

subtilis) associates specifically with origin-proximal *parS* sites and also “spreads” in a poorly understood way by interacting nonspecifically with adjacent chromosomal DNA. Spo0J complexes in *B. subtilis* are required for early segregation of newly replicated origins and facilitate loading of the bacterial condensin homolog SMC. using *in vitro* single-molecule imaging, we have studied the mechanism of Spo0J nucleoprotein complex formation by simultaneously observing Spo0J binding to DNA and motion of site-specific labels on the DNA chain. Our results suggest that Spo0J forms complexes by trapping long-distance loops between consensus *parS* sites and distal nonspecific segments of DNA. Detailed *in vitro* and *in vivo* analysis of mutants has allowed us to define the molecular determinants of DNA bridging by Spo0J.

3003-Pos Board B158

Acto-Myosin Contractility Rotates the Cell Nucleus

Abhishek Kumar¹, Ananyo Maitra², Madhuresh Sumit¹, Sriram Ramaswamy², Shivashankar G.V.¹.

¹Mechanobiology Institute, Singapore, Singapore, ²Indian Institute of Science, Bangalore, India.

The stiffest and largest organelle of the eukaryotic cell – the nucleus – is coupled to active cytoskeletal filaments. But how this organelle responds to stresses in the surrounding cytoplasm is poorly investigated. We report here the results of studies of the translational and rotational dynamics of the nuclei of single fibroblast cells, with the effects of cell migration suppressed by plating onto fibronectin-coated micro-fabricated patterns. Patterns of the same area but different shapes and/or aspect ratio were used to study the effect of cell geometry on the dynamics. On circles, squares and equilateral triangles, the nucleus undergoes persistent rotational motion, while on high-aspect-ratio rectangles of the same area it moves only back and forth. The circle and the triangle showed respectively the largest and the smallest angular speed. We rationalize our observations through a hydrodynamic approach in which the nucleus is treated as a highly viscous inclusion residing in a less viscous fluid of orientable filaments endowed with active stresses. Lowering actin contractility selectively by introducing blebbistatin at low concentrations drastically reduced the speed and persistence time of the angular motion of the nucleus. Time-lapse imaging of actin revealed a correlated hydrodynamic flow around the nucleus, with profile and magnitude consistent with the results of our theoretical approach. Coherent intracellular flows and consequent nuclear rotation thus appear to be a generic property that cells must balance by specific mechanisms in order to maintain nuclear homeostasis.

3004-Pos Board B159

Cellular Geometry Mediated Apical Stress Fibers Dynamically Couples Nucleus to Focal Adhesion

Qingsen Li, Abhishek Kumar, Shivashankar G.V.
Mechanobiology Institute, Singapore, Singapore.

Cells sense their physical microenvironment and transduce these signals through actin-nuclear links to regulate nuclear functions including gene expression. However, the spatio-temporal coupling between actin cytoskeleton and the nucleus and its modulation by cell geometry are still unclear. Using micro-patterned substrates to control cell geometry, we show that perinuclear actin organization at the apical plane remodels from mesh-like structure to stress fibers. The formation of these apical stress fibers (ASF) correlated with significant reduction in nuclear height and was found to exert an active compressive load on the nucleus via direct contact with mature focal adhesion sites. We further show, using quantitative fluorescence spectroscopy experiments, that these ASFs were dynamically coupled to the nucleus via outer nuclear membrane proteins nesprin2G. Taken together, our work provides direct evidence of physical links between the nucleus and focal adhesion sites via ASFs. We suggest that such direct links may underlie nuclear mechanotransduction to regulate genomic programs.

3005-Pos Board B160

Geometric Constraints on Cells Induce Cytoplasmic to Nuclear Redistribution of Transcription Co-Factors to Regulate Gene Expression

Nikhil Jain, K. Venkatesan Iyer, Abhishek Kumar, Shivashankar G.V.
Mechano-biology Institute, Singapore, Singapore.

