Image Credit

Cover: False colour image of a 2 mm section of a chemically cleared mouse brain. Image composed of 35 single images. Sample prepared by Oriane Blanqui (group of Heiko Luhmann, University Medicine Mainz) and imaged by IMB's Microscopy Core Facility. Image credit: Sandra Ritz.

Portraits of Group Leaders and Core Facility heads (p2, 5-40, 44-51), pictures of IMB researchers (p 52, 56-58, 63, 64, 69), IMB building (inside cover, p3, p73, p76-77): Thomas Hartmann

Microscopy images: (p3) Stage 38 Xenopus larvae stained for 5hmC (red) and yoyo1 (green). Image credit Victoria Hatch (Niehrs Group). (p4) Stained section of mouse intestine. Image credit Bernadette Mekker. (p61) Monolayer section of whole murine colon after DNA damage. Image credit Konrad Gronke (Diefenbach group, Charite, Berlin), image taken at the AF7000 Fluorescence Microscope (Leica) at 10x magnification by the Microscopy and Histology CF, IMB.

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FOREWORD

OVERVIEW

2018 brought some excellent news for the future of IMB with an additional commitment from the Boehringer Ingelheim Foundation and the State of Rhineland-Palatinate to jointly fund IMB with €106 million until 2027. Three new Group Leaders joined IMB in the last year with one more joining in 2019. During the year, IMB hosted several successful international events including an EMBO workshop. IMB has been heavily involved in preparing an application for a Collaborative Research Center (CRC), which was greenlit by the DFG in November. The developments of 2018 are shaping the future of IMB, consolidating IMB’s position as a leading international research centre.

NEW DEVELOPMENTS AT IMB

This year saw two major developments at IMB. First and foremost, IMB’s future was consolidated with the announcement from the Boehringer Ingelheim Foundation and the state of Rhineland-Palatinate of the continuation of IMB’s core funding beyond its first funding period. Starting in autumn 2020, the foundation will contribute €54 million and the state €52 million. This second round of financing secures IMB’s core funding until mid-2027. The funding follows on from the generous donation of €100 million by the Boehringer Ingelheim Foundation in 2010, which provided core funding for IMB through the period 2010 to 2020. With this funding secured, IMB can continue recruiting top talents in the life sciences while providing state of the art facilities and technology to its researchers. In November this year, the DFG announced €12.4 million in funding for a Collaborative Research Center on “DNA Repair and Genome Stability”, which will be coordinated by IMB and comprises 8 projects from IMB group leaders, 3 support services provided by IMB, and an Integrated Research Training Group that will link in with the existing International PhD Programme at IMB. The initiative brings together researchers from IMB, Johannes Gutenberg University Mainz, University Medical Center Mainz, University of Technology Darmstadt, Ludwig Maximilian University Munich, and Goethe University Frankfurt to create a research network on DNA repair and genome stability in which IMB will be a central hub.
2018 saw three new Group Leaders join IMB: Peter Baumann, Edward Lemke and Anton Khmelinskii. Peter joins us from the Stowers Institute, Kansas as an Adjunct Director and an Alexander von Humboldt Professor at JGU. Peter’s research focuses on both telomere biology and chromosomal inheritance. Edward is also a new Adjunct Director and Professor of synthetic biophysics at JGU. Edward and his lab will be moving to Mainz from EMBL, Heidelberg upon completion of the second new university biology building (Biocenter II) in late 2019. Edward’s group probes the structure and function of intrinsically disordered proteins. Anton joins IMB as a new Junior Group Leader from the Center for Molecular Biology in Heidelberg. Anton investigates protein quality control systems and the contributions of protein ubiquitylation to these. Furthermore, Martin Möckel has been appointed as the new head of our Protein Production Core Facility in March 2018.

We are also delighted to announce that Joan Barau from the Institut Curie in Paris will be joining IMB in 2019. Joan was a recipient of the French Academy of Sciences prize for great advances in biological sciences in 2015. He specialises in developmental epigenetics and genome stability and will fit in extremely well with the current research focus at IMB. A further round of recruitment for two additional group leader positions will commence next year. We also say goodbye to three of our members this year: Miguel Andrade, Holger Richly and Natalia Soshnikova. I wish them all the best with their future plans.

2018 was one of the busiest but most productive years for IMB to date. Three international events were held at IMB this year. In March, we hosted a one-day symposium on “Gene Regulation in Evolution” which aimed at bridging the gap between researchers focusing on the molecular side of gene regulation and evolutionary biologists. In October, IMB held a four-day EMBO workshop on “RNA and Genome Maintenance: Cooperation and Conflict Management”. The workshop featured 25 speakers and 15 short talks, with 187 participants from 30 countries attending. In November, we had a workshop on “Molecular Mechanisms of Circadian Clocks”, which featured talks from 16 renowned international speakers. In addition to these events, IMB also hosted 17 seminars from distinguished speakers, including three organised by IMB’s postdocs, and several outreach activities.

IMB continued with its trend of publishing excellent research with 81 research publications in 2018. Highlight articles from this year include publications from the Ketting group in Developmental Cell, the Butter group in Science and the Niehrs group in Nature Genetics. Furthermore, Natalia Soshnikova was accepted into the prestigious Heisenberg Programme of the DFG. Christof Niehrs was the recipient of an ERC Advanced Grant to pursue research into epigenetic regulation via R-loops. My own research was supported with the awarding of an ERC Proof of Concept Grant to explore the translation of a ubiquitin toolkit, developed in my group, into a commercial product.

Our community of PhD students and postdocs continues to thrive at IMB. The international PhD Programme now consists of 128 students from 31 different countries and this year celebrated its 40th graduate. As part of the IMB Postdoc Programme, we also launched our first coordinated recruitment call for postdoctoral researchers. With interviews taking place in early 2019, we expect this format to recruit exceptional junior scientists to IMB. The 7th International Summer School at IMB retained its track record of offering great training and hosted 17 international students this summer.

For 2019 we have already planned our IMB Conference on “Chromosome Territories and Nuclear Architecture” in October. This event is in honour of Christoph Cremer who will be retiring at the end of next year. The Collaborative Research Center goes into full swing next year and IMB will host the kick-off meeting for the new programme at the end of summer. In March, we will be holding a practical course in the context of a pan-European initiative, UbiCODE. Finally, in May, Christof Niehrs will organise a public event in cooperation with the Academy of Science and Literature, Mainz on the ethics of CRISPR/Cas technology.

I would like to thank the Boehringer Ingelheim Foundation for their support and generous continued funding of our institute. I wish to say a special thank you to the members of our Scientific Advisory Board (SAB) who have been instrumental in the growth and success of IMB. In particular, I wish to sincerely welcome Geneviève Almouzni from the Institute Curie who has joined our SAB this year. Finally, I am very grateful to all the staff at IMB who have made my first year as Executive Director as smooth as possible.

Helle Ulrich Executive Director
EDUCATION

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

POSITIONS HELD

Since 2014  Adjunct Director, Institute of Molecular Biology (IMB), Mainz
            Professor of Bioinformatics, Johannes Gutenberg University Mainz
Since 2008  Affiliate Investigator, Ottawa Health Research Institute
2007 – 2014 Group Leader, Max Delbruck 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 Postdoc, National Centre for Biotechnology, Madrid
1994 – 1995 Postdoc, European Molecular Biology Laboratory (EMBL), Heidelberg

GROUP MEMBERS

Gregorio Alanis-Lobato Postdoc; since 02/2015
Steffen Albrecht PhD Student; since 03/2017
Tommaso Andreani PhD Student; since 01/2016
Jean-Fred Fontaine Postdoc; since 04/2014
Jonas Ibn-Salem PhD Student; 09/2014 – 02/2018
Pablo Mier Postdoc; since 01/2015
Enrique Muro Staff Scientist; since 04/2014
Sweta Talyan PhD Student; 12/2014 – 06/2018
Katerina Taškova Postdoc; 11/2014 – 05/2018
Kristina Kastano PhD Student; since 07/2018
Michael Lenz Postdoc; since 07/2018

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 gene expression profiles. 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. Carrying out these projects within the same group allows each project to benefit from and complement each other.

RESEARCH HIGHLIGHTS

Protein interaction networks

Geographical maps are useful because distances in them are informative. We are working to create similarly meaningful (hyperbolic) maps of protein-protein interaction networks, where the geometric properties of the map correspond to biological properties: distance to likelihood of interaction, radial distance to protein age, and angular coordinates to function. Our maps have predictive power and can be explored using an associated web server (GAPI). We evaluated the properties of disease modules (DMs) in these maps. DMs are sets of proteins associated to a single disease that agglomerate in the network because they tend to interact with common components (explaining why they associate to the same disease). Indeed, when compared to groups of proteins of similar size and connectivity but not related to the same disease, we observe that DMs form ensembles with significantly large connectivity and short spatial distances (in both the network and hyperbolic maps).
Protein sequence analysis
Following our work in the development of tools to support the study of protein evolution and function, we developed a tool (ProteinPathTracker) that aids the investigation of the evolutionary path of a protein. It is based on the retrieval of homologs (if possible, orthologues) in a series of species at increasing taxonomic distances from a central species of interest. These homologs should give information on the ancestral sequences that lead to the sequence of interest. The tool allows for testing a series of evolutionary paths, for example, from human as central species to bacteria. Beginning with a protein in the central species, the user can start a step-wise search for orthologues from species to species or, if a sequence is provided, from the closest homologue.

Transcript prediction from sequence analyses of genomes
Comparing the genomic regions of DNA that apparently do not code for proteins with their protein coding counterparts can reveal either ncRNAs that are complementary to coding RNAs, thereby potentially interacting with them, or actual coding regions that might have escaped previous detection. With this goal in mind, we developed an alignment method specifically to detect regions in non-coding DNA with similarity to protein coding genes (Figure 1). This method benefits from a substitution matrix that we use to compare three-frame translations of non-coding DNA against proteins. The similarity score is modelled for random mutations. Application of this method to human lincRNAs detected 203 transcripts with significant similarity to protein-coding genes, suggesting regulatory functions for these lincRNAs. We also contributed to a method and associated web tool (AnABlast) that detects potential coding regions in DNA by running the standard BLASTX algorithm to compare all translated frames of the query DNA sequence against a protein database. Graphical display of the accumulated hits can be used to indicate potential coding regions or their remnants.

Figure 1. Alignment of non-overlapping fragments of a three-frame translated non-coding DNA region to a protein they are similar to. This was obtained using our similarity matrix that accounts for random DNA evolution.

**FUTURE DIRECTIONS**

We are expanding our approaches to aid the prediction of gene and protein function. For example, we are working on tools that evaluate multiple sequence alignments of proteins to find amino acids or motifs conserved in particular species. To further study low complexity protein regions, we are developing new measures for repetitiveness, which we will apply to hundreds of species whose proteomes are complete.

Finally, we are trying to explain the function of genomic regions, called Topologically Associating Domains (TADs), which are defined as having more contacts within them than with other genomic regions. To do this, we will explore the distributions of genes with different functions and breadth of expression within TADs and their dependence on the size of the TAD.
EDUCATION
1998  PhD in Biochemistry, University College London
1994  MPhil, University of Cambridge

POSITIONS HELD
Since 2018  Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Since 2017  Alexander von Humboldt Professor, Johannes Gutenberg University Mainz
Since 2013  Professor, Kansas University Medical Center
2013 – 2018  Investigator, Howard Hughes Medical Institute, Kansas City
2013 – 2018  Priscilla Wood-Neaves Endowed Chair in the Biomedical Sciences, Stowers Institute for Medical Research, Kansas City
2013 – 2018  Investigator, Stowers Institute for Medical Research, Kansas City
2009 – 2013  Early Career Scientist, Howard Hughes Medical Institute, Kansas City
2009 – 2013  Associate Professor, Kansas University Medical Center
2009 – 2012  Associate Investigator, Stowers Institute for Medical Research, Kansas City
2004 – 2009  Assistant Professor, Kansas University Medical Center
2002 – 2008  Assistant Investigator, Stowers Institute for Medical Research, Kansas City
2000 – 2002  Research Associate, Howard Hughes Medical Institute, University of Colorado, Boulder
1998 – 2000  Wellcome Trust Travelling Research Fellow, University of Colorado, Boulder

GROUP MEMBERS
David Ho  PhD Student; since 04/2018
Kristi Jensen  Lab Manager; since 05/2018
Katja Kratz  Staff Scientist; since 10/2017
Abinaya Manivannan  PhD Student; since 08/2018
Aaron Odell  Bioinformatician; since 01/2017
Alex Orioli  PhD Student; since 01/2018
Diego Paez-Moscoso  PhD Student; since 06/2014
Lili Pan  Postdoc; since 02/2015
Ute Sideris  Personal Assistant; since 10/2017
Chi-Kang Tseng  Postdoc; since 09/2013
Hui-Fang Wang  Technician; since 07/2014

OVERVIEW
Elucidating the mechanisms of chromosome end maintenance has far-reaching implications for the treatment of cancer and certain degenerative diseases characterised by the premature depletion of stem cell pools. Overall, our research goals in this area are guided by the conviction that a better understanding of telomerase biogenesis and the dynamic interactions that occur at telomeres will enable us to identify compounds that modulate telomere length. Such reagents will have therapeutic use either to limit the lifespan of tumour cells or to boost the proliferative potential of desired cell populations counteracting many of the detrimental phenotypes associated with ageing.

Our interest in chromosome dynamics and evolution has led us to a group of lizard species with fascinating attributes: They arose by interspecific hybridisation and reproduce clonally via parthenogenesis. We have revealed deviations from the normal meiotic program that permit such unisexual reproduction, and have generated cell, biological, and genomic resources to elucidate the molecular basis of clonal reproduction in a vertebrate species. Such research carries the promise of furthering our knowledge of evolution and gene regulation in the context of hybridisation, ploidy variation, and parthenogenesis.

