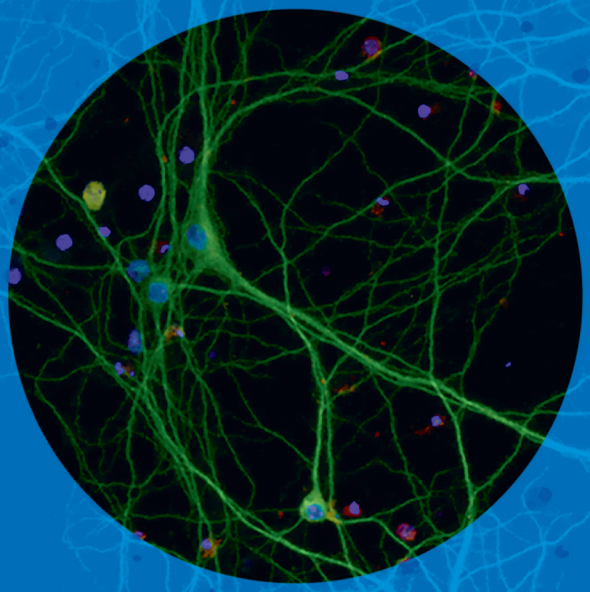
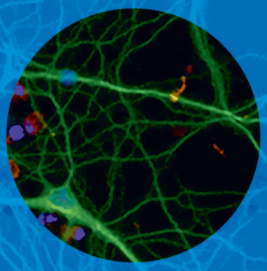
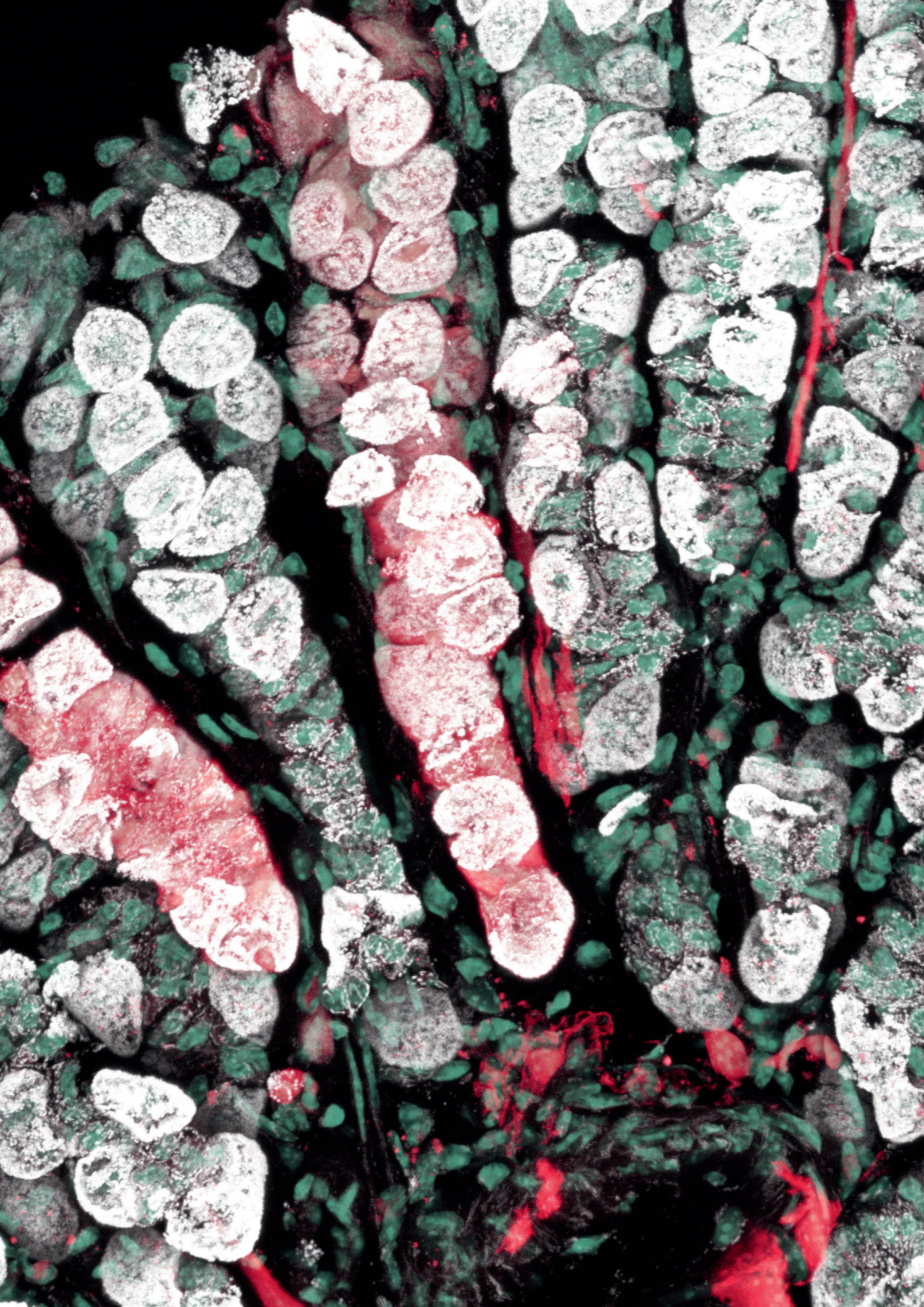
Institute of Molecular Biology



