BP 39: Optogenetics for the Cytoskeleton - Focus Session organized by Ulrich Schwarz

Time: Wednesday 9:30–12:30 Location: SCH A251

BP 39.1 Wed 9:30 SCH A251

Synthetic reconstitution of morphogenetic processes in naïve embryonic tissues — Emiliano Izquierdo and •Stefano De Renzis — EMBL-Heidelberg, Germany

Morphogenesis of multicellular organisms is characterized by changes in cell and tissue behaviours that occur at specific space and time in a coordinated manner. This stereotypic and genetically encoded spatiotemporal organization makes it difficult to determine the extent to which individual cell behaviour drive morphological remodelling. In my talk, I will present a novel optogenetic-based synthetic approach that allowed us to reconstitute complex morphogenetic processes in naïve Drosophila embryonic tissues independent of any pre-determined physical and biochemical conditions or other tissue-scale properties that accompany endogenous morphogenetic processes.

BP 39.2 Wed 10:00 SCH A251

Collective dynamics determine selection and regulation of leaders during epithelial wound healing — •Medhavi Vishwakarma, Tamal Das, and Joachim P. Spatz — Department New Materials and Biosciences, Max-Planck-Institute for Intelligent Systems, Stuttgart

Collective migration involves coordinated movement of several cells, and influences many biological processes including embryogenesis, wound healing, and cancer metastasis. The prevalent view on collective cell migration, especially in the context of epithelial cells during wound healing, assumes a hierarchical leader-follower organization and belittles the contribution of follower cells in choosing or regulating the leaders. Here, we report and analyse distinct phases of collective migration during wound closure and demonstrate how collective dynamics influence selection and regulation of leader cells in these phases. We found that in the preparatory phase, before the initiation of migration (Phase 0), the selection of leader cells at the epithelial wound margin is largely governed by dynamic heterogeneity of the followers in the monolayer. Long before the prospective leaders actually start displaying their phenotypic peculiarities, cells behind them manifest stochastic augmentations in the traction forces and monolayer stresses, and display large perimeter-to-area ratio indicating a local unjamming. Strikingly, the length scale of this collective dynamics matches with the distance between two emerging leaders. Furthermore, It is also possible to control the leader cell formation by introducing followers with high contractile forces at the back. For that, we used an optogenic technique involving a photo-excitable form of RhoA to transiently increase the RhoA activation and hence the cellular traction stresses. Upon photoactivation of RhoA in the followers, we could spatially bias the formation of leaders at the interface. As the migration progresses from the phase 1 to the phase 2, the number of followers per leader is limited by formation of new leaders at the margin and this limit is again set by the length scale of cell-cell force transmission. Any perturbations in mechanical forces that modifies the force correlation lengths and hence the collective dynamics of the system, invariably enforces a change in the number of followers per leader thereby modifying the time required to transit from one phase to the other. Together, these findings provide a novel insight into formation and regulation of leader cells, and indicate integrative leader-follower interactions during wound closure.

BP 39.3 Wed 10:15 SCH A251

Optogenetic switches to control cellular actin dynamics — •Robert Grosse — University of Marburg

Actin dynamics is essential for cellular functions such as adhesion, motility and spatial organization. The Rho-GTPase effector proteins of the formin family are tightly regulated through autoinhibitory interactions. Formins are potent actin assembly factors that can nucleate and elongate linear actin filaments for multiple cellular functions including transcriptional control. Here we discuss tools and approaches to rapidly and reversibly modulate actin assembly by light by targeting Rho-GTPases, formins and beyond at the plasma membrane as well as in the somatic cell nucleus.