RESEARCH HIGHLIGHTS
Telomerase biogenesis and regulation
Progressive telomere shortening eventually limits the replicative potential of cells by triggering senescence. However, replenishing telomeric sequences is a double-edged sword. It is vital for tissue homeostasis especially in long-lived species such as humans, but it also permits the continued proliferation of malignant cells. Thus, tight regulation of the enzyme telomerase is of pivotal importance. The isolation of the telomerase RNA subunit (TER1) from fission yeast in our laboratory has provided a key
tool for studying the biogenesis and regulation of the enzyme in a genetically tractable organism. This has led to a series of discoveries in telomere and RNA biology. We demonstrated that the RNA splicing machinery has a second, previously overlooked, function in RNA 3' end processing. Instead of removing an intronic sequence in a two-step process, the first transesterification reaction alone generates the mature 3' end of TER1. Our work also defined roles for several RNA chaperones in telomerase biogenesis. Most interestingly, we found that the Sm proteins and a related protein complex (LSm - Like-Sm) sequentially associate with telomerase RNA and play distinct roles in telomerase maturation. Sm and LSm proteins are members of an ancient family of RNA binding proteins that affect virtually every aspect of RNA metabolism. In contrast to the established view that they have distinct sets of RNA targets, our work revealed the first example of an RNA that requires sequential binding by the two complexes and documents specific functions for each during maturation.

While our studies on telomerase and telomeres in fission yeast have provided fundamental insights into chromosome end maintenance, they are ultimately a stepping stone towards understanding telomere maintenance in human cells. Recent work in our group uncovered roles of the cap-binding complex, the nuclear exosome targeting complex, the Trf4/Air2/Mtr4 polyadenylation complex, the exosome, and poly(A) ribonuclease in the biogenesis of human telomerase. Importantly, our studies showed that human telomerase RNA (hTR) processing is in kinetic competition with degradation (Figure 1), an observation that hints at potential treatment options for telomerase insufficiency disorders and for delaying or reversing certain degenerative processes associated with ageing.

Mechanism of parthenogenesis

Although all-female species of whiptail lizards were already described in the early 1960s, how mature eggs are produced in the absence of fertilisation, remained an enigma until we showed that a transient doubling of chromosomes prior to the meiotic divisions produces diploid rather than haploid eggs. To gain a deeper mechanistic understanding, we have sequenced and de novo assembled ovarian transcriptomes and cloned key meiotic regulators. These reagents enabled us to show that the doubling of chromosomes affects only a small fraction of oocytes and have provided insights into the underlying cellular processes.

Figure 1. Telomerase biogenesis is a highly regulated multi-step process. The long precursor of human telomerase RNA is either degraded or processed into a shorter precursor that undergoes further quality control for folding and ribonucleoprotein complex assembly. Only those molecules that pass QC are processed into the mature form that is part of the active telomerase complex.

FUTURE DIRECTIONS

To gain a comprehensive understanding of human telomerase biogenesis, regulation and turnover, future studies are aimed at identifying additional factors and using biochemical and genetic means to elucidate their functions. Unravelling how telomerase is made and regulated has led us to several exciting questions: Can we modulate telomerase activity by manipulating RNA processing events? Is increasing the levels of telomerase offering a path towards the treatment of telomeropathies? Does increased telomerase activity contribute to resilience and delay processes associated with ageing?

With respect to parthenogenesis, future studies will be focused on the mechanism of chromosome doubling and the regulation of cellular functions despite the presence of multiple genomes in hybrids. These studies will inform subsequent experiments aimed at understanding the molecular events through which barriers to unisexuality are overcome. Such research has far-reaching implications for areas as diverse as evolutionary biology and agriculture.

SELECTED PUBLICATIONS


OVERVIEW

The genome integrity of living organisms is challenged by by-products of cellular metabolism and external factors such as ultraviolet (UV) light and ionising radiation. To maintain genome integrity, mammalian cells have evolved elaborate mechanisms, jointly known as the DNA damage response (DDR), that regulates DNA repair and cell cycle checkpoints. Germline or somatic mutations in DDR genes that affect the cellular capacity to respond to DNA damage result in the accumulation of mutations. This consequently leads to syndromes that manifest as premature ageing and the development of cancer. Protein phosphorylation mediated by Ataxia-telangiectasia mutated kinase (ATM), Ataxia telangiectasia and Rad3 related (ATR) and DNA-dependent protein kinases (DNA-PKcs) play essential roles during the cellular response to DNA damage. We are employing quantitative mass spectrometry (MS) based proteomics to identify and characterise phosphorylation-and ubiquitin-dependent signalling networks that regulate the DDR in human cells.

RESEARCH HIGHLIGHTS

Recent studies revealed that exposure of human cells to UV light affects transcription, splicing and translation. These findings raised a number of questions: Are these changes a regulated process that is important for genome stability maintenance? If so, which signalling pathways and molecular mechanisms link DNA damage with the regulation of RNA metabolism? In addition to ATR-Chk1, studies have shown that p38 and JNK MAPK signalling is activated after UV light, however, the substrates and functions of these pathways remained poorly understood.

We combined kinase inhibition and quantitative phosphoproteomics to analyse kinase-dependent signalling in response to UV light. We defined the cellular phosphorylation events dependent on canonical DNA damage signalling mediated by the ATR-Chk1.
and the p38 MAPK pathways and determined the functional contributions of these pathways to the UV light-induced DDR. Whereas ATR primarily phosphorylates proteins that function in DNA repair and cell cycle regulation, the p38-MK2 signalling axis phosphorylates a multitude of RNA-binding proteins (RBPs). We identified 138 phosphorylation sites on 122 proteins to be dependent on p38-MK2 signalling. We showed that p38-MK2-dependent phosphorylation of cellular proteins triggers the recruitment of 14-3-3 dimers, thus providing a general mechanism that rapidly regulates RBPs after UV light exposure.

Furthermore, we demonstrated that the negative elongation factor (NELF) complex, which plays an essential role in promoting RNA polymerase II (RNA pol II) promoter-proximal pausing, is a substrate of p38-MK2. Biochemical studies and X-ray crystallography revealed that NELF complex phosphorylation leads to the recruitment of 14-3-3 to the RNA binding subunit NELFE. We employed SILAC-based MS to systematically probe the composition of chromatin after UV light exposure as well as to identify p38-dependent changes in protein recruitment to or dissociation from chromatin. Phosphorylation and subsequent interaction of the NELF complex with 14-3-3 promotes its rapid release from chromatin, which correlates with RNA pol II elongation.

Taken together, the results of our study provided insights into the function of the p38-MK2 signalling pathway in the regulation of RBPs and revealed a phosphorylation-dependent mechanism that promotes RNA pol II elongation and DNA repair in response to UV light (Figure 1). We anticipate that the provided datasets of UV light-induced phosphorylation sites and p38-dependent 14-3-3 interactions will enable further studies focusing on the functions of the p38-MK2 pathway in the regulation of different RNA metabolic processes after UV light exposure.

**FUTURE DIRECTIONS**

Posttranslational modifications of proteins by phosphorylation and ubiquitylation play important regulatory roles in the cellular response to DNA damage. However, the complete picture of posttranslational regulatory events that control the DNA damage response and its interplay with other cellular processes is missing. We will employ quantitative proteomics, biochemistry and cell biology to identify and characterise phosphorylation and ubiquitin-dependent processes that govern the DNA damage response. Furthermore, we aim to develop proteomics methods that will enable us to obtain an unbiased view of the chromatin proteome at sites of DNA damage.

**SELECTED PUBLICATIONS**


EDUCATION

2010  PhD in Biochemistry, Ludwig Maximilian University (LMU), Munich
2006  Diploma in Biochemistry, University of Leipzig

POSITIONS HELD

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

GROUP MEMBERS

Alina Bluhm  PhD Student; since 01/2014
Hanna Braun  PhD Student; since 06/2015
Núria Casas Vila  PhD Student; 11/2013 – 12/2018
Sabrina Dietz  PhD Student; since 01/2015
Albert Fradera Sola  PhD Student; since 03/2018
Teresa Laguna  Postdoc; 03/2016 – 04/2018
Michal Levin  Postdoc; since 09/2018
Katarina Luke  PhD Student; since 12/2017
Tony May  Master’s Student; 11/2017 – 09/2018
Emily Nischwitz  PhD Student; since 09/2018
Merve Öztürk  PhD Student; since 11/2015
Lara Perez  PhD Student; since 10/2015
Marion Scheibe  Postdoc; since 06/2013
Marian Scherer  Bachelor’s Student; since 11/2017
Vivien Schoonenberg  PhD Student; since 02/2018
Nikenza Viceconte  Postdoc; since 01/2018

OVERVIEW

Mass spectrometry is a powerful tool for studying proteins in an unbiased and global manner. The current improvements in identification accuracy, sample throughput, and data analysis allow the streamlined application of proteomics in answering diverse biological questions. Our group applies quantitative approaches, such as label-free quantitation (LFQ), reductive demethylation (DML) or stable isotope labelling with amino acids in cell culture (SILAC), which enables us to directly compare thousands of proteins in complex mixtures. These technologies allow us to study changes in protein expression and are also applied in 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.

RESEARCH HIGHLIGHTS

Phylointeractomics reveals evolutionary changes in protein binding

We developed a new experimental workflow for comparative evolutionary biology, termed “phylointeractomics”. Built upon the ability of mass spectrometry-based proteomics to identify proteins from sequence information, it is a perfect technique for cross-species comparison. In phylointeractomics, we interrogate a bait of interest with the proteome of evolutionarily related species in a systematic manner to uncover similarities and differences in protein binding. In a first application, we studied the telosome of 16 different vertebrate species ranging from zebrafish to human, which span a timeframe of 450 million years of evolution. While the telomeric sequence in vertebrates is a conserved TTAGGG repeat, there are some known variations of the interacting proteins, e.g. a Pot1 gene duplication in the rodent lineage and absence of TIN2 in several bird genomes. In our phylointeractomics screen, we recapitulated these evo-
Characterisation of new telomeric proteins

We use quantitative interactomics to identify new telomeric proteins. Apart from HOT1, we recently reported that the zinc finger protein ZBTB48 is a telomeric protein in mammals. The extension of our workflow to other model species resulted in the identification of novel telomere binding proteins in Trypanosoma brucei. We characterised one of these candidates, which we termed TelAP1 as a dynamic telomere binding protein that is differentially expressed between the two life cycle stages of the parasite.

Systems approaches to study developmental gene regulation

To study proteome dynamics during development, we generated two large developmental proteomic datasets of Drosophila melanogaster: a full life cycle dataset encompassing 15 different time points and a highly temporally-resolved proteome of its embryogenesis. As both datasets match the previously published modENCODE developmental transcriptome, we systematically compared developmental transcriptome and proteome expression, showing that for selected cases protein stability is the major determinant of protein levels. Additionally, we identified maternally loaded proteins, uncovered peptides originating from small open reading frames in lncRNAs and resurrected the pseudogene (Cyp9f3). The data is available to the research community via our web interface (www.butterlab.org/flydev).

Further, we teamed up with the Legewie group to use our large-scale transcriptomics and proteomics datasets to investigate posttranslational gene regulation by mathematical modelling.

**FUTURE DIRECTIONS**

We will continue to apply quantitative proteomics to diverse biological questions with a focus on differentiation, epigenetics, development and evolution. To this end, we are currently improving several parts of the proteomics and interactomics workflow established during the last few years in our group. Combining omics studies with classical biology, we are at the moment characterising novel telomeric proteins in diverse model species and investigating gene regulation in several eukaryotes.

**SELECTED PUBLICATIONS**


EDUCATION

1983  Habilitation in General Human Genetics and Experimental Cytogenetics, University of Freiburg

1976  PhD in Biophysics and Genetics, University of Freiburg

1970  Diploma in Physics, Ludwig Maximilian University (LMU), Munich

POSITIONS HELD

Since 2015  Research Associate, Max Planck Institute for Chemistry, Mainz

Since 2013  Honorary Professor, Johannes Gutenberg University Mainz

Since 2011  Group Leader, Institute of Molecular Biology (IMB), Mainz

Since 2005  Director, Cooperation Unit Biophysics, 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

GROUP MEMBERS

Shih-Ya Chen  PhD Student; since 05/2016

Marton Gelleri  Postdoc; since 05/2018

Maria Contreras Gerenas  Postdoc; since 02/2018

Xiaomin Liu  Postdoc; since 11/2018

Renata Pandolfo  PhD Student; since 11/2017

Florian Schock  PhD Student; since 03/2014

Felix Schreiber  Bachelor’s Student; since 07/2018

Fuguang Zhao  PhD Student; since 11/2018

OVERVIEW

A complete mechanistic understanding of gene regulation in space and time requires a detailed insight into the spatial constraints of gene activity control in the cell nucleus, along with sequence and biochemical information. For this, it is necessary to enhance the resolution of the spatial DNA distribution and other relevant molecules substantially beyond the conventional resolution level. This has become possible by various super-resolution light microscopy (SRM) approaches. SRM of the nuclear genome nanostructure is expected to contribute to fundamental questions including how does the complex organisation of chromosome territories in the mammalian cell nucleus arise, and how is this organisation related to epigenetic gene regulation and its dynamics? We have shown that Single Molecule Localisation Microscopy (SMLM), a type of SRM, is particularly useful for enhanced analysis of the cell nucleus. In 2018, we applied a recently developed SMLM approach to nuclear genome nanostructure imaging, designated DNA Structure Fluctuation Assisted Binding Activated Localisation Microscopy (fBALM), to analyse the mammalian cell nucleus in three dimensions at unprecedented resolution (optical and structural 3D single molecule resolution of few tens of nm). Present application fields include neurobiology (genome nanostructure of neurons) and human cancer tissues.

