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Combined Confocal and Atomic Force Microscopy Studies of Force Transmission, Force Generation and Strain Dynamics in Living Cells

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It is now clear that many significant and medically relevant biological processes (for example, muscle (re)generation, stem cell differentiation and cancer metastasis) can be modulated and controlled through mechanical forces arising in the microenvironment (ME). It is also evident that the immediate response of a cell to mechanical force is complex cytoskeletal and nuclear deformation, organelle movements and focal adhesion remodelling which all takes place far upstream any other biological events. Our work has demonstrated how cells display extremely complex responses to mechanical forces and often exhibit unexpected time-dependent anisotropic mechanical properties that are intimately linked to cell function [1]. The dynamic mechanical properties of cells during physiological processes, such as mitosis, metastasis and cell death [2-4], also play an important regulatory role.

We employ a variety of cell line models to study the earliest effects of mechanical stimulation on living cells in health and disease. In general, my laboratory focuses on the effects of physical force on mouse embryonic stem cell fate, the differentiation of muscle precursor cells into myotubes, and the onset and progression of cancer metastasis. Details of cell lines have been described previously [1-4].

In order to study the effects of local mechanical stimulation we combine Atomic Force microscopy (AFM) (JPK NanoWizard II), Optical Stretching, Planar Bi-Axial Stretching and Traction Force Microscopy with simultaneous high-speed confocal (Nikon TiE-A1R), phase contrast and fluorescence microscopies (Nikon TiE). Cells expressing fluorescently tagged structures of interest (cytoskeleton, nucleus, plasma membrane, focal adhesions, etc) are placed in these commercial and custom-built devices and are subjected to nano/micro-indentation or physical stretch while simultaneously imaging stress-induced sub-cellular deformation and force propagation.

Computational image tracking routines written in my lab are used to quantify and characterize the response of cells to physical force. Combined with optical approaches, such as photobleaching, we quantify the nanoscale deformation ad response of living cells to physical stimuli with high spatial and temporal resolution.

In this talk I will describe our work utilizing simultaneous confocal and AFM to investigate the dynamic and timescale dependent mechanical properties of cells. In combination with molecular biology and physical/computational approaches we physically touch and mechanically stimulate living cells in order to examine the transmission of force through the cytoarchitecture (plasma membrane, cytoskeleton, focal adhesions and the nucleus), which results in complex elastic and viscoelastic three-dimensional relaxation and remodelling.

Physical forces and the mechanical properties of cells and their microenvironment, play a critical role in cell biology. The mechanical properties of cells are distinctly controlled by time and cell type dependent

mechanisms that, in turn, are governed by the mechanical microenvironment. The interesting physical response of cells to mechanical stimuli is not merely a side-product of biology but is a key component of a biological and physical feedback loop governing the life of a cell.

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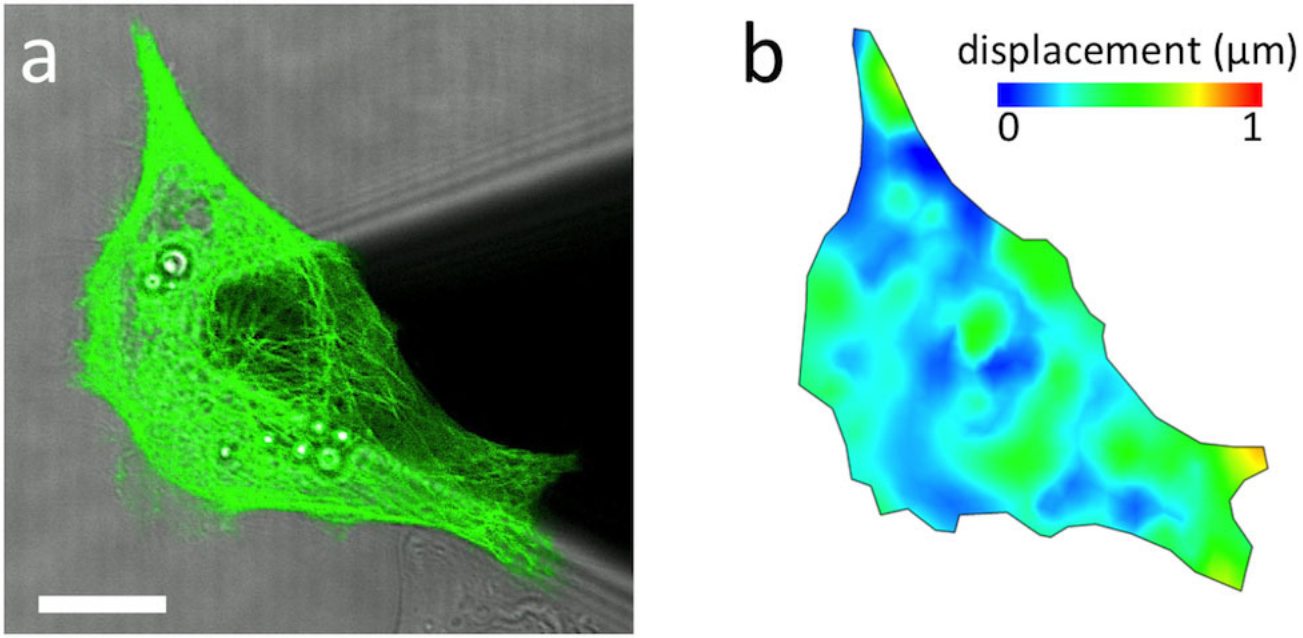

Figure 1. (a) An AFM cantilever touching and mechanically stimulating a living cell expressing EGFP-alpha-tubulin [3] (scale bar = 15 μm). (b) The resulting deformation heat map reveals complex and heterogeneous displacements taking place throughout the cell body in response to a local nanomechanical indentation. For more information see www.pellinglab.net.

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