



**ANNUAL
REPORT
2019**





ANNUAL REPORT 2019

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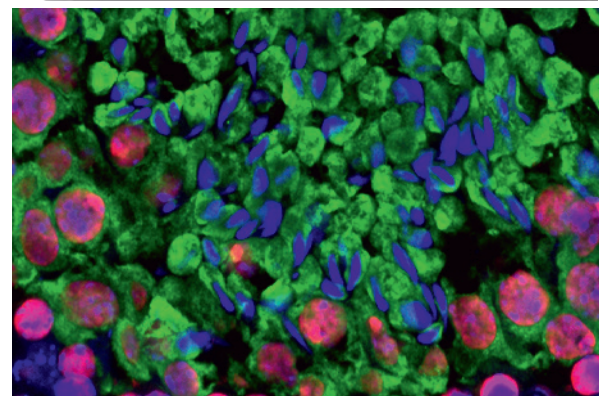
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FOREWORD

2019 was an excellent year for IMB as we continued to make our mark as a leading international research centre in the life sciences. In a first for IMB, we are now a driver of two exceptional research initiatives, a Collaborative Research Center (CRC) and a Research Training Group (RTG), both of which were launched in 2019.

The CRC “DNA Repair & Genome Stability” is funded by the German Research Foundation (DFG) with €12.4 million in its 1st funding period, which will run for 4 years until December 2022. The initiative was initiated and is led by IMB and focuses on understanding the molecular mechanisms modulating the activities of genome maintenance pathways in the cell. It features 18 projects from investigators across 6 institutions: IMB, Johannes Gutenberg University Mainz (JGU), University Medical Center Mainz, University of Technology Darmstadt, Ludwig Maximilian University Munich, and Goethe University Frankfurt. It brings together experts in structural biology, organic chemistry, biochemistry, cell & molecular biology, and genetic toxicology. This interdisciplinary alliance will firmly establish Mainz as a leading research hub in DNA repair and genome maintenance for the foreseeable future. The initiative is supported by 3 dedicated service projects, centralised management, and an Integrated Research Training Group (IRTG). The IRTG will ensure that students participating in projects within the programme receive the best possible training and career development while completing their PhDs.

IMB, in partnership with the Faculty of Biology of JGU, is now also co-leading the RTG “GenEvo – Gene Regulation in Evolution: From Molecular to Extended Phenotypes”. The DFG announced €5.8 million in funding for the 1st period of 4.5 years for this programme, which launched in July. The initiative provides PhD students with the structured, high-calibre research and training programme necessary to acquire interdisciplinary qualifications and obtain autonomy at an early stage of their careers. The research within GenEvo aims at a better understanding of the evolution of complex and multi-layered genetic regulation systems and brings together researchers from the fields of evolutionary and molecular biology. The 14 individual projects link across fields which are seldomly combined, such as evolutionary biology, epigenetics, gene regulation, omics technologies, and bioinformatics. The programme, headed by Susanne Foitzik (JGU) and René Ketting (IMB), will further promote both IMB and Mainz as one of the most attractive locations for life sciences research internationally.



IMB continued its tradition of hosting an annual conference on emerging themes in biology. This year, the focus of our conference “Chromosome Territories & Nuclear Architecture” was chosen in honour of Christoph Cremer, a pioneer of super-resolution microscopy, who has been with IMB since its founding and will retire at the end of the year, aged 75. The conference was a resounding success with 21 invited speakers, 178 participants from 20 countries, and a packed public lecture by Christoph on the history of super-resolution microscopy. This year, we also kept up our tradition of involving the wider public in scientific discussions. We held a combined public event and scientific workshop on the CRISPR/Cas method and its implications for human health. This event was very well attended, with 120 guests arriving at IMB from a variety of disciplines.

There were several successes for IMB’s Group Leaders in 2019. Peter Baumann, who is a Humboldt Professor at JGU and an Adjunct Director at IMB, was elected as an EMBO Member. This prestigious award is thoroughly deserved and reflects the exceptional research on telomere biology that Peter is performing. Once again, our talented Group Leaders demonstrated the quality of their science and ideas through their publications and grant funding. In total, IMB Group Leaders raised over €2.3 million in funding for the next 3 years beyond that included in the two research initiatives mentioned above. On the publications side, there were 65 IMB publications this year. Highlights include papers from the Lemke group in *Science*, the Niehrs group in *Nature Genetics*, the Beli group in *Blood*, and from the Roukos and Ulrich groups in *Molecular Cell*.

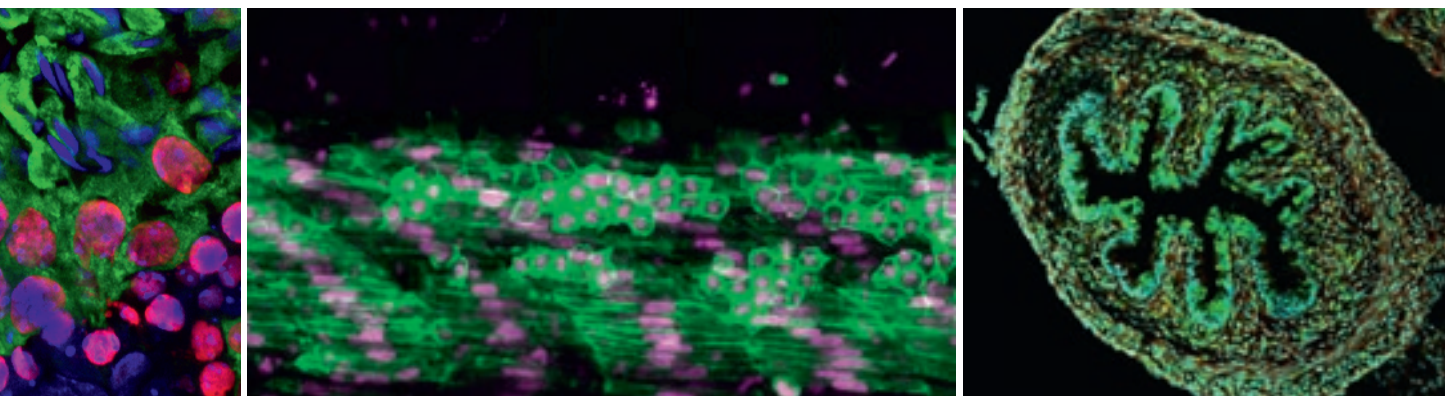
As IMB grows, so does our roster of exceptional scientists. This year we welcome Joan Barau from the Institut Curie in Paris. Joan was the 2015 recipient of the French Academy of Science prize for great advances in biological sciences. His research focuses on the conflict between transposable elements and genome stability during development and evolution, an excellent complement to

many of IMB’s current research themes. This year we also say farewell to Jean-Yves Roignant who has taken up an associate professorship at the Center for Integrative Genomics at the University of Lausanne. We wish him the very best of success in his new position. We are also delighted to announce that following a very successful recruitment call, 4 new Group Leaders will join IMB in 2020.

IMB’s thriving community of PhD students and postdocs continues to grow and succeed. Our International PhD Programme (IPP) is still growing following the arrival of new students as part of the RTG and the CRC’s IRTG. The IPP now has 144 students across 52 research groups and celebrated 25 successful PhD defences this year. IMB’s Postdoc Programme (IPPro) brought in more exceptional junior scientists to IMB through its 1st coordinated call this year. The programme now consists of 36 postdocs and will grow further with a 2nd recruitment round in early 2020. Lastly, 18 enthusiastic young scientists visited IMB for the 8th iteration of our International Summer School. Here they gained hands-on research experience with projects at the forefront of biological science. Due to the success of this programme, it has now received a continuation of its funding for the 9th round in summer 2020.

Finally, I would like to thank the Boehringer Ingelheim Foundation for their generous and continued support in the funding of our institute. I wish to also say a special thank you to the members of our Scientific Advisory Board (SAB) who, through their excellent guidance, continue to foster the growth and success of IMB. As I pass IMB’s rotating executive directorship to Christof Niehrs at the beginning of 2020, I would like to express how very grateful I am to everyone at IMB. These last two years have been very successful and rewarding. It could not have been so without all those who made this time as productive as possible. Thank you!

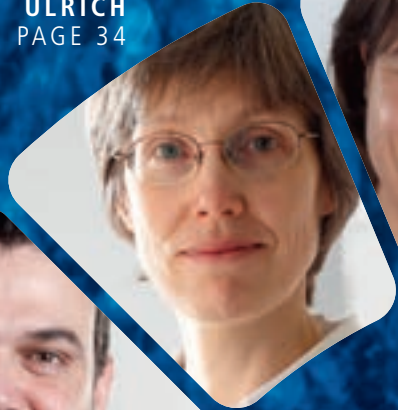
Helle Ulrich Executive Director



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RESEARCH GROUPS

*“We study the impacts
of intragenomic conflict
on genome regulation &
stability”*

JOAN BARAU

EDUCATION

- 2012** PhD in Genetics and Molecular Biology, University of Campinas
- 2005** BS in Biology, University of Campinas

POSITIONS HELD

- Since 2019** Group Leader, Institute of Molecular Biology (IMB), Mainz
- 2013 – 2019** Postdoc, Institut Curie, Paris

GROUP MEMBERS

- Jessica Leissmann** PhD Student; since 11/2019
- Violeta Morin** Lab Manager; since 10/2019
- Abishek Prakash** PhD Student; since 05/2019

OVERVIEW

Selfish intragenomic parasites such as transposable elements (TEs) are thought to have evolved shortly after the first genetic molecules that were able to self replicate. Across evolution, genomes constantly develop ways of isolating and protecting their hereditary material from TEs and TEs counter-evolve ways to escape, invade, and proliferate – effectively an arms-race running since the dawn of life. The more we understand how genomes work to shape an organism, the more we appreciate how this ratcheted co-evolutionary turnover contributed to novel ways of regulating the genome and its output. Our lab’s focus is in the main arena of this conflict – the germline – identifying key components ‘under dispute’ as a powerful approach to uncover new mechanisms affecting gene regulation, genome stability and inheritance. We apply genomics and biochemistry to mouse and cell culture models to explore key questions at the intersection of transposon and genome biology.

RESEARCH HIGHLIGHTS

Sorting the wheat from the chaff: How are transposons specifically silenced in the germline?

Germ cells have the demanding task of distinguishing what is a ‘normal’ functioning gene from what is an active TE that should be inactivated. Failure to do so can result in damage to the heritable genome, germ cell death and infertility. Information about active TEs is recorded by processing their mRNA into piwi-interacting RNAs (piRNAs, named after their interaction with PIWI Argonaute proteins). PIWIs constitute the core of genome defence against TEs in the germline: they connect biogenesis of piRNAs with the effector mechanism that uses cytosine DNA methylation for the life-long epigenetic repression of TE transcription. The DNA methyltransferase enzyme DNMT3C spe-

cifically methylates active TE promoters in germ cells, suggesting that DNMT3C operates downstream of piRNAs. However, the mechanism connecting piRNAs to the stable epigenetic silencing of TE promoters remains unknown, despite being paramount to genome stability and reproduction. One of the projects in our lab aims to close this knowledge gap by uncovering the proteome associated with the effectors of piRNA-guided transcriptional silencing. To do this we: (i) developed novel immunoprecipitation approaches in mouse embryonic germ cells and (ii) are developing novel targeted proteomic approaches tailored for low-input, rare primary cell populations. We are now using genome editing and biochemistry to study the candidates and understand the steps required to use a small RNA to stop and heritably shut down an active promoter in the mammalian germline.

What are the genetic and epigenetic determinants of germline transcription?

Genome-wide epigenetic reprogramming in the germline happens in every mammalian generation. It erases the somatic patterns of

genome regulation, allowing the germline-specific transcriptional program to unfold. To survive throughout evolution, TEs need to effectively hijack germline transcription. The timely (gametogenesis) and spatial (germline) regulation of germline genes and the capture of this regulatory program by TEs is a prime example of how genetic and epigenetic layers of regulation are used to control development and genome stability. Our lab is interested in understanding the rules and factors governing germline transcription during sex-specific gametogenesis. Also, how the pressure TEs exert on these networks helps shape germline genome regulation during development. We use genomics techniques to characterise the regulatory changes during sexually-dimorphic waves of transposon and germline gene expression. We are also developing biochemistry approaches to discover factors binding to the promoters of these sequences. Studying how these factors integrate into the dynamic landscape of germline chromatin during gametogenesis will provide unprecedented insights into the genetic and epigenetic layers that ensure mammalian reproductive capacity.

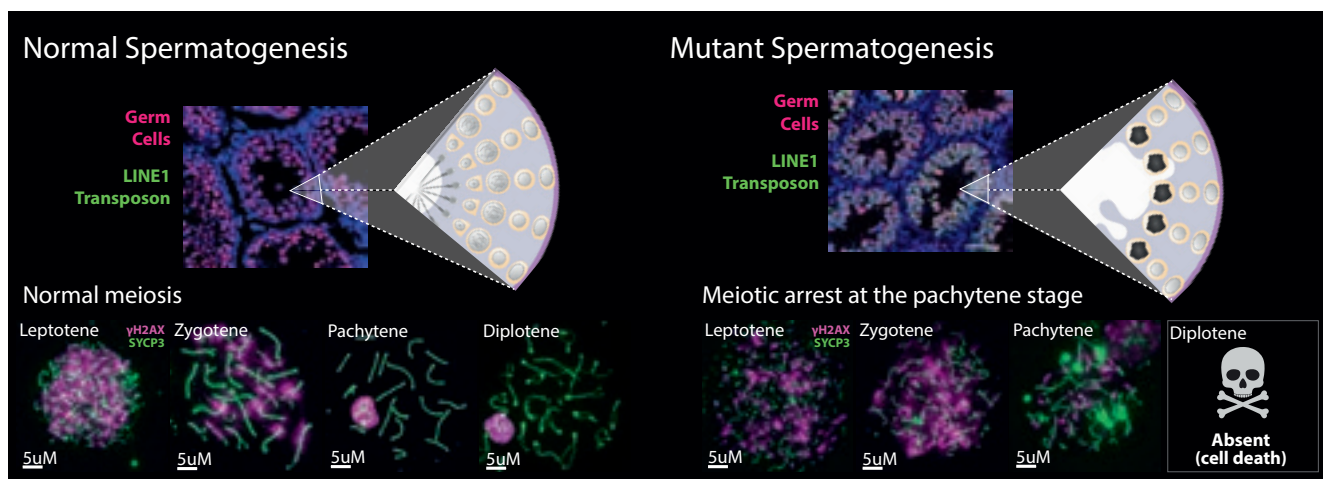


Figure 1. Immunofluorescence from wild-type and mutant *Dnmt3C* male gonads (top panels) and meiotic chromosome spreads (bottom panel), showing LINE1 retrotransposon reactivation leading to genome instability in meiosis and subsequent infertility.

FUTURE DIRECTIONS

In the upcoming year, we want to narrow down the results obtained in cell culture systems and extend the analysis to *in vivo* mouse models, thereby gaining mechanistic insights into how these protein complexes function stepwise. We will also expand

our team and projects to validate and further develop novel genomic and proteomic techniques to be applied to these problems, generating insight into the genetic and epigenetic control of gametogenesis in mammals.

SELECTED PUBLICATIONS

Barau J, Teissandier A, Zamudio N, Roy S, Nalesso V, Héroult Y, Guillou F and Bourc'his D (2016) The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science*, 354:909–912

Barau J, Grandis A, Carvalho VMA, Teixeira GS, Zapparoli GHA, do Rio MCS, Rincones J, Buckeridge MS and Pereira GAG (2015) Apolastic and intracellular plant sugars regulate developmental transitions in witches' broom disease of cacao. *J Exp Bot*, 66:1325–1337

Zamudio N, Barau J, Teissandier A, Walter M, Borsos M, Servant N and Bourc'his D (2015) DNA methylation restrains transposons from adopting a chromatin signature permissive for meiotic recombination. *Genes Dev*, 29:1256–1270

“Insufficient telomerase activity is associated with the hallmarks of ageing”

PETER BAUMANN



EDUCATION

- 1998** PhD in Biochemistry, University College London
- 1994** MPhil, University of Cambridge

POSITIONS HELD

- Since 2018** Adjunct Director, Institute of Molecular Biology (IMB), Mainz
- Since 2017** Alexander von Humboldt Professor, Johannes Gutenberg University Mainz
- 2013 – 2019** Professor, Kansas University Medical Center
- 2013 – 2018** Investigator, Howard Hughes Medical Institute, Kansas City
- 2013 – 2018** Priscilla Wood-Neaves Endowed Chair in the Biomedical Sciences, Stowers Institute for Medical Research, Kansas City
- 2013 – 2018** Investigator, Stowers Institute for Medical Research, Kansas City
- 2009 – 2013** Early Career Scientist, Howard Hughes Medical Institute, Kansas City
- 2009 – 2013** Associate Professor, Kansas University Medical Center
- 2009 – 2012** Associate Investigator, Stowers Institute for Medical Research, Kansas City
- 2004 – 2009** Assistant Professor, Kansas University Medical Center
- 2002 – 2008** Assistant Investigator, Stowers Institute for Medical Research, Kansas City
- 2000 – 2002** Research Associate, Howard Hughes Medical Institute, University of Colorado, Boulder
- 1998 – 2000** Wellcome Trust Travelling Research Fellow, University of Colorado, Boulder

GROUP MEMBERS

- Lukas Ende** PhD Student; since 09/2019
- Thomas Faust** Personal Assistant; since 05/2019
- Yasmin Greiner** Project Manager; since 03/2019
- David Ho** PhD Student; since 04/2018
- Kristi Jensen** Lab Manager; since 05/2018
- Katja Kratz** Staff Scientist; since 10/2017
- Abinaya Manivannan** PhD Student; since 08/2018
- Philip McNamara** Staff Scientist; since 04/2019
- Aaron Odell** Bioinformatician; since 01/2017
- Alex Orioli** PhD Student; since 01/2018
- Diego Paez-Moscoso** PhD Student; since 06/2014
- Lili Pan** Postdoc; since 02/2015
- Valentine Patterson** PhD Student; since 10/2019

OVERVIEW

Elucidating the mechanisms of chromosome end maintenance has far-reaching implications for the treatment of cancer and a group of degenerative diseases characterised by the premature depletion of stem cell pools. Overall, our research goals in this area are guided by the conviction that a better understanding of telomerase biogenesis and regulation will enable us to identify compounds that modulate telomere length. Such reagents will have therapeutic uses, either to limit the lifespan of tumour cells or to boost the proliferative potential of desired cell populations, counteracting many of the detrimental phenotypes associated with ageing. Our interest in chromosome dynamics and stability in an evolutionary context has led us to a group of lizard species with fascinating attributes: They arose by interspecific hybridisation and reproduce clonally via parthenogenesis. We have revealed deviations from the normal meiotic program that permit such unisexual reproduction, and have begun to elucidate the molecular basis of clonal reproduction in vertebrate species. This line of research carries the promise of furthering our understanding of gene regulatory processes in the context of hybridisation and ploidy variation, and provides new insights into the plasticity of reproductive strategies.

