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PCP and septins compartmentalize cortical actomyosin to direct collective cell movement

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Abstract

Despite our understanding of actomyosin function in individual migrating cells, we know little about the mechanisms by which actomyosin drives collective cell movement in vertebrate embryos. The collective movements of convergent extension drive both global reorganization of the early embryo and local remodeling during organogenesis. We report here that Planar Cell Polarity (PCP) proteins control convergent extension by exploiting an evolutionarily ancient function of the septin cytoskeleton. By directing septin-mediated compartmentalization of cortical actomyosin, PCP proteins coordinate the specific shortening of mesenchymal cell-cell contacts, which in turn powers cell interdigitation. These data illuminate the interface between developmental signaling systems and the fundamental machinery of cell behavior and should provide insights into the etiology of human birth defects such as spina bifida and congenital kidney cysts.

Convergent extension (CE) is an essential morphogenetic process that shapes tissues and organs during embryonic development (1), and defective convergent extension is implicated in structural birth defects ranging from spina bifida to congenital kidney cysts (2–4). The longest-standing model of CE is vertebrate gastrulation (1, 5, 6), during which mediolaterally-oriented cell intercalations elongate the body axis and drive the internalization of mesoderm and endoderm within the ectoderm (Fig. S1A) (7,8). Pioneering experiments in *Xenopus* frogs revealed that mediolaterally-polarized protrusions on gastrula mesenchyme cells make stable attachments to neighboring cells, and these protrusions are thought to exert traction, effecting interdigitation by a cell crawling mechanism (Fig. S1B, left) (7,8). PCP proteins are essential for polarization and stabilization of mediolateral protrusions, and thus for CE (9, 10). These cell behaviors, and their regulation by PCP proteins, are conserved across vertebrates, including mammals (1, 6, 11).

PCP proteins also control CE in vertebrate epithelial cells, where cell movement is accomplished not by crawling, but rather by active shortening of cell-cell junctions (Fig. S1B, right)(3, 12). This junction-shrinking mechanism is reminiscent of that observed during CE in the *Drosophila* germband epithelium, which does not require PCP proteins (13–15). In light of these findings, we addressed two fundamental questions concerning CE in vertebrate gastrula mesenchyme cells. We asked where and how actomyosin-based

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contraction acts to drive cell intercalation; and we asked how PCP proteins act to spatially organize such contraction.

As an initial proxy for actomyosin-based contraction, we examined phosphorylation of myosin regulatory light chains (MRLCs)(16). Phosphorylated Myosin II (pMyoII) was enriched along mediolaterally-aligned cell-cell junctions where the anterior and posterior faces of neighboring cells abut (Fig. 1A–A'', C; Fig. S2). These so-called “v-junctions” (nomenclature of ref. 17) displayed significant enrichment of pMyoII (Fig. 1B, C, gray) as compared to adjoining, less-mediolaterally aligned cell edges (so-called “t-junctions”)(Fig. 1B, C, red). Levels of cortical pMyoII correlated significantly with junction orientation (Fig. S3A), but such correlation was not observed for the generalized cell junction marker β -catenin (Fig. S3B–D''). This enrichment of pMyoII suggests that actomyosin-based contraction along v-type junctions may drive mediolateral cell intercalation, and live imaging revealed the consistent association of shrinking v-junctions with cell rearrangement (Fig. S4, Movies 1, 2).

To ask directly if enriched pMyoII at v-type junctions contributes to cell intercalation, we assessed patterns of cell cortex tension using laser microdissection, where retraction following laser cutting indicates relative tension in the cell edge (Fig. S5A–B') (17, 18). Consistent with the pattern of pMyoII, mediolaterally-aligned v-junctions displayed significantly higher cortical tension than did adjacent, less mediolaterally-aligned t-junctions (Fig. 1D; S5C, Movies 3, 4). Moreover, tension in v-junctions correlated with cell edge length (Fig. 1E, gray) and also correlated with changes in neighboring cell shapes as captured by the angle ϕ (Fig. 1B, F, gray; Fig. S6). Neither correlation was observed for t-junctions (Fig. 1E, F, red). Together, these data suggest that accumulating tension in shrinking v-type junctions exerts a pulling force on adjacent t-junctions to drive mediolateral cell intercalation during CE in the vertebrate gastrula. A similar mechanism drives CE in *Drosophila* epithelial cells (17).

To further test this model, we explored actin dynamics using live imaging of mosaic embryos expressing different colors of an actin biosensor in neighboring cells (Fig. 2A; Fig. S7A). This assay illuminated the actin-rich protrusions at mediolateral cell vertices (Fig. 2A', arrowheads)(7, 9). It also revealed pulses of actin assembly along shrinking v-type junctions (Fig. 2A', arrows; 2B; S7A; Movie 5). Similar pulses characterize *Drosophila* epithelial CE (19). Accumulation of actin significantly correlated with v-junction shrinkage (Fig. 2C).