RESEARCH HIGHLIGHTS

In the last year, we focused on the following topics: What are the differences in DNA content between the low density (active) and the high density (inactive) compartments? What is the spatial nanoscale distribution of such small chromatin domains? How many nucleosomes do they contain? What is the variability of such nucleosome clusters? Information on these questions is essential for a quantitative, mechanistic and dynamic understan-
ing of epigenetic gene regulation. As such, we have applied a new technique (fBALM), developed in our group, for localisation microscopy of nuclear DNA at single molecule resolution. This substantially enhanced the structural resolution, down to a few tens of nm in 3D. The fBALM localisation microscopy confirmed that the nuclear genome is organised into distinct, presumably inactive, high-density domains with a size substantially below 100 nm in diameter, and, presumably active, low-density compartments (Figure 1). It was possible to obtain estimates of the absolute number of nucleosomes (DNA content) in such nanoclusters. New software packages were developed for an improved quantitative nanocluster analysis. To further enhance resolution, we completed the construction of a new 3D Single Molecule Localisation Microscope (3D-SMLM) in the last year. Using a spatial light modulator system, it is now possible to illuminate specific cells or intracellular regions and to enhance the homogeneity of the illumination. In addition, the 3D localisation mode of the new 3D-SMLM system allowed measuring up to 5.5 million different DNA sites throughout an entire three-dimensional nucleus, with a precision of about 10 nm. We also combined the fBALM method with Single Molecule Localisation Analysis using immunostaining. In addition, we constructed and implemented a multiplex micropump system; this system now makes it possible to prolong fBALM experiments up to around 10 hours. This will allow us to register up to 1 million image frames and thus map the nuclear 3D DNA distribution at unprecedented resolution (up to 30 million DNA sites/nucleus corresponding to 1 DNA position per nucleosome).

In collaboration with wet lab scientists, the new fBALM approaches were used to address a variety of research questions such as studying genome nanostructure in tissue sections from human colon cancer and in mouse neuronal cells. Our first experiments with Light Sheet Microscopy allowed us to identify almost all nuclei in thick mouse brain tissue sections. Finally, in collaboration with the Max Planck Institute for Chemistry, allergy-related membrane clusters (TLR4) and the translocation of NfkB to the nucleus were studied.

**FUTURE DIRECTIONS**

In addition to the continuation of the projects described above, the SRM instrumentations and the imaging and data evaluation methods developed will be applied in further collaborative projects. In one such example, in collaboration with the MPI for Polymer Research, an extremely fast switchable laser system will be adapted for SRM to facilitate multiplexed localisation microscopy.

The long-term perspectives of SRM of the nuclear landscape will be the integration of the collected collaborative data into a quantitative, mechanistic, predictive and dynamic space-time model of functional nuclear organisation and its integration into molecular biology approaches. Using super-resolution techniques, it should become possible to directly measure transcription induced condensation changes in space and time on the level of very small, individual nuclear domains in single nuclei and to study the nanostructural consequences of chromatin modifiers, e.g. histone/DNA methylases and demethylases.

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**RESEARCH GROUPS**

**SELECTED PUBLICATIONS**

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. 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 miRNAs) to full-blown shutdown of loci at the transcriptional level (piRNAs). We focus on mechanisms related to piRNA and siRNA biology, two species of small RNAs that are particularly abundant in, and important for, the germline. These small RNA pathways have a major role in maintaining genome integrity through controlling the activity of transposable elements. We use zebrafish and C. elegans as model systems to understand the molecular mechanisms governing these pathways and to understand how these pathways contribute to normal development. Questions including, how do small RNA pathways distinguish transposable elements from regular genes, how are these pathways organised at a sub-cellular level, and how can small RNA populations be inherited across generations are at the heart of our research.

RESEARCH HIGHLIGHTS

Flexible usage of a conserved protein in diverse small RNA pathways

In studying the factors required for small RNA pathways in different organisms, we found that the proteins acting in these pathways tend to evolve quickly. In many cases, factors that appear to be very species-specific are essential for such pathways. This likely reflects the biological niche in which these pathways operate: the control of endogenous parasites. We studied one of the few strongly conserved proteins that has been described
to act in the Piwi pathway of the fruit fly, GTSF1. Strikingly, we found that even though this protein is conserved at the sequence level, its molecular function is not. This protein has been found to trigger transcriptional silencing in flies, whereas we find it stimulates small RNA biogenesis in C. elegans. This protein allows the assembly of a larger molecular machinery that drives small RNA biogenesis. This principle may well apply to the fly, where GTSF1 might drive the assembly of a larger complex that instigates chromatin modification. This research emphasises that we can learn a lot from studying one factor in different organisms. Whereas one would have intrinsically coupled GTSF1 to chromatin biology based on the data from the fruitfly, our work, which was published this year in The EMBO Journal, shows that instead, it acts as a protein that allows the assembly of larger protein, or protein-RNA complexes.

Control of phase separation during germ cell development

We have been studying the effect of a piRNA-pathway component, named Tdrd6, on germ cell formation in zebrafish. Maternal loss of this protein leads to less efficient germ cell specification, but the molecular reasons behind this phenotype have remained unclear. We have now been able to show, using single-cell RNA sequencing, that Tdrd6 plays a role in ensuring every germ cell specified receives a certain ratio of mRNA species from different germ cell-specifying genes. Tdrd6 is itself present in granules that also bind such mRNA species, as can be visualised by single molecule FISH studies on zebrafish embryos. Tdrd6 drives the loading of these transcripts into germ cells by helping the fusion of small RNA-protein aggregates into larger units. This enables larger aggregates of these germ cell specifying mRNAs, also known as germ plasm, to be loaded into the future germ cells. Finally, we found that Tdrd6 performs this function by controlling the phase separation behaviour of a core germ plasm component named Bucky Ball (Figure 1). In absence of Tdrd6, Bucky Ball aggregates into amyloid-like structures that are no longer functional. This work demonstrates how, within a living animal, phase separation can be controlled and what the implications are if this control fails. This work was published this year in Developmental Cell.

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 begin to describe their mechanisms on a more molecular level. Both C. elegans and zebrafish will continue to play important roles in these studies. For instance, we will focus on a protein complex newly identified in our laboratory that is essential for the generation of piRNAs. This complex is also required for an as yet unidentified but essential pathway that acts during early embryogenesis. We will also further study phase separation and how it can be controlled in vivo. We recently identified a novel phase-separated structure that is specific to sperm and is essential for paternal inheritance of small RNA mediated gene control. We aim to unravel the molecular details behind this new intriguing structure and how it is controlled.
OVERVIEW

The functional state of a cell is ultimately defined by the state of its proteome, i.e. abundance, localisation, turnover and mobility of all proteins and their organisation in complexes and organelles. Numerous cellular systems contribute to proteome homeostasis through prevention, detection and removal of abnormal proteins. Selective protein degradation by the ubiquitin-proteasome systems plays a key role in proteome turnover and quality control. When degradation is not possible, the impact of abnormal proteins can be minimised through their asymmetric partitioning during cell division. Despite the activity of such systems, proteome homeostasis declines with ageing and in numerous diseases, resulting in accumulation of abnormal proteins and loss of cell functionality.

Working in yeast and human cells, we aim to systematically identify substrates for the various components of the ubiquitin-proteasome system and explore the functions of this system in replicative ageing and genome stability. We are using genetics and proteomic approaches that exploit fluorescent timers to follow protein trafficking, inheritance and degradation down to subcellular resolution. Our goals are to understand how protein quality control is coordinated with protein synthesis, folding and trafficking, to elucidate how cells recognise abnormal proteins and how they adapt to challenges in proteome homeostasis.

RESEARCH HIGHLIGHTS

Selective protein degradation is involved in most cellular processes and contributes to proteome homeostasis through the removal of unnecessary or abnormal proteins. The ubiquitin-proteasome system (UPS) plays a key role in selective protein degradation, whereby a cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-protein ligase (E3) enzymes mark proteins with polyubiquitin chains for degradation by the protea-
some. Deubiquitinating enzymes, which remove ubiquitin marks and replenish the pool of free ubiquitin, are involved at various stages of the targeting and degradation processes. Despite the central role of the UPS in protein degradation and its association with various diseases and ageing, many UPS components remain poorly characterised and our understanding of specificity in the UPS is inadequate.

We made some progress in identifying substrates and functions for various UPS components in the budding yeast *Saccharomyces cerevisiae*. Using a proteomic approach that relies on fluorescent timers as reporters of protein turnover, we were able to examine the impact of inactivating individual UPS components on the yeast proteome. This led us to the discovery of a protein quality control pathway that operates specifically at the inner nuclear membrane (INM). This pathway involves the Asi E3 ligase and appears to have a dual function: to control the abundance of specific INM-resident proteins, in a manner resembling classical endoplasmic reticulum-associated protein degradation pathways, and to maintain the identity of the INM, through recognition and targeting for degradation proteins that mislocalise to the INM.

In addition, we developed methods to facilitate further studies of the yeast proteome and address questions of specificity in the ubiquitin-proteasome system. Genome-wide libraries of strains in which each open reading frame (ORF) is fused to the same tag at its endogenous chromosomal locus are very useful tools to study the yeast proteome. But their construction is laborious and expensive, hindering the use of new tags in proteomic studies. To address these limitations, we developed the SWAp-Tag (SWAT) approach for high-throughput tagging of yeast ORFs (Figure 1). Using SWAT we are now able to endogenously tag 86% of yeast ORFs with virtually any tag in approximately three weeks, greatly expanding the possibilities of proteome-wide studies in yeast. Furthermore, we contributed to the development of multiplexed protein stability (MPS) profiling, a method to study protein degradation signals. These signals, also known as degrons, are typically short linear motifs. Proteins with exposed degrons are recognised by cellular quality control machinery and sent for degradation. With MPS profiling, it is possible to identify and dissect degrons by examining how the turnover of a reporter protein is influenced by fusing it to thousands of different protein fragments in parallel. This method will help us understand specificity in the ubiquitin-proteasome system.

**FUTURE DIRECTIONS**

We will continue our systematic search for substrates of E3 ubiquitin ligases in yeast by combining our reporter-based screens with mass spectrometry and expand this search to human cells. We will also apply genetic and proteomic approaches to identify redundancies in the ubiquitin-proteasome system and in this way, find substrates for overlapping degradation pathways. Finally, we will build on our work on INM-associated protein degradation to understand how cells recognise mislocalised proteins. We are eager to test the importance of such quality control pathways under stress and during the ageing process.

**SELECTED PUBLICATIONS**


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 implicated in disease, their detailed investigation is fundamental to our understanding of human biology in general and disease processes in particular.

The information in the RNA sequence and how it is read and interpreted by RNA-binding proteins (RBPs) is commonly referred to as the “splicing code”. Cracking this code remains a long-standing goal of RNA biologists. To further advance these efforts, our group uses functional genomics approaches to dissect the underlying regulatory networks. We developed new technologies to study the role of cis-regulatory RNA sequence elements and protein-RNA interactions at an unprecedented level of detail and resolution. Currently, we are addressing the following key questions: What is the role of cis-regulatory elements in alternative splicing? How do proteins assemble into functional ribonucleoprotein (RNP) complexes? How do RNPs act in gene expression quality control?

RESEARCH HIGHLIGHTS

Decoding a cancer-relevant splicing decision in the RON proto-oncogene using high-throughput mutagenesis

Mutations causing aberrant splicing are frequently implicated in human diseases including cancer. Together with Kathi Zarnack’s group at Goethe University Frankfurt and Stefan Legewie’s group at IMB, we established a high-throughput screen of randomly mutated minigenes (Figure 1) to decode the cis-regulatory landscape that determines alternative splicing of exon 11 in the proto-oncogene MST1R (RON). Mathematical modelling of splicing kinetics enabled us to identify more than 1000
Mutations affecting RON exon 11 skipping, which corresponds to the pathological isoform RONΔ165. Importantly, the measured effects correlated with RON alternative splicing in cancer patients bearing the same mutations. Moreover, we highlighted heterogeneous nuclear ribonucleoprotein H (HNRNPH) as a key regulator of RON splicing in healthy tissues and cancer. Using iCLIP and synergy analysis, we pinpointed the functionally most relevant HNRNPH binding sites and demonstrated how cooperative HNRNPH binding facilitates a splicing switch of RON exon 11. Our results thereby offer insights into splicing regulation and the impact of mutations on alternative splicing in cancer.

In vitro iCLIP-based modelling uncovers how the splicing factor U2AF2 relies on regulation by co-factors

Alternative splicing generates distinct mRNA isoforms and is crucial for proteome diversity in eukaryotes. The RBP U2AF2 is central to splicing decisions, as it recognises 3’ splice sites and recruits the spliceosome. Together with Stefan Legewie’s group at IMB, we established in vitro iCLIP experiments, in which recombinant RBPs are incubated with long transcripts, to study how U2AF2 recognises RNA sequences and how this is modulated by trans-acting RBPs. We measured U2AF2 affinities at hundreds of binding sites, and compared in vitro and in vivo binding landscapes by mathematical modelling. We found that trans-acting RBPs extensively regulate U2AF2 binding in vivo, including enhanced recruitment to 3’ splice sites and clearance of introns. Using machine learning, we identified and experimentally validated novel trans-acting RBPs (including FUBP1, CELF6 and PCBP1) that modulate U2AF2 binding and affect splicing outcomes. Our study offers a blueprint for the high-throughput characterisation of in vitro mRNP assembly and in vivo splicing regulation.

Selected Publications


Figure 1. The high-throughput mutagenesis screen provides quantitative splicing information across the RON minigene. Mutagenic PCR creates a library of mutated minigene variants that upon transfection into human cell lines give rise to alternatively spliced transcripts. A unique 15-nt barcode sequence in each minigene (coloured nucleotides) links mutated minigenes and corresponding splicing products, which are characterised by next-generation DNA and RNA sequencing, respectively. A mathematical model allows for a high-throughput identification of splicing-effective mutations.

Future Directions

Quantitative description of protein-RNA interactions with the in vivo and in vitro iCLIP technologies offers an attractive system for understanding the forces of competition and synergy that govern RNP complexes. We focus our efforts on elucidating 3’ splice-site definition, which was previously identified as a hotspot for cancer-associated mutations. To achieve this, we are combining in vivo and biochemical approaches on a genome-wide scale, which will yield a systemic understanding of RNP function in splicing regulation.

