DIVISION OF CELL FUSION (ADJUNCT)

Professor: *Issei Mabuchi*
Associate Professor: *Hiroshi Abe*
Research Associate: *Hirotaka Fujimoto*
Insttitute Research Fellow: *Izuru Yonemura*
Graduate Students: *Tatsuhiko Noguchi (University of Tokyo)*
Sawako yamashiro (University of Tokyo)
Ryutaro Yanase (University of Tokyo)

Cytokinesis in animal and some primitive eukaryotic cells is achieved by the progressive contraction of the cleavage furrow. The cleavage furrow contains a contractile apparatus, called the contractile ring, which is composed of a bundle of actin filaments that lies in the furrow cortex beneath the plasma membrane. It has been established that the contractile ring contracts as the result of interaction between actin filaments and myosin. However, little is known about process of its formation, mechanism that controls its formation, protein constituents, and its ultrastructure. The goal of our research is to solve these problems and thereby clarify the molecular mechanism of cytokinesis. For this purpose, we use three kinds of cells, namely, sea urchin egg, *Xenopus* egg, and the fission yeast *Schizosaccharomyces pombe*.

S. pombe is an excellent system to investigate the changes in the actin cytoskeleton during cell cycle since F-actin patches, F-actin cables and F-actin ring are only visible structures in the cell (Fig. 1). The F-actin ring is considered to correspond to the contractile ring in animal cells. It is formed during anaphase in this organism.

S. pombe cells have two II-type myosin heavy chains called Myo2 and Myo3/Myp2. Recently, we studied how myosin accumulates at the division site. First, we showed that Cdc4, an EF-hand protein, appears to be a common myosin light chain associated with both Myo2 and Myo3. Loss of function of both Myo2 and Myo3 caused defect in the F-actin ring (contractile ring) assembly at the division site, like the phenotype of *cdc4* null cells. It is suggested that Myo2, Myo3 and Cdc4 function in a cooperative manner in the formation of the F-actin ring during mitosis.

Next, we investigated dynamics of myosin-II during mitosis in *S. pombe* cells. In early mitosis, Myo2 was detected primarily as dots widely located in the medial cortex. Myo2 fibers also became visible following the appearance of the dots. The Myo2 dots and fibers then fuse with each other to form a medial cortical network. Finally the network is packed into a thin contractile ring. In mutant cells that cannot form the F-actin ring such as *cdc3*, *cdc8* and *cdc12*, Myo2 is able to accumulate as the dots in the medial cortex, whereas no accumulation of the Myo2 dots was detected in *cdc4* cells. Moreover, F-actin did not seem to be required for the accumulation of the Myo2 dots.

A truncated Myo2 which lacks putative Cdc4-binding sites (Myo2ΔIQs) was able to rescue *myo2* null cells, *myo3* null cells, *cdc4* mutant cells and *cdc4* null cells. The Myo2ΔIQs could assemble into a normal-shaped ring in these cells. Thus, its assembly at the division site does not require function of either Cdc4 or Myo3.

On the other hand, we studied reorganization of actin-myosin cytoskeleton at the growing ends of the cleavage furrow of *Xenopus* eggs. At the the furrow formation, a cortical movement towards the division plane occurs at the growing ends of the furrow. Immunofluorescence microscopy demonstrated that myosin II assembles at the growing end as spots probably as a result of the cortical movement. Actin filaments assemble a little later after the formation of the myosin spots as small clusters which we call "F-actin patches", at the same positions as the myosin spots. The F-actin patches seemed to be formed and grow through new actin polymerization rather than assembly of pre-existing cortical F-actin. This was substantiated by microinjection of rhodamine-G-actin near the growing end: the microinjected G-actin was rapidly incorporated in the F-actin patches. The F-actin patches then align tandemly to form short F-actin bundles, and then the short bundles form long F-actin bundles which compose the contractile ring. The myosin spots are aligned on the long F-actin bundles and fused each other to show fibrous appearance.

