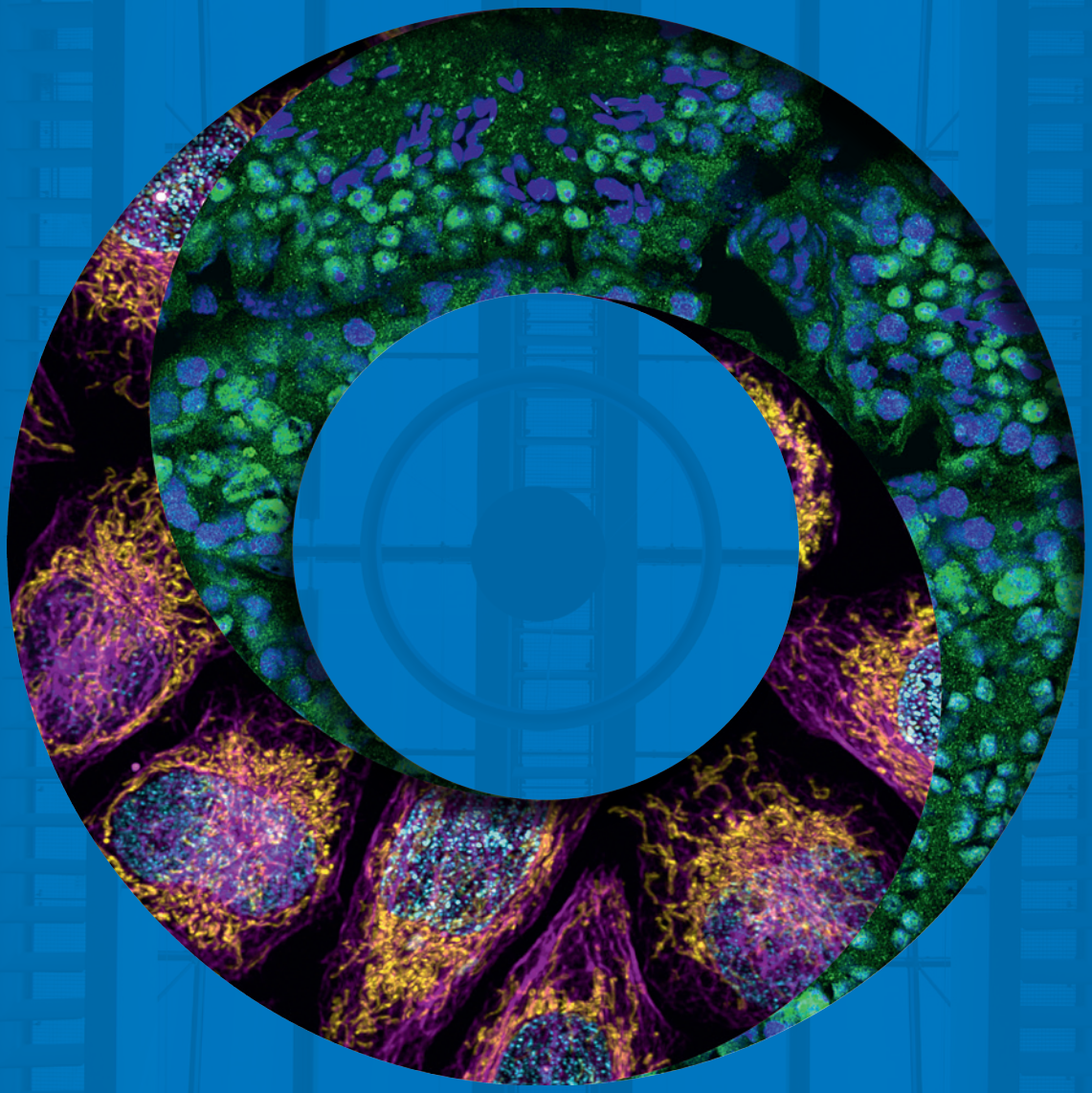
Institute of Molecular Biology



ANNUAL REPORT 2022

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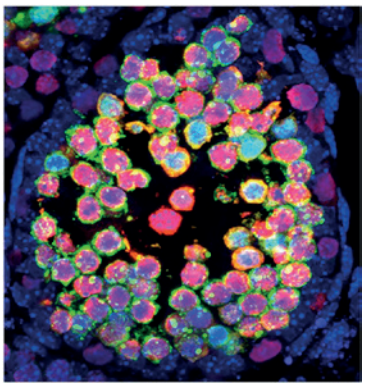
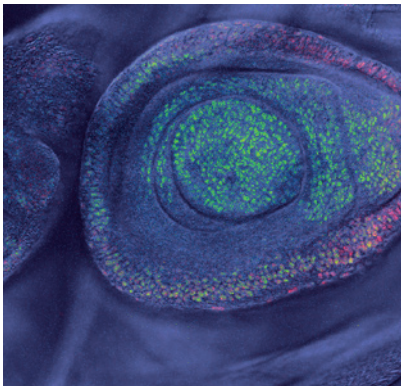
FOREWORD

Welcome to the IMB Annual Report 2022. This year saw a return to onsite international conferences and seminars, as well as confirmation of funding for continuing and new major research initiatives.





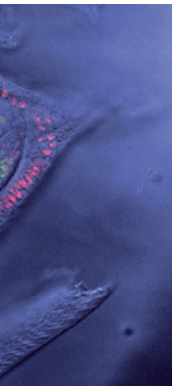
The last year at IMB has seen a culmination of successes, despite the many COVID-19 restrictions of the past few years. For instance, we are delighted that the DFG has funded two Collaborative Research Centre (CRC) networks with strong IMB involvement. The first of these is the CRC 1361 on "Regulation of DNA Repair & Genome Stability", which was first launched in 2019. This initiative, which is led by IMB's Scientific Director Helle Ulrich, aims to decipher the molecular mechanisms modulating the activities of genome maintenance in the cell. It features 18 projects led by investigators across 7 institutions (IMB, Mainz University and its University Medical Center, Darmstadt University, Munich University, Frankfurt University and Jena University), is supported by 3 dedicated service projects and incorporates an Integrated Research Training Group to ensure that participating students receive the best possible training and career development. In July, the CRC 1361 was externally reviewed and received excellent reviews. As a result, the DFG has now prolonged its funding with another €12.9 million until December 2026. By bringing together experts in structural biology, organic chemistry, biochemistry, cell and molecular biology and genetic toxicology from across Germany, this interdisciplinary network will establish Mainz as a leading research hub for DNA repair and genome maintenance.

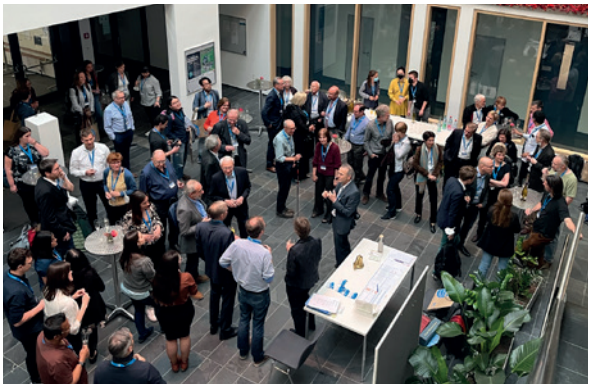
In addition to renewing the existing CRC 1361, we were extremely pleased that the DFG approved €12 million for the establishment of a new CRC on "Cell Function Driven by Polymer Concepts" in Mainz starting in 2023. The CRC 1551, which has been set up by IMB's Adjunct Director Edward Lemke, will bring together 31 group leaders from IMB, Mainz University, its University Medical Center and the Max Planck Institute for Polymer Research (Mainz), with the aim of building an interdisciplinary think tank for polymer scientists and life scientists to stimulate the incorporation of liquid-liquid phase separation and other polymer science concepts into biological research questions. This initiative will incorporate 16 research projects on biological processes ranging from transcription and protein aggregation to the formation of higher-order complexes and organelles and runs for an initial 4 years.

Further to these accomplishments, IMB has continued to expand its research focus on cellular and organismic ageing. In November, we were honoured by a visit from the state Science Minister Clemens Hoch, who presented a state grant of €1.56 million for the second phase of the Science of Healthy Ageing Research Programme (SHARP), which was launched last year and runs until 2025. This joint PhD training programme between IMB, Mainz University and its Medical Centre brings together 25 investigators in collaborative projects bridging basic and clinical research and places Mainz as an internationally visible beacon in the field of ageing research.

Due to our excellent COVID-testing platform, as well as the exemplary behaviour of our employees, we have been able to provide a safe environment throughout the pandemic, but we also noticed that the restrictions on personal contact did have an impact on the institute. As the COVID-19 pandemic gradually subsided over the past year, on-site and in-person events started to take off again with all their associated positive effects. These included the IMB Conference on "Epigenetics of Ageing: Responses to Adversity Across Scales" with 29 speakers and 114 participants from 12 countries at the end of June, and the IMB/CRC 1361 Conference on "Restore, Reorganise, Repurpose: the many faces of DNA repair" with 41 speakers and 149 participants in September.

IMB welcomed two new group leaders in 2022. First, Jan Padeken joined IMB as a Junior Group Leader focusing on understanding how heterochromatin is altered in response to persistent stress and how this contributes to genome stability and the maintenance of tissue integrity. Sara Vieira-Silva also joined us as an Adjunct Director with a joint appointment as a professor at Mainz University. Sara is an expert in microbiome research and studies the contribution of gut microbes to human metabolism and disease.





Furthermore, IMB welcomed four Adjunct Clinicians from Mainz University's Medical Center to intensify our clinical research collaborations. Stephan Grabbe is the Director of Dermatology and studies skin immunology and tumour immunotherapy, while Susann Schweiger is the Director of Human Genetics and studies diseases of the central nervous system. Oliver Tüscher is an expert in neurodegeneration and healthy brain ageing, while Philipp Wild is the Head of Preventative Cardiology and Preventative medicine and develops systems medicine approaches to treat heart failure, obesity, diabetes and thrombotic disease. This year, we also bid a fond farewell to Vassilis Roukos, who took up a position as an Assistant Professor at the University of Patras, Greece, and Falk Butter, who is leaving for the Friedric Loeffler Institute. We are glad, however, that both of them will retain a part-time affiliation with IMB.

IMB's researchers produced 76 publications in 2022. The groups of Helle Ulrich and Petra Beli published a paper in *Molecular Cell*, where they created tailor-made ubiquitin ligase enzymes to manipulate polyubiquitin chains in cells and proved that tagging a protein with different polyubiquitin chains can regulate its degradation and function. My own lab collaborated with the Butter Group to publish a *Nature Cell Biology* paper where we reported that small RNAs can be passed from fathers to offspring in phase-separated granules in sperm cells. Another notable mention comes from the Dormann Group, who published a paper in *Nature Neuroscience* detailing their discovery that clearance of TDP-43 condensates and lipid droplets in microglia is required to support regeneration in the brain.

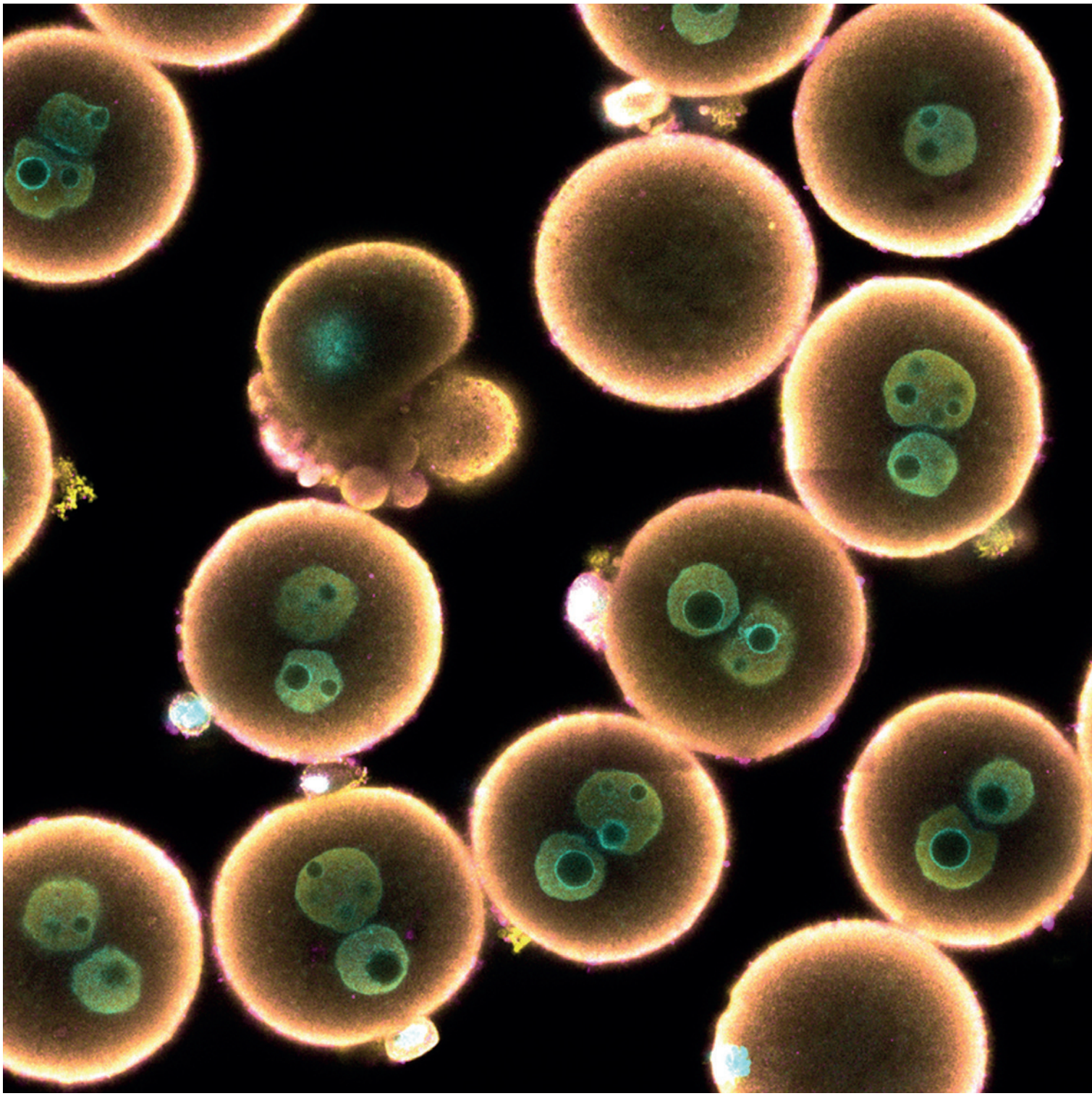
The year 2022 also brought other signs of recognition for our researchers. For instance, Edward Lemke was elected as an EMBO member, marking his outstanding contributions to understanding how intrinsic disorder in proteins plays a key role in regulating the traffic of biomolecules in and out of the cell nucleus. In addition, Sara Vieira-Silva was awarded the Antoine Faes Prize for her postdoctoral research showing that humans have at least four distinct patterns of gut microbes and that one of these is associated with inflammatory disease.

IMB's community continues to thrive as we expand our training programme for young scientists. Our International PhD Programme (IPP) has now grown to include 189 students from 44 countries and celebrated 18 defences this year. IMB's International Summer School also took place online for the second time to minimise COVID-19 infection risks, bringing together 22 undergraduate participants from 10 countries in a 3-week course comprising lectures, workgroups and discussions to train them in scientific skills.

Finally, I would like to thank the Boehringer Ingelheim Foundation and the State of Rhineland-Palatinate for their support and generous funding, and the members of our Scientific Advisory Board, whose advice and feedback have been instrumental in developing IMB. I would also like to thank all my colleagues at IMB for their contributions to IMB's successes this year and for managing their research so well throughout the pandemic.

René Ketting
Executive Director

RESEARCH GROUPS



Joan **BARAU**

"We discovered that transposable elements have a fascinating, bivalent relationship with their host genomes."

POSITIONS HELD

Since 2019 Group Leader, Institute of Molecular Biology (IMB), Mainz

2013 – 2019 Postdoc, Institut Curie, Paris

EDUCATION

2012 PhD in Genetics and Molecular Biology, University of Campinas

2005 BS in Biology, University of Campinas

GROUP MEMBERS

Ishita Amar	PhD Student; since 11/2022
Hannah Gonzalez Dammers	Student Assistant; since 05/2022
Styliani Eirini Kanta	PhD Student; since 04/2020
Jessica Leissmann	PhD Student; since 11/2019
Liana Marengo	Staff Scientist; since 09/2022
Violeta Morin	Lab Manager; since 10/2019
Srinivasa Abishek Prakash	PhD Student; since 05/2019
Anna Szczepinska	PhD Student; since 09/2021
Julia Isabet Zamora Interiano	Student Assistant; since 10/2022

OVERVIEW

Transposable elements, or TEs, are abundant genomic repeats linked to genome instability and regulatory perturbations that can lead to phenotypic consequences. In addition, TE-encoded proteins can be co-opted into functional components of our genomes, and their genomic sequences into elements that instruct genomic regulation. Our lab's work focuses on understanding transposon biology as a proxy to uncover new mechanisms that affect gene regulation, genome stability and inheritance. In the past year, our lab has been working on three fronts aimed at discovering (i) how transposons are targeted for epigenetic silencing in mouse germ cells, (ii) how transposon sequences and their epigenetic status impact their regulatory potential in mouse germ cells, and (iii) novel regulators of the transposon 'life cycle' in pluripotent and differentiated stages of mammalian development.

RESEARCH HIGHLIGHTS

How transposons are targeted for epigenetic silencing in mouse germ cells

Germ cells have the demanding task of distinguishing 'normal' functioning genes from active TEs, which should be inactivated. This is achieved by processing TE mRNAs into small PIWI-interacting RNAs (piRNAs). Production of piRNAs allows germ cells to specifically degrade TE mRNA and guide nuclear silencing factors to active TE loci, which leads to stable, life-long epigenetic silencing by DNA methylation. We now know that the final, stable step of TE transcriptional silencing in germ cells depends on the epigenetic modifier DNMT3C. In the past year, we have experimentally connected DNMT3C with the core components of the piRNA pathway. We are now focusing on understanding the

mechanisms of piRNA-guided DNA methylation and how chromatin modifications and nuclear organisation impact this process.

How transposon-induced DNA damage can trigger development

Transposons live dual lives in mammalian genomes: an individual transposon can act as a developmentally important enhancer of gene expression, but it can also promote its own transposition, with potentially dire consequences for genome stability. We hypothesise that DNA methylation at TE promoters can tip the scale towards either of these opposing roles by impacting the binding of regulatory factors and interactions with readers and writers of chromatin modifications. In the past year, we learned a lot about DNA-binding factors that may drive the activity of a TE as a selfish promoter or an enhancer of gene expression. We are now investigating how these factors are important for genome regulation during development and how TEs exploit them for their selfish activity.

FUTURE DIRECTIONS

The achievements outlined above will allow us to dive deeper into mechanistic studies focused on understanding how epigenetic settings are laid out at TE promoters in mouse germ cells and how this impacts the behaviour of germ cells during gametogenesis. The lab has developed and optimised tools for applying epigenomics to very specific cell types across gametogenesis. We will develop mouse models to couple these genome-wide studies with functional genomic studies. We are also excited to continue exploring the relationship between TEs and DNA damage and repair using cell lines in which TE activity can be controlled and hope to present exciting novel data about this relationship in stem cells in the upcoming year.

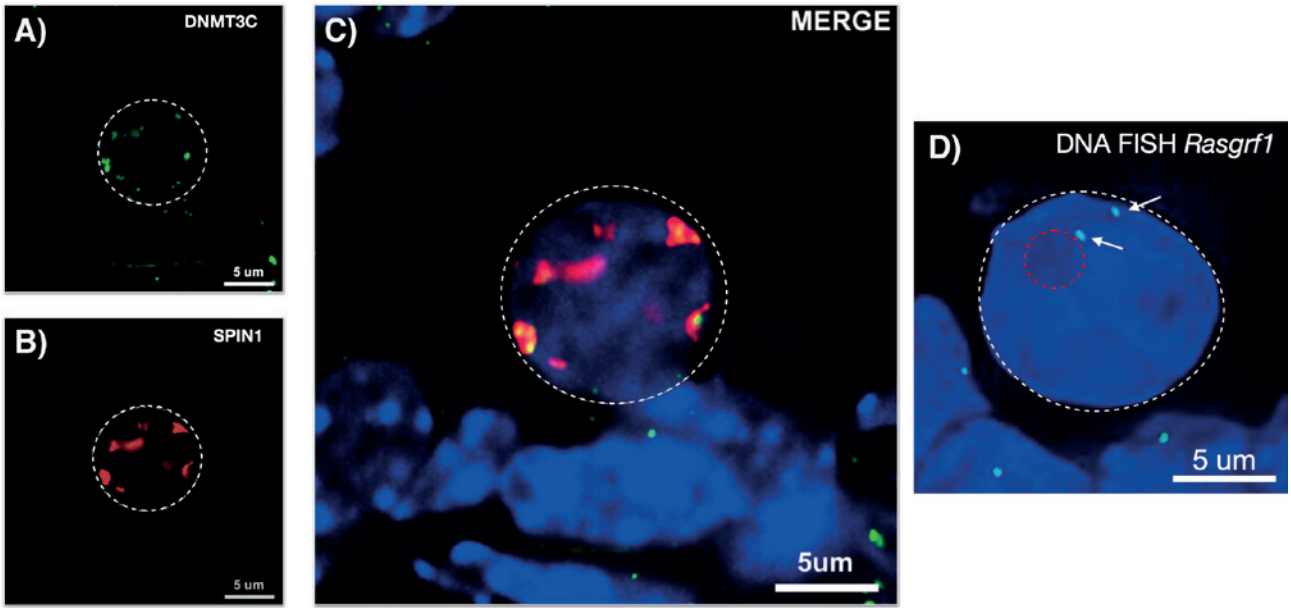


Figure 1. piRNA-guided DNA methylation in the nucleus of embryonic germ cells. Cryosections of mouse embryonic gonads at 17.5 days after fertilisation, the stage when piRNA-guided DNA methylation peaks.
A) Immunofluorescence detection of 2XMYC-DNMT3C foci.
B) Immunofluorescence detection of SPIN1, a core factor of nuclear piRNA targets discovered in our DNMT3C IP-MS experiments.
C) Co-localisation between SPIN1 and DNMT3C foci.
D) DNA FISH detection of a unique piRNA-targeted locus, *Rasgrf1*, showing its association with the nuclear lamina and the nucleolus of mouse embryonic germ cells.

SELECTED PUBLICATIONS

Dura M, Teissandier A, Armand M, Barau J, Lapoujade C, Fouchet P, Bonneville L, Schulz M, Weber M, Baudrin LG, Lameiras S and Bourc'his D (2022) DNMT3A-dependent DNA methylation is required for spermatogonial stem cells to commit to spermatogenesis. *Nat Genet*, 54:469–480

Mosler T, Conte F, Longo GMC, Mikicic I, Kreim N, Möckel MM, Petrosino G, Flach J, Barau J, Luke B, Roukos V and Beli P (2021) R-loop proximity proteomics identifies a role of DDX41 in transcription-associated genomic instability. *Nat Commun*, 12:7314

Prakash SA and Barau J (2021) Chromatin profiling in mouse embryonic germ cells by CUT&RUN. Pages 253–264 in: Epigenetic reprogramming during mouse embryogenesis. Methods in Molecular Biology, vol 2214 (eds. Ancelin K & Borensztein M), *Springer US*, New York

Peter **BAUMANN**

"We find that accumulating DNA damage and telomere erosion are key drivers of ageing."

POSITIONS HELD

Since 2021	Member of the Emergent AI Center Mainz
Since 2021	Board Member for the Science of Healthy Ageing Research Programme (SHARP)
Since 2021	Coordinator, Quantitative and Computational Biology Postdoc Programme
Since 2021	Director, Centre for Healthy Ageing, Mainz
Since 2020	Member of the Senate of JGU
Since 2018	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Since 2017	Alexander von Humboldt Professor, Johannes Gutenberg University (JGU), 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
1998 – 2002	Research Associate, University of Colorado, Boulder

EDUCATION

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

GROUP MEMBERS

Wafa Abuhashem	PhD Student; since 10/2021
Nadine Bobon	PhD Student; since 03/2020
Nathaniel Deimler	PhD Student; since 09/2022
Lukas Ende	PhD Student; since 09/2019
Martin Fahr	Animal Caretaker; since 2018
Thomas Faust	Personal Assistant; since 05/2019
David Ho	PhD Student; since 04/2018
Yu-Chia Ku	PhD Student; since 08/2022
Yasmin Luke	Project Manager; since 03/2019
Abinaya Manivannan	PhD Student; since 08/2018
Alex Orioli	PhD Student; since 01/2018
Lili Pan	Postdoc; since 02/2015
Valentine Patterson	PhD Student; since 10/2019
Jayaprakash Srinivasan	PhD Student; since 03/2020
Elisa Thomas	Technician; since 03/2021

OVERVIEW

Elucidating the mechanisms by which chromosome ends are protected and maintained is a matter of great interest with far-reaching implications for a broad spectrum of age-related diseases including cancer, cardiovascular disease and immune senescence. Our telomere-related work centres around two key topics: (i) the mechanistic basis of chromosome end protection, and (ii) the biogenesis and regulation of telomerase. Our research is guided by the conviction that a better understanding of telomerase biogenesis and its regulation will lead to the identification of compounds that modulate telomere length. Such reagents will have therapeutic uses either to limit the proliferation of tumour cells or to boost the proliferative potential of desired cell populations. The latter could not only help patients with insufficient telomerase activity, but may also counteract many of the manifestations associated with normal ageing, such as increased susceptibility to infections and diminished response to vaccinations. To reach these goals, we employ genetic, molecular and cell biological approaches, and have built a network of collaboration partners to examine telomere dynamics in the physiological contexts of immune senescence, frailty and ageing of the enteric nervous system.

RESEARCH HIGHLIGHTS

Telomerase biogenesis and regulation

Progressive telomere shortening is intrinsically linked to cell division and critically short telomeres trigger cellular senescence to prevent further proliferation. Mechanisms that replenish telomeric sequences are a double-edged sword. On one hand, they extend the replicative lifespan of a cell population and are thus vital for tissue homeostasis. On the other hand, replenishing telomeres also permits the continued proliferation of malignant cells. Consequently,

telomere addition must be tightly regulated in multicellular organisms. The isolation of the telomerase RNA subunit (TER1) from fission yeast by our laboratory 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 and has established a paradigm for telomerase assembly. Continuing our characterisation of the stepwise assembly process of telomerase, we recently showed that Pof8 is a La-related protein and a constitutive component of telomerase. We identified two additional factors that form a complex with Pof8 and participate in telomerase assembly. One shares structural similarity with the nuclear cap-binding complex, the other is the orthologue of the methyl phosphate capping enzyme (Bin3/MePCE). Based on functional characterisation and structural similarities, we named the factors Thc1 (Telomerase Holoenzyme Component 1) and Bmc1 (Bin3/MePCE 1), respectively. Thc1 and Bmc1 function together with Pof8 in recognising correctly folded telomerase RNA and promoting the recruitment of the Lsm2-8 complex and the catalytic subunit to assemble functional telomerase.

It has been known for many years that short telomeres are susceptible to end-to-end fusions, as the distinction between chromosome end and DNA break site is increasingly difficult the shorter the telomere. Contrary to this conventional thinking that only critically short telomeres are at risk of being mistaken for DNA breaks, our recent work revealed that under certain conditions long telomeres can also be susceptible to uncapping and thus become a source of genomic instability (Figure 1). These observations provide a conceptional framework for why cells maintain telomeres within a clearly defined size range and why overly long telomeres may undergo rapid deletion events. We are now extending these studies to characterise the effects of an above-average telomere length on genome stability, cancerogenesis and tissue homeostasis.

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? Complementing these avenues of enquiry are projects aimed at understanding how chromosome end protection is accomplished across a naturally-occurring telomere length distribution and how different repair pathways engage denuded chromosome ends and contribute to genome instability.

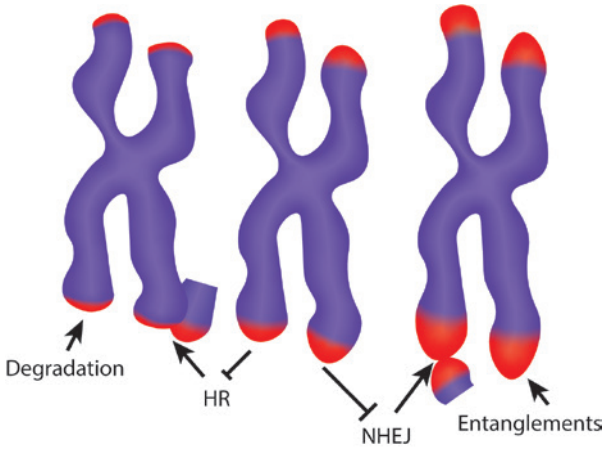


Figure 1. Telomere length naturally varies in a species and cell-type specific range. Work by a number of groups including our own revealed years ago how illicit recombination events engage critically short telomeres. We only recently found that long telomeres can also contribute to genome instability due to an increased vulnerability to chromosome fusions mediated by the non-homologous end joining pathway.

SELECTED PUBLICATIONS

Cole CJ, Baumann DP, Taylor HL, Bobon N, Ho DV, Neaves WB and Baumann P (2022) Reticulate Phylogeny: a new tetraploid parthenogenetic whiptail lizard derived from hybridization among four bisexual ancestral species of *Aspidoscelis* (Reptilia: Squamata: Teiidae). *Bull Mu Com Zoo*, *in press*

Pan L, Tormey D, Bobon N and Baumann P (2022) Rap1 prevents fusions between long telomeres in fission yeast. *EMBO J*, 41:e110458

Páez-Moscoso DJ, Ho DV, Pan L, Hildebrand K, Jensen KL, Levy MJ, Florens L and Baumann P (2022) A putative cap binding protein and the methyl phosphate capping enzyme Bin3/MePCE function in telomerase biogenesis. *Nat Commun*, 13:1067

Petra **BELI**

"Our work shows that R-loops are a cell-intrinsic source of genomic instability."

POSITIONS HELD

Since 2020 Adjunct Director and Group Leader, Institute of Molecular Biology (IMB) & Full Professor for Quantitative Proteomics, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz

2013 – 2020 Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz

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

EDUCATION

2011 PhD in Biology, Goethe University Frankfurt

2007 MSc in Molecular Biology, University of Zagreb

GROUP MEMBERS

Georges Blattner	PhD Student; since 07/2020
Christian Blum	PhD Student; since 06/2020
Francesca Conte Caio Almeida Batista	PhD Student; since 01/2019
De Oliveira	PhD Student; since 12/2020
Justus Gräf	Student Assistant; 04/2021 -08/2022
Rebecca Hobrecht	PhD Student; since 11/2022
Ekaterina Isaakova	PhD Student; since 10/2020
Katharina Mayr	Lab Manager; since 01/2020
Ivan Mikicic	PhD Student; since 04/2019
Thorsten Mosler	Postdoc; since 07/2022
Claudia Scalera	PhD Student; since 09/2019
Eric Schmitt	PhD Student; since 01/2022
Ute Sideris	Personal Assistant; since 01/2021
Aldwin Suryo Rahmanto	Postdoc; since 11/2019
Juanjuan Wang	PhD Student; until 06/2022

OVERVIEW

Genome maintenance is essential for gene expression fidelity, as well as for the prevention of cancer and premature ageing. The research in our group focuses on identifying and characterising proteins and signalling pathways that counteract genomic instability. A complex network of proteins and signalling pathways ensures genome maintenance in response to external stressors and DNA lesions introduced through cellular metabolism, as well as DNA replication and transcription. We develop and employ quantitative mass spectrometry-based approaches to obtain systematic insights into the proteins and signalling pathways that counteract genomic instability arising from cell-intrinsic sources, as well as in response to external stressors such as ultraviolet irradiation.

RESEARCH HIGHLIGHTS

Transcription by RNA polymerases is essential for all cellular processes and the adaptive response of cells to internal and external stimuli. Dysregulated transcription and increased frequency of transcription-replication conflicts is observed in many tumours. R-loops are three-stranded nucleic acid structures formed by an RNA-DNA hybrid core and a displaced non-template DNA strand. In addition to the regulatory functions of R-loops in transcription, DNA repair, telomere maintenance and chromosome segregation, these non-B DNA structures can be a driver of genomic instability. In cycling cells, R-loops constitute an obstacle for the replication machinery and can cause transcription-replication conflicts that lead to replication fork breakage and DNA double-strand breaks. Furthermore, R-loops can tether transcription complexes to chromatin, triggering the recruitment of nucleotide-excision repair endonucleases that process R-loops into DNA double-strand breaks. Thus, tight

regulation of R-loop levels is essential for their function as regulatory elements and for preventing R-loop-dependent genomic instability.

To gain insights into the protein networks that regulate R-loop homeostasis, we probed R-loop-proximal proteins in their native chromatin environment using RNA-DNA proximity proteomics (RDProx). We fused the hybrid-binding domain of RNaseH1 to the ascorbate peroxidase APEX2 and identified previously known R-loop regulators such as TOP1, AQR, the single-stranded DNA-binding proteins RPA1/2 and RNaseH2A, as well as components of the THO complex and the nuclear exosome. These R-loop proximal proteins showed functional interactions, as demonstrated by the identification of different protein clusters involved in splicing, m6A regulation, mRNA 3' end processing, mRNA export, transcription regulation, chromatin organisation and DNA replication/repair. They were also enriched in domains typical for RNA- and DNA-binding proteins, including RRM, helicase, DEAD/DEAH, CID, MCM N-terminal, RNA polymerase II-binding, SAP, MCM OB and K homology domains. Using RDProx, we identified a role for the tumour suppressor DEAD box protein 41 (DDX41) in opposing R-loop-dependent genomic instability. Loss of DDX41 resulted in an accumulation of R-loops and DNA double-strand breaks at gene promoters. Germline loss-of-function mutations in DDX41 predispose to acute myeloid leukaemia (AML) in adulthood. We propose that the accumulation of R-loops at gene promoters, DNA double-strand breaks and the inflammatory response contribute to the development of familial AML with mutated DDX41 (Mosler *et al*, *Nature Communications*, 2021).