Reaching into translational research, we are extending our high-throughput mutagenesis approach to study splicing-mediated resistance in paediatric cancer therapy. The knowledge of splicing-effective mutations will enable us to develop prognostic biomarkers and may ultimately lead to new therapy strategies.
EDUCATION
2008 PhD in Biophysics, Humboldt University, Berlin
2004 Diploma in Biochemistry, University of Witten/Herdecke

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

GROUP MEMBERS
Alex Anyaegbunam PhD Student; since 08/2016
Kolja Becker PhD Student; 08/2013 – 07/2018
Mihaela Enculescu Postdoc; since 10/2013
Rishi Horn Student Assistant; since 10/2018
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Lorenz Ripka PhD Student; since 05/2017

OVERVIEW
Eukaryotic cells sense and process information in order to respond to environmental changes. While the signalling pathways relaying information from the membrane to the nucleus are well characterised, much less is known about decision making at the level of gene expression responses. One focus of our group is to derive a systems-level understanding of gene regulation, which describes: (i) the interplay of signalling pathways and transcription factors in complex gene-regulatory networks; (ii) how the gene expression is co-ordinately controlled at the transcriptional and post-transcriptional levels. We tackle these questions by integrating systematic perturbation screens and multi-OMICS data to derive predictive mathematical models.

A second focus of our group is the quantitative description of cellular heterogeneity. Even genetically identical cells frequently respond in different ways to the same external stimulus, leading to differences in differentiation programs, drug resistance and viral pathogenesis. Together with experimental partners, we employ live-cell imaging approaches to calibrate stochastic and deterministic models of cell population heterogeneity. We employ these models to: (i) derive experimentally testable hypotheses about the causes and consequences of cellular heterogeneity; (ii) better understand therapeutic intervention strategies.

RESEARCH HIGHLIGHTS

Single-cell dynamics of signalling and transcription
We investigated cell-to-cell variability in TGFβ/SMAD signalling, which plays a key role in tumorigenesis and metastasis. Together with Alexander Löwer (Darmstadt University), we monitored SMAD2 nuclear translocation in thousands of living MCF10A cells and explained heterogeneous signalling using a quantitative modelling approach, thereby gaining insights into why only a subset of cells induce a migratory response. Along similar
lines, using quantitative imaging and stochastic modelling, we characterised the heterogeneous growth of breast cancer cells by studying the stochastic transcription of a key regulator of estrogen-induced cell proliferation. We found that conventional therapeutic inhibitors of estrogen signalling have limited efficacy, as they induce pronounced gene expression heterogeneity at the single-cell level. This heterogeneity can be overcome by the co-application of small-molecule inhibitors of epigenetic processes. Hence, therapeutic success in cancer treatment could be greater when estrogen receptor antagonists would be applied in combination with other inhibitors.

**Reconstruction of alternative splicing networks**

Alternative splicing increases protein diversity in eukaryotic cells thereby playing an important role in development and tissue identity but also in diseases such as cancer. Splicing reactions are catalysed by the spliceosome and moderated by auxiliary RNA-binding proteins (RBPs). These guide splicing activity, thereby promoting splicing specificity. The RBP U2AF2 is central to alternative splicing decisions as it recruits the spliceosomal machinery to 3’ splice sites. To study how U2AF2 recognises RNA sequences, we, together with the König group, established "in vitro" iCLIP experiments in which recombinant RBPs are incubated with long transcripts. Based on titration experiments, we determined U2AF2 affinities at hundreds of binding sites and compared the in vitro and in vivo binding landscapes by mathematical modelling. Surprisingly, purified U2AF65 does not exhibit any intrinsic binding preference for 3’ splice-sites, suggesting extensive modulation of U2AF2 binding by trans-acting RBPs in living cells. Using machine learning, we identified RNA-binding motifs that are predictive for enhanced or reduced U2AF2 binding and experimentally validated novel trans-acting RBPs controlling U2AF2 binding and splicing outcomes. Our study provided insights into the specificity of alternative splicing decisions.

Mutations causing aberrant splicing are frequently implicated in human diseases including cancer. Together with the König and Zarnack groups, we established a high-throughput screen of randomly mutated minigenes to decode the cis-regulatory landscape that determines alternative splicing of exon 11 in the proto-oncogene MST1R (RON). A combination of kinetic and linear regression modelling enabled us to identify the effects of individual point mutations and to understand how these individual mutations interact to control splicing outcomes. In fact, we identified more than 1,000 mutations affecting RON exon 11 skipping, which corresponds to the pathological isoform RONA165 (Figure 1). Importantly, the effects correlate with RON alternative splicing in cancer patients bearing the same mutations. Our results, therefore, offer insights into splicing regulation and the impact of mutations on alternative splicing in cancer. In the future, we plan to further characterise trans-acting RBPs that recognise cis-regulatory elements defined by the mutagenesis screen.

**Figure 1.** Regression modelling determines more than 1,900 single mutation effects in the MST1R (RON) proto-oncogene. Experimental data (left). Using RNA-Seq, splice isoforms were quantified for ~5,000 minigene variants, each harbouring a unique set of combined point mutations. A kinetic model of splicing reactions (middle) reveals that point mutation effects show additive behaviour if the data is properly normalised. Thus, a linear regression model (right) was formulated, in which the measured effect of combined mutations equals the sum of single mutation effects. The single mutation effects are inferred by fitting this model to the data. See Braun et al (2018) for details.

**FUTURE DIRECTIONS**

We plan to further refine and develop existing models of signalling and gene expression. For instance, we are investigating the stochastic dynamics of TGFβ/SMAD signalling and studying additional prototypical splicing events. With this, we hope to convert these general insights into a splice code, which describes splicing outcomes based on sequence information and RBP expression patterns. Moreover, we have begun to model the dynamics of DNA (de)methylation to better understand how this important epigenetic mark can be set and erased to tune gene activity.

**SELECTED PUBLICATIONS**


EDUCATION

2005  PhD, Max Planck Institute for Biophysical Chemistry & University of Göttingen

2001  Diploma in Chemistry, Technical University of Berlin

2001  MSc in Biochemistry, University of Oklahoma

POSITIONS HELD

Since 2018  Adjunct Director, Institute of Molecular Biology (IMB), Mainz

Professor of Synthetic Biophysics, Johannes Gutenberg University Mainz

Since 2009  Group Leader, European Molecular Biology Laboratory (EMBL), Heidelberg

2005 – 2008  Research Associate (Postdoc), The Scripps Research Institute, La Jolla

GROUP MEMBERS

Joana Caria  Lab Manager; since 04/2017

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Giulia Paci  PhD Student; since 10/2014

Panagiotis Patsis  PhD Student; since 11/2018

Christopher Reinkemeier  PhD Student; since 09/2016

Piau Siong Tan  Postdoc; since 04/2015

Miao Yu  Postdoc; since 10/2018

OVERVIEW

We focus on studying intrinsically disordered proteins (IDPs), which constitute up to 50% of the eukaryotic proteome. IDPs are found in many vital biological processes, such as nucleocytoplasmic transport, transcription and gene regulation. The ability of IDPs to exist in multiple conformations is considered a major driving force behind their enrichment during evolution in eukaryotes. Studying biological machineries containing such dynamic proteins is a major hurdle for conventional technologies. Because of this and as they are hard to visualise, IDPs are termed the dark proteome. Using a question-driven, multidisciplinary approach paired with novel tool development, we have made major strides in understanding the biological dynamics of such systems from the single molecule to the whole cell level. Fluorescence tools are ideally suited to study the plasticity of IDPs, since their non-invasive character permits smooth transition between in vitro (biochemical) and in vivo (in cell) studies. In particular, single molecule and super-resolution techniques are powerful tools for studying the spatial and temporal heterogeneities that are intrinsic to complex biological systems. We synergistically combine this effort with advanced tool developments in chemical biology, microfluidics and microscope engineering to increase the throughput, strength and sensitivity of the approach as a whole.

RESEARCH HIGHLIGHTS

Our strong focus on the mechanistic understanding of IDPs using single molecule and super-resolution tools is both driven and driving novel tool developments for "in-cell/in situ structural biology". This comprises of a synergistic effort of chemical/synthetic biology and precision fluorescence-based technology/nanoscopy/microfluidics development (Figure 1).
A major technical breakthrough of my lab was the ability to engineer “click”able functionalities into any protein both in vitro and in vivo. This genetic code expansion (GCE) approach has the potential to become a true GFP (fusion protein) surrogate strategy, with the major advantages being that superior synthetic dyes can be coupled with residue-specific precision anywhere in a protein. This opens up new avenues in single-molecule fluorescence and super-resolution microscopy.

These precision tools enable us to make even the most complex molecular machinery visible to our core methodologies, which are based on time-resolved multiparameter and nanoscopy tools. This enables innovative approaches to study the heterogeneity of IDPs in biology. More recently, we discovered a distinct ultrafast protein-protein interaction mechanism that can explain how nuclear pore complexes (NPCs) can efficiently fulfill their central role in cellular logistics and how nuclear transport can be both fast and selective at the same time. We also determined how this function can coexist with other nuclear transport mechanisms that provide a platform for cargo undocking. These findings provided a leap forward in our understanding of how IDPs maintain different functionalities through conformational changes despite the normal requirement for rigid molecular specificity.

Despite our advancing technologies for in situ science, we always consider it important to perform studies on reconstituted systems (in vitro/biochemical) to understand our biological problems in a bottom-up fashion and complement our results from our in situ studies. To achieve this, we worked on various aspects e.g. i) utilising microfluidics to integrate Lab-on-chip technology into our workflows and ii) developing a versatile baculovirus-based platform, which combines the benefits from GCE technology and the versatility of click chemistry with the strength of recombinant protein engineering. This technology now enables in vitro/bottom up/reconstitution based biological project design that was previously unachievable in situ. Our work is accompanied by rigorous analysis on the physicochemical properties of IDPs and examines to what extent simple, known polymer concepts, such as phase separation, can be used to describe the function of such biopolymers in vivo.

**FUTURE DIRECTIONS**

IDPs are highly multifunctional. Due to their multivalency and large disordered regions they can function as dynamic scaffold platforms. We combine chemical and synthetic biology approaches to enable non-invasive, multi-color high- and super-resolution studies of activity-dependent changes of protein conformation in living cells, enabling fluorescence driven in situ structural biology. The key point is that the enhanced spatial and temporal resolution offered by our fluorescent methods will enable us to detect rare events and unexpected behaviours inside cells. We want to use this to identify and understand IDP multifunctionalities that are clearly distinguishable from their normal mode of action, for example nucleoporins (Nups) in the nuclear pore complex (NPC). In fact, many IDP-Nups have roles in pathogen-host interactions and have been suggested to shuttle away from the NPC to function in gene regulatory processes. A prominent example of IDP-Nup action distinct from its normal mode in the NPC is Nup98, since several genetic fusions of Nup98 with transcription factors are related to leukaemia.

**SELECTED PUBLICATIONS**


OVERVIEW

It has become increasingly clear that RNA plays a critical role in a myriad of DNA transactions ranging from transcription regulation to DNA repair and chromosome 3D structure. RNA has the ability to form hybrid molecules with DNA in the form of R-loops, RNA-DNA triplexes and ribonucleoside monophosphate (rNMP) insertions directly into the DNA strands. We are only beginning to understand how these RNA moieties are regulated as well as the consequences that arise when they are misregulated. We are currently focused on understanding how the RNase H enzymes are coordinated in RNA-DNA hybrid removal in terms of both time and space. Currently, there is very little understanding regarding the regulation of these critical RNA-DNA hybrid regulatory enzymes. In addition to the regulation of the RNase H enzymes, we are trying to understand how the replication machinery reacts when RNA-DNA hybrids are not adequately eliminated. In this respect, a recently described RNase H2 separation of function allele is allowing us to interrogate the differences between rNMPs and R-loops. We use a variety of biochemical, proteomic and genetic techniques to address these mechanistic questions. The recent implication of RNase H mutations in human disease makes these studies pertinent to eventual medical applications.

RESEARCH HIGHLIGHTS

We have had a longstanding interest in understanding how the ubiquitin ligase, Rtt101^mms1^ contributes to genome stability and DNA replication fidelity. We have demonstrated that the conserved Rtt101 ligase becomes essential when rNMPs accumulate (Figure 1A). This genetic interaction is relevant, as mutations in RNase H2, which removes rNMPs from the genome, have been implicated in neurological disorders as well as human cancer. We used a mass spectrometry-coupled ubiquitin remnant profiling approach to identify the relevant target(s) of Rtt101 when
rNMPs accumulate. We identified and confirmed that the DNA polymerase epsilon subunit, Dpb2, gets ubiquitylated in a Rtt101 dependent manner when ribonucleotides accumulate (Figure 18). Follow-up biochemical fractionations have demonstrated that Rtt101 promotes the removal of Dpb2, as well as other DNA polymerase epsilon subunits, from chromatin. Based on these results, we propose a hypothesis whereby DNA damage-induced replication stress on the leading strand triggers the eviction of DNA polymerase epsilon in a Rtt101 ubiquitylation dependent manner. Based on our previous studies, we speculate that polymerase removal is essential to promote homologous recombination-mediated restart of stalled replication forks.

RNase H enzymes are dedicated to the nucleolytic removal of RNA that is hybridised to DNA. RNase H1 is a monomeric enzyme that degrades RNAs hybridised to DNA with the involvement of 4 or more base pairs, and is largely limited to R-loop metabolism. RNase H2, on the other hand, is a heterotrimeric enzyme that can eliminate both R-loops and rNMPs. By creating cell cycle-regulated alleles of the RNase H enzymes we have been able to determine that RNase H1 removes R-loops in the S phase of the cell cycle. RNase H2, however, functions in the G2 phase of the cell cycle where it carries out ribonucleotide excision repair as well as R-loop removal. These results suggest that RNase H2 may also promote a post-replicative repair function.

Telomeres are transcribed into a non-coding RNA, referred to as TERRA (Telomeric repeat-containing RNA). We have demonstrated that TERRA R-loops accumulate at short telomeres and promote homology-directed repair due to the inability of RNase H2 to localise to those telomeres. We have used a proteomics approach to assess which proteins may localise to short telomeres in an RNA dependent manner. We identified Npl3, an hnRNP-like protein, as a novel telomere binding factor. Npl3 is specifically recruited to short telomeres in an R-loop dependent manner. In the absence of Npl3, cells enter replicative senescence with accelerated kinetics, suggesting the recruitment of Npl3 to short telomeres is essential to regulate senescence onset by directly regulating telomeres. Our preliminary data indicate that Npl3 may be promoting the formation of R-loops, which in turn drives telomeric recombination.

