Institute of Molecular Biology

ANNUAL REPORT 2015

TABLE OF CONTENTS

Foreword 2

Research Groups

Miguel Andrade 4
Petra Beli 6
Falk Butter 8
Christoph Cremer 10
René Ketting 12
Julian König 14
Stefan Legewie 16
Brian Luke 18
Christof Niehrs 20
George Reid 22
Holger Richly 24
Jean-Yves Roignant 26
Vassilis Roukos 28
Natalia Soshnikova 30
Vijay Tiwari 32
Helle Ulrich 34
Eva Wolf 36

Core Facilities

Overview 39
Bioinformatics 40
Flow Cytometry 41
Genomics 42
Microscopy & Histology 43
Proteomics 44
Media Lab 45

Facts & Figures

Staff 48
Extramural Funds 49
Research & Training 50
Training Courses 52
Invited Speakers 54
Selected Scientific Events 56
Publications 57
Awards & Recognition 61
Research Environment 62
Scientific Advisory Board 63
Campus Map & Contact 64
Imprint 65

FOREWORD

This year has been exciting for IMB and marks 5 years since its foundation. It also brings my 5-year term as Executive Director to a close. Before I hand over, I would like to take this opportunity to review some of this year's progress. IMB is an unusual basic research centre in the molecular life sciences, as it is set up in a public-private partnership between the Boehringer Ingelheim Foundation, which supports the research and the state of Rhineland-Palatinate, which provided a state-of-the-art research building located on the Campus of the Johannes Gutenberg University Mainz.

Milestones

In October, we had our first 5-year institute-wide evaluation, which was carried out by an international review board of leading scientists. The board was extremely positive about the progress we have made in the short time since IMB opened. Their very encouraging feedback has rewarded our endeavours and confirmed that we are on the right path to establishing IMB as a world-class research institute in the life sciences.

As well as the evaluation of IMB as a whole, in 2015 our International PhD Programme (IPP) on "Gene Regulation, Epigenetics & Genome Stability" was evaluated for the first time. In this review, the external experts, who represented leading life sciences PhD programmes in Germany, concluded that IMB has established an internationally competitive and highly attractive programme for graduate students in Mainz. In this context, I would like to offer congratulations to the first IPP students to have defended their theses this year: Claire Mestdagh, Bernadette Mekker, Yuliya Mikhed and Sudhir Thakurela.

Finally, 2015 also saw the first external evaluations of Group Leaders at IMB: I am happy to say that both Stefan Legewie and Christoph Cremer were very positively evaluated and will continue their exciting research at our institute.

I thank all external scientists who dedicated their precious time to reviewing IMB, its PhD programme, Group Leaders and students. Their feedback has been most helpful and is very much appreciated.




New Staff

IMB has continued to grow this year and has breached the 200-staff mark with employees and students in 17 research groups. We were very pleased to welcome two new Group Leaders: Brian Luke, who moved with his lab from the Centre for Molecular Biology (ZMBH) in Heidelberg in March; and Vassilis Roukos, who started his group here in April having moved from a postdoc position at the National Cancer Institute in Bethesda, USA. The Luke group conducts research into the maintenance of telomeres and their role in maintaining chromatin integrity, while the Roukos lab investigates double strand break repair using high-throughput imaging techniques. Together, they complement and expand the research at IMB in these exciting areas of genome stability.

Events

The 2015 IMB Conference on "DNA Repair & Genome Stability in a Chromatin Environment" in June featured talks from some of the top researchers in this field. We already look forward to next year's IMB Conference on "Epigenetics in Development", which will take place on 20-22 October 2016.

As well as the IMB Conference, we have hosted a highly acclaimed international workshop on "Breakthroughs in Epigenetics", which featured presentations by many of the pioneers in this field, as well as an EMBO workshop on the iCLIP technique. Further to these meetings, IMB organised a range of other scientific as well as outreach events, some of which are detailed in this report.

The 4th International Summer School, reserved mostly for undergraduate students, was a great success. It received more applications than ever before and the 20 students who took part came from 16 different countries.

Outlook

The end of 2015 marks the end of my 5-year term as IMB's 1st Executive Director, a position which will now rotate between René Ketting, Helle Ulrich and myself. It is with pleasure that I now hand over the reins to René Ketting, who will hold this position for the next 2 years. I am confident that he will continue to guide IMB to further successes as the institute moves from its foundation to its consolidation phase.

I would like to thank the Boehringer Ingelheim Foundation for their generous funding of the institute. I would also like to thank our dedicated Scientific Advisory Board (SAB), who have been instrumental in getting IMB to where it is today. Finally, I welcome Professors Ruth Lehmann and Peter Becker to IMB's SAB as of 2016 and look forward to their input on the future direction of IMB.

Christof Niehrs
Founding and Executive Director

MIGUEL ANDRADE

"Our evolutionary studies of disordered regions in proteins show their functional importance."

POSITIONS HELD

Since 2014	Adjunct Director, IMB, Mainz
Since 2014	Professor of Bioinformatics, Johannes Gutenberg University (JGU), Mainz
Since 2008	Affiliate Investigator, Ottawa Health Research Institute
2007 - 2014	Group Leader, Max Delbrück Center for Molecular Medicine, Berlin
2006 - 2008	Senior Scientist, Ottawa Health Research Institute
2003 - 2006	Scientist, Ottawa Health Research Institute
1998 - 2003	Staff Scientist, European Molecular Biology Laboratory (EMBL), Heidelberg
1996 - 1998	Postdoc, EMBL-EBI, Hinxton
1995 - 1996	National Centre for Biotechnology, Madrid
1994 - 1995	Postdoc, EMBL, Heidelberg

EDUCATION

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

GROUP MEMBERS

Gregorio Alanis Lobato Postdoc; since 02/2015
Jean-Fred Fontaine Postdoc; since 04/2014
Marie Gebhardt PhD Student; since 04/2014
Jonas Ibn-Salem PhD Student; since 09/2014
Desireé Kaufmann PhD Student; 11/2014 - 08/2015
Pablo Mier Postdoc; since 01/2015
Enrique Muro Staff Scientist; since 04/2014
Sweta Talyan PhD Student; since 12/2014
Katerina Taškova Postdoc; since 11/2014

OVERVIEW

Our group develops and applies methods that integrate data at different levels of molecular biology to investigate biological questions, including the function of genes and proteins, and the mechanisms that control cell identity or cause disease. Our projects often overlap, both in terms of the resources and methods they use. For example, we develop data mining methods that associate keywords to therapeutic drugs, which we can then apply to the interpretation of profiles of gene expression. In a different project, we created particular phylogenetic analyses of protein families that we can then use to study the evolution of the human protein interaction network. By being carried out within the same group, our projects benefit from and complement each other.

RESEARCH HIGHLIGHTS

STUDY OF PROTEIN INTERACTION NETWORKS

We are continuing to develop HIPPIE, a database of human protein interactions that are scored according to experimental evidence. From these data, we noticed that different proteins tend to be studied for interactions with variable intensity, which generates a bias in the protein interaction network that need to be accounted for. For example, proteins involved in cancer are studied more often, so appear to have more interaction partners.

PROTEIN SEQUENCE AND STRUCTURE ANALYSIS

Homorepeats, tracts of repeated amino acids in proteins (e.g. polyQ), are present in approximately 10% of all human proteins and in most organisms (Figure 1). However, their function is

unclear and they are difficult to study because methods of studying protein function rely on homology searches that do not work well with these repetitive sequences. Thus, we are using methods to study homorepeats in the context of the protein families where they evolve, and looking for the features with which they coevolve, as a means to finding out their biological relevance. For this, we take advantage of automated clustering of the protein database that we are developing. Such clusters allow us to simplify the results of protein sequence similarity searches. In addition, they enable us to search for protein families with particular properties, e.g. regarding their taxonomic distribution and function.

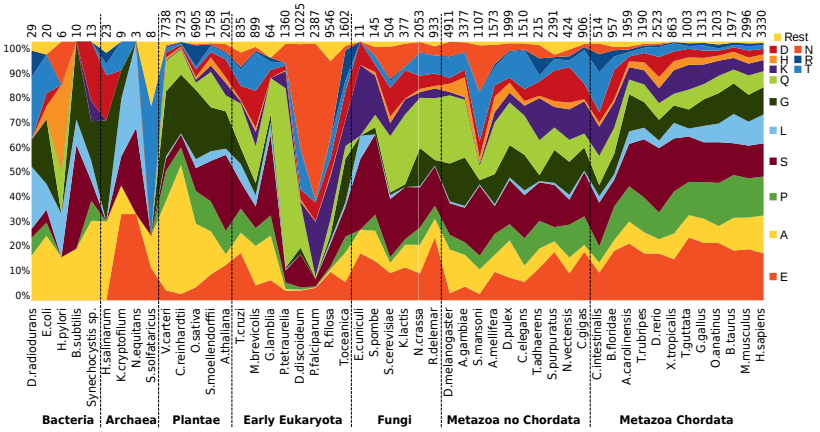


Figure 1. Fraction of homorepeats present in proteins (clustered) of each of 50 species. Different colours indicate homorepeats of different amino acids. The numbers at the top indicate the number of clusters with homorepeats per species. Numbers are low for prokaryotic organisms and correlate with organismal complexity. PolyQ (light green) is present only in eukaryotic organisms. PolyL (light blue) is mostly restricted to Chordata. *P. falciparum*, *D. discoideum* and *R. filosa* are unicellular organisms with very unusual frequencies of homorepeats (the latter has three times more repeats than humans).

TRANSCRIPT REGULATION AND PREDICTION

We hypothesised that there are groups of genes that need to be repressed together and that this repression can occur in different cell types or states, both at the transcriptional level and at the post-transcriptional level, by a transcriptional repressor and a miRNA, respectively. We proved this assumption by observing significant enrichment of miRNA targets for particular miRNAs in the genes targeted by the neural repressor REST, as derived from multiple ChIP-seq experiments. The set of miRNA targets that overlap with REST targets was enriched in experimentally-verified targets and the corresponding set of miRNAs was significantly associated with the suppression of glioblastoma (a neural-related tumour). We have made the method available as a web tool (mBISON).

DATA AND TEXT MINING

Interaction between computational methods and human expert annotators is crucial for making better algorithms, but it is not easy to implement. We showed that the annotation of diseases with words describing their phenotypes by a clinician can be supported by a text mining procedure. We then showed that its application to a small number of neuropsychiatric disorders can be successfully expanded for the automated annotation of a larger set of disorders. This led to the discovery of novel associations between genes/drugs and disorders, as well as a correlation between a gene's functional class and the specificity of its association to the set of disorders.

As part of our increasing involvement in editorial practices, we edited a special issue on text mining of the biomedical literature with an introduction where we mention current copyright issues that obstruct text mining and how the apparently open access policies from publishers are sometimes not so open. Additionally, Jean-Fred Fontaine in our group is the editor of a new peer-reviewed journal, *Genomics and Computational Biology*, which covers all aspects of genome biology; the first issue was published in September 2015.

FUTURE DIRECTIONS

With our current work in genomics, we aim to increase the integration of gene expression and evolutionary data with growing amounts of new data on the 3D structure of the genome. In this way, we want to learn if the structural data is consistent with the genomic data, and understand further the evolutionary forces that shape genomic structure. Regarding protein structure and function, we want to further exploit the power of using clustered groups of protein families. This will allow us to answer questions regarding the evolution of protein interaction networks and the function of disordered regions in proteins (half of all proteins have at least one such region). In the field of data and text mining, we are striving to develop tools that use very complex data to find elegant solutions to answer very simple questions (e.g. do proteins X and Y interact? Does protein A bind gene B?). The ultimate goal is to bring computational analyses up to the level of expert human analyses to the normal user.

SELECTED PUBLICATIONS

Schaefer MH, Serrano L and Andrade-Navarro MA (2015). Correcting for the study bias associated with protein-protein interaction measurements reveals differences between protein degree distributions in different cancer types. *Front Genet*, 6, 260

Fontaine JF, Priller J, Spruth E, Perez-Iratxeta C and Andrade-Navarro MA (2015). Assessment of curated phenotype mining in neuropsychiatric disorder literature. *Methods*. 74, 90-96

Gebhardt ML, Reuter S, 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

PETRA BELI

"We employ mass spectrometry-based proteomics to understand the complexity of ubiquitin signalling in the nucleus."

POSITIONS HELD

Since 2013	Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz
2010 - 2013	Postdoc, The Novo Nordisk Foundation Center for Protein Research, University of Copenhagen

EDUCATION

2011	PhD in Biology, Goethe University Frankfurt
2007	Diploma in Molecular Biology, University of Zagreb

GROUP MEMBERS

Marina Borisova PhD Student; since 12/2013
Jan Heidelberger PhD Student; since 04/2014
Matthias Ostermaier PhD Student; since 05/2015
Andrea Voigt Lab Manager; 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. Protein phosphorylation and ubiquitination play an essential role in the regulation of the cellular response to DNA damage. 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

UBIQUITIN IN THE REGULATION OF PROTEIN HOMEOSTASIS ON CHROMATIN

We have previously shown that DNA damage induces site-specific ubiquitination and deubiquitination of DNA repair factors, highlighting the regulatory role of protein ubiquitination in processes that maintain genome integrity after genotoxic stress. Ku heterodimer binds DNA ends and promotes the repair of double strand DNA breaks by non-homologous end joining. Although components of the non-homologous end-joining pathway have been well characterised, the mechanisms that promote their dis-

sociation from repair sites remain poorly understood. In collaboration with the group of Steve Jackson (University of Cambridge), we have identified a ubiquitin-dependent mechanism that mediates the dissociation of the Ku heterodimer from repair sites. We have established that DNA damage induces ubiquitination of Ku heterodimer by Cullin-RING ligase CUL4, thereby leading to the extraction of the Ku heterodimer from chromatin after DNA repair has taken place. This study thus delivers insights into the mechanisms that underlie Ku dissociation from chromatin after repair and highlights a pervasive role of ubiquitin in the dynamic assembly and disassembly of protein complexes on chromatin after DNA damage.

IDENTIFICATION OF CELLULAR SIGNALLING INDUCED BY DNA REPLICATION STRESS

DNA replication is the predominant source of DNA damage in human cells and the mechanisms for protection and repair of damaged replication forks are essential for maintenance of genome stability and suppression of cancer. The slowing down or stalling of replication forks is commonly known as replication stress and arises from multiple causes such as DNA lesions, repetitive DNA sequences, RNA-DNA hybrids and oncogene activation. The ataxia telangiectasia and Rad3-related kinase (ATR) plays an essential role in the cellular response to replication stress and inhibition of ATR has emerged as a therapeutic strategy for the treatment of cancer. However, cellular signalling during replication stress is poorly studied and the complete spectrum of proteins that are targeted by ATR remains unclear. We employed quantitative mass spectrometry-based proteomics to define cellular signalling after nucleotide depletion-induced replication stress and replication fork collapse following ATR inhibition. We demonstrate that replication stress results in increased phosphorylation of a subset of proteins, many of which are involved in RNA splicing and transcription and have previously not been associated with the cellular replication stress response (Figure 1). Furthermore, our data reveal the ATR-dependent phosphorylation following replication stress and novel ATR target sites on MCM6, TOPBP1, RAD51AP1 and PSMD4. We have established that ATR inhibition rewires cellular signalling networks induced by replication stress and leads to the activation of the ATM-driven double strand break repair signalling.

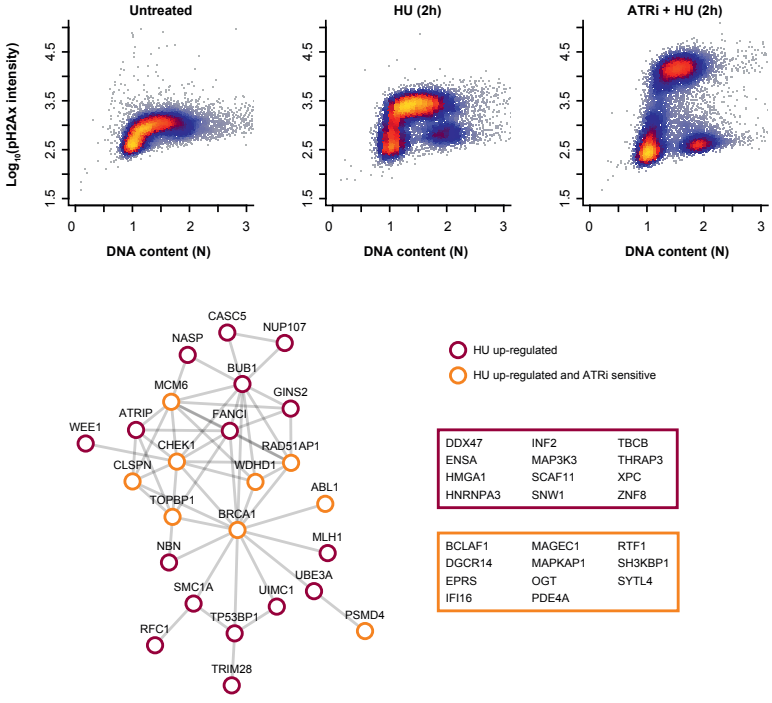


Figure 1. ATR inhibition increases DNA damage in U2OS cells after replication stress. The extent of DNA damage is analysed by monitoring the phosphorylation of histone variant H2AX by flow cytometry (upper panel). Analysis of functional associations among proteins with up-regulated phosphorylation sites after replication stress induced by nucleotide depletion. The proteins phosphorylated in an ATR-dependent or ATR-independent manner are colour coded in orange and red, respectively (lower panel).

FUTURE DIRECTIONS

Ubiquitin can regulate the function of proteins in the nucleus by promoting recycling or degradation of proteins through the ubiquitin-proteasome system. Components of the ubiquitin-proteasome system, mainly studied in the context of cytoplasmic processes, including co-chaperones and proteasome receptors, are also present on chromatin. Many of these proteins are dynamically recruited to lesion-flanking chromatin after DNA damage, thereby supporting the existence of protein quality control mechanisms in the nucleus that regulate the homeostasis of chromatin-associated proteins after cellular stress such as DNA damage. So far, little is known about the role of these proteins on chromatin as well as their interplay with ubiquitin ligases and deubiquitinating enzymes. Future efforts will be focused on studying the interplay between the ubiquitin-proteasome system and DNA repair on chromatin.

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*, 14, 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 for RNA processing factor THRAP3 in the 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 joint authorship

FALK BUTTER

"We apply innovative proteomics approaches to diverse organisms and biological areas."

POSITIONS HELD

Since 2013	Group Leader, Institute of Molecular Biology (IMB), Mainz
2010 - 2013	Postdoc, Max Planck Institute for Biochemistry, Martinsried

EDUCATION

2010	PhD in Biochemistry, Ludwig Maximilians University, Munich
2006	Diploma in Biochemistry, University of Leipzig

GROUP MEMBERS

Alina Bluhm PhD Student; since 01/2014
Hanna Braun PhD Student; since 08/2015
Núria Casas-Vila PhD Student; since 11/2013
Sabrina Dietz PhD Student; since 01/2015
Daniel Dowling PhD Student; since 11/2015
Anja Freiwald Engineer, since 04/2013
Merve Öztürk PhD Student; since 11/2015
Lara Perez PhD Student; since 10/2015
Marion Scheibe Postdoc; since 06/2013

OVERVIEW

Mass spectrometry is a powerful tool to study proteins in an unbiased and global manner. The current improvements in identification accuracy, sample throughput, and data analysis allow streamlined application of proteomics to answer diverse biological questions. Our group applies quantitative approaches, such as label free quantitation (LFQ), reductive dimethylation (DML) or stable isotope labelling with amino acids in cell culture (SILAC), 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 within a vast number of background binders. We apply mass spectrometry in several biological areas to advance our knowledge of cellular processes in a diverse range of organisms such as cattle, birds and fungi.

RESEARCH HIGHLIGHTS

PROTEOME COMPOSITION OF BOVINE GERM CELLS

In close collaboration with the Ketting group, which is interested in piRNA regulation, we applied label-free quantitative proteomics to compare protein expression levels in bovine sperm and oocytes. In single run mass spectrometric experiments, we quantified up to 5,359 proteins in three different cell types: oocytes, sperm and cumulus cells. We were able to show that one of the piwi proteins (PIWIL3) is strongly expressed in oocytes, while two other piwi proteins (PIWIL1 and PIWIL2) are more prominent in testis. Together with their functional characterisation of piRNAs, our proteomic analysis underscores the existence of a piRNA pathway

in the female germline of a mammalian species. The application of mass spectrometry as a generic method to detect protein expression, circumvents the requirement of affinity reagents and can be applied to non-classical model species such as cow.

TELOMERIC PROTEINS IN *NEUROSPORA CRASSA*

The ends of chromosomes consist in most cases of repetitive repeats that are maintained by telomerase and protected by telomere binding proteins from recognition by the DNA damage repair machinery. In contrast to *Saccharomyces cerevisiae* (baker's yeast) and *Schizosaccharomyces pombe* (fission yeast), which have degenerated TG-rich repeat telomeres, *Neurospora crassa* features TTAGGG repeat telomeres also found in vertebrates. Using label-free interactomics, we identified TTAGGG-binding proteins in this fungus and could demonstrate that at least six proteins in this species are able to bind directly to the telomeric sequence, among them evolutionarily conserved factors such as ncTbf1, ncTbf2 and ncTay1 (Figure 1). Additionally, we identified three currently uncharacterised proteins of which two (NCU02644 and NCU05718) are not conserved in yeast, hinting at possible unique telomeric features in TTAGGG repeat containing fungi, in comparison to the well-studied model systems *S.cerevisiae* and *S. pombe*.

