Effects of Actin-Nucleus Connections on the Vascular Smooth Muscle Cell Differentiation
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Vascular smooth muscle cells (VSMCs) differentiate from contractile to synthetic phenotype under pathological conditions. To understand smooth muscle pathophysiology, it is important to understand the mechanism of their differentiation and dedifferentiation. We recently found that the actin stress fibers (SFs) have a mechanical interaction with the nucleus, and the internal forces of SFs were transmitted directly to the nucleus. Thus, it is possible that the mechanical interaction between SFs and nuclei could be associated with gene transcription and cell differentiation. Here we investigated the alterations in the mechanical interaction between actin cytoskeleton and the nucleus during the differentiation and dedifferentiation processes of VSMCs.

What is the molecular mechanism of stress fiber disassembly caused by mechanical cyclic stretch?
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The mechanism underlying selective disassembly of stress fibers (SFs) oriented in the direction of cyclic stretch remains unclear. Here, we show that fast shortening of cells that exceeds the intrinsic contraction speed of SFs causes disassembly of SFs. SFs that contained myosin light chain mutants whose actin-myosin-II interactions were restricted were more prone to disassembly upon fast cell shortening. We overexpressed active LIM-kinase mutants to inactivate cofilin, and found that disassembly of SFs still occurred in a manner similar to that of controls. We suggest that the disassembly is caused by the unbundling of constituent actin filaments in a manner critically dependent on myosin II but independent of the severing activity of cofilin.

Responses of fibroblasts against fluid flow stimuli in a three-dimensional collagen gel culture system
Cells in vivo are bathed in the interstitial fluid and it has been said that the fluid flow-derived stimulus is one of the basic constraints that determine cells’ basic phenotypes in vivo. Cells in the three-dimensional (3D) collagen gel culture system are known to recapitulate their in vivo phenotypes. The effects of medium flow-stimulus on human fibroblasts were studied using this culture method. The cells were supplied with medium through a pump-driven syringe at a regulated velocity (0.012 μm/s to 1.5 μm/s) and characterized for their various phenotypes. The medium flow-stimulus activated the cell growth in a velocity-dependent manner, the expression of hyaluronan synthetase 2 gene, and markedly increased the gel’s wet volume.

Cell sheet延伸における細胞核変形量の測定
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Cells have an ability to activate their cell functions in response to external forces. Mechanical properties of cells have been widely studied in single cells, but less in cell population. Here, we investigated how cell nucleus deforms in response to uniaxial external forces, using cell sheet of COS-Fucci (RIKEN) [1] by monitoring the fluorescence of nucleus depending on cell phase. We found that the deformation of nucleus was proportional to that of the whole cell sheet, but the former value was much less than the latter indicating a heterogeneous deformation of intracellular structures. Detailed results for the cell cycle dependence will be presented. We thank Dr. Atsushi Miyawaki for COS-Fucci and NMuMG-Fucci cells. [1] A. Sakau-e-Sawano et al., Cell 132, 487 (2008)

Microfluidics analyses of coordinated dynamics of F-actin and cAMP signaling in Dictyostelium chemotaxis
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In migrating Dictyostelium, chemotactic field is dynamic, self-generated and amplified by the moving cells themselves. How the cell movement and the amplification of guidance cue are coordinated in space and time remains elusive. Here we study the role of actin polymerization on the chemotactic CAMP-induced elevation of cAMP. We show by combining FRET-based live-cell imaging of cytosolic cAMP and microfluidics that the amplitude and the timescale of the response are altered when actin polymerization is pharmacologically inhibited. The population-level oscillations of cAMP also diminished under latrunculin treatment, suggesting that self-generation of chemotactic cAMP field is linked strictly to cell movement.