PSF Simulation Johann von Hase, KIP

Symposium on Laserscanning Microscopy

October 9/10, 2008

Kirchhoff-Institute of Physics, Kleiner Hörsaal

Program

Thursday, October 9, 2008

13.30 h Welcome und Introduction (C. Cremer)

Session 1: Single Molecule Measurements

13.45 h Single molecule tracking in cell nuclei
Ulrich Kubitscheck, Bonn

14.15 h Dual-focus and dual-intensity confocal microscopy and spectroscopy
Jörg Enderlein, Göttingen

14.45 h Multiparameter Fluorescence Imagespectroscopy
Claus Seidel, Düsseldorf

15.15 h Coffee Break

15.45 h – 18 h **SPP 1128 Postersession**

18.30 h SPP1128 Businessmeeting (Principal Investigators)

Friday, October 10, 2008

Session 2: Improvement of Optical Resolution

9.00 h *"Laser Confocal Microscopy and its Applications"*
Tony Wilson, Oxford

9.45 h Far-Field Fluorescence Nanoscopy
Stefan Hell, Göttingen

10.30 h Coffee Break

11.00 h Structured Illumination and Interferometric Resolution Improvement
Rainer Heintzmann, London

11.45 h Light sheet based Fluorescence microscopes (LSFM, SPIM, DSLM) reduce phototoxic effects by several orders of magnitude
Ernst Stelzer, Heidelberg

12.30 h Lunch (Mensa, DKFZ Kantine, Brasserie Fritz)

13.45 h Photoswitches: Key Molecules for Super-Resolution Fluorescence Imaging and Molecular Quantification

Markus Sauer, Bielefeld

Session 3: Applications of Light Optical Nanosopy Approaches

14.30 h Optical nanoscopy and nuclear architecture studies: where does the field stand and where should we go

Thomas Cremer, München

15.15 h Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy

Heinrich Leonhardt, München

15,45 h Kaffeepause

16.15 h The application of localisation microscopy to cardiac calcium signalling

David Baddely, Auckland

17.00 h The human kinetochore and its mitotic control

Stephan Diekmann, Jena

17.30 h Perspectives of Light Optical Nanoscopy,

Christoph Cremer, Heidelberg

LIGHT SHEET BASED FLUORESCENCE MICROSCOPES (LSFM, SPIM, DSLM) REDUCE PHOTOTOXIC EFFECTS BY SEVERAL ORDERS OF MAGNITUDE

Ernst H. K. Stelzer
EMBL-Heidelberg, Meyerhofstrasse 1, D-69117 Heidelberg, Germany
stelzer@embl.de.

Most optical technologies (microscopy, optical tweezers [1], laser nanoscalpel [2]) are applied to two-dimensional cellular systems, i.e. they are used in a cellular context that is defined by hard and flat surfaces. However, physiological meaningful information relies on the morphology, the mechanical properties, the media flux and the biochemistry of a cell's context found in live tissue [3, 4]. A physiological context is certainly not found in single cells cultivated on cover slips. It requires the complex three-dimensional relationship of cells cultivated e.g. in an ECM-based gel or in naturally developing small embryos of flies or embryos and, of course, in tissue sections [4]. However, the observation as well as the optical manipulation of extended biological specimens suffers from at least two severe problems. 1) The specimens are optically dense, i.e. they scatter and absorb light. Thus, the delivery of the probing light and the collection of the signal light tend to become inefficient. 2) Many biochemical compounds apart from fluorophores also absorb light and suffer degradation of some sort (photo-toxicity), which induces malfunction or death of a specimen [4]. The situation is particularly dramatic in conventional and confocal fluorescence microscopy. Even though only a single plane is observed, the entire specimen is illuminated. Recording stacks of images along the optical z-axis thus illuminates the entire specimen once for each plane. Hence cells are illuminated 10-20 and fish embryos even 100-300 times more often than they are observed. Surprisingly, this can be avoided by a simple change of the optical arrangement. The basic idea is to use light sheets, which are fed into the specimen from the side and which overlap with the focal plane of a wide-field fluorescence microscope [5]. Thus, in contrast to an epi-fluorescence arrangement, which uses the same lens, our azimuthal arrangement uses two independently operated lenses for illumination and detection. Optical sectioning and no photo-toxic damage outside a small volume around the focal plane are its intrinsic properties. Light sheet based fluorescence microscopes (LSFM) take advantage of modern camera technologies, which provide them with a signal to noise ratio that is at least thirty times better than that of a confocal microscope [6]. LSFM can be combined with essentially every contrast and specimen manipulation tool to operate in a truly three-dimensional fashion. In a current application, they are used to record early zebrafish (*Danio rerio*) development *in vivo* and *in toto* from the early 32-cell stage until late neurulation with sub-cellular resolution and very short sampling periods (60-90 sec/stack) [7]. The recording speed is more than 30 Million voxels/sec or more than five very large frames/sec with a dynamic range of 12-14 bit. We follow the cell movements during gastrulation and reveal its development during the cell migration processes. We can show that an LSFM exposes an embryo to 200 times less energy than a conventional, 5,000-6,000 times less than a confocal and about one million times less than a two-photon fluorescence microscope. Based on this outstanding performance, we claim that our novel, truly three-dimensional approach will have a dramatic impact on developmental and cell biology as well as on biophysics [8].