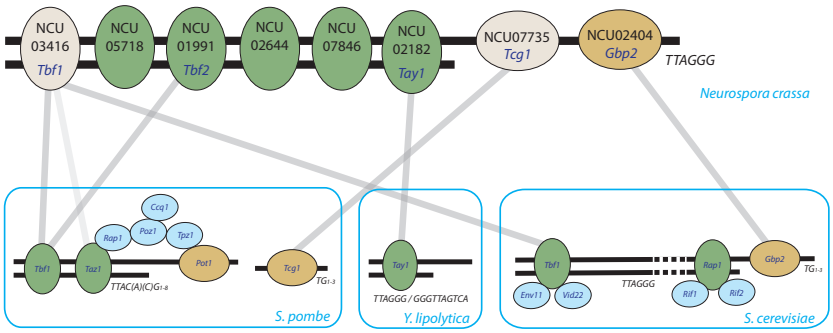


Figure 1. Comparison of protein homologues between three different yeasts and *Neurospora crassa* shows a set of homologous telomeric proteins between these species, but also non-conserved TTAGGG-binding proteins. As neurospora features the TTAGGG-repeat telomere also found in mammals, it may serve as an emerging model to study telomere regulation (figure from Casas-Vila *et al.*, 2015).

READING THE HISTONE CODE IN BIRDS

The DNA in eukaryotic cells is wrapped around small basic proteins called histones. The tails of these proteins are highly modified by a variety of posttranslational modifications such as methylation, acetylation and phosphorylation. These modifications influence the transcriptional activity of each gene. For example, methylation of lysine 4 of histone 3 is linked to active promoters while methylation of lysine 9 of this histone marks inactive genes. Extending previous studies that focused on the elucidation of the histone methyl interactome in human and mouse cells, we adapted this approach to study the interactome in avian cells. While the overall methyl interactome is strongly conserved between mammals and birds, the binding properties of individual proteins show a few distinct differences. While the mammalian N-PAC protein readily bound to the H3K36me3 modified peptide, its avian homologue showed no specificity for this modification using the same methylated peptide. This indicates at least biochemical differences for the recognition of the mark between protein homologues in different species.

FUTURE DIRECTIONS

We will continue to work on telomeric proteins in other organisms and try to understand the functional consequences of the difference in protein composition of the telosome. We also aim to extend our proteomic analyses to other species with the focus on a more systematic description of proteome and interactome evolution.

SELECTED PUBLICATIONS

Casas-Vila N, Scheibe M, Freiwald A, Kappei D and Butter F (2015). Identification of TTAGGG-binding proteins in *Neurospora crassa*, a fungus with vertebrate-like telomere repeats. *BMC Genomics*, 16, 965

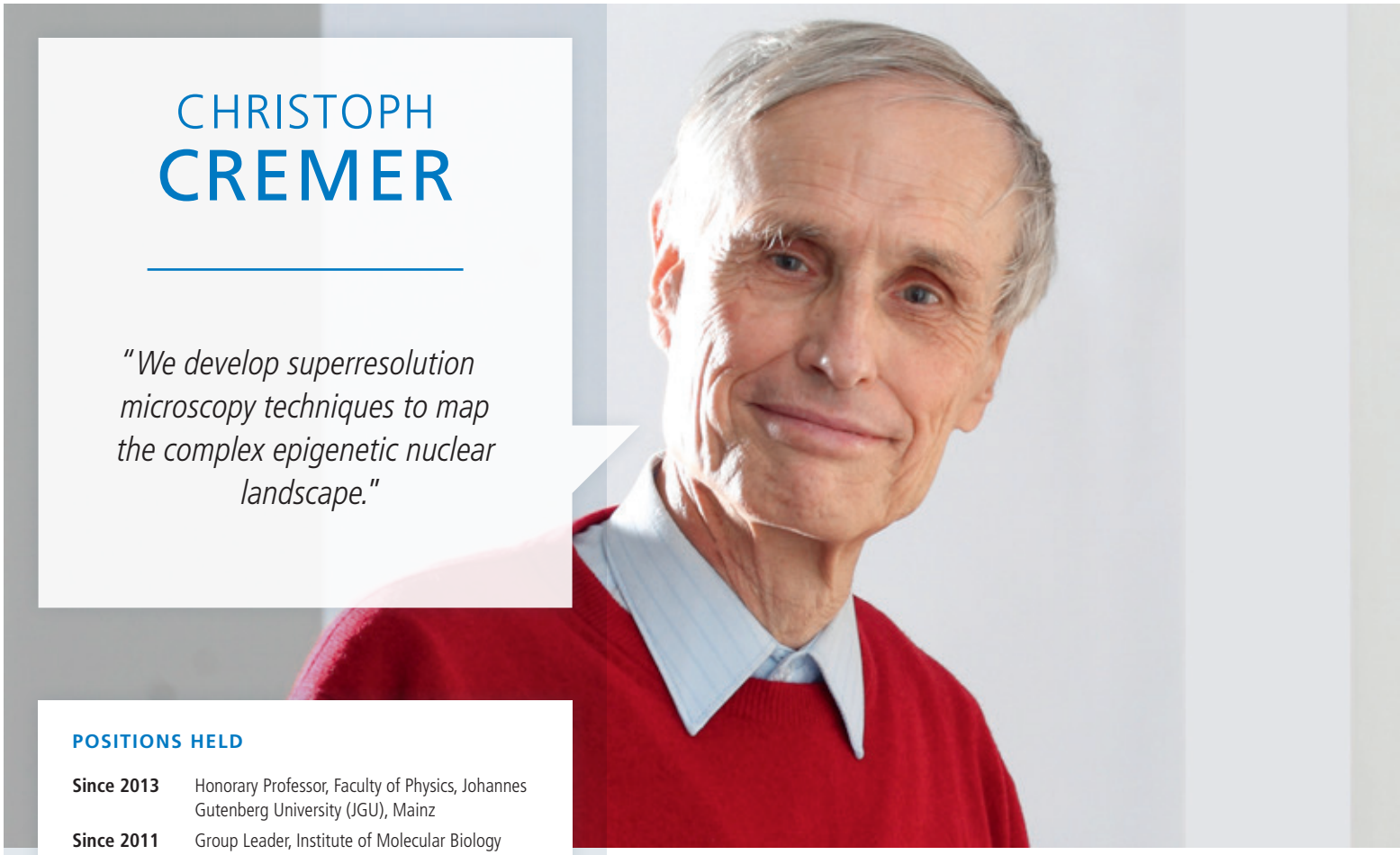
Viturawong T, Meissner F, Butter F* and Mann M* (2013). A DNA-centric protein-interaction map of ultra-conserved elements reveals major contribution of transcription factor binding hubs to conservation. *Cell Rep*, 5, 531-545

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 joint authorship

CHRISTOPH CREMER

"We develop superresolution microscopy techniques to map the complex epigenetic nuclear landscape."



POSITIONS HELD

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

EDUCATION

1983	Habilitation in Human Genetics, University of Freiburg
1976	PhD in Biophysics and Genetics, University of Freiburg
1970	Diploma in Physics, Ludwig Maximilian University, Munich

GROUP MEMBERS

Udo Birk Postdoc; since 02/2012
Gerrit Best Postdoc; 11/2014 - 06/2015
Songchao Cui Master's Student; 02/2015 - 09/2015
Amine Gourram Master's Student; since 06/2014
Jan Neumann PhD Student; since 03/2014
Kirti Prakash PhD Student; since 02/2013
Aleksander Szczurek PhD Student; since 09/2013
Jun Xing Master's Student; 03/2015 - 11/2015

OVERVIEW

The human genome has been decoded, but we are still far from understanding the regulation of all gene activities. A largely unexplained role in these regulatory mechanisms is played by the three-dimensional arrangement of the genetic material; i.e. the spatial organisation of the genome in the cell nucleus has far-reaching functional consequences for gene regulation. Since the molecular machinery of nuclear epigenetics works on the nanoscale, it is important to further develop tools to investigate such control mechanisms using spatial analyses at enhanced resolution in single cells. Until recently, it appeared to be impossible to achieve this goal by light microscopy. However, novel developments in optical imaging technology have allowed us to radically surpass the limited resolution of conventional far-field fluorescence microscopy (ca. 200 nm). In this novel field of super-resolution microscopy (SRM), our laboratory has made special contributions to the development of highly facilitated SRM approaches that use standard fluorophores to explore the functional nuclear nanostructure. This allows us to study the general distribution of DNA and its replication, of DNA and RNA oligonucleotides, of epigenetic histone markers and of proteins involved in replication, transcription, splicing and repair, down to the single molecule level.

RESEARCH HIGHLIGHTS

On the methodological side, we further developed the SRM technique of Spectral Precision Distance/Position Determination Microscopy (SPDM). SPDM, a variant of localisation microscopy,

makes use of conventional fluorescent proteins or single standard organic fluorophores in combination with standard (or only slightly modified) specimen preparation conditions, allowing to use the same laser frequency for both photoswitching and fluorescence read out of a given type of molecule. In particular, various standard DNA dyes were adapted to a highly efficient use in the SPDM analysis of nuclear DNA nanoscale distribution. In a single optical nuclear section, up to several tens of thousands of DNA sites/μm^2 can now be visualised, resulting in an unprecedented light-optical structural resolution of nuclear chromatin. SPDM also allowed sequence-specific mapping by fluorescence *in situ* hybridisation using probes only a few oligonucleotides long, as well as the cellular localisation of individual microRNA molecules. In 2015, these achievements were applied to perform quantitative analyses of the distribution of individual small chromatin domains. These included the nanoscale distribution of epigenetic histone markers and repair related factors, as well as of newly replicated DNA. Our experimental results support recent models of functional nuclear structure, which postulate that the nucleus contains spatially separated active and inactive compartments. As a novel biomedical application perspective, we applied dual-colour SPDM to monitor the environmental effects of ischaemia on chromatin nanostructure in cell nuclei of mouse cardiomyocytes (Figure 1). Short-term oxygen and nutrient deprivation (OND) induced a previously undescribed nuclear architecture, consisting of large, chromatin sparse voids interspersed between DNA-dense hollow helicoid structures of the order of 40 to 700 nm. The OND induced chromatin compaction was reversible, and upon restitution of normoxia and nutrients, chromatin transiently adopted a significantly more open nanostructure than in untreated cells, exemplifying the dynamic capacity of nuclear genome architecture to physically respond to environmental conditions. In another recent application, we combined dual-colour localisation microscopy with statistical evaluation methods to image at the nanoscale the epigenetic landscape of individual pachytene chromosomes of meiotic prophase

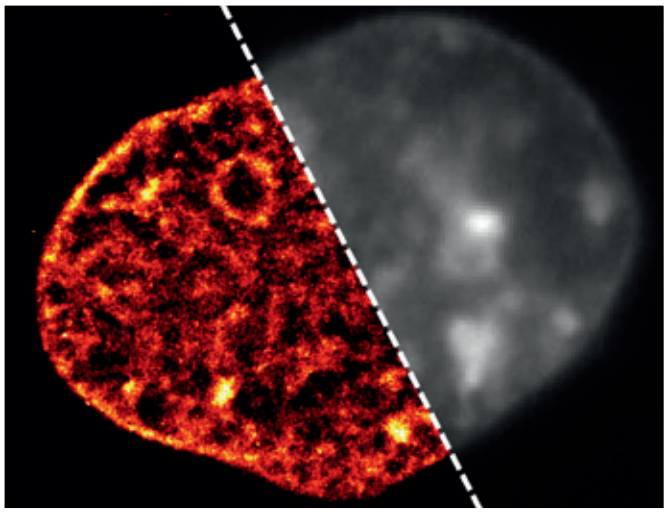


Figure 1. DNA in a myocardial cell nucleus taken with SPDM (left) or conventional widefield (right) microscopy. The superresolution SPDM technique reveals an organisation of DNA in the nucleus that is not apparent with conventional techniques.

in female mouse oocytes. The DNA was found to be non-randomly distributed along the length of the synaptonemal complex (SC) in condensed clusters. Periodic clusters of repressive chromatin were observed at 500-nm intervals along the SC, whereas one of the ends of the SC displays a large and dense cluster of centromeric histone marks. Chromatin associated with active transcription was arranged in a radial hair-like loop pattern emerging laterally from the SC. The SPDM-based findings indicate that the nanoscale structure of the pachytene chromosomes is constrained by periodic patterns of chromatin marks. These SRM approaches open an avenue to study the epigenetic landscape directly on the individual cell level at unprecedented spatial resolution. They allow us to validate predictions by molecular high throughput sequencing methods, and to perform quantitative tests of numerical models of nuclear genome nanostructure down to the single molecule level.

FUTURE DIRECTIONS

The methodological focus of the Cremer group will be to further improve SRM methods, in particular in terms of resolution, multicolour and *in vivo* imaging capability. In addition to localisation microscopy based approaches, this includes structured illumination microscopy and optical tomography techniques which are particularly suited for enhanced resolution imaging of extended tissue specimens. The SRM methods will be applied to study the epigenetic nuclear landscape in various cell types and organisms, including its relevance for genome stability and repair, cancer research and other disease conditions. This will be done in the context of collaborative projects with partners from IMB, Mainz University (JGU), the Max Planck Institutes for Chemistry and for Polymer Research (Mainz), Heidelberg University and Munich University (LMU), as well as the University of Michigan/Ann Arbor and the University of Washington/Seattle.

SELECTED PUBLICATIONS

Kirmes I, Szczurek A, Prakash K, Charapits I, Heiser, Musheev M, Schock F, Fornalczy K, Ma D, Birk U, Cremer C and Reid G (2015). A transient ischemic environment induces reversible compaction of chromatin. *Genome Biol*, 16, 246

Prakash K, Fournier D, Redl S, Best G, Borsos M, Tiwari VK, Ketting K, Tachibana-Konwalski K, Cremer C and Birk U (2015). Superresolution imaging reveals structurally distinct periodic patterns of chromatin along pachytene chromosomes. *Proc Natl Acad Sci USA*, 112, 47

Lang M, Jegou T, Chung I, Richter K, Münch S, Udvarhelyi A, Cremer C, Hemmerich P, Engelhardt J, Hell S and Rippe K (2010). On the three-dimensional organization of promyelocytic leukemia nuclear bodies. *J Cell Sci*, 123, 392-400

RENÉ KETTING

"We work on small RNAs, which are remarkably versatile regulators of gene activity."

POSITIONS HELD

Since 2015	Executive Director, Institute of Molecular Biology (IMB), Mainz
Since 2012	Scientific Director, IMB, Mainz
	Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz
Since 2010	Professor of Epigenetics in Development, University of Utrecht
2005 - 2012	Group Leader, Hubrecht Institute, Utrecht
2000 - 2004	Postdoc, Hubrecht Institute, Utrecht
2000	Postdoc, Cold Spring Harbor Laboratories

EDUCATION

2000	PhD in Molecular Biology, Netherlands Cancer Institute, Amsterdam
1994	MSc in Chemistry, University of Leiden

GROUP MEMBERS

Bruno de Albuquerque PhD Student; 10/2012 - 07/2015
Miguel Almeida PhD Student; since 06/2013
Walter Bronkhorst Postdoc; since 01/2015
Holger Dill Postdoc; since 02/2013
Antonio Domingues Postdoc; since 12/2014
Yasmin El Sherif Technician; since 10/2014
Lucas Kaaij Postdoc; since 11/2013
Monika Kornowska Animal Caretaker; since 03/2015
Svetlana Lebedeva Postdoc; since 05/2013
Maria Placentino PhD Student; since 11/2013
Stefan Redl PhD Student; since 04/2013
Anke Ries Lab Manager; 12/2012 - 11/2015
Ricardo Rodrigues PhD Student; since 12/2012
Elke Roovers PhD Student; since 07/2013
Patricia Rostan Master's Student; since 11/2015
Jan Schreier PhD Student; since 05/2015
Saskia Weiß Animal Caretaker; since 05/2012
Nadine Wittkopp Postdoc; since 11/2012

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 start 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). While we aim to work out regulatory networks involving miRNAs, we mainly focus on mechanisms related to piRNA and siRNA biology, two species of small RNAs that are particularly abundant in, and important for the germline. We do so mostly within the setting of (embryonic) development and are using both zebrafish and *C. elegans* as model systems for these studies. In addition, we aim to understand the conserved features of the mechanisms we are discovering and describing in these two model systems, by analysing small RNA pathways also in non-model system animals.

RESEARCH HIGHLIGHTS

PARENTAL SMALL RNA POPULATIONS ARE ESSENTIAL FOR FERTILITY

In the past few years, we have been analysing the effects of the piRNA pathway on the germline of *C. elegans*. Strikingly, whereas loss of piRNAs in most other animals results in acute sterility, in *C. elegans* this is not the case. Animals lacking piRNAs are still fertile, and do not show the major activation of transposable elements

that is observed in other species. We have described that piRNAs can induce a stably inherited chromatin state on targeted loci, and that this in fact can be maintained in absence of piRNAs. In these cases, the heritable silencing is completely taken over by a nuclear siRNA response. We reasoned that in *C. elegans*, transposons may be controlled by such heritable siRNA populations, and no longer by the piRNAs themselves. In order to test this, we genetically removed these inherited siRNAs, and asked whether in these animals, piRNAs would be required for transposon silencing. We found that this is indeed true, but to our surprise, we found that animals that lack inherited siRNAs, fully depend on piRNAs for fertility. This sterility is not due to activation of transposons, but to off-target activities of the zygotic siRNA machinery. Hence, inherited small RNA are not only required for a proper transposon-silencing response, but also to keep the transposon-silencing mechanisms away from genes that should be expressed.

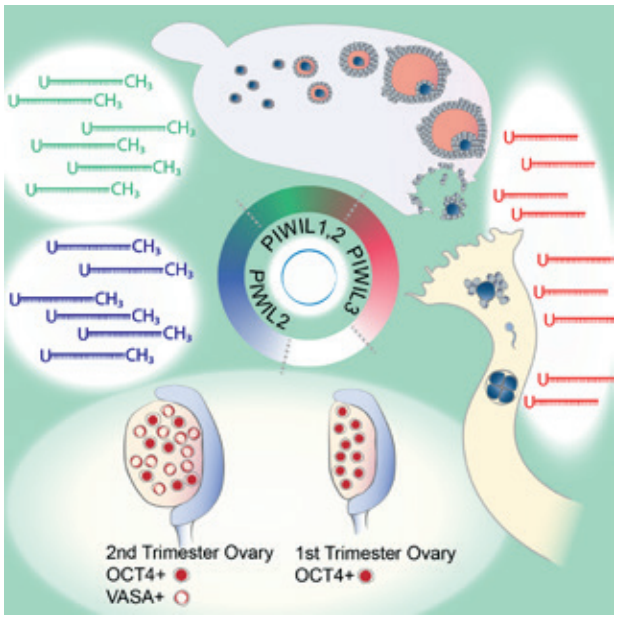


Figure 1. Diagram depicting the dynamic expression of Piwi proteins during mammalian oogenesis and development. Right: ovulated oocytes express Piwil3. Piwil3 is maintained during early development, but then disappears. Bottom and left: germ cells start to re-express Piwi proteins during the second trimester. In this embryonic stage only Piwil2 can be detected. Top: Oocytes in the adult ovary mainly express Piwil1. Piwil1- and Piwil2-bound piRNAs are methylated at their 3' ends, Piwil3-bound piRNAs are not.

PARENTALLY TRANSMITTED SMALL RNA IN MAMMALS

In the past year, we also performed experiments that address whether such parental inheritance of small RNAs occurs in mammals. In particular, we asked whether piRNAs are made in mammalian oocytes and whether they are loaded into early embryos. While previous reports have shown that in mice, piRNAs are expressed at very low levels in oocytes, and that they are not needed for female fertility, we found that piRNAs are abundantly expressed in oocytes from non-rodent mammals, such as pig, cow, macaque and human (see Figure 1 for summary). Interestingly, we found that piRNA populations change during oogenesis in these species. During early oogenesis, piRNAs are likely bound by the well-studied Piwi proteins Piwil1 and Piwil2, and the targets of these piRNAs are similar to those observed in the testis, meaning that they are not particularly enriched for transposon-sequences. However, during further oocyte maturation the piRNA repertoire changes drastically. The targets of these late oocyte-piRNAs become strongly enriched for transposons and they appear no longer bound by Piwil1 or Piwil2, but by the poorly studied Piwi protein Piwil3. We found that this Piwil3 protein is highly oocyte specific, suggesting a dedicated role for this Piwi protein during late oogenesis or early embryogenesis. Consistent with a role in embryogenesis, we have found piRNAs in early bovine embryos, obtained through *in vitro* fertilisation. These results indicate that parental loading of small RNAs into zygotes also occurs in mammals. Hence, trans-generational effects that are mediated by small RNAs, may not be restricted to model systems such as *C. elegans*, but may also occur in mammals, including humans.

FUTURE DIRECTIONS

Our future work will continue to mechanistically unravel the molecular pathways that are steered by small RNA guides. We are performing a genetic screen in order to identify novel factors, and are increasingly using biochemical approaches to start to describe the mechanisms on a more molecular level. We even plan to extend our studies to include structural biology, in order to be able to design specific point-mutations that disrupt specific aspects of the identified mechanisms. *C. elegans* and zebrafish will continue to play important roles in these studies. We will have a focus on chromatin-related effects of small RNAs and want to understand how and when a transient small RNA-mediated response can be transmitted into a stably inherited response. In addition, we will start to work on how small RNA pathways are connecting to other aspects of the cell's gene-regulatory programmes, including those active during germ cell specification and differentiation.

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

Kaaij LJ, van de Wetering M, Fang F, Decato B, Molaro A, van de Werken HJ, van Es JH, Schuijers J, de Wit E, de Laat W, Hannon GJ, Clevers HC, Smith AD and Ketting RF (2013). DNA methylation dynamics during intestinal stem cell differentiation reveals enhancers driving gene expression in the villus. *Genome Biol*, 14, R50

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 J*, 31, 3422-3430

JULIAN KÖNIG

"Our aim is to crack the splicing code."

POSITIONS HELD

Since 2013	Group Leader, Institute of Molecular Biology (IMB), Mainz
2008 - 2013	Postdoc, MRC Laboratory of Molecular Biology, Cambridge

EDUCATION

2008	PhD in Biology, Max Planck Institute for Terrestrial Microbiology and Phillipps University, Marburg
2003	Diploma in Biology, Ludwig Maximilian University, Munich

GROUP MEMBERS

Maximilian Bach Master's Student; since 07/2015
Simon Braun PhD Student; since 11/2013
Stefanie Ebersberger Postdoc; since 04/2014
Heike Hänel Lab Manager; since 11/2013
Andrea Hildebrandt PhD Student; since 04/2014
Reymond Sutandy PhD Student; since 12/2013

OVERVIEW

Posttranscriptional regulation of gene expression at the level of splicing and translation plays a critical role in development and tissue identity. Since these processes are often targeted during disease, their detailed investigation is fundamental to our understanding of human biology and disease.