FUTURE DIRECTIONS

We will focus our future efforts on obtaining a proteomics view of the mechanisms that counteract genomic instability arising from cell-intrinsic sources. More than 10% of the human genome can fold into non-canonical (non-B) DNA structures, including R-loops and G-quadruplexes, which can regulate different cellular processes. Non-canonical DNA structures also obstruct DNA replication and transcription, thereby creating an intrinsic source of DNA double-strand breaks and genomic instability. Another direction will be to employ multiplexed phospho- and ubiquitin-proteomics to delineate the interplay between genomic instability and immune signalling pathways.

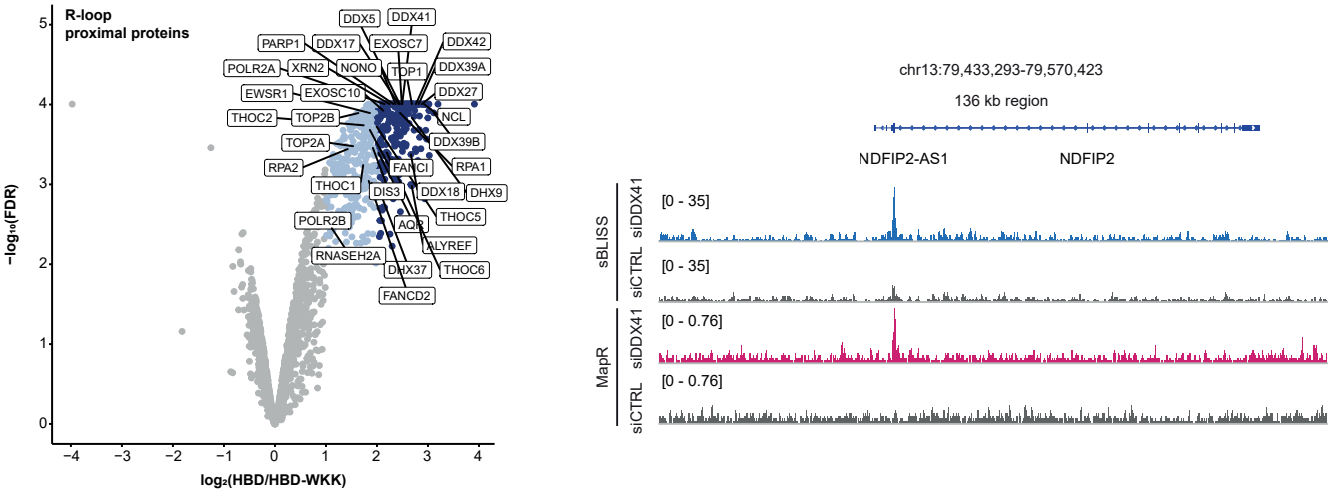


Figure 1. Proteins that regulate R-loops in human cells were mapped using quantitative mass spectrometry. Among the proteins that regulate R-loops, we identified DDX41; loss of function in this protein leads to familial AML. DDX41 loss results in the accumulation of R-loops and DNA double-strand breaks in the promoters of active genes.

SELECTED PUBLICATIONS

Mosler T*, Baymaz HI*, Gräf JF, Mikicic I, Blattner G, Bartlett E, Ostermaier M, Piccinno R, Yang J, Voigt A, Gatti M, Pellegrino S, Altmeyer M, Luck K, Ahel I, Roukos V and Beli P (2022) PARP1 proximity proteomics reveals interaction partners at stressed replication forks. *Nucleic Acids Res*, 50:11600–11618

Mosler T, Conte F, Longo GMC, Mikicic I, Kreim N, Möckel MM, Petrosino G, Flach J, Barau J, Luke B, Roukos V and Beli P (2021) R-loop proximity proteomics identifies a role of DDX41 in transcription-associated genomic instability. *Nat Commun*, 12:7314

Borisova ME, Voigt A, Tollenaere MAX, Sahu SK, Juretschke T, Kreim N, Mailand 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

*indicates joint contribution

Falk **BUTTER**

"We use mass spectrometry to study protein expression changes in development and evolution."

POSITIONS HELD

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

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

EDUCATION

2010 PhD in Biochemistry, Ludwig Maximilian University (LMU), Munich

2006 Diploma in Biochemistry, University of Leipzig

GROUP MEMBERS

Alexandra Blake	PhD Student; since 04/2022
Hanna Braun	PhD Student; 08/2015 - 08/2022
Alejandro Ceron	PhD Student; since 10/2019
Mario Dejung	Bioinformatician; since 05/2014
Albert Fradera Sola	PhD Student; since 03/2018
Carisa Goh Sho Yee	PhD Student; since 10/2019
Michal Levin	Postdoc; since 09/2018
Liudmyla Lototska	Postdoc; 06/2019 - 12/2022
Rachel Mullner	Lab Technician; since 02/2021
Emily Nischwitz	PhD Student; since 09/2018
Sarah Pawusch	PhD Student; since 10/2021
Franziska Roth	Lab Technician; since 01/2019
Marion Scheibe	Postdoc; since 06/2013
Vivien Schoonenberg	PhD Student; since 02/2018
Patricia Schupp	PhD Student; since 04/2021
Jan Sluka	PhD Student; since 03/2022
Lars Teschke	PhD Student; since 04/2022
Varvara Verkhova	PhD Student; since 11/2020
Maya Wilkens	PhD Student; since 10/2019

OVERVIEW

Mass spectrometry is a powerful tool for studying proteins in an unbiased and global manner. Current improvements in identification accuracy, sample throughput and data analysis allow the streamlined application of proteomics to answer diverse biological questions. Our group applies quantitative approaches such as label-free quantitation (LFQ), reductive demethylation (DML) and stable isotope labelling with amino acids in cell culture (SILAC), which enable 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 protein interactions 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, which we termed "phylointeractomics". In phylointeractomics, we interrogate a bait of interest with the proteomes 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 humans, which spans an evolutionary timeframe of 450 million years. 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 an absence of TIN2 in bird genomes. In our phylointeractomics screen, we recapitulated these evolutionary differences for the shelterin complex and discovered that, contrary to predictions, not all homologs of TRF1 (a direct TTAGGG repeat-binding

subunit of the complex) associated with our telomeric baits. Using recombinant TRF1 DNA-binding domains of even more vertebrate species, we located 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 exchanging selected amino acid residues in the platypus TRF1-DNA-binding domain, we could recreate a gain-of-binding switch *in vitro* that recapitulates a possible evolutionary scenario. Our phylointeractomics study therefore underscores that sequence homologs, 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. In addition to HOT1, 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 more novel telomere-binding proteins. For example, we have just identified the long-sought double-strand telomere-binding proteins TEBP-1 and TEBP-2 in the nematode *C. elegans*. We showed that both proteins modulate telomere length, but TEBP-1 CRISPR knockout strains have longer telomeres while TEBP-2 knockout strains have shorter telomeres and exhibit a mortal germline phenotype. Notably, both double-strand telomere-binding proteins form a complex with the known *C. elegans* single-strand binder POT-1, defining the first known telomere complex in nematodes.

Systems approaches to studying gene regulation

To study proteome dynamics during development, we generated two large developmental proteomic datasets for *Drosophila melanogaster*: a full life cycle dataset encompassing 15 different time points and a high temporal resolution proteome of embryogenesis. As both datasets match the previously published modENCODE developmental transcriptome, we systematically compared the developmental transcriptome and proteome and showed that in some 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). Recently, we published one of the biggest proteome datasets to date by analysing proteome changes in one of our yeast knockout collections (see Figure 1).

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 in our group during the last few years. By combining omics studies with classical biology, we are currently characterising novel telomeric proteins in diverse model species and investigating gene regulation in several eukaryotes.

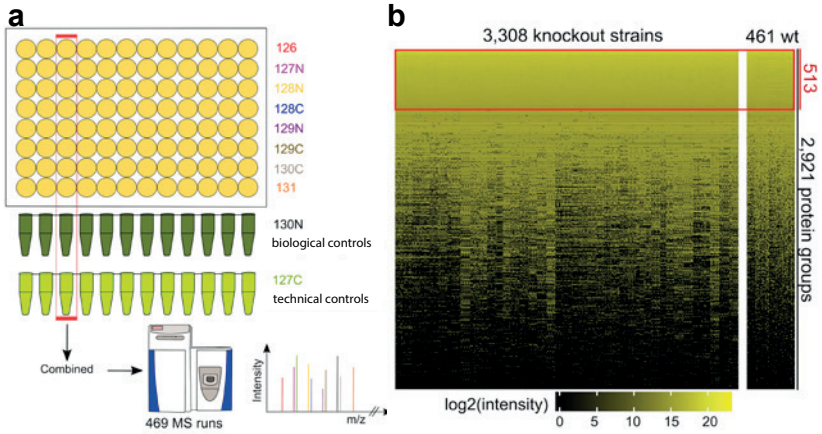


Figure 1. Studying the proteome across a complete knockout library. A) Schematics of the experimental design for quantifying protein expression levels in the *S. pombe* knockout collection by quantitative proteomics using 10plex tandem mass tags. B) Normalised protein expression levels of 3,308 knockout strains and 461 wild-type replicates. The complete dataset contains the quantification of 2,921 protein-coding genes, of which 513 proteins were quantified across all samples. (Öztürk *et al*, 2022, *Nat Commun*).

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Öztürk M, Freiwald A, Cartano J, Schmitt R, Dejung M, Luck K, Al-Sady B, Braun S, Levin M and Butter F (2022) Proteome effects of genome-wide single gene perturbations. *Nat Commun*, 13:6153

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Harris CJ*, Scheibe M*, Wongpalee SP, Liu W, Cornett EM, Vaughan RM, Li X, ..., Butter F# and Jacobsen SE# (2018) A DNA methylation reader complex that enhances gene transcription. *Science*, 362:1182–1186

*indicates joint contribution, #indicates joint correspondence

Dorothee **DORMANN**

"We study the molecular ageing processes that drive neurodegeneration."

POSITIONS HELD

Since 2021 Adjunct Director, Institute of Molecular Biology (IMB) and Professor of Molecular Cell Biology, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz

2014 – 2021 Emmy Noether Group Leader at LMU Munich, Biomedical Center (BMC), Cell Biology

2007 – 2014 Postdoctoral Fellow at LMU Munich, Adolf-Butenandt Institute

EDUCATION

2007 PhD, Rockefeller University, New York

2002 Diplom in Biochemistry, Eberhard-Karls-Universität Tübingen

GROUP MEMBERS

Lara Gruijs da Silva	PhD Student; since 10/2016
Saskia Hutten	Postdoc; since 04/2016
Bernhard Lieb	Staff Scientist; since 04/2021
Simone Mosna	PhD Student; since 08/2021
Thomas Schubert	Research Assistant; since 04/2021
Francesca Simonetti	PhD Student; since 09/2019
Erin Sternberg	Postdoc; since 07/2019
Yongwon Suk	PhD Student; since 08/2020
Irene Yiallouros	Staff Scientist; since 04/2021
Fatmanur Tiryaki Yildiz	PhD Student; since 11/2021
Yelyzaveta Zadorozhna	PhD Student; since 01/2022

OVERVIEW

Our research focuses on the molecular mechanisms of age-associated neurodegenerative diseases, most notably ALS (amyotrophic lateral sclerosis), FTD (frontotemporal dementia) and Alzheimer's disease. Existing therapies are designed to treat only the symptoms of disease, and no therapies are available to slow down or stop disease progression. Our main objective is to obtain a molecular understanding of the mechanisms underlying these devastating disorders. We seek to unravel how RNA-binding proteins (RBPs) such as TDP-43 and FUS become mislocalised and aggregated in these disorders, and how dysregulation of these RBPs causes a decline in cellular function and eventually neurodegeneration. We previously demonstrated that RBP mislocalisation and aggregation in ALS and FTD are intimately linked to 1) disturbed nuclear import, 2) aberrant phase separation and molecular ageing processes, and 3) altered post-translational modifications (PTMs). We therefore study how nuclear transport, phase separation and PTMs of these disease-linked RBPs are normally regulated, how they are misregulated in disease and how these pathological changes could be overcome. By understanding the molecular mechanisms of protein mislocalisation and aggregation, we hope to help develop new therapeutic approaches to treat neurodegenerative diseases.

RESEARCH HIGHLIGHTS

The neurodegeneration-linked RBPs TDP-43 and FUS harbour extended intrinsically disordered regions (IDRs) that allow them to undergo weak multivalent self-self interactions, leading to liquid-liquid phase separation (LLPS) and partitioning into cellular condensates, such as stress granules. Subsequent liquid-to-solid state transition is a molecular ageing process believed to underlie the formation of

pathological protein aggregates, however such aberrant phase transitions are normally suppressed by cellular quality control mechanisms. We have uncovered two important quality control mechanisms: regulation of RBP phase transitions by nuclear import receptors and PTMs. Using *in vitro* reconstitution and cellular experiments, we showed that the nuclear import receptor of FUS (Transportin, TNPO1) efficiently suppresses LLPS and stress granule recruitment of FUS by interacting with the C-terminal nuclear localisation signal and RGG/RG-rich regions, both of which are crucial drivers of FUS LLPS. We also found that other importins (e.g. TNPO3 and importin ß) bind directly to FUS via RGG/RG motifs and can suppress LLPS and stress granule recruitment of FUS, and that TNPO3 and importin ß can also import FUS into the nucleus, albeit with lower efficiency than Transportin. Based on these findings, we propose that FUS utilises a network of import receptors for chaperoning and import, similar to histones, ribosomal proteins and other RBPs we have investigated. We also found that importins can directly bind and "shield" toxic arginine-rich dipeptide repeat proteins (poly-GR and -PR) that arise in the most common inherited form of ALS and FTD due to a hexanucleotide (GGGGCC) repeat expansion in the *C9orf72* gene. Poly-GR and -PR aberrantly interact with nucleic acids and promote LLPS of various RBPs, including TDP-43; however, these pathological interactions can be suppressed by elevated importin levels. Together, our work established an important role for importins in protein quality control as suppressors of aberrant phase transitions linked to disease. Thus, elevating importin levels or enhancing the binding of importins to aggregation-prone proteins could be a novel treatment for protein aggregation disorders. A second key regulator of RBP phase transitions we have uncovered is disease-associated PTMs. PTMs frequently occur in IDRs and influence phase separation, RNA-binding properties and RNA-processing functions. Abnormal PTMs often arise in neurodegenerative diseases, e.g. TDP-43 is hyperphosphorylated in ALS and FTD, and we found that arginine methylation of FUS RGG/RG motifs is reduced in FTD patients. Using *in vitro* methylation of purified FUS and phase separation assays, we found that FUS hypomethylation promotes LLPS and stress granule accumulation, suggesting that loss of this PTM may promote FUS aggregation in disease. More recently, we found that disease-associated C-terminal TDP-43 hyperphosphorylation reduces TDP-43 phase separation and aggregation, renders TDP-43 condensates more dynamic and liquid-like, and suppresses its recruitment into cellular condensates that TDP-43 phosphorylation may therefore be a protective cellular mechanism for preventing its aggregation and a physiological mechanism for regulating TDP-43 condensation.

FUTURE DIRECTIONS

We aim to further decipher how RBPs become dysfunctional in the context of neurodegenerative disorders and what consequences impaired importin function, aberrant RBP phase transitions and disease-linked PTMs have in cells. For instance, we plan to globally explore the cytosolic "chaperoning" function of importins by determining which proteins in the human proteome become insoluble upon acute importin depletion and better understand the interaction of the nuclear transport machinery (importins and nucleoporins) with TDP-43 and FUS. We also plan to identify and study potential new regulators/modifiers of FUS and TDP-43 phase separation and molecular ageing (e.g. interacting proteins, nucleic acids, PTMs, antibodies or small molecules). Moreover, we will address whether the main physiological functions of TDP-43 and FUS (e.g. regulation of splicing, translation, R loops and DNA damage repair) require phase separation or are altered by aberrant phase transitions, and to what extent their molecular sociology (i.e. protein and RNA interactions) is affected by altered self-assembly and phase separation behaviour.

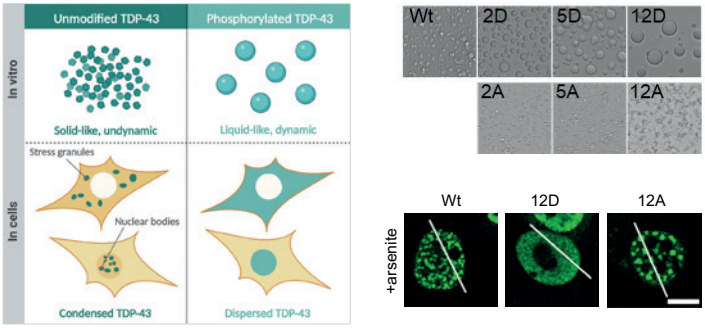

***Figure 1.* Suppression of TDP-43 condensation by phosphorylation.** Unphosphorylated TDP-43 shows a high propensity to condense and aggregate, whereas disease-associated C-terminal hyperphosphorylation, as seen in ALS and FTD patients, solubilises TDP-43 and suppresses its aggregation and accumulation in stress-induced cellular condensates. These findings are illustrated by *in vitro* reconstitution and cellular experiments comparing the condensation behaviour of wild-type TDP-43 (Wt) and TDP-43 harbouring 2, 5 or 12 phospho-mimetic serine-to-aspartate (D) or corresponding serine-to-alanine (A) mutations.

SELECTED PUBLICATIONS

Gruijs da Silva LA, Simonetti F, Hutten S, Riemenschneider H, Sternburg EL, Pietrek LM, Gebel J, Dötsch V, Edbauer D, Hummer G, Stelzl LS and Dormann D (2022) Disease-linked TDP-43 hyperphosphorylation suppresses TDP-43 condensation and aggregation. *EMBO J*, 41:e108443

Hutten S#, Usluer S, Bourgeois B, Simonetti F, Odeh HM, Fare CM, Czuppa M, Hruska-Plochan M, Hofweber M, Polymenidou M, Shorter J, Edbauer D, Madl T and Dormann D# (2020) Nuclear import receptors directly bind to arginine-rich dipeptide repeat proteins and suppress their pathological interactions. *Cell Rep*, 33:108538

Hofweber M*, Hutten S*, Bourgeois B, Spreitzer E, Niedner-Boblenz A, Schifferer M, Ruepp MD, Simons M, Niessing D, Madl T and Dormann D (2018) Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. *Cell*, 173:706-719.e13

*indicates joint contribution, #indicates joint correspondence

Claudia **KELLER VALSECCHI**

"We show how a balanced genome allows faithful development and prevents diseases."

POSITIONS HELD

Since 2020 Group Leader, Institute of Molecular Biology (IMB), Mainz

2013 – 2020 Postdoctoral Researcher, Max Planck Institute of Immunobiology & Epigenetics, Freiburg

2012 – 2013 Postdoctoral Researcher, Friedrich Miescher Institute (FMI), Basel

EDUCATION

2012 PhD in Biochemistry, Friedrich Miescher Institute (FMI)

2008 MSc in Molecular Biology, Friedrich Miescher Institute (FMI)

2007 BSc in Molecular Biology, Biozentrum, University of Basel

GROUP MEMBERS

M. Felicia Basilicata Senior Research Associate; since 09/2020

Anna Einsiedel Student Assistant; since 09/2022

Annika Maria Fox Student Assistant; since 07/2022

José Fritz Garcia PhD Student; since 04/2022

Agata Izabela Kalita PhD Student; since 07/2020

Feyza Polat PhD Student; since 08/2021

Anna Szczepinska PhD Student; since 09/2021 (co-supervised with P Baumann and J Barau)

Frederic Zimmer PhD Student; since 07/2022

OVERVIEW

A characteristic feature of diploid organisms is that the chromosomes are present in proportional amounts with respect to one another. For example, a principal factor of miscarriage in humans is aneuploidy, a condition where chromosomes occur in different numbers than the normal two. Furthermore, exome sequencing studies reveal a growing number of rare developmental disorders caused by heterozygous mutations in individual genes. Hence, having exactly twofold gene dosage appears to be highly relevant for proper cellular and organismal function. Paradoxically, gene dosage changes also occur naturally, for example in the form of polyploidy, which is a widespread phenomenon in plants and also occurs in fish, amphibians, reptiles and insects. Another example is the heteromorphic sex chromosomes, where often only a single functional allele is present in the heterogametic sex. Such dosage alterations can be buffered by cellular mechanisms such as dosage compensation. Using genome engineering, epigenomics, biochemistry and imaging, we investigate the causes and consequences of gene dosage alterations and their impact on developmental, pathogenic and evolutionary processes.

RESEARCH HIGHLIGHTS

Dosage regulation in sex chromosomes

Sex chromosomes and their regulatory pathways show extremely high evolutionary turnover. We have recently compared DC in *Anopheles* mosquitos and fruit flies, two closely related dipteran insects with similar gene content and whose X chromosomes have evolved from the same ancestral autosome. Interestingly, we find that DC in *Anopheles* is achieved by an unknown molecular mechanism entirely different from that in *Drosophila*. Through functional genomics, we have now identified a previously uncharacterised gene that is specifically expressed in males.

This gene, which we called *SOA*, encodes a nucleic acid-binding protein that associates with a distinct subnuclear territory. Interfering with the function of SOA *in vivo* results in dysregulation of the sex chromosomal genes. Parallel to this work in insects, we also aim to understand the complex biology of the sex chromosomes in other systems, for example in mammals where females inactivate one of their two X chromosomes. Despite X inactivation, some genes escape silencing and hence are expressed from both X chromosomes. This may provide an advantage to females by providing an increase in gene dosage and higher allelic diversity. Together with colleagues from JGU and the University Medical Center in Mainz, we have now developed a novel cellular system to understand this poorly characterised phenomenon in the context of ageing and neurodevelopment. Finally, we also study sex chromosome regulation in the crustacean *Artemia*, which lives in waters with very high salinity (up to 25%). This will be the first characterisation of sex chromosome regulation in a ZW species, where females are heterogametic (i.e. contain only one functional gene copy of the genes located on the Z chromosome) and males are homogametic (ZZ, with two gene copies).

Gene paralogues and dosage-sensitivity of RNA-binding proteins

Dosage alterations occur not only at a chromosome-wide level, but also at the level of individual genes. Gene duplication events are a key process in evolution because they create redundancy, which liberates DNA sequences from previous selective constraints. Gene duplication was responsible for creating the two paralogues SNRPB and SNRPN, which are tissue-specific components of the spliceosome. Mutations in these genes can lead to disorders in humans that manifest with remarkably distinct phenotypes. We have created a heterologous system in which we characterised the dosage responses of these two paralogues. We detected crosstalk between these paralogues and furthermore found that they display intrinsic differences in their cellular behaviour, despite their sequences being almost identical. These differences manifest at a posttranscriptional level and appear to involve distinct protein turnover rates.

FUTURE DIRECTIONS

Our goal is to characterise DC mediated by SOA. Using genomics, we will identify how it regulates the X chromosome and create mutants to understand its relevance for DC. In addition to being only the 4th DC mechanism ever to be identified, these findings could ultimately inform novel strategies for fighting infectious diseases such as malaria by vector control. We also plan to develop tools to comprehensively identify dosage-sensitive genes and cellular responses in mammals, for example those in SNRPB/N. We will also expand our work on the mammalian X chromosome and study the mechanisms of reactivation during development, as well as age-related chromosomal mosaicism during ageing.

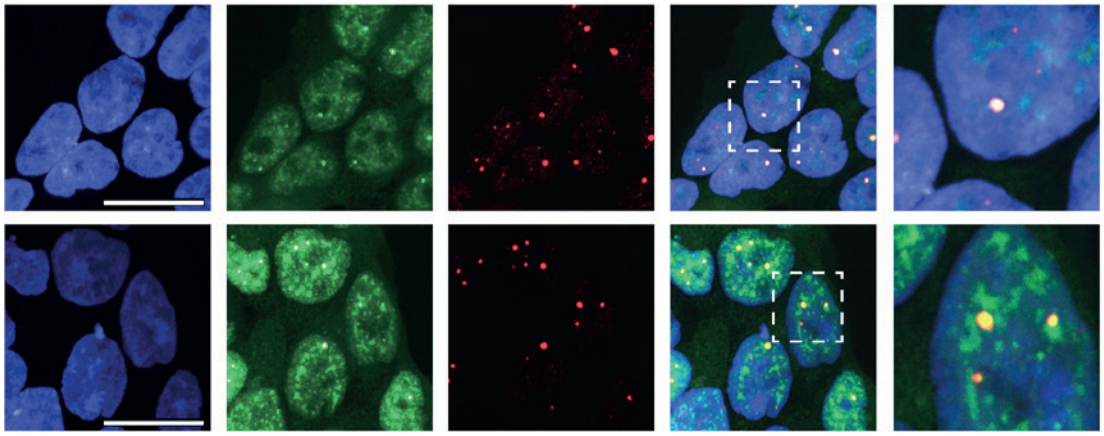

Figure 1. Immunofluorescence of Snrpb and Snrpn splicing paralogues (green) upon ectopic expression in human cells. The nucleus is visualised in blue. The two paralogues co-localise with coilin (red), which marks Cajal bodies. A merged picture with magnification is shown at the very right.

SELECTED PUBLICATIONS

Basilicata MF# and Keller Valsecchi CI# (2021) The good, the bad and the ugly: Evolutionary and pathological aspects of gene dosage alterations. *PLOS Genet*, 17:e1009906

Keller Valsecchi CI*, Basilicata MF*, Georgiev PG, Gaub A, Seyfferth J, Kulkarni T, Panhale A, Semplicio G, Dasmeh P and Akhtar A (2021) RNA nucleation by MSL2 induces selective X chromosome compartmentalization. *Nature*, 589:137-142

Keller Valsecchi CI*#, Marois E*, Basilicata MF*, Georgiev P and Akhtar A# (2021) Distinct mechanisms mediate X chromosome dosage compensation in *Anopheles* and *Drosophila*. *Life Sci Alliance*, 4:e202000996

*indicates joint contribution, #indicates joint correspondence

René **KETTING**

"We seek to understand epigenetic inheritance by identifying the molecular mechanisms."

POSITIONS HELD

Since 2022	Executive Director, Institute of Molecular Biology (IMB), Mainz
Since 2012	Scientific Director, Institute of Molecular Biology (IMB), Mainz
	Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz
2015 – 2017	Executive Director, Institute of Molecular Biology (IMB), Mainz
2010 – 2013	Professor of Epigenetics in Development, University of Utrecht
2005 – 2012	Group Leader, Hubrecht Institute, Utrecht
2000 – 2004	Postdoc, Hubrecht Institute, Utrecht
2000	Postdoc, Cold Spring Harbor Laboratories

EDUCATION

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

GROUP MEMBERS

Walter Bronkhorst	Postdoc; since 01/2015
Fiona Carey	PhD Student; since 10/2021
Edoardo Caspani	PhD Student; since 01/2017
Alessandro Consorte	PhD Student; since 01/2018
Joana Sofia Costa Pereirinha	PhD Student; since 01/2018
Yasmin El Sherif	Lab Manager; since 10/2014
Svena Hellmann	Technician; since 11/2016
Adrian Hepp	Student Assistant; since 08/2021
Ida Josefine Isolehto	PhD Student; since 08/2020
Joanna Michowicz	PhD Student; since 09/2022
Regina Otto	Personal Assistant; since 09/2015
Diego Páez Moscoso	Postdoc; since 08/2021
René-Maurice Pfeifer	Student Assistant; since 12/2018
Nadezda Podvalnya	PhD Student; since 01/2018
Lizaveta Pshanichnaya	PhD Student; since 10/2021
Shéraz Sadouki	Lab Technician; since 10/2020
Jan Schreier	Postdoc; since 05/2021 (PhD Student since 05/2015)
Ann-Sophie Seistrup	PhD Student; since 07/2019
Shamitha Shamitha	PhD Student; since 11/2019
Nadine Wittkopp	Senior Research Associate; since 11/2012

OVERVIEW

The major focus of my lab is gene regulation by small RNA molecules acting through 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 the 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 by 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 such as how small RNA pathways distinguish transposable elements from regular genes, how these pathways are organised at a sub-cellular level and how small RNA populations can be inherited across generations are at the heart of our research.

RESEARCH HIGHLIGHTS

Epigenetic inheritance via sperm through a newly-identified germ granule

Epigenetic inheritance describes the inheritance of genetic traits independent of the genomic sequence. This mode of inheritance is increasingly recognised as an important aspect of development, but the molecular mechanisms that may be behind such cases of inheritance are only poorly understood. This lack of knowledge about how this works precisely is a major problem for the field, because without credible hypotheses about the molecular mechanisms, the

phenomena covered by the term "epigenetic inheritance" will remain just that: phenomena.

One mode of epigenetic inheritance is driven by small non-coding RNAs and it has previously been shown that such inheritance can be surprisingly effective via the male. The reason why this is surprising is that during the process of sperm cell development the majority of cellular components are discarded, leaving the sperm with just a nucleus and some mitochondria. How then can cytoplasmic small RNAs be inherited? We identified a novel subcellular compartment that is specifically found in the sperm cells of the nematode *C. elegans*. These granules, named PEI granules (for Paternal Epigenetic Inheritance), can absorb a specific protein, named WAGO-3. This protein binds small RNAs and as a complex it can trigger gene regulation. We could show that WAGO-3 is indeed required for the inheritance of small RNA-mediated gene regulation via the male. Furthermore, we could show that the PEI granules are needed for this as well. Without PEI granules, WAGO-3 is still normally expressed during spermatogenesis, but it is lost from the maturing sperm cells, just like much of the rest of the sperm cell cytoplasm.

We continued to resolve how PEI granules are retained in sperm cells and identified two PEI granule proteins named PEI-1 and PEI-2. While PEI-1 is mostly needed for PEI granule formation and the recruitment of WAGO-3, PEI-2 appears to be primarily required to ensure PEI granule maintenance within the maturing sperm cells. Our experiments suggest that PEI-2 may be modified with fatty acid chains and that these mediate interactions with membranes of so-called FB-MOs. FB-MOs are organelles derived from the Golgi that resemble human acrosomes (membrane structures required during the process of fertilisation). These FB-MOs are maintained in maturing sperm by a cellular motor protein named myosin VI, and indeed, myosin VI is also required for PEI granule functionality.

These studies have revealed for the first time a detailed molecular mechanism behind the inheritance of small RNAs through sperm. At the same time, this work has identified a novel germ granule and coupled it with a very specific function. In general, germ granules can be seen as great systems to study the relevance and impact of a process called phase separation on biological processes in a biologically-relevant setting. Thus far, PEI granules appear to consist of rather few components and hence they present great opportunities to dissect germ granule properties and function in unprecedented detail.

FUTURE DIRECTIONS

Future work will continue to mechanistically unravel the molecular pathways that are steered by small RNA molecules. One current emphasis will be on the further characterisation of small RNA inheritance via the above-described PEI granules. This mechanism likely involves a process known as phase separation, and a future aim will be to understand how this mechanism can release the silencing information in the fertilised oocyte. We will also study additional piRNA-related condensates and how they are regulated during germ cell development and embryogenesis in *C. elegans* and zebrafish. We will increasingly use biochemistry to look at phase separation-related processes, as well as the processing of small RNAs. Finally, we will continue to use genetic screens and are currently planning one to better understand the embryonic function of a piRNA biogenesis complex that we recently identified, as our results indicate that this complex has a much broader function.

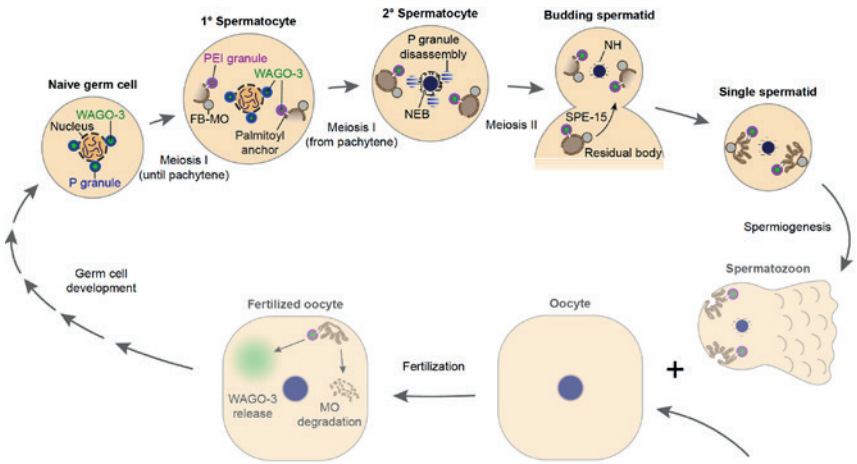

Figure 1. A model for how PEI granules mediate the inheritance of gene-regulatory small RNAs, which are bound to the Argonaute protein WAGO-3.

SELECTED PUBLICATIONS

Schreier J, Dietz S, Boermel M, Oorschot V, Seistrup AS, de Jesus Domingues AM, Bronkhorst AW, Nguyen DAH, Phillis S, Gleason EJ, L'Hernault SW, Phillips CM, Butter F and Ketting RF (2022) Membrane-associated cytoplasmic granules carrying the Argonaute protein WAGO-3 enable paternal epigenetic inheritance in *Caenorhabditis elegans*. *Nat Cell Biol*, 24:217-229

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Roovers EF*, Kaaij LJT*, Redl S, Bronkhorst AW, Wiebrands K, de Jesus Domingues AM, Huang H, Han C, Riemer S, Dosch 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

*indicates joint contribution

Anton **KHMELINSKII**

"We work to dissect the protein degradation code."

