様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　３０　年　　６　月　　８　日現在

機関番号：　８２４０１
研究種目：　基盤研究(B)（一般）
研究期間：　2015～2017
課題番号：　１５Ｈ０４３７３

研究課題名（和文）Elucidating the mechanisms underlying epithelial cell height change mediated by modifications of apical-basal polarity

研究課題名（英文）Elucidating the mechanisms underlying epithelial cell height change mediated by modifications of apical-basal polarity

研究代表者

ＷＡＮＧ　ＹＵＣＨＩＵＮ（Wang, Yuchiun）

国立研究開発法人理化学研究所・多細胞システム形成研究センター・チームリーダー

研究者番号：８０７２５９９５

交付決定額（研究期間全体）：（直接経費）　　12,400,000円

研究成果の概要（和文）：本研究は、ネットワークを形成した微小管が細胞の極性の変化に依存して能動的に細胞の形態変化を制御する機構を明らかにした。この微小管ネットワークはPatroninにより頂端膜に裏打ちされ、Dyneinモーターの駆動力により頂端面を押し上げる力を生じ、ドーム様構造の形成に貢献する。このネットワークはPatroninとKataninとの拮抗による急速なリモデリング下にあり、組織に形態変化が起こる以前の細胞の大きさ・形態の恒常性を維持する。この後頂端面が胚の表面から沈み込み折りたたみ構造を形成する際には、細胞の極性変化に応答したPatroninの再分布が細胞形態の変形に重要な役割を果たすことを示した。

研究成果の概要（英文）：In the Drosophila gastrula, dorsal fold formation occurs despite a lack of localized myosin changes, while the fold-initiating cells reduce cell height following basal shifts of polarity via an unknown mechanism. We show that cell shortening that initiate dorsal fold formation depends on an apical microtubule network that is organized by the CAMSAP protein Patronin. Prior to gastrulation, microtubule forces generated by the minus-end motor dynein scaffold the apical cell cortex into a dome-like shape, while the severing enzyme Katanin facilitates network remodelling to ensure tissue-wide cell size homeostasis. During fold initiation, Patronin redistributes following basal polarity shifts in the initiating cells, apparently weakening the scaffolding forces to allow dome descent. The homeostatic network that ensures size/shape homogeneity is thus repurposed for cell shortening, mechanistic linking epithelial polarity and folding via a microtubule-based mechanical mechanism.

研究分野：　Cell Biology

キーワード：　Epithelial folding　Apical-basal polarity　Microtubule mechanics

１．研究開始当初の背景

The dorsal side of the gastrulating *Drosophila* embryo forms two epithelial folds, called the anterior and the posterior dorsal folds (DFs). Dorsal fold formation has emerged as a crucial model system for alternative folding mechanisms since the levels of myosin, the actin motor that produces contractile forces to induce apical cell shape changes in most of the epithelial folding processes examined thus far, low and uniform across the tissue. Following the completion of cellularization that forms the first embryonic epithelial layer, DF formation begins as two stripes of initiating cells straddling across the dorsal surface at stereotypical locations become shorter than their neighbors, leading to the formation of anterior and posterior DFs eventually. The initiation of cell shortening is stereotypical and occurs prior to global tissue rearrangement, called germband extension (GBE), and thus must be controlled locally, rather than a result of passive buckling due to external compression. Prior to cell shortening, Par-1, the MARK family kinase that specifies the basal-lateral membrane, retracts its apical margin and becomes downregulated, resulting in basal repositioning of adherens junctions (AJs) in the initiating cells. Following such basal polarity shifts, the apices of initiating cells shrink and descend below the embryonic surface. How basal polarity shifts result in shrinkage and descent of the apices to reduce cell height was not known, however.

２．研究の目的

The epithelial cells are typically polarized along the apical-basal axis and manifest distinct cell heights that are critical for their physiological function. The connection between polarity and cell height, albeit intuitive, has never been rigorously examined. We proposed to use an epithelial folding process that is driven by differential changes of apical-basal polarity to investigate how these changes influence the dynamics of microtubule and the apical cell shape. Our goal was to establish a mechanistic understanding of the intricate interplay between cell polarity, cytoskeleton and cell surface mechanics to resolve the long-standing question of cell height control in the context of epithelial morphogenesis.

３．研究の方法

Drosophila genetics, immunofluorescence, confocal microscopy, two-photon scanning microscopy, superresolution imaging, image processing and quantitative RT-PCR.

４．研究成果

To better understand how basal polarity shifts result in cel height reduction, we began by investigating the function of the CAMSAP family microtubule minus-end protector Patronin because recent work showed the cortical localization of Patronin is controlled by Par-1, raising the question whether Patronin may underlie cell shortening in response to Par-1 downregulation in the initiating cells. We found that Patronin organizes a previously undescribed, non-centrosomal microtubule network at the apical cortex, and Patronin is required for the smoothening of apical contour for the formation of the spherical apical membrane domain that we called the “apical dome”. This apical network consists primarily of the unstable form of microtubules, contains both the minus ends (Patronin) and the plus ends (EB1), and likely is anchored to the cortex via Patronin. We hypothesized that the minus-end directed motor Dynein, via bivalent crosslinking and sliding of microtubule filaments anchored at the cortex at their minus end, could generate pushing forces to scaffold the dome shape given that the apical network contains microtubule filaments arranged in a disordered manner and that microtubule filaments are known to possess high bending rigidity. Genetic loss-of-function analysis of Dynein supports this hypothesis that the apical surface becomes jagged and protrusive, similar to the phenotype caused by RNAi of Patronin. Thus, we propose that minus-end anchorage coupled with minus-end motor dependent crosslinking generate outward pushing forces that scaffold the apical cortex to form a dome-like, spherical shape.