The information in the RNA sequence and how it is read and interpreted by RNA-binding proteins (RBPs) is commonly referred to as the "ribonucleoprotein (RNP) code". Cracking this code and understanding the underlying regulatory networks remain long-standing goals of RNA biologists. To further these efforts, I previously developed the iCLIP technique, which provides genome-wide maps of protein-RNA interactions with single-nucleotide resolution. Building on this expertise, our group will help to decipher the RNP code using functional genomics approaches. Currently, we are addressing the following key questions: What is the role of *cis*-regulatory elements in alternative splicing? How do proteins act together in RNP assembly? How do RNPs control gene regulation in early development?

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 RNP 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 suggest them as 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. These highly abundant retrotransposons pose a great threat to transcriptome integrity as they can be erroneously recognised as exons by the splicing machinery in a process called *Alu* exonisation. 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.

INTERGENIC *ALU* EXONISATION CREATES NEW TRANSCRIPT 3' ENDS

Considerable attention has been paid to the interference of intronic *Alu* elements with splicing, however the effect of *Alu* elements in the intergenic regions in the neighbourhood of genes remained largely unexplored. We could recently show that the exonisation of intergenic *Alu* elements can introduce new terminal exons and polyadenylation sites from intergenic regions (Figure 1). These events are repressed in most tissues by the RNA-binding protein hnRNP C, thereby ensuring the formation of *bona fide* 3' ends. Surprisingly, the *Alu*-derived isoforms show considerable tissue-specific expression. Our comparative genomics analysis suggests that intergenic *Alu* exonisation shaped human transcript 3' ends during evolution.

At the mechanistic level, we observe the kinetic competition of *Alu* exonisation with splicing and polyadenylation of the preceding terminal exon. This means that the polyadenylation machinery must race to cleave at the canonical polyadenylation site before splicing at the *Alu* exon occurs. However, we find that polyadenylation is not fast enough to match the increased efficiency of intergenic *Alu* exon splicing upon hnRNP C knockdown. The resulting inclusion of intergenic *Alu* exons can change the composition of 3' UTRs, modifying posttranscriptional regulation of the newly emerging isoforms, e.g. at the level of transcript stability. Intriguingly, we provide evidence that intergenic *Alu* exonisation served as a mechanism to evolve new tissue-specific transcript isoforms. We thus present a novel mechanism by which transposable elements within intergenic regions can function in driving the evolution of 3' UTRs in the human genome.

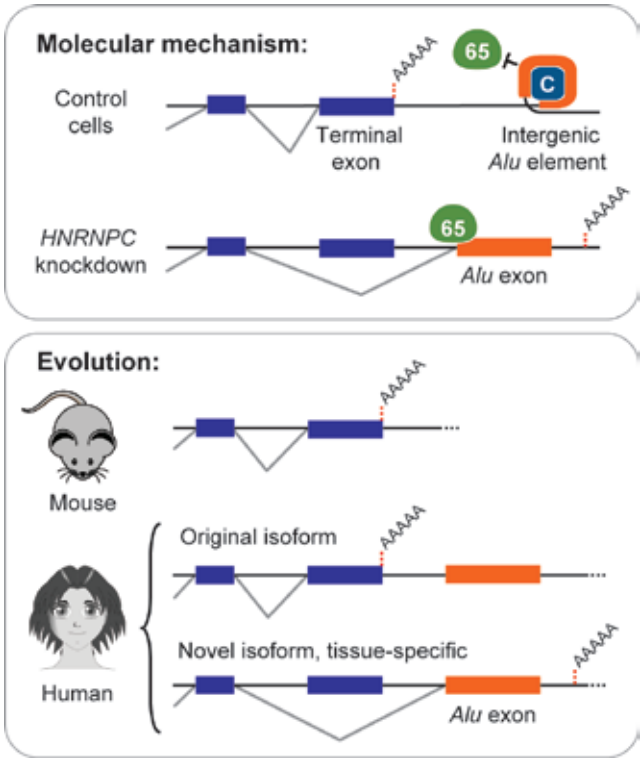


Figure 1. Intergenic *Alu* exonisation facilitated the formation of alternative transcript ends during human genome evolution. Under normal conditions, the terminal exon is correctly spliced. In the absence of hnRNP C, the splice sites of the intergenic *Alu* element are recognised by the splicing machinery to promote *Alu* exonisation. Comparison of the human and mouse genomes reveals human orthologues that were extended by an *Alu* exon during evolution.

FUTURE DIRECTIONS

The quantitative description of protein-RNA interactions with the iCLIP technology in combination with the clinically relevant model of *Alu* exonisation offer an attractive system for understanding the forces of competition and synergy that govern RNP 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 RNP 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 the complex and interactive environment within cells. These aspects will be addressed in the context of cryptic splice sites as they are present in *Alu* elements, which will be an important contribution to the understanding of genetic disease.

SELECTED PUBLICATIONS

Tajnik M, Vigilante A, Braun S, Hanel H, Luscombe NM, Ule J, Zarnack K[#] and Konig J[#] (2015). Intergenic Alu exonisation facilitates the evolution of tissue-specific transcript ends. *Nucleic Acids Res*, 43, 10492-10505

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

*indicates joint authorship
[#] indicates corresponding authors

STEFAN LEGEWIE

"We explore how robustness is maintained in biological systems."

POSITIONS HELD

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

EDUCATION

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

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 Technician; since 10/2012
Uddipan Sarma Postdoc; since 08/2012

OVERVIEW

Cellular processes are coordinated by networks of interacting molecules, and many diseases are caused by perturbations in these networks. Neither can be fully understood by studying single genes or proteins. To better understand cellular function in health and disease, my group analyses the dynamic interplay of cellular components with special emphasis on how cells sense environmental changes. One recent focus was to investigate how biological signalling networks function robustly despite internal and external fluctuations. We apply systems biology approaches, and combine mathematical modelling with quantitative experimental data from collaborators to understand the wiring and dynamics of signalling and gene regulatory networks.

RESEARCH HIGHLIGHTS

ROBUST TIMING OF EVENTS IN MITOSIS

Cellular signalling networks function reliably despite intracellular noise and fluctuating environments. We study design principles of signalling pathways that promote robustness. In our recent work, we cooperated with Silke Hauf (Virginia Tech, USA) to investigate how the temporal order of events is robustly maintained in anaphase.

The segregation of chromosomes and their subsequent distribution to the daughter cells are critical events that need to be completed in the correct order to maintain genome stability. Chromosome segregation requires the degradation of securin, whereas chromosome distribution relies on cyclin B degradation and involves a series of events collectively known as mitotic exit (Figure 1).

Using live-cell imaging in *S. pombe*, we found that chromosome segregation and cyclin B-dependent events are temporally coordinated with high precision even if the system is perturbed strongly.

A combination of modelling and experimentation revealed that robust temporal coordination is maintained by two complementary mechanisms (Figure 1). First, securin and cyclin B compete for a common degradation machinery, so that an excess of one protein slows down degradation of the other. Second, the threshold amount of securin that needs to be degraded for chromosome segregation to occur is not fixed, but adapts dynamically to variations in securin and cyclin B abundance. This adaptation allows the system to compensate for fluctuations in the kinetics of securin degradation. Taken together, we identified a mechanism that coordinates highly dynamic parallel pathways, thereby buffering timing against fluctuations in signalling proteins.

SINGLE-CELL DYNAMICS OF SIGNALLING AND TRANSCRIPTION

Single-cell datasets are often multivariate in nature and escape intuition. Thus, quantitative mathematical models are required to fully understand dynamic phenomena in single cells.

The TGFβ/SMAD signalling pathway plays a key role in tumourigenesis. Together with Alexander Loewer (MDC Berlin, Germany), we have monitored the nuclear translocation of SMAD2/4-GFP fusion proteins in thousands of living cells, and found that the phenotypic response of the cell is determined by the temporal dynamics of signalling. We have established a predictive mathematical model describing the average signalling response, and are currently extending this model to a quantitative description of heterogeneity. To this end, we derived sub-population models reflecting representative cells with qualitatively different dynamic behaviour. We are currently testing model predictions concerning the sources of heterogeneity by assessing fluctuations in signalling protein expression levels.

Transcription is 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 chromatin modifications which are typically only measurable at the cell population level using chromatin immunoprecipitation (ChIP). Together with the Reid group, we have established an imaging pipeline for visualising nascent transcripts in living MCF7 cells in response to estrogen stimulation using the PP7-PCP system, and perform ChIP assays under the same conditions. We have derived stochastic models of estrogen-dependent promoter regulation that describe the sequential progression through multiple ON and OFF states ("transcriptional ratchet"), and hope to infer the role of epigenetic events in single cell bursting.

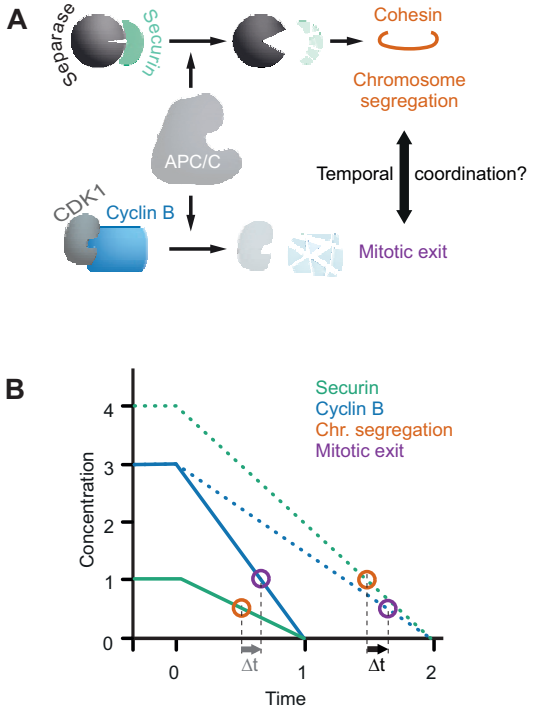


Figure 1. Robust temporal coordination of anaphase events. (A) The APC/C ubiquitin ligase triggers securin and cyclin B degradation. (B) Substrate competition for the APC/C temporally coordinates the degradation of securin (green lines) and cyclin B (blue lines) despite perturbations (solid lines: wildtype; dashed lines: securin overexpression). Robust temporal coordination (Δt constant) additionally requires that the thresholds of chromosome segregation (orange circles) and mitotic exit (purple circles) are not fixed, but adapt to the regulator degradation rate.

FUTURE DIRECTIONS

Cells can respond to external cues by inducing changes in gene expression. How these gene expression responses are coordinated by complex gene regulatory networks remains poorly understood. Together with the König and Tiwari groups, we are employing perturbation approaches and mathematical modelling to characterise the topology and the dynamics of transcription factor and splicing networks controlling oncogenic transformation. Using our models, we hope to develop effective intervention strategies in order to perturb tumour growth and metastasis.

SELECTED PUBLICATIONS

Kamenz J, Mihaljev T, Kubis A, Legewie S* and Hauf S* (2015). Robust ordering of anaphase events by adaptive thresholds and competing degradation pathways. *Mol Cell*, 60, 446-459

Kallenberger S, 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 Comput Biol*, 10, e1003421

*indicates joint authorship

BRIAN LUKE

"Non-coding RNA plays a surprisingly critical role at telomeres."

POSITIONS HELD

Since 2014	Group Leader, Institute of Molecular Biology (IMB), Mainz
2009 - 2014	Group Leader, Centre for Molecular Biology (ZMBH), University of Heidelberg
2005 - 2009	Postdoc, Swiss Federal Institute of Technology in Lausanne (EPFL)
2005	Postdoc, Biochemistry, Swiss Federal Institute of Technology in Zürich

EDUCATION

2005	PhD in Biochemistry, ETH Zürich
1999	BSc in Biology, Queen's University, Ontario

GROUP MEMBERS

Katharina Bender PhD Student; since 03/2015
Diego Bonetti Postdoc; since 03/2015
Marco Graf PhD Student; since 03/2015
Stefanie Grimm Technician; since 01/2015
Arianna Lockhart PhD Student; since 03/2015
Vanessa Kellner PhD Student; since 03/2015
Julia Klermund Postdoc since 08/2015
René Schellhaas PhD Student; since 03/2015
Tina Wagner PhD Student; since 11/2015

OVERVIEW

Telomeres are protective caps at the ends of linear chromosomes. When telomeres shorten, replicative senescence occurs as a result of DNA damage checkpoint activation. Cells that have undergone more divisions have a higher likelihood of becoming senescent. The accumulation of senescent cells may contribute to ageing in multi-cellular organisms. On the other hand, replicative senescence acts as a barrier to tumour development by limiting proliferative potential. Therefore cancer cells must overcome the checkpoint barrier and furthermore, re-elongate their telomeres, in order to achieve immortality. We are interested in understanding how cells transition through replicative senescence when short/dysfunctional telomeres arise and checkpoints become activated. We have observed that the long non-coding RNA, TERRA, has an important influence on senescence dynamics. Moreover, the 3D conformation of the telomere becomes altered at shortened telomeres. The downregulation of the checkpoint continues to be a focus in the lab. Checkpoint adaptation is one way that cells can overcome the DNA damage checkpoint. Our observations indicate that by altering nutrient conditions it is possible to influence the frequency of checkpoint adaptation events. Finally, we have demonstrated that TERRA also plays a role in the re-elongation of telomeres via homologous recombination following the escape from replicative senescence.

RESEARCH HIGHLIGHTS

We feel that we have made important steps forward in the understanding of TERRA regulation and function. We have now demonstrated that TERRA and R-loops specifically accumulate at short telomeres where they trigger homologous recombination and hence promote telomere length maintenance. This has the effect of preventing early onset senescence when critically short telomeres spontaneously arise. Moreover, we have added mechanistic details regarding how this regulation is achieved. Our results indicate that RNase H (an enzyme that resolves R loops) is only recruited to telomeres when they are long, and hence limits TERRA/R loop accumulation at these telomeres. As telomeres shorten, TERRA hybrids accumulate, in part, due to the diminishing local concentration of RNase H. These data have important implications for the regulation of replicative senescence.

When cells escape senescence they may re-elongate their telomeres via recombination, as is the case for ALT (Alternative lengthening of telomeres) cancer cells. We have now shown that when cells use the ALT mechanism they significantly up-regulate TERRA levels. We speculated that TERRA R loops may also promote telomere recombination in these cells. Upon RNase H overexpression, we were able to reduce telomere length in cells using ALT and subsequently impair their growth capacity. Importantly, the RNase H effects were only seen to affect ALT cells and not telomerase positive cells. Taken together, our results suggest that TERRA function depends on the state of the telomere. We propose that TERRA R loops promote recombination at critically short telomeres during replicative senescence, and through a similar mechanism maintain telomere length in cells using the ALT mechanism. In cells with "normal" length telomeres, we believe that TERRA levels are suppressed as recombination is not desired when length is sufficient (Figure 1).

Telomeres loop back onto themselves and form a lariat structure. Using the 3C (chromosome conformation capture) technique we have been able to monitor telomere looping in a quantitative manner. We have demonstrated that the telomere loop occurs in a recombination-independent manner. Furthermore we have shown that the loop can interact with multiple subtelomeric loci, up to 15 kbp away from the telomere. Importantly, the loop structure is lost as telomeres shorten, e.g. in telomerase negative cells. Furthermore we have demonstrated that both transcription (via RNA polymerase II) as well as RNA are required to maintain telomere loops in a similar manner to canonical gene loops. It will be interesting to determine if the RNA required for looping is TERRA. We speculate that TERRA transcription may trigger a loop structure, which limits further TERRA transcription, specifically at "normal" length telomeres. At short telomeres, this regulation would be lost and thereby allow increased TERRA transcription, which may account for the TERRA increase at short telomeres (see Figure 1).

Finally, we have now determined that nutrient limitation prevents checkpoint adaptation by promoting the degradation of the polo-like kinase (Cdc5). This occurs in a proteasome independent manner in the presence of persistent DNA damage and has important implications for genome stability and healthy ageing.

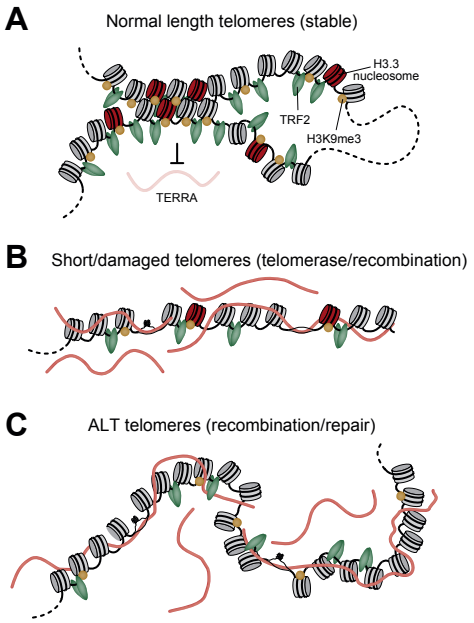


Figure 1. (A) At normal length telomeres, long-range telomere back-folding and physical interaction with other chromosome domains may help to ensure that TERRA levels are kept low. (B) At short/damaged telomeres TERRA and R-loops are upregulated, which increases the likelihood that they will be extended by recombination. (C) The features of ALT telomeres are correlated with high TERRA expression and telomere association, which may promote recombination through an R-loop intermediate. From Rippe and Luke (2015). *Nat Struct Mol Biol*, 22, 853-858

FUTURE DIRECTIONS

We continue to try to understand the function of telomere looping into the sub-telomere. It has previously been demonstrated that sub-telomeric genes become up regulated as telomeres shorten. We will test the hypothesis that telomere loops keep these genes silent when telomeres are long, but allow expression upon shortening due to loss of the loop structure. Furthermore, we will further investigate the relationship between TERRA and the telomere loop.

We will focus on the how TERRA is differentially regulated in telomerase positive cells compared to ALT positive cells. We will try to understand, in molecular detail, how TERRA and R-loops are regulated in a cell cycle manner and importantly, determine the biological relevance of such a regulation.

Lastly, we will continue to explore the relationship between chemotherapeutic agents and nutrient status, in both yeast and human cells. Our long-term goals are to propose improvements to current chemotherapeutics through dietary intervention.

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 Rep*, 9, 324-35

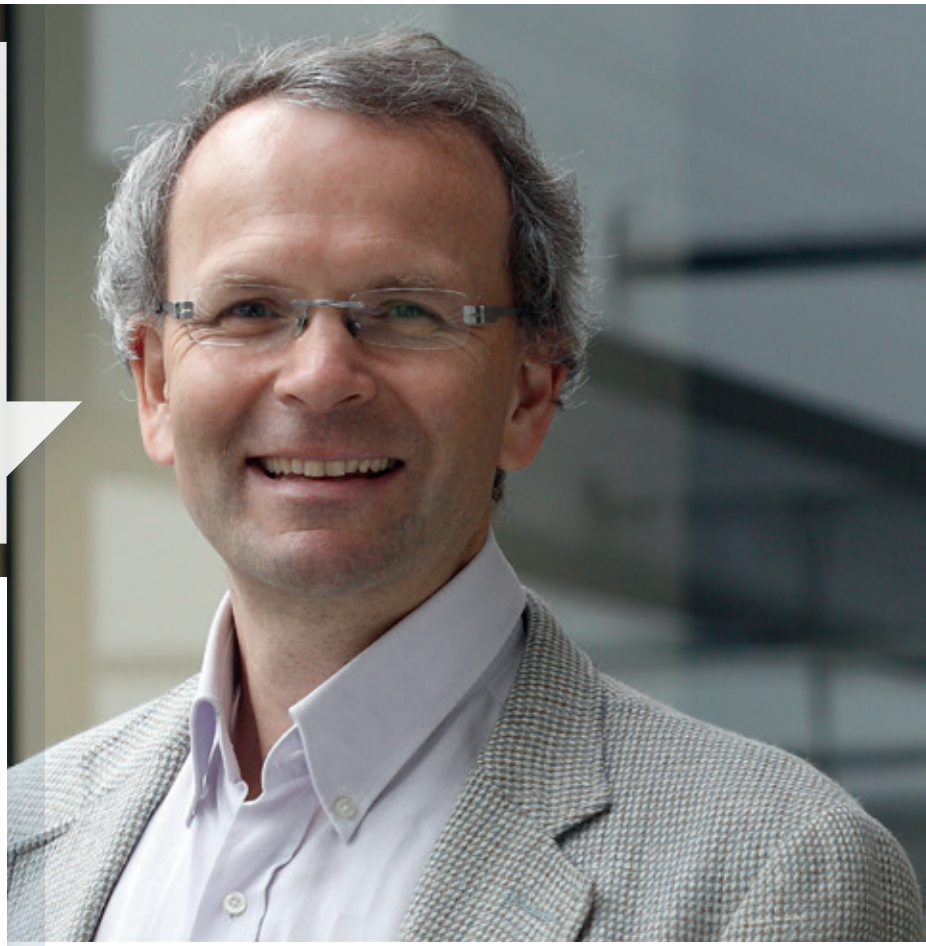
Balk B*, Maicher A*, Klermund J, Luke-Glaser S, Dees M, Bender K and Luke B (2013). Telomeric RNA-DNA hybrids affect telomere length dynamics and senescence. *Nat Struct Mol Biol*, 10, 1199-205

Poschke H*, Dees M*, Chang M, Kaderali L, Amberkar S, Rothstein R and Luke B (2012). Rif2 promotes a telomere fold-back structure through Rpd3L recruitment in budding yeast. *PLoS Genet*, 8, e1002960

*indicates joint authorship

CHRISTOF NIEHRS

"We study Neil DNA glycosylases, which pass on the baton during DNA demethylation."



