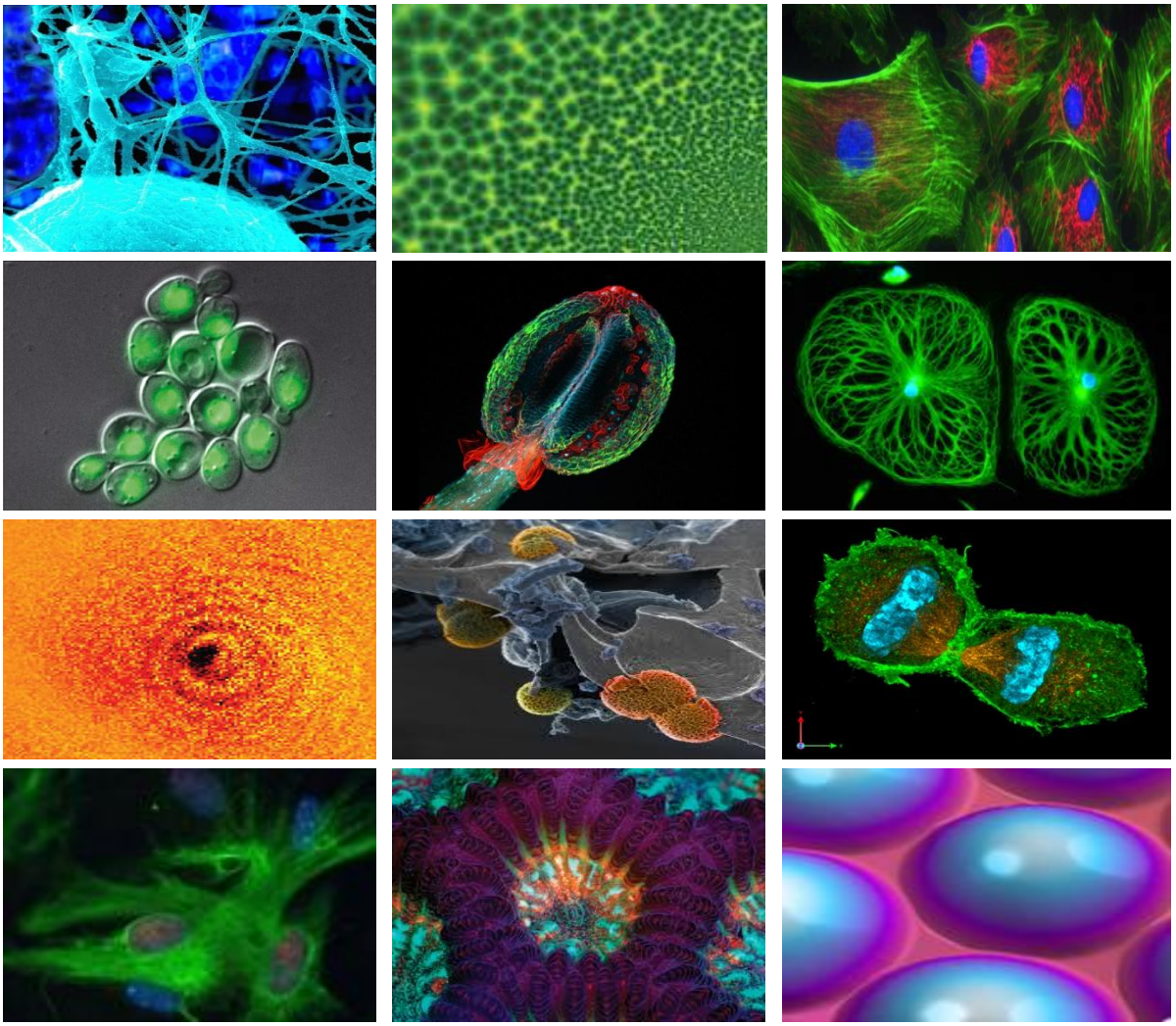
Workshop

"Live Cell Imaging Microscopy"

May 23 – 25, 2013



Faculty of Medicine, University of Rijeka

Braće Branchetta 20, Rijeka, Croatia

Workshop organiser

Professor Siniša Volarević

Faculty of Medicine, University of Rijeka

Department of Molecular Medicine and Biotechnology

Croatia

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Capacities - REGPOT

"Upgrading the capacities for research in translational medicine at the Faculty of Medicine University of Rijeka"

TransMedRi (2010 – 2013)

Project Coordinator: Professor Bojan Polić

Thursday, May 23, 2013 (Lectures)

