
# ANNUAL REPORT 2014

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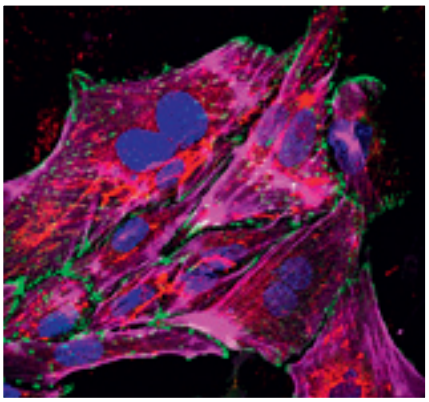
# FOREWORD

This is the 4th annual report of the Institute of Molecular Biology (IMB), which opened its doors in 2011 on the Campus of the Johannes Gutenberg University Mainz. IMB is an unusual basic research centre in that it was set up as a public-private partnership between the state of Rhineland-Palatinate, which provided the state-of-the-art research building, and the Boehringer Ingelheim Foundation, which supports the research. IMB is now reaching "cruising speed" and research activity is picking up as Junior Group Leaders are publishing their first key papers. Our International PhD Programme and Summer School are well established and attracting very good students. Scientific meeting activities are in full swing and IMB is continuing to form strong connections with the local and international scientific communities. With 16 research groups and close to 170 people now working at IMB, the institute is filling up and developing into a very lively place.



## NEW APPOINTMENTS

This year has seen the appointment of two Adjunct Directors at IMB, Eva Wolf and Miguel Andrade. They have taken up these positions in conjunction with Professorships in the Faculty of Biology at Mainz University, and their groups are located at IMB. Eva has joined us from Ludwig Maximilians University, Munich. Her exciting work, which uses structural biology to investigate chronobiology and the regulation of circadian genes, brings new facets to our work at IMB. Miguel was formerly at the Max Delbrück Center for Molecular Medicine in Berlin. His strong expertise in bioinformatics, computational biology and data mining greatly boosts these fields here in Mainz.

We see these joint appointments with Mainz University as a blueprint for the future and we are actively working on more joint recruitments of outstanding researchers. We hope that this will also help to foster the generational change that the Faculty of Biology will undergo over the next 10 years.

Two new Junior Group Leaders have recently been appointed at IMB following a call for applications earlier this year. Brian Luke arrived from the Centre for Molecular Biology (ZMBH) at the University of Heidelberg, and continues his research into the roles of telomeres in genome stability and DNA damage. In 2013, he was elected as an EMBO Young Investigator, the only scientist working in Germany to receive this honour that year. We also welcome Vassilis Roukos from the National Cancer Institute in Bethesda, USA, where he is currently working as a Research Fellow. Vassilis will join us in early 2015. At IMB, he will investigate the cell biological aspects of genome maintenance using high-throughput live cell imaging.

## RESEARCH ACTIVITIES

IMB continues to pursue research into its main themes of epigenetic gene regulation and genome stability, particularly within the context of development. Our groups tend to work at the interfaces of these topics, giving our research a unique angle. Several groups at IMB have had their efforts rewarded this year with original publications in renowned journals and by receiving scientific awards. René Ketting, one of IMB's Scientific Directors, was recently chosen for a prestigious EMBO Membership.

One of our missions is to network with the local life science community in Mainz. With this in mind, we have joined forces with the Faculty of Biology of Mainz University in the research initiative "Gene Regulation in Evolution and Development" (GeneRED). In this collaborative project, the Ministry of Research of Rhineland-Palatinate is funding PhD fellowships with the aim to expand and strengthen research into gene regulation in Mainz by creating a critical mass of competitive research groups. GeneRED builds on our existing International PhD Programme and Summer School, which also bring together groups with similar research foci across IMB, the University, the University Medical Centre and the Max Planck Institute for Polymer Research in Mainz.

## EVENTS AT IMB AND BEYOND

This year saw the first "All IMB" retreat. A total of 110 staff and students attended the two day retreat, which was held at Schloss Buchenau, a 16th century castle sitting on top of rolling hills in the heart of Germany. This retreat proved to be a great opportunity for our scientists to share their research and exchange ideas, as well as enjoy social interactions with colleagues.

The 3rd IMB Conference, "Nuclear RNA in Gene Regulation & Chromatin Structure", in October again hosted many distinguished international speakers and participants. As well as this, several scientific events have forged further links between IMB and the international scientific community. In the interests of networking with the local scientific community within and beyond the University campus, IMB has organised a range of mini-symposia in the Mainz region with institutions such as: the Cluster of Excellence for Individualised Immune Intervention (Ci3); the Institute for Translational Oncology (TRON), a non-profit company located in Mainz; the University Medical Centre; and the Boehringer Ingelheim company. It has also organised the 1st meeting of local research groups working with *Drosophila* as a model organism and the 1st regional zebrafish meeting.

## LOOKING AHEAD

There is much in store for IMB in 2015. Our 4th IMB Conference "DNA Repair & Genome Stability in a Chromatin Environment", will be held on 4–7 June 2015 and promises to continue the tradition of bringing outstanding speakers to Mainz to talk on cutting-edge topics. On 19–20 March, IMB will also organise a workshop on "Breakthroughs in Epigenetics", which will feature presentations from many of the pioneers and leaders in the field of epigenetics. Next year also brings IMB's first international evaluation, and we look forward to welcoming the review committee for their on-site visit.

I would like to take this opportunity to thank the members of our Scientific Advisory Board for their continued support over the years. Their advice helped to get IMB off to a flying start. My special thanks go to Ingrid Grummt, Jörg Michaelis, and Ernst Ludwig Winnacker, who will rotate out of the SAB, for their critical input, which has been crucial in shaping the institute during its early phase. Of course, none of this could have been realised without the generous support of the Boehringer Ingelheim Foundation, to whom we are indebted.

**Christof Niehrs**,
Founding and Executive Director

# MIGUEL ANDRADE

*We predict the function of proteins and genes by integrating all sorts of biological data*

## EDUCATION

| | |
|---|---|
| 1989 | MSc in Chemistry, Complutense University of Madrid, Spain |
| 1994 | PhD in Computational Biology, Complutense University of Madrid, Spain |

## POSITIONS HELD

| | |
|---|---|
| 1994 – 1995 | Postdoc, EMBL, Heidelberg, Germany |
| 1995 – 1996 | Postdoc, EMBL-EBI, Hinxton, UK |
| 1998 – 2003 | Staff Scientist, EMBL, Heidelberg, Germany |
| 2003 – 2006 | Scientist, Ottawa Health Research Institute, Canada |
| 2006 – 2008 | Senior Scientist, Ottawa Health Research Institute, Canada |
| 2007 – 2014 | Group Leader, Max Delbrück Center for Molecular Medicine, Berlin, Germany |
| Since 2008 | Affiliate Investigator, Ottawa Health Research Institute, Canada |
| Since 2014 | Professor of Bioinformatics, University of Mainz, Germany |
| Since 2014 | Adjunct Director, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Jean-Fred Fontaine / Postdoc; since 04/2014
- Marie Gebhardt / PhD Student; since 04/2014
- Jonas Ibn-Salem / PhD Student; since 09/2014
- Susanne Klingenberg / Administrative Assistant; since 09/2014
- Nancy Mah / Postdoc; since 04/2014
- Enrique Muro / Staff Scientist; since 04/2014
- Katerina Taškova / Postdoc; since 11/2014

## OVERVIEW

Our group focuses on the development and application of computational methods for the study of gene and protein function, with a focus on the molecular causes of human disease. In collaboration with experimental groups, we are investigating a range of clinically relevant topics including cell pluripotency, cancer, neurodegeneration and asthma. One of our goals is to make our methods available as public web tools that are free and simple to use.

## RESEARCH HIGHLIGHTS

### STUDY OF PROTEIN INTERACTION NETWORKS

The HIPPIE database, created by our group, integrates and scores human protein-protein interaction (PPI) data according to experimental evidence. We recently showed that the reliability of experimentally measured interactions is higher if the interacting proteins are expressed in the same tissue or cellular location, and are involved in similar processes. These properties are useful to focus on relevant PPI subnetworks and mechanisms to do this were implemented in HIPPIE.

Expanded polyglutamine (polyQ) stretches are observed in the proteins of patients with different neurodegenerative diseases and are thought to trigger disease. We have recently carried out research that integrated genomic, phylogenetic, PPI and functional information to add evidence about the role of polyQ tracts in the modulation of protein interactions (Figure 1). It is known that

polyQ expansion results in an increased number of abnormal interactions, leading to pathological effects such as protein aggregation. We proposed that interactions of coiled-coil proteins with polyQ regions might increase aggregation; whereas the interaction of proteins with other regions might reduce protein aggregation and neurodegeneration.

## PROTEIN SEQUENCE ANALYSIS

We have developed a neural network method to identify protein repeats, such as HEAT and Armadillo, which form structural domains composed of alpha-helices. We show that these repeats have emerged repeatedly in evolution in distant taxa and have an increased frequency in organisms of high cellular complexity such as eukaryotes, and cyanobacteria and planctomycetes within prokarya.

We have studied the predictive power of amino acid composition at variable ranges of exposure to the surrounding media to predict the protein's subcellular location. An optimised two step predictor trained with vectors of amino acid composition in different ranges of exposure and using a Support Vector Machine followed by a Neural Network (NYCE), reaches an accuracy of 62% when predicting nuclear, nucleocytoplasmic, cytoplasmic or extracellular location of eukaryotic proteins.

## TRANSCRIPT REGULATION AND PREDICTION

We are developing computational methods to predict which genetic sequences are transcribed as ncRNAs and to evaluate experimental results identifying ncRNAs. These include the study of transcripts arising from pseudogenes and the characterisation of miRNA function based on the overlap of miRNA targets to those of particular transcriptional repressors, as derived from ChIP-seq experiments.

## FUTURE DIRECTIONS

In the coming years, we will take advantage of novel data types of increasing complexity to answer computational problems at the forefront of the field of computational biology. We will face challenges related to the increasing amount of information and computational time required to store, integrate and analyse such data, but these new data types offer the keys to the solution of increasingly relevant inter-related biological problems. Such problems include our complete knowledge of the transcriptional networks in humans and model organisms, the prediction of the function of all human genes and proteins, the association of all genes and other genomic elements to all inherited diseases, the knowledge of all protein structures, a deep understanding of the biological evolution of all species, and the computational annotation of all biomedical literature.

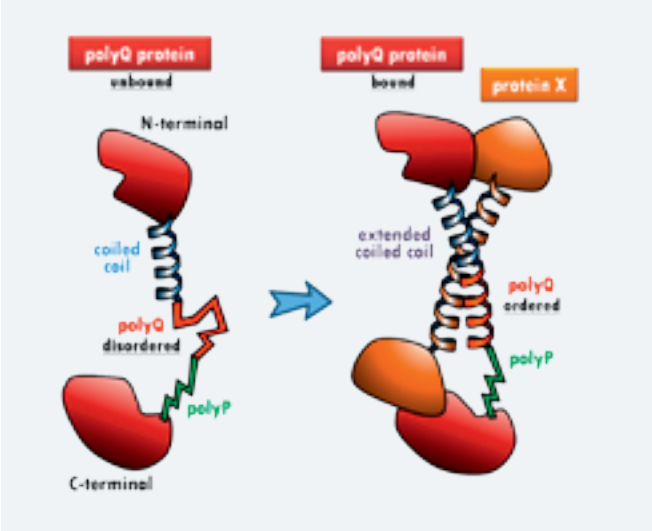



**Figure 1.** Model for polyQ modulation of protein-protein interactions. Left: unbound state of a protein containing a polyQ region (red) in disordered state, N-terminally preceded by a coiled-coil region (blue), and C-terminally followed by a disordered polyP region (green). Right: upon interaction of the polyQ protein with a protein X (brown) through a coiled-coil interaction, the polyQ region adopts a coil conformation extending the preceding (blue) coiled coil, therefore increasing the strength of the interaction. The polyP region cannot adopt a coiled coil conformation, effectively providing a cap to the interaction dependent conformational change induced by the polyQ.

## ANALYSIS OF GENE EXPRESSION

In a meta-analysis of DNA microarray gene expression data, we showed that induction of pluripotency is favoured by the induction of mesenchymal to epithelial transition (MET). We subsequently studied a time series following gene expression during the first three days of reprogramming of human fibroblasts into induced pluripotent stem cells. We observed the activation of particular pluripotency markers and the suppression of genes favoring MET, which points to targets that may enable us to improve the reprogramming protocol.

## DATA AND TEXT MINING

We are continually developing web tools that assist researchers exploring the biomedical literature to understand the molecular basis of disease. Génie is a tool that ranks the genes of an organism according to their relevance to user-defined biomedical topics based on the bibliography associated with those genes (in PubMed) or with their orthologues. More recently we developed the Alkemio web server, which ranks thousands of chemicals for their relevance to any topic using a similar principle.

## SELECTED PUBLICATIONS

Schaefer MH, Wanker EE and Andrade-Navarro MA (2012). Evolution and function of CAG/polyglutamine repeats in protein-protein interaction networks. *Nucleic Acids Research*, 40, 4273-4287.

Fontaine JF, Priller F, Barbosa-Silva A and Andrade-Navarro MA (2011). Génie: literature based gene prioritization at multi genomic scale. *Nucleic Acids Research*, 39, W455-W461.

Muro EM and Andrade-Navarro MA (2010). Pseudogenes as an alternative source of natural antisense transcripts. *BMC Evolutionary Biology*, 10, 338.

# PETRA BELI

*We employ mass spectrometry-based proteomics to understand the complexity of the cellular response to DNA damage*

## EDUCATION

| | |
|---|---|
| 2007 | Diploma in Molecular Biology, University of Zagreb, Croatia |
| 2011 | PhD in Biology, Goethe University Frankfurt, Germany |

## POSITIONS HELD

| | |
|---|---|
| 2010 – 2013 | Postdoc, NNF Center for Protein Research, University of Copenhagen, Denmark |
| Since 2013 | Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Marina Borisova / PhD Student; since 12/2013
- Jan Heidelberger / PhD Student; since 04/2014
- Andrea Voigt / Research Technician; since 01/2014
- Jiwen Yang / Postdoc; since 12/2014

## OVERVIEW

Cells have evolved complex DNA repair mechanisms to counteract DNA damage, which is continuously imposed by metabolic activity and environmental factors. An in-depth understanding of the cellular response to DNA damage is essential to understand cancer development and to design novel targeted cancer therapies. Recent studies have shown that, in addition to DNA repair, other cellular processes including chromatin remodelling, transcription and RNA metabolism, are regulated after genotoxic stress to ensure cellular homeostasis. We are employing state-of-the-art quantitative mass spectrometry-based proteomics to decipher the regulatory mechanisms that cells employ to preserve genome and chromatin integrity and to understand the complexity of the cellular response to DNA damage.

## RESEARCH HIGHLIGHTS

### CELLULAR DNA DAMAGE RESPONSE MECHANISMS

The cellular response to DNA damage is regulated through dynamic changes in posttranslational modifications of proteins and protein-protein interactions. In recent years, mass spectrometry (MS)-based proteomics has become an indispensable tool for the investigation of cellular signalling. We have employed quantitative MS-based proteomics to investigate the cellular phosphorylation signatures in response to genotoxic stress induced by ionising radiation and topoisomerase II inhibition. This study has revealed novel regulators of the DNA damage response and highlighted the link between transcription and DNA damage signalling. Furthermore, we have shown that protein kinases ATM, ATR

and DNA-PK phosphorylate hundreds of protein targets in response to DNA damage, highlighting the regulatory role of these enzymes in DNA damage signalling. Interestingly, many identified DNA damage-induced phosphorylation sites do not match the ATM/ATR/DNA-PK consensus motif and are therefore, most likely, targets of protein kinases acting downstream and/or independently of ATM/ATR/DNA-PK. Recent studies have shown that the p38 MAPK-MK2 pathway is activated by drugs that damage DNA and suggested that this pathway acts in parallel with ATM/ATR/DNA-PK to regulate the cellular response to DNA damage. In an ongoing project, we have employed quantitative MS-based proteomics to identify the targets of p38 MAPK in cells irradiated with UV light. We anticipate that these experiments will provide insights into the proteins and cellular processes that are regulated by p38 MAPK in response to genotoxic stress.

## SPECIFICITY IN NUCLEAR UBIQUITIN SIGNALLING

Protein ubiquitylation is involved in diverse cellular processes including protein degradation, DNA damage repair and chromatin remodelling. We have previously developed mass spectrometry-based methods for analysis of protein ubiquitylation. Using these methods we were among the first to demonstrate proteome-wide identification of ubiquitylation sites and quantification of ubiquitylation sites in response to cellular perturbations, including proteasome inhibition and genotoxic stress. We have shown that DNA damage induces site-specific ubiquitylation and deubiquitylation of DNA repair factors highlighting the regulatory role of protein ubiquitylation in processes that maintain genome integrity after genotoxic stress. Ubiquitin ligases are enzymes that mediate the attachment of ubiquitin to lysine residues of target proteins and thereby play an essential role in the ubiquitin system by defining its substrate specificity. Despite the importance of this class of enzymes, the protein targets of most ubiquitin ligases in human cells remain elusive. At present, we are using genome editing tools to knockout ubiquitin ligases that are implicated in DNA repair and chromatin remodelling in human cells. We will employ these model systems to identify the targets of ubiquitin ligases and to deepen our understanding of the regulatory roles of ubiquitin ligases in the cellular response to DNA damage.

## FUTURE DIRECTIONS

Recent studies have shown that the complexity of the DNA damage response extends far beyond DNA damage repair. In response to DNA damage, diverse cellular processes including chromatin remodelling, transcription and RNA metabolism, are coordinated to ensure cellular homeostasis. Posttranslational modification of proteins by phosphorylation and ubiquitylation is an essential regulatory mechanism in the DNA damage response. Future research efforts will focus on the following questions: (i) How do protein phosphorylation and ubiquitylation coordinate cellular processes in response to genotoxic stress? (ii) How do basic DNA and RNA metabolic processes, e.g. replication, transcription and splicing, integrate with DNA repair to ensure cellular homeostasis?

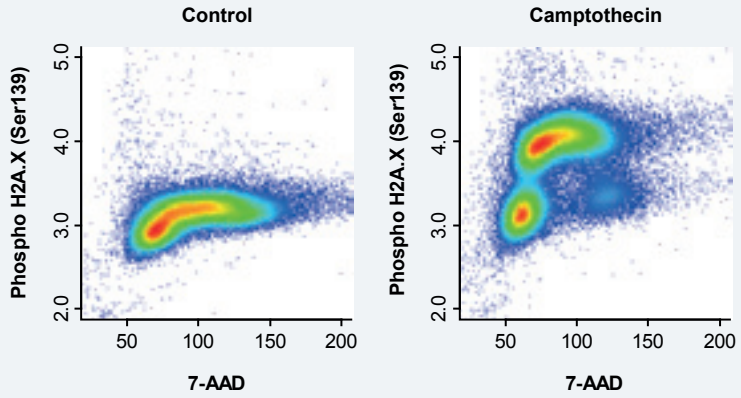



**Figure 1.** Flow cytometric analysis of DNA content (7-AAD) and H2A.X phosphorylation in human osteosarcoma cells treated with the topoisomerase I inhibitor camptothecin. Camptothecin induces phosphorylation of histone variant H2A.X and arrests cells in S phase. Phosphorylation of H2A.X is critical for the assembly of DNA repair factors at DNA damage sites.

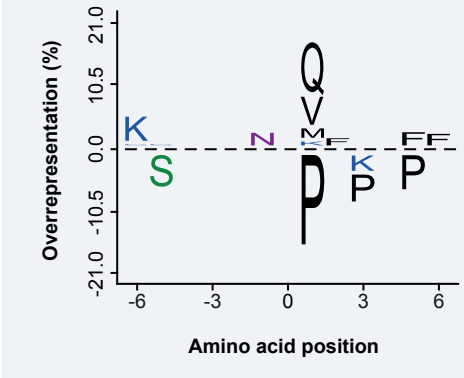



**Figure 2.** Sequence motif analysis of phosphorylation sites induced by treatment of cells with camptothecin. Protein kinases of the PIKK family ATM, ATR and DNA-PK phosphorylate substrates on serines or threonines that are followed by a glutamine residue (S/TQ). The S/TQ sequence motif is overrepresented in phosphorylation sites that are induced by camptothecin highlighting the importance of ATM/ATR/DNA-PK.

## SELECTED PUBLICATIONS

Povlsen LK*, Beli P*, Wagner SA, Poulsen SL, Sylvestersen KB, Poulsen JW, Nielsen ML, Bekker-Jensen S, Mailand N and Choudhary C (2012). Systems-wide analysis of ubiquitylation dynamics reveals a key role for PAF15 ubiquitylation in DNA-damage bypass. *Nat Cell Biol*, 23, 1089-1098.

Beli P*, Lukashchuk N*, Wagner SA, Weinert BT, Olsen JV, Baskcomb L, Mann M, Jackson SP and Choudhary C (2012). Proteomic investigations reveal a role of THRAP3 in DNA damage response. *Mol Cell*, 46, 212-225.

Wagner SA*, Beli P*, Weinert BT, Nielsen ML, Cox J, Mann M and Choudhary C (2011). A proteome-wide, quantitative survey of *in vivo* ubiquitylation sites reveals widespread regulatory roles. *Mol Cell Proteomics*, 10, M111.013284.

* indicates equal contribution

# FALK BUTTER

*We use quantitative proteomics to identify telomere binding proteins*

## EDUCATION

| | |
|---|---|
| 2006 | Diploma in Biochemistry,<br>University of Leipzig, Germany |
| 2010 | PhD in Biochemistry,<br>Ludwig Maximilians University,<br>Munich, Germany |

## POSITIONS HELD

| | |
|---|---|
| 2010 – 2013 | Postdoc, Max Planck Institute of Biochemistry,<br>Martinsried, Germany |
| Since 2013 | Group Leader,<br>Institute of Molecular Biology (IMB),<br>Mainz, Germany |

## GROUP MEMBERS

- Alina Bluhm / PhD Student; since 01/2014
- Núria Casas Vila / PhD Student; since 11/2013
- Anja Freiwald / Engineer; since 04/2013
- Marion Scheibe / Postdoc; since 06/2013

## OVERVIEW

Mass spectrometry has evolved into a powerful tool to study proteins in an unbiased and global manner. The current improvements in identification, accuracy, sample throughput, and data analysis, allow us to observe changes in the proteome with unprecedented speed and detail. Our group applies quantitative approaches such as label free quantitation or SILAC (Stable Isotope Labelling with Amino acids in Cell culture) that enable us to directly compare thousands of proteins in complex mixtures. This lets us study changes in protein expression, and we also use this approach for interactomics to identify specific interactions of proteins with targets of interest within a vast number of background binders. We apply this technology in several biological areas to advance our knowledge of cellular processes, such as telomere biology and RNA-mediated gene regulation.

## RESEARCH HIGHLIGHTS

### HOT1 IS A DYNAMIC TELOMERE BINDING PROTEIN

The ends of chromosomes consist in most cases of repetitive repeats that are maintained by telomerases and protected by telomere binding proteins from recognition by the DNA damage repair machinery. Using SILAC-based quantitative mass spectrometry, we identified HOT1 as a direct double strand telomere binding protein in human and mouse. Despite the known significance of telomeres in ageing and cancer, this finding was a surprise and opens up new avenues to study the maintenance of telomeres. In contrast to the well established shelterin complex which binds constitutively at telomeres, HOT1 is a dynamic telomere binding protein with increased telomeric localisation that is dependent

on higher telomerase *in situ* activity. Indeed, experiments suggest its involvement in telomerase-dependent telomere homeostasis by a putative function in telomerase recruitment. In collaboration, we established a knock-out mouse model for HOT1 which we are currently investigating.

## TELOMERE BINDING PROTEINS IN FUNGI

We extended our telomere interactomics screen to identify direct telomere binding proteins in other species. For example, we identified the telomere binding proteins of *Neurospora crassa*, a fungus with classical TTAGGG repeat telomeres. Based on these findings, we are now able to begin evolutionary analysis of telomere binding proteins by comparing other fungi to the well-studied baker's yeast and fission yeast telomere model systems and thus uncover new insights into the development of telomere regulation.

## FUTURE DIRECTIONS

Based on our previous investigations, we aim to further demonstrate the molecular mechanism by which HOT1 executes its function in the telomerase recruitment pathway. Furthermore, we are continuing to use our expertise in quantitative mass spectrometry to study nucleic acid-protein interactions in diverse biological pathways and systems.

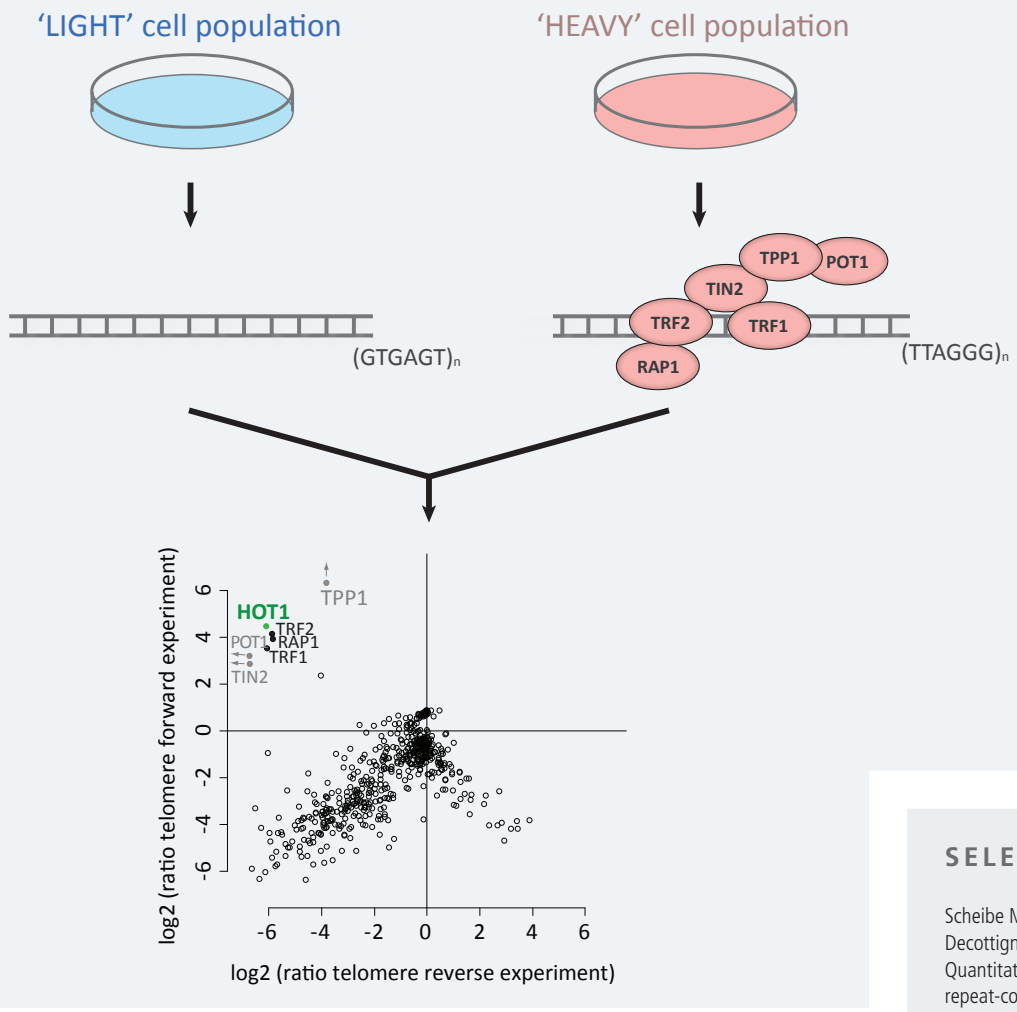



**Figure 1.** Schemata of SILAC-based interactomics. The cellular proteome is labelled with selected amino acids differing in their mass. These encoded extracts are used in purification experiments to quantify proteins that are enriched at the bait of interest in comparison to a control employing quantitative mass spectrometry. In this example, proteins binding to the human telomeric TTAGGG repeat sequence were identified.

## QUANTITATIVE INTERACTOME OF THE lncRNA TERRA

Recently, it was recognised that telomeric repeats are actively transcribed, giving rise to telomeric repeat-containing RNA (TERRA). TERRA is involved in telomere homeostasis and telomere maintenance and contributes to chromatin organisation at the chromosome end. We reported the first quantitative interactome of TERRA using state-of-the-art high-resolution mass spectrometry and characterised over a dozen new TERRA-associated proteins for their effect on global TERRA levels and TERRA localisation at telomeres using RNA interference. Nearly all candidates had an effect on at least one of the investigated features. We thus greatly increased the number of proteins known to influence this long non-coding RNA, providing a basis for further in-depth studies.