POSITIONS HELD

Since 2018	Group Leader, Institute of Molecular Biology (IMB), Mainz
2013	Visiting Scientist, Donnelly Centre for Cellular & 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
2010 – 2011	Postdoc, European Molecular Biology Laboratory (EMBL), Heidelberg

EDUCATION

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

GROUP MEMBERS

Karla Blöcher-Juárez	PhD Student; since 04/2019
Andrea Coti	PhD Student; since 12/2019
Jia Jun Fung	PhD Student; since 01/2018
Anika Haschke	Bachelor Student; since 07/2022
Elena Ivanova	PhD Student; since 03/2019
Alina Jenn	Student Assistant; since 10/2021
Vincent Klösgen	Student Assistant; since 11/2021
Ka Yiu Kong	Postdoc; since 08/2019
Zhaoyan Li	PhD Student; since 12/2018
Rocío Nieto-Arellano	Postdoc; since 04/2018
Christian Ochs	PhD Student; since 09/2021
Anke Salzer	Lab Manager; since 01/2018
Susmitha Shankar	PhD Student; since 06/2020

OVERVIEW

Proteome integrity is maintained by a complex network that controls the synthesis, folding, transport and degradation of proteins. Numerous quality control systems operate throughout the protein lifecycle to prevent, detect and remove abnormal proteins, thus contributing to proteome homeostasis. Selective protein degradation by the ubiquitin-proteasome system (UPS) plays a key role in proteome turnover and quality control. When degradation is not possible, abnormal proteins can eventually be removed via asymmetric partitioning during cell division. Despite the activity of such systems, proteome homeostasis declines with ageing and in numerous diseases, resulting in the accumulation of abnormal proteins and loss of cell functionality. Working in yeast and human cells, we aim to systematically examine how cells deal with different types of abnormal proteins. We use genetic and proteomic approaches that exploit fluorescent timers to identify UPS substrates and explore the functions of this system in replicative ageing and genome stability. Our goals are to understand the coordination between protein biogenesis and quality control, decipher how abnormal proteins are recognised and elucidate how cells adapt to challenges in proteome homeostasis.

RESEARCH HIGHLIGHTS

Selective protein degradation is involved in most cellular processes and contributes to proteome homeostasis by removing unnecessary or abnormal proteins. The UPS is the key system of selective protein degradation, whereby a cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-protein ligase (E3) enzymes marks proteins with polyubiquitin chains for proteasomal degradation. Deubiquitinating enzymes (DUBs), 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, the functions of many UPS components are unclear and the substrate specificities of E3s and DUBs are not well defined. To address these limitations, we systematically assessed the roles of UPS components in proteome turnover using budding yeast as a model. We established a proteomic approach based on fluorescent timers to examine how the inactivation of individual UPS components affects proteome abundance and turnover (Figure 1A). Using this approach, we analysed the state of the yeast proteome in mutants of almost all known E2s, E3s and DUBs and observed phenotypes for 76% of them (Kong *et al*, 2021). This effort yielded a rich dataset to explore the functions of the UPS from the perspective of a protein of interest or with a UPS component as a starting point (Figure 1B).

We used this dataset to gain insights into the functions of a poorly studied E3 called GID/CTLH. The yeast GID complex is a large multisubunit E3 that appears to use interchangeable receptor subunits for substrate recognition via N-degrons, i.e. degradation signals located at the N-termini of proteins. Previously described GID substrates carry N-degrons specified by an N-terminal proline. Most notably, GID targets four gluconeogenic enzymes (Mdh2, Pck1, Fbp1 and Icl1) for proteasomal degradation when yeast are switched from ethanol to glucose as a carbon source and transiently induces expression of the Gid4 receptor subunit.

Surprisingly, we identified 30 potential GID substrates that have an N-terminal threonine or serine instead of a proline. How are such substrates recognised by GID? Genetic screens for turnover regulators of two such substrates, the nucleotidase Phm8 and the carbamoyl phosphate synthetase Cpa1, led us to the uncharacterised protein Ylr149c/Gid11. Using genetic and biochemical approaches, we found that Gid11 is a GID subunit that likely functions as a receptor recognising substrates via threonine N-degrons (Figure 1C). Our work shows that Gid11 expression is induced during a variety of metabolic transitions, including the switch from glucose to ethanol as a carbon source, suggesting a model whereby the GID^{Gid11} complex downregulates glycolysis during this transition. Thus, whereas GID^{Gid4} targets gluconeogenic enzymes for proteasomal degradation when cells switch from ethanol to glucose, GID^{Gid11} is potentially involved in downregulation of glycolytic enzymes during the reverse transition (Figure 1C). We are eager to identify the factors involved in conditional expression of the Gid4 and Gid11 substrate receptors, thus defining this so-far unique regulatory network, to determine the mechanisms by which GID^{Gid11} controls glycolysis and explore the possibility that GID regulates bidirectional control of other metabolic transitions.

FUTURE DIRECTIONS

We will continue our work on the GID complex to define its substrate repertoire, its different modes of substrate recognition and its physiological functions. In addition, we will expand our systematic characterisation of the UPS by combining our timer-based screens with mass spectrometry and adapting these approaches to human cells. We will apply these 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 exploit our high-throughput approaches to understand how eukaryotic cells dispose of different types of abnormal proteins, including misfolded, mislocalised and orphan molecules.

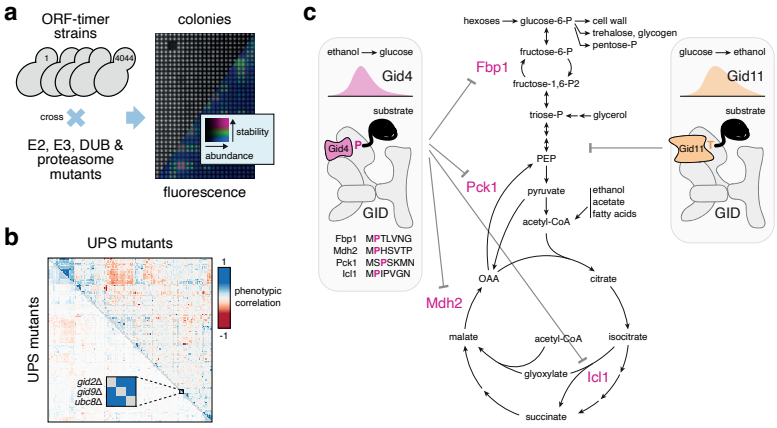

Figure 1. Systematic characterisation of the ubiquitin-proteasome system. A) Proteomic profiling of the yeast UPS in colony arrays expressing different fluorescent timer-tagged proteins and carrying mutations in individual UPS components. B) Correlations between the proteome turnover profiles of UPS mutants highlight functional relationships between UPS components. C) Model of bidirectional control of the switch between glycolysis and gluconeogenesis by GID complexes.

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*indicates joint contribution, #indicates joint correspondence

Julian **KÖNIG**

"We investigate the epigenetics of RNA."

POSITIONS HELD

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

2008 – 2013 Postdoc, MRC Laboratory of Molecular Biology, Cambridge

EDUCATION

2008 PhD in Biology, Max Planck Institute for Terrestrial Microbiology & Philipps University, Marburg

2003 Diploma in Biology, Ludwig Maximilian University (LMU), Munich

GROUP MEMBERS

Miona Corovic	PhD Student; since 10/2021
Stefanie Ebersberger	Postdoc; since 04/2014
Peter Hoch-Kraft	Postdoc; 10/2020
Nadine Körtel	PhD Student; since 11/2018
Nicolas Melchior	Master Student; since 02/2022
Mikhail Mesitov	Postdoc; since 10/2019
Miriam Mulorz	PhD Student; since 11/2019
Anna Orekhova	Lab Manager; since 07/2020
Tim Preißendörfer	Master Student; since 05/2022
Cornelia Rückle	PhD Student; since 04/2018
Kerstin Tretow	PhD Student; since 12/2018

OVERVIEW

Posttranscriptional gene regulation plays an important role not only in development and tissue identity but also in neurodegenerative diseases and cancer. The fate of mRNA is regulated by the cooperative action of RNA-binding proteins (RBPs), which recognise specific RNA sequences to form messenger ribonucleoprotein complexes (mRNPs). The information in the RNA sequence and how it is interpreted by RBPs is commonly referred to as the 'mRNP code'. However, the molecular features that define this code remain poorly understood. My main goal is to significantly contribute to cracking the mRNP code.

RESEARCH HIGHLIGHTS

An autoinhibitory intramolecular interaction proofreads RNA recognition by the essential splicing factor U2AF2

In a recent study together with the Sattler group at the Technical University of Munich, we combined our *in vitro* iCLIP approach with NMR structural biology to identify a novel molecular mechanism for U2AF2 splicing regulation. The recognition of splice sites is initiated by U2AF2 binding stably to the poly-pyrimidine tract (Py-tract) upstream of exons to assemble the spliceosome. However, it remains unclear how U2AF2 discriminates between weak and strong Py-tract RNAs. We found that the intrinsically disordered linker region connecting the two RNA recognition motif (RRM) domains of U2AF2 mediates autoinhibitory intramolecular interactions that reduce non-productive binding to weak Py-tract RNAs. This proofreading favours the binding of U2AF2 at stronger Py-tracts, which is required to define 3' splice sites at the early stages of spliceosome assembly. Mutations that impair the linker autoinhibition enhance U2AF2 affinity for weak Py-tracts, resulting in promiscuous binding along mRNAs and impacting splicing fidelity. Our

findings highlight an important role for intrinsically disordered linkers in modulating the RNA interactions of multi-domain RBPs.

RNA stability controlled by m6A methylation drives X-to-autosome dosage compensation in mammals

In mammals, X-chromosomal genes are expressed from a single copy since males (XY) possess a single X chromosome while females (XX) undergo X inactivation. To compensate for this reduction in dosage relative to the two active copies of autosomes, it has been proposed that genes from the active X chromosome exhibit dosage compensation ("Ohno's hypothesis"). However, the existence and mechanism of X-to-autosome dosage compensation are still under debate. Here, we show that dosage compensation is achieved via differential N6-methyladenosine (m6A) RNA modification. X-chromosomal transcripts are deficient in m6A modifications and more stable compared to their autosomal counterparts. Acute depletion of m6A using a small molecule inhibitor selectively stabilises autosomal transcripts across sexes, cell types, tissues and species, resulting in perturbed dosage compensation. We propose that increased stability of X-chromosomal transcripts is directed by lower levels of m6A, indicating that mammalian dosage compensation occurs via epitranscriptomic RNA regulation.

FUTURE DIRECTIONS

My research will focus on deciphering the regulatory code of splicing and quality control mechanisms in human physiology and disease. To this end, we will build on the iCLIP technology to map protein-RNA interaction sites throughout the transcriptome. We will use our approaches to predict mutations that cause mis-splicing in cancer and neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). We will also take a closer look at critical RNA regulators that are relevant in neurodegeneration. For instance, we recently showed that small alterations in the cellular concentration of the RNA-binding protein HNRNPH can have a strong impact on alternative splicing events in diseases caused by nuclear aggregation. In a parallel project, we investigate the role of m6A modifications in splicing regulation. The aim is to compile the full catalogue of m6A-dependent splicing events in the transcriptome and reliably map all m6A sites that may impact these events.

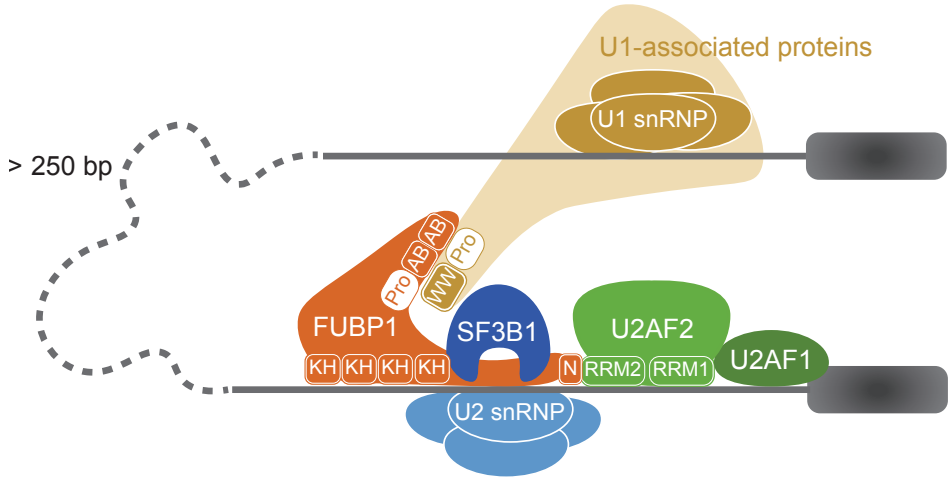

Figure 1. Early spliceosome assembly at the 5′ and 3′ splice sites.

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*indicates joint contribution, #indicates joint correspondence

Nard **KUBBEN**

"We elucidate the origins of ageing through a high-throughput microscopic lens."

POSITIONS HELD

Since 2021 Group Leader, Institute of Molecular Biology (IMB), Mainz

2015 – 2019 NIH Research Fellow, National Cancer Institute, NIH, Bethesda

2011 – 2015 NIH Postdoctoral Fellow, National Cancer Institute, NIH, Bethesda

EDUCATION

2004 – 2010 PhD in Molecular Biology, Maastricht University

2001 – 2004 MSc in Biological Health Sciences, Maastricht University

2000 – 2001 BS in Health Sciences, Maastricht University

GROUP MEMBERS

Lukas Mann PhD Student; since 05/2021
Felix Etienne van der Walt PhD Student; since 05/2021
Verena Wilhelm Lab Manager; since 11/2021

OVERVIEW

Ageing is a prime pathological component of most prevalent diseases. At the cellular level, it is characterised by various hallmarks, including epigenetic alterations, genomic instability and loss of protein homeostasis, all of which contribute to an organism-wide decline in function. Unfortunately, our current knowledge of the molecular pathways that drive cellular ageing and the formation of ageing hallmarks is severely limited. Our lab's work focuses on uncovering the fundamental biological mechanisms of ageing that can be manipulated to slow down the progression of ageing-related diseases, including the rare, lethal premature ageing disease Hutchinson-Gilford Progeria Syndrome (HGPS). Our group employs unbiased genomics, proteomics and high-throughput microscopy-based screening approaches to 1) identify novel pathways that slow down the onset of cellular ageing, 2) investigate cellular pathways that help reverse ageing defects that have already formed, and 3) validate the therapeutic potential of identified ageing mechanisms across various model systems of ageing-related diseases. The overarching goal of our research is to uncover fundamental biological mechanisms of ageing, which can help to improve human healthspan.

RESEARCH HIGHLIGHTS

A novel model system to identify drivers of ageing

One of the major challenges of ageing research is that ageing manifests as a slow build-up of relatively low percentages of aged cells in our bodies. Molecular techniques that aim to identify causes of ageing by directly comparing young and aged biological tissue samples therefore have the disadvantage of only detecting the most robust ageing-correlated changes, many of which turn out to be a consequence rather than a cause of ageing. It is therefore key to establish a technical approach that excludes these passive bystander

effects of ageing and focuses directly on identifying mechanisms that actively drive ageing. We have therefore established an HGPS-based system to functionally screen for events that drive ageing. HGPS is predominantly caused by a silent mutation in the *LMNA* gene, which encodes the nuclear lamina-localised protein lamin A, a key organiser of the mammalian nucleus. The silent mutation in HGPS results in the accumulation of an alternatively spliced lamin A mutant termed progerin. A more modest accumulation of progerin also occurs during physiological ageing, which suggests that HGPS and physiological ageing have a common mechanistic basis. Unfortunately, the mechanisms by which progerin exerts its dominant negative effects remain largely unknown. We have now generated a cellular system in which we can inducibly express progerin and study the formation of many cellular ageing defects within a convenient time frame of only 4 days, using a semi-automated high-throughput microscopy pipeline to visualise and quantify ageing defects. This system enables us to investigate whether any type of drug or gene-targeting intervention can prevent ageing upon progerin expression, thereby identifying pathways that are directly involved in driving the cellular ageing process.

High-throughput identification of new anti-ageing targets

We have previously provided proof of principle that we can use our progerin-inducible cell system for high-throughput identification of molecular pathways that drive progerin-induced ageing and small molecular compounds that can remedy these defects. By screening a library targeting 320 human ubiquitin ligases for their capacity to prevent the formation of ageing defects, we identified defective NRF2 pathway activity as an early driver of progerin-induced ageing, which over time drives genomic instability and the formation of other ageing hallmarks. NRF2 is a transcriptional master regulator of pathways that protect the cellular proteome by promoting anti-oxidative, autophagy and ubiquitin proteasome (UPS) activity. Furthermore, we discovered that the main reason why NRF2 pathway activity becomes repressed in HGPS patients is that progerin forms intra-nuclear aggregates that can entrap the NRF2 protein. Encouraged by these findings, we propose that HGPS is primarily a proteostatic disease, unlike other premature ageing diseases such as Werner syndrome, where initial defects in DNA repair ultimately are thought to drive ageing pathologies. Initial results from a genome-wide RNAi screen on our progerin-inducible cell system support the idea of HGPS being predominantly driven by proteostatic defects, as we have now identified a second pathway that is altered in HGPS and whose misregulation drives proteostatic collapse in Parkinson's disease.

FUTURE DIRECTIONS

Our future work will continue to mechanistically unravel the molecular pathways that regulate ageing. We have started with validating candidate genes from our genome-wide RNAi screen to identify novel factors that prevent the formation of proteostatic and DNA damage ageing defects upon expression of progerin. We will further investigate these candidates for their ability to prevent and reverse the formation of a wide variety of ageing defects in both HGPS and physiologically aged cells. Complementary to this screen, which investigates the anti-ageing effects of reduced expression in target genes, we plan on establishing a new CRISPR-Cas9 based model system that will determine which genes have anti-ageing effects upon increased expression. We will further deepen our mechanistic understanding of how progerin affects cellular proteostasis through a variety of genomics- and proteomics-driven studies. Lastly, we plan on investigating the role of the newly-identified ageing mechanisms in animal models of ageing.

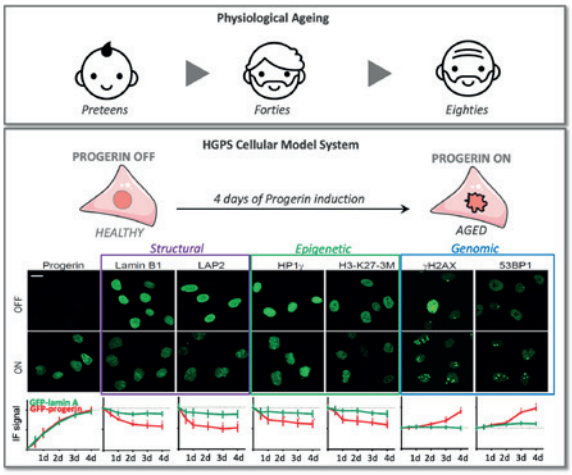

Figure 1. An overview of the inducible cellular HGPS model in comparison to other forms of ageing. The manifestation of ageing defects on average takes decades during physiological ageing, years in the premature ageing disease HGPS, and 4 days in the HGPS cellular system. Structural, epigenetic and genomic defects are induced by progerin, but not by wild-type lamin A expression.

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*indicates joint contribution, *indicates joint correspondence

Edward **LEMKE**

"We study how disorder allows proteins to be specific and selective."

POSITIONS HELD

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

Professor of Synthetic Biophysics, Johannes Gutenberg University (JGU), Mainz

2009 - 2017 Group Leader, European Molecular Biology Laboratory (EMBL), Heidelberg (visiting since 2018)

2005 - 2008 Postdoc, The Scripps Research Institute, La Jolla

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

GROUP MEMBERS

Rajanya Bhattacharjee PhD Student; since 10/2020
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Sabrina Giofrè Postdoc; since 09/2021
Nike Heinss Technology Manager; since 05/2022
Cosimo Jann Postdoc; since 06/2021
Cathrin Lutz Group Administrator; since 05/2019
Sara Mingu PhD Student; since 09/2020
Hao Ruan Postdoc; since 09/2020
Lukas Schartel PhD Student; since 02/2022
Tom Scheidt Postdoc; since 01/2020
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 most famous for their involvement in neurodegenerative diseases of ageing like Alzheimer's, Parkinson's and Huntington's disease. The ability of IDPs to exist in multiple conformations is considered a major driving force behind their enrichment during evolution in eukaryotes, but it also comes with the risk of molecularly 'ageing' into states that ultimately cause disease. Studying biological machineries containing such dynamic proteins is a huge hurdle for conventional technologies. 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, as their non-invasive character permits a 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 and synthetic biology, microfluidics and microscope engineering to increase the throughput, strength and sensitivity of the approach as a whole.

RESEARCH HIGHLIGHTS

Our strong focus on understanding the mechanisms of IDP function and molecular ageing 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/single-molecule/super-resolution/microfluidics development. 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 advantage 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. More recently, we have been able to merge our understanding of protein disorder and synthetic biology to design new membraneless organelles dedicated to protein engineering *in situ* (Figure 1). These custom organelles do not just execute a distinct second genetic code inside the cells; their bottom-up design also enables us to learn how phase separation can be used to generate new functions in eukaryotes. Our findings also have wider implications for understanding gene regulatory and stress-based mechanisms carried out by distinct, naturally-occurring organelles that play vital roles in regular cell function, as well as in ageing mechanisms. 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 *in vitro* and *in vivo*. We discovered a distinct ultrafast protein-protein interaction mechanism that can explain how nuclear pore complexes (NPCs) efficiently fulfil their central role in cellular logistics and how nuclear transport can be both fast and selective at the same time. We also used microfluidics to show how the permeability barrier of the nuclear envelope could be formed by liquid-liquid phase separation of a single disordered protein species and how they molecularly age over time. These findings provide a leap forward in our understanding of how IDPs can perform multiple functions through conformational changes, despite the normal requirement for rigid molecular specificity.

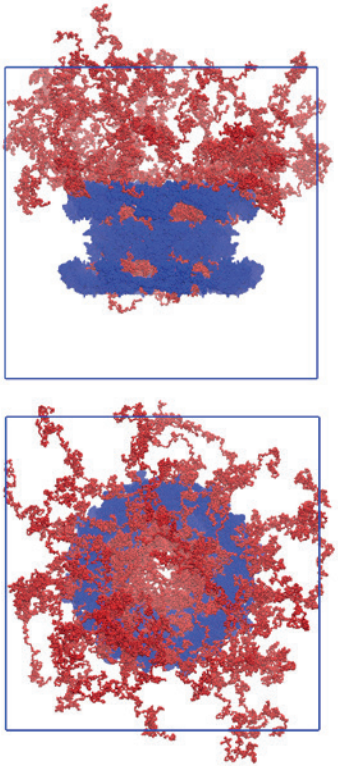

Figure 1. The known electron tomogram of the nuclear pore complex (blue) overlaid with a measured model of the disordered proteins that constitute more than a third of the total molecular weight of this vital machinery (red). Upper panel, side view; bottom panel, top view. Figure adapted from Yu *et al, BioRxiv* doi: 10.1101/2022.07.07.499201.

FUTURE DIRECTIONS

IDPs lack a stable structure and can easily misfold to the amyloid state and aggregate, resulting in their prominent role in many age-related diseases. This intrinsic risk must be outweighed by multiple advantages to explain their enrichment in the evolution of more complex species, but we are only at the beginning of understanding this. IDPs are highly multifunctional and 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-colour high- and super-resolution studies of activity-dependent protein conformation changes 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, which we will then use to identify and understand IDP multifunctionalities that are clearly distinguishable from their normal mode of action. For example, nucleoporins (Nups) normally function in the nuclear pore complex (NPC), but in fact many IDP-Nups have diverse roles, such as in pathogen-host interactions, and can even shuttle away from the NPC to function in gene regulatory processes. Moreover, fusions of Nup98 with transcription factors are known to be linked to leukaemia. 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*. Vice versa, we are particularly interested in how disordered proteins play key roles in gene regulation and cellular ageing.

SELECTED PUBLICATIONS

Yu M*, Heidari M*, Mikhaleva S*, Tan PS*, Mingu S, Ruan H, Reinkemeier CD, Obarska-Kosinska A, Siggel M, Beck M, Hummer G# and Lemke EA# (2022) Deciphering the conformations and dynamics of FG-nucleoporins *in situ*. *BioRxiv*, doi: 10.1101/2022.07.07.499201

Reinkemeier CD and Lemke EA (2021) Dual film-like organelles enable spatial separation of orthogonal eukaryotic translation. *Cell*, 184:4886-4903.e21

Reinkemeier CD, Girona GE and Lemke EA (2019) Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes. *Science*, 363:eaaw2644

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Katja **LUCK**

"We decipher how protein interactions drive cell function in health and disease."

POSITIONS HELD

Since 2020	Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz
2013 – 2019	Postdoctoral Fellow, Dana-Farber Cancer Institute and Harvard Medical School
2007 – 2008	Research Assistant, EMBL, Heidelberg

EDUCATION

2012	PhD in Bioinformatics, University of Strasbourg
2007	Diploma in Bioinformatics, Friedrich Schiller University Jena

GROUP MEMBERS

Milena Djokic	PhD Student; since 04/2021
Kristina Hintz	Master Student; since 10/2021
Johanna Lena Geist	Master Student; 09/2021 – 09/2022
Chop Yan Lee	PhD Student; since 08/2020
Dalmira Merzhakupova	PhD Student; since 12/2020
José Naveja Romero	Postdoc; since 04/2022
Christian Schäfer	Postdoc; since 08/2020
Jonas Schönfeld	PhD Student; since 07/2022
Eric Rudi Schumbera	Master Student; 06/2021 – 04/2022
Mareen Welzel	Lab Manager; since 08/2020

OVERVIEW

Cells function because their molecular components, i.e. DNA, RNA, proteins, etc. interact with each other. This complex network of molecular interactions mediates all cellular functions and organisation. Genetic and environmental insults perturb these interactions, causing disease. Because of technical limitations, we still lack a comprehensive structural and functional understanding of all the protein interactions in a human cell, hindering our ability to understand physiological and pathological molecular mechanisms. To tackle these limitations, my lab develops novel computational and experimental methods to identify protein interaction interfaces, and based on this, obtain information on the molecular function of the interaction. Furthermore, we use protein interaction interface information to predict the pathogenicity of genetic variants and develop integrative omics data approaches to generate testable mechanistic hypotheses. We apply our approaches to proteins associated with neurodevelopmental disorders (NDDs), fibroblast growth factor receptors and their role in cancer, proteins involved in mRNA splicing and BAF chromatin remodelling complexes and their implications in genome stability.

RESEARCH HIGHLIGHTS

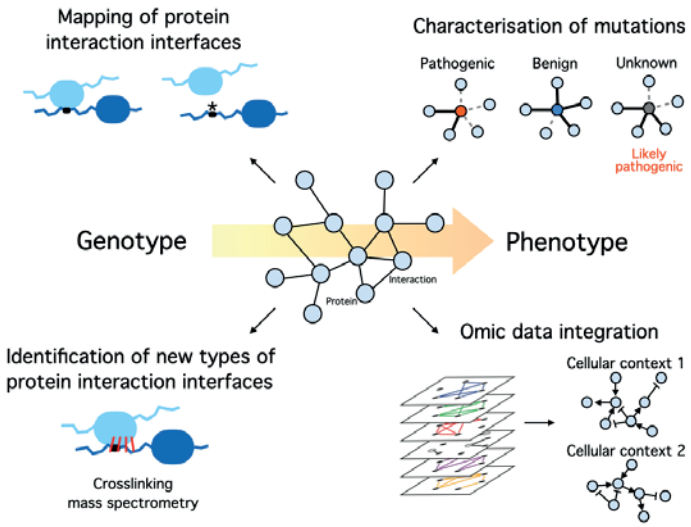
Identification of protein interaction interfaces

Proteins exhibit a modular architecture consisting of folded regions, or domains, and disordered regions, which can carry short linear motifs. Proteins commonly mediate interactions with other proteins via domain-domain or domain-linear motif interaction interfaces. We have built tools to predict domain-domain and domain-motif interfaces in known protein interactions using information on known interface types. However, most interface types are still unknown, which is why we heavily benchmarked the ability of AlphaFold Multimer to accurately predict novel types of

interfaces in interacting proteins and developed a prediction pipeline. Using our tools, we predicted interfaces for protein interactions identified in a human proteome-wide systematic interactome mapping effort (Luck *et al*, 2020, *Nature*) focusing on interactions involving NDD-associated proteins. We are now analysing the data to select candidates of interest for experimental validation. As part of this, we integrated predicted interfaces with genetic variation data to select interfaces that overlap with pathogenic or uncharacterised variants, which may indicate interfaces that cause disease when disrupted. We anticipate that experimental validation of these predictions using a high throughput cloning protocol and bioluminescence resonance energy transfer (BRET) assay that we operate in a 96-well format will aid the functional characterisation of NDD-associated proteins and their variants, as well as understanding the underlying mechanisms of disease. To this end, we collaborate with IMB's Adjunct Clinician, Susann Schweiger (UMC).

Experimental mapping of protein interaction interfaces using XL-MS

We explore the use of crosslinking mass spectrometry (XL-MS) to advance the experimental mapping of interfaces in known protein interactions. We are playing with various crosslinkers and selected interactions with structurally-resolved interfaces to develop and benchmark our XL-MS pipeline. We have made progress in obtaining the first crosslinks between tested protein pairs and are now increasing the sensitivity of the method. We are pursuing this project in close collaboration with IMB's Proteomics Core Facility.



Molecular mechanisms that regulate mRNA splicing

In collaboration with the König (IMB) and Sattler labs (TU Munich), we have used the BRET assay to decipher protein interactions and interfaces for a given protein of interest. Combined with other data, we provide strong evidence that this protein is a novel core splice factor that is particularly important for the splicing of long introns. While the manuscript is in preparation, we successfully applied for IMB collaborative research funds to support a PhD student who will continue exploring the functional roles of various protein interactions in mRNA splicing.

Integrative systems biology

Integrating various omics data resources is a powerful strategy for deciphering the systems properties of cells and allows us to employ a data-driven approach to identify new cellular mechanisms. We integrate protein interaction, gene expression and mutation data with the aim of predicting the molecular mechanisms that mediate brain-specific phenotypes in neurodevelopmental disorders. We are also collaborating with the Schick lab (IMB) to gain a systematic and global understanding of the role of BAF chromatin remodelling complexes in genome protection and repair.

FUTURE DIRECTIONS

We will continue developing our tools to predict and experimentally characterise protein interaction interfaces with the primary goal of studying protein interactions that involve disordered regions of proteins. Such protein interactions are often involved in the formation of liquid-like condensates, which we aim to study in the context of viral infections, mRNA splicing and protein homeostasis in the nucleus. To this end, we have initiated collaborations with the Lemke, Dormann, König and Beli labs (all at JGU/IMB) and have or are in the process of applying for funding. An intriguing idea is to use identified critical interaction interfaces for the development of proximity-induced drugs to modulate so-far undruggable biological processes.

Figure 1. Interactions between proteins are central to cellular function. To improve our ability to predict phenotypes from genotypes, we develop computational and experimental methods to structurally and functionally characterise protein interactions, use interaction profiling to characterise mutations and use omics data integration to predict cellular mechanisms for selected biological processes.

SELECTED PUBLICATIONS

Luck K*, Kim DK*, Lambourne L*, Spirohn K*,..., Hill DE#, Vidal M#, Roth FP# and Calderwood MA# (2020) A reference map of the human binary protein interactome. *Nature*, 580:402–408

Kovács IA, Luck K, Spirohn K, Wang Y, Pollis C, Schlabach S, Bian W, Kim DK, Kishore N, Hao T, Calderwood MA, Vidal M and Barabási AL (2019) Network-based prediction of protein interactions. *Nat Commun*, 10:1240

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*indicates joint contribution, #indicates joint correspondence

Brian **LUKE**

“We identified synthetic genetic interactions that reveal alternative ways to remove rNMPs.”

POSITIONS HELD

Since 2017	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Heisenberg Professor, Johannes Gutenberg University (JGU), Mainz
2014 – 2017	Group Leader, Institute of Molecular Biology (IMB), Mainz
2009 – 2014	Group Leader, Centre for Molecular Biology (ZMBH), University of Heidelberg
2005 – 2009	Postdoc, Swiss Federal Institute of Technology Lausanne (EPFL)
2005	Postdoc, Swiss Federal Institute of Technology Zurich (ETH)

EDUCATION

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

GROUP MEMBERS

Fábio Bento	PhD Student; since 04/2017
Rodolfos Danalatos	PhD Student; since 11/2020
Eduardo Gameiro	PhD Student; since 09/2021
Sacha Heerschop	Postdoc; since 10/2021
Kristi Jensen	PhD Student; since 01/2021
Sana’a Khraisat	PhD Student; since 09/2021
Dennis Knorr	Technician; since 06/2021
Nina Lohner	PhD Student; since 09/2021
Vanessa Pires	PhD Student; since 08/2017
Stefanie Reimann	Technician; since 01/2015
Natalie Schindler	Postdoc; since 12/2017
Christiane Stürzbecher	Personal Assistant; since 04/2017
Matthias Tonn	PhD Student; since 04/2017
Olga Vydzhak	Postdoc; since 08/2020
Carolin Wagner	PhD Student; since 03/2020

OVERVIEW

R-loops are three-stranded structures consisting of an RNA-DNA hybrid and a displaced strand of ssDNA. They have previously been depicted as by-products of transcription that can lead to genomic instability, especially when confronted with the DNA replication machinery (Niehrs and Luke, 2020). Recently, it has become evident that R-loops also have important regulatory functions and can affect processes such as transcription, DNA repair, telomere protection and centromere function (Niehrs and Luke, 2020). Hence, it is important that R-loops are tightly regulated in a manner that allows them to be formed, while at the same time ensuring that they are efficiently removed to prevent the accumulation of “unscheduled” R-loops. RNase H enzymes are conserved throughout evolution and promote cleavage of RNA that is base-paired to DNA. They are considered the major regulatory enzymes that control R-loop levels. RNase H2, which provides the majority of the RNase H activity in the cell, is a heterotrimer that removes R-loops, as well as single ribonucleotide insertions in dsDNA. Loss of RNase H2 leads to severe human diseases, including Aicardi Goutiers Syndrome and cancer. Understanding synthetic interactions will be important for the future treatment of such diseases (Figure 1).