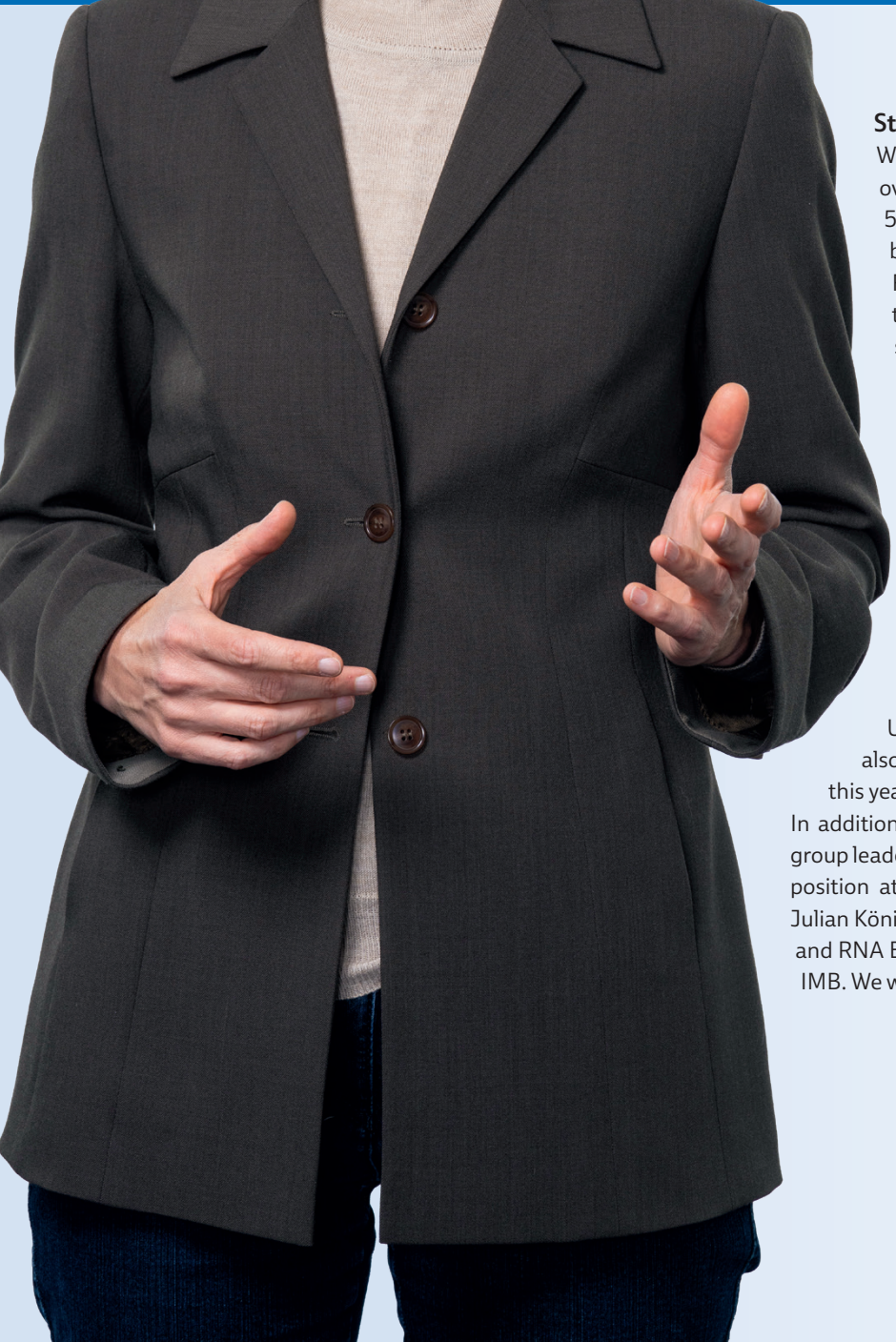


ANNUAL REPORT 2024



ANNUAL REPORT 2024

Welcome to IMB's 2024 Annual Report!



Staff changes

We pride ourselves on attracting top scientists from all over the world, with 308 staff and students hailing from 50 countries in 2024. Earlier this year, we were joined by two new group leaders: Roopesh Anand from the Francis Crick Institute, who studies the mechanisms that underlie homology-directed repair of double-strand breaks in DNA, and Katharina Papsdorf from Stanford University, whose research focuses on understanding how specific lipids can protect against the cellular changes underlying ageing and how they might be used to promote longevity. Lukas Stelzl, previously an Adjunct Group Leader, was promoted to Professor of Biomolecular Simulations at Mainz University and accordingly appointed as an Adjunct Director at IMB.

In 2024, our International PhD Programme (IPP) recruited 47 new PhD students, bringing the total to 203 students spread across groups at IMB, Mainz University and the University Medical Center. We are also pleased to have celebrated 32 successful defences this year.

In addition to these new starts, we bid farewell to two of our group leaders. Joan Barau will be taking up an Associate Director position at BioNTech Cell & Gene Therapies in Mainz, while Julian König has accepted the position of Chair for Biochemistry and RNA Biology at the University of Würzburg after 11 years at IMB. We wish them both the best of success in their new roles.

We are excited to share our researchers' achievements with you. 2024 was marked by high-impact publications, new grants and the establishment of new groups at IMB.

New publications

