Institute of Molecular Biology

ANNUAL REPORT 2017

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FOREWORD

Welcome to the 7th edition of the Annual Report for the Institute of Molecular Biology.

Having gone through its initial growth phase, IMB has now firmly set its foundations and continues to thrive and mature. For instance, building on our previous successes, we have recruited more outstanding scientists this year, notably with two new Adjunct Directors and one new Group Leader who will join us over the next few months. We also extended our presence on the international research scene, hosting three major conferences this year alone. As I rotate out of the Executive Director position and hand the reigns over to Helle Ulrich, I want to thank all our staff for continuing to make IMB an exceptional place to work on exciting projects as well as feel like home.

STAFF CHANGES

This year saw Brian Luke transition from being a group leader at IMB to become our 3rd Adjunct Director in a joint appointment with Mainz University. Brian and his lab will remain based at IMB where he will continue to unravel the mechanisms of telomere maintenance and repair. Brian was awarded a Heisenberg Professorship in 2016 and showcased his outstanding research with an article published in Cell this year.

In addition, I am delighted to welcome three new researchers who will be joining IMB in 2018, Peter Baumann, Edward Lemke and Anton Khmelinskii. Peter Baumann is the recipient of a prestigious Alexander von Humboldt Professorship and will be joining us as an Adjunct Director in April. He comes to us from the Stowers Institute in Kansas where he is an investigator for the Howard Hughes Medical Institute. His research expertise on telomere biology and chromosomal inheritance provides an excellent fit to IMB's current research strategy.

Edward Lemke (from EMBL, Heidelberg) will join us in January as our 5th Adjunct Director. Edward is a specialist in combining chemical techniques with biology to probe the structure and function of intrinsically disordered proteins. His expertise in protein engineering, fluorescent labelling and super-resolution microscopy will be a great addition to IMB's research portfolio.

Finally, Anton Khmelinskii will join us as a new Junior Group Leader from the Center for Molecular Biology (ZMBH), Heidelberg. Anton has just been awarded an ERC Starting Grant in the area of protein quality control and will start his group at IMB in January. His research focuses on selected protein degradation, the quality control of mislocalised proteins and asymmetric protein inheritance.

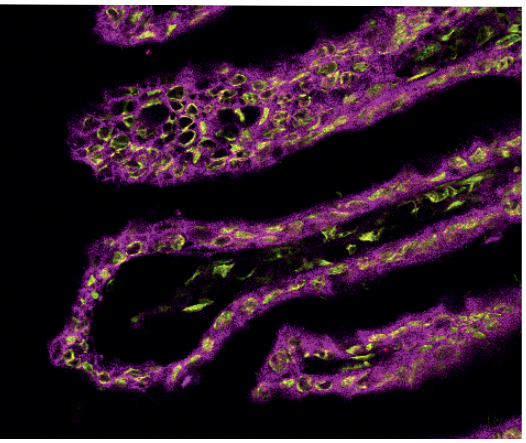
On the management side, from January 2018 Helle Ulrich will be taking over from me and rotating in as the new Executive Director for a period of two years. I am sure IMB will continue to flourish under her guidance.

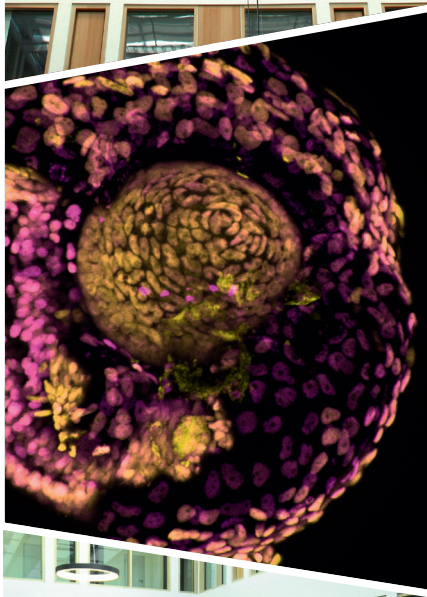
RESEARCH ACTIVITIES AND EVENTS

2017 was a very productive year at IMB. In a continuing trend of superb publications, in this year alone IMB published 75 papers (with a further 6 currently in print). These articles included papers from the Luke group in *Cell*, from the Butter group in *Nature Communications* and from the Richly group in *Genes & Development*. Our International PhD Programme (IPP) continues to grow with currently 128 students (89 of whom are at IMB) from 25 different countries. In addition, eight of its students successfully defended this year. The International Summer School retained its track record of offering great training to 20 international students and 6 Masters students from JGU this summer.

IMB organised 8 major scientific events this year as well as a range of outreach and training activities. We had the privilege of hosting three scientific conferences that were wonderfully successful. In May, we held a Symposium on Translational Epigenetics with 210 participants from 11 countries. June brought around the annual IMB conference, which this year focused on "Gene Regulation By The Numbers", featured 19 renowned speakers, and brought in 103 participants from 20 different countries. In September, we hosted the final conference of the year: the 1st Symposium on Nucleic Acid Modifications with 19 speakers and 150 participants from 21 countries.

2017 also saw a flurry of workshops including an RNA salon on "Genomics Approaches in RNA Biology" and a second EMBO-sponsored course on iCLIP in Protein-RNA Interactions. In addition to these events, IMB hosted 26 seminars from renowned speakers, including three seminars organised and hosted by our IPP students. Our outreach activities brought young scientists and the public into closer contact with research and included our annual Girl's Day, an international biology olympiad for Swiss and German high school students, and an interdisciplinary discussion event on the topic "Mainstream: Source or Barrier to Novelty".





OUTLOOK

2018 is right now shaping up to be as busy as ever with a number of events already planned. The annual IMB conference will be replaced by an EMBO workshop next year. This event (10-13 Oct) will be on the topic of "RNA & Genome Maintenance: Cooperation & Conflict Management" – an increasingly relevant topic in research. March will see IMB host a symposium on Gene Regulation & Evolution jointly with Mainz University's Faculty of Biology and in April we will have a "Beyond Science" workshop for postdocs that will provide information regarding the transition from postdoc to group leader positions.

As always, I would like to thank the Boehringer Ingelheim Foundation for their support and generous funding of our institute. I wish to say a special thank you to the members of our Scientific Advisory Board (SAB) who this year once again gave us excellent and constructive feedback on the progress of IMB. In particular, I wish to express my deep gratitude to the chair of the SAB, Renato Paro, who is stepping down this year. Renato has been with us from the start and has been instrumental in IMB getting to where it is today. He will be replaced as chair by Peter Becker from Ludwig Maximilian University (LMU). I also want to warmly welcome Geneviève Almouzni from the Institute Curie who has agreed to join our SAB as of next year. Last but by no means least, I want to thank all the people at IMB and outside whose work continues to make this institute an exceptional place to be.

René Ketting Executive Director

RESEARCH GROUPS

MIGUEL ANDRADE

COMPUTATIONAL BIOLOGY AND DATA MINING

POSITIONS HELD

Since 2014 Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Professor of Bioinformatics, Mainz University
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, EMBL, Heidelberg

EDUCATION

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

GROUP MEMBERS

Gregorio Alanis Lobato Postdoc; since 02/2015
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; since 09/2014
Susanne Klingenberg Personal Assistant, 06/2014 – 08/2017
Pablo Mier Postdoc; since 01/2015
Enrique Muro Staff Scientist; since 04/2014
Birgit Reubert Personal Assistant; since 09/2017
Sweta Talyan PhD Student; since 12/2014
Katerina Taškova Postdoc; since 11/2014

OVERVIEW

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

RESEARCH HIGHLIGHTS

Data mining mechanisms of drug toxicity

Resources are available that describe the effect of a toxic compound on gene expression and its associated phenotypes. These toxicogenomic resources compile data from system models, which can be either *in vitro* (including human cell lines) or *in vivo* (including rat and mouse whole organs). However, a difficulty remains in determining if these system models really reflect the effects of these compounds in humans and, if so, what aspects of toxicity they reproduce. Faithful recapitulation of toxic effects in systems other than human beings is essential to explore the pathology and mechanics of these toxins. To overcome this limitation, we proposed a new approach that would enable an evaluation of different toxicogenomic models through the use of the available literature on human toxicity. We take a set of compounds, already associated with a particular toxicity, apply them to a system model and examine gene expression and phenotypic changes. We compare these changes with those already published for humans and only consider

"We discovered that life on Earth uses 3.75 million different proteins."

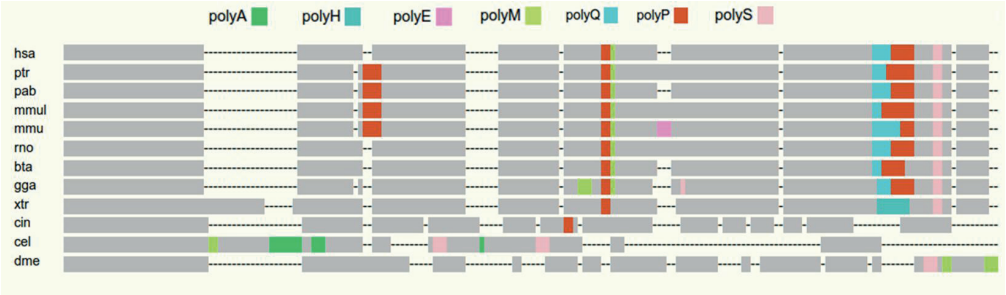


Figure 1. dAPE overview of a protein family. The view shows a simplified multiple sequence alignment where homorepeats (consecutive tracts of the same amino acid) are represented as coloured blocks. dAPE can be used to examine the variation of homorepeats in protein families at http://cbdm-01.zdv.uni-mainz.de/~munoz/polyx/.

the system a good model when there is a high correlation between both systems. Once identified, these systems allow for the exploration of both direct and indirect mechanisms of specific toxicities. We have used this approach to determine that gene expression changes in response to toxic exposure in the liver are well recapitulated in the rat liver but not in other system models such as human hepatocytes and rat kidney.

Protein sequence analysis

Ever wondered if we will ever know all of the different proteins out there? And how many are there? To address these questions, we studied the increasing redundancy in the new protein sequences deposited in the databases using a pragmatic clustering method. We concluded that by 2020 few proteins will enter the databases that will have no known homolog, and estimated the number of diverse proteins (proteins with a unique sequence or function) on Earth to be 3.75 million. This suggests that while the diversity of proteins is staggering, we are finally approaching the end of the frontier of novel protein discovery.

In a collaboration with Ulrich Stelzl (Graz University), we developed an approach to evaluate human non-receptor tyrosine kinases (NRTKs) in a high-throughput fashion using yeast. Yeast lack a system for the phosphorylation of tyrosine residues on proteins, a function often carried out by NRTKs in humans. By introducing human NRTKs into yeast, we could reproduce NRTK activity on the corresponding conserved substrate orthologs. Using conservation analysis, we examined which tyrosine sites were being phosphorylated in yeast. Our results suggest that protein phosphorylation recognition motifs are more variable and play a lesser role than previously thought.

Homorepeats (or polyX) in protein sequences are stretches of consecutive repetitions of a single amino acid residue. The length of a repeat determines its function but this length is variable from protein to protein making it hard to identify when a repeat becomes functional. To facilitate the study and detection of homorepeats, we created a web server that annotates homorepeats in multiple sequence alignments (dAPE; Figure 1). Our studies of the use of homorepeats suggest that homorepeats modulate protein-protein interactions in a context dependent manner.

Genomic analysis and gene expression

Topologically associating domains (TADs) are genomic self-interacting regions containing multiple genes. We provided evidence for the functional importance of TADs by interpreting the pathologies of 17 individuals with chromosomal abnormalities (translocations and inversions) apparently not affecting coding genes, but suffering from abnormal developmental and cognitive phenotypes. We used known chromatin contact information to predict the genes whose expression could be disrupted by the rearrangements, providing computational evidence of a pathological mechanism by which structural variants disrupt 3D genome architecture and thus gene regulation.

FUTURE DIRECTIONS

We are translating some of our research into stronger prediction methods, for example for protein-protein interactions using the geometric properties of the protein interaction network, and in the field of genomic structure, for the prediction of contacts using information from phantom peaks from ChIP-seq. After introducing a series of concepts describing the context of homorepeats, we will focus on the evolutionary study of particular types of homorepeats to understand how they emerge and enlarge over evolutionary time. We will apply a new computational method to find remains of protein coding sequences within non-protein-coding RNA sequences to aid in the evaluation of their function. Finally, we want to take advantage of the increasingly large ChIP-seq datasets that should provide information on the combinations of epigenetic marks and transcription factors that result in the control of gene expression. Towards this goal, we are working on the extraction and integration of ChIP-seq data from different databases and we are creating algorithms that measure similarity between ChIP-seq datasets.

SELECTED PUBLICATIONS

Corwin T, Woodsmith J, Apelt F, Fontaine JF, Meierhofer D, Helmuth J, Grossmann A, Andrade-Navarro MA, Ballif BA and Stelzl U. (2017). Defining human tyrosine kinase phosphorylation networks using yeast as an *in vivo* Model Substrate. *Cell Syst*, 5: 128–139.

Mier P and Andrade-Navarro MA. (2017). dAPE: A web server to detect homorepeats and follow their evolution. *Bioinformatics*, 33: 1221–1223.

Taškova K, Fontaine J-F, Mrowka R and Andrade-Navarro MA. (2018). Evaluation of *in vivo* and *in vitro* models of toxicity by comparison of toxicogenomics data with the literature. *Methods*, 132: 57–65.

PETRA **BELI**

CHROMATIN BIOLOGY AND PROTEOMICS

POSITIONS HELD

Since 2013 Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz

2010–2013 Postdoc, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen

EDUCATION

2011 PhD in Biology, Goethe University Frankfurt

2007 Masters in Molecular Biology, University of Zagreb

GROUP MEMBERS

Hamdiye Irem Baymaz Postdoc; since 11/2016
Marina Borisova PhD Student; since 12/2013
Jan Heidelberger PhD Student; since 04/2014
Thomas Juretschke PhD Student; since 03/2017
Thorsten Mosler PhD Student; since 04/2017
Matthias Ostermaier PhD Student; since 05/2015
Andrea Voigt Lab Technician; since 01/2014
JuanJuan Wang PhD Student; since 04/2017

OVERVIEW

The function of cellular proteins is dynamically regulated by different posttranslational modifications, among which protein ubiquitylation plays a central role in human cells. Ubiquitin is a 76 amino acid long protein that is attached to lysine residues of substrate proteins in an orchestrated enzymatic cascade consisting of E1, E2 and E3 enzymes. More than 500 ubiquitin E3 ligases target ubiquitylation to specific substrate proteins and thus regulate different cellular processes. Ubiquitylation is a dynamic modification that can be reversed by deubiquitylating enzymes. Although ubiquitylation is a subject of intensive investigations, the functions of many ubiquitin ligases and deubiquitylating enzymes have not been characterised yet.

Proteins can be modified by monoubiquitylation or by eight different types of ubiquitin chains: K48- and K11-linked ubiquitin chains target proteins for degradation by the proteasome, whereas K63-linked and linear chains are involved in cellular signalling by mediating protein-protein interactions. The functions and substrates of other types of ubiquitin chains remain poorly understood. Ubiquitin binding proteins read the ubiquitin code and thereby play an important role in determining the fate of ubiquitylated proteins. We develop and employ mass spectrometry (MS)-based proteomic methods to characterise ubiquitin-dependent signalling networks in human cells. In particular, we are focusing on identifying the ubiquitin ligase – substrate relations during the DNA damage response as well as on developing approaches to study the functions of atypical ubiquitin chains in nuclear processes.

RESEARCH HIGHLIGHTS

We have previously developed a mass spectrometry-based approach to identify and quantify endogenous ubiquitylation sites on a proteome-wide scale. In this approach,

"We study ubiquitin-dependent signalling in the DNA damage response."

termed ubiquitin remnant profiling, proteins extracted from cells or tissues are digested into peptides using trypsin. This results in the generation of the ubiquitin remnant peptides that contain a di-glycine tag covalently linked to a previously ubiquitylated lysine. Ubiquitin remnant peptides are enriched by di-glycine-lysine-specific antibodies and subsequently analysed by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) (Figure 1). If combined with a quantitative proteomics approach such as stable isotope labelling with amino acids in cell culture (SILAC), this enables relative quantification of thousands of endogenous ubiquitylation sites in a single experiment. We have used this method to analyse cellular ubiquitylation in immune signalling and in the DNA damage response, as well as to identify novel ubiquitin-dependent mechanisms that regulate these processes.

Valosin-containing protein (VCP, also known as p97) is an evolutionary conserved ubiquitin-dependent ATPase that mediates the degradation of proteins through the ubiquitin-proteasome pathway. The most well studied function of VCP is in the endoplasmic reticulum-associated degradation (ERAD) pathway, where it extracts misfolded proteins from the ER membrane before their degradation by the proteasome. More recent studies have shown that VCP also remodels proteins on chromatin and thus regulates DNA repair. Despite the central role of VCP in the regulation of protein homeostasis, the identity and nature of its cellular substrates remain poorly defined. We combined chemical inhibition of VCP and quantitative ubiquitin remnant profiling to assess the effect of VCP inhibition on the ubiquitin-modified proteome and to probe the substrate spectrum of VCP in human cells. We demonstrate that inhibition of VCP perturbs global cellular ubiquitylation patterns and increases ubiquitylation of a different subset of proteins compared to proteasome inhibition. We report 450 putative VCP substrates, many of which function in nuclear processes, including gene expression, DNA repair and cell cycle regulation.

Ubiquitin remnant profiling

Ubiquitin chain topology profiling

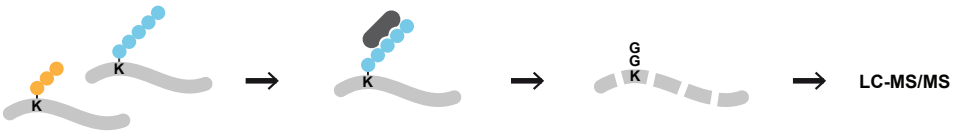


Figure 1. Characterising ubiquitin signalling by MS-based proteomics. Ubiquitin remnant profiling allows proteome-wide identification of endogenous ubiquitylation sites. If combined with SILAC, ubiquitin remnant profiling can be used for the relative quantification of ubiquitylation sites in different conditions. To gain insights into the topology of ubiquitin chains, binders such as antibodies or recombinant ubiquitin binding domains can be used to enrich proteins modified with a specific type of ubiquitin chains. Enriched proteins are then digested in-gel into peptides and analysed by LC-MS/MS.

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 regulate 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 to 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

Wagner SA, Oehler H, Voigt A, Dalic D, Freiwald A, Serve H and Beli P. (2016). ATR inhibition rewires cellular signaling networks induced by replication stress. *Proteomics*, 16: 402–416.

Schmidt CK, Galanty Y, Sczaniecka-Clift M, Coates J, Jhujh S, Demir M, Cornwell M, Beli P and Jackson SP. (2015). Systematic E2 screening reveals a UBE2D–RNF138–CtIP axis promoting DNA repair. *Nat Cell Biol*, 17: 1458–1470.

Beli P, Lukashchuk N, Wagner SA, Weinert BT, Olsen J V., Baskcomb L, Mann M, Jackson SP and Choudhary C. (2012). Proteomic investigations reveal a role for RNA processing factor THRAP3 in the DNA damage response. *Mol Cell*, 46: 212–225.