RESEARCH HIGHLIGHTS

Telomerase biogenesis and regulation

Progressive telomere shortening eventually limits the replicative potential of cells by triggering senescence. However, replenishing telomeric sequences is a double-edged sword. It is vital for tissue homeostasis, especially in long-lived species such as humans, but it also permits the continued proliferation of malignant cells. Thus, tight regulation of the enzyme telomerase is of pivotal

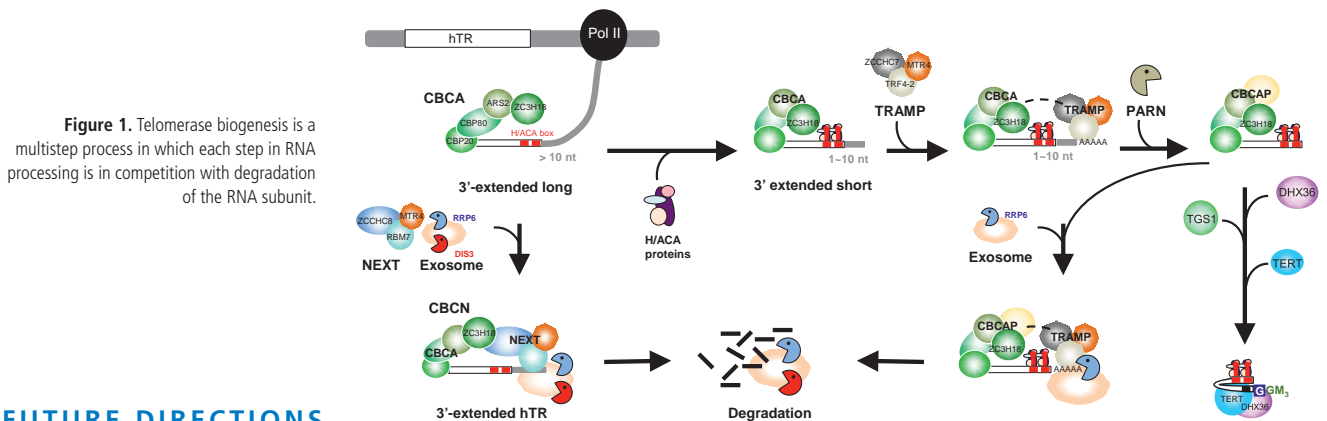
importance. The isolation of the telomerase RNA subunit (TER1) from fission yeast by our laboratory has provided a key tool for studying the biogenesis and regulation of the enzyme in a genetically tractable organism. This has led to a series of discoveries in telomere and RNA biology. We demonstrated that the RNA splicing machinery has a second, previously overlooked, function in RNA 3' end processing. Instead of removing an intronic sequence in a two-step process, the first transesterification reaction alone generates the mature 3' end of TER1. Our work also defined roles for several RNA chaperones in telomerase biogenesis. Most interestingly, we found that the Sm proteins and a related protein complex (LSm – Like-Sm) sequentially associate with telomerase RNA and play distinct roles in telomerase maturation. Sm and LSm proteins are members of an ancient family of RNA binding proteins that affect virtually every aspect of RNA metabolism. In contrast to the established view that they have distinct sets of RNA targets, our work revealed the first example of an RNA that requires sequential binding by the two complexes and documents specific functions for each during maturation.

While our studies on telomerase and telomeres in fission yeast have provided fundamental insights into chromosome end

maintenance, they are ultimately a stepping-stone towards understanding telomere maintenance in human cells. Recent work in our group uncovered roles for several RNA processing factors and an unusual RNA structure, called a triple helix, in the biogenesis of human telomerase. Importantly, our studies showed that maturation of human telomerase RNA (hTR) is in kinetic competition with its degradation (Figure 1). This observation hints at potential treatment options for telomerase insufficiency disorders and for delaying or reversing certain degenerative processes associated with ageing.

Mechanism of parthenogenesis

Although all-female species of whiptail lizards were already described in the early 1960s, how mature eggs are produced in the absence of fertilisation remained an enigma until we showed that a transient doubling of chromosomes prior to the meiotic divisions produces diploid rather than haploid eggs. To gain a deeper mechanistic understanding, we have sequenced, assembled, and annotated the genomes of several species of whiptail lizards. These resources have enabled us to clone key meiotic regulators and to gain insights into the underlying cellular processes.



FUTURE DIRECTIONS

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

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

SELECTED PUBLICATIONS

Páez-Moscoso DJ, Pan L, Sigauke RF, Schroeder MR, Tang W and Baumann P (2018) Pof8 is a La-related protein and a constitutive component of telomerase in fission yeast. *Nat Commun*, 9:587

Tseng CK, Wang HF, Schroeder MR and Baumann P (2018) The H/ACA complex disrupts triplex in hTR precursor to permit processing by RRP6 and PARN. *Nat Commun*, 9:5430

Newton AA, Schnitker RR, Yu Z, Munday SS, Baumann DP, Neaves WB and Baumann P (2016) Widespread failure to complete meiosis does not impair fecundity in parthenogenetic whiptail lizards. *Development*, 143:4486–4494

"We use quantitative proteomics to study nuclear protein quality control"

PETRA BELI

EDUCATION

- 2011** PhD in Biology, Goethe University Frankfurt
- 2007** MSc in Molecular Biology, University of Zagreb

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

GROUP MEMBERS

- Irem Baymaz** Postdoc; since 11/2016
- Francesca Conte** PhD Student; since 01/2019
- Sofia Lobato Gil** Postdoc; since 10/2018
- Jan Heidelberg** PhD Student; since 04/2014
- Thomas Juretschke** PhD Student; since 09/2016
- Ivan Mikicic** PhD Student; since 04/2019
- Thorsten Mosler** PhD Student; since 04/2017
- Patrick Müller** Master's Student; since 08/2019
- Matthias Ostermaier** PhD Student; since 05/2015
- Claudia Scalerà** PhD Student; since 09/2019
- Aldwin Suryo Rahmanto** Postdoc; since 11/2019
- Andrea Voigt** Lab Manager; since 01/2014
- Juanjuan Wang** PhD Student; since 08/2016

OVERVIEW

Protein misfolding and aggregation is linked to human disorders, including Alzheimer's and Parkinson's disease. Human cells have evolved protective RNA and protein quality control mechanisms to recognise misfolded and aggregated proteins or aberrantly assembled complexes and limit their toxic effects. For instance, terminally misfolded proteins or protein aggregates are degraded through the ubiquitin-proteasome or autophagy-lysosomal pathway. Modification of proteins with ubiquitin, mediated by ubiquitin E3 ligases, plays an essential role in the regulation of different protein quality control pathways. These E3 ligases are able to modify cellular proteins with different types of "ubiquitin signals" to determine the fate of the modified substrate. We are employing quantitative mass spectrometry-based proteomics to study the substrates and functions of ubiquitin E3 ligases and different types of ubiquitin chains in the maintenance of protein homeostasis.

RESEARCH HIGHLIGHTS

During gene expression, quality control pathways monitor each step to detect and degrade aberrant mRNAs and proteins. These mechanisms ensure protein homeostasis and are essential to prevent neurodegenerative diseases. A common source of aberrant mRNAs is premature polyadenylation, which can result in non-functional protein products. Therefore, mechanisms are in place that recognise such homopolymeric adenosine (poly(A)) sequences and abrogate their translation. Translating ribosomes that encounter poly(A) sequences are terminally stalled, followed by ribosome recycling and decay of the truncated nascent polypeptide via ribosome-associated quality control (RQC). Upon splitting of the 60S and 40S ribosomal subunits, the RQC complex assembles on the 60S subunit to initiate the release and rapid degradation

of the truncated tRNA-bound polypeptide. The E3 ubiquitin ligase Listerin (LTN1) modifies the truncated polypeptide with K48-linked ubiquitin chains to target it for degradation through the proteasome. Whereas peptide release and ribosome recycling by the RQC complex are relatively well understood, less is known about the mechanisms that promote poly(A) recognition and initial ribosome stalling.

In collaboration with Julian König (IMB) and Kathi Zarnack (Goethe University Frankfurt), we demonstrated that the conserved RNA-binding E3 ubiquitin ligase Makorin Ring Finger Protein 1 (MKRN1) promotes ribosome stalling at poly(A) sequences

during ribosome-associated quality control. We showed that MKRN1 directly binds to the cytoplasmic poly(A)-binding protein (PABPC1) and associates with polysomes. MKRN1 is positioned upstream of poly(A) tails in mRNAs in a PABPC1-dependent manner. Ubiquitin remnant profiling and *in vitro* ubiquitylation assays uncovered PABPC1 and ribosomal protein RPS10 as direct ubiquitylation substrates of MKRN1. We propose that MKRN1 mediates the recognition of poly(A) tails to prevent the production of erroneous proteins from prematurely polyadenylated transcripts, thereby maintaining proteome integrity.

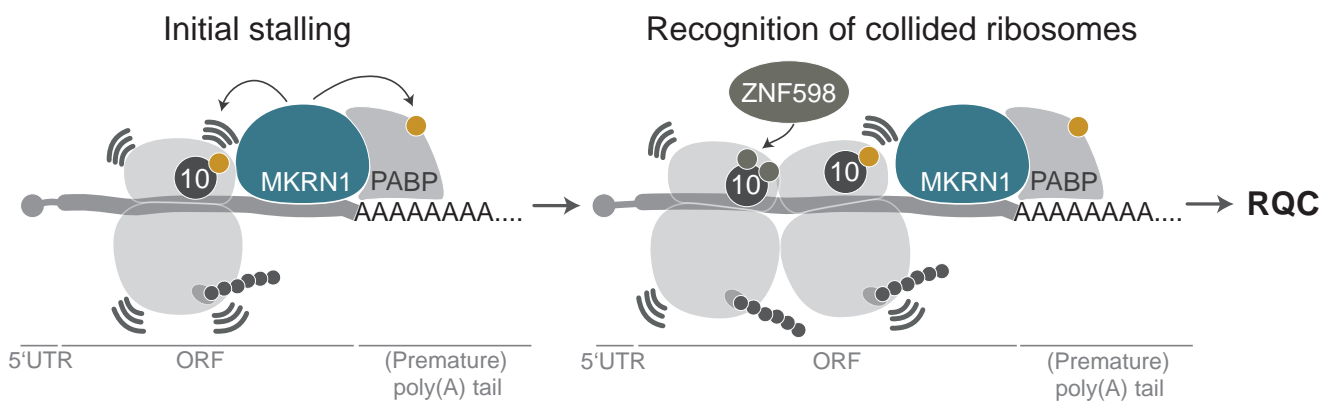


Figure 1. MKRN1 is a sensor for poly(A) sequences that stalls ribosomes on messenger RNA to initiate ribosome quality control.

FUTURE DIRECTIONS

We will study protein quality control pathways that function in the nucleus of human cells. To this end, we aim to identify ubiquitin ligases, deubiquitinating enzymes and ubiquitin-binding proteins that function in the maintenance of nuclear protein homeostasis. In particular, we will follow the hypothesis that phase-separated

membraneless organelles act as centres of protein quality control in the nucleus. Quantitative mass spectrometry-based proteomics based on proximity biotinylation such as APEX2 and TurboID will be employed to determine the protein composition of these nuclear sub-compartments.

SELECTED PUBLICATIONS

Hildebrandt A, Brüggemann M, Rücklé C, Boerner S, Heidelberg JB, Busch A, Hänel H, Voigt A, Möckel MM, Ebersberger S, Scholz A, Dold A, Schmid T, Ebersberger I, Roignant JY, Zarnack K[#], König J[#] and Beli P[#] (2019) The RNA-binding ubiquitin ligase MKRN1 functions in ribosome-associated quality control of poly(A) translation. *Genome Biol*, 20:216

Borisova ME, Voigt A, Tollenaere MAX, Sahu SK, Juretschke T, Kreim N, Mailland N, Choudhary C, Bekker-Jensen S, Akutsu M, Wagner SA and Beli P (2018) p38-MK2 signaling axis regulates RNA metabolism after UV-light-induced DNA damage. *Nat Commun*, 9:1017

Heidelberg JB, Voigt A, Borisova ME, Petrosino G, Ruf S, Wagner SA and Beli P (2018) Proteomic profiling of VCP substrates links VCP to K6-linked ubiquitylation and c-Myc function. *EMBO Rep*, 19:e44754

[#] indicates joint correspondence

“Mass spectrometry allows us to analyse thousands of proteins in a single experiment”

FALK BUTTER

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

- Miguel Almeida** Postdoc; since 04/2019
- Alina Bluhm** PhD Student; 01/2014 – 05/2019
- Hanna Braun** PhD Student; since 08/2015
- Alejandro Ceron** PhD Student; since 10/2019
- Emilia Rita De Caro** Research Assistant; since 05/2019
- Sabrina Dietz** PhD Student; since 01/2015
- Albert Fradera Sola** PhD Student; since 03/2018
- Carisa Goh** Research Assistant; since 10/2019
- Michal Levin** Postdoc; since 09/2018
- Liudmyla Lototska** Postdoc; since 06/2019
- Katarina Luko** PhD Student; since 12/2017
- Emily Nischwitz** PhD Student; since 09/2018
- Merve Öztürk** PhD Student; since 11/2015
- Lara Perez** PhD Student; 10/2015 – 12/2019
- Franziska Roth** Student Assistant; since 01/2019
- Marion Scheibe** Postdoc; since 06/2013
- Marian Scherer** Student Assistant; since 11/2019
- Vivien Schoonenberg** PhD Student; since 02/2018
- Nikenza Viceconte** Postdoc; 01/2018 – 03/2019
- Maya Wilkens** PhD Student; since 10/2019

OVERVIEW

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

RESEARCH HIGHLIGHTS

Phylointeractomics reveals evolutionary changes in protein binding

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

rodent lineage and absence of *TIN2* in several bird genomes. In our phylointeractomics screen, we recapitulated these evolutionary differences for 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 recreate a gain-of-binding switch *in vitro* recapitulating a possible evolutionary scenario. Our phylointeractomics study, therefore, underscores that sequence homologues, as determined by phylogenomics, do not necessarily need to equate to functional homology.

Characterisation of new telomeric proteins

We use quantitative interactomics to identify new telomeric proteins. Apart from HOTA1, we reported that the zinc finger protein ZBTB48 is a telomeric protein in mammals. Recently, we characterised ZBTB10 as a telomeric protein that preferentially binds to telomeres elongated by the alternative lengthening

pathway. The extension of our workflow to other model species resulted in the identification of novel telomere binding proteins in *Trypanosoma brucei*. We characterised one of these candidates, which we termed TelAP1 as a dynamic telomere binding protein that is differentially expressed between the two life cycle stages of the parasite.

Systems approaches to study developmental gene regulation

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

FUTURE DIRECTIONS

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

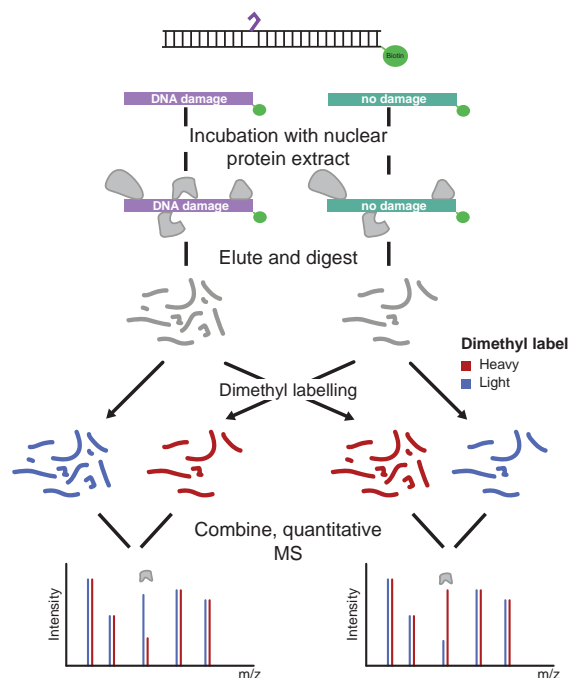


Figure 1. Workflow of a dimethyl labelling experiment to catch proteins interacting with specific DNA lesions. Oligonucleotides either carrying the lesion or the non-damaged control are incubated with a cellular extract. The bound protein fractions are purified independently and labelled with different isotope-encoded dimethyl adducts that result in a specific mass shift in the mass spectrometer. From the different intensities of the peptide pairs in the mass spectrometer, a list of proteins enriched at the DNA lesion is generated.

SELECTED PUBLICATIONS

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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

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“The 3D structure of the nuclear genome is decisive for cellular function”

CHRISTOPH CREMER

EDUCATION

- 1983** Habilitation in General Human Genetics and Experimental Cytogenetics, University of Freiburg
- 1976** PhD in Biophysics and Genetics, University of Freiburg
- 1970** Diploma in Physics, Ludwig Maximilian University (LMU), Munich

POSITIONS HELD

- Since 2015** Research Associate, Max Planck Institute for Chemistry, Mainz
- Since 2013** Honorary Professor of Physics, Johannes Gutenberg University Mainz
- Since 2011** Group Leader, Institute of Molecular Biology (IMB), Mainz
- Since 2005** Director, Cooperation Unit Biophysics, Institute for Pharmacy and Molecular Biotechnology, University of Heidelberg
- Since 1992** Co-Director, Interdisciplinary Center for Scientific Computing, University of Heidelberg
- 2005 – 2007** Deputy Director, Kirchoff Institute of Physics, University of Heidelberg
- 1983 – 2011** Professor of Applied Optics and Information Processing, University of Heidelberg
- 1983 – 1999** Managing/Deputy Director, Institute of Applied Physics I, University of Heidelberg
- 1970 – 1983** Staff Scientist, Institute of Human Genetics, University of Freiburg

GROUP MEMBERS

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OVERVIEW

A full mechanistic understanding of gene regulation in space and time requires a detailed quantitative insight into the spatial constraints of gene activity control in the cell nucleus, along with sequence and biochemical information. For this, it is necessary to enhance the resolution of spatial DNA distribution and other relevant molecules substantially beyond the conventional resolution level. This has become possible by various super-resolution light microscopy (SRM) approaches. SRM of the nuclear genome nanostructure is expected to contribute to fundamental questions including how does the complex organisation of chromosome territories in the mammalian cell nucleus arise, and how is this organisation related to epigenetic gene regulation and its dynamics? We have shown that Single Molecule Localisation Microscopy (SMLM), a type of SRM, is particularly useful for enhanced analysis of the cell nucleus. In 2019, we applied a recently developed SMLM approach to nuclear genome nanostructure imaging, designated DNA Structure Fluctuation Assisted Binding Activated Localisation Microscopy (fBALM), to analyse the mammalian cell nucleus in three dimensions at unprecedented resolution. Under appropriate conditions, using fBALM, we were able to resolve spatial position differences of single fluorophores bound to DNA down to ca. 3 nm, corresponding to the diameter of one single protein molecule.

RESEARCH HIGHLIGHTS

In 2019, we continued to focus on the following topics in nuclear genome landscape topology: what are the differences in DNA content between the low density (active) and the high density (inactive) compartments? What is the spatial nanoscale distribution of such small chromatin domains? How many nucleosomes do they contain? What is the variability of such

nucleosome clusters? What is the DNA density in these 'nanodomains'? Answering these questions is essential for a quantitative, mechanistic, dynamic, and (eventually) predictive understanding of epigenetic gene regulation. As such, we have applied a new technique (fBALM), developed in our group, for localisation microscopy of nuclear DNA at single-molecule resolution. The absolute DNA density estimates obtained by fBALM in small compacted chromatin domains (sub 100 nm diameter range) suggest maximum densities far above those conducive to accessibility by nuclear protein complexes. However, they fit well to our earlier measurements on specific FISH labelled domains using another approach (axially structured illumination) and support recent models on the functional nanoscale compartmentalisation of nuclear genome structure.

To improve SMLM analysis of specific chromatin nanodomains, in a collaboration with Heidelberg University, oligo-probe-FISH was applied in combination with immunostaining to realise a three colour SMLM of *Alu* consensus, L1 sequences, and H3K9me3 sites. To further enhance the possibility of long term SMLM measurements (required for highest resolution 3D measurements), we also developed a precise calibration method (better than ca. 10 nm in all 3 spatial directions). Finally, in collaboration with the Max Planck Institute for Polymer Research (Mainz), we investigated the use of nanographenes (large polycyclic aromatic hydrocarbons) as a new class of fluorophores for SMLM. These findings open the door for the widespread application of nanographenes in super-resolution fluorescence microscopy.

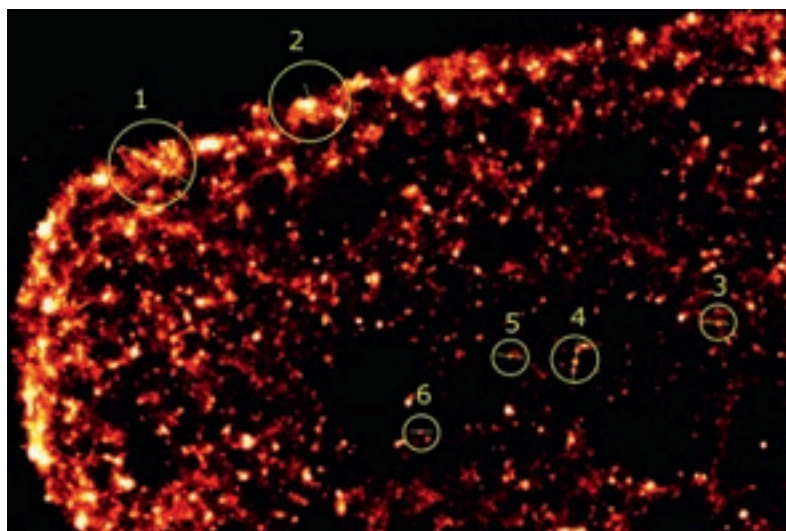


Figure 1. Nanoscale chromatin distribution in part of a HeLa cell nucleus. Section recorded with SMLM-fBALM and is composed of about 240,000 DNA bound, fluorescent Sytox Orange molecules. The image shows small, highly compact chromatin clumps (1-6) of different sizes, down to diameters in the 60 - 70 nm range. Preliminary estimates of absolute DNA densities in such small "nanodomains" yielded figures above 50 Mb/ μm^3 , indicating substantial accessibility barriers for macromolecule complexes.