2024 was a respectable year for IMB, with our researchers publishing a total of 87 papers in journals such as *Nature Communications*, *Nature Cell Biology* and *Molecular Cell*. Notable mentions include the Niehrs lab's *Cell* paper describing their finding that 5-formylcytosine functions as an activating epigenetic mark in zygotic genome activation, and the Roukos lab's *Nature Biotechnology* paper in which they developed a high-throughput method called BreakTag to assess the location and structure of DNA double-strand breaks genome-wide. This method will be extremely useful for improving the precision and predictability of CRISPR/Cas9 genome editing tools. The Vieira-Silva group published a paper in *Gastroenterology*, describing how gut microbiota composition in patients with inflammatory bowel disease can be used to predict treatment outcomes.

Grants & awards

Collectively, IMB researchers received a total of €10.3 million in extramural funding in 2024. We would especially like to congratulate Stamatis Papathanasiou and Sandra Schick, who both got ERC Starting Grants. I was awarded an ERC Advanced Grant, while Ralf Dahm and Christof Niehrs acquired funding from the Ministry of Health and Science of Rhineland-Palatinate to establish the "Cohorts for Healthy Ageing" (CoAGE) doctoral programme, which seeks to investigate why many diseases occur more frequently in old age and how we can age more healthily.

We are also proud of the honours and awards won by our researchers this year. In particular, Claudia Keller Valsecchi was selected as an EMBO Young Investigator, while Edward Lemke became a Fellow of the Biophysical Society, and Siyao Wang was awarded an Excellence Award from the Federation of European Biochemistry Society (FEBS).