## SELECTED PUBLICATIONS

Scheibe M, Arnoult N, Kappei D, Buchholz F, Decottignies A, Butter F* and Mann M* (2013). Quantitative interaction screen of telomeric repeat-containing RNA reveals novel TERRA regulators. *Genome Res*, 23, 2149-2157.

Viturawong T, Meissner F, Butter F* and Mann M* (2013). A DNA-centric protein-interaction map of ultra-conserved elements reveals major contribution of transcription factor binding hubs to conservation. *Cell Rep*, 5, 531–545.

Kappei D*, Butter F*, Benda C, Scheibe M, Draškovič I, Stevense M, Novo CL, Basquin C, Araki M, Araki K, Krastev DB, Kittler R, Jessberger R, Londoño-Vallejo JA, Mann M and Buchholz F (2013). HOT1 is a mammalian direct telomere repeat-binding protein contributing to telomerase recruitment. *EMBO J*, 32,1681-1701.

*indicates equal contribution

# CHRISTOPH CREMER

*Visualising nuclear compounds in their natural environment helps us to understand their biological function*

## EDUCATION

| | |
|---|---|
| 1970 | Diploma in Physics, Ludwig-Maximilians University, Munich, Germany |
| 1976 | PhD in Biophysics and Genetics, University of Freiburg, Germany |
| 1983 | Habilitation, University of Freiburg, Germany |

## POSITIONS HELD

| | |
|---|---|
| 1970 – 1983 | Staff Scientist, Institute of Human Genetics, University of Freiburg, Germany |
| 1983 – 1999 | Managing/Deputy Director, Institute of Applied Physics I, University of Heidelberg, Germany |
| 1983 – 2011 | Professor of Applied Optics & Information Processing, University of Heidelberg, Germany |
| 2005 – 2007 | Deputy Director, Kirchhoff-Institute of Physics, University of Heidelberg, Germany |
| Since 2005 | Director Biophysics of Genome Structure, Institute for Pharmacy and Molecular Biotechnology, University of Heidelberg, Germany |
| Since 2011 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |
| Since 2013 | Honorary Professor (Physics), University of Mainz, Germany |

## GROUP MEMBERS

- Alexander Al Saroori / Student Assistant; 12/2013 – 08/2014
- Margund Bach / Research Assistant; 04/2014 – 6/2014
- Udo Birk / Postdoc; since 02/2012
- Hyun-Keun Lee / Student Assistant; 08/2013 – 10/2014
- Dongy Ma / Masters Student; 03/2014 – 08/2014
- Jan Neumann / PhD Student; since 03/2014
- Kirti Prakash / PhD Student; since 02/2013
- Aleksander Szczurek / PhD Student; since 09/2013

## OVERVIEW

A functionally compartmentalised arrangement of chromatin has been proposed as another level of epigenetic gene regulation. Due to the limitations of light microscopy, conclusive tests of models on the nanoscale are very difficult, hampering a full mechanistic understanding of transcription/splicing and repair. To overcome this difficulty, we established a variety of superresolution ("nanoscopy") methods. Using a recently developed Localisation Microscopy technique termed Spectral Precision Distance/Spatial Position Determination Microscopy (SPDM) with Physically Modifiable Fluorophores (SPDM-Phymod), we presently achieve an optical resolution of biostructures of a few 10 nm laterally and a few 100 nm axially. In addition we have succeeded in performing Localisation Microscopy (SPDM) of nuclear DNA distribution at a molecule signal density up to ~25,000 signals/$\mu m^2$ using standard DNA dyes, which also provides a dramatically enhanced structural resolution within the cell nucleus.

## RESEARCH HIGHLIGHTS

### SUPERRESOLUTION LIGHT MICROSCOPY

During 2014, we set up an improved version of a 3D Localisation Microscope with a horizontal axis. This system allows Single Molecule Localisation Microscopy (SMLM) of an optical section through a 3D specimen with a thickness of few tens of nm. In comparison, Confocal Laser Scanning Microscopy allows optical sectioning with a thickness of 600 nm, more than ten times thicker. In addition, we implemented further improvements to our multicolour Localisation Microscope with a vertical optical axis. This instrument (featuring the option to include axially structured illumination) provides a 2D optical single molecule resolution of a few

tens of nm in the object plane and several hundred along the optical axis. The instrument is now routinely used for a large number of collaborative projects.

The multicolour combinatorial microscope or "COMBI-Microscope" system for fast switching from laterally structured illumination microscopy to Single Molecule Localisation Microscopy realised in 2013 is now routinely used in research projects. In 2014, the hardware and control software of this system was further enhanced. A second Structured Illumination Microscope developed in the form of an Ophthalmoscope is currently used for retina diagnostics in human patients, a collaborative project with Prof. Stefan Dithmar, University Hospital, Heidelberg. Presently, this system provides the best resolved live images of the retina in human patients ever obtained.

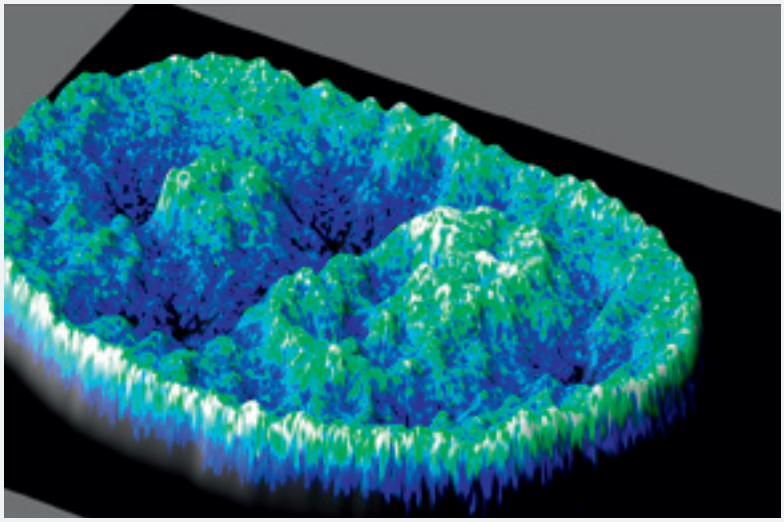


**Figure 1.** Superresolution Microscopy of DNA Distribution in a HeLa Nucleus. SPDM Intensity Image of DNA distribution in an optical section after labelling with a DNA specific fluorochrome (average ~ 5,000 DNA-signals/μm²). The image indicates the large differences in the nanoscale distribution of DNA predicted from the Interchromatin Compartment Model.

## BIOLOGICAL APPLICATIONS OF SUPERRESOLUTION MICROSCOPY

Within IMB as well as in collaboration with research partners from all over the world we are presently applying our superresolution microscopes to a variety of biological questions. In principle, these studies are covering all aspects of the IMB core research areas. A deeper understanding of nuclear architecture also requires us to include evolutionary aspects and to study chromatin nanostructure of various species and of diverging cell types down to the nanometer resolution range.

In collaborative projects, we apply our microscopy and image analysis methods to the following areas: 1) functional nuclear architecture and epigenetics of the chromatin nanostructure of active or inactive genes on the single cell level, 2) consequences of repair/genome instability on the nuclear nanoscale, 3) the distribution of plasma membrane bound receptor molecules. In our research, priority is given to collaborative projects with IMB members, IMB associates and their partners.

## SELECTED PUBLICATIONS

Cremer T, Cremer C and Lichter P (2014). Recollections of a scientific journey published in Human Genetics: from chromosome territories to interphase cytogenetics and comparative genome hybridization. *Human Genetics*, 133, 403-416.

Szczurek AT, Prakash K, Lee HK, Żurek-Biesiada DJ, Best G, Hagmann M, Dobrucki JW, Cremer C and Birk U (2014). Single molecule localization microscopy of the distribution of chromatin using Hoechst and DAPI fluorescent probes. *Nucleus,* 5, 1-10.

Cremer C and Masters BR (2013). Resolution enhancement techniques in microscopy. *Eur Phys J H*, 38, 281-344.

## FUTURE DIRECTIONS

In the context of collaborative projects, we plan on continuing and extending our biological applications of superresolution microscopy at IMB. The long-term goal of these efforts is to integrate light-optical superresolution approaches with the results of ultrastructure (electron/X-ray) microscopy techniques, with molecular biology, and with biocomputing approaches of IMB. It is hoped that this will particularly develop a fully quantitative, mechanistic understanding of the spatial epigenetic landscape and its relation to biological function. To achieve this, specific improvements in superresolution microscopy methods, especially in terms of resolution, multicolour and *in vivo* imaging capability as highlighted below are planned.

Another goal of our work will be to advise users in the Microscopy Core Facility of complementary microscopy methods (e.g. STED, GSDIM, Light Sheet Microscopy, high throughput microscopy). As a general strategy, we plan on providing the full spectrum of superresolution methods, with applications according to the specific biological questions posed.

Two specific technical goals of our work are the further development of our microscopy setups with respect to multicolour single Molecule 3D-Localisation Microscopy (3D SPDM) and Structured Excitation Illumination Microscopy of fixed specimens. One long term goal is to enhance the optical resolution from the present few tens of nm laterally and few hundred axially to ~5 nm laterally and 20 nm axially (assuming optimal conditions). This would give us the possibility to study specifically labelled nuclear nanostructures with a 3D resolution approaching the single nucleosome level.

Since the 1990s, the members of the Cremer Lab have been developing approaches to realise 3D imaging of biological specimens by optical tomographic approaches to image entire small organisms. So far, the optical resolution of routine meso-scale tomography with a working distance of several cm is in the μm-range. We plan on establishing a correspondingly enhanced nano-scale imaging technique by employing structured excitation illumination (SEI).

# RENÉ KETTING

*The diversity among piRNA pathways is astonishing. Yet, they all silence dangerous elements in our genomes*

## EDUCATION

| | |
|---|---|
| 1994 | Masters in Chemistry and Molecular Biology, University of Leiden, The Netherlands |
| 2000 | PhD in Molecular Biology, Netherlands Cancer Institute Amsterdam and University of Utrecht, The Netherlands |

## POSITIONS HELD

| | |
|---|---|
| 2000 | Postdoc, Cold Spring Harbor, USA |
| 2000 – 2005 | Postdoc, Hubrecht Institute, Utrecht, The Netherlands |
| 2005 – 2013 | Group Leader, Hubrecht Institute, Utrecht, The Netherlands |
| Since 2010 | Professor of Epigenetics and Development, University of Utrecht, The Netherlands |
| Since 2012 | Professor, Faculty of Biology, University of Mainz, Germany |
| Since 2012 | Director, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Bruno Albuquerque / PhD Student; since 10/2012
- Miguel Almeida / PhD Student; since 06/2013
- Yi-Yen Chen / Postdoc; 03/2013 – 9/2014
- Jana Deckelmann / Student Assistant; 7/2014 – 10/2014
- Holger Dill / Postdoc; since 02/2013
- Sabine Dominitzk / Research Technician; 10/2012 – 9/2014
- Yasmin El Sherif / Research Technician; since 10/2014
- Lucas Kaaij / Postdoc; since 17/2013
- Laura Krebs / Student Assistant; 5/2013 – 7/2014
- Svetlana Lebedeva / Postdoc; since 03/2013
- Philipp W Meyer / Masters Student; since 9/2014
- Antonio Miguel de Jesus Domingues / Postdoc; since 12/2014
- Maria Placentino / PhD Student; since 11/2013
- Anke Ries / Lab Manager; since 12/2012
- Ricardo Rodrigues / PhD Student; since 12/2012
- Elke Roovers / PhD Student; since 05/2013
- Stefan Redl / PhD Student; since 04/2013
- Saskia Weiß / Animal Caretaker; since 01/2013
- Nadine Wittkopp / Postdoc; since 11/2012
- Steven Zenner / Student Assistant; since 7/2014

## OVERVIEW

The major focus of my lab is on gene regulation by small RNA molecules acting in RNAi-related pathways. Since their discovery at the beginning of the 21st century many different RNAi-related pathways have been identified and it is now evident that although all of these pathways depend on proteins from the Argonaute family, each pathway has its own unique characteristics and effects on gene expression. These can range from relatively minor effects on translation (in the case of many miRNAs) to the full-blown shut down of loci at the transcriptional level (piRNAs). We mainly focus on mechanisms related to piRNA biology, but we also aim to work out regulatory networks involving miRNAs. We do so mostly within the setting of (embryonic) development and we are using both zebrafish and *C. elegans* as model systems for these studies.

## RESEARCH HIGHLIGHTS

During 2014 we have started to make good progress on the question of how piRNAs are being generated and how and when they are being employed to silence piRNA targets. Four highlights are briefly presented below:

### piRNA BIOGENESIS IN *C. ELEGANS*

In *C. elegans*, piRNAs (also named 21U RNAs) are made through a process that differs from that found in flies and vertebrates. Instead of residing in clusters, 21U RNAs are found in small, individual transcription units and none of the known vertebrate piRNA biogenesis factors are conserved in this nematode. In fact, besides the Argonaute that they bind to (PRG-1), no gene has been identified

that is essential for their production. Through our screens for mutants in the *C. elegans* 21U RNA pathway (also see below) we have identified a novel gene, pid-1, that is essential for making 21U RNAs. The gene encodes a small, cytoplasmic protein with no known domains. Its protein product does not seem to interact directly with the Piwi protein PRG-1. RNA-Seq experiments have led us to suggest PID-1 acts in between 21U RNA transcription and processing. Identification of PID-1 also led to the finding that the piRNA pathway has a very strong maternal component: without maternal piRNAs, silencing cannot be initiated. This suggests that piRNAs initiate silencing very early in development. We are currently further dissecting other proteins that PID-1 is interacting with. Using label-free mass spectrometry, we have already identified four strong candidates that we are now following up.

## FACTORS INVOLVED IN THE NUCLEAR EFFECTS OF piRNAS

We are performing mutant screens based on GFP-encoding transgenes, in order to identify genes required for the above processes. We are also designing customised experiments for individual genes that are known to play a role in this silencing process. This has resulted in the identification of a number of mutants which we are currently identifying and analysing. One gene we identified already is pid-2, which likely acts between recognition of target RNA by PRG-1:21U RNA complexes, and the recruitment of RdRP enzymes to mediate 22G RNA generation. This is a completely unresolved step in any of the *C. elegans* small RNA pathways, and we are currently further analysing this gene and its encoded protein.

## A ZEBRAFISH SYSTEM WITH TRANSGENIC piRNAS

So far we have characterised the zebrafish piRNA system using a reverse genetic strategy, consisting of trying to decipher the roles of Piwi pathway components. In relation to these ongoing efforts, we are now creating transgenic lines that should allow us to better study the effect of the Piwi pathway on individual loci. The main problems in doing this using wild-type vs. mutant comparisons are that a) germ cells mutant for piRNA factors have strong developmental defects and b) natural piRNAs by their very nature recognise repetitive loci, making it hard to draw definitive statements. Hence, piRNAs produced from a single locus and targeting an artificial, single-copy locus, will allow a much finer dissection of the effects of Piwi proteins. Based on the idea that transposons get "caught" in piRNA-producing loci, we have been able to generate lines that produce GFP-derived piRNAs by simply having transposons containing fragments of GFP inserted randomly into the genome and selecting for GFP-silencing activity in germ cells. The insertion sites of the GFP-piRNA generating transposon copies is currently performed using a 4C-related approach. The genetics of these strains show that again, like in *C. elegans*, maternally provided piRNAs are essential to set-up silencing in germ cells during early development. Interestingly, this strain also reveals mosaicism of piRNA silencing within individuals. Some germ cells are fully silenced while others are not at all. This suggests a binary state of action, rather than a continuous scale of silencing by piRNAs. The reasons behind this are currently being traced.

## MECHANISMS OF MATERNAL TRANSMISSION

Maternally deposited piRNAs appear to be essential for setting up silencing responses. Presumably, the silencing is set up during early development, while later developmental stages merely propagate the established silencing responses. We have established that Tdrd6 in zebrafish is a platform that loads maternal piRNAs into the developing primordial germ cells (PGCs). Interestingly, Tdrd6 also brings other maternal RNA species to the PGCs, including mRNAs for vasa, dazl, nanos and other mRNAs known to be required for PGC specification. Consistently, Tdrd6 mutants display a disturbed PGC specification phenotype, emphasising the relevance of this maternal transmission. Through label-free, quantitative mass spectrometry, we have established that Tdrd6 interacts very strongly with Tdrd7. Tdrd7, another tudor-domain protein, also contains a LOTUS domain, an RNA-binding domain also found in the *Drosophila* Oskar protein. Notably, Oskar acts in maternal transmission of mRNAs. We are therefore testing the hypothesis that Tdrd6 on the one hand brings Piwi into PGCs and on the other hand binds Tdrd7 in order to properly deliver specific mRNA molecules into developing PGCs.

## FUTURE DIRECTIONS

Our future experiments will be mainly aimed at the further elucidation and characterization of piRNA biogenesis and piRNA silencing mechanisms. Following the genetic identification of factors involved, we are now also moving into more biochemical approaches towards answering these questions. Also, the reasons behind the apparent binary decisions made by the piRNA system are among our central themes. Finally, now that we have a handle on the efficiency of germ cell specification in the zebrafish, efforts will be undertaken to reveal more details about germ cell specification and maintenance in this animal.

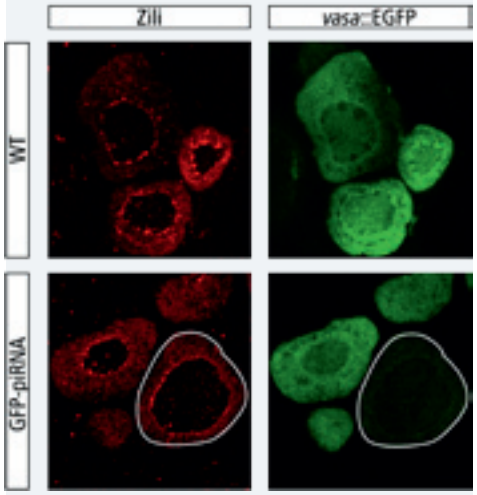



**Figure 1.** Zebrafish oocytes stained for the Piwi protein Zili (red) and GFP (green). The top panels are from a wild-type animal, lacking piRNAs targetting GFP. The bottom panels reflect oocytes from an animal expressing GFP-piRNAs and shows the mosaic silencing observed in these animals (only one out of four oocytes lacks GFP).

## SELECTED PUBLICATIONS

de Albuquerque BFM, Luteijn MJ, Cordeiro Rodrigues RJ, van Bergeijk P, Waaijers S, Kaaij LJT, Klein H, Boxem M and Ketting RF (2014). PID-1 is a novel factor that operates during 21U-RNA biogenesis in *Caenorhabditis elegans*. *Genes Dev*, 28, 683-688.

Luteijn MJ, van Bergeijk P, Kaaij LJ, Almeida MV, Roovers EF, Berezikov E and Ketting RF (2012). Extremely stable Piwi-induced gene silencing in *Caenorhabditis elegans*. *EMBO Journal*, 31, 3422-3430.

van Wolfswinkel JC, Claycomb JM, Batista PJ, Mello CC, Berezikov E and Ketting RF (2009). CDE-1 affects chromosome segregation through uridylation of CSR-1-bound siRNAs. *Cell*, 139, 135-148.

# JULIAN KÖNIG

*Splicing defects are a major cause of genetic disorders*

## EDUCATION

| | |
|---|---|
| 2003 | Diploma in Biology, Ludwig Maximilians University, Munich, Germany |
| 2008 | PhD in Biology, Max Planck Institute for Terrestrial Microbiology and Phillipps University, Marburg, Germany |

## POSITIONS HELD

| | |
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| 2008 – 2013 | Postdoc, MRC Laboratory of Molecular Biology, Cambridge, UK |
| Since 2013 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Simon Braun / PhD Student; since 11/2013
- Heike Hänel / Research Technician; since 11/2013
- Andrea Hildebrandt / PhD Student; since 04/2014
- Reymond Sutandy / PhD Student; since 12/2013
- Stefanie Tauber / Postdoc; since 04/2014

## OVERVIEW

Pre-messenger RNA (pre-mRNA) splicing – the removal of introns and joining of exons – is a main pillar of gene expression in higher eukaryotes. Splicing is regulated by activators and repressors that recognise cis-elements at their target exons. It is clear that the recruitment of these RNA-binding proteins (RBPs) is not solely dependent on the underlying RNA sequence, but is configured by their interactive environment, including direct competition or cooperative recruitment of RBPs as well as modulations of secondary structure. However, the molecular rules that govern the splicing outcome remain to be discovered. Intriguingly, this so-called "splicing code" is strongly shaped by the interactive assembly of pre-messenger ribonucleoprotein (pre-mRNP) complexes, co-transcriptional recruitment and the local chromatin environment.

Our lab unites a systemic analysis of pre-mRNP assembly during splicing with a molecular understanding of the underlying processes. Employing cutting-edge genomic RNA biology techniques, such as iCLIP and RNA-seq, in combination with biochemical and genetic tools. We aim to dissect the code of selected splicing events at an unprecedented depth. Disease-relevant alternative exons serve as a paradigm to identify cis-regulatory elements and trans-acting factors and thereby disclose the blueprint of the regulatory network. We hope that in the future our work will significantly advance our understanding of genetic disorders connected to splicing and of splicing regulation in general.

## RESEARCH HIGHLIGHTS

### UNCOVERING hnRNP C AS A GUARDIAN OF THE TRANSCRIPTOME

A particularly interesting protein family to study in the context of splicing regulation and interactive pre-mRNP assembly are the heterogeneous nuclear ribonucleoproteins (hnRNPs). Rivalling histones in their abundance, hnRNP proteins have been described to form hnRNP particles, which have – in analogy to nucleosomes – been referred to as "ribonucleosomes". Their high abundance and presence along most transcripts suggests they are major players in guiding the binding and function of other RBPs.

We have recently shown that hnRNP C, the core component of hnRNP particles, prevents the binding of the splicing factor U2AF65 to the uridine tracts of thousands of Alu elements that cut across the human genome (Figure 1). More than half a million of these retrotransposons reside within transcribed regions of the human genome. The Alu elements contain numerous cryptic splice sites, and they can be erroneously recognised as exons by the splicing machinery in a process called Alu exonisation. However, despite posing a major threat to transcriptome integrity, little was known about the molecular mechanisms preventing their inclusion. Since U2AF65 is a major player in early 3' splice-site definition, hnRNP C's competition with this protein ensures that cryptic splice sites within the Alu elements are kept silent, which is a vital mechanism to preserve human health. Consistently, loss of hnRNP C leads to formation of previously suppressed Alu exons, which severely disrupt transcript function. This is also reflected in the existence of disease-associated mutations in Alu elements that hinder hnRNP C-dependent repression.

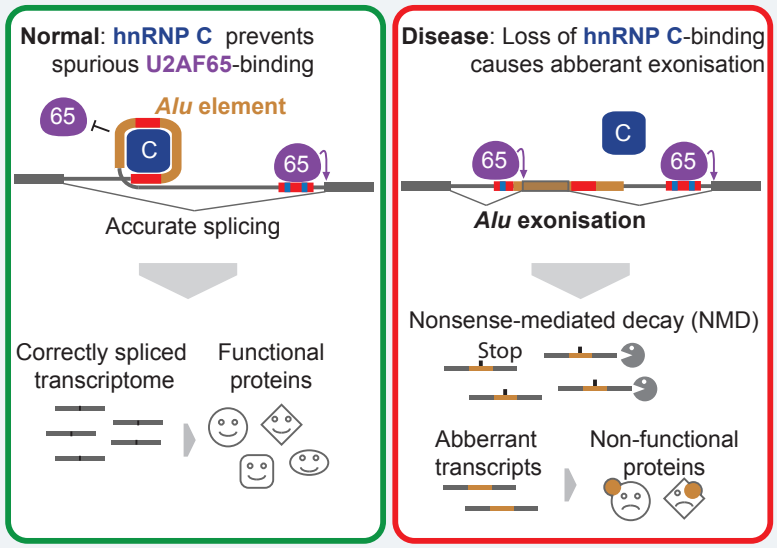


**Figure 1.** hnRNP C acts as a guardian of the transcriptome by preventing binding of the splicing factor U2AF65 to Alu elements, retrotransposons that are abundant in the human genome, and ensuring that splicing occurs correctly. In the absence of hnRNP C binding, the splicing machinery erroneously recognises these Alu elements as exons, and the resultant transcripts give rise to non-functional proteins (Zarnack *et al*, 2013).

### DEVELOPING iCLIP, A METHOD TO MAP PROTEIN-RNA INTERACTIONS WITH UNPRECEDENTED RESOLUTION

The development of quantitative individual-nucleotide resolution CLIP (iCLIP) was key for the understanding of hnRNP C's role in the regulation of Alu exonisation, and enabled us to obtain the first genome-wide and quantitative data on the interaction between two RBPs. This innovative approach allows mapping of protein-RNA interactions at single-nucleotide resolution on a genome-wide scale. It overcomes a number of critical limitations of previous ribonomic methods and offers unprecedented insights into RBP function, as evidenced by the large number of studies already using the technique.

## FUTURE DIRECTIONS

The quantitative description of protein-RNA interactions using iCLIP in combination with the clinically relevant model of Alu exonisation offers an attractive system for understanding the forces of competition and synergy that govern pre-mRNP complexes and splicing regulation. We will focus our efforts on elucidating the machinery for 3' splice-site definition, which has previously been identified as a hotspot for cancer-associated mutations. To achieve this, we will combine *in vivo* and biochemical approaches on a genome-wide scale, which will yield a systemic understanding of pre-mRNP function in splicing regulation. Our group's core competencies are the qualitative, quantitative, and comparative description of RBP binding, which will provide knowledge on how RBPs behave in their complex and interactive environment. These aspects will be addressed in the context of intronic cryptic splice sites as they are present in Alu elements, which will be an important contribution to the understanding of genetic disease.

## SELECTED PUBLICATIONS

Zarnack K*, König J*, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, Anders S, Luscombe NM and Ule J (2013). Direct Competition between hnRNP C and U2AF65 Protects the Transcriptome from the Exonization of *Alu* Elements. *Cell*, 152, 453-466.

König J, Zarnack K, Luscombe NM and Ule J (2012). Protein-RNA interactions: new genomic technologies and perspectives. *Nat Rev Genet*, 13, 77-83.

König J*, Zarnack K*, Rot G, Curk T, Kayikci M, Zupan B, Turner DJ, Luscombe NM and Ule J (2010). iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. *Nat Struct Mol Biol*, 17, 909-915.

* indicates equal contribution

# STEFAN LEGEWIE

*Mathematical models are required to fully exploit complex single-cell datasets*

## EDUCATION

| | |
|---|---|
| 2004 | Diploma in Biochemistry, University of Witten/Herdecke, Germany |
| 2008 | PhD in Biophysics, Humboldt University Berlin, Germany |

## POSITIONS HELD

| | |
|---|---|
| 2008 | Postdoc, Institute for Theoretical Biology, Humboldt University Berlin, Germany |
| 2009 – 2010 | Group Leader "Theoretical Systems Biology", German Cancer Research Center (DKFZ), Heidelberg, Germany |
| Since 2010 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Stephan Baumgärtner / PhD Student; since 11/2011
- Kolja Becker / PhD Student; since 08/2013
- Mihaela Enculescu / PostDoc; since 10/2013
- Lu Huang / PostDoc; since 10/2012
- Monika Kuban / Research Technician; since 10/2012
- Tamara Mihaljev / PostDoc; 11/2010 – 10/2014
- Shankar Patil / PhD Student; 5/2013 – 6/2014
- Uddipan Sarma / PostDoc; since 08/2012
- Marcel Schniedermann / Bachelor Student; 9/2013 – 4/2014

## OVERVIEW

Our group investigates how biological regulatory networks function robustly despite internal and external fluctuations. We tackle this question by analysing single-cell measurements and systematic perturbation screens in close collaboration with experimental partners in order to derive mechanistic and predictive mathematical models of biological processes. Our research focuses on cell-to-cell variability in cellular signal transduction and on quantitative modelling of gene expression responses. We employ deterministic, ODE-based modelling approaches as well as stochastic simulation algorithms. We develop computational tools for improved parameter estimation and model discrimination based on multivariate single-cell datasets, and for network reconstruction from perturbation datasets.