POSITIONS HELD

2010 - 2015	Executive Director, Institute of Molecular Biology (IMB), Mainz
Since 2010	Founding & Scientific Director, IMB, Mainz
	Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz
2000	Professor of Molecular Embryology, German Cancer Research Center (DKFZ), Heidelberg
Since 1994	Head of Division „Molecular Embryology", DKFZ, Heidelberg
1990 - 1993	Postdoc, University of California Los Angeles (UCLA)

EDUCATION

1997	Habilitation in Biology, University of Heidelberg
1990	PhD in Biology, European Molecular Biology Laboratory (EMBL) and University of Heidelberg
1985	Diploma in Biochemistry, Free University of Berlin

GROUP MEMBERS

Khelifa Arab Postdoc; since 11/2011
Tamara Dehn Animal Caretaker; since 06/2011
Anna Luise Ernst PhD Student; since 04/2015
Dandan Han PhD Student; since 12/2013
Victoria Hatch Postdoc; since 11/2014
Laura Krebs Technician; since 09/2015
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
Sandra Rölle Technician; since 04/2011
Andrea Schäfer Postdoc; since 09/2010
Lars Schomacher Postdoc; since 07/2011
Carola Scholz Technician; since 05/2015
Ulrike Stapf Technician; 05/2011 - 07/2015
Viviana Vastolo Postdoc; since 09/2015
Annika von Seggern Technician; 06/2011 - 07/2015

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 and cell biological approaches, employing the mouse and frog model 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 a 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.

One prominent mode of enzymatic DNA demethylation involves oxidation of 5mC by the Ten-eleven translocation (TET) family of dioxygenases, which interact with Gadd45a in demethylation e.g. of the TCF21 tumor suppressor. Tet enzymes iteratively oxidise 5mC to form 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). The oxidation products act as intermediates in DNA demethylation that engage base excision repair (BER) to restore unmodified cytosines. BER is initiated by thymine DNA glycosylase (TDG), which recognises and excises higher oxidation products. A vulnerable intermediate in this TET/TDG demethylation is the abasic (AP) site. We have discovered that NEIL1 and NEIL2 DNA glycosylases coordinate AP site processing during TET/TDG DNA demethylation. In an siRNA screen intended to discover novel components of DNA demethylation, we identified the DNA glycosylases NEIL1 and NEIL2, whose knockdown inhibited the ability of HeLa cell extracts to excise 5fC and 5caC excision from oligonucleotides. NEILs are bifunctional enzymes, which excise the damaged base and introduce a DNA strand break via their AP-lyase activity. Interestingly, purified NEIL1 and NEIL2 neither process nor specifically bind 5fC or 5caC. Instead NEIL1 and NEIL2 stimulate 5fC and 5caC excision in the presence of TDG, which functions together with Tet enzymes in the currently best understood DNA demethylation pathway. Our data support a model where TDG and NEIL1/NEIL2 act in the coordinated substrate hand-over during the processing of 5fC or 5caC. TDG hydrolyses the modified base, and becomes displaced by NEILs, whose lyase activity cleaves the baseless sugar (AP site).

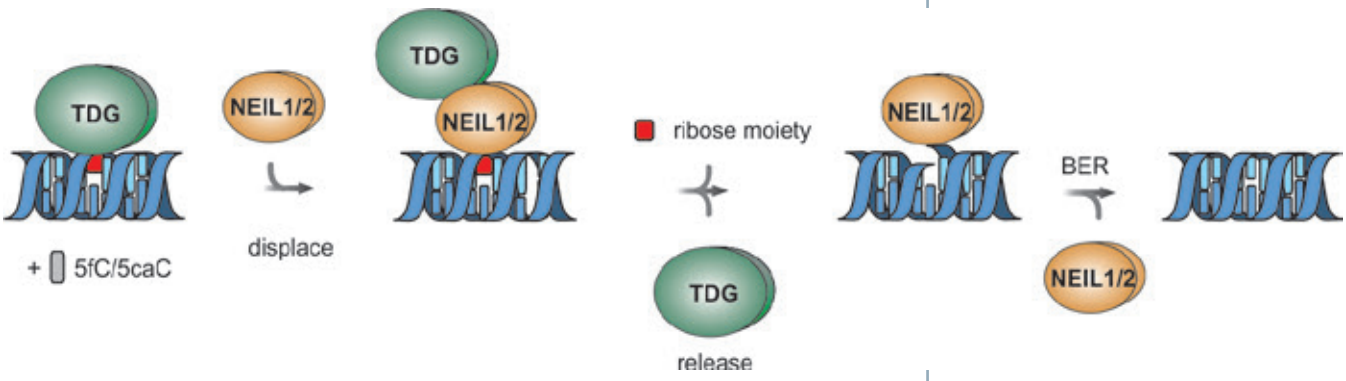


Figure 1. NEIL DNA glycosylases coordinate substrate hand-over during oxidative DNA demethylation. In this model for NEIL in TET/TDG-mediated DNA demethylation, TDG is product-inhibited and stalls after 5fC and 5caC excision at the resulting AP site (red). NEIL proteins displace TDG from the AP site by transiently contacting TDG and competing for AP site binding. The lyase activity of NEIL enzymes then generates a strand break, removing the sugar moiety and leaving behind phosphate termini at the former AP site.

Experiments in *Xenopus* embryos corroborate that neil2 cooperates with tdg to remove oxidised cytosines and to specify neural crest development together with tet3. Our results support the notion that the potentially harmful repair intermediates arising during TET/TDG/Gadd45 demethylation are managed by a dedicated mechanism involving NEILs. In this way NEIL glycosylases coordinate the substrate hand-over during oxidative DNA demethylation, where vulnerable intermediates are passed on between successively acting enzymes, like a baton is passed on in a relay race (Figure 1).

FUTURE DIRECTIONS

Our discovery of NEIL proteins as components of TET/TDG/Gadd45 demethylation raises new questions. Which target genes are regulated by NEIL proteins? What are the sequence determinants that recruit NEILs to specific genes during demethylation? What is the role of Neil-mediated demethylation in mammals? To investigate these questions, we will analyse mouse embryonic stem cells which are mutant for Neil1, 2, and 3. We plan to monitor oxidised cytosine levels in their genome, analyse DNA repair activity in Neil-mutant cells, analyse the differentiation capacity of Neil-mutant cells, and identify Neil target genes.

SELECTED PUBLICATIONS

Schomacher L, Han D, Musheev M, Kienhöfer S, von Seggern A and Niehrs C (2016). NEIL DNA glycosylases coordinate substrate hand-over during oxidative DNA demethylation. *Nat Struc Mol Biol*, doi: 10.1038/nsmb.3151

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

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

*indicates joint authorship

GEORGE REID

"We investigate chromatin architecture: the structural and topological product of epigenetic regulation."

POSITIONS HELD

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

EDUCATION

1988	PhD in Biochemistry, University of Strathclyde
1984	BSc in Biochemistry, University of Strathclyde

GROUP MEMBERS

Iryna Charapitsa Postdoc; since 07/2011
Christoph Fritzsch PhD Student; since 09/2012
Wolf Henning Gebhardt Postdoc; 08/2011 - 12/2015
Christina Heiser Master's Student; 01/2015 - 11/2015
Ina Caroline Kirmes PhD Student; since 06/2012

OVERVIEW

Our research focuses on developing an understanding of transcriptional regulation in contrasting conditions, in particular through exploiting the kinetics of estrogen induced changes in gene expression profiles, in chromatin marks and structure and in DNA methylation. We have three areas under investigation: the effect of estrogen on the transcription of estrogen-dependent genes; the effect of ischaemia upon chromatin compaction and transcriptional output; and the effect of a novel inhibitor that blocks the interaction of a sub-class of RNAs with the fragile X mental retardation class of proteins.

RESEARCH HIGHLIGHTS

The expression of estrogen-dependent genes is a dynamic, structured and cyclical process consisting of the assembly of intermediate transcription factors that in turn provoke conscription of the basal transcription machinery. These events then initiate activation of the polymerase II complex, which is subsequently followed by limitation of productivity through the action of repressive transcription complexes. These reset the target promoter, through acting on chromatin structure, such that a subsequent transcriptional cycle can be initiated. Transcription is therefore dependent upon *cis*-acting elements (DNA and nucleosomes) that either interact with, or are modified by, *trans*-acting factors. Induced local structural changes to chromatin encompassing regulatory elements of gene promoters include alterations in the positional phasing of nucleosomes, substitution by variant histones, post-translational modification of nucleosomes, changes in the methylation of CpG

dinucleotides and breaks in the sugar-phosphate backbone of DNA. One of our primary research aims is to determine the role of covalent modification of chromatin in driving a sequential progression of reversible interactions, which collectively act to regulate gene expression.

ISCHAEMIC CONDITIONS PROVOKE THE REVERSIBLE COMPACTION OF CHROMATIN

Cellular oxygen insufficiency, hypoxia, occurs in physiological and developmental processes and in disease, such as tumour growth, stroke and cardiac infarction. Hypoxia in pathological situations often results from ischaemia and is associated with a reduced availability of glucose. Cells detect, accommodate and adapt to changes in oxygen and nutrient availability, particularly through transcriptional, translational and metabolic responses. In conjunction with the Cremer group, we have employed super-resolution optical microscopy to describe the effects of ischaemia on chromatin nanostructure (Figure 1) and the resulting transcriptional capacity of chromatin during and upon recovery from ischaemia.

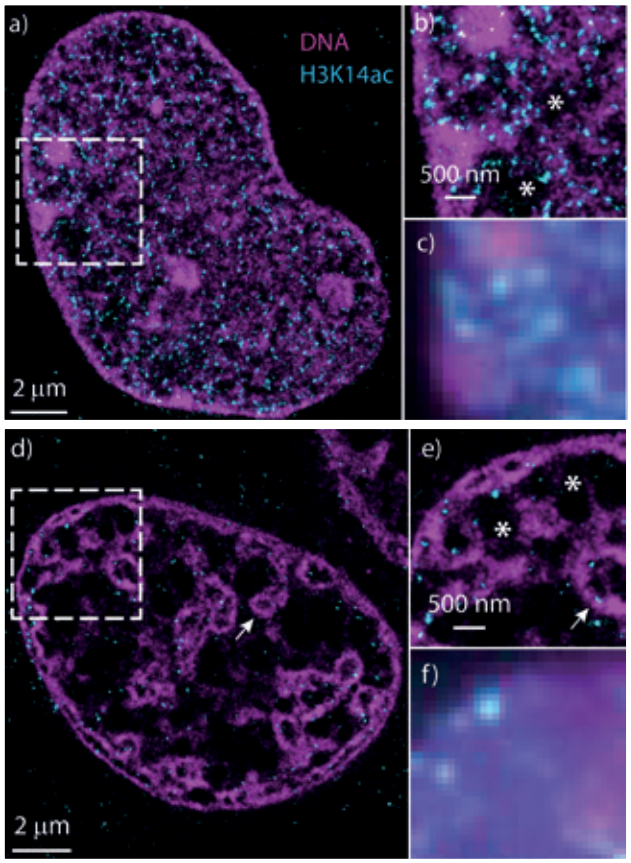


Figure 1. Oxygen and nutrient depletion (OND) induces compaction of chromatin. Cardiomyocytes were fixed, permeabilised, immunostained with anti-acetylated histone H3K14 and then with Vybrant DyeCycle Violet. Two colour single molecule localisation microscopy (SMLM) was performed on untreated cardiomyocytes (a and inset b) or on cells exposed for one hour of OND (d and inset e). Wide-field images of the inset regions are shown in c and in f (Kirmes *et al*., 2015).

THE DISCOVERY AND CHARACTERISATION OF ISOXAZOLES THAT MODULATE THE TRANSCRIPTIONAL OUTPUT OF CELLS

We performed a phenotypic screen to discover small molecules that could reinstate expression driven by a fully methylated promoter. This provided two sets of small molecules that achieve over-activation of methylated promoters. Alongside this phenotypic screen, we used multiple complementary approaches to characterise the response of cells to our compounds and to identify the molecular target interacting with these small molecules. These approaches include RNA-seq, candidate evaluation, a global shRNA screen and affinity purification using a biotinylated derivative of our series.

FUTURE DIRECTIONS

Analysis and perturbation of transcriptional bursting: We will optically observe labelled GREB1 loci, where the reporter is embedded either in the first or in the last transcribed exon. These will quantitatively describe transcription of the 160 kbp GREB1 locus. In conjunction with modelling, these datasets will provide a unique insight into transcriptional dynamics.

Modulating chromatin compaction, a new strategy to mitigate the consequences of catastrophic ischaemic events: Attenuating ischaemia-induced chromatin condensation could be beneficial in stroke and cardiac infarction. We have discovered that inhibiting HDAC activity prior to an ischaemic challenge reduces the extent of compaction and may offer interventions that limit the effect of ischaemia.

Completing definition of the molecular target of IMB363: We have shown that IMB363 has a major impact on cellular biochemistry, provokes extensive modification of chromatin, a general increase in transcriptional output and activation of several kinase signalling pathways. The interacting complex contains the RNA binding protein FXR1, in association with a number of known interaction partners. These discoveries allow exploration of the therapeutic potential interfering with the regulation of RNA stability.

SELECTED PUBLICATIONS

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Honkela A, Peltonen J, Topa H, Charapitsa I, Matarese F, Grote K, Stunnenberg HG, Reid G, Lawrence ND and Rattray M (2015). Genome wide modelling of transcription kinetics reveals patterns of RNA processing delays. *Proc Natl Acad Sci USA,* 112, 13115

wa Maina C, Honkela A, Matarese F, Grote K, Stunnenberg HG, Reid G, Lawrence ND, Rattray M (2014). Inference of RNA polymerase II transcription dynamics from chromatin immunoprecipitation time course data. *PLOS Comput Biol*, 10, e1003598

HOLGER RICHLY

"We aim to decipher the molecular mechanisms of epigenetic pathways."

POSITIONS HELD

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

EDUCATION

2005	PhD in Biology, Ludwig Maximilian University, Munich
2000	Diploma in Biochemistry, Ruhr University, Bochum

GROUP MEMBERS

Jonathan Byrne PhD Student; since 08/2011
Shalaka Chitale PhD Student; since 02/2013
Faranak Fassihianifard Master's Student; since 07/2014 - 03/2015
Johannes Geisinger Master's Student; since 10/2015
Ekaterina Gracheva PhD Student; since 08/2011
Aysegül Kaymak PhD Student; since 02/2013
Rebeca Medina 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. Our scientific approach relies largely on dissecting the function of diverse epigenetic players biochemically but also employs genetics and semi-automated screening techniques (Figure 1).

RESEARCH HIGHLIGHTS

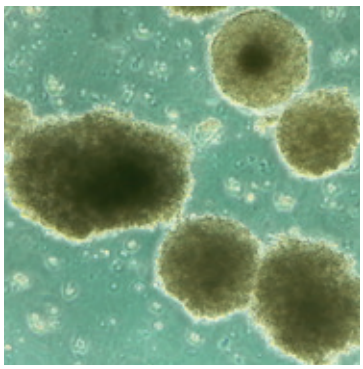
Epigenetic systems govern most cellular processes that take place in a chromatin environment, for example differentiation, DNA repair and replication. Our research provides evidence for how epigenetic factors act in concert with DNA repair factors. We have unravelled the identity and function of a novel E3-ligase complex that sets the mono-ubiquitin mark at lysine 119 of histone H2A in the course of nucleotide excision repair (NER). We provide further evidence that this epigenetic mark constitutes a recruitment platform for the H2A-ubiquitin binding protein ZRF1, which remodels DNA repair protein complexes at the damage site and thereby impacts on DNA repair. Additionally, we have delineated a sequence of E3 ligases operating at the DNA lesion site that form a ubiquitin signalling cascade, which eventually causes polyubiquitylation of the lesion recognition factor XPC. We are currently investigating the molecular function of ubiquitylated XPC and whether its particular ubiquitin chain represents a means of linking lesion recognition with the subsequent unwinding of damaged DNA. We have identified proteins that specifically read ubiquitylated XPC and we

are in the process of characterising their function in NER. Moreover, we have investigated the function of epigenetic factors downstream of DNA lesion recognition, involving the endoribonuclease DICER and setting of specific histone methylation marks.

Another interest of my lab is the sub-nuclear localisation of NER, which is controlled through DNA damage-dependent setting of histone marks. We have isolated and characterised a multi-protein complex, which catalyses the specific modification of histones at damaged chromatin upon UV irradiation. We have demonstrated that this specific histone mark is read by a tailor-made protein leading to accumulation of damaged chromatin at sub-nuclear structures and generation of DNA repair foci.

In parallel, we are interested in understanding the complex network regulating the activation of gene expression at the onset of cellular differentiation. To this end we have been studying the function of activating non-coding RNAs (ncRNAs) and epigenetic components during the epigenetic programming that occurs during stem cell differentiation. We have unravelled how ncRNAs, Mediator and Polycomb complexes together regulate the expression of genes during both pluripotency and differentiation of embryonic stem cells. Our research shows that at gene promoters gene silencing is a function of ncRNA-containing protein complexes, which are converted into gene-activators by chromatin remodelling machines during cellular differentiation. Altogether, our research indicates the importance of ncRNA-protein complexes in transcriptional regulation and their impact on the chromatin architecture.

A



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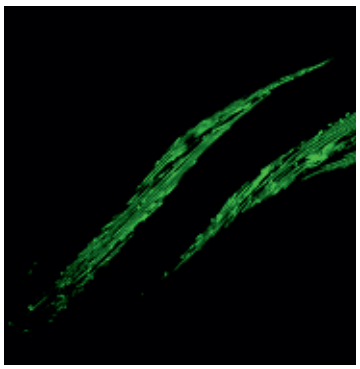

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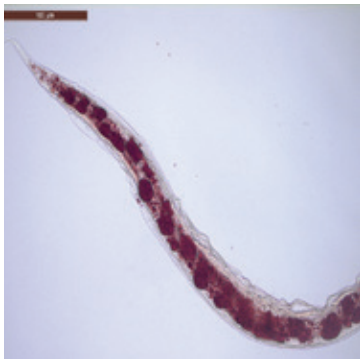

Figure 1.
(A) Embryoid bodies from mouse embryonic stem cells serve as a model system to study developmental processes *in vitro*.
(B) Visualisation of body wall muscles in *C. elegans* employing a GFP reporter strain.
(C) Deposition of lipids in aged animals is visualised by Oil Red staining.

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 to isolate novel factors involved in the ageing of the worms. We have analysed in detail the function and molecular mechanism of one epigenetic factor identified by the screen and we have started to investigate its impact on ageing in the fruit fly. In the near future, we will characterise the function of other factors using physiological assays and, more importantly, we will start to investigate how they reprogramme the epigenome in the course of ageing.

FUTURE DIRECTIONS

In the future, we will prioritise the research on DNA repair in the NER pathway and the investigation of organismal ageing. One of our main aims is to understand how epigenetic components and in particular ubiquitylation processes, govern DNA damage pathways. To that end, we will look into the function of epigenetic players in the partitioning of chromatin during DNA repair and into the underlying mechanisms of the nuclear localisation of NER. Furthermore, we will investigate the function of K63-linked polyubiquitylation in NER, which provides a means of recruiting repair factors. To this end, we will identify readers of specific polyubiquitin chains that decorate factors at the DNA damage sites. To extend our studies in organismal ageing, we plan to study how epigenetic factors and environmental cues extend the lifespan and the health span of *C. elegans*. In particular, we will study how the identified late life factors crosstalk with other ageing-related pathways. Further, we will use specific drugs to inhibit the function of the aforementioned factors to study their impact on the life and health span of the animals. We will also extend our studies employing the fruit fly *D. melanogaster* to identify conserved epigenetic mechanisms of ageing.

SELECTED PUBLICATIONS

Ribeiro J, Morey L, Mas A, Gutierrez A, Luis N, Mejetta D, Richly H, Benitah S, Keyes WM and Di Croce L (2012). ZRF1 controls oncogene-induced senescence through the INK4-ARF locus. *Oncogene*, 32, 2161-8

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*, 468, 1124-8

JEAN-YVES ROIGNANT

"We are studying epigenetics and epitranscriptomics, which are fundamental to unlocking the splicing code."

POSITIONS HELD

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

EDUCATION

2003	PhD in Developmental Biology, Jacques Monod Institute, Paris
1998	MSc in Developmental Biology, Paris IV
1997	BSc in Molecular and Cellular Biology, Rennes University

GROUP MEMBERS

Junaid Akhtar Postdoc; since 02/2012
Marc Bayer Master's Student; since 04/2015
Nadja Dinges PhD Student; since 03/2014
Annabelle Dold PhD Student; since 01/2015
Laura Götzinger Student Assistant; since 08/2013
Giriram Kumar PhD Student; since 04/2014
Tina Lence PhD Student; since 07/2013
Claire Mestdagh PhD Student; 01/2012 - 04/2015
Violeta Morin Technician; since 05/2015

OVERVIEW

The process of pre-mRNA splicing is crucial for the regulation of gene expression and generation of protein diversity. Most human genes undergo alternative splicing, and deregulation of this process is associated with a large number of disorders including cancer. Despite its relatively early discovery, the precision and complexity of intron removal is not yet fully understood. Recent work has shown that most splicing events occur co-transcriptionally, allowing novel layers of regulation by the transcription machinery, chromatin structure and RNA modifications. Using several approaches including molecular biology and classical genetics combined with high throughput techniques and computational tools, my lab investigates the contribution of epigenetics and epitranscriptomics to the regulation of pre-RNA splicing during *Drosophila* development. To address these questions, we are following two main angles: the first one aims to decipher the mechanisms of the exon junction complex in pre-mRNA splicing and the second deals with the m^{6}A RNA modification and its roles in RNA processing during *Drosophila* development.