FUTURE DIRECTIONS

At the end of 2019, the Cremer Lab at IMB will close. From 2020, the research topics of the Cremer Lab will be continued through collaborations between Christoph Cremer and IMB's Microscopy Core Facility; the Max Planck Institutes for Polymer Research and Chemistry; Mainz University Medicine; and Heidelberg University, in particular at the Institute of Pharmacy & Molecular Biotechnology/IPMB, the Institute of Physical Chemistry/PCI, and the Interdisciplinary Center for Scientific Computing (IWR).

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

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

SELECTED PUBLICATIONS

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“Mechanisms that control repetitive DNA often build on existing gene-regulatory circuitries”

RENÉ KETTING

EDUCATION

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

POSITIONS HELD

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GROUP MEMBERS

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- Elke Roovers** PhD Student; 07/2013 – 02/2019
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OVERVIEW

The major focus of my lab is gene regulation by small RNA molecules acting in RNAi-related pathways. Since their discovery at the start of the 21st century, many different RNAi-related pathways have been identified. It is now evident that although all of these pathways depend on proteins from the Argonaute family, each pathway has its own unique characteristics and effects on gene expression. These can range from relatively minor effects on translation (in the case of miRNAs) to full-blown shutdown of loci at the transcriptional level (piRNAs). We focus on mechanisms related to piRNA and siRNA biology, two species of small RNAs that are particularly abundant in, and important for, the germline. These small RNA pathways have a major role in maintaining genome integrity through controlling the activity of transposable elements. We use zebrafish and *C. elegans* as model systems to understand the molecular mechanisms governing these pathways and how they contribute to normal development. Questions including, how do small RNA pathways distinguish transposable elements from regular genes, how are these pathways organised at a sub-cellular level, and how can small RNA populations be inherited across generations are at the heart of our research.

RESEARCH HIGHLIGHTS

A novel protein complex that makes piRNAs in worms

The nematode *C. elegans* makes a special class of small RNAs that, when bound to a protein of the Piwi-family, scan the genome for the presence of foreign sequences, triggering their silencing. In this way, these small RNAs act as specificity factors in a genomic immune system. These small RNAs, named 21U RNAs or piRNAs, are produced as tiny non-coding transcripts from tens of thousands of individual transcription units clustered

in two large regions of the genome. This type of organisation resembles how other non-coding transcripts are made, e.g. tRNAs, rRNAs and snRNAs. To mature, the precursor piRNAs of *C. elegans* first need to be processed, losing nucleotides at both the 5' and 3' ends. While the enzyme responsible for processing the 3' ends has been identified, nothing is known about 5' end processing. In the past year, we identified a protein complex that binds to piRNA precursors and is essential for their maturation. We named this complex PETISCO –Portuguese for *tapas*. PETISCO consists of a number of subunits with different activities, one of which binds 5' cap structures. Most *C. elegans* mRNAs carry a modified cap structure: a tri-methyl cap. On the other hand, piRNAs contain a 'regular cap' structure. PETISCO can bind to 'regular caps' but not to tri-methylated caps, which provides the possibility for substrate selection. Furthermore, a second PETISCO subunit is predicted to bind to 5'-phosphate ends. This combination of cap and 5'P binding suggests that it is via PETISCO's binding to piRNA precursors that they lose their 5'-cap.

The sub-cellular localisation of PETISCO indicates that it is present in peri-nuclear granules, which are close to, or are

possibly the same as, P-granules. Indeed, these granules have already been shown to house mature piRNAs and Piwi proteins. Interestingly, in addition to defects in piRNA biogenesis, mutants that lack PETISCO also display a strong maternal lethality effect. This indicates that PETISCO is involved in loading something essential into embryos. We found that these two functions are accompanied by two different proteins that can bind to PETISCO: PID-1 and TOST-1. PID-1 was previously described by us as a factor needed for piRNA generation, whereas TOST-1, despite having sequence similarity to PID-1 – i.e. binds to the same PETISCO subunit – is not required for piRNA generation. Instead, mutants lacking TOST-1 display the same maternal effect lethality as PETISCO mutants. Sequence conservation of PETISCO subunits in other nematodes indicates it is highly likely that, of the two roles, the maternal lethality effect is the more ancient. This work, which was published in *Genes & Development* this year, identifies a platform for piRNA maturation and suggests that this process was likely built upon a pre-existing molecular mechanism. However, the nature of this ancestral function is not yet clear.

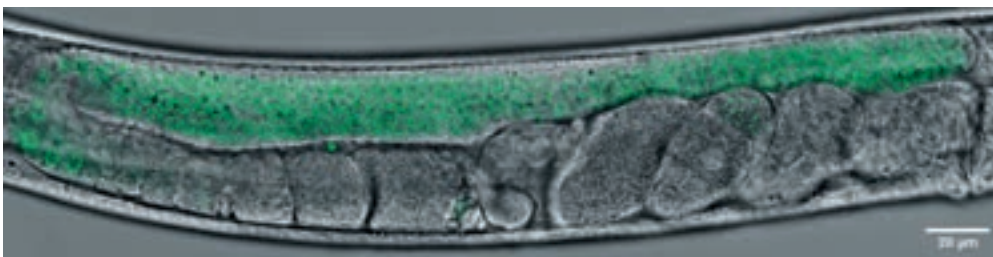


Figure 1. A confocal image showing P-granules in the *C. elegans* germline, visualised by a GFP-tag integrated into the endogenous locus of the *pgl-1* gene. These structures also contain mature piRNAs and Piwi proteins.

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 to identify novel factors and are increasingly using biochemical approaches to describe their mechanisms on a more molecular level. Both *C. elegans* and zebrafish will continue to play important roles in these studies. For instance, we will focus on further mechanistic dissection of the PETISCO complex aiming to identify and understand the process

that renders it essential to embryonic function. Having also identified a novel phase-separated structure that is specific to sperm and essential for paternal inheritance of small RNA-mediated gene control, we aim to unravel the molecular details behind this new, intriguing structure and how it is controlled. Phase-separation-related mechanisms are also studied in our zebrafish system, where we study their role in germ cell specification.

SELECTED PUBLICATIONS

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Roovers EF, Kaaij LJT, Redl S, Bronkhorst AW, Wiebrands K, de Jesus Domingues AM, Huang H, Han C, Riemer S, Dösch R, Salvenmoser W, Grün D, Butter F, van Oudenaarden A and Ketting RF (2018) Tdrd6a regulates the aggregation of Buc into functional subcellular compartments that drive germ cell specification. *Dev Cell*, 46:285–301

de Albuquerque BFM, Placentino M and Ketting RF (2015) Maternal piRNAs are essential for germline development following *de novo* establishment of endo-siRNAs in *Caenorhabditis elegans*. *Dev Cell*, 34:448–456

*"We unravel how cells
recycle their proteins"*

ANTON KHMELINSKII

EDUCATION

- 2010** PhD in Biology, University of Heidelberg
- 2005** Licenciatura degree in Biochemistry, University of Lisbon

POSITIONS HELD

- Since 2018** Group Leader, Institute of Molecular Biology (IMB), Mainz
- 2013** Visiting Scientist, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto
- 2011 – 2017** Postdoc, Center for Molecular Biology (ZMBH), University of Heidelberg
- 2011 – 2016** Visiting Scientist, European Molecular Biology Laboratory (EMBL), Heidelberg
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GROUP MEMBERS

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- Elena Ivanova** PhD Student; since 03/2019
- Ka Yiu Kong** Postdoc; since 08/2019
- Zhaoyan Li** PhD Student; since 12/2018
- Rocío Nieto-Arellano** Postdoc; since 04/2018
- Anke Salzer** Lab Manager; since 01/2018
- Simone Snead** Technician; since 10/2018

OVERVIEW

The functional state of a cell is ultimately defined by the state of its proteome, i.e. abundance, localisation, turnover, and mobility of all proteins and their organisation in complexes and organelles. Numerous cellular systems contribute to proteome homeostasis through prevention, detection, and removal of abnormal proteins. Selective protein degradation by the ubiquitin-proteasome system plays a key role in proteome turnover and quality control. When degradation is not possible, the impact of abnormal proteins can be minimised through their asymmetric partitioning during cell division. Despite the activity of such systems, proteome homeostasis declines with ageing and in numerous diseases, resulting in accumulation of abnormal proteins and loss of cell functionality. Working in yeast and human cells, we aim to systematically identify substrates for the various components of the ubiquitin-proteasome system and explore the functions of this system in replicative ageing and genome stability. We are using genetic and proteomic approaches that exploit fluorescent timers to follow protein trafficking, inheritance, and degradation down to subcellular resolution. Our goals are to understand how protein quality control is coordinated with protein synthesis, folding and trafficking, to elucidate how cells recognise abnormal proteins, and how they adapt to challenges in proteome homeostasis.

RESEARCH HIGHLIGHTS

Selective protein degradation is involved in most cellular processes and contributes to proteome homeostasis through the removal of unnecessary or abnormal proteins. The ubiquitin-proteasome system (UPS) plays a key role in selective protein degradation, whereby a cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin-protein ligase (E3) enzymes mark

proteins with polyubiquitin chains for degradation by the proteasome. Deubiquitinating enzymes, which remove ubiquitin marks and replenish the pool of free ubiquitin, are involved at various stages of the targeting and degradation processes. Despite the central role of the UPS in protein degradation and its association with various diseases and ageing, many UPS components remain poorly characterised and our understanding of specificity in the UPS is inadequate.

We develop and deploy proteomic approaches to gain insights into selective protein degradation. Using fluorescent timers as reporters of protein turnover (Figure 1), we established screening pipelines to identify substrates for various components of the ubiquitin-proteasome system and to define signals involved in substrate recognition. To apply these methods in yeast, we developed the SWAp-Tag (SWAT) approach for high-throughput tagging of yeast open reading frames (ORFs). Genome-wide libraries of strains in which each ORF is fused to the same tag at its

endogenous chromosomal locus are very useful tools to study the yeast proteome. But their construction is laborious and expensive, hindering the use of new tags in proteomic studies. Using SWAT we are now able to endogenously tag virtually every yeast ORF with any tag in approximately three weeks, greatly expanding the possibilities of proteome-wide studies.

Using our screening approaches, we are searching for substrates of E3 ubiquitin ligases. This led us to the discovery of a protein quality control pathway that operates specifically at the inner nuclear membrane (INM). This pathway appears to have an important role in maintaining the identity of the INM, whereby proteins that mislocalise to the INM are recognised and targeted for degradation. Moreover, our recent work on endoplasmic reticulum-associated protein degradation (ERAD) revealed an unexpected role of ERAD in the destruction of proteins that otherwise mislocalise to the outer mitochondrial membrane.

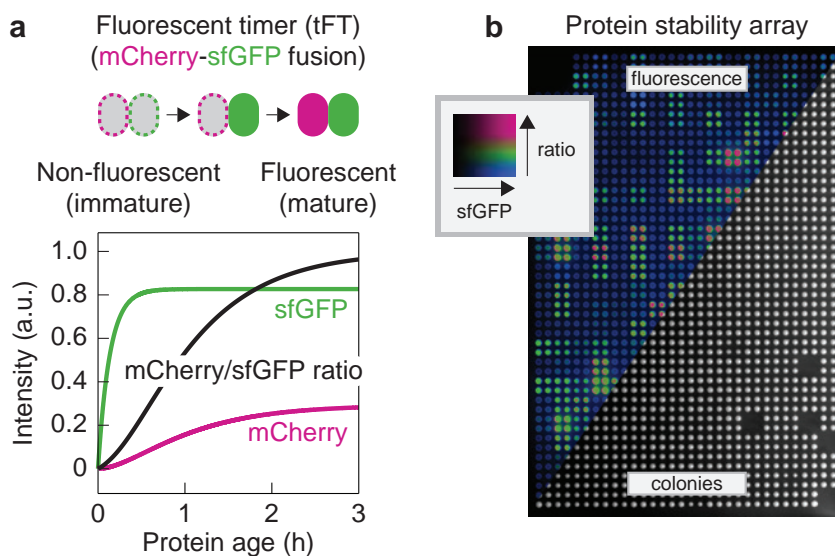


Figure 1. Analysis of protein dynamics with tandem fluorescent protein timers (tFTs). (a) A tFT is a fusion of two conventional fluorescent proteins: sfGFP (green) and mCherry (red). Due to different maturation kinetics of mCherry (slow) and sfGFP (fast), the mCherry/sfGFP ratio is a measure of protein age and can be used to follow trafficking, inheritance and degradation of tFT-tagged proteins. (b) Analysis of protein degradation with tFTs. Fluorescence measurements of yeast colonies expressing tFT-tagged proteins.

FUTURE DIRECTIONS

We will continue our systematic search for substrates of E3 ubiquitin ligases in yeast by combining our reporter-based screens with mass spectrometry and expand this search to human cells. We will also apply genetic and proteomic approaches to identify redundancies in the ubiquitin-proteasome system and, in this way,

find substrates for overlapping degradation pathways. Finally, we will build on our work on mislocalised proteins to understand how such molecules are recognised as abnormal in the cell. We are eager to test the importance of such quality control pathways under stress and during the ageing process.

SELECTED PUBLICATIONS

Dederer V, Khmelinskii A, Huhn AG, Okreglak V, Knop M and Lemberg MK (2019) Cooperation of mitochondrial and ER factors in quality control of tail-anchored proteins. *eLife*, 8:e45506

Meurer M, Duan Y, Sass E, Kats I, Herbst K, Buchmuller BC, Dederer V, Huber F, Kirrmaier D, Štefl M, Van Laer K, Dick TP, Lemberg MK, Khmelinskii A[#], Levy ED[#] and Knop M[#] (2018) Genome-wide C-SWAT library for high-throughput yeast genome tagging. *Nat Methods*, 15:598–600

Kats I, Khmelinskii A, Kschonsak M, Huber F, Knieß RA, Bartosik A and Knop M (2018) Mapping degradation signals and pathways in a eukaryotic N-terminome. *Mol Cell*, 70:488–501.e5

[#] indicates joint correspondence

“Artificial intelligence will help us crack the splicing code”

JULIAN KÖNIG



EDUCATION

- 2008** PhD in Biology, Max Planck Institute for Terrestrial Microbiology & Philipps University, Marburg
- 2003** Diploma in Biology, Ludwig Maximilian University (LMU), Munich

POSITIONS HELD

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

GROUP MEMBERS

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- Mariela Cortes Lopez** PhD Student; since 02/2017
- Antonella Di Liddo** Staff Scientist; since 07/2019
- Stefanie Ebersberger** Postdoc; since 04/2014
- Heike Hänel** Lab Manager; since 11/2013
- Andrea Hildebrandt** PhD Student; since 04/2014
- Nadine Körte** PhD Student; since 11/2018
- Mikhail Mesitov** Postdoc; since 10/2019
- Miriam Mulorz** PhD Student; since 11/2019
- Holger Mutter** Student Assistant; since 07/2018
- Cornelia Rückle** PhD Student; since 04/2018
- Laura Schulz** PhD Student; since 04/2017
- Kerstin Tretow** PhD Student; since 12/2018

OVERVIEW

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

RESEARCH HIGHLIGHTS

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

Mutations causing aberrant splicing are frequently implicated in human diseases including cancer. Together with Kathi Zarnack’s group at Goethe University Frankfurt and Stefan Legewie’s group at IMB, we established a high-throughput screen of randomly mutated minigenes to decode the *cis*-regulatory landscape that determines alternative splicing of exon 11 in the proto-oncogene *MST1R* (*RON*). Mathematical modelling of splicing kinetics enabled us to identify more than 1000 mutations affecting *RON* exon 11 skipping, which corresponds to the pathological

isoform RONΔ165. Importantly, the measured effects correlated with *RON* alternative splicing in cancer patients bearing the same mutations. Moreover, we highlighted heterogeneous nuclear ribonucleoprotein H (HNRNPH) as a key regulator of *RON* splicing in healthy tissues and cancer. Using iCLIP and synergy analysis, we pinpointed the functionally most relevant HNRNPH binding sites and demonstrated how cooperative HNRNPH binding facilitates a splicing switch of *RON* exon 11. Our results thereby offer insights into splicing regulation and the impact of mutations on alternative splicing in cancer.

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

Alternative splicing generates distinct mRNA isoforms and is crucial for proteome diversity in eukaryotes. The RBP U2AF2 is

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

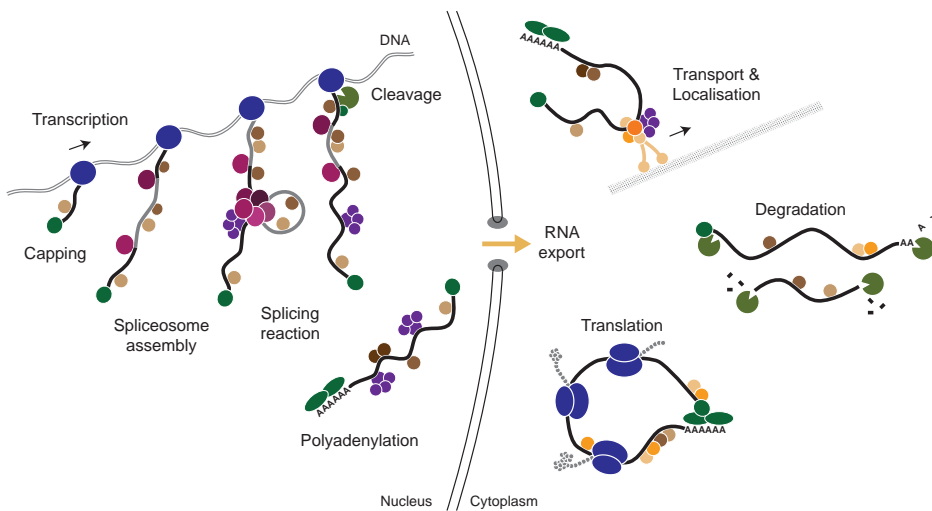


Figure 1. Overview of the life of an mRNA.

FUTURE DIRECTIONS

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

will yield a systemic understanding of RNP function in splicing regulation.

Reaching into translational research, we are extending our high-throughput mutagenesis approach to study splicing-mediated resistance in paediatric cancer therapy. The knowledge of splicing-effective mutations will enable us to develop prognostic biomarkers and may ultimately lead to new therapy strategies.

SELECTED PUBLICATIONS

Hildebrandt A, Brüggemann M, Rücklé C, Boerner S, Heidelberger JB, Busch A, Hänel H, Voigt A, Möckel MM, Ebersberger S, Scholz A, Dold A, Schmid T, Ebersberger I, Roignant JY, Zarnack K*, König J* and Beli P* (2019) The RNA-binding ubiquitin ligase MKRN1 functions in ribosome-associated quality control of poly(A) translation. *Genome Biol*, 20:216

Braun S*, Enculescu M*, Setty ST*, Cortés-López M, de Almeida BP, Sutandy FXR, Schulz L, Busch A, Seiler M, Ebersberger S, Barbosa-Morais NL, Legewie S*, König J* and Zarnack K* (2018) Decoding a cancer-relevant splicing decision in the *RON* proto-oncogene using high-throughput mutagenesis. *Nat Commun*, 9:3315

Sutandy FXR*, Ebersberger S*, Huang L*, Busch A, Bach M, Kang HS, Fallmann J, Maticzka D, Backofen R, Stadler PF, Zarnack K, Sattler M, Legewie S* and König J* (2018) *In vitro* iCLIP-based modeling uncovers how the splicing factor U2AF2 relies on regulation by cofactors. *Genome Res*, 28:699–713

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“We investigate the kinetic principles of alternative splicing”



STEFAN LEGEWIE

EDUCATION

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POSITIONS HELD

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- Timur Horn** Research Assistant; since 02/2019
- Sofya Lipnitskaya** PhD Student; since 08/2017
- Lorenz Ripka** PhD Student; since 05/2017

OVERVIEW

Eukaryotic cells sense and process information in order to respond to environmental changes. While the signalling pathways relaying information from the membrane to the nucleus are well-characterised, much less is known about decision making at the level of gene expression responses. One focus of our group is to derive a systems-level understanding of gene regulation, which describes (i) the interplay of signalling pathways and transcription factors in complex gene-regulatory networks, and (ii) how gene expression is co-ordinated 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 and (ii) better understand therapeutic intervention strategies.

