Spatiotemporal control of epithelial remodeling by regulated myosin phosphorylation

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Edited by Ruth Lehmann, New York University Medical Center, New York, NY, and approved June 27, 2014 (received for review January 12, 2014)

Spatiotemporally regulated actomyosin contractility generates the forces that drive epithelial cell rearrangements and tissue remodeling. Phosphorylation of the myosin II regulatory light chain (RLC) promotes the assembly of myosin monomers into active contractile filaments and is an essential mechanism regulating the level of myosin activity. However, the effects of phosphorylation on myosin localization, dynamics, and function during epithelial remodeling are not well understood. In *Drosophila*, planar polarized myosin contractility is required for oriented cell rearrangements during elongation of the body axis. We show that regulated myosin phosphorylation influences spatial and temporal properties of contractile behavior at molecular, cellular, and tissue length scales. Expression of myosin RLC variants that prevent or mimic phosphorylation both disrupt axis elongation, but have distinct effects at the molecular and cellular levels. Unphosphorylatable RLC produces fewer, slower cell rearrangements, whereas phosphomimetic RLC accelerates rearrangement and promotes higher-order cell interactions. Quantitative live imaging and biophysical approaches reveal that both phosphovariants reduce myosin planar polarity and mechanical anisotropy, altering the orientation of cell rearrangements during axis elongation. Moreover, the localized myosin activator Rho-kinase is required for spatially regulated myosin activity, even when the requirement for phosphorylation is bypassed by the expression of phosphomimetic myosin RLC. These results indicate that myosin phosphorylation influences both the level and the spatiotemporal regulation of myosin activity, linking molecular properties of myosin activity to tissue morphogenesis.

Contractile assemblies of actin filaments and the nonmuscle myosin II motor protein produce mechanical forces that generate changes in cell shape during cytokinesis, cell movements during cell migration, and the dynamic remodeling of multicellular tissues (1–3). During development, spatiotemporal patterns of actomyosin contractility remodel simple epithelia into functional tissues with complex form and structure. Contractile force generation requires the assembly of inactive myosin monomers into active bipolar filaments (4). Phosphorylation of the myosin II regulatory light chain promotes myosin filament assembly and the movement of the myosin motor along actin filaments in vitro (5, 6) and is necessary for cytokinesis (7–9), oogenesis (7, 10, 11), and tissue morphogenesis (12–15). However, the in vivo effects of regulatory light chain phosphorylation on myosin localization, dynamics, and force generation, and how these properties are integrated to achieve complex changes in the shapes of tissues, are not well understood.

Epithelial elongation in the *Drosophila* embryo is driven by cell rearrangements that are coordinated by spatiotemporal patterns of actomyosin contractility. Myosin contractility is spatially regulated in the plane of the epithelium (termed planar polarity), driving the planar polarized contraction of cell interfaces oriented close to perpendicular to the anterior–posterior axis (AP edges) (16–20). New interfaces preferentially form between dorsal and ventral cells (DV edges), resulting in oriented cell rearrangements that rapidly elongate the body axis of the animal from head to tail (16–18, 21). The myosin II activator Rho-associated protein kinase (Rho-kinase) localizes to regions of cells that display strong actomyosin activity during development (14, 15, 22) and promotes

myosin contractility by phosphorylating the myosin regulatory light chain (RLC) and inactivating myosin phosphatase (23). Rho-kinase is required for localized myosin contractility during *Drosophila* axis elongation (14), neural tube closure in chick (22), and in other epithelia that undergo planar polarized cell behaviors (24, 25). Localized phosphorylation by Rho-kinase could therefore serve as an instructive cue that determines where and when myosin is activated within the cell. However, constitutively active myosin variants that evade this upstream regulation are sufficient to support normal myosin function during cytokinesis (26, 27), oogenesis (7), and wing hair formation (12), indicating that in some contexts regulated myosin phosphorylation is dispensable for morphogenesis. This raises the alternative possibility that the primary requirement for phosphorylation is to activate myosin, and additional mechanisms regulate the spatiotemporal patterns of myosin localization within epithelia.

Here we use time-lapse imaging and biophysical approaches to analyze the role of myosin phosphorylation in polarized cell rearrangements during *Drosophila* axis elongation. Myosin II regulatory light chain variants that prevent or mimic phosphorylation both disrupt axis elongation, but have distinct effects on myosin localization, dynamics, and cell behavior. These results indicate that regulated myosin phosphorylation provides an instructive cue that directs both the magnitude and spatiotemporal pattern of myosin activity during epithelial remodeling, linking molecular-scale myosin activity to cell behavior and tissue morphogenesis.