[1] Rohrbach, A, Tischer, C, Neumayer, D, Florin, EL, Stelzer, EHK (2004) Trapping and tracking a local probe with a PFM, R*ev Sci Instr* **75**:2197-2210. **[2]** Colombelli J, Reynaud EG, Stelzer EHK (2007) Investigating relaxation processes in cells and developing organisms, *Methods in Cell Biology* (Edt. Berns M & Greulich KO), **82**:267-91. **[3]** Keller PJ, Pampaloni F, Stelzer EHK (2007) 3D preparation and imaging reveal intrinsic MT properties, *Nature Methods*, **4**(10):843-846. **[4]** Pampaloni F, Reynaud EG, Stelzer EHK (2007) The third dimension bridges the gap between cell culture and live tissue, *Nature Rev MCB*, **8**(10):839-845. **[5]** Huisken J, Swoger J, Del Bene F, Wittbrodt J, Stelzer EHK (2004) Optical sectioning deep inside live embryos by SPIM, *Science* **305**:1007-9. **[6]** Verveer P, Swoger J, Pampaloni F, Greger K, Marcello M, Stelzer EHK (2007) High-resolution 3D imaging of large specimens with light-sheet based microscopy, *Nature Methods*, **4**:311-313. [**7**] Keller PJ, Schmidt A, Wittbrodt J, Stelzer EHK (2008) In toto reconstruction of *Danio rerio* embryonic development, submitted. **[8]** Keller PJ, Pampaloni F, Lattanzi G, Stelzer EHK (2008) Three-dimensional microtubule behaviour in *Xenopus* egg extracts reveals four dynamic states and state-dependent elastic properties, *Biophys. J.*, **95**(3):1474-86.

Dynamic actin patterns formed by self-organization in live cells

Till Bretschneider[1], Silke Gerwig[2], Britta Schroth-Diez[2], Mary Ecke[1], Stefan Diez[2], and Günther Gerisch[1]

[1] Max-Planck-Institut für Biochemie, Martinsried
[2] Max Planck Institute for Molecular Cell Biology and Genetics, Dresden

Typically, actin polymerization is locally initiated by membrane-bound protein complexes, and the resulting structures are involved in spezialized cellular functions, such as migration, particle uptake, or mitotic division. To disclose the potential of the actin system to self-organize into dynamic complexes, we analyzed the structure and dynamics of actin waves that propagate on the planar, substrate-attached membrane of a cell. We show that self-assembly involves the ordered recruitment of proteins from the cytoplasmic pool and relate the 3-dimensional organization of actin waves to their capacity of applying force.

Dual-emission TIRF microscopy combined with confocal z-scanning revealed three proteins to form disctinct 3-dimensional patterns: myosin-IB enriched at the wave front and close to the plasma membrane, the Arp2/3 complex distributed throughout the waves, and coronin that forms a sloping layer on top of them. FRAP revealed that wave propagation is based, similar to leading-edge protrusion, on an actin treadmilling mechanism. The formation of actin waves does not depend on external signals transmitted by heterotrimeric G-proteins. The role in wave formation of the pentameric SCAR complex, a regulator upstream of Arp2/3, has been studied in single and double mutants deficient in SCAR-associated proteins.