RESEARCH HIGHLIGHTS

Kinetic modelling of alternative splicing events

Alternative splicing increases protein diversity in eukaryotic cells and therefore plays an important role in development and tissue identity. Mutations causing aberrant splicing are frequently implicated in human diseases including cancer. Together with the König and Zarnack groups, we established high-throughput

screens of randomly mutated minigenes to decode the *cis*-regulatory landscape controlling selected disease- and therapy-relevant splicing events. We use a combination of kinetic and linear regression modelling to identify the effects of individual point mutations from measured, combined mutations and to understand how these individual mutations interact to control splicing outcomes.

Although splicing is perturbed in many diseases, we currently lack insight into the regulatory mechanisms promoting its precision and efficiency. We derived mechanistic mathematical models describing the recruitment of spliceosomes to splice sites and subsequent execution of splicing reactions. We show that alternative splicing control is facilitated if spliceosomes recognise exons as functional units ('exon definition') and that exon definition is crucial to prevent the accumulation of partially spliced retention products during alternative splicing regulation. Furthermore, exon definition modularises splicing control, as multiple regulatory inputs are integrated into a common net input irrespective of the location and nature of the corresponding *cis*-regulatory elements in the pre-mRNA. These predictions of our model are qualitatively and quantitatively supported by high-throughput mutagenesis data obtained for an alternatively spliced exon in the proto-oncogene *RON* (*MST1R*). Our analysis provides insights into the kinetic mechanisms of splicing regulation and suggests that exon definition has evolved as the dominant splice-regulatory mechanism in higher organisms to promote robust and reliable splicing outcomes.

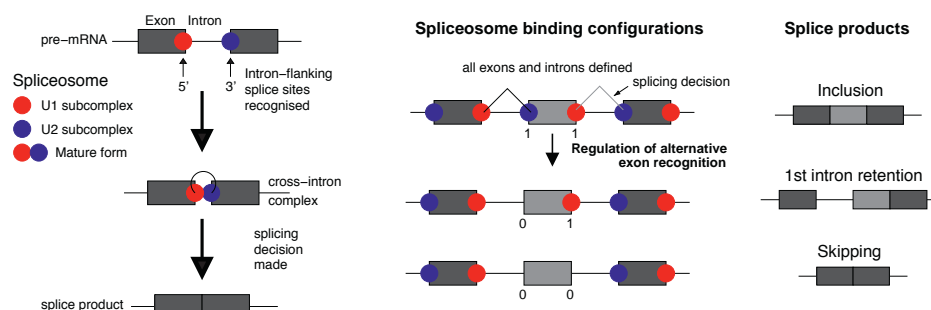


Figure 1. Kinetic modelling of splicing decisions. Splice sites in the nascent pre-mRNA are recognised by spliceosome subunits (red and blue dots). Higher-order spliceosome complexes then catalyse intron removal. The kinetic model describes all possible spliceosome binding configurations in a three-exon gene (three shown) and links them to splicing products. Thereby, it provides quantitative insights into coordinated splice isoform changes upon sequence mutations or regulation of splicing by RNA-binding proteins.

FUTURE DIRECTIONS

We plan to further refine and develop existing models of signalling and gene expression. For instance, we are investigating the stochastic dynamics of TGF β /SMAD signalling and modelling downstream gene expression networks based on time-resolved RNA sequencing data. Using publicly available data from cancer patients, we will employ machine learning to predict novel

Single-cell dynamics of cell cycle networks

The segregation of chromosomes during mitosis and their subsequent distribution into daughter cells are critical events that need to be completed in the correct order to maintain genome stability. Using live-cell imaging, we found that chromosome segregation and other mitotic events in *S. pombe* are temporally coordinated with high precision, even if the system is strongly perturbed. Using a combination of kinetic modelling and experimentation, we identified mechanisms that coordinate highly dynamic parallel pathways to buffer timing against signalling protein fluctuations. In our current work, we developed a quantitative modelling approach in which we describe a heterogeneous population of dividing cells using an ensemble of single-cell models. Fitting this *in silico* cell population to measured protein degradation and cell cycle data from single cells during anaphase, enabled us to simulate rare outlier cells in which chromosome segregation is no longer coordinated with other mitotic events. These cells could potentially undergo chromosome mis-segregation, genomic instability, or cell death. Using the calibrated model, we predicted experimental perturbations that lead to enrichment of the mis-segregating subpopulation and subsequently validated these predictions experimentally. Our models provide insights into the robustness and vulnerability of cellular networks and the molecular determinants of successful cell cycle completion.

RNA-binding proteins controlling selected splicing decisions and follow up on predicted candidates using knockdown approaches. Moreover, we have begun to model the dynamics of DNA methylation and de-methylation to better understand how this important epigenetic mark can be set and erased to tune gene activity.

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* indicates joint contribution # indicates joint correspondence

*“Floppy but not sloppy!
Evolution selected dis-
ordered proteins for complex,
yet specific, functionality”*

EDWARD LEMKE



EDUCATION

- 2005** PhD in Chemistry, Max Planck Institute for Biophysical Chemistry & University of Göttingen
- 2001** Diploma in Chemistry, Technical University of Berlin
- 2001** MSc in Biochemistry, University of Oklahoma

POSITIONS HELD

- Since 2018** Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Professor of Synthetic Biophysics, Johannes Gutenberg University Mainz
- Since 2009** Group Leader, European Molecular Biology Laboratory (EMBL), Heidelberg (visiting since 2018)
- 2005 – 2008** Postdoc, The Scripps Research Institute, La Jolla

GROUP MEMBERS

- Joana Caria** Lab Manager; since 04/2017
- Nike Heiness** PhD Student; since 05/2019
- Christine Koehler** PhD Student; since 01/2017
- Cathrin Lutz** Group Administrator; since 05/2019
- Sofya Mikhaleva** PhD Student; since 09/2015
- Panagiotis Patsis** PhD Student; since 11/2018
- Christopher Reinkemeier** PhD Student; since 09/2016
- Piau Siong Tan** Postdoc; since 04/2015
- Mikhail Sushkin** PhD Student; since 09/2019
- Miao Yu** Postdoc; since 10/2018

OVERVIEW

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

RESEARCH HIGHLIGHTS

Our strong focus on the mechanistic understanding of IDPs using single molecule and super-resolution tools is both driven by and driving novel tool developments for “in-cell/*in situ* structural biology.” This comprises a synergistic effort of chemical/synthetic biology and precision fluorescence-based technology/nanoscopy/microfluidics development (Figure 1).

A major technical breakthrough of my lab was the ability to engineer “click”able functionalities into any protein *in vitro* and *in vivo*. This genetic code expansion (GCE) approach has the potential to become a true GFP (fusion protein) surrogate strategy, with the major advantages being that superior synthetic dyes can be coupled with residue-specific precision anywhere in a protein. This opens up new avenues in single-molecule fluorescence and super-resolution microscopy.

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

our understanding of how IDPs maintain different functionalities through conformational changes despite the normal requirement for rigid molecular specificity.

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

FUTURE DIRECTIONS

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

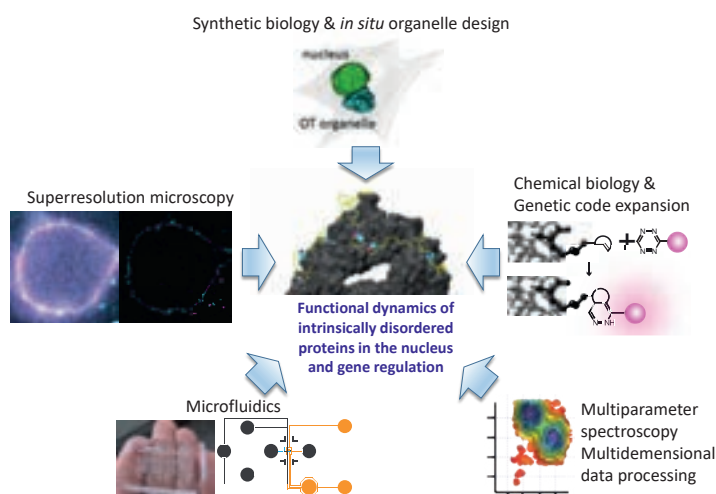


Figure 1. Schematic representation of technologies used in the Lemke Lab to study intrinsically disordered proteins.

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“Regulation of RNA:DNA hybrids is relevant in human disease & ageing”

BRIAN LUKE



EDUCATION

- 2005** PhD in Biochemistry, Swiss Federal Institute of Technology (ETH), Zurich
- 1999** BSc in Biology, Queen's University, Ontario

POSITIONS HELD

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- 2005 – 2009** Postdoc, Swiss Federal Institute of Technology Lausanne (EPFL)
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- Dennis Knorr** Student Assistant; 09/2019
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- Vanessa Pires** PhD Student; since 08/2017
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- Tina Wagner** PhD Student; since 11/2015

OVERVIEW

RNA:DNA hybrids are now recognised as important regulatory entities that influence gene expression, the chromatin environment, and DNA repair activities. R-loops are RNA:DNA hybrids that, for example, act as guides for regulating gene expression. However, RNA:DNA hybrids must be tightly regulated as misregulation can quickly lead to DNA replication stress, DNA damage, and genome instability. We have previously demonstrated how RNA:DNA hybrids at telomeres fine-tune the telomere shortening process and ensure proper entry into replicative senescence. This is achieved in part by RNase H enzymes, which degrade the RNA moiety of an RNA:DNA hybrid. RNase H exists in two forms, RNase H1 and RNase H2. H1 degrades consecutive stretches of RNA nucleotides (at least 4) hybridised to DNA, whereas H2 can additionally remove single ribonucleotides that have been introduced into dsDNA in a reaction referred to as ribonucleotide excision repair (RER). Recently, it has been demonstrated that RNase H2 enzymes are frequently mutated in certain types of cancer, and are even considered to be driver mutations. Moreover, 50% of patients with the severe neurodegenerative disease Aicardi-Goutières Syndrome have mutations in one of the three RNase H2 subunits. In the last year, we have made important discoveries with respect to how RNase H enzymes are regulated. Through the course of this work, we may have revealed an alternative RER pathway that will be the focus of future studies.

RESEARCH HIGHLIGHTS

We used budding yeast to create alleles of the RNase H enzymes where their expression could be restricted to a specific cell cycle stage (either S or G2/M phase). We observed that restricting expression of RNase H2 to G2/M phase was sufficient to complement

the phenotypic defects associated with both faulty R-loop metabolism as well as defective RER. Importantly, restricting expression of H2 to S phase could not complement these phenotypes and, in the case of RER, even exacerbated phenotypes. Many of the phenotypes associated with defective RER are a result of aberrant topoisomerase 1 (Top1) activity at ribonucleoside monophosphates (rNMPs). Accordingly, the loss of RNase H2 phenotypes can largely be rescued upon further inactivation of *TOP1*. Strikingly, however, the RER defects associated with restricting RNase H2 to S phase are not improved when *TOP1* is deleted. We were able to demonstrate that RNase H2 causes DNA damage in S phase that requires *RAD52* for repair. This exciting result suggests that there may be two modes for rNMP removal by RNase H2: the canonical RER pathway, which appears to be optimally executed in a post-replicative manner, and an alternative pathway, which occurs when RNase H2 acts in S phase (Figure 1). Currently, we are only aware that the alternative RER pathway requires *RAD52* and *RTT101*. Future experiments will be aimed at further characterising this repair pathway.

RNase H1 can be expressed either in S phase or post-replicatively to complement the phenotypes of defective R-loop processing. Unlike RNase H2, RNase H1 responds to R-loop

accumulation by associating readily to chromatin. Together, and in combination with other work, these results suggest that RNase H2 is the primary RNA:DNA hybrid removal nuclease at R-loops and rNMPs, and that its activity is largely, but not exclusively, restricted to a post-replicative time point. This ensures that the majority of the RER nickase activity does not occur during DNA replication, which would result in double-strand breaks (DSBs). Nonetheless, residual rNMPs that were not removed in the previous cell cycle may utilise this alternative pathway, which depends on *RAD52* and *RTT101*. When R-loops accumulate and induce stress (likely replication stress), RNase H1 is efficiently recruited to chromatin to remove them. It makes sense that H1, and not H2, is the responder to R-loop stress so that H2 does not aberrantly nick DNA in S phase.

In addition and complementary to this work, we discovered that the Ubiquitin E3 ligase Rtt101^{Mms1} ubiquitylates the DNA polymerase epsilon subunit Dpb2 in response to high rNMP load. We hypothesise that this ubiquitylation may be important to allow efficient replication past nicked rNMPs. Indeed, restricting RNase H2 expression to S phase is toxic in the *rtt101* mutant background.

FUTURE DIRECTIONS

We will try to get a more complete picture of how RNase H1 is activated following R-loop-induced stress. We have employed genome-wide screens aimed specifically at elucidating both positive and negative regulators of RNase H1 under stress. Promising candidates will be further characterised in both biochemical and functional assays. Similar screens have been performed with RNase H2, and a proteomics screen will be performed to understand the dynamic interactome with respect to the cell cycle. We will continue to characterise the alternative RER pathway we have stumbled upon, especially with respect to how Rtt101 and replisome regulation is involved.

Work on the regulation of TERRA R-loops at telomeres continues to be an important project in the lab and proteomic screens have elucidated additional candidates that may be involved in R-loop regulation at telomeres.

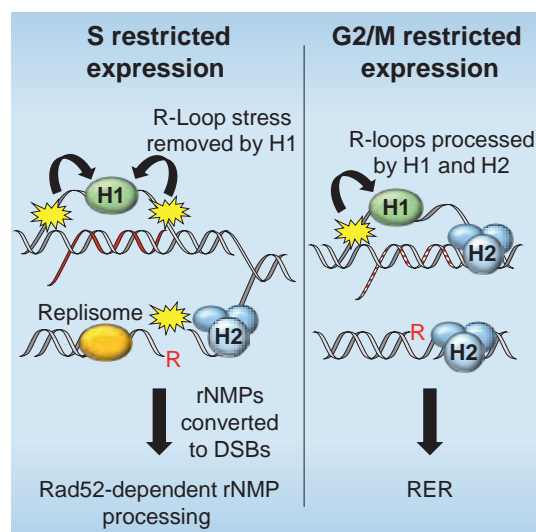


Figure 1. RNase H2 expression exclusively in the G2/M phase is both necessary and sufficient to properly process RNA:DNA hybrids (both R-loops and rNMPs). Expression of RNase H2 exclusively in S phase leads to DNA damage that requires recombination-dependent repair. RNase H1 responds to RNA:DNA hybrid-induced stress through chromatin recruitment in all cell cycle phases.

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* indicates joint contribution # indicates joint correspondence

*“Our three ‘R’s:
antisense RNAs, R-loops,
gene Regulation”*

CHRISTOF NIEHRS

EDUCATION

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

POSITIONS HELD

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

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- Andrea Schäfer** Postdoc; since 09/2010
- Carola Scholz** Technician; since 05/2015
- Lars Schomacher** Postdoc; since 07/2011
- Katrin Schüle** PhD Student; since 01/2016
- Philipp Trnka** PhD Student; since 11/2016
- Viviana Vastolo** Postdoc; since 09/2015

OVERVIEW

In the genome of many multicellular organisms, DNA methylation is a common epigenetic mark associated with gene silencing. DNA methylation is a dynamic process and can be reversed by enzymatic demethylation, a process that is still incompletely understood. DNA demethylation is a widespread phenomenon occurring in plants as well as in animals during development, in the adult, and during the somatic cell reprogramming of pluripotency genes. We showed that Growth arrest and DNA damage 45A (GADD45A) is a key player in active DNA demethylation and acts via DNA repair. One goal of our research is to analyse the mechanism of DNA demethylation as well as the role played by GADD45A in development. We established that GADD45A acts as an adapter protein, which directs DNA methylation machinery to specific loci. To address GADD45A's function, we use biochemical, molecular biology and cell biological approaches, employing the mouse and frog model systems as well as embryonic stem cells.

RESEARCH HIGHLIGHTS

DNA methylation plays important roles in regulating gene expression, genomic imprinting, X-chromosome inactivation, genomic instability, embryonic development, and cancer. DNA methylation is reversible by active DNA demethylation, with examples in plants, animal development, cancer, and immune cells. Yet, the molecular mechanisms underlying active demethylation are still unclear.

We showed that the stress response protein GADD45A mediates active DNA demethylation. GADD45 proteins regulate a range of cellular processes, including DNA repair, proliferation, apoptosis, and differentiation. GADD45A directly interacts with the two key enzymes of the DNA demethylation machinery, TET

(Ten-eleven translocation) and TDG (thymine-DNA glycosylase), to enhance turnover of oxidised cytosines. Therefore, GADD45A acts as an adapter that recruits DNA modifying enzymes to specific sites in the genome and promotes local demethylation.

To target specific genomic loci, GADD45A relies on bridging factors, which include RNA. We previously showed that expression of the tumour suppressor *TCF21* is activated by a lncRNA named TARID (*TCF21* antisense RNA inducing demethylation), which is transcribed antisense to *TCF21*. TARID recruits the DNA demethylation machinery to the *TCF21* promoter, leading to decreased DNA methylation and increased *TCF21* expression. How does TARID interact with the *TCF21* promoter? In a collaboration with Ingrid Grummt, we find that R-loops target GADD45A to specific loci. R-loops are DNA-RNA hybrids enriched at CpG islands (CGIs) that regulate chromatin states. How R-loops are recognised and interpreted by specific epigenetic readers is unknown. We found that GADD45A binds directly to R-loops and mediates local DNA demethylation by recruiting TET1. TARID indeed forms an R-loop at the *TCF21* promoter and binding of GADD45A to the R-loop triggers local DNA demethylation and *TCF21* expression. We showed that TARID transcription, R-loop formation, DNA demethylation, and *TCF21* expression proceed sequentially during the cell cycle. Oxidised DNA demethylation intermediates are enriched at R-loops and increase upon RNase H1 depletion. Using genomic profiling in embryonic stem cells, we identified thousands of R-loop-dependent TET1 binding sites at CGIs. This work indicates that GADD45A is an epigenetic R-loop reader that recruits the demethylation machinery to promoter CGIs.

The GADD45 protein family contains several members, including GADD45A, GADD45B, and GADD45G. While our work supports that these proteins are adaptors for TET/TDG mediated DNA demethylation, it remains poorly understood in which physiological processes and at which genomic loci they mediate demethylation. In addition, they show overlapping expression and hence may be functionally redundant, complicating their analysis. To address these issues, we analysed *Gadd45a,b,g* triple-knock-out (TKO) mouse embryonic stem cells (mESC) and *Gadd45a,b* DKO mice. mESC cultures contain a rare population of '2C-like' cells resembling 2-cell embryos, the key stage of zygotic genome activation (ZGA). Little is known about positive regulators of the 2C-like state and 2-cell stage embryos. We found that GADD45

proteins promote both states. Methylome analysis of *Gadd45* TKO mESCs revealed hypermethylation of ~7,000 sites, which are enriched for enhancers and loci undergoing TET-TDG mediated demethylation. Gene expression is misregulated in TKO cells, notably upon neural differentiation, and displays signatures of expression changes of DNMT and TET targets. While *Gadd45* TKO mESCs remain pluripotent and self-renew, they manifest impaired transition into the 2C-like state and exhibit DNA hypermethylation and downregulation of 2C-like state specific genes. *Gadd45a,b* double mutant mouse embryos display embryonic sublethality, deregulated ZGA gene expression, and developmental arrest. These results reveal an unexpected role of GADD45 proteins in embryonic 2-cell stage regulation.

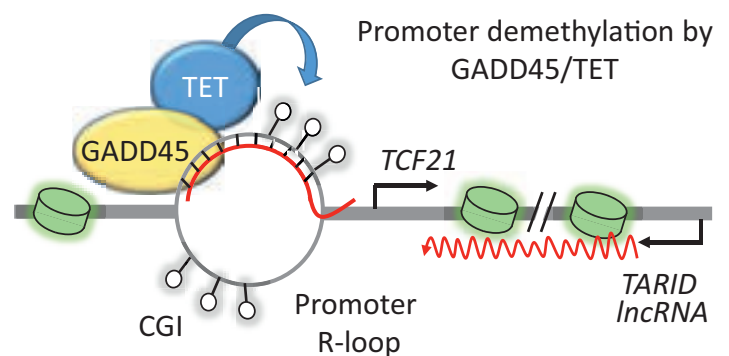


Figure 1. Model showing how the lncRNA TARID acts as a guide RNA to form an R-loop at the *TCF21* promoter. This regulatory R-loop is recognised by the R-loop reader GADD45A, which recruits the DNA demethylation machinery (e.g. TET DNA demethylase). Demethylated CpGs (lollipops) at a CpG island are indicated.