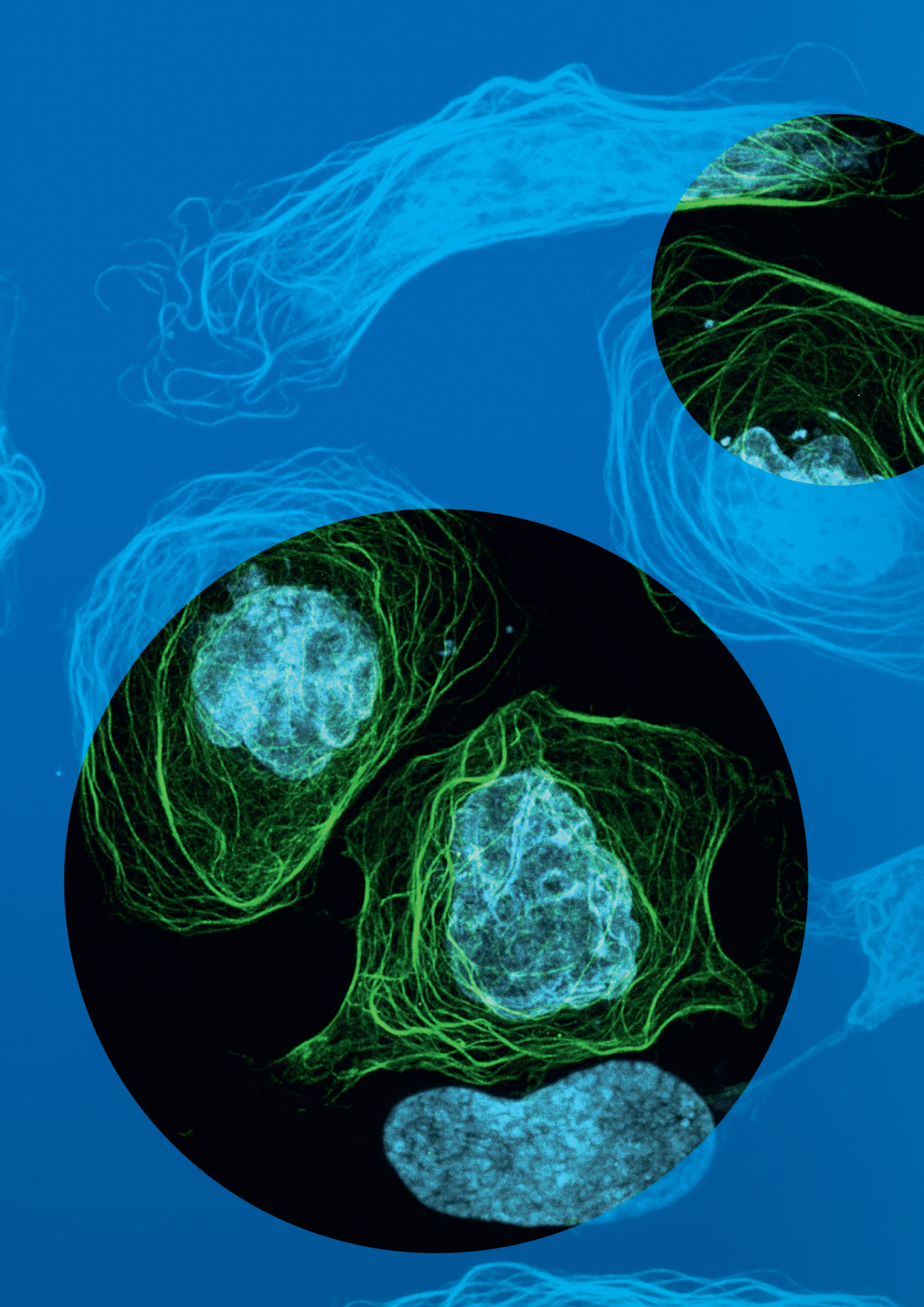
Events at IMB

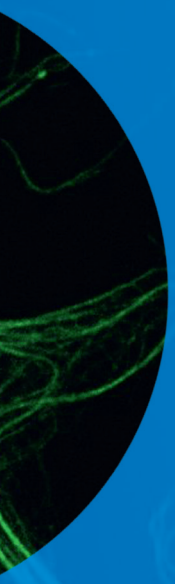
IMB continues to promote cooperation and exchange in ageing research through scientific events. In June, IMB researchers co-organised the Annual Meeting of the German Association for Aging Research, which was held at IMB. The event had over 120 attendees, including 29 speakers from Singapore, Denmark, Switzerland, the UK and all over Germany. This was followed by the Centre for Healthy Ageing (CHA) Workshop in November, which featured keynote speakers from Heidelberg and the US and brought together 80 scientists in Mainz working on diverse fields of ageing research for an intense two-day session of talks, posters and discussions.