In conclusion, the actin waves formed in live cells are highly organized supramolecular structures recruited from molecular motors and proteins controlling actin polymerization and depolymerization. A program coupling actin assembly at their front to disassembly at their back is responsible for their propagation. These actin waves are self-sustained molecular networks capable of applying force to change the shape of a cell.

Publications on actin dynamics

Gerisch, G., Bretschneider, T., Müller-Taubenberger, A., Simmeth, E., Ecke, M., Diez, S., and Anderson, K. (2004). Mobile actin clusters and traveling waves in cells recovering from actin depolymerization. Biophys. J., 87:3493-3503.

Diez, S., Gerisch, G., Anderson, K., Müller-Taubenberger, A., and Bretschneider, T. (2005). Subsecond reorganization of the actin network in cell motility and chemotaxis. PNAS 102:7601-7606.

Medalia, O., Beck, M., Ecke, M., Weber, I., Neujahr, R., Baumeister, W., and Gerisch, G. (2007). Organization of actin networks in intact filopodia. Curr. Biol. 17:79-84.

Dalous, J., Burghardt, E., Müller-Taubenberger, A., Bruckert, F., Gerisch, G., and Bretschneider, T. (2008). Reversal of cell polarity and actin-myosin cytoskeleton reorganization under mechanical and chemical stimulation. Biophys.J. 94:1063-1074.

Dynamics of the actin cytoskeleton in response to periodic stimuli

C. Westendorf[1], H. Ishikawa-Ankerhold[2], G. Gerisch[2], E. Bodenschatz[1], C. Beta[1,3]

[1] Max Planck Institute for Dynamics and Selforganisation, Göttingen.

[2] Max Planck Institute for Biochemistry, Martinsried.

[3] Institute of Physics and Astronomy, University of Potsdam.

The dynamic properties of the actin cytoskeleton provide the basis for motility, phagocytosis, and division of eukaryotic cells [1]. A widely used model system for the study of actin dynamics *in vivo* is the social amoeba *Dictyostelium discoideum*. Under starvation, *D. discoideum* senses extracellular cAMP by a G-protein coupled signaling cascade [2], which stimulates actin polymerization and re-organization of the cytoskeleton. Polymerization of actin fibers within the branched cortical network exerts a force at the membrane of the leading edge resulting in the formation of pseudopods and, finally, cell motion. It is the aim of this study to characterize intrinsic time scales of the actin cytoskeleton in chemotactic *Dictyostelium* cells. This is achieved by investigating cellular responses to periodic stimuli of cAMP.

We observe filamentous actin using a LimE-GFP mutant strain in AX-2 background [3]. Microfluidic chambers are used to produce controlled and well-defined environments. In the micro-flow, we generate pulses of cAMP by laser induced photochemical release of cAMP from a biologically inert, caged form [4]. Series of periodic photoactivation events were applied with periods ranging from 6 sec to 40 sec. Responses of the cytoskeleton were recorded by fluorescence imaging of LimE-GFP using confocal laser scanning microscopy. We have performed both frequency analysis and computer automated cell tracking and find an optimal response regime of the actin system around 20 sec. For longer forcing periods, a frequency doubled resonant response could be observed. For short forcing periods no entrainment was found. Furthermore, first experiments were performed on single-pulse stimulation of cytoskeletal mutant strains with deficiencies in Aip1 and coronin. Our results indicate changes in the characteristic time scale of actin depolymerization. Future work will focus on periodic stimulation of these and other mutant strains.

[1] **Pollard, T.D. BORISY, 2003**: Nature, Vol. 422 p741 – 745.
[2] **Stacey, S., et al., 2006**: European Journal of Cell Biology Vol. 85 p897 – 904.
[3] **Schneider, N., et al., 2003**: Cell Motility and the Cytoskeleton, Vol. 56 p130 – 139.
[4] **Beta, C., et al., 2007**: Analytical Chemistry, Vol. 79 p3940 – 3944.