BP 39.4 Wed 10:45 SCH A251

Optogenetic manipulation of membrane signaling and cytoskeletal dynamics in the social amoeba *Dictyostelium discoideum* — •Sven Flemming, Kirsten Sachse, and Carsten Beta — University Potsdam, Department of Physics and Astronomy, Potsdam, Germany

Motile cells such as macrophages, mesenchymal stem cells, or cancer cells show complex spatiotemporal pattern formation in the actin cytoskeleton. These patterns can be influenced by external cues such as chemoattractant signals, which lead to directed movement but can also occur without an external stimulus, for example as self-sustained actin oscillations or waves. We use the social amoeba *Dictyostelium discoideum* as a model organism to elucidate how different components of the signaling pathways contribute to these dynamics. To this end, we establish an optogenetic approach to recruit downstream targets of cAMP signaling — agonists as well as antagonists of actin polymerization — to distinct areas of the plasma membrane. We will show to what extent these downstream targets affect the actin dynamics in the targeted regions and will use successful constructs for the mechanistic characterization of dynamic actin structures. Ultimately, our approach will allow us to manipulate and control the formation of complex spatiotemporal actin patterns in the cell cortex.

15 min break

Invited Talk BP 39.5 Wed 11:15 SCH A251

Navigating the cytoskeleton: new tools to dissect and direct intracellular transport — •Lukas Kapitein — Utrecht University

Cellular organization depends on the cytoskeleton, a mechanical network of biopolymers that controls cell shape and strength, as well as on motor proteins that can move over these biopolymers to deliver cargo to specific subcellular compartments. Nevertheless, the precise mechanisms that control cytoskeletal organization, the function and dynamics of different motor proteins, and the precise functions of subcellular positioning are still poorly understood. In my lecture, I will highlight novel light-based technologies that enable addressing these questions with unprecedented precision. First of all, we successfully engineered a system to control the transport and positioning of intracellular components with light through the controlled recruitment of specific motor proteins. This allows us to directly explore the intracellular activity of motor proteins and the functional consequences of organelle mislocalization. In addition, we have engineered novel probes and methodology for the super-resolution imaging of the cytoskeleton. These approaches allow us to better resolve cytoskeletal organization in dense cellular compartments, such as the axons and dendrites of neurons. Together, these technologies hold great promises for exploring cellular organization and dynamics in health and disease.

BP 39.6 Wed 11:45 SCH A251

Controlling and modelling contractility in adherent cells — •Dimitri Probst[1], Christoph A. Brand[1], Marco Linke[1], Patrick W. Oakes[2], Elizabeth Wagner[3], Michael Glotzer[3], Margaret L. Gardel[2], and Ulrich S. Schwarz[1] — [1]Institute for Theoretical Physics & BioQuant, Heidelberg University, Germany — [2]Institute for Biophysical Dynamics, James Franck Institute and the Department of Physics, University of Chicago, USA — [3]Department of Molecular Genetics and Cell Biology, University of Chicago, USA

Cellular contractility is known to be controlled by a small GTPase called RhoA, whose active form promotes both actin polymerization and myosin II motor activity. For example, a global increase of active RhoA in adherent tissue cells leads to the formation of focal adhesions and stress fibers. However, how localized RhoA signals translate into cell-level responses is not well understood. Here we address this question through experiments and modelling using an optogenetic approach. Local activation of RhoA stimulates local contraction that is quickly propagated over the whole cell through the stress fibers. It also drives F-actin and myosin towards the region of heightened RhoA. Surprisingly, the flow reverses direction when local RhoA activation stops. We explain our experimental findings with a viscoelastic physical model that demonstrates that stress fibers are elastic-like structures. We show that the elasticity of the stress fibers is preserved even at time scales exceeding turnover of constituent proteins. Our model furthermore allows to identify the repair molecule zyxin as a key regulator of stress fiber mechanics, as they become fluid-like in its absence.

BP 39.7 Wed 12:00 SCH A251

Towards in vivo optomechanical control of actomyosin — •Stephan Grill — BIOTEC, Technische Universität Dresden

In the nematode *Caenorhabditis elegans*, actomyosin-based active tension and active torque generation drive morphogenetic processes such as cell polarization and left/right symmetry breaking. I will report on our ongoing activities towards perturbing and remote-controlling active tension and active torque by light.