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研究課題名（和文）The counterbalancing forces underlying polarity-coupled epithelial cell height control prior to and during epithelial folding

研究課題名（英文）The counterbalancing forces underlying polarity-coupled epithelial cell height control prior to and during epithelial folding

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研究成果の概要（和文）：上皮の折り畳みは、細胞と組織の力の一時的な不均衡から生じる面外変形プロセスである。ショウジョウバエの胎生期に形成される背側の折り畳みは、細胞の極性が基底部で移動することで始まり、最終的に組織を変形させるために細胞を短縮させる。最近発表された研究では、細胞の短縮は、極性の変化に応じて、CAMSAPタンパク質のパトロニンによって先端側に固定されている皮質の微小管ネットワークの再分配に依存していることが明らかになった。また、理研BDRの柴田研究室との共同研究により、膜テザーによる微小管ネットワークの乱れをシミュレーションするための粗視化分子動力学モデルを構築しました。

研究成果の学術的意義や社会的意義
Proper cell shapes are crucial for effective execution of cellular functions. Transmission of neuronal signals requires elongated cells, while gas exchange in the lung need flat cells. Revealing mechanics underlying cell shape can elucidate how cells acquire their physiological functions.

研究成果の概要（英文）：Epithelial folding is an out-of-plane deformation process resulting from transient imbalance of cellular and tissue forces. The dorsal folds that form during Drosophila gastrulation are initiated by basal shifts of cell polarity leading to cell shortening for the eventual deformation of the tissue. Our recently published work showed that cell shortening depends on the redistribution of a cortical microtubule network that is anchored apically by the CAMSAP protein Patronin in response to the polarity shifts. During this granting period, we established a robust set of imaging probes that can be used to investigate dorsal fold mechanics, employed optogenetic tools to manipulate morphogenetic forces, identified α-Spectrin as a candidate that confers membrane elasticity during apical dome decent, and constructed a coarse-grained molecular dynamics model for simulation of disorder microtubule network with membrane tethers through collaboration with the Shibata lab at RIKEN BDR.

研究分野：Cell biology

キーワード：microtubule forces　epithelial cell shape　CAMSAP/Patronin　optogenetics

１．研究開始当初の背景

Epithelial folding is an out-of-plane deformation process resulting from transient imbalance of cellular and tissue forces. The dorsal folds that form during *Drosophila* gastrulation are initiated by basal shifts of cell polarity leading to cell shortening for the eventual deformation of the tissue. Dorsal fold formation occurs despite lack of overt changes in myosin contractility and has emerged as a crucial model system for the investigation of non-canonical folding mechanisms. Our recently published work showed that cell shortening depends on the redistribution of a cortical microtubule network that is anchored apically by the CAMSAP protein Patronin in response to the polarity shifts, raising the questions as to how microtubule forces are generated to control cell shape, and what counteracting forces act in concert to orchestrate cell shortening. In the proposed project, we aimed to employ a multi-scale approach to identify and characterize the counterbalancing forces prior to and during epithelial folding.

２．研究の目的

The overarching goal of our lab is to elucidate the molecular and mechanical mechanisms that govern the homeostatic and transformative states of cell and tissue morphology during epithelial folding. Epithelial folding is a 3D, out-of-plane deformation process that can be induced by local forces that actively bend the tissue, or by global stresses that cause passive buckling of the epithelia due to mechanical instabilities. Both types of folding processes have been primarily associated with modulation of myosin-dependent contractility. During *Drosophila* gastrulation, however, dorsal fold formation occurs despite lack of overt myosin changes, whereas the initiating cells display a characteristic, stereotypical basal shifts of cell polarity, which leads subsequently to the reduction of cell height. The dorsal fold system has thus emerged as a crucial model system for the investigation of non-canonical folding mechanisms, promising to reveal not only the alternative cellular forces that might otherwise be overlooked in contexts in which myosin contractility dominated, but also to link apical-basal polarity mechanistically to the control of epithelial cell height.

There are three specific aims associated with the proposed project:

1) Generating imaging tools and probes to visualize components related to the function of Patronin during dorsal fold initiation.
2) Developing optogenetic tools and methodologies to manipulate epithelial folding mechanics in the contexts of dorsal fold formation and beyond.
3) Identifying the mechanical mechanism that counteracts microtubule pushing forces for the shortening of dorsal fold initiating cells

３．研究の方法

This project employs the following methodologies.

1) BAC recombineering for the generation of fluorescent tagged components related to the dorsal fold mechanics: The use of the BAC genomic construct allows us to express the fluorescent tagged protein at its endogenous level, utilizing its native genomic regulatory environment, thereby avoiding the potential issue of non-physiological or toxic effect of overexpression. The well-established BAC recombineering approach also allows us to generate and test several tagging strategies more rapidly than direct genomic editing approach using the CRISPR-Cas9 system.