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 on mature transcripts. The EJC controls several posttranscriptional functions, including RNA localisation, translation and nonsense-mediated decay. We have previously identified a novel nuclear role for the

EJC in preventing exon skipping in a subset of transcripts, many of which harbour large introns and are preferentially expressed from heterochromatin. A major goal in my lab is to characterise the underlying mechanisms of the EJC in pre-mRNA splicing. We have recently demonstrated that one mechanism by which the EJC controls the splicing of "weak" introns is by its prior deposition to flanking exon junctions after rapid splicing of *bona fide* introns. During its binding to maturing mRNAs, the EJC helps the recognition of neighboring introns that contain non-canonical splice sites via its splicing subunits RnpS1 and Acinus. However, the precise mechanism by which this function is achieved still remains to be discovered. In addition, we recently noticed that this mechanism does not explain all EJC splicing functions as knockdown of EJC core components gave additional splicing defects in comparison to knockdown of the splicing subunits RnpS1 and Acinus. We now provide evidence that one mechanism by which core components control splicing is by modulating the rate of transcription elongation. We found that the loss of EJC core components, but not of the splicing subunit RnpS1, is associated with global changes in nucleosome occupancy and in the phosphorylation state of RNA polymerase II (Pol II). We showed that pausing of Pol II at promoters was strongly reduced upon EJC knockdown. Furthermore, restoring pausing by knocking down the transcription elongation kinase CDK9 was sufficient to rescue *MAPK* splicing and the eye phenotype associated with the loss of the EJC. Thus, our data suggest a model in which the EJC maintains promoter-proximal pausing in order to facilitate splicing. This mechanism can be particularly important to ensure faithful splicing of challenging introns such as heterochromatic introns, which are usually large and rich in repeated elements.

FUNCTIONS OF m^{6}A RNA MODIFICATION IN *DROSOPHILA*

Analogous to DNA methylation and histone modifications, mRNA modifications represent another layer of epigenetic regulation of gene expression. The most common modification of mRNA in mammals is the N6-methyladenosine RNA (m^{6}A). m^{6}A is a highly dynamic modification that plays crucial roles in several physiological processes including germline development, embryonic cell differentiation, neurogenesis and the circadian clock. A multicomponent methyltransferase complex composed of at least three subunits (METTL3, METTL14 and WTAP) catalyses the methylation of adenosines on mRNA at consensus sequence RRACH. While m^{6}A was proposed to affect mRNA decay and alternative splicing, the exact mechanism of this modification in these processes still remains to be determined. We have started to characterise m^{6}A in *Drosophila* and shown that a conserved functional methyltransferase complex is enriched in the nervous system and contributes to different physiological functions. We have identified the target genes of the complex using RNA-seq and meRIP-seq (methylated RNA immunoprecipitation sequencing) and found that they are highly enriched for neuronal functions. Collectively, these results indicate that m^{6}A RNA modification in flies plays a major role in the nervous system, in part by regulating pre-mRNA splicing of key neuronal targets. The particular abundance of m^{6}A in the brain in vertebrates suggests that mRNA methylation is a conserved mechanism of neuronal mRNA regulation contributing to brain development and function.

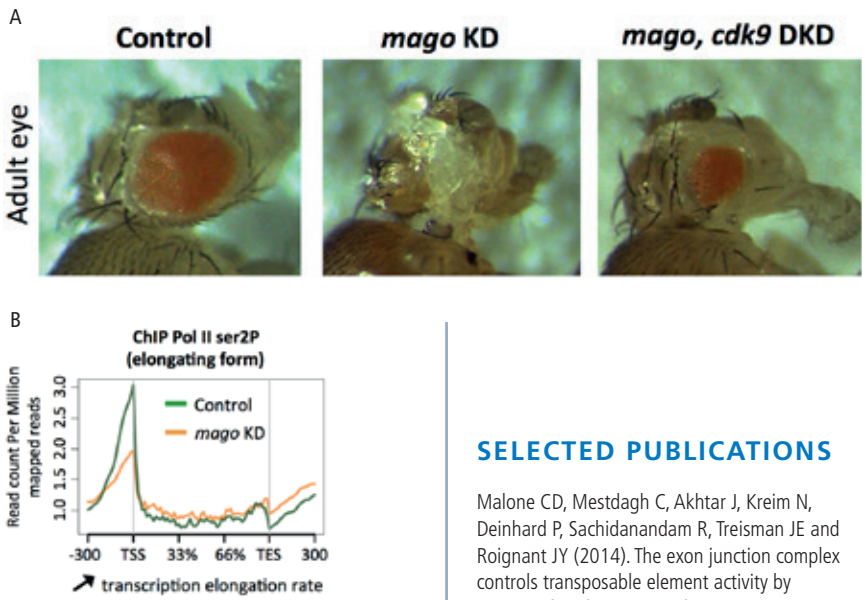

Figure 1. The EJC controls transcription elongation rate

(A) Adult *Drosophila* eye of wild type and *mago* knockdown. Loss of *mago* results in defects in photoreceptor differentiation, which is partially rescued upon simultaneous depletion of Cdk9.

(B) Metagene profile of ser2P level (elongating form of RNA Pol II) in control and mago-depleted cells. Loss of Mago results in a decrease of ser2P at transcription start sites and an increase along the gene body.

FUTURE DIRECTIONS

Our work has identified a critical role for the EJC in controlling transcriptional kinetics and demonstrated the importance of the m^{6}A RNA modification in alternative splicing and brain function. We plan to continue and extend our studies on the interplay of epigenetics, epitranscriptomics and pre-mRNA splicing. Specifically, we aim to understand the mechanism by which the EJC controls the rate of transcription elongation using cutting-edge molecular and biochemical approaches. In addition, we have initiated a genetic screen to identify new genes required in this poorly studied transcriptional process. Finally, we plan to pursue the characterisation of m^{6}A RNA methylation in *Drosophila* and its role in posttranscriptional gene regulation. Altogether, our studies have the potential to provide fundamental knowledge into the contribution of epigenetics and epitranscriptomics in pre-mRNA processing and will impact on our general understanding of gene regulation and diseases.

SELECTED PUBLICATIONS

Malone CD, Mestdagh C, Akhtar J, Kreim N, Deinhard P, Sachidanandam R, Treisman JE 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

VASSILIS ROUKOS

"We use high-throughput microscopy to shed light on the molecular and cellular mechanisms that safeguard genome integrity."

POSITIONS HELD

Since 2015	Group Leader, Institute of Molecular Biology (IMB), Mainz
2013 - 2014	NIH Research Fellow, National Cancer Institute, National Institutes of Health (NIH), Bethesda
2008 - 2013	Postdoc, National Cancer Institute, NIH, Bethesda

EDUCATION

2005 - 2008	PhD in Molecular Biology and Cytogenetics, Medical School, University of Patras
2002 - 2005	MSc in Applications in Medical Sciences, Medical School, University of Patras
1998 - 2002	BSc in Biology, University of Patras

GROUP MEMBERS

Marta Cipinska PhD Student; since 05/2015
Rossana Piccinno PhD Student; since 04/2015
Ulrike Stapf Lab Manager; since 07/2015

OVERVIEW

Maintaining the integrity of genetic information is essential for cell survival. Mechanisms that counteract DNA damage are important to help maintain cellular homeostasis by suppressing mutagenic events and genome rearrangements that may lead to disease, particularly cancer. One of the most severe forms of genome rearrangements are chromosome translocations, which represent the end product of a series of cellular mistakes after cells suffer from DNA double-strand breaks (DSBs) at different chromosomes. Rather than being accurately repaired, translocating DSBs are illegitimately joined to form aberrant fusions, which often show oncogenic potential. Although translocations have been extensively characterised using cytological methods and their pathological relevance in initiating several haematological malignancies and solid tumours is well established, how translocations form in the context of the intact cell nucleus is only poorly understood. Our research aims to shed light on the basic principles of translocation biogenesis by uncovering molecular pathways and cellular mechanisms that contribute to their formation.

RESEARCH HIGHLIGHTS

SPATIAL DYNAMICS OF CHROMOSOME TRANSLOCATIONS IN LIVING CELLS

Different models have been proposed to explain how chromosome breaks lead to tumorigenic chromosome translocations within the mammalian cell nucleus. However, the lack of a direct assessment using experimental systems that directly probe these events in living cells, has led to contradicting ideas. To shed light on the timing and sequence of the events that lead to the formation of chromosome translocations, we have established the first cell-based experimental system to visualise the formation of translocations in real time. We have employed high-throughput microscopy and automated image analysis that has enabled us to track individual chromosome double-strand breaks (DSBs) during the formation of these highly rare translocations with single-cell resolution (Figure 1). Using this approach, we have demonstrated that the rate of the formed DSBs, their motion properties, their illegitimate pairing and joining efficiency are important determinants of the observed translocation frequency.

We have used this system to monitor the spatial position of DSBs during the course of translocation formation. Our studies have shown that translocations are formed predominantly from spatially proximal DSBs, providing a definite and direct answer to this long-standing question. By tracking DSBs through time, we measured the motion properties of DSBs. Our analysis unravelled a faster component in the motion of DSBs destined to form translocations compared to non-translocating breaks, arguing for a key role of DSB-motility in the biogenesis of translocations. In combination with chemical inhibition or loss-of-function experiments for key players of the DNA damage response, we identified distinct DSB-repair factors as promoters or suppressors of chromosome translocations. Our findings provide a comprehensive spatial and temporal framework to understand the formation of chromosome translocations in living cells.

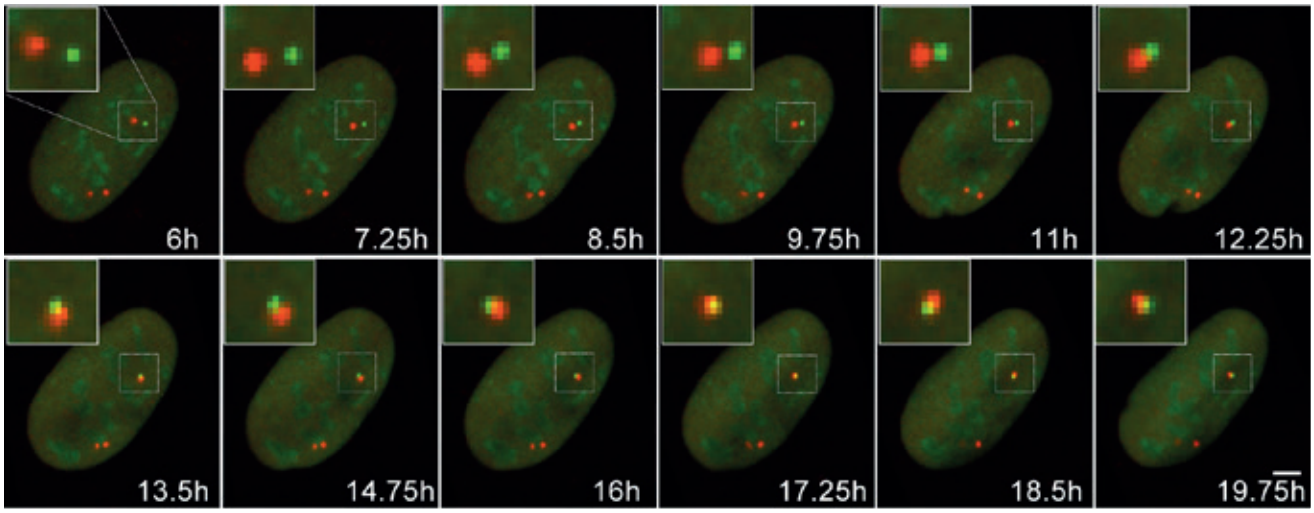

Figure 1. Rare chromosome translocations can be visualised in living cells as joining events where the two individual chromosome breaks (marked by red and green spots) become persistently superimposed as identified by automated image analysis. Scale bar: 5µm.

In parallel, we have developed novel imaging-based tools and analysis pipelines to combine the phenotypic analysis of breakage and translocations in single cells with the subcellular localisation and patterning of relevant proteins (e.g. repair foci), with the cell cycle stage of the cells (DAPI staining) or with expression analysis of relevant proteins. These multi-parametric imaging tools allow the in depth analysis of general mechanisms governing the formation of chromosome translocations *in vivo*.

FUTURE DIRECTIONS

Our goal is to use our established high-throughput microscopy tools in combination with novel recombineering approaches in unbiased and targeted screens to identify novel factors that govern key steps of the translocation process and systematically elucidate molecular pathways underlying their formation. Of particular interest to us is the identification of factors influencing the motion properties of DSBs or the interchromosomal synapsis with potential translocation partners. We are also in the process of developing novel high-throughput imaging-based methodologies that allow the modelling and quantification of rare, recurrent, cancer-initiating translocations frequently found in cancer patients. Our future research is now focused on identifying the molecular pathways underlying the formation of specific oncogenic chromosome translocations, which will advance our understanding of fundamental principles of cancer etiology.

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Roukos V, Pegoraro G, Voss TC and Misteli T (2015). Cell cycle staging of individual cells by fluorescence microscopy. *Nat Protoc*, 10, 334-48

Roukos V and Misteli T (2014). The biogenesis of chromosome translocations. *Nat Cell Biol*, 16, 293-300

Roukos V, Voss T, Lee S, Meaburn K, Wangsa D and Misteli T (2013). Spatial dynamics of chromosome translocations in living cells. *Science*, 341, 660-4

NATALIA SOSHNIKOVA

"We use intestinal stem cells to investigate the enigmas of embryogenesis."

POSITIONS HELD

From 2012	Group Leader, Institute of Molecular Biology (IMB), Mainz
2004 - 2011	Postdoc, University of Geneva

EDUCATION

2004	PhD in Molecular Biology, Humboldt University, Berlin
1998	Diploma in Molecular Biology, Novosibirsk State University

GROUP MEMBERS

Marius Baeken Master's Student; 02/2014 - 01/2015
Sandra Katharine Dauer Master's Student; since 07/2015
Margarita Dzama PhD Student; since 09/2014
Juri Kazakevych PhD Student; since 09/2012
Berith Messner Technical Assistant; since 03/2015
Lira Nigmatullina PhD Student; since 04/2013
Maxim Norkin PhD Student; since 08/2015

OVERVIEW

Our research addresses the principles underlying the formation of adult intestinal stem cells (ISCs). The adult small intestinal epithelium is composed of two compartments: differentiated villi and proliferating crypts. Rapidly dividing cells located within the crypt units constantly give rise to various terminally differentiated cells: absorptive enterocytes, secretory goblet, entero-endocrine, tuft and Paneth cells. Gene expression and lineage tracing studies revealed multiple markers of the adult ISCs and defined their position at the bottom of the crypts. In contrast to our broad understanding of the identity, location, and functional hierarchies between the adult ISCs, their developmental origin remained unknown. To understand how the ISCs are defined and integrated into the future stem cell compartment, we are investigating the functional and molecular properties of different epithelial cell populations within the embryonic small intestine. We use a combination of system-wide approaches, state-of-the-art mouse genetics, viral technologies and *ex vivo* 3D organoid assays to functionally characterise different epithelial cell populations within the embryonic small intestine during development, disease or injury.

RESEARCH HIGHLIGHTS

The embryonic small intestine is a simple tube composed of highly proliferative epithelial cells surrounded by layers of mesenchyme. However, little is known about functional and molecular heterogeneity of the embryonic intestinal epithelium. In mouse, at late foetal stages (by E15.5), the gut epithelium differentiates, which coincides with the formation of villi and intervilli (future crypts). Cells within the intervillus regions proliferate and begin to express adult ISC markers, suggesting that progenitors of the adult ISCs are established at E15.5 in the intervillus compartment. Although a few lineage-tracing studies had been performed previously, none of these studies were able to identify early embryonic progenitors of the adult ISCs. Moreover, little was known about the molecular mechanisms of adult ISC formation during embryogenesis.

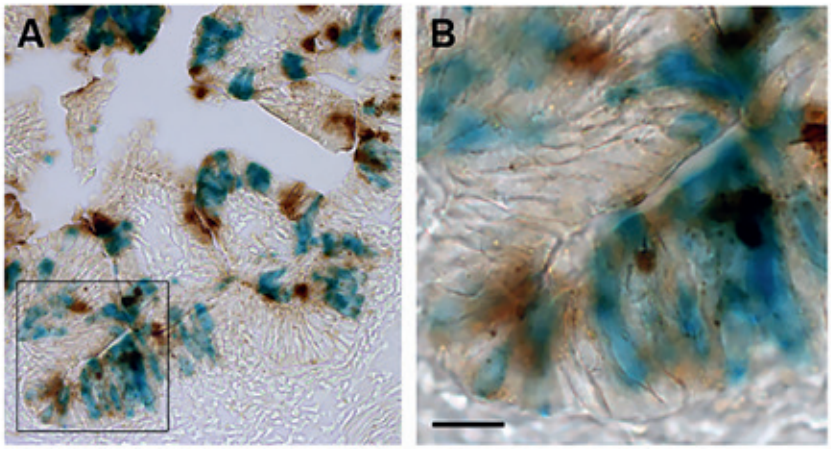

Figure 1. (A) A section of mouse small intestine stained for β-galactosidase activity (blue) and EGFP expression (brown) showing distribution of two different cell populations at E15.5. (B) Enlargement of the area boxed in panel (A). Scale bar: 40 μm (A), 10 μm (B).

We focused on the molecular characterisation of various embryonic epithelial cell populations, adult ISCs and their differentiated progenies using low cell number RNA-sequencing. Our results support a model in which the embryonic intestinal epithelium consists of transcriptionally heterogeneous cell populations, which are in turn different from the adult ISCs. To define the role of chromatin during ISC formation, maintenance and differentiation, we have generated genome-wide chromatin state maps for two embryonic stages (E12.5 and E14.5), adult ISCs, enterocytes and Paneth cells using ChIP and methyl-CpG binding domain (MBD) sequencing for low amounts of FACS purified cells. Specifically, we assessed the distribution of 5meC, H3K4me3, H3K27me3, H3K27Ac and H2Az marks. Clustering and meta-analysis of all transcriptome and chromatin data revealed potential functions for each chromatin mark in the establishment of ISC identity. Furthermore, it identified a large number of potential regulatory elements, including distal, proximal and intergenic enhancers.

To answer questions about the functional heterogeneity of the embryonic intestinal epithelium, we performed *in vivo* cell fate mapping analyses and *in vitro* organoid-forming assays (Figure 1). From these, we identified and characterised, at the molecular and phenotypic levels, distinct populations of embryonic intestinal epithelial progenitors that contribute to the adult ISC pool. Using a lineage tracing approach to fluorescently mark the different cell types of the embryonic intestinal epithelium, we defined the cellular hierarchy that controls intestinal development.

FUTURE DIRECTIONS

The presence of various cell populations within the embryonic intestinal epithelium suggests that they may be able to functionally substitute for each other *in vivo*. Currently, we are performing genetic cell ablation studies using mice expressing diphtheria toxin. Furthermore, we are establishing a hierarchical relationship between different progenitor cell populations within the embryonic gut by characterising them at molecular and phenotypic levels using RNA-sequencing and *ex vivo* 3D intestinal organoid cultures.

Transcriptome analysis of various cell populations isolated from E13.5 embryos provided us with a number of genes encoding transcription factors, receptors and ligands, which could be essential for the acquisition of the adult ISC identity. To test functions of the candidate genes, we are performing a phenotypic screen using organoid cultures. To genetically ablate the candidate genes we are applying the CRISPR/CAS9 technique using an inducible *Rosa26*$^{Cas9\text{-}P2A\text{-}EGFP}$ mouse model.

SELECTED PUBLICATIONS

Soshnikova N, Dewaele R, Janvier P, Krumlauf R and Duboule D (2013). Duplication of hox gene clusters and the emergence of vertebrates. *Dev Biol*, 378, 194-199

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

VIJAY TIWARI

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

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

Yael Fonseca PhD Student; 01/2015 - 08/2015
David Fournier Postdoc; since 07/2014
Angela Garding Postdoc; since 04/2012
Johannes Jung PhD student; since 05/2013
Markus Muckenhuber PhD student; since 01/2015
Abhijeet Pataskar PhD student; since 06/2014
Sanjeeb Kumar 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
Yuan Zhuang Technician; 09/2014 - 05/2015

OVERVIEW

Cell-fate specification during mammalian development involves extensive remodelling of gene expression programmes. The role of epigenetic mechanisms and transcription factors in this process has been increasingly appreciated. The research in our lab is aimed at understanding the mechanisms by which epigenetic regulators and transcription factors contribute to the 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

EPIGENETIC REGULATION OF NEURONAL DEVELOPMENT AND FUNCTION

The nervous system is the most complex organ in all mammalian organisms. The last decade has seen extensive research into how this complexity is generated during neuronal development. Despite exciting progress, very little is known about the function of epigenetic mechanisms in neurogenesis and neuronal activity. Using epigenetics, genomics and molecular biology tools in combination

with extensive computational biology approaches, we have been investigating the crosstalk of transcription factors with chromatin during specification of neuronal fate and the dynamics of epigenetic state during neurogenesis and neuronal activity. We recently discovered the gene regulatory programme through which a critical proneural transcription factor, NeuroD1, specifies neuronal fate. We further revealed how this function involves reprogramming the transcription factor and chromatin landscapes at its target sites (Figure 1). Our data further show that a transient activity of NeuroD1 during development is sufficient to induce a permanent neuronal gene expression programme that is stably maintained by epigenetic memory. In another study, we investigated the dynamics of the distal regulatory landscape during neurogenesis. Here we uncovered novel regulatory elements that function in concert with epigenetic mechanisms and transcription factors to generate the transcriptome-dynamics underlying neuronal development and activity.

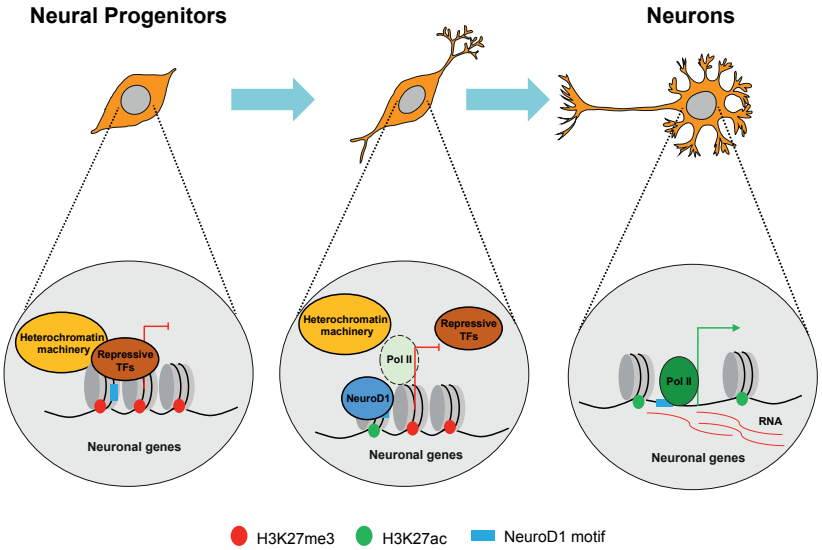

Figure 1. NeuroD1 reprogrammes chromatin and transcription factor landscapes to induce the neuronal programme. Upon onset of neurogenesis, NeuroD1 directly binds regulatory elements of neuronal genes that are developmentally silenced by epigenetic mechanisms. This targeting is sufficient to initiate events that confer transcriptional competence, including reprogramming of transcription factor landscape, conversion of heterochromatin to euchromatin and increased chromatin accessibility, indicating potential pioneer factor ability of NeuroD1. The transcriptional induction of neuronal fate genes is maintained via epigenetic memory despite a transient NeuroD1 induction during neurogenesis. From Pataskar *et al.*, 2015.