We then took a bioinformatic approach to identify specific MRLCs that may act during CE (Fig. S8A, A'). We identified Myl9, which we found to be essential for gastrulation (Fig. S8B, C). Expression of a functional GFP fusion revealed pulses of Myl9 accumulation at v-junctions that correlated with actin pulses at these sites (Fig. S9A–C). Neighboring t-junctions displayed neither actin nor Myl9 pulses, and Myl9 did not associate with the actin present at mediolateral cell vertices (Fig. S9C–E).

Because cell movement frequently involves spatially and functionally distinct actomyosin populations (19–22), we further interrogated actin dynamics using a photoactivatable actin

biosensor. We found that cortical actin was constrained at mediolateral vertices but spread rapidly along anteroposterior cell edges (Fig. 3A (blue), Fig. S10A). Thus, v-type junctions in the *Xenopus* gastrula are characterized by cortical actin flow, coordinated pulses of actin and Myl9, enriched pMyoII, and enhanced cell cortex tension. These data argue that actomyosin contraction along anteroposteriorly-apposed cell edges results in their preferential shrinkage, thereby driving mediolateral cell intercalation during vertebrate gastrulation.

In this model, dynamic actomyosin must be actively partitioned to v-type cell edges. The septin cytoskeleton plays an ancient role in compartmentalizing cortical actomyosin during cytokinesis (23, 24), and we previously showed that the PCP protein WPCP/Fritz controls cell cortex dynamics and CE via septins (25). We therefore assessed Septin localization using a functional Sept7-GFP-fusion construct (25). Sept7-GFP was preferentially enriched at mediolateral cell vertices (Fig. 3B, B'), and the intense population of actin filaments at these locations was specifically eliminated following Sept7 knockdown (Fig. 3C–E; S7B). Moreover, the lateral spread of actin from mediolateral vertices was significantly increased following Sept7-KD (Fig. 3A (red), S10B; S7C) though knockdown did not alter actin dynamics along cell edges (Fig. S10C). These data suggest that Sept7 maintains the stable actin population at mediolateral cell vertices. Sept7 knockdown also abolished the planar polarization of pMyoII, such that the normally significant difference between pMyoII levels in v-type and t-type junctions was lost in morphants (Fig. 4A–A'', B; S11A)

These changes in actomyosin had substantial functional consequences, as the normally significant difference in cortex tension between v- and t-type junctions was also eliminated by Sept7 knockdown (Fig. 4C). There was not, however, a global defect in actomyosin contraction, as overall cortex tension (the mean tension for all junctions) was not different between Sept7 morphants and control embryos (Fig. 4D). Most significantly, v-type junctions failed to accumulate tension and shrink following Sept7 knockdown, and convergent extension was severely inhibited (Fig. S11B–D''). Thus, both the accumulation of pMyoII and the mediolaterally-directed increase in cell cortex tension along v-junctions are dependent upon Sept7 function. Together with the observed effects on local actin dynamics (Fig. 3A, S10B), these data suggest that Sept7 at cell vertices acts to restrict actomyosin contraction to v-type cell edges and is thereby central to their preferential shrinkage and to convergent extension.

Finally, we sought to link Sept7 and actomyosin to the PCP proteins, known regulators of CE (11). First, we demonstrated that pharmacological manipulation of pMyoII disrupted CE and that loss of pMyoII reduced cell cortex tension (Fig. S12), as these parameters had not yet been explored in this system. Then, we showed that disruption of the core PCP protein Dishevelled (Dvl) in *Xenopus* gastrula mesoderm reduced overall pMyoII levels (Fig. S11E), consistent with data from *Drosophila* (26). By contrast, Sept7 knockdown elicited a marked increase in pMyoII (Fig. S11E), suggesting a model in which Sept7 at mediolateral vertices suppresses the action of PCP proteins which, conversely, act at anteroposteriorly-apposed cell faces (e.g. (27, 28)). Finally, interference with Dvl function also perturbed the normal localization of Sept7 to cell vertices (Fig. 5A–C) and disrupted the correlation between edge orientation and pMyoII intensity (Fig. S13A–D). Together, our data suggest

that Dvl function is required to localize Sept7 to cell vertices, which in turn compartmentalizes cortical actomyosin and directs the shrinkage of v-type cell edges that ultimately drives cell intercalation and convergent extension (Fig. S14).

We propose here that significant force for CE in *Xenopus* mesoderm is generated by contraction of mediolaterally-oriented cell edges that join the anteroposterior faces of neighboring cells (Fig. S14). This new model is parsimonious at many levels. First, the pattern of pMyoII-based contraction reported here is consistent with reports of core PCP protein localization at anteroposterior cell faces (12, 27, 28) and with the positive role for core PCP proteins in phosphorylating MyoII (Fig. S11E; refs. 12, 26). This model is also consistent with embryological studies showing that CE in *Xenopus* requires precise anteroposterior patterning (29), as it does in *Drosophila* (13). Finally, the model reconciles the long-standing archetype of vertebrate gastrulation with more recent results in *Drosophila* (14, 15) and in vertebrate neuroepithelia and kidney tubules (3, 12), suggesting that contraction of cell junctions via compartmentalized actomyosin is an ancient and unifying feature of CE.