FALK BUTTER

QUANTITATIVE PROTEOMICS

POSITIONS HELD

Since 2013 Group Leader,
Institute of Molecular Biology (IMB),
Mainz

2010–2013 Postdoc,
Max Planck Institute for Biochemistry,
Martinsried

EDUCATION

2010 PhD in Biochemistry,
Ludwig Maximilian University, Munich

2006 Diploma in Biochemistry,
University of Leipzig

GROUP MEMBERS

Alina Bluhm PhD Student; since 01/2014
Hanna Braun PhD Student; since 08/2015
Núria Casas-Vila PhD Student; since 11/2013
Sabrina Dietz PhD Student; since 01/2015
Daniel Dowling PhD Student; 11/2015 – 01/2017
Teresa Laguna Postdoc; since 03/2016
Katarina Luko PhD Student; since 12/2017
Tony May Masters Student; since 11/2017
Merve Öztürk PhD Student; since 11/2015
Lara Perez PhD Student; since 10/2015
Marion Scheibe Postdoc; since 06/2013

OVERVIEW

Mass spectrometry is a powerful tool 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, we 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 evolutionary differences for

"We use proteomics to study genome stability & developmental gene regulation."

the shelterin complex and additionally uncovered that, in contrast to predictions, not all homologues of TRF1, a direct TTAGGG-repeat binding subunit of the complex, associated with our telomeric baits. Using recombinant expressed TRF1 DNA-binding domains of even more vertebrate species, we could locate a gain-of-binding event at the branch point of the therian lineage, where mammals and marsupials diverged from monotremes such as the platypus. While TRF1 is present in most vertebrates, it seems to have obtained its telomeric function only later during vertebrate diversification. By exchange of selected amino acid residues in the platypus TRF1-DNA binding domain, we could recapitulate a gain-of-binding switch *in vitro* positing a possible evolutionary scenario. Our phylointeractomics study therefor underscores that sequence homologues, as determined by phylogenomics, do not necessarily need to equate to functional homology.

Characterisation of ZBTB48 as a new telomeric protein in vertebrates

In addition to studying the evolution of the shelterin complex, our phylointeractomics screen also revealed further putative telomeric proteins. Among the most frequent proteins binding to TTAGGG repeats in our screen was ZBTB48, showing enrichment in 13 of 16 species. Together with our collaboration partner (Dennis Kappei, National University Singapore), we confirmed that ZBTB48 is indeed a telomeric protein in human cells and acts as a negative regulator of telomere length (Figure 1). It acts on telomeres of human cancer cells regardless of the mode of their telomere maintenance. Furthermore, using a combination of omics techniques (ChIP-seq, RNA-seq and quantitative proteomics) we demonstrated that ZBTB48 is a transcriptional activator on a small set of target genes, including mitochondrial fission process 1 (*MTFP1*). This discovery places ZBTB48 at the interface of telomere length regulation, transcriptional control and mitochondrial metabolism.

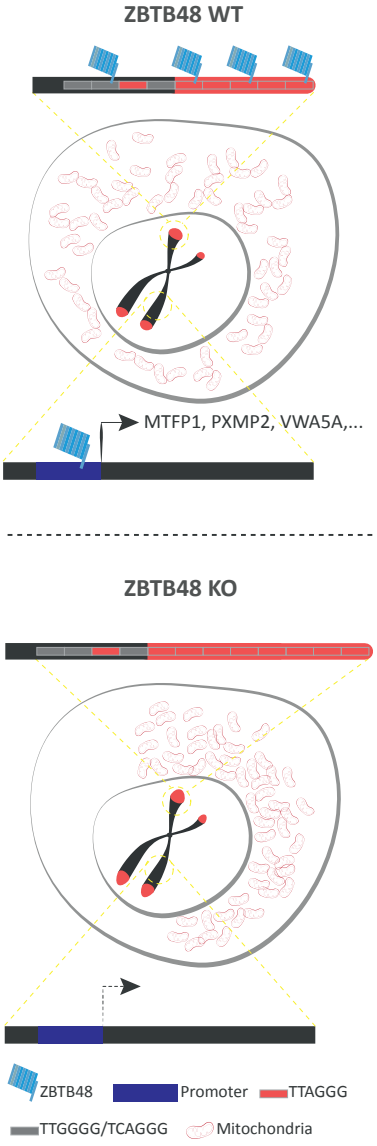


Figure 1. ZBTB48 is a vertebrate telomeric protein that acts as a negative regulator of telomere length. It acts as a transcription factor regulating the expression of mitochondrial fission process 1, a protein shaping mitochondrial morphology. These findings indicate that ZBTB48 may act as a link between telomere homeostasis and cellular metabolism. (Jahn, Rane *et al.*, 2017, *EMBO Rep*).

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 high temporal-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).

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.

SELECTED PUBLICATIONS

Casas-Vila N*, Bluhm A*, Sayols S*, Dinges N, Dejung M, Altenhein T, Kappei D, Altenhein B, Roignant JY and Butter F. (2017). The developmental proteome of *Drosophila melanogaster*. *Genome Res*, 27: 1273–1285.

Jahn A*, Rane G*, Paszkowski-Rogacz M, Sayols S, Bluhm A, Han C, Draškovič I, Londoño-Vallejo JA, Kumar AP, Buchholz F#, Butter F# and Kappei D# (2017). ZBTB48 is both a vertebrate telomere-binding protein and a transcriptional activator. *EMBO Rep*, 18: 929–946.

Kappei D*, Scheibe M*, Paszkowski-Rogacz M, Bluhm A, Gossmann TI, Dietz S, Dejung M, Herlyn H, Buchholz F#, Mann M# and Butter F#. (2017). Phylointeractomics reconstructs functional evolution of protein binding. *Nat Commun*, 8: 14334.

*indicates joint contribution;
#indicates co-corresponding authors

CHRISTOPH **CREMER**

SUPER-RESOLUTION MICROSCOPY

POSITIONS HELD

Since 2015 Research Associate, Max Planck Institute for Chemistry, Mainz
Since 2013 Honorary Professor, Department of Physics, Mainz University
Since 2011 Group Leader, Institute of Molecular Biology (IMB), Mainz
Since 2005 Director, Biophysics of Genome Structure, Institute for Pharmacy & Molecular Biotechnology, University Heidelberg
2005–2007 Deputy Director, Kirchhoff-Institute of Physics, University Heidelberg
1983–2011 Professor of Applied Optics & Information Processing, Heidelberg University
1983–1999 Managing/Deputy Director, Institute of Applied Physics I, Heidelberg University
1970–1983 Staff Scientist, Institute of Human Genetics, Freiburg University

EDUCATION

1983 Habilitation in General Human Genetics & Experimental Cytogenetics, University of Freiburg
1976 PhD in Biophysics and Genetics, University of Freiburg
1970 Diploma in Physics, Ludwig Maximilian University, München

GROUP MEMBERS

Udo Birk Postdoc; 02/2012–10/2017
Shih-Ya Chen PhD Student; since 05/2016
Amine Gourram Guest Researcher; 11/2016–01/2017
Jan Neumann Guest Researcher; since 03/2014
Aleksander Szczurek PhD Student; 09/2013–03/2017
Irma Gryniuk Masters Student; 08/2017–09/2017
Renata Vaz Pandolfo Guest Researcher; since 11/2017
Ada Wawrzyniak Masters Student; 03/2017–08/2017
Julita Weleskowa Guest Researcher; 08/2017–09/2017

OVERVIEW

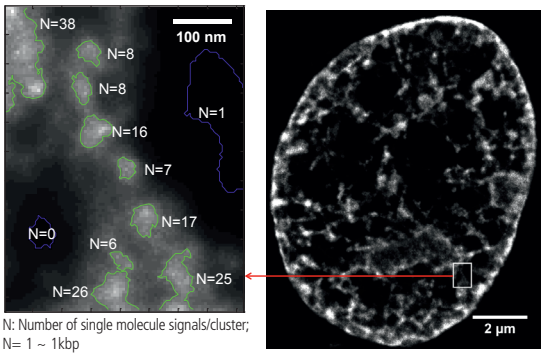
A complete mechanistic understanding of gene regulation in space and time requires a detailed topological insight into the epigenetic landscape of the cell nucleus; for this, it is necessary to enhance our ability to resolve the spatial DNA distribution substantially beyond the conventional level (~ 200 nm). This is now possible by advances in various super-resolution light microscopy (SRM) approaches. There are many areas where the advances in SRM are expected to, or already have, contributed include: How does the complex organisation of chromosome territories in the mammalian cell nucleus arise from the interaction of individual nucleosomes? How is this organisation related to epigenetic gene regulation and its dynamics? We have shown that single molecule localisation microscopy (SMLM), a type of super-resolution microscopy (SRM), is particularly useful for enhanced SRM analysis of the cell nucleus due to its superlative resolving capability combined with very specific fluorescent labelling. Recently we developed a novel SMLM approach for nuclear genome nanostructure imaging, designated DNA Structure Fluctuation Assisted Binding Activated Localisation Microscopy (fBALM). Presently, fBALM allows the imaging and analysis of chromatin nano-architecture at an optical and structural resolution of a few tens of nm.

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 chromatin domains? How many nucleosomes do they contain? To what degree can the size of interacting nuclear protein factories be determined on the nano-scale? These data are essential for a quantitative, mechanistic and dynamic understanding of epigenetic gene regulation. To address these

"Nuclear nanostructure is key to understanding gene regulation."

questions, we have developed a new technique (fBALM) for localisation microscopy of nuclear DNA at single molecule resolution, which substantially enhanced structural resolution. The fBALM localisation microscopy allowed us to quantitatively analyse variations in nuclear DNA distributions with an estimated structural resolution down to about 30 – 40 nm, localising up to several millions of single molecule signals in one optical nuclear section. Through this, we discovered evidence that nuclear DNA density may vary up to two orders of magnitude between nuclear regions. This gives further support to the notion that the mammalian nuclear genome is organised into distinct (active) high density regions and (inactive) low density regions. Furthermore, the inspection of small regions of interest in such nuclear fBALM images revealed nanoclusters of high DNA density, which would otherwise not be resolved in conventional resolution images of the same regions (Figure 1). A closer inspection indicated that the basic units of such domains are DNA clusters corresponding to a relatively small number of nucleosomes; DNA clusters only 100 nm apart were clearly distinguished. An essential parameter for a quantitative spatiotemporal understanding of nuclear organisation is the size of gene domains or of interacting protein factories under different conditions. In collaboration with Helle Ulrich (IMB) we performed measurements of specific DNA repair domains in yeast nuclei undergoing genotoxic treatment. The formation of such repair foci started at a minimum size of about 20 nm and extended 90 minutes later to a diameter 10 times as large or a thousand fold increase of the proteins involved. Using this super-resolution technique, it should be possible to directly measure transcription induced condensation changes on the level of very small, individual nuclear domains in single nuclei.



N: Number of single molecule signals/cluster;
N= 1 ~ 1kbp

Figure 1. Single-molecule localisation microscopy (fBALM) indicates a basic organisation of chromatin into small DNA clusters
The fBALM image at the right presents a murine cardiac muscle cell nucleus following oxygen nutrition depletion (OND). The inspection of small insets in such images (left) revealed nanoclusters of high DNA density. A closer inspection indicates that the basic units of such domains are DNA clusters corresponding to a relatively small number of nucleosomes; each molecule signal N corresponds to about 1 kbp. Domains only 100 nm apart are clearly distinguished (Szczurek *et al.*, 2017, *Nucleic Acids Res*).

To further enhance our optical resolution capacity, in the last year we completed the construction of a second EPI fluorescent localisation microscope. Using DNA Origamis for calibration, the average localisation precision achieved was 2 nm, indicating a structural resolution limit of about 5 nm. We also set up a novel inverse system for 3D Localisation Microscopy that allowed us to substantially enhance the general optical resolution and even more so in the axial direction (along the optical axis). This will allow for quantitative analysis of 3D DNA distribution in whole intact nuclei at unprecedented structural resolution.

FUTURE DIRECTIONS

The SRM instrumentations and the imaging methods developed will be applied in a number of collaborative projects: Experimental tests of nuclear genome nanostructure models (collaboration: Thomas Cremer/Univ. München); Imaging of nuclear nanostructure modifications induced by allergic signalling (Collaboration: MPI for Chemistry/Mainz); High precision measurements of the spatial nuclear organisation of the NR3C1 gene region after Cortisol treatment (collaboration: Univ. Michigan/Ann Arbor); Super-Resolution Microscopy of Hodgkin Cancer cells (collaboration: Univ. Manitoba/Winnipeg); Structured Illumination Microscopy of small protein aggregates in Retina tissue (collaboration: Univ. Heidelberg); Super-Resolution Microscopy of Smurf2-mediated chromatin re-arrangements following genotoxic treatment (collaboration Bar Ilan Univ./ Israel); Super-Resolution microscopy of chromatin remodelling by modified histone demethylases (collaboration: Univ. Oxford, UAB Barcelona, Michigan Technical University); and Super-Resolution Microscopy of mouse brain tissue with stress induced epigenetic differences (collaboration Neurosciences Univ. Mainz/Core Facility Microscopy IMB).

The long-term perspectives of SRM of the nuclear landscape will be the integration of these data into a quantitative, mechanistic, predictive and dynamic space-time model of functional nuclear organisation.

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Prakash K, Fournier D, Redl S, Best G, Borsos M, Ketting R, Tachibana-Konwalski K, Cremer C and Birk U. (2015). Super-resolution imaging reveals structurally distinct periodic patterns of chromatin along pachytene chromosomes *Proc Natl Acad Sci,* 112: 14635–14640.

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RENÉ KETTING

BIOLOGY OF NON-CODING RNA

POSITIONS HELD

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EDUCATION

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Nadine Wittkopp Postdoc; since 11/2012

OVERVIEW

The major focus of my lab is on gene regulation by small RNA molecules acting in RNAi-related pathways. Since their discovery at the start of the 21st century, many different RNAi-related pathways have been identified and it is now evident that although all of these pathways depend on proteins from the argonaute family, each pathway has its own unique characteristics and effects on gene expression. These can range from relatively minor effects on translation (in the case of many miRNAs) to the full-blown shutdown of loci at the transcriptional level (piRNAs). We mainly focus on mechanisms related to piRNA and siRNA biology, two species of small RNAs that are particularly abundant in, and important for, the germline. We do so mostly within the setting of (embryonic) development and are using both zebrafish and *C. elegans* as model systems for these studies. In addition, we aim to understand the conserved features of the mechanisms we are discovering and describing in these two model systems through analysis of small RNA pathways in other non-model animals. Finally, we have a strong interest in general germ cell development and early embryogenesis.

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 in the Piwi pathways, GTSF1. Strikingly, we found that even though this protein is conserved at the sequence level, its molecular function is not. This protein has been

"Small RNAs are at the heart of precision gene control."

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 assembly that drives small RNA biogenesis. This principle may well apply to the fly, where GTSF1 might drive the assembly of a larger complex that drives chromatin modification. Furthermore, we could show that the Zinc-finger domains present in GTSF-1 do not, as would be expected, drive RNA binding, but rather act in direct protein-protein interaction. This research emphasises that we can learn a lot from studying one factor in different organisms. Whereas one might have intrinsically coupled GTSF1 to chromatin biology, our work shows that it is much more likely to act as a protein that allows the assembly of larger protein, or protein-RNA complexes.

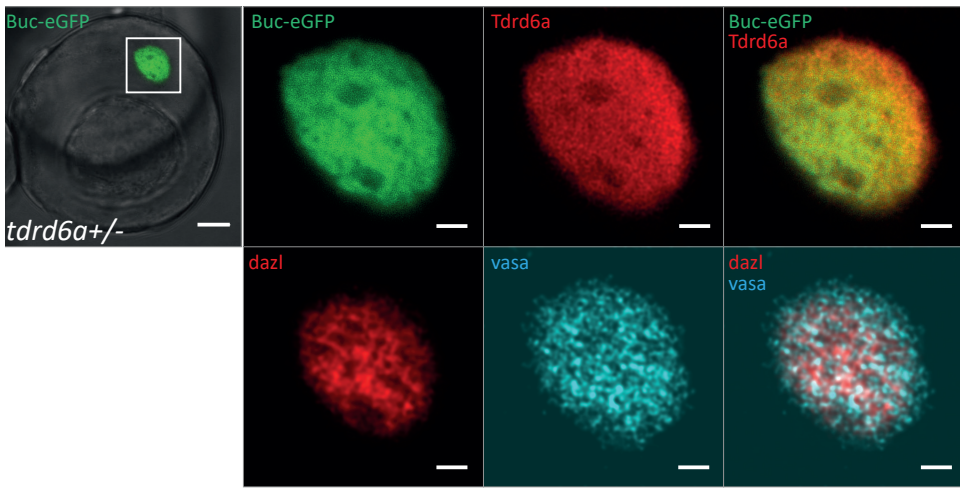

Figure 1. The Balbiani body in a zebrafish oocyte. Buc is a protein that is known to be present in germplasm. TDRD6a is visualised through immune fluorescence. *Vasa* and *dazl* represent two mRNAs that are visualised through single molecule FISH. The scale bar represents 2µm.

Coordinated deposition of mRNAs into the germ cells of zebrafish

We have been studying the effect of a protein 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 immune-single molecule FISH studies on zebrafish embryos. The way Tdrd6 drives loading of these transcripts into germ cells is by helping the fusion of small, RNA-protein aggregates into bigger units. This enables larger aggregates of these germ cell specifying mRNAs, also known as germ plasm, to be loaded into the future germ cells. In Figure 1 a particularly large TDRD6-dependent aggregate is shown, the Balbiani body. This structure is present in the oocyte and is a precursor to the embryonic germ plasm.

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. In order to perturb specific aspects of the identified mechanisms, we have started to extend our studies to include structural biology, allowing for the design specific disruptive point-mutations. *C. elegans* and zebrafish will continue to play important roles in these studies. We will focus on how a piRNA response can be initiated de novo and want to understand how and when a transient small RNA-mediated response can be transformed into a stably inherited response. In addition, we will start to work on how small RNA pathways are connecting to other aspects of the cell's gene-regulatory programmes, including those active during germ cell specification and differentiation.

SELECTED PUBLICATIONS

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

JULIAN KÖNIG

GENOMIC VIEWS OF SPLICING REGULATION

POSITIONS HELD

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OVERVIEW

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

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

RESEARCH HIGHLIGHTS

Splicing repression allows the gradual emergence of new Alu-exons in primate evolution

Alu elements are retrotransposons that frequently form new exons (Alu-exons) during primate evolution. Together with Jernej Ule's lab at University College London, we have assessed the interplay of splicing repression by the RBP hnRNPC and the nonsense-mediated mRNA decay (NMD) pathway in the quality control and evolution of Alu-exons. We identified 3,100 new Alu-exons and showed that NMD recognises Alu-exons more efficiently than exons containing premature termination codons. However, some Alu-exons escape NMD, especially when an adjacent intron is retained, highlighting the

"We're uncovering the enigma of the splicing code."

importance of concerted repression by the NMD and splicing pathways. Cross-species analysis of Alu-exons within primates showed that as Alu splice sites become better targets for the splicing machinery over evolutionary time there is a coupled, concurrent appearance of longer U-tracts, which are more efficient at repression. Ancient Alu-exons have short U-tracts, which decreases their splicing repression by hnRNPC, but they generally remain sensitive to NMD. We conclude that repressive motifs are under the strongest positive selection in the *Alu* elements that are evolving into new exons. Additionally, we hypothesise that the eventual, gradual removal of these repressive motifs might be crucial for the evolutionary emergence of new exons.

