View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

238-Pos Board B24

Self-Organized Protein Localization and DNA Segregation Inside Bacterial Cells

Saeed Saberi Modaber, Eldon Emberly.
sfu, Burnaby, BC, Canada.

Asymmetric cell division in C.Crescentus relies on differentially localizing certain proteins to the poles where they bind to the scaffolding protein PopZ that displays a bipolar pattern. On the other hand, expressing PopZ in *E. coli* favours unipolar patterning. Additionally, the aggregation of misfolded proteins as well as plasmids in bacteria also display unipolar and biopolar patterns, similar to that of PopZ. These different systems have led to the hypothesis that chromosome free regions + biomolecular aggregation can be sufficient to drive localization in bacterial cells. We have performed Monte-Carlo simulations to show that the entropic force provided by the self-avoiding chromosome confined in the cylindrical geometry of the cell and the energy gained from aggregation results in phase separation between proteins and the polymer. We fully explore the phase space showing how patterning depends on protein concentration, chromosome density, cell shape and aggregation strength. The exploration results in a rich phase diagram of patterns which the observed systems can be fit into. Additionally, the dynamics of pattern formation depends somewhat on the rate at which proteins are added to the system. When proteins are added very slowly to the cell, the unipolar phase dominates, whereas at faster rates the bipolar phase dominates and at yet faster still rate, aggregation at nonpolar locations occurs. Such a rate dependency may explain differences in the observed patterns reported for PopZ induced expression in *E. coli* cells. Lastly, we show how such a localization process may aid the segregation of other cellular components such as the replication origin which anchors to a pole. We find that adding extra interactions does not destabilize the polar patterning, nor is it sufficient to drive the localization of the origin and indeed other localization and stabilization mechanisms are required.

239-Pos Board B25

Computational Modeling of Protein Dynamics in Eukaryotic Cells

Vesa Aho[1], Thomas Kühn[2], Kateryna Filippovych[1], Jussi Timonen[1], Maija Vihinen-Ranta[1].
[1]University of Jyväskylä, Jyväskylä, Finland, [2]University of Eastern Finland, Kuopio, Finland.

Proteins have important functions inside the cell, traveling diffusively or being actively transported to various cellular sites where their activity is needed. Protein motion in the cellular environment is therefore an important topic to understand. However, the cell provides a very complex environment for that motion, which poses problems especially for any modeling effort designed to interpret experimentally observed features. So as to gain a realistic picture of protein dynamics inside the cell, we have recently introduced advanced numerical methods for describing that dynamics [1]. The starting point is an accurate numerical duplicate of the cell determined by LSCM, which can be used as a simulation geometry. Interpreting the internal cellular structures that obstruct the protein motion as the solid component of a porous structure, and treating the motion in the same way as in the case of a porous medium (distinction between effective and apparent diffusion), we can rather accurately take into account the cellular environment in which the motion takes place. The numerical simulations of protein motion in the model cell are based on the lattice-Boltzmann method. Here we use these methods to exactly reproduce photobleaching experiments on the diffusion of Enhanced Yellow Fluorescent Protein (EYFP) in HeLa cells. Comparison of simulated and real experiments can be used to characterize protein motion and the related diffusion coefficients. We pay particular attention to the nuclear translocation of EYFP in these cells.

[1] Kühn T., Ihalainen T. O., Hyväluoma J., Dross N., Willman S. F., Langovski J., Vihinen-Ranta M. and Timonen J. 2011 *Protein Diffusion in Mammalian Cell Cytoplasm*. PLoS ONE 6(8): e22962.

240-Pos Board B26

A Barrier to Diffusion of Opsins but not Peripheral Membrane Proteins at the Disk Rims of Cone Photoreceptor Sensory Cilia

Ivayla I. Geneva, Cherry G. Ignacio, Barry E. Knox, Peter D. Calvert.
SUNY Upstate Medical University, Syracuse, NY, USA.

Localization and retention of transduction cascade proteins within ciliary signaling compartments are thought to be mediated, in part, by diffusion barriers at the transition zone. Mutations in cone opsins and other phototransduction cascade proteins often result in their mislocalization from the ciliary cone outer segment, and can lead to a spectrum of diseases, ranging from color blindness to progressive cone dystrophy, often followed by rod dystrophy, and eventually blindness.