## RESEARCH HIGHLIGHTS

### CONTROL OF CELL-TO-CELL VARIABILITY IN INTRACELLULAR REGULATORY NETWORKS

Cellular signalling networks function reliably despite noise from intracellular events and fluctuating environments. We study design principles of signalling pathways that promote robustness. In a recent study, we applied analytical theory and numerical simulations to systematically analyse how fluctuations in signalling protein concentrations give rise to cell-to-cell variability in protein kinase signalling. We found that even simple signalling systems can be inherently robust to protein concentration fluctuations, and identified points of control that allow for tuning the variability. The theoretical predictions were compared to single-cell measurements of the SMAD signalling pathway.

In an ongoing collaboration with Alexander Loewer (MDC Berlin), we study the heterogeneity of the TGFβ/SMAD signalling pathway, and characterise the nucleo-cytoplasmic shuttling of SMAD2-GFP fusion proteins using live-cell imaging. Based on this quantitative dataset, we employed clustering approaches, correlation analyses and single-cell modelling to identify sources of heterogeneity, and to characterise cellular subpopulations showing qualitatively distinct signalling dynamics. Our models could successfully predict the adaptation dynamics of the pathway at the cell population level. Model predictions concerning the sources of single-cell heterogeneity will be tested using FISH and immunofluorescence against selected signalling proteins.

In addition, we study the temporal robustness of the metaphase-anaphase transition in collaboration with Silke Hauf (Virginia Tech). In this system, the anaphase-promoting complex (APC) triggers the degradation of securin and cyclin B to induce chromosome segregation and cytokinesis, respectively. Using live-cell imaging in fission yeast, our collaborators could show that the temporal order of APC-induced events is maintained precisely in single cells even if the system is perturbed strongly. We employed mechanistic modelling to explain this remarkable invariance, and found that strong substrate competition for the APC may establish robust timing.

## HARNESSING THE MULTIVARIATE NATURE OF SINGLE-CELL DATA TO DERIVE MEANINGFUL MODELS

Single-cell datasets are often multivariate in nature and escape intuition. Hence, quantitative mathematical models are required to fully understand dynamic phenomena in single-cells. Currently however, most quantitative models are calibrated based on population average data which hides information about nonlinear dynamics in single-cells.

Our recently published work is a step towards a quantitative description of single-cell events. We investigated activation mechanisms of caspase-8 in the plasma membrane using a single-cell modelling approach: individual single-cell simulations were quantitatively fitted to measured single-cell caspase-8 activity time courses. At the same time, the average over many single-cell simulations was fitted to bulk measurements of caspase-8 cleavage fragments. Our modelling approach thus accommodates that comprehensive single-cell measurements are typically restricted to certain signalling intermediates, while less informative, population-based assays can be done on a higher throughput. By linking these two complementary experimental scales, our modelling approach takes into account all available information, thereby resulting in more profound insights into the underlying biochemical mechanisms. In fact, our results show that simultaneous fitting to single-cell and cell population data allowed for better discrimination of biochemical mechanisms, and resulted in improved predictive power when compared to conventional fitting to population average data. In this study, we could identify a positive feedback loop emanating from death receptor clustering in the plasma membrane which amplifies apoptosis signalling.

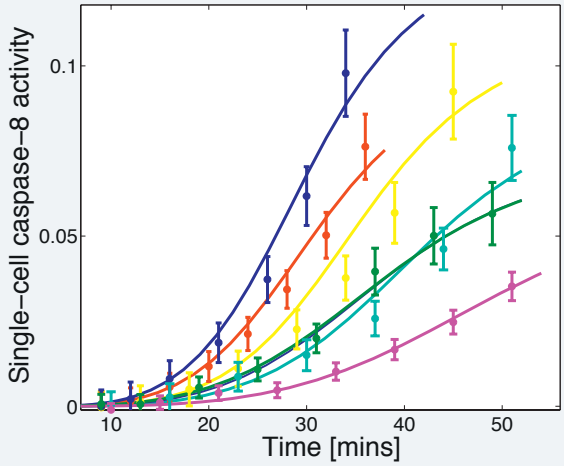


**Figure 1.** Single-cell modelling reveals mechanisms of caspase-8 activation and identifies sources of heterogeneity. Single-cell caspase-8 activities measured using fluorescent cleavage probes (shown as data points for 6 cells), and a model was fitted assuming that individual cells differ in the concentrations of signalling proteins (lines).

## RECONCILING SINGLE-CELL AND POPULATION DYNAMICS OF TRANSCRIPTION

Transcription is a prime example of a biological process where multi-scale models linking single-cell and cell population data are required for a full mechanistic understanding. At the single cell level, transcription is a stochastic process and occurs in bursts. These bursts are regulated by epigenetic chromatin modifications which are typically only measurable at the cell population level using chromatin immunoprecipitation (ChIP). In cooperation with George Reid (IMB), we have derived ODE-based models of estrogen-dependent promoter regulation by fitting to ChIP time course data, and derived insights into the life times, order and combinatorial complexity of epigenetic events. Model predictions concerning the single-cell behavior and regulation of transcription by changing estrogen concentrations are currently tested experimentally by the Reid group. In the future, we plan to implement more realistic models of transcriptional regulation by taking into account combinatorial binding of multiple factors to promoter sites.

## FUTURE DIRECTIONS

In the future, we plan to extend our single-cell modelling activities, and will combine stochastic and deterministic approaches. Clustering approaches will be employed to classify single cells into groups of recurrent dynamic behavior. Such clustering reduces the dimensionality of single-cell datasets, thereby simplifying the model fitting problem. By comparing our models to data, we hope to gain insights into how heterogeneity arises in cellular systems. Perturbations will be introduced to study the effect of heterogeneity on cell fate decisions. In this way, we plan to understand how cells buffer heterogeneity and how reliable cell fate decisions can be made in the face of intracellular and external fluctuations.

## SELECTED PUBLICATIONS

Kallenberger SM, Beaudouin J, Claus J, Fischer C, Sorger PK, Legewie S* and Eils R* (2014). Intra- and Interdimeric Caspase-8 Self-Cleavage Controls Strength and Timing of CD95-Induced Apoptosis. *Sci Signal,* 7, ra23.

Jeschke M, Baumgärtner S and Legewie S (2013). Determinants of cell-to-cell variability in protein kinase signalling. *PLoS Comput Biol*, 9, e1003357.

Paulsen M*, Legewie S*, Eils R, Karaulanov E and Niehrs C (2011). Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signalling range and canalizes development. *PNAS*, 108, 10202-10207.

*indicates equal contribution

# BRIAN LUKE

*Learning how to control DNA damage checkpoints may be the key to keeping chromosomes fit during ageing*

## EDUCATION

| | |
|---|---|
| 1999 | BSc in Biology, Queen's University, Canada |
| 2005 | PhD, ETH Zürich, Switzerland |

## POSITIONS HELD

| | |
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| 2005 | Postdoc, Biochemistry, ETH Zürich, Switzerland |
| 2005 - 2009 | Postdoc, EPFL Lausanne, Switzerland |
| 2009 – 2014 | Group Leader, ZMBH, Universität Heidelberg, Germany |
| Since Nov 2014 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Katharina Bender / PhD Student; relocating to IMB in March 2015
- Marco Graf / PhD Student; relocating to IMB in March 2015
- Arianna Lockhart / PhD Student; relocating to IMB in March 2015
- Vanessa Kellner / PhD Student; relocating to IMB in March 2015
- René Schellhaas / PhD Student; relocating to IMB in March 2015

## OVERVIEW

Telomeres make up the ends of linear chromosomes and are composed of TG-rich repetitive DNA. They ensure that the chromosome ends are not recognised as DNA damage, which could result in their aberrant repair, leading to the formation of chromosome end-to-end fusions and in turn, genome instability. Telomeres shorten following each round of cell division due to the "end replication problem". Telomere shortening plays a critical role in the increase in chromosomal abnormalities linked with ageing. It is important to understand how telomere maintenance is upheld to prevent premature cellular senescence and preserve genome integrity, which constitutes the main aim of our research goals. We focus on multiple aspects of telomere biology that are relatively new to the field and as a result, poorly characterised. Indeed, we have discovered that checkpoint adaptation, chromatin looping and the non-coding telomere repeat containing RNA (TERRA), have significant influences on telomere dynamics and rates of cellular senescence.

## RESEARCH HIGHLIGHTS

### CHECKPOINT ADAPTATION

Dysfunctional telomeres result in checkpoint activation, which leads to cell cycle arrest at the G2/M transition. Following prolonged telomere dysfunction cells eventually down-regulate the checkpoint response and progress through mitosis even in the presence of damaged telomeres – a phenomenon referred to as "checkpoint adaptation". We observed that the inhibition of the metabolically regulated TORC1 signalling pathway, through the addition of rapamycin, drastically

improves the viability of cells experiencing prolonged telomere dysfunction. We have further shown that the addition of rapamycin prevents checkpoint adaptation and that the inhibition of checkpoint adaptation is required for the viability rescue of telomere capping mutants. Whereas cells with telomere dysfunction eventually pass into G1 and resume the cell cycle, TORC1 inhibition holds the cells for a longer period of time in the G2/M block in a Rad53/Chk2 dependent manner (Figure 1). We believe these results could have important implications for human pathologies (e.g. dyskeratosis congenita) associated with chronic telomere dysfunction and increased genomic instability as well as for cancer therapy. Further studies will focus on characterising checkpoint adaptation in yeast and human cells, as well as elucidating the role of adaptation during telomere induced cellular senescence. Moreover, we are trying to understand how rapamycin regulates Cdc5 (PLK1) during checkpoint adaptation. Finally, we will investigate how nutrient regulation can potentially be exploited for therapeutic purposes.

## TERRA; A NON-CODING TELOMERE REPEAT CONTAINING RNA

Although telomeres are heterochromatic they get transcribed, producing a long non-coding RNA, TERRA. There have been many hypotheses regarding TERRA function, however it remains an unsolved mystery and will require continued investigation. Using chromatin immunoprecipitation (ChIP) directed against RNA: DNA hybrids we found that telomeres were enriched, suggesting that TERRA RNA-DNA hybrids (R-loops) exist at chromosome ends. The number of R-loops at telomeres increases when we deplete RNase H activity from the cell. We found that RNase H depleted cells senesce rapidly and have increased rates of telomere loss in the absence of telomerase and homologous recombination (HR). Importantly, in the presence of HR, telomere lengthening occurred with increasing RNA-DNA hybrids and replicative senescence was delayed, consistent with R-loops being hyper-recombinogenic substrates. These results led us to our current working model: that TERRA containing R-loops at telomeres lead to either telomere lengthening or shortening depending on the HR status of the cell. Further work on TERRA will focus on trying to understand how telomere transcription is regulated during the senescence process both in terms of R-loop and transcription regulation. In addition, in telomerase positive cells we are trying to understand the mechanistic details regarding how TERRA promotes telomerase function. Finally, we are elucidating the role of TERRA in ALT positive tumor cells, which exclusively use recombination to maintain their telomere length.

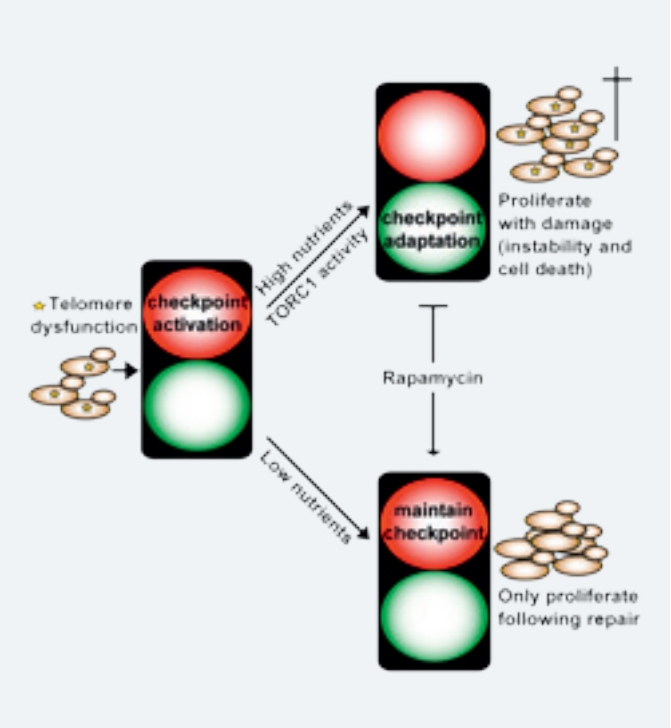



**Figure 1.** Telomere dysfunction activates the DNA damage checkpoint leading to cell cycle arrest. With high nutrient levels, cells adapt and proliferate in the presence of damage which leads to cell death and genome instability. Rapamycin (nutrient deprivation) can prevent adaptation and uphold the checkpoint until repair has been completed (Klermund *et al*, 2014).

## TELOMERE STRUCTURE ANALYSIS

Telomeric chromatin folds back onto itself and forms a lariat structure, which is conserved from yeast to man. It has been proposed that this structure protects the chromosome ends from degradation; much like the plastic cap on a shoelace protects the lace from fraying. We have recently developed a 3C-based approach to detect the loop structure in budding yeast. We plan on exploring how telomere looping is regulated in terms of cell cycle, telomere length and genetic context. Furthermore, we are testing possible links between telomere loops and telomere (TERRA) transcription. Our data suggest that the telomere loop requires a minimal telomere length to be maintained. Indeed, telomeres in telomerase negative pre-senescent and senescent cells are looping defective, which may explain why they are more prone to nucleolytic resection and unscheduled DNA repair events.

## FUTURE DIRECTIONS

Using yeast as a model organism we have elucidated new regulatory factors that influence telomere function, genome stability and replicative senescence in the absence of telomerase. We will continue to focus on how TERRA RNA-DNA hybrids are regulated and will expand our studies to telomerase positive cells where TERRA appears to also be playing a critical, yet undefined, role. Furthermore, we are planning to initiate genetic screens to find novel regulators of checkpoint adaptation. Finally, future experiments will focus on how TERRA regulation and checkpoint adaptation affect telomere metabolism in human cancer cells and as well as in cells with telomere dysfunction.

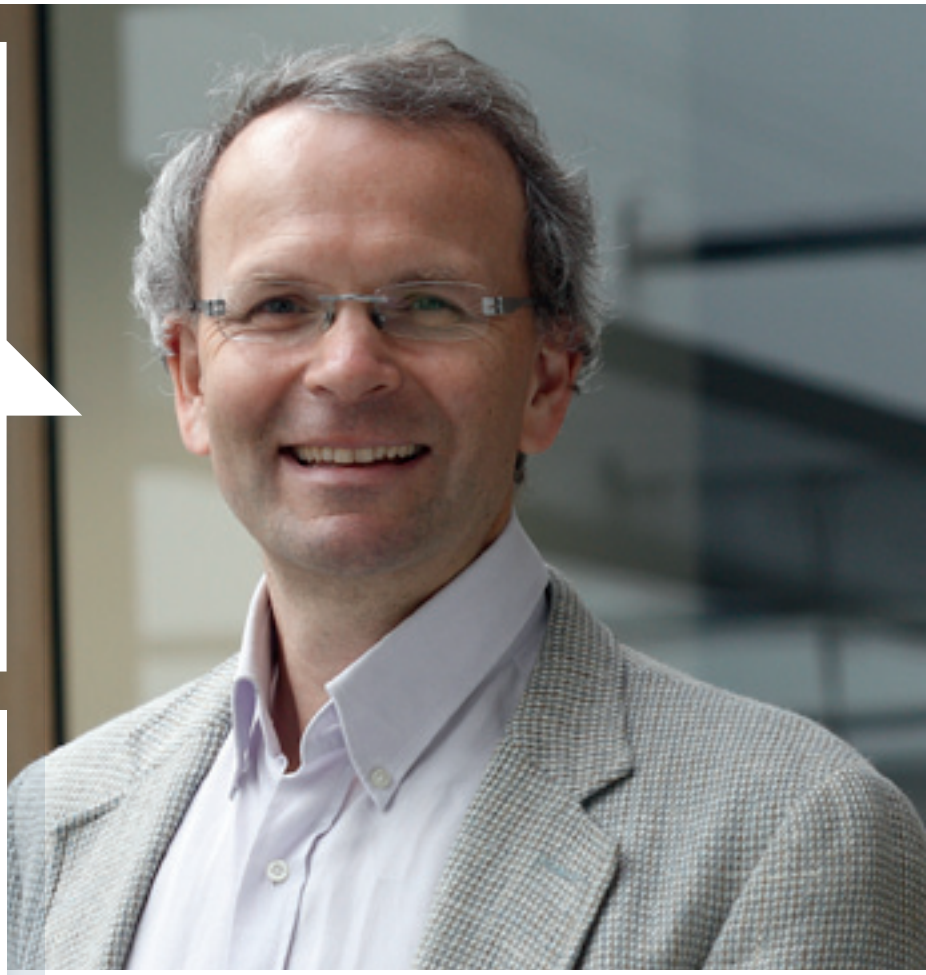
## SELECTED PUBLICATIONS

Klermund J, Bender K and Luke B (2014). High nutrient levels and TORC1 activity reduce cell viability following prolonged telomere dysfunction and cell cycle arrest. *Cell Reports*, 9, 324-335.

Balk B, Dees M, Bender K and Luke B (2014). The differential processing of telomeres in response to increased telomeric transcription and RNA-DNA hybrid accumulation. *RNA Biol,* 2, 95-100.

Balk B*, Maicher A*, Klermund J, Luke-Glaser S, Dees M, Bender K and Luke B (2013). Telomeric RNA-DNA hybrids affect telomere length dynamics and senescence. *Nat Struct Mol Biol*, 10, 1199-1205.

# CHRISTOF NIEHRS



*Location, Location, … DNA demethylation has an RNA ZIP code*

## EDUCATION

| | |
|---|---|
| 1985 | Diploma in Biochemistry, Free University of Berlin , Germany |
| 1990 | PhD, EMBL, Heidelberg, Germany |
| 1997 | Habilitation, Faculty of Biology, University of Heidelberg, Germany |

## POSITIONS HELD

| | |
|---|---|
| 1990 – 1993 | Postdoc, UCLA, USA |
| Since 1994 | Head of Division "Molecular Embryology", German Cancer Research Center (DKFZ), Heidelberg, Germany |
| Since 2000 | Professor of Molecular Embryology, DKFZ, Heidelberg, Germany |
| Since 2010 | Professor, Faculty of Biology, University of Mainz, Germany |
| Since 2010 | Founding Director, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Khelifa Arab / Postdoc; since 11/2011
- Mathias Gierl / Postdoc; 07/2011 – 10/2014
- Victoria Halch / Postdoc; since 11/2014
- Dandan Han/ PhD Student; since 12/2013
- Sabine Kienhöfer / Postdoc; since 07/2011
- Manuel Leichsenring / PhD Student; since 04/2011
- Medhavi Mallick / PhD Student; since 08/2012
- Bernadette Mekker / PhD Student; since 07/2010
- Svetlana Melcea / PhD Student; since 07/2013
- Michael Musheev / Postdoc; since 07/2011
- Andrea Schäfer / Postdoc; since 09/2010
- Lars Schomacher / Postdoc; since 07/2011
- Annika von Seggern / Research Technician; since 06/2011
- Susanne Spengler / Lab Manager; since 09/2013
- Ulrike Stapf / Research Technician; since 05/2011

## OVERVIEW

In the DNA of many multicellular organisms, DNA methylation is a common epigenetic mark associated with gene silencing. DNA methylation is a dynamic process and can be reversed by enzymatic demethylation, a process that is still incompletely understood. DNA demethylation is a widespread phenomenon and occurs in plants as well as in animals, during development, in the adult, and during somatic cell reprogramming of pluripotency genes. We have shown that growth arrest and DNA damage 45a (Gadd45a) is a key player in active DNA demethylation and acts via DNA repair. The goal of our research is to analyse the mechanism of DNA demethylation as well as the role played by Gadd45 in development. We study these questions using biochemical, molecular biological as well as cell biological approaches, and employ the frog and mouse models as genetic systems.

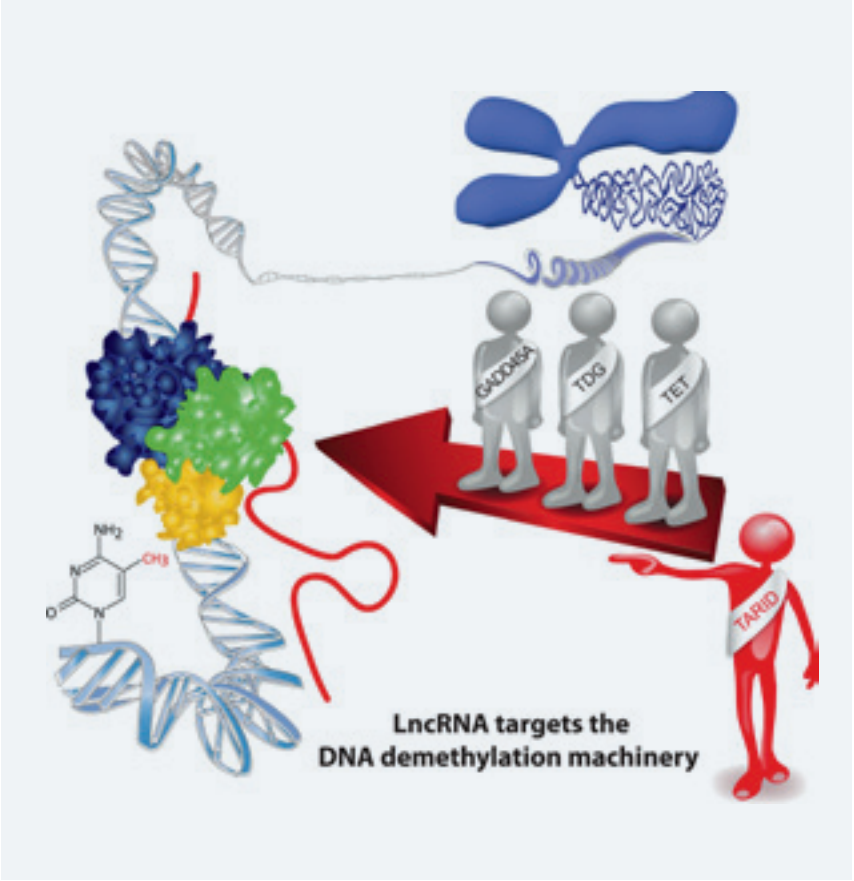
## RESEARCH HIGHLIGHTS

DNA methylation at 5-methylcytosine (5mC) of CpGs is a common epigenetic mark in metazoa, and plays important roles in regulating gene expression, genomic imprinting, X-chromosome inactivation, genomic instability, embryonic development, and cancer. It has become clear that DNA methylation is reversible by enzymatic active DNA demethylation, with examples in plants, animal development, cancer, and immune cells. Yet, the molecular mechanisms underlying active demethylation are only beginning to be understood. We have shown that growth arrest and DNA damage protein 45a (Gadd45a) mediates active DNA demethylation. Gadd45a is member of a small gene family of stress

response genes encoding histone fold proteins. Gadd45a proteins are multifunctional and regulate a range of cellular processes, including DNA repair, proliferation, apoptosis, and differentiation. Gadd45a mediated demethylation involves recruitment of the nucleotide excision repair and/or base excision repair machineries.

DNA demethylation of target genes by Gadd45a proteins is a highly selective process: not only is it gene-specific, but within a given gene it typically affects distinct mCpGs, often in the promoter region. This specificity highlights a set of general, unresolved key questions in DNA demethylation. What determines the target site specificity of DNA demethylation? Is there a relationship between site-specific DNA demethylation and the epigenetic landscape? What are the cofactors involved?

Last year we reported that p33ING1b (ING1b) protein serves as a cofactor for Gadd45a, essential for targeting DNA demethylation. Specifically, the results showed that histone methylation (H3K4me3) regulates DNA demethylation. However, while ING1b is necessary, it is not sufficient to explain Gadd45a targeting, not least because there are many more H3K4me3 sites in the genome than are becoming demethylated. This raises the question of what may be additional targeting mechanisms. In a collaboration with the laboratories of Ingrid Grummt and Christoph Plass (DKFZ)




we now provide evidence that gene-specific DNA demethylation may be directed by long non-coding RNAs. We have studied the tumor suppressor, *transcription factor 21 (TCF21)*, and discovered a promoter at a downstream CpG island within the *TCF21* gene that expresses a 4.5 kb long non-coding RNA (lncRNA) in antisense direction. Expression of this transcript, termed *TCF21 antisense RNA inducing promoter demethylation (TARID)*, decreases DNA methylation at the transcriptional start site of *TCF21* and induces expression of *TCF21* mRNA. We had previously discovered that Gadd45a is an RNA binding protein and indeed Gadd45a binds directly to *TARID*. Moreover, *TARID* associates with the *TCF21* promoter through a complementary antisense interaction. We also find that this ribonucleoprotein complex recruits two key enzymes required for DNA demethylation – thymine DNA glycosylase (TDG) and ten-eleven translocation methylcytosine dioxygenases (TET) – to the TCF21 promoter. The results indicate that *TARID*, and likely other lncRNAs, may serve as sequence-specific ZIP codes to target the DNA demethylation machinery to specific gene loci.

## FUTURE DIRECTIONS

Our discovery of an lncRNA as a targeting factor for Gadd45a has raised new questions. By which type of molecular interaction does TARID associate with its cognate *TCF21* promoter? Future studies will also need to address the question of which specific demethylation intermediates and which chromatin modifying enzymes contribute to *TCF21* demethylation. What other lncRNAs are bound by Gadd45a and what may be their target genes regulated by DNA demethylation? What are the sequence determinants which allow Gadd45 to bind to specific lncRNAs? How does lncRNA function in DNA demethylation relate to the biological processes in which Gadd45 has been implicated, such as autoimmunity, ageing, and cancer? The results also suggest that Gadd45 can interact with Tet enzymes, which are key players in the oxidation of cytosines and in DNA demethylation. It will therefore be important to analyse if Tet and Gadd45 proteins can interact directly and what the consequence of such an interaction is, *in vitro* and *in vivo*.

**Figure 1.** lncRNA can act as ZIP code for gene-specific DNA demethylation. TARID lncRNA assembles an RNP consisting of the RNA binding protein GADD45a, Thymine-DNA glycosylase (TDG) and Ten-eleven translocation methylcytosine dioxygenase (TET) to achieve TCF21 promoter specific DNA demethylation. These results demonstrate that an lncRNA can serve as a genomic address label for GADD45a mediated, gene-specific DNA demethylation.

## SELECTED PUBLICATIONS

Arab K, Park YJ, Lindroth AM, Schäfer A, Oakes C, Weichenhan D, Lukanova A, Lundin E, Risch A, Meister M, Dienemann H, Dyckhoff G, Herold-Mende C, *Grummt I, *Niehrs C and *Plass C (2014). Long Noncoding RNA TARID Directs Demethylation and Activation of the Tumor Suppressor TCF21 via GADD45A. *Mol Cell*, 55, 604-614.

Schäfer A, Karaulanov E, Stapf U, Döderlein G and Niehrs C (2013). Ing1 functions in DNA demethylation by directing Gadd45a to H3K4me3. *Genes Dev*, 27, 261-273.

Niehrs C and Schäfer A (2012). Active DNA demethylation by Gadd45 and DNA repair. *Trends Cell Biol*, 22, 220-227.

*indicates equal contribution

# GEORGE REID

*Epigenetic marks on chromatin function as environmental sensors*

## EDUCATION

| | |
|---|---|
| 1984 | BSc in Biochemistry, University of Strathclyde, Glasgow, UK |
| 1988 | PhD in Biochemistry, University of Strathclyde, Glasgow, UK |

## POSITIONS HELD

| | |
|---|---|
| 1984 – 1985 | Software developer, Turing Institute, Glasgow, UK |
| 1988 – 1993 | Postdoc, MRC Retrovirus Research Laboratory, University of Glasgow, UK |
| 1994 – 1996 | Senior Scientist, Pfizer Central Research, Sandwich, UK |
| 1996 – 1998 | Postdoc, Beatson Institute for Cancer Research, Glasgow, UK |
| 1999 – 2004 | Postdoc, EMBL, Heidelberg, Germany |
| 2004 – 2010 | Staff Scientist, EMBL, Heidelberg, Germany |
| Since 2006 | Co-founder and Executive of Elara Pharmaceuticals, Heidelberg, Germany |
| Since 2010 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Stephan Baumgärtner / PhD Student; since 09/2012
- Susan Bertes / Masters Student; since 04/2014
- Iryna Charapitsa / Postdoc; since 07/2011
- Christof Fritzsch / PhD Student; since 09/2012
- Wolf Gebhardt / Postdoc; since 08/2011
- Ina Kirmes / PhD Student; since 06/2012
- Monica Kuban / Research Technician; since 10/2012
- Martha Paluschinski / Diploma Student; 01/2013 – 11/2013

## OVERVIEW

The functional template of transcription is chromatin, which generates multiple regulatory barriers that have to be overcome prior to the initiation of RNA synthesis. Our group has demonstrated that dynamic cycling between permissive and non-permissive chromatin states can be an inherent process in the expression of tightly regulated genes. We have utilised state of the art experimental methodologies, allied with cutting edge bioinformatics and mathematical modelling to develop comprehensive, quantitative and predictive understanding of estrogen dependent gene regulatory networks. Additionally, we have discovered novel small molecules that provoke activating epigenetic changes in chromatin and in consequence, act to amplify the output of kinase cascades that signal to chromatin. Finally, we have demonstrated that chromatin acts as an oxygen sensor in response to hypoxia and undergoes profound epigenetic changes in response to oxygen deprivation and upon reperfusion with oxygen following a period of hypoxia.

