# CDB SEMINAR


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**Title:** " Probing cell morphogenesis dynamics at the molecular level: Actin turnover regulation at the leading edge and processive actin capping by a Formin homology protein, mDial "

**Date:** Tuesday, October 5

**Time:** 16:00 P.M.～17:00 P.M.

**Place:** 7th floor Conference Room of Building A, CDB


## Summary

Despite our knowledge of the molecular mechanisms involved in morphogenesis and motile process of cells, much remains unclear about how cells orchestrate such complex molecular systems to drive their shape change. Here I describe single-molecule speckle microscopy, a potent tool for probing on-going cytoskeletal reorganization process at the molecular level.

Fluorescent speckle microscopy (FSM), which was introduced by Waterman-Storer and Salmon in 1997, has been an excellent imaging method for following polymer movement. FSM provides fiduciary marks on cytoskeletal polymers with dilute fluorescent tags. During the course of the development of actin FSM in Dr. Tim Mitchison's laboratory, I realized that it was possible to visualize speckles of a single EGFP-actin molecule in cells by further lowering the label concentration (ref. 1). As only immobilized EGFP-actin gives rise to discrete "speckle" signals, the single-molecule speckle microscopy allowed actin polymerization mapping as well as actin filament lifetime measurement with high precision. It was then revealed that more actin polymerized in the lamellipodium body than at the leading edge of fibroblasts.

In order to solve how this fast actin turnover is regulated, we are extending application of the single-molecule speckle method to actin regulators. One example is our recent discovery of the long-range directional movement of a Formin protein, mDia1 (ref. 2). Formin homology proteins (Formins) play critical roles in cytokinesis and cell polarization in many eucaryotes. After nucleating actin filaments, Formins interact with the fast-growing barbed end of the filaments. We captured images of mDia1 moving along with the growing actin filaments in living cells, and it provided direct evidence of processive actin capping by Formins. We are also analyzing molecular kinetics of major actin filament-end interacting proteins such as the Arp2/3 complex and the capping protein. I will discuss how actin filament lifetime is regulated at the leading edge of living cells.


**Reference**


1) Watanabe N. and Mitchison T. J., *Science* **295**: pp1083 (2002)
2) Higashida C. et al., *Science* **303**: pp2007 (2004)



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