Finally, we have shown that when repair defective yeast cells are exposed to DNA damaging agents, they undergo checkpoint adaptation. The adapted cells acquire drug resistance and are aneuploid. We have now shown that the combination of genotoxic agents together with targeting of adaptation and aneuploidy, leads to a synergistic cytotoxicity specifically of repair defective cells. These results are highly relevant for cancer chemotherapy.

**FUTURE DIRECTIONS**

The function of Dpb2 ubiquitylation will be investigated with regards to how it affects DNA replication and repair dynamics by creating non-ubiquitylatable Dpb2 alleles and analysing replication and repair. We will also try to determine whether the ubiquitylation of Dpb2 leads to degradation of the protein. Finally, we will investigate upstream signalling events that may trigger the modification of Dpb2.

The genetic data that we have generated with the RNase H cell cycle alleles will be substantiated with biochemical experiments to determine levels of R-loops and rNMPs in the genome when the alleles are expressed. Using a combination of genetic experiments and imaging we will test the potential post-replicative repair function of RNase H2. We will use the telomere as a model locus to elucidate how R-loops drive recombination. We will test the hypothesis that for recombination at telomeres an R-loop must be present but then eventually removed. Npl3 and Rnh1 are candidates to examine in this context.

**SELECTED PUBLICATIONS**


DNA DEMETHYLATION AND REPROGRAMMING

CHRISTOF NIEHRS

“WE ARE A STEP CLOSER TO UNDERSTANDING HOW R-LOOPS ACT AS EPIGENETIC GENE REGULATORS”

EDUCATION

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

POSITIONS HELD

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

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Tamara Dehn Animal Caretaker; since 06/2011
Anna Luise Ernst PhD Student; since 04/2015
David Fournier Postdoc; since 09/2018
Dandan Han PhD Student; since 12/2013
Laura Krebs Technician; since 09/2015
Medhavi Mallick PhD Student; 08/2012 – 09/2018
Carmen Meyer Technician; since 05/2018
Michael Musheev Postdoc; since 07/2011
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Eleftheria Parasyraki PhD Student; since 09/2018
Mihika Pradhan PhD Student; since 12/2016
Sandra Rölle Lab Manager; since 04/2011
Andrea Schäfer Postdoc; since 09/2010
Carola Scholz Technician; since 05/2015
Lars Schomacher Postdoc; since 07/2011
Katrin Schüle PhD Student; since 01/2016
Philipp Trnka PhD Student; since 11/2016
Viviana Vastolo Postdoc; since 09/2015

OVERVIEW

In the genome 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 occurring in plants as well as in animals during development, in the adult and during the somatic cell reprogramming of pluripotency genes. We showed that Growth arrest and DNA damage 45A (GADD45A) is a key player in active DNA demethylation and acts via DNA repair. One goal of our research is to analyse the mechanism of DNA demethylation as well as the role played by GADD45A in development. Our results indicate that GADD45A acts as an adapter protein, which directs DNA methylation machinery to specific loci. To address GADD45A function, we use biochemical, molecular biology and cell biological approaches, employing the mouse and frog model systems as well as embryonic stem cells.

RESEARCH HIGHLIGHTS

DNA methylation at 5-methylcytosine (5mC) of CpGs plays important roles in regulating gene expression, genomic imprinting, X-chromosome inactivation, genomic instability, embryonic development, and cancer. 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 previously that the stress response protein Gadd45a mediates active DNA demethylation. GADD45 proteins are multifunctional and regulate a range of cellular processes, including DNA repair, proliferation, apoptosis, and differentiation. GADD45A directly interacts with the two key enzymes of the DNA
demethylation machinery, TET (Ten-eleven translocation) and TDG (thymine-DNA glycosylase), to enhance turnover of oxidized cytosines. Therefore, GADD45A acts as an adapter, which recruits DNA modifying enzymes to specific sites in the genome and promotes local demethylation.

To be directed to specific genomic loci, GADD45A relies on bridging factors, which include RNA. We previously showed that expression of the tumour suppressor TCF21 is activated by a lncRNA, termed TARID (for TCF21 antisense RNA inducing demethylation), which is transcribed in antisense orientation to TCF21. TARID recruits the DNA demethylation machinery to the TCF21 promoter, leading to decreased DNA methylation and increased expression of TCF21. TARID-dependent activation of TCF21 transcription is brought about by recruitment of GADD45A, which tethers TDG and TET proteins to specific genomic sites to direct base excision repair mediated DNA demethylation.

How does TARID interact with the TCF21 promoter? We hypothesised that being an antisense transcript, TARID could form an R-loop at a CpG island (CGI) in the promoter of TCF21, which is recognised by GADD45A. R-loops are naturally occurring three-stranded DNA:RNA hybrids and have recently gained increasing attention, since they play important roles in transcription, RNA processing, DNA damage, and genome stability. Genome-wide mapping showed that they exist throughout the genome under physiological conditions in diverse organisms. R-loops can be detrimental to cells because they can induce DNA damage and genome instability and have been associated with neurodegenerative disease. Conversely, R-loops also play a regulatory role in various nuclear processes, notably in the regulation of gene expression.

We now found that TARID indeed forms an R-loop at the TCF21 promoter, which is bound by GADD45 to trigger local DNA demethylation and TCF21 expression. TARID transcription, R-loop formation, DNA demethylation, and TCF21 expression proceed sequentially during the cell cycle to allow both the sense and antisense RNAs to be expressed without hindrance. In support of a generic action of GADD45A we found that oxidized DNA demethylation intermediates are enriched at genomic R-loops and that their levels increase upon RNase H1 depletion. Genomic profiling in embryonic stem cells identified thousands of R-loop-dependent TET1 binding sites at CGIs. Our study supports to notion, that R-loops associated with certain classes of RNAs can act as bona fide epigenetic regulators. Conceptually, R-loops are attractive candidates because analogous to bacterial guide RNAs, which form R-loops to direct CAS9 endonuclease, mammalian R-loops may direct epigenetic modifiers in a sequence-specific manner via base pairing to genomic loci. Our study indicates that GADD45A is an epigenetic reader of such regulatory R-loops at CGIs (Figure 1).

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**FUTURE DIRECTIONS**

Our discovery of GADD45A as an R-loop reader raises new questions. How are R-loops decoded in embryonic stem cell pluripotency and differentiation? Which R-loop regions in ESCs are engaged in epigenetic regulation and what are their molecular determinants? To which R-loops is TET1 guided? How are regulatory R-loops erased? Are there other specific R-loop readers and what are their functions? We will approach these questions in embryonic stem cells where R-loops have been well documented and which are easily amenable to genetic experimentation.

**SELECTED PUBLICATIONS**


EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

Daniel Henning  Master’s Student; 04/2018 – 11/2018
Aysegül Kaymak  PhD Student; 02/2013 – 04/2018
Rebeca Medina  Technician; 08/2013 – 11/2018
Jens Stadler  PhD Student; since 09/2014

OVERVIEW

The research undertaken in my laboratory aims at deciphering molecular pathways that underlie chromatin signalling networks that regulate physiological processes such as DNA repair and organismal ageing. Our scientific approach relies largely on dissecting the functions of diverse chromatin components, as for example epigenetic players, biochemically in cell culture systems and by applying high resolution microscopy. We complement our in vitro approach by employing genetics and RNAi screening techniques in Caenorhabditis elegans.

RESEARCH HIGHLIGHTS

Epigenetic networks 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. In our investigations studying DNA repair, we have largely concentrated on one particular histone mark, the mono-ubiquitylation of histone H2A at lysine 119 (H2A-ubiquitin). H2A-ubiquitylation is a hallmark of signalling cascades as part of the DNA damage response. We have demonstrated that timing of DNA repair specific E3 ligases is an important feature of nucleotide excision repair (NER) and we have discussed a new concept of remodelling E3 ligase complexes at chromatin during DNA lesion recognition. In brief, we discovered that H2A-ubiquitin is catalysed predominantly by a novel E3 ligase complex (UV-RING1B complex) that operates early during lesion recognition (Figure 1). ZRF1 tethers to the H2A-ubiquitin mark at the damage site and mediates the remodelling of the UV-RING1B complex, a process that we have coined on-site remodelling.

We have further demonstrated that on-site remodelling in the global genomic branch of NER is confined to specific nuclear
regions. More recently, we have shown that ZRF1, apart from remodelling multi-protein complexes, is also engaged in the decondensation of chromatin. ZRF1 recruits the endoribonuclease DICER to the DNA damage sites and both proteins in conjunction with PARP1 facilitate the remodelling of chromatin (Figure 1). We have further demonstrated that DICER recruits the methyltransferase MMSET to the DNA damage site, which catalyzes the dimethylation of histone 4 at lysine20 (H4K20me2). This chromatin mark tethers the DNA repair factor XPA via the adaptor proteins 53BP1 and RPA2 (Figure 1). More recently, we have examined the concerted function of ubiquitylation events and DNA incision at the DNA damage site.

Furthermore, we are interested in understanding the impact of gene regulation and DNA repair mechanisms during organismal ageing. To this end we investigate ageing in the nematode *C. elegans*, employing sophisticated RNAi screening techniques, genetics and high resolution microscopy. We performed the first late-life RNAi screen designed to discover novel longevity genes that exhibit antagonistic pleiotropy in *C. elegans*. We identified that post-reproductive inactivation of genes required for autophagosome nucleation, such as the *Atg6/VPS30/beclin-1* ortholog *bec-1*, led to a strong lifespan increase of up to 60% post-RNAi initiation. We could further show that while the process of vesicle nucleation is still active and possibly enhanced in old worms, the process is blocked downstream of autophagosome biogenesis at the step of autolysosomal degradation. Moreover, we found that post-reproductive inactivation of autophagosome nucleation extends lifespan primarily through the neurons. Currently we are exploring the functions of DNA repair factors in organismal ageing. To this end we have carried out a late-life RNAi screening of factors from different DNA repair systems. We are now investigating candidate DNA repair factors in detail to reveal their significance in the ageing process.

**Future Directions**

In the future, we will further prioritise the research on DNA repair in the NER pathway and the investigation of organismal ageing. One of our main aims is to understand the chromatin signalling network underlying DNA damage recognition in the NER pathway and the transition from recognition to the verification of DNA damage. In particular, we will analyse how ubiquitin signalling cascades crosstalk to other chromatin factors and histone marks. Furthermore, we will investigate the function of K63-linked polyubiquitylation in NER, which presumably provides a means of recruiting repair factors. To extend our studies in organismal ageing, we plan to study how chromatin factors and environmental cues extend the lifespan and the health span of *C. elegans*. In particular, we will examine late life functions of metabolic genes.

**Selected Publications**


**OVERVIEW**

Our work focuses on elucidating the functions and mechanisms of RNA modifications, a novel layer of post-transcriptional gene regulation also known as epitranscriptomics. Modifications of RNA are very abundant, as more than 170 types have been detected in the last sixty years. However, understanding of their biological significance has been hampered by a lag in the technology needed for their identification. As a result, very little is known about their role in messenger and non-coding RNAs. Over the past few years, work from us and other groups has contributed to deciphering the role of mRNA modifications in gene regulation, revealing their importance in several physiological processes and diseases. Current projects in the lab deal with the m6A mRNA modification as well as less characterised modifications. For our research, we use *Drosophila melanogaster* as a model organism and employ state of the art approaches including molecular biology and classical genetics combined with high throughput techniques and computational tools.

**RESEARCH HIGHLIGHTS**

**Elucidation of the roles, mechanisms and targets of the m6A mRNA modification**

The widespread roles of m6A RNA modification in the regulation of post-transcriptional gene expression have recently been brought to light. From yeast to mammals, m6A has been shown to regulate pre-mRNA splicing, translation and mRNA decay. However, the precise mechanisms of this modification in these processes still remain to be determined. Furthermore, the physiological functions of m6A in multi-cellular organisms have not yet been fully investigated. To address these questions, we have characterised the m6A pathway in *Drosophila*. We found that a conserved m6A methyltransferase complex controls alternative splicing in both *Drosophila* cells and in vivo. As in mammals, components
of the complex are ubiquitously expressed but show significant enrichment in the nervous system, which is consistent with the high level of m^6^A in this tissue. We find that flies mutant for \textit{Mettl3} are viable but suffer from severe locomotion defects due to impaired neuronal functions. A synaptic overgrowth was observed at neuromuscular junctions (Figure 1). Components of the m^6^A methyltransferase complex also control the female-specific splicing of the Sex lethal (Sxl) transcript and of its downstream targets, revealing a role for this modification in sex determination and dosage compensation. We have recently identified a new member of the methyltransferase complex that we have named Flacc for Fl(2)d Associated Complex Component. Our work suggests that Flacc serves as an adapter to link Spenito with other components of the methyltransferase complex. We demonstrated that this association is critical for m^6^A deposition. In addition, we have developed a new approach to map m^6^A in vivo as current techniques were not adapted for in vivo studies. We are currently taking advantage of this approach to characterise m^6^A molecular targets and their functions during organismal development.

**Functions of Pseudouridine synthase 7 (\textit{Pus7}) in neuronal behaviour**

For decades, pseudouridine has been known to be the most abundant modification on transfer RNA (tRNA) and ribosomal RNA (rRNA) and more recently was found to be also widespread in mRNA molecules. In humans, pseudouridine is catalysed via 13 distinct pseudouridine synthases (PUSs), each of which has distinct specificities, most of them only poorly understood. In collaboration with the groups of Schraga Schwartz (Weizmann Institute) and Arjan de Brouwer (Radboud University) we have identified several human patients with distinct \textit{Pus7} mutations. All these individuals have intellectual disabilities with speech delay, short stature, microcephaly, and aggressive behaviour. We showed that the disease-related variants lead to abolishment of \textit{PUS7} activity on both tRNA and mRNA substrates. Moreover, we generated a \textit{Pus7} knockout in \textit{Drosophila melanogaster}. Mutant flies are viable but display a number of behavioural defects, including increased activity, disorientation, and aggressiveness supporting the idea that neurological defects are caused by \textit{PUS7} variants. Therefore, our findings demonstrate that RNA pseudouridylation by \textit{PUS7} is essential for proper neuronal development and function. We are currently looking for the relevant targets of \textit{Pus7} in mediating its neuronal defects, both in \textit{Drosophila} and in patient cells.