Lecture hall 3rd floor
Faculty of Medicine, University of Rijeka

09:30 – 10:20	Jiri Bartek *Danish Cancer Society Research Center, Copenhagen, Denmark*
10:20 – 10:30	Questions
10:30 – 10:50	Coffee break
10:50 – 11:40	Martin Eduard van Royen *Department of Pathology, Josephine Nefkens Institute, Erasmus MC, Rotterdam, The Netherland*
11:40 – 11:50	Questions
11:50 – 12:40	Himjyot Jaiswal *Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden*
12:40 – 12:50	Questions
12:50 – 13:50	Lunch (catering)

Moderator: Professor Siniša Volarević

Friday, May 24, 2013 (Practical part)

Department of Molecular Medicine and Biotechnology
Faculty of Medicine, University of Rijeka

10:00 – 16:00

Maja Cokarić Brdovčak

Martin Pfannkuchen

Department of Molecular Medicine and Biotechnology
Faculty of Medicine, University of Rijeka, Croatia

II Abstracts

BIOLOGICAL RESPONSES TO DNA DAMAGE; MECHANISMS AND RELEVANCE FOR CANCER

Jiri Bartek

Danish Cancer Society Research Center, Copenhagen, Denmark

Through work in many laboratories including ours, it has become evident that the mechanisms of DNA damage sensing, signaling and repair rely on multiple protein modifications that operate in concert with the phosphorylation (and dephosphorylation) network governed by the ATM/ATR-regulated DNA damage response (DDR). This lecture will summarize our recently published and unpublished data documenting the biological and pathophysiological role of the emerging ubiquitylation/deubiquitylation cascade, including the RNF8, RNF168, HERC2 and BRCA1 ubiquitin ligases, in DNA damage signaling after ionizing and laser radiation, as well as checkpoint pathways and repair in human cells. The data will also include results from high-throughput RNAi-based screens for novel DDR components, live-cell imaging of human cells to analyze the spatiotemporal orchestration of the key DDR pathways, and mechanistic insights into responses to DNA double strand breaks. Emphasis will be on dynamic recruitment of DDR factors to sites of damage, responses to laser-induced DNA damage, ionizing radiation and replication stress, the latter identified in our laboratory as the key trigger of a DDR barrier against activated oncogenes and tumor progression. Unpublished data on a novel mechanism regulating RNF168, critical to limit cellular responses to ionizing radiation, will be discussed.

In addition, our recent results exploitation of DDR defects in human pathology, with focus on a new radiosensitivity syndrome (mimicking ataxia-telangiectasia) caused by a homozygous defect of the RNF168 ubiquitin ligase, and on tumor-associated DDR aberrations as predictive markers to guide individualized cancer therapy, will be presented. The latter topic extends our concept of DDR as a biological anti-cancer barrier and selection for DDR defects that allow the nascent tumor cells to escape from the DDR-imposed checkpoints, thereby facilitating tumor progression and also affecting responses to standard-of-care genotoxic therapies as well as the emerging DDR-targeted drugs such as PARP inhibitors.

Selected references:

Jackson SP, Bartek J. *Nature*, 461, 1071-8 (2009); Bartkova J, et al. *Nature*, 434: 864-70 (2005); Bartek J, Mistrik M, Bartkova J. *Nature Struct.Mol. Biol*., 19: 5-7 (2012); Doil C et al. *Cell,* 136, 435-446 (2009); Lukas C et al. *Nature Cell Biol*; 13, 243-253 (2011); Bartkova J, et al. *Nature*, 444: 633-7 (2006); Halazonetis, T. D., Gorgoulis, V. G., and Bartek, J. *Science,* 319, 1352-1355 (2008); Bouwman P et al. *Nature Struct Mol Biol*.17, 688-95 (2010); Lukas J, Lukas C, Bartek J. *Nature Cell Biol.* 13, 1161-1169 (2011); Takacova S. et al. *Cancer Cell* , 21: 517-31 (2012); Gudjonsson T et al., *Cell* 150: 697-709, (2012), Burrell R. et al. *Nature* 494: 492-496 (2013); Velimezi G. Et al. *Nature Cell Biol*. (in press, 2013).

SPATIOTEMPORAL ORGANIZATION OF ANDROGEN RECEPTOR REGULATED GENE TRANSCRIPTION

Martin Eduard van Royen

Department of Pathology, Josephine Nefkens Institute, Erasmus MC, Rotterdam, The Netherlands

Androgens exert their key function in development and maintenance of the male phenotype via the androgen receptor (AR). This ligand-activated transcription factor also play a role in prostate cancer. AR-regulated gene expression is a highly coordinated dynamic process mediated by AR ligand binding and DNA binding, and by specific AR protein–protein interactions. The latter include DNA-binding domain (D-box) interactions in AR homodimers, and the interaction of the FQNLF motif in the AR N-terminal domain and the coactivator groove in the ligand-binding domain (N/C interaction).