Results

Myosin II Regulatory Light Chain Phosphovariants Disrupt *Drosophila* Axis Elongation. To systematically examine the effects of myosin II RLC phosphorylation on cell behavior in a multicellular tissue, we

Significance

Cells in the fruit fly *Drosophila* require forces generated by the myosin II motor to reorganize the embryo and elongate the body axis from head to tail. It has been proposed that regulated myosin phosphorylation generates global patterns of myosin activity that shape tissues. However, the mechanisms that determine where and when forces are generated are not well understood. We show that myosin variants that evade upstream regulation by preventing or mimicking phosphorylation disrupt the spatiotemporal pattern of forces during axis elongation. Mimicking myosin phosphorylation accelerates cell rearrangements, but these rearrangements are poorly oriented and produce less efficient elongation. These results indicate that regulated myosin phosphorylation provides an instructive cue that determines where and when myosin generates force to shape multicellular tissues.

Author contributions: K.E.K. and J.A.Z. designed research; K.E.K. performed research; D.L.F. contributed new reagents/analytic tools; K.E.K., D.L.F., and J.A.Z. analyzed data; and K.E.K. and J.A.Z. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1400520111/-DCSupplemental.

generated a collection of transgenic *Drosophila* lines that express RLC variants with altered molecular properties. Phosphorylation of the *Drosophila* RLC (Sqh, encoded by *spaghetti squash*) at S21 and T20 is required for myosin filament assembly and contractile activity, with S21 being the primary site (7, 12). We generated GFP-tagged RLC variants with negatively charged glutamate substitutions at one or both sites to mimic phosphorylation (RLC-EE, RLC-E20, RLC-E21) or alanine substitutions to mimic the unphosphorylated form (RLC-AA, RLC-A21) (Fig. S14) (28). Transgenes were expressed at comparable levels in the progeny of females bearing *sqh*¹ mutant germ lines that have a 90% reduction in endogenous RLC levels (referred to as *sqh*¹ mutants) (29) (Fig. S1 B and C), making it possible to modulate myosin activity without eliminating it. Phosphomimetic RLC is expected to be constitutively active, whereas only a fraction of wild-type RLC is predicted to be in the phosphorylated form (28, 30). Therefore, phosphomimetic variants are predicted to shift the myosin population toward assembled, contractile filaments, and unphosphorylatable variants should shift the population toward the noncontractile, monomeric form (Fig. 1A), with potential consequences for cell behavior and tissue structure.

We analyzed the effects of myosin RLC phosphovariants on axis elongation in *sqh*¹ mutants. Embryos mutant for *sqh*¹ display severely reduced elongation (Fig. 1C and D), whereas *sqh*¹ mutants expressing wild-type RLC (RLC-WT) elongated at the same rate and to the same extent as wild-type embryos, indicating that RLC-WT functions normally in elongation (Fig. 1B–D) (21, 31). These embryos were therefore used as wild-type controls. In contrast, *sqh*¹ embryos expressing unphosphorylatable RLC-AA or RLC-A21 failed to elongate, whereas phosphomimetic RLC-EE or RLC-E21 produced an intermediate amount of elongation (Fig. 1B–D). Particle image velocimetry analysis of tissue deformation in confocal movies showed that embryos expressing RLC-WT elongated 1.70 ± 0.10-fold, RLC-EE elongated 1.55 ± 0.17-fold, and RLC-AA elongated 1.15 ± 0.05-fold (Fig. 1D). Thus, RLC variant expression effectively varies the extent of tissue elongation over a broad range, providing an opportunity

to investigate the mechanisms by which regulated myosin activity contributes to efficient elongation.

Myosin II RLC Variants Display Altered Localization and Dynamics.

Actomyosin contractility is required for polarized cortical contraction and junctional remodeling during axis elongation. Dynamic myosin structures are present at cell–cell boundaries near adherens junctions and at the apical cell cortex (19, 20, 32–34). To assess the localization of myosin RLC phosphovariants to these force-generating structures, we first examined myosin II localization in fixed embryos. We focused on RLC-EE and RLC-AA, which produced epithelia that were morphologically intact but displayed significantly reduced tissue elongation. In confocal sections, RLC-WT was present in the cytoplasm, at the medial cell cortex, and near adherens junctions at the apicolateral boundaries of cells, where it was preferentially enriched (2.2 ± 0.1 -fold) (Fig. 2A and D and Fig. S24). RLC-AA was less enriched at adherens junctions compared with RLC-WT (1.4 ± 0.1 -fold, $P = 0.003$) (Fig. 2C and F and Fig. S24), with residual junctional accumulation likely due to coassembly into filaments with low levels of endogenous RLC (26). In contrast, RLC-EE was more strongly enriched at junctions relative to the medial/cytoplasmic population (2.8 ± 0.4 -fold, $P = 0.002$) (Fig. 2B and E and Fig. S24), consistent with results in cultured cells (35). In addition, RLC-EE displayed an overall reduction in apical levels and accumulated at the basal side of the epithelium, where it appeared to localize to the central yolk cell (Fig. S2B–E). Myosin II heavy chain displayed a similar localization to the RLC variants, suggesting that the RLC variants reflect the distribution of myosin motor complexes (Fig. S3).