We also concentrate our study on function of actin-regulatory proteins, including ADF/cofilin family proteins, during cytokinesis using *Xenopus* eggs and embryos. ADF/cofilin family proteins exist in all animals and plants examined and have been shown to be essential. We found that ADF/cofilin family proteins are essential for cytokinesis (Abe, Obinata, Minamide, and Bamburg, J. Cell Biol. 132: 871-875, 1996). Recent studies revealed that ADF/cofilin accelerates turnover of actin filaments both in vitro and in vivo. Most recently, we found a novel actin-regulatory protein which induces disassembly of actin filaments cooperatively with ADF/cofilin. cDNA analysis revealed that this protein is a *Xenopus* homologue of yeast actin interacting protein 1 (AIP1). Thus, we designated this protein as *Xenopus* AIP1 (XAIP1). Purified XAIP1 itself binds to pure actin filaments to some extent, but it induces a rapid, drastic disassembly of actin filaments associated with cofilin. Microinjection of this protein into *Xenopus* embryos arrested development by the resulting actin cytoskeletal disorder. XAIP1 represents the first case of a protein cooperatively disassembling actin filaments with ADF/cofilin family proteins.

Publication List:

Suda, M., Fukui, M., Sogabe, Y., Sato, K., Morimatsu, A., Arai, R., Motegi, F., Miyakawa, T., Mabuchi, I., and Hirata, D. (1999). Overproduction of elongation factor 1a, an essential translational component, causes aberrant cell morphology by affecting the control of growth polarity in fission yeast. *Genes to Cells* **4**, 517-527.

Sayers, Lee G., Katayama, S., Nakano, K., Mellor, H., Mabuchi, I., Toda, T., and Parker, P. J. Rho-dependence of Schizosaccharomyces pombe Pck2. *Genes to Cells* in

press.

Kusano, K., Abe, H. and Obinata, T. (1999). Detection of a sequence involved in actin-binding and phosphoinositide-binding in the N-terminal side of cofilin. *Mol. Cell. Biochem.* **190**, 133-141.

Tsubakimoto, K., Matsumoto, K., Abe, H., Ishii, J., Amano, M., Kaibuchi, K. and Endo, T. (1999). Small GTPase RhoD suppresses cell migration and cytokinesis. *Oncogene* **18**, 2431-2440.

Okada, K., Obinata, T. and Abe, H. (1999). XAIP1: a *Xenopus* homologue of yeast actin interacting protein 1 (AIP1), which induces disassembly of actin filaments cooperatively with ADF/cofilin family proteins. *J. Cell Sci.* **112**, 1553-1565.

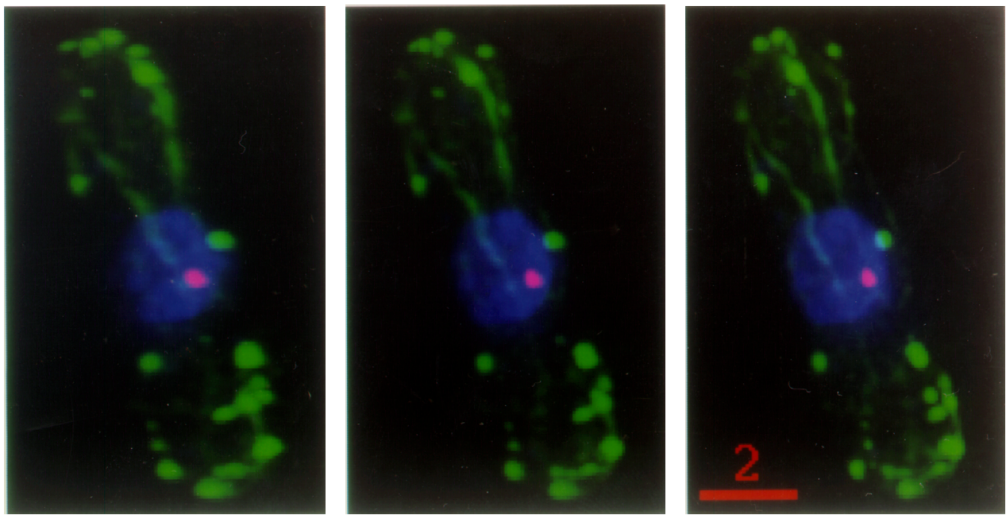

Fig. 1 3-D images of an interphase *S.pombe* cell. Each image is rotated by 12 degree from the neighbor. Green, F-actin. Blue, DNA. Red, spindle pole body. Bar, 2 μm.