Identification of stable protein interaction partners of six RNA-binding E3 ubiquitin ligases

Many RBPs display catalytic activities, thereby enabling crosstalk between posttranscriptional regulation and various other molecular pathways. Among these, RNA-binding E3 ubiquitin ligases (RBULs) harbour the potential to link RNA-mediated regulatory mechanisms to the ubiquitin system, which is involved in regulating almost every process in the cell. Yet few RBULs have been characterised in depth to date.

Together with the Beli and Andrade groups at IMB, we used quantitative mass spectrometry-based proteomics to identify the core interaction partners of six different RBULs. In order to distinguish stable from transient interactions, we developed an adapted SILAC approach based on 'lysis after mixing'. Differentially isotope-labelled cells expressing either GFP-tagged RBULs or GFP alone were mixed prior to cell lysis. In this setup, only interactions that are highly stable and were already present in the native cellular environment can retain high SILAC ratios, whereas dynamically exchanging proteins will reach an equilibrium of both labels similar to background levels (Figure 1). We exploited gene ontology (GO) similarity measures to benchmark the specificity of our approach by evaluating the interactors' participation in shared cellular processes.

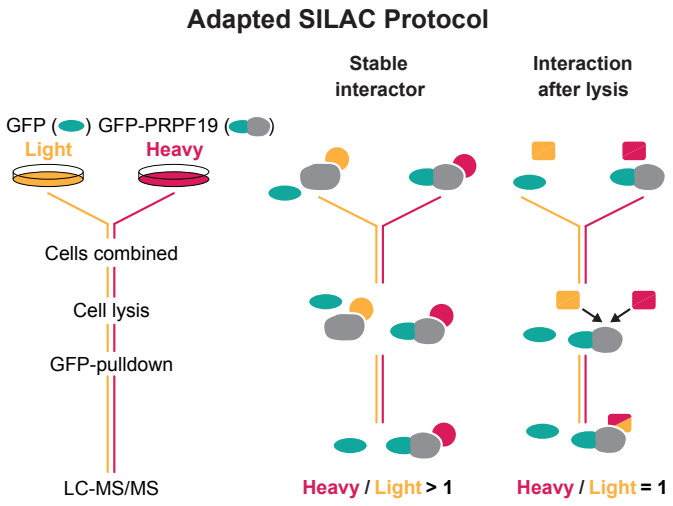

Figure 1. The adapted SILAC approach specifically enriches for stable protein-protein interactions. To this end, isotopically labelled cells expressing either GFP (Light) or a GFP-tagged protein of interest (Heavy) are mixed prior to cell lysis. Only stable interactions that persist from before lysis until the end of the purification result in high SILAC ratios (Heavy/Light >1). In contrast, interactions in the lysate remain at background levels (Heavy/Light=1).

Using our adapted sample preparation strategy and bioinformatics analysis, we identified the interactomes of the human RBULs ARIH2, MEX3B, MKRN1, MKRN2, RNF17 and PRPF19 proteins. We recovered known complex compositions and described novel functional links. For instance, our data indicated that RNF17 is involved in translation, but also regulates transcription. Globally, we found that all RBULs are extensively linked both to posttranscriptional processes and to the ubiquitin system. In summary, our adapted SILAC approach aids the identification of RBP interactomes and enables us to link them to new molecular functions.

FUTURE DIRECTIONS

The quantitative description of protein-RNA interactions with iCLIP technology, in combination with the clinically relevant model of *Alu* exonisation offer an attractive system for understanding the forces of competition and synergy that govern RNP complexes and splicing regulation. We will focus our efforts on elucidating the machinery for 3' splice-site definition, which has previously been identified as a hotspot for cancer-associated mutations. To achieve this, we will combine *in vivo* and biochemical approaches on a genome-wide scale, which will yield a systemic understanding of RNP function in splicing regulation. Our group's core competencies are the qualitative, quantitative, and comparative description of RBP binding, which will provide knowledge on how RBPs behave in the complex and interactive environment within cells.

These aspects will be addressed in the context of cryptic splice sites as they are present in *Alu* elements, which will be an important contribution to the understanding of genetic disease.

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Zarnack K*, König J*, Tajnik M, Martincorena I, Eustermann S, Stévant I, Reyes A, Anders S, Luscombe NM and Ule J. (2013). Direct competition between hnRNP C and U2AF65 protects the transcriptome from the exonization of Alu elements. *Cell*, 152: 453–466.

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STEFAN LEGEWIE

MODELLING OF BIOLOGICAL NETWORKS

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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; and (ii) how the gene expression is coordinately 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) optimise therapeutic intervention strategies.

RESEARCH HIGHLIGHTS

Single-cell dynamics of signalling and transcription

We investigated the cell-to-cell variability in the TGFβ/SMAD signalling pathway which plays a key role in tumourogenesis and metastasis. Together with Alexander Loewer (Darmstadt University), we have monitored the nuclear translocation of SMAD2/4-GFP fusion proteins in thousands of living MCF10A cells. To derive a quantitative description

"Understanding heterogeneous cell populations requires quantitative approaches."

of heterogeneous signalling, we devised a three-tiered, modelling strategy, in which we initially describe the population-average dynamics, then refine the description to the level of six subpopulations, and finally develop a model describing the complete heterogeneous cell population. CRISPR/Cas9-mediated knockouts confirmed the model prediction that the concentration fluctuations of certain pathway regulators are major sources of heterogeneity. In the future, we plan to investigate the dynamics and heterogeneity of TGFβ/SMAD signalling by systematically perturbing pathway components or neighbouring signalling cascades. We hope to understand why certain cells induce a migratory response and potentially metastasis, whereas others fail to do so.

To characterise the heterogeneous growth of estrogen-dependent breast cancer, we are studying the stochastic transcription of a key regulator of estrogen-induced cell proliferation. We established an imaging pipeline for visualising nascent transcripts in living MCF7 cells using the PP7-PCP system (Figure 1). Using a stochastic model fitting framework, we investigated how varying estrogen concentrations or small-molecule inhibitors control the heterogeneity of transcription. 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 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.

Role of post-transcriptional regulation during Drosophila development

Cells respond to changing environments by adjusting their protein expression patterns. Even though proteins are produced from mRNA, mRNA levels are generally a poor predictor of protein expression. In many cases, it is unclear whether this low mRNA-protein correlation is due to extensive post-transcriptional regulation or simply arises from the delayed dynamics of protein relative to mRNA. Together with the Butter and Roignant groups, we determined mechanisms of protein expression regulation during fruit fly development using a combination of time-resolved transcriptome and proteome measurements, together with mathematical modelling. We found that simple mathematical models of protein translation and degradation fit approximately 80% of mRNA-protein pairs, without assuming a complex post-transcriptional regulation. The remaining unexplained class of potentially post-transcriptionally regulated proteins contained various RNA binding protein motifs. We are currently validating candidate post-transcriptional regulators to confirm that they regulate translation during embryonic development as predicted by our model. In summary, we developed a systems biology framework for the identification of post-transcriptional gene regulation mechanisms from large-scale time-resolved analyses of mRNA and protein expression patterns.

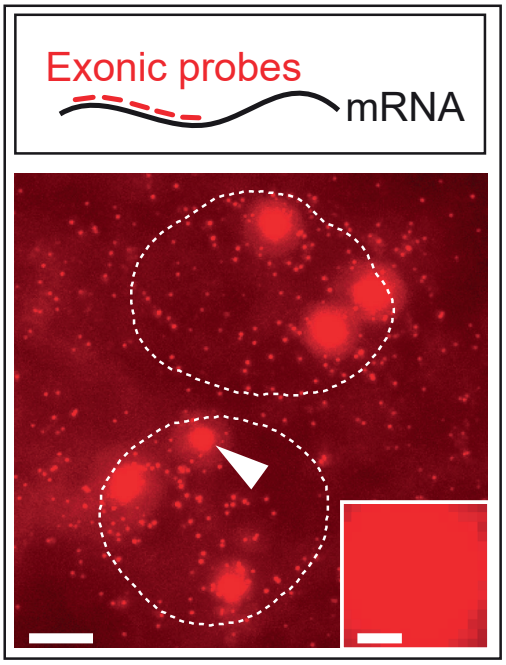

Figure 1. Imaging of estrogen-induced transcription. An estrogen-sensitive transcript was visualised using single-molecule fluorescence *in-situ* hybridisation (smFISH) using probes targeting the mRNA exons. Three high-intensity foci (one marked by arrowhead) within each nucleus (dashed line) reflect sites of nascent transcription at three endogenous gene loci in MCF7 cells. Smaller surrounding dots are diffusing single mRNA molecules.

FUTURE DIRECTIONS

Cells respond to external cues by inducing changes in gene expression. How these gene expression responses are coordinated by complex gene regulatory networks remains poorly understood. Together with the König group (IMB) and Kathi Zarnack (BMLS, Frankfurt), we are employing perturbation approaches, and mathematical modelling to characterise the topology and the dynamics of post-transcriptional splicing regulatory networks. Using our models, we hope to contribute to a better understanding of how deregulated splicing contributes to tumour growth.

SELECTED PUBLICATIONS

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

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Kallenberger SM, Beaudouin J, Claus J, Fischer C, Sorger PK, Legewie S# and Eils R#. (2014). Intra- and interdimeric caspase-8 self-cleavage controls strength and timing of CD95-induced apoptosis. *Sci Signal*, 7: ra23.

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TELOMERE BIOLOGY

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Tina Wagner PhD Student; since 11/2015

OVERVIEW

Telomeres are protective caps at the ends of linear chromosomes. When telomeres shorten, replicative senescence occurs as a result of DNA damage checkpoint activation. Cells that have undergone more divisions are prone to becoming senescent, due to telomere shortening. The accumulation of senescent cells contributes to ageing in multi-cellular organisms. On the other hand, replicative senescence counteracts tumour development by limiting proliferative potential. Therefore, cancer cells must overcome the checkpoint barrier and re-elongate their short telomeres in order to achieve immortality. We are interested in understanding how cells surmount replicative senescence by elongating their short telomeres. We have observed that the long non-coding RNA, TERRA, with its ability to form RNA-DNA hybrids, marks the shortest telomeres for elongation by homology-directed repair (HDR) in budding yeast.

Short/dysfunctional telomeres elicit a DNA damage checkpoint response. If the damage can be repaired, i.e. the telomeres are elongated, the checkpoint is turned off and the cell continues through the cell cycle. In the case of irreparable damage, the cells attempt to overcome the cell cycle arrest via checkpoint adaptation. The process of checkpoint adaptation is conserved from yeast to human cells and in the latter has been proposed to contribute to genome instability and drug resistance.

RESEARCH HIGHLIGHTS

We have progressed in the understanding of TERRA regulation and function. We have demonstrated that TERRA and R-loops specifically accumulate at short telomeres where they trigger homologous recombination and hence promote telomere length maintenance. TERRA and its R-loops are regulated by the cell-cycle and are formed in the early stages of S phase. Shortly thereafter, the ribonucleases Rat1 and RNase H2 associate with telomeres and degrade TERRA and its R-loops, respectively. This coordinated action

"The non-coding RNA TERRA promotes the elongation of short telomeres."

ensures TERRA removal before passage of the DNA replication machinery. Strikingly, when telomeres become critically short, Rat1 and RNase H2 are no longer recruited to telomeres and TERRA is not removed from telomeres. This deregulation likely results in the replication machinery encountering TERRA R-loops and subsequent recombination at short telomeres. We believe that this TERRA regulation may explain how cells distinguish short from long telomeres *in vivo*. (Figure 1).

When cells escape senescence (adapt) they may re-elongate their telomeres via recombination, as is the case for ALT (alternative lengthening of telomeres) cancer cells. We have now shown that when yeast cells use the ALT mechanism they up-regulate TERRA levels and activate DNA damage signalling. We speculated that TERRA R-loops also promote telomere recombination in these cells. Upon RNase H overexpression, their growth was strongly inhibited, whereas no effect was observed in telomerase positive cells. We propose that TERRA R-loops promote recombination at critically short telomeres during replicative senescence and similarly maintain telomere length in cells using the ALT mechanism. In cells with "normal" length telomeres, we believe that TERRA levels are suppressed because recombination is not desired.

We have expanded our research on RNA-DNA hybrid regulation and have investigated how the yeast Mph1 (Mutator Phenotype 1) helicase, the homolog of human Fanconi Anemia protein M (FancM), regulates R-loops. We have observed that regulation of Mph1 activity by the Smc5/6 complex is crucial during senescence. Mph1 seems to play a broader role in R-loop metabolism as its inactivation leads to synthetic lethality with loss of RNase H1 and H2 function.

RNA-DNA hybrids do not only occur as long stretches of interconnected bases but also as single nucleotide interactions, e.g. following mistakes made by the replicative DNA polymerase. We found that the E3 ubiquitin ligase Rtt101 is crucial in regulating these single ribonucleotide insertions.

Using a dysfunctional telomere model, we have previously demonstrated that interventions which reduce metabolism also inhibit checkpoint adaptation. We have expanded this line of research to repair defective cells lacking the central recombination protein Rad52 and have obtained similar results. The *rad52* cells that became resistant to DNA damaging agents exhibit aneuploidy following adaptation. In line with previous reports showing that aneuploid cells have a higher energy demand, these cells are sensitive to rapamycin, an agent mimicking starvation. Aneuploidy has been associated with proteotoxic stress. Consistently, we could demonstrate that adapted *rad52* cells showed increased sensitivity to heat shock and proteasome inhibitors. We hypothesise that agents interfering with energy metabolism and protein homeostasis could prove valuable in preventing the occurrence of repair defective cells, such as tumour cells, becoming resistant to chemotherapeutics.

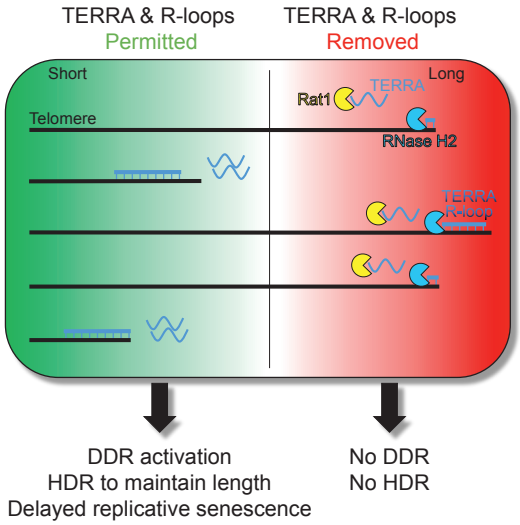

Figure 1. TERRA marks short telomeres for elongation via HDR. In the absence of telomerase, telomeres progressively shorten; replicative senescence ensues as does activation of the DNA damage response (DDR). It remained enigmatic how cells recognise the shortest telomere for elongation. We showed that the shortest telomeres are elongated by homology directed repair (HDR), as they accumulate TERRA R-loops. At long telomeres, there is no DDR activation, no TERRA accumulation and therefore no elongation by HDR. (Graf *et al.*, 2017, *Cell*)

FUTURE DIRECTIONS

We continue to investigate the role of TERRA and R-loops in respect to telomere elongation, especially in the context of ALT telomere maintenance mechanisms. We will focus on modulating TERRA and TERRA R-loop levels with the goal of specifically affecting the viability of ALT cells. Additionally, we are interested in the regulation of R-loops by the yeast helicase Mph1 and by its human homolog, FANCM. We are equally excited to discover how the E3 ubiquitin ligase Rtt101 regulates the response to single ribonucleotides that are mistakenly incorporated during DNA replication. Finally, we are characterising chemical compounds that affect checkpoint adaptation with the goal of combining them with genotoxic chemotherapeutics to enhance cytotoxicity and prevent resistance. We aim to gain a mechanistic understanding of how these reagents act and modulate the response to chemotherapeutic agents.

SELECTED PUBLICATIONS

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CHRISTOF **NIEHRS**

DNA DEMETHYLATION AND REPROGRAMMING

POSITIONS HELD

2010–2015 Executive Director, Institute of Molecular Biology (IMB), Mainz

Since 2010 Founding & Scientific Director, IMB, Mainz
Professor, Faculty of Biology, Mainz University

Since 2000 Professor of Molecular Embryology, German Cancer Research Center (DKFZ), Heidelberg

Since 1994 Head of Division "Molecular Embryology", DKFZ, Heidelberg

1990–1993 Postdoc, University of California, Los Angeles (UCLA)

EDUCATION

1997 Habilitation in Biology, University of Heidelberg

1990 PhD in Biology, European Molecular Biology Laboratory (EMBL) & University of Heidelberg

1985 Diploma in Biochemistry, Free University of Berlin

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Andrea Schäfer Postdoc; since 09/2010
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Carola Scholz Lab Technician; since 05/2015
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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 and occurs in plants as well as in animals, during development, in the adult, and during 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 GADD45 in development. Our results indicate that GADD45a acts as an adapter protein, which directs DNA methylation machinery to specific loci. To address GADD45 function, we use biochemical, molecular biological and cell biological approaches, employing the mouse and frog model systems.

RESEARCH HIGHLIGHTS

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

We have shown that *Gadd45a*, a member of a small gene family of stress response genes encoding histone-fold proteins, 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

"We are beginning to understand the biology of DNA methylation."

two key enzymes of the DNA demethylation machinery, TET (Ten-eleven translocation) and TDG (thymine-DNA glycosylase), to enhance turnover of oxidized cytosines (Figure 1). 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 loci, GADD45a relies on bridging factors including the histone reader ING1. ING1 contains a PHD finger motif, which recognizes trimethylated histone H3 at Lysine 4 (H3K4me3), a promoter-specific mark anti-correlated with DNA methylation. ING1 is involved in chromatin remodelling, transcriptional regulation, regulating cell growth, apoptosis, senescence, and tumorigenesis. *Gadd45a* and *Ing1* are both stress response genes and mice deficient for either gene are viable, though radiation sensitive and tumour prone.

We characterized *Gadd45a/Ing1* homozygous double mutant (DKO) mice to reveal synthetic phenotypes, since the function of proteins acting in a complex is often phenotypically buffered. Strikingly, *Gadd45a/Ing1* DKO mice exhibit symptoms of premature ageing. For a molecular analysis, we carried out whole-genome bisulphite sequencing in cells from DKO mice identifying around 1000 differentially methylated regions (DMRs). The majority of the DMRs are hypermethylated, consistent with a role of *Gadd45a/Ing1* in promoting DNA demethylation. DMRs are greatly enriched for lowly methylated regions (LMRs) in WT cells. LMRs are known hotspots for active DNA demethylation marked by a high density of 5-hydroxymethylcytosine, the first product in the demethylation of 5mC. Indeed, overlaying 5-hydroxy-, 5-formyl- and 5-carboxyl-cytosine data from mouse embryonic stem cells revealed that in the DKO context, DMRs are enriched for 5hmC and gain 5fC and 5caC upon Tdg knockdown, corroborating that these are sites undergoing active TET/TDG-mediated demethylation. Moreover, hypermethylated DMRs are highly enriched in enhancers, supporting the model that *Gadd45a/Ing1* cooperate in gene-specific DNA demethylation.