Myosin phosphorylation decreases myosin turnover in cultured cells (27, 30, 35), but the effect of phosphorylation on myosin dynamics in multicellular tissues in vivo has not been examined. To investigate how phosphorylation influences myosin dynamics, we performed fluorescence recovery after photobleaching (FRAP) experiments on the junctional myosin population at cell–cell boundaries. RLC-WT is highly dynamic (20), with a recovery half-time of ~6 s and a mobile fraction of $68 \pm 3\%$ (Fig. 2G–I). RLC-EE displayed a similar recovery half-time, but the mobile fraction was reduced to $47 \pm 3\%$ (Fig. 2G–I), suggesting that a larger fraction of RLC-EE protein is assembled into filaments that are stably associated with the cortex. In contrast, RLC-AA turned over faster, with a recovery half-time of ~4 s, but a similar mobile fraction to RLC-WT (Fig. 2G–I). These results support the idea that phosphorylation stabilizes myosin association with the cortex.

Myosin II RLC Variants Alter the Number and Speed of Cell Rearrangements.

We next investigated the effects of changes in myosin regulation and dynamics on the cell rearrangements that drive axis elongation. First, we examined whether the RLC phosphovariants influence the number of cell rearrangements during elongation (Fig. 3A and B). A similar fraction of AP edges contracted in RLC-WT and RLC-EE embryos ($96 \pm 1\%$ and $95 \pm 1\%$) (Fig. 3F), indicating that similar numbers of rearrangements are initiated in these embryos. In contrast, fewer edges contracted in RLC-AA and *sqh*¹ ($88 \pm 2\%$ and $85 \pm 2\%$, $P \leq 0.002$), as expected for reduced myosin activity. The fraction of contracting edges was positively correlated with the extent of elongation on an embryo-by-embryo basis (Fig. 3F), consistent with an important contribution of cell rearrangement to elongation.

We next tested whether RLC variants alter rearrangement speed by measuring the rates of the two steps of cell rearrangement, interface contraction and vertex resolution (Fig. 3A and B). In RLC-WT, the median interface contraction rate was $0.83 \mu\text{m}/\text{min}$ (Fig. 3C). This rate was increased by 11% in RLC-EE ($0.91 \mu\text{m}/\text{min}$) and decreased by 40% in RLC-AA and *sqh*¹ (0.51 and $0.49 \mu\text{m}/\text{min}$). In addition, the median time required for a vertex to resolve through formation of a new interface $>1 \mu\text{m}$ in length was reduced by 30% in RLC-EE compared to RLC-WT (Fig. 3D). As a result, the median time

Fig. 1. Myosin II regulatory light chain phosphovariants disrupt *Drosophila* axis elongation. (A) Regulatory light chain (RLC) phosphorylation promotes myosin assembly and contractility. (B, Left) Schematic of *Drosophila* axis elongation. The germ band (dark gray) narrows and elongates along the anterior–posterior (AP) axis, folding over onto the dorsal side. Myosin II and Rho-kinase (green) are asymmetrically localized (planar polarized) in germ-band cells. (Right) Stills from bright-field movies of embryos expressing RLC variants in a *sqh*¹ background. The germ band at $t = 60$ min is outlined (arrowheads, posterior end). (C) Fraction of embryos that fully elongated after 2 h (black) and hatched after 1 d (gray) (11–38 embryos per genotype). (D) Germ band AP length normalized to AP length at $t = 0$ for individual embryos (3–9 embryos per genotype), quantified from particle image velocimetry analysis of confocal movies.