Live cell imaging and computer modeling of PML nuclear body assembly

Stefanie Weidtkamp-Peters, Thorsten Lenser, Dmitri Negorev, Norman Gerstner, Thomas Hofmann, Georg Schwanitz, Christian Hoischen, Gerd Maul, Peter Dittrich, and Peter Hemmerich

Leibniz-Institute of Age Research, Fritz-Lipman-Institute, Beutenbergstr. 11, 07745 Jena, Germany

PML nuclear bodies (NBs) are involved in the regulation of key nuclear pathways such as transcriptional regulation, apoptosis, the DNA damage response and senescence. The precise biochemical function of these macromolecular assemblies in nuclear metabolism is still unknown.
In order to elucidate the molecular basis for PML NB assembly and maintenance we have investigated factor exchange at these subnuclear domains by quantitative live cell imaging and mathematic modelling of its ten constitutive protein components. We show that all six nuclear PML protein isoforms exhibit individual exchange rates at NBs and identify PML V as a scaffold subunit. Sp100 exchanges at least 5-fold faster at NBs than PML proteins. Using computer modeling based on biophysical dynamics parameters obtained by FRAP and FCS we also demonstrate that SUMOylation provides a mechanism to fine-regulate the residence time and the exchange rates of PML and Sp100 at nuclear bodies. Factor exchange at PML NBs is not temperature-dependent indicating a diffusion-only mechanism in nuclear body maintenance. The PML NB component HIPK2 requires an active kinase domain for PML NB targeting and elevated levels of PML IV increase HIPK2's residence, thus stongly suggesting that HIPK2-mediated phosphorylation activity occurs at PML NBs. DAXX and BLM turn over rapidly and completely at PML NBs within seconds indicating very fast binding and un-binding reactions of these two components at PML nuclear bodies.
In summary, our findings provide a kinetic model for factor exchange at PML nuclear bodies and reveal potential mechanisms to regulate intranuclear trafficking of specific factors at these domains (1).

(1) Weidtkamp-Peters et al., (2008) *J. Cell Sci*., in press

Observation of single transport events through single pore complexes of the nuclear envelope by means of near-field optical microscopy

N. Neuberth,[a)] M. Herrmann,[b)] J. Pérez,[a)] J. Wissler,[a)] D. Gradl,[b)] and A.Naber [a)]

a) Institut für Angewandte Physik, Universität Karlsruhe (TH)
b) Zoologisches Institut II, Universität Karlsruhe (TH)

A method for applying scanning near-field optical microscopy (SNOM) to freestanding biological membranes in physiological solution has been established. An unfixed membrane spanned over 20-µm-sized holes in a flat surface is imaged at a resolution of < 50 nm by scanning a SNOM probe across the unsupported membrane patch. The membrane integrity is neither disrupted by axial forces exerted by the tip nor is the membrane noticeably sheared by lateral forces during scanning. The capabilities of this method are demonstrated by imaging the densely packed pores in a freestanding nuclear envelope of *Xenopus laevis* oocytes. The nuclear pore complexes (NPCs), which are embedded in the membrane, were fluorescently double stained with antibodies specific for different proteins in the NPC and visualized with the same tip at different emission wavelengths. The freestanding membrane separates two different fluid compartments and thus enables us to investigate transport processes of fluorescently labeled proteins binding to and passing through the NPCs. We placed the near-field probe just above a pore and measured the fluorescence intensity as a function of time. For the first time, the fast diffusional motion of single molecules could be resolved on a nanoscale by using SNOM in combination with fluorescence correlation spectroscopy (FCS). Thus, we were able to determine the characteristic diffusion time of the transport factor NTF2 within the transport channel of the nuclear pore complex. Furthermore, we could determine the dwell time of NTF2 at the nuclear basket and found a good correspondence with results of previous studies based on single molecule tracking techniques [2].

References:

[1] C. Höppener, J. P. Siebrasse, R. Peters, U. Kubitscheck, and A. Naber, "High-Resolution Near-Field Optical Imaging of Single Nuclear Pore Complexes under Physiological Conditions", Biophys. J. 88, 3681-8 (2005).

[2] U. Kubitscheck et al., Nuclear transport of single molecules: dwell times at the nuclear pore complex, J. Cell Biol. 168 (2005).

Cryo-electron tomography of neuronal synapses

Ruben Fernandez, Geoffrey Kong, Benoit Zuber, Wolfgang Baumeister, Vladan Lucic

We have used cryo-electron tomography (cryo-ET) to investigate the architecture of macromolecular complexes present at the excitatory synapses of the central nervous synapses. In addition to providing full three-dimensional picture, cryo-ET allows for the preservation of the biological sample in a near-to-physiological state, thereby avoiding staining, fixation and dehydration artefacts. The application of cryo-ET to isolated synapses shows distinct, stimulation-dependent organization of synaptic vesicles. Using our automated segmentation and analysis procedure we have detected and characterized molecular connections between synaptic vesicles in a comprehensive manner. Furthermore, we have investigated the morphology of synaptic adhesion complexes under different pharmacological and gentic treatments.