2) Quantitative live imaging via multiple imaging modalities to bridge across scales: We have previously utilized standard confocal scanning microscopy and multi-photon excitation scanning microscopy to analyze dorsal fold dynamics in living embryos, as well as Zeiss Airsyscan super-resolution system on fixed samples. During this granting period, we have expanded our imaging modality to include a) the use of the multi-photon system for multi-view, embryo-scale imaging in order to track and correlate major morphogenetic events across the entire embryo; 2) a newly established spinning disc confocal system, based on Yokogawa CSU-SORA, that enables both standard confocal and super resolution imaging at high frame rates.

3) Optogenetic manipulation of morphogenetic forces: During this granting period, we have successfully executed optogenetic manipulation of morphogenetic mechanics, using primarily the

CIBN-CRY2 photodimerization system. We employed existing constructs to cause increased or decreased actomyosin contractility. In addition, we have been actively developing novel optogenetic tools for manipulation of protein function and mechanics.

4) Laser ablation with pulsed IR laser: We have been using this approach to cause laser damage in the developing embryo. This experimental approach allows us to perform the following three types of manipulations: a) Laser microsurgery to reveal tensile stress at the cellular and tissue level; b) Tissue-scale damage for reduction of cell and tissue stiffness that reveals the existence of compressive stress at the embryo scale; c) Cell elimination during cellularization resulting from nuclear fall-out of the damaged cells, used in experiments that test the mechanical contribution of a given region in the embryo.

４．研究成果

During this granting period, we have achieved the following objectives.

1) Establishment of a robust set of imaging probes that can be used to investigate dorsal fold mechanics:

Using BAC recombineering, we have generated genomic constructs that can be used to image α-Spectrin, a component of the Spectin membrane skeleton; Dhc64C, the canonical Dynein heavy chain in *Drosophila*; and Shot, the Spectraplackin dual cytoskeleton linker. In addition, we have produced a) a bright microtubule probe, EMTB-3XGFP, based on the established probe in the vertebrate, and a 3XmScarlet version, a bright red fluorescent protein of the same probe; b) a superior membrane marker with either a GFP or a mScarlet tag that labels the cell surface along the entire apical-basal axis; and c) our first BFP-tagged construct, Histone-BFP, allowing us to, for the first time, performing three-color live imaging in the fly embryo. These probes, in conjunction with other published probes that we have acquired from other laboratories, including E-Cadherin-3XGFP, Myosin-3XmKate2, Centrosomin-GFP (for centrosome), Utrophin-Venus and Utrophin-mCherry (for F-actin), EB1-GFP (for microtubule plus ends) and PATj-GFP (for basal membrane), will allow us to comprehensively observe and describe cellular dynamics including cell shape, cell-cell adhesion, cytoskeletal reorganization and cellular mechanical forces.

Using these newly established probes, we have observed that α-Spectrin is initially localized to the basal junction during cellularization, while beginning to enrich at the apical junction, including a more scattered localization in the contact-free region apical to the junction, towards the end of cellularization. The junctional localization is rather continuous, as compared to the discontinuous pattern of E-Cadherin. Furthermore, we confirmed that the apical region of the cells assembles a microtubule network structure, using EMTB-3XGFP and EB1-GFP, consistent with our previous description using immunofluorescence of microtubule antibodies.

2) Employment of optogenetic tools to disrupt actomyosin contractility:

During the granting period, we have endeavored into a separate project that concerns the mechanism and the spatial-temporal precision of cephalic furrow formation. We employed optogenetic manipulation developed within the framework of tool developed envisioned in this grant, the results of which had been incorporated into a paper published in Developmental Cell, entitled “Tissue-scale mechanical coupling reduces morphogenetic noise to ensure precision during epithelial folding”.

Briefly, in this project we conducted a systematic, multiscale characterization of cephalic furrow formation. We showed that a spatially precise Btd/Eve positional code with single-cell row resolution specifies the cephalic furrow by activating lateral myosin contractility to shorten the initiating cells. However, myosin contractility is spatially and temporally heterogeneous, and cell shortening obeys the Btd/Eve positional code with only 80% accuracy. Remarkably, however, the initiating cells precisely align among themselves so that spatial linearity of the furrow structure is maintained throughout furrow initiation. This observation suggests the existence of a “denoising” mechanism.