FUTURE DIRECTIONS

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

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* indicates joint contribution # indicates joint correspondence

“RNA modifications are dynamic & have the potential to alter RNA metabolism”

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EDUCATION

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

POSITIONS HELD

- 2012 – 2019** Group Leader, Institute of Molecular Biology (IMB), Mainz
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- Jessica Leismann** Master's Student; 04/2018 – 09/2019
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- Chiara Paolantoni** PhD Student; since 10/2017
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- Michael Stock** PhD Student; since 06/2018
- Minh Anh Vu** Master's Student; 04/2018 – 09/2019
- Lina Worpenberg** PhD Student; since 08/2017

OVERVIEW

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

RESEARCH HIGHLIGHTS

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

The widespread roles of m⁶A RNA modification in the regulation of post-transcriptional gene expression have recently been brought to light. From yeast to mammals, m⁶A has been shown to regulate pre-mRNA splicing, translation, and mRNA decay. However, the precise mechanism of this modification in these processes still remains to be determined. Furthermore, the physiological functions of m⁶A in multi-cellular organisms have not yet been fully investigated. To address these questions, we have characterised the m⁶A pathway in *Drosophila*. We found that a

conserved m⁶A methyltransferase complex controls alternative splicing both in *Drosophila* cells and *in vivo*. As in mammals, components of the complex are ubiquitously expressed but show significant enrichment in the nervous system, which is consistent with the high level of m⁶A in this tissue. We find that flies mutant for *Mettl3* are viable but suffer from severe locomotion defects due to impaired neuronal functions. A synaptic overgrowth was observed at neuromuscular junctions. Components of the m⁶A methyltransferase complex also control the female-specific splicing of the Sex lethal (*Sxl*) transcript and its downstream targets, revealing a role for this modification in sex determination and dosage compensation. We have recently identified a new member of the methyltransferase complex that we have named Flacc for Fl(2)d Associated Complex Component. Our work suggests that Flacc serves as an adapter to link Spenito with other components of the methyltransferase complex. We demonstrated that this association is critical for m⁶A deposition. In addition, we have developed a new approach to map m⁶A *in vivo* as current techniques were not adapted for *in vivo* studies. We are currently taking advantage of this approach to characterise m⁶A molecular targets and their functions during organismal development.

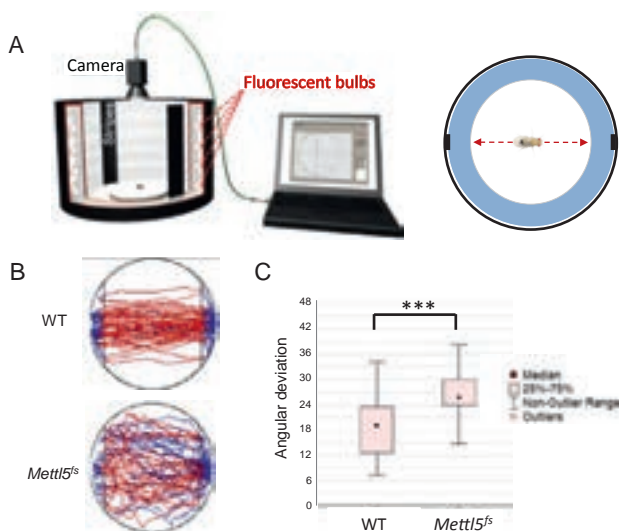


Figure 1. (A) Cartoons depicting (left) the Buridan's paradigm (from Colomb et al, 2012), which is used to assess behavioural function, and (right) the normal behaviour of a wild-type (WT) fly. Black rectangles represent the visual landmarks and the red arrow the main trajectory undertaken by WT flies. (B) Representative trajectories of WT and *Mettl5* mutant flies analysed by Buridan's Paradigm. The blue lines indicate when the fly stops and changes direction. (C) Quantification of orientation in WT and *Mettl5* mutant flies.

Functions of Pseudouridine synthase 7 (Pus7) in neuronal behaviour

For decades, pseudouridine has been known to be the most abundant modification on transfer RNA (tRNA) and ribosomal RNA (rRNA). More recently, it has also been found to be widespread on mRNA. In humans, pseudouridine modifications are catalysed via 13 distinct pseudouridine synthases (PUSs), each of which has distinct specificities, most of them only poorly understood. In collaboration with the groups of Schraga Schwartz (Weizmann Institute) and Arjan de Brouwer (Radboud University), we have identified several patients with distinct *Pus7* mutations. All these individuals have intellectual disabilities with delayed speech, short stature, microcephaly, and aggressive behaviour. We showed that the disease-related variants lead to abolishment of PUS7 activity on both tRNA and mRNA substrates. Moreover, we generated a *Pus7* knockout in *Drosophila melanogaster*. Mutant flies are viable but display a number of behavioural defects (Figure 1), including increased activity, disorientation, and aggressiveness, supporting the idea that neurological defects are caused by *PUS7* variants. Therefore, our findings demonstrate that RNA pseudouridylation by PUS7 is essential for proper neuronal development and function. We are currently looking for the *Pus7* targets that mediate neuronal defects in both *Drosophila* and patient cells.

FUTURE DIRECTIONS

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

SELECTED PUBLICATIONS

Akhtar J, Kreim N, Marini F, Mohana G, Brüne D, Binder H and Roignant JY (2019) Promoter-proximal pausing mediated by the exon junction complex regulates splicing. *Nat Commun*, 10:521

Knuckles P*, Lence T*, Haussmann IU, Jacob D, Kreim N, Carl SH, Masiello I, Hares T, Villaseñor R, Hess D, Andrade-Navarro MA, Biggiogera M, Helm M, Soller M, Bühler M and Roignant JY (2018) Zc3h13/Flacc is required for adenosine methylation by bridging the mRNA-binding factor Rbm15/Spenito to the m⁶A machinery component Wtap/Fl(2)d. *Genes Dev*, 32:415–429

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* indicates joint contribution

*"We investigate how
oncogenic genome
rearrangements arise"*

VASSILIS ROUKOS

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

- Marta Cipinska** PhD Student; 05/2015 – 05/2019
- Henrike Gothe** PhD Student; since 05/2016
- Monika Kuban** Lab Manager; since 01/2017
- Marie Kube** Research Assistant; since 10/2019
- Gabriel Longo** PhD Student; since 09/2019
- Vera Minneker** PhD Student; since 04/2016
- Rossana Piccinno** PhD Student; since 04/2015
- Gabrielle Sant** PhD Student; since 11/2018

OVERVIEW

Maintaining the integrity of genetic information is essential for cell survival. Mechanisms that counteract DNA damage are important for maintaining 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 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 chromosome fusion and what are the factors that favour interchromosomal fusion (translocation) over intrachromosomal repair? By using a combination of molecular biology techniques, genetics, and high-throughput imaging and sequencing approaches, we aim to shed light on the basic molecular mechanisms underlying the formation of oncogenic chromosome translocations.

RESEARCH HIGHLIGHTS

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

Modelling the formation of recurrent cancer-initiating genome rearrangements of interest requires a versatile approach that can probe rare events with high sensitivity. We have now established a method called C-Fusion 3D that uses fluorescence *in situ* hybridisation (FISH) to probe the position of individual chromosome ends of potential translocation partners in interphase cells in 3D. High-throughput microscopy and automated image analysis is

then used to identify single cells with chromosome breakage and translocations. This methodology complements existing approaches and offers several advantages in detecting and quantifying translocations. It is: (i) suitable for detecting translocations without the requirement to map the precise translocation breakpoints or fusion product; (ii) 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); (iii) efficient in detecting translocations in interphase cells at frequencies down to 10^{-4} without the need for metaphase spread preparation. 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

Cancers are commonly treated with anticancer drugs called topoisomerase poisons. Unfortunately, treatment with topoisomerase poisons can also cause chromosome translocations in healthy cells that disrupt gene regulation and lead to the development of leukaemia. However, it is unclear why these leukaemia-promoting translocations are so common after treatment with topoisomerase poisons.

We are interested in combining cutting-edge genomics and single-cell imaging methods to determine why these leukaemia-promoting translocations arise. Our current work has shown that certain sites with highly active genes tend to be close to

regions of DNA folding into chromatin loops that are under more mechanical strain. This makes them susceptible to DNA breaks caused by topoisomerase poisons, producing translocations that drive leukaemia. We have also identified factors involved in the repair of these DNA breaks that actively suppress the formation of these translocations. In another direction that may have clinical implications, we are performing unbiased siRNA-based screens to identify factors that suppress these types of translocations, while leaving the cytotoxic effect of topoisomerase poisons intact. Our findings highlight how gene activity and the arrangement of DNA within the nucleus can have a profound impact on events that trigger genomic instability to promote cancer.

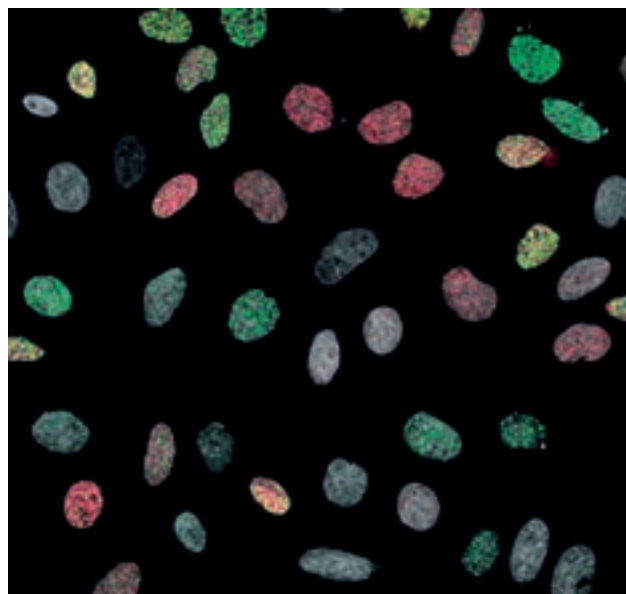


Figure 1. Genomic instability in human U2OS cells treated with the topoisomerase poison Etoposide, showing the DNA break markers γ H2AX (red), phospho-RPA (green), and DNA (grey).

FUTURE DIRECTIONS

Central to our focus is shedding light on the events promoting genomic instability in the context of chromatin and chromosome organisation. We, therefore, intend to profile endogenous DNA breaks across the genome in various cell types, with the aim of identifying common or cell type-specific signatures of DNA fragility. We will then focus on identifying mechanistically how these endogenous DNA breaks form and evaluate how DNA break

repair efficiency is influenced by the genomic and chromatin context. These studies will directly highlight the link between cell type-specific DNA fragility and repair in the formation of tissue-specific, recurrent oncogenic translocations. Taken together, our research will shed light on the mechanisms of cancer-initiating translocations, which will advance our knowledge of the fundamental principles of cancer aetiology.

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Gothe HJ, Bouwman BAM, Gusmao EG, Piccinno R, Petrosino G, Sayols S, Drechsel O, Minneker V, Josipovic N, Mizi A, Nielsen CF, Wagner EM, Takeda S, Sasanuma H, Hudson DF, Kindler T, Baranello L, Papantonis A, Crosetto N and Roukos V (2019) Spatial chromosome folding and active transcription drive DNA fragility and formation of oncogenic MLL translocations. *Mol Cell*, 75:267–283.e12

Lucic B, Chen HC, Kuzmann M, Zorita E, Wegner J, Minneker V, Wang W, Fronza R, Laufs S, Schmidt M, Stadhouders R, Roukos V, Vlahovicek K, Filion GJ and Lusic M (2019) Spatially clustered loci with multiple enhancers are frequent targets of HIV-1 integration. *Nat Commun*, 10:4059

Roukos V. (2018). Actin proteins assemble to protect the genome. *Nature*, 559: 35–37

“Lesions in the replication template are processed in dedicated compartments”

HELLE ULRICH



EDUCATION

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

POSITIONS HELD

- Since 2018** Executive Director, Institute of Molecular Biology (IMB), Mainz
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- 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), University of Heidelberg

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- Christian Renz** Postdoc; since 08/2014
- Axel Schäfer** PhD Student; 10/2017 – 07/2019
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- Vera Tröster** PhD Student; since 01/2017
- Maria Villagomez-Torres** PhD Student; since 01/2018
- 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

A robust response to DNA replication stress is an important defence mechanism against genome instability and serves as a last barrier against the development of cancer. In order to avoid permanent arrest when replication forks encounter lesions in the replication template, cells have developed DNA damage bypass mechanisms. 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 cell survival in the presence of genotoxic agents. However, as lesion bypass is often associated with damage-induced mutations, the pathway is also a potential source of genome instability in itself and therefore needs to be tightly controlled. We are interested in elucidating how DNA damage bypass is organised in the context of chromatin and how the process is coordinated with genome replication and global damage signalling.

RESEARCH HIGHLIGHTS

Tracing DNA repair factors by fluorescence microscopy provides valuable information about how DNA damage processing is orchestrated within the nucleus. Most repair pathways involve single-stranded ssDNA. As such, the ssDNA-binding Replication Protein A (RPA) complex is a hallmark of DNA damage and replication stress. For example, DNA double-strand breaks and damaged replication forks are known to give rise to distinct RPA foci that move to nuclear pores or the nuclear periphery in budding yeast. In contrast, RPA foci emerging in response to tolerable loads of polymerase-blocking lesions during S phase are generally

thought to indicate stalled replication intermediates rather than strand breaks. We previously showed that DNA damage bypass is, in principle, separable from genome replication, causing regions of ssDNA to accumulate behind replication forks. These so-called daughter-strand gaps could thus also contribute to damage-induced RPA foci. We, therefore, asked whether RPA could serve as a reporter of DNA damage bypass events in replicating cells and how such events are coordinated with overall genome replication.

Using live-cell imaging and localisation of fluorescently tagged RPA in fixed cells, we quantified the emergence and resolution of spontaneous as well as damage-induced RPA foci over the course of the cell cycle. We analysed the dynamic properties of these foci and their relationship to other replication and repair proteins, such as recombination factors and helicase, as well as newly synthesised DNA. We found that RPA foci predominantly represent DNA damage bypass tracts induced by polymerase-blocking lesions both spontaneously and under conditions of replication stress. These are largely resolved via the RAD6 damage bypass pathway, involving translesion synthesis and template switching, but also classical

homologous recombination. Surprisingly, the RPA foci form far away from sites of ongoing replication (Figure 1A, B), and they do not overlap with any of the repair centres associated with collapsed replication forks or double-strand breaks. Instead, they represent postreplicative repair territories (or 'PORTs', as we now call them) that arise from polymerase re-priming in replication centres and subsequent expansion of daughter-strand gaps over the course of S phase.

Our observations challenge the prevalent view that spontaneous or replication stress-induced repair foci generally represent stalled replication forks. Instead, they suggest a model where these foci originate in the wake of replication forks as local clusters of ssDNA due to multiple replisome re-priming events in spatial proximity (Figure 1C). Subsequent expansion over time contributes to the gradual emergence of discernible RPA foci in regions of replicated DNA that are resolved locally by DNA damage bypass activity and homologous recombination. Thus, it appears that processing of DNA lesions not involving double-strand breaks in budding yeast is both temporally and spatially well-segregated from ongoing replication.

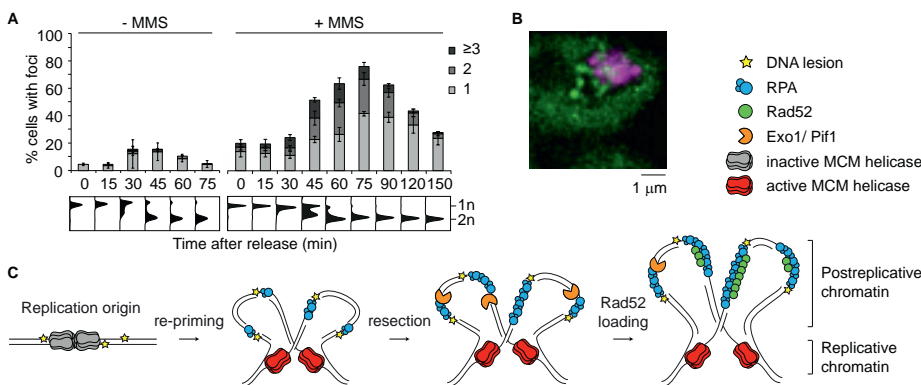


Figure 1. Coordination of DNA damage bypass with genome replication. (A) Distribution of spontaneous and damage (MMS)-induced RPA foci in budding yeast cells over the course of a cell cycle. (B) Fluorescently tagged replicative helicase (Mcm44GFP, green) and damage-induced RPA (Rfa1mRuby, magenta) do not co-localise during S phase. (C) Model for the emergence of daughter-strand gaps and their expansion to RPA foci in postreplicative repair territories. (Figure adapted from Wong *et al.*, *Mol Cell*, 2019).

FUTURE DIRECTIONS

Having demonstrated that the response to replication-blocking lesions and spontaneous replication stress largely occurs at a significant distance from active replication forks in budding yeast, it will be instructive to examine to what extent our findings apply to vertebrate cells, where both fork-associated and postreplicative modes of translesion synthesis have been reported. Despite the higher complexity of chromatin in vertebrate cells, most of the principles of genome maintenance are fundamentally conserved among eukaryotes. This suggests that attempts to manipulate the

response to replication stress for therapeutic purposes should not exclusively focus on the replication fork as a target. In addition, we are currently establishing an experimental system to enzymatically introduce defined non-strand-break lesions in a specific region of the yeast genome in order to follow the progression of the replisome over a damaged sequence with high spatial and temporal resolution. This will enhance our understanding of the mechanisms by which the replisome reacts to obstacles and the influence of the chromatin environment on damage bypass.

SELECTED PUBLICATIONS

Wong RP, García-Rodríguez N, Zilio N, Hanulová M and Ulrich HD (2019) Processing of DNA polymerase-blocking lesions during genome replication is spatially and temporally segregated from replication forks. *Mol Cell*, doi: 10.1016/j.molcel.2019.09.015

García-Rodríguez N, Morawska M, Wong RP, Daigaku Y and Ulrich HD (2018) Spatial separation between replisome- and template-induced replication stress signaling. *EMBO J*, 37:e98369

García-Rodríguez N[#], Wong RP and Ulrich HD[#] (2018) The helicase Pif1 functions in the template switching pathway of DNA damage bypass. *Nucleic Acids Res*, 46:8347–8356

[#] indicates joint correspondence

“We are interested in molecular connections between the circadian clock & genome maintenance”

EVA WOLF



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

POSITIONS HELD

- Since 2013** Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Professor of Structural Chronobiology, Faculty of Biology, Johannes Gutenberg University Mainz
- 2012 – 2013** Group Leader, Ludwig Maximilian University (LMU), 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

GROUP MEMBERS

- Arne Börgel** PhD Student; since 01/2016
- Marcel Conrady** PhD Student; since 10/2019
- Archit Garg** PhD Student; since 11/2014
- Tim Grimmelsmann** PhD Student; since 01/2015
- Silke Helmke** Technician; since 05/2014
- Shruti Krishnan** PhD Student; since 10/2016
- Torsten Merbitz-Zahradnik** Postdoc; since 01/2014
- Sebastien Moniot** Postdoc; since 06/2019
- Ruth Schaupp** Personal Assistant; since 05/2014

OVERVIEW

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

RESEARCH HIGHLIGHTS

Accumulating evidence suggests a functional interconnection between mammalian circadian clocks and genome maintenance. For example, DNA damage induces phase advances of circadian rhythms, likely involving the Timeless (TIM) protein and ATM-mediated damage signalling. Furthermore, Cryptochrome1 (CRY1) modulates ATR/Chk1-mediated DNA damage checkpoint responses in a circadian manner. To elucidate the molecular mechanisms

underlying these functional connections between the circadian clock and genome maintenance, we structurally, biochemically, and biophysically analyse interactions of the mammalian clock proteins CRY1/2 with TIM and the Timeless-interacting protein Tipin. Additionally, we analyse interactions of TIM and Tipin with replication fork-associated proteins such as RPA.