Fig. 2. Myosin II RLC phosphovariants display altered localization and dynamics. (A–F) RLC variants visualized with antibodies to GFP (white, green) and cell outlines visualized with antibodies to Nrt (red). (Scale bar, 5 μ m.) (A–C) Early elongation (stage 7). (D–F) Late elongation (stage 8). (G) The fraction of prebleach fluorescence intensity recovered after photobleaching GFP-tagged RLC variants at AP interfaces (18–38 edges in three to six embryos per genotype). (H) Kymographs showing fluorescence recovery in FRAP experiments. A 1- μ m region (vertical bars) was bleached at $t = 0$. Horizontal bars, time at which fluorescence recovered to half the final postbleach intensity ($t_{1/2}$). (I) RLC-EE has a reduced mobile fraction (% of prebleach fluorescence intensity recovered at $t = 30$ s) and RLC-AA has a reduced $t_{1/2}$ compared with RLC-WT.

for a rearrangement was 14.4 min for RLC-WT and 16 min for RLC-AA and *sqh*¹ (Fig. 3E), consistent with the expectation that RLC-AA is inactive and does not further increase myosin activity in *sqh*¹ mutants. In contrast, cell rearrangement time was reduced to 11.3 min for RLC-EE, due to both faster contraction and resolution (Fig. 3C–E). Thus, RLC-AA and *sqh*¹ display slowed cell rearrangement and RLC-EE accelerates rearrangement, indicating that the speed of cell rearrangement is tuned by the cellular levels of active myosin.

Phosphomimetic Myosin II RLC Promotes Higher-Order Rearrangements. Tissue elongation occurs through both local (T1 process) and higher-order (rosette) cell interactions, which are mediated by myosin-driven contraction of single or multiple cell interfaces, respectively (Fig. 3A and B) (17, 18, 36). To test whether RLC phosphovariants influence the frequency or relative contributions of these two behaviors, we quantified the frequency of T1 processes and rosettes in these embryos. On average in RLC-WT, contracting AP edges participated relatively equally in T1 processes ($48 \pm 1\%$ of AP edges) and rosettes ($47 \pm 2\%$). We exploited natural variations in the embryo population (Fig. 1D) to analyze the relationship between cell behaviors and tissue elongation on an embryo-by-embryo basis. In RLC-WT, rosette behaviors were positively correlated with tissue elongation—embryos with more rosettes elongated to a greater extent (Fig. 3H). In contrast, the number of T1 processes showed a negative correlation with the extent of elongation (Fig. 3G). This indicates that a shift in the cell rearrangement distribution toward higher-order rearrangements is associated with increased elongation.

In RLC-EE, a larger fraction of contracting AP edges formed rosettes ($54 \pm 1\%$, $P = 0.003$), accompanied by a decrease in T1 processes ($41 \pm 1\%$, $P = 0.001$). In contrast, fewer T1 processes occurred in RLC-AA ($38 \pm 1\%$, $P < 10^{-3}$) and a greater fraction of edges completely failed to contract (Fig. 3F). Variations in the number of contracting AP edges and the nature of cell rearrangements in RLC-AA and *sqh*¹ embryos did not correlate with differences in tissue elongation (Fig. 3F–H), suggesting that both types of rearrangement are ineffective in these embryos. Therefore, the cell rearrangement distribution is shifted toward fewer rearrangements in RLC-AA and more rosettes in RLC-EE, suggesting that increased myosin activity in RLC-EE embryos promotes higher-order cell interactions.

Although tissue elongation is positively correlated with increased rosette formation in embryos expressing wild-type myosin, RLC-EE embryos did not follow this trend—embryos with more rosettes elongated less (Fig. 3H). Moreover, while an increase in the speed of cell rearrangements would be expected to enhance tissue elongation, faster rearrangements are not translated into efficient elongation in RLC-EE (Figs. 1D and 3C–E). These results indicate that additional changes in cell behavior contribute to the tissue elongation defects in RLC-EE embryos.

Myosin II RLC Variants Disrupt Planar Polarized Myosin Localization and Activity. The myosin activator Rho-kinase is present in a planar polarized distribution during axis elongation (14) (Fig. 1B), raising the possibility that myosin contractility is spatially controlled by localized myosin phosphorylation. If this is the case, then RLC phosphovariants would be predicted to disrupt this spatial regulation. To test this, we developed computational methods to analyze the planar polarized localization of myosin RLC phosphovariants in living embryos (Fig. 4A–F and Movies S1–S3). RLC-WT became enriched at AP edges ~ 10 min before the onset of elongation and reached a maximum relative AP enrichment of 2.34 ± 0.15 (Fig. 4A, B, and D and Fig. S4). RLC-EE planar polarity initiated around the same time prior to elongation, but RLC-EE displayed a 25% reduction in peak planar polarity (2.01 ± 0.13) (Fig. 4A, B, and E and Fig. S4). In contrast, RLC-AA displayed an 83% reduction in peak planar polarity (1.23 ± 0.03) (Fig. 4A, B, and F and Fig. S4). These

