Apical and Basal Matrix Remodeling Control Epithelial Morphogenesis

Highlights

- Apical and basal extracellular matrices are degraded to elongate Drosophila limbs
- Apical matrix is degraded by the Stubble protease and basal matrix by MMPs
- Limbs elongate via convergent extension and cell flattening, driven by Myosin-II
- In the haltere, Ultrabithorax prevents matrix remodeling and tissue elongation

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In Brief

Diaz-de-la-Loza et al. show that morphogenetic elongation of Drosophila limbs occurs via both convergent extension and columnar-to-cuboidal cell shape change. These processes are spatially organized by Myosin-II and temporally organized by remodeling of the extracellular matrix, including both apical (ZP-domain-containing) and basal (Collagen IV/Laminin/Perlecan-containing) matrices.
Apical and Basal Matrix Remodeling Control Epithelial Morphogenesis

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SUMMARY

Epithelial tissues can elongate in two dimensions by polarized cell intercalation, oriented cell division, or cell shape change, owing to local or global actomyosin contractile forces acting in the plane of the tissue. In addition, epithelia can undergo morphogenetic change in three dimensions. We show that elongation of the wings and legs of Drosophila involves a columnar-to-cuboidal cell shape change that reduces cell height and expands cell width. Remodeling of the apical extracellular matrix by the Stubb protease and basal matrix by MMP1/2 proteases induces wing and leg elongation. Matrix remodeling does not occur in the haltere, a limb that fails to elongate. Limb elongation is made anisotropic by planar polarized Myosin-II, which drives convergent extension along the proximal-distal axis. Subsequently, Myosin-II relocates to lateral membranes to accelerate columnar-to-cuboidal transition and isotropic tissue expansion. Thus, matrix remodeling induces dynamic changes in actomyosin contractility to drive epithelial morphogenesis in three dimensions.

INTRODUCTION

The generation of form in living organisms is one of the most fascinating unsolved problems in biology (Dreher et al., 2016; Pasakarnis et al., 2016). Genetic analysis of epithelial tissue morphogenesis in model organisms has revealed that epithelia can elongate by either polarized cell intercalation (Pare et al., 2014; Blankenship et al., 2006; Bertet et al., 2004; Zallen and Wieschaus, 2004; Heisenberg et al., 2000; Irvine and Wieschaus, 1994; Keller, 1980) or oriented cell division (Campinho et al., 2013; Gibson et al., 2011; Mao et al., 2011; da Silva and Vincent, 2007; Baena-Lopez et al., 2005; Gong et al., 2004; Wei and Mikawa, 2000; Concha and Adams, 1998). These two general mechanisms for elongation of epithelial sheets are also observed during elongation of epithelial tubules in Drosophila and vertebrates (Saxena et al., 2014; Lienkamp et al., 2012; Saburi et al., 2008; Voiculescu et al., 2007). Both epithelial cell intercalation or oriented cell division can be driven either by local forces arising from planar polarized Myosins or by global forces acting across entire tissues (Collinet et al., 2015; Etournay et al., 2015; Lye et al., 2015; Ray et al., 2015; Legoff et al., 2013; Mao et al., 2013; Lye and Sanson, 2011; Vichas and Zallen, 2011; Lecuit and Le Goff, 2007).

A third general mechanism of epithelial morphogenesis is cell shape change. Recent research has been focused mainly on forces acting to shape the apical domain in two dimensions (Dreher et al., 2016; Pasakarnis et al., 2016; Paluch and Heisenberg, 2009). However, epithelial cells can also undergo three-dimensional shape changes to drive morphogenesis. One example is the columnar-to-cuboidal shape change that reduces apical-basal cell height and expands the apical surface to drive expansion and elongation of the Drosophila wing and leg (Fristrom and Fristrom, 1975; Poodry and Schneiderman, 1970). This mechanism was found to be intrinsic to the tissue itself, rather than driven by external forces, as it can occur ex vivo (Fristrom, 1988; Fristrom and Fristrom, 1975). Later work identified similar cell shape flattening events occurring during embryonic development of the fishes Fundulus heteroclitus and Danio rerio, the frog Xenopus laevis, and the sea anemone Nematostella vectensis, indicating that this morphogenetic mechanism is widespread in the animal kingdom (Fritz et al., 2013; Behrndt et al., 2012; Fristrom, 1988; Keller and Trinkaus, 1987; Keller, 1980). How columnar-to-cuboidal shape change might be developmentally controlled remains poorly understood.

One possible control mechanism has been observed in the Drosophila wing and leg, where an overlying layer of cells known as the peripodial (“around the foot”) layer is removed and discarded prior to the onset of columnar-to-cuboidal shape change and tissue elongation (Fristrom, 1988; Milner et al., 1984). The removal of the peripodial layer was found to be driven by Myosin-II contractility in the peripodial cells (Aldaz et al., 2013), yet whether removal of this layer is strictly causative for the subsequent wing expansion and elongation remains unclear. Here we show that remodeling of the extracellular matrix (ECM), rather
RESULTS

Matrix Remodeling Controls Columnar-to-Cuboidal Cell Shape Change, which Drives Tissue Expansion and Elongation

We began by applying modern live-imaging methods (Aldaz et al., 2010) to reproduce the seminal work of early investigators who characterized the morphogenesis of the Drosophila wing and leg epithelia by transmission electron microscopy (Friestrom and Fristrom, 1975; Mandaron, 1970, 1971; Poody and Schneiderman, 1970). Imaging of GFP-tagged E-cadherin (E-cad-GFP) confirms their key finding that morphogenetic expansion and elongation of the wing occurs by columnar-to-cuboidal cell shape change, a process that flattens the wing as it increases in both length and width (Figures 1A–1C). The key events take place between 4 and 7 hr after puparium formation (APF), prior to cuticle secretion, when the tall pseudo-stratified columnar epithelial cells become dramatically shorter along their apico-basal axis, such that initially densely packed nuclei become neatly aligned side by side and the apical area of each cell expands (Figures 1B and 1C).