RESEARCH HIGHLIGHTS

We have previously demonstrated that the E3 ubiquitin ligase Rtt101 interacts with the replisome to promote the repair of DNA lesions. Cells with deletions of any component of the Rtt101 complex show increased sensitivity to DNA-damaging agents and have impaired growth when RNase H2 activity is compromised. Importantly, the growth defect in the absence of RNase H2 is caused by the loss of ribonucleoside monophosphate (rNMP) removal, and not

by a failure to remove R-loops. We have demonstrated that when rNMPs accumulate, Rtt101 is required specifically in the S phase to promote cell survival. We hypothesised that when rNMPs are hydrolysed before S phase (resulting in a ss nick) they are converted into double-strand breaks upon DNA replication, and it is at this stage that Rtt101 is required to promote homology-directed repair (HDR). Indeed, when we used cell cycle-regulated alleles of RNase H2, we were able to demonstrate that Rtt101 becomes essential when rNMPs are nicked in S phase. In order to look for genes that are also required for viability upon rNMP nicking in S phase, we performed and verified a genome-wide SGA (synthetic genetic array) screen to identify other genetic factors that, like Rtt101, are required for survival in the presence of S-phase nicks at rNMPs. We found that HDR factors and histone chaperones are both important for repairing nicks. Importantly, we were able to demonstrate epistatic genetic interactions between loss of RAD51 and loss of RTT101, further supporting our initial hypothesis. In addition, we have identified relevant ubiquitin targets of Rtt101, including Dpb2 (a DNA polymerase subunit) and histone H3. Importantly, mutation of the ubiquitylation sites on H3 is able to phenocopy the effects of Rtt101 loss in the presence of high rNMP loads. In summary, our recent data shows that Rtt101-mediated ubiquitylation of histone H3 is required to tolerate rNMP accumulation in the absence of RNase H2. This project is funded by the CRC 1361 on "Regulation of DNA Repair and Genome Stability".

FUTURE DIRECTIONS

Accidentally inserted rNMPs are normally removed by RNase H2 in a reaction referred to as ribonucleotide excision repair (RER). When RNase H2 is mutated, as is frequently the case in chronic lymphatic leukaemia and prostate cancer, Top1 removes the rNMPs, which can in turn lead to 2-5 bp deletion mutations, especially in repetitive regions. We will use yeast genetics and mutagenesis assays to understand why Top1 only becomes mutagenic when RNase H2 is absent. This will be important in terms of cancer biology, as cells lacking RNase H2 show Top1-dependent genomic instability. Moreover, we will be verifying the synthetic interactions that we have found between RTT101 and RNASE H2 in human cells, to test whether this would be a viable avenue for therapeutic intervention. Finally, we will be trying to elucidate the mechanistic details of the HDR defect in rtt101 cells when rNMPs accumulate.

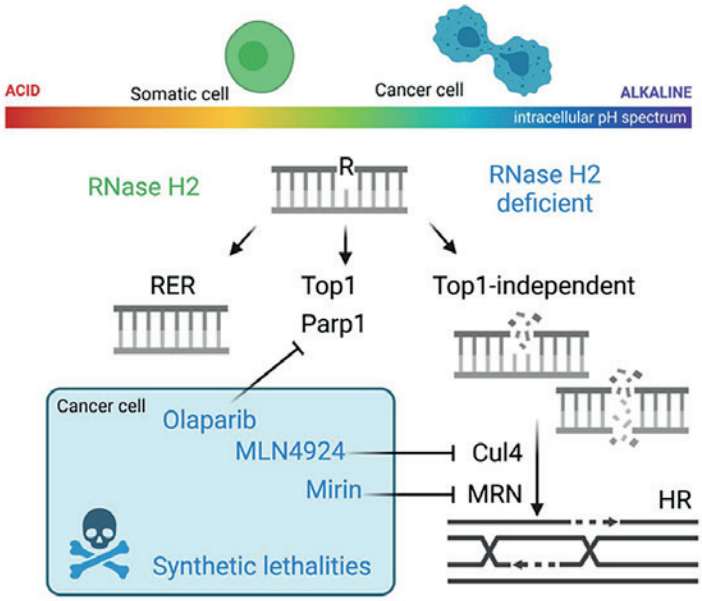

Figure 1. When RNase H2 activity is defective, as occurs in some human cancers, Topisomerase 1 can cleave the rNMPs. This reaction renders cells sensitive to Parp inhibitors such as Olaparib. Some rNMPs, however, are nicked in a Top1-independent-manner, hence opening up other potential targets in RNase H2-defective cells. In yeast, we have shown that the Top1-independent nicks are repaired through HR and require the Rtt101 E3 ubiquitin ligase, as well as the MRX complex. If these relationships are true in human cells, then treatment with MLN4924 and Mirin may represent therapeutic possibilities to inhibit Cul4 and MRN, respectively.

SELECTED PUBLICATIONS

Misino S, Busch A, Wagner C, Bento F and Luke B (2022) TERRA increases at short telomeres in yeast survivors and regulates survivor associated senescence (SAS). *Nucleic Acids Res*, in press

Wagner T, Pérez-Martínez L, Schellhaas R, Barrientos-Moreno M, Öztürk M, Prado F, Butter F and Luke B (2021) Chromatin modifiers and recombination factors promote a telomere fold-back structure, that is lost during replicative senescence. *PLOS Genet*, 16:e1008603

Pérez-Martínez L, Öztürk M, Butter F[#] and Luke B[#] (2020) Npl3 stabilizes R-loops at telomeres to prevent accelerated replicative senescence. *EMBO Rep*, 21:e49087

*indicates joint contribution, #indicates joint correspondence

Christof **NIEHRS**

"70 years after Watson & Crick, we find that DNA continues to hold surprises."

POSITIONS HELD

Since 2010	Founding & Scientific Director, Institute of Molecular Biology (IMB), Mainz Professor, Johannes Gutenberg University (JGU), 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
2020 – 2022	Executive Director, Institute of Molecular Biology (IMB), Mainz
2010 – 2015	Executive Director, Institute of Molecular Biology (IMB), Mainz
1990 – 1993	Postdoc, University of California Los Angeles (UCLA)

EDUCATION

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

GROUP MEMBERS

Khelifa Arab	Postdoc; since 11/2011
Sudeshna Banerjee	Postdoc; since 08/2020
Amitava Basu	Postdoc; since 03/2018
Jasmin Dehnen	PhD Student; since 04/2021
Deepa Jayaprakashappa	PhD Student; since 05/2022
Gaurav Joshi	PhD Student; since 07/2019
Yulia Kargapolova	Postdoc; since 07/2022
Laura Krebs	Technician; since 09/2015
Ivan Laptev	Postdoc; since 02/2022
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Debasish Mukherjee	Postdoc; since 03/2019
Michael Musheev	Postdoc; since 07/2011
Regina Otto	Personal Assistant; since 09/2015
Eleftheria Parasyraki	PhD Student; since 09/2018
Sandra Rölle	Lab Manager; since 04/2011
Carola Scholz	Research Technician; since 05/2015
Lars Schomacher	Senior Research Associate; since 07/2011
Umut Taşdelen	PhD Student; since 06/2021
Philipp Trnka	PhD Student 11/2016-06/2022
Rintu M Umesh	Postdoc; since 03/2022

OVERVIEW

Although cellular DNA is commonly perceived as a static molecule that carries genetic information in the form of nucleotide sequences, genomic nucleobases are in fact physiologically modified by a variety of chemical modifications. These DNA modifications are deposited in the genome in a site-specific manner and are known or suspected to epigenetically regulate gene expression. Typically, DNA modifications are recognised by specific reader proteins and can be reversed by a variety of enzymatic mechanisms. We study which DNA modifications occur in the mammalian genome, how and where in the genome they are deposited, what biological role they play, and how they are recognised and removed. We use ultrasensitive mass spectrometry to identify and quantify DNA modifications in mammalian cells. We employ next-generation sequencing and computational analysis to identify modification sites genome-wide. We characterise the roles of proteins involved in depositing, reading and removing modifications in embryonic stem cells, *Xenopus* embryos and mice.

RESEARCH HIGHLIGHTS

DNA poly-ADP-ribosylation

Poly-ADP-ribosylation (PARylation) is a widespread post-translational modification of proteins where an ADP-ribose from NAD^+ is transferred to the amino acid residues of target polypeptides. PARylation is catalysed by poly(ADP-ribose) polymerases (PARPs), which play important roles in many biological processes and diseases such as DNA repair and cancer. In mammalian cells, PARylation is regarded as a protein-specific modification. However, some PARPs were recently shown to modify DNA termini *in vitro*. These observations raise the intriguing possibility that PARylation is a novel DNA modification and pose the questions of whether mammalian DNA is PARylated, where in the genome the modification occurs and what its physiological role could be.

Employing PAR antibodies, PAR-binding reagents and stable isotope dilution mass spectrometry (LC-MS/MS), we recently discovered that mammalian DNA is PARylated *in vivo*, including in mouse embryonic stem cells, mouse heart, muscle, brain, thymus, liver and placenta, and in human liver. Notably, treating mESCs with an inhibitor of PAR glycohydrolase (PARG), the primary enzyme responsible for degrading cellular ADP-ribose moieties, strongly enhanced DNA PARylation. The results indicate that PARylation is a rare and reversible DNA modification occurring in primary mammalian tissues and cell lines. Using siRNA knockdowns, we established that both Parp1 and Parp2 are required for DNA PARylation. Since PARP1 plays a key role in the DNA damage response, we examined whether DNA PARylation may generally localise to DNA strand breaks (DSB). However, inducing DSBs in a mammalian cell line did not lead to PARylation and we conclude that DNA PARylation is not a general feature of DNA breaks.

We established an *in vitro* assay for PARylation of DNA by PARP1 and found that the modification occurs mostly at a nucleobase, with less at the DNA ends. To identify the PAR acceptor site on ssDNA, we enzymatically degraded the PAR chains to leave a single ribose remnant on the PAR acceptor molecule. Screening the products by LC-MS/MS for ribosylated nucleosides, i.e. containing both a ribose moiety remaining from PARylation and a deoxyribose, we identified a positively charged ion with a mass over charge ratio (m/z) of 385 ('nucleoside 385') and mass transitions that fit the loss of a ribose and a deoxyribose (m/z = 385→137), as well as loss of a single deoxyribose (m/z = 385→269), supporting the presence of both sugars on the nucleobase. To identify the PARylated base, we used ssDNA oligos homopolymeric for all four canonical nucleosides, but could not detect PARylation. Moreover, the reaction was specific for single-stranded (ss) DNA. These results suggests a requirement of base PARylation for certain sequence contexts and/or secondary structures in ssDNA.

By labelling the DNA with stable isotopes and LC-MS/MS analysis, we discovered that nucleoside '385' is ribosyl-deoxy-inosine, a deamination product of deoxy-adenine. Subsequent analysis revealed that the primary modification indeed occurs on the N1 position of adenine. Through partial hydrolysis of milligram amounts of mammalian DNA, we identified PAR-DNA *in vivo* via the diagnostic deamination product N1-ribosyl-deoxyinosine. We conclude that N1-adenosine PARylation is a novel, reversible DNA modification in mammals.

FUTURE DIRECTIONS

Our results indicating that PARylation is a physiologically-relevant DNA modification in mammalian cells raise many questions. Which loci are PARylated? What is common to such loci? What determines the specificity of locus PARylation? What is the physiological role of PARylation at these loci? To start addressing these questions, we will profile DNA PARylation genome-wide by developing next-generation sequencing protocols for its detection. A challenge here is that standard sample workup during next generation sequencing precludes amplification and detection of the PARylated DNA strand because of steric hindrance by PAR chains that stall DNA polymerases. A challenge in addressing the biological function of PARylation by *PARP1* is the enzyme's pleiotropy – the vast number of substrates and processes in which this protein is involved, including transcription and DNA repair – which renders it difficult to interpret loss-of-function effects and establish causality. Hence, a separation-of-function *PARP1* mutant that is deficient in PARylation of DNA but not of proteins will be an ideal tool in the future.

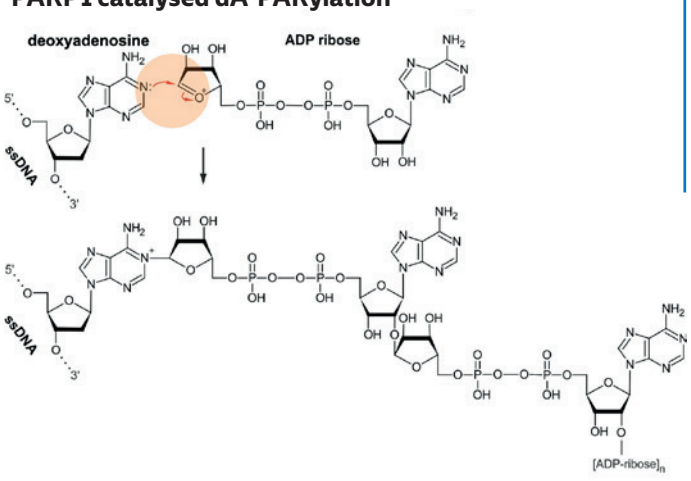


Figure 1. **Poly-ADP-ribosylation occurs on mammalian DNA**
Proposed reaction mechanism for DNA PARylation. PARP1 catalyses a nucleophilic attack of N1 from dA to C1 of the oxonium ion of ADP ribose, followed by polymerisation of PAR chains via PARP1.

SELECTED PUBLICATIONS

Musheev MU*, Schomacher L*#, Basu A*, Han D, Krebs L, Scholz C and Niehrs C# (2022) Mammalian N1-adenosine PARylation is a reversible DNA modification. *Nat Commun*, 13:6138

Musheev MU#, Baumgärtner A, Krebs L and Niehrs C# (2020) The origin of genomic N6-methyl-deoxyadenosine in mammalian cells. *Nat Chem Biol*, 16:630–634

Arab K, Karaulanov E, Musheev M, Trnka P, Schäfer A, Grummt I# and Niehrs C# (2019) GADD45A binds R-loops and recruits TET1 to CpG island promoters. *Nat Genet*, 51:217–223

*indicates joint contribution, #indicates joint correspondence

Jan **PADEKEN**

"We investigate the impact of genotoxic stress on the epigenome and its role in ageing."

POSITIONS HELD

Since 2022 Group Leader, Institute of Molecular Biology (IMB), Mainz

2013 – 2022 Postdoctoral Researcher, Friedrich Miescher Institute, Basel

EDUCATION

2013 PhD in Cell Biology, Max Planck Institute of Immunobiology and Epigenetics, Freiburg

2009 Diploma in Biology, Albert-Ludwigs University, Freiburg

2006 Vordiploma in Biology, RWTH Aachen University

GROUP MEMBERS

Valerie Arz PhD Student; since 10/2022
Daniel Helmkamp Master Student; since 10/2022
Rosa Herrera Rodriguez PhD Student; since 06/2022
Sheraz Sadouki Lab Manager; since 06/2022

OVERVIEW

The epigenetic memory of a cell is shaped by pathways that establish, erase and maintain chromatin marks. Lysine 9 methylation on histone H3 (H3K9me) is a defining modification of heterochromatin. In multicellular eukaryotes, heterochromatin has two main functions. First, it silences repetitive sequences to ensure genome stability; second, it maintains the silencing of genes during and post development to ensure a stable differentiated state. The unprogrammed transcription of repetitive sequences leads to an accumulation of toxic R-loops and a dependence on BRCA1 and DNA repair proteins for survival. Thus, it is not surprising that loss of appropriately targeted heterochromatin is associated with cancer and ageing. In our lab, we are interested in this interface between the mechanisms that establish or alter heterochromatin and the DNA damage response, particularly in the context of premature ageing.

RESEARCH HIGHLIGHTS

How does persistent DNA damage alter heterochromatin after acute exposure and are these changes maintained in old cells?

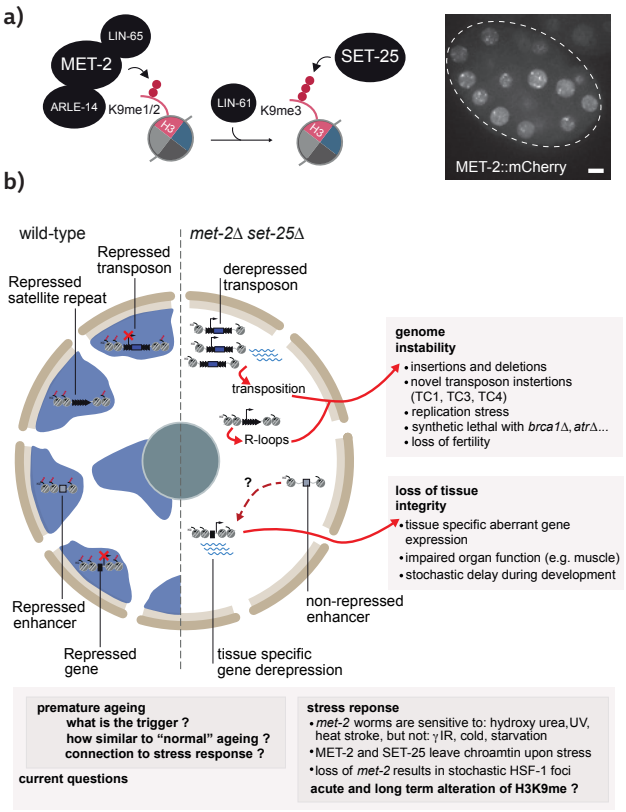
Rare genetic diseases have been central in linking DNA damage to ageing. Cockayne syndrome (CS) is caused by autosomal recessive mutations in either the *CSA* or *CSB* gene and results in persistent DNA damage. *CSA* and *CSB* are essential for initiating transcription-coupled nucleotide excision repair (TC-NER), a DNA damage response pathway that repairs DNA lesions (e.g. UV-induced pyrimidine dimers) blocking RNA polymerase II at sites of active transcription. CS patients therefore accumulate persistent DNA damage in transcribed genes. This manifests in a complex, multi-organ set of clinical features, including premature ageing, neurodegeneration, dysfunctional mitochondria, retarded development and loss of subcutaneous fat and

muscle function. This progressive, multi-tissue pathology requires a simpler, but well-characterised model organism such as *C. elegans*, which in contrast to the mouse mimics the clinical features of CS patients. Survival of persistent UV damage is tightly linked to genome-wide chromatin changes. Interestingly, the phenotypes observed in CS patients (or the worm model) are mimicked by the loss of H3K9me. Indeed, H3K9me and the histone methyltransferase (HMT) MET-2 are essential in the CS model.

Using our expertise in chromatin biology, we describe the acute and persistent changes in heterochromatin upon persistent UV damage to ultimately answer how H3K9me protects an organism from the persistent DNA damage and premature ageing characteristic of CS.

What are the mechanisms that mediate *de novo* establishment of heterochromatin?

The importance of H3K9me in the stress response, as well as its role in silencing tissue-specific genes and potentially active transposable elements, imply that H3K9me can be highly dynamic. To understand the *de novo* establishment and maintenance of heterochromatin domains on a mechanistic level, we developed a unique system to identify sequences that are sufficient to trigger *de novo* recruitment of the two H3K9-specific HMTs and identify the proteins essential for establishing of the H3K9me domain. We will use this to screen for factors essential for the establishment vs. maintenance of heterochromatin and ultimately link these pathways to the stress response and premature ageing.



What regulates chromatin compaction and transcriptional noise of heterochromatic genes in parallel to H3K9me?

We previously showed that loss of H3K9me results in cell-type-specific gene derepression (Methot *et al*, 2021). Interestingly, loss of H3K9me was not sufficient to establish an open, decondensed chromatin state at the promoter and enhancer regions of the derepressed genes. We also observed that this specific form of derepression was characterised by high cell-to-cell transcriptional variability, even between cells of the same tissue. Because this stochasticity in gene expression mirrors the stochastic phenotypes associated with loss of heterochromatin across evolution and has also been repeatedly observed in old or senescent cells, we are currently establishing imaging-based methods to quantitatively screen for mediators of both chromatin compaction and transcriptional noise.

FUTURE DIRECTIONS

Ultimately, the projects above will give us a comprehensive understanding of the pathways that regulate heterochromatin and enable us to ask how these processes are (mis)regulated during ageing and persistent DNA damage. It will also give us a basis to further explore how DNA damage response pathways impact chromatin states beyond the acute response at the site of damage and link this to progeria models such as CS, Hutchinson-Gilford progeria and normal ageing.

Figure 1. Overview of H3K9-specific histone methyl transferases (HMTs) and their phenotypes in *C. elegans*. MET-2 and its co-factors LIN-65 (ATF7IP) and ARLE-14 (ARLE14EP) H3K9me1/me2. H3K9me2 and MET-2 are essential for repression of satellite and simple repeats to prevent the accumulation of R-loops. Transposons and tissue-specific genes are further marked with H3K9me3 by SET-25, the MBT domain protein LIN-61. Maintaining the repression of tissue-specific genes during and post-development maintains tissue identity and function.

SELECTED PUBLICATIONS

Delaney CE, Methot SP, Kalck V, Seebacher J, Hess D, Gasser SM and Padeken J (2022) SETDB1-like MET-2 promotes transcriptional silencing and development independently of its H3K9me-associated catalytic activity. *Nat Struct Mol Biol*, 29:85-96

Methot SP*, Padeken J*, Brancati G, Zeller P, Delaney CE, Gaidatzis D, Kohler H, van Oudenaarden A, Großhans H and Gasser SM (2021) H3K9me selectively blocks transcription factor activity and ensures differentiated tissue integrity. *Nat Cell Biol*, 23:1163-1175

Padeken J, Methot S, Zeller P, Delaney CE, Kalck V and Gasser SM (2021) Argonaute NRDE-3 and MBT domain protein LIN-61 redundantly recruit an H3K9me3 HMT to prevent embryonic lethality and transposon expression. *Genes Dev*, 35:82-101

*indicates joint contribution, #indicates joint correspondence

Vassilis **ROUKOS**

"We use imaging and sequencing to study when, where and why chromosomes break."

POSITIONS HELD

Since 2022 Assistant Professor, Medical School, University of Patras, Greece & Affiliated Group Leader, Institute of Molecular Biology (IMB), Mainz

2015 – 2022 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

EDUCATION

2008 PhD in Molecular Biology & Cytogenetics, University of Patras Medical School

2005 MSc in Applications in Medical Sciences, University of Patras Medical School

GROUP MEMBERS

Andriana Kotini Postdoc; since 06/2022
Gabriel Longo PhD Student; since 09/2019
Waheba Mohamed Postdoc; since 05/2021

OVERVIEW

Maintaining the integrity of genetic information is essential for cell survival. Mechanisms that counteract DNA damage maintain cellular homeostasis by suppressing mutagenic events and genomic rearrangements that may lead to diseases such as cancer. Chromosome translocations are one of the most severe forms of genomic rearrangements. Translocations form through 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 molecular features define recurrent chromosome breakpoints? How do the broken chromosome ends find each other within the nuclear space? What are the DNA repair mechanisms that mediate chromosome fusion and which factors 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 understand when, where and why chromosomes break and identify molecular mechanisms that lead to the formation of cancerous rearrangements.

RESEARCH HIGHLIGHTS

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

Modelling the formation of recurrent, cancer-initiating genomic rearrangements requires a versatile approach that can probe rare events with high sensitivity. We have now established a method called CRI3D that uses fluorescence in situ hybridisation (FISH) to probe the position of individual chromosome ends of potential rearrangements in interphase cells in 3D. High-throughput microscopy and automated image analysis are then used to identify single cells with chromosome breakages and different rearrangements

(deletions, inversions, fusions, etc.). This methodology complements existing approaches and offers several advantages: it is (i) suitable for detecting and quantifying rearrangements without the need for mapping the precise breakpoints or rearrangement product; (ii) compatible with both site-specific induction of breaks (mediated by endonucleases, ZNFs or CRISPR), as well as more physiological methods of inducing DNA damage, such as ionising radiation and chemotherapeutics (see below); and (iii) able to detect rearrangements in interphase cells at frequencies down to 10^{-4} without the need for metaphase spread preparation. CRI3D is a powerful tool that can be used to dissect the molecular and cellular mechanisms that contribute to the formation of any oncogenic genome rearrangement of interest.

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

Cancers are commonly treated with anticancer drugs called topoisomerase poisons. Treatment with topoisomerase poisons, however, can also cause chromosome translocations in healthy cells that disrupt gene regulation and lead to the development of leukaemia. 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 where the DNA folds into chromatin loops and is under more mechanical strain. This makes them susceptible to DNA breaks caused by topoisomerase poisons such as etoposide, producing translocations that drive leukaemia. We have also identified factors involved in the repair of these DNA breaks that actively suppress the formation of 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 and thereby promote cancer.

FUTURE DIRECTIONS

Central to our focus is shedding light on cellular events that promote DNA fragility intrinsically or upon treatment with cancer therapy and the use of programmed genome-editing nucleases, such as CRISPR/Cas9 (Figure 1). In one of our future directions, we 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 genomic, chromatin and chromosome organisation 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.

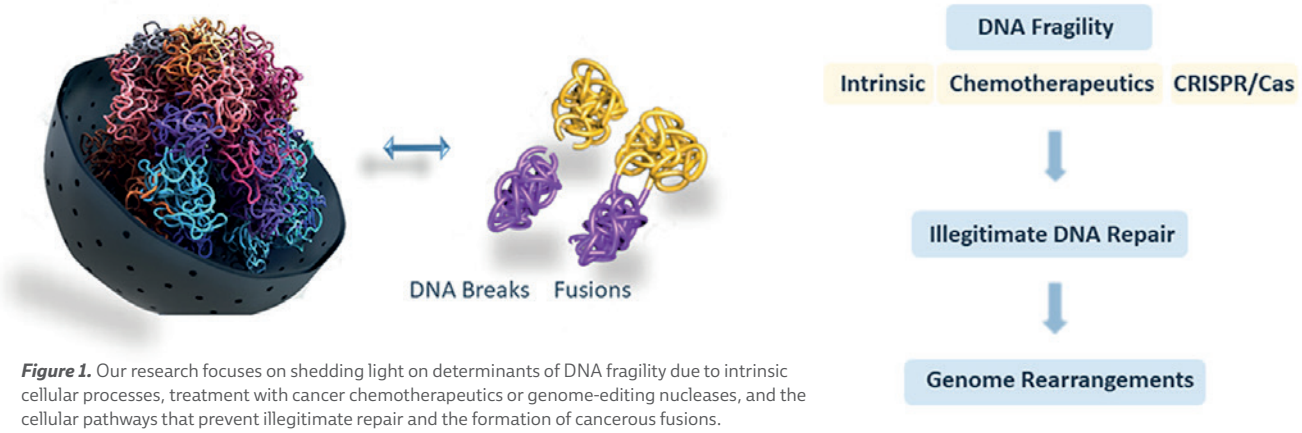


Figure 1. Our research focuses on shedding light on determinants of DNA fragility due to intrinsic cellular processes, treatment with cancer chemotherapeutics or genome-editing nucleases, and the cellular pathways that prevent illegitimate repair and the formation of cancerous fusions.

SELECTED PUBLICATIONS

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

Longo GMC and Roukos V (2021) Territories or spaghetti? Chromosome organization exposed. *Nat Rev Mol Cell Biol*, 22:508–508

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

Sandra **SCHICK**

"We investigate how chromatin misregulation affects cellular and developmental processes."

POSITIONS HELD

Since 2020 Group Leader, Institute of Molecular Biology (IMB), Mainz

2016 - 2020 Postdoctoral Fellow, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna

EDUCATION

2016 Doctorate in Molecular Biology, Institute of Molecular Biology (IMB), Mainz

2012 Master in Biomedicine and Diploma in Biology, Johannes Gutenberg University Mainz (JGU)

2008 Bachelor in Molecular Biology, Johannes Gutenberg University Mainz (JGU)

GROUP MEMBERS

Lukas Mann PhD Student; since 05/2021
Felix Etienne van der Walt PhD Student; since 05/2021
Verena Wilhelm Lab Manager; since 11/2021

OVERVIEW

Compaction of the genome into higher-order chromatin structures requires a variety of dynamic regulatory mechanisms for the temporal and spatial control of genomic processes. These regulatory mechanisms are particularly important for ensuring proper gene expression and thus the appropriate execution of all cellular processes. Various regulators act in an integrative and coordinated fashion, resulting in a highly complex and fine-tuned system. Therefore, it is not surprising that mutations in genes encoding these regulators are frequently associated with various diseases. In order to uncover how these regulators integrate and contribute to gene regulation, genome stability and other genomic processes, we employ mammalian – especially human – cellular model systems in combination with genome editing, epigenomics, proteomics and various molecular and biochemical approaches. Moreover, we explore the cellular and molecular consequences of mutations in these regulators in order to unravel the mechanisms underlying their associated diseases and identify potential therapeutic approaches.

RESEARCH HIGHLIGHTS

One class of chromatin regulators that is essential for modulating chromatin structure is the BRG1/BRM associated factor (BAF) chromatin remodellers. These remodellers are pleomorphic complexes comprised of multiple subunits that are encoded by more than 30 genes and assembled in a combinatorial fashion. There are three subtypes of BAF complexes, each with a few distinct subunits: the canonical BAF complexes (BAF/cBAF), the polybromo-associated BAF complexes (PBAF) and the non-canonical GLTSCR1/1L-BAF complexes (GBAF/ncBAF). These remodellers utilise energy from ATP hydrolysis to slide or eject nucleosomes and thereby modulate DNA accessibility. They control gene regulatory regions and consequently regulate a multitude

of cellular functions, as well as developmental processes such as lineage specification and differentiation. Moreover, BAF complexes contribute to genomic processes such as the DNA damage response, DNA replication and sister chromatid cohesion, as well as chromatin topology and organisation. The unexpectedly high mutation rate in genes encoding various BAF subunits in cancer and neurodevelopmental disorders highlights the importance of these remodellers. Therefore, it is of key importance to elucidate the functions of the diverse BAF complexes and the molecular consequences of mutations in genes encoding BAF complex subunits. These novel insights would likely enable the development of targeted therapeutics for BAF-associated diseases.

To address these questions, we use two different approaches in the laboratory. On the one hand, we systematically investigate the role of distinct BAF complexes in different cellular processes in a conventional 2D cell line using a wide variety of experimental approaches, ranging from genomics and proteomics to image analysis. Using this approach, we observed subtype-specific regulatory mechanisms, with sometimes opposing effects. On the other hand, we have established complex human cell culture models, so-called organoids that reflect the development and cellular heterogeneity of human organs. Since BAF complex composition and function can differ by cell type, these heterogeneous models are suitable for addressing their cell type-specific roles. Having these model systems in hand allows us to study diseases that are caused by mutations in BAF complex-encoding genes at the molecular and cellular level *in vitro*. Here, our studies show time- and cell type-dependent phenotypic, cellular and molecular alterations following BAF perturbations, which may mimic disease-related alterations in patients with BAF mutations. In particular, developmental processes and tissue homeostasis are impaired, leading, for example, to altered cell composition.

FUTURE DIRECTIONS

We will further explore the molecular function and regulation of individual BAF complex subtypes, the processes they are involved in and how they integrate with other regulatory mechanisms, using a number of different experimental and computational approaches. We will also continue to study context-dependent functions of BAF complexes, including their role in developmental processes and disease. Ultimately, our research aims to unravel pathogenic mechanisms that can be targeted for therapy.

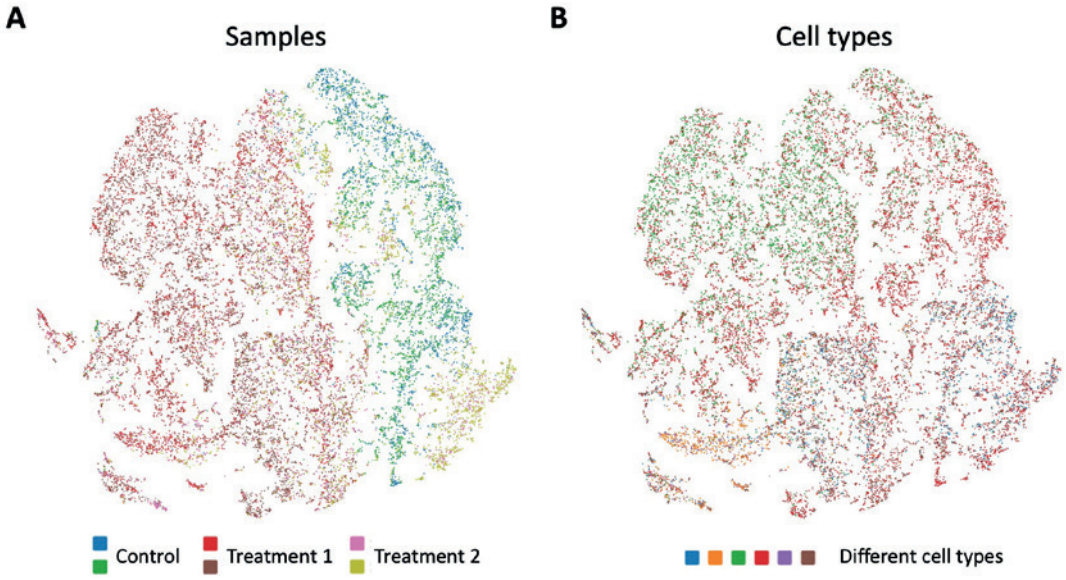


Figure 1. Single-cell RNA-Seq (scRNA-Seq) reveals the altered cellular composition of organoids after BAF perturbation. A) t-SNE plot of scRNA-Seq data of two organoid cell lines without treatment and under two different BAF perturbation conditions. B) t-SNE plot showing cell-type annotation based on a published reference dataset.

SELECTED PUBLICATIONS

Schick S*#, Grosche S*, Kohl KE*, Drpic D, Jaeger MG, Marella NC, Imrichova H, Lin JMG, Hofstätter G, Schuster M, Rendeiro AF, Koren A, Petronczki M, Bock C, Müller AC, Winter GE and Kubicek S# (2021) Acute BAF perturbation causes immediate changes in chromatin accessibility. *Nat Genet*, 53:269–278

Varga J, Kube M, Luck K and Schick S (2021) The BAF chromatin remodeling complexes: structure, function, and synthetic lethalities. *Biochem Soc Trans*, 49:1489–1503

Schick S, Rendeiro AF, Runggatscher K, Ringler A, Boidol B, Hinkel M, Májek P, Vulliard L, Penz T, Parapatics K, Schmidl C, Menche J, Boehmelt G, Petronczki M, Müller AC, Bock C and Kubicek S (2019) Systematic characterization of BAF mutations provides insights into intracomplex synthetic lethalities in human cancers. *Nat Genet*, 51:1399–1410

*indicates joint contribution, #indicates joint correspondence

Lukas **STELZL**

"We study how phase separation regulates gene expression and how it is impaired in ageing."