IMB's International Summer School took place for the 12th time in August and September, with 10 undergraduate students from 9 countries coming to participate in a 6-week programme at IMB. This year, IMB also launched an Internship Programme and welcomed its first 17 undergraduate research interns. In addition, our postdocs in the IMB Postdoc Programme held their first retreat in November, where they networked, participated in career discussions and listened to talks on the world beyond academia.

As always, I would like to thank the Boehringer Ingelheim Foundation and the State of Rhineland-Palatinate for their continued support and generous funding, as well as our Scientific Advisory Board for providing excellent advice and feedback to guide IMB as we develop as a research institute. Thank you also to all the wonderful researchers at IMB, whose efforts have contributed so much to making IMB an amazing place for research and discovery.

Helle Ulrich
Executive Director





1 RESEARCH GROUPS

Roopesh **Anand**

"We study the mechanisms of DNA double-strand break repair pathways."

POSITIONS HELD

Since 2024	Group Leader, Institute of Molecular Biology (IMB), Mainz
2019 – 2024	Postdoc, The Francis Crick Institute, London
2016 – 2019	Postdoc, Institute for Research in Biomedicine (IRB), Bellinzona

EDUCATION

2016	PhD in Tumour Biology, University of Zurich
2011	MSc in Transfusion and Transplantation Sciences, University of Bristol
2009	BSc in Medical Laboratory Technology, Punjab Technical University

GROUP MEMBERS

Postdoc Lepakshi Ranjha

PhD Student Swaroopa Nakeeran

BSc Student Reem Ali

Technician Sanabel Chehab

Research Assistant Maxin Bakalo

OVERVIEW

The primary focus of my lab is to investigate the mechanisms of DNA double-strand break (DSB) repair pathways. DSBs are highly toxic and their incorrect repair can lead to genome instability, resulting in cancer. DSBs are predominantly repaired by non-homologous end joining (NHEJ) and homologous recombination (HR) repair pathways. While NHEJ is template-independent and error-prone, HR uses a homologous template to guide DSB repair and is therefore error-free. Additionally, alternative repair pathways in cells, such as single-strand annealing (SSA), microhomology-mediated end-joining (MMEJ), and break-induced replication (BIR), can act as "backup" repair pathways. These alternative pathways use varying degrees of homology, but unlike HR they are error-prone. In many cancers, cells rely heavily on these alternative pathways to survive increased DNA damage. We specifically focus on elucidating the molecular mechanisms of HR and other homology-directed repair (HDR) pathways. We aim to determine how key steps like homology search, DNA strand invasion, DNA synthesis and DNA annealing are carried out by individual HDR factors or an ensemble of factors. We employ biochemical analysis using purified proteins and DNA substrates, combined with single-molecule imaging techniques, to uncover crucial details of these HDR mechanisms.

RESEARCH HIGHLIGHTS

Role of DNA helicase HELQ in HR and alternative HDR pathways

Cancer cells experience constant DNA damage, including DSBs, due to their rapid proliferation. To deal with this increased DNA damage and sustain growth, cancer cells utilise both primary and "backup" repair pathways. HELQ, a DNA helicase, promotes HR and has been shown to be important for tumour suppression in mouse models. Additionally, HELQ loss is linked to premature ovarian insufficiency in women. Recently, we identified a novel role for HELQ in alternative repair pathways such as SSA, MMEJ and single-strand template repair (SSTR). Surprisingly, we also found that HELQ exhibits a robust ability to anneal complementary DNA, in

contrast to its known function of unwinding DNA. These discoveries have expanded the scope of HELQ's cellular functions, as well as raised important questions about its role in maintaining genomic stability and its connection to cancer development. For instance, HELQ overexpression correlates either positively or negatively with cancer cell growth, depending on the cancer type. It remains unclear how and which specific HELQ activities contribute to these distinct effects. We aim to mechanistically define HELQ's cellular functions by biochemically characterising its enzymatic activities, both alone and in conjunction with protein partners like RAD51, RPA and BCDX2 (RAD51B-RAD51C-RAD51D-XRCC2).