## RESEARCH HIGHLIGHTS

### ANALYSING AND MODELLING ESTROGEN DEPENDENT TRANSCRIPTIONAL CYCLING

A bioinformatic and mathematical modelling analysis of estrogen dependent gene transcription at multiple timepoints determined that an estrogen receptor responsive gene (ATAD3B) is associated with a decrease in the survival of breast cancer patients when expressed at high levels. Moreover, these projects led to the development of several new computational tools for the analysis and modelling of deep sequencing data. Key processes developed through these activities

are (1) SPINLONG, which identifies estrogen responsive genes; (2) GROK, for fast analysis and processing of deep sequence data; (3) BitSeq, a tool for transcript-level deconvolution of RNA-Seq data and differential expression analysis across conditions; (4) Gaussian methods for testing temporal changes in high-throughput sequencing data, especially splicing, using RNA-Seq data; (5) Gaussian methods that model the dynamics of RNA-Pol II and linking these dynamics to mRNA production and (6) A novel Bayesian method for inferring targets of multiple co-regulating transcription factor proteins from time course data. Additionally, we have shown at single base resolution on a genome wide basis, that the methylation status of a considerable number of CpG dinucleotides, generally associated with transcriptional start sites, cycle. We have also established clonal reporter cell lines that contain a stable integration of an estrogen sensitive transgene which expresses RNA stem-loops that recruit RNA binding protein labelled with GFP to nascent RNA upon transcription. Consequently, active transcription is visualised as a fluorescent spot over the background of unbound protein and can be followed over time. We find that estrogen dependent expression induces transcriptional bursting, and in conjunction with the Legewie group, we are developing models that reconcile transcriptional bursting with the transcriptional cycling observed in synchronised cell populations when evaluated by ChIP.

## CHARACTERISATION OF NOVEL SMALL MOLECULES THAT PROVOKE ACTIVATING EPIGENETIC CHANGES IN CHROMATIN

We have identified, through chemigenetic screening, two series of small molecules that up-regulate, by two orders of magnitude, expression driven by the SV40 immediate early promoter. Remarkably, these compounds induce a global increase in the occurrence of activating histone marks, in particular histone acetylation and H3K4 trimethylation. Mechanistically, it is necessary to identify target proteins that bind to, and to characterise biological pathways that are modulated by, these novel small molecules. We have performed a global phenotypic shRNA screen, to identify pathway components that are essential in mediating the effect of these compounds. This has revealed that our compounds have a primary influence on the epigenetic state of chromatin, which acts to enhance signalling events that up-regulate the expression of a limited range of primary targets, for example, EGR1 expression. Additionally, we have shown that a phosphoinositol dependent kinase cascade, initiated through the action of an orphan tyrosine kinase, LTK (leukocyte tyrosine kinase), is the initiating process that is enhanced by our compounds. We have also screened an additional 300 compounds with structural similarity to one of our initial series of hits. Structure activity analysis of this dataset identified key aspects of the core structure of active molecules, which has enabled us to rationally construct biotinylated molecules. This will allow direct affinity purification of the target protein or complex, which will then be identified by SILAC mass spectroscopy. In addition to providing biotools that probe signalling to chromatin, these discoveries may have practical use in in the treatment of cancer, in ageing and development, in cognitive neuroscience and in inherited hematological disorders.

## CHROMATIN AS AN OXYGEN SENSOR

We utilise a mouse model of cardiac ischaemia reperfusion and a cell model of murine cardiomyoctes under defined hypoxic exposure to evaluate the epigenetic response of cells to reduced oxygen tension. Hypoxia induces profound changes in chromatin, characterised by a rapid loss of activating histone marks and an acquisition of repressive marks. Additionally, we observe that chromatin undergoes general condensation upon hypoxia. In conjunction with the Cremer group, we are using super-resolution microscopy to describe chromatin compaction and to characterise histone modifications at a single nucleotide resolution. Upon recovery from hypoxia, chromatin responds by acquiring globally permissive histone alterations, which results in a dramatic change in transcriptional output. Transient ischaemia, followed by reperfusion, of the area of the heart supplied by the left descending artery induces a local increase in permissive histone marks as observed by immunofluorescence and in the production of a local inflammatory response, predominantly driven by the transcription factors EGR1 and AP1. Collectively, this work provides a novel framework for potential therapeutic interventions that protect cells from the deleterious effects of hypoxia.

## FUTURE DIRECTIONS

We will continue to explore how the epigenetic status of chromatin is influenced by external signalling, such as hormonal activation, environmental changes or through modulation of signalling pathways by novel small molecules. This work, in addition to providing insight into the interplay between chromatin and cellular function, also describes novel approaches to modulate gene responses in normal cellular differentiation and physiology and in diseases with a strong epigenetic influence, such as cancer and inflammation.

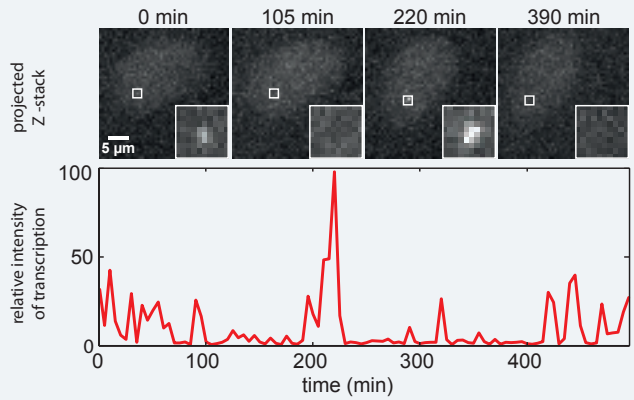



**Figure 1.** A reporter cell line was established that expresses an estrogen sensitive transgene containing RNA stem-loops that recruit GFP-labelled proteins to nascent RNA. Consequently, active transcription is visualised as a local fluorescent spot. Real-time, live-cell imaging reveals short bursts of transcription interspersed between inactive periods.

## SELECTED PUBLICATIONS

Ovaska K, Matarese F, Grote K, Charapitsa I, Cervera A, Liu C, Reid G, Seifert M, Stunnenberg HG and Hautaniemi S (2013). Integrative analysis of deep sequencing data identifies estrogen receptor early response genes and links ATAD3B to poor survival in breast cancer. *PLoS Comput Biol*, 9, e1003100.

Metivier R, Gallais R, Tiffoche C, Le Peron C, Jurkowska RZ, Carmouche RP, Ibberson D, Barath P, Demay F, Reid G, Benes V, Jeltsch A, Gannon F and Salbert G (2008). Cyclical DNA methylation of a transcriptionally active promoter. *Nature*, 452, 45-50.

Kangaspeska S, Stride B, Metivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F and Reid G (2008). Transient cyclical methylation of promoter DNA. *Nature*, 452, 112-115.

# HOLGER RICHLY

*We decipher the molecular mechanisms of epigenetic pathways*

## EDUCATION

| | |
|---|---|
| 2000 | Diploma in Biochemistry, Ruhr University, Bochum, Germany |
| 2005 | PhD in Biochemistry, Ludwig-Maximilians University, Munich, Germany |

## POSITIONS HELD

| | |
|---|---|
| 2005 – 2011 | Postdoc, Center for Genomic Regulation, Barcelona, Spain |
| Since 2011 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Jonathan Byrne / PhD Student; since 08/2011
- Shalaka Chitale / PhD Student; since 02/2013
- Faranak Fassihianifard / Masters Student; since 07/2014
- Ekaterina Gracheva / PhD Student; since 08/2011
- Aysegül Kaymak / PhD Student; since 02/2013
- Rebeca Medina / Research Technician; since 08/2013
- Thaleia Papadopoulou / PhD Student; since 10/2011
- Jens Stadler / PhD Student; since 09/2014
- Thomas Wilhelm / PhD Student; since 11/2011

## OVERVIEW

The research undertaken in my laboratory aims at deciphering molecular pathways that underlie epigenetic networks to regulate physiological processes such as cellular differentiation, DNA repair and organismal ageing. Environmental cues and endogenous stimuli have great impact on the composition of chromatin and alter the function of epigenetic systems. We are particularly interested in how epigenetic factors contribute to the DNA Damage Response after irradiation with UV light. Working at the crossroads of epigenetic research and DNA repair we try to understand the interplay of epigenetic factors and components of the DNA repair machinery. Inaccurate DNA repair causes an accumulation of gene mutations and epimutations and is considered one of the major determinants of organismal ageing. The development of animals is a highly complex process and it has become clear that it relies substantially on epigenetic programming. In contrast, ageing commences post-developmentally after a reproduction phase. We intend to understand how epigenetic programming determines the life expectancy of animals and how the environement impacts on health and life span through epigenetic mechanisms. Our scientific approach relies largely on dissecting the functions of diverse epigenetic players either biochemically or by sophisticated next generation sequencing techniques. We also employ genetics and have recently developed a semi-automated screening technique to isolate novel players of organismal ageing. Hence, we combine mechanistic and physiological research approaches to gain insight into the mechanisms underlying DNA repair and ageing.

## RESEARCH HIGHLIGHTS

Our research has provided evidence as to how epigenetic factors act in concert with DNA repair factors. We have unravelled the identity and function of a novel E3-Ligase complex that sets the mono-ubiquitin mark at lysine 119 of histone H2A in the course of Nucleotide Excision Repair (NER). Our findings provide insight into how epigenetic factors regulate DNA lesion recognition and how assembly of multi protein complexes is accomplished at DNA lesion sites. Furthermore, we have found that ubiquitin signalling at chromatin predominantly orchestrates DNA repair through polyubiquitylation of repair factors. We are particularly interested in deciphering the function of different polyubiquitin-linkages and are screening for specific ubiquitin binding factors involved in NER. Our findings indicate that chromatin is partitioned during NER and that repair is carried out at defined nuclear regions, and we are currently investigating which epigenetic factors impact on the localisation and dynamics of damaged chromatin. We have also investigated the function of epigenetic factors downstream of DNA lesion recognition involving the endoribonuclease DICER. In the future we will address the function of small RNAs produced by DICER in more detail.

In parallel, we are interested in understanding the complex network regulating the activation of gene expression at the onset of cellular differentiation. To that end we have been studying the function of activating non-coding RNAs (ncRNAs) and epigenetic components during the epigenetic programming that occurs in stem cell differentiation. Importantly, we have identified a novel role of Polycomb group proteins in establishing defined chromatin architecture during stem cell pluripotency. We are currently generating a genome-wide picture of the dynamics of certain epigenetic factors and will in the future deduce a model for the concerted function of ncRNAs and epigenetic players in gene activation of Polycomb target genes. Moreover, we are interested in the function of DICER during pluripotency and in differentiation of embryonic stem cells. Our findings suggest that DICER is recruited to specific chromatin regions during the differentiation of embryonic stem cells. In this context we are currently investigating the role of small RNAs during epigenetic programming of chromatin.

We combine our mechanistic research approach with sophisticated screening techniques to understand the epigenetic mechanisms underlying ageing. Classically, ageing research has been carried out with the nematode *C. elegans* owing to its relatively short lifespan. In particular, genetic approaches facilitated the discovery of metabolic and signalling pathways, which impact on the longevity of the nematode. Importantly, these pathways establish a link to the environment and hence to epigenetic mechanisms. We have generated a semi-automated siRNA screening technique isolating novel factors involved in the ageing of the worms. We are currently concentrating our efforts on analysing the epigenetic factors identified by the screen. In the near future, we will characterise the function of these factors using physiological assays and more importantly, we will set out to investigate how they reprogramme the epigenome in the course of ageing.

## FUTURE DIRECTIONS

In the future we will prioritise research on DNA repair in the NER pathway and the investigation of organismal ageing. One of our main aims is to understand how epigenetic information is maintained at damaged chromatin. To that end, we will look into the function of epigenetic players during and after DNA repair. We believe that specific ubiquitylation and phosphorylation events at chromatin might play a decisive role in the maintenance of the epigenome.

We are also planning to investigate how epigenetic factors and environmental cues extend the lifespan and the healthspan of C. elegans and will extend our studies employing the fruit fly D. melanogaster to identify conserved epigenetic mechanisms of ageing.

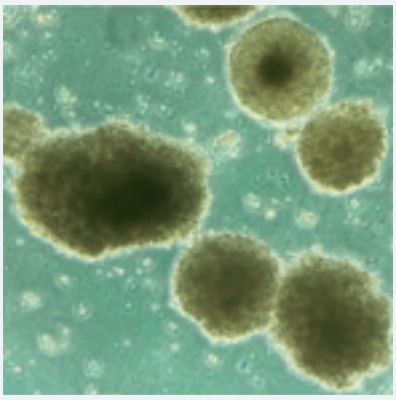


**Figure 1.** Embryoid bodies from mouse embryonic stem cells serve as a model system to study developmental processes *in vitro*.

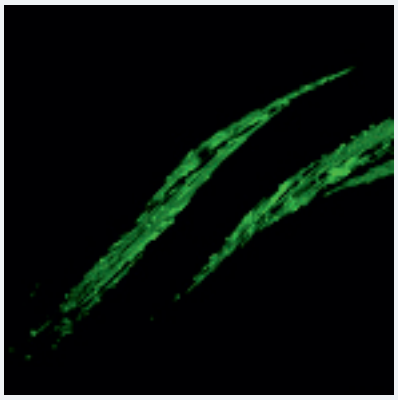


**Figure 2.** Visualisation of body wall muscles in *C. elegans* employing a GFP reporter strain.

**Figure 3.** Deposition of lipids in aged animals is visualised by Oil Red staining.

## SELECTED PUBLICATIONS

Ribeiro J, Morey L, Mas A, Gutierrez A, Luis N, Mejetta D, Richly H, Benitah S, Keyes WM and Di Croce L (2013). ZRF1 controls oncogene-induced senescence through the INK4-ARF locus. *Oncogene*, 32, 2161-2168.

Richly H and Di Croce L (2011). The flip side of the coin: role of ZRF1 and histone H2A ubiquitination in transcriptional activation. *Cell Cycle*, 10, 745-750.

Richly H, Rocha-Viegas L, Ribeiro Domingues J, Demajo S, Gundem G, Lopez-Bigas N, Nakagawa T, Ito T, Rospert S and Di Croce L (2010). Transcriptional Activation of Polycomb-repressed genes by ZRF1. *Nature*, Dec 23, 468, 1124-1128.

# JEAN-YVES ROIGNANT

*We've found a novel function of the exon junction complex in controlling the rate of transcription elongation*

## EDUCATION

| | |
|---|---|
| 1997 | BSc in Molecular and Cell Biology, University of Rennes 1, France |
| 2003 | PhD in Developmental Biology, Jacques Monod Institute, Paris, France |

## POSITIONS HELD

| | |
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| 2003 – 2008 | Postdoc, Skirball Institute of Biomolecular Medicine, New York University, USA |
| 2008 – 2011 | Research Associate, Skirball Institute of Biomolecular Medicine, New York University, USA |
| Since 2012 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Junaid Akhtar / Postdoc; since 02/2012
- Maria-Carla Antonelli / Masters Student; since 04/2014
- Nadja Dinges / PhD Student; since 03/2014
- Laura Götzinger / Student Assistant; since 08/2013
- Giriram Kumar Mohana / PhD Student; since 04/2014
- Anna Lena Leifke / Research Technician; since 07/2013
- Tina Lence / PhD Student; since 07/2013
- Claire Mesdagh / PhD Student; since 01/2012

## OVERVIEW

The process of pre-mRNA splicing is crucial for regulating gene expression and generating protein diversity. Intriguingly, despite its relative early discovery, the precision and complexity of intron removal is not yet fully understood. Until recently, whether an exon was alternatively or constitutively spliced was thought to be mainly influenced by sequences in the pre-mRNA and by binding of splicing factors. However, recent work has shown that most splicing events occur co-transcriptionally, allowing novel layers of regulation by the transcription machinery and chromatin structure. The research in my lab aims to uncover the intricate relationships between these processes.

## RESEARCH HIGHLIGHTS

### MECHANISMS OF THE EXON JUNCTION COMPLEX IN PRE-mRNA SPLICING

Pre-mRNA splicing results in the deposition of the exon junction complex (EJC) upstream of exon-exon boundaries. The EJC controls several posttranscriptional functions including RNA localisation, translation and nonsense mediated decay. Additionally, the EJC prevents exon skipping in a subset of transcripts, many of which harbour large introns and are expressed from a heterochromatic location. The importance of these features for EJC dependency is not known. We have made some progress towards the role of EJC during splicing by investigating EJC function during ovarian development. We have discovered that the EJC prevents accumulation of transposable elements in the ovaries by facilitating the splicing of the piwi transcript, which encodes a member of the Argonaute family. We

have found that the retained intron contains a weak polypyrimidine tract which is necessary and sufficient to confer EJC dependency. We have further observed that the retained intron requires the flanking introns to increase its removal. We propose a model in which the EJC is rapidly deposited to exon junctions after the splicing of bona fide introns and subsequently facilitates the splicing of flanking introns containing divergent canonical cis-acting sequences. This dependency of splicing on the EJC might be exploited as a means to control the temporal order of splicing events.

How the EJC facilitates intron recognition after its binding to flanking exon junctions remains to be discovered. Furthermore, it is not clear whether this EJC function in intron recognition is similar to its function in exon definition involved in splicing large heterochromatin transcripts. One clue to these questions came from our observations that EJC loss is accompanied with a decrease in nucleosome occupancy and a gain in C-terminal domain phosphorylation of RNA Polymerase II (Pol II) at the MAPK locus, one of the large EJC heterochromatic targets. As several studies have previously shown that the rate of transcription elongation can affect exon definition, our data suggest that the EJC might prevent exon skipping from heterochromatic transcripts by decreasing the phosphorylation state of Pol II, in order to reduce the rate of transcription elongation. Consistent with this hypothesis, we have found that decreasing the speed of Pol II rescues exon skipping associated with the absence of the EJC *in vivo*. This effect appears specific since mutations in other

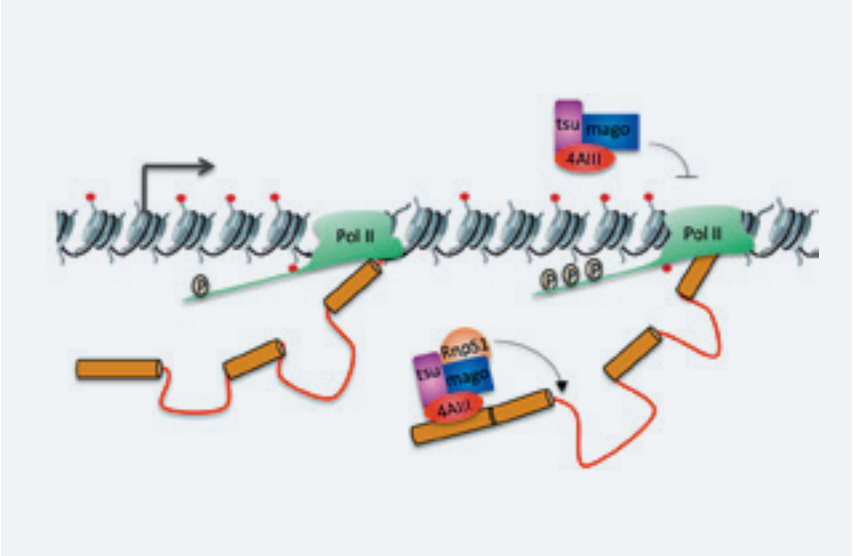



**Figure 1.** Mechanisms of the exon junction complex (EJC) in pre-mRNA splicing. The EJC facilitates splicing of weak introns following its initial deposition at adjacent exon junctions. This function is mediated via the splicing subunit RnpS1. In addition, the EJC helps splicing of large introns by decreasing the rate of transcription elongation.

spliceosome components do not increase Pol II phosphorylation. Therefore, our results support a mechanism in which the EJC influences exon definition through the modulation of transcription elongation. This mechanism might be particularly important for ensuring proper splicing of challenging introns such as heterochromatic introns, which are usually large and rich in repeated elements.

## MECHANISMS OF GENE EXPRESSION FROM A HETEROCHROMATIC ENVIRONMENT

Organisation of chromosomes into euchromatin and heterochromatin is one of the most enigmatic aspects of genome evolution. In flies, several coding genes are expressed from constitutive heterochromatin at pericentromeric regions. It remains unknown how expression can occur from this repressive environment. When euchromatic genes are moved within heterochromatin they fail to be expressed. Conversely, chromosomal rearrangements resulting in relocation of heterochromatic genes within a euchromatic environment prevent their expression. This strongly suggests that heterochromatic genes are expressed by a distinct mechanism that is dependent on the structure of heterochromatin itself. Consistent with this, decreasing the level of H3K9me2/3 reduces heterochromatic gene expression. We now show that germline knock down of Piwi also affects expression of a subset of heterochromatic genes. This is associated with a specific decrease in the silencing marks H3K9me2/3 at the affected loci. The nature of this specificity is currently under investigation.

## FUTURE DIRECTIONS

We have shown that the EJC controls nucleosome occupancy and Pol II phosphorylation at the MAPK locus, which in turn influence pre-mRNA splicing. We will next address the mechanisms underlying this function. What are the direct partners of the EJC in controlling transcription elongation? What is the link with nucleosome organisation? Is the increase in transcription elongation specific to a subset of target genes? Proteomic and genome wide studies should answer these questions. We will also investigate the role of chromatin modifications during cell fate decisions. Which marks are more predictive of alternative splicing events? How are they specifically deposited? Finally, we will pursue our investigations of the mechanisms of transcription from heterochromatin domains. These studies should provide key insights into the role of chromatin on transcription and RNA processing events.

## SELECTED PUBLICATIONS

Malone CD, Mestdagh C, Akhtar J, Kreim N, Deinhard P, Sachidanandam R, Treisman J and Roignant JY (2014). The exon junction complex controls transposable element activity by ensuring faithful splicing of the piwi transcript. *Genes Dev*, 28, 1786-1799.

Roignant JY and Treisman JE (2010). Exon junction complex subunits are required to splice *Drosophila* MAP kinase, a large heterochromatic gene. *Cell*, 143, 238-250.

Roignant JY, Legent K, Janody F and Treisman JE (2010). The transcriptional cofactor Chip acts with LIM-homeodomain proteins to set the boundary of the eye field in *Drosophila*. *Development*, 137, 273-281.

# NATALIA SOSHNIKOVA

*A central question in developmental biology is how tissues and organs acquire their unique shapes and functions*

## EDUCATION

| | |
|---|---|
| 1998 | Diploma in Molecular Biology, Novosibirsk State University, Russia |
| 2004 | PhD in Molecular Medicine, Max-Delbrück-Centre for Molecular Medicine (MDC), Berlin, Germany |

## POSITIONS HELD

| | |
|---|---|
| 2004 – 2011 | Postdoc, Department of Genetics and Evolution, University of Geneva, Switzerland |
| Since 2012 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Marius Baeken / MSc Student; since 02/2014
- Margarita Dzama / PhD Student; since 09/2014
- Stefanie Grimm / Research Technician; 01/2012 – 08/2014
- Juri Kazakevych / PhD Student; since 09/2012
- Christina Krienke / PhD Student; 02/2013 – 08/2014
- Liron-Mark Lavitas / Postdoc; since 07/2013
- Burkhard Mayer / Student Assistant; 01/2014 – 07/2014
- Berith Messner / MSc Student; since 09/2014
- Lira Nigmatullina / PhD Student; since 04/2013

## OVERVIEW

We are interested in the regulatory mechanisms underlying vertebrate pattern formation during embryogenesis and tissue homeostasis in adults. Our current focus is the mouse small intestine. Homeostasis of the intestinal epithelium requires rapid and continuous regeneration of specific cell types from the intestinal stem cells (ISCs). This process is governed by the interplay of signalling pathways that impose developmental restrictions on progenitor cells. Transcription factors and histone-modifying complexes are instrumental for the maintenance of stem cell potential and for a stable determination of cell fate. We are investigating when and how the ISCs are defined and integrated into the future stem cell compartment during embryogenesis. To functionally characterise different epithelial cell populations within the embryonic small intestine we are using a combination of system-wide approaches, mouse models and *ex vivo* 3D organoid assays.

## RESEARCH HIGHLIGHTS

### DECIPHERING MOLECULAR MECHANISMS RESPONSIBLE FOR THE FORMATION OF ADULT ISCS

Transcription profiles of intestinal tumours and differentiated enterocytes gave multiple markers for adult ISCs, including Lgr5. Further lineage tracing analysis using Lgr5-Egfp-Cre-ERT2 mice confirmed that Lgr5 positive cells are adult ISCs. In contrast to well-characterised adult intestinal cell populations, nothing was known about the embryonic intestinal epithelium. Based on molecular signatures we found that there are several cell populations within the embryonic intestinal

epithelium. We further functionally characterised some of these cell populations using genetic cell fate mapping analysis. We have determined that certain cell populations within the embryonic intestinal epithelium serve as precursors of the adult ISCs. To assess the temporal and hierarchical relationship between these cell types, we performed transcriptome profiling as well as lineage tracing analyses.

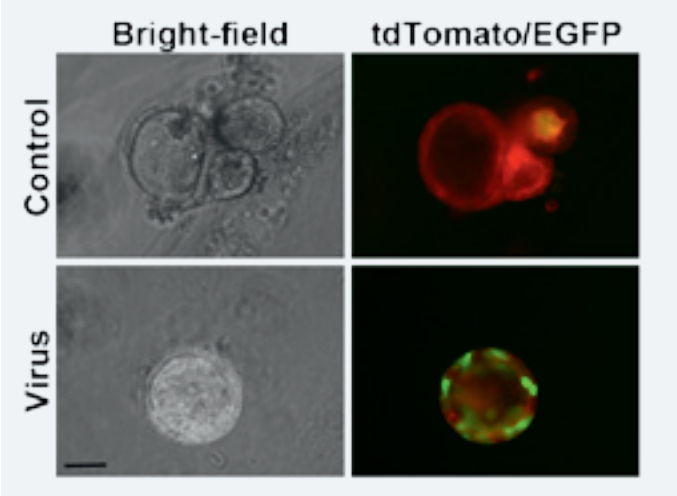


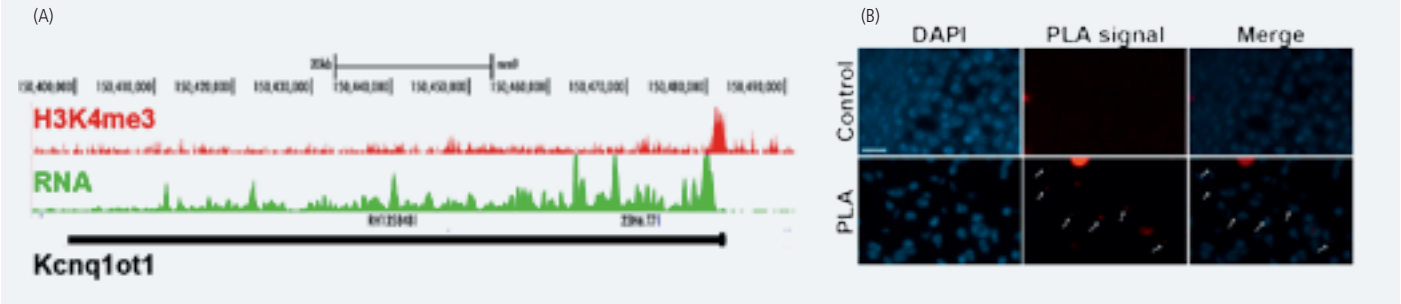

**Figure 1.** Embryonic intestinal organoids from tdTomato mice (red) cultured in control media or media containing the EGFP-expressing virus. Scale bar, 30 µM.