Fig. 3. Myosin II RLC phosphovariants influence the number, speed, and nature of cell rearrangements. (A) Stills from time-lapse movies showing a T1 process (Upper) and rosette (Lower). (Scale bar, 5 μ m.) (B) Schematics of cell rearrangements showing interface contraction and vertex resolution. (C) AP interface contraction rates are increased in RLC-EE and decreased in RLC-AA and *sqh*¹ compared with RLC-WT ($P < 0.003$). (D) Vertex resolution times are reduced in RLC-EE ($P < 0.001$) and not significantly affected in RLC-AA and *sqh*¹. (E) Total rearrangement times are reduced in RLC-EE and increased in RLC-AA and *sqh*¹ ($P < 0.001$). (C–E) Each data point represents one AP edge (C and E) or vertex (D). $n = 115$ –467 edges in 3 embryos per genotype (RLC variants) or 1 *sqh*¹ embryo. (F–H) Extent of tissue elongation vs. the percentage of AP interfaces that contract to a vertex (F) and participate in T1 processes (G) or rosettes (H). (F–H) Each data point represents one embryo ($n = 3$ –9 embryos per genotype). (F) Line, guide to the eye. (G and H) Lines, best linear fits. Correlations: T1 processes, RLC-WT ($R = -0.55$) and RLC-EE ($R = 0.93$); rosettes, RLC-WT ($R = 0.79$) and RLC-EE ($R = -0.81$).

Fig. 6. Myosin II RLC phosphovariants disrupt planar polarized cell behaviors. (A) Fraction of contracting edges that are poorly oriented (0° – 75° relative to the AP axis). (B) Fraction of vertices with errors in new edge formation, including: a DV edge forms but is unstable and contracts to a vertex (orange) or an AP edge reforms (red). Resolution errors are increased in RLC-EE and RLC-AA ($P < 0.001$) (317–914 edges in 3–6 embryos per genotype). (C) In *Rok*² embryos expressing RLC-WT or RLC-EE, vertex resolution is disrupted compared with wild-type controls expressing gap43: mCherry ($P < 0.05$). *Rok*² embryos expressing RLC-EE also displayed increased interface contraction errors ($18 \pm 2\%$ of shrinking edges were oriented at 0 – 45° , compared to $2 \pm 1\%$ in controls, $P < 0.05$) (144–340 edges in 2–3 embryos per genotype). (D) Extent of tissue elongation vs. the percentage of AP cell edges with correctly oriented contraction and vertex resolution, resulting in productive cell rearrangement. $R^2 = 0.86$. Each point represents one embryo. (E and F) Stills of cell rearrangements from movies of RLC-WT (E) or RLC-EE (F). (G) Schematic showing contributions of cell rearrangement number and orientation to tissue elongation.

The spatial organization of cell behaviors was further disrupted when RLC-EE was expressed in *Rok*² maternal mutants, in which RLC-EE protein was cortically localized but not planar polarized (Fig. 4J). These embryos displayed increased errors in both contraction and resolution (Fig. 6C), consistent with an essential role for Rho-kinase in controlling spatially localized actomyosin contractility.

The total fraction of AP edges that productively contribute to tissue elongation through correctly oriented interface contraction and vertex resolution was well correlated with elongation on an embryo-by-embryo basis (Fig. 6D). This correlation across genotypes suggests that the number and orientation of cell rearrangements can account for the major effects of RLC variants on tissue elongation (Fig. 6G).

Discussion

The levels and spatiotemporal patterns of myosin contractility are actively regulated to generate tissue shape and structure. Here we show that both properties of myosin contractility are controlled at the level of myosin II regulatory light chain phosphorylation. We investigated the role of myosin phosphorylation in a multicellular system by expressing myosin RLC phosphovariants in *Drosophila* embryos with reduced levels of endogenous RLC. Variants that prevent or mimic phosphorylation both disrupt axis elongation, but differ in their effects on myosin dynamics and cell behavior. Phosphomimetic RLC-EE accelerates and *sqh*¹ and RLC-AA slow cell rearrangement, indicating that the levels of active myosin are rate limiting in this context. Strikingly, RLC-EE reduces myosin planar polarity and mechanical anisotropy, and RLC-AA abolishes both. Thus, whereas cells rearrange faster in RLC-EE, interface contraction and resolution are poorly oriented, resulting in reduced tissue elongation. These results highlight that even partial perturbations to planar polarized myosin activity can significantly disrupt the spatial organization of cell behaviors.