**Figure 1.** Lack of m^6^A modification results in axonal growth at neuromuscular junctions. Representative confocal images of muscle – 6/7 NMJ synapses of abdominal hemisegement A2 for the indicated genotypes labelled with anti-Synaptotagmin (green) and HRP (red) to reveal the synaptic vesicles and the neuronal membrane. \textit{Mettl3}Δcat/\textit{Mettl3}null mutants display synaptic outgrowths. Scale bar: 20 μm.

**FUTURE DIRECTIONS**

Our long-term goal is to decipher the chemical code decorating mRNA and how it impacts gene regulation during development and diseases. We are currently performing CRISPR/Cas9 based-screens to identify novel functions for specific RNA modifications. Our study should expose a new layer of regulation of gene expression that may have important implications for understanding key cellular processes that dictate cell fate.

**SELECTED PUBLICATIONS**


EDUCATION

2008  PhD in Molecular Biology & Cytogenetics, Medical School, University of Patras
2005  MSc in Applications in Medical Sciences, Medical School, University of Patras

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, National Institutes of Health (NIH), Bethesda

GROUP MEMBERS

Marta Cipinska  PhD Student; since 05/2015
Henrike Gothe  PhD Student; since 05/2016
Monika Kuban  Lab Manager; since 01/2017
Vera Minneker  PhD Student; since 04/2016
Rossana Piccinno  PhD Student; since 04/2015
Gabrielle Sant  PhD Student; since 11/2018

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. Translocations form by the illegitimate joining of chromosome breaks and often play key roles in the initial steps of tumorigenesis. Despite their prevalence and importance, our understanding of their genesis is, however, still rudimentary. Which are the molecular features that define recurrent chromosome breakpoints? How do the broken chromosome ends find each other within the nuclear space? Which are the DNA repair mechanisms that mediate the chromosome fusion and which are the factors that favour the interchromosomal fusion (translocation) over the intrachromosomal repair? By using a combination of molecular biology techniques, genetics and high-throughput imaging and sequencing approaches, we aim to shed light on the basic molecular mechanisms underlying the formation of oncogenic chromosome translocations.

RESEARCH HIGHLIGHTS

Novel imaging-based tools to probe rare, cancer-initiating genome rearrangements

Modelling the formation of recurrent cancer-initiating genome rearrangements of interest requires a versatile approach that can probe rare events with high sensitivity. We have now established a methodology, C-Fusion 3D, that uses fluorescence in situ hybridisation (FISH) to probe the position of individual chromosome ends of potential translocation partners in interphase cells in 3D. High-throughput microscopy and automated image analysis is
then used to identify single cells with chromosome breakage and translocations. This methodology complements existing approaches and offers several advantages in detection and quantification of translocations. It is: (a) suitable for detection of translocations without the requirement to map the precise translocation breakpoints or fusion product; (b) compatible with both site specific induction of breaks (mediated by endonucleases, ZNFs, CRISPR) and more physiological methods of inducing DNA damage, such as ionising radiation and chemotherapeutics (see below); (c) efficient in detecting translocations in interphase cells without the need of metaphase spread preparation with frequencies down to 10⁻⁴. C-Fusion 3D is a powerful tool that can be used to dissect molecular and cellular mechanisms that contribute to the formation of any oncogenic chromosome translocation of interest.

Mechanistic insights into the formation of therapy-related, oncogenic translocations

A major problem following successful chemotherapy is the appearance of a second primary cancer, which is a direct consequence of the treatment due to the formation of cancer-initiating translocations. As an example, therapy-induced acute myeloid leukaemias (t-AMLs) often develop after treatment with alkylating agents or topoisomerase-inhibitors and are characterised by distinct chromosome abnormalities that drive the occurrence of the secondary malignancies (Figure 1). Sequencing analysis of translocation junctions found in patients revealed recurrent fusion hotspots, indicative of defined molecular mechanisms that contribute to their formation. To identify molecular features that define recurrent breakpoint sites upon treatment with chemotherapeutic agents, we are using state-of-the-art genomic methodologies that map double-strand breaks (DSBs) across the genome with single nucleotide resolution. We then compare the high-resolution break-enrichment maps of chemotherapeutics with genomic, chromatin and topological features and associate recurrent break patterns with translocation hotspots. In combination with C-Fusion 3D, our efforts are focusing on: (1) identifying cellular and molecular pathways that contribute to the formation of recurrent DNA breaks at translocation hotspots found in patients; (2) understanding how the chromatin environment may predispose susceptibility to breakage and chromosome translocations; (3) assessing the influence of 3D genome organisation on chromosome breakage and fusion formation; and (4) identifying molecular players of the DNA damage response and novel factors that promote or inhibit the illegitimate fusion of chromosomes.

Figure 1. Treatment with chemotherapeutics, such as alkylating agents or topoisomerase poisons has been associated with increased risk of developing secondary, therapy-induced cancers, driven by recurrent chromosome translocations.

FUTURE DIRECTIONS

We will extend our analysis to identifying mechanisms governing the formation of different chromosome translocations leading to a variety of secondary malignancies. Central to our focus is shedding light on the events leading to the genesis of recurrent chromosome translocations involving the mixed lineage leukaemia (MLL) gene, which frequently occur upon treatment with the topoisomerase poison Etoposide. Moreover, in an effort to link DNA fragility with cellular physiology, we plan to profile endogenous DSBs across the genome in different cell types, with the aim to identify common or cell type-specific signatures of DNA fragility. We will also develop novel tools that allow us to perform siRNA and CRISPR-based, unbiased and targeted screens to identify novel factors that govern key steps of the formation of translocations. Taken together, our research will shed light on the mechanisms of cancer-initiating translocations, which will advance our knowledge of the fundamental principles in cancer aetiology.

SELECTED PUBLICATIONS


OVERVIEW

In the adult organism, millions of cells are replaced every day. Adult stem cells are essential for the maintenance and regeneration of our complex organs. Yet, with age or in pathological conditions adult stem cells cease functioning properly. Understanding the natural path of adult stem cell specification during embryogenesis would allow for the generation of patient-specific adult stem cells. These could either be used for transplantation or for the screening of pharmacological compounds to develop personalised treatments. We use the mouse small intestine as a model system to decipher mechanisms of the intestinal stem cell (ISC) specification. We aim to identify signals and cells that trigger specification of embryonic ISCs. Moreover, we determine the degree of heterogeneity within the embryonic epithelial progenitors. Does their molecular heterogeneity reflect an early determination of distinct stem cell sub-types with potentially distinct functions? To address our questions, we employ mouse genetics, single-cell genomics and ex vivo 3D organoid assays.

RESEARCH HIGHLIGHTS

Until recently, the identity of embryonic epithelial cells giving rise to ISCs was unknown. In contrast to the morphologically distinct cells of the adult intestine, the embryonic small intestine is composed of morphologically identical, highly proliferative epithelial cells. Furthermore, adult ISCs can be identified based on the expression of a specific set of genes, so-called ISC signature genes. However, cells within the embryonic intestinal epithelium do not express these genes until 13.5 days of embryonic development (E13.5).

Using genetic cell fate mapping analysis we found that adult ISCs originate from molecularly distinct embryonic progenitors. For example, duodenal ISCs are specified from embryonic progenitors expressing Foxa2, whereas ISCs of the jejunum and...
ileum come from cells expressing Axin2. Moreover, these distinct embryonic progenitors have different capacities to generate adult ISCs. This suggests that ISC progenitors are molecularly heterogeneous and might be different not only on the transcriptional but also on the functional level. To test this hypothesis, we performed single-cell RNA-sequencing analysis of the small intestines from mouse embryos. Based on the expression of markers and our genetic lineage tracing analysis, we separated the embryonic ISC progenitors into several groups. Currently, we are establishing a functional role for this heterogeneity during epithelial homeostasis and cancer.

To date, the signalling molecules triggering specification of the embryonic Lgr5+ ISC progenitors remain unknown. Wnt/β-catenin signalling is a crucial regulator of adult ISCs maintenance and proliferation. Ablation of β-catenin or the Wnt receptors, Lrp5/6, in the embryonic intestinal epithelium leads to reduced cell proliferation and a loss of ISC progenitors at E15.5. Further genetic experiments demonstrated that the secretion of Wnt ligands from mesenchymal cells is required for the maintenance of embryonic ISC progenitors at E15.5. Before this developmental stage, the expression of Wnt genes (that activate β-catenin dependent signalling) was not detected in either the embryonic intestinal epithelium or mesenchyme. BMP signalling negatively controls self-renewal of adult ISCs and attenuates intestinal tumour formation. During embryonic development, several Bmp genes are expressed in either the intestinal mesenchyme or epithelium. We found that the Id2 transcription factor, which is a target of TGF-β/BMP signalling, controls the timing of Lgr5+ progenitor specification during embryogenesis. In Id2-deficient embryos, Lgr5+ cells appear several days earlier and are much more abundant. Ectopic expression of Wnt ligands is required for the precocious activation of Wnt/β-catenin target genes and earlier specification of Lgr5+ ISC progenitors in Id2 mutant intestine. To identify factors essential for the specification of the embryonic ISC progenitors we analysed our single-cell RNA-sequencing data for the expression of various signalling molecules, including Rspo, Wnt, Bmp/Tgf-β and Jag, along with their receptors (Figure 1). Using mouse genetics and organoid assays we validated a function for these pathways during specification of the embryonic ISC progenitors.

**Figure 1.** Visualising Rspo/Wnt signalling during mouse development. The expression pattern of Rspo3 (blue) and its receptor Lgr5 (pink) in a paraffin section of a mouse embryo at E13.5 Scale bar: 30 mm.

**FUTURE DIRECTIONS**

Our immediate goal is to understand the relationship of intestinal stem cells to cancer. We will examine the mechanisms underlying intestinal epithelial progenitor heterogeneity and whether some of these heterogeneous cells are more likely to become cancer forming later in life. Furthermore, our long-term plan is to understand how the gut as an organ is maintained as a whole. Specifically, how both neurons and immune cells inside of the intestine maintain and communicate with the epithelium.

**SELECTED PUBLICATIONS**


OVERVIEW

Ubiquitin, as a posttranslational protein modifier, affects most aspects of cellular metabolism by modulating the stability, localisation or molecular interactions of its targets. Its versatility as a signalling molecule derives in part from its ability to form polymeric chains of varying topologies. Ubiquitin receptors with a basal affinity for the substrate along with one or several ubiquitin-binding domains that recognise the modification in a more or less linkage-selective manner, usually mediate the biological effects of polyubiquitylation. Structural biology has provided detailed insight into the principles of ubiquitin recognition by such receptors, while a wide range of analytical tools, including mass spectrometry, linkage-selective affinity probes, and selective inhibitors have facilitated the characterisation of ubiquitin conjugates. However, in many cases, the relevance of a particular chain linkage for its biological function is still unclear. Our poor understanding arises largely from a lack of suitable tools to directly manipulate the ubiquitin chain linkage. We have approached this problem by a new strategy of in vivo polyubiquitin chain engineering that involves the design and application of tailor-made ubiquitin protein ligases (E3s) for the purpose of “linkage mutagenesis”.

RESEARCH HIGHLIGHTS

Polyubiquitin chain linkage is controlled by the combination of the ubiquitin-conjugating enzymes (E2s) and E3s contributing to their assembly and is, therefore, an invariable feature of any ubiquitylation event. The experimental system underlying our approach is based on a well-defined K63-linked chain assembled on the replication factor PCNA in response to replication stress in budding yeast. Modification of PCNA at a single invariant lysine facilitates DNA replication in the presence of lesions and thereby contributes to genome stability. Monoubiquitylation by a specific E2-E3 pair promotes a mutagenic pathway of damage
bypass called translesion synthesis (TLS). Alternatively, extension of the modification to a K63-polyubiquitin chain by a dedicated E3, Rad5, initiates an alternative, error-free pathway known as template switching (TS). While the mechanism of TLS activation by PCNA monoubiquitylation is well understood, the function of the polyubiquitin chain in the TS pathway is still a matter of debate.

In order to examine the relevance of the K63-linkage in the context of DNA damage bypass, we designed three tailor-made E3s with defined, but distinct linkage specificities to replace Rad5 (Figure 1A). Selectivity for PCNA as a substrate was achieved by means of a conserved PCNA-interacting peptide (PIP), and well-characterised domains from unrelated E3s were used to install K63-, K48- and M1-specificity. Using a fusion of monoubiquitin to PCNA as a surrogate substrate, we found that in combination with the appropriate E2, all PIP-E3s generated PCNA-linked polyubiquitin chains in vitro (Figure 1B). The intended specificities were confirmed by linkage-specific antibodies or by using relevant ubiquitin mutants. Moreover, we found that the enzymes were E2- and substrate-specific and required prior monoubiquitylation of PCNA for activity. Kinetic analysis revealed substrate-binding properties very similar to Rad5 itself but with a range of different catalytic rate constants (Figure 1B). Expression of the PIP-E3s in a yeast rad5Δ mutant (Figure 1C) and monitoring of DNA damage sensitivity was used to assess their in vivo function in the TS pathway (Figure 1D). Whereas the K63-specific E3 fully complemented the damage sensitivity of a rad5Δ mutant, PIP-E3(48) conferred a dominant negative effect that was attributable to an inhibition of the TLS pathway. We were able to show that this was due to proteasomal degradation of monoubiquitylated PCNA after its K48-linked polyubiquitylation. Surprisingly, the M1-specific E3 partially restored damage resistance to rad5Δ, and this was indeed mediated by PCNA modification and activation of TS. The partial nature of this effect is consistent with the lower intrinsic activity of this E3. Thus, our study has revealed that polyubiquitin chains of distinct linkages can sometimes substitute for each other, but can also convey alternative fates to the modified protein. At the same time, it is the first example of an in vivo manipulation of polyubiquitin chain linkage that illustrates a novel approach to analysing ubiquitin signalling in a physiological context.