Transcriptome analysis of adult ISC precursors isolated from embryos provided us with a number of transcription factors, receptors and ligands, which could be essential for the acquisition of adult ISC identity. To test their functions, we have established a culture system that recapitulates, in 3D, the development and maintenance of the intestinal epithelium. In these cultures embryonic cells grow and give rise to the adult intestinal stem cells, which then differentiate to all lineages found *in vivo*, thus generating mini-guts. We explored methods to efficiently infect embryonic intestinal epithelial cells using virus-based systems (Figure 1). Currently we perform both loss-of-function studies applying the CRISPR/CAS9 system, and overexpression analyses using viral transduction.

## FUTURE DIRECTIONS

Using 3D organoid cultures we will further test candidate genes responsible for the formation of the adult ISCs during mouse embryogenesis. Candidate genes whose gain- or loss-of-function leads to improved or reduced organoid formation, would be characterised in mouse models. Moreover, we are interested in learning whether other cell types within the embryonic epithelium could function as precursors of the adult ISCs or whether they could function as signalling centres essential for the growth and patterning of the embryonic gut. To answer these questions we will perform genetic cell ablation studies using mice that express diptheria toxin.

**Figure 2.** (A) Transcription and chromatin profiles along the Kcnq1ot1 locus in the intestinal cells at E14.5. (B) PLA detection *in situ* in sections of mouse embryos at E14.5. DAPI marks nuclei (blue), PLA amplification marks H3K27Ac at the Kcnq1ot1 promoter (red). Scale bar, 20 µM. PLA: proximity ligation assay.




## *IN SITU* ANALYSIS OF CHROMATIN MARKS AT SPECIFIC GENE LOCI

We performed whole transcriptome and chromatin profiling of embryonic intestinal epithelial cells, enterocytes, and Lgr5+ adult ISCs using RNA-, MBD- and ChIP-sequencing (Figure 2A). Based on clustering and meta-analysis of all transcriptome and chromatin data we found that many genes employ alternative transcription start sites (TSS) for their expression. Very dynamic changes in histone marks at the promoters of the target genes faithfully reflect the transcriptional status of cell populations during development. However, these yield only the average epigenetic state of a population, not showing cell to cell differences. Indeed, RNA *in situ* hybridisation on tissue sections revealed that a large number of genes are expressed in a "salt and pepper pattern" within the embryonic epithelium. To understand how developmental genes are epigenetically regulated on the single cell, and possibly also single allele, level we established a proximity ligation assay (PLA), which allows for mapping of a certain histone mark to a specific promoter region in each cell on tissue sections at 40 nm precision (Figure 2B).

## SELECTED PUBLICATIONS

Montavon T, Soshnikova N, Mascrez B, Joye E, Thevenet L, Splinter E, de Laat W, Spitz F and Duboule D (2011). A Regulatory Archipelago Controls Hox Genes Transcription in Digits. *Cell*, 147, 1132-1145.

Soshnikova N, Montavon T, Leleu M, Galjart N and Duboule D (2010). Functional analysis of CTCF during mammalian limb development. *Dev Cell*, 19, 819-830.

Soshnikova N and Duboule D (2009). Epigenetic temporal control of mouse Hox genes *in vivo*. *Science*, 324, 1320-1323.

# VIJAY TIWARI

*We study epigenetic mechanisms of gene regulation underlying cell-fate specification during development and misspecification in disease*

## EDUCATION

| | |
|---|---|
| 2002 | MSc in Molecular and Human Genetics, Banaras University, Varanasi, India |
| 2006 | PhD in Developmental Biology, Uppsala University, Sweden |

## POSITIONS HELD

| | |
|---|---|
| 2006 – 2008 | Postdoc, Johns Hopkins University School of Medicine, Baltimore, USA |
| 2008 – 2011 | Postdoc, Friedrich Miescher Institute (FMI) for Biomedical Research, Basel, Switzerland |
| Since 2012 | Group Leader, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Pablo Bora / Research Assistant; since 04/2014 – 10/2014
- Sarbashis Das / Postdoc; 02/2013 – 06/2014
- Angela Garding / Postdoc; since 04/2012
- Johannes Jung / PhD Student; since 05/2013
- Abhijeet Pataskar / PhD Student; since 11/2014
- Sanjeeb Sahu / PhD Student; since 05/2013
- Anke Salzer / Lab Manager; since 07/2012
- Sandra Schick / PhD Student; since 06/2012
- Sudhir Thakurela / PhD Student; since 01/2012
- Alicia Villatoro / Student Assistant; 04/2014 – 12/2014
- Yuan Zhuang / Research Assistant; since 09/2014

## OVERVIEW

The research in our lab is aimed at understanding the mechanisms by which epigenetic machinery and transcription factors contribute to transcriptional reprogramming that defines cell-fate during development and how this communication is altered in diseases such as cancer. We employ a multidisciplinary approach combining cutting-edge epigenetics and genomics together with computational biology tools in sophisticated and defined models of cellular differentiation and carcinogenesis. Our primary research interests include:

- Signalling to chromatin crosstalk in gene regulation
- Transcription factors and lineage specification
- Epigenomics of cell-fate specification
- Epigenetic regulation of neurogenesis
- Chromatin and cancer
- Systems biology of gene regulatory networks

## RESEARCH HIGHLIGHTS

### DISCOVERING NOVEL EPIGENETIC REGULATORS OF NEURONAL DEVELOPMENT

Cell-fate specification during mammalian development involves stable resetting of transcriptional programmes and the role of chromatin-mediated regulation in this process has been increasingly appreciated. The nervous system is the most complex organ in all mammalian organisms. The last decade has observed extensive research in understanding how this complexity is generated

during neural development. Despite exciting progress, very little is known about epigenetic regulatory networks during embryonic neurogenesis. Using extensive computational biology tools for prediction in combination with analysis of multiple tissues from all three lineages during embryonic development, we have identified new candidate epigenetic regulators of neurogenesis. Preliminary analysis suggests that a set of these factors are critically required for neuronal development (Figure 1). By employing a multidisciplinary approach, we next aim to generate a mechanistic insight into how these potential novel epigenetic regulators contribute to the transcriptional reprogramming underlying neurogenesis.

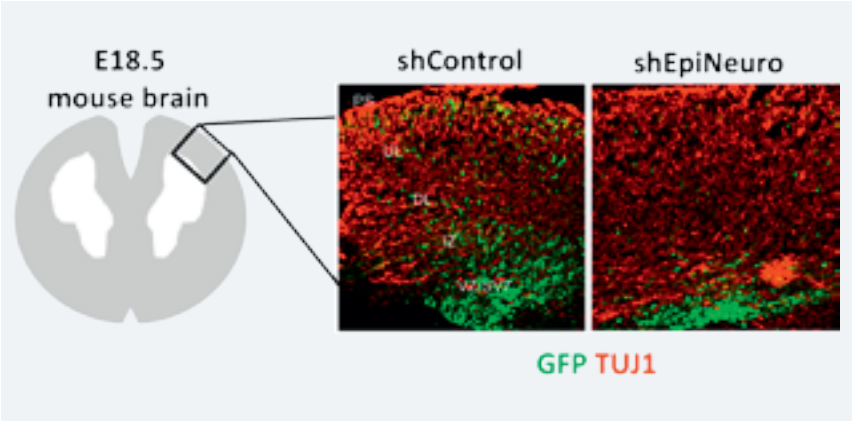



**Figure 1.** Candidate novel epigenetic regulators are essential for brain development. (a) Schematic representation of the E18.5 (embryonic day 18.5) mouse brain. (b) Micrographs of sections of the cerebral cortex analysed at E18.5 four days post-electroporation with either pSUPER.GFP. control shRNA (shControl) or pSUPER.GFP.candidate shRNA (shEpiNeuro) plasmid. Immunostaining was performed using antibodies against TUJ1 (neuronal marker). Cortical layers are marked as VZ = ventricular zone, SVZ = sub-ventricular zone, IZ = intermediate zone, DL = deep layer neurons, UL = upper layer neurons, PS = pial surface.

## CROSSTALK BETWEEN TRANSCRIPTION FACTORS AND EPIGENETIC MACHINERY DURING EPITHELIAL TO MESENCHYMAL TRANSITION

The epithelial to mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion, and gain migratory and invasive properties to become mesenchymal cells. EMT plays crucial roles in generating the body plan by contributing to the morphogenesis of multiple tissues and organs during embryonic development. It further contributes to wound healing and tissue regeneration in adults. However, its aberrant activation is known to cause organ fibrosis and promote carcinoma progression through a variety of mechanisms. While the field has witnessed tremendous advances, the complexity of transcriptional regulatory network during EMT remains poorly understood, in significant part due to yet unidentified regulators of this process. Our recent findings have uncovered a role for distinct signalling pathways in defining the transcriptome that underlies specification of mesenchymal fate and at the same time, revealed epigenetic mechanisms and a new repertoire of transcription factors that mediate these responses. We plan on gaining a molecular understanding of how these novel transcription factors and epigenetic regulators contribute to the transcriptional reprogramming that drives cell-fate changes during EMT (Figure 2).

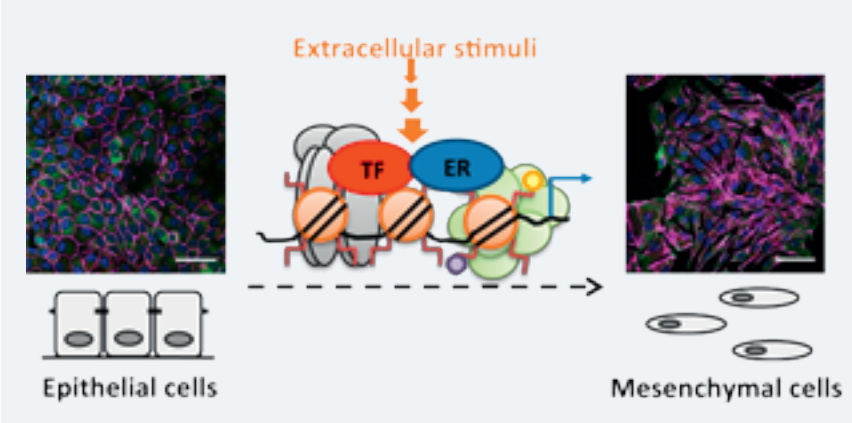



**Figure 2.** In response to certain extracellular cues, a defined set of transcription factors (TFs) and epigenetic regulators (ERs) modulate transcription of key EMT genes to drive phenotypic changes underlying epithelial to mesenchymal transition. Scale bars for immunofluorescence images are 100 µm.

## FUTURE DIRECTIONS

Our work has identified novel proteins and pathways critically involved in neuronal development and epithelial to mesenchymal transition. Using a multidisciplinary approach, we aim to attain an integrated molecular and systems-level understanding of the mechanisms by which they contribute to the gene regulatory programme underlying cell-fate specification during these processes. These studies, therefore, have the potential to provide fundamental knowledge of relevance to a broad range of research disciplines encompassing basic and translational research.

## SELECTED PUBLICATIONS

Thakurela S*, Garding A*, Jung J, Schübeler D, Burger L and Tiwari VK (2013). Gene regulation and priming by topoisomerase IIα in embryonic stem cells. *Nat Commun*, 4, 2478.

Tiwari VK*, Burger L, Nikoletopoulou V, Deogracias R, Thakurela S, Wirbelauer C, Kaut J, Terranova R, Hoerner L, Mielke C, Boege F, Murr R, Peters AH, Barde YA and Schübeler D* (2012). Target genes of Topoisomerase IIβ regulate neuronal survival and are defined by their chromatin state. *PNAS*, 190, E934-E943.

Tiwari VK, Stadler M, Wirbelauer C, Paro R, Beisel C and Schübeler D (2012). A Chromatin-modifying Function of JNK during Stem Cell Differentiation. *Nat Genet*, 44, 94-100.

* indicates equal contribution

# HELLE ULRICH

*In many chromatin-associated ubiquitylation events, DNA itself plays an active role in the conjugation reaction*

## EDUCATION

| | |
|---|---|
| 1992 | Diploma in Biology, Georg-August-University Göttingen, Germany |
| 1996 | PhD in Chemistry, University of California, Berkeley, USA |
| 2004 | Habilitation, Faculty of Biology (Genetics), Philipps University Marburg, Germany |

## POSITIONS HELD

| | |
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| 1997 – 1998 | Postdoc, University of Heidelberg, Germany |
| 1998 – 2000 | Postdoc, Max Planck Institute for Biochemistry, Martinsried, Germany |
| 2000 – 2004 | Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany |
| 2004 – 2012 | Group Leader, Cancer Research UK London Research Institute, Clare Hall Laboratories, UK |
| Since 2013 | Professor, Faculty of Biology, University of Mainz, Germany |
| Since 2013 | Director, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Liliana Batista / Postdoc; since 02/2013
- Sabrina Batke / PhD Student; since 11/2013
- Heike Brinkman / Lab Manager; since 02/2013
- Stephanie Freiß / PhD Student; since 11/2014
- Néstor García-Rodríguez / Postdoc; since 05/2013
- Laure Gonzalez / Postdoc; since 04/2013
- Nataliia Gralievska / PhD Student; since 01/2014
- Christian Renz / Postdoc; since 08/2014
- Hanna Windecker / Postdoc; since 05/2013
- Hans-Peter Wollscheid / Postdoc; since 09/2014
- Ronald Wong / Postdoc; since 04/2013
- Nicola Zilio / Postdoc; since 08/2014

## OVERVIEW

Ubiquitin and SUMO are small proteins that act as posttranslational modifiers. When attached to a target, they modulate its properties and interactions, thus serving as a rapid and reversible means of regulating protein function. Our research aims at understanding the mechanisms by which ubiquitin and SUMO contribute to the maintenance of genome stability. To this end we are investigating the modifications of selected chromatin-associated proteins, the consequences for their association with DNA and other interaction partners, and the biological impact of the modifications. One of the main focuses of our lab is the system of DNA damage tolerance, which promotes the replication of damaged DNA and thereby ensures that cells can proliferate even in the presence of genotoxic agents. The pathway contributes to the cell's overall resistance to DNA damage, but as it is often associated with mutations, it is also a potential source of genome instability in itself and therefore needs to be tightly controlled by ubiquitin and SUMO.

## RESEARCH HIGHLIGHTS

### AN EXPERIMENTAL SYSTEM FOR THE ANALYSIS OF DNA DAMAGE BYPASS IN A CELLULAR ENVIRONMENT

How DNA damage bypass is regulated in the context of the cellular environment, particularly how it is coordinated with the cell's overall damage response, is still an open question. Likewise, we still have little insight into the influence of chromatin dynamics on the efficiency of damage processing and the choice between error-free and mutagenic pathways. Using budding yeast as a

model system, we have previously demonstrated that the ubiquitin-dependent damage tolerance system is separable from bulk genome replication, such that its action can be delayed until G2/M phase without any detrimental consequence for the cell. This important observation has allowed us to devise an experimental tool for the dissection of damage bypass in the course of a single cell cycle, which we are now employing to address the above mentioned questions.

Our analysis is based on a system for the inducible expression of Rad18, the ubiquitin ligase that controls the activity of DNA damage bypass in eukaryotes by monoubiquitylating the replication factor PCNA. When G1-arrested cells are exposed to DNA-damaging agents in the absence of Rad18 and allowed to progress through S phase, they accumulate in G2/M phase with small tracts of unreplicated DNA, which can subsequently be filled by postreplicative action of the damage bypass pathway upon induction of Rad18. The efficiency of bypass can then be monitored either by measuring cell survival or by quantifying the amount of a nucleotide analogue that is incorporated during gap filling. Based on this approach we have established several new projects addressing the contributions of a range of damage processing factors that have either been implicated genetically in ubiquitin-dependent DNA damage bypass or are assumed to be of general importance for dealing with replication stress.

We are now particularly interested in understanding the impact of checkpoint signalling on cellular damage tolerance. Importantly, while checkpoint function is dispensable for both PCNA ubiquitylation and downstream damage processing, we found a requirement for maintaining damage bypass competency during S phase. Consistent with a fork-independent action of the damage tolerance pathway, this requires a functional Rad53- and Rad9-dependent damage checkpoint, but not the Mrc1-dependent replication checkpoint that monitors the intactness of the replisome. Our preliminary results indicate that the damage checkpoint prevents inappropriate recombination events at the single-stranded gaps that accumulate in the absence of Rad18. Hence, ablation of checkpoint function results in cell death via catastrophic recombination and chromosome fragmentation.

## FUTURE DIRECTIONS

We will continue to explore damage bypass both *in vivo* and in biochemical approaches with recombinant proteins. On the one hand, we will further exploit the inducible damage bypass system for analysing how checkpoint signalling and chromatin dynamics impinge on ubiquitin-dependent lesion tolerance. On the other hand, our mechanistic analysis of Rad5 will be expanded by designing simplified versions of the protein that will permit the separation of its ubiquitin ligase function from its ATPase activity. Their analysis will not only give us mechanistic information about the protein, but at the same time allow us to prepare polyubiquitylated PCNA on a large scale for the identification of relevant interaction partners that might act as downstream effectors of the template switching pathway.

## MECHANISTIC ANALYSIS OF THE UBIQUITIN LIGASE RAD5

In an effort to gain better insight into the molecular mechanism of DNA damage bypass, we are performing biochemical characterisations of important enzymes involved in the process. In this context, we have analysed functional domains within the ubiquitin ligase Rad5, which mediates PCNA K63-polyubiquitylation and thereby triggers an error-free template switching pathway of damage tolerance. The protein is of particular interest because its ubiquitin ligase domain, a RING finger, is embedded in a helicase-like domain, and the protein exhibits DNA-dependent ATPase activity (Figure 1A). How this is related to Rad5's role in DNA damage bypass is not well understood.

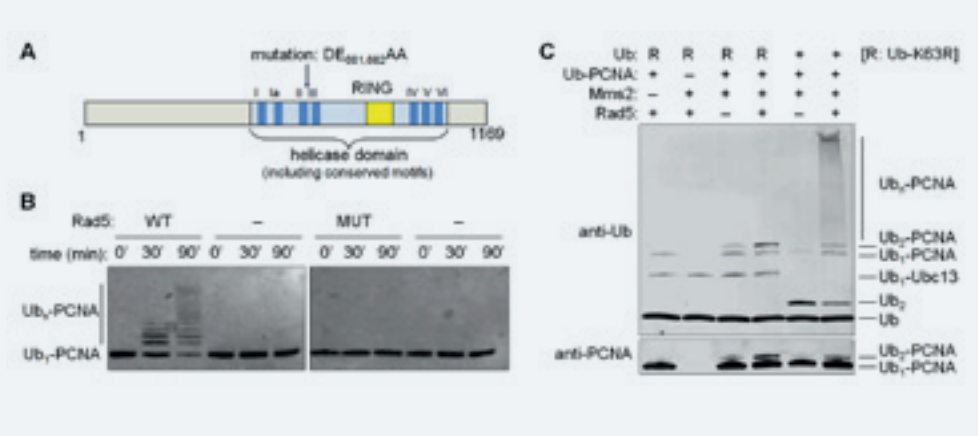



**Figure 1.** Significance of the Rad5 helicase-like domain. (A) Rad5 domain structure. (B) Mutation of the helicase motif (MUT) abolishes ligase activity towards PCNA. (C) Rad5 is active despite apyrase-mediated depletion of ATP when ubiquitin is provided by pre-charged Ubc13 thioester. Linear $Ub^1$-PCNA was used as a substrate for polyubiquitylation.

In collaboration with Xiaolan Zhao at the Memorial Sloan Kettering Cancer Center in New York, USA, we have characterised a mutant in one of its conserved helicase consensus motifs. We unexpectedly found that this motif, responsible for Mg2+ binding and ATP hydrolysis, is required for ligase activity towards PCNA (Figure 1B). Using an *in vitro* ubiquitylation assay in which the general, ATP-dependent activation of ubiquitin was separated from its transfer to the substrate, we were able to show that ATP hydrolysis is dispensable for the ubiquitin transfer reaction per se (Figure 1C). Instead, we found that mutation of the motif reduced interactions between Rad5 and both PCNA and the ubiquitin conjugating enzyme Ubc13, thus likely indicating a structural importance.

## SELECTED PUBLICATIONS

Saugar I, Parker JL, Zhao S and Ulrich HD (2012). The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Res*, 40, 245-257.

Daigaku Y, Davies AA and Ulrich HD (2010). Ubiquitin-dependent DNA damage bypass is separable from genome replication. *Nature*, 465, 951-955.

Zhao S and Ulrich HD (2010). Distinct consequences of post-translational modification by linear versus K63-linked polyubiquitin chains. *Proc Natl Acad Sci USA*, 107, 7704-7709.

# EVA WOLF

*We investigate high-resolution crystal structures to understand circadian clock mechanisms*

## EDUCATION

| | |
|---|---|
| 1992 | Diploma in Biophysics, Genetics, Biochemistry and Physics, University of Heidelberg, Germany |
| 1992 – 1996 | PhD in Structural Biology, EMBL and University of Heidelberg, Germany |

## POSITIONS HELD

| | |
|---|---|
| 1996 – 2000 | Postdoc, Rockefeller University New York, USA |
| 2000 – 2009 | Group Leader at the Max Planck Institute of Molecular Physiology, Dortmund, Germany |
| 2009 – 2011 | Group Leader, Max Planck Institute for Biochemistry, Martinsried, Germany |
| 2012 – 2013 | Group Leader, Adolf-Butenandt-Institute, Ludwig-Maximilians University, Munich, Germany |
| Since 2013 | Professor of Structural Biology, University of Mainz, Germany |
| Since 2013 | Adjunct Director, Institute of Molecular Biology (IMB), Mainz, Germany |

## GROUP MEMBERS

- Archit Garg / PhD Student; since 11/2014
- Silke Helmke / Research Technician; since 05/2014
- Markus Kiebisch / Lab Manager; since 01/2014
- Torsten Merbitz-Zahradnik / Postdoc; since 01/2014

## OVERVIEW

Many physiological, metabolic and behavioural processes are regulated in a day-time dependent (circadian) manner. Circadian rhythms are generated by endogenous circadian clocks, which are operated by gene regulatory feedback loops and involve daily rhythmic changes in quality (chemical modifications, activity) and quantity (expression rate and degradation) of clock components. Our goal is to acquire an atomic resolution picture and quantitative understanding of the molecular processes governing circadian (24h) rhythms and their synchronisation with the environmental light-dark cycle. To this end we apply a highly interdisciplinary structural biology approach, which includes structural (X-ray crystallography), biochemical and biophysical studies on purified clock proteins as well as structure-based functional assays in cell cultures and living organisms. We are also pursuing the structure-function analysis of mammalian Timeless, which is involved in DNA replication and checkpoint signalling.

## RESEARCH HIGHLIGHTS

### STRUCTURE-FUNCTION ANALYSES OF PERIOD AND CRYPTOCHROME CLOCK PROTEINS AND THE MAMMALIAN CRY1-PER2 COMPLEX

So far, our group has determined and functionally validated X-ray crystal structures of the *Drosophila* and mammalian PERIOD (d/mPER) clock proteins, the circadian blue-light photoreceptor *Drosophila* Cryptochrome (dCRY) and the mammalian clock protein and transcriptional repressor Cryptochrome1 (mCRY1). In 2014, we have reported the crystal structure of the mouse Cryptochrome1-PERIOD2 (mCRY1-mPER2) complex, which is essential for mammalian circadian

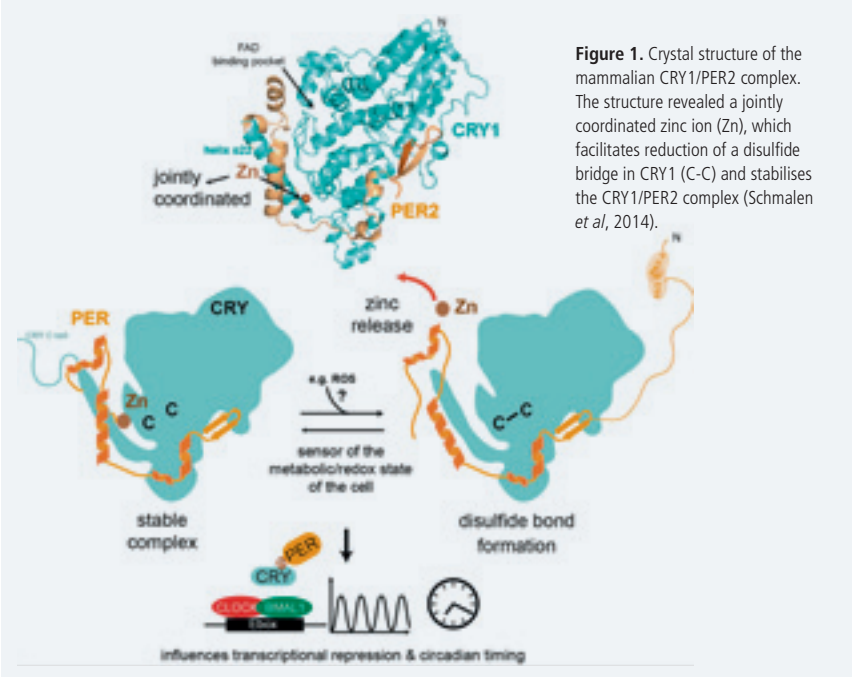



**Figure 1.** Crystal structure of the mammalian CRY1/PER2 complex. The structure revealed a jointly coordinated zinc ion (Zn), which facilitates reduction of a disulfide bridge in CRY1 (C-C) and stabilises the CRY1/PER2 complex (Schmalen *et al*, 2014).

clock operation (Figure 1). Mammalian Cryptochromes repress the BMAL1/CLOCK transcription factor complex, which regulates many clock-controlled genes and thereby mammalian physiology in a daily rhythmic manner. Our mCRY1-mPER2 structure shows how mPER2 stabilises mCRY1 by preventing binding of the E3 ligase FBXL3 and hence proteasomal degradation of the Cryptochrome. Our studies also revealed that mCRY1-mPER2 complex formation is modulated by the binding of a zinc ion and by disulfide bond formation, which may act as sensors for the redox state of the cell and thereby link the circadian clock to metabolism. Since mammalian CRYs are also involved in glucose homeostasis, our apo-mCRY1 and mCRY1-mPER2 structures are likely to catalyse the development of CRY chemical probes and metabolic modulators that could be used in the therapy of clock-related disorders, type II diabetes as well as anti-inflammatory glucocorticoid treatments.

## FUTURE DIRECTIONS

We will continue our interdisciplinary structure-function analyses of circadian clock proteins, their specific interactions, activities and conformational dynamics as well as the light-regulation of circadian photoreceptors with two main goals in mind:
1) to provide a detailed mechanistic understanding of the circadian clockwork and its role in aligning biochemical, metabolic, physiological and ultimately behavioural processes with the environmental light/dark cycle and
2) to guide the (structure-based) design of small-molecule ligands with diagnostic or therapeutic potentials in the treatment of clock-related disorders. We will also continue our structural and biochemical analyses of mammalian Timeless as a component of the replication machinery as well as the circadian clock.

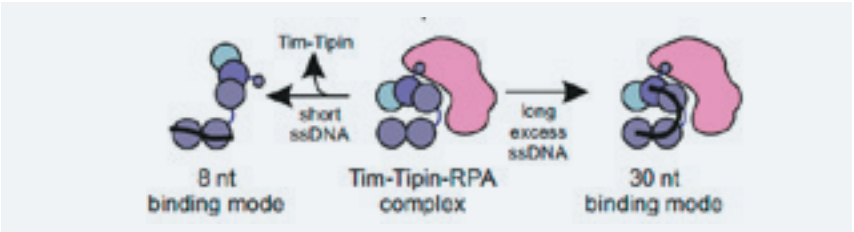



**Figure 2.** Architecture and ssDNA binding activities of the Timeless-Tipin-RPA complex. In the DNA-free complex (middle) RPA adopts a compact conformation resembling its 30 nt ssDNA binding mode. The Timeless-Tipin-RPA complex binds to ssDNA with RPA in its 30 nt binding mode (right), but not in its 8 nt binding mode (left; Witosch *et al*, 2014).

