細胞内のミオシン Is を介して誘導されるキラル力の定量分析

Quantitative analysis of chiral force induced through Myosin Is in cells

Rensselaer Polytechnic Institute　Wai Cheng Wong

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滞在研究機関	Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy Troy, NY 12180, United States
共同研究者等	Prof. Leo Q. Wan
区分	個人 A

Chirality is an intrinsic property of cells. In *Drosophila*, MyosinIC (MyoIC) and MyosinID (MyoID) are known to determine cellular and organ chirality, causing left-handed twist and right-handed twist respectively. We hypothesize that mechanical forces deforming organs are driven by cellular chirality. However, these chiral forces have not been quantitatively analyzed. This study aims to quantify the direction and magnitude of the chiral mechanical forces induced in a MyoIC- and MyoID-dependent manner using genetically encoded multimeric nanoparticles (GEMs). We are collaborating with Wan Lab who are investigating MyoID and chirality in mammalian cells, to learn analytical methods during a 1-month stay to apply in our *Drosophila* system.

海外研究活動概要

The objective of this collaboration is to learn quantitative analytic skills from Wan Lab in Rensselaer Polytechnic Institute (RPI). I worked closely with Frank Peters, a Ph.D. candidate in Wan Lab for one month. My daily activities in Wan Lab included discussion with Frank and Prof. Wan, data processing and data analysis. Additionally, I was also invited to join the host lab’s group meeting once to twice a week.

Due to the time constraint and institutional regulation, I was not able to get the necessary safety training for conducting wet experiments in time. However, I had the opportunity to shadow members of the Wan Lab occasionally and learn about their method of culturing cells on micropatterned slides as well as basic stem cell culture practices. We are not currently using this technology, but this system might be useful in our future work.

For my collaboration work, I learned how to optimize my image post-processing flow through active discussion with Frank. Additionally, we also discussed how best to improve the data quality and what type of algorithms we can apply to calculate chiral mechanical force in the future.

Outside of lab work, I had the chance to attend a Ph.D. public defense. There were no seminars during my stay because July is summer holiday in the institute.

成果

The current data consists of hemocytes with expression of Myosin II (MyoII) and GEM particles. To analyze the relationship between MyoII and GEM, we leveraged MOSAIC plugin in ImageJ for particle tracking of GEM and PIVLab library in MATLAB for analysis of MyoII motion. Using the current analysis method, we have learned that MyoII and GEM particles show similar directional rotation in the single cell hemocyte of *Drosophila melanogaster* (Figure 1).

However, the generated results contain background noise due to the quality of the videos being analyzed. In details, the time-lapse videos were captured with a confocal microscope at a frame rate of two minutes. We

think that our current frame rate is too long causing loss of valuable information for trajectory tracking. There is also a chance that the particles move in and out of the observation plane during this two-minute interval. Based on the results from published research, the shortest frame rate that have been tested was 10 milliseconds (ms), albeit for a different purpose.

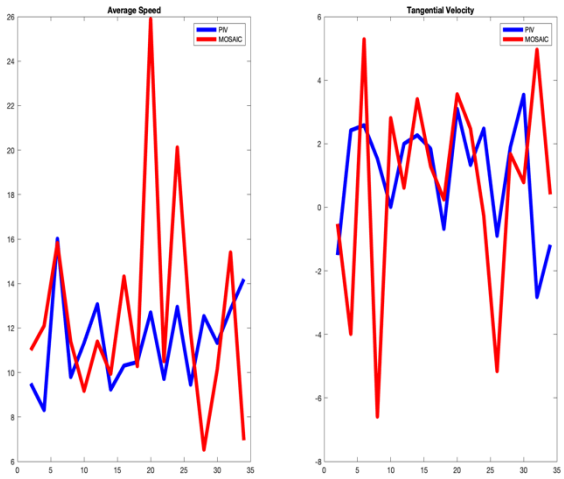

Figure 1. Results from analysis of GEM and MyoII. Blue graph represents analysis of GEM particles and red graph represents analysis of MyoII. Left graphs shows the average speed of GEM and MyoII. Right graph shows the tangential velocity, where positive value represents clockwise direction and negative value represents counterclockwise direction.

Another obstacle that we have encountered during analysis was the detection of cell centroid. We need to detect cell centroid as a parameter for the calculation of average speed and tangential velocity. Currently, we are manually drawing the outline of cell membrane and then calculating the centroid coordinates in ImageJ. To have a more accurate centroid, we can stain the nucleus with DAPI. We can then possibly use the same method to outline the nucleus periphery and then obtain the center position of each cell.

今後の展望

From the initial analysis, we have learned that the quality of the data can be improved. I plan to improve the quality of data acquisition by doing the following:

To-do	Reason
Capture only one slice instead of a stack of Z-planes	1. To avoid risk of x-y-z drifting 2. To speed up image acquisition rate
Lower the interval between frames (30 seconds or lower)	To reduce noise due to inability to connect trajectories of particles between frames
DAPI staining of hemocyte's nucleus	To allow calculation of cell center more easily and accurately

Parallel to improving image acquisition quality, I am also creating fly lines that overexpress Myosin Is and GEM particles together. We hope to use these fly lines to analyze the chiral force in the hemocytes in the future because actin have been shown to exhibit directional flow when MyoID and MyoIC is overexpressed.

Additionally, my collaborator in Wan Lab is working on the algorithm and code for calculating the mechanical force. Briefly, we are adapting a Newtonian fluid model. We start by assuming the cytoplasm as a viscous fluid. The Navier-Stokes equation is commonly used to describe fluid motion, and a chiral force term can be introduced to account for asymmetric forces arising from cytoskeleton (actin and myosins). Solving the velocity field while considering these parameters, we can quantify the chiral force contributions by analyzing velocity deviations or deformation patterns.

研究の発表

口頭発表

1. 無し

誌上発表

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