GENE REGULATORY MECHANISMS UNDERLYING EPITHELIAL TO MESENCHYMAL TRANSITION (EMT)

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. We find that the induction and progression of EMT involves massive transcriptional and epigenetic reprogramming. Interestingly, a number of transcription factors and epigenetic regulators are also upregulated during EMT. We also find that while the Smad pathway is required for initiation of EMT, the JNK pathway is required for the progression of phenotypic changes associated with EMT. Such dependency is the result of JNK-driven transcriptional reprogramming of critical EMT genes and changes in their chromatin state. Furthermore, we identified eight novel JNK-induced transcription factors that were required for proper EMT. Three of these factors were also highly expressed in invasive cancer cells, where they function in gene regulation to maintain mesenchymal identity. These factors were also induced during neuronal development and function in neuronal migration *in vivo*. These comprehensive findings uncovered kinetically distinct roles for various signalling pathways in defining the transcriptome that underlies mesenchymal identity and revealed novel transcription factors that mediate these responses during development and disease.

FUTURE DIRECTIONS

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. By employing a multidisciplinary approach, we next aim to generate a mechanistic insight into how these potential novel epigenetic regulators function in the gene regulation programme underlying neurogenesis. Furthermore, our recent findings have also uncovered a role for distinct signalling pathways in defining the transcriptome that specifies mesenchymal fate and revealed epigenetic mechanisms and a new repertoire of transcription factors that mediate these responses. We next plan on gaining a molecular understanding of the crosstalk between transcription factors and epigenetic machinery in organising the gene expression programme that drives cell-fate changes during EMT.

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Sahu SK, Garding A, Tiwari N, Thakurela S, Toedling J, Gebhard S, Ortega F, Schmarowski N, Berninger B, Nitsch R, Schmidt M and Tiwari VK (2015). JNK-dependent gene regulatory circuitry governs mesenchymal fate. *EMBO J*, 34, 2162-2181

HELLE ULRICH

"We are developing tools to dissect the consequences of protein polyubiquitylation."

POSITIONS HELD

Since 2013	Scientific Director, Institute of Molecular Biology (IMB), Mainz
	Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz
2004 - 2012	Group Leader, Clare Hall Laboratories, Cancer Research UK London Research Institute
2000 - 2004	Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
1998 - 2000	Postdoc, Max Planck Institute for Biochemistry, Martinsried
1997 - 1998	Postdoc, Centre for Molecular Biology (ZMBH), Heidelberg

EDUCATION

2004	Habilitation in Genetics, Philipps University Marburg
1996	PhD in Chemistry, University of California, Berkeley
1992	Diploma in Biology, Georg August University Göttingen

GROUP MEMBERS

Liliana Batista Postdoc; since 02/2013
Sabrina Batke PhD Student; since 11/2013
Heike Brinkman Lab Manager; 02/2013 - 11/2015
Karolin Eifler-Olivi Postdoc; since 10/2015
Stephanie Freiß PhD Student; since 11/2014
Néstor García-Rodríguez Postdoc; since 05/2013
Laure Gonzalez Postdoc; 04/2013 - 09/2015
Nataliia Gralievska PhD Student; since 01/2014
Christian Renz Postdoc; since 08/2014
Annie Sriramachandran Postdoc; since 03/2015
Laura Tomini Research Assistant; since 01/2015
Hanna Windecker Postdoc; since 05/2013
Hans-Peter Wollscheid Postdoc; since 09/2014
Ronald Wong Postdoc; since 04/2013
George Yakoub PhD Student; since 01/2015
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. We aim to understand 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

In eukaryotes, DNA damage bypass is activated via posttranslational modifications of the replication factor PCNA. While monoubiquitylation triggers mutagenic translesion synthesis by damage-tolerant DNA polymerases, attachment of a lysine (K)-63-linked polyubiquitin chain to PCNA is required for an error-free pathway of template switching. Hence, the balance between the two pathways has a significant impact on the overall fidelity with which damaged DNA is replicated.

Insight into the mechanism and regulation of PCNA polyubiquitylation is particularly important for understanding the choice between monoubiquitin-dependent translesion synthesis and polyubiquitin-dependent template switching. In an attempt to dissect the features of polyubiquitylated PCNA relevant for activating template switching, we have explored the use of linear fusions between ubiquitin and PCNA as mimics of genuine K63-polyubiquitylated PCNA. We had previously shown that attachment of a single ubiquitin moiety to the N-terminus of PCNA supports translesion synthesis in yeast cells, and that extension of this N-terminal ubiquitin to a K63-chain is sufficient to activate template switching. Hence, the attachment site of ubiquitin on PCNA is irrelevant for its activity in damage bypass. However, in the context of a linear polyubiquitin chain, variations in the chain length or the identity or length of the linker between the ubiquitin moieties did not support template switching. Likewise, mutation of the C-terminal amino acid of ubiquitin in order to vary the cleavability of the chain did not restore activity to the linear constructs. These negative results strongly imply the existence of an effector protein that recognises modified PCNA by means of a highly K63-selective ubiquitin binding domain. Alternatively, presence of the ubiquitin ligase (E3) Rad5 itself, whose catalytic RING domain is embedded into a SWI/SNF-like ATPase domain, at the site of PCNA polyubiquitylation could be required for proper activation of template switching.

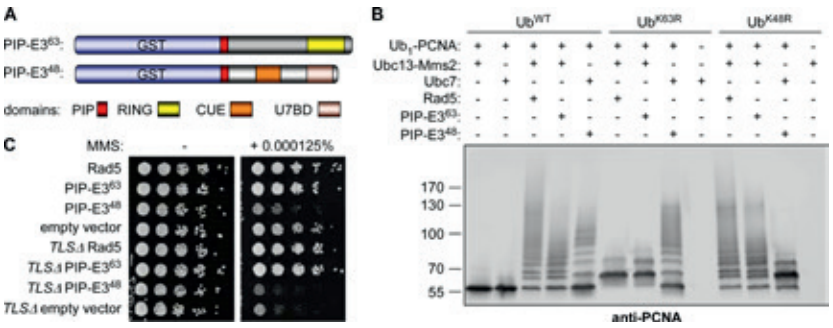

Figure 1. Design and function of PCNA- and linkage-specific ubiquitin ligases. (A) Domain structures of recombinant PIP-E3 constructs. PIP-E3^{63} harbors a Ubc13-Mms2-specific RING domain; PIP-E3^{48} contains parts of the Cue1 protein. PIP: PCNA-interacting peptide; U7BD: Ubc7-binding domain. (B) PIP-E3^{63} and PIP-E3^{48} polyubiquitylate Ub_1-PCNA in a linkage-specific manner *in vitro*, cooperating with Ubc13-Mms2 and Ubc7, respectively. (C) PIP-E^{63}, but not PIP-E3^{48}, complements the methyl methanesulfonate (MMS) sensitivity of a *rad5* deletion in a translesion synthesis (TLS)-dependent manner.

In order to differentiate between these models, we designed simplified analogues of Rad5 by fusing a PCNA-interacting peptide to unrelated E3 domains capable of assembling polyubiquitin chains of defined linkages (Figure 1A). In this manner, we managed to produce tailor-made enzymes for K63- and K48-specific polyubiquitylation of PCNA, whose activity is dependent on prior PCNA monoubiquitylation (Figure 1B). Importantly, the K63-specific E3 successfully complements the damage sensitivity of a *rad5* deletion mutant, indicating that Rad5 can be replaced by an artificial enzyme without ATPase activity (Figure 1C). On the other hand, the K48-specific E3 not only failed to activate template switching, but further sensitised the *rad5* mutant in a translesion synthesis-dependent manner, indicating an inhibitory effect (Figure 1C). Given that K48-linked polyubiquitylation usually serves as a signal for proteasomal degradation, depletion of the monoubiquitylated form of PCNA is the most likely explanation for this phenomenon. Our observations thus indicate that indeed the K63-linkage rather than the presence of Rad5 is required for activation of template switching. In a more general context, we have for the first time unambiguously shown that polyubiquitin chains of different linkages, assembled at the same target site, confer distinct biological consequences for the modified protein.

FUTURE DIRECTIONS

Having demonstrated the strikingly different effects of K63- and K48-linked polyubiquitin chains on PCNA *in vivo*, we will now attempt to generalise our findings by designing additional ubiquitin ligases, capable of assembling even different linkages and potentially targeting other model substrates. At the same time, we will use our insight in order to identify potential downstream effectors of template switching. While a number of candidates have been postulated to fulfil this function based on their ability to interact with polyubiquitylated PCNA, we found that none of them are selective for K63-linked chains. In order to identify such factors, we will therefore use our tailor-made K63-specific E3 for producing large amounts of recombinant polyubiquitylated PCNA in order to directly isolate relevant interaction partners from yeast and mammalian cell extracts and identify them by mass spectrometry. In this manner, we expect to gain insight into how template switching is controlled and activated.

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 study molecular links between circadian clocks, metabolism and epigenetics."

POSITIONS HELD

Since 2013	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz
2012 - 2013	Group Leader, Ludwig Maximilan University, Munich
2009 - 2011	Group Leader, Max Planck Institute for Biochemistry, Martinsried
2000 - 2009	Group Leader, Max Planck Institute for Molecular Physiology, Dortmund
1996 - 2000	Postdoc, Rockefeller University, New York

EDUCATION

2007	Habilitation in Biochemistry, Ruhr University, Bochum
1996	PhD in Biology, European Molecular Biology Laboratory (EMBL), Heidelberg
1991	Diploma in Biology, University of Heidelberg

GROUP MEMBERS

Archit Garg PhD Student; since 11/2014
Tim Grimmelsmann PhD Student; since 01/2015
Silke Helmke Technician; since 05/2014
Markus Kriebisch Technician; since 01/2014
Torsten Merbitz-Zahradnik Postdoc; since 01/2014
Roberto Orru Postdoc; since 10/2015

OVERVIEW

Most organisms have circadian clocks, which coordinate essential physiological functions with the environmental light-dark cycle. Circadian clocks are operated by gene regulatory feedback loops. In mammals, the transcription factor complex BMAL1/CLOCK activates the clock genes cry1,2 and per1,2,3, as well as many clock-controlled genes, which orchestrate mammalian physiology in a day-time dependent manner. CRY proteins act as potent repressors of BMAL1/CLOCK. Additionally, epigenetic factors affect the activity of clock and clock-controlled genes, with different epigenetic modifiers being active at different times of the day. Accumulating evidence, from us and others, suggests that mammalian CRYs also play important roles in coordinating circadian clocks with physiology and metabolism. The connection between circadian clocks and cellular metabolism plays important roles in human physiology and health, as it underlies the resetting of our peripheral body clocks by food intake. Irregular food intake arising, for example, from frequent shift work or jet lag misaligns our body clocks and thereby favours diseases such as cancer, metabolic syndrome and obesity. In the past year, we have continued our work on the mammalian Cryptochromes as metabolic sensors and transcriptional repressors of BMAL1/CLOCK and started to work on epigenetic activators of the BMAL1/CLOCK transcription factor complex.

RESEARCH HIGHLIGHTS

MAMMALIAN CRYPTOCHROMES AS TRANSCRIPTIONAL REPRESSORS OF BMAL1/CLOCK AND LINKERS BETWEEN CIRCADIAN CLOCKS AND CELLULAR METABOLISM

Our crystal structure of the mouse Cryptochrome1-PERIOD2 (CRY1-PER2) complex suggested that formation of this central clock protein complex is affected by the cell's metabolic state via the regulation of disulphide bond formation and zinc binding (Figure 1). This poses several new questions: Are disulphide bond formation in CRY1 and cellular zinc concentrations fluctuating in a daily regulated manner? Does Flavin Adenine Dinucleotide (FAD) – a light-absorbing cofactor of photoreceptor-type Cryptochromes – play a role in mammalian CRY1-dependent redox sensing? If yes, is there an interplay between zinc binding and redox changes of the disulphide bond and FAD? Do reactive oxygen species (ROS) play a role in CRY redox sensing and signalling? To define the roles of Cryptochromes in metabolic sensing and to facilitate the design of CRY-based drugs against clock-related diseases, we are further analysing the molecular mechanisms underlying CRY1-PER2 complex formation and CRY1 redox changes, also in presence of FAD, using X-ray crystallography, UV-VIS and fluorescence spectroscopy as well as mass spectrometry. Preliminary studies suggest that FAD binds to CRY1 and the CRY1-PER2 complex with a weak (µM) affinity and can change its redox state in a reversible manner. Crystallographic analyses of FAD complexes will shed light on conformational and redox changes that may be correlated with FAD binding.

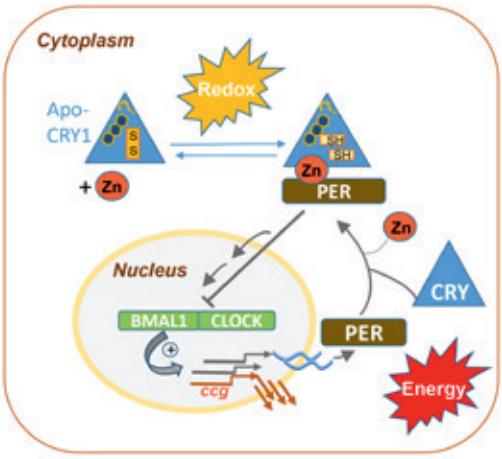

Figure 1. Metabolic, transcriptional and epigenetic regulation of the mammalian circadian clock. CRY1-PER2 complex formation is regulated by zinc binding, disulphide bond formation in CRY1 and potentially FAD ( ). This defines CRY1's transcriptional repression activity towards BMAL1/CLOCK, in accordance with the cell's metabolic state.

Moreover, our CRY-PER crystal structure showed that PER binding to CRY1 occupies essential binding sites of the BMAL1 transcription factor, suggesting that PER has to be displaced before CRY can act as a transcriptional repressor. We are now pursuing the structural analyses of CRY-BMAL1 complexes in order to understand the alternative interactions of mammalian CRYs with PER as a stabilising binding partner and with BMAL1 during the transcriptionally repressive state.

EPIGENETIC REGULATION OF CIRCADIAN CLOCKS

While daily fluctuations in the occurrence of certain histone modifications have been described, it is unknown how, at a molecular level, epigenetic regulators such as histone acetyltransferases or (de)methylases affect circadian gene expression of clock- and clock-controlled genes. Initial insights, obtained by us and others, suggest that BMAL1-acetylation enhances CRY1 binding during the repressive phase, while p300/CBP displaces CRY in the active state. In the early transcriptional repression phase (about CT12-CT20), large complexes including CRY, PER and other gene silencing factors, which affect chromatin structure and transcription termination, are recruited to BMAL1/CLOCK.

To provide the missing mechanistic insights into epigenetic control of circadian rhythms, we are pursuing the structural analyses of interactions of the BMAL1/CLOCK transcription factor complex with selected epigenetic regulators. We will compare these structures with 3D-structures and biochemical properties of the repressive mCRY-mCLOCK/BMAL1 complex.

FUTURE DIRECTIONS

A deeper mechanistic understanding of mammalian Cryptochromes and the CRY1-PER2 complex as transcriptional repressors and redox sensors will facilitate the structure and mechanism based design of small molecule compounds that affect this interaction and thereby circadian regulation. We will also continue our work on the structure determination of complexes between epigenetic regulators and the BMAL1/CLOCK transcription factors. This will allow us to understand the mechanisms that underlie how daily changes between transcriptionally repressive and activating states orchestrate circadian human physiology. Newly determined high-resolution 3D structures will be validated *in vivo* in terms of their implications for cellular function.

SELECTED PUBLICATIONS

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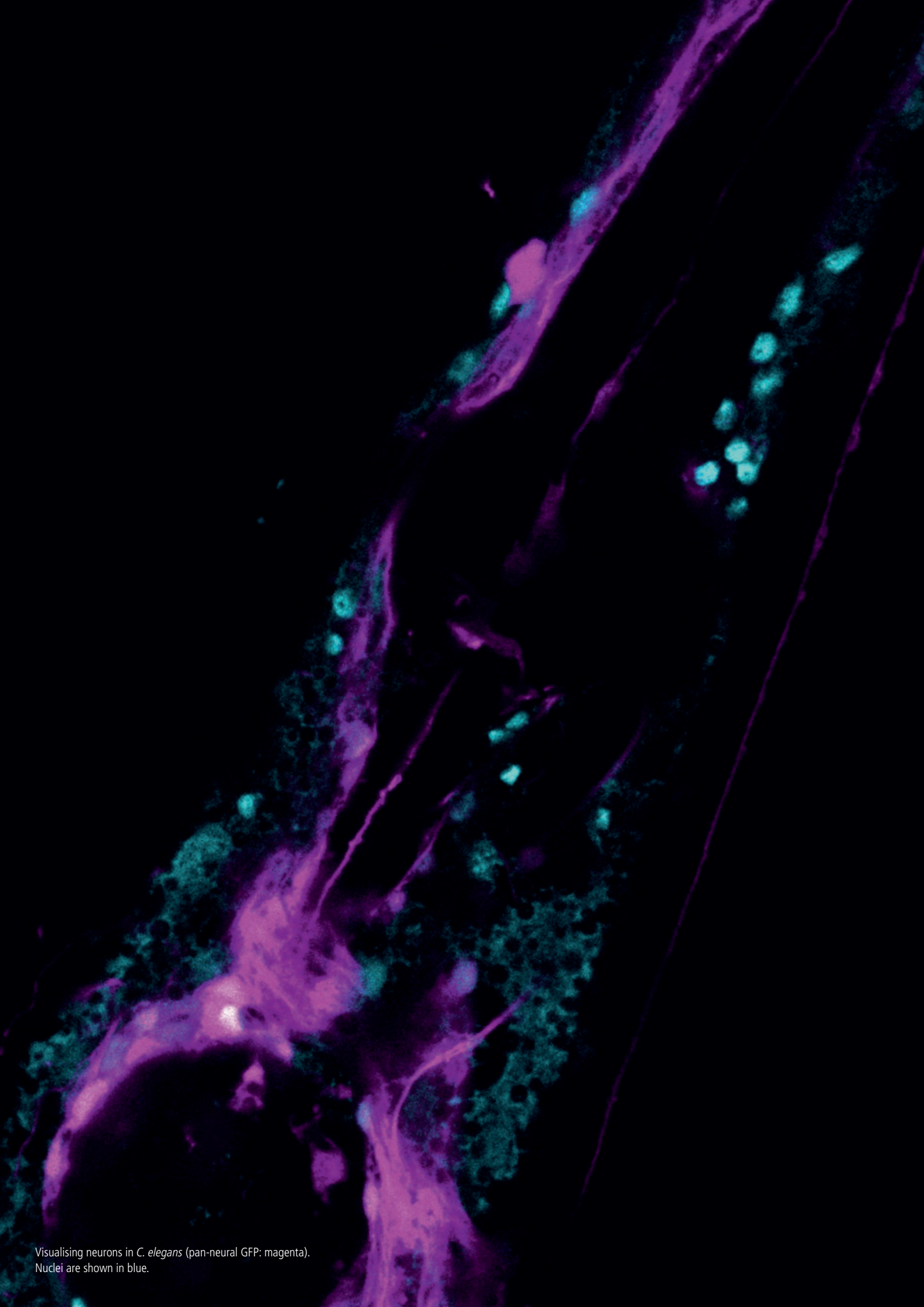
Kucera N, Schmalen I, Hennig S, Öllinger R, Strauss HM, Grudziecki A, Wieczorek C, Kramer A and Wolf E (2012). Unwinding the differences of the mammalian PERIOD clock proteins from crystal structure to cellular function. *Proc Natl Acad Sci USA*, 109, 3311-3316



Visualising neurons in *C. elegans* (pan-neural GFP: magenta). Nuclei are shown in blue.

CORE FACILITIES
OVERVIEW

The Core Facilities (CFs) at IMB provide access to state-of-the-art technology, and offer services and training from expert staff. In addition, the CFs organise lectures and courses to tutor researchers in new techniques and instrumentation, as well as experimental design and data processing.

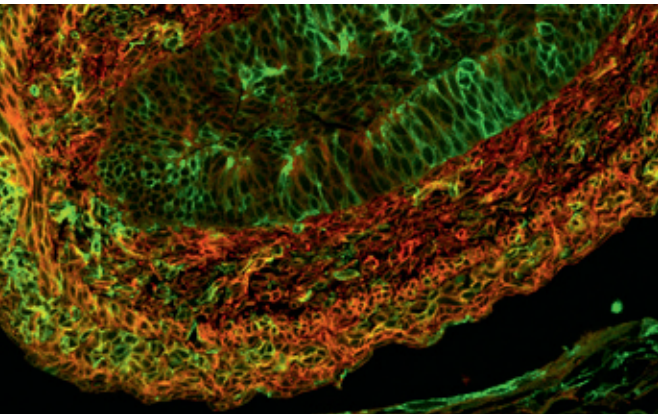
There are currently six Core Facilities at IMB: Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics and a Media Lab. The Bioinformatics, Genomics and Proteomics CFs provide users with a "full service", from quality control of samples to data production and analysis. The Flow Cytometry and Microscopy/Histology CFs provide an "assisted service", where researchers work independently on CF equipment after introductory training by CF staff. Whether receiving full or assisted service, the CFs' staff are available for consultation and troubleshooting. Furthermore, CF staff often collaborate with researchers to provide customised or specialised services.