Quantitative imaging of kinetochore assembly in living human cells

Sandra Orthaus, Steffi Weidtkamp-Peters, Christian Hoischen, Peter Hemmerich, Daniela Hellwig, Stephan Diekmann

Leibniz Institute for Age Research, Molecular Biology, Beutenbergstr. 11, D-07745 Jena/Germany

Analysis of the interaction between the hypoxia-inducible factor 1α and the prolyl hydroxylase 1 by means of fluorescence resonance energy transfer

S Gneuß, U Berchner-Pfannschmidt, C Wotzlaw, J Fandrey

Institute of Physiology, University of Duisburg-Essen

The hypoxia-inducible factor 1α (HIF-1α) is one of two subunits of the transcription factor HIF-1 and central to adaptation to low oxygen availability. Three prolyl hydroxylases (PHDs), namely PHD 1, 2 and 3, which belong to the enzyme family of dioxygenases have been identified as oxygen sensors. In the presence of oxygen PHDs catalyze the hydroxylation of HIF-1α which initiates its degradation in the proteasome. In hypoxia PHDs are inactivated resulting in stabilization of HIF-1α protein. Stabilized HIF-1α translocates to the nucleus where it heterodimerizes with HIF-1β to form HIF 1, which regulates transcription of many genes involved in responses to hypoxia.
In this study we analysed the interaction between HIF-1α and PHD1 in living human cells by means of fluorescence resonance energy transfer (FRET). For studying this radiation less energy transfer between two fluorescent proteins we used a laser scanning microscope. We used the fusion protein pECFP-C1-HIF-1α as donor molecule and pEYFP-C1-PHD1 as acceptor molecule.
Our results confirm that HIF-1α directly interacts with PHD 1. We could also show that this interaction takes place in the nucleus and that the distance between the two interaction partners is 5.67 nm.
This work was supported by DFG SPP1128 (FA225/19-3)

Correlated Microscopy: From Dynamics to Structure

S. Lepper[1], M. Kudryashev[1], A. Sartori[2], S. Bohn[3], M. Cyrklaff[1] and F. Frischknecht[1]

1. Department of Parasitology, Hygiene Institute, University of Heidelberg Medical School, INF 324, 69120 Heidelberg, Germany
2. Institut Pasteur, Imagopole, Dynamic Imaging and Ultrastructural Microscopy Platform, 25-28 rue du Dr. Roux, 75015 Paris, France
3. Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany

Simone.Lepper@med.uni-heidelberg.de
Keywords: Correlation, Cryo-Electron Tomography, Malaria

It has always been the aim of functional studies in biology to establish structure-function relationships of the molecular machineries involved in the process of interest. A powerful tool within this field has always been electron microscopy continuously adapting to answer latest questions [,] and therefore, principally shaping our view on cell architecture. Correlative approaches now allow to bridge the gap of information by combining dynamic data obtained by fluorescent light microscopy (LM) with the structural information of cryo-electron microscopy (CEM) or cryo-electron tomography (CET) and vice versa. Plasmodium berghei sporozoites are an ideal system for such studies. They are the reactive agents transmitting malaria from mosquitoes to rodents, and due to their close relation to the human pathogen Plasmodium falciparum they are widely used as a model organism. Since they are only 1 μm thick they can be viewed in toto with ET. The sporozoites represent both, a motile, highly polarized eukaryotic cell and a devastating pathogen.