We found that the purified TIM-Tipin complex directly interacts with CRY1 and CRY2. The TIM-Tipin-CRY complex may recruit CRY proteins to the replication fork, for example, to modulate checkpoint responses. Conversely, the TIM-Tipin complex may be recruited to the circadian clock by its interaction with CRY1 or CRY2. While TIM, Tipin, and the TIM-Tipin complex are known to be involved in checkpoint signalling, fork protection, and PARP1-dependent DNA repair (Figure 1), Tipin and the TIM-Tipin complex have, so far, not been implicated in circadian regulation. Hence, the biological role of the mammalian TIM-Tipin-CRY complex in the circadian clock is unclear. We observed that CRY binding to the purified TIM-Tipin complex, to BMAL1 or PERIOD (PER) is mutually exclusive, implying overlapping binding sites. We, therefore,

speculate that the TIM-Tipin-CRY or TIM-CRY complex impacts on circadian BMAL1/CLOCK transcriptional activity by interfering with the formation of BMAL1-CRY complexes in the repressive phase of the circadian oscillator (Figure 1).

We further set out to identify molecular regions involved in TIM-Tipin-CRY interactions. We identified a short region in the mammalian TIM protein that modulates interactions with our purified CRY1 and CRY2 proteins. Deletion of this TIM region differentially affects CRY1 and CRY2 interactions *in vitro*, suggesting different biological roles of the TIM-(Tipin)-CRY1 and TIM-(Tipin)-CRY2 complexes. The identification of this TIM deletion construct enabled us to further dissect the distinct roles of the TIM-(Tipin)-CRY1 and TIM-(Tipin)-CRY2 complexes in the circadian clock and in genome maintenance and to search for new TIM functions. By combining 3D-structural, biochemical and biophysical analyses of TIM-Tipin-CRY interactions, we aim to provide insights into the interaction network and architecture of this complex and to understand its roles in circadian regulation and genome maintenance.

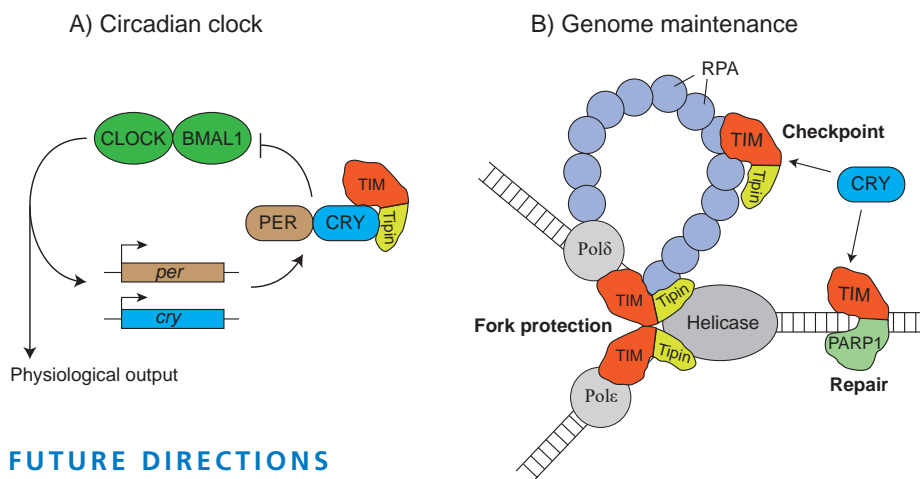


Figure 1. (A) Main gene regulatory feedback loop of the mammalian circadian clock. The transcription factor BMAL1/CLOCK activates CRY1/2 and PER1/2 clock protein expression. CRY and PER feedback repress BMAL1/CLOCK. (B) Roles of TIMELESS (TIM) and Tipin in DNA replication, checkpoint signalling and DNA-repair (via PARP1). TIM-Tipin-CRY1/2 complexes may connect the circadian clock and genome maintenance e.g. by affecting BMAL1/CLOCK, checkpoint signalling or DNA repair.

FUTURE DIRECTIONS

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

and the distinct roles of TIM-CRY or TIM-Tipin-CRY interactions in these processes? Finally, it will be interesting to find out if TIM-CRY or TIM-Tipin-CRY interactions play a role in mammalian DNA repair pathways.

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

SELECTED PUBLICATIONS

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

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CORE FACILITIES



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PROTEOMICS
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MEDIA LAB
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OVERVIEW

CORE FACILITIES

"The Core Facilities at IMB provide access to key technologies and offer services & training from experts"



There are currently seven Core Facilities (CFs) at IMB: Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics, Protein Production, and a Media Lab. The Bioinformatics, Genomics and Proteomics CFs can provide users with a "full service", from experimental design and quality control of samples to data generation, analysis, and data presentation. The Flow Cytometry and Microscopy/Histology CFs provide an "assisted service", where researchers work independently on CF equipment after introductory training by CF staff. Whether receiving full or assisted service, the CFs' staff are available for consultation and troubleshooting. Furthermore, CF staff often collaborate with researchers to provide customised or specialised services. For IMB researchers, all seven CFs are available for use. Beyond that, Flow Cytometry, Genomics, Microscopy/Histology and Proteomics CFs also offer their services to the larger research community in Mainz. CF services provided are based on user demand. For each facility, a user committee gives feedback on the equipment and user experience and helps define the services that each CF provides. These committees also help shape the implementation of applicable new services within the CFs. The overall Core Facility functions as a service axis by aligning and combining individual services in its units to innovate new workflows, for example, single cell sequencing, which requires a service overlap between flow cytometry and genomics. In addition to technical services, the CFs offer lectures on a variety of methods, as well as practical courses to instruct researchers in new techniques and instrumentation, data acquisition, experimental design, data processing, and analysis. These allow researchers to keep up-to-date with and broaden their knowledge of current and emerging technologies. Lectures are generally open to everyone.

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

Andreas Vonderheit

Director of Core Facilities and Technology

BIOINFORMATICS

CORE FACILITY

THE BIOINFORMATICS CORE FACILITY (BCF) supports researchers at IMB with computing infrastructure, web services, system administration, software training, and consulting on experimental design and statistics. In addition, BCF members actively participate in the computational processing, analysis, visualisation and interpretation of high-throughput “omics” data generated in the course of research projects. The BCF also provides bioinformatics expertise to the Collaborative Research Center “Regulation of DNA Repair & Genome Stability”.

SERVICES OFFERED

The BCF staff offer support on different levels depending on project needs, ranging from basic IT and bioinformatics services to full-scale scientific collaborations in the context of “big data” research projects:

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

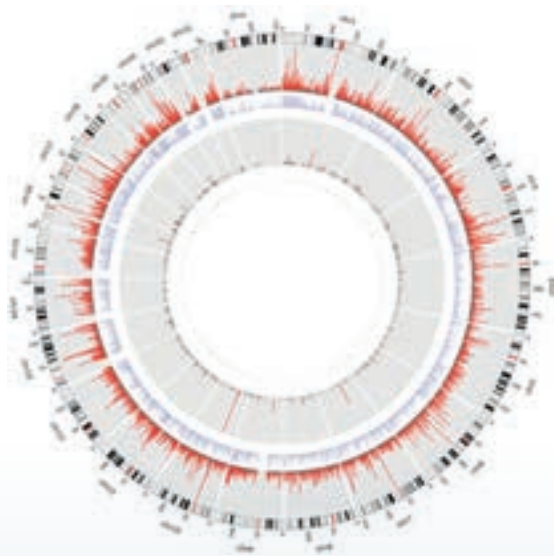


Figure 1. Circos plot depicting double-strand break (DSB) hotspots detected by the NGS method sBLISS in control (inner two circles) and Etoposide-treated (outer two circles) human TK6 cells; published in Gothe *et al.* (*Mol Cell*, 2019).

The BCF operates several computing and storage servers, and maintains web services providing user-friendly access to various analytical resources. The Facility maintains a GitHub repository (github.com/imbforge) with software tools and pipelines dedicated

to different types of next-generation sequencing (NGS) assays. The BCF also offers customised solutions and long-term analytical support for numerous data-intensive projects on a collaborative basis.



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Since 06/2019

Pascal Silberhorn System Administrator

Since 12/2015

FLOW CYTOMETRY CORE FACILITY

THE FLOW CYTOMETRY CORE FACILITY (FCCF) offers high-throughput measurements, analysis, and separation of biological units through four different systems: a large particle sorter, a cell sorter, and two analysers. With this equipment, the FCCF can analyse and sort particles of 0.5 μm to 1,000 μm in diameter.

SERVICES OFFERED

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



Stefanie Möckel Head

Since 10/2016

Jesús Gil Pulido Staff Scientist

Since 09/2018

Figure 1. Phosphorylation of histone H3-Ser10 during mitosis.

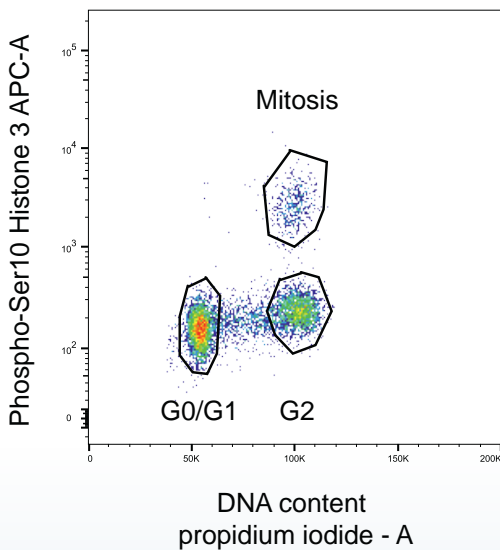


Figure 2. BD FACSAria, a high-speed benchtop cell sorter used to sort fluorescent cells into tubes and multiwell plates.



GENOMICS

CORE FACILITY

THE GENOMICS CORE FACILITY (GCF) offers next-generation sequencing (NGS) services based on the Illumina NextSeq500 and MiSeq platforms.

SERVICES OFFERED

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

After submission of RNA or DNA samples, the GCF performs initial quality control of the samples, library preparation, quality control of the prepared libraries, sequencing, and raw data generation. Currently, the GCF supports library preparation for more than twenty applications as a standard service and develops new protocols to accommodate the user's needs for their specific projects.

In 2019, the GCF acquired a Mosquito HV genomics liquid handler (TTP Labtech) to assist in library preparation. Furthermore, the GCF also added a 10x genomics chromium system to its instrument line-up to enable further development in single-cell genomic analysis.

RNA:

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

DNA:

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

User-prepared libraries:

- + iCLIP-Seq
- + Amplicon-Seq
- + ATAC-Seq
- + 4C / Capture-C
- + RR-MAB-Seq
- + GLOE-Seq
- + LAM-HGTS



Maria Mendez-Lago Head

Since 04/2016

Hanna Lukas Technician

Since 01/2013

Clara Werner Technician

07/2015 – 09/2019

Regina Zimmer Technician

Since 12/2019

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



MICROSCOPY & HISTOLOGY

CORE FACILITY

THE MICROSCOPY & 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.

SERVICES OFFERED

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

In 2019, the MHCF updated and increased the Columbus database for high-content image storage and analysis from 100 TB to 200 TB. The facility is now using applications, both open source and commercial from the fields of machine learning and artificial intelligence to improve the quality of the quantitative data produced in the facility.

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



Sandra Ritz Head

Since 01/2016

Márton Gelléri Staff Scientist

Since 06/2019

Mária Hanulová Staff Scientist

02/2014 – 03/2019

Jonas Schwirz Staff Scientist

06/2016 – 03/2019

Petri Turunen Staff Scientist

Since 08/2019

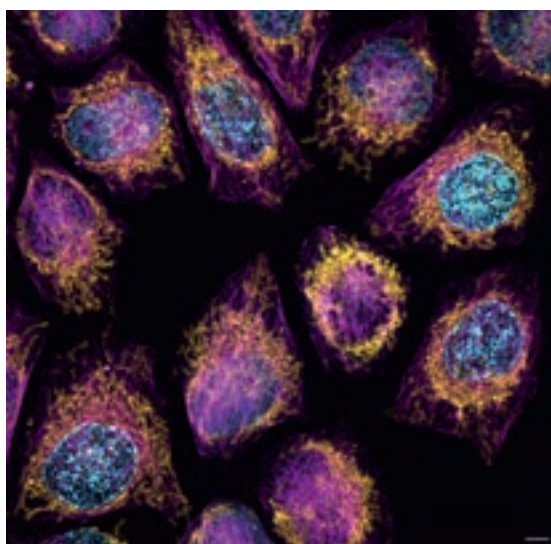


Figure 1. Maximum intensity projection of an 11.4 μm deep confocal z-stack of live U2Os cells stably expressing a cell cycle marker, a nanobody fused to TagRFP and targeting PCNA (Cyan). The cells are stained with a mitochondria marker MitoView™ Green (Yellow) and a microtubulin marker SiR-Tubulin (Magenta). The image was acquired using Visiscope 5-Elements spinning disc confocal system from Visitron Systems (60x/1.2 NA water immersion objective) and the image was further processed using Huygens Deconvolution software from Scientific Volume Imaging. The scale bar is 5 μm .

PROTEIN PRODUCTION

CORE FACILITY

THE PROTEIN PRODUCTION CORE FACILITY (PPCF) provides support with the design, expression, purification, and assay development of recombinant proteins that are used in IMB's research. The facility also offers a variety of common protein tools that are routinely used by IMB researchers on a day-to-day basis.

SERVICES OFFERED

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

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



Martin Möckel Head
Since 03/2018



Figure 1. The Biorad NGC Quest Plus chromatography system is the new workhorse FPLC within the PPCF. It is located inside a cooling cabinet and offers a high degree of versatility. Semi-independent workflows are possible due to an additional sample pump, multiple inlet and column switching valves, an air detection system, as well as a fractionator that allows sample collection from microtiter plates to 250 ml bottles.

PROTEOMICS CORE FACILITY

THE PROTEOMICS CORE FACILITY (PCF) operates an EASY nLC 1000 ultraHPLC coupled online to a Q Exactive Plus mass spectrometer to perform proteomic measurements.

SERVICES OFFERED

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



Falk Butter Head

Since 05/2013

Jasmin Cartano Technician

Since 02/2014

Jiaxuan Chen Staff Scientist

Since 03/2019

Mario Dejung Bioinformatician

Since 05/2014

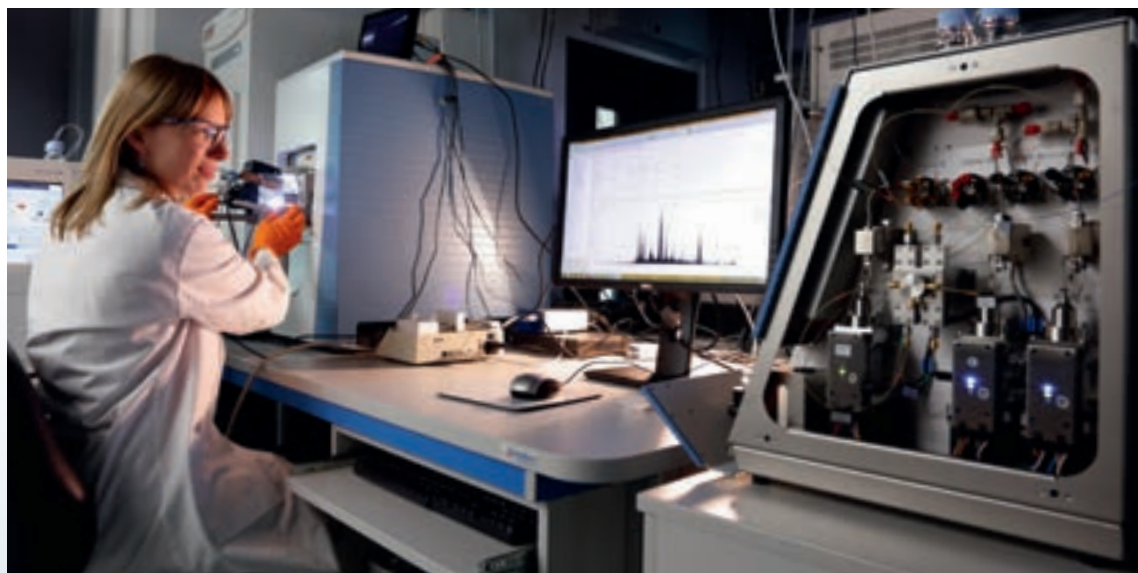
Anja Freiwald Engineer

04/2013 – 08/2019

Ramona Schmitt Technician

Since 12/2018

Figure 1. The mass spectrometry platform uses a top of the line Q Exactive Plus machine to support IMB researchers with their proteomics experiments.



MEDIA LAB

CORE FACILITY

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

SERVICES OFFERED

The Media Lab provides the following services:

- + 24/7 supply of routinely-used buffers, solutions, liquid media, and agar plates for molecular biology research and for culturing bacteria, yeast, insect cells, and *C. elegans*
- + Production of made-to-order media
- + Management of three supply centres for enzymes, kits, and cell culture media
- + Administration of a vector data bank, human ORF clone collection, and cell line bank
- + Overnight cultures for plasmid preparation
- + Sterilisation of solutions/media
- + Cleaning and sterilisation of glassware and lab equipment
- + Autoclaving of S1/S2 waste
- + Maintenance of in-house transport system

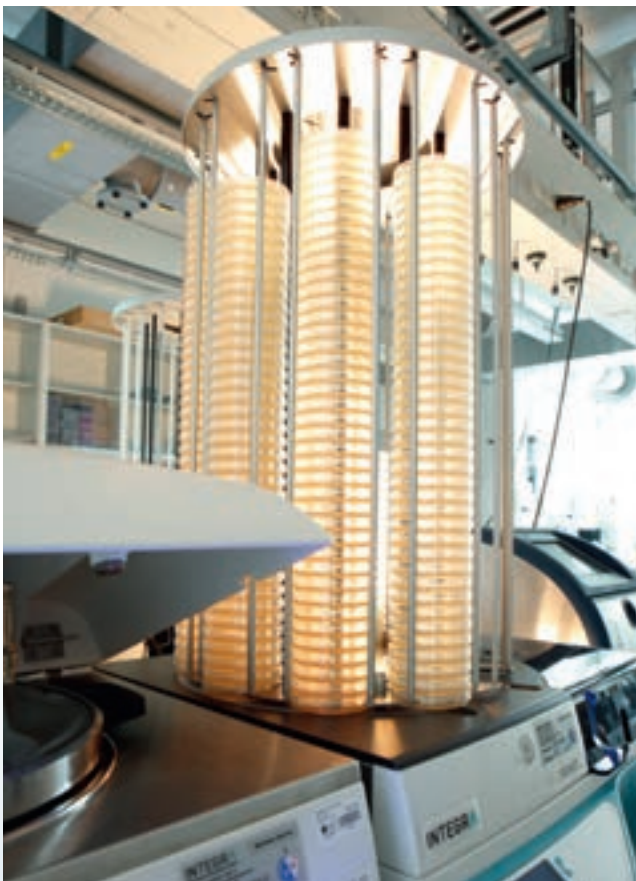


Figure 1. The media lab produces Petri dishes for different experimental setups with an output of about 1,700 plates per week.