## MAMMALIAN TIMELESS AS A COMPONENT OF THE REPLICATION FORK PROTECTION COMPLEX

We are also working on mammalian Timeless (mTIM), which, different from its *Drosophila* Timeless homolog (dTIM), only plays a minor role in the circadian clock, but has additional functions in DNA replication and cell cycle control. We have studied mammalian Timeless in complex with the Timeless interacting protein Tipin and the ssDNA binding protein RPA (Replication protein A) using biochemical analyses as well as electron microscopy (negative stain and Cryo-EM in collaboration with Dr. Naoko Mizuno). Fitting the known RPA crystal structures into the EM envelope of the Timeless-Tipin-RPA complex and antibody labelling revealed that RPA adopts a compact conformation resembling its 30 nucleotide binding mode, in which all four DNA binding domains of RPA contact ssDNA. Furthermore our biochemical studies showed that the Tim-Tipin-RPA complex binds ssDNA only when RPA adopts the 30 nt binding mode. The Tim-Tipin subcomplex dissociates from RPA, when RPA adopts the 8 nt binding mode with only two DNA-binding domains contacting ssDNA (Figure 2). We therefore propose that conformational rearrangements of RPA regulate the recruitment of the Timeless-Tipin complex to the replication fork to fulfill its functions in normal replication (coordinate helicase and polymerase activity) or in checkpoint signalling upon replicative stress and DNA damage.

## SELECTED PUBLICATIONS

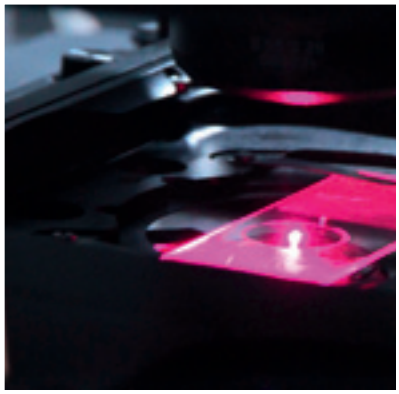
Schmalen I, Reischl S, Wallach T, Klemz R, Grudziecki A, Prabu JR, Benda C, Kramer A and Wolf E (2014). Interaction of circadian clock proteins CRY1 and PER2 is modulated by zinc binding and disulfide bond formation. *Cell*, 157, 1203-1215.

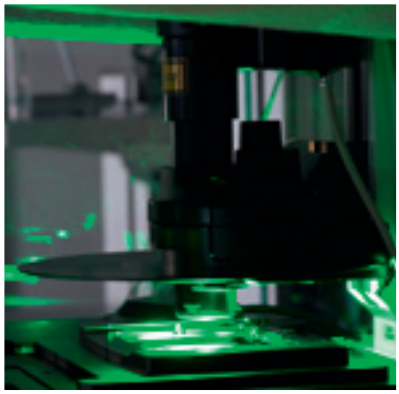
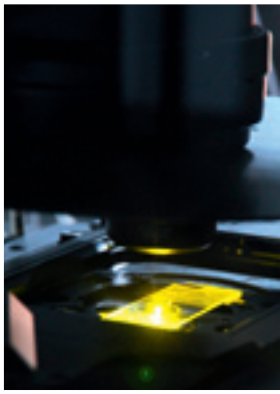
Witosch J, Wolf E and Mizuno N (2014). Architecture and ssDNA interaction of the Timeless-Tipin-RPA complex. *Nucleic Acids Res*, 42, 12912-12927.

Czarna A, Berndt A, Singh HR, Grudziecki A, Ladurner A, Timinszky G, Kramer A and Wolf E (2013). Structures of *Drosophila* Cryptochrome and mouse Cryptochrome1 provide insight into circadian function. *Cell*, 153, 1394-1405.

# CORE FACILITIES

Sample on Fluorescence Microscope,
Images by Andreas Vonderheit



# OVERVIEW

The IMB Core Facilities (CF) provide access to state-of-the-art technology, hands-on-training or services and organise lectures and courses to train researchers in new techniques and instrumentation, proper experimental setup, and data processing. There are seven different CF units. Bioinformatics, Genomics, Media Lab, and Proteomics provide a full service: the researcher provides samples, which are quality controlled by the CF, and a complete service is then provided up to the point of data production. Basic data analysis is also conducted to ensure good quality and accuracy of the information before it is returned to the researcher.

Cytometry, Histology, and Microscopy provide an assisted service, meaning that the individual researcher is trained on the specific instrument. After an introduction and a supervised session, the researcher is able to work on the instrument independently. Of course, the specific Core Facility trained personnel can always be consulted and are ideally involved from the planning phase of an experiment. All Core Facility employees are specialists in their fields, have a profound knowledge, and give advice on choosing the right instrument or method. A fee finances the service provided, this is either an hourly fee for the use of equipment or a fee per sample processed and analysed.

Some services are not only offered to the researchers of IMB, but also to the whole scientific community in Mainz. In the past year, non-IMB groups accounted for 30% of the time booked on instruments in the Cytometry and Microscopy CFs.

For each CF unit we established a user committee (UC), consisting of three to four IMB Group Leaders. The UC meets with the head and the staff of the CF unit at least twice a year. Thus, the further development of the CF unit is driven by user demand. Together they defined in detail the standard services provided by each core facility, which will result in acknowledging the CF in a publication. Beyond these standard services, customised or specialised services can be performed by the CF in collaboration with the researcher.

In addition to the seven CF units, the core facilities are responsible for further services, such as the hot lab, the S2 lab, and the infrastructure of the animal facilities. Last year, CF coordinated the opening of the IMB in-house mouse facility, consisting of 210 cages.

Beyond all these obvious services offered by the different CF units, CF is also responsible for the infrastructure "behind the scenes" making the work of every researcher at IMB more effective. For example, the management of an in-house clone/vector/cell line storage facility, 24/7 access to media and buffers from an on-site supply centre, or restriction enzymes and polymerases from an e-freezer, and access to a huge range of consumables via the plastic storage facility, are all coordinated by IMB's Core Facilities.

**Andreas Vonderheit**
Director of Core Facilities and Technology

# BIOINFORMATICS
## CORE FACILITY

## CORE FACILITY MEMBERS

- **Emil Karaulanov / Core Facility Head; since 10/2014**
- Anke Busch / Bioinformatician; since 01/2014
- Matthias Koch / System Administrator; since 05/2011
- Holger Klein / Bioinformatician; 05/2011 – 02/2014
- Nastasja Kreim / Bioinformatician; since 04/2012
- Sergi Sayols Puig / Bioinformatician; since 10/2013
- Sebastian Uhrig / System Administrator; since 11/2011

## OVERVIEW

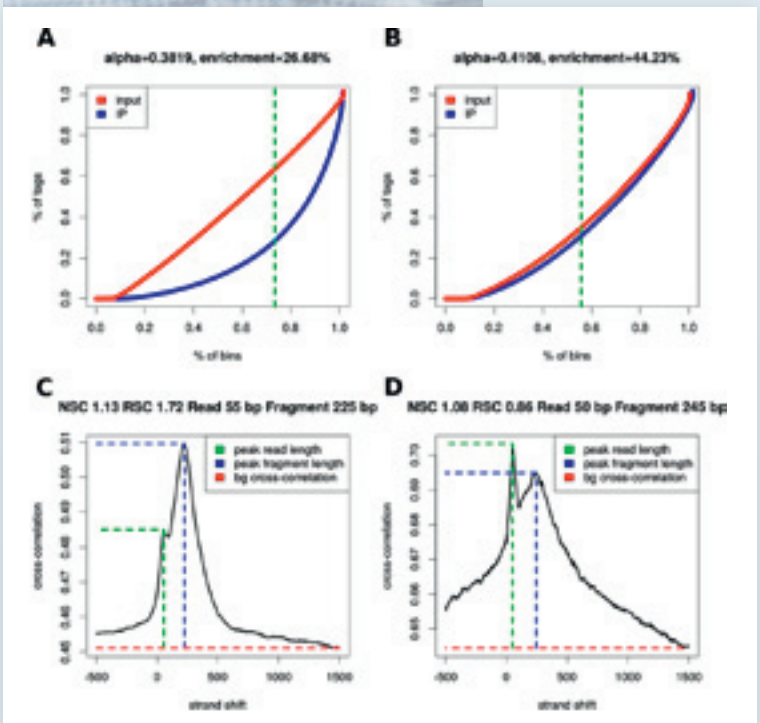
The Bioinformatics Core Facility (BCF) provides access to advanced data analysis tools and supports IMB researchers in project planning, experimental design, software training, as well as in the computational processing, analysis, visualisation, interpretation and publication of high-throughput "omics" data.

## SERVICES OFFERED

BCF offers know-how and support on different levels from basic services to full-scale scientific collaborations:

- » Consulting on experimental design of high-throughput projects
- » In-house training on bioinformatics tools and databases
- » Implementation and customisation of various software tools
- » Setup and development of automated data analysis pipelines for common assays
- » Data quality assessment, processing, visualisation, interpretation and presentation of results
- » Development of novel tools and custom methods for specific analysis tasks
- » Data mining of published datasets, correlation and integration of results
- » Assistance with the preparation of manuscripts and grant proposals

In the last year, BCF in cooperation with the University of Mainz Data Center has continued to maintain and extend the IT infrastructure of IMB. In order to achieve fair and efficient resource usage, an LSF job queuing system has been installed on the IMB compute servers. Furthermore, BCF has been maintaining online services such as Galaxy, Chipster, Genomatix, R-Studio and OMERO, which provide user-friendly interfaces to various analytical tools to all interested researchers in house. A new online service, Huygens Remote Manager, enables users to enhance images from fluorescent microscopes with the help of deconvolution algorithms. Additionally, BCF has been developing and testing customised data processing pipelines for the automation of common analysis tasks. On the IT side, IMB's intranet has been constantly updated to assist the internal communications of the institute and a customised Galaxy-based laboratory information management system facilitates sample submission, documentation and tracking of sequencing projects.




Customised R implementation of two published quality control methods allows rigorous and objective evaluation of good (A, C) as well as suboptimal (B, D) ChIP-seq datasets from in-house projects.

# FLOW CYTOMETRY CORE FACILITY

## CORE FACILITY MEMBERS

- **Jens Hartwig / Core Facility Head; since 10/2013**
- Heinz Eipel / Research Technician; since 06/2011
- Ina Schäfer / Biotechnologist; since 08/2011

## OVERVIEW

The Flow Cytometry Core Facility (FCCF) houses state-of-the-art equipment for sorting and analysing cells and even larger particles or small organisms, up to 1000µm. Users from IMB, Mainz University (JGU) and its Medical Center take advantage of the wide range of applications and the knowledge offered by this service facility.

## SERVICES OFFERED

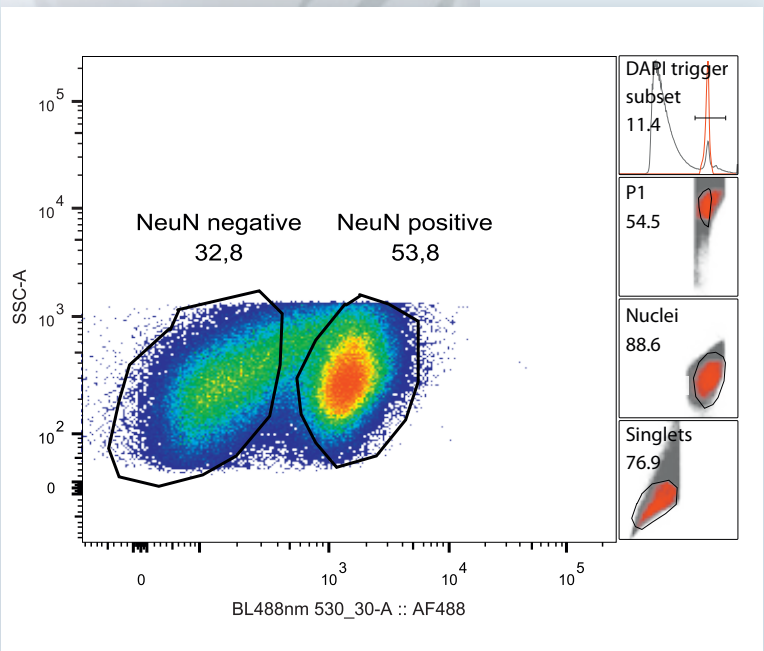
The FCCF is equipped with two FACS Sorters, Becton Dickinson (BD) FACS Aria II SORP and Union Biometrica BioSorter, and one analyser, BD LSR Fortessa SORP. The BD machines are optically identical and harbour a five laser excitation suite and an 18 parameter detector bench (Excitation laser: 355/405/488/561/640 nm; Emission detection: number of PMTs: 2/5/3/5/3). The BioSorter has 2 lasers, 488 nm and 561 nm, installed and the capability to detect 3 parameters in parallel.

With this equipment we are able to offer the user the possibility to sort and analyse cells and particles with a very broad size spectrum, from 0.5µm to 1000µm. Moreover, many cytometric applications can be carried out in our service unit, ranging from proliferation assays, like cell cycle or CFSE cell division assays, multi-parametric application, like stem cell isolation or cancer research, high-througput screenings, for drugs or cytotoxic reagents, examinations of smaller organisms, e.g. *C. elegans*, to nanoparticle science and studies of subcellular compartments.

The FCCF provides a superior training scheme to educate researchers in the practical and theoretical aspects of flow cytometry and familiarise them with its possible uses and their requirements. Additionally, we offer assistance with every step of an experiment using flow cytometry – planning, designing, measuring, trouble shooting and analysing the data – in order to ensure the best possible scientific outcome.

This service is rounded up by daily quality controls to ensure consistent measurements.

Currently the FCCF has served more than 60 different users from the whole campus of the University of Mainz at consistently short waiting times.




Isolation of neuronal cell nuclei: A mouse brain was digested and cell nuclei were extracted. An unstained control sample was measured to determine NeuN-AlexaFluor488 positive neuronal nuclei. Additional DAPI staining was used as the trigger channel and the suspension media, PBS, was checked for contaminations prior to the sorting (not shown).

# GENOMICS CORE FACILITY

## CORE FACILITY MEMBERS

- **Chung-Ting "Tina" Han / Core Facility Head; since 04/2013**
- Christoph Bast / Research Technician; 06/2014 – 08/2014
- Jasmin Cartano / Research Technician; 07/2012 – 02/2014
- Stefanie Grimm / Research Technician; since 09/2014
- Hanna Lukas / Research Technician; since 01/2013

## OVERVIEW

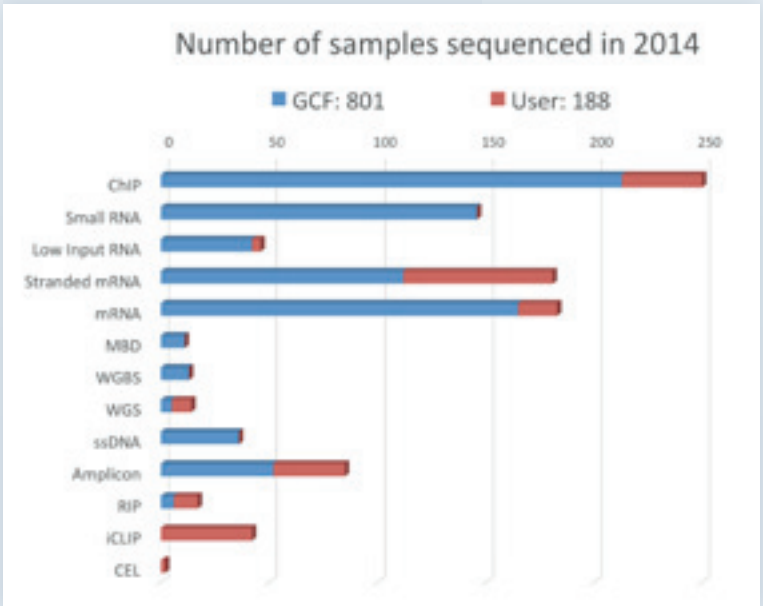
The Genomics Core Facility offers Next-Generation Sequencing (NGS) services based on the Illumina HiSeq 2500 and MiSeq platforms.

## SERVICES OFFERED

Genomics Core Facility undertakes all of the quality control processes required for NGS, which entail sample preparation, library construction, and raw sequencing data generation. Currently we support these types of library preparation as our standard operating procedure (SOP):

- » mRNA-Seq with poly-A selection (mRNA)
- » Total RNA-Seq with RiboZero rRNA depletion
- » Stranded mRNA-Seq with poly-A selection
- » Stranded total RNA-Seq with RiboZero rRNA depletion
- » Low-input RNA-Seq
- » RIP-Seq
- » Small RNA-Seq
- » ChIP-Seq / MBD-Seq
- » Whole genome sequencing (WGS)
- » Whole genome bisulfite sequencing (WGBS)

With these established SOPs the turn around time from sample submission to library preparation to sequencing raw data is less than 6 weeks. Besides these SOPs, we put a lot effort into customising protocols according to users' needs. Since March we have successfully applied a random barcoding (aka molecular indexing) strategy to quantify absolute levels of small RNAs and to remove PCR duplicates from small RNA sequencing libraries. In July we established a new workflow for whole genome bisulfite sequencing (WGBS). Our next step is to apply oxidative bisulfite technology into our WGBS workflow in order to accurately and quantitatively distinguish between 5mC and 5hmC in DNA at single-base resolution. To accomadate users' needs for their specific sample type, we recently developed a new workflow for library preparation and sequencing from single-stranded DNA (ssDNA) and bisulfite-converted amplicons.




An overview of sample type sequenced in 2014, either prepared by Genomics CF (GCF) or users.

# HISTOLOGY CORE FACILITY


## OVERVIEW

The Histology Core Facility offers a broad range of standard techniques comprising semi-automated fixation and paraffin embedding as well as microtomes for paraffin, cryo or gelatine/agarose sectioning (Vibratome). Since October, the Histology Core Facility has been managed by the Microscopy Core Facility staff.

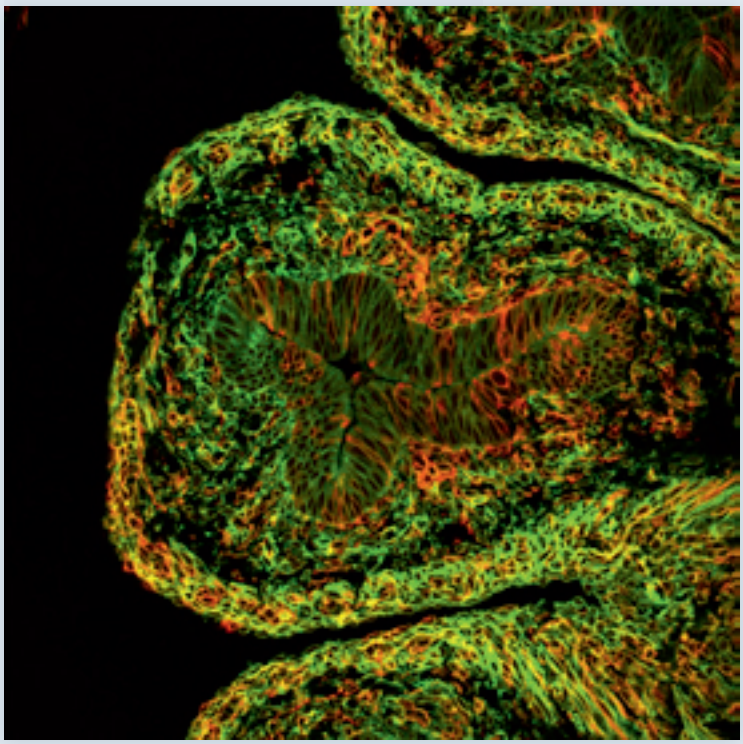
## SERVICES OFFERED

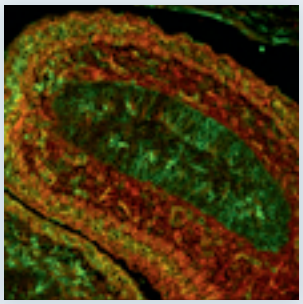
The Histology Core Facility provides equipment and support for tissue embedding, sectioning and staining. Currently, machines are available for automated fixation, embedding (TP1020, EG1150, Leica) and sectioning of paraffin (Microtom RM2255, Leica) and frozen tissue (Cryostat CM3050-S, Leica). For the sectioning of "soft objects", like organotypic slice cultures or gelatine/agarose embedded objects a Vibratome (VT1000-S) is provided.

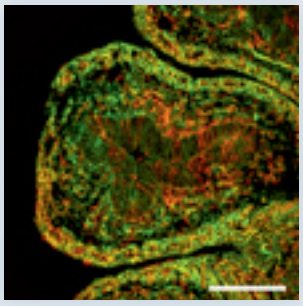
IMB members can use the equipment of the Core Facility after a mandatory introduction and training session. Subsequently, the machines can be booked and used independently. In addition to the maintenance and hands-on machine training, the Histology Core Facility provides basic protocols (e.g. H&E staining, *in situ* hybridisation, immuno-stainings) and the reagents needed to perform routine histological stainings (e.g. H&E).

## CORE FACILITY MEMBERS

- **Mathias Gierl / Core Facility Head; 07/2011 – 09/2014**
- Maria Hanulova / Scientific staff; since 09/2014
- Sandra Ritz / Scientific staff; since 09/2014







Confocal images of the small intestine from the Rosa-Cre-ERT2:tdTomato-EGFP mouse embryos administered tamoxifen at E11.5 and analysed at E15.5. The majority of the cells are labelled by EGFP (green), however cells are still tdTomato positive (red). Bar scale 100 µm. Image courtesy of the Soshnikova group.

# MEDIALAB

## OVERVIEW

The MediaLab provides support for the day-to-day work of IMB's scientists, by producing more than 40 different solutions and culture media, managing sterilisation and waste disposal services, and maintaining a stock of equipment and reagents.

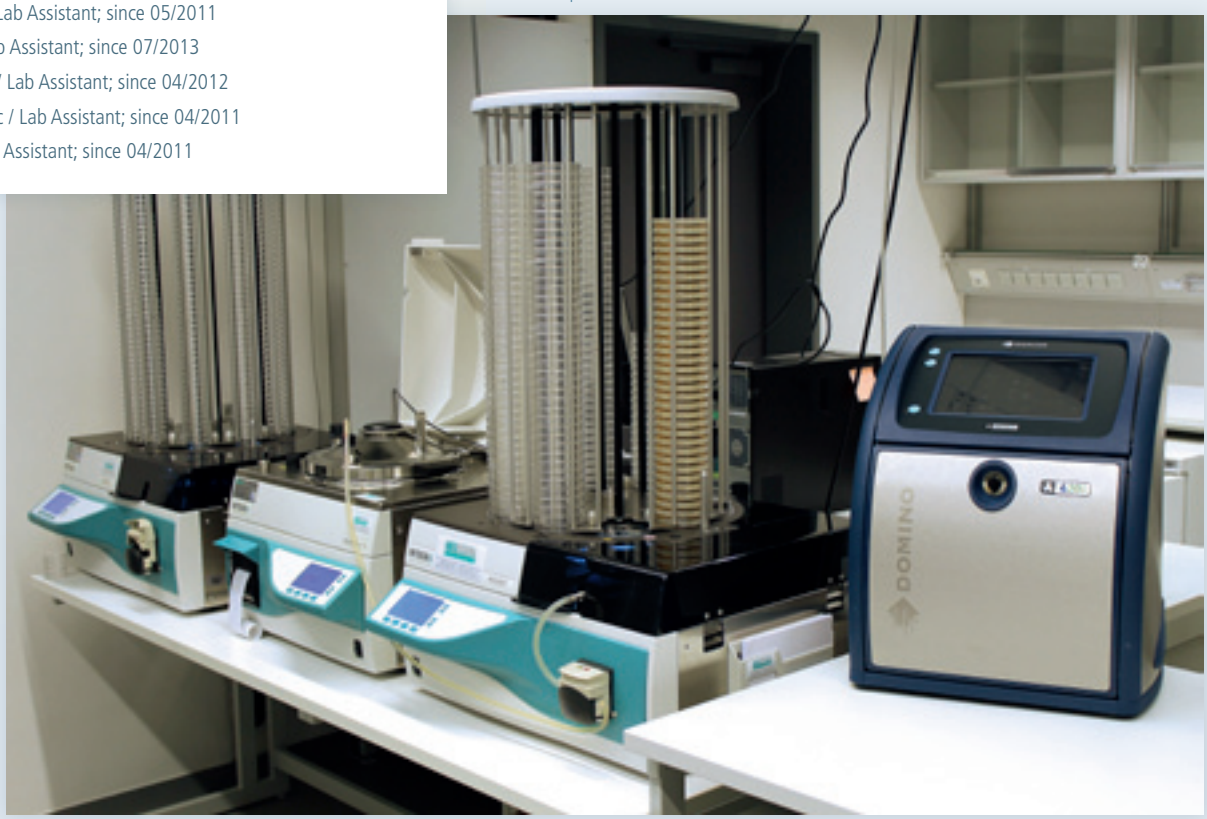
## SERVICES OFFERED

The MediaLab offers a daily service of media production, sterilisation and waste management. In addition, this Core Facility provides and maintains a vector database, human ORF cDNA clone collection and cell line bank, which are authorised by the Gene Technological Authority and are available for use by all IMB scientists. The MediaLab also offers a New England BioLabs eFreezer (Hitchin, UK), which provides researchers with quick and easy access to restriction enzymes, DNA ladder and other genetic tools. In November 2014, a LifeTechnologies™ Supply Centre was established in the Facility to provide cell culture equipment in house, including enzymes, media and media supplements. The needs of scientists at IMB are regularly assessed to ensure that equipment and reagents of consistent quality are always in stock, so that these are readily available when required.

## MEDIALAB MEMBERS

- **Ina Schafer / Head of MediaLab; since 03/2013**
- Doris Beckhaus / Lab Assistant; since 05/2011
- Alwina Eirich / Lab Assistant; since 07/2013
- Annette Holstein / Lab Assistant; since 04/2012
- Valentina Jankovic / Lab Assistant; since 04/2011
- Johann Suss / Lab Assistant; since 04/2011

Automated petri dish filling station (Integra Biosciences GmbH; left) linked with an inkjet printer for labelling (Domino Deutschland GmbH; right). Currently, the MediaLab prepares around 2000 petri dishes per week.



# MICROSCOPY
## CORE FACILITY

## OVERVIEW

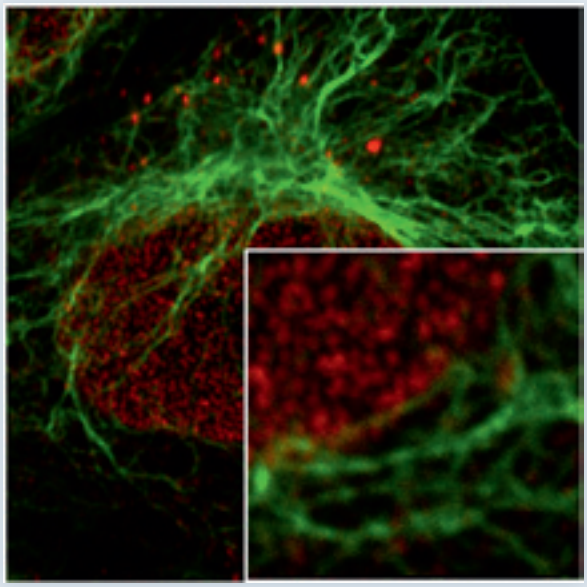
The Microscopy Core Facility offers supervised access to state-of-the-art equipment, ranging from stereo macroscopes and widefield microscopes to confocal and super-resolution microscopes. In total more than eight different microscopes are available.

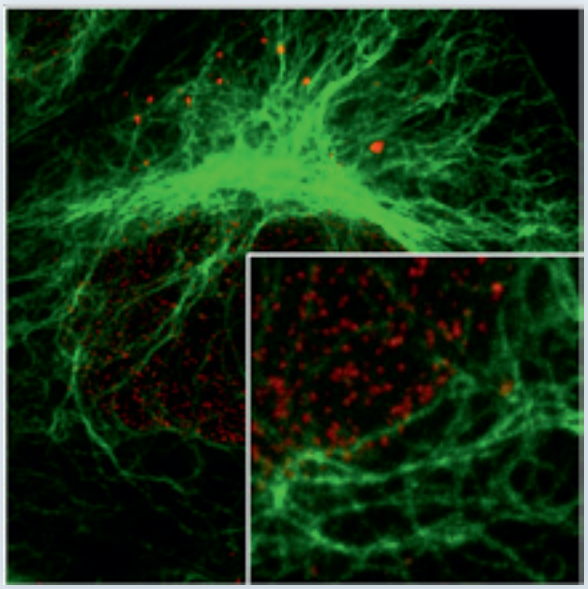
## SERVICES OFFERED

The Microscopy Core Facility provides hands-on training and access to various advanced microscopes. After an introduction and training on the specific systems by Microscopy Core Facility staff, users can book and use the microscopes and image processing stations and a variety of imaging and image processing software. The Microscopy Core Facility also offers advanced training through practical courses and a variety of lectures. Lectures include a general "Introduction to Microscopy", "Pitfalls in Image Acquisition", "Image Processing", and "Ethics in Image Acquisition and Processing". The courses range from practical classes about confocal, live-microscopy and super-resolution microscopy to software courses such as image processing: "Hands on ImageJ", "Using OMERO", and "Using Huygens Deconvolution".