Elucidating the molecular mechanisms of BIR and alternative lengthening of telomeres (ALT)

BIR is an HDR pathway that primarily repairs one-ended DSBs, which frequently occur in cancer cells due to high replication stress. BIR's mutagenic nature stems from its conservative mode of replication, inefficient mismatch repair, the absence of an S-phase replisome, and the prolonged presence of ssDNA as a BIR intermediate product. BIR not only helps cancer cells cope with high replication stress but can also allow them to adapt and evolve by rapidly accumulating novel mutations, enabling survival under stressful conditions. BIR also drives pathological processes like microhomology-mediated BIR, chromothripsis, mitotic DNA synthesis (MiDAS) and ALT, all of which can contribute to cancer formation and progression. Notably, all cancer cells must overcome the "end-replication problem", which limits unrestricted cellular proliferation due to a critical shortening of telomeres. Therefore, to extend telomere lengths, most cancers reactivate a reverse transcriptase telomerase, while 10-15% of all cancers utilise BIR-mediated ALT. ALT-positive cancers such as glioblastoma and osteosarcoma can be aggressive and may also show resistance to therapy due to the rapid accumulation of novel mutations. Despite decades of research revealing critical aspects of BIR and ALT pathways, the molecular mechanisms of human BIR and ALT remain poorly understood. The heterogeneity of ALT+ cancers poses significant challenges for studying ALT mechanisms through cell-based approaches. We elucidate BIR and ALT mechanisms by reconstituting their entire repair pathways using an ensemble of purified proteins to circumvent the problems associated with cell-based studies.

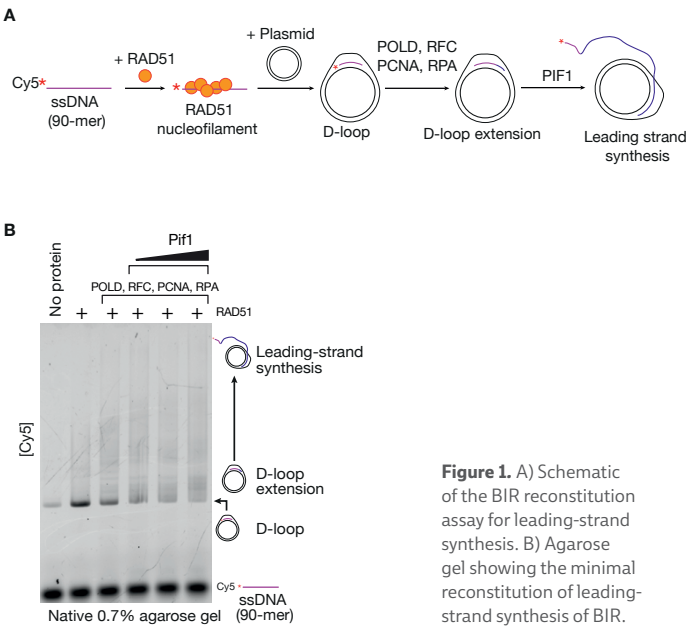


Figure 1. A) Schematic of the BIR reconstitution assay for leading-strand synthesis. B) Agarose gel showing the minimal reconstitution of leading-strand synthesis of BIR.

FUTURE DIRECTIONS

We are producing various BIR/ALT factors at high yields to reconstitute these repair pathways *in vitro*. While we can already reconstitute the leading-strand synthesis for BIR (Figure 1), we will generate resources to achieve lagging-strand synthesis in the future. We will also develop resources to reconstitute minimal ALT *in vitro*. To gain unprecedented insights, we will study these reactions at the single-molecule level using dual optical tweezers (C-Trap, Lumicks) and a TIRF-based setup (Nanoimager, ONI).