To identify the mechanism controlling columnar-to-cuboidal cell shape change in the elongating Drosophila wing, we considered the possible role of the ECM. Previous work has implicated the basal Collagen IV-based ECM in the process of maintaining columnar cell shape in the wing (Pastor-Pareja and Xu, 2011). Since the wing epithelium also produces an apical ECM composed of the ZP-domain protein Dumpy, which has an important role in attachment of the epithelium to the exoskeleton at later stages of development (Ray et al., 2015), we examined the distribution of both types of matrix during the process of wing expansion and elongation. We have analyzed the localization of fluorescent-tagged versions of ECM components: apical Dumpy (Dumpy-YFP, Dp-YFP); and the main components of the basal matrix, Collagen IV (Collagen IV z2-subunit, Vkg-GFP, bECM), which distribute homogeneously in the developing wing from 2 to 4 hr (h) after puparium formation (APF), but are degraded from 5 hr APF, concomitant with peripodial membrane release. The entire wing disc is shown for Dp-YFP, and only the basal surface for Vkg-GFP. Scale bar, 50 μm. (B) Cross-sections of developing wings at 4 and 7 hr APF. At 4 hr APF, Dp-YFP and Vkg-GFP cover the apical and the basal surface of the wing epithelia, respectively and Vkg-GFP also surrounds the basal side of the peripodial membrane. At 7 hr APF the matrix has been removed and only some traces of Vkg-GFP are still detected. Nuclei (DNA) are shown in blue. Scale bar, 50 μm. (C) High-magnification view of epithelial cells showing that, at 4 hr APF, columnar epithelial cells distributed in a pseudo-stratified epithelium are in contact with apical Dp-YFP and basal Vkg-GFP (orange arrowhead), whereas by 7 hr APF the matrix is absent and cells adopt a more cuboidal morphology. The layer of Vkg-GFP that covers the basal surface of the peripodial membrane at 4 hr APF is indicated by a white arrowhead. Scale bar, 25 μm. (D) Quantification of the percentage of epithelium covered with apical Dp-YFP and basal Vkg-GFP in developing wings from 2 to 7 hr APF. Average and SD are presented; n > 4 for each developmental stage. Statistically significant differences are indicated (*p < 0.05, ***p < 0.001). (E) Quantification of wing blade area and epithelial cell height from 4 to 7 hr APF. Average, SD, and individual data points are presented; n > 8 for each developmental stage. Statistically significant differences are indicated (*p < 0.05, **p < 0.005, ***p < 0.001).
starts metamorphosis, initiating the transformation of the imaginal disc into the adult appendages (Riddiford, 1993). The transcription factor Broad is one of the early genes that controls the response to ecdysone, and is essential for the elongation of Drosophila appendages at that stage (Kiss et al., 1988). Since defects in appendage morphogenesis in broad mutants are enhanced when combined with mutations in Stubble (Beaton et al., 1988; Kiss et al., 1988), it is likely that Broad controls wing elongation at least in part by inducing degradation of the apical ECM via Stubble. Similar to broad and Stubble, MMPs could be regulated in a similar way to the Stubble protease. These observations point to the expression of apical and basal ECM degrading enzymes as a key part of the response to ecdysone that mediates wing elongation during metamorphosis.

**Wing Shape Change Occurs through Early Convergent Extension and Late Isotropic Expansion**

Although the columnar-to-cuboidal transition drives wing surface expansion, it does not explain how the wing also elongates.
A

**Live imaging**

| 4h APF | 5h APF | 6h APF | 7h APF | 8h APF |
|--------|--------|--------|--------|--------|

**Fixed samples**

| 4h APF | 5h APF | 6h APF | 7h APF | 8h APF |
|--------|--------|--------|--------|--------|

B

**Graphs showing measurements**

- Width (μm)
- Length (μm)
- Ratio length/width

C

**DNA PH3**

D

**Convergent-extension (4h30min-5h APF)**

- T1 transitions
- T2 transitions and rosettes

E

**Expansion (6-8h APF)**

- Apical area increase

F

**Graph showing rearrangements per total cell hour**

G

**Graph showing cell shape anisotropy (μm²)**

(legend on next page)
along the proximal-distal (PD) axis. We therefore observed the process of wing morphogenesis in both live imaging and fixed samples, which reveal that elongation occurs during the initial stage of wing expansion, while the late stages of wing expansion are nearly isotropic (Figure 3A and Video S1). Quantification of wing width and length over time reveals convergence (a decrease in width) and extension (an increase in length) occurring between 4 and 5 hr APF, following which both width and length increase isotropically (Figure 3B). Notably, oriented cell divisions cannot account for the elongation of the wing, because few if any mitotic cells are observed between 4 and 7 hr APF (Figure 3C). These results confirm that the wing elongates via convergent extension movements that occur prior to the process of isotropic wing expansion.

Live imaging of the early wing-extension phase reveals cell shape changes, and cell intercalation events in which cells rearrange via classic “T1” neighbor exchange, “T2” extrusion, and “rosette” formation (Blankenship et al., 2006) (Figures 3D–3F and S2A; Videos S2–S4). Live imaging of the later wing-expansion phase reveals a progressive isometric increase in apical cell area and wing surface area as cells become more cuboidal (Figures 3E and 3F; Video S5). To analyze the contribution of cell shape and apical area to changes in wing shape and size during convergent extension and expansion, we performed segmentation of the apical surface of cells within wing fixed samples from 4 to 7 hr APF, using E-cad-GFP to detect apical cell membranes (Figures 3G and S2). We found that the relative increase in apical cell area is nearly equal to the relative increase in wing area, indicating that the tissue area expansion is driven by cell area increase (Figures S2D–S2F). Both cell and wing area dramatically increased at the end of the process, from 6 to 7 hr APF. We then quantified the average cell elongation in the wing using a nematic tensor of cell elongation, obtained from a triangulation of the network of cellular junctions (Etournay et al., 2015). We found that before peripodial membrane release, cells in the wing disc are elongated in the anterior-posterior (AP) direction (Figure S2E). During convergent extension the cell shape anisotropy decreases, resulting in roughly isotropic cells. During expansion, the anisotropy of both cell shape and wing shape remains approximately constant (Figures S2D and S2E). We then asked whether changes in cell shape anisotropy account for tissue convergent extension, by comparing the rate of cell elongation with the rate of tissue elongation (Figure S2G). In the absence of tissue deformation arising from cellular rearrangements, these quantities should be equal (Popovic et al., 2017; Etournay et al., 2015), but we found instead that tissue elongation occurs at a faster rate than cell elongation, indicating that cellular rearrangements do contribute to wing elongation. The maximum rate of shear growth due to cell rearrangements is ~0.17 per hour, higher than the one observed in high-resolution live-imaging experiments (compare Figures 3F and S2G).

One possibility is that imaging conditions were associated with increased phototoxicity to the tissue, causing the wings to elongate less well than that observed in lower-resolution videos or in fixed samples (compare Figure 3A and Video S1 with Video S6).

Overall, our data show that the increase in wing anisotropy during convergent extension relies on both the change in cell shape and the cumulative effect of cell intercalations, while the increase in wing area during expansion is entirely accounted for by the increase in cell area. Convergent extension is thought to be brought about by planar polarized junctional tension (Blankenship et al., 2006; Bertet et al., 2004), a point supported by computer simulations in two dimensions (Lan et al., 2015; Rauzi et al., 2008). We therefore sought to identify possible planar polarizing mechanisms that can drive tissue elongation.