POSITIONS HELD

Since 2020 ReALity Junior Group Leader and IMB Associate Group Leader, Faculty of Biology and KOMET1, Institute of Physics, Johannes Gutenberg University Mainz and Institute of Molecular Biology (IMB), Mainz

2015 – 2020 Postdoctoral fellow, Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Frankfurt am Main

EDUCATION

2015 PhD in Biochemistry, University of Oxford

2010 MSc, Molecular and Cellular Biochemistry, University of Oxford

GROUP MEMBERS

Kumar Gaurav PhD Student; since 03/2021
Xiaofei Ping PhD Student; since 03/2022
Arya Changiarath Sivadasan PhD Student; since 04/2021
Yehor Tuchkov Student Assistant; since 03/2022
Emanuele Zippo PhD Student; since 09/2022

OVERVIEW

Our aim is to elucidate how liquid-liquid phase separation and phase-separated condensates of proteins and nucleic acids provide specific regulation and how such specific regulation is lost in pathologies. We are a computational biophysics and computational biology group and use particle- based multi-scale simulations in our research. The discovery that liquid-liquid phase separation and phase-separated condensates of proteins and nucleic acids are important regulators in cells is revolutionising our understanding of cell biology. Through liquid-liquid phase separation, biomolecules can form distinct liquid phases – a dilute phase and a protein- and nucleic acid-rich phase. Phase separation helps to organise biological functions in time and space. Thus, phase separation not only plays an important role in regulating genes at the transcriptional level, but also at the post-transcriptional level in developmental biology. Dysregulation of liquid-liquid phase separation is hypothesised to be an important driver of ageing and age-related diseases.

RESEARCH HIGHLIGHTS

To understand the biological roles of liquid-liquid phase separation and phase-separated biomolecular condensates, we have developed multi-scale simulations of disordered proteins, their condensates and liquid-liquid phase separation. Simulations provide important insights into the conformational dynamics of biomolecules (Stelzl*, Erlenbach* *et al*, 2017) and their biomolecular function (Stelzl *et al*, 2020) and can thus complement experiments. In particular we performed atomistic modelling for disordered tau, which is critical in Alzheimer's disease, and found not only agreement with many different experimental datasets, but importantly we also resolved biologically relevant conformations in our highly diverse and representative ensemble of tau (Stelzl* & Pietrek*, 2022). We found that the two aggregation-prone

hexapeptide motifs sample conformations, similar to tau fibrils from patient samples. These two hexapeptide motifs flank a PGGG motif. P301 to L/S/T mutations give rise to devastating disease and are used in mouse models of Alzheimer's disease. These mutations shift the conformational equilibrium of this turn towards extended structures. Turns between the two aggregation- prone hexapeptide motifs may be essential for preventing the formation of extended beta-strands and pathological aggregation. Our ensemble agrees with biophysical (e.g. NMR) data on its local and long-range structure.

In collaboration with Dorothee Dormann (IMB/JGU), we studied TDP-43 phosphorylation using multi-scale simulations. Phosphorylation of TDP-43 is a hallmark of neurodegenerative disease. The paradigm of the field has been that phosphorylation induces liquid-liquid phase separation and pathological aggregation of TDP-43. Experiments by the Dormann lab have questioned this prevailing dogma and offered a new interpretation: her research has shown that phosphorylation and phosphomicking mutations reduce propensity to phase separation and aggregation. We have started to complement these experiments with multi-scale simulations (Gruijs da Silva *et al*, 2022). Using multi-scale simulation methods that we developed (Pietrek*, Stelzl* *et al*, 2020; Benayad, 2021; Stelzl, in preparation), we showed that phosphomicking S→D mutations favour increased solvation of TDP-43 and thus provided a mechanistic basis for the proposed cyto-protective effects (Figure 1). Moreover, we showed that hyperphosphorylation dissolves TDP-43 condensates, in line with the anti-aggregation role of phosphorylation proposed by Dorothee Dormann. Our simulations also revealed that aromatic residues engage in "sticker-sticker" interactions in accordance with the "sticker-spacer" model, and thus our study provides a first step towards understanding such interactions on the molecular scale with atomic resolution.

FUTURE DIRECTIONS

We want to understand disease-linked modifications and mutations on a much larger scale. In collaboration with the Dormann lab, we will simulate large numbers of neurodegeneration-linked posttranslational modifications and mutations to better understand how they modulate phase behaviour and aggregation in disease. We are increasingly dealing with complex multicomponent condensates in our simulations to understand gene regulation. To understand how phase separation can underpin essential cell biological processes, we are working together with the Ketting lab (IMB). We will collaborate to investigate multi-component protein-RNA condensates in posttranscriptional gene regulation. Currently, we are writing up an initial simulation study on the mutator foci. We will also continue to further develop our simulation methods to improve our models and better match experimental complexity. These future directions will be greatly enhanced by new CRC 1551, where our group has two projects to support our collaborative work with the Dormann and Ketting labs. We are also developing new simulation methods to study ATP-driven processes in cells (in our new project in the CRC/TRR 146). Longer term, our simulation methods will be vital for understanding cellular homeostasis, including the proper functioning of phase-separated condensates.

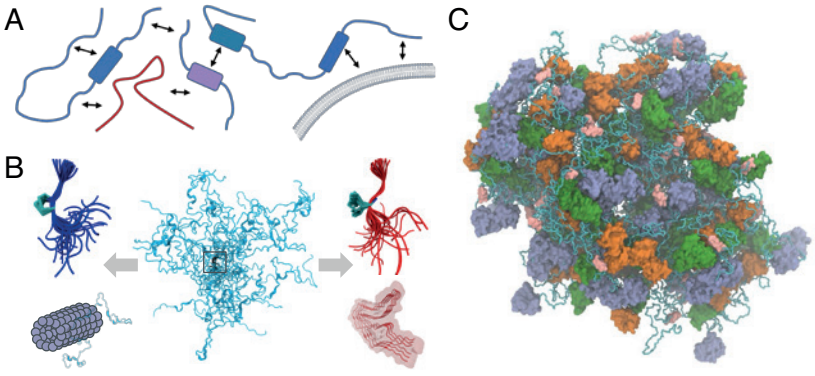


Figure 1. Disordered proteins and their condensates. A) Diversity of interactions of multi-domain proteins, including folded and disordered regions, which determine self assembly, phase behaviour and aggregation, as well as interaction with biological membranes. Nucleic acids are indicated in red. B) Atomic-resolution modelling results in highly diverse and representative ensembles. Left: Turn conformations enable microtubule binding in healthy neurons. Disease mutations shift the ensemble to extended structures prone to forming fibrils. (from Stelzl*, Pietrek* *et al*, 2022, *JACS Au*). C) Phase-separated condensate of full-length TDP-43 from a molecular dynamics simulation with a near-atomic resolution coarse-grained model. Disordered regions are shown in cyan. Folded N-terminal, RRM1 and RRM2 domains are shown in blue, green and orange, respectively. Ping, Stelzl, *unpublished*.

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Stelzl LS, Mavridou DAI, Saridakis E, Gonzalez D, Baldwin AJ, Ferguson SJ, Sansom MSP and Redfield C (2020) Local frustration determines loop opening during the catalytic cycle of an oxidoreductase. *eLife*, 9:e5466

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Helle **ULRICH**

"Our work shows that actin-dependent motor activity drives replication fork protection."

POSITIONS HELD

Since 2013	Scientific Director, Institute of Molecular Biology (IMB), Mainz Professor, Faculty of Biology, Johannes Gutenberg University (JGU), Mainz
2018 – 2019	Executive Director, Institute of Molecular Biology (IMB), Mainz
2004 – 2012	Group Leader, Clare Hall Laboratories, Cancer Research UK London Research Institute
2000 – 2004	Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg
1998 – 2000	Postdoc, Max Planck Institute for Biochemistry, Martinsried
1997 – 1998	Postdoc, Centre for Molecular Biology (ZMBH), University of Heidelberg

EDUCATION

2004	Habilitation in Genetics, Philipps University Marburg
1996	PhD in Chemistry, University of California, Berkeley
1994	Diploma in Biology, Georg August University Göttingen

GROUP MEMBERS

Marwah Al-Hushail	Technician/Lab Manager; since 10/2019
Kezia Ann	PhD Student; since 10/2021
Evrydiki Asimaki	PhD Student; since 09/2018
Claudia Carulla Capdevila	Student Assistant; since 10/2022
Nádia Da Silva Fernandes	PhD Student; since 11/2021
Philipp Elleringmann	Master Student; 09/2021 – 07/2022
Louis Hammer	Bachelor Student; since 10/2022
Kristine Hauschulte	PhD Student; since 09/2020
Wiktoria Kabza	PhD Student; since 10/2021
Yogita Mallu Kattimani	PhD Student; since 06/2022
Nils Christian Krapoth	PhD Student; since 01/2022
Cindy Meister	Postdoc; since 01/2020
Regina Otto	Personal Assistant; since 09/2015
Zhi-Hoon Park	Bachelor Student; 05/2022 – 09/2022
Kirill Petriukov	PhD Student; since 08/2018
Christian Renz	Postdoc; since 08/2014
Maximilian Reuter	Postdoc; since 02/2022
Aina Mas Sanchez	PhD Student; since 10/2021
Patrick Schardey	Bachelor Student; since 10/2022
Philipp Schönberger	PhD Student; since 11/2019
Ulrike Seeburg	Technician; since 11/2022
Virender Kumar Sharma	Postdoc; since 02/2022
Jie Shi	PhD Student; since 10/2018
Nadia da Silva Fernandes Lucas	PhD Student; since 11/2021
Tina Strauch	PhD Student; since 09/2019
Laura Tomini	Lab Manager; 01/2015 – 09/2022
Maria Villagomez-Torres	PhD Student; 01/2018 – 12/2022
Hans-Peter Wollscheid	Postdoc; since 09/2014
Ronald Wong	Postdoc; since 04/2013
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 cancer. Our lab studies the regulatory mechanisms that contribute to ensuring the complete and accurate duplication of a cell's genetic information, especially as they relate to the posttranslational protein modifiers ubiquitin and SUMO. We aim to understand how cells choose between alternative processing pathways for replication-blocking lesions in the DNA template, e.g. between error-prone translesion synthesis and accurate recombination-mediated template switching, or between fork-associated and postreplicative modes of damage bypass. Posttranslational modifications of the replication clamp protein PCNA have proven to be critical determinants of these pathways in eukaryotes. Another aspect of replication stress management is the stabilisation of reversed replication forks. Such structures have been postulated to serve as protective measures against fork breakdown in human cells and are processed by factors that include homologous recombination proteins. Work from our lab led by Hans-Peter Wollscheid has now implicated an actin-dependent motor protein, myosin 6, in replication fork protection and homology-directed DNA double-strand break repair, thus linking the nuclear actin system to organising the dynamics of the DNA damage and replication stress response.

RESEARCH HIGHLIGHTS

The actin cytoskeleton is best known for its cytoplasmic role in supporting cell motility and mechanics. Whether actin filaments also form in the nucleus and how they might impinge on genome maintenance has long remained uncertain. An actin-directed nanobody improved the visualisation of nuclear actin filaments and revealed a connection between

polymerised nuclear actin and the DNA repair and replication stress pathways. However, the mechanisms and relevance of myosins as actin-based motor proteins in these pathways remain elusive. Myo6 is one of the few myosins reported to have nuclear functions, mainly in transcription. In collaboration with Petra Beli (IMB), we identified novel interaction partners of Myo6 in a mass spectrometry screen. Our follow-up on these interactors now implicates Myo6 in two major genome maintenance pathways: the protection of stalled or reversed replication forks and the homology-directed repair of DNA double-strand breaks (DSBs).

Reversed replication forks form as a protective response to replication stress, and involves reannealing of the parental strands and joining of the newly synthesised strands. The resulting four-way structure requires protection from nuclease-mediated degradation. We found that Myo6 interacts with the well-established fork protector WRNIP1. This interaction is strongly enhanced by replication stress, suggesting a functional connection (Figure 1A). Accordingly, depletion of Myo6 leads to enhanced degradation of newly synthesised DNA upon fork stalling (Figure 1B), and a dependency on the nuclease DNA2 places Myo6 in the same pathway as WRNIP1. Based on our observations that Myo6 depletion impairs WRNIP1 localisation to forks and that the motor domain of Myo6 appears to be crucial for fork protection, we hypothesise that under replication stress Myo6 mediates the recruitment of WRNIP1 to reversed replication forks in an actin-dependent manner to prevent fork erosion via nucleolytic attack from DNA2 (Figure 1C).

In addition to a defect in replication fork protection, loss of Myo6 also results in impaired DSB repair. Using traffic light reporter assays, we could attribute this defect specifically to the homology-directed repair (HDR) pathway, similar to what has recently been described for nuclear actin filaments. The latter has been proposed to play roles in two key steps of the HDR pathway, namely DNA end resection and mobilisation of the break ends for homology search. Consistent with a cooperation between Myo6 and actin in HDR, we observed similar defects upon Myo6 depletion, namely a reduction in the number of Replication Protein A (RPA) foci upon treatment with the DSB-inducing agent camptothecin, indicating decreased levels of single-stranded DNA (Figure 1D), and reduced mobility of DSBs induced by restriction endonuclease digestion in live cells (collaboration with Alexander Löwer, TU Darmstadt; Figure 1E).

Taken together, our data not only reveal unexpected functions of a molecular motor in DNA replication and DSB repair, but in effect implicate the actin cytoskeleton in governing the dynamics of multiple aspects of genome maintenance.

FUTURE DIRECTIONS

Given the complexity of the actin cytoskeleton, we envision roles for additional cytoskeletal components beyond actin and Myo6 in genome maintenance. We therefore plan to explore the potential contributions of regulators of actin polymerisation, branching and capping, as well as other myosins, to DNA repair and replication. Moreover, we aim to understand if actin/myosin functions are required for higher-order chromatin dynamics. Is the actin cytoskeleton involved in chromatin remodelling or the (re-)organisation of chromatin? To better characterise the temporal and spatial engagement of the nuclear actin system with chromatin, we plan to combine live-cell imaging with next-generation sequencing techniques. Finally, we will explore the interplay between ubiquitin signalling and the regulation of the nuclear actin cytoskeleton.

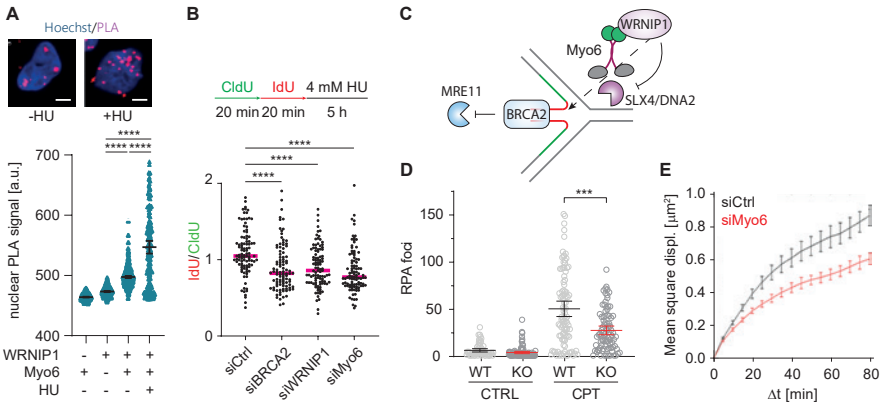

Figure 1. Myo6 acts in replication fork protection and DSB repair. A) Proximity ligation assays (PLA) show enhanced Myo6·WRNIP1 interaction upon HU-induced replication stress (scale bar: 5 µm). B) Fibre assays show nascent strand degradation in BRCA2-, WRNIP1- and Myo6-depleted cells upon HU treatment. C) Model of Myo6´s role in fork protection. D) Myo6 deletion reduces the number of camptothecin (CPT)-induced RPA foci. E) Myo6 depletion reduces DSB mobility in live-cell imaging.

SELECTED PUBLICATIONS

Wegmann S*, Meister C*, Renz C, Yakoub G, Wollscheid HP, Takahashi DT, Mikicic I, Beli P and Ulrich HD (2022) Linkage reprogramming by tailor-made E3s reveals polyubiquitin chain requirements in DNA damage bypass. *Mol Cell*, 82:1589-1602.e5

Sriramachandran AM, Petrosino G, Méndez-Lago M, Schäfer AJ, Batista-Nascimento LS, Zilio N# and Ulrich HD# (2020) Genome-wide nucleotide-resolution mapping of DNA replication patterns, single-strand breaks, and lesions by GLOE-Seq. *Mol Cell*, 78:975-985.e7

Wong RP, García-Rodríguez N, Zilio N, Hanulová M and Ulrich HD (2020) Processing of DNA polymerase-blocking lesions during genome replication is spatially and temporally segregated from replication forks. *Mol Cell*, 77:3-16.e4

*indicates joint contribution, #indicates joint correspondence

Sara **VIEIRA-SILVA**

"We study how the human microbiota contributes to disease risk through health perturbations and with age."

POSITIONS HELD

Since 2022 Professor, University Medical Center, Johannes Gutenberg University (JGU), Mainz & Adjunct Director, Institute of Molecular Biology (IMB), Mainz

2022 Group Leader, University Medical Center, Johannes Gutenberg University (JGU), Mainz

2015 – 2022 Postdoctoral Researcher, Catholic University of Leuven

2011 – 2015 Postdoctoral Researcher, Free University of Brussels

EDUCATION

2007 – 2010 PhD in Genomics, Université Pierre et Marie Curie Institut Pasteur, Paris

2005 – 2006 Postgraduate studies in Computational Biology (PDBC), Instituto Gulbenkian de Ciência, Oeiras

2003 Diploma in Biology, University of Lisbon

GROUP MEMBERS

Javier Centelles-Lodeiro PhD Student; since 10/2018
Tanine Daryoush PhD Student; since 10/2019

OVERVIEW

The human body hosts microbial communities that have an essential role in health. My lab focuses on understanding the ecological dynamics of human gut-associated microbial communities in healthy host-microbiome homeostasis and how their disturbance contributes to the risk of disease onset or progression. We apply quantitative approaches in population cohorts and intervention trials to identify the mechanisms that drive the dynamics of the gut ecosystem in health, what determines its resilience to perturbations, and which alterations contribute to disease (dysbiosis). We focus on tracking the metabolic capacity of these complex communities and their symbiotic or deleterious interactions with the host and its immune system. Our objectives are to identify and quantify the contribution of the gut microbiome as a risk factor for disease development and help develop strategies for microbiota remediation in (combined) therapeutic interventions. For this aim, we favour hypothesis-driven experimental design and invest in the development of experimental and computational approaches to study human-associated microbial communities.

RESEARCH HIGHLIGHTS

How the human gut microbiota contributes to ischaemic heart disease development

We studied the involvement of the human gut microbiota in the progression of ischemic heart disease (IHD) using a comprehensively phenotyped cohort covering disease initiation, escalation and response to treatment (METACARDIS). We found that the microbiota alterations (dysbiosis) and associated metabolome profiles that distinguished patients with IHD from healthy controls were apparent before disease onset, i.e. in the early stages of initial metabolic dysregulation. This dysbiotic state, characterised by low microbial

loads and depletion of key symbiotic bacteria (butyrogens), is associated with a higher inflammatory burden sustained by adverse pro-inflammatory feedback between an opportunistic faction of microbiota and the host immune system. The prevalence of this dysbiotic state among patients increased with disease escalation. We derived microbiome- and metabolome-based signatures to discriminate IHD patients from healthy controls. These outperformed conventional clinical markers, supporting their pathophysiological relevance.

How commensal bacteria are transmitted within families

The bacteria that comprise our commensal microbiota can be acquired by vertical (maternal) or horizontal (social) transmission, or by environmental exposure. Characterisation of how the human gut microbiota is acquired and matures over a lifespan and the transmissibility of different bacterial strains is of key importance for clinical research. We studied a multigenerational family cohort to investigate the transmission and persistence of familial microbiome patterns. We found that microbiome community composition is significantly associated with kinship, resulting in family-bound microbiome community profiles. While our analyses do not exclude cross-generational transmission of strains resulting from maternal inheritance, the transmission or co-acquisition of bacterial strains appeared to be strongly linked to cohabitation, suggesting horizontal transmission or co-acquisition from a shared environmental source. While rare, we detected potential transmission events spanning three and four generations, primarily involving species of the genera *Alistipes* and *Bacteroides*. In the future, assessing the transmissibility of bacterial strains will inform the development of guidelines to facilitate (or prevent) their vertical transmission, depending on their beneficial or deleterious impact on host health.

How human gut microbiota composition relates to gastrointestinal transit time

Gut microbiota composition is the result of a process comprising the ecological maturation of the community from the moment of ingestion, with a selection bottleneck in the stomach, followed by progressive growth of the community along the small intestine and colon. We have reported in a large population cohort that proxies of total transit time are the highest driver of microbiota variation, higher than any other recorded physiological or lifestyle parameter. Now, we have used a wireless motility capsule that records passage-related variation in pH and pressure to link segmental transit times to gut microbiota composition in more detail. We found that different community states – enterotypes – are characterised by the parameters of different segments: the Rum-enterotype by longer colonic transit time, and the dysbiotic Bact2-enterotype by faster small intestine passage rate. Both small intestinal pH and colonic transit time influenced the abundance of specific bacteria. This trial serves as a pilot to study the *in vivo* parameters that modulate the ecological maturation of the microbiota.

FUTURE DIRECTIONS

Our group will continue to study the role of human gut microbiota in health and disease throughout life, diving deeper into its contribution to the risk of cardiovascular disease development in collaboration with clinical partners. We are also exploring how gut microbiota composition modulates therapeutic efficacy and patient outcome. We will continue to focus on studying the ecological maturation of the gut microbiota under varying conditions, within and outside the boundaries of health, in order to solidify our mechanistic understanding of gut microbiota-host interactions.

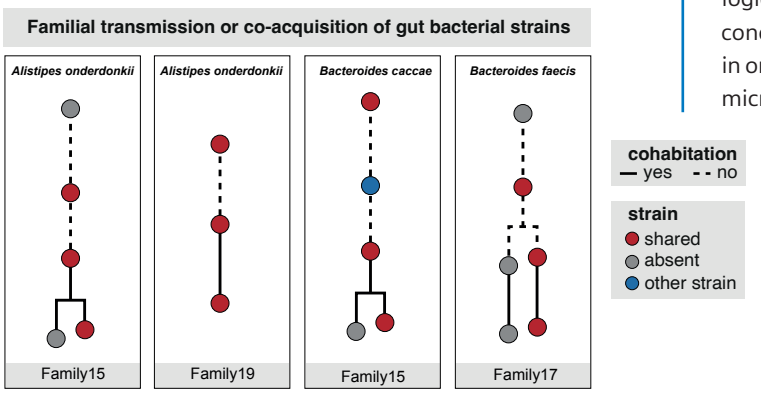

Figure 1. Tracking transmission of microbiota strains in multigenerational families. Using shotgun metagenomics to track gut bacterial strains in a cohort of multiple multigenerational families allowed us to detect potential transmission events spanning multiple generations. Our analyses do not exclude that these could result from maternal transmission and persistence, however these transmission events were strongly linked to cohabitation, suggesting horizontal transmission or co-acquisition from shared environmental source(s).

SELECTED PUBLICATIONS

Vieira-Silva S*, Falony G*, Belda E*, Nielsen T, Aron-Wisnewsky J, Chakaroun R, Forslund SK, Assmann K, Valles-Colomer M, ..., Stumvoll M, Vestergaard H, Zucker JD, Bork P, Pedersen O, Bäckhed F, Clément K and Raes J (2020) Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. *Nature*, 581: 310–315

Vieira-Silva S*, Sabino J*, Valles-Colomer M, Falony G, Kathagen G, Caenepeel C, Cleynen I, van der Merwe S, Vermeire S and Raes J (2019) Quantitative microbiome profiling disentangles inflammation- and bile duct obstruction-associated microbiota alterations across PSC/IBD diagnoses. *Nat Microbiol*, 4:1826–1831

Valles-Colomer M*, Falony G*, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C, Claes S, Van Oudenhove L, Zhernakova A, Vieira-Silva S* and Raes J* (2019) The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat Microbiol*, 4:623–632

*indicates joint contribution, #indicates joint correspondence

Eva **WOLF**

"We investigate the molecular mechanisms of circalunar timing."

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

Baris Cakilkaya	PhD Student; since 09/2022
Marcel Conrady	PhD Student; since 10/2019
Maxim Emelianov	Bachelor Student; 03/2022 – 09/2022
Constantin Hammer	Bachelor Student; 04/2022 – 10/2022
Florian Hof	PhD Student; since 04/2021
Torsten Merbitz-Zahradnik	Postdoc/Lab Manager; since 01/2014
Ruth Schaupp	Personal Assistant; since 05/2014
Hong Ha Vu	PhD Student; since 09/2021
Lars Peter Wittig	Bachelor Student; 03/2022 – 09/2022

OVERVIEW

Many marine animals including corals, brown algae, fish, turtles and bristle worms time their maturation and reproduction according to the phases of the moon. These monthly (~29.5 days) circalunar rhythms are essential to ensure their successful reproduction, which in turn is crucial to maintaining the marine ecosystem. Over the past years, anthropogenic disturbances (e.g. light pollution and climate change) have increasingly endangered the survival of marine organisms by desynchronising their moon-controlled reproduction cycles, amongst other reasons. However, despite these emerging ecological threats and the importance of nocturnal moonlight for the timely coordination of the physiology and reproduction of marine animals, the molecular architecture of circalunar timing systems is still completely unknown. The marine bristle worm *Platynereis dumerilii* possesses an inner monthly calendar (referred to as circalunar clock or circalunar oscillator) to control its monthly reproduction cycles. Using the bristle worm as a model system, we have now provided the first molecular-mechanistic insights into a circalunar timing system. Specifically, we have discovered that a light-sensitive cryptochrome called L-Cry can discriminate between moonlight and sunlight and even between different moon phases, which enables the marine worm *Platynereis dumerilii* to synchronise its reproduction to the full moon.

RESEARCH HIGHLIGHTS

To gain molecular-mechanistic insights into circalunar oscillators, we characterised the light-sensitive cryptochrome L-Cry of the marine annelid *Platynereis dumerilii* (*Pdu*) *in vitro* by performing protein biochemistry and UV-VIS spectroscopy, as well as *in vivo* (in collaboration with Kristin Tessmar-Raible, Vienna) by employing genetics, cell biology and behavioural studies. Interestingly, knockdown of L-Cry

led to a pronounced phenotype in the circalunar behaviour of the worms, indicating an important role for L-Cry in monthly timing. Subsequent spectroscopic analyses of the purified L-Cry protein revealed that L-Cry can sense very dim moonlight, forming a distinct moonlight state. Under darkness, L-Cry forms homodimers with a fully oxidised flavin adenine dinucleotide (FAD_{ox}) cofactor. Sunlight illumination leads to complete photoreduction of FAD to $FAD^{\bullet-}$ within minutes, whereas only about half of the FAD molecules are photoreduced after at least 6 hours of dim moonlight illumination (Pöhn, Krishnan *et al*, 2022; Figure 1). This allows L-Cry to form distinct sunlight, moonlight and dark states. We propose that the two flavin cofactors in the dark-state L-Cry dimer have different quantum yields ϕ for FAD photoreduction, where one monomer with a high quantum yield ("A" in Figure 1) acts as a low-light sensor for very dim nocturnal moonlight, whereas the second monomer ("B") with a lower quantum yield ($\phi_B < \phi_A$) can only be photoactivated by strong sunlight (number of photons N_P(sun) >> N_P(moon)). These three distinct molecular states allow L-Cry to distinguish moonlight from sunlight and to recognise the full moon (the moon phase to which the worms synchronise their reproduction) as the moon phase with the highest intensity and longest duration in the night sky. Hence, our study revealed a novel molecular mechanism for how dim light-sensing photoreceptors can decode moon phases. Notably, moonlight illumination also leads to L-Cry having a different cellular localisation and stability than in sunlight: while moonlight state L-Cry is mostly nuclear and stable, sunlight shifts the L-Cry protein to the cytosol and triggers its degradation. This suggests different signalling functions of moonlight and sunlight state L-Cry, which are still completely unknown and subject to further research. Furthermore, it remains a mystery how the distinct cellular localisations and molecular states of L-Cry translate into the different behaviour and physiology of the worms at specific moon phases. Finally, our spectroscopic analyses suggest that the dark, moonlight and sunlight states of L-Cry not only differ by the degree of photoreduction of their flavin cofactors, but also by their conformations and activities. The structural properties of the distinct sunlight, moonlight and dark states of L-Cry that define their distinct cellular functions and molecular interactions also remain to be investigated.

FUTURE DIRECTIONS

Our long-term goal is to elucidate the molecular mechanism of the circalunar oscillator that generates monthly rhythms of reproduction and behaviour in animals and synchronises these biological rhythms to the lunar cycle. Following on from our molecular-mechanistic characterisation of the L-Cry protein, we will now elucidate L-Cry's signalling to the circalunar clock. To this end, we will search for L-Cry interactors in worm tissues using pulldown and mass spectrometry approaches under dark, moonlight and sunlight conditions. Putative L-Cry interactors will then be analysed *in vivo* and *in vitro*. Furthermore, we will pursue structural analyses of L-Cry in its dark, moonlight and sunlight states. Solving the structure of the dark-state L-Cry dimer will enable us to design dimer interface mutations to further probe the role of the L-Cry dimer in generating the half-reduced moonlight state.

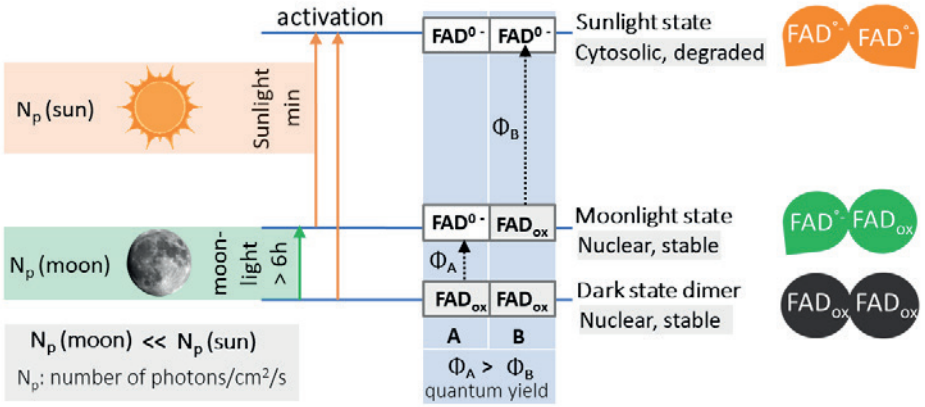

Figure 1. Model of L-Cry responses to sunlight (orange), moonlight (green) and darkness (black). Dim moonlight (> 6 h) only photoreduces the flavin (FAD) cofactor in L-Cry monomer A with high quantum yield Φ_A, generating the half-reduced nuclear moonlight state (FAD_{ox} $FAD^{\bullet-}$). Strong sunlight also photoreduces FAD in monomer B ($\Phi B < \Phi A$) within minutes, generating a fully photoreduced cytosolic sunlight state ($FAD^{\bullet-}$ $FAD^{\bullet-}$). Dark-state L-Cry is a fully oxidised dimer (FAD_{ox} FAD_{ox}). Adapted from Pöhn, Krishnan *et al*, 2022.

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Garg A, Orru R, Ye W, Distler U, Chojnacki JE, Köhn M, Tenzer S, Sönnichsen C and Wolf E (2019) Structural and mechanistic insights into the interaction of the circadian transcription factor BMAL1 with the KIX domain of the CREB-binding protein. *J Biol Chem*, 294:16604-16619

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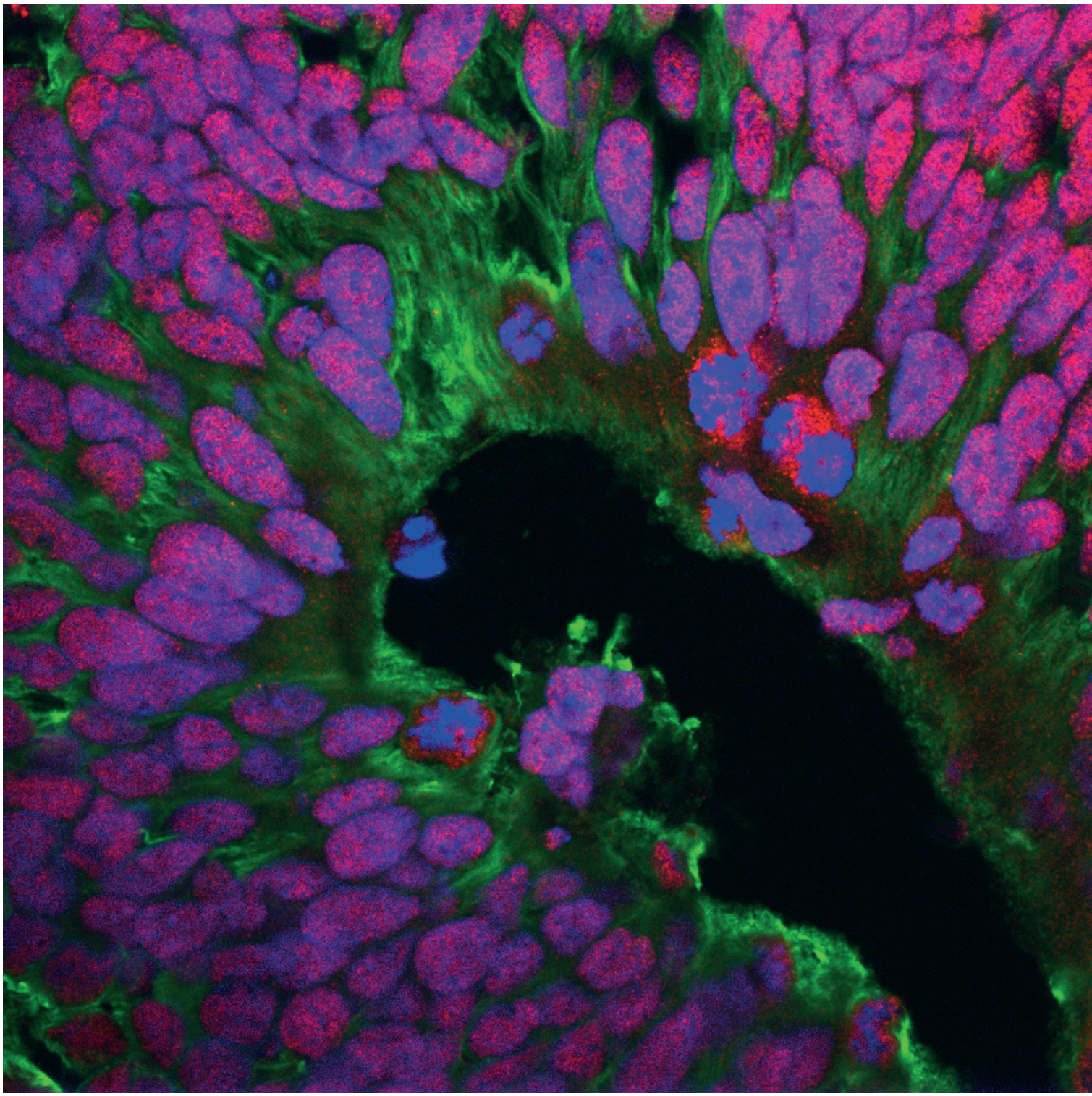
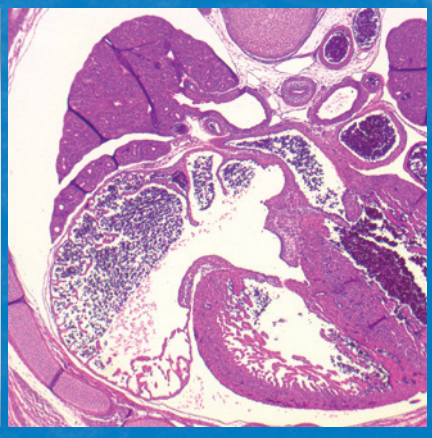
ADJUNCT CLINICIANS





STEPHAN **GRABBE**

"We develop immunotherapies to treat autoimmune diseases and cancer."