A series of quantitative live cell imaging approaches, including single molecule microscopy, fluorescent recovery after photobleaching (FRAP), time lapse imaging and fluorescence resonance energy transfer (FRET) has shown to be very instrumental to unravel the spatiotemporal organization and the details of these events [1-3]. We showed that the initial cytoplasmic intramolecular AR N/C interaction after ligand binding is followed by a D-box-dimerization-dependent transition to intermolecular N/C interaction in a proportion of nuclear ARs. The consecutive steps leading to homodimerization are initiated prior to DNA binding [4].

By combining three different fluorescence microscopy assays: single-molecule microscopy, photo¬bleaching (FRAP) and correlation spectroscopy (FCS), we could distinguish two types of AR-DNA binding: very brief interactions, in the order of a few hundred milliseconds, and hormone-induced longer-lasting interactions in foci partly overlapping transcription sites, with a characteristic binding time of several seconds [5]. In addition, freely mobile ARs were slowed down in the presence of hormone, suggesting the formation of large AR-coregulator complexes, including AR dimerization, in the nucleoplasm upon hormone activation. Simultaneous FRET and FRAP showed that N/C interactions are largely lost when AR transiently binds to DNA allowing AR-coregulator interactions with the DNA bound ARs [6].

Together, our data unravels the details of a model in which mobile, hormone-induced transcription factor dimer-coregulator complexes probe DNA by briefly binding at random sites, and only bind specific coregulators when bound to specific recognition sequences to form relatively stable transcription initiation complexes.

Papers to be discussed:

Van Royen, M.E., et al., FRAP and FRET methods to study nuclear receptors in living cells, in The nuclear receptor superfamily, I.J. McEwan, Editor 2009, Humana Press / Springer: Totowa. p. 69-96.; Van Royen, M.E., et al., Fluorescence recovery after photobleaching (FRAP) to study nuclear protein dynamics in living cells, in The nucleus, R. Hancock, Editor 2009, Humana Press / Springer: Totowa. p. 363-85.; Van Royen, M.E., et al., Nuclear proteins: finding and binding target sites in chromatin. Chromosome Research, 2011. 19(1): p. 83-98.; Van Royen, M.E., et al., Stepwize androgen receptor dimerization. Journal of Cell Science, 2012. 125(125): p. 1970-9.; Van Royen, M.E., et al., Androgen receptor complexes probe DNA for recognition sequences by short random interactions. submitted for publication.; Van Royen, M.E., et al., Compartmentalization of androgen receptor protein-protein interactions in living cells. Journal of Cell Biology, 2007. 177(1): p. 63-72.

SPATIO-TEMPORAL DYNAMICS OF ATM KINASE ACTIVITY IN SINGLE CELLS

Himjyot Jaiswal

Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden

ATM kinase is a central player in the double strand break (DSB) mediated DNA damage response (DDR). The activation of ATM kinase upon DSBs initiates cell cycle arrest through a cascade of phosphorylation events, involving the effector kinases Chk1, Chk2 and MK2. However, how the DDR is deactivated to allow cellcycle resumption after repair of damaged DNA is poorly understood. Moreover, whether spatial regulation of ATM-target phosphorylation affects a cell cycle arrest remains unclear.

We have generated a biosensor that can monitor the phosphorylation of an ATM substrate in single live cells. Using this biosensor we follow ATM target phosphorylation from the initiation of DDR to resumption of the cell cycle and subsequent mitotic entry. Our data indicate that ATM activity is dynamic and correlates to the amount of DSBs. In addition, there is a clear difference between the phosphorylation of a chromatin bound and a soluble nuclear substrate. We speculate that the phosphorylation of chromatin bound substrates controls a cell cycle arrest after DSBs.

Publication:

Himjyot Jaiswal, Charlotte Conz, Hendrik Otto, Tina Wölfle, Edith Fitzke, Matthias Mayer and Sabine Rospert. The chaperone network connected to human ribosome---associated complex. Mol Cell Biol. (2011), Mar; 31(6):1160-°©-73. PMID: 21245388; Julia Benedix, Patrick Lajoie, Himjyot Jaiswal, Carsten Burgard, Markus Greiner, Martin Jung, Richard Zimmerman, Sabine Rospert, Erik L. Snapp and Johanna Dudek. Bip modulates the affinity of its co---chaperone Erj1 to ribosomes. J Biol Chem. (2010), 285(47): 36427-°©-33. PMID: 20864538