Dynamic Changes in the Localization of Myosin II Drive Convergent Extension and Expansion

An important polarizing mechanism for convergent extension is the planar polarization of Myosin-II, first discovered in the Drosophila embryo (Bertet et al., 2004; Zallen and Wieschaus, 2004). We analyzed the distribution of the Myosin-II regulatory light chain (encoded by the spaghetti squash or sqh gene in Drosophila) using a GFP-tagged transgene expressed from the endogenous promoter in a genetic background mutant for the endogenous gene (sqh<AV2> (sqh-GFP)). We found that Myosin-II is planar polarized during convergent extension between 4 and 5 hr APF (2-fold increase in Myosin-II fluorescence intensity along the PD axis compared with the AP axis), and that it later relocalizes from the apical to the lateral sides of the cell as the tissue expands isotropically from 6 to 8 hr APF (Figures 4A, 4B, S3A, and S3B). Thus, initial planar polarization of Myosin-II...
A. Developmental Cell 46, 23–39, July 2, 2018

B. sqh^{A02}; sqh-GFP

C. nub-Gal4>UAS.venus-Rok^{K118A}

D. sqh^{A02}; sqh-GFP + Rok inhibitor 30min

E. sqh^{A02}; sqh-GFP + Rok inhibitor 1h30 min

(legend on next page)
correlates with initial anisotropic convergent extension, and subsequent lateral relocalization of Myosin-II correlates with isotropic tissue expansion as cell height decreases.

Rho-kinase (Rok) is responsible for activating Myosin-II contractility by directly phosphorylating the Myosin-II regulatory light chain. We found that Rok localized in precisely the same fashion as Myosin-II in third instar larval wing discs and in the early pupal wing at 4 hr APF and also dissipated apically by 7 hr APF (Figure 4C). Rok activity is required for convergent extension and expansion of the wing, since the addition of the Rok inhibitor Y-27632 to cultured wings completely abolished the elongation of the wing between 4 hr 30 min and 5 hr APF and wing expansion between 5 hr 30 min and 7 hr APF in culture (Figure 4D and Video S7). By adjusting parameters in a continuum model (Popovic et al., 2017) to experimental data of cell and tissue elongation, we found that Myosin-II polarization can account for anisotropic cell shape changes and cellular rearrangements during wing elongation (Figure S3). These results define an essential role for planar polarized Rok and Myosin-II in driving axial convergent extension to elongate the tissue.

Classical Planar Polarization Systems Are Not Required for Wing Convergent Extension

We next considered how Myosin-II undergoes dynamic changes in its localization during wing development. We considered two hypotheses for the initial planar polarization of Myosin-II leading to convergent extension: (1) a developmentally programmed pattern of gene expression that orients a planar polarity system or (2) a developmentally programmed pattern of tissue growth that generates global tensile forces within the constraining environment of the ECM.

We found that none of the known planar polarity systems, Dachsous-Fat cadherin, Frizzled, or Par/3/Bazooka (Baz), affects the anisotropic growth of the wing. fat mutant wings complete the process of expansion and elongation normally during pupal development, although they are rounded prior to expansion and elongation (Figure S4A). Mutants in frizzled or genes encoding other pathway components of the Frizzled planar polarity system, such as flamingo/starry night or van gogh/strabismus, do not affect limb elongation in Drosophila (Chae et al., 1999; Lu et al., 1999; Taylor et al., 1998; Wolff and Rubin, 1998). Similarly, depletion of Baz, which is necessary for embryo germ-band extension (Zallen and Wieschaus, 2004), does not affect wing elongation, and is not planar polarized in the elongating wing (Figures S4B and S4C), where it instead localizes to adherens junctions (Figure S4D). Thus, Drosophila wing convergent extension, mediated by Myosin-II polarization, does not depend on any known planar polarity system.

Myosin-II planar polarization could conceivably result from the circumferential stretch pattern induced by a differential cell proliferation rate along the future PD axis, which is sufficient to planar polarize Myosin-II orthogonal to the PD axis by the third larval instar stage (Figure 4B) (Legoff et al., 2013; Mao et al., 2013). Myosin-II remains polarized during disc eversion between the third larval instar and 4 hr APF, at which point the ECM is released and Myosin-II is able to produce convergent extension movements.

We next considered how Myosin-II relocalizes from the apical ring of adherens junctions to lateral membranes to drive wing expansion from 6 to 8 hr APF. We noticed that GFP-tagged Rok also relocalizes to lateral membranes, as do the adherens junctions themselves, as marked by β-Catenin/Armadillo (Arm) (Figures 4C and S4E). Myosin-II localizes adjacent to adherens junctions in the lateral membrane at 7 hr APF (Figure S4D). This result suggests that relocalization of Rok, Myosin-II and adherens junctions might be linked events. One potential mechanism for repositioning adherens junctions involves the Par-3/Baz protein, shown to be important for junctional movement laterally in Drosophila embryos (Wang et al., 2012). However, we found that Baz does not relocalize laterally with Rok/Myosin-II or Arm during the phase of wing expansion at 7 hr APF (Figures S4C–S4E). We favor the simplest model for wing expansion, which is that after removal of the ECM the columnar cells begin to return to their more energetically favorable cuboidal form, gradually expanding the apical surface and dissipating the entire apical actomyosin ring, which is known to be tension dependent (Lecuit and Yap, 2015; Fernandez-Gonzalez et al., 2009). In the absence of an apical actomyosin ring, adherens junctions are no longer restricted apically and can spread.
along the entire lateral membrane, taking some remaining clusters of contractile actomyosin (Rok/Myosin-II) with it (Figures 4A–4C and 4E). The lateral actomyosin clusters then contribute actively to cell shape change from columnar to cuboidal, since Rok-inhibitor treatment prevents transformation to a cuboidal cell shape by 7 hr APF (Figure 4D).