POSITIONS HELD

Since 2022 Adjunct Clinician, Institute of Molecular Biology (IMB), Mainz

Since 2007 Director, Dept. of Dermatology, University Medical Center (UMC), Mainz

2003 – 2007 Director, Dept. of Dermatology, University of Essen Medical Center

2000 – 2003 Professor of Dermatology and Dermato-Oncology, University of Münster

1998 – 1999 Heisenberg Scholarship: Visiting Scientist at the "Skin Disease Research Center", Brigham and Women's Hospital, Harvard University, Boston

1992 – 1998 Research Associate, later 'University Assistant', 'Senior University Assistant', Dept. of Dermatology, University of Münster

1989 – 1992 Postdoctoral Research Fellow, Wellman Laboratories of Photomedicine and MGH-Harvard Cutaneous Biology Research Center, Massachusetts General Hospital, Harvard University, Boston

1987 – 1989 Research Associate, University of Münster

EDUCATION

1996 Habilitation, University of Münster

1996 Dermatology, Allergology & Phlebology National Boards (Germany)

1987 Doctoral degree in Human Medicine, University of Münster

1987 Human Medicine, University of Münster

RESEARCH HIGHLIGHTS

Within my research group, we pursue several aspects of cutaneous and general immunology research. Our projects centre on cellular immunology, with a focus on dendritic cells and regulatory T cells. The group is tightly embedded into two DFG-funded collaborative research centres: the CRC 1066 on "Nanoparticle-mediated tumour immunotherapy", of which I am the Speaker, and the CRC TRR156 on the "Skin immune system", of which I am the Site Coordinator for Mainz. Moreover, we are part of the JGU "Research Center for Immunotherapy *(Forschungszentrum für Immuntherapie, FZI)*" (Speakers: Stephan Grabbe and Tobias Bopp).

Dendritic cells: master controls of adaptive immunity

Dendritic cells (DCs) play a central role in maintaining self-tolerance by presenting self antigens and harmless environmental antigens (peptides) in the absence of stimulatory signals to T cells. T cells that bind to these antigens are inactivated or reprogrammed to so-called (immuno) regulatory T cells (Treg). In contrast, when DCs encounter an antigen within an inflamed tissue, they undergo a functional switch that enables them to activate immune-effector T cells. Activated cytotoxic T cells (CTL) can directly kill infected cells and tumour cells, while other activated T cells exert helper functions (Th cells) and promote CTL activation.

Due to their versatile role, DCs are interesting immunotherapeutic targets to treat autoimmune and allergic diseases, or to mount profound and sustained anti-tumour responses. We work to test multi-functionalised nanovaccines for their ability to activate DC and stimulate DC-mediated T cells, as well as to test candidate vaccines in tumour mouse models. In addition, we study where immunotherapeutic nanoparticles travel in the body after

intravenous injection, and elucidate the mechanisms by which they are retained in the liver.

β_2 integrins: leukocyte adhesion molecules with multiple immune functions

β_2 integrin receptors are expressed specifically by leukocytes. They have many functions in the immune system; some bind ICAMs, providing a scaffold for interactions between immune cells, while others enable leukocytes to roll along the endothelium in search of inflammation sites or function as phagocytic receptors for complement-opsonised pathogens and immune complexes.

We study the roles that β_2 integrins play in maintaining tolerance and how their dysregulation contributes to autoimmune disease in order to discover therapeutic treatments. For this purpose, we recently generated a mouse strain with a floxed CD18 gene locus, which will enable us to study the distinct roles of β_2 integrins in DC, Treg and neutrophil cells.

Tumour immunotherapy

Tumours can be recognised and destroyed by the immune system, but often manage to escape destruction. Using murine melanoma models and patient-derived tumour samples, we work to understand key elements of the interaction between the immune system and tumours and develop anti-cancer immunotherapeutic strategies using nanoparticle-based approaches or by modulating the tumour microenvironment with β_2 integrins.

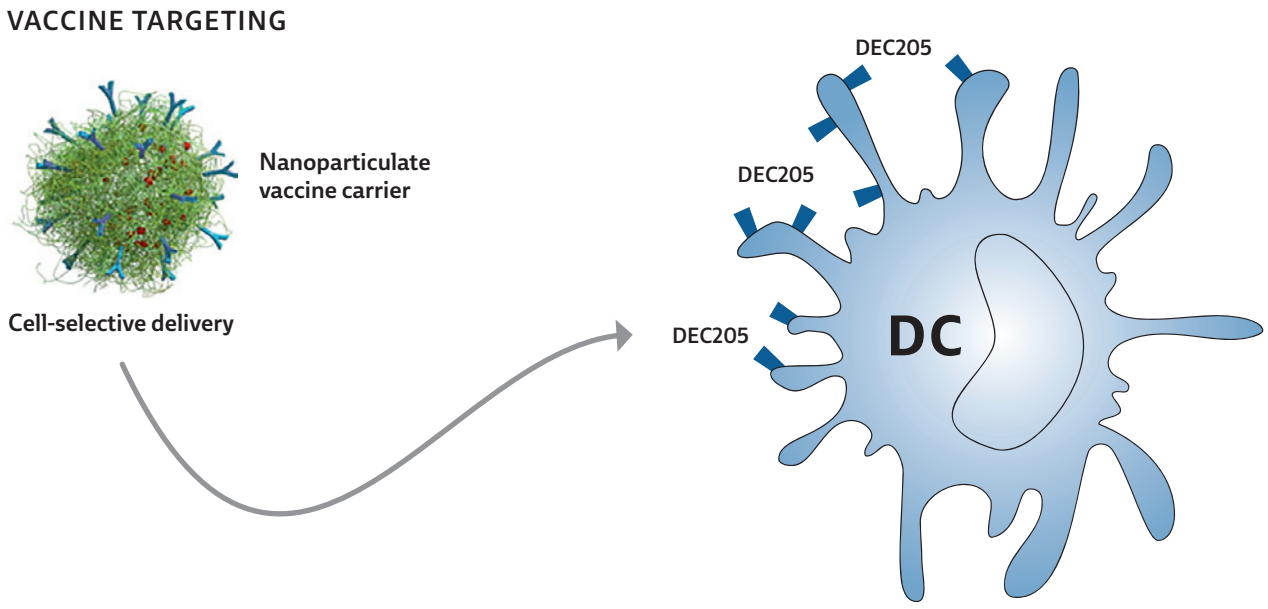


Figure 1. Nanoparticle-based modulation of dendritic cells *in vivo*.

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Kappel C, Seidl C, Medina-Montano C, Schinnerer M, Alberg I, Leps C, Sohl J, Hartmann AK, Fichter M, Kuske M, Schunke J, Kuhn G, Tubbe I, Paßlick D, Hobernik D, Bent R, Haas K, Montermann E, Walzer K, Diken M, Schmidt M, Zentel R, Nuhn L, Schild H, Tenzer S, Mailänder V, Barz M, Bros M# and Grabbe S# (2021) Density of conjugated antibody determines the extent of Fc receptor dependent capture of nanoparticles by liver sinusoidal endothelial cells. *ACS Nano*, 15:15191-15209

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Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, Maurus D, Schwarck-Kokarakis D, Kuhn AN, Omokoko T, Kranz LM, Diken M, Kreiter S, Haas H, Attig S, Rae R, Cuk K, Kemmer-Brück A, Breitkreuz A, Tolliver C, Caspar J, Quinckhardt J, Hebich L, Stein M, Hohberger A, Vogler I, Liebig I, Renken S, Sikorski J, Leierer M, Müller V, Mitzel-Rink H, Miederer M, Huber C, Grabbe S, Utikal J, Pinter A, Kaufmann, R, Hassel JC, Loquai C and Türeci Ö (2020) An RNA vaccine drives anti-tumor immunity in patients with checkpoint-inhibition experienced melanoma. *Nature*, 585:107-112

SUSANN **SCHWEIGER**

"We study the molecular basis of rare diseases to develop therapies."

POSITIONS HELD

Since 2022	Adjunct Clinician, Institute of Molecular Biology (IMB), Mainz
Since 2020	Group Leader, Leibniz Institute for Resilience Research (LIR), Mainz
Since 2012	Director, Institute of Human Genetics, University Medical Center (UMC), Mainz
2007 – 2012	Professor for Molecular Medicine, Dundee Medical School
2010 – 2012	Vice Chair, Wellcome Trust Center for Molecular Medicine, Dundee
2005 – 2010	Lichtenberg Professor, Charité-Berlin
2001 – 2005	Group Leader, MPI for Molecular Medicine, Berlin

EDUCATION

2006	Board Certificate in Human Genetics, Charité-Berlin
1993	MD in Biochemistry, University of Freiburg
1993	Medical School, University of Freiburg
1989	Medical School, University of Innsbruck

RESEARCH HIGHLIGHTS

In our genetics clinic, we see a large variety of patients with rare diseases, with a focus on neurodevelopmental and neurodegenerative disorders. We study the mutations in our patients in combination with their phenotypes in order to understand gene function in humans. We also use reprogramming of patients' cells and differentiate induced pluripotent stem cells into neural precursor cells, neurons and cerebral organoids to study gene function and the mechanisms of disease. We put a particular emphasis on understanding the molecular mechanisms underlying variability of clinical phenotypes. Mouse models and analysis in patient cohorts complete our methodological repertoire. With all these attempts, we aim to develop experimental therapies for patients with rare disorders.

Early processes in Huntington's Disease

Huntington's Disease (HD) is a late-onset and devastating neurodegenerative disorder that is very hard to detect in the early stages. However, once the disease has reached the symptomatic phase, neurodegeneration is already far advanced and therapy is likely to be too late. Using mouse models of HD, we have found aberrations in the cortical network at a very early stage before disease onset, which were associated with subtle behavioural abnormalities. We had found that the synthesis of disease-causing protein in Huntington's Disease is driven by a protein complex that contains the mTOR kinase (mammalian target of rapamycin). Metformin inhibits formation of this complex and, as we could show, substantially reduces the production of disease-causing protein in an animal model of Huntington's Disease. We are currently investigating whether early treatment with metformin can improve later disease progression in the mouse and have put together a clinical trial for patients before disease onset.

Epigenetic enhancement of non-canonical gene expression in Birk-Barel Syndrome

Mutations in the imprinted gene *KCNK9* cause Birk-Barel mental retardation syndrome. Using a mouse model of the disease, we characterised the allele-specific expression of *Kcnk9* in various regions of the brain and correlated this with brain function and behaviour. We observed partial expression of the paternal allele in several brain regions and intermediate phenotypes in animals with a maternal-only knockout of *Kcnk9*, leading us to hypothesise that stimulating paternal gene expression could rescue the phenotype. Using specific histone deacetylase inhibitors, we induced upregulation of the paternal *Kcnk9* allele in primary cortical neurons and in the brains of *Kcnk9*KO^{mat} mice. This fully rescued the cognitive deficits, suggesting that this may be a promising experimental therapy for patients with Birk-Barel mental retardation syndrome.

Dynamic X-chromosomal reactivation enhances female brain resilience

Sexual dimorphism is well-documented in neurodevelopmental disorders, but the underlying molecular mechanisms are not well understood. One of the most important differences between male and female mammals is the sex chromosomes. In order to allow dosage compensation between the sexes, large parts of one X chromosome are randomly inactivated in females. We use induced pluripotent stem cells, neural precursor cells, neurons and brain organoids as models to study how X chromosomal gene expression and inactivation are regulated during neurodevelopment.

In collaboration with the ReALity community and with Claudia Keller-Valsecchi (IMB), Felicia Basilicata (IMB), Joan Barau (IMB) and Peter Baumann (IMB/JGU), we also plan to study X chromosomal gene re-activation in the developing immune system and work to understand the molecular mechanisms underlying X chromosomal re-activation.

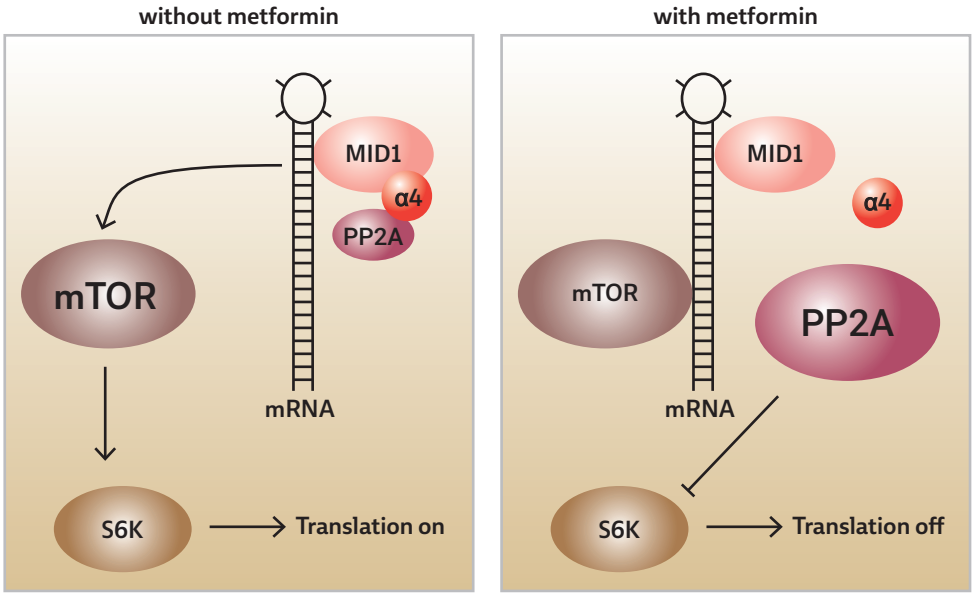

Figure 1. Protein synthesis of aberrant protein in Huntington's Disease is induced by an mTOR-containing protein complex that binds to a hairpin made by the RNA containing the expanded CAG repeat. Metformin destroys this complex and thereby inhibits the synthesis of aberrant protein in Huntington's Disease.

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Arnoux I*, Willam M*, Griesche N*, Krummeich J, Watari H, Offermann N, Weber S, Narayan Dey P, Chen C, Monteiro O, Buettner S, Meyer K, Bano D, Radyushkin K, Langston R, Lambert JJ, Wanker E, Methner A, Krauss S#, Schweiger S# and Stroh A# (2018) Metformin reverses early cortical network dysfunction and behavior changes in Huntington's disease. *eLife*, 7:e38744

Deciphering Developmental Disorders Study (2017) Prevalence and architecture of de novo mutations in developmental disorders. *Nature*, 542:433–438

*indicates joint contribution, #indicates joint correspondence

OLIVER **TÜSCHER**

"We unravel the mechanisms of resilience that enable healthy human ageing."

POSITIONS HELD

Since 2022	Adjunct Clinician, Institute for Molecular Biology (IMB), Mainz
Since 2020	Founding member, Research Group Leader and Head of the Clinical Investigation Center (CIC), Leibniz Institute for Resilience Research (LIR), Mainz
Since 2018	Spokesperson of the Centre for Rare Diseases of the Nervous System, University Medical Center (UMC) of Johannes Gutenberg University (JGU)
Since 2016	Professor of Mental Health and Cognitive Resilience in Old Age, UMC/JGU and German Resilience Center (DRZ), Mainz
Since 2015	Vice-chair, Dept. of Psychiatry, UMC/JGU, Mainz
Since 2013	Attending in Psychiatry and Psychotherapy, UMC/JGU, Mainz
2010 – 2013	Residency in Psychiatry and Psychotherapy, UMC/JGU, Mainz
2009 – 2010	Residency in Psychiatry and Psychotherapy, University of Freiburg
2006 – 2010	Head of the Emotion Regulation and Impulse Control Imaging Group (ERIC), Freiburg Brain Imaging, University of Freiburg
2006 – 2009	Residency in Neurology, University of Freiburg
2003 – 2006	Postdoc and supervised consultant service, Weill Medical College Cornell University, New York
2001 – 2003	Residency in Neurology, UKE, University of Hamburg

EDUCATION

2013	Board certification for Psychiatry and Psychotherapy
2010	Board certification for Neurology
2011	Habilitation for Neurology, University of Freiburg
2002	MD/PhD in Neurobiology, University of Heidelberg
2000	Medical School, University of Heidelberg
1995	Medical School, University of Bochum

RESEARCH HIGHLIGHTS

Within our research area, "Healthy ageing, neurodegeneration and neuropsychiatry", led by Kristina Endres, Katharina Geschke/Isabel Heinrich and Oliver Tüscher, we use a broad methodological spectrum spanning from preclinical lab work to clinical studies to investigate the mechanisms of healthy ageing. Based on these findings, we aim to develop preventive, disease-modifying and therapeutic interventions. Our interdisciplinary research group includes biologists, chemists, psychologists, physicians and computer scientists, enabling us to implement research from molecular mechanisms to clinical use. The results of our investigations are evaluated using a translational cycle, with the ultimate goal of fostering an ageing process that is as cognitively healthy and free of ailments as possible.

We work in close cooperation with the Centre for Healthy Ageing (CHA), Mainz to identify and investigate biomarkers and mechanisms of (healthy) ageing in neuronal tissues. Intervention strategies are tested on animal models ranging from *C. elegans* to mice. Using neuroimaging techniques, we translate this research to the human brain and study neural network mechanisms of resilient ageing – a conceptual framework we recently developed to explicitly understand and target those biological mechanisms which protect the brain and body against functional loss caused by ageing and ageing-related diseases. Studies in our lab include:

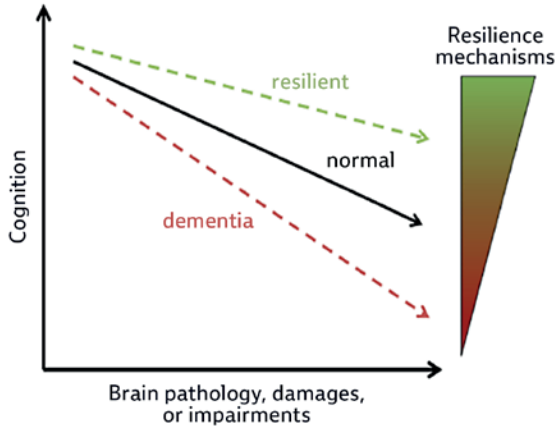
Resilient ageing: ReALizing healthy body & brain ageing (ReALity HBBA)

We are investigating the mechanism(s) conveying resilience to body and brain ageing by comprehensively phenotyping the (epi)genomic, proteomic, cellular-immunologic and cardiovascular phenotypic levels of participants in the AgeGain study (with the Bopp Lab, FZI/UMC and the Wild Lab, CTH/UMC & IMB). We will also uncover genetic and cellular

senescence mechanisms by comparing resilient and non-resilient participants (with the Baumann Lab, JGU/IMB).

Gut-brain-axis in ageing

Recent studies suggest that certain bacterial commensals may cause accelerated or diseased ageing. We study the gastrointestinal system in mouse models of Alzheimer's disease and accelerated ageing (with the Baumann and Schick labs (IMB) through the CHA and SHARP initiative), to identify pathways that can serve as new therapeutic treatment options to ameliorate cognitive decline in ageing.



Signatures of vulnerability in the ageing brain

Certain brain regions maintain function throughout ageing and even diseased ageing, while others are highly vulnerable. Together with the Dormann (JGU/IMB), Gerber (UMC) and Bopp (UMC) labs, we analyse how different brain areas and cellular subpopulations in the brain are affected by normal and accelerated ageing. With the Krämer-Albers lab (JGU), we also analyse neuronal extracellular vesicles (EVs) in humans to unravel novel biomarkers of cognitively healthy ageing.

Anti-brain-ageing therapeutics

We are evaluating the use of sarcopenia (the progressive loss of strength and functionality of skeletal muscles) as an external measure of healthy ageing in rodent models and humans, and are using it to assess the efficacy of therapeutic interventions for Alzheimer's disease and preventing cognitive decline in normal and accelerated ageing.

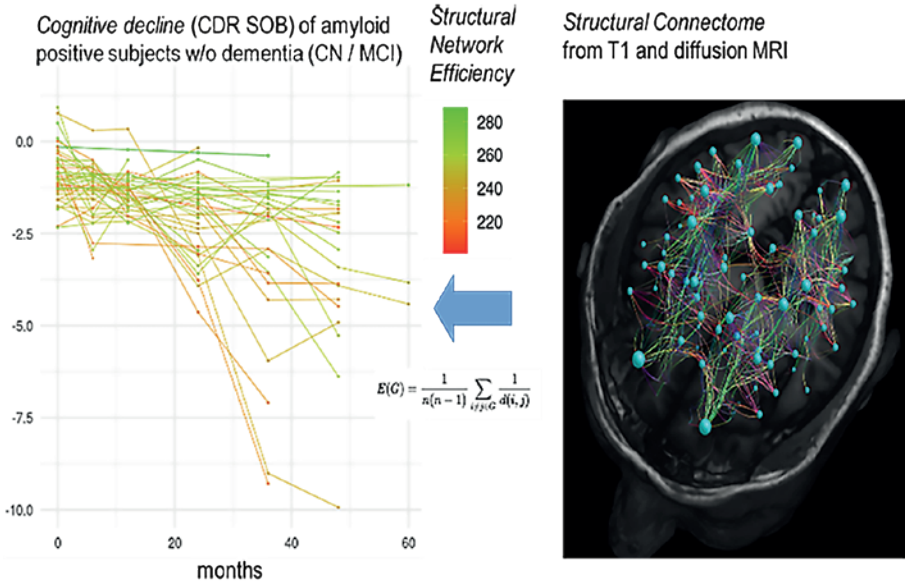


Figure 1. Resilient ageing: concept (top panel). Example: Resilience to cognitive decline despite high amyloid burden (scatter plot, bottom left panel). Structural Network Efficiency (SNE) predicts functional outcomes (bottom right panel; Fischer *et al*, 2021, Front Aging Neurosci).

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Schäfer SK, Kunzler AM, Kalisch R, Tüscher O and Lieb K (2022) Trajectories of resilience and mental distress to global major disruptions. *Trends Cogn Sci*, 26:1171–1189

Ahrens KF, Neumann RJ, Kollmann B, Plichta MM, Lieb K#, Tüscher O# and Reif A# (2021) Differential impact of COVID-related lockdown on mental health in Germany. *World Psychiatry*, 20:140–141

Schaum M, Pinzuti E, Sebastian A, Lieb K, Fries P, Mobascher A, Jung P, Wibral M# and Tüscher O# (2021) Right inferior frontal gyrus implements motor inhibitory control via beta-band oscillations in humans. *eLife*, 10:e61679

#indicates joint correspondence

PHILIPP **WILD**

"We use systems medicine to obtain a granular understanding of age-related diseases."

POSITIONS HELD

Since 2022	Adjunct Clinician, Institute of Molecular Biology (IMB), Mainz
Since 2020	Deputy Site Speaker RheinMain, German Center for Cardiovascular Research (DZHK)
Since 2015	Head of Liquid Biobank, UMC, Mainz
Since 2015	Speaker, Research Center for Translational Vascular Biology (CTVB), UMC, Mainz
Since 2013	Head of Preventive Cardiology and Preventive Medicine, Center for Cardiology, UMC, Mainz
Since 2012	W3 Professor of Clinical Epidemiology, Center for Thrombosis and Hemostasis Mainz (CTH), UMC, Mainz
Since 2012	Head of Clinical Epidemiology and Systems Medicine, Center for Thrombosis and Hemostasis (CTH), UMC, Mainz
Since 2011	Coordinating Principal Investigator and Steering Committee Member of the Gutenberg Health Study (GHS), UMC, Mainz
2010 - 2012	Senior Physician, Dept. of Medicine 2, UMC, Mainz

EDUCATION

2022	Board certification in Cardiology
2012	MSc in Epidemiology, IMBEI, University Medical Center (UMC), Mainz
2009	Board certification in Internal Medicine
2004	MD, Philipps University Marburg
2002	Medical School, University Leipzig and Medical School, Philipps University Marburg

RESEARCH HIGHLIGHTS

Systems medicine – a holistic approach to promoting healthy ageing

The Systems Medicine Group has comprehensive experience in molecular epidemiology and systems medicine research, with a focus on investigating complex common diseases, which are strongly driven by the ageing process. Our research themes range from cardiovascular diseases, such as thrombotic disease and heart failure to cardiometabolic conditions like obesity and type 2 diabetes mellitus, as well as infectious diseases with system-wide sequelae (SARS-CoV-2 and COVID-19) and cancers. The study of how the ageing process induces pathological changes is highly clinically relevant and a key priority for our group.

Developing tailor-made therapeutic treatments for disease

By using artificial intelligence (AI) methods and state-of-the-art high-throughput omics profiling techniques, we holistically integrate multi-omics data with environmental exposures, (sub)clinical parameters and advanced imaging data to discover new biomarkers and biosignatures, detect diseases at an earlier stage and predict their further progression. This is the basis for the development of tailor-made therapies, diagnostics and prognostics, and therapy monitoring tools to reflect a patient's response to therapy. A key strength of our group is our large multidisciplinary team, spanning expertise from molecular biology, biomedicine and clinical and molecular epidemiology to bioinformatics and biostatistics/AI and disciplines critical to maintaining a large clinical research infrastructure (e.g. IT, biobanking, regulatory affairs).

Exemplary highlights

The year 2022 featured notable successes for our department. One rapidly growing research area is clinical epigenetics. In the EpiHF project (collaborators: Christoph Niehrs, IMB, and Steve Horvath, Altos lab, USA), a ReALity/SHARP network collaboration, we investigate the role of DNA methylation as a readout of environmental exposures for the development and progression of heart failure by analysing global (whole genome), regional (gene region-specific) and specific methylation (epigenome-wide association studies, EWAS). The recent expansion of the clinical epigenetics unit and its resources (MethylationEPIC 850k array in nearly 4,000 individuals, as well as sequential sampling) now puts it among the top 5 largest cohorts in the world for analysing DNA methylation in cardiovascular disease.

In the area of thrombotic disease, our team initiated a multinational GWAS consortium to discover additional genetic loci implicated in hypercoagulability. Our latest success in the area of thrombotic disease is our leading participation in the trail-blazing excellence cluster curATime (curatime.org; funding volume for the first 3 years: €15m), in which we collaborate with basic researchers, drug developers (TRON, BioNTech), as well as several large and small-to-medium enterprises to identify and evaluate new therapeutic targets for atherothrombosis and convert these into actionable interventions using innovative RNA-based immunotherapy.

Figure 1. Research focuses of the Systems Medicine group.

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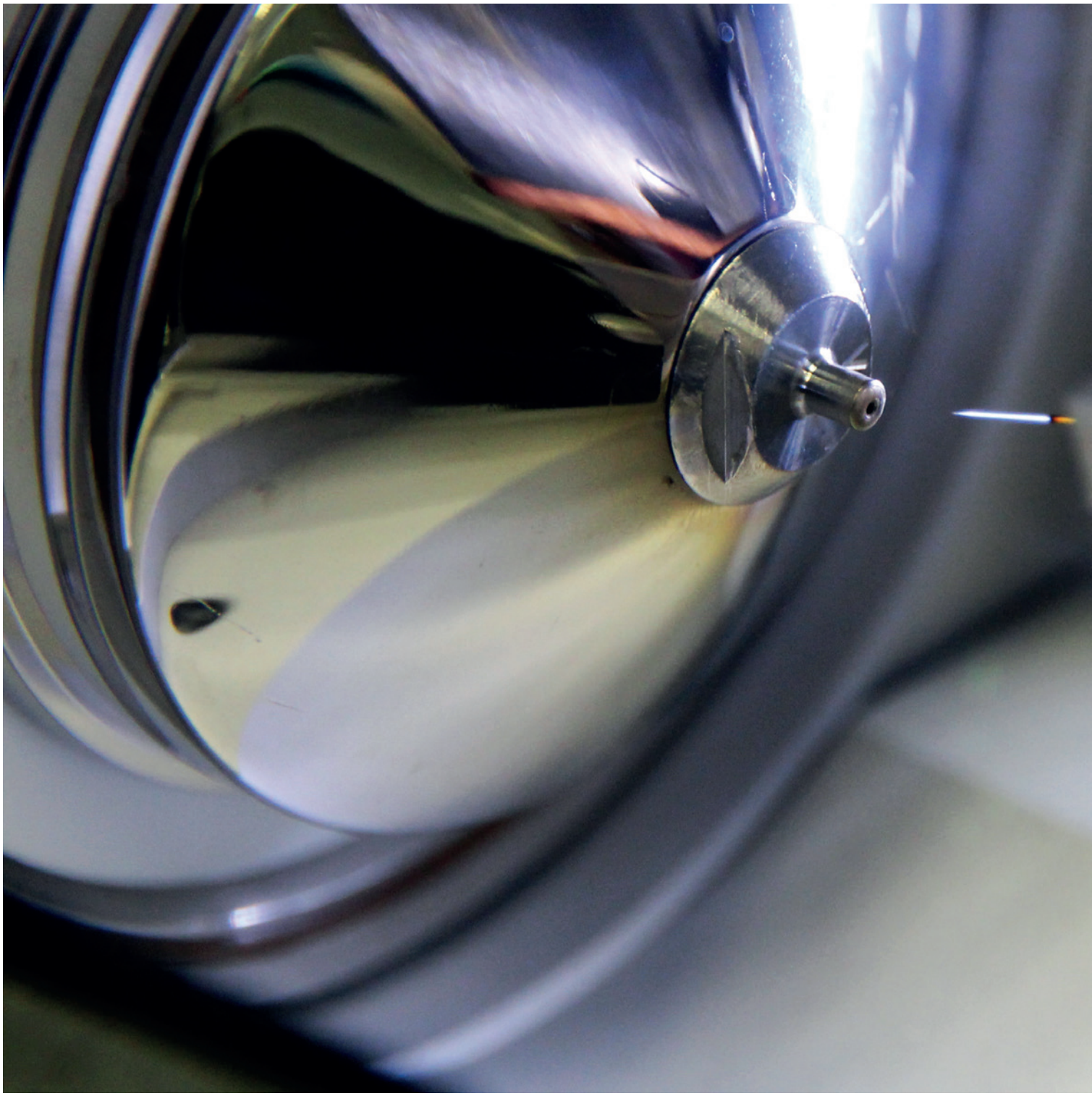
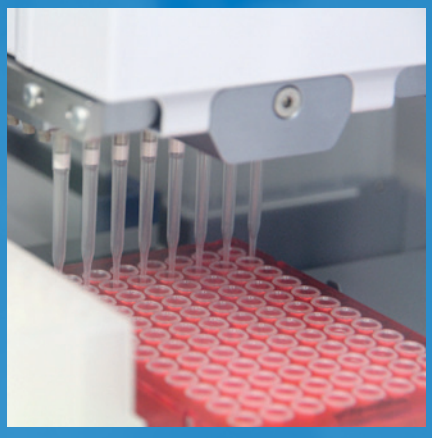
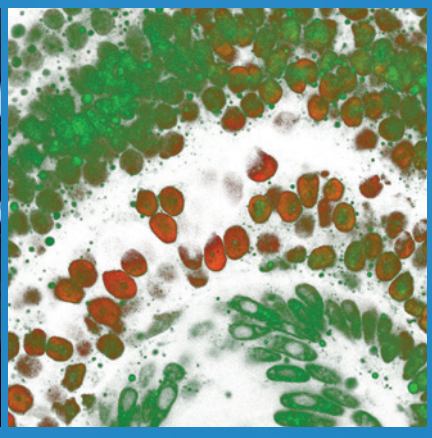
CORE FACILITIES







OVERVIEW

“Our Core Facilities provide access to key technologies, as well as services & training by experts.”

IMB has seven Core Facilities (CFs): Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics, Protein Production and a Media Lab. The Bioinformatics, Genomics and Proteomics CFs provide a “full service”, encompassing experimental design and quality control to data generation, analysis and 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. The CFs’ staff are available for consultation and trouble-shooting for all users, whether they receive a full or assisted service. Furthermore, CF staff can collaborate with researchers to provide customised or specialised services. IMB researchers have access to all seven CFs. In addition, the Flow Cytometry, Genomics, Microscopy/Histology and Proteomics CFs are open to external users in Mainz.