These results are very significant, since although changes in DNA methylation are among the best-documented epigenetic alterations accompanying organismal ageing, if and how altered DNA methylation is causally involved in ageing has remained elusive. Hallmarks of ageing are global hypomethylation and local hypermethylation, similar to that seen in cancer cells. Our study reveals for the first time a causal nexus between DNA demethylation, metabolism and organismal ageing.

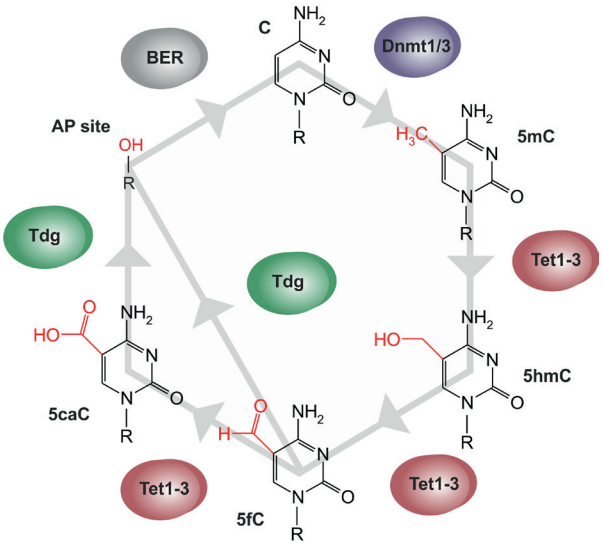

Figure 1. TET-TDG mediated active DNA demethylation. Unmodified cytosine is methylated by DNMTs (DNMT3a/b for de novo or DNMT1 for maintenance methylation) to 5 methylcytosine (5mC), then iteratively oxidized by TET(1-3) to 5-hydroxymethycytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), respectively. 5fC and 5caC are excised by TDG and the generated abasic (AP) site is repaired by base excision repair to restore the DNA with an unmodified cytosine (from Schomacher et al. 2017).

FUTURE DIRECTIONS

Our discovery of the GADD45a/ING1 interaction in demethylation and ageing raises new questions. Which target genes are regulated by GADD45a/ING1? What are the sequence determinants that recruit GADD45a to specific genes during demethylation? To investigate these questions, we will screen for GADD45a/ING1 target genes and analyse GADD45a interacting proteins. Moreover, we will study mouse embryonic stem cells (mESC) that have mutated *Gadd45α*, *β* and *γ* genes and continue analysing the function of Neil DNA glycosylases during DNA demethylation and their role in mESC differentiation.

SELECTED PUBLICATIONS

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HOLGER **RICHLY**

MOLECULAR EPIGENETICS

POSITIONS HELD

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

2005–2011 Postdoc,
Centre for Genomic Regulation (CRG), Barcelona

EDUCATION

2005 PhD in Biology,
Ludwig Maximilian University, Munich

2000 Diploma in Biochemistry,
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GROUP MEMBERS

Jonathan Byrne PhD Student; 08/2011–07/2017
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Thomas Wilhelm PhD Student; 11/2011–08/2017

OVERVIEW

The research undertaken in my laboratory aims at deciphering molecular pathways that underlie chromatin signalling networks that regulate physiological processes such as cellular differentiation, DNA repair and organismal ageing. Our scientific approach relies largely on dissecting the functions of diverse chromatin components, as for example epigenetic players, in the cell culture system biochemically and by applying high resolution microscopy. We complement our *in vitro* approach by employing genetics and RNAi screening techniques in *C. 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 recently 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 have 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*. In analogy, ZRF1 also remodels multiprotein complexes during differentiation of mouse embryonic stem cells. During cellular differentiation, ZRF1 is recruited to regulatory regions of genes that are silenced by PRC1 and Mediator complexes.

"We aim to decipher chromatin signalling pathways."

We have further demonstrated that *on-site remodelling* in the global genomic branch of NER is confined to specific nuclear regions (Chitale et al, 2017a). 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) (Chitale et al., 2017b). In our latest publication we demonstrate 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).

Further, we are interested in understanding gene regulation during organismal aging. To this end we investigate aging 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*. Through this novel screening approach, we found that post reproductive inactivation of the prominent forkhead box (FOX) A transcription factor PHA-4 results in longevity, while its inactivation in early life shortens lifespan. Previous studies have shown that PHA-4 governs the expression of autophagy genes and thereby mediates longevity in diet-restricted and germline-less animals. We discovered that postreproductive inactivation of genes required for the 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. Just like pha-4, these autophagy genes reduced lifespan when inactivated early in life, which is in line with previous observations (Wilhelm et al, 2016). Interestingly, post-reproductive inactivation genes that control later steps of the autophagic cascade (e.g. vesicle elongation, maturation, and cargo degradation) did not positively affect *C. elegans* lifespan. 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 (Byrne et al, 2016). We moreover found that post-reproductive inactivation of autophagosome nucleation extends lifespan primarily through the neurons.

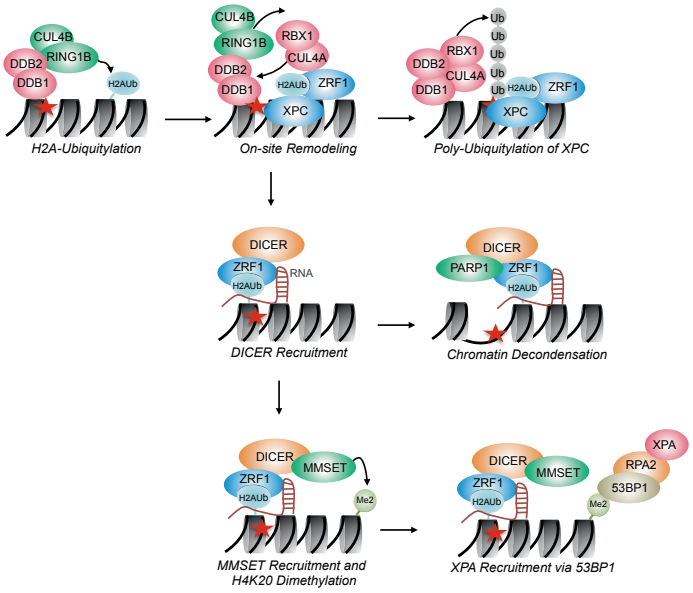

Figure 1. Spatiotemporal regulation of chromatin factors and ubiquitylation events during NER. (top) The assembly of the UV-RING1B complex at DNA damage sites causes mono-ubiquitylation of histone H2A. ZRF1 is recruited to the damage site via XPC and tethers to H2A-ubiquitin causing on-site remodelling of the UV-RING1B E3 ligase complex. The newly-established DDB-CUL4A complex catalyses the poly-ubiquitylation of various substrates and most importantly XPC, which is thereby stabilised at the damage site. (middle) ZRF1 recruits DICER to DNA damage sites and decondenses chromatin in conjunction with PARP1. (bottom). DICER recruits MMSET to catalyze H4K20methylation at the DNA damage site. H4K20me2 tethers XPA via 53BP1 and RPA2.

FUTURE DIRECTIONS

In the future, we will prioritise the research on DNA repair in the NER pathway and the investigation of organismal ageing. One of our main aims is to understand 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 chromatinc factors and environmental cues extend the lifespan and the health span of *C. elegans*. In particular, we will examine late life functions of DNA repair factors and metabolic genes.

SELECTED PUBLICATIONS

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Gracheva E, Chitale S, Wilhelm T, Rapp A, Byrne J, Stadler J, Medina R, Cardoso MC and Richly H. (2016). ZRF1 mediates remodeling of E3 ligases at DNA lesion sites during nucleotide excision repair. *J Cell Biol*, 213: 185–200.

Chitale S and Richly H. (2018). DICER and MMSET-catalyzed H4K20me2 recruits the nucleotide excision repair factor XPA to DNA damage sites. *J Cell Biol*, 217: 201704028.

* indicates joint authorship

JEAN-YVES ROIGNANT

RNA EPIGENETICS

POSITIONS HELD

Since 2012 Group Leader, Institute of Molecular Biology (IMB), Mainz

2008–2011 Research Associate, Skirball Institute of Biomolecular Medicine, New York University

2003–2008 Postdoc, Skirball Institute of Biomolecular Medicine, New York University

EDUCATION

2003 PhD in Developmental Biology, Jacques Monod Institute, Paris

1998 MSc in Developmental Biology, Paris IV

1997 BSc in Molecular & Cellular Biology, Rennes University

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Tina Lence PhD Student; since 07/2013
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Violeta Morin Lab Technician; since 05/2015
Chiara Paolantoni PhD Student; since 10/2017
Michael Stock Guest Researcher; 03/2017–09/2017
Lina Worpenberg PhD Student; since 08/2017

OVERVIEW

Our work is centered on elucidating the functions and mechanisms of co-transcriptional splicing during development. In the post-genome era, it has become evident that the complexity of higher eukaryotes involves multiple layers of gene regulatory mechanisms. Alternative splicing (AS) increases the number of proteins generated from a single pre-mRNA and therefore is one of the most important mechanisms to control protein expression and function post-transcriptionally. As most splicing events occur co-transcriptionally, this process can be regulated at many levels, from chromatin and the transcription machinery through to modifications on RNA. The ever-growing number of human diseases associated with splicing deregulation highlights the fundamental importance of alternative splicing in generating and maintaining a functional cell. With our current research, we aim to decipher the mechanisms of gene regulation, with a specific focus on the interplay between transcription, RNA modifications and alternative splicing in the context of animal development and behaviour.

RESEARCH HIGHLIGHTS

Comprehensive characterisation of the *lola* locus

Lola is a transcription factor, which plays essential roles in axon guidance during embryogenesis. Its gene is among the most complex loci in *Drosophila*, giving rise to at least 80 different isoforms through alternative splicing and the activity of multiple promoters. lola spans over a large region of the genome and consists of a constitutive N-terminal region and alternatively spliced 3' terminal exons. Ultimately, *lola* encodes for 20 different protein isoforms, comprising a common BTB protein-protein interaction domain fused to different zinc finger DNA binding C-terminal motifs (Figure 1). Most experiments aimed at investigating *lola* function were performed using loss of function alleles, which contained mutations in the N-terminal region that is conserved across isoforms.

"The vast diversity of RNA modifications exposes a new world of gene regulation."

Besides defects in axon growth, these mutations give rise to pleiotropic effects *in vivo* including impairment in germline stem cell maintenance, photoreceptor differentiation, and maintenance of differentiated neurons in the adult brain. To systematically address the developmental roles of Lola isoforms, we generated isoform-specific deletions in their unique 3' terminal exons using the recently developed CRISPR/Cas9 system. We obtained at least one loss-of-function mutation for every isoform. Altogether, we have identified 8 Lola isoforms which give rise to a visible defect upon depletion, with 4 previously uncharacterised. We focused on the Lola-O isoform. Knock out of *lola O* results in homozygous viable animals, yet adult flies display several abnormalities. They suffer from locomotion defects, reduced lifespan, as well as the formation of melanotic tumours. We demonstrated that these defects arise from dysfunctions in the tyrosine-octopamine pathway. Octopamine is homologous to the vertebrate norepinephrine, which acts as a neurotransmitter, neuromodulator and neurohormone. We found that the transcript levels of *Tyramine B-hydroxylase (TBH)* required for the synthesis of octopamine was significantly reduced in *lola O* mutant. Taken together, we have uncovered a novel function for the transcription factor Lola in regulating the octopamine pathway in *Drosophila*. Furthermore, we demonstrated that despite high sequence homology of their zinc finger domain, the loss of Lola isoforms cannot be compensated by others. These results imply that alternative splicing of *lola* must be tightly controlled to ensure proper development and behaviour.

Functions of RNA modification in *Drosophila*

The widespread roles of m^{6}A RNA modification in the regulation of post-transcriptional gene expression have recently been brought to light. From yeast to mammals, m^{6}A 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 m^{6}A in multi-cellular organisms have not yet been fully investigated. To address these questions, we have characterised the m^{6}A pathway in *Drosophila*. We found that a conserved m^{6}A methyltransferase complex, composed of four proteins, controls alternative splicing in Drosophila cells and *in vivo*. Furthermore, we identified the split ends (SPEN) family protein, Spenito (Nito), as an additional bona fide subunit of the complex. 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 *Mettl3* and for the second catalytic subunit are viable but suffer from severe locomotion defects due to impaired neuronal functions. 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. Currently, we are developing new approaches to map m^{6}A *in vivo* as current techniques are not adapted for *in vivo* studies, which prevent the characterisation of m^{6}A molecular targets and their functions during organismal development.

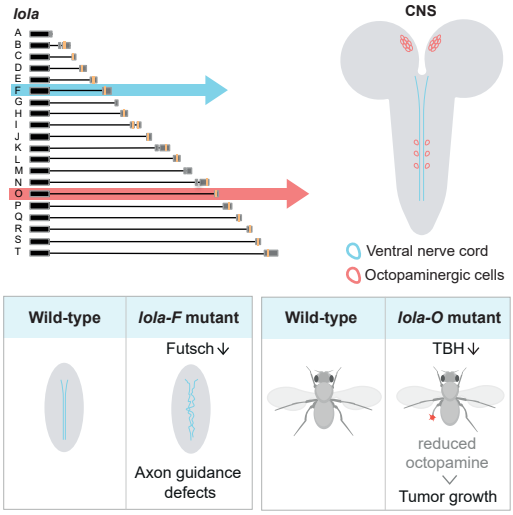

Figure 1. The complex *lola* locus in *Drosophila*. lola encodes 80 mRNA isoforms, giving rise to 20 unique proteins. Boxes indicate alternative 5' non-coding exons (pink), common exons (blue) and alternative 3' coding exons (grey). Letters indicate alternative exons of each isoform. (from Dinges *et al.* 2017)

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 going deeper into the mechanisms and functions of m^{6}A modification and have also started the characterisation of novel modifications *in vivo*.

SELECTED PUBLICATIONS

Lence T, Akhtar J, Bayer M, Schmid K, Spindler L, Ho CH, Kreim N, Andrade-Navarro MA, Poeck B, Helm M and Roignant JY. (2016). m(6)A modulates neuronal functions and sex determination in *Drosophila*. *Nature*, 540: 242–247.

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

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

VASSILIS **ROUKOS**

CELL BIOLOGY OF GENOME MAINTENANCE

POSITIONS HELD

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EDUCATION

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Monika Kuban Lab Technician; since 01/2017
Vera Minneker PhD Student; since 04/2016
Rossana Piccinno PhD Student; since 04/2015

OVERVIEW

Maintaining the integrity of genetic information is essential for cell survival. Mechanisms that counteract DNA damage are important to help maintain cellular homeostasis by suppressing mutagenic events and genome rearrangements that may lead to disease, particularly cancer. One of the most severe forms of genome rearrangements are chromosome translocations. 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 tools to probe rare, cancer-initiating chromosome translocations

Modelling the formation of recurrent cancer-initiating translocations 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 (Figure 1). High-throughput imaging and automated image analysis is used to probe and quantify individual cells with intra-chromosomal separation and rare cancer-initiating translocations. This methodology complements existing approaches and offers several advantages in detection and quantification of translocations. It is: (a)

"We use microscopy & genomics to understand oncogenic translocations."

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 as low as 10^{-3} 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-AML) 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. 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 (Figure 1), our efforts are focusing on: (1) understanding which are the cellular and molecular pathways that contribute to the formation of recurrent DNA breaks upon treatment with various chemotherapeutic agents; (2) on how the chromatin environment may predispose susceptibility to breakage and chromosome translocations; and (3) on identifying molecular players of the DNA damage response and novel factors that promote or inhibit the illegitimate fusion of chromosomes'.

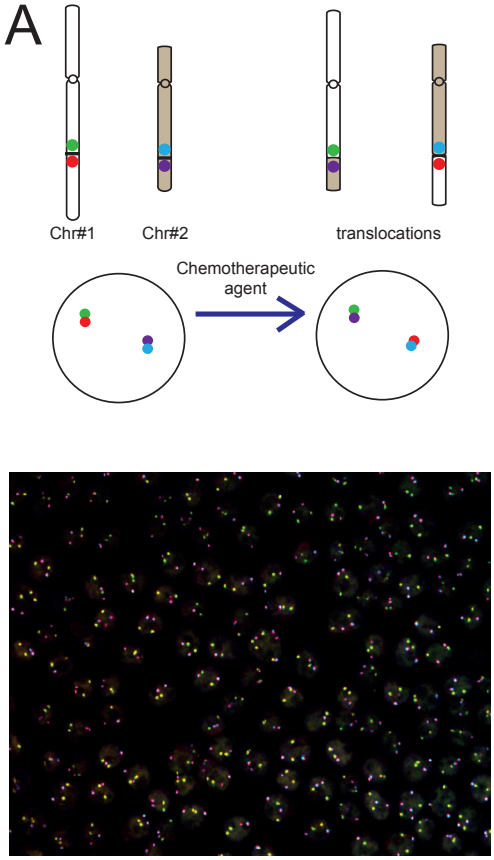

Figure 1. (A) Rational of C-Fusion 3D, a methodology we have developed to detect with single cell resolution rare chromosome translocations. Fluorescence in situ hybridisation (FISH) is used to probe the position of chromosome ends of potential translocation partners. Translocations are detected by the spatial co-localisation in 3D of chromosome ends of the respective translocation partners identified by distinct probes.

FUTURE DIRECTIONS

We will extend our analysis in identifying mechanisms governing the formation of different chromosome translocations leading to a variety of secondary malignancies. As chromosome breaks need to come to spatial proximity and physically interact before illegitimate repair, the spatial arrangement of the chromosome partners is an important contributor to translocation frequency. We would like to integrate genomic maps of breakage with genome-wide maps of preferential translocation partners and high-resolution chromosome organisation maps, to comprehensively assess the contribution of spatial genome organisation in the translocation process. We also plan to develop 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

Roukos V, Pegoraro G, Voss TC and Misteli T. (2015). Cell cycle staging of individual cells by fluorescence microscopy. *Nat Protoc*, 10: 334–348.

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

Roukos V, Voss TC, Schmidt CK, Lee S, Wangsa D and Misteli T. (2013). Spatial dynamics of chromosome translocations in living cells. *Science*, 341: 660–664.

NATALIA SOSHNIKOVA

DEVELOPMENTAL AND STEM CELL BIOLOGY

POSITIONS HELD

Since 2012 Group Leader, Institute of Molecular Biology (IMB), Mainz

2004–2011 Postdoc, University of Geneva

EDUCATION

2004 PhD in Molecular Biology, Humboldt University, Berlin

1998 Diploma in Molecular Biology, Novosibirsk State University

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Jakub Klassek Lab Technician; since 08/2016
Lira Nigmatullina PhD Student; 04/2013–04/2017
Maxim Norkin PhD Student; 08/2015–04/2017

OVERVIEW

Developmental and stem cell biology are two tightly linked disciplines. Stem cells are found in adult tissues, yet their progenitors are specified during embryogenesis. Gene expression and lineage tracing studies revealed LGR5 as a marker of the adult intestinal stem cells (ISCs) and defined their position at the bottom of the crypts, the space between intestinal villi. Although the adult ISCs are among the best characterised tissue-specific stem cells, nothing was known about their embryonic progenitors until our discovery of a factor crucial for their correct specification. Our studies showed that various populations of the early intestinal epithelial progenitors give rise to Lgr5$^+$ cells. The ISC specification process is accompanied by dramatic changes in chromatin and transcriptional states. To further understand the mechanisms of ISC specification during mouse development we are addressing the following questions: What triggers the specification of Lgr5$^+$ progenitors during embryogenesis? What is the identity of cells triggering specification of Lgr5$^+$ progenitors? How does patterning of the small intestine during embryogenesis influence Lgr5$^+$ cell formation? Do Lgr5$^+$ cells influence patterning of the embryonic intestine? To answer these questions, we apply tools of mouse genetics, single-cell genomics and molecular cellular (*ex vivo* 3D organoids) biology.