Tissue-Specific Control of Matrix Remodeling by the Hox Gene Ultrabithorax

To confirm the generality of our findings in the wing, we examined whether the same mechanism operates during elongation of the leg. Once again, we find that both apical and basal matrices remain in place while leg cells are still columnar at or before 4 hr APF, but that the matrix begins to be degraded at 5 hr APF as the leg expands and extends (Figures 5A, 5B and 5E). These results confirm that matrix remodeling immediately precedes morphogenetic change in both the wings and legs of Drosophila. One limb of Drosophila that famously fails to extend is the haltere, a tissue that was once a wing in the four-winged ancestors of Drosophila, but that evolved into a vestigial stigmal upon the evolutionary selection for two-winged insects (order Diptera). We therefore characterized the ECM distribution and cell shapes of the haltere at 4–7 hr APF. We found that the haltere is initially composed of pseudo-stratified columnar cells at 4 hr APF and strikingly remains so all the way through to 7 hr APF, such that the haltere does not flatten or extend (Figures 5C–5E). Notably, the ECM remains present at both the apical and basal surface of the haltere throughout this process, despite the removal of the overlying peripodial layer. To confirm our findings with Collagen IV, we examined GFP-tagged forms of two other basal ECM components, Laminin (Laminin [1]-subunit encoded by LanB1, Lan[1]-GFP), and Perlecan (Pcan-GFP). We found that, similar to Collagen IV, both are degraded between 4 and 7 hr APF in the wing and leg, but not in the haltere (Figures 6A–6G and S5).

To test whether degradation of the ECM is sufficient to allow the haltere to flatten and extend, we added the serine protease enzyme trypsin to cultured halteres. We found that addition of this enzyme for 15 min allowed the haltere to flatten and induced loss of both the apical and basal matrix (Figures 7A and 7B). The result is a “winglet”-like structure that is much smaller than the wing itself at this stage due to the fact that the haltere comprises fewer cells than the wing throughout its early growth phase. These data show that inducing degradation of the ECM can drive flattening and expansion of the haltere epithelia, although we cannot rule out that degradation of additional proteins by trypsin could contribute to change cell shape. To examine why matrix degradation fails to occur in the haltere, which like the wing and leg expresses both broad and MMP1 (Figure S1), we considered the role of the Hox transcription factor Ultrabithorax (Ubx), a master control gene governing the switch between wing and haltere development whose loss-of-function mutation produces four-winged Drosophila (Pavlopoulos and Akam, 2011; Lewis, 1978).

To test whether Ubx controls the decision not to degrade the ECM in the haltere, we inactivated Ubx after the growth phase by expressing RNAi against Ubx in late larval imaginal discs with the Gal4/UAS conditional expression system. Loss of Ubx in the pupal stages resulted in degradation of both apical and basal ECM, allowing the flattening of the pseudo-stratified columnar epithelium to a cuboidal one, which expands and extends the haltere into a “winglet” (Figures 7B and 7C). Thus, Ubx is specifically required for restricting haltere morphogenetic expansion and elongation, independently of its known roles in restricting haltere growth and patterning. We next tested the gain-of-function phenotype of Ubx expression in the wing, which has been reported to cause transformation into a haltere-like structure but whose mechanism of action has remained unclear (Pavlopoulos and Akam, 2011). We find that overexpression of Ubx is sufficient to prevent apical and basal matrix degradation in the wing such that the wing fails to expand and extend by 7 hr APF, resulting in a tiny adult structure that resembles the haltere (Figures 7D and 7E). This phenotype is similar to that caused by culturing the wing discs ex vivo in the presence of a protease inhibitor cocktail, such that the tissue fails to elongate at 7 hr APF (Figure 2). Thus, matrix remodeling can be controlled in a tissue-specific fashion by a master developmental control gene, whose increased expression at the beginning of metamorphosis (Figures 7F and S1) blocks matrix remodeling to prevent columnar-to-cuboidal shape change and tissue morphogenesis.

Comparison of Myosin-II Localization Dynamics in the Wing, Leg, and Haltere

We next asked whether the dynamic changes in Myosin-II localization we observe during elongation of the wing also occur during elongation of the leg. We examined Myosin-II-GFP and...
Rok-Venus localization in the larval leg imaginal disc and in pupal legs at 4–7 hr APF (Figures S6A–S6C). We find that, as in the wing, planar polarization of Myosin-II and Rok begins in the larval stages in a circumferential pattern around the central region of the leg disc. After eversion, this centralmost region becomes the distalmost point of the elongating leg. Upon leg elongation, the planar polarization of Myosin-II dissipates, and Myosin-II then relocalizes laterally and then basally as cells undergo columnar-to-cuboidal transition, again similar to the wing. Differences between the wing and leg include the overall flat blade versus round tube form, and the more extensive folding of the leg while still encapsulated within the ECM, which appears to involve early apical matrix degradation and PD elongation preceding release of the basal matrix (Figure S7). This early PD elongation of the apical surface and consequent folding provides an explanation for why Myosin-II planar polarization dissipates more rapidly in the leg (Figures S6A–S6C). Notably a similar folded morphology is induced in the wing upon expression of Timp to prevent basal matrix degradation, which is rescued when Dumpy degradation is inhibited by adding the protease inhibitor mix, supporting the view that folding arises from elongation of the apical surface while the basal surface remains attached to the matrix (Figure S7).

We also examined the localization of Myosin-II in the haltere, which remains encapsulated within the ECM. Firstly, the initial planar polarization of Myosin-II is much weaker in the halteres than the wing at the same stage, presumably due to the much-reduced growth of the halteres compared with the wing (Figures S6D–S6F). Secondly, unlike the wing and leg, Myosin-II does not relocalize laterally or basally by 7 hr APF in the haltere. These findings support the notion that matrix remodeling in the wing and leg is the trigger than induces subsequent dynamic changes in Myosin-II localization to drive morphogenetic elongation.

**DISCUSSION**

Our results show that morphogenetic elongation of *Drosophila* limbs occurs via two processes: convergent extension and expansion. Convergent extension is driven by planar polarized localization of Myosin-II, which drives both anisotropic cell shape change and intercalation of cells such that the tissue contracts along the AP axis and extends along the PD axis. Expansion involves relocalization of Myosin-II from the apical ring to lateral membranes, which promotes columnar-to-cuboidal transition and thus isotropic tissue expansion. These processes are sequentially induced following release of the constraining force of the ECM. Remodeling of the matrix is subject to at least two distinct forms of developmental control. First there is temporal control of matrix removal, which is timed to coincide with removal of the peripodial membrane, and is mediated by hormonal signals including ecdysone, which induces the apical matrix protease Stubble and the basal matrix proteases MMP1 and MMP2 (Figures 2D, 2E, and S1). The transcription factor Broad is induced by ecdysone at pupariation (Figures 2D and S1) and is likely to mediate induction of protease expression as broad mutants fails to undergo limb elongation (Karim et al., 1993; Beaton et al., 1988; Kiss et al., 1988; Mandaron, 1970). Second, there is tissue-specific control of matrix removal, which occurs in the wings and legs but not in the halteres (despite simultaneous removal of the peripodial layer from all three limbs) due to the haltere-specific master control gene Ubx (Figures 7F and S1) (Lewis, 1978).