CF services are adjusted based on user demand. Each facility has a user committee that provides feedback on the equipment and user experience. This also helps determine the implementation of new CF services. The overall CF functions as a service axis by aligning and combining individual services to create new, innovative workflows (e.g. single-cell sequencing, which requires an overlap between Flow Cytometry and Genomics). The CFs also offer lectures and practical courses on new techniques and instrumentation, experimental design, statistics and data acquisition, processing and analysis to allow researchers to keep up-to-date with current and emerging technologies. Lectures are open to everyone, including those outside of IMB.

In order to offer users the best and most modern research equipment, several CFs purchased new state-of-the-art instrumentation this year. In addition, the CFs now have a shared postdoc in a collaboration with Joan Barau’s lab to develop novel tools for mouse transgenesis and genome editing, which could lead to the establishment of a new transgenic mouse core facility.

The CFs also maintain and provide training for IMB’s core equipment and are responsible for managing the radioactivity lab, the S2 lab and IMB’s in-house animal facilities (mouse, zebrafish and *Xenopus*).

Furthermore, the CFs are responsible for institute-wide aspects of occupational health and safety, including all safety measures relating to the COVID-19 pandemic. We offer all IMB employees a COVID-19 qPCR test twice a week so that IMB can remain a safe place to work.

Andreas Vonderheit
Director of Core Facilities and Technology

BIOINFORMATICS

OVERVIEW

The Bioinformatics Core Facility (BCF) supports researchers with computing infrastructure, web services, system administration, software training, designing experiments, biostatistics and data analysis. In addition, BCF members participate in the analysis, visualisation and interpretation of high-throughput data from research projects. Genome-wide assays employing next-generation sequencing (NGS) and other omics methods have become indispensable for elucidating the molecular mechanisms of gene regulation and the BCF strives to offer tailored bioinformatics solutions for these. The BCF also provides bioinformatics expertise to the CRC 1361 on "Regulation of DNA Repair & Genome Stability", as well as the "Science of Healthy Ageing Research Programme" (SHARP).

MEMBERS

Emil Karaulanov
Head; since 10/2014
Anke Busch
Bioinformatician; since 01/2014
Sivarajan Karunanithi
Bioinformatician; since 07/2021
Fridolin Kielisch
Biostatistician; since 06/2020
Nastasja Kreim
Bioinformatician; since 04/2012
Antonella di Liddo
Bioinformatician; since 03/2022
Martin Oti
Bioinformatician; 12/2017 – 03/2022
Giuseppe Petrosino
Bioinformatician; since 03/2017
Frank Rühle
Bioinformatician; since 01/2019
Sergi Sayols
Bioinformatician; since 06/2019

SERVICES OFFERED

BCF staff offer support at different levels depending on the project needs, ranging from basic bioinformatics services to full-scale scientific collaborations in the context of "big data" research projects.

Services include:

- Consulting on biostatistics and the experimental design of genomics projects
- Data quality assessment, processing, analysis, visualisation and interpretation
- Implementation of NGS pipelines and customising them for individual projects
- Development of novel tools and custom methods for specific analysis tasks
- Testing, implementation and customisation of software tools and online services
- Data mining of published datasets, correlation and integration of results
- Assistance with preparing manuscripts, presentations and grant proposals
- Workshops and tutorials on bioinformatics topics to facilitate data access and analysis
- Biostatistics course for IPP PhD students, which is also open to IMB employees

The facility maintains GitLab and GitHub repositories with software tools and pipelines for advanced NGS data analysis, which are also used by the computational biologists embedded in the research groups. In addition to standard tools and pipelines, the BCF offers customised bioinformatics solutions and long-term analytical support for numerous data-intensive omics projects that require expert handling for optimal results.

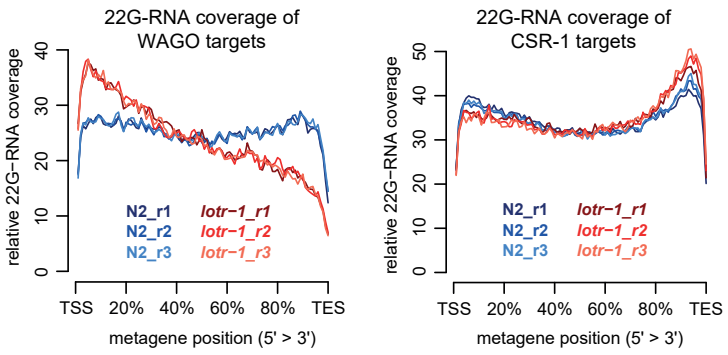


Figure 1. Metagene profiles of 22G endo-siRNAs over two sets of Argonaute target genes in wild-type (N2) and *lotr-1* mutant *C. elegans* reveal selective changes in 5'>3' coverage of 22G-RNAs (published in Marnik *et al*, 2022, doi:10.1371/journal.pgen.1010245).

FLOW CYTOMETRY

OVERVIEW

The Flow Cytometry Core Facility (FCCF) offers high-throughput measurements, analysis and separation of biological units using 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 with training for the analysers. Additionally, its staff are available for collaboration in 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. The FCCF works with many 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 and an annual practical course for basic flow cytometry analysis, as well as an advanced practical course for cell sorting.

In 2022, the facility purchased a new analytical flow cytometer, the Novocyte Quanteon. Furthermore, the FCCF submitted a major instrument grant proposal to the German Research Foundation (DFG) for the acquisition of a new cell sorter, which was successful. The new full-spectrum jet-in-air instrument with integrated biosafety cabinet will allow cell sorting on a BSL-2 level and broaden the range of applications within the facility.

MEMBERS

Stefanie Möckel
Head; since 10/2016
Stephanie Nick
Staff Scientist; since 01/2021

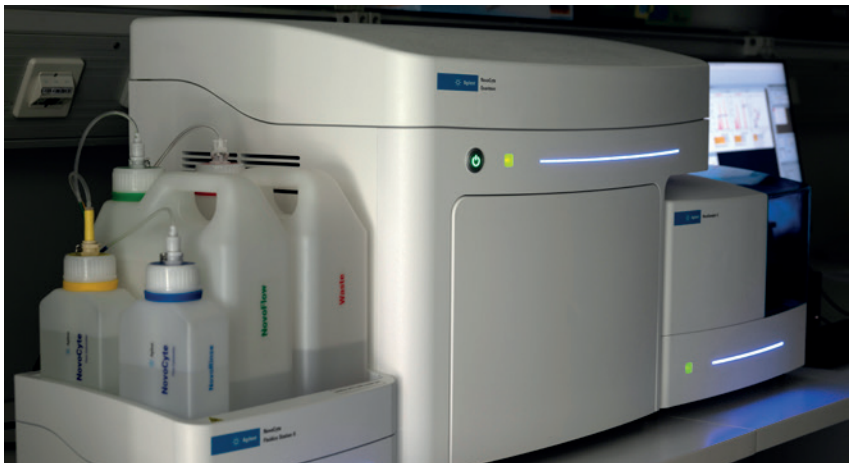
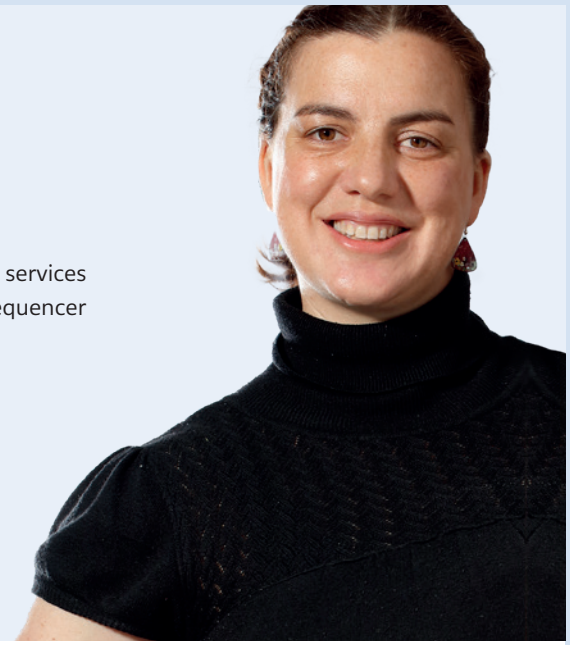


Figure 1. The latest addition to the FCCF: the Novocyte Quanteon

The Novocyte Quanteon is an analytical four-laser system equipped with a high-throughput loader for tubes and multiwell plates (24 to 384 well formats).

GENOMICS



OVERVIEW

The Genomics Core Facility (GCF) offers next-generation sequencing (NGS) services based on the Illumina NextSeq500, MiniSeq and MiSeq platforms. A MinION sequencer (Oxford Nanopore Technologies) is also available.

MEMBERS

Maria Mendez-Lago
Head; since 04/2016
Annabelle Dold
Staff Scientist; since 01/2020
Pablo Llavona
Deputy Head, Staff Scientist; since 11/2021
Hanna Lukas
Technician; since 01/2013
Robert Pyne
Staff Scientist; since 11/2021
Ramona Rohde
Technician; since 05/2022
Regina Zimmer
Technician; since 12/2019

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

SERVICES OFFERED

The GCF provides a full service for NGS, beginning with the experimental design of the project and continuing to the generation of sequencing data. In addition, the GCF also sequences self-prepared libraries from researchers at IMB, Mainz University and the University Medical Center, as well as others from outside Mainz. 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 20 applications as a standard service. New protocols are also developed to accommodate users' needs for their specific projects.

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
- BrdU-Seq
- SLAM-Seq
- APEX-Seq
- cDNA library preparation
- circRNA
- GRO-Seq
- STARR-Seq
- 3' Quant-seq

DNA:
- ChIP-Seq
- DIP-Seq
- DRIP-Seq
- MBD-Seq
- Whole genome sequencing
- Whole genome bisulfite sequencing
- Single-stranded DNA library preparation
- Hi-C
- Amplicon-Seq (low diversity)
- GLOE-Seq (from November 2022)

Single cell sequencing:
- RNA-Seq
- Multiplex RNA-Seq
- ATAC-Seq
- Multiome (ATAC + RNA) sequencing
- SmartSeq2 protocols

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

MICROSCOPY & HISTOLOGY

OVERVIEW

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

SERVICES OFFERED

The MHCF has 13 instruments ranging from stereo and widefield to confocal, high-content screening and super-resolution microscopes. Eight setups (three widefield, one holotomography, two 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 for assistance.

In 2022, MHCF acquired a line-scanning confocal microscope with a fluorescence-lifetime option (Stellaris 8 & Falcon, Leica). It can be used for sensitive confocal imaging, FRAP and detecting changes in the fluorescence lifetime (FLIM) of fluorophores, enabling molecular interaction studies using FLIM-FRET. To meet the data storage requirements of the MHCF's high-content screening microscope (OperaPhenix, Perkin Elmer), we acquired a 400 TB server.

The MHCF has four high-power workstations with licensed software for image restoration functions like deconvolution (Huygens Essential, SVI) and 3D visualisation and analysis (Imaris, Harmony, Vision 4D, LAS-X, VisiView). In addition, we develop custom solutions with users by macro programming in open source software (e.g. Fiji, ImageJ, or ilastik) or assembling predefined building blocks in Columbus, a database and analysis software specifically designed for high-content imaging data. Most of these software tools can also analyse images with machine learning and artificial intelligence.

In addition to the "Image Analysis and Processing" course, we offered a practical "Super-resolution Microscopy" course for the second time, which teaches sample preparation, super-resolution techniques and data analysis.

The MHCF also provides histology techniques. In addition to semi-automated fixation and paraffin embedding, machines for sectioning paraffin-embedded tissue (microtome), frozen tissue (cryotome) and gelatine/agarose embedded or fresh tissue (vibratome) are available. Users can access optimised protocols for immunodetection and tissue clearing, as well as solutions for classical tissue staining.

MEMBERS

Sandra Ritz
Head; since 01/2016
Márton Gelléri
Staff Scientist; since 06/2019
Petri Turunen
Staff Scientist; since 08/2019
Eric Schumbera
Image Analysis Support, 05/2022 – 12/2022

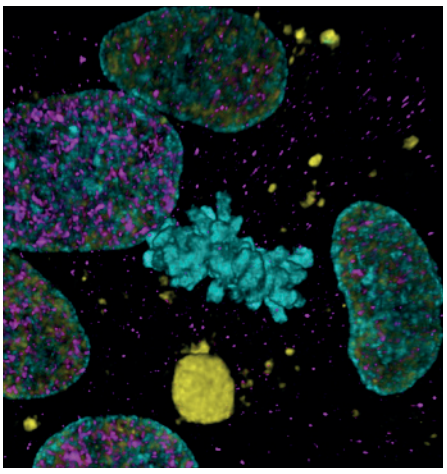


Figure 1. Snapshot of a multicolour 4D video showing the temporal interplay between chromatin and chromatin binding proteins in interphase and mitotic nuclei of HAP1 cells. Nuclear puncta of transcriptional coactivators are in pink, chromatin modifiers in yellow and chromatin in cyan. Fast live cell multicolour 4D videos were acquired on the Stellaris 8 Falcon confocal microscope (Leica Microsystems). Sample kindly provided by Samuel Shoup (Schick group, IMB). Imaged and reconstructed by Petri Turunen (MHCF). Two videos showing fast time-lapse sequences (35 x 35 x 20 µm, 2.8 sec, 5 min, Dynamic Signal Processing (DSE, rolling average over time) and deconvolution (LIGHTNING)) in 3D are available at https://seafile.rlp.net/d/5f4d2bfb4c0e4cf1bd99 and https://seafile.rlp.net/d/50fd4e0a2e014229a85c.

Video 1

Video 2

PROTEIN PRODUCTION

OVERVIEW

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

MEMBERS

Martin Möckel
Head; since 03/2018
Claire Mestdagh
Technical Assistant; 03/2020 – 08/2021
Sabine Ruegenberg
Staff Scientist; since 10/2021

SERVICES OFFERED

The PPCF supports researchers throughout the process of protein production. This includes screening 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 four automated chromatography systems, which enable the use of the latest chromatographic methods for state-of-the-art protein purification strategies.

Another key task of the PPCF is to generate and perform functional quality control of routine laboratory enzymes and affinity probes for IMB researchers. The PPCF currently offers 30 products to IMB scientists, matching the most frequently used protein tools at the institute.

The demand by IMB's research groups for PPCF support in producing recombinant proteins and developing assays has been steadily increasing over the years. The facility has a head and a full-time staff scientist, who assist researchers with their project needs and offer services tailored to specific user requests.

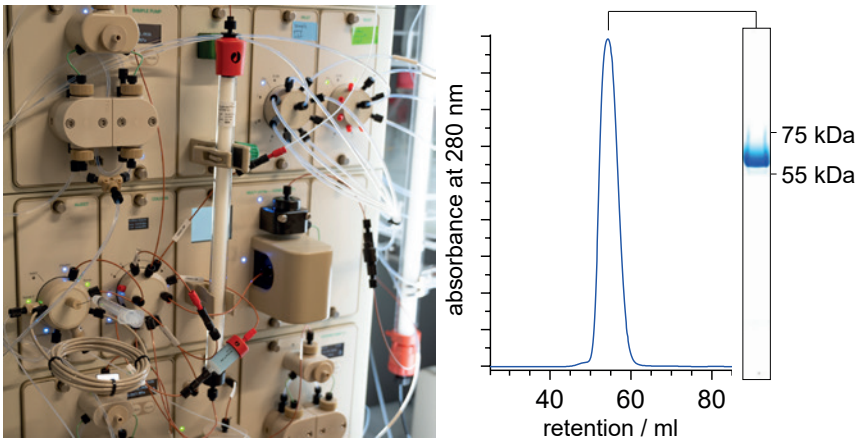
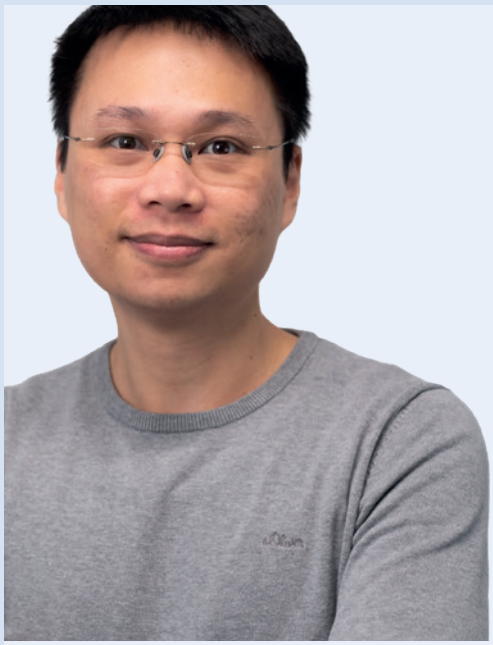


Figure 1. *Left:* Most recombinant protein purifications are performed using various fast protein liquid chromatography systems (FPLCs) in the facility. *Right:* Elution profile of a recombinant protein during the final gel filtration step, showing the absorbance at 280 nm over the column volume (retention). The purity of the protein is visualised by Coomassie-stained SDS-PAGE next to the elution profile.



PROTEOMICS

OVERVIEW

The Proteomics Core Facility (PCF) operates high-resolution mass spectrometers (Q Exactive Plus and Exploris 480, the latter exclusively for CRC 1361 projects) coupled to nanoflow ultra-high performance liquid chromatography (UHPLC) systems (EASY-nLC 1000 and 1200) and provides services to the entire scientific research community in Mainz. The PCF is staffed by both wet- and dry-lab scientists who possess a broad range of research experience and technical expertise.

SERVICES OFFERED

The PCF actively participates in the experimental design of each user project and offers tailored services that range from simple gel band identification to quantitative analysis of complex samples. The PCF currently supports label-free, dimethyl-labelled, TMT and SILAC-based quantitative proteomic experiments. The PCF also works closely with users on downstream bioinformatic data analysis to support users in making discoveries from the data. As part of the annual Modern Techniques in Life Sciences lecture series offered by the Core Facilities, the PCF delivers a theoretical lecture on proteomics technologies. Additionally, the PCF offers an annual practical training course on proteomics sample preparation and related bioinformatic data analysis.

MEMBERS

Jiaxuan Chen
Head; since 04/2022
(Staff Scientist since 03/2019)
Falk Butter
Head; 05/2013 – 03/2022
Jasmin Cartano
Technician; since 02/2014
Mario Dejung
Bioinformatician; since 05/2014
Amitkumar Fulzele
Staff Scientist; since 06/2020

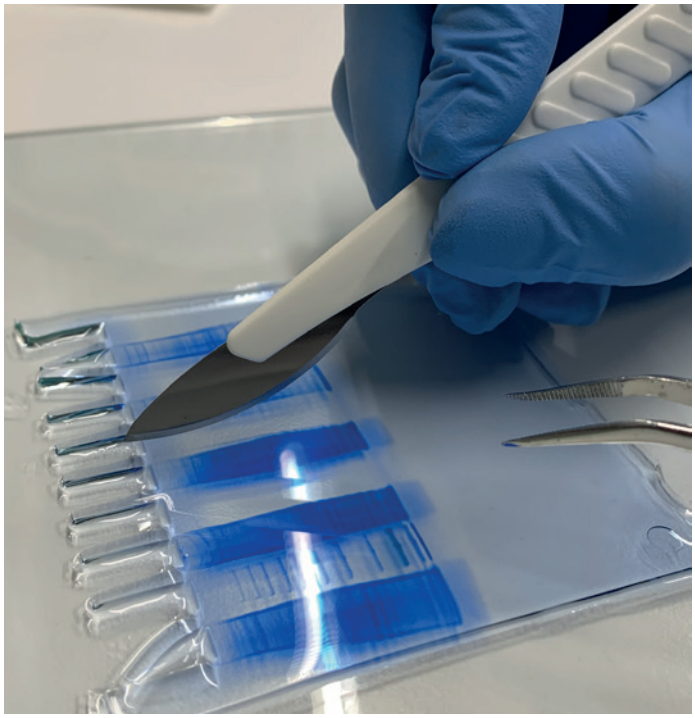


Figure 1. Gel excision step of an in-gel digestion workflow for proteomics sample processing.

MEDIA LAB

OVERVIEW

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, S1/S2 waste management and the cleaning and sterilisation of glassware.

MEMBERS

Andrea Haese-Corbit
Head; since 01/2018
Doris Beckhaus
Assistant; since 05/2011
Alwina Eirich
Assistant; since 07/2013
Pascal Hagebölling
Assistant; since 01/2015
Annette Holstein
Assistant; since 04/2012
Marion Kay
Assistant; since 04/2016
Johann Suss
Assistant; 04/2011 – 06/2022
Abraham Welday Gebre
Assistant; since 06/2022

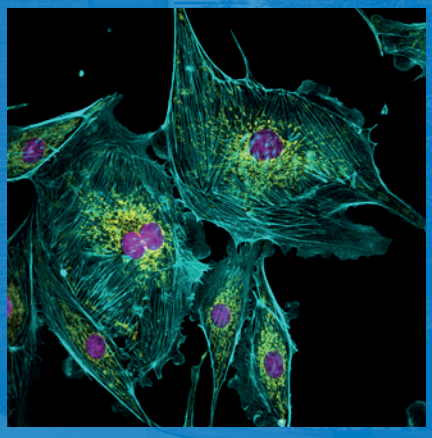
SERVICES OFFERED

The Media Lab provides the following services:

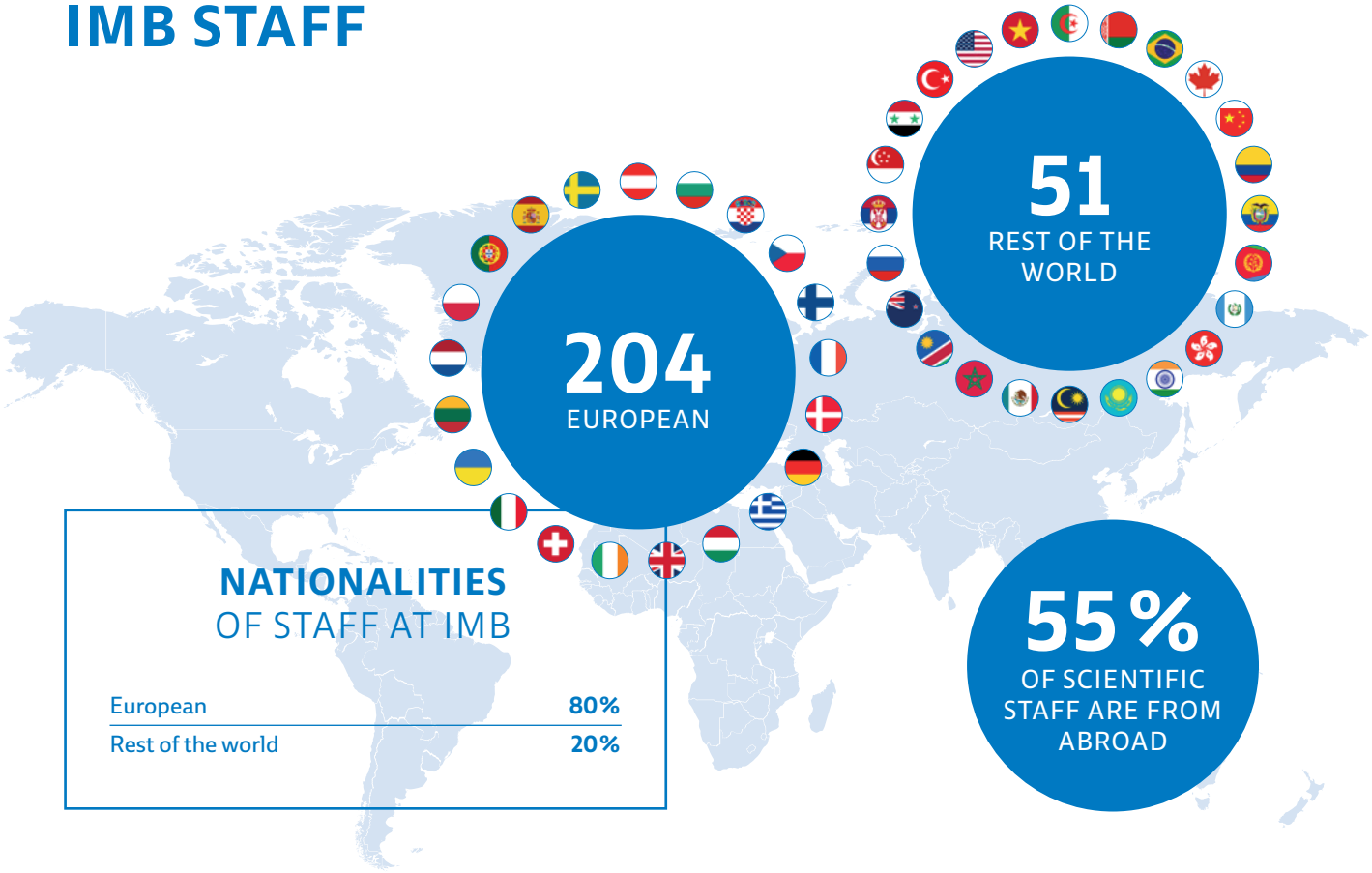
- 24/7 supply of routinely used buffers, solutions, liquid media and agar plates for molecular biology research and for culturing bacteria, yeast, insect cells and *C. elegans*
- Production of made-to-order media
- Management of three supply centres for enzymes, kits and cell culture media
- Administration 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 the in-house transport system

Figure 1. The Media Lab produces 120L of buffers and media per week.

FACTS & FIGURES



IMB STAFF



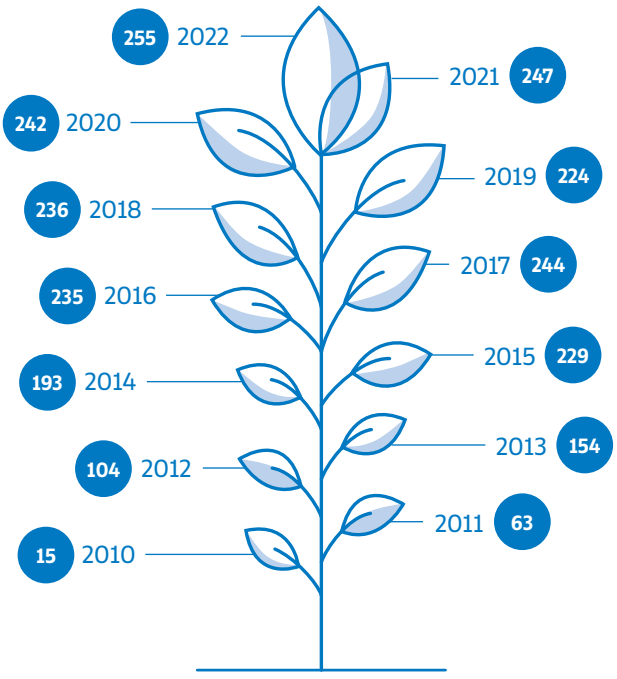
NATIONALITIES OF STAFF AT IMB

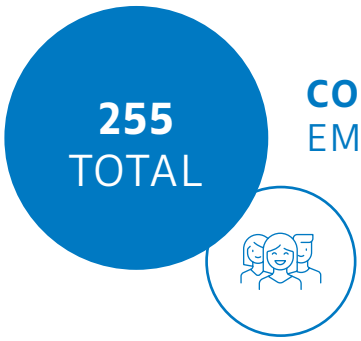
European	80%
Rest of the world	20%

NUMBER OF SCIENTIFIC STAFF AT IMB BY CATEGORY

Master/Bachelor Students	4
PhD Students	84
Postdocs	60
Group Leaders	25
Technical Staff	48

STAFF GROWTH SINCE 2010

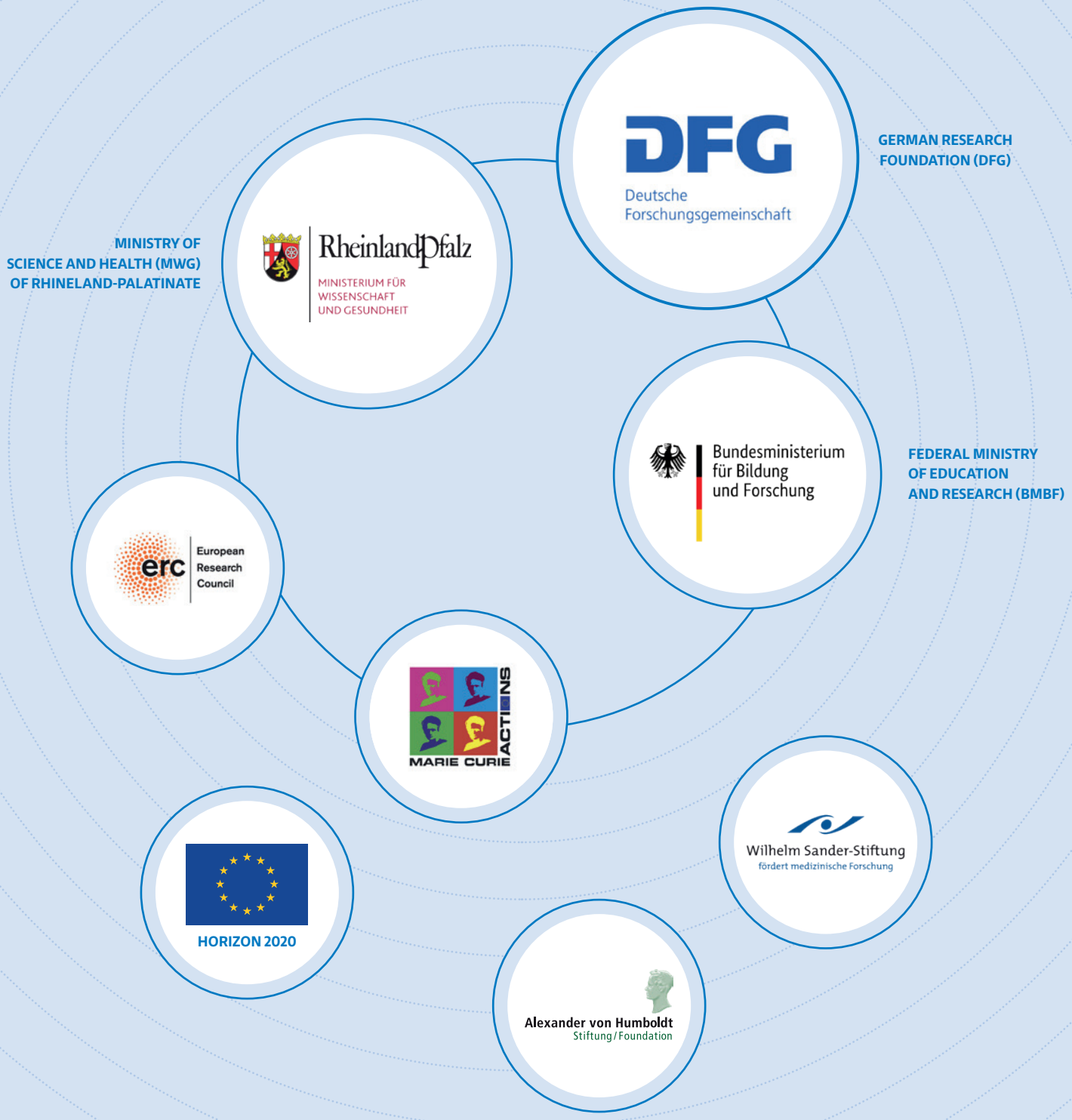




COUNT OF EMPLOYEES

EXTRAMURAL FUNDING

in addition to core funding from the Boehringer Ingelheim Foundation and the State of Rhineland-Palatinate, IMB is grateful for funding from the following:



SCIENTIFIC MANAGEMENT

Ralf Dahm
Director of Scientific Management

"We support our scientists across a range of areas so they can focus on their research."

At IMB, we foster our scientists' success by providing an excellent environment for their research. This is the main responsibility of IMB's Scientific Management team.

We know that research involves a lot of time-consuming administrative work, from screening job applications, organising training for students and writing reports to managing funds and publicising key achievements. To relieve our scientists of these tasks, Scientific Management takes on such non-scientific duties, freeing up their time for quality research.

Another important way we support our scientists is to build a friendly atmosphere in which scientists enjoy working. This encourages collaborations that spark innovative ideas and build a strong community spirit. Through a wide range of scientific events, we also allow our scientists to engage with outstanding leaders in research from around the world at IMB.

Scientific Management achieves these goals through a range of services in, for example, the following areas:

- Communications & Outreach
- Event Management
- Scientific Writing
- Fundraising & Grant Management
- Recruitment, Training & Career Development
- Technology Transfer
- Reporting, Research Evaluations & Strategic Planning

Communications & Events

At IMB, we recognise that building a strong collaborative spirit and encouraging frequent social interactions between our scientists are key for productive scientific exchange. To facilitate this, we organise a wide range of events for our scientists to share their research with each other, with the scientific community, and with the general public. We also invite top experts from all over the world to speak about their work in Mainz. Events we organise include:

- Seminars, conferences, workshops & symposia
- Public outreach activities
- Institutional reviews
- Social events & retreats

We also maintain connections between current staff and IMB's alumni by keeping them informed of the latest developments via newsletters and IMB's social media channels.

Fundraising & Grant Management

Getting funding is essential for scientists to embark on ambitious projects and advance their careers. The Grants Office maximises our scientists' chances of getting funded by helping them find, obtain and manage extramural funding. Our services include:

- Screening & informing scientists of calls for grants & fellowships
- Supporting scientists in the application process
- Administering existing project grants

Science Writing

For junior scientists, it is extremely important that their breakthroughs are seen by the scientific community. We increase the visibility of our scientists' work to a broad audience by producing and distributing scientific and general texts about IMB's research. We help with writing texts for:

- Press releases, IMB's website & social media posts
- Annual Reports
- Institutional grant applications

Recruitment, Training & Career Development

IMB has a vibrant and international population of young scientists who are the driving force behind our exciting research. New students and postdocs are recruited to IMB through the Professional Development Office. We work to attract talented young researchers and manage IMB's structured training programmes, ensuring that each provides a comprehensive range of courses, lectures and career events to target the specific needs of our scientists and prepare them for the next step of their career. IMB's programmes in this area include:

- The International Summer School (ISS) for undergraduate students
- The International PhD Programme (IPP) for graduate students
- The IMB Postdoc Programme (IPPro) for more experienced scientists
- The Career Development Programme for Junior Group Leaders

You can learn more about these programmes on the following pages.