The misoriented cell rearrangements in embryos expressing myosin RLC phosphovariants provide direct evidence that spatiotemporally regulated phosphorylation is essential for localized actomyosin contractility. In the *Drosophila* embryo, Rho-kinase is planar polarized and required for axis elongation (14). The reduced RLC-EE planar polarity in *sqh*¹ mutants, and the lack of RLC-EE planar polarity in *Rok*² mutants, argue that Rho-kinase is an essential spatial input regulating localized actomyosin contractility during epithelial remodeling. Supporting this idea, phosphomimetic myosin RLC also disrupts myosin dynamics and radial cell polarity in apically constricting cells (40). It is also necessary to consider that mutating phosphorylation sites could fail to fully mimic the structure of phosphorylated myosin or

eliminate dynamic cycling between phosphorylated and dephosphorylated states that may be important for spatial control of contractility. Notably, eliminating myosin phosphorylation by expression of RLC-AA abolishes the ability of cortical myosin to respond to planar spatial cues. Thus, although several inputs influence myosin planar polarity during axis elongation, including the actin cytoskeleton (18), the polarity protein Par-3 (14), cell adhesion (41), and mechanical tension (20), the spatial systems that generate planar polarity require regulated RLC phosphorylation to control myosin localization. These results demonstrate that regulated myosin phosphorylation is essential for spatiotemporally controlled cell behaviors during epithelial elongation, linking molecular properties of myosin contractility to tissue morphogenesis.

Higher-order rosette behaviors are a conserved feature of tissue elongation (18, 20, 22, 42–45). In *Drosophila*, several mutants with reduced elongation have fewer rosettes (18, 44), but phosphomimetic RLC-EE is the only known perturbation that enhances rosette formation. Because the myosin cables that lead to rosette formation sustain increased tension (20), increased rosette formation is likely to be an outcome of enhanced myosin activity in RLC-EE embryos. Notably, we find that the extent of axis elongation in wild-type embryos is positively correlated with rosette formation, revealing that rosettes are closely associated with the essential mechanisms that drive elongation. Embryos expressing RLC-EE do not follow this trend. This may arise from two independent consequences of increased myosin activation: more rosettes form due to increased myosin activity, but more errors in rearrangement orientation occur because myosin is not correctly localized. Alternatively, too many overlapping rosettes could disrupt the coherence of cell rearrangements, impeding efficient remodeling. These results highlight the role of regulated myosin phosphorylation in controlling both the overall levels and patterns of myosin activity to direct cell behaviors.

Myosin contractility is a conserved mechanism for cortical contraction and junctional remodeling in epithelia (3). The increase in vertex resolution errors in embryos expressing RLC phosphovariants reveals a role for regulated myosin activity in the formation of new interfaces between cells, in addition to the well-appreciated role for myosin in cell interface contraction. These errors may result from ectopic myosin activity, which could promote contraction and oppose adhesion at newly formed interfaces. In the *Drosophila* wing, aberrant myosin accumulation at new contacts that form during cell rearrangement in *PTEN* mutants is associated with disrupted cell packing (46). Alternatively, errors in vertex resolution could be a consequence of the increased

rate of rearrangements in RLC-EE, if faster rearrangements do not allow enough time for cells to respond to biochemical signals that mediate cell–cell recognition. This model suggests a tradeoff between the speed and the precision of rearrangement, yielding specific timescales of cell rearrangement that are optimal for elongation. Finally, RLC phosphovariants could produce changes in the mechanical properties of the epithelium that constrain cell rearrangements, altering both their speed and orientation. Increased mechanical tension is associated with increased stiffness in cells and tissues (47), and RLC phosphorylation is therefore likely to alter tissue mechanics (39, 48). Future studies of how myosin activity influences the mechanical properties of epithelia in vivo will be required to understand the extent to which intrinsic properties of myosin contractility account for emergent patterns of tissue structure and mechanics.

Materials and Methods

Unless otherwise noted, embryos were maternally mutant for *sqh*¹ (29) or *Rok*² (12) and had one maternal copy each of a GFP-tagged myosin

RLC variant and gap43:mCherry (49) expressed from the *sqh* promoter. Fixed embryo imaging and FRAP were performed on a laser scanning confocal microscope. Live imaging and laser ablation were performed on a spinning disk confocal microscope outfitted with a Micropoint laser. Time-lapse movies were analyzed computationally using custom MATLAB routines (Figs. 4 and 6A) complemented with manual analysis (Figs. 3 and 6B–D). Tissue elongation was quantified by particle image velocimetry. Error bars are the SEM. Details can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We are grateful to F. Wirtz-Peitz for pSqh-attB-mEGFP; A. Mainieri and L. Fairchild for movie segmentation; O. Weitz for computational methods; R. Fernandez-Gonzalez, S. Simões, M. Tamada, and A. Vichas for helpful discussions; and R. Fernandez-Gonzalez, A. Martin, C. Vasquez, D. Robinson, D. Warshaw, and R. Zallen for critical manuscript readings. This work was supported by National Institutes of Health/National Institute of General Medical Sciences R01 Grant GM102803 (to J.A.Z.) and a Howard Hughes Medical Institute–Helen Hay Whitney Fellowship and Burroughs Wellcome Fund Career Award at the Scientific Interface (to K.E.K.). J.A.Z. is an Early Career Scientist of the Howard Hughes Medical Institute.