A correlative approach is opening new doors for the understanding of pathogenesis and dynamic cell architecture. Not only the spatial organisation of the cell organelles remains barely understood, but so does also one crucial factor for pathogenesis: the motility of the parasite. Unlike the immobile sporozoites from the mosquito midgut, only the motile ones isolated from the salivary glands of the mosquito are infectious [ ]. During a mosquito bite a very variable number of parasites is transmitted [ ]. Any regulation of this process is unrecognized, but might be triggered by the spatial organisation within the salivary glands. Once inoculated into the dermis the parasite uses his highly adapted gliding machinery to start his destructive journey through the host. Some key players of the gliding machinery are characterized, but their dynamic interaction stays largely unclear. Various drugs such as cytochalasin D and jasplakinolide inhibit actin dynamics and modulate the motility of the parasite [ ]. The distribution of actin is reorganized under the influence of jasplakinolide (Figure 1A, B), a drug that stabilizes actin in the filamentous form. However, the better characterization of the role of actin within the gliding machinery is still necessary. In order to answer this question the behaviour of sporozoites expressing fluorescently tagged actin was monitored by LM (Figure 1A, B) and the different, drug-concentration dependent phenotypes were quantified. Further structural investigations have been hampered so far due to the known shortness of the actin filaments and because only a minor part of the total actin pool of the cell occurs as filaments [ ].

Our aim now is to complete the description of the different phenotypes with structural information on the EM level. Plasmodium sporozoites are drug treated and filmed on EM grids in LM followed by plunge freezing in liquid ethane within a few seconds. This preserves even sensitive structures like the gliding machinery (Figure 1C), thus making higher resolution data accessible (Figure 1D, E, F). To investigate the spatial organisation within the salivary glands and of certain organelles, vitreous cryosections are obtained from intact salivary glands and fluorescently tagged organelles are retrieved in cryo-LM and CEMs are taken. This leads to a new understanding of host-vector interaction and gives new insights into the highly adapted, but still unravelled, architecture of Plasmodium sporozoites.

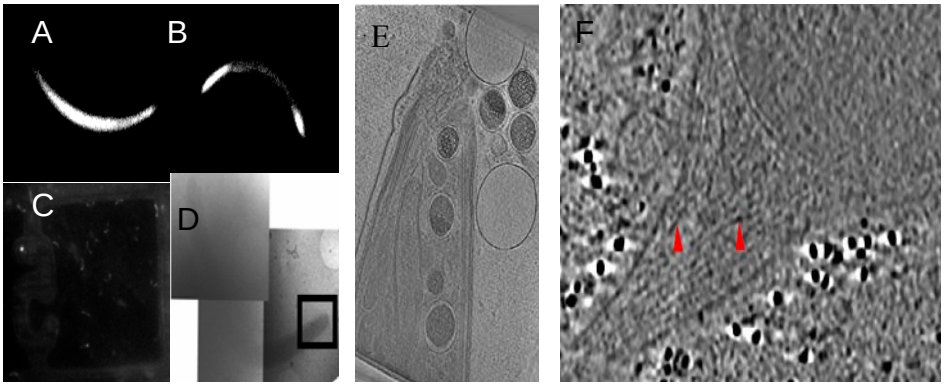

Figure 1: Massive rearrangement of the spatial actin distribution within the sporozoite occurs under jasplakinolide (A and B, fluorescent LM of a sporozoite expressing mCherry tagged actin treated with jasplakinolide (B) and without jasplakinolide (A)). The fluorescent signal can be preserved in cryo-LM (C) allowing the access to higher resolution data (D, low magnification CEM). Parts of the sporozoite of special interest can be further examined and CETs are taken (E and F, cross-sections from a tomogram of the apical (E) and back (F) end of a jasplakinolide treated sporozoite; arrowheads indicate the actin filaments).

1. W. Baumeister, FEBS 579 (2005) 933.
2. A. Al-Amoudi, J.J. Chang, A. Leforestier, A. McDowall, L. M. Salamin, L . P. O. Norlén, K. Richter, B. N. Sartori, D. Studer and J. Dubochet, EMBO Journal 23 (2004) 3583.
3. K. Matuschewski, Journal of Cellular Microbiology 8 (2006) 1547.
4. F. Frischknecht. P. Baldacci, B. Martin, C. Zimmer, S. Thiberge, J. C. Olivo-Marin, S.L. Shorte and R. Ménard, Cell Microbiol. 6 (2004) 687.
5. D. M. Wetzel, S. Håkansson, K. Hu, D. Roos, L. D. Sibley, Molecular Biology of the Cell 14 (2003) 396.
6. H. Schüler, A.-K. Müller, K. Matuschewski, FEBS Letters 579 (2005) 655.
7. Funding by the German Ministry of Education and Research (BMBF: BioFuture) and the German Research Foundation (DFG: SPP 1128) are acknowledged.