Placing these results in the context of the earlier stages of limb development, we note that both the apical and basal ECM components are present as limbs grow during the larval “imaginal disc” stages of life (Ray et al., 2015; Pastor-Pareja and Xu, 2011). We propose that the matrix provides an elastic constraining force along the apical and basal surface of the epithelium, but not along the lateral sides of each cell. This constraining force pattern promotes columnar epithelial cell shape, because the lateral sides are able to grow longer than the apical or basal sides of each cell as the tissue increases in mass (Pastor-Pareja and Xu, 2011). Our data show that release of this constraining force by developmentally controlled matrix remodeling allows each epithelial cell to return to a more cuboidal form, which involves shrinkage of the lateral sides and expansion of the apical and basal sides to expand the entire tissue.

In the presence of a chitinous exoskeleton in late wing development, Dumpy has an essential role as a mediator of the epidermal-cuticle junction (Etournay et al., 2015; Ray et al., 2018).
Developmental Cell 46, 23–39, July 2, 2018
During early pupal wing convergent extension and expansion, however, the cuticle has not yet been secreted, which indicates that Dumpy may act instead as a component of the apical ECM itself, a role supported by the structure of Dumpy protein. Each Dumpy molecule is a gigantic structure anchored to the cell membrane at its carboxy-terminal end and arranged into fibers by its epidermal growth factor modules, with less organized regions that confer elasticity. Also, its ZP domain could crosslink different Dumpy molecules with other ECM components (Wilkin et al., 2000). Dumpy’s size, physical properties, and capacity to polymerize could build an apical ECM capable of redistributing and resisting tension, providing mechanical strength during morphogenesis of the wing from larval stages.

At the apical surface of the epithelium, differential growth of the larval imaginal disc tissue within the ECM leads to a global stretch pattern that causes planar polarization of Myosin-II orthogonal to the PD axis (Legoff et al., 2013; Mao et al., 2013). This phenomenon of stretch-induced Myosin-II accumulation has also been observed in the embryo (Fernandez-Gonzalez et al., 2009; Fernandez-Gonzalez and Zallen, 2009). Release of the ECM then allows polarized Myosin-II to contract junctions to drive cell shape changes and cell intercalation events, and thus generate convergent extension of the entire tissue.

This elegant “biological spring” mechanism of accumulating mass within an elastic tensile matrix and then inducing its release to trigger morphogenetic change in three dimensions is a classic example of the internal storage of potential energy that is subsequently released to do work, and represents an important function for matrix remodeling in controlling cell shape and tissue morphogenesis (Bonnans et al., 2014; Rodriguez-Fraticelli and Martin-Belmonte, 2014).

Drawing comparisons with other tissue types and other species, we note that the columnar-to-cuboidal transition in Drosophila limbs is a process driven entirely by forces intrinsic to each individual cell: cortical actomyosin contractility that is initially weak at lateral membranes but then strengthens upon re-localization of Myosin-II laterally. In this respect, it contrasts with other examples of tissue flattening that are simply driven by external stretching forces imposed by neighboring tissues. Examples include stretching of the Drosophila ovarian follicle cells by the force of the growing egg (Haigo and Bilder, 2011), pulling of the Drosophila embryonic ectoderm over the contracting amnioserosa (Solon et al., 2005), spreading of the zebrafish enveloping cell layer over the yolk cell by a contractile ring (Xiong et al., 2014; Behrmtd et al., 2012; Solnica-Krezel, 2005), or flattening of the trophoderm layer by accumulation of fluid inside the mammalian blastocyst. Instead, Drosophila limb elongation more closely resembles elongation of the primitive gut (archenteron) of the sea urchins Strongylocentrotus purpuratus and Lytechinus pictus (Hardin and Cheng, 1986; Ettensohn, 1985) as well as elongation of the tentacles of the sea anemone N. vectensis (Fritz et al., 2013).

The process of convergent extension in Drosophila limbs appears similar to other examples of convergent extension movements in the Drosophila embryo and vertebrate primitive streak and kidney tubules, which are also driven by intrinsic local forces driven by planar polarized Myosin-II (Pare et al., 2014; Lienkamp et al., 2012; Voiculescu et al., 2007; Blankenship et al., 2006; Sepich et al., 2005; Bertet et al., 2004; Zallen and Wieschaus, 2004; Irvine and Wieschaus, 1994). However, global forces can also contribute to convergent extension in Drosophila and vertebrate embryos (Collinet et al., 2015; Lye et al., 2015; Campinho et al., 2013; Behrmtd et al., 2012; Butler et al., 2009; Keller and Trinkaus, 1987; Keller, 1980). There are no extrinsic pulling forces acting globally to stretch Drosophila limbs during the early stages of pupal development investigated here, making limb elongation a bona fide example of local Myosin-II planar polarization being solely responsible for convergent extension. Nevertheless, we and others previously showed that global stretch forces do arise much later in pupal development due to redeposition of ECM for patterned attachment of the limbs to the exoskeleton, which serve as anchor points for shaping the tissue into its precise final form (Etournay et al., 2015; Ray et al., 2015).

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
EXPERIMENTAL MODEL AND SUBJECT DETAILS
- Drosophila Melanogaster Genetics

METHOD DETAILS
- Adult Haltere and Wing Preparations
- Immunohistochemistry
- Ex Vivo Culture of Pupal Imaginal Wing Discs
- Live-Imaging and Imaging of Fixed Samples
- Rok Inhibitor Assay
- Metalloprotease Treatment
- Protease Inhibitor Treatment
- Modeling of Convergent Extension. A Continuum Model for Autonomous Convergent Extension of the Imaginal Disc

QUANTIFICATION AND STATISTICAL ANALYSIS
- Quantification of Wing Size and Shape (Figures 1E, 3B, and S2C)
- Quantification of Cell and Wing Elongation and Area (Figures 3D–3G, S2A, S2B, S2D–S2G, and S3)
- Cell Segmentation
- Quantification of Cell Rearrangements
- Quantification ECM Degradation
- Quantification of Myosin Intensity along the Apico-Basal Axis
- Statistical Analysis

SUPPLEMENTAL INFORMATION
Supplemental Information includes seven figures and seven videos and can be found with this article online at https://doi.org/10.1016/j.devcel.2018.06.006.

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AUTHOR CONTRIBUTIONS
M.D.C.D.D.L.L designed and performed the experimental work and part of the data analysis (wing size and shape, cell rearrangements, and fluorescence quantification). R.P.R. contributed to the initial experimental work. P.S.G. segmented epithelial cells and performed the analysis of the segmented data and the continuum model. J.R.D., A.H., and N.T. developed the segmentation software. S.A. and G.S. supervised the segmentation analysis and the continuum model of wing elongation. B.J.T. designed the experiments and wrote the manuscript with input from the other authors.