- Green RA, Paluch E, Oegema K (2012) Cytokinesis in animal cells. *Annu Rev Cell Dev Biol* 28:29–58.
- Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR (2009) Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol* 10(11):778–790.
- Heisenberg CP, Bellaïche Y (2013) Forces in tissue morphogenesis and patterning. *Cell* 153(5):948–962.
- Tan JL, Ravid S, Spudich JA (1992) Control of nonmuscle myosins by phosphorylation. *Annu Rev Biochem* 61:721–759.
- Suzuki H, Onishi H, Takahashi K, Watanabe S (1978) Structure and function of chicken gizzard myosin. *J Biochem* 84(6):1529–1542.
- Sellers JR, Spudich JA, Sheetz MP (1985) Light chain phosphorylation regulates the movement of smooth muscle myosin on actin filaments. *J Cell Biol* 101(5 Pt 1):1897–1902.
- Jordan P, Kares R (1997) Myosin light chain-activating phosphorylation sites are required for oogenesis in *Drosophila*. *J Cell Biol* 139(7):1805–1819.
- Komatsu S, Yano T, Shibata M, Tuft RA, Ikebe M (2000) Effects of the regulatory light chain phosphorylation of myosin II on mitosis and cytokinesis of mammalian cells. *J Biol Chem* 275(44):34512–34520.
- Dean SO, Rogers SL, Stuurman N, Vale RD, Spudich JA (2005) Distinct pathways control recruitment and maintenance of myosin II at the cleavage furrow during cytokinesis. *Proc Natl Acad Sci USA* 102(38):13473–13478.
- Wang Y, Riehmman V (2007) The role of the actomyosin cytoskeleton in coordination of tissue growth during *Drosophila* oogenesis. *Curr Biol* 17(15):1349–1355.
- He L, Wang X, Tang HL, Montell DJ (2010) Tissue elongation requires oscillating contractions of a basal actomyosin network. *Nat Cell Biol* 12(12):1133–1142.
- Winter CG, et al. (2001) *Drosophila* Rho-associated kinase (Drok) links Frizzled-mediated planar cell polarity signaling to the actin cytoskeleton. *Cell* 105(1):81–91.
- Dawes-Hoang RE, et al. (2005) folded gastrulation, cell shape change and the control of myosin localization. *Development* 132(18):4165–4178.
- Simões S, et al. (2010) Rho-kinase directs Bazooka/Par-3 planar polarity during *Drosophila* axis elongation. *Dev Cell* 19(3):377–388.
- Mason FM, Tworoger M, Martin AC (2013) Apical domain polarization localizes actin-myosin activity to drive ratchet-like apical constriction. *Nat Cell Biol* 15(8):926–936.
- Zallen JA, Wieschaus E (2004) Patterned gene expression directs bipolar planar polarity in *Drosophila*. *Dev Cell* 6(3):343–355.
- Bertet C, Sulak L, Lecuit T (2004) Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* 429(6992):667–671.
- Blankenship JT, Backovic ST, Sanny JS, Weitz O, Zallen JA (2006) Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Dev Cell* 11(4):459–470.
- Rauzi M, Verant P, Lecuit T, Lenne PF (2008) Nature and anisotropy of cortical forces orienting *Drosophila* tissue morphogenesis. *Nat Cell Biol* 10(12):1401–1410.
- Fernandez-Gonzalez R, Simoes S, Röper JC, Eaton S, Zallen JA (2009) Myosin II dynamics are regulated by tension in intercalating cells. *Dev Cell* 17(5):736–743.
- Irvine KD, Wieschaus E (1994) Cell intercalation during *Drosophila* germband extension and its regulation by pair-rule segmentation genes. *Development* 120(4):827–841.
- Nishimura T, Honda H, Takeichi M (2012) Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell* 149(5):1084–1097.
- Amano M, Fukata Y, Kaibuchi K (2000) Regulation and functions of Rho-associated kinase. *Exp Cell Res* 261(1):44–51.
- Robertson F, Pinal N, Fichelson P, Pichaud F (2012) Atonal and EGFR signalling orchestrate rok- and Drak-dependent adherens junction remodelling during ommatidia morphogenesis. *Development* 139(18):3432–3441.
- Bulgakova NA, Grigoriev I, Yap AS, Akhmanova A, Brown NH (2013) Dynamic microtubules produce an asymmetric E-cadherin-Bazooka complex to maintain segment boundaries. *J Cell Biol* 201(6):887–901.