RESEARCH HIGHLIGHTS

When and how are Lgr5$^+$ intestinal stem cell progenitors specified during embryogenesis?

Using lineage tracing analysis we defined the time of Lgr5$^+$ progenitor specification (Figure 1). Interestingly, Lgr5$^+$ cells represent only a minor fraction of the small intestinal epithelium in mouse embryos. We found that ID2 is a key factor controlling the timing of Lgr5$^+$ progenitor specification during embryogenesis. In ID2-deficient embryos, Lgr5$^+$

"We use intestinal stem cells to investigate the enigma of embryogenesis"

cells appear several days earlier and are much more abundant. Ectopic expression of WNT ligands is required for the precocious activation of the WNT/β-catenin target genes and earlier specification of Lgr5$^+$ progenitors in the *Id2* mutant intestine. Moreover, ID2 negatively regulates transcription of WNT/β-catenin dependent genes by affecting the stability of the β-catenin protein. The precocious ID2-deficient Lgr5$^+$ embryonic progenitors give rise to adult ISCs. Yet, in *Id2* mutants, the number of LGR5-EGFP$^+$ cells and their transcriptional signature differ from those in wild-type mice. This indicates that the timing of ISC progenitor specification is an important factor for molecular and phenotypic characteristics of the adult ISCs.

Role of chromatin modifications during specification and differentiation of ISCs

We established transcriptome and chromatin profiles for the embryonic intestinal epithelium prior to and after the initial specification towards stem cells, as well as for the adult ISCs and their most abundant differentiated progenies, enterocytes. We found that already during embryogenesis, major chromatin changes at the transcription start site (TSS) and distal regulatory elements prepare appropriate transcriptional landscapes for later activation of the enterocyte specific genes during ISC differentiation. Interestingly, strong changes in the distribution of H3K27me3 take place during the transition from embryonic progenitors to adult ISCs (Figure 1). A large proportion of genes essential for the development of the small intestine acquire H3K27me3 in adult ISCs, suggesting its role during ISCs specification. In contrast, the majority of ISC signature and enterocyte specific genes lose DNA methylation during adult ISCs specification. In the adult gut, the loss of H2A.Z accompanies activation of many enterocyte specific genes during ISCs differentiation.

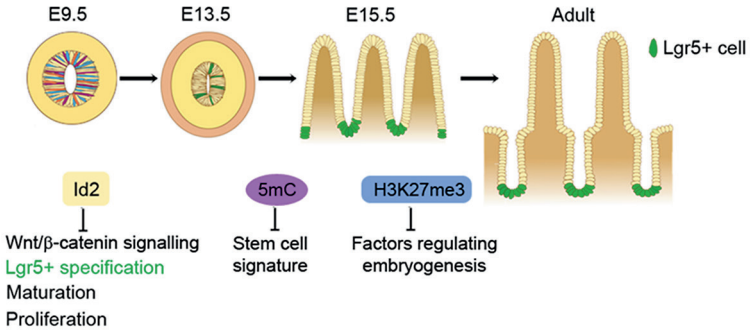

Figure 1. ISC specification during mouse embryogenesis. Various early embryonic epithelial progenitors (multiple colours) give rise to Lgr5$^+$ ISCs. ID2 prevents maturation and commitment of the small intestinal epithelial cells towards Lgr5$^+$ progenitors (green) by repressing WNT signalling. Upon specification, ISC signature genes lose 5mC, whereas developmental genes get silenced and acquire H3K27me3. By unknown mechanisms, Lgr5$^+$ cells reside within the inter-villi domain at E15.5, which become intestinal crypts in the adult gut. Modified from Nigmatullina *et al.* 2017

What is the source of transcriptional heterogeneity found in Lgr5$^+$ ISCs?

The adult ISCs are transcriptionally heterogeneous. Whether this heterogeneity reflects an early determination of distinct cellular sub-types with potentially distinct physiological functions remained an open question. We found that the early midgut epithelium is comprised of heterogeneous cell populations (Figure 1). On the one hand, epithelial cells have different transcriptional signatures according to their positions along the anterior-posterior axis. On the other hand, there is heterogeneity in the levels of WNT signalling within the midgut epithelium. Does this transcriptional heterogeneity translate into functional heterogeneity? Cell fate mapping analysis showed that early progenitors have different capacities to generate Lgr5$^+$ ISC progenitors depending on their molecular profile. Furthermore, the origin of the molecularly distinct early precursors along the anterior-posterior axis defines the transcriptional signature of embryonic Lgr5$^+$ ISC progenitors and subsequently adult ISCs.

FUTURE DIRECTIONS

We are continuing our studies on the cellular and molecular mechanisms of intestinal patterning and stem cell specification during embryogenesis. Using single-cell transcriptomics we have learned about cellular heterogeneity within both the small intestinal epithelium and mesenchyme. We have defined signals which could be essential for triggering ISC specification. We are performing functional studies *in vivo* and *ex vivo* to test the involvement of those signalling pathways in embryonic gut patterning.

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Kazakevych J, Sayols S, Messner B, Krienke C and Soshnikova N. (2017). Dynamic changes in chromatin states during specification and differentiation of adult intestinal stem cells. *Nucleic Acids Res*, 45: 5770–5784.

Nigmatullina L, Norkin M, Dzama MM, Messner B, Sayols S and Soshnikova N. (2017). Id2 controls specification of Lgr5(+) intestinal stem cell progenitors during gut development. *EMBO J*, 36: 869–885.

HELLE ULRICH

UBIQUITIN, SUMO AND GENOME STABILITY

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

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Sonja Braun Lab Technician; since 02/2016
Karolin Eifler-Olivi Postdoc; since 10/2015
Néstor García-Rodríguez Postdoc; since 05/2013
Nataliia Gralievska PhD Student; since 01/2014
Martin Möckel Postdoc; since 11/2016
Julia Müller Research Assistant; since 10/2017
Stephanie Nick PhD Student; since 11/2014
Regina Otto Personal Assistant; since 09/2015
Vanessa Patronelli Student Assistant; since 04/2017
Christian Renz Postdoc; since 08/2014
Axel Schäfer PhD Stundent; since 10/2017
Annie Sriramachandran Postdoc; since 03/2015
Laura Tomini Lab Technician since 01/2015
Vera Tröster PhD Student; since 01/2017
Sabrina Wegmann PhD Student; since 11/2013
Hanna Windecker Postdoc; 05/2013–09/2017
Hans-Peter Wollscheid Postdoc; since 09/2014
Ronald Wong Postdoc; since 04/2013
George Yakoub PhD Student; since 01/2015
Nicola Zilio Postdoc; since 08/2014

OVERVIEW

Dealing with DNA damage during genome replication is particularly important because the replication machinery cannot cope with defective templates. In order to avoid a permanent arrest in this situation, cells have developed mechanisms of damage bypass. In contrast to DNA repair systems, which usually rely on the excision and subsequent re-synthesis of the damaged region to restore the original sequence information, DNA damage bypass allows the replication machinery to tolerate lesions without their actual removal. This ensures the completion of DNA replication on damaged templates and thus contributes to the survival of a cell in the presence of genotoxic agents. As lesion bypass is often associated with damage-induced mutations, however, the pathway is also a potential source of genome instability in itself and therefore needs to be tightly controlled. In eukaryotic cells, DNA damage bypass is modulated via ubiquitylation of the essential replication factor PCNA. In order to understand the factors that determine the efficiency and accuracy of damage processing, we have focused on investigating how the pathway is coordinated with global cellular damage signalling.

RESEARCH HIGHLIGHTS

Replication stress as an initiator of a checkpoint response can arise from various sources. Under conditions specifically affecting the replicative DNA polymerases, such as nucleotide depletion or lesions in the template, the checkpoint signal is thought to arise from single-stranded (ss)DNA accumulating at stalled replication forks by an uncoupling between helicase and polymerase movement. However, as an alternative to persistent fork stalling, re-priming of DNA synthesis downstream of a lesion can give rise to daughter-strand gaps behind the replication fork. Indeed, we and others had previously shown that inhibition of the ubiquitin ligase Rad18, a rate-limiting factor for DNA damage bypass in budding yeast, causes accumulation of such structures, but

"As initiators of DNA damage signalling, replication forks are overrated."

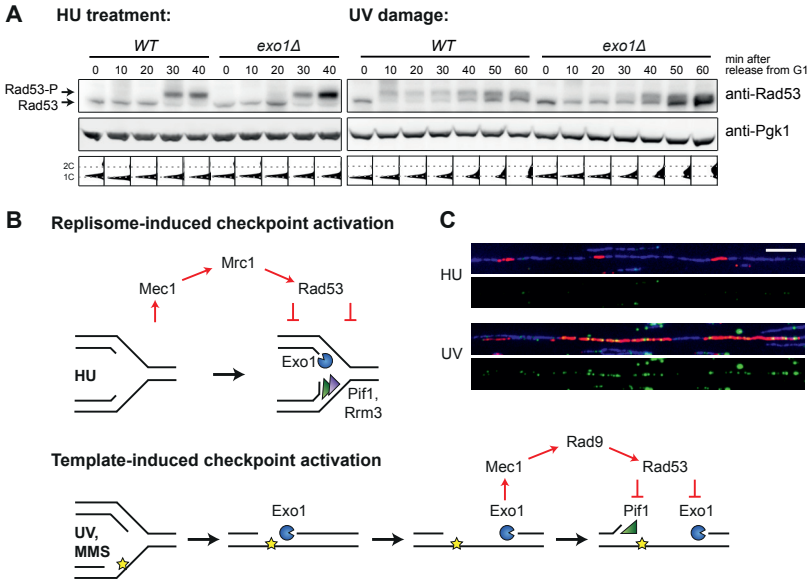

Figure 1. ssDNA triggers checkpoint signalling in two distinct modes. A) Checkpoint activation (measured by Rad53 phosphorylation on western blots) is Exo1-dependent in response to DNA damage (UV: ultraviolet irradiation), but not after nucleotide depletion (HU: hydroxyurea). PGK: loading control. B) Model for fork- versus gap-associated checkpoint activation. C) ssDNA accumulates within EdU-labelled tracts of newly synthesised DNA in response to UV-induced lesions, but not after HU treatment. DNA fibres were stained with YOYO-1 (total DNA, blue), anti-EdU (red) and anti-ssDNA (green). Scale bar: 10 kbp.

re-expression of Rad18 in the G2/M phase allows cells to recover by postreplicative gap filling. We had found that under these conditions Rad53-mediated checkpoint signalling is responsible for preventing premature decay of daughter-strand gaps. As a consequence, checkpoint mutants accumulate irreversible chromosome damage during S phase when Rad18 is inhibited, largely mediated by the excessive action of an exonuclease, Exo1. Thus, inhibition of Exo1 appears to be the predominant function of the checkpoint that stabilises daughter-strand gaps and allows postreplicative damage bypass.

We have now identified an additional, critical role for Exo1 in initiating replication stress signalling. We found that in response to DNA-damaging agents, Exo1 is actually required for mounting a timely checkpoint response in wild-type cells. This is in stark contrast to conditions of nucleotide depletion or polymerase defects, where checkpoint activation is Exo1-independent (Figure 1A). Our data suggest a model whereby polymerase problems give rise to genuine fork uncoupling, which generates sufficient ssDNA to initiate damage signalling at the stalled fork in an Exo1-independent manner, while template problems – even in a wild-type setting – give rise to daughter-strand gaps rather than fork stalling, and a robust checkpoint response requires the expansion of those gaps via Exo1 (Figure 1B). In agreement with this model, we detected significant stretches of ssDNA within tracts of newly replicated DNA upon treatment of cells with DNA-damaging agents, but not after nucleotide depletion (Figure 1C). This spatial separation between Exo1-independent checkpoint signalling at forks by polymerase-inherent problems *versus* Exo1-dependent signalling at gaps in response to template disturbances provides for the first time a satisfying mechanistic explanation for the existence of two distinct mediators of the replication stress response. While the claspin homologue Mrc1 as an integral replisome component is responsible for transmitting the Mec1 (ATR) signal to the effector kinase Rad53 (CHK1) at stalled forks, the 53BP1 homologue Rad9 fulfils an analogous function at daughter-strand gaps in response to genuine DNA damage. The notion that Rad9 is recruited via binding to methylated histone H3 is consistent with its action in a chromatinised environment at some distance from the replication fork.

FUTURE DIRECTIONS

Exo1 has previously been implicated in the widening of postreplicative daughter-strand gaps in preparation for DNA damage bypass by template switching. This notion, combined with our findings concerning its role in checkpoint activation at these structures, raises the question of how the nuclease is recruited to its sites of action during DNA replication. In human cells, Exo1 is known to interact with PCNA in the context of mismatch repair. This suggests that its localisation to daughter-strand gaps could also be mediated by a direct association with PCNA. The interaction involves the C-terminal region of Exo1, which is not conserved between humans and yeast. In ongoing experiments, we are therefore investigating whether the association of budding yeast Exo1 with daughter-strand gaps requires an interaction with PCNA. In addition, we are examining whether the Exo1-dependent mode of replication stress signalling that we observed in budding yeast also applies to human cells. Conservation of the effect in both species would indicate a fundamental contribution of Exo1 to the coordination of DNA replication and the cellular stress response.

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Morawska M and Ulrich HD. (2013). An expanded tool kit for the auxin-inducible degron system in budding yeast. *Yeast*, 30: 341–351.

#indicates co-corresponding authors

EVA WOLF

STRUCTURAL CHRONOBIOLOGY

POSITIONS HELD

Since 2013 Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz

2012–2013 Group Leader, Ludwig Maximilian University, Munich

2009–2011 Group Leader, Max Planck Institute for Biochemistry, Martinsried

2000–2009 Group Leader, Max Planck Institute for Molecular Physiology, Dortmund

1996–2000 Postdoc, Rockefeller University, New York

EDUCATION

2007 Habilitation in Biochemistry, Ruhr University, Bochum

1996 PhD in Biology, European Molecular Biology Laboratory (EMBL), Heidelberg

1991 Diploma in Biology, University of Heidelberg

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Silke Helmke Lab Technician; since 05/2014
Shruti Krishnan PhD student; since 10/2016
Torsten Merbitz-Zahradnik Postdoc; since 01/2014
Roberto Orru Postdoc; since 10/2015
Ruth Schaupp Personal Assistant; since 05/2015

OVERVIEW

Many physiological processes such as sleep-wake cycles, body temperature, blood pressure, hormone production or the immune system underlie circadian regulation. In mammals, circadian clocks are operated via cell-autonomous gene-regulatory feedback loops, where the transcription factors BMAL1/CLOCK activate three period (*per1,2,3*) and two cryptochrome (*cry1,2*) genes as well as many clock controlled genes (*ccgs*) affecting circadian physiology. The CRY1/2 and PER1/2 proteins act as repressors of BMAL1/CLOCK, partly within large multi-subunit repressive complexes. Daily rhythmic acetylation of BMAL1 enhances recruitment of CRY1 to the C-terminal transactivation domain (TAD) of BMAL1 and thereby engenders transcriptional repression. In contrast, binding of the histone acetyltransferase CBP (CREB-binding protein) and its homologue p300 to the BMAL1-TAD leads to transcriptional activation of circadian genes through histone H3K9/K14 acetylation. Alternative and temporally separated interactions of BMAL1/CLOCK with large CRY/PER containing "early" repressive complexes (after sunset), with CRY1 ("late" repressive complex, after sunrise) or CBP (active transcription, during daytime) therefore regulate circadian gene activity in a day/time-dependent manner. To further our mechanistic understanding of the transcriptional and epigenetic regulation of the mammalian circadian clock and of clock controlled genes, we pursue structure-function analyses of clock protein interactions with co-activators or co-repressors of BMAL1/CLOCK.

RESEARCH HIGHLIGHTS

Transcriptional and epigenetic regulation of circadian genes

In mammals, the heterodimeric BMAL1/CLOCK transcription factor complex regulates clock genes (e.g. *per1/2/3, cry1/2*) and many clock controlled genes (*ccgs*) in a day/time-dependent manner. Genome-wide analyses in mouse liver revealed temporally

"We study molecular mechanisms underlying circadian gene regulation."

separated phases of BMAL1/CLOCK dependant circadian gene regulation (Figure 1):

1) A transcriptionally active state between circadian time (CT) 4 and CT12 (CT0 = sunrise, CT12 = sunset), where BMAL1/CLOCK recruits co-activators such as the histone acetyltransferases CBP/p300 and the histone methyltransferase Mixed Lineage Leukemia 1 (MLL1).
2) An early repressive state between CT12 and CT20. Here, BMAL1/CLOCK is repressed by a large CRY/PER containing complex that includes additional proteins such as histone deacetylases (HDAC), NONO and WDR5.
3) A late repressive state in which CRY alone represses BMAL1/CLOCK within an inactive DNA-bound BMAL1/CLOCK/CRY complex (CT0 to CT4, after sunrise)

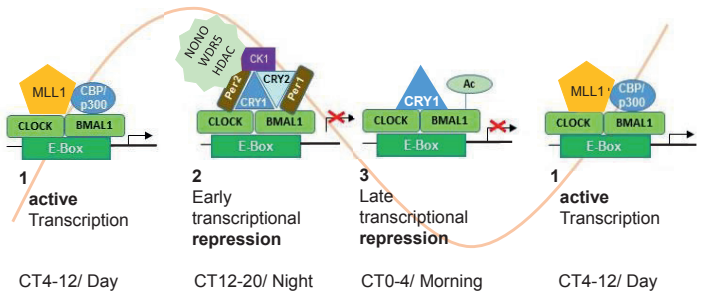

Figure 1. Day-time dependent transcriptional regulation of the mammalian circadian clock in mouse liver. (1) CBP/p300 and MLL1 co-activate BMAL1/CLOCK. (2) BMAL1/CLOCK is repressed by a MDa size CRY/PER containing "early repressive complex", that also includes CK1, NONO, WDR5 and HDACs. (3) Late repressive CRY/BMAL1/CLOCK complex. BMAL1-K537 acetylation enhances CRY1 dependent repression of BMAL1/CLOCK. CT = Circadian time, CT0 = sunrise, CT12 = sunset

The transition from the late repressive state to the transcriptionally active state of BMAL1/CLOCK correlates with the competition of CRY1 and the co-activator p300/CBP for overlapping binding sites in the C-terminal transactivation domain (TAD) of BMAL1. Moreover, the repressive CRY1-BMAL1/CLOCK interaction is enhanced by daily rhythmic BMAL1-K537 acetylation. Our data suggests an allosteric mechanism, whereby K537 acetylation unmasks negatively charged BMAL1 residues for electrostatic interactions with positively charged CRY1 residues. It will be interesting to further characterise the role of BMAL1-K537 acetylation in controlling BMAL1/CLOCK activity within the circadian cycle.