DECLARATION OF INTERESTS
The authors declare no competing or financial interests.

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# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Goat polyclonal anti-GFP | Abcam | Cat# ab6662; RRID: AB_305635 |
| Rabbit anti-Bazooka | Andreas Wordaz laboratory | RRID: AB_2570125 |
| Mouse anti-Armadillo | DSHB | Cat# 25E9.D7; RRID: AB_528104 |
| Mouse anti-MMP1     | DSHB   | Cat# 3A6B4; RRID: AB_579780 |
| Mouse anti-MMP1     | DSHB   | Cat# 3B8D12; RRID: AB_579781 |
| Mouse anti-MMP1     | DSHB   | Cat# 5H7B11; RRID: AB_579779 |
| Mouse anti-Ubx      | DSHB   | Cat# FP3.38; RRID: AB_10805300 |
| Goat anti-mouse Alexa fluor 546 | Invitrogen | Cat# A11030; RRID: AB_144695 |
| Goat anti-rabbit Alexa fluor 546 | Invitrogen | Cat# A11035; RRID: AB_143051 |
| **Chemicals, Peptides, and Recombinant Proteins** |        |            |
| Shield and Sang M3 insect medium | Sigma | S365 |
| 2% Fetal bovine serum | Sigma | F3018 |
| Streptomycin/ampicillin antibiotics mix | Invitrogen | 15140-122 |
| Ec dysone | Sigma | E9004 |
| Insulin | Sigma | 5500 |
| Methyl-cellulose | Sigma | M0387 |
| Rock inhibitor | Sigma | Y-27632 |
| Trypsin-EDTA | Gibco | 15400-054 |
| DAPI | Sigma | D9542 |
| Phalloidin-Atto 647N | Sigma | 65906 |

**Experimental Models: Organisms/Strains**

* D. melanogaster: E-cadherin-GFP. *w; shg-GFP*
  Huang et al., 2009 N/A

* D. melanogaster: Dumpy-YFP. *w[118]; P{Bac[681.P.FSVD-1]dp}[CPTI001769]*
  DGGR (Kyoto) Cat# 115238; RRID: DGGR 115238

* D. melanogaster: Vkg-GFP. *w[7]; P{PTT-un1}vkg02055*
  FlyTrap G205

* D. melanogaster: Lan[1]-GFP. *w[7]; LanB1-GFP/TM2*
  Sarov et al., 2016 N/A

* D. melanogaster: Perlecan-GFP. *w[7]; P[w+mC=PTT-un1]ZCL1700*
  DGGR (Kyoto) Cat# 110807; RRID: DGGR_110807

* D. melanogaster: Myosin-II-GFP. *sqtAX3; sqh-Sqh-GFP*
  Royou et al., 2002 N/A

* D. melanogaster: MMP2 overexpression. *w[7]; P[w+mC]=UAS-Mmp2.P2*
  Bloomington Cat# 58705; RRID: BDSC_58705

* D. melanogaster: Temp overexpression. *w[7]; P[w+mC]=UAS-Timp.P3*
  Bloomington Cat# 58708; RRID: BDSC_58708

* D. melanogaster: Ubx allele overexpression. *w[7]; P[w+mC]=UAS-Ubx[4,6]/36.2/TM3, Ser[7]*
  Bloomington Cat# 911; RRID: BDSC_911

* D. melanogaster: RNAi of Bazooka. *w[7]; baz[NGF,5055R] /TM3*
  NIG-Fly 5055R-1

* D. melanogaster: RNAi of Ultrabithorax *w[118]; P{GD5049}vo37823*
  VDRC Cat# 37823; RRID: Flybase_FBst0462184

* D. melanogaster: Rak[K116A] allele overexpression *w[7]; UAS-venus-Rak[K116A] /TM6B*
  Simoes Sde et al., 2010 N/A

* D. melanogaster: Dil-Gal4 *w[7]; Dil-Gal4/CyO*
  Calleja et al., 1996 N/A

(Continued on next page)
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Barry J. Thompson (barry.thompson@crick.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Drosophila Melanogaster Genetics

Flies were grown at 25°C using standard procedures. The following fluorescent-tagged proteins were used: E-cadherin-GFP (Huang et al., 2009), Dp-YFP (Drosophila Genomics and Genetic Resources (Kyoto), 115238), Collagen IV-GFP (a2-subunit, Vkg-GFP; (Sarov et al., 2016)), Laminin-GFP (Laminin b1-subunit, Lanb1-GFP; (Sarov et al., 2016)), Perlecan-GFP (Pcan-GFP, Kyoto DGGR, 110807) and Myosin-II-GFP (sqh-GFP construct) in the sqhAX3 null mutant background (Royou et al., 2002). Gene expression mediated by the Gal4/UAS system was performed at 25°C. To decrease Ubx levels in the haltere, UAS-UbxRNAi (VDRC, 37823) was combined with the Ubx-Gal4 driver (kindly provided by Gregory Gibson). To ectopically express Ubx or bazooka in the wing, the UAS-UbxIa (Bloomington, 911, (Pavlopoulos and Akam, 2011)) or UAS-bazRNAi constructs (NIG-Fly, 5055R-1) were combined with the nubbin-Gal4 (nub-GAL4) driver, respectively. Overexpression of MMP2 in the haltere was mediated by combining the UAS-MMP2 construct (Bloomington, 58705) and Ubx-GAL4; and Timp was overexpressed in the wing by combining UAS.Timp (Bloomington, 58708) and nub-GAL4. UAS.venus-RokK116A (Simoes Sde et al., 2010) expression was mediated using specific drivers for the different imaginal discs: nub-GAL4 in the wing, Dil-Gal4 (Calleja et al, 1996) in the leg, and Ubx-Gal4 in the haltere. Fat mutant condition consists of the heterozygous combination of the loss of function alleles Fat8 and FatGrV (Mao et al., 2006; Matakatsu and Blair, 2006).

METHOD DETAILS

Adult Haltere and Wing Preparations

Halteres and wings were dissected from the adult fly, fixed in 3:1 ethanol glycerol and mounted in Hoyer’s mounting media. Images were acquired on a Zeiss axioplan microscope using a LeicaDFC420c digital camera and processed with Adobe Photoshop software.

