Mechanical adaptability of cell migration in 3D collagen gels

for a dozen or so downstream proteins. Fluorescence recovery after photobleaching (FRAP) experiments have shown that the Z ring is highly dynamic, with recovery half time of 8~30 seconds, yet has a rather persistent overall structure. But the mechanism of how a single narrow dynamic Z ring emerges from a big pool of cytoplasmic FtsZ molecules remains elusive. In this work, we developed a rule-based molecular model with FtsZ and ZipA/FtsA molecules, by explicitly taking into account the elementary assembling events of these two types of molecules and their diffusion on membrane. Our model can not only efficiently reproduce the Z ring with statistical properties in accordance with experimental observation, but provide a convenient way to combine biochemical dynamic and physical assembling processes within the same spatio-temporal modeling framework. Our results indicate that the FtsZ and ZipA can spontaneously self-assemble into a ring structure. And as observed in experiments, our model also shows that either high or low FtsZ to ZipA/FtsA ratios would result in multiple Z rings or aggregated bundles. Our in silico FRAP experiment further yields a recovery half time comparable to experimental results. Our systematic simulation unveils that it is the rapid turnover dynamics that prevents the FtsZ molecules from being sequestered by small FtsZ bundles dispersed over the membrane, therefore allowing one single Z ring to emerge and mature. This compete-and-survive mechanism provides cells a simple way for spatial regulation.

2283-Pos Board B420

Fluorescence Microscopy and Solution NMR Studies of Cytoskeletal Proteins from Tetrahymena

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The cytoskeleton of single-cell eukaryotes such as the alveolates is remarkably complex, and very little is known about the molecular and structural bases of its organization and regulation. We have previously identified several proteins during proteomic analysis of calcium-dependent contractile fibers isolated from the membrane-associated cytoskeleton of the ciliated protozoan *Tetrahymena thermophila*. These include a putative calcium-binding protein (Tcb2), a filament-forming structural protein (Epc1), and a protein kinase (Epk1), among others. The function of these proteins is unclear, but it has been proposed that they play regulatory and structural roles in the calcium-dependent regulation of ciliary movement. We are using a combination of localization and structural methods, in particular confocal laser scanning microscopy and solution NMR spectroscopy, to understand how these proteins interact to form intricate cytoskeleton structures and contractile filaments, and gain insight into their structure-function relationship. Confocal microscopy studies of GFP-tagged constructs indicate that the proteins concentrate into a very thin submembranous layer called the epiplasm. Interestingly, we are able to reconstitute calcium-triggered contractile fabrics *in vitro* with Tcb2 alone. Full-length Tcb2 forms filamentous structures and becomes insoluble in the presence of calcium and/or upon concentration, but its C-terminal domain is highly soluble and amenable to biophysical/structural characterization. NMR spectra show that this domain is well folded both in the presence and absence of calcium, and undergoes a dramatic conformational change upon calcium addition. We are currently using NMR spectroscopy to quantify the calcium-binding properties of this domain, and to investigate its structure and dynamics. Future NMR studies will probe Tcb2 interactions with other cytoskeletal proteins, in particular Epk1 and subdomains of Epc1. We will also investigate the relative cellular distributions of these proteins through fluorescence double-labeling and FRET experiments.

2284-Pos Board B421

Temporal Response of Bacterial Cells to High Pressures

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Cells encounter physicochemical fluctuations in their environment on a routine basis. Bacterial cells have developed regulatory mechanisms to cope with many of these stress fluctuations. The response of the cells to different stresses may depend on the type, magnitude and the duration of the stress [1]. While the stress response of bacterial cells to temperature fluctuations is relatively known, the stress response to fluctuations in high pressures is not explored. We experimentally study the effect of pressure fluctuations on a mesophilic bacterium, Escherichia coli. Specifically, we study the cell morphology, cell division, and gene expression of the one of the major cytoskeletal proteins MreB, which is implicated in maintaining both the cell shape and cell division, as a function of temporal fluctuations in pressure. We find that the probability of high pressure induced elongation of bacterial cells or lack of cell division decreases as the cells are allowed to relax at lower hydrostatic pressure. Moreover, we find that for a given pressure, the rate of cell division depends on the ratio of doubling time and the time scale of relaxation to normal pressure. From our data we can quantify the critical time-scale over which cell morphology and cell division does not exhibit any apparent changes compared to bacterial cells at normal growth conditions. Our experimental results can be explained by a two state stochastic model of bacterial cell elongation at high pressure and relaxation to normal morphology at normal pressures [3]. Our results shed light on the cellular response of bacterial cells to novel stress fluctuations.

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2285-Pos Board B422

Development of a Microfluidic Platform to Study Effects of Physical Stresses on Microglial Activation

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Brain cells are surrounded by bodily fluids such as cerebral interstitial and cerebrospinal fluid and exposed to continuous electrical stimulations. These physical microenvironments are known to play an important role in the mechanical protection of the brain cells and regulation of their functions in both physiological and pathological states. Among many cell types, microglia cells sensitively respond to the changes in physical stimuli and are transformed into different activation states featuring distinct morphological and migration patterns. To study microglial activation in a brain-like environment, we developed a microfluidic channel to realize physiologically relevant physicochemical microenvironments of controlled interstitial flow, electric field, and chemical gradients. Under various shear stress conditions, bipolar cells are transformed into an amoeboidal cells with enhanced spreading movement. Similarly, electric field promotes transformation of microglial phenotype with enhanced migration as well as phagocytic activity. Since the change in the activation state of microglia is closely related to neurodegenerative diseases, this work might provide unconventional perspectives for pathological outbreaks in the brain.

Acknowledgement: This research was supported by the fundamental research project (A0601003001) and high risk high return project (N10140037) funded by KAIST.

Cell Mechanics, Mechanosensing, and Motility III

2286-Pos Board B423

Mechanical Adaptability of Cell Migration in 3D Collagen Gels

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Migration of cells across tissues with diverse biophysical environments plays a crucial role in a wide variety of physiological functions and pathological processes, such as in embryonic development, wound healing, haemostasis, tumor and cancer progression. Indeed, one of the most devastating features of cancer is metastasis_the ability of cancer cells to escape from the primary tumor and invade and colonize a distant tissue. Understanding the biophysical and biochemical mechanisms underlying cell migration remains a challenge, however, partly because it has been only recently realized that cells employ different strategies and molecular mechanisms in three-dimensional (3D) environments, compared to on traditional 2D glass surfaces.

In this work, we examined cell migration, simultaneously at the individual cell and cell population levels, in a 3D collagen hydrogel model mimicking the connective tissue topology confronted by malignant breast cancer cells. Our findings revealed two distinct migration patterns that depend specifically on the location of the individual cells within the population: a rapid and directionally persistent migration of the ''leader cells'' and a more randomized migration of the ''follower cells''. This disparity, strikingly, occurred with minimal cell-cell contacts. Rather, this heterogeneity is associated with local remodeling of the pericellular matrix and results in an apparent independence of the inherent migration on matrix condition. Despite such robustness, effects of anti-migratory drugs were interestingly observed to vary strongly with matrix stiffness and architecture. Specifically, cytoskeletal contractility-targeting drugs reduced migration speed in sparse gels, whereas migration in dense gels was retarded effectively by inhibiting proteolysis. Our results therefore corroborate a mechanistic plasticity that allows cells to actively adapt their invasion machinery depending on the local biophysical microenvironment.