To uncover HELQ's cellular functions, we will create separation-of-function mutants of HELQ to distinguish the roles of its DNA unwinding and annealing activities. After identifying these roles, we will use cell-based studies to examine the effects of HELQ mutants on cell growth under normal and stressed conditions. Additionally, we have evidence that while HELQ's N-terminus is autoinhibitory, the C-terminus is critical for its overall enzymatic activities. We will investigate HELQ's self-regulation mechanisms and validate our findings using cell-based studies.

SELECTED PUBLICATIONS

Belan O, Greenhough L, Kuhlen L, Anand R, Kaczmarczyk A, Gruszka DT, Yardimci H, Zhang X, Rueda DS, West SC and Boulton SJ (2023) Visualization of direct and diffusion-assisted RAD51 nucleation by full-length human BRCA2 protein. *Mol Cell*, 83:2925-2940.e8

Fleury H, MacEachern MK, Stiefel CM, Anand R, Sempeck C, Nebenfuehr B, Maurer-Alcalá K, Ball K, Proctor B, Belan O, Taylor E, Ortega R, Dodd B, Weatherly L, Dansoko D, Leung JW, Boulton SJ and Arnoult N (2023) The APE2 nuclease is essential for DNA double-strand break repair by microhomology-mediated end joining. *Mol Cell*, 83:1429-1445.e8

Anand R*, Buechelmaier E*, Belan O, Newton M, Vancevska A, Kaczmarczyk A, Takaki T, Rueda DS, Powell SN and Boulton SJ (2022) HELQ is a dual-function DSB repair enzyme modulated by RPA and RAD51. *Nature*, 601:268-273

*indicates joint contribution

Joan **Barau**

"We decipher how transposons impact evolution, development & disease."

POSITIONS HELD

Since 2024 Associate Director, BioNTech Cell & Gene Therapies, Mainz

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 BSc in Biology, University of Campinas

GROUP MEMBERS

PhD Students Ishita Amar, Styliani Eirini Kanta, Jessica Leismann, Srinivasa Abishek Prakash, Anna Szczepinska

Lab Manager Violeta Morin

Student Assistant Carl Weile

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 1) how transposons are targeted for epigenetic silencing in mouse germ cells, 2) how transposon sequences and their epigenetic status impact their regulatory potential in mouse germ cells, and 3) novel regulators of the transposon 'life cycle' in pluripotent and differentiated stages of mammalian development.

RESEARCH HIGHLIGHTS

How transposons are specifically 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. Specificity is a key factor for the piRNA pathway: mistargeting can lead to potent and stable silencing of protein-coding genes. Using the chromatin profiling protocols we established in mouse embryonic germ cells, we have collaborated to show that active retrotransposons acquire an H3K9me3-H3K4me3 bivalent signature and that two major components involved in specifying piRNA pathway targets, SPIN1 and SPOCD1, co-localise to these signatures (Figure 1A). The combination of these unique chromatin signatures with their binding by SPIN1 and SPOCD1 bookmarks active retrotransposons as piRNA targets prior to piRNA production and piRNA-guided transcriptional silencing (Mirandela *et al*, 2024).

How the most dangerous retrotransposon becomes active in germ cells

TEs are increasingly in the spotlight as the main drivers of evolutionary innovation; however, they are also an immediate threat to germline integrity. The piRNA pathway and TEs are engaged in an antagonistic co-evolutionary cycle: The silencing machinery is constantly evolving to detect newly active TEs, while TEs are under constant pressure to escape and proliferate to avoid extinction. A defective piRNA pathway and the resulting loss of DNA methylation releases TEs from silencing, leading to meiotic failure, germ cell death and male sterility. While the biochemical systems responsible for the silencing of TEs are well studied, it remains unknown how TEs hijack the host activation machinery when silencing by DNA methylation is not in place.

We identified NRF1 as a potential transcriptional regulator of TEs using a proteomics approach designed to discover transcription factors binding to unmethylated TE promoters in germ cells. Using low-input chromatin profiling by CUT&Tag in sorted germ cells, we found increased NRF1 binding to unmethylated TE promoters, which suggested a direct correlation between TE expression patterns and NRF1 binding. We then genetically tested the impact of NRF1 on TE activity using elegant mouse conditional knockouts of *Nrf1* either combined with the hypomethylated *Dnmt3C* knockout background or in a wild-type background. Our *in vivo* experiments showed that unmethylated TEs had reduced transcriptional output in the absence of NRF1. More strikingly, the germline conditional *Nrf1* knockout completely rescued the patterns of reactivation of the most mutagenic TE in mice - Intracisternal A-particle retrotransposons (IAPs; Figure 1B).