Immunohistochemistry

White pupae were collected and aged, and then imaginal discs were dissected from the puparium in PBS and transferred to 4% paraformaldehyde for fixation. After 30 minutes of fixation, tissues were immunostained as described in (Ray et al., 2015). Anti-GFP antibody (Abcam, ab6662, 1:400) was used to amplify E-cad-GFP, Dp-YFP, Vkg-GFP, Lanb1-GFP, Pcan-GFP, and Myosin-II-GFP immunofluorescence signals; rabbit anti-Bazooka was used at 1:250 (A. Wordaz), mouse anti-Armadillo at 1:100 (DSHB), and mouse anti-Broad at 1:100 (DSHB, 25E9.D7), mouse anti-MMP1 1:1:1 antibodies cocktail (DSHB; 3A6B4, 3B8D12 and 5H7B11) at 1:1, and mouse anti-Ubx at 1:10 (DSHB, FP3.38). Secondary antibodies, goat Alexa fluor 488, 546 or 647 (Invitrogen), were used at 1:500. DAPI and rhodamine phallodin (Sigma, 65906) were used at 1:250. Samples were mounted in Vectashield (Vector Labs, H1000) using different separators depending on the thickness of the sample.

Ex Vivo Culture of Pupal Imaginal Wing Discs

Pupal wing discs of the appropriate age were cultured as described in (Bell et al., 2016; Aldaz et al., 2010). Wing discs were dissected from the puparium in Shield and Sang M3 insect medium (Sigma, S3652) supplemented with 2% fetal bovine serum (Sigma, F3018), 1% streptomycin/ampicillin antibiotics mix (Invitrogen, 15140-122), 0.1 μg/mL ec dysone (Sigma, E9004) and 0.14 μg/mL insulin...
Live-Imaging and Imaging of Fixed Samples

In vivo and ex vivo samples images were acquired with a Leica SP5 confocal using 20x/0.70 NA or 40x/1.25 NA immersion objectives, controlled by the Leica Las AF software. Alternatively, a Zeiss LSM 880 confocal controlled by the ZEN software was used to perform high-resolution live imaging experiments, using a 40x/1.3 NA and applying a 2x zoom magnification. Images were analysed and processed using Fiji, Adobe Photoshop and Adobe Illustrator software. Live imaging experiments were performed at room temperature and an average of 50 Z-sections at 1 to 2 μm interval were acquired every 5 minutes.

Rok Inhibitor Assay

To inhibit myosin contraction during convergent extension, 4 hours APF sqh\textsuperscript{AX3}; sqh-GFP wing discs were dissected and transferred, right after peripodial membrane release, to supplemented Shield and Sang medium plus 2.5% methyl-cellulose containing 2.5mM Rock inhibitor (Sigma, Y-27632), and filmed immediately, or alternatively fixed and immunostained after 30 min of inhibitor treatment at 25 C. To inhibit myosin contraction during wing expansion, 5 hours 30 min sqh\textsuperscript{AX3}; sqh-GFP wing discs were dissected and transferred to supplemented Shield and Sang medium containing 2.5mM Rock inhibitor, incubated for 3 hours at 25C, fixed and immunostained.

Metalloprotease Treatment

Dp-YFP and Vkg-GFP haltere imaginal discs of the correct age were dissected from the puparium in PBS and transferred to PBS containing 0.001% Trypsin-EDTA (Gibco, 15400-054). Tissues were treated for 15 minutes at room temperature, and transferred to supplemented Shield and Sang medium to stop the trypsin reaction, fixed and immunostained.

Protease Inhibitor Treatment

dp-YFP, nub-Gal4>Timp and vkg-YFP, nub-Gal4>Timp wing imaginal discs were dissected from the puparium at 4 hours APF and transferred to supplemented Shield and Sang medium including a protease inhibitors mixture (2ml of media containing 1 tablet of cOmplete Protease Inhibitor Cocktail, 04693116001, Roche). Wing disc were incubated for 3 hours and 30 minutes at 25C, fixed and immunostained.
2K\langle Q_{\alpha \beta} \rangle + \zeta Q_{\alpha \beta} + 2\mu \frac{d(Q_{\alpha \beta})}{dt} = 0, \quad \text{(Equation 4)}

which can be solved for \langle Q_{\alpha \alpha}(t) \rangle:

\langle Q_{\alpha \alpha}(t) \rangle = e^{-\frac{t}{\tau_S}} \left( \langle Q_{\alpha \alpha}(t_0) \rangle e^{\frac{\zeta}{2K}} \int_{t_0}^{t} dt' e^{\zeta / \tau_S} q_{\alpha \alpha}(t') \right). \quad \text{(Equation 5)}

The anisotropic diagonal component of the velocity gradient, \( \bar{v}_{\alpha \alpha} \), is related to the natural strain rate in the tissue:

\[ \bar{v}_{\alpha \alpha} = \frac{1}{2} \log(\frac{L}{H}) \] \quad \text{(Equation 6)}

with

\[ L = \log\left( \frac{l}{l_0} \right), \quad H = \log\left( \frac{h}{h_0} \right) \] \quad \text{(Equation 7)}

where \( l \) and \( h \) are the length and height of the tissue respectively and \( l_0 \) and \( h_0 \) the corresponding values at \( t = t_0 \).

Using Equations 1 and 4, the natural strain rate of the tissue is then given by:

\[ (L - H)(t) = (L - H)(t_0) - 2 \int_{t_0}^{t} dt' \left( \frac{1}{\tau_S} \langle Q_{\alpha \alpha}(t') \rangle + \left( \frac{\zeta}{2\mu} - \lambda \right) q_{\alpha \alpha}(t') \right). \] \quad \text{(Equation 8)}

2. Adjustment to Experimental Data

We choose the following form for the evolution of the myosin anisotropy \( q_{\alpha \alpha} \) (t):

\[ q_{\alpha \alpha}(t) = \begin{cases} 1 & t_0 < t < t_1 \\ t_2 - t & t_1 < t < t_2 \\ 0 & t > t_2 \end{cases} \] \quad \text{(Equation 9)}

\[ q_{\alpha \alpha}(t) = \begin{cases} t_0 - t & t_0 < t < t_1 \\ t_2 - t_1 & t_1 < t < t_2 \end{cases} \] \quad \text{(Equation 10)}

\[ q_{\alpha \alpha}(t) = 0 \quad t > t_2 \] \quad \text{(Equation 11)}

with \( t_0 = 4h \text{ APF} \), \( t_1 = 5h \text{ h APF} \) and \( t_2 = 6h \text{ APF} \). This choice of time evolution of the magnitude of \( q_{\alpha \alpha} \) is in accordance with measurements of Myosin-II anisotropy (Figure S3A).