## CORE FACILITY MEMBERS

- **Andreas Vonderheit / Core Facility Head; since 04/2011**
- Katharina Böse / Staff Scientist Advanced Light Microscopy; since 01/2013
- Maria Hanulova / Staff Scientist Advanced Light Microscopy; since 02/2014
- Sandra Ritz / Staff Scientist Advanced Light Microscopy; since 10/2013





Proof of concept: comparison between confocal laser scanning microscopy (CLSM, left) and stimulated emission depletion microscopy (STED, right) for two fluorescent colours. The green fibers are vimentin (Atto 425) and the red spots are nucleoporin (Chromeo 505). Images taken by Sandra Ritz, Microscopy Core Facility.

# PROTEOMICS
## CORE FACILITY

## OVERVIEW

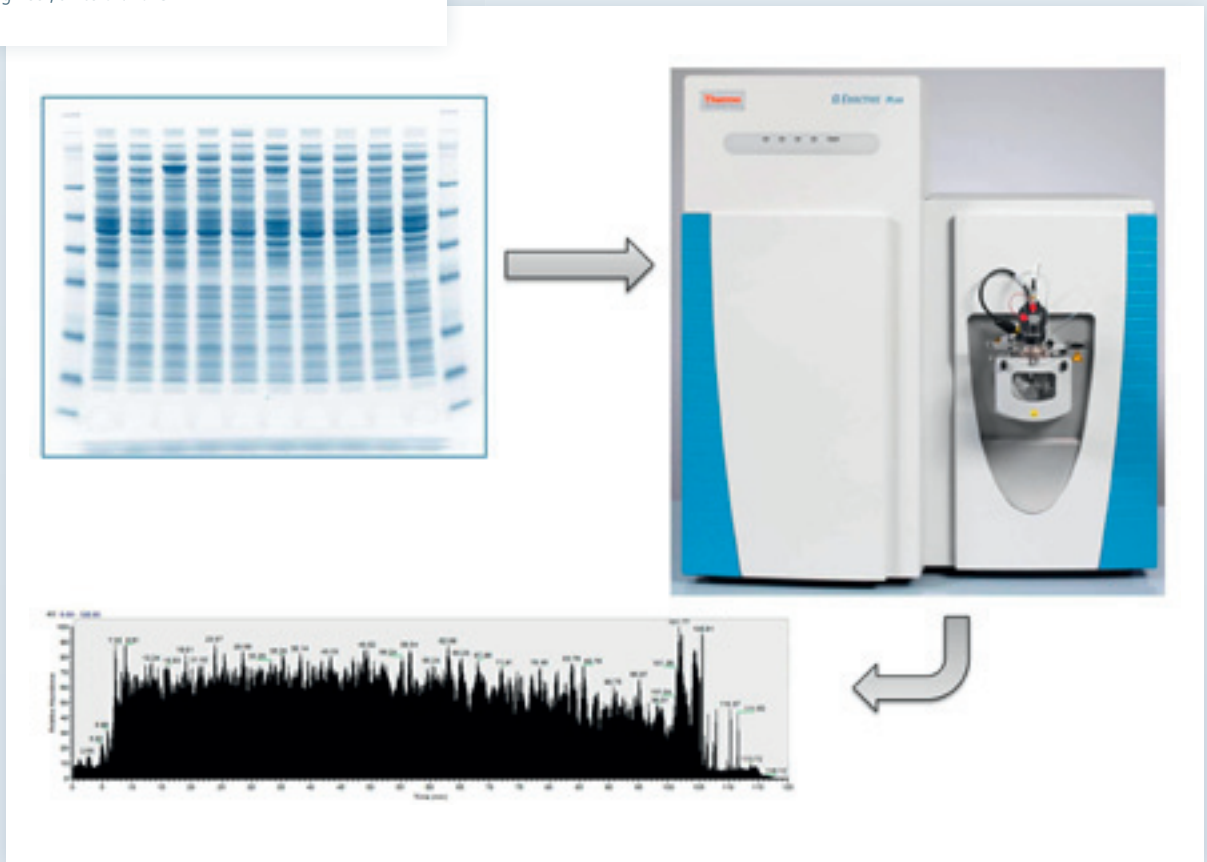
The Proteomics Core Facility operates an EASY nLC 1000 ultraHPLC coupled online to a Q Exactive Plus mass spectrometer to perform basic proteomic measurements.

## SERVICES OFFERED

The Facility provides 1500 measurement hours per year to IMB and the surrounding research centers in Mainz. Current services include band identification, analysis of posttranslational modifications on single proteins and measurement of SILAC (stable isotope labelling with amino acids in cell culture) experiments. Mass spectrometry service is provided as a full service including initial consultation, sample preparation and basic proteomics data analysis by the Core Facility. Furthermore, we offer a lecture on Proteomics and provide researchers with hands-on experience during our practical courses.

## CORE FACILITY MEMBERS

- **Falk Butter / Core Facility Head; since 05/2013**
- Jasmin Cartano / Research Technician; since 02/2014
- Mario Dejung / Bioinformatician; since 05/2014
- Anja Freiwald / Engineer; since 04/2013



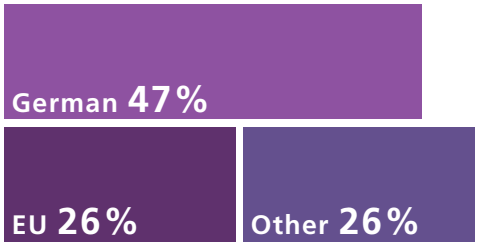
Overview of the mass spectrometry sample workflow. Proteins are visualised in a Coomassie Brilliant Blue-stained SDS polyacrylamide gel and subsequently digested to peptides for measurement using a high-resolution mass spectrometer platform (Q Exactive Plus). The chromatogram shows the analysis of a highly complex protein sample.

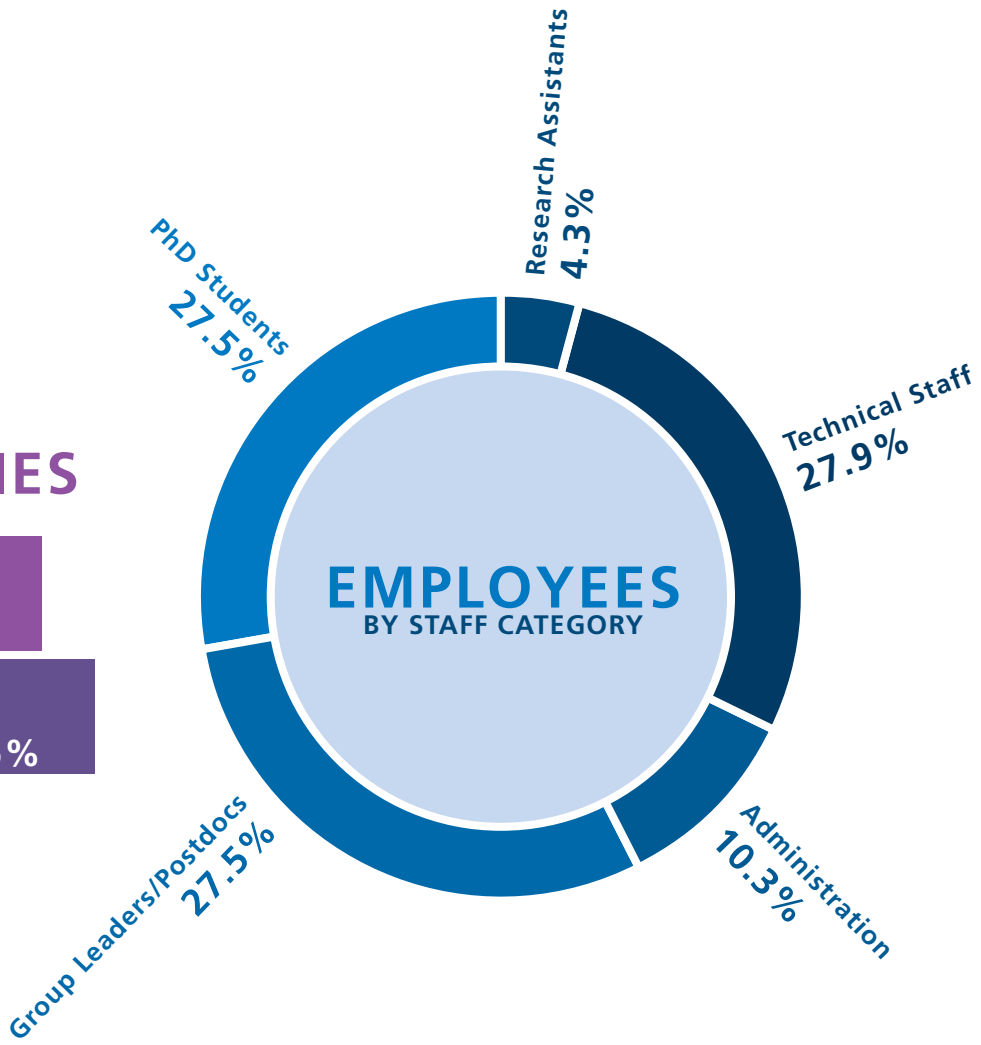
Olive trees in IMB's atrium

# FACTS & FIGURES

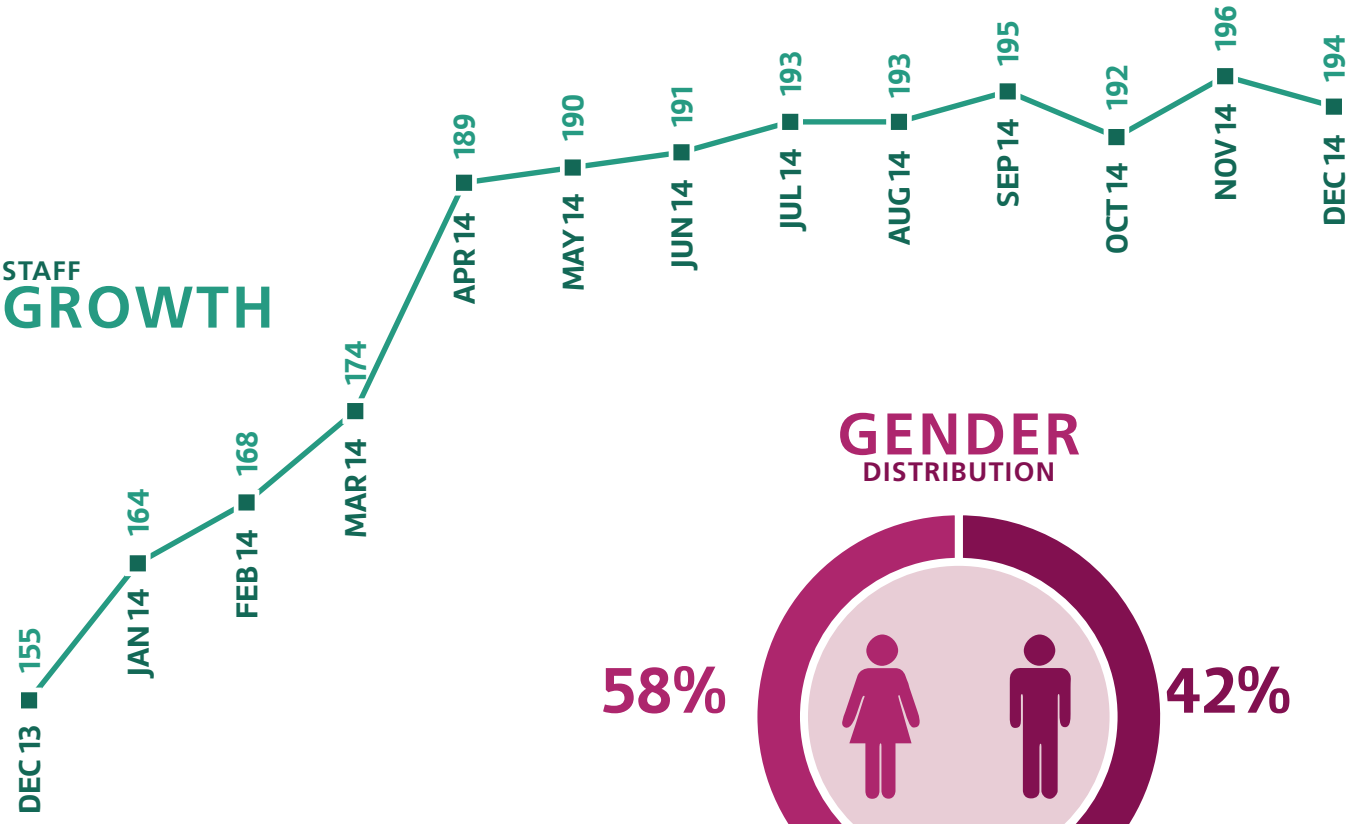
# STAFF

## NATIONALITIES
OF SCIENTIFIC STAFF



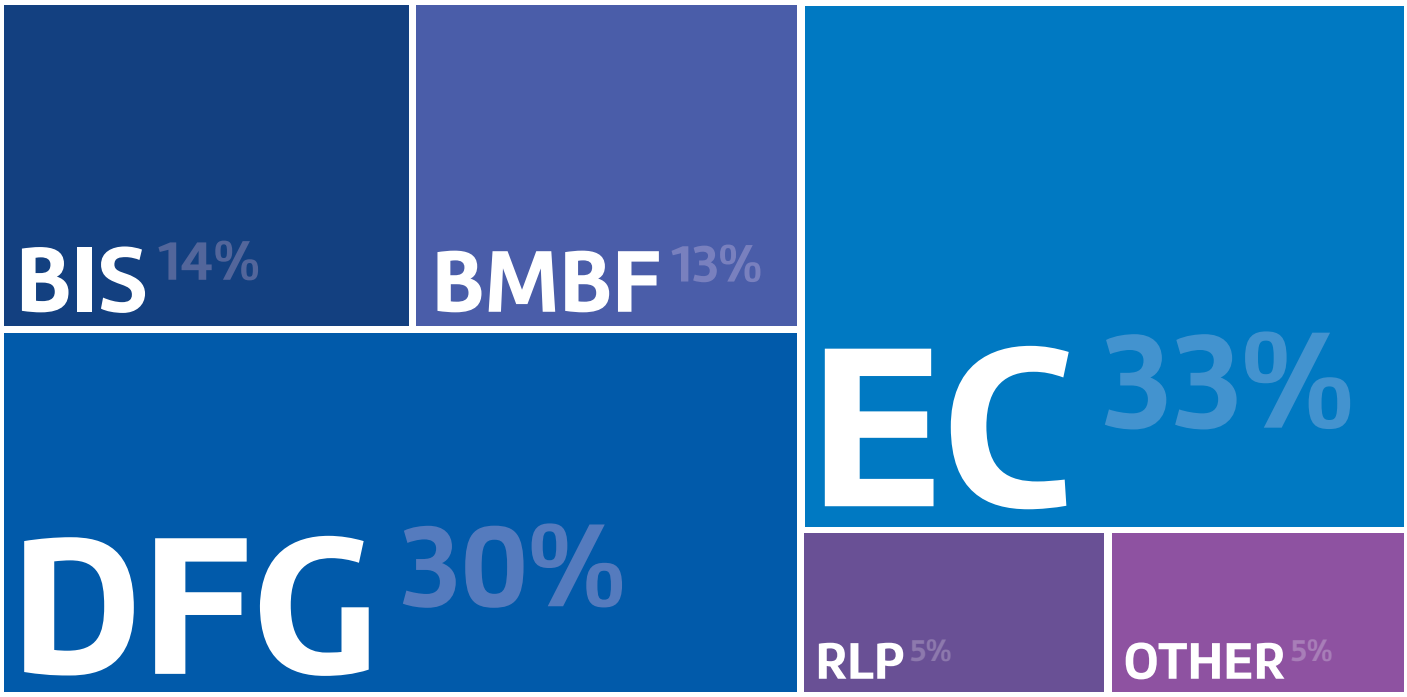




## STAFF GROWTH




## GENDER DISTRIBUTION

# EXTRAMURAL FUNDS




## MAJOR FUNDERS

Boehringer Ingelheim Stiftung (BIS)

Federal Ministry of Education and Research (BMBF)

Deutsche Forschungsgemeinschaft (DFG)

Forschungsinitiative Rheinland-Pfalz (RLP)

## EUROPEAN COMMISSION (EC)

European Research Council

Marie Curie Actions

EpiGeneSys Network of Excellence

## FURTHER SUPPORT

Deutscher Akademischer Austauschdienst

European Molecular Biology Organisation

German National Academy of Sciences Leopoldina

Naturwissenschaftlich-Medizinisches Forschungszentrum

Netherlands Organisation for Scientific Research

Projektträger Jülich

Stiftung Rheinland-Pfalz für Innovation

International Society for Differentiation

VDI Technologiezentrum

Volkswagen Stiftung

Wilhelm Sander-Stiftung

# PUBLICATIONS

## Andrade


Louis-Jeune C, Andrade-Navarro MA and Perez-Iratxeta C (2014). FASTA Herder: a web application to trim protein sequence sets. *ScienceOpen Research*, doi:10.14293/A2199-1006.01.SOR-LIFE.67837.v1.

Suratanee A, Schaefer MH, Betts MJ, Soons Z, Mannsperger H, Harder N, Oswald M, Gipp M, Ramminger E, Marcus G, Männer R, Rohr K, Wanker E, Russell RB, Andrade-Navarro MA, Eils R and König R (2014). Characterizing protein interactions employing a genome-wide siRNA cellular phenotyping screen. *PLoS Comp Biol*, 10, e1003814.

Bader M, Alenina N, Andrade-Navarro MA and Santos RA (2014). Mas and its related G protein-coupled receptors, Mrgprs. *Pharmacol Rev*, 66, 1080-1105.

Gijón-Correas JA, Andrade-Navarro MA and Fontaine JF (2014). Alkemio: association of chemicals with biomedical topics by text and data mining. *Nucleic Acids Res*, 42, W422-W429.

Voss F, Ullrich F, Münch J, Lazarow K, Lutter D, Mah N, Andrade-Navarro MA, von Kries JP, Stauber T and Jentsch TJ (2014). Identification of LRRC8 heteromers as essential component of the volume-regulated anion channel VRAC. *Science*, 344, 634-638.

Gebhardt ML, Reuter A, Mrowka R and Andrade-Navarro MA (2014). Similarity in targets with REST points to neural and glioblastoma related miRNAs. *Nucleic Acids Res*, 42, 5436-5446.

Bollen S, Leddin M, Andrade-Navarro MA and Mah N (2014). CAFE: an R package for the detection of gross chromosomal abnormalities from gene expression microarray data. *Bioinformatics*, 30, 1484-1485.

Fehlker M, Huska, Jöns T, Andrade-Navarro MA and Kemmner W (2014). Concerted down-regulation of immune-system related genes predicts metastasis in colorectal carcinoma. *BMC Cancer*, 14, 64.

Norton M, Cheuk-Him Ng A, Baird S, Dumoulin A, Shutt T, Mah N, Andrade-Navarro MA, McBride HM and Screaton RA (2014). Genome-wide RNAi screen identifies ROMO1 as an essential redox-dependent regulator of mitochondrial cristae dynamics. *Sci Signal*, 7, ra10.

Stachelscheid H, Seltmann S, Lekschas F, Fontaine JF, Mah N, Neves M, Andrade-Navarro MA, Leser U and Kurtz A (2014). CellFinder: a cell data repository. *Nucleic Acids Res*, 42, D950-D958.

Wethmar K, Barbosa da Silva A, Andrade-Navarro MA and Leutz A (2014). uORFdb – a comprehensive literature database on eukaryotic uORF biology. *Nucleic Acids Res*, 42, D60-67.


## Beli


Oka Y, Varmark H, Vitting-Seerup K, Beli P, Waage J, Hakobyan A, Mistrik M,Choudhary C, Rohde M, Bekker-Jensen S and Mailand N (2014). UBL5 is essential for pre-mRNA splicing and sister chromatid cohesion in human cells. *EMBO Rep*, 15, 956-964.


## Butter


Ericson M, Janes MA, Butter F, Mann M, Ullu E and Tschudi C (2014). On the extent and role of the small proteome in the parasitic eukaryote Trypanosoma brucei. *BMC Biol*, 12, 14.

Sharma NL, Massie CE, Butter F, Mann M, Bon H, Ramos-Montoya A, Menon S, Stark R, Lamb AD, Scott HE, Warren AY, Neal DE and Mills IG (2014). The ETS family member GABPα modulates androgen receptor signalling and mediates an aggressive phenotype in prostate cancer. *Nucleic Acids Res*, 42, 6256-6269.

Hornburg D, Drepper C, Butter F, Meissner F, Sendtner M and Mann M (2014). Deep proteomic evaluation of primary and cell line motoneuron disease models delineates major differences in neuronal characteristics. *Mol Cell Proteomics*, 13, 3410-3420.

Machyna M, Kehr S, Straube K, Kappei D, Buchholz F, Butter F, Ule J, Hertel J, Stadler PF and Neugebauer KM (2014). Global identification of coilin binding partners reveals hundreds of small non-coding RNAs that traffic through Cajal bodies. *Mol Cell*, 56, 389-399.

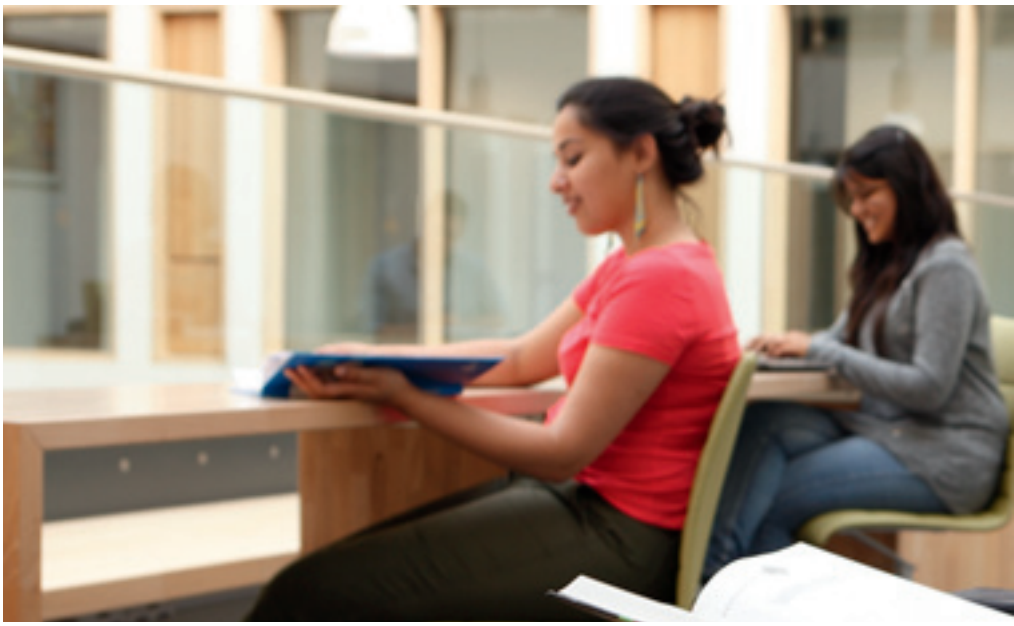

## Cremer

Cremer T, Cremer C and Lichter P (2014). Recollections of a scientific journey published in Human Genetics: from chromosome territories to interphase cytogenetics and comparative genome hybridization. *Hum Genet*, 133, 403-416.

Szczurek AT, Prakash K, Lee HK, Żurek-Biesiada DJ, Best G, Hagmann M, Dobrucki JW, Cremer C and Birk U (2014). Single molecule localization microscopy of the distribution of chromatin using Hoechst and DAPI fluorescent probes. *Nucleus*, 5, 1-10.

Wang Q, Dierkes R, Kaufmann R and Cremer C (2014). Quantitative analysis of individual Hepatocyte Growth Factor Receptor Clusters in Influenza A virus infected human epithelial cells using localization microscopy. *Biochim Biophys Acta*, 1838, 1191-1198.

Cremer C, Kaufmann R, Gunkel M, Polanski F, Müller P, Dierkes R, Degenhard S, Wege C, Hausmann M and Birk U (2014). Application Perspectives of localization microscopy in Virology. *Histochem Cell Biol*, 142, 43-59.



Müller P, Lemmermann NA, Kaufmann R, Gunkel M, Paech D, Hildenbrand G, Holtappels R, Cremer C and Hausmann M (2014). Spatial distribution and structural arrangement of a murine cytomegalovirus glycoprotein detected by SPDM localization microscopy. *Histochem Cell Biol*, 142, 61-67.

Cremer C (2014). Far-field Light Microscopy. *Encyclopedia of Life Sciences* (eLS), Wiley-Blackwell.

Cremer C (2014). Vorstoß in atomare Dimensionen. *Laborjournal*, 7-8, 65-67.

## Ketting

Swarts DC, Makarova K, Wang Y, Nakanishi K, Ketting RF, Koonin EV, Patel DJ and van der Oost J (2014). The evolutionary journey of Argonaute proteins. *Nat Struct Mol Biol*, 21, 743-753.

de Albuquerque BFM, Luteijn MJ, Cordeiro Rodrigues RJ, van Bergeijk P, Waaijers S, Kaaij LJT, Klein H, Boxem M and Ketting RF (2014). PID-1 is a novel factor that operates during 21U-RNA biogenesis in Caenorhabditis elegans. *Genes Dev*, 28, 683-688.

Phillips CM, Montgomery BE, Breen PC, Roovers EF, Rim YS, Ohsumi TK, Newman MA, van Wolfswinkel JC, Ketting RF, Ruvkun G and Montgomery TA (2014). MUT-14 and SMUT-1 DEAD Box RNA Helicases have overlapping roles in germline RNAi and endogenous siRNA formation. *Curr Biol*, 24, 839-844.

Kellner S, Neumann J, Rosenkranz D, Lebedeva S, Ketting RF, Zischler H, Schneider D and Helm M (2014). Profiling of RNA modifications by multiplexed stable isotope labeling. *Chem Commun (Camb)*, 50, 3516-8.

Huang HY and Ketting RF (2014). Isolation of zebrafish gonads for RNA isolation. *Methods Mol Biol*, 1093, 183-94.

## König

Huppertz I, Attig J, D'Ambrogio A, Easton LE, Sibley CR, Sugimoto Y, Tajnik M, *König J and *Ule J (2014). iCLIP: protein-RNA interactions at nucleotide resolution. *Methods*, 653, 274-287.

Rossbach O, Hung LH, Khrameeva E, Schreiner S, König J, Curk T, Zupan B, Ule J, Gelfand MS and Bindereif A (2014). Crosslinking-immunoprecipitation (iCLIP) analysis reveals global regulatory roles of hnRNP L. *RNA Biol*, 11, 146-155.

## Legewie

Kallenberger SM, Beaudouin J, Claus J, Fischer C, Sorger PK, *Legewie S and *Eils R (2014). Intra- and Interdimeric Caspase-8 Self-Cleavage Controls Strength and Timing of CD95-Induced Apoptosis. *Sci Signal*, 7, ra23.

Casanovas G, Banerji A, d'Alessio F, *Muckenthaler M and *Legewie S (2014). A multi-scale model of hepcidin promoter regulation reveals factors controlling systemic iron homeostasis. *PLoS Comp Biol*, 10, e1003421.

## Luke

Klermund J, Bender K and Luke B (2014). High nutrient levels and TORC1 activity reduce cell viability following prolonged telomere dysfunction and cell cycle arrest. *Cell Reports*, 9, 324-335.

Balk B, Dees M, Bender K and Luke B (2014). The differential processing of telomeres in response to increased telomeric transcription and RNA-DNA hybrid accumulation. *RNA Biol*, 2, 95-100.

## Niehrs

Huang YL and Niehrs C (2014). Polarized Wnt Signalling Regulates Ectodermal Cell Fate in Xenopus. *Dev Cell*, 29, 250-257.

Perez-Acebron S, Karaulanov E, Berger BS, Huang YL and Niehrs C (2014). Mitotic Wnt signalling promotes protein stabilization and regulates cell size. *Mol Cell*, 54, 663-774.

