ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
令和　2 年　7 月　1 日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

採 用 年 度　平成30年度
受 付 番 号　201860572
氏　　名　山下琢己

（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：　パリ　（国名：　フランス　）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
クラスタ解析を用い形態形成において細胞の挙動と力を制御する仕組みを解明する

3. 派遣期間：~~令和~~ 平成30 年　4 月　1 日　～　令和　2 年　3 月　26 日

4. 受入機関名及び部局名
フランス国立科学研究センター (CNRS), Université de Paris Diderot, Laboratory Matiere et Systemes Complexes

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

所期の目的の遂行状況及び成果

During embryo and tissue morphogenesis, different regions inside a tissue acquire different mechanical properties, resulting in different deformations. The deformations of the regions are orchestrated across the tissue, making resultant complex shape. Thanks to large improvements in imaging techniques and image analysis tools, we are now able to observe each cell and track them across a tissue, and seeing the cells behaving differently inside the tissue. However, we do not know how the cellular behaviors are spatially regulated, nor know how the tissue should be divided into the regions. To challenge this problem, I tried two approaches, 1) developing a method to divide a heterogeneous tissue into homogeneous regions in terms of tissue deformation and underlying cellular processes; 2) simulating epithelial cell mono-layer deforming in apical-basal direction using a cellular Potts model.

For the development of tissue-segmentation method, I employed an image segmentation method, clustering algorithm, and cellular Potts model to analyze fields of tensor representing tissue morphogenesis. In preceding studies, our team established a method to quantify local tissue deformation rate and deformation rate derived from each cellular process, cell divisions, cell rearrangements, cell shape changes, and cell delamination. The deformation can be decomposed into an isotropic part (change in area) and an anisotropic part (elongation in one direction and contraction in the orthogonal direction). By comparing the deformation rate between the tissue and each cellular process, one can measure how much the cellular process contributes to the tissue morphogenesis. With the qualification method, *Drosophila* pupal dorsal thorax and wing blade had been shown heterogeneous in tissue deformation rate and underlying cellular processes.

Therefore I first divided the tensor fields with an image segmentation method called region growing algorithm, which divide an image into a given number of regions. In each of the resultant region, points were similar to the average of the region. However, it relies on a randomly given initial condition, and the results varied largely among different trials. For an application to the study of morphogenesis, we need a unique segmentation.

Next I integrated the multiple results of the region growing algorithm by a consensus clustering algorithm called label propagation on a consensus matrix. Consensus clustering is a genre of clustering where objects are partitioned into clusters not based on similarity between the objects like in common clustering algorithms but based on how they were partitioned by other clustering algorithms. The label propagation algorithm returned a unique segmentation. To assess if the resultant regions were homogeneous or not, the regions were tested by silhouette analysis, a tool of cluster analysis measuring how objects were well partitioned. Comparison of the silhouette value between the resultant regions and randomly made control segmentations showed that the resultant regions were significantly more homogeneous than the controls. However, the resultant regions might include disconnected points. For the application to a study of mechanical interaction between the regions and for a comparison with gene expression patterns, we need every region connected.

To get homogeneous and connected regions, I smoothed boundaries of the regions with cellular Potts model simulation. In the simulation, the regions deformed so that it reduce boundary length and increase the silhouette value. It also include a

random process and its result is dependent on parameters. Therefore I made a parameter screening program so that smoothed regions have the highest homogeneity and an enough small ratio between boundary length and area. Also, results of iterated boundary smoothing were again integrated by the label propagation algorithm. Resultant regions were enough homogeneous and connected.

With the pipeline of the region growing, the label propagation, and the cellular Potts model, now a heterogeneous tissue can be divided into regions homogeneous in terms of a given quantity. The quantity can be anything as long as it can measure a similarity between points. To divide the *Drosophila* dorsal thorax and wing blade, I tested two quantities, 1) time-evolution of tissue local deformation rate, and 2) time-evolution of cellular processes effective contributions. For the time-evolution, it summed up similarity between points at each time point.

The dorsal thorax was divided into regions which corresponded to scutum, scutellum, and boundary between them. Interestingly, the regions based on the two quantities largely overlapped with each other. The regions were significantly homogeneous compared to the controls, and showed distinct patterns of underlying cellular processes.