Apart from the clock proteins CRY1/2, PER1/2, casein kinase I (CKI), BMAL1 and CLOCK, several other proteins including e.g. histone deacetylases (HDAC), NONO and WDR5 were identified as components of the MDa multi-subunit early repressive complex of BMAL1/CLOCK. NONO reportedly modulates transcriptional repression of clock genes via PER interactions, is involved in coupling circadian clocks to cell cycle regulation and participates in RNA processing and paraspeckle formation. The WDR5 protein is also known to be part of several other multi-subunit protein complexes such as the MLL1 histone methyltransferase activating complex. HDACs can repress BMAL1/CLOCK regulated circadian genes by reversing activating acetylation events. To dissect the roles of the early repressive complex in circadian gene regulation and chromatin modification, we pursue biochemical, biophysical and 3D-structural analyses of CRY and PER interactions with additional components of the early repressive complex. Combined with our previous structural and biophysical analyses of CRY, PER, the CRY-PER complex and CRY-BMAL1 interactions, these studies will further our understanding of the architecture and assembly of the early repressive complex as well as the mechanisms underlying the transition to the late repressive complex, where only CRY binds to BMAL1/CLOCK.

FUTURE DIRECTIONS

The BMAL1/CLOCK transcription factor complex regulates many clock controlled genes (*ccgs*) that define our daily changing physiology and behaviour. A deeper mechanistic characterisation of the distinct day-time dependant interactions of BMAL1/CLOCK with different transcriptional activators, repressors and histone modifiers is therefore important not only to understand how the cellular circadian oscillator keeps its 24-hour rhythm but also to understand how circadian genes affect our physiology and health. Understanding the daily changing interactions of BMAL1/CLOCK in molecular and (through 3D structural studies) atomic resolution detail will inspire new directions for functional *in vivo* studies and also facilitate the development of drugs targeting circadianly regulated gene activities.

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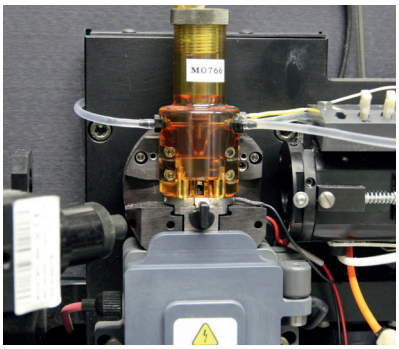
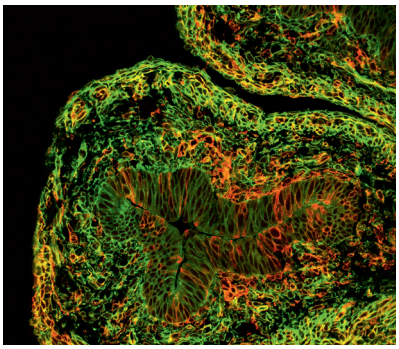
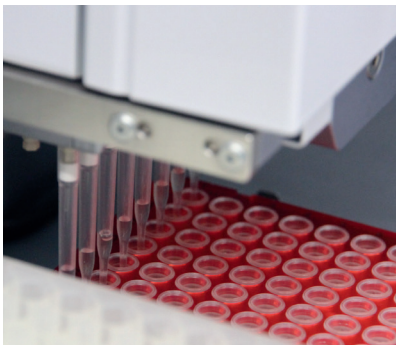


CORE FACILITIES

OVERVIEW

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

In addition, the Core Facilities organise lectures and courses to instruct researchers in new techniques and instrumentation, as well as experimental design and data processing. There are currently seven Core Facilities 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. The CFs are open to all IMB researchers, and the Flow Cytometry, Genomics, Microscopy/Histology and Proteomics CFs also offer services to external users at Mainz University and further afield. The services provided are based on user demand. For each CF, a user committee gives feedback on the equipment and user experience and helps to define the services that a CF provides.

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

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

Andreas Vonderheit
Director of Core Facilities and Technology

BIOINFORMATICS

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

Emil Karaulanov Head
Since 10/2014
Anke Busch Bioinformatician
Since 01/2014
Jan Bockelmann System Administrator
08/2015 – 07/2017
Sri Dewi Student Assistant
10/2016 – 03/2017
Christian Dietrich System Administrator
Since 04/2017
Nastasja Kreim Bioinformatician
Since 04/2012
Giuseppe Petrosino Bioinformatician
Since 03/2017
Sergi Sayols Puig Bioinformatician
Since 10/2013
Pascal Silberhorn System Administrator
Since 12/2015

SERVICES OFFERED

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

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

The BCF maintains a small computer cluster and online services such as Galaxy, R-Studio and OMERO, which provide IMB researchers with a user-friendly interface for various analytical tools. The BCF also offers customised solutions and long-term analytical support for numerous computational projects on a collaborative basis. Additionally, the BCF develops and tests novel quality control methods and customised NGS pipelines for the automation of data processing and analysis. It also offers user training and access to popular software tools and computing resources.

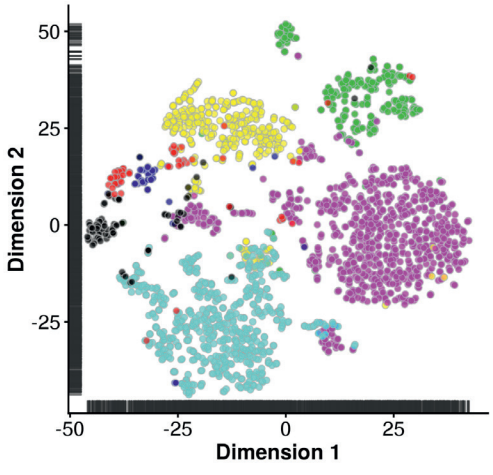


Figure 1. A dimensionality reduction t-SNE plot of a published single-cell RNA-seq dataset (GSE60361) with colours matching different subpopulations of cells in the mouse brain.

FLOW CYTOMETRY

The Flow Cytometry Core Facility (FCCF) offers high-throughput measurements, analysis and separation of biological units through three different systems: a large particle sorter, a cell sorter, and an analyser. With this equipment, the FCCF can analyse and sort particles of 0.5 µm to 1,000 µm in diameter.

Stefanie Bürger Head
Since 10/2016
Ina Schäfer Biotechnologist
Since 08/2011
Noah Weingart Technician
01/2017 – 09/2017

SERVICES OFFERED

The FCCF offers a full service for sorting and an assisted service and training for the analyser. Additionally, its staff collaborates in terms of analysing flow cytometrical 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. Additionally, the FCCF successfully established single cell sorting with subsequent single-cell RNA sequencing and analysis in collaboration with the Genomics and Bioinformatics Core Facilities. The FCCF works with different types of material: nuclei, stem cells, *C. elegans*, 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 including multicolour setups (partly shown in Figure 1) and an advanced practical course for cell sorting.

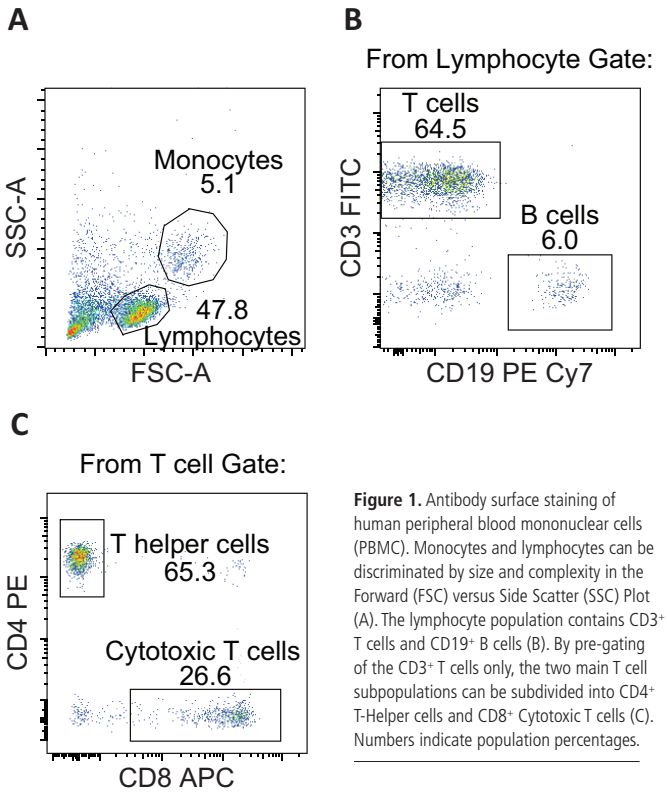


Figure 1. Antibody surface staining of human peripheral blood mononuclear cells (PBMC). Monocytes and lymphocytes can be discriminated by size and complexity in the Forward (FSC) versus Side Scatter (SSC) Plot (A). The lymphocyte population contains $CD3^+$ T cells and $CD19^+$ B cells (B). By pre-gating of the $CD3^+$ T cells only, the two main T cell subpopulations can be subdivided into $CD4^+$ T-Helper cells and $CD8^+$ Cytotoxic T cells (C). Numbers indicate population percentages.

GENOMICS

The Genomics Core Facility (GCF) offers next-generation sequencing (NGS) services based on the Illumina NextSeq500 and MiSeq platforms.

Maria Méndez-Lago Head
Since 04/2016
Hanna Lukas Technician
Since 01/2013
Clara Werner Technician
Since 07/2015

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 (Table 1) and develops new protocols to accommodate user needs for their specific projects. In 2017, this included the successful testing and implementation of the Smart-Seq2 protocol for single-cell RNA-seq, from cells FACS sorted into a 96-well plate, using Formulatrix's Mantis and Agilent's Bravo liquid handlers for library preparation. Single-cell RNA-Seq is now offered as standard protocol at the GCF.

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
- GROseq
- single-cell RNA (Smart-seq2)

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
- NickSeq
- LAM-HGTS

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

MICROSCOPY & HISTOLOGY

The Microscopy and Histology Core Facility (MHCF) provides state-of-the-art microscopes and histology instruments, as well as expertise in sample preparation and data post-processing. Users benefit from a broad range of lectures and hands-on training and can choose from independent, assisted, or full service.

Sandra Ritz Head
Since 01/2016
Mária Hanulová Staff Scientist
Since 02/2014
Jonas Schwirz Staff Scientist
Since 06/2016

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. Three of the set-ups 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 (Imaris, Bitplane), custom-made solutions are developed together with the users e.g. by macro programming in open source software (e.g. Fiji, ImageJ). Super-resolution microscopy is offered on a full service or 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 2017, MHCF established several tissue clearing protocols for the 3D imaging of whole spheroids or small organs, as well as an iodide-based contrast staining of soft-tissues for µCT-applications. MHCF also submitted a DFG proposal for major instrumentations and applied for a spinning disc microscope equipped with an ablation laser for the localised induction of DNA damage and a photomanipulation module (FRAP/TIRF).

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 instruments available. Users may furthermore utilise optimised protocols for immunodetection and solutions for classical tissue stainings (H&E, Masson Goldner Trichrome, PAS, and Azan).

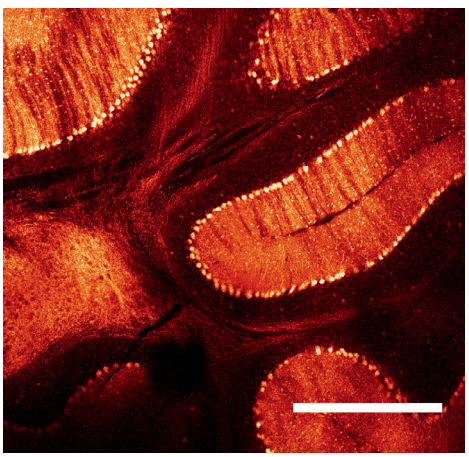


Figure 1. Detail of an adult mouse brain imaged ca. 2 mm inside a chemically cleared brain. Image composed of 35 single images (scale = 1 mm). Sample prepared by Oriane Blanqui (group of Heiko Luhmann, University Medicine Mainz) and imaged by MHCF.

PROTEIN PRODUCTION

The Protein Production Core Facility (PPCF) provides support with the design, expression and purification of recombinant proteins.

Markus Matthes Head
Since 02/2016

SERVICES OFFERED

The PPCF was established in February 2016 as a joint effort between IMB and JGU. The facility maintains *E. coli* and insect cell cultures, which are used for protein expression. It is fully equipped with an Äkta chromatography system for protein purification using different chromatography methods. The PPCF supports researchers throughout the process of protein production. A central part of the service is providing up-to-date information and individual assistance to researchers regarding their protein expression experiments. Services offered by the PPCF include:

- The design of DNA constructs and baculoviruses for cloning
- Assistance with the amplification of high-titer virus stocks for insect cell culture
- Large-scale expression of recombinant proteins
- Protein purification and analysis

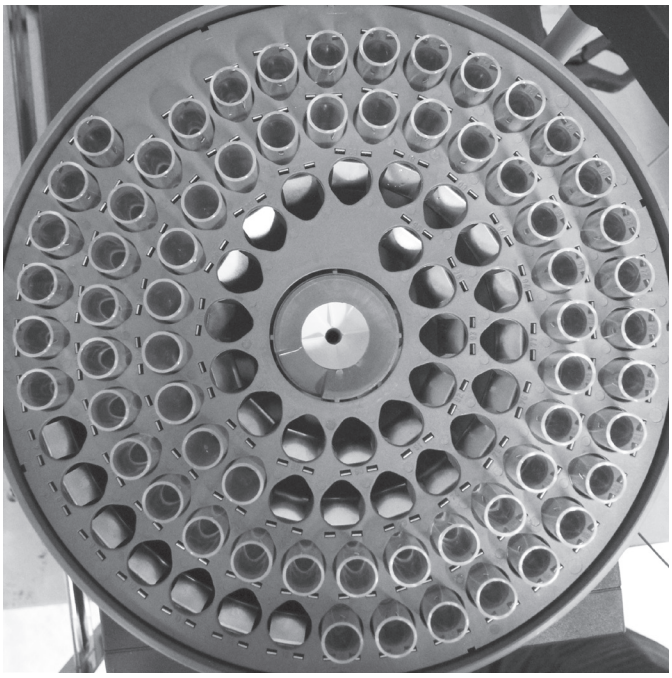


Figure 1. Image of the fraction collection system of an ÄKTAprime fast protein liquid chromatography system.

PROTEOMICS

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

Falk Butter Head
Since 05/2013
Jasmin Cartano Technician
Since 02/2014
Mario Dejung Bioinformatician
Since 05/2014
Anja Freiwald Engineer
Since 04/2013

SERVICES OFFERED

The PCF provides 1,500 measurement hours annually to IMB and the surrounding research centres in Mainz using a state-of-the-art mass spectrometry platform. Current services include band identification, analysis of posttranslational modifications on single proteins and measurement of SILAC (stable isotope labelling with amino acids in cell culture) experiments. Additionally, the PCF offers reductive dimethylation and for large-scale quantitation tandem mass tagging (TMT). TMT can be applied for quantifying up to 10 samples in parallel. 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.

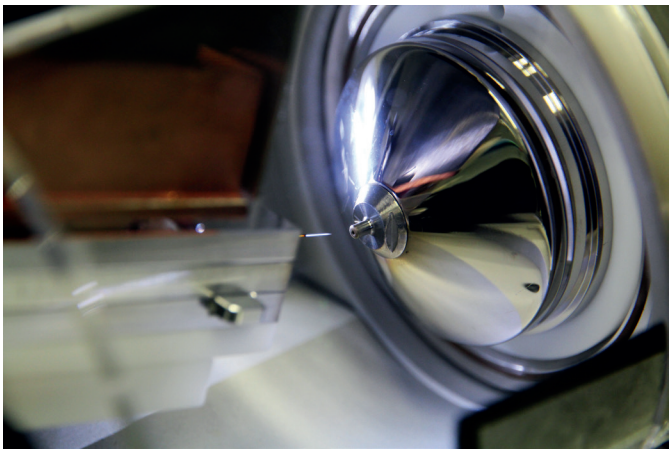


Figure 1. Electrospray ionisation (ESI) is used to ionize peptides for mass spectrometry measurement. The peptides are separated on a chromatography column and sprayed directly into the mass spectrometer.

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 two supply centres, plasmid/cell line banks, general waste management and sterilisation of glassware.

Andreas Vonderheit Head
Since 05/2016
Doris Beckhaus Media Lab Assistant
Since 05/2011
Alwina Eirich Media Lab Assistant
Since 07/2013
Andrea Haese-Corbit Operations Manager
Since 05/2016
Pascal Hagebölling Media Lab Assistant
Since 01/2015
Annette Holstein Media Lab Assistant
Since 04/2012
Marion Kay Media Lab Assistant
Since 04/2016
Johann Suss Media Lab Assistant
Since 04/2011

SERVICES OFFERED

The Media Lab provides the following services:

- 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*
- Management of a central supply for New England Biolabs and Thermo Fisher Scientific products
- Administration of a vector data bank, human ORF clone collection and cell line bank
- Overnight cultures for plasmid preparation
- Sterilisation of solutions/equipment
- Cleaning and sterilisation of glassware and lab equipment
- Autoclaving of S1/S2 waste
- Maintenance of in-house transport system

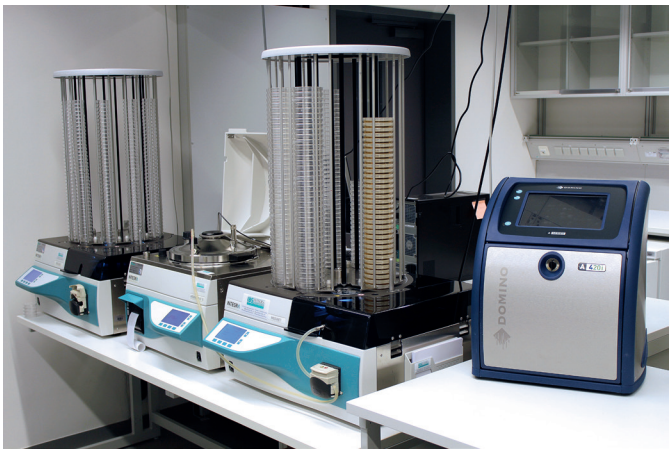
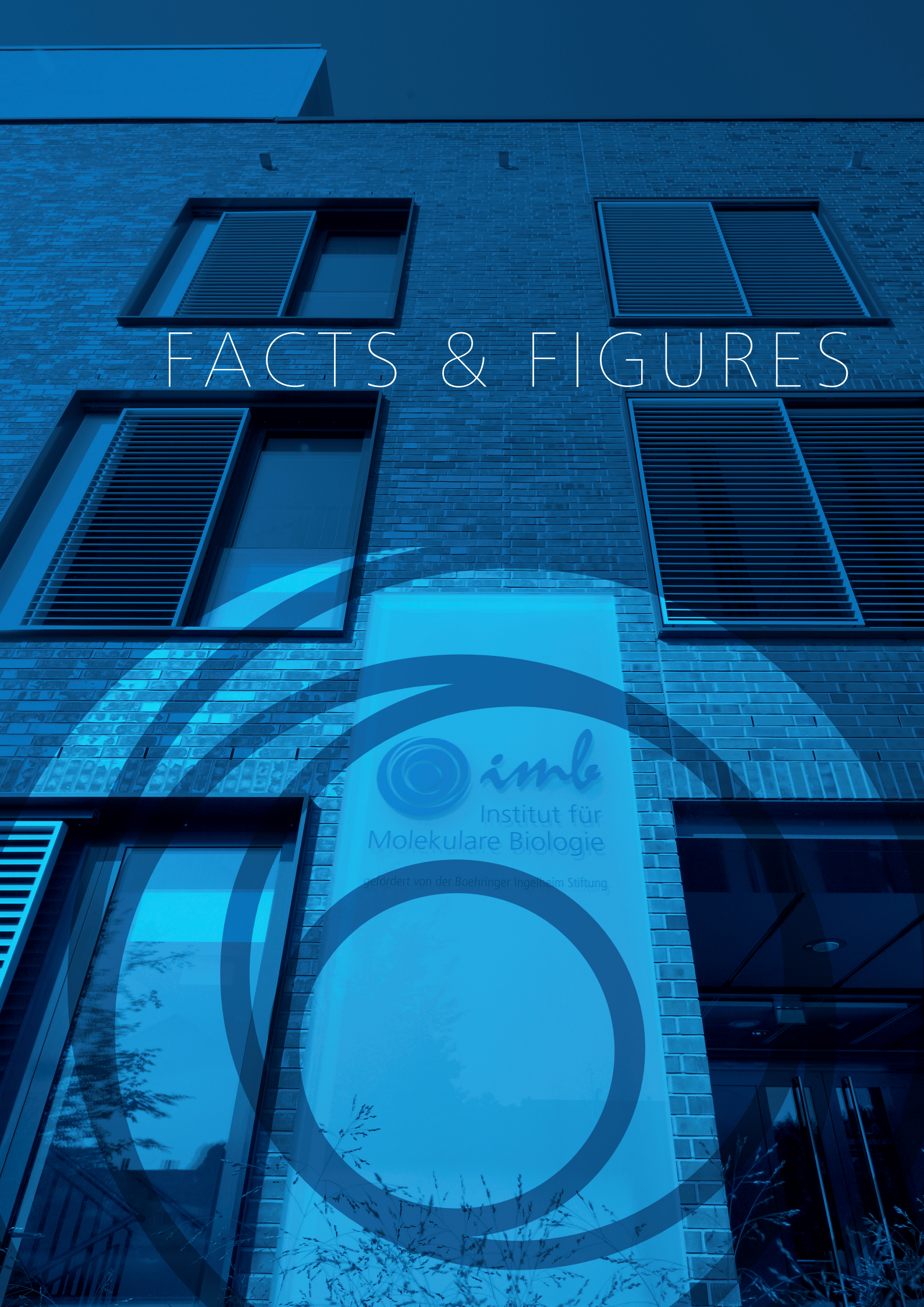


Figure 1. The media lab produces more than 1,700 plates per week and produces Petri dishes of different kinds for different experimental setups.