Andrea Haese-Corbit Head
Since 01/2018

Doris Beckhaus Assistant
Since 05/2011

Alwina Eirich Assistant
Since 07/2013

Pascal Hageböling Assistant
Since 01/2015

Annette Holstein Assistant
Since 04/2012

Marion Kay Assistant
Since 04/2016

Johann Suss Assistant
Since 04/2011



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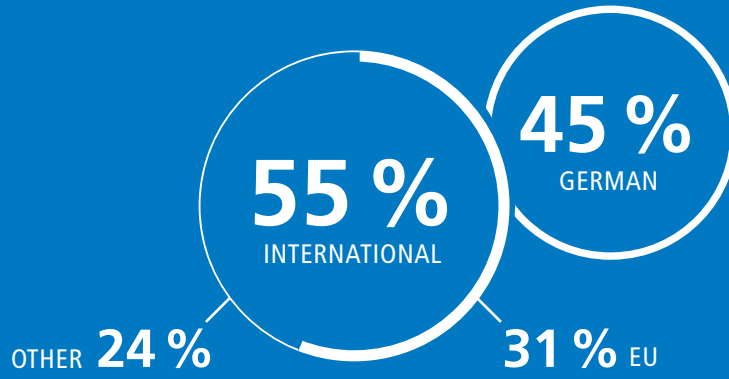
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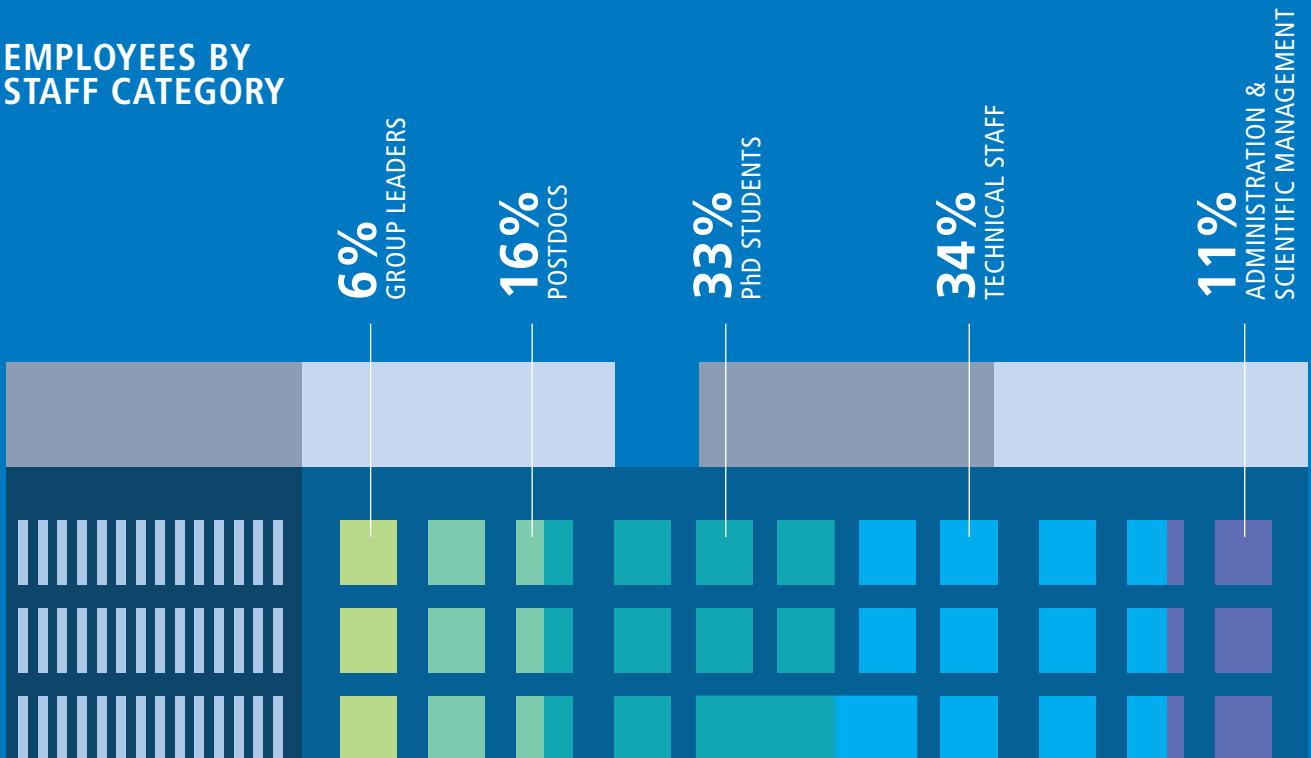


IMB STAFF

NATIONALITIES OF ALL IMB STAFF



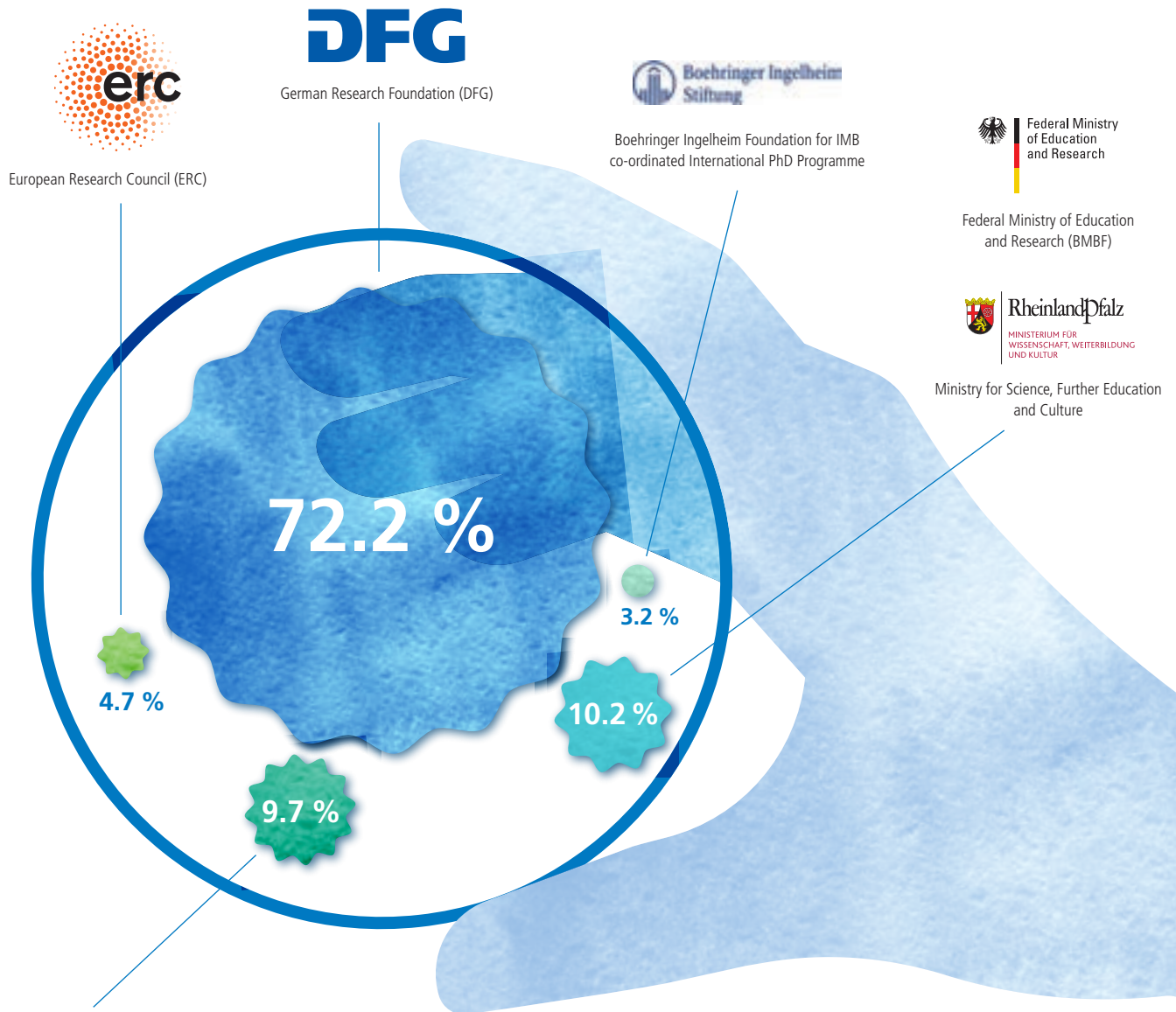
EMPLOYEES BY STAFF CATEGORY





EXTRAMURAL FUNDING

IN ADDITION TO BOEHRINGER INGELHEIM STIFTUNG CORE FUNDING



FURTHER SUPPORT

Boehringer Ingelheim Fonds (BIF)
Stiftung für medizinische Grundlagenforschung

DAAD
German Academic Exchange Service (DAAD)

Deutsche Krebshilfe
HELFFEN. FORSCHEN. INFORMIEREN.
German Cancer Aid

Natural Science Medical Research Center (NMFZ)

DZHK
DEUTSCHES ZENTRUM FÜR HERZ-KREBLAUF-FORSCHUNG E.V.
German Center for Heart and Circulation Research (DZHK)

Peter und Traudl Engelhorn Stiftung
Peter and Traudl Engelhorn Foundation

German Scholars Organization (GSO)

International Society of Differentiation

Leopoldina
National Academy of Sciences Leopoldina



RESEARCH AND TRAINING

As a thriving international research centre, IMB focuses on giving our researchers the best possible environment in which to do their science.

Here, scientists work at the cutting-edge of their fields to answer key questions on how organisms grow, age, and develop disease. Through the discoveries already made at IMB, we are beginning to transform our understanding of gene regulation, epigenetics, and genome stability.

Our talented scientists come from over 30 countries across the world, giving us a vibrant international atmosphere. We currently host 13 active research groups with over 95 PhD students and 36 postdocs. The collaborative and friendly environment at IMB drives our research and enabled us to produce over 348 publications in the last 5 years, with 65 in 2019.

We actively support our scientists as their careers develop. We do this by providing comprehensive training in scientific, technical, and complementary skills. A key part of this training comes from our state-of-the-art Core Facilities in which experts train and support researchers in implementing the latest techniques and skills needed for their projects. To further develop their professional expertise, we host specialist workshops relevant to careers in both academic and commercial settings. These include: presentation skills, scientific writing, project management, fundraising, career development, negotiation, and leadership. Through this dedicated training, our scientists gain a competitive edge at all stages of their career.



Our PhD students are key to the research carried out at IMB. In order to provide the structure, training, and supervision necessary to excel during a PhD, IMB created the International PhD Programme (IPP) thanks to funding from the Boehringer Ingelheim Foundation. The IPP is comprised of PhD students from IMB together with students at Mainz University & its Medical Centre. The IPP currently has 144 PhD students in 52 research groups and 63 successful graduates. The interdisciplinary faculty of the IPP is chosen for complementing research themes, facilitating connections between groups and students across Mainz. Within this network, our students tackle ambitious research projects, receive a broad and diverse education, and have easy access to the expertise and equipment needed to drive their projects forward. With the comprehensive scientific and technical training the IPP provides, students are prepared to meet the challenges of establishing a career in the quickly evolving field of the life sciences.

www.imb.de/PhD



IMB's International Summer School (ISS) is a 6-week programme on "Gene Regulation, Epigenetics & Genome Stability" that brings talented undergraduate and Masters' students from around the world to Mainz every summer. Through the ISS, these enthusiastic young scientists have the chance to work hands-on with projects at the forefront of biological research. The informal and international environment of the ISS gives the students an excellent framework in which to develop their practical and professional skills. Courses develop valuable abilities needed as scientists, while lectures give students comprehensive insights into the latest research. At the end of the ISS the students can identify key open questions in the fields of gene regulation, epigenetics & genome stability and are well prepared to tackle ambitious Master's or PhD projects.

www.imb.de/ISS



IMB POSTDOC PROGRAMME



IMB established its Postdoc Programme (IPPro) to meet the specific needs of our postdoctoral community. It aims to provide our ambitious, early-career scientists with the skill sets and guidance necessary to become future scientific leaders. The IPPro builds upon our community of 36 international postdocs who work on cutting-edge research projects in a fully interdisciplinary environment. The IPPro actively supports our young professionals as their careers develop. We provide advanced training in scientific methods and professional skills through a range of expert lectures, focused workshops, and tailored events. Alongside guidance from IMB's group leaders, our postdocs receive mentoring from IMB's directors and via career events with leading external scientists. As well as offering fully funded positions, we also guide and support postdocs in raising funds for their research as a means of becoming independent researchers. Collectively, the IPPro ensures that our postdocs have access to the training and information needed to effectively carry out their research projects and advance their prospects in building successful careers.

www.imb.de/postdocs





TRAINING COURSES

IMB provides our scientists with comprehensive training spanning both scientific and non-scientific skills. This ensures they have the expertise to perform top-quality research and succeed in their careers.

CORE FACILITIES TRAINING

LECTURES

CORE FACILITY	DATE	TITLE
GENERAL	15 Apr	Molecular & Biochemistry Techniques
	08 Jul	Nuclear Magnetic Resonance (NMR)
BIOINFORMATICS	27 May	Databases in Bioinformatics
FLOW CYTOMETRY	01 Apr	Advanced Flow Cytometry: Principles of Cell Sorting
	01 Jul	Flow Cytometry
	21 Oct	Flow Cytometry: Introduction I
	28 Oct	Flow Cytometry: Introduction II
GENOMICS	20 May	Genomics (NGS)
MICROSCOPY & HISTOLOGY	29 Apr	Introduction to Microscopy
	06 May	Microscopy: F-Techniques & Super-Resolution
	13 May	Histology & Fluorescent Labelling
	24 Jun	Electron Microscopy
	25 Nov	Image Manipulation: The Slippery Slope to Misconduct
PROTEOMICS	03 Jun	Proteomics
PROTEIN PRODUCTION	17 Jun	Protein Production & Crystallography

PRACTICAL COURSES

CORE FACILITY	DATE	TITLE
BIOINFORMATICS	27 Mar	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part I)
	04 Apr	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part II)
	10 Apr	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part III)
	17 Apr	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part IV)
	08 May	Bioinformatics: ChIP-Seq & RNA-Seq Analysis with R/Bioconductor (Part I)
	15 May	Bioinformatics: ChIP-Seq & RNA-Seq Analysis with R/Bioconductor (Part II)
	20 May	Bioinformatics: ChIP-Seq & RNA-Seq Analysis with GALAXY
FLOW CYTOMETRY	08 – 11 Apr	Advanced Flow Cytometry: Principles of Cell Sorting
	11 – 22 Nov	Flow Cytometry: Practical Course
MICROSCOPY & HISTOLOGY	28 – 30 Jan	Histology Crash Course
	19 – 21 Mar	Microscopy: Image Processing & Analysis Practical Course
PROTEOMICS	25 – 27 Feb	Proteomics: Practical Course



LECTURES

DATE	TITLE
18 Feb	How to Manage Your Research Data
01 Apr	How to Get Funded
15 – 16 May	CRISPR/Cas – The Designed Human?
22 May	The Reproducibility Crisis: Can You Trust Your Data?
12 – 16 Aug	Advanced Lectures on “Gene Regulation, Epigenetics & Genome Stability”
09 Sep	Good Scientific Practice
19 Nov	Ethics in the Life Sciences: How to Take Responsibility in Research Practice

PRACTICAL COURSES

DATE	TITLE
14 – 15 Jan	Critical Reasoning & Logic
19 – 20 Feb	Writing for the Public
26 Feb	Adobe Illustrator (Part I)
27 Feb	Adobe Illustrator (Part II)
06 – 07 Mar	Introduction to Biostatistics
12 – 13 Mar	Presentation Skills
19 – 21 Mar	Image Analysis & Processing
25 – 29 Mar	UbiCODE Training Course: SUMOylation and Ubiquitylation
26 – 27 Mar	Professional Communication Skills
28 – 29 Mar	Career Development & Application
10 Apr	Getting Your Paper “Through the Hoops”
11 – 12 Apr	Scientific Writing
08 – 15 May	ChIP-Seq & RNA-Seq Analysis with R/Bioconductor
03 Jun	Negotiation for Scientists
17 – 18 Sep	Introduction to Biostatistics
16 – 17 Oct	Convincing Scientific Presentations
11 – 12 Nov	Find Your Career Path
18 Nov	Agile Project Management
02 Dec	Proposal Writing
10 – 11 Dec	Convincing Scientific Presentations
16 Dec	Strategic Application



INVITED SPEAKERS

IMB hosts regular talks with prestigious international leaders to promote networking and exchange of novel scientific ideas.

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
10 Jan	Ad Hoc Seminar	FLORIAN STEINER	University of Geneva, CH	The role of histone H3 variants in development and disease
12 Feb	TechTalk	ANGELA GARDING	Covaris, Frankfurt, DE	Robust sample preparation solutions for chromatin-based applications
13 Feb	IMB Seminar	JOVICA NINKOVIC	Ludwig Maximilian University, Munich, DE	Regulation of progenitor fate in the adult brain
14 Feb	IMB Seminar*	BORIS PFANDER	Max Planck Institute of Biochemistry, Martinsried, DE	Double strand break repair – control by chromatin and the cell cycle
20 Feb	Ad Hoc Seminar	BASANT KUMAR THAKUR	University Hospital Essen, DE	DNA in extracellular vesicles (exosome): Functional potential in cell-to-cell communication in cancer and metastasis
21 Feb	IMB Seminar*	ANNE DONALDSON	University of Aberdeen, UK	Lessons from yeast on human chromosome maintenance
21 Mar	IMB Seminar*	LORENZA PENENGO	University of Zurich, CH	Role of ubiquitin and ubiquitin-like modifiers in the regulation of genome stability
04 Apr	IMB Seminar	JACCO VAN RHEENEN	Netherlands Cancer Institute, Amsterdam, NL	Imaging (cancer) stem cell dynamics in living mice
05 Apr	Ad Hoc Seminar	ABRAHAM FAINSOD	The Hebrew University, Jerusalem, IS	Alcohol drinking and its negative effects on retinoic acid signalling
17 Apr	Ad Hoc Seminar	LEOPOLD PARTS	Wellcome Sanger Institute, Hinxton, UK	Predicting the mutational outcomes of CRISPR/Cas9-induced double strand break repair
07 May	TechTalk	THORSTEN LEMKER	10x Genomics, Stuttgart, DE	10x Genomics chromium technology: Multiple features in single cells - biology at true resolution
09 May	IMB Seminar	KIM SNEPPEN	University of Copenhagen, DK	Theoretical tool bridging cell polarities with development of morphologies
28 May	Ad Hoc Seminar	NARAYANA H. MOHAN	Indian Council of Agricultural Research, New Delhi, IN	Climate change: Physiogenomic responses to heat stress
06 Jun	IMB Seminar*	CAROLINE KISKER	Julius Maximilian University, Würzburg, DE	News from TFIIF – Putting together the pieces
11 Jun	Ad Hoc Seminar	UDO BIRK	University of Applied Sciences, Chur, CH	Image analysis using deep learning
18 Jun	IMB Seminar	DOROTHEE DORMANN	Ludwig Maximilian University, Munich, DE	RNA-binding proteins in neurodegeneration – From nuclear transport defects to aberrant phase separation
12 Sep	Ad Hoc Seminar	LIESBETH VEENHOFF	European Research Institute for the Biology of Ageing, Groningen, NL	Poor old pores: The cell's challenge to make nuclear pore complexes in ageing
19 Sep	IMB Seminar*	AMIR AHARONI	Ben Gurion University of the Negev, Beersheba, IS	Live cell measurements of eukaryotic DNA replication kinetics
24 Oct	IMB Seminar*	THOMAS KUNKEL	National Institute of Environmental Health Sciences, Durham, USA	Studies of eukaryotic DNA replication fidelity
07 Nov	IMB Seminar*	BEAT FIERZ	Swiss Federal Institute of Technology Lausanne (EPFL), CH	A synthetic and single-molecule exploration of the dynamic chromatin landscape
11 Nov	IMB Seminar*	THANOS HALAZONETIS	University of Geneva, CH	DNA replication stress in cancer
14 Nov	IMB Seminar*	MATTHIAS ALTMAYER	University of Zurich, CH	Evaluating PARP inhibitor activities beyond BRCA mutations
20 Nov	Ad Hoc Seminar	MEELAD M DAWLATY	Albert Einstein College of Medicine, New York, USA	Epigenetic regulation of stem cells, development, and cancer by Tet enzymes: Lessons from Mice
21 Nov	IMB Seminar	ALFRED NORDHEIM	University of Tübingen and Fritz Lipmann Institute, Jena, DE	Murine liver cancer: Insights into metabolic reprogramming and anti-fibrotic microRNA functions
28 Nov	IMB Seminar	JEFFREY CHAO	Friedrich Miescher Institute for Biomedical Research (FMI), Basel, CH	Imaging the life and death of mRNAs in single cells
12 Dec	Ad Hoc Seminar	ALEJANDRO ROJAS FERNANDEZ	University of Valdivia, CI	The best of alpacas: From wool to nanobodies

*These seminars were part of the SFB 1361 seminar series on the "Regulation of DNA Repair & Genome Stability"



RESEARCH INITIATIVES



**REGULATION OF DNA REPAIR
& GENOME STABILITY**

Spokesperson: Helle Ulrich

This initiative brings together 18 projects from investigators across 6 institutions (IMB, Johannes Gutenberg University, University Medical Center Mainz, Darmstadt University, Munich University, and Frankfurt University) with the goal of understanding the molecular mechanisms modulating the activities of genome maintenance in the cell. Supported by 3 dedicated service projects and centralised management, the network comprises experts in structural biology, organic chemistry, biochemistry, molecular & cell biology, genetic toxicology, and clinical sciences. The SFB's Integrated Research Training Group is designed to ensure that participating students receive the best possible training and career development while completing their PhDs. The SFB 1361 was launched in January 2019 and is funded by the DFG with €12.4 million in its first funding period until December 2022.

www.sfb1361.de



**GENE REGULATION
IN EVOLUTION**

Spokespersons: Susanne Foitzik (JGU) & René Ketting

GenEvo is a DFG-funded Research Training Group organised in a collaboration between IMB and Johannes Gutenberg University's Faculty of Biology. The initiative is centred around the core question of how complex and multi-layered gene regulatory systems have both evolved and driven evolution. Mixing both junior and senior researchers, GenEvo brings together 12 outstanding scientists in 14 projects, fusing expertise in evolutionary and molecular biology and utilising tools such as evolutionary biology, epigenetics, gene regulation, omics technologies, and bioinformatics. The programme also focuses on training a new generation of PhD students to work on ambitious research projects at the interface of these two themes, while also receiving a broad, interdisciplinary education. GenEvo launched in June 2019 and is funded by the DFG with €5.8 million in its first funding period until December 2023.

www.imb.de/genevo

PETER BAUMANN

Elected as a Member of the European Molecular
Biology Organisation (EMBO)



AWARDS

MIAO YU
(POSTDOC, LEMKE GROUP)

Alexander von Humboldt Fellowship

ELENA IVANOVA
(PHD STUDENT, KHMELINSKII GROUP)

Helmsley Scholarship (Helmsley Charitable Trust)



INTERNATIONAL SCIENTIFIC EVENTS

ORGANISED BY IMB IN 2019

INVITATION

**CRISPR/Cas
The Designed Human?**

Wednesday, 15 May 2019
 Institut für Molekulare Biologie (IMB)
 Ackermannweg 4
 55128 Mainz

EINE KOOPERATION DES IMB MIT DER AKADEMIE DER WISSENSCHAFTEN UND DER LITERATUR

www.adwmainz.de

AKADEMIE DER WISSENSCHAFTEN UND DER LITERATUR | MAINZ

15 – 16 May

OUTREACH EVENT & SCIENTIFIC WORKSHOP CRISPR/CAS – THE DESIGNED HUMAN?