![Image](image-url)

**Figure 1.** Tailor-made E3s provide insight into the function of polyubiquitin chain linkage in DNA damage bypass. A) Linkage-specific polyubiquitylation of monoubiquitylated PCNA (Ub-PCNA) by designed PIP-E3s in vitro, detected by anti-PCNA immunoblotting. B) Kinetic parameters of PCNA ubiquitylation reactions, determined by fitting of initial rates to Michaelis Menten kinetics. C) Expression of PIP-E3(1) in S. cerevisiae (Hta2: histone H2A). D) Distinct effects of PIP-E3s on the sensitivity of rad5Δ cells towards an alkylating agent, methyl methanesulfonate (MMS).

**FUTURE DIRECTIONS**

Our strategy of linkage manipulation by tailor-made E3s would lend itself to the development of a promising and generally applicable research tool if the problems of substrate recognition and chain initiation can be overcome. While substrate recognition could, in principle, be generalised by replacing the PIP-PCNA interaction with an inducible dimerisation system, efficient chain initiation is more challenging, as our E3s were designed to only extend existing ubiquitin moieties on the substrate. In the case of PCNA, the proximal ubiquitin moiety is attached by a distinct E2-E3 pair, which bypasses the need for direct modification of the substrate. In order to generalise this feature, we are currently developing a universal ubiquitin acceptor site that can be fused to the substrate of choice in combination with a dimerisation domain that would provide an interaction site for the E3. In this manner, we hope to generate a molecular tool analogous to existing degron systems that would allow the induced, linkage-specific polyubiquitylation of appropriately tagged substrates in vivo.

**SELECTED PUBLICATIONS**


OVERVIEW

Circadian clocks — operated by cell-autonomous transcription/translation feedback loops — affect many essential cellular, physiological and behavioural processes. In mammals, the transcription factors BMAL1/CLOCK activate three period (per1,2,3) and two cryptochrome (cry1,2) genes. The CRY1/2 and PER1/2 clock proteins repress BMAL1/CLOCK, whereas recruitment of co-activators to BMAL1/CLOCK leads to transcriptional activation. The mammalian Timeless (TIM) protein interacts with CRY but its role in the circadian clock is unclear. However, TIM together with its binding partner Tipin are implicated in DNA replication, checkpoint signalling and DNA repair. Hence, mammalian TIM is a multifunctional protein that may connect the circadian clock with genome maintenance by interacting with either CRY (circadian clock) or with replication fork associated proteins. To elucidate direct molecular links between circadian clocks and genome maintenance, we pursue 3D-structural, biochemical and quantitative biophysical analyses of complexes formed between CRY, TIM and Tipin and investigate their interplay within the circadian clock. These studies will advance our mechanistic understanding of how circadian gene regulation and genome maintenance are interconnected by time of day dependent changes in protein interaction networks.

RESEARCH HIGHLIGHTS

Accumulating evidence suggests a functional interconnection between mammalian circadian clocks and genome maintenance. For example, DNA damage induces phase advances of circadian rhythms, likely involving the Timeless (TIM) protein and ATM mediated damage signalling. Furthermore, Cryptochrome1 (CRY1) modulates ATR/Chk1-mediated DNA damage checkpoint responses in a circadian manner. To elucidate the molecular mechanisms underlying functional connections between the circadian clock and genome maintenance, we structurally, biochemically...
and biophysically analyse interactions of the mammalian clock proteins CRY1/2 with TIM and the Timeless-interacting protein Tipin. Additionally, we analyse interactions of TIM and Tipin with replication fork associated proteins such as RPA.

We found, that the purified TIM-Tipin complex directly interacts with CRY1 and CRY2. The TIM-Tipin-CRY complex may recruit CRY proteins to the replication fork, for example, to modulate checkpoint responses. Conversely, the TIM-Tipin complex may be recruited to the circadian clock by its interaction with CRY1 or CRY2. While TIM, Tipin and the TIM-Tipin complex are known to be involved in checkpoint signalling and fork protection (Figure 1), Tipin and the TIM-Tipin complex have so far not been implicated in circadian regulation. Hence, the biological role of the mammalian TIM-Tipin-CRY complex in the circadian clock is unclear. We observed that CRY binding to the purified TIM-Tipin complex, to BMAL1 or PERIOD (PER) is mutually exclusive, implying overlapping binding sites. We, therefore, speculate that the TIM-Tipin-CRY or TIM-CRY complex impacts on the circadian BMAL1/CLOCK transcriptional activity by interfering with the formation of PER-CRY or BMAL1-CRY complexes in the repressive phase of the circadian oscillator (Figure 1).

We further set out to identify molecular regions involved in TIM-Tipin-CRY interactions. We identified a short region in the mammalian TIM protein that modulates interactions with our purified CRY1 and CRY2 proteins. Deletion of this TIM region differentially affects CRY1 and CRY2 interactions in vitro, suggesting different biological roles of the TIM-(Tipin)-CRY1 and TIM-(Tipin)-CRY2 complexes. The identification of this TIM deletion construct now enables us to dissect the distinct roles of the TIM-(Tipin)-CRY1 and TIM-(Tipin)-CRY2 complexes in the circadian clock and in genome maintenance.

Using single particle Electron Microscopy (EM), we determined an initial negative stain EM structure from our purified TIM-Tipin-CRY complex samples. Compared to our previously reported TIM-Tipin-RPA Cryo-EM structure, the EM map of the TIM-Tipin-CRY sample has similar dimensions but lacks the regions that we assigned to RPA in the TIM-Tipin-RPA complex (Figure 1). We are currently pursuing 3D structural analyses of the TIM-Tipin-CRY complex at higher resolution. This will enable us to design additional mutants for the targeted functional analyses of TIM-Tipin-CRY and TIM-CRY interactions.

**FUTURE DIRECTIONS**

The roles of mammalian Timeless (TIM) in the circadian clock are vaguely defined and it is not known if or how the TIM-binding protein Tipin and the TIM-Tipin complex contribute to circadian regulation. Do TIM-CRY- or TIM-Tipin-CRY interactions affect circadian timing by altering CRY’s activity as a transcriptional repressor of BMAL1/CLOCK? Furthermore, CRY and TIM are implicated in time of day dependent regulation of checkpoint signalling and advancing the circadian clock in phase in response to DNA damage. What are the underlying molecular mechanisms and the distinct roles of TIM-CRY- or TIM-Tipin-CRY interactions in these processes? Finally, it will be interesting to find out if TIM-CRY- or TIM-Tipin-CRY interactions play a role in mammalian DNA repair pathways.

Our structural and biophysical protein-protein interaction analyses uncover molecule regions and amino acids involved in CRY-TIM-Tipin interactions. In future, this will enable us to design mutants and deletion constructs to address these interesting open questions in a targeted manner.

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**SELECTED PUBLICATIONS**


The Core Facilities at IMB provide access to state-of-the-art technology and offer services and training from experts.

There are currently seven Core Facilities (CFs) at IMB: Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics, Protein Production, 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. For IMB researchers, all seven CFs are available for use. Beyond that, Flow Cytometry, Genomics, Microscopy/Histology and Proteomics CFs also offer their services to the larger research community in Mainz. CF services provided are based on user demand. For each facility, a user committee gives feedback on the equipment and user experience and helps define the services that each CF provides.

In addition to technical services, the CFs offer lectures on a variety of methods, as well as practical courses to instruct researchers in new techniques and instrumentation, data acquisition, experimental design, data processing, 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.

Furthermore, IMB’s CFs are 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, web services, system administration, software training, and consulting on experimental design and statistics. In addition, BCF members actively participate in the computational processing, analysis, visualisation and interpretation of high-throughput "omics" data generated in the course of research projects.

SERVICES OFFERED

With their accumulated professional expertise, BCF staff offer support on different levels depending on project needs, ranging from basic IT and bioinformatics services to full-scale scientific collaborations in the context of "big data" research projects:

- Consulting on the statistics and experimental design of genomics projects
- Data quality assessment, processing, visualisation, interpretation and presentation of results
- Development of analytical pipelines and their customisation for individual projects
- Data mining of published datasets, correlation and integration of results
- Assistance with the preparation of manuscripts, presentations and grant proposals
- Workshops and tutorials on bioinformatics topics to facilitate data access and analysis
- Testing, implementation and customisation of various software tools and online services
- System administration and IT support in cooperation with the University of Mainz Data Center

The BCF operates a small computer cluster, a storage server, and web services such as Galaxy and R-Studio, which provide IMB researchers with a user-friendly interface to bioinformatics tools and databases. The Facility maintains a GitHub repository (github.com/imbforge) with software tools and pipelines dedicated to different types of next-generation sequencing (NGS) assays. BCF also offers customised solutions and long-term analytical support for numerous data-intensive IMB projects on a collaborative basis.

Figure 1. Time-course of RNA-Seq transcriptome profiling of human MCF10A cells. A) Principal component analysis plot of global sample similarity. B) Volcano plot depicting gene expression changes at a single time point with labelled top differential genes.
The Flow Cytometry Core Facility (FCCF) offers high-throughput measurements, analysis and separation of biological units through four different systems: a large particle sorter, a cell sorter, and two analysers. With this equipment, the FCCF can analyse and sort particles of 0.5 μm to 1,000 μm in diameter.

SERVICES OFFERED

The FCCF offers a full service for sorting and an assisted service along with training for the analysers. Additionally, its staff collaborates in terms of analysing flow cytometry data and sample preparation. During the past year, the FCCF has performed various types of experiments including multicolour measurements, cell separation for next generation sequencing, sorting of isolated neuronal nuclei, classical enrichments for subsequent cell culture, qPCR analysis, mass spectrometry, and microscopic investigations. Moreover, the FCCF performed reagent validation for the Protein Production Core Facility. The FCCF works with different types of material including: nuclei, stem cells, yeast, C. elegans, Arabidopsis seeds, and lipid droplets as well as various cultured cell lines and primary cells from humans, mice, zebrafish, and Drosophila. To educate and train users, the FCCF offers three different lectures per year, as well as an annual practical course for basic flow cytometry analysis and an advanced practical course for cell sorting.

Figure 1. Analysis of apoptosis in Jurkat T cells treated with Camptothecin. Annexin V staining is used for analysis of exposed phosphatidylserine residues. Changes in mitochondrial membrane potential can be monitored with JC-1 staining. Both assays were performed in the FCCF’s annual practical course.
GENOMICS
CORE FACILITY

The Genomics Core Facility (GCF) offers next-generation sequencing (NGS) services based on the Illumina NextSeq500 and MiSeq platforms. In 2018, the GCF acquired a MinIon sequencer (Oxford Nanopore).

SERVICES OFFERED

The GCF provides a full service for NGS, starting with the experimental design of the project and continuing up to the generation of sequencing data. In addition, the GCF also sequences self-prepared libraries from researchers at IMB.

After submission of RNA or DNA samples, the GCF performs initial quality control of the samples, library preparation, quality control of the prepared libraries, sequencing and raw data generation. Currently, the GCF supports library preparation for more than twenty applications as a standard service and develops new protocols to accommodate the user’s needs for their specific projects. For example, in 2018, GCF supported members of Helle Ulrich’s research group in establishing GLOE-Seq, a method for genome-wide detection of nicks, gaps, breaks and lesions.

The GCF also offers training in genomics techniques, including principles of NGS and the application of NGS in translational epigenetics.

RNA:
- Strand specific mRNA-Seq, with poly-A selection
- Strand specific total RNA-Seq, with rRNA depletion
- Low input RNA-Seq
- Small RNA-Seq
- RIP-Seq
- Bru-Seq
- cDNA library preparation
- circRNA
- GRO-Seq
- single-cell RNA (Smart-Seq2)
- STARR-Seq

DNA:
- ChIP-Seq
- MBD-Seq
- Whole genome sequencing
- Whole genome bisulfite sequencing
- Single-stranded DNA library preparation
- Hi-C
- MeDIP
- 8-oxoG
- DamID

User-prepared libraries:
- iCLIP-Seq
- Amplicon-Seq
- ATAC-Seq
- 4C / Capture-C
- RR-MAB-Seq
- GLOE-Seq
- LAM-HGTS

Figure 1. NextSeq 500 benchtop, high throughput sequencer from Illumina.
SERVICES OFFERED

Microscopy users can select from 10 different instruments ranging from stereo microscopes and widefield microscopes to confocal, high-content screening and super-resolution microscopes. Four of the set-ups (one widefield, one scanning confocal, and two spinning disk confocal microscopes) are equipped for live cell imaging. Users are trained to work independently on the microscopes, although MHCF staff are always available to assist with sample preparation, image acquisition, as well as image processing, deconvolution and analysis (quantification). Beside licensed software programs for image deconvolution (Huygens Essential, SVI) and 3D visualisation/analysis (Imaris from Bitplane, Harmony from PerkinElmer), custom-made solutions are developed together with the users (e.g. by macro programming in open source software such as Fiji or ImageJ) or by the assembly of predefined building blocks in Columbus, a database and analysis software designed for high content imaging data (PerkinElmer). Super-resolution microscopy is offered as a full service or on a collaborative basis. User training for both microscopy and histology takes place throughout the year via practical courses and lectures, ranging in emphasis from confocal, live cell microscopy and super-resolution microscopy to image processing, as well as basics in histology and staining techniques.

In 2018, a new spinning disc microscope was co-funded by the DFG and put into operation. The system is devoted to live cell imaging of different samples. It is equipped with an ablation laser for the localised induction of DNA damage, a photomanipulation module for FRAP and TIRF, and two cameras for fast parallel imaging. Further, a 100TB server was installed to keep up with the increased demand for data storage and analysis on the Opera Phenix Screening Microscope.

For histology purposes, the MHCF provides a variety of histology techniques. In addition to semi-automated fixation and paraffin embedding, machines for the sectioning of paraffin-embedded tissue (microtome), frozen tissue (cryotome), and for gelatine/agarose embedded tissue and fresh tissue (vibratome) are part of the available instruments. Users may furthermore utilise optimised protocols for immunodetection, tissue clearing and solutions for classical tissue stainings (H&E, Masson Goldner Trichrome, PAS, and Azan).