While the mechanisms for protein transport to and retention within rod outer segments have received considerable attention, little is known about these processes in cones. Retention of opsins in the rod outer segment is achieved by physical separation of the disc membranes from the plasma membrane. Cone discs, however, are contiguous with the plasma membrane, and therefore retention of opsin would require a diffusion barrier either at the cilium base, as has been demonstrated for primary cilia, or at the disc rim. Here we examine the diffusion of the transmembrane red cone opsin-GFP fusion protein and the peripheral membrane protein double geranylgeranyl-GFP expressed in cones of *Xenopus laevis* using multiphoton FRAP. We show that although both proteins diffuse laterally in the disc membranes with similar rates, diffusion of opsin-GFP between discs is significantly retarded compared with double geranylgeranyl-GFP.

Our results are consistent with cone opsin retention in the outer segment being mediated by a selective barrier to free diffusion for trans-membrane proteins located at the end loops between adjacent discs. Peripheral membrane proteins, on the other hand, are not prevented from crossing this barrier.

241-Pos Board B27

Lamin-A/C is a Nuclear Rheostat that Couples Microenvironment Rigidity to Cell Lineage

Joe Swift, Takamasa Harada, Joel Pinter, Jae-Won Shin, Irena Ivanovska, Amnon Buxbiom, Dennis E. Discher.
University of Pennsylvania, Philadelphia, PA, USA.

A solid tissue can be soft like fat or brain, stiff like striated muscle and heart, or rigid like bone. Proteomic profiling of tissue nuclei shows that Lamin-A/C expression increases more than 30-fold and in near-proportion to micro-elasticity of tissue, while other nuclear envelope components such as Lamin-B exhibit small variations. Lamin-A/C has been implicated in aging syndromes that affect muscle and fat but not brain, and we find nuclei in brain-derived cells are indeed dominated by Lamin-B and are much softer than nuclei derived from muscle cells with predominantly Lamin-A/C. In vitro, matrix elasticity can affect expression of nuclear envelope components in adult stem cells, and major changes in Lamin-A/C are indeed shown to direct lineage with lower levels favoring soft tissue and higher levels promoting rigid tissue lineage. At a molecular level, tagging of cryptic sites while physically stressing isolated nuclei reveals stress-driven, mass spectrometry-mapped changes in various nuclear proteins including Lamin-A/C, consistent with cell and tissue evidence that the nucleus transduces physical stress.

242-Pos Board B28

FRAP Analysis of Proteins Diffusion and Binding in Inhomogeneous Media

Darya Yu. Orlova[1,2], Eva Bártová[1], Valeri P. Maltsev[2,3], Stanislav Kozubek[1], Andrei V. Chernyshev[2].
[1]Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Brno, Czech Republic, [2]Institute of Chemical Kinetics and Combustion, Novosibirsk, Russian Federation, [3]Novosibirsk State University, Novosibirsk, Russian Federation.

Fluorescence recovery after photobleaching (FRAP) is used for the study of the diffusion and binding of target proteins in biological cells. But the approach poses problems in analytical tractability of measurements in an inhomogeneous medium. Here, we present a method to solve the problem applying an integral transform of the kinetics of the entire cellular two-dimensional spatial pattern of recovered fluorescence. The experimental data were obtained with the Leica TCS SP5X confocal microscope. The method was demonstrated by the evaluation of the association and the dissociation rate constants and the diffusion coefficient of GFP-tagged heterochromatin protein 1 and GFP-tagged c-Myc protein in the nuclei of mouse embryonic fibroblasts. The main novelty of the approach is that it takes into account the inhomogeneous distribution of binding sites and does not require the complete mathematical solution of a corresponding system of diffusion-reaction equations that is typical for conventional FRAP data processing.

This work was supported by the Russian Ministry of Education and Science (P1039, 14.740.11.1194, 14.740.11.0921), by the European Union project (COST TD09/05) and Marie Curie project PIRSES-GA-2010-269156-LCS, by the Grant Agency of the Czech Republic (P302/10/1022). The work also has been supported by the Academy of Sciences of the Czech Republic, and by the Ministry of Education Youth and Sports of the Czech Republic: the research projects Nos. LC535, LC06027, ME 919, AVOZ50040702, AVOZ50040507, and COST-CZ LC11020.