundary that in turn biases junctional rearrangements during cell intercalations to maintain the characteristic straight shape of the compartment boundary. The large-scale shape of the AP boundary is thus in part the result of a difference in Hedgehog signal transduction between anterior and posterior cells that induces a pattern of mechanical tension that in turn influences junctional rearrangements.

A key question to be addressed in the future is how the difference in Hedgehog signal transduction leads to a local increase in mechanical tension confined to cell junctions along the AP boundary? Activation of the transcription factor Cubitus interruptus (Ci) by Hedgehog signal transduction is required for the separation of anterior and posterior cells.[17] Thus, it is likely that the difference in Hedgehog signal transduction increases cell bond tension along the AP boundary by inducing the expression of one or more target genes in anterior cells. Since Ci is activated in a strip of approximately 10-cells width along the AP boundary, but mechanical tension is only increased at cell bonds along the AP boundary, this target gene(s) need to act indirectly. One possibility is that this target gene might encode a membrane-bound receptor (or ligand) that by interacting with its ligand (or receptor) in posterior cells induces an increase in myosin II-dependent mechanical tension at that cell bond. Eph receptor tyrosine kinases and their membrane-bound ligands appear to be prime candidates in this context. Eph/ephrin signaling is known to influence actomyosin organization, Eph receptors and ephrins are expressed in complementary patterns in compartments in vertebrate embryos and are required to maintain their straight shape (reviewed in[1]). The single *Drosophila* Eph receptor was recently shown to be required for the separation of anterior and posterior cells in wing imaginal discs, however, the *eph* gene is expressed uniformly throughout the wing imaginal discs.[28] It will be interesting to reveal whether the *Drosophila* Eph receptor, and its ephrin ligand, mediate Hedgehog's role in increasing cell bond tension along the AP boundary.

A further question that needs to be addressed in the future regards the mechanism by which the Hedgehog-independent pathway of Engrailed contributes to the shaping of the AP boundary. Apparently, this pathway does not involve the local modulation of mechanical tension along the compartment boundary.[27] Prior work

based on theoretical models indicate that also global anisotropic tissue stresses can contribute to the straight shape of compartment boundaries.[25] This raises the possibility that the Hedgehog-independent pathway of Engrailed contributes to the shaping of the AP boundary by globally influencing mechanical tissue stress.

Furthermore, it will be interesting to test whether signaling pathways commonly regulate cell bond tension at compartment boundaries. Maintaining the characteristic straight shape of the DV boundary in wing imaginal discs, for example, also involves a local increase in cell bond tension and signaling by Notch.[25,31,32] It might be worthwhile to investigate the role of Notch signaling in modulating cell bond tension at the DV boundary.

The local modulation of cellular force generation in response to biochemical signals is a hallmark of epithelial organization during animal development. Further elucidating the mechanisms of compartment boundary formation promises to shed light on the general principles underlying cellular organization within epithelia.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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