Figure 1. 3D reconstruction of double smFISH against dazl (red) and boc (cyan) in a zebrafish stage II oocyte. They mark the germinal granules that will accumulate into germ plasm after fertilisation, a structure that is required for induction of primordial germ cell fate in the progeny and therefore fertility. Image acquired by Elke Roovers (Ketting group) with the spinning disk microscope “VisiScope” (Visitron, 40x water objective) at the IMB’s Microscopy & Histology Core Facility.
PROTEIN PRODUCTION CORE FACILITY

The Protein Production Core Facility (PPCF) provides support with the design, expression, purification and assay development of recombinant proteins that are in the focus of IMB’s research. The facility also offers a variety of common protein tools that are routinely used by IMB researchers on a day-to-day basis.

SERVICES OFFERED

The PPCF supports researchers throughout the process of protein production. This includes the screening of suitable expression systems and vectors, optimisation of purification steps, upscaling of protein production and purification, as well as functional analysis and assay development with the purified products. The facility is equipped with two automated chromatography systems. These enable the use of the latest chromatographic methods required for state-of-the-art protein purification strategies.

Another key function of the PPCF is the generation and functional quality control of routine laboratory enzymes and affinity probes for IMB researchers. The facility is aiming to offer ready-to-use enzyme kits for all frequent applications at the institute.

Figure 1. Side view of the Äkta Prime Plus Chromatography system, showing inlet, injection and gradient valves, as well as the UV and conductivity monitor.

Martin Möckel Head
Since 03/2018
Markus Matthes Head
02/2016 – 01/2018
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 proteomic measurements.

SERVICES OFFERED

As a general service, the PCF provides band identification, analysis of posttranslational modifications on single proteins and measurement of SILAC (stable isotope labelling with amino acids in cell culture) experiments. Additionally, the PCF also offers reductive dimethylation as a labelling technique and tandem mass tagging (TMT) for large-scale quantitation. TMT can be applied for quantifying up to 10 samples in parallel. In total, 1,500 measurement hours annually are provided to IMB and the surrounding research centres in Mainz using a state-of-the-art mass spectrometry platform. The mass spectrometry service is provided as a full service, including initial consultation, sample preparation and basic proteomics data analysis by the PCF. Advanced proteomic workflows, label-free quantitation measurements, in-depth statistical and bioinformatics analysis are available in a collaborative context. The PCF offers lectures on proteomics and data analysis as well as providing researchers with hands-on experience during our practical courses.

Figure 1. The inside of an electrospray ionization (ESI) source showing ESI needle (upper right) pointing towards the mass spectrometry entrance. A fluid sample is transferred to the gaseous phase by the application of high voltage resulting in a fine spray. The same source housing can be modified for other ionization methods. Corona discharge needle (lower left) is necessary for atmospheric pressure chemical ionization (APCI).
MEDIA LAB

The Media Lab primarily supports scientific groups and other Core Facilities by producing media, buffers, and agar plates. In addition, the Media Lab is responsible for the administration of three supply centres, plasmid/cell line banks, general waste management and sterilisation of glassware.

SERVICES OFFERED

+ Supply of routinely-used buffers, solutions, liquid media and agar plates for molecular biological research and for culturing bacterial, yeast, and insect cells, as well as *C. elegans*
+ Production of made-to-order media
+ Management of three supply centres for enzymes, kits, and cell culture media
+ Administration of a vector data bank, human ORF clone collection and cell line bank
+ Overnight cultures for plasmid preparation
+ Sterilisation of solutions/media
+ Cleaning and sterilisation of glassware and lab equipment
+ Autoclaving of S1/S2 waste
+ Maintenance of in-house transport system

Figure 1. The media lab produces approximately 1,500 agar plates per week of different sizes and compositions depending on the needs of the user.

Andrea Haese-Corbit  Head  Since 01/2018
Doris Beckhaus  Assistant  Since 05/2011
Alwina Eirich  Assistant  Since 07/2013
Pascal Hagebölling  Assistant  Since 01/2015
Annette Holstein  Assistant  Since 04/2012
Marion Kay  Assistant  Since 04/2016
Johann Suss  Assistant  Since 04/2011
In 2018 IMB welcomed 457 guests from 45 countries as participants in our scientific events.

**IMB STAFF NATIONALITIES**

- 55% International
- 31% EU
- 24% Other

**IMB STAFF**

**Group Leaders** 6%

**Technical Staff** 34%

**Administration & Scientific Management** 11%

**PhD Students** 33%

**Postdocs** 16%

**EMPLOYEES BY STAFF CATEGORY**
EXTRAMURAL FUNDING

MAJOR FUNDERS

Boehringer Ingelheim Foundation (BIS)  
German Research Foundation (DFG)  
European Research Council (ERC)  
Marie Curie Actions

FURTHER SUPPORT

Boehringer Ingelheim Fonds (BIF)  
Federal Ministry of Education and Research (BMBF)  
German Academic Exchange Service (DAAD)  
German Cancer Aid  
European Molecular Biology Organisation (EMBO)  
Joachim Herz Foundation  
Natural Science Medical Research Center (NMFZ)
RESEARCH AND TRAINING

IMB is a thriving international research centre in Mainz. Our researchers explore science at the cutting edge of gene regulation, genome stability and other aspects of nuclear biology, ranging from the structural and molecular to the organismic level.

Currently, IMB supports 18 research groups with around 90 PhD students and 50 postdocs coming from 28 countries across the globe. This international environment has allowed ideas and expertise to be shared across different research priorities and has resulted in over 350 publications with 81 in 2018 alone. The individual research topics pursued at IMB centre on key questions in chromatin and RNA biology; epigenetics; DNA damage and genome stability; protein homeostasis; and related biomedical fields. These areas are producing exciting results that are transforming our understanding of how we develop and adapt to our environment, and how we age or develop disease.

IMB aims to ensure the best possible environment for its scientists by providing training in core competencies and professional skills. We thereby enable our researchers to tackle ambitious projects and develop their careers. A key part of the support offered comes through IMB’s Core Facilities. Each facility is staffed by experts who advise, train and assist our researchers in the techniques and skills needed for cutting-edge science. Moreover, scientists at IMB are offered a wide range of professional skills courses that help push their competencies at all stages of their career. Courses offered cover topics such as presentation skills, scientific writing, project management, fundraising, career development, negotiation skills, and global leadership.
INTERNATIONAL PHD PROGRAMME

PhD students are a key part of our research at IMB. All of them participate in the International PhD Programme (IPP) on "Gene Regulation, Epigenetics & Genome Stability". This very successful programme was established with generous funding from the Boehringer Ingelheim Foundation and is supported by the participating institutions: IMB, Johannes Gutenberg University Mainz and the University Medical Center Mainz. Students benefit from the regular scientific exchange and networking across 46 research groups. In addition, they receive state-of-the-art scientific and professional skills training to support their personal development as a scientist.

www.imb.de/PhD

INTERNATIONAL POSTDOC PROGRAMME

IMB’s Postdoc Programme (IPPro) 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 provides sound scientific training through a variety of lectures, workshops and events, and offers postdocs full support with raising funds for their research. In addition to the guidance given by group leaders, the Postdoc Programme also offers mentoring discussions with IMB’s scientific directors and invited speakers, career events, 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 topics. The programme also organises company site visits 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 and Masters’ students. Research groups participating in the ISS include group leaders at IMB, Johannes Gutenberg University and Mainz’s University Medical Center. The ISS offers an attractive framework for training prospective scientists in an informal and international atmosphere. 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 offers courses on complementary skills, such as presentation and communication techniques, that are required for a successful career as a scientist. Most importantly, each student works on a cutting-edge research project within the lab of one of the participating research groups.

www.imb.de/ISS
In 2018, IMB offered the following training courses in scientific, technical and professional skills:

## Lectures

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<td>14 May</td>
<td>Introduction to Molecular &amp; Biochemistry Techniques</td>
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<td>02 Jul</td>
<td>Nuclear Magnetic Resonance (NMR)</td>
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<td>Bioinformatics</td>
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<td>Databases in Bioinformatics</td>
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<td>Flow Cytometry</td>
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<td>Advanced Flow Cytometry: Principles of Cell Sorting</td>
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<td>Flow Cytometry: Introduction I</td>
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<td>Flow Cytometry: Introduction II</td>
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<td>Genomics</td>
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<td>Microscopy &amp; Histology</td>
<td>23 Apr</td>
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<td>30 Apr</td>
<td>Microscopy: F-Techniques &amp; Super-Resolution</td>
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<td>Histology &amp; Fluorescent Labeling</td>
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<td>Image Manipulation: The Slippery Slope to Misconduct</td>
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<td>Proteomics</td>
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<tr>
<td>Protein Production</td>
<td>04 Jun</td>
<td>Protein Production &amp; Crystallography</td>
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## Practical Courses

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<td>Bioinformatics: Introduction to R (Part I)</td>
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<td>Bioinformatics: Introduction to R (Part III)</td>
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<td>Bioinformatics: Introduction to R (Part IV)</td>
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<td>Bioinformatics: ChIP-seq &amp; RNA-seq Analysis with R (Part I)</td>
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<td>Bioinformatics: ChIP-seq &amp; RNA-seq Analysis with R (Part II)</td>
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<td>Bioinformatics: ChIP-seq &amp; RNA-seq Analysis with GALAXY</td>
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<td>Flow Cytometry: Practical Course</td>
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<td>26 – 29 Mar</td>
<td>Microscopy: Image Processing &amp; Analysis Course</td>
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<td>Proteomics</td>
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<td>Proteomics: Practical Course</td>
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## LECTURES

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<td>Lecture Series: Modern Techniques in Life Sciences</td>
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<td>06 – 15 Aug</td>
<td>Advanced Lectures on Gene Regulation, Epigenetics &amp; Genome Stability</td>
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<td>03 Sep</td>
<td>Good Scientific Practice</td>
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<td>15 Oct</td>
<td>Reaching your Readers Better in the Digital Age:</td>
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<td>From Cognitive Psychology to Practical Measures</td>
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<tr>
<td>16 Oct 2018 – 05 Feb 2019</td>
<td>Lecture Series: Introduction to Epigenetics</td>
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<td>30 Oct</td>
<td>Intellectual Property Rights in Life Sciences –</td>
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<td>Basics, Strategies &amp; Specialties, Pitfalls &amp; Troubleshooting for Scientists</td>
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## PRACTICAL COURSES

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<td>How to Design Scientific Figures</td>
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<td>Project Management for Postdocs</td>
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<td>Time Management</td>
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<td>Project Management with Certification 1a</td>
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<td>08 – 09 Mar</td>
<td>Biostatistics</td>
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<td>Project Management with Certification 1b</td>
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<td>Professional Communication</td>
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<td>20 – 21 Mar</td>
<td>Project Management with Certification 2a+b</td>
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<td>Convincing Scientific Presentations</td>
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<td>Presenting Scientific Data</td>
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<td>Intellectual Property Rights</td>
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<td>Conflict Management</td>
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<td>How to Create One’s Job</td>
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AWARDS

CHRISTOF NIEHRS

Advanced Grant (ERC)
To pursue research into epigenetic gene regulation via R-loops

HELLE ULRICH

Proof of Concept Grant (ERC)
To develop a tool that allows adding specific ubiquitin modifications to proteins in cells

NATALIA SOSHNIKOVA

Heisenberg Fellowship (DFG)
To continue her research into stem cell specification during gut development

CHIARA PAOLANTONI

(Roignant Group)

PhD Fellowship from the Boehringer Ingelheim Fonds (BIF),
For research into the molecular mechanisms of m6A in Drosophila neurogenesis

WALTER BRONKHorST

(Ketting Group)

Postdoctoral Fellowship from the Peter und Traudl Engelhorn Stiftung
For research into the regulation of phase transition during zebrafish germ cell development and insights this may provide for neurodegenerative diseases
15 March

SYMPOSIUM

GENE REGULATION IN EVOLUTION:
FROM MOLECULAR TO EXTENDED
PHENOTYPES

Scientific organisers: Joachim Burger, Falk Butter, Susanne Foitzik,
Thomas Hankeln, Martin Kaltenpoth and René Ketting

10-13 October

EMBO WORKSHOP

RNA AND GENOME
MAINTENANCE:
COOPERATION AND
CONFLICT MANAGEMENT

Scientific organisers: Andrés Aguilera, Petra Beli,
Fabrizio d’Adda di Fagagna, Brian Luke and Helle Ulrich

15-16 November

IMB WORKSHOP

MOLECULAR MECHANISMS
OF CIRCADIAN CLOCKS

Scientific organisers: Achim Kramer and Eva Wolf
PUBLICATIONS

**ANDRADE**


**BAUMANN**


**BELI**


* indicates joint contribution *indicates joint correspondence
CREMER


KETTING


KHMELENSKII


* indicates joint correspondence
KÖNIG


LEGEWIE


LEMKE


* indicates joint contribution * indicates joint correspondence


**LUKE**


**NIEHRS**


PUBLICATIONS

RICHLY


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* indicates joint contribution  *indicates joint correspondence
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RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located on the leafy campus of Johannes Gutenberg University, just west of Mainz city centre.

With 10 departments, more than 150 institutes and 32,000 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 over 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 Strungmann 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.
CAMPUS MAP
OF JOHANNES GUTENBERG UNIVERSITY MAINZ

[Map of the campus with various buildings and amenities labeled, including University of Applied Sciences, Max Planck Institute for Chemistry, Max Planck Institute for Polymer Research, Helmholtz Institute, and others.}

[Legend for map markers: Car entrance, bus stop, tram stop, International Student Housing, University Library, University Administration, etc.]

[Key features: MZ-Bretzenheim, MZ-Lerchenberg, Car entrance, Frankfurt Airport (30 km), City Centre and Train Station (2 km), CAMPUS OF THE UNIVERSITY OF MAINZ, Frankfurt Airport (30 km), University of Applied Sciences, Max Planck Institute for Chemistry, Helmholtz Institute, etc.]
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Portraits of Group Leaders and Core Facility heads (p2, 5-40, 44-51), pictures of IMB researchers (p 52, 56-58, 63, 64, 69), IMB building (inside cover, p3, p73, p76-77): Thomas Hartmann

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