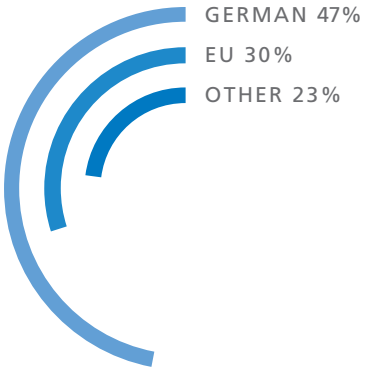
FACTS & FIGURES

STAFF

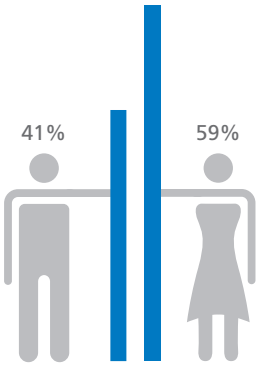
EMPLOYEES BY STAFF CATEGORY

GROUP LEADERS	8%	
TECHNICAL STAFF	26%	
ADMINISTRATION & SCIENTIFIC MANAGEMENT	11%	
PHD STUDENTS	38%	
POSTDOCS	17%	

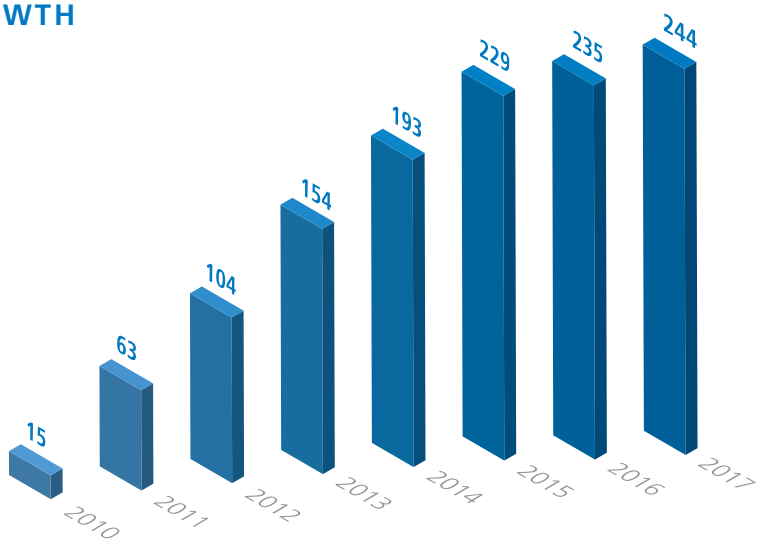
NATIONALITIES OF SCIENTIFIC STAFF



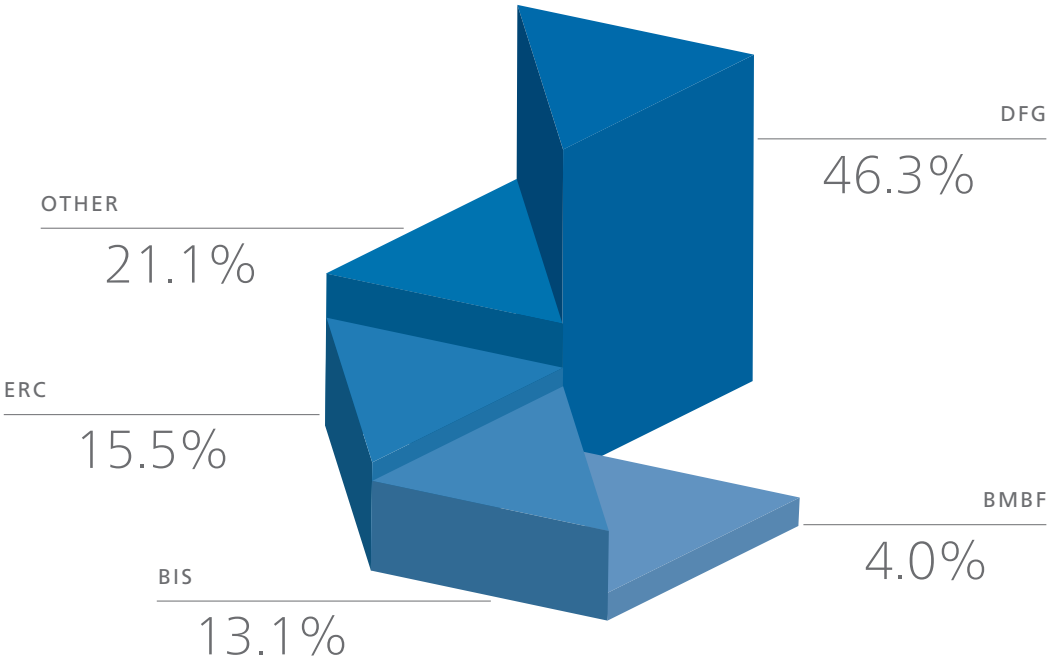
GENDER DISTRIBUTION



STAFF **GROWTH**



EXTRAMURAL FUNDS



MAJOR FUNDERS

Boehringer Ingelheim Foundation (BIS)

German Research Foundation (DFG)

EUROPEAN COMMISSION (EC)

European Research Council (ERC)

Marie Curie Actions

FURTHER SUPPORT

Alexander von Humboldt Foundation

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 Stiftung

National Research Foundation of Korea

Naturwissenschaftlich-Medizinisches Forschungszentrum (NMFZ)

Wilhelm Sander-Stiftung

RESEARCH AND TRAINING

The scientists at IMB carry out research in epigenetics, developmental biology, genome stability and at the interfaces between these fields. In 2017, the international community of researchers at IMB included 89 PhD students, 45 postdocs and 16 group leaders, from over 25 different countries.

IMB's scientists come from a range of backgrounds, including biochemistry and genetics, as well as molecular, cell and developmental biology. They study, for example, the molecular mechanisms of embryonic development, evolution, ageing or diseases. In addition, our bioinformaticians and systems biologists analyse high-throughput datasets and model regulatory gene networks, and applied physicists develop new super-resolution microscopes. This variety of expertise and the interactive atmosphere at IMB encourages collaborations and innovative research.

IMB ensures that its scientists can work productively. A key part of the support offered comes through the Core Facilities. They provide services in bioinformatics, flow cytometry, genomics, microscopy and histology, protein production and proteomics. Each facility is staffed by experts to advise and assist scientists during every step of their experiments, from the initial conception to the analysis of data. As part of the collaborative spirit at IMB, all key equipment is shared between research groups and looked after by staff in the Core Facilities. This means our scientists have access to up-to-date and well-maintained equipment that is required for their experiments. In addition to the training available through our Core Facilities, IMB offers training through scientific events and theoretical, as well as hands on instruction in technical and methodological skills. Moreover, junior scientists at IMB learn transferable skills required for a successful career both within and outside of academia. Courses offered cover topics such as presentation skills, scientific writing and project management, as well as fundraising. IMB also organises an annual Life Sciences Career Day.

INTERNATIONAL PHD PROGRAMME

PhD students are a key part of our research teams at IMB. Our International PhD Programme (IPP) on Gene Regulation, Epigenetics and Genome Stability, supported by the Boehringer Ingelheim Foundation, gives talented and enthusiastic students the opportunity to undertake PhD research at the cutting-edge of modern biology. Projects within the IPP address key questions of fundamental biological importance through basic research. IPP's unique profile and expertise provides students with an interdisciplinary education in biochemistry, genetics, evolution, cell and developmental biology, bioinformatics and systems biology. The fields we study range from embryonic development, evolution, ageing and disease to RNA and chromatin biology, the analysis of high-throughput datasets in genomics and proteomics and modelling of regulatory gene networks. In addition, students receive state-of-the-art scientific and transferable skills training to support their personal development as a scientist.

The IPP is coordinated by IMB and participating groups are located in Mainz at the:

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

www.imb.de/PhD

POSTDOC PROGRAMME

The IMB Postdoc Programme has been established to meet the specific needs of postdocs, and to ensure that they are able to build the strongest possible foundation for success in their future careers. The programme ensures sound scientific training through a variety of lectures, workshops and events available at IMB, and offers postdocs full support with raising funds for their research. IMB also recognises the need for career development. In addition to the guidance given by Group Leaders, who provide postdocs with day-to-day scientific and career advice, the Postdoc Programme also offers mentoring discussions with IMB's Scientific Directors and invited speakers, Career Days, and preparation for applications and interviews. To succeed in today's competitive job market, postdocs must have excellent presentation, writing, project management and time management skills. As such, IMB provides courses and lectures on these elements. The programme also organises talks by representatives from local scientific companies, and sets up company site visits and job shadowing initiatives, so that IMB postdocs have the opportunity to learn more about a range of future career opportunities.

www.imb.de/postdocs

INTERNATIONAL SUMMER SCHOOL

IMB's International Summer School (ISS) is a six-week programme for outstanding and enthusiastic undergraduate, masters and PhD students who want to acquire excellent practical skills and hands-on training from leading scientists in molecular biology. Research groups participating in the ISS include Group Leaders at IMB, Johannes Gutenberg University and Mainz's University Medical Centre. The ISS offers an attractive framework for training prospective scientists in an informal and international atmosphere. This includes theory modules (lectures and discussion groups) and practical research projects. The lectures give students comprehensive insights into the latest research findings and identify key open questions in gene regulation, epigenetics and genome stability. Furthermore, the ISS teaches students the complementary skills, such as presentation and communication techniques, that are required for a successful career as a scientist. Beyond these specific events, ISS participants are also fully integrated into scientific life at IMB by participating in lab meetings and journal clubs. Furthermore, each student works on a cutting-edge research project within the lab of one of the participating research groups.

www.imb.de/ISS

TRAINING COURSES

In 2017, IMB offered the following training courses in scientific and transferable skills

CORE FACILITIES TRAINING

LECTURES

CORE FACILITY	DATES	TITLE
GENERAL CORE FACILITIES	18 Apr	Introduction to Molecular & Biochemistry Techniques
	11 Jul	Nuclear Magnetic Resonance (NMR)
BIOINFORMATICS	23 May	Databases in Bioinformatics
	30 May	Design & Analysis of NGS Experiments
FLOW CYTOMETRY	25 Apr	Advanced Flow Cytometry: Principles of Cell Sorting
	27 Jun	Flow Cytometry
	17 Oct	Flow Cytometry: Introduction I
	24 Oct	Flow Cytometry: Introduction II
GENOMICS	16 May	Genomics (NGS)
MICROSCOPY & HISTOLOGY	25 Apr	Introduction to Microscopy
	02 May	Microscopy: F-Techniques & Super-Resolution
	09 May	Histology & Fluorescent Labeling
	20 Jun	Electron Microscopy
	04 Jul	Image Processing
	28 Nov	Microscopy: Ethics in Image Acquisition & Processing
PROTEOMICS	13 Jun	Proteomics
PROTEIN PRODUCTION	06 Jun	Protein Production & Crystallography

PRACTICAL COURSES

CORE FACILITY	DATE	TITLE
BIOINFORMATICS	05 Apr	Bioinformatics: Introduction to R (Part I)
	12 Apr	Bioinformatics: Introduction to R (Part II)
	19 Apr	Bioinformatics: Introduction to R (Part III)
	26 Apr	Bioinformatics: Introduction to R (Part IV)
	10 May	Bioinformatics: ChIP-seq & RNA-seq Analysis with R (Part I)
	17 May	Bioinformatics: ChIP-seq & RNA-seq Analysis with R (Part II)
	28 – 29 Jun	Bioinformatics: ChIP-seq & RNA-seq Analysis with GALAXY
FLOW CYTOMETRY	03 – 05 May	Advanced Flow Cytometry: Principles of Cell Sorting
	13 – 21 Nov	Flow Cytometry: Practical Course
MICROSCOPY & HISTOLOGY	13 – 16 Mar	Microscopy: Image Processing & Analysis Course
	10 – 12 Jul	Histology Crash Course
PROTEOMICS	06 – 08 Mar	Proteomics: Practical Course



SCIENTIFIC & TRANSFERABLE SKILLS TRAINING

LECTURES

DATES	TITLE
25 Oct 2016 – 07 Feb 2017	Lecture Series: Introduction to Epigenetics
14 Mar	Good Scientific Practice
14 - 25 Aug	Block Lecture Weeks of IMB's International PhD Programme

PRACTICAL COURSES

DATE	TITLE
09 – 10 Jan	Career Planning & Job Application Training
16 – 17 Jan	Grant Writing
25 – 26 Jan	How to Design Scientific Figures
26 Jan	Proposal Writing
31 Jan – 01 Feb	Scientific Writing
06 – 07 Feb	Project Management for Postdocs
13 Feb	Proposal Writing
21 – 22 Feb	Time Management
06 – 07 Mar	Project Management with Certification 1a
08 – 09 Mar	Biostatistics
13 – 14 Mar	Project Management with Certification 1b
13 – 14 Mar	Leadership Skills
20 – 21 Mar	Professional Communication
20 – 21 Mar	Project Management with Certification 2a+b

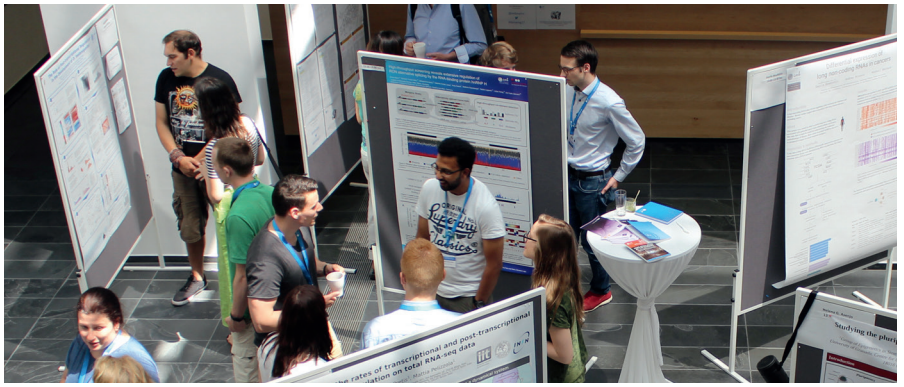
DATE	TITLE
22 – 23 Mar	Convincing Scientific Presentations
27 – 28 Mar	Presenting Scientific Data
30 Mar	Intellectual Property Rights
30 – 31 Mar	Conflict Management
20 + 27 Apr	How to Create One's Job
13 – 14 Jun	Introduction to Biostatistics
25 Aug	Time & Self-Management
04 – 05 Sep	Writing for the Public
06 – 07 Sep	Convincing Scientific Presentations
18 – 19 Oct	Professional Communication
22 – 23 Nov	Time & Self-Management
28 – 29 Nov	Scientific Writing
11 Dec	Proposal Writing

GRANTS OFFICE TRAINING

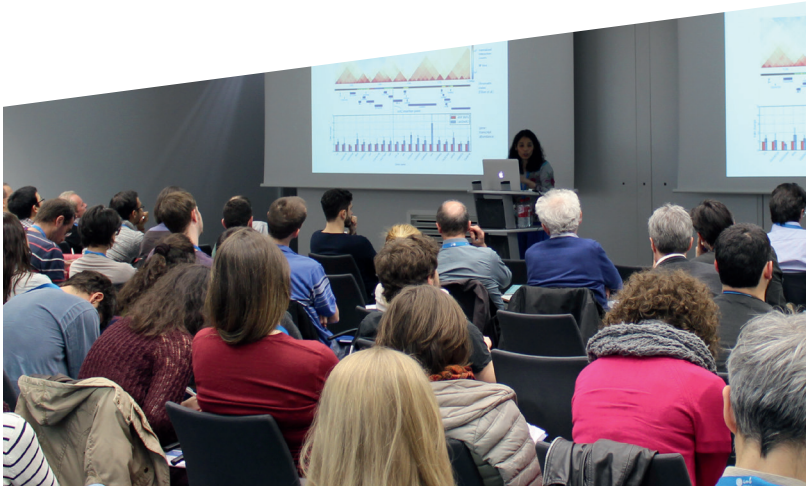
DATE	TITLE
16 – 17 Jan	Grants Writing Course for PhD Students
11 Sep	HORIZON 2020: ERC* (informational event about ERC funding schemes)