How is this apical scaffold involved in DF initiation? We found that during fold initiation, Patronin redistributes following basal polarity shifts in the initiating cells in response to the downregulation of Par-1. Such a basal redistribution apparently weakens the scaffolding forces to allow dome descent.

We showed that overexpression of Patronin at saturating levels blocks dome descent and DF formation, likely because of the loss of local discrepancies of Patronin cortical distribution. These results thus support our model that basal shifts of Patronin cortical distribution upon Par-1 downregulation underlies dome descent.

We reasoned that the apical network must undergo constant, rapid remodeling to respond to shifts in cell polarity. In support of this, we found that Patronin recruits the microtubule severing enzyme Katanin to the apical cortex and that the Patronin-Katanin circuit facilitates network remodeling. Prior to the onset of gastrulation at which the shifts of basal polarity begin in the initiating cells, the rapidly remodeling network appears to constitute a mechanism that dampens the mechanical noises that may arise within the tissue, ensuring tissue-wide cell size homeostasis, as evidenced by an increase in the inhomogeneities of cell size/shape in embryos in which either Patronin or Katanin activity is reduced. At the onset of DF initiation, however, the homeostatic network that ensures size/shape homogeneity during cellularization is repurposed for cell shortening during gastrulation by coupling to the basal polarity shifts (Figure 1).

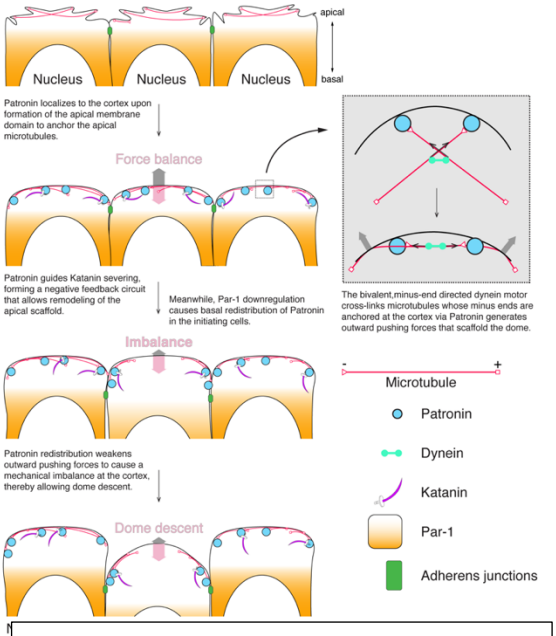

Figure 1. Schematic representation of the cellular and mechanical models for dorsal fold initiation involving the polarity-coupled microtubule network its modulators, including Patronin, Dynein and Katanin.

In summary, our results establish a novel mechanism underlying cell shortening following the initiation of basal polarity shifts. This mechanism links apical-basal polarity to epithelial folding via a microtubule-based mechanical mechanism.

The existence of a microtubule network that buffers mechanical pre-stress present within the tissue to ensure size/shape homeostasis raises the possibility that release of such pre-stress via a localized, spatially programmed of force imbalance may represent a general mechanism for rapid, efficient reorganization of tissue architecture.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計1件）

Takeda, M., Sami, M. M., and Wang, Y.-C. (2018) A homeostatic apical microtubule network shortens cells for epithelial folding via a basal polarity shift. *Nature Cell Biology* 20: 36-45. (refereed)

〔学会発表〕（計5件）

1. Wang, Y.-C.: 2017 ASCB/EMBO meeting, Philadelphia, USA. Dec, 2017
2. Wang, Y.-C.: Mechanical Forces in Biology, EMBL symposium, Heidelberg, Germany. July, 2017
3. Wang, Y.-C.: 12th Japanese Drosophila Research Conference (JDRC12), Rikkyo University, Tokyo, Japan. Sep, 2016
4. Wang, Y.-C.: From Genes to Growth and Form, KITP Program, Kavli Institute for Theoretic Physics, Santa Barbara, USA. Aug, 2016
5. Wang, Y.-C.: 56th Annual *Drosophila* Research Conference. Chicago, USA. March, 2015

〔図書〕（計0件）

〔産業財産権〕
○出願状況（計0件）

○取得状況（計0件）

〔その他〕
ホームページ等

http://www.cdb.riken.jp/epm/index-jp.html

６．研究組織

(1)研究代表者

ワン　ヨウチュン（Wang, Yu-Chiun）

国立研究開発法人理化学研究所・多細胞システム形成研究センター・チームリーダー

研究者番号：80725995

(2)研究分担者

N/A

(3)連携研究者

N/A

(4)研究協力者

N/A