In the same way, the wing blade was divided into anterior, middle, posterior, and distal regions with significant homogeneity and distinct underlying cellular processes by the two quantities.

In conclusion, we developed the method with which a heterogeneous tissue can be divided into homogeneous regions based on any quantity. With the method, *Drosophila* pupal dorsal thorax and wing blade were divided into regions which showed distinct local tissue deformation rate and underlying cellular processes.

This study is under submission (preprint: https://doi.org/10.1101/696252), and Matlab scripts developed in this study are available at https://doi.org/10.5281/zenodo.3626111.

For the simulation of epithelial tissue morphogenesis with cellular Potts model, I developed a Potts model which marks subcellular locations such as apical, basal, lateral cell surface and apico-lateral site where adherens junction localizes. It can also include subcellular components such as nucleus and filopodia.

The cellular Potts model simulates a tissue deformation in a stochastic process with thermal fluctuation according to an increment/decrement of system energy. Generally, the energy is composed of contact energy and volume constraint which produce surface contractility and pressure. In addition to them, I included surface constraint which gives surface elasticity, composing a surface tension along with the surface contractility, and a potential energy of subcellular locations and subcellular components to reproduce an effect of supracellular actomyosin bundles contraction.

With the epithelia model, I simulated two types of invagination, one driven by apical constriction, and another driven by contractile ring of supracellular actomyosin bundles.

The apical constriction has long been studied and simulated using a vertex model and a finite element model. However, these models allows only small curvature on the cell surface. On the other hand, the cellular Potts model allows a large fluctuation on the cell surface. When I simulated a deformation of epithelial tissue with two cell types of different apical surface contractility, apico-lateral sites between cells with different apical surface contractility were moved largely but the other apico-lateral

sites remained not so moved. The apico-lateral sites shift in the boundary cells was accompanied by a bending of cell lateral surface, making the cells from columnar to drop shape, not to wedge shape. This result can be interpreted that the apico-lateral position was moved due to an imbalance in the apical surface tension between the cells, while the decrease of apical surface made a cell volume decreased and the pressure increased, and then the imbalance in the pressure made the cell lateral surface curved. If the lateral cell surface were restricted to straight line (flat plane), change in the apical surface is inevitably accompanied by large deformation of the entire cell shape, and thus an actual apical surface tension (derivative of energy for the apical surface area) differed from that of contact energy. In contrast to it, the flexible cell lateral surface in our model allows various shape of the cell, gives a degree of freedom to the apical surface, and thus the apico-lateral sites move according to a local force balance between attaching apical and lateral surfaces tension and the pressures.

In a set of simulations, the apical surface elasticity, which may correspond to a remodeling of the apical cortex, was shown to play important role in the apical constriction along with the apical contractility. Plus, for the cell shape transformation from the columnar to the wedge shape, it was suggested that the deformation and change in pressure must be coordinated between the cells.

In contrast to the apical constriction, a morphogenesis by a contraction of the supracellular actomyosin bundle is a relatively new concept and has no established model. When the supracellular bundle is formed in circular shape, its contraction generate a force in the radial direction. Our experiments indicated that a *Drosophila* pupa neck invagination was driven by the supracellular bundles. There I simulated a deformation of epithelial tissue with apico-lateral sites in a cell pulled basally by the potential. When there was no apicals surface constraint, most of the epithelial cells remained attached to an apical extracellular matrix and a motion of the pulled apico-lateral sites made a narrow cut in the epithelium. With a strong apical surface constraint but without an affinity between the cells and the apical extracellular matrix, the epithelium moved basally while largely keeping its initial flat shape, resulting in a wide invagination. For the invagination, the cells must having the sufficient apical surface constraint and affinity with the apical extracellular matrix. The affinity with the extracellular matrix is equivalent to the change in apical surface contractility in the apical constriction. However, we did not observed the cell shape change from the columnar to the wedge in experiments and simulations. In this case the apical surface tension was balanced with the pulling force from the apico-lateral sites.

Together, the simulations of apical constriction and the supracellular bundles contraction elucidated a significant role of the mechanical coordination between the cells during tissue morphogenesis.