We found that lateral contractility is planar polarized, linking lateral membranes into a supracellular “ribbon” structure, suggesting that tissue-scale mechanical coupling aligns the initiating cells to overcome myosin noise. We tested this hypothesis first by reducing cell-cell mechanical coupling using RNAi knockdown of the junctional force transducer α-Catenin and next with an optogenetic approach in which we locally disrupted mechanical coupling. In both cases, we observed increased furrow misalignment, supporting our hypothesis.

In conclusion, our study reveals that patterning precision and information richness per se are insufficient to ensure invariant morphology, if downstream mechanics is intrinsically noisy. Furrow positioning in different embryos deviates from gene expression pattern to varying degrees, suggesting that spatial patterning is not fully deterministic. Irrespective of adhering to, or deviating from, the positional code, the initiating cells align among themselves linearly, displaying features of self-organization that ensures the spatial precision of furrow positioning.

The successful implementation of the optogenetic approach is crucial in providing not only a strong support that cephalic furrow formation depends on actomyosin contractility, but also an elegant manipulation that shows that reduced tissue-scale mechanical coupling and force transmission causes a deviation of furrow linear alignment.

3) Identification of α-Spectrin as a candidate that confers membrane elasticity during apical dome decent

In search of mechanical mechanism that counteracts the microtubule pushing force to orchestrate apical dome descent during dorsal fold initiation, we tested several components of the Spectrin membrane skeleton and found that RNAi knockdown of α-Spectrin causes a delay in apical dome descent. We followed up on these observations and found that adherens junctions are not formed properly. Specifically, in the wild-type embryo the E-Cadherin puncta normally coalesce into more continuous pattern of localization in the junctional region as adherents junctions mature from the initial spot junction like structures to form a bell-like adhesive complex. In the α-Spectrin RNAi embryo, E-Cadherin puncta retains spotty appearance for a prolonged period of time. These data suggest a possibility that apical dome fails to descend in the α-Spectrin RNAi embryo in part due to defective junctional coalescence. We are in the process of further characterizing this phenotype.

4) Construction of a coarse-grained molecular dynamics model for simulation of disorder microtubule network with membrane tethers.

In collaboration of the Shibata lab at RIKEN BDR, we have constructed a mechanical model of coarse-grained molecular dynamics for the microtubule filaments and motors within a cell. The filaments, motors and cell membrane are modeled as particles connected by elastic springs. The current version of the model is a prototypical version where the filaments and motors are confined in a 2D space with a rigid membrane. Since the motors can bind to, and unbind from, the filaments, while the filaments themselves undergo turnover, the network structures can change dynamically with time. Our preliminary simulation indicates that the network evolves from an initial condition of randomly distributed filaments and motors to a non-uniform pattern that shows separation of areas with high density of filaments near the membrane from centeral area with low filament density. Such a separation implies that the network exhibits an extensile property. Indeed, our force measurement indicates that the network generates extensile forces, which increase with the forces generated by the individual motors.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Eritano Anthony S.、Bromley Claire L.、Bolea Albero Antonio、Schuetz Lucas、Wen Fu-Lai、Takeda Michiko、Fukaya Takashi、Sami Mustafa M.、Shibata Tatsuo、Lemke Steffen、Wang Yu-Chiun	４．巻 53
２．論文標題 Tissue-Scale Mechanical Coupling Reduces Morphogenetic Noise to Ensure Precision during Epithelial Folding	５．発行年 2020年
３．雑誌名 Developmental Cell	６．最初と最後の頁 212～228.e12
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.devcel.2020.02.012	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計5件（うち招待講演　5件／うち国際学会　2件）

１．発表者名 Yu-Chiun Wang
２．発表標題 Force (im)balance during homeostatic and transformative states of epithelial folding
３．学会等名 Institute of Cellular and Organismal Biology, Academia Sinica, Taiwan（招待講演）
４．発表年 2018年

１．発表者名 Yu-Chiun Wang
２．発表標題 The novel mechanism and unexpected roles of mechanical polarity during epithelial out-of-plane deformation
３．学会等名 APDRC5 (the 5th Asia Pacific Drosophila Research Conference), Pune, India.（招待講演）（国際学会）
４．発表年 2020年

１．発表者名 Yu-Chiun Wang
２．発表標題 Tissue-scale mechanical coupling reduces morphogenetic noise to ensure precision during epithelial folding.
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１．発表者名 Yu-Chiun Wang
２．発表標題 The novel mechanism and unexpected roles of mechanical polarity during epithelial out-of-plane deformation
３．学会等名 National Centre for Biological Sciences, Bangalore, India（招待講演）
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１．発表者名 Yu-Chiun Wang
２．発表標題 The novel mechanism and unexpected roles of mechanical polarity during epithelial out-of-plane deformation
３．学会等名 Tata Institute of Fundamental Research, Mumbai, India（招待講演）
４．発表年 2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考
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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関