Physical forces in the form of substrate rigidity or geometrical constraints have been shown to alter gene expression profile and differentiation programs. However, the underlying mechanism of gene regulation by these mechanical cues is largely unknown. In this work, we use micropatterned substrates to alter cellular geometry (shape, aspect ratio and size) and study the nuclear mechanotransduction to regulate gene expression. We show that geometric

constraints result in differential modulation of nuclear morphology, actomyosin contractility, histone acetylation and the activity of transcription co-factor, MRTF-A. In addition, genome-wide transcriptome analysis revealed cell geometry dependent alterations in chromosomal activity and actin dependent gene expression. Promoter analysis of these differentially regulated genes showed that serum response factor (SRF) was an essential regulatory factor sensitive to geometric cues. Further, we show that geometric constraints resulted in nuclear translocation of MRTF-A and enhanced serum response element (SRE) promoter activity. Interestingly, nuclear accumulation of MRTF-A by geometric constraints also modulated NF- κ B activity. Taken together, our work provides mechanistic insights underlying the regulation of gene expression by cellular geometry.

Nucleic Acid Biophysics in vivo

3006-Pos Board B161

Multi-Scale Models of Genomic Bacterial DNA

Liang Fang, Michael Feig

Michigan State University, East Lansing, MI, USA.

Detailed structural models of genomic bacterial DNA were developed using multi-scale modeling methods. Initial models were generated using a coarse-grained representation of supercoiled plectonemic DNA informed by experimental data. Conformational sampling was carried out using a Monte Carlo procedure to generate ensembles of nucleoid structures for complete genomic DNA within the constraints of known nucleoid sizes. The resulting models suggest that nucleoids are porous structures that may allow the diffusion of most proteins and protein complexes, in particular those involved in transcription and replication. The coarse-grained models were further refined with increasingly detailed representations of helical DNA up to quasi-atomistic models to serve as starting structures for realistic models of cellular environments.

3007-Pos Board B162

The Escherichia Coli Chromosome is Segregated by Biased Diffusion

Nathan J. Kuwada, Paul A. Wiggins.

University of Washington, Seattle, WA, USA.

The physical mechanism responsible for accurately partitioning newly replicated Escherichia coli chromosomes into daughter cells remains a mystery. We present a quantitative characterization of the dynamical motion of the origin of replication (*oriC*) using a large ensemble of trajectories generated by automated complete-cell-cycle imaging. In contrast to the dynamics of chromosome segregation in eukaryotic cells, we find that the motion in this bacterium is dominated by sub-diffusive dynamics before, throughout and after the segregation process rather than processive (ballistic) motion. Instead, to maintain accurate partitioning without processive motion, we find that *oriC* sub-diffusion is subject to a small diffusional bias (or drift velocity). Prior to *oriC* replication, we find that the drift velocity profile is analogous to a damped spring with equilibrium position at mid-cell. After two replicated *oriC* loci are distinguishable, the equilibrium position moves immediately to the quarter-cell positions and stays relatively constant for the remainder of the cell cycle, suggesting the mechanism responsible for maintaining chromosome structure may also be responsible for *oriC* segregation.

3008-Pos Board B163

Effect of Capsid Tail on the Ejection Dynamics of Semiflexible Polymers

Isam Hasan, Afaf Al Lawati, Muataz Al Barwani.

Sultan Qaboos University, Al Khod, Oman.

We present simulations investigating the role of the tail of a spherical viral capsid (mimicking a bacteriophage) on the ejection dynamics of a semiflexible polymer (representing viral dsDNA). We compare the ejection dynamics of a neutral polymer with that of a charged one. We find that the presence of the tail markedly slows down ejection. Our simulations suggest that this is because the last few polymer sections are trapped in the tail. Such trapping is particularly efficient for a charged polymer where the entropy of the part of the polymer outside the capsid is greatly reduced making complete ejection of the last few polymer sections difficult. Lowering the temperature further enhances this trapping.

3009-Pos Board B164

Conformational Fluctuations of Chromosomal DNA in Escherichia Coli

Rudra P. Kafle, Jens-Christian Meiners.

University of Michigan, Ann Arbor, MI, USA.

The cell is a very crowded structure, consisting of various organelles, proteins, nucleic acids, and cellular inclusions. It is the site of active, motor-driven