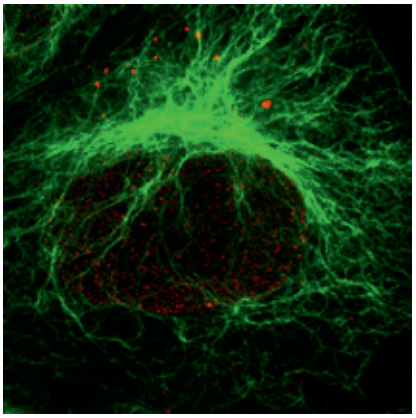
The CFs are open to all IMB researchers, and the Flow Cytometry, Microscopy/Histology and Proteomics CFs also offer services to external users at Mainz University and further afield. The services provided are based on user demand. For each CF, a user committee gives feedback on the equipment and user experience and helps to define the services that a CF provides.

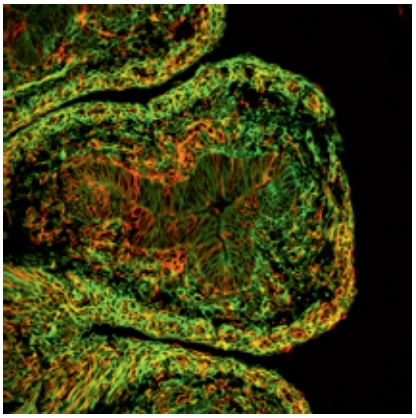
In addition to technical services, the CFs offer lectures on a variety of methods, as well as practical courses on data acquisition and analysis. These allow researchers to keep up-to-date with and broaden their knowledge of current and emerging technologies. Lectures are generally open to everyone, including the wider scientific community in Mainz.

IMB's CFs are also responsible for maintaining and providing training on core equipment that is available at IMB, as well as the radioactivity lab, the S2 lab, and IMB's in-house animal facilities (mouse, zebrafish, *Xenopus* and *Drosophila*).

Andreas Vonderheit
Director of Core Facilities and Technology







BIOINFORMATICS
CORE FACILITY

The Bioinformatics Core Facility (BCF) supports researchers at IMB with computing infrastructure, software training and consulting on experimental design and statistics. In addition, the BCF participates in the computational processing, analysis and interpretation of genomic data generated in the course of research projects.

CORE FACILITY MEMBERS

Emil Karaulanov Head
since 10/2014

Jan Bockelmann System Administrator
since 08/2015

Anke Busch Bioinformatician
since 01/2014

Oliver Drechsel Bioinformatician
since 04/2015

Matthias Koch System Administrator
05/2011-12/2015

Nastasja Kreim Bioinformatician
since 04/2012

Sergi Sayols Puig Bioinformatician
since 10/2013

Pascal Silberhorn System Administrator
since 12/2015

Sebastian Uhrig System Administrator
11/2011-10/2015

SERVICES OFFERED

The BCF offers know-how and support on different levels from basic services to full-scale scientific collaborations in the context of "big data" research projects:

- Consulting on statistics and experimental design of genomic projects
- Data quality assessment, processing, visualisation, interpretation and presentation of results
- Implementation and customisation of software tools, online services and analysis platforms
- Development of automated NGS data processing pipelines
- Development of novel approaches for individual projects
- Data mining of published datasets, correlation and integration of results
- Assistance with the preparation of manuscripts, presentations and grant proposals
- In-house training (workshops and lectures) on bioinformatics topics

The BCF maintains online services such as Galaxy, Chipster, Genomatix, R-Studio and OMERO, which provide interfaces to various analytical tools to researchers in-house. The BCF also offers customised solutions and long-term analytical support for projects on a collaborative basis. Additionally, the BCF develops and tests novel quality control methods and customised NGS pipelines for the automation of data processing and analysis. It also offers training and access to popular software tools and computing resources.

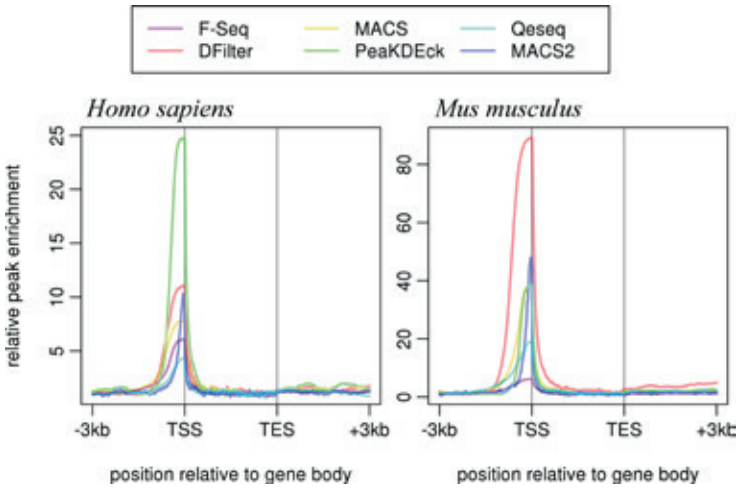


Figure 1. Test of several popular ChIP-seq peak callers on human and mouse FAIRE-seq datasets reveal strong enrichments of peaks around gene transcription start sites (TSS), which is indicative of open chromatin environment.

FLOW CYTOMETRY
CORE FACILITY

The Flow Cytometry Core Facility (FCCF) offers high-throughput measurements, analysis and separation of biological units through three different systems: a large particle sorter, a cell sorter, and an analyser. With this equipment, the FCCF can analyse and sort particles of 0.5 µm to 1,500 µm in diameter under S1 conditions, covering nearly the whole target material spectrum.

CORE FACILITY MEMBERS

Jens Hartwig Head
since 10/2013

Dieter Bork Mechanical Technician
since 05/2015

Ina Schäfer Biotechnologist
since 08/2011

SERVICES OFFERED

The FCCF offers a full service for sorting; for the analyser, assisted service is provided. Additionally, the staff collaborate in terms of analysing flow cytometrical data and sample preparation. During the past year, the FCCF has performed various types of experiments, including for example, multicolour measurements, single cell separation for sequencing RNA content, sorting of isolated neuronal nuclei, classical enrichments for subsequent cell culture or qPCR analysis, and cell sorting for microscopic investigations. The FCCF works with different types of material: nuclei, stem cells, *C. elegans*, as well as different cultured cell lines and primary cells from humans, mice, zebrafish, and *Drosophila*. To educate and train users, the FCCF offers three different lectures and a practical course.

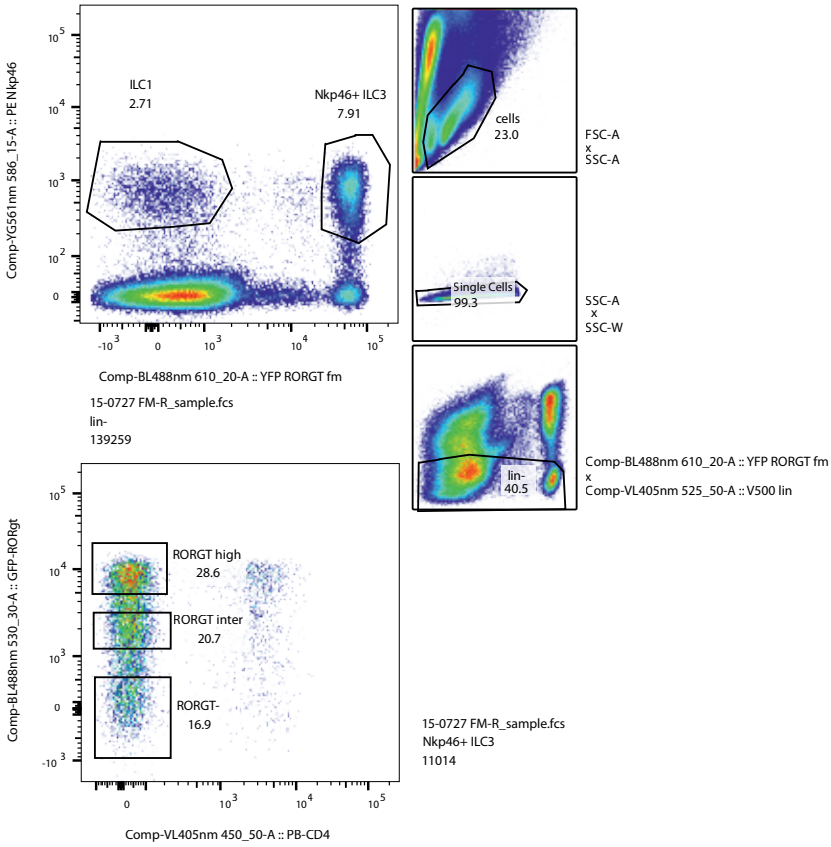


Figure 1. A 9-colour-panel for studying group 3 innate lymphoid cells based on two transgenic markers: GFP and YFP. Group 3 innate lymphoid cells (ILC3s) are characterised by being developmentally dependent on the expression of the lineage-defining transcription factor RORγt. However, it has become apparent through the use of genetic fate-mapping techniques that some of the natural killer receptor (NKR) positive ILC3s lose the expression of RORγt. The establishment of a RORγt-YFP fatemap x RORγt-GFP reporter mouse enables us to isolate ILC3 by FACS and interrogate the functional consequences of the loss of RORγt expression. Data courtesy of Michael Kofoed-Nielsen, Mainz University Medical Center.

GENOMICS
CORE FACILITY

The Genomics Core Facility (GCF) offers next-generation sequencing (NGS) services based on the Illumina HiSeq 2500 and MiSeq platforms.

CORE FACILITY MEMBERS

Chung-Ting "Tina" Han Head
since 04/2013

Hanna Lukas Technician
since 01/2013

Clara Sophie Werner Technician
since 07/2015

SERVICES OFFERED

The GCF provides full service from DNA or RNA samples, including quality control required for NGS, which entails sample preparation and library construction, to sequencing and raw data generation. Currently, the GCF supports more than 15 different types of library preparation as standard services (Figure 1). The GCF develops new protocols to accommodate user needs for their specific sample type. In 2015, the GCF implemented and validated two automation systems for library preparation using microfluidics and a liquid handling robot.

The GCF offers a two-day practical course on NGS, in which participants learn about preparing their own RNA or small RNA-seq libraries. Additionally, the GCF holds lectures on the principles of NGS and its application in translational epigenetics. As NGS is a fast-changing field, the GCF hosted six TechTalks in 2015, which covered topics ranging from long-read sequencing, oxidised bisulfite sequencing, genome editing, single-cell RNA sequencing, droplet digital PCR, and DNA shearing. In November, the GCF hosted an Illumina User Day to showcase various NGS applications to an audience from Mainz and Frankfurt.

	Application	Since
Fully	Small RNA-Seq	05.2013
	RNA IP-Seq (Ultralow-input)	05.2013
	Whole genome sequencing	08.2013
	Strand-specific mRNA-Seq	08.2013
	Strand-specific total RNA-Seq	12.2013
	ChIP-Seq (Ultralow-input)	01.2014
	Small RNA-Seq with molecular indexing	04.2014
	Whole genome bisulfite sequencing	07.2014
	Single-stranded DNA-Seq	08.2014
	Low diversity amplicon sequencing	10.2014
	Smart-Seq2 (Ultralow-input)	04.2015
	Bru-Seq	07.2015
Partially	HiC (Low-input)	02.2015
	Circular RNA-Seq	03.2015
Experimental	ATAC-Seq	
	CEL-Seq	
	iCLIP-Seq	

Figure 1. In order to accommodate user needs for their specific sample type, the GCF continuously develops new workflows for library preparation and sequencing. The GCF *fully* supports various types of library preparation as SOP. The GCF *partially* supports its users with library preparation for HiC and circular RNA-seq. For *experimental* applications, the GCF provides troubleshooting for these user-made libraries so that the highest quality of sequencing output can be achieved.

MICROSCOPY & HISTOLOGY
CORE FACILITY

The Microscopy and Histology Core Facility (MHCF) offers supervised access to microscopy equipment, ranging from stereo macroscopes (Figure 1) and widefield microscopes to confocal and super-resolution microscopes.

CORE FACILITY MEMBERS

Andreas Vonderheit Head
since 04/2011

Katharina Böse Staff Scientist
since 01/2013

Maria Hanulova Staff Scientist
since 02/2014

Sandra Ritz Staff Scientist
since 10/2013

SERVICES OFFERED

In 2015, the MHCF acquired a rotational unit for multiple view image acquisition, which is compatible with most of the MHCF's microscopes. The MHCF also offers a broad range of histology techniques, comprising semi-automated fixation and paraffin embedding as well as microtomes for paraffin, cryo- or gelatine/agarose sectioning (Vibratome). After an introduction, users independently work on the MHCF's microscopes, the image processing stations, and the histology equipment. The MHCF staff assist with sample preparation, image acquisition and image analysis, processing, and deconvolution. Additionally, the MHCF offers super-resolution microscopy on a full service or on a collaborative basis.

The MHCF also offers training through practical courses and lectures. The latter include an "Introduction to Microscopy", "Pitfalls in Image Acquisition", "Image Processing", and "Ethics in Image Acquisition and Processing". The courses range from practical classes on confocal, live cell microscopy and super-resolution microscopy to image processing.

Figure 1. Extended focus stereo micrograph of a mouse intestine with lacZ staining. Scale bar length is 75 µm. Image courtesy of Natalia Soshnikova.

PROTEOMICS
CORE FACILITY

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

CORE FACILITY MEMBERS

Falk Butter Head
since 05/2013

Jasmin Cartano Technician
since 02/2014

Mario Dejung Bioinformatician
since 05/2014

Anja Freiwald Engineer
since 04/2013

SERVICES OFFERED

The PCF provides 1,500 measurement hours annually to IMB and the surrounding research centres in Mainz using a state-of-the-art mass spectrometry platform (Figure 1). 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. Additionally, we recently introduced reductive dimethylation for quantitative analysis. The mass spectrometry service is provided as a full service, including initial consultation, sample preparation and basic proteomics data analysis by the PCF. In-depth statistical and bioinformatics analysis is provided as a collaboration. Additionally, we offer a lecture on proteomics and provide researchers with hands-on experience during our practical courses.

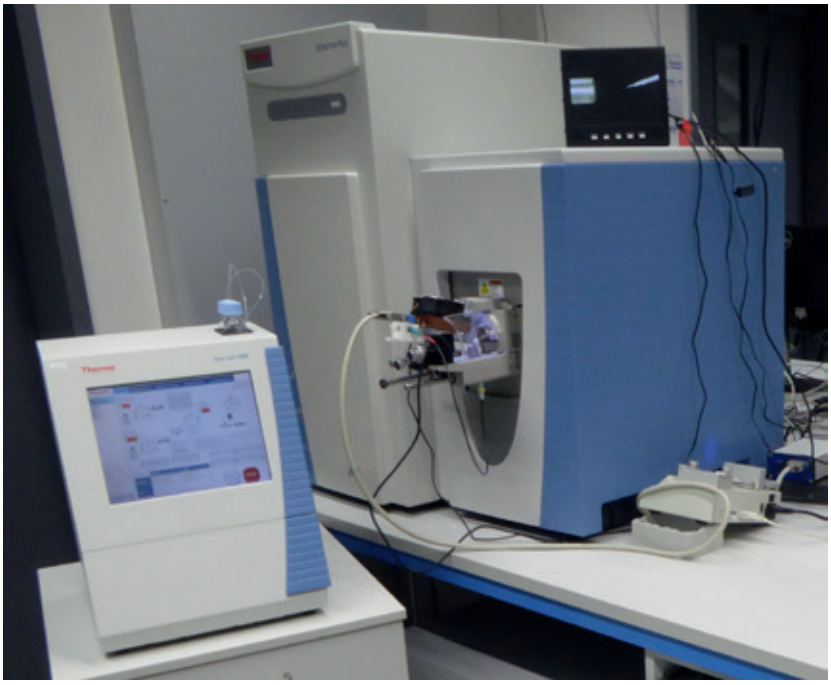


Figure 1. EASY nLC 1000 coupled via electrospray ionisation to a Q Exactive Plus mass spectrometer providing high resolution and excellent sensitivity for streamlined shot-gun protein identification and quantitation.

MEDIA LAB

The Media Lab produces buffers and agar plates and runs a supply center of media and other molecular products, such as enzymes and antibodies.

CORE FACILITY MEMBERS

Ina Schäfer Head
since 03/2013

Doris Beckhaus Lab Assistant
since 05/2011

Alwina Eirich Lab Assistant
since 07/2013

Pascal Hagebölling Lab Assistant
since 01/2015

Valentina Helmus Lab Assistant
since 04/2011

Annette Holstein Lab Assistant
since 04/2012

Johann Suss Lab Assistant
since 04/2011

SERVICES OFFERED

The Media Lab provides the following services:

- Preparation of different buffers, media, and plates (Figure 1)
- Autoclaving of waste, as well as cleaning and sterilisation of glassware
- Management of a New England Biolabs freezer and a Supply Center for Thermo Fisher Scientific products

The Media Lab also provides and maintains a vector database, a human ORF clone collection and a cell line bank. Catalogues of these collections are maintained in a central database, so that they are readily available to IMB's scientists.

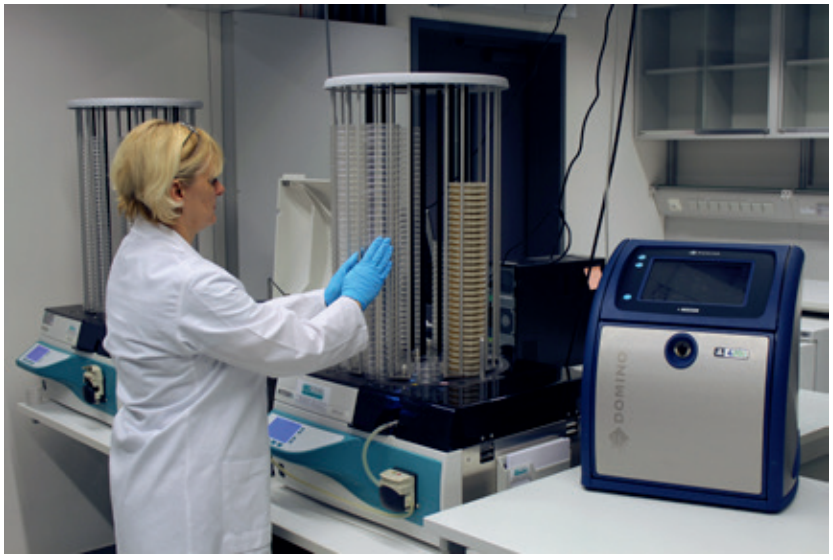
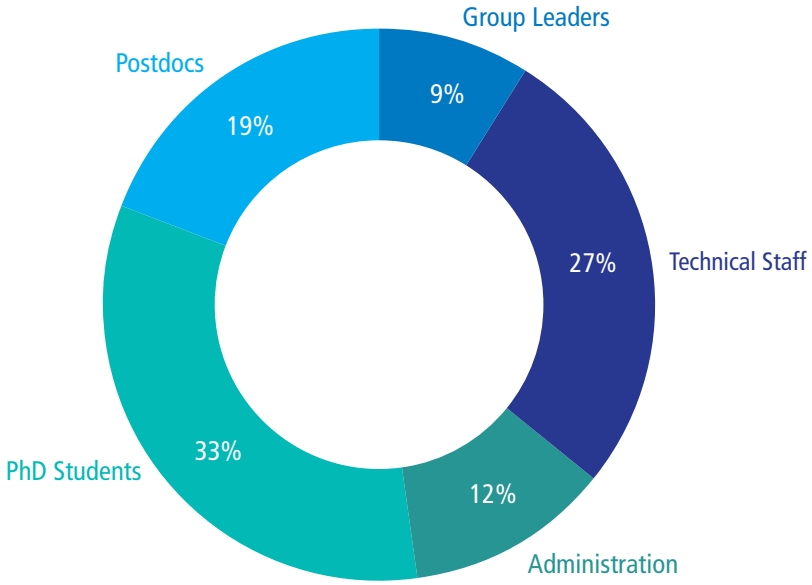


Figure 1. The Media Lab works with the Mediajet system from INTEGRA Biosciences and ibs tecnomara, allowing for continuous filling of petri dishes of different sizes up to several litres per week.