RESEARCH & TRAINING

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

At IMB, scientists work at the forefront of their fields to answer key questions in 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.

IMB Postdoc Programme

International PhD Programme

International Summer School

Training Courses

FACTS & NUMBERS

IMB's scientists produced **346** publications in the last 5 years,
with **76** in 2022 (of which 36 % had an IF of 10 or higher).

We actively support our scientists as their careers develop by providing comprehensive training in scientific, technical and complementary skills, including:

- **Scientific & technical training** in state-of-the-art equipment by experts, as well as technical support in implementing the latest techniques
- **Professional skills training** in presentation, scientific writing, project management, fundraising, career development, negotiation and leadership by qualified trainers

Through this dedicated training, our scientists gain a competitive edge at all stages of their career in both academic and commercial settings.

IMB POSTDOC PROGRAMME

IMB's Postdoc Programme (IPPro) was established to meet the specific needs of our postdoctoral community. The programme provides ambitious, early-career scientists with the skills and guidance necessary to develop into future scientific leaders.

The IPPro actively supports our young professionals as their careers develop.

IMB POSTDOC PROGRAMME PRO

www.imb.de/postdocs

WE PROVIDE:

- Advanced training in **scientific methods** and **professional skills** through a range of lectures, focused workshops and tailored events
- **Guidance** from leading scientists and **mentoring** from IMB's structured Mentoring Programme for Junior Scientists
- Networking at **career events** and **symposia** with leading external scientists from industry and academia

As well as offering fully funded positions, we also support our postdocs to raise funds for their research to help them become more independent. Collectively, the IPPro ensures that our postdocs have access to the training and technology needed to effectively carry out their research projects and advance their prospects in building successful careers.

INTERNATIONAL PHD PROGRAMME

PhD students are key to our research at IMB. To provide the structure, training and supervision necessary to excel during a PhD, IMB created the International PhD Programme (IPP) with the help of funding from the Boehringer Ingelheim Foundation.

Within this programme, 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.

INTERNATIONAL PhD PROGRAMME MAINZ

www.imb.de/PhD

THIS TRAINING INCLUDES:

- **Regular supervision** from 3 or more experts
- Training in **scientific, professional & technical skills**
- **Networking** opportunities at symposia, retreats & seminars

With the comprehensive scientific and technical training the IPP provides, our students are prepared for successful careers in the quickly evolving field of life sciences.

In 2021, a panel of independent reviewers representing leading PhD programmes at German and international institutions considered the IPP **"as a model for a structured PhD programme, which is on par with the most prominent schools in Europe"**

INTERNATIONAL SUMMER SCHOOL

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, enthusiastic students get the chance to work on their own hands-on project at the forefront of biological research. In 2022, the ISS was held as a 3-week online event due to the COVID-19 pandemic.

The informal and international environment of the ISS gives participants an excellent framework in which to develop their practical and professional skills.

www.imb.de/ISS

THIS INCLUDES:

- **Training by leading experts** in scientific and transferable skills needed as a scientist
- **Lectures** to learn comprehensive insights into the latest research
- **Networking** with leading international researchers

The ISS teaches students to identify key open questions in the fields of gene regulation, epigenetics and genome stability and prepares them to tackle ambitious Masters or PhD projects. This gives our ISS alumni a head start in their careers, with many going on to study and work at prestigious institutions around the world.

IMB INTERNATIONAL SUMMER SCHOOL

FACTS & NUMBERS:

Currently **41** postdocs from **16** countries

22 % Europe — 27 % Germany

51 % Rest of the world

Alumni work in industry, academia and beyond as:
» Assistant Professors » Lab Heads » Senior Research Scientists » Managers » Policy & Governance Officers » Consultants

IN A 2021 SURVEY:

81 % of IMB postdocs felt supported by the IPPro and **70 %** were satisfied or very satisfied with their training

FACTS & NUMBERS:

Includes students from 3 institutions (IMB, JGU & UMC) plus 3 affiliated research networks

500 applications per call (2 calls per year)

Currently **189** PhD students from **44** countries in **77** research groups

34 % Europe — 34 % Germany

32 % Rest of the world

112 graduates since **2011**

FACTS & NUMBERS:

300 applicants for **20** positions each year
176 participants from **38** countries since 2012

Rated as **"excellent"** or **"very good"** by **99 %** of participants

70 % "excellent" — 29 % "very good"

1 % "as expected"

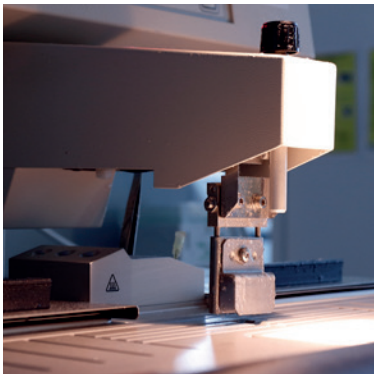
TRAINING COURSES

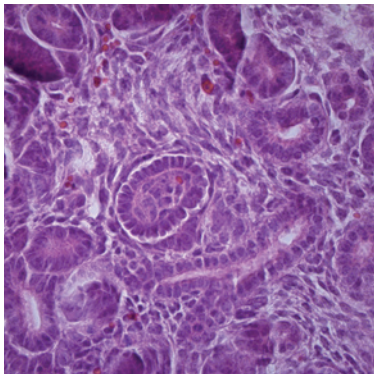
CORE FACILITIES & TRAINING

IMB's Core Facilities staff provide our scientists with training in key scientific techniques and a wide range of cutting-edge methodologies to ensure they can consistently perform top-quality research. In 2022, IMB offered the following training courses:

LECTURES

CORE FACILITY	DATES	TITLE
GENERAL	25 Apr	Molecular & Biochemistry Techniques
BIOINFORMATICS	30 May	Databases in Bioinformatics
	27 Jun	Design & Analysis of NGS Experiments
FLOW CYTOMETRY	01 Mar	Flow Cytometry: Introduction I
	08 Mar	Flow Cytometry: Introduction II
	05 Apr	Advanced Flow Cytometry: Principles of Cell Sorting
	04 Jul	Flow Cytometry
GENOMICS	20 Jun	Genomics (NGS)
MICROSCOPY & HISTOLOGY	02 May	Introduction to Microscopy
	09 May	Microscopy: F-Techniques & Super-Resolution
	16 May	Histology & Fluorescent Labelling
	11 Jul	Electron Microscopy
	14 Nov	Image Manipulation: The Slippery Slope to Misconduct
PROTEOMICS	13 Jun	Proteomics
PROTEIN PRODUCTION	23 May	Protein Production & Crystallography





PRACTICAL COURSES

CORE FACILITY	DATES	TITLE
BIOINFORMATICS	27 Apr	Bioinformatics: Introduction to Biostatistics
	04 May	Bioinformatics: Introduction to Biostatistics
	11 May	Bioinformatics: Introduction to Biostatistics
	18 May	Bioinformatics: Introduction to Biostatistics
	25 May	Bioinformatics: Introduction to Biostatistics
	01 Jun	Bioinformatics: Introduction to Biostatistics
	06 Oct	Bioinformatics: Introduction to R (Part I)
	10 Oct	Bioinformatics: Introduction to R (Part II)
	13 Oct	Bioinformatics: Introduction to R (Part III)
	17 Oct	Bioinformatics: Plotting with R (Part I)
	20 Oct	Bioinformatics: Plotting with R (Part II)
	24 Oct	Bioinformatics: Introduction to RNA-seq Analysis (Part I)
	27 Oct	Bioinformatics: Introduction to RNA-seq Analysis (Part II)
	07 Nov	Bioinformatics: Introduction to ChIP-seq Analysis (Part I)
	10 Nov	Bioinformatics: Introduction to ChIP-seq Analysis (Part II)
	08 Nov	Bioinformatics: Introduction to Biostatistics
	15 Nov	Bioinformatics: Introduction to Biostatistics
	22 Nov	Bioinformatics: Introduction to Biostatistics
	29 Nov	Bioinformatics: Introduction to Biostatistics
	06 Dec	Bioinformatics: Introduction to Biostatistics
	13 Dec	Bioinformatics: Introduction to Biostatistics
	15 Dec	Bioinformatics: Efficient HPC Usage and Version Control with GitLab
FLOW CYTOMETRY	09 – 10 Mar	Basic Flow Cytometry Practical Course
	16 – 17 Mar	Basic Flow Cytometry Practical Course
	23 – 24 Mar	Basic Flow Cytometry Practical Course
	30 – 31 Mar	Basic Flow Cytometry Practical Course
	06 – 07 Apr	Advanced Flow Cytometry Practical Course: Principles of Cell Sorting
	05 – 06 May	Advanced Flow Cytometry Practical Course: Principles of Cell Sorting
MICROSCOPY	07 – 11 Feb	Image Processing & Analysis
	26 – 30 Sep	Super-Resolution Imaging
PROTEOMICS	28 – 29 Mar	Proteomics Data Analysis
	24 – 26 Oct	Proteomics Practical Course

INVITED SPEAKERS

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

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
27 Jan	IMB Seminar ■	**Johan Auwerx**	EPFL, Lausanne, CH	Cross-species genetics to map new players in mitochondria and ageing
10 Mar	IMB Seminar ▲	**Tugce Aktas**	Max Planck Institute for Molecular Genetics, Berlin, DE	To splice or not to splice? That is not the only question
14 Mar	IMB Seminar ◆ ■	**Karla Neugebauer**	Yale University School of Medicine, New Haven, US	Pre-mRNA processing and the organisation of the cell nucleus
24 Mar	IMB Green Seminar ■	**Joan Suris**	EPFL, Lausanne, CH	Nobody is against sustainability – Greening EPFL's School of Life Sciences
31 Mar	IMB Seminar ▲●	**Ian Hickson**	University of Copenhagen, DK	Mechanisms to copy difficult-to-replicate regions of the human genome
07 Apr	IMB Seminar ■	**Oded Rechavi**	Tel Aviv University, IL	Long and really long molecular memories in *C. elegans*
25 Apr	IMB Seminar ▲	**Ruth Lehmann**	Whitehead Institute, Cambridge, US	Guiding germ cells toward immortality
28 Apr	IMB Seminar ▲●	**Olivier Hyrien**	IBENS, Paris, FR	Using nanopore sequencing to study eukaryotic DNA replication
19 May	IMB Seminar ▲●	**Helen Walden**	Institute of Molecular, Cell and Systems Biology, University of Glasgow, UK	Regulation of DNA interstrand crosslink repair by monoubiquitin
02 Jun	IMB Green Seminar ■	**Noemi Bender & Hollyn Hartlep**	German Cancer Research Center (DKFZ), Heidelberg, DE	The DKFZ and Helmholtz: on their way to sustainable research
30 Jun	IMB Seminar ◆	**Gilles Travé**	IGBMC, Strasbourg, FR	Multifunctional E6 oncoproteins produced by oncogenic HPVs: atomic and interactomic insights into viral and tumour hijacking of human cells

● Seminars which were part of the CRC 1361 seminar series on "Regulation of DNA Repair and Genome Stability"

◆ Seminars which were part of the Research Training Group GenEvo seminar series on "Gene Regulation in Evolution: From Molecular to Extended Phenotypes"

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
14 Jul	IMB Seminar ▲●	**Agnel Sfeir**	Sloan Kettering Institute / MSKCC, New York City, US	DNA repair: A tale of two genomes
15 Sep	IMB Seminar	**Maria Hondele**	University of Basel, CH	DEAD-box ATPases are global regulators of RNA-containing membraneless organelles
29 Sep	IMB Seminar ■	**Juri Rappsilber**	Technical University of Berlin, DE	Integrative modelling with in-cell crosslinking mass spectrometry and AlphaFold
04 Oct	IMB Seminar ◆	**Toni Goßmann**	Bielefeld University, DE	BigData meets Darwin: Understanding function and disease through evolutionary approaches
18 Oct	Ad Hoc Seminar	**Florian Altegoer**	Heinrich Heine University Düsseldorf, DE	Kiwellin proteins in host-microbe interactions
03 Nov	IMB Seminar	**Jolanda van Leeuwen**	Center for Integrative Genomics, University of Lausanne, CH	Systematic analysis of genotype-to-phenotype relationships: from yeast to human
08 Nov	Ad Hoc Seminar	**Michael Meyer-Hermann**	Helmholtz Centre for Infection Research, Braunschweig, DE	Digital immunology in the context of physiology and ageing
17 Nov	IMB Seminar ▲●	**Joachim Lingner**	Swiss Institute for Experimental Cancer Research (ISREC), Lausanne, CH	Challenges of telomere maintenance and TERRA long non-coding RNA
24 Nov	IMB Seminar ▲●	**Gaëlle Legube**	Centre for Integrative Biology (CBI), Toulouse, FR	Chromosome and chromatin dynamics at DNA double-strand breaks
01 Dec	IMB Seminar ■	**Anne Brunet**	Stanford University, US	Understanding and modeling ageing
06 Dec	Ad Hoc Seminar	**Siyao Wang**	CECAD Cluster of Excellence, University of Cologne, DE	Deciphering the epigenetic code of genome stability, from soma to germline

■ Online seminars ▲ Hybrid seminars



RESEARCH INITIATIVES

IMB is a driver in four major research initiatives, which bring together scientists from multiple centres across Germany.

CRC 1361 "REGULATION OF DNA REPAIR & GENOME STABILITY"

Spokesperson: Helle Ulrich

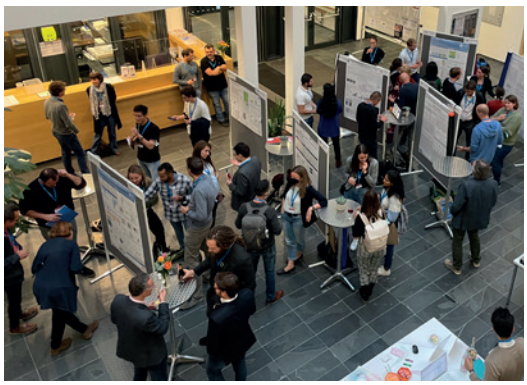
The CRC 1361 was launched in January 2019. During its first four-year funding period, it was funded by the DFG with €12.4 million. In July 2022, the CRC was evaluated by an external review board and received excellent reviews. As a result, the DFG has now prolonged its funding and granted another €12.9 million for a second funding period until December 2026.

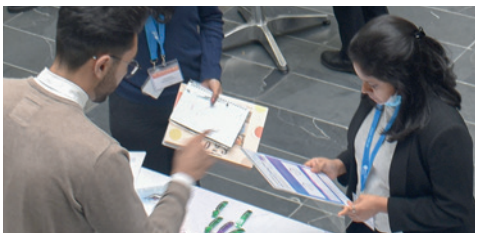
This initiative consolidated 18 projects from investigators across 7 institutions (IMB, Mainz University and its University Medical Center, Darmstadt University, Munich University, Frankfurt University and Jena University) with the goal of understanding the molecular mechanisms modulating the activities of genome maintenance in the cell.

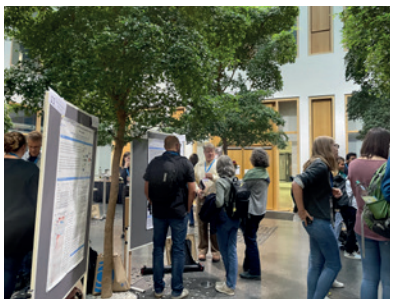
Supported by 3 dedicated service projects and centralised management, the network comprises experts in structural biology, organic chemistry, biochemistry, molecular & cell biology, genetic toxicology and clinical sciences. The CRC's Integrated Research Training Group is designed to ensure that participating students receive the best possible training and career development while completing their PhDs.

Since the beginning of 2022, researchers in the CRC 1361 have published a total of 29 papers that were directly funded by projects in this initiative. A highlight was the IMB/CRC 1361 Conference "Restore, Reorganise, Repurpose: The many faces of DNA repair", which took place from 20-23 September at IMB. The conference was attended by 149 international participants and brought together leading scientists in the field of DNA repair, damage signalling and related research areas. It focused on the diverse aspects of cellular metabolism that genome maintenance mechanisms impinge on, thereby approaching the classical "3R" (Replication - Recombination - Repair) theme from a new angle. Apart from the lively scientific exchanges and discussions, the programme was rounded off by a boat trip along the Rhine River to the picturesque town of Rüdesheim and a public lecture by Björn Schumacher (CECAD Cologne) entitled "The mystery of human ageing: New insights from the biology of ageing".

www.sfb1361.de



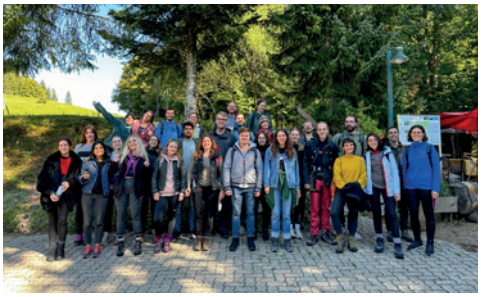





RTG GENEVO: GENE REGULATION IN EVOLUTION

Spokespersons: Susanne Foitzik (JGU) and René Ketting (IMB)





The Research Training Group GenEvo launched in June 2019 and is funded by the DFG with €5.8 million until December 2023. This initiative was organised in a collaboration between IMB and JGU's Faculty of Biology. GenEvo 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. The programme 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.

In 2022, 14 new PhD students joined GenEvo, while an additional 3 students joined as associated members. GenEvo also held its first student retreat from 21-23 September in the Black Forest, Baden-Württemberg, where 30 GenEvo PhD students came together to share their research with one another. Another highlight was the 3rd GenEvo Symposium in November, which marked the official start of the second cohort of GenEvo PhD students. The first two GenEvo-associated PhD students successfully completed their PhDs in 2022, and to date researchers in GenEvo have published a total of 12 papers connected to this initiative.

www.imb.de/genevo

SCIENCE OF HEALTHY AGEING RESEARCH PROGRAMME (SHARP)

SHARP was launched in 2021 as a joint PhD training programme between IMB, Johannes Gutenberg University and the University Medical Center Mainz (UMC). The goal of SHARP is to strengthen cooperation between research groups at IMB and UMC in projects focusing on ageing and longevity. This programme brings together 25 group leaders, including clinical/preclinical researchers from the fields of stem cell biology, epidemiology, immunology, cardiology, neurobiology and cancer biology together with basic molecular biology researchers in the fields of epigenetics, proteomics, telomere biology, RNA biology, DNA repair and autophagy.

By combining the complementary skills of basic and clinical/translational researchers, SHARP will gain new insights into the underlying causes of ageing and discover new ways to successfully prevent them. SHARP is funded by Rhineland-Palatinate's Ministry of Science, Education and Culture. The first SHARP PhD students began their studies in September 2021, and in November 2022 the Ministry officially awarded €1.56m in funding for the second phase of the SHARP project.

www.cha-mainz.de/SHARP

CRC 1551 "CELL FUNCTION DRIVEN BY POLYMER CONCEPTS"

Spokesperson: Edward Lemke

In November 2022, the DFG announced the funding of the CRC 1551 in Mainz with €9.5 million. This initiative will build an interdisciplinary think tank for polymer scientists and life scientists to further stimulate the incorporation of liquid-liquid phase separation and other polymer science concepts into biological research questions. The CRC 1551 will bring together 31 group leaders from IMB, Mainz University, its University Medical Center and the Max Planck Institute for Polymer Research (Mainz) in 16 interdisciplinary research projects on biological processes ranging from transcription and protein aggregation to the formation of higher-order complexes and organelles.

EVENTS

Scientific events organised by IMB in 2022 include:

14-16 March

INTERNATIONAL MEETING OF THE SPP 1935 & RTG 2355

"RNA: BEYOND ITS GENETIC CODE"

Scientific organisers: Albrecht Bindereif (University of Giessen), Sutapa Chakrabarti (Free University of Berlin), Utz Fischer (Julius Maximilian University of Würzburg), Niels Gehring (University of Cologne), Julian König (IMB), Katja Sträßer (Justus Liebig University Giessen)

28 June - 1 July

IMB CONFERENCE

"EPIGENETICS OF AGEING:
RESPONSES TO ADVERSITY ACROSS SCALES"

Scientific organisers: Joan Barau (IMB), Peter Baumann (IMB/Mainz University), René Ketting (IMB), Beat Lutz (University Medical Center of Mainz University), Meng Wang (Baylor College of Medicine, Houston, USA)

20-23 September

IMB/CRC 1361 CONFERENCE

"RESTORE, REORGANISE, REPURPOSE:
THE MANY FACES OF DNA REPAIR"

Scientific organisers: Cristina Cardoso (Technical University of Darmstadt), Thomas Hofmann (University Medical Center of Mainz University), Karl-Peter Hopfner (Ludwig Maximilian University of Munich), Andriy Khobta (University Medical Center of Mainz University), Brian Luke (IMB/Mainz University), Helle Ulrich (IMB)

16-17 November

GENEVO RTG 2526 SYMPOSIUM

Scientific organisers: Susanne Foitzik (Mainz University) and René Ketting (IMB)

AWARDS

M. FELICIA BASILICATA
Senior Research Associate, Keller Valsecchi group
High Potentials Grant of the University Medicine Mainz

DOROTHEE DORMANN
Funding for a collaborative project supported by the Generet Award for Rare Disease (awarded to Rosa Rademakers, Mayo Clinic, Florida, USA)

SABRINA GIOFRÈ
Postdoc, Lemke group
Humboldt Fellowship

CHRISTINE KOEHLER
PhD Student, Lemke group
Boehringer Ingelheim Foundation PhD Award

EDWARD LEMKE
Elected EMBO Member

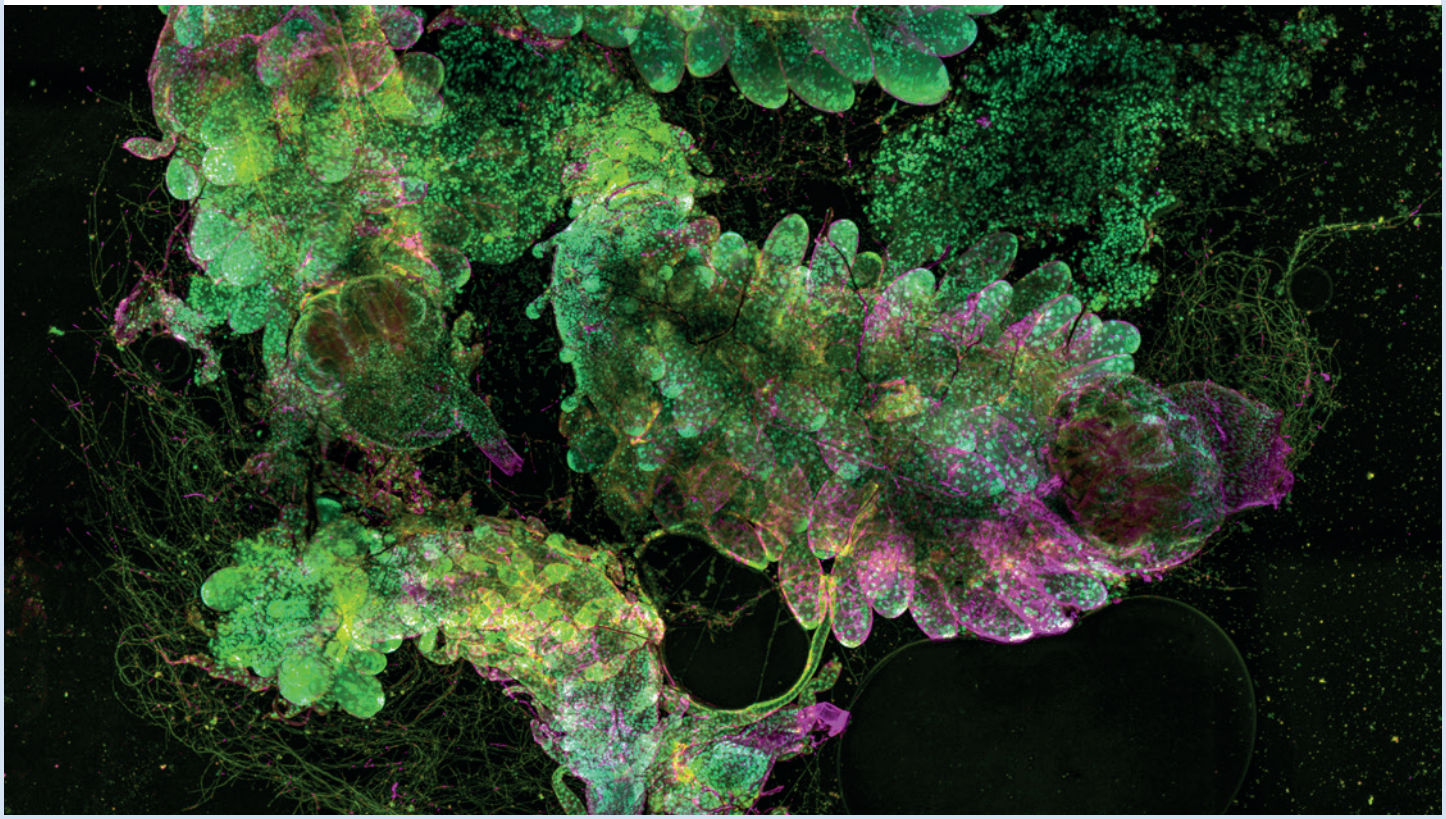
CHRISTOPHER REINKEMEIER
PhD Student, Lemke group
JGU (Chemistry) Doctoral Award

JOSÉ NAVEJA ROMERO
Postdoc, Luck group
Alexander von Humboldt Postdoc stipend

SARA VIEIRA-SILVA
Antoine Faes Triennial Award for Biomedical Research 2019-2021

MAX REUTER
Postdoc, Ulrich group
Funding for own position (DFG)

JAN SCHREIER
PhD Student, Ketting group
Elisabeth Gateff Prize (Gesellschaft für Genetik)



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BARAU

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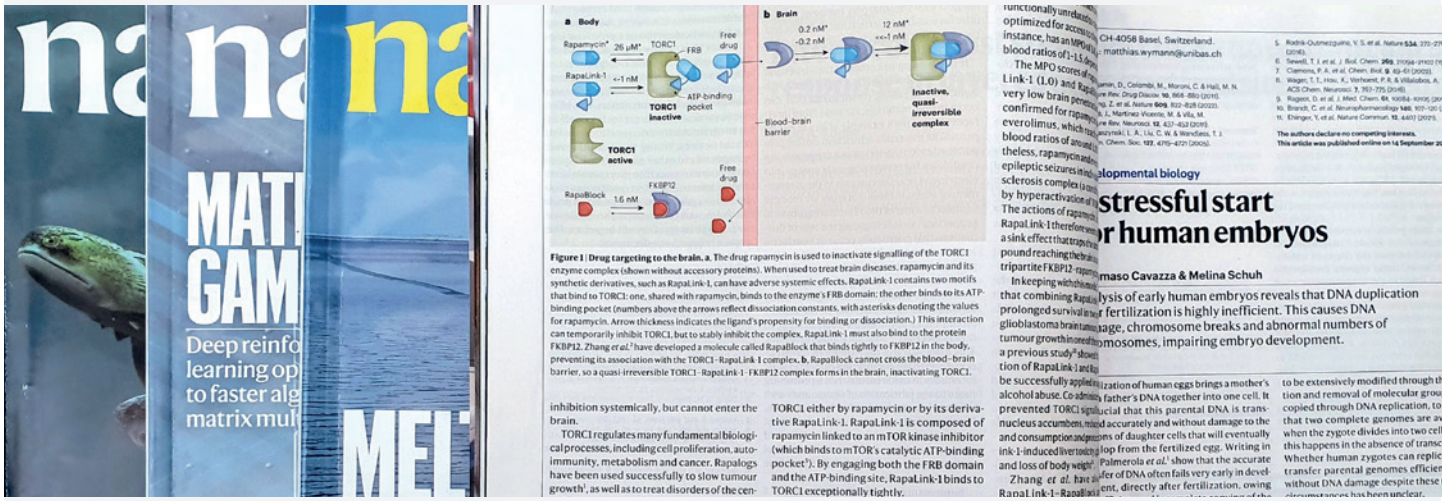

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RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located on the leafy campus of Johannes Gutenberg University, just west of the 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), the Leibniz Institute for Resilience 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.

In addition, there is an extensive industry R&D presence, with, for example, the headquarters of Boehringer Ingelheim, BioNTech, Translational Oncology (TRON) and the Merck Group in close vicinity.

WHERE WE ARE

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

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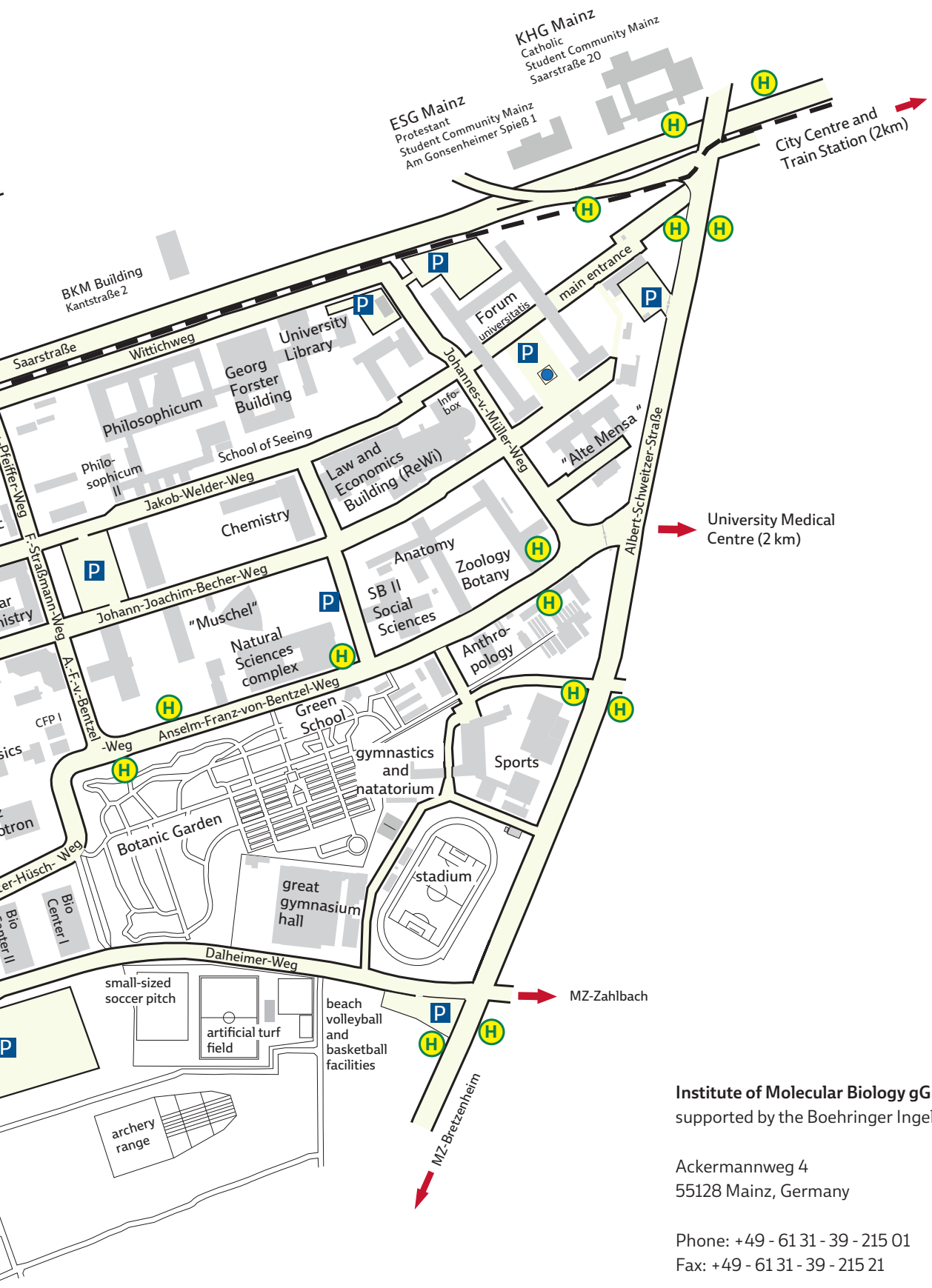

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Portraits of group leaders and Core Facility heads: Thomas Hartmann (p5, 8-14, 20-24, 28-34, 38-40, 44, 48, 62-67, 69, 74) and Anton Pfurtschneller (p16-18, 26, 36, 42, 46, 52-58, 68).
Pictures of IMB researchers and students: Thomas Hartmann (p 51, 65, 75-80, 82-83, 95).
(p4) Left: A burst of L1 activity (L1 ORF1p stained in green) in germ cells (GCNA stained in magenta) of mouse mutants with defective DNA methylation. Image credit: Joan Barau.
(p6) Mouse zygotes at the pronuclear stage just prior to becoming a two-cell stage embryo, stained with DAPI. Image credit: Viviana Vastolo (Niehrs Lab).
(p50) 16 µm cryo-section of a cerebral organoid derived from hiPSCs (HMGU1), with staining for neuron-specific class III beta-tubulin (TUJ1, green), ARID1A (red) and DNA (blue). Image credit: Marie Kube (Schick group).
(p61) Section of a *Xenopus* stage 38 embryo eye, stained with 5hmC antibody (red) and yoyo1 DNA stain (green), with the background subtracted. Image credit: Victoria Hatch (Niehrs group).
(p71) BPAE cells stained with MitoTracker Red, AlexaFluor488 phalloidin (green) and DAPI (blue). Image credit: Andreas Vonderheit (Microscopy Core Facility).
(p81) H&E staining of a cholangiocarcinoma in mouse liver.
(p87) Fluorescence image showing midgut caeca of the grain weevil *Sitphilus oryzae*, stained for nuclei (cyan), *Sodalis pierantonius* endosymbionts (yellow) and wheat germ agglutinin (magenta). Image credit: Petri Turunen (Microscopy Core Facility) & Tobias Engl (Institute of Organismic and Molecular Evolution, JGU).
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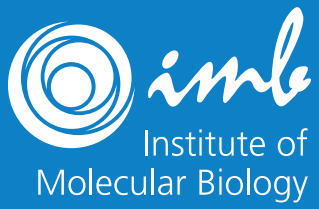
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