Our data are also significant because spatially distinct populations of actomyosin are central to cell movement but little is known about such populations in vertebrate embryos. In *Drosophila*, CE involves a complex interplay between cortical actomyosin at v-junctions and a distinct “medial” network of actomyosin (13, 14, 17–19, 21, 22). A similar picture is emerging in vertebrates, as Dvl governs not only the polarization of cortical actomyosin deep in the cell (Fig. S13), but also the oscillatory behavior of a system of actomyosin nodes and struts (30, 31).

Finally, the mechanistic link between core PCP proteins and septins remains unclear, but we previously discovered that WDPCP (aka Fritz) binds to septins and controls their localization during both ciliogenesis and cell movement in *Xenopus* (25); a later study confirmed these results in mice (32). Because mutations in WDPCP/Fritz were found in human ciliopathy patients (25), our data here on septin function may provide new insights into the molecular links between CE, neural tube birth defects (2), and cystic kidney disease (3, 4).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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General audience paragraph

Mechanisms controlling the migration of individual cells have been studied for decades, and it is well known that the same basic machinery used for muscle contraction (actomyosin) is also deployed to generate force for cell movement. In contrast to such individual cell migrations, the sculpting of embryos during development involves coordinated movement of cells in large groups. How actomyosin is controlled during such collective cell movement remains poorly understood. In this issue of *Science*, Shindo and Wallingford elucidate the mechanism by which a well-known molecular system for cell-cell communication interfaces with the actomyosin machinery to direct collective cell movement.

Figure 1. Myosin-mediated cell cortex tension is planar polarized in *Xenopus* mesoderm (A–A'') pMyoII immunostaining of notochord in vivo (bar=20 μm) (B) Illustration defining T- and V-type junctions, and vertex angle ϕ (gray: ϕ for v-junction, red: ϕ of t-junction). (C) Normalized intensity of pMyoII, defined as intensity relative to the maximum (=100%) and minimum (=0%) raw intensity for each image ($n=177(v)$, $n=221(t)$, 3 embryos). (D) Average vertex position change following ablation. (E, F) Scatter plots showing correlation between tension and edge length (E) or vertex angle (F) ($n=84(v)$, $n=24(t)$, 54 embryos).

Figure 2. Pulsed actin assembly at v-junctions during edge shortening

(A) Mosaic expression of two colors of the actin biosensors Utrophin-FP. (A') Time-lapse following the junction in box A'. Two populations of actin are visible: bipolar lamellipodia (green, arrowheads) and actin at the v-junction (magenta, arrows). (B) Mean intensity of Utrophin (Utr, pink line) at v-junctions during edge length shortening (black line). (C) Edge length change is correlated with increase of Utrophin intensity (red), but not with reduction in Utrophin intensity (blue) (n=133 cycles, 6 explants).

Figure 3. Septin 7 compartmentalizes cortical actin dynamics

(A) Quantification of the lateral spread of paGFP-Utr at a cell vertex or edge (see Fig. S10A) (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, $n = 7-11$ explants). (B) GFP-sept7 localization in explant. (B') GFP-sept7 co-localizes with Utr-RFP at vertices. (C, C') Utr-GFP normally localizes at vertices. (D, D') Sept7 knockdown disrupts Utraphin accumulation at the vertices. (E) Quantification of C and D (see also Fig. S7B). (bar = 20 μm)

Figure 4. Sept7 controls planar polarization of cell cortex tension

(A–A'') pMyoII immunostaining of notochord in sept7 knockdown embryos (compare with Figure 1A) (bar=20 μm). (B, C) The normal differences in pMyoII or vertex shift following laser ablation between v- and t- junctions are lost in sept7 morphants (B, n=74(v), n=188(t), 3 embryos) (C, n=50(v), n=28(t), 39 explants). (D) Average vertex shift for all junctions is not changed following sept7 knockdown, but is significantly reduced by treatment with a myosin kinase inhibitor (control: n=108, sept7-MO: n=78, ML-7: n=36, 111 explants).

Figure 5. PCP signaling regulates Sept7 localization

(A, A') GFP-sept7 localizes at vertices in control explants. (B, B') Dominant negative Dishevelled (Xdd1) promotes ectopic GFP-sept7 localization along edges (arrowheads). (C) Quantification of GFP-sept7 localization (control: $n=67$, 6 explants, sept7-MO: $n=50$, 6 explants, bar=20 μ m) (see also Fig. S7B).