Simonetti M, Agarwal N, Stösser S, Bali KK, Karaulanov E, Kamble R, Pospisilova B, Kurejova M, Birchmeier W, Niehrs C, Heppenstall P and Kuner R (2014). Wnt-Fzd signalling sensitizes peripheral sensory neurons via distinct noncanonical pathways. *Neuron*, 83, 104-121.

Arab K, Park YJ, Lindroth AM, Schäfer A, Oakes C, Weichenhan D, Lukanova A, Lundin E, Risch A, Meister M, Dienemann H, Dyckhoff G, Herold-Mende C, *Grummt I, *Niehrs C and *Plass C (2014). Long Noncoding RNA TARID Directs Demethylation and Activation of the Tumor Suppressor TCF21 via GADD45A. *Mol Cell*, 55, 604-614.

## Reid

wa Maina C, Honkela A, Matarese F, Grote K, Stunnenberg HG, Reid G, Lawrence ND and Rattray M (2014). Inference of RNA Polymerase II Transcription Dynamics from Chromatin Immunoprecipitation Time Course Data. *PLOS Comp Biol*, 10, e1003598.

## Roignant

Malone CD, Mestdagh C, Akhtar J, Kreim N, Deinhard P, Sachidanandam R, Treisman J and Roignant JY (2014). The exon junction complex controls transposable element activity by ensuring faithful splicing of the piwi transcript. *Genes Dev*, 28, 1786-1799.

## Soshnikova

Montavon T and Soshnikova N (2014). Hox gene regulation and timing in embryogenesis. *Semin Cell Dev Biol*, 34, 76-84.

Jung H, Mazzoni EO, Soshnikova N, Hanley O, Venkatesh B, Duboule D and Dasen JS (2014). Evolving Hox activity profiles govern diversity in locomotor systems. *Dev Cell*, 29, 171-187.

Soshnikova N (2014). Hox genes regulation in vertebrates. *Dev Dyn*, 243, 49-58.

## Ulrich

Parker JL and Ulrich HD (2014). SIM-dependent enhancement of substrate-specific SUMOylation by a ubiquitin ligase *in vitro*. *Biochem J*, 457, 435-440.

Ulrich HD (2014). Two-way communications between ubiquitin-like modifiers and DNA. *Nat Struct Mol Biol*, 21, 317-324.

## Wolf

Schmalen I, Reischl S, Wallach T, Klemz R, Grudziecki A, Prabu JR, Benda C, Kramer A and Wolf E (2014). Interaction of circadian clock proteins CRY1 and PER2 is modulated by zinc binding and disulfide bond formation. *Cell*, 157, 1203-1215.

Witosch J, Wolf E and Mizuno N (2014). Architecture and ssDNA interaction of the Timeless-Tipin-RPA complex. *Nucleic Acids Research*, 42, 12912-12927.

## Core Facilities

Fröhlich D, Kuo WP, Frühbeis C, Sun JJ, Zehendner CM, Luhmann HJ, Pinto S, Toedling J, Trotter J and Krämer-Albers EM (2014). Multifaceted effects of oligodendroglial exosomes on neurons: impact on neuronal firing rate, signal transduction and gene regulation. *Philos Trans R Soc Lond B Biol Sci*, 369, 2013510.

Malone CD, Mestdagh C, Akhtar J, Kreim N, Deinhard P, Sachidanandam R, Treisman J and Roignant JY (2014). The exon junction complex controls transposable element activity by ensuring faithful splicing of the piwi transcript. *Genes Dev*, 28, 1786-1799.

Simonetti M, Agarwal N, Stösser S, Bali KK, Karaulanov E, Kamble R, Pospisilova B, Kurejova M, Birchmeier W, Niehrs C, Heppenstall P and Kuner R (2014). Wnt-Fzd signalling sensitizes peripheral sensory neurons via distinct noncanonical pathways. *Neuron*, 83, 104-121.

Acebron SP, Karaulanov E, Berger BS, Huang YL and Niehrs C (2014). Mitotic wnt signalling promotes protein stabilization and regulates cell size. *Mol Cell*, 54, 663-674.

de Albuquerque BF, Luteijn MJ, Cordeiro Rodrigues RJ, van Bergeijk P, Waaijers S, Kaaij LJ, Klein H, Boxem M and Ketting RF. PID-1 is a novel factor that operates during 21U-RNA biogenesis in Caenorhabditis elegans. *Genes Dev*, 28, 683-688.

Holloschi A, Ritz S, Schäfer I and Kioschis P (2014). Analysis of huntingtin aggregation by fluorescence and FRET microscopy, *Microscopy: advances in scientific research and education* [A Méndez-Vilas, Ed]. Volume 1, 217-225.

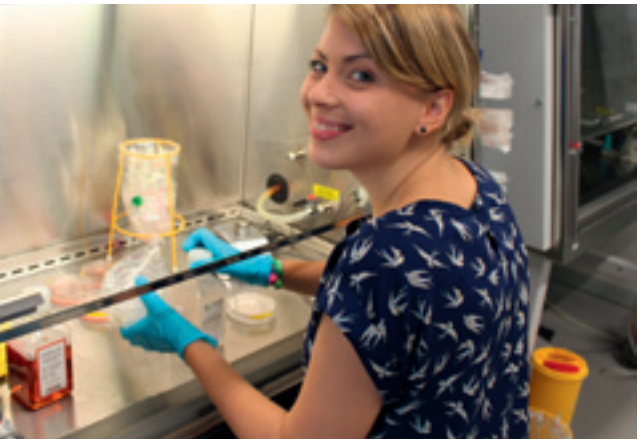
*indicates joint authorship

## Awards and Recognition

**René Ketting**
Elected EMBO Member in 2014
MicroRNAs Innovator Award 2014

**Brian Luke**
Chica and Heinz Schaller Research Award for Young Scientists

# RESEARCH AND TRAINING



IMB is the place to be for scientists who want to carry out cutting-edge research in epigenetics, developmental biology, genome stability and the interfaces between these fields. Our multinational team of scientists addresses key questions within these areas. They cover a broad range of expertise and include leading biochemists, geneticists, molecular cell and developmental biologists who study, for example, the molecular mechanisms of embryonic development, evolution, ageing and disease. In addition, our bioinformaticians and systems biologists analyse high-throughput datasets and model regulatory gene networks, and applied physicists develop ground breaking superresolution microscopes. This variety of expertise and the open and vibrant atmosphere at IMB encourages multidisciplinary collaborations and innovative research.

At IMB we make sure our scientists can work productively. A key part of the support we offer comes from our state-of-the-art Core Facilities. They provide services in bioinformatics, cytometry, genomics, histology, microscopy, and proteomics. Each facility is staffed by experts who are there to advise and assist our scientists during every step of their experiments, from the initial conception to the analysis of data. As part of the collaborative spirit at IMB, all key equipment is shared between research groups and looked after by trained staff in the Core Facilities. This means our scientists always have access to the most up-to-date and well-maintained equipment that is required for their experiments.

In addition to the training available through our Core Facilities, IMB offers sound scientific training in the form of involvement and attendance at scientific events at IMB and Johannes Gutenberg University, and theoretical as well as hands on instruction in technical and methodological skills (e.g. statistics, analysis of large datasets (including those generated by next-generation sequencing) and advanced live and superresolution microscopy). We also make sure our junior scientists learn the transferable skills required for a successful career in an increasingly competitive scientific world both within and outside of academia. Courses we offer cover topics such as presentation skills, scientific writing and project management, as well as fundraising and leadership.

## INTERNATIONAL PHD PROGRAMME

PhD Students are a key part of our research teams at IMB and work on projects of fundamental biological importance or of relevance to human disease. Their research is conducted in a vibrant and highly interdisciplinary environment with leaders in their respective fields.

Our International PhD Programme (IPP) on Gene Regulation, Epigenetics and Genome Stability gives talented and enthusiastic students the opportunity to undertake PhD research at the cutting edge of modern biology.

The IPP has a unique profile that provides students with interdisciplinary education in the following fields:

- Epigenetics
- Gene Regulation
- DNA Repair & Genome Stability
- Functional Morphology of the Nucleus
- Systems Biology and Bioinformatics

The Programme is coordinated by IMB and participating groups are located at the:

- Institute of Molecular Biology (IMB)
- Johannes Gutenberg University
- University Medical Centre
- Max Planck Institute for Polymer Research

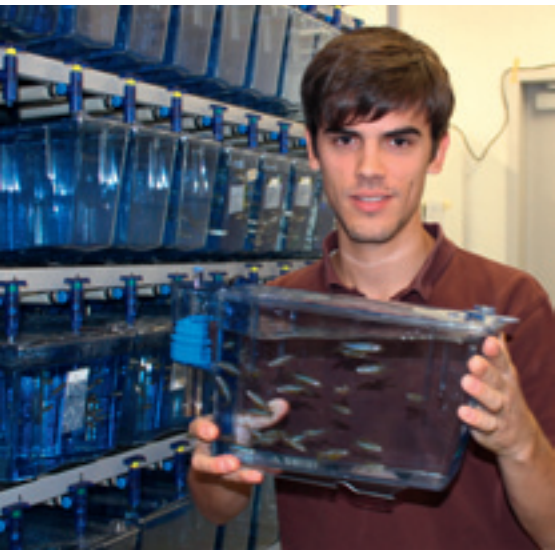
www.imb.de/PhD

## POSTDOC PROGRAMME

The IMB Postdoc Programme has been established to meet the specific needs of postdocs, and to ensure that they are able to build the strongest possible foundation for success in their future careers. The programme ensures sound scientific training through a variety of lectures, workshops and events available at IMB, and offers postdocs full support with raising funds for their research.

IMB also recognises the need for career development. In addition to the guidance given by Group Leaders, who provide postdocs with day-to-day scientific and career advice, the Postdoc Programme also offers mentoring discussions with IMB's Scientific Directors, Career Days, and preparation for applications and interviews. To succeed in today's competitive job market, postdocs must have excellent presentation, writing, project management and time management skills. As such, IMB provides courses and lectures on these elements. The programme also organises talks by representatives from local scientific companies, and sets up company site visits and job shadowing initiatives, so that IMB postdocs have the opportunity to learn more about a range of future career opportunities.

www.imb.de/Postdocs



## INTERNATIONAL SUMMER SCHOOL

IMB's International Summer School (ISS) is a six-week programme for outstanding and enthusiastic undergraduate, masters and PhD Students who want to acquire excellent practical skills and hands-on training from leading scientists in molecular biology. Research Groups participating in the ISS include Group Leaders at IMB, Johannes Gutenberg University and Mainz's University Medical Centre.

The ISS offers an attractive framework for training prospective scientists in an informal and international atmosphere. This includes theory modules (lectures and discussion groups) and practical research projects. The lectures give students comprehensive insights into the latest research findings and identify key open questions in gene regulation, epigenetics and genome stability. Furthermore, the ISS teaches students the complementary skills, such as presentation and communication techniques, that are required for a successful career as a scientist.

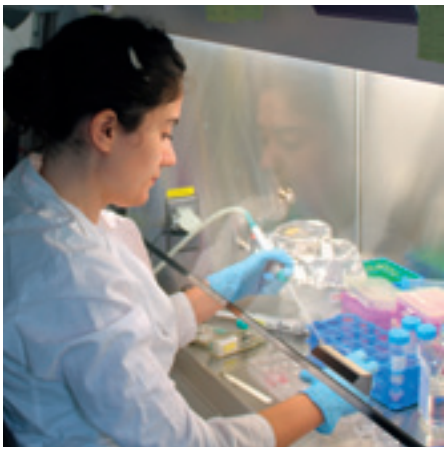
Beyond these specific events, ISS participants are also fully integrated into scientific life at IMB by participating in lab meetings and journal clubs. Furthermore, each student works on a cutting-edge research project within the lab of one of the participating research groups.

www.imb.de/ISS

Looking up at the roof of IMB

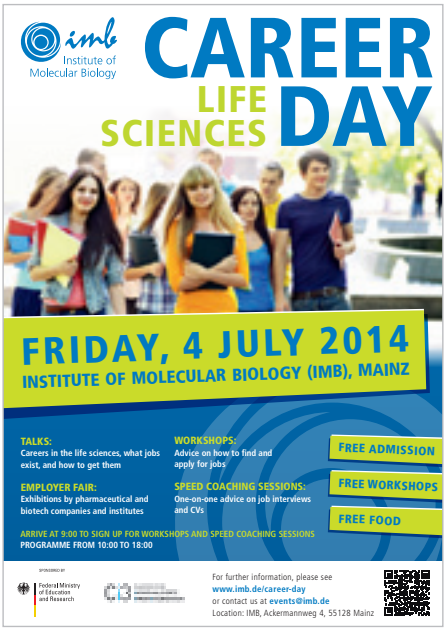
# TRAINING COURSES

## CORE FACILITIES TRAINING



| CORE FACILITY | LECTURES | PRACTICAL COURSES |
|---|---|---|
| Bioinformatics | Experimental Design and Quality Control | R: Practical Course |
| | ChIP Seq | Galaxy Course |
| | RNA Seq | |
| | DNA Methylation | |
| Flow Cytometry | Cytometry I - Basics of Flow Cytometry | Cytometry: Practical Flow Cytometry |
| | Cytometry II - Advanced Flow Cytometry | |
| | Data Analysis | |
| Genomics | Introduction to Next-Generation Sequencing | |
| Microscopy | Microscopy I | Microscopy: Practical Course |
| | Microscopy II | |
| | Pitfalls in Image Acquisition | |
| | Image Processing | |
| | Ethics in Image Acquisiiton & Processing | |
| Proteomics | Proteomics | Proteomics: Practical Course |

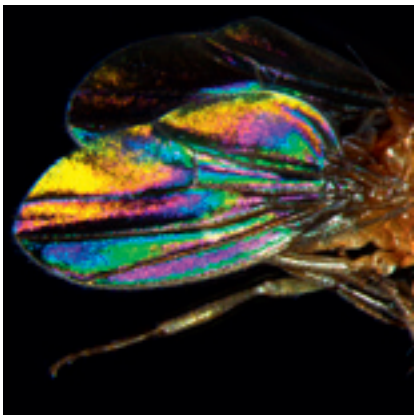
## SCIENTIFIC AND TRANSLATIONAL SKILLS TRAINING




| DATE | TITLE |
|---|---|
| 22 JAN | Project Management for PhD Students |
| 03 APRIL | Grants Writing Workshop |
| 17 APRIL | Application Training |
| 05 – 06 MAY | Scientific Writing: Optimising Writing Strategies for Getting Research Published in English |
| 04 JULY | 2014 Career Day |
| 14 – 17 JULY | Presentation Skills: How to Give Successful Scientific Talks |
| 28 JULY – 08 AUG | Block Lecture Weeks on the topics of IMB's International PhD Programme |
| 15 SEP | Intellectual Property Rights |
| 15 – 16 OCT | Statistics: Introduction into Biostatistics |
| FROM 28 OCT | Introduction to Epigenetics Lecture Series |

# EVENTS

## SELECTION OF SCIENTIFIC EVENTS ORGANISED BY IMB



29 Jan 2014

### DROSOPHILA MEETING

Scientific organisers: Jean-Yves Roignant

7 May 2014

### SYMPOSIUM OF IMB AND THE FACULTY OF BIOLOGY, MAINZ UNIVERSITY (JGU)

Scientific organisers: Christof Niehrs, Hans Zischler and Ralf Dahm

12 June 2014

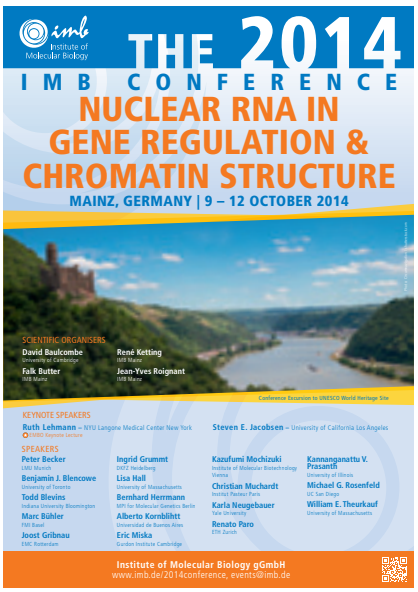
### IMB-TRON (TRANSLATIONAL ONCOLOGY MAINZ) SYMPOSIUM

Scientific organisers: Ugur Sahin (TRON) and Christof Niehrs

8 Sep 2014

### 1ST REGIONAL ZEBRAFISH MEETING

Scientific organisers: René Ketting, Didier Steiner (Max Planck Institute for Heart and Lung Research, Bad Nauheim) and Joachim Wittbrodt (Centre for Organismal Studies, Heidelberg)




9 – 12 Oct 2014

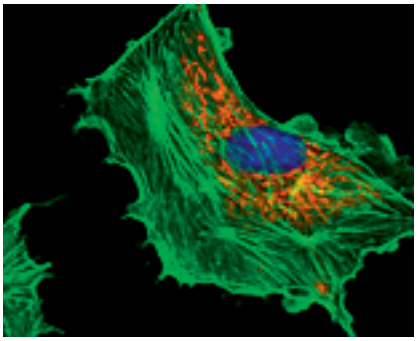
### 2014 IMB CONFERENCE "NUCLEAR RNA IN GENE REGULATION & CHROMATIN STRUCTURE"

Scientific organisers: David Baulcombe (University of Cambridge), Falk Butter, René Ketting and Jean-Yves Roignant

6 Nov 2014

### RESEARCH WORKSHOP ON "CELL FOCUSSED DATA: INTEGRATION, ORGANISATION AND APPLICATIONS"

Scientific organisers: Miguel Andrade and Andreas Kurtz (Berlin-Brandenburg Centre for Regenerative Therapies, Berlin)



7 – 8 Nov 2014

### NEUROKINE GENOMICS AND BIOINFORMATICS WORKSHOP

Scientific organisers: John Castle (TRON), Ari Waisman (UMC) and Ralf Dahm

# INVITED SPEAKERS

| DATE | EVENT | SPEAKER | INSTITUTION | TITLE |
|---|---|---|---|---|
| 23 Jan 2014 | Seminar | Magdalena Götz | Helmholtz Center & Ludwig-Maximilians University, Munich, Germany | Novel regulators of neural stem cells |
| 04 Feb 2014 | Seminar | Brian Luke | University of Heidelberg, Germany | Non-coding RNA, checkpoint adaptation and cellular senescence: A telomere perspective |
| 04 Feb 2014 | TechTalk | Peter Rhein | Merck Chemicals GmbH, Life Science Division, Germany | Applications of high speed high content image analysis of cells in suspension using Amnis® imaging flow cytometry |
| 05 Feb 2014 | Seminar | Francois Spitz | European Molecular Biology Laboratory, Heidelberg, Germany | Managing long-distance regulatory interactions in development and disease |
| 11 Feb 2014 | TechTalk | Mathias Holpert | Merck Millipore, Germany | Take control of your cells' micro-environment for better live cell imaging |
| 20 Feb 2014 | Seminar | Antoine Peters | Friedrich Miescher Institute, Basel, Switzerland | Epigenetic control of mammalian germ line and early embryonic development |
| 26 Feb 2014 | TechTalk | Christina Wege | University of Stuttgart, Germany | Light-optical nanoscopy rulers based on plant viral assemblies: from imaging tools to biodetection and multifunctional hybrids |
| 04 Mar 2014 | Seminar | Jonas Ries | European Molecular Biology Laboratory Heidelberg, Germany | Superresolution microscopy of protein structures *in situ* |
| 06 Mar 2014 | Seminar | Baris Tursun | Max Delbrück Center for Molecular Medicine, Berlin, Germany | Safeguarding cellular identities - identifying mechanisms that counteract direct reprogramming |
| 13 Mar 2014 | Seminar | Didier Stainier | Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany | Imaging organ formation and function in zebrafish |
| 13 Mar 2014 | TechTalk | Karl-Heinz Brenner | University of Heidelberg, Germany | Scanning microscopy with diffractive microlenses |
| 20 Mar 2014 | Seminar | Adele Murrell | University of Bath, UK | Genomic imprinting: merely parental conflict or a model system of epigenetic paradigms for cellular reprogramming? |
| 28 Mar 2014 | Seminar | Christian Eckmann | Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany | C elegans germline development – a GoLD mine to unearth RNA regulatory networks |
| 15 Apr 2014 | TechTalk | Anina Moritz | Takara Bio Europe | SMARTer® solutions for low input transcriptome sequencing |
| 22 Apr 2014 | Seminar | Robert Schneider | Institute of Genetics and Molecular and Cellular Biology, Strasbourg, France | Novel players in the regulation of genome function |
| 23 Apr 2014 | Seminar | Maria Elena Torres-Padilla | Institute of Genetics and Molecular and Cellular Biology, Strasbourg | Epigenetic mechanisms in early mammalian development |
| 05 May 2014 | Seminar | Javier F Caceres | University of Edinburgh, UK | Cellular functions of the microprocessor |
| 20 May 2014 | TechTalk | Douglas Amorese | NuGEN Technologies Inc | Technologies for efficient generation of transcriptome data by next generation sequencing |
| 22 May 2014 | Seminar | Juan Valcarcel Juarez | ICREA and Center for Genomic Regulation (CRG), Barcelona, Spain | Networks of alternative splicing regulation in cancer |
| 05 June 2014 | Seminar | Christoph Plass | German Cancer Research Centre, Heidelberg, Germany | Epigenetic reprogramming in cancer |
| 05 June 2014 | TechTalk | Malathi Raman | Takara Bio Europe | Cloning without compromise…discover In-Fusion®! |
| 26 June 2014 | Seminar | Thomas Tuschl | Howard Hughes Medical Institute, Rockefeller University, New York, USA | Principles of posttranscriptional gene regulation |
| 03 July 2014 | Seminar | Stéphane Ronsseray | Developmental Biology Laboratory (IBPS-LBD), Paris, France | piRNAs and epigenetic conversion in *Drosophila* |
| 08 July 2014 | Seminar | Helge Grosshans | Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland | The ticking of a (developmental) clock: keeping time with miRNAs and oscillations |
| 31 July 2014 | Seminar | Julian Sale | MRC Laboratory of Molecular Biology, Cambridge, UK | Replication stress, structured DNA and epigenetic instability |
| 27 Nov 2014 | Seminar | Rolf Backofen | Albert-Ludwigs-University Freiburg, Germany | How to determine binding motifs for RNA-binding proteins |

# RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located within the leafy campus of the Johannes Gutenberg University, just west of Mainz city centre. With 10 departments, 150 institutes and 38,000 students, Johannes Gutenberg University is one of the largest German universities. In biomedical research, the University has built strong, interdisciplinary centres dedicated to neuroscience, cardiovascular medicine, immunology and oncology.

The University Medical Centre, which is located near the main university campus, has a strong focus on clinical and translational research and has researchers who also work in close contact with IMB. In addition to the University, IMB has two Max Planck Institutes (the Max Planck Institute for Chemistry and the Max Planck Institute for Polymer Research) and Mainz's University of Applied Sciences as immediate neighbours.

Mainz is also surrounded by a number of towns and cities with extensive research activities. For instance, Frankfurt is only 35 km away and is home to Goethe University, which has a total of 38,000 students and 10 research institutes within the Biochemistry, Chemistry and Pharmacy Department alone. Furthermore, there are several Max Planck Institutes in Frankfurt (including the Max Planck Institute for Biophysics, the Max Planck Institute for Brain Research and the Ernst Strüngmann Institute for Cognitive Brain Research). In addition to Frankfurt, nearby Darmstadt is home to both a Technical University, whose Department of Biology has a focus on synthetic biology and the biology of stress responses, and a University of Applied Sciences that includes a focus on biotechnology.

Furthermore, there is an extensive industry R&D presence, with, for example, the headquarters of Boehringer Ingelheim and the Merck Group both in close vicinity.

## WHERE WE ARE

IMB is located in the city of Mainz, a charming, open-minded city that dates back 2,000 years to Roman times and still has a historic centre with a magnificent medieval cathedral. It was also here, in 1450, that Johannes Gutenberg invented modern book printing. The city is located at the confluence of two of the most important rivers in Germany, the Rhine and the Main, and has spectacular esplanades. Mainz is within easy reach of both cosmopolitan Frankfurt, with its famous opera house, avant-garde museums and glass-and-steel banking district, and the Rhine valley region with its castles, vineyards and nature reserves that offer great outdoor activities. With Frankfurt airport – one of the largest airports in Europe – only 25 minutes away, countless European and overseas destinations are within easy reach.

# SCIENTIFIC ADVISORY BOARD

IMB IS VERY GRATEFUL TO THE FOLLOWING OUTSTANDING SCIENTISTS FOR THE INSIGHT, GUIDANCE AND ADVICE THAT THEY HAVE PROVIDED TO HELP US BECOME A WORLD-LEADING RESEARCH CENTRE.

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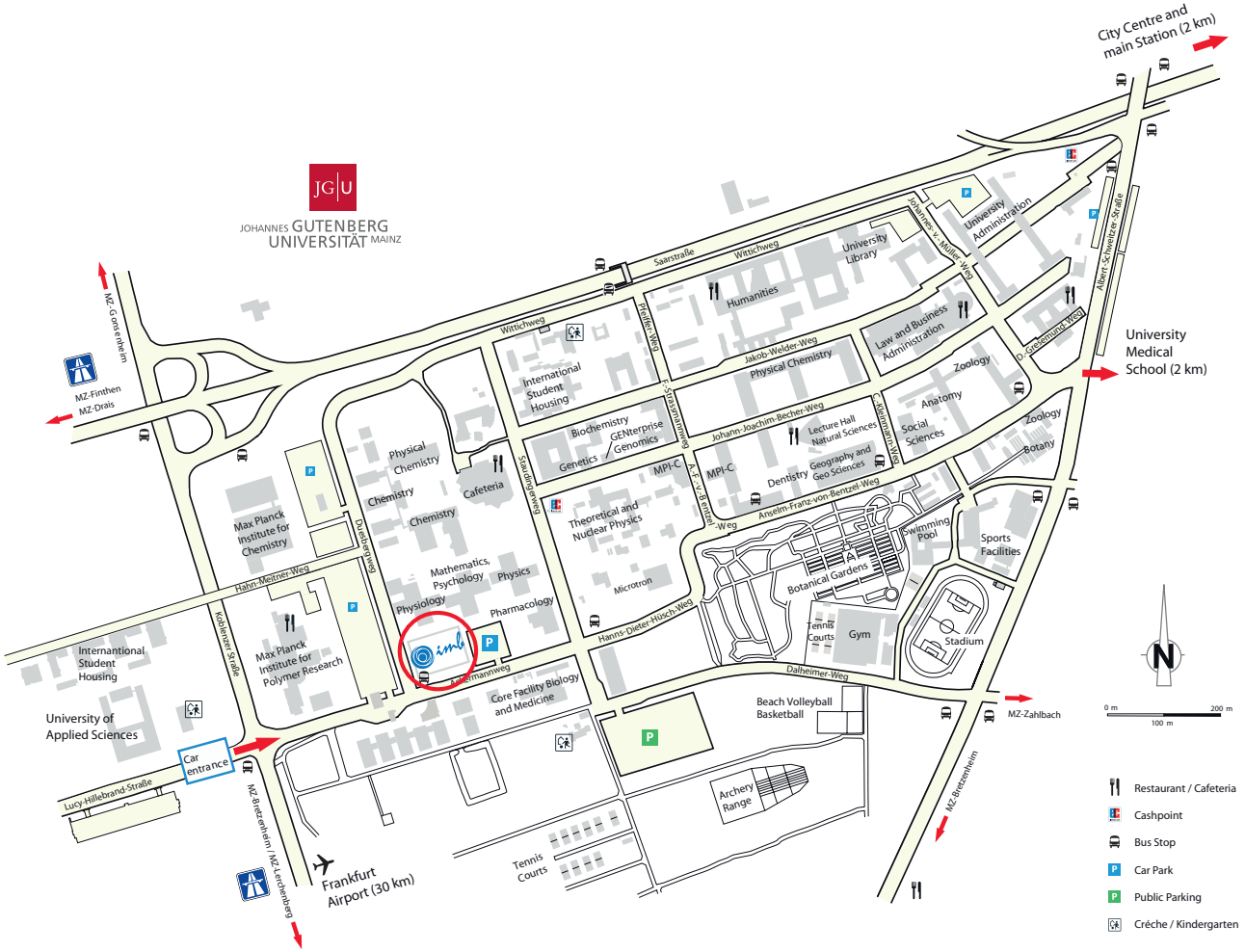
# CAMPUS MAP

## OF JOHANNES GUTENBERG UNIVERSITY MAINZ

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