High-Frequency Imaging of MyoB Dynamics in D. Discoideum

Silke Gerwig [1], Britta Schroth-Diez [1], Mary Ecke [2], Günther Gerisch [2], and Stefan Diez [1]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
[2] Max-Planck-Institut für Biochemie, Martinsried

Dictyostelium discoideum Myosin IB (MyoB) is a single-headed class I myosin that has been implicated in the regulation of pseudopod formation in general and in the suppression of lateral pseudopod formation in particular [1-4]. MyoB has been shown to associate with the plasma membrane during locomotion [5] and has been localized to actin-rich regions, as well as to the filopods of Dictyostelium cells. However, no data on how single MyoB molecules might be organized into molecular complexes and recruited to its place of action have been reported.
Using Total Internal Reflection Fluorescence (TIRF) microscopy with highly sensitive EMCCD detection, we imaged GFP-MyoB mutants in both AX2 wild-type cells and in cells having a MyoB null background. To visualize the dynamics of the single headed motor we acquired time-lapse movies using high frame rates.
Data analysis using kymographs revealed MyoB clusters along filopods as well as at the substrate attached surface of the cell body. In both cases we found the MyoB clusters moving randomly, showing no particular direction. Especially in the area of the cell body, MyoB molecules seem to diffuse as aggregates in and out of the region near the substrate attached membrane.

1. Jung, G., et al., The Dictyostelium CARMIL protein links capping protein and the Arp2/3 complex to type I myosins through their SH3 domains. Journal of Cell Biology, 2001. 153(7): p. 1479-1497.
2. Wessels, D., et al., Myosin Ib Null Mutants of Dictyostelium Exhibit Abnormalities in Motility. Cell Motility and the Cytoskeleton, 1991. 20(4): p. 301-315.
3. Wessels, D., M. Titus, and D.R. Soll, A Dictyostelium myosin I plays a crucial role in regulating the frequency of pseudopods formed on the substratum. Cell Motility and the Cytoskeleton, 1996. 33(1): p. 64-79.
4. Wessels, D., et al., 3-Dimensional Dynamics of Pseudopod Formation and the Regulation of Turning during the Motility Cycle of Dictyostelium. Cell Motility and the Cytoskeleton, 1994. 27(1): p. 1-12.
5. Senda, S., et al., Recruitment of a specific amoeboid myosin I isoform to the plasma membrane in chemotactic Dictyostelium cells. Journal of Biological Chemistry, 2001. 276(4): p. 2898-2904.

Thibaud Jegou[*,§], Inn Chung[*,§], Gerrit Heuvelman[*,§], Malte Wachsmuth[||], Sabine M. Görisch[†], Karin M. Greulich-Bode[‡], Petra Boukamp[‡], Peter Lichter[†] and Karsten Rippe[*,§]

Deutsches Krebsforschungszentrum, [*] Research Group Genome Organization & Function, [†] Division of Molecular Genetics and [‡] Division of Genetics of Skin Carcinogenesis, Im Neuenheimer Feld 280, and [§] BioQuant, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany, [||] European Molecular Biology Laboratory, Cell Biology/Biophysics Unit, Meyerhofstr. 1, 69117 Heidelberg, Germany

Telomerase-negative tumor cells maintain their telomeres via an alternative lengthening of telomeres (ALT) mechanism. This process involves the association of telomeres with promyelocytic leukemia nuclear bodies (PML-NBs). Here, the mobility of both telomeres and PML-NBs as well as there interactions were studied in human U2OS osteosarcoma cells, in which the ALT pathway is active. An U2OS cell line was constructed that had *lac* operator repeats stably integrated adjacent to the telomeres of chromosomes 6q, 11p and 12q. By fluorescence microscopy of autofluorescent LacI repressor bound to the *lac*O arrays the telomere mobility during interphase was traced and correlated with the telomere repeat length. A confined diffusion model was derived that describes telomere dynamics in the nucleus on the time scale from seconds to hours. Two telomere groups were identified that differed with respect to the nuclear space accessible to them. Furthermore, translocations of PML-NBs relative to telomeres and their complexes with telomeres were evaluated. Based on these studies a model is proposed, in which the shortening of telomeres results in an increased mobility that could facilitate the formation of complexes between telomeres and PML bodies.