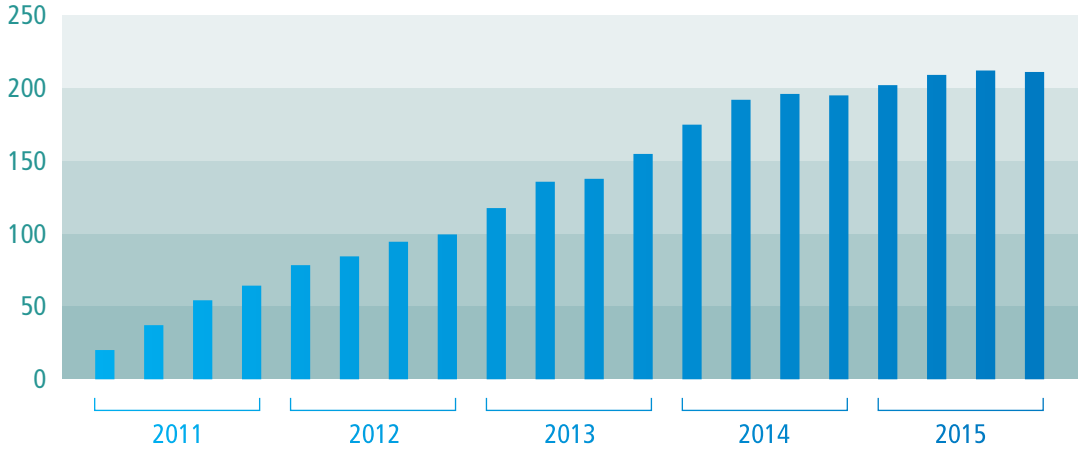
FACTS & FIGURES

STAFF

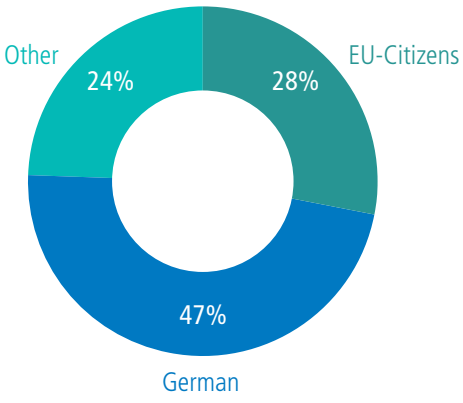
Staff by Category



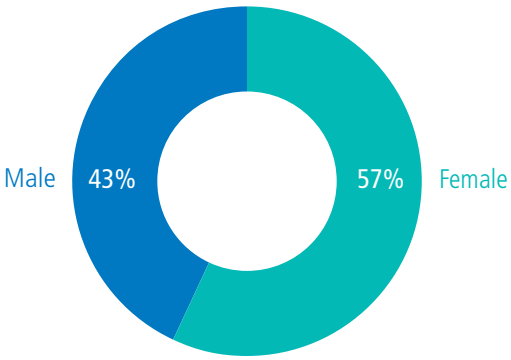
Staff Growth



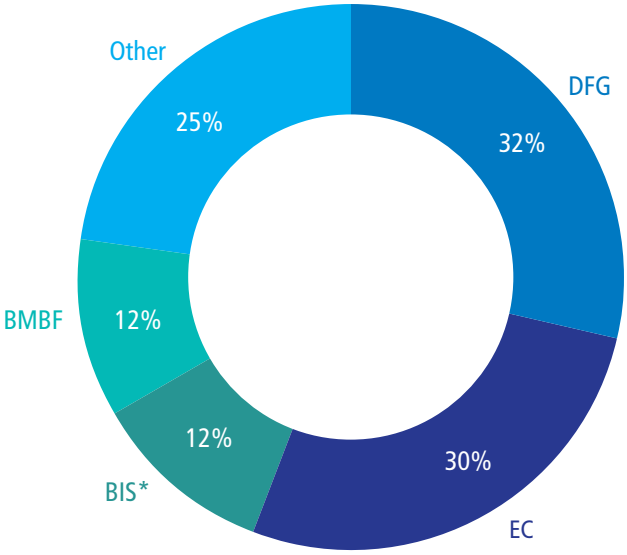
Nationalities of Scientific Staff



Gender Distribution



EXTRAMURAL FUNDS



*Grant funding for the International PhD Programme awarded by the BIS in addition to IMB's core funding

Major Funders

Boehringer Ingelheim Stiftung (BIS)

Federal Ministry of Education and Research (BMBF)

German Research Foundation (DFG)

European Commission (EC)

European Research Council (ERC)

EpiGeneSys Network of Excellence

Marie Curie Actions

Further Support

European Molecular Biology Organisation (EMBO)

German Academic Exchange Service (DAAD)

German National Academy of Sciences Leopoldina

International Society for Differentiation

Naturwissenschaftlich-Medizinisches Forschungszentrum (NMFZ)

Netherlands Organisation for Scientific Research (NWO)

Projektträger Jülich

Rhineland Palatinate Foundation for Innovation

VDI Technologiezentrum

Volkswagen Stiftung

Wilhelm Sander-Stiftung

RESEARCH & TRAINING

The scientists at IMB carry out research in epigenetics, developmental biology, genome stability and the interfaces between these fields. In 2015, the international community of researchers at IMB included 64 PhD students, 41 postdocs and 17 group leaders. IMB's scientists come from a range of backgrounds, including biochemistry and genetics, as well as molecular, cell and developmental biology. They study, for example, the molecular mechanisms of embryonic development, evolution, ageing or diseases. In addition, our bioinformaticians and systems biologists analyse high-throughput datasets and model regulatory gene networks, and applied physicists develop new superresolution microscopes. This variety of expertise and the interactive atmosphere at IMB encourages collaborations and innovative research.

IMB ensures that its scientists can work productively. A key part of the support offered comes through the Core Facilities. They provide services in bioinformatics, flow cytometry, genomics, microscopy and histology, and proteomics. Each facility is staffed by experts to advise and assist 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 staff in the Core Facilities. This means our scientists have access to 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 training through scientific events and theoretical, as well as hands on instruction in technical and methodological skills. Moreover, junior scientists at IMB learn transferable skills required for a successful career both within and outside of academia. Courses offered cover topics such as presentation skills, scientific writing and project management, as well as fundraising. IMB also organises an annual Life Sciences Career Day.

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, supported by the Boehringer Ingelheim Foundation, 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 & 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

TRAINING COURSES

In 2015, IMB offered the following training courses in scientific and transferable skills.

CORE FACILITIES TRAINING

LECTURES

CORE FACILITY	DATES	TITLE
BIOINFORMATICS	12 May	ChIP-Seq
	19 May	RNA-Seq
	26 May	DNA Methylomics
	02 Jun	Experimental Design & Quality Control
	23 Jun	Systems Biology & Modelling
FLOW CYTOMETRY	14 Apr & 03 Nov	Introduction to Flow Cytometry I
	28 Apr & 10 Nov	Introduction to Flow Cytometry II
GENOMICS	24 Mar	What can NGS do for you?
MICROSCOPY	13 Jan & 29 Sep	Introduction to Microscopy I
	20 Jan & 6 Oct	Introduction to Microscopy II
	3 Mar & 13 Oct	Introduction to Microscopy III
	10 Mar	Pitfalls in Image Acquisition
	30 Jun	Ethics in Image Acquisiiton & Processing
	07 Jul	Image Processing
PROTEOMICS	24 Feb	Proteomics

PRACTICAL COURSES

CORE FACILITY	DATES	TITLE
BIOINFORMATICS	26 Feb, 5 Mar, 12 Mar & 19 Mar	R Practical Course (I - IV)
	9 - 10 Jun	ChIP-Seq & RNA-Seq with Galaxy
	21 Jul	ChIP-Seq & RNA-Seq with Genomatix
FLOW CYTOMETRY	4 - 11 May & 16 - 23 Nov	Flow Cytometry Practical Course
	16 Jun	Flow Cytometry Data Analysis
GENOMICS	25 - 27 Mar	NGS Practical Course on RNA Library Preparation
MICROSCOPY	13 - 16 Jul	Image Pro Practical Course
PROTEOMICS	9 - 11 Feb & 26 - 23 Oct	Proteomics Practical Course

SCIENTIFIC AND TRANSFERABLE SKILLS TRAINING

LECTURES

DATE	TITLE
19 Jan, 28 May	Scientific Publishing Lectures *(EMBO Journal, Nature Biotechnology)*
27 Jan	Good Scientific Practice Lecture
31 Mar	Introduction to Grants Options for PhD Students
27 Jul - 7 Aug	Block Lecture Weeks on the Topics of IMB's International PhD Programme
27 Oct 2015 - 2 Feb 2016	Lecture Series: Introduction to Epigenetics

PRACTICAL COURSES

DATE	TITLE
13 Jan - 12 Feb, 17 Nov - 17 Dec	PERL Course (10 units; twice a week)
2 - 3 Feb, 19 - 20 Aug	Presentation Skills
4 Feb, 9 Feb, 14 - 15 Aug	Project Management
10 Apr	Time Management
20 - 21 Apr, 28 - 29 Sep	Scientific Writing
23 Apr	Application Training
27 Apr	Grants Writing Workshop for PhD Students
7 - 8 May	Project Management in Science for Postdocs
20 - 21 May, 2 - 3 Dec	Introduction to Biostatistics
1 - 2 Jun, 14 - 15 Dec	Grant Writing Workshop for Group Leaders & Late Stage Postdocs

OTHER EVENTS

DATE	TITLE
7 May	Research Careers in Europe*
2 Jul	HORIZON2020: EU Funding Opportunities for Life Sciences & Nanotechnologies*
15 Oct	HORIZON2020: Future & Emerging Technologies (FET)*
2 Nov	HORIZON2020: ERC Funding Schemes*
6 Nov	2015 Career Day

* organised in cooperation with JGU, JGU's University Medical Center and the Max Planck Institute for Polymer Research

INVITED SPEAKERS

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
22 Jan 2015	Seminar	**JIRI LUKAS**	University of Copenhagen	Spatial and temporal regulation of genome integrity maintenance
05 Feb 2015	Seminar	**UTZ FISCHER**	University of Würzburg	Neurological disorders: lessons from RNA biology
26 Feb 2015	Seminar	**HENNING URLAUB**	Max Planck Institute for Biophysical Chemistry, Göttingen	Mapping RNA-binding sites in RNA-binding proteins
17 Mar 2015	TechTalk	**RALPH VOGELSANG**	Pacific Biosciences	Single-molecule, real-time (SMRT™) DNA sequencing: technology update and recent applications
21 Apr 2015	TechTalk	**OLIVER STEPHAN**	Covaris, Inc.	Empower chromatin shearing with Covaris AFA technology
23 Apr 2015	Seminar	**THOMAS CREMER**	Ludwig Maximilian University of Munich	Evidence for functional nuclear organization based on spatially co-aligned active and inactive nuclear compartments
30 Apr 2015	Seminar	**JAMES ADJAYE**	Düsseldorf University Hospital	Human iPS cells as cellular models for studying hepatogenesis and non-alcoholic fatty liver disease
30 Apr 2015	TechTalk	**HANNA SCHUTZ**	Cambridge Epigenetix Ltd.	Next generation epigenetics – innovative research tools for the accurate analysis of DNA modifications
05 May 2015	Seminar	**DAN LARSON**	Center for Cancer Research, National Cancer Institute, Maryland	Regulation of transcription and RNA processing in single cells: understanding heterogeneity in gene expression
07 May 2015	Seminar	**HANS REINKE**	University of Düsseldorf	A conserved role for Period proteins in autophagy regulation
21 May 2015	Seminar	**MARC BÜHLER**	Friedrich Miescher Institute for Biomedical Research (FMI), Basel	siRNA-mediated assembly of heterochromatin - a kiss to remember
28 May 2015	Seminar	**JESPER SVEJSTRUP**	The Francis Crick Institute/ Cancer Research UK, London	Transcription-associated genome instability
29 May 2015	Seminar	**EDDY DE ROBERTIS**	University of California, Los Angeles	Integration of growth factor signalling gradients during development
09 Jun 2015	TechTalk	**STEFAN BORNSCHEIN & PIA SCHEU**	Bio-Rad Laboratories GmbH	Digital PCR: improving nucleic acid quantification - precision, accuracy and sensitivity are among the benefits reported by researchers
11 Jun 2015	Seminar	**JAN-MICHAEL PETERS**	Institute of Molecular Pathology (IMP), Vienna	How cohesin controls sister chromatid cohesion and chromatin structure

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
02 Jul 2015	Seminar	**TEWIS BOUWMEESTER**	Novartis Institutes for BioMedical Research, Basel	Drug discovery at NIBR and new insights into the role of Wnt and Yap pathways in tissue regeneration
06 Jul 2015	Seminar	**MASSIMO LOPES**	University of Zürich	Fork remodelling upon replication stress in cancer and stem cells
10 Aug 2015	Seminar	**VALENTIN BÖRNER**	Cleveland State University	A role of the proteasome in the repair of meiotic chromosome breaks
22 Sep 2015	TechTalk	**DANIEL LACKNER**	Horizon Genomics GmbH	Haploid cell lines empower genetics in human cells
24 Sep 2015	Seminar	**RAY DESHAIES**	Caltech, Pasadena	Regulation of cullin–RING ubiquitin ligase assembly: what we can learn from analytical mass spectrometry
24 Sep 2015	Seminar	**ANA POMBO**	Max Delbrück Center for Molecular Medicine, Berlin	Complex multi-enhancer contacts captured in ES cells by genome architecture mapping, a novel ligation-free approach
08 Oct 2015	Seminar	**MARTIN VINGRON**	Max Planck Institute for Molecular Genetics, Berlin	Computing networks for histone modifications and modifiers
22 Oct 2015	Seminar	**STEVEN BROWN**	University of Zürich	Genetic and epigenetic mechanisms of circadian plasticity
27 Oct 2015	TechTalk	**MEDHANIE A. MULAW**	University of Ulm	Long non-coding RNAs associated with *in vivo* engraftment potential of acute myeloid leukemia
05 Nov 2015	Seminar	**ANNE EPHRUSSI**	European Molecular Biology Laboratory (EMBL), Heidelberg	Assembly and transport of oskar mRNPs in the *Drosophila* oocyte
24 Nov 2015	TechTalk	**ILLIP BURMESTER**	Quanterix Corporation	Using single molecule arrays (Simoa): technology and applications
26 Nov 2015	Seminar	**KARSTEN RIPPE**	German Cancer Research Center (DKFZ), Heidelberg	Integrative analysis of epigenetic networks: from cell lines to primary tumor cells
03 Dec 2015	Seminar	**KYUNG-MIN NOH**	European Molecular Biology Laboratory (EMBL), Heidelberg	Read to act: histone reading module in chromatin writer and remodeler
07 Dec 2015	Seminar	**MANOLIS PAPAMICHOS-CHRONAKIS**	Institute Curie, Paris	Killing to survive: chromatin control of protein degradation in the nucleus
10 Dec 2015	Seminar	**TERESA TEIXEIRA**	The Laboratory of Molecular and Cellular Biology of Eukaryotes, Paris	How telomeres determine the route to senescence

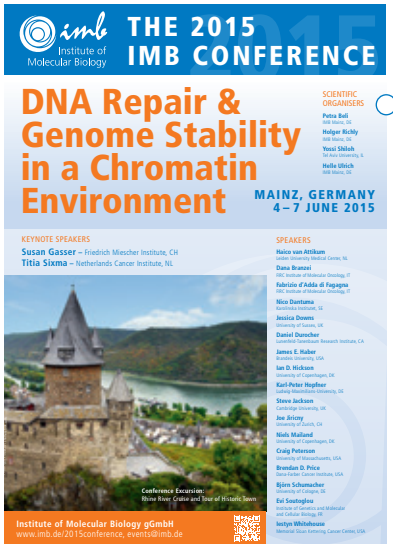
SELECTED SCIENTIFIC EVENTS
ORGANISED BY IMB

24 Feb 2015

"*SEEING IS BELIEVING*" MICROSCOPY WORKSHOP
Scientific organisers: Christoph Cremer and Carsten Sönnichsen (JGU, Mainz)

19-20 Mar 2015

IMB WORKSHOP "*BREAKTHROUGHS IN EPIGENETICS*"
Scientific organiser: Vijay Tiwari



4-7 Jun 2015

2015 IMB CONFERENCE "*DNA REPAIR & GENOME STABILITY IN A CHROMATIN ENVIRONMENT*"
Scientific organisers: Yossi Shiloh (Tel Aviv University), Petra Beli, Falk Butter, Holger Richly and Helle Ulrich

30 Jun 2015

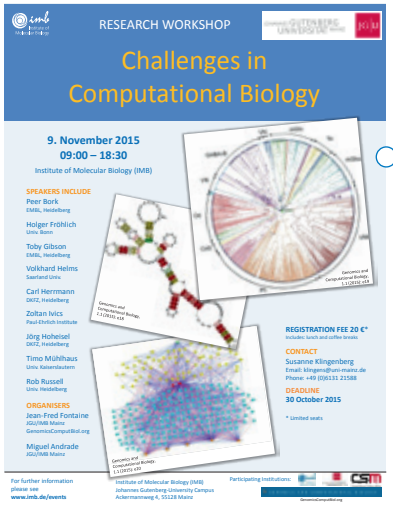
JOINT SYMPOSIUM OF THE MAX PLANCK INSTITUTE FOR POLYMER RESEARCH, JGU & IMB
Scientific organisers: Mischa Bonn (Max Planck Institute for Polymer Research, Mainz) and Ralf Dahm

8 Sep 2015

SYMPOSIUM ON TRANSLATIONAL EPIGENETICS FOR GROUP LEADERS AT JGU, UMC & IMB
Scientific organisers: Ralf Dahm and Christof Niehrs

29 Sep 2015

LECTURE AND DISCUSSION EVENT "*DIE VERZAUBERUNG DER WELT (THE ENCHANTMENT OF THE WORLD)*"
Featuring talks by Peter Fischer (Professor of Science History, University of Heidelberg) and Jörg Lauster (Professor for Systematic Theology and Philosophy of Religion, Philipps University Marburg), organised in cooperation with Mainz Academy of Science and Literature



3 Nov 2015

IMB-JGU FACULTY OF BIOLOGY SYMPOSIUM ON RNA BIOLOGY
Scientific organisers: René Ketting and Hans Zischler (JGU, Mainz)

9 Nov 2015

CHALLENGES IN COMPUTATIONAL BIOLOGY
Scientific organiser: Miguel Andrade

15-21 Nov 2015

iCLIP EMBO COURSE "*GENOMIC VIEWS OF PROTEIN-RNA INTERACTIONS*"
Scientific organisers: Elias Bechara (Center for Genomic Regulation, Barcelona), Julian König, Michaela Müller-McNicoll (Goethe University Frankfurt), Chris Sibley (University College London), Jernej Ule (University College London), Kathi Zarnack (Buchmann Institute for Molecular Life Sciences, Frankfurt)

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Miguel Andrade

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Petra Beli

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George Reid

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Vijay Tiwari

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Helle Ulrich

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(* indicates equal contribution, # indicates corresponding authors)

AWARDS AND RECOGNITION
2015

Petra Beli
Nominated by the German Research Foundation (DFG) for AcademiaNet internet database of excellent female scientists

Christoph Cremer
Appointment as Research Associate at the Max Planck Institute for Chemistry, Mainz

Brian Luke
Awarded Heisenberg Fellowship

Vijay Tiwari
Wilhelm Sander-Stiftung Award for recognition of outstanding contributions in biomedical research
F1000 Faculty Member of the Year Award in Developmental Biology

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 35,000 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 46,000 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.

Bradley Cairns
Huntsman Cancer Institute, University of Utah, Salt Lake City, USA

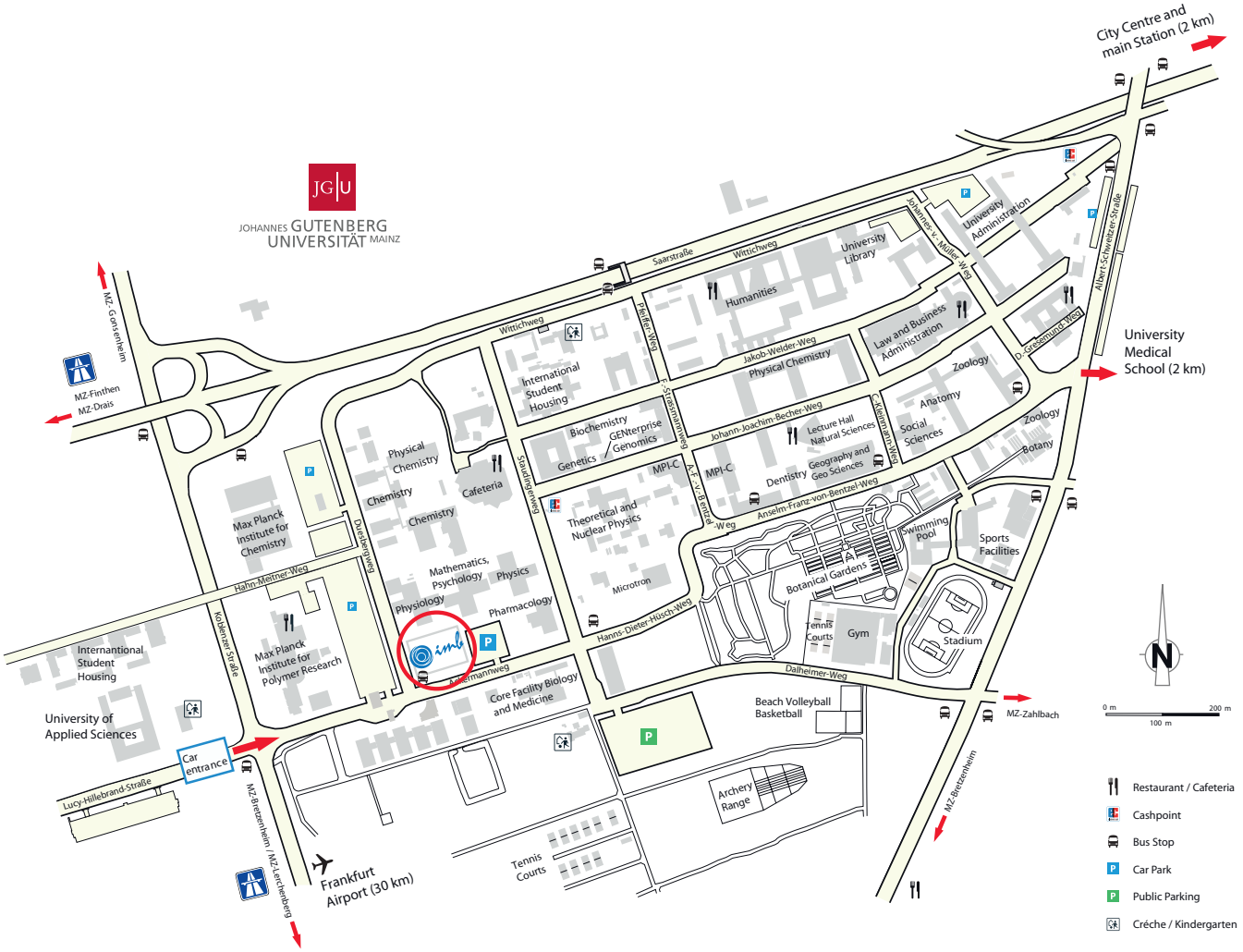
Rudolf Jaenisch
The Whitehead Institute for Biomedical Research, Cambridge, USA

Renato Paro (Chair)
Department of Biosystems Science and Engineering, Swiss Federal Institute of Technology, Basel, Switzerland

Josef Jiricny
Institute of Molecular Cancer Research University of Zurich, Zurich, Switzerland

Jan-Michael Peters
Research Institute of Molecular Pathology (IMP), Vienna, Austria

CAMPUS MAP
OF JOHANNES GUTENBERG UNIVERSITY MAINZ



CONTACT

Institute of Molecular Biology gGmbH
supported by the Boehringer Ingelheim Foundation
Ackermannweg 4
55128 Mainz
Phone: +49-6131-39-21501
Fax: +49-6131-39-21521
www.imb.de
info@imb.de

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