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

INVITED SPEAKERS 2017



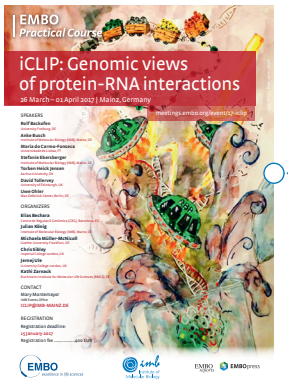
DATE	EVENT	SPEAKER	INSTITUTION	TITLE
19 Jan	Seminar	**KARL-PETER HOPFNER**	Ludwig Maximilian University (LMU), Munich	Structural mechanisms of DNA double-strand break repair
02 Feb	Seminar	**ANJA GROTH**	University of Copenhagen	Chromatin replication and epigenome maintenance
09 Feb	Seminar	**TONY KOUZARIDES**	University of Cambridge	Function of RNA modifications and their role in cancer
09 Mar	Seminar	**SVEN ROTTENBERG**	University of Bern	PARP inhibitor resistance in BRCA1/2-mutated mouse mammary tumors
04 Apr	TechTalk	**ANDREAS MEYER**	Dharmacon	A workflow for CRISPR-Cas9 high throughput arrayed screening with synthetic crRNA
06 Apr	Seminar	**EVI SOUTOGLOU**	Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch	Nuclear organisation of genome maintenance
11 Apr	TechTalk	**MAIK PRÜSS**	Nanostring	3D Biology - simultaneous quantification of proteins, SNVs and gene expression in one experiment
27 Apr	Seminar	**ANDREAS LADURNER**	Ludwig Maximilian University (LMU), Munich	Cellular ADP-ribosylation and nuclear dynamics
02 May	Seminar	**KENNETH ZARET**	University of Pennsylvania, Philadelphia	Overcoming chromatin barriers to control cell fate
02 May	TechTalk	**JÜRGEN COX**	Max Planck Institute for Biochemistry, Martinsried	Computational biology for large-scale omics data analysis
04 May	Seminar	**MARKUS LANDTHALER**	Max Delbrück Center for Molecular Medicine (MDC), Berlin	Exploring protein-mRNA interactomes
11 May	Seminar	**MICHIEL VERMEULEN**	Radboud Institute for Molecular Life Sciences, Nijmegen	Deciphering chromatin biology using integrative omics approaches



DATE	EVENT	SPEAKER	INSTITUTION	TITLE
18 May	Seminar	**PABLO HUERTAS SÁNCHEZ**	Andalusian Molecular Biology and Regenerative Medicine Centre (CABIMER), Seville	Controlling homologous recombination
06 Jun	TechTalk	**THORSTEN LEMKER**	10X Genomics	A transformative upgrade to short-read sequencers that fits easily into existing lab infrastructures
09 Jun	Seminar	**WIELAND HUTTNER**	Max Planck Institute of Molecular Cell Biology and Genetics, Dredsen	Human-specific gene ARHGAP11B, neural stem and progenitor cells, and neocortex expansion in development and evolution
30 Jun	Seminar	**ANDRE NUSSENZWEIG**	National Institutes of Health (NIH), Bethesda	Chromosome organisation drives genome instability
19 Oct	Seminar	**RALF JUNGMANN**	Max Planck Institute of Biochemistry, Martinsried	Super-resolution microscopy with DNA molecules
26 Oct	Seminar	**CARL PETERSEN**	Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne	Neural circuits for goal-directed sensorimotor transformation
07 Nov	Seminar	**JOSEF JIRICNY**	Institute of Biochemistry (ETH), Zurich	FAN1 - nuclease with a phenotype but without a function?
21 Nov	Seminar	**FYODOR KONDRASHOV**	Institute of Science and Technology Austria (IST Austria), Vienna	Experimental characterisation of the genotype to phenotype connection on a macroevolutionary scale
05 Dec	Seminar	**TANJA WEIL**	Max Planck Institute for Polymer Research, Mainz	Polymer chemistry meets biology - new concepts in drug delivery, bioimaging and sensing
07 Dec	Seminar	**THOMAS CARELL**	Ludwig Maximilian University (LMU), Munich	DNA bases beyond Watson and Crick
14 Dec	Seminar	**CEDRIC NOTREDAME**	Centre for Genomic Regulation (CRG), Barcelona	Large scale *in-silico* biology: costly, non-reproducible and complicated. Why bother?

SELECTED SCIENTIFIC EVENTS

ORGANISED BY IMB



23 March 2017

CI3 TECHNOLOGY WORKSHOP

Scientific organiser: Ralf Dahm

24 March – 01 April 2017

LIFE SCIENCES CAREER DAY

Organiser: Mary Montemayor

26 March 2017

iCLIP: GENOMIC VIEWS OF PROTEIN-RNA INTERACTIONS

Scientific organisers: Elias Bechara, Julian König, Michaela Müller-McNicoll, Chris Sibley, Jernej Ule, Kathi Zamack

08 May 2017

SYMPOSIUM ON TRANSLATIONAL EPIGENETICS

Scientific organisers: Andreas Diefenbach, Peter Galle, Vijay Tiwari

19 – 21 June 2017

GENE REGULATION BY THE NUMBERS: QUANTITATIVE APPROACHES TO STUDY TRANSCRIPTION

Scientific organisers: Julian König, Stefan Legewie, Bertil Schmidt, Sarah Teichmann, Vijay Tiwari

17 – 21 July 2017

INTERNATIONAL BIOLOGY OLYMPIAD: TRAINING CAMP

Scientific organiser: Falk Butter

04 – 06 September 2017

1ST SYMPOSIUM ON NUCLEIC ADIC MODIFICATIONS

Scientific organisers: Thomas Carell, Michaela Frye, Mark Helm, Jean-Yves Roignant

22 – 24 November 2017

SPP1935 – DECIPHERING THE MRNP CODE: RNA-BOUND DETERMINANTS OF POST-TRANSCRIPTIONAL GENE REGULATION

Scientific organiser: Utz Fischer

29 – 30 November 2017

MAINSTREAM: BREMSE ODER BASIS DES NEUEN?

Scientific organiser: Christof Niehrs

PUBLICATIONS 2017

MIGUEL ANDRADE

Alanis-Lobato G, Andrade-Navarro MA and Schaefer MH. (2017). HIPPIE v2.0: Enhancing meaningfulness and reliability of protein-protein interaction networks. *Nucleic Acids Res*, 45: D408–D414.

Cerdá-Esteban N, Naumann H, Ruzittu S, Mah N, Pongrac IM, Cozzitorto C, Hommel A, Andrade-Navarro MA, Bonifacio E and Spagnoli FM. (2017). Stepwise reprogramming of liver cells to a pancreas progenitor state by the transcriptional regulator Tgif2. *Nat Commun*, 8: 14127.

Corwin T, Woodsmith J, Apelt F, Fontaine JF, Meierhofer D, Helmuth J, Grossmann A, Andrade-Navarro MA, Ballif BA and Stelzl U. (2017). Defining human tyrosine kinase phosphorylation networks using yeast as an *in vivo* model substrate. *Cell Syst*, 5: 128–139.

Hildebrandt A, Alanis-Lobato G, Zarnack K, Andrade-Navarro MA, Beli P and König J. (2017). The interaction profile of RNA-binding ubiquitin ligases reveals a link between posttranscriptional regulation and the ubiquitin system. *Sci Rep,* 7: 16582.

Ibn-Salem J, Muro EM and Andrade-Navarro MA. (2017). Co-regulation of paralog genes in the three-dimensional chromatin architecture. *Nucleic Acids Res*, 45: 81–91.

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Paladin L, Hirsh L, Piovesan D, Andrade-Navarro MA, Kajava AV and Tosatto SCE. (2017). RepeatsDB 2.0: Improved annotation, classification, search and visualization of repeat protein structures. *Nucleic Acids Res*, 45: 3613.

Totzeck F, Andrade-Navarro MA and Mier P. (2017). The Protein structure context of PolyQ regions. *PLoS One*, 12: e0170801.

Zepeda-Mendoza CJ, Ibn-Salem J, Kammin T, Harris DJ, Rita D, Gripp KW, MacKenzie JJ, Gropman A, Graham B, Shaheen R, Alkuraya FS, Brasington CK, Spence EJ, Masser-Frye D, Bird LM, Spiegel E, Sparkes RL, Ordulu Z, Talkowski ME, Andrade-Navarro MA, Robinson PN and Morton CC. (2017). Computational prediction of position effects of apparently balanced human chromosomal rearrangements. *Am J Hum Genet*, 101: 206–217.

PETRA BELI

Borisova ME, Wagner SA and Beli P. (2017). Mass spectrometry-based proteomics for quantifying DNA damage-induced phosphorylation. *Methods Mol Biol*, 1599: 215–227.

Diamanti MA, Gupta J, Bennecke M, De Oliveira T, Ramakrishnan M, Braczynski AK, Richter B, Beli P, Hu Y, Saleh M, Mittelbronn M, Dikic I and Greten FR. (2017). IKKα controls ATG16L1 degradation to prevent ER stress during inflammation. *J Exp Med*, 214: 423–437.

Hildebrandt A, Alanis-Lobato G, Zarnack K, Andrade-Navarro MA, Beli P and König J. (2017). The interaction profile of RNA-binding ubiquitin ligases reveals a link between posttranscriptional regulation and the ubiquitin system. *Sci Rep,* 7: 16582.

Mahendrarajah N, Borisova ME, Reichardt S, Godmann M, Sellmer A, Mahboobi S, Haitel A, Schmid K, Kenner L, Heinzel T, Beli P and Krämer OH. (2017). HSP90 is necessary for the ACK1-dependent phosphorylation of STAT1 and STAT3. *Cell Signal*, 39: 9–17.

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FALK BUTTER

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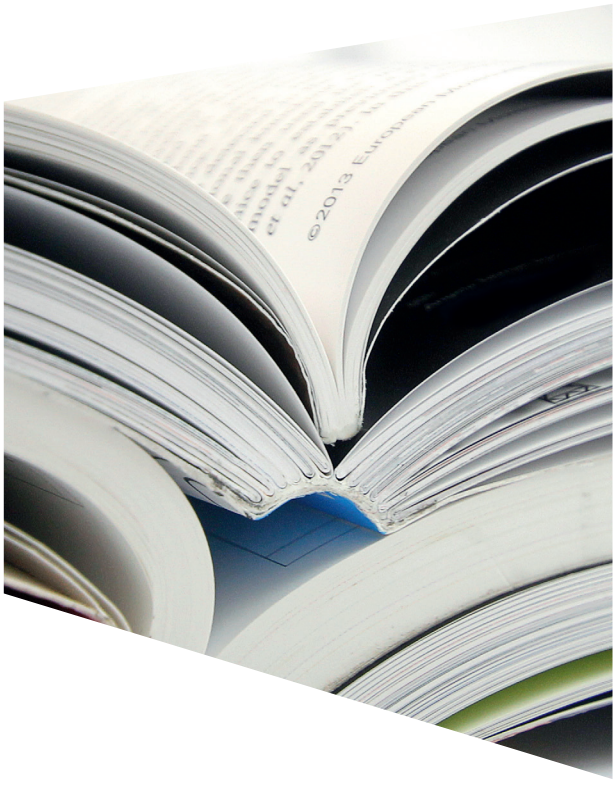

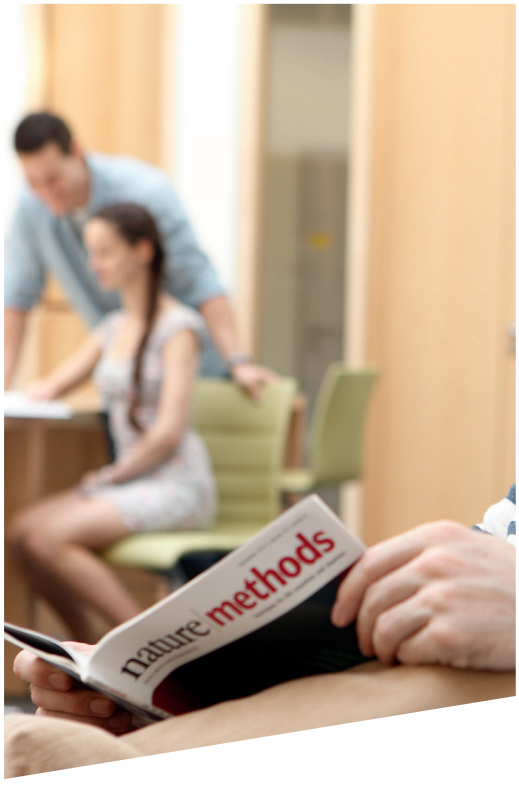

RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located within the leafy campus of the Johannes Gutenberg University, just west of Mainz city centre. With 10 departments, 150 institutes and 33,000 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.

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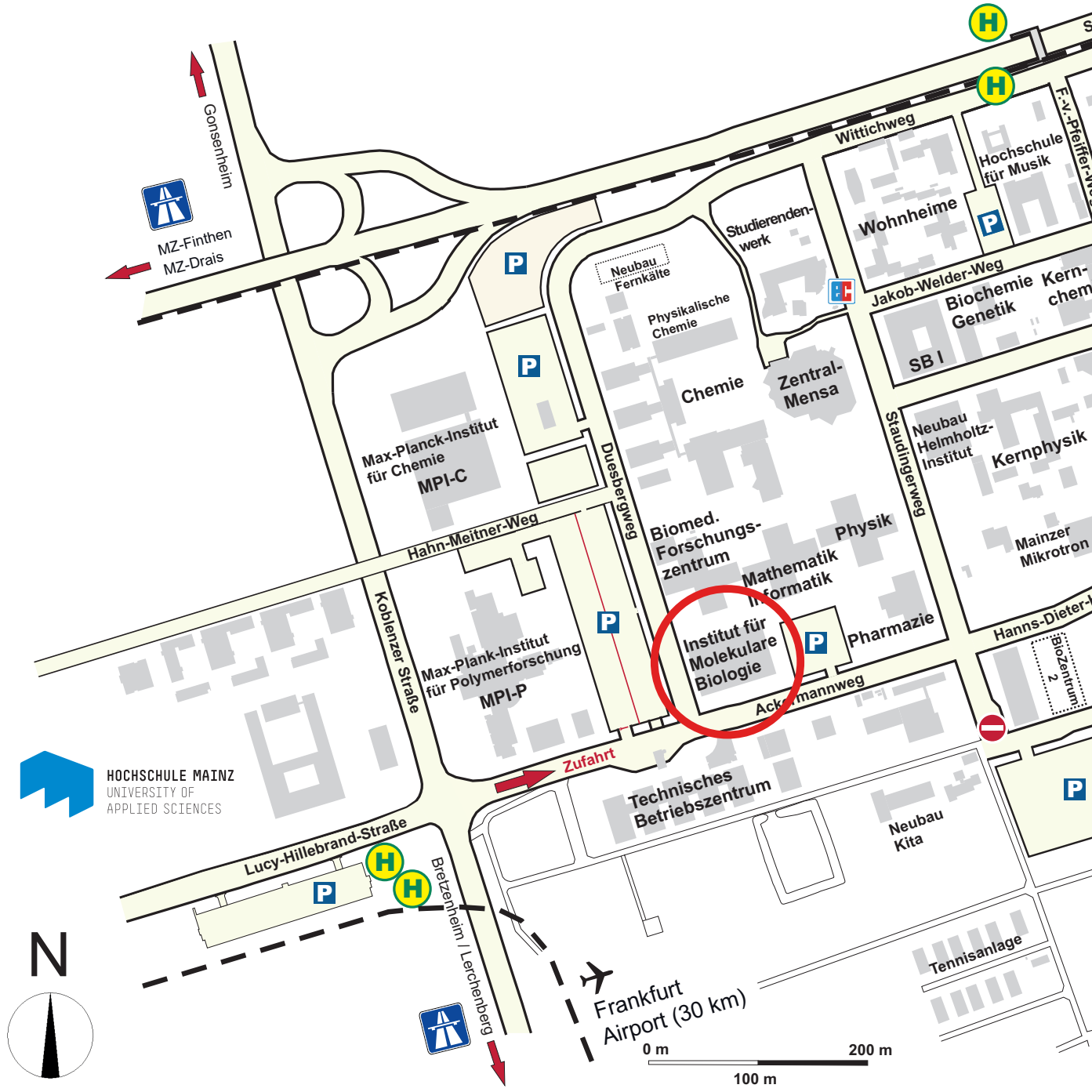

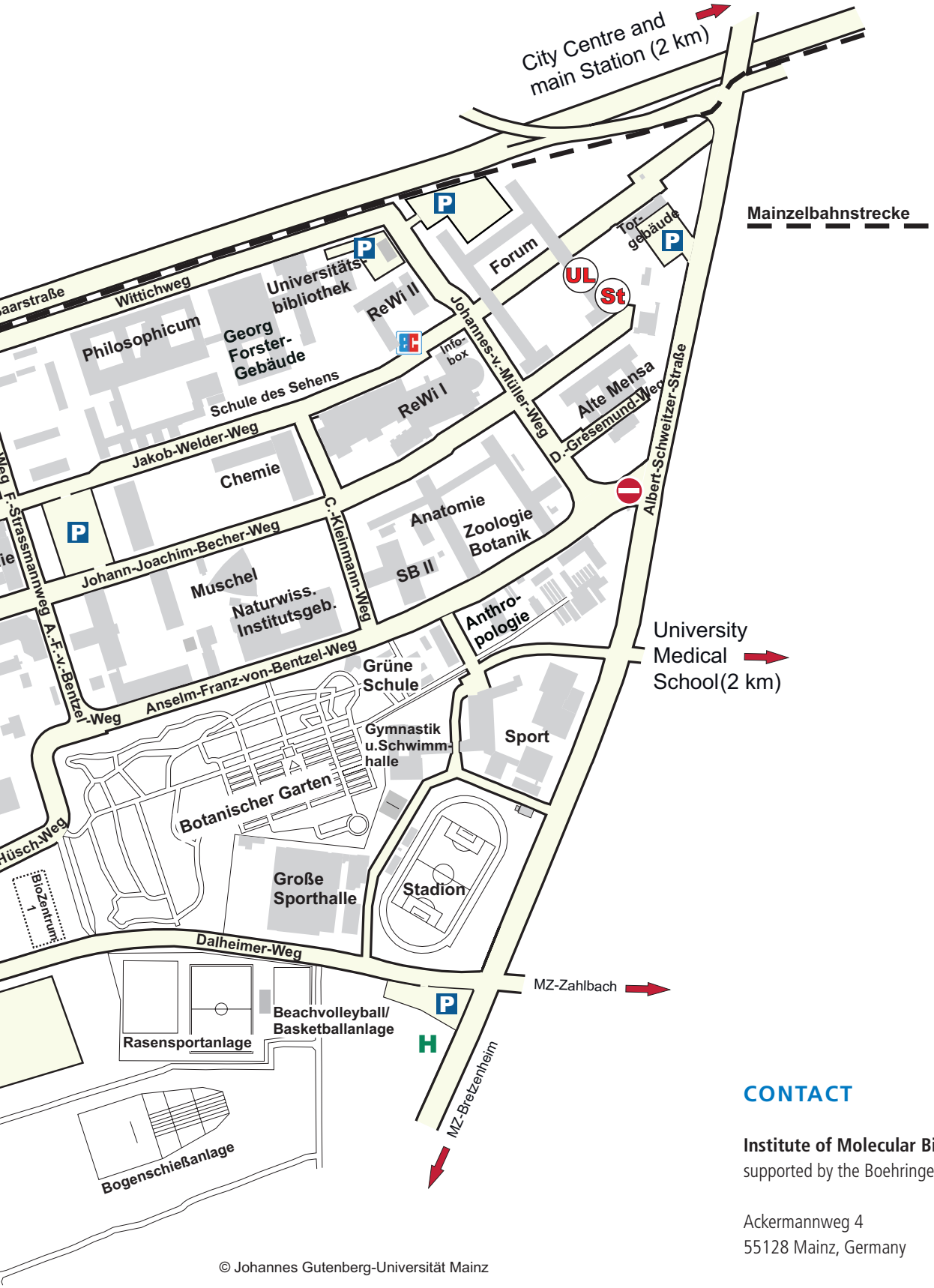

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