- Dean SO, Spudich JA (2006) Rho kinase's role in myosin recruitment to the equatorial cortex of mitotic *Drosophila* S2 cells is for myosin regulatory light chain phosphorylation. *PLoS ONE* 1:e131.
- Uehara R, et al. (2010) Determinants of myosin II cortical localization during cytokinesis. *Curr Biol* 20(12):1080–1085.
- Sweeney HL, Yang Z, Zhi G, Stull JT, Trybus KM (1994) Charge replacement near the phosphorylatable serine of the myosin regulatory light chain mimics aspects of phosphorylation. *Proc Natl Acad Sci USA* 91(4):1490–1494.
- Kares RE, et al. (1991) The regulatory light chain of nonmuscle myosin is encoded by spaghetti-squash, a gene required for cytokinesis in *Drosophila*. *Cell* 65(7):1177–1189.
- Vicente-Manzanares M, Horwitz AR (2010) Myosin light chain mono- and di-phosphorylation differentially regulate adhesion and polarity in migrating cells. *Biochem Biophys Res Commun* 402(3):537–542.
- Butler LC, et al. (2009) Cell shape changes indicate a role for extrinsic tensile forces in *Drosophila* germ-band extension. *Nat Cell Biol* 11(7):859–864.
- Rauzi M, Lenne PF, Lecuit T (2010) Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature* 468(7327):1110–1114.
- Fernandez-Gonzalez R, Zallen JA (2011) Oscillatory behaviors and hierarchical assembly of contractile structures in intercalating cells. *Phys Biol* 8(4):045005.
- Sawyer JK, et al. (2011) A contractile actomyosin network linked to adherens junctions by Cnca/afadin helps drive convergent extension. *Mol Biol Cell* 22(14):2491–2508.
- Watanabe T, Hosoya H, Yonemura S (2007) Regulation of myosin II dynamics by phosphorylation and dephosphorylation of its light chain in epithelial cells. *Mol Biol Cell* 18(2):605–616.
- Weaire D, Rivier N (1984) Soap, cells and statistics—random patterns in two dimensions. *Contemp Phys* 25(1):59–99.
- Hutson MS, et al. (2003) Forces for morphogenesis investigated with laser microsurgery and quantitative modeling. *Science* 300(5616):145–149.
- Zhou J, Kim HY, Davidson LA (2009) Actomyosin stiffens the vertebrate embryo during crucial stages of elongation and neural tube closure. *Development* 136(4):677–688.
- Fischer SC, et al. (2014) Contractile and mechanical properties of epithelia with perturbed actomyosin dynamics. *PLoS ONE* 9(4):e95695.
- Vasquez CG, Tworoger M, Martin AC (2014) Dynamic myosin phosphorylation regulates contractile pulses and tissue integrity during epithelial morphogenesis. *J Cell Biol* 10.1083/jcb.201402004.
- Levayer R, Lecuit T (2013) Oscillation and polarity of E-cadherin asymmetries control actomyosin flow patterns during morphogenesis. *Dev Cell* 26(2):162–175.
- Nishimura T, Takeichi M (2008) Shroom3-mediated recruitment of Rho kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. *Development* 135(8):1493–1502.
- Lienkamp SS, et al. (2012) Vertebrate kidney tubules elongate using a planar cell polarity-dependent, rosette-based mechanism of convergent extension. *Nat Genet* 44(12):1382–1387.
- Tamada M, Farrell DL, Zallen JA (2012) Abl regulates planar polarized junctional dynamics through β -catenin tyrosine phosphorylation. *Dev Cell* 22(2):309–319.
- Williams M, Yen W, Lu X, Sutherland A (2014) Distinct apical and basolateral mechanisms drive planar cell polarity-dependent convergent extension of the mouse neural plate. *Dev Cell* 29(1):34–46.
- Bardet PL, et al. (2013) PTEN controls junction lengthening and stability during cell rearrangement in epithelial tissue. *Dev Cell* 25(5):534–546.
- Kasza KE, et al. (2007) The cell as a material. *Curr Opin Cell Biol* 19(1):101–107.
- Davidson LA (2011) Embryo mechanics: Balancing force production with elastic resistance during morphogenesis. *Curr Top Dev Biol* 95:215–241.
- Martin AC, Gelbart M, Fernandez-Gonzalez R, Kaschube M, Wieschaus EF (2010) Integration of contractile forces during tissue invagination. *J Cell Biol* 188(5):735–749.