Scientific organisers: Mita Banerjee (JGU and Obama Institute), Christof Niehrs (IMB and Academy of Science & Literature) and Ruben Zimmermann (JGU and e/ac)

17 – 18 June

SFB KICK-OFF MEETING REGULATION OF DNA REPAIR & GENOME STABILITY

Scientific organiser: Helle Ulrich (IMB)

17 - 18 JUNE 2019

**SFB Kick-off Meeting
Regulation of
DNA Repair &
Genome Stability**

www.sfb1361.de

imb
Institute of
Molecular Biology

IMB Conference
30 October - 1 November 2019

**Chromosome
Territories &
Nuclear
Architecture**

Celebrating the 75th birthday of
CHRISTOPH CREMER
- a pioneer of
super-resolution
microscopy

SPEAKERS

WENDY BICKMORE Memorial Sloan-Kettering, USA	LEONID MIRNY Memorial Sloan-Kettering, USA	KARSTEN RIPPE Max Planck Institute of Biophysics, Germany
ALISTAIR BOETTIGER University of Cambridge, UK	TOM MISTELI Harvard Medical School, USA	JANE SKOK NIH, USA
KERSTIN BYSTRICKY University of Cambridge, UK	STEFAN MUNDLOS University of Cambridge, UK	AMOS TANAY Weizmann Institute, Israel
RAFAEL CASELLAS National Institutes of Health, USA	NICOLA NERETTI University of Cambridge, UK	BAS VAN STEENSEL The Netherlands Cancer Institute, Amsterdam, The Netherlands
THOMAS CREMER Harvard Medical School, USA	MARIO NICODEMI University of California, San Diego, USA	NADINE VASTENHOUW The Netherlands Cancer Institute, Amsterdam, The Netherlands
CHRISTOF NIEHRS IMB, Mainz, Germany	JOB DEKKER Harvard Medical School, USA	CLODAGH O'SHEA Cell Biology and Biophysics, Dublin, Ireland
ANA POMBO Max Delbrück Center for Molecular Medicine, Berlin, Germany	PETER FRASER Harvard Medical School, USA	JAN-MICHAEL PETERS Harvard Medical School, Boston, USA
VASSILIS ROUKOS National Institutes of Health, USA	RAINER HEINTZMANN IMB, Mainz, Germany	TING WU Harvard Medical School, Boston, USA

SCIENTIFIC ORGANISERS
CHRISTOF NIEHRS (IMB, Mainz, Germany)
ANA POMBO (Max Delbrück Center for Molecular Medicine, Berlin, Germany)
VASSILIS ROUKOS (National Institutes of Health, USA)

Institute of Molecular Biology (IMB), Ackermannweg 4, 55128 Mainz, Germany
events@imb.de, www.imb.de/2019conference

30 October – 1 November

IMB CONFERENCE CHROMOSOME TERRITORIES & NUCLEAR ARCHITECTURE

Scientific organisers: Christof Niehrs (IMB), Ana Pombo (Max Delbrück Center for Molecular Medicine) and Vassilis Roukos (IMB)

2 – 3 December

GENEVO KICK-OFF MEETING GENE REGULATION IN EVOLUTION: FROM MOLECULAR TO EXTENDED PHENOTYPES

Scientific organisers: Susanne Foitzik (JGU) and René Ketting (IMB)

Genevo

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KICK-OFF MEETING
2 - 3 DECEMBER 2019

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Johannes Gutenberg University,
Mainz, DE
René Ketting
Institute of Molecular Biology (IMB),
Mainz, DE

IMB: Institute of Molecular Biology (IMB), Ackermannweg 4, Mainz University Campus
www.imb.de/2018genevo, events@imb.de



In 2019, IMB welcomed

457 guests from

45 countries



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RESEARCH ENVIRONMENT

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

With 10 departments, more than 150 institutes and 32,000 students, Johannes Gutenberg University is one of the largest German universities. In biomedical research, the university has built strong, interdisciplinary centres dedicated to neuroscience, cardiovascular medicine, immunology and oncology.

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

Mainz is also surrounded by a number of towns and cities with extensive research activities. For instance, Frankfurt is only 35 km away and is home to Goethe University, which has over 46,000 students and 10 research institutes within the Biochemistry, Chemistry and Pharmacy Department alone. Furthermore, there are several Max Planck Institutes in Frankfurt (including the Max Planck Institute for Biophysics, the Max Planck Institute for Brain Research and the Ernst Strungmann

Institute for Cognitive Brain Research). In addition to Frankfurt, nearby Darmstadt is home to both a Technical University, whose Department of Biology has a focus on synthetic biology and the biology of stress responses, and a University of Applied Sciences that includes a focus on biotechnology.

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



WHERE WE ARE

IMB is located in the city of Mainz, a charming, open-minded city that dates back 2,000 years to Roman times and still has a historic centre with a magnificent medieval cathedral. It was also here, in 1450, that Johannes Gutenberg invented modern book printing. The city is located at the conflu-

ence of two of the most important rivers in Germany, the Rhine and the Main, and has spectacular esplanades. Mainz is within easy reach of both cosmopolitan Frankfurt, with its famous opera house, avant-garde museums and glass-and-steel banking district, and the Rhine valley region with its

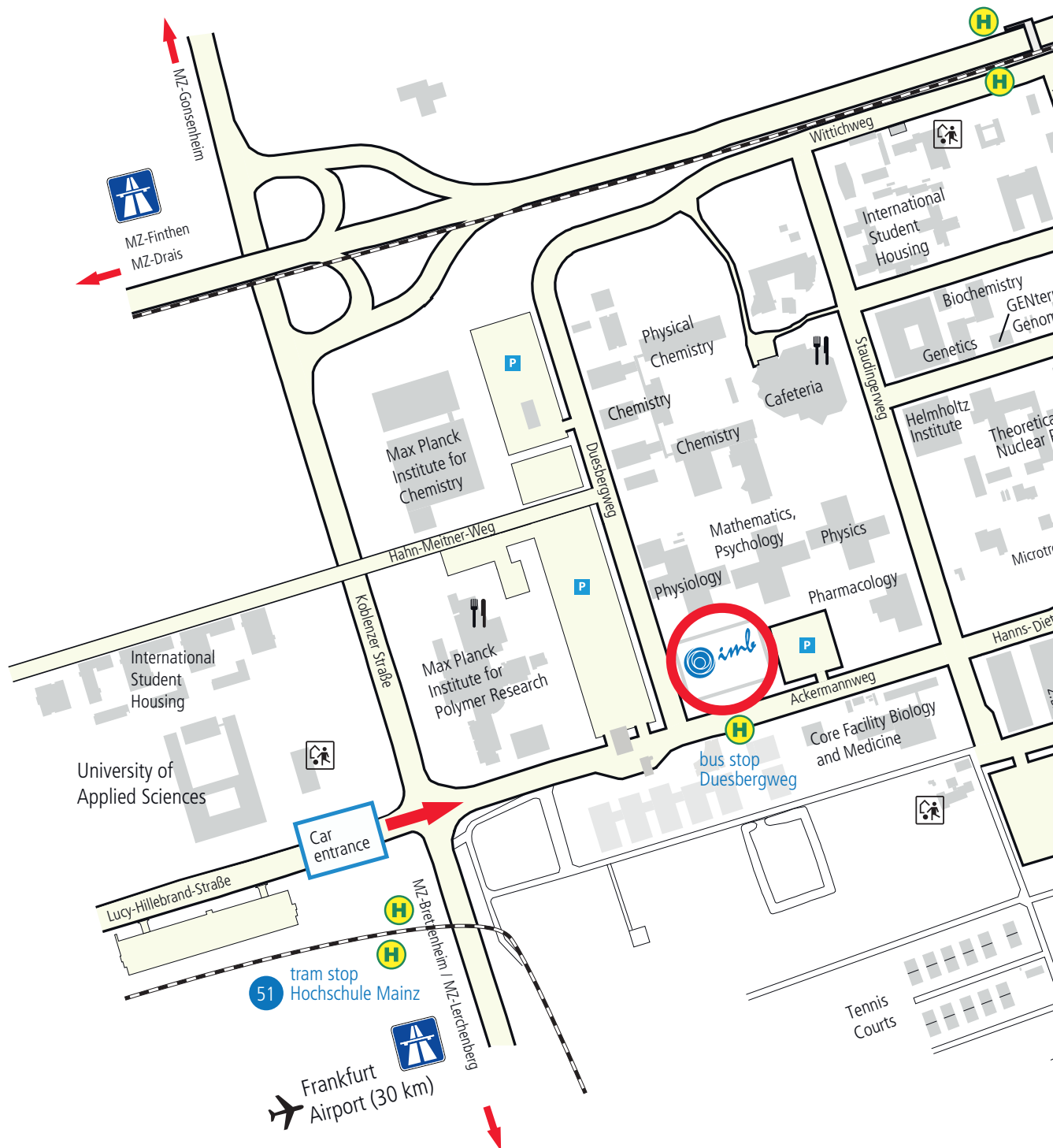
castles, vineyards and nature reserves that offer great outdoor activities. With Frankfurt airport – one of the largest airports in Europe – only 25 minutes away, countless European and overseas destinations are within easy reach.





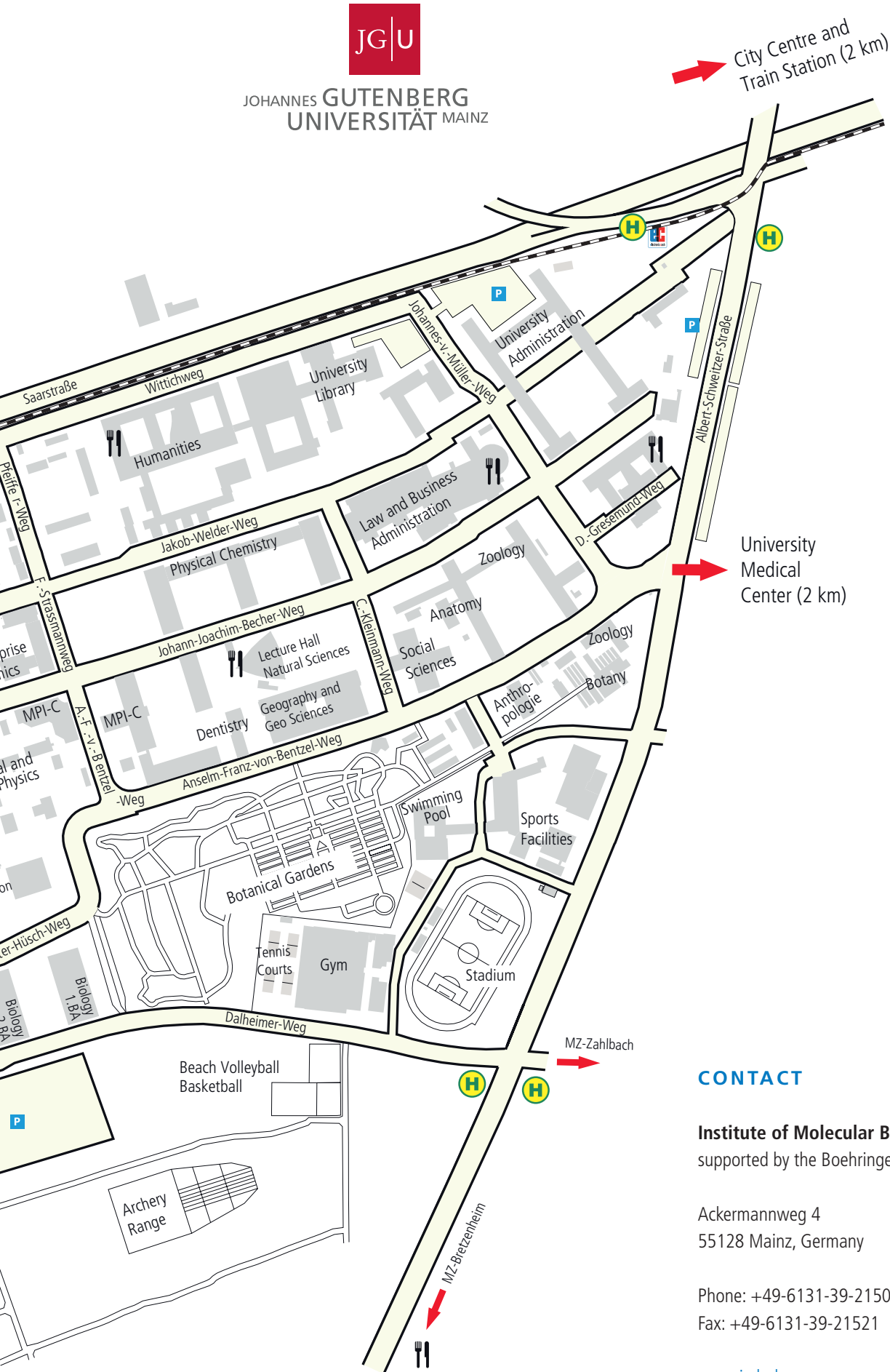
CAMPUS MAP

OF JOHANNES GUTENBERG UNIVERSITY MAINZ





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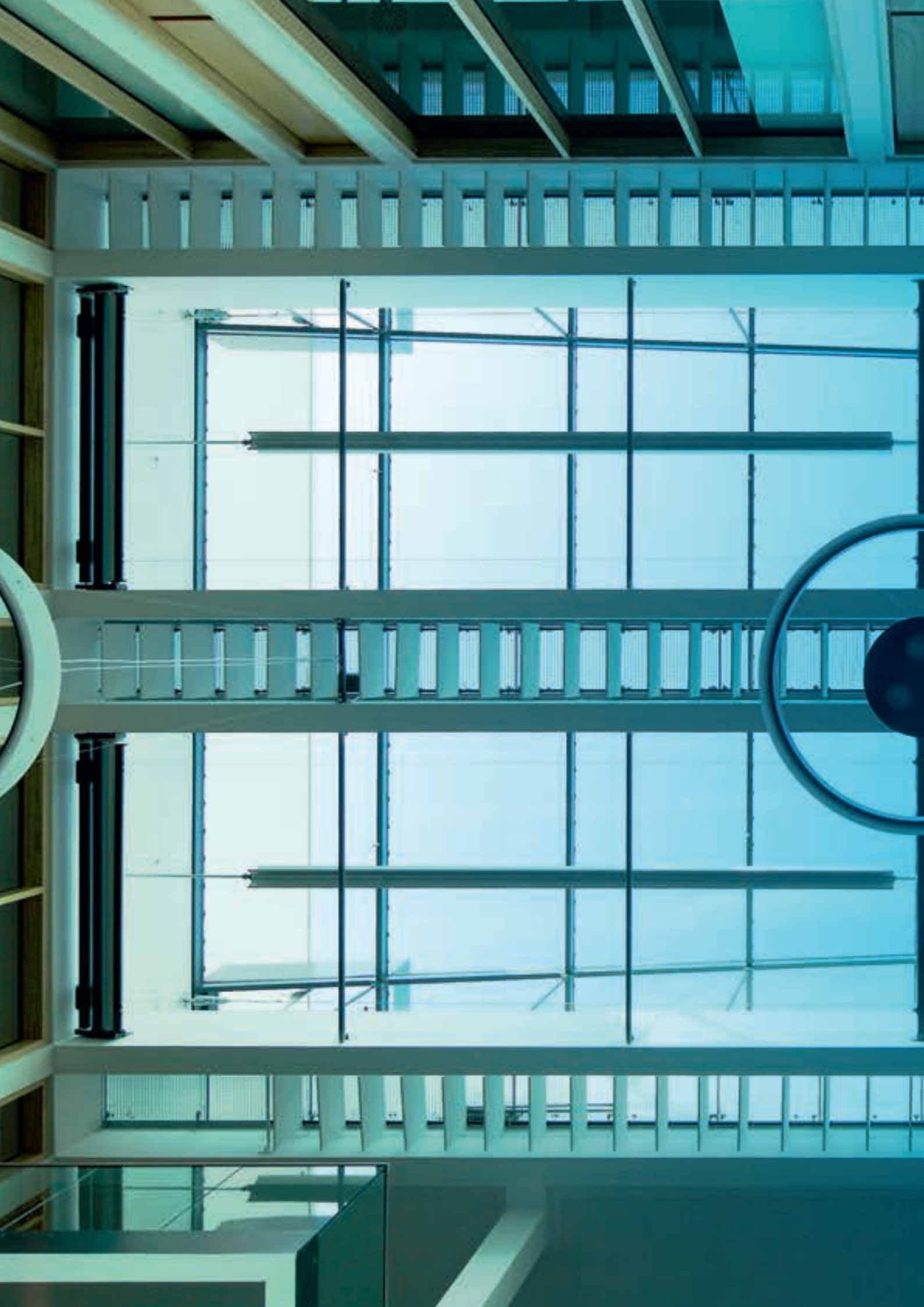
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Cover: Staining of the eye of a stage 38 *Xenopus* embryo. Sample is stained for 5hmC (yellow) and yoyo1 (blue). Image credit Victoria Hatch (Niehrs Group).

Portraits of Group Leaders and Core Facility heads (p2, 4-36, 39-47), pictures of IMB researchers (p 52-55, 61-64): Thomas Hartmann

Microscopy images: (p1) Phase-separated structures in the cytoplasm of *C. elegans* germ cells, stained with mCherry-Tdrd6cABCpld (red) and GFP-DCP1 (blue).

(p3) Left: Cross-section of a mouse seminiferous tubule at stage XI of spermatogenesis.

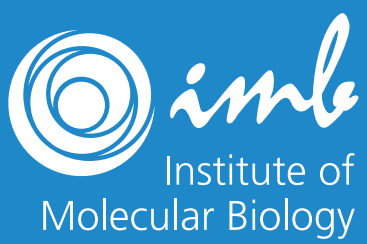
Ezh2 is stained in green, germ cell-specific antigen GCNA in magenta, and DNA in blue. Image credit Abishek Srinivasa (Barau Group). Middle: *Xenopus tropicalis* embryo at tail-bud stage. The membranes are stained green and the nuclei (H2B) are shown in magenta. Image credit: Eleftheria Parasyraki (Niehrs Group). Right:

Immunostaining showing Lgr5-GFP+ cells, EPCAM-RFP+ cells and DAPI staining in the intestinal tube of embryonic stage E15.5 mouse embryos. Image credit: Margarita Dzama (Kühn group, UMC Mainz).

(p4&5) False colour image of H&E staining of a cholangiocarcinoma in mouse liver.

Mainz (p68): Left: View of the west tower of St Martin's Cathedral, Mainz, © Landeshauptstadt Mainz. Middle: The Gutenberg Bibles in the "strong room" of Gutenberg Museum, Mainz, © Landeshauptstadt Mainz. Right: Image of Frankfurt am Main skyline during twilight blue hour by Rudy Balasko (www.shutterstock.com/g/rudi1976).

SAB (p66): All images courtesy of SAB members
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