We then varied the parameter \( \tau_S \) within a range of values that are physiologically relevant (from 0.1 to 1 hour). For each value of \( \tau_S \), we calculated the average cell elongation using Equations 5 and 8, taking the initial values \( \langle Q_{\alpha \alpha}(t_0) \rangle \) to be equal to the experimental measured average cell elongation at \( t = 4h \text{ APF} \), and \( (L - H)(t_0) = 0 \). We then fitted the parameters \( \zeta/2K \) and \( \lambda \) to experimental measurements of cell and tissue elongation (Figure S3D).

Figure S3E shows the parameters values obtained for \( \zeta/2K \) and \( \lambda \) from the fits as a function of \( \tau_S \). We find that the parameter \( \lambda \) takes a value around \( \sim 0.1h^{-1} \) for all values of \( \tau_S \). The value of \( \zeta/2K \) is negative and varies between \( -0.06 \) and \( -0.14 \). The negative sign of the coefficient \( \zeta \) corresponds to an overall anisotropic stress driving a contractile force along the anterior-posterior direction. This sign is consistent with experimentally observed myosin polarization along junctions oriented along the anterior-posterior direction.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Quantification of Wing Size and Shape (Figures 1E, 3B, and S2C)**

Up to 8 wing discs of each stage were analysed, and DNA and actin cytoskeleton dyes (DAPI and rhodamine phalloidin) allowed us to detect wing shape. The entire wing disc was imaged by acquiring Z stacks each 1.72 microns. After generating the maximum intensity projection from the Z stacks, wing area, maximal length \( l \) (along PD axis), and width \( h \) (along AP axis), were calculated manually using the ROI tool from Fiji. The average height of each wing was calculated manually using measurements from different regions of the epithelium in apico-basal cross sections, using the ROI tool from Fiji.

**Quantification of Cell and Wing Elongation and Area (Figures 3D–3G, S2A, S2B, S2D–S2G, and S3)**

Cell area was quantified from cell segmentation of fixed samples by averaging the area of all segmented cells. Wing area was measured in Fiji taking the whole blade as the region of interest. Cell elongation was obtained by triangulating the network of cell junctions, as described in (Merkel et al., 2017; Etournay et al., 2015). Briefly, triangles were obtained by connecting the cell centers of neighbouring cells. Triangular shapes were compared to the shape of an equilateral triangle, resulting in the definition of a tensor of nematic elongation \( Q \). The tensor of nematic elongation was then averaged over all triangles to obtain the average elongation \( \langle Q \rangle \).
Alternatively, to obtain a measure of overall cell shape anisotropy, the magnitude of tensor $|Q| = \sqrt{(Q_{xx})^2 + (Q_{xy})^2}$ was averaged over all triangles to obtain an average cell shape anisotropy $|Q|$ (Figure S3G).

Tissue elongation was obtained by defining the natural strain variables $L = \log (l/l_0)$ and $H = \log (h/h_0)$, with $l_0$ and $h_0$ the length and width at 4 hours APF, and taking the difference $L - H$ (Figure S2D).

The difference between tissue elongation and average cell elongation defines a cell rearrangement tensor $R$, whose diagonal component along the x (proximal-distal) axis is given by:

$$R_{xx} = \frac{1}{2} \frac{d(L - H)}{dt} - \frac{d(Q_{xx})}{dt}$$

which corresponds to the contribution of cellular rearrangements to the deformation of the tissue along the proximal-distal axis. The data was obtained from 2, 3, 6 and 2 fixed wings disc samples at 4, 5, 6 and 7 hours APF, respectively.

In live imaging experiments, wing and cell area (n = 10) were calculated manually using the freehand ROI tool from Fiji.

**Cell Segmentation**

Segmentation was performed in Wolfram Mathematica, whereby images were initially blurred through convolution with a Shen-Castan matrix, in order to smooth pixel intensities whilst preserving edges. Cell membranes were highlighted with a ridge filter, which is a principal-curvature based region detector, and the cells segmented with a watershed algorithm. For triangulation analysis cells were highlighted with a unique hexadecimal colour code that was randomly generated. After the segmentation step, an interactive skeleton correction process was introduced. For this, a new graphical tool was developed in MATLAB (Mathworks Inc.) which allowed overlaying the original and the segmented image sequences with the purpose of making corrections by drawing or deleting skeleton segments in an easy and intuitive manner. The editing options were accessible through keyboard shortcuts. This allowed for efficient switching between editing options while advancing through the sequence. The actual drawing or deletion of skeleton segments could be performed with a Wacom Pen and Graphics Tablet which made this process highly intuitive. The corrected skeletons were then exported for the subsequent analysis.

**Quantification of Cell Rearrangements**

Four high resolution videos of E-cadherin-GFP wing discs cultured ex vivo from 4 hours 30 min to 5 hours APF (convergent extension), and from 6 hours 30 min to 7 hours APF (expansion) were analysed. The number of cells involved in cell rearrangements, as well as the type of event (T1, rosettes and T2) and the total number of cells (>1500 cells per wing) were manually analysed using Image J.

**Quantification ECM Degradation**

To analyse ECM degradation in the different tissues and experimental conditions, up to 12 imaginal discs were analysed. Several confocal Z stacks acquired each 1.72 microns were selected and projected to include the complete epithelial surface and the adjacent ECM, and their areas were calculated manually using ROI measurement tool in Fiji. The percentage of apical or basal epithelial surfaces (marked by actin), covered by apical Dp-YFP or basal Vkg-GFP, Lanb1-GFP and Pcan-GFP ECM components were calculated.

**Quantification of Myosin Intensity along the Apico-Basal Axis**

To analyse how Myosin-II distributes along the epithelial apico-basal axis in the imaginal wing disc, apico-basal cross sections of the appropriate stages were acquired by confocal scanning in ZXY, and up to 20 cells were analysed. sqh-GFP fluorescence intensity was measured along linear ROIs perpendicular to the apico-basal axis, and separated approximately each 2 μm, using Fiji. Intensity values along each ROI were normalised in respect with the average of total intensity fluorescence to calculate the average fluorescence for each ROI. To analyse the planar polarisation of myosin along the AP axis in the wing disc, apical cross sections were acquired from 5 to 8 hours APF, and up to 20 cells were analysed. sqh-GFP fluorescence intensity at the edges of each cell was calculated manually using Fiji, and the fluorescence intensity corresponding to the boundaries along the AP axis was normalised in respect with the ones parallel to the PD axis.

**Statistical Analysis**

Experiments were performed with at least three biological replicas. The average, standard deviation and individual data points were represented. To determine which values were significantly different, p values were obtained using two-tailed Student’s t tests. p<0.05, statistically significant; p<0.005, very statistically significant; p<0.00, extremely statistically significant.