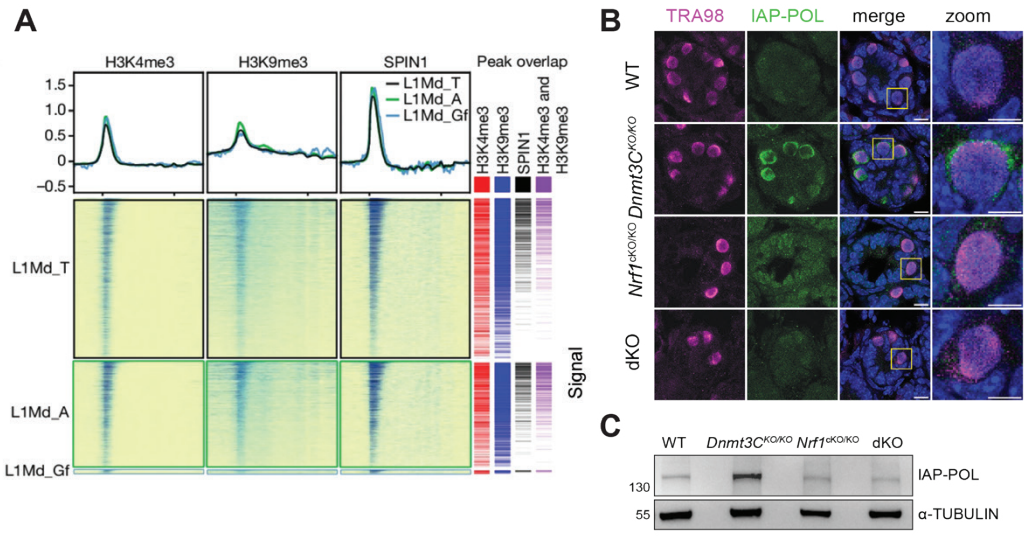


Figure 1. A) CUT&Tag data for H3K4me3, H3K9me3 and SPIN1 from E14.5 fetal germ cells. Metaplot and heatmaps of signal over elements of different transposon copies in the L1Md_T, L1Md_A and L1Md_Gf families. Columns adjacent to the heatmaps show statistically significant peaks called for SPIN1 and the indicated histone modifications. B) Representative immunostaining of wild-type, *Dnmt3C* KO/KO, *Nrf1* cKO/KO and double (dKO) *Dnmt3C* KO/KO-*Nrf1* cKO/KO germ cells, showing rescue of the IAP-POL activity in germ cells (TRA98-positive) of dKO (Scale bars, 10µm in full and 5µm in cropped images). C) Western blot analysis using protein extracts from wild-type, *Dnmt3C* KO/KO, *Nrf1* cKO/KO and dKO testes at P5, showing rescue of IAP-POL protein expression in dKO.

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.

We have now two manuscripts in revision and expect the publication of our discoveries of NRF1 as an IAP transcriptional regulator and DNMT3C as a piRNA-guided DNA methyltransferase within the next year.

SELECTED PUBLICATIONS

Dias Mirandela M, Zoch A*, Leismann J*, Webb S*, Berrens RV, Valsakumar D, Kabayama Y, Auchynnikava T, Schito M, Chowdhury T, MacLeod D, Xiang X, Zou J, Rappsilber J, Allshire RC, Voigt P, Cook AG, Barau J and O'Carroll D (2024) Two-factor authentication underpins the precision of the piRNA pathway. *Nature*, 634:979-985

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

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

*indicates joint contribution

Peter **Baumann**

"We study age-related decline in the context of evolution & disease."

POSITIONS HELD

Since 2023	Founding Director, Institute for Quantitative and Computational Biosciences (IQCB)
Since 2021	Director, Centre for Healthy Ageing (CHA), Mainz
Since 2018	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Since 2017	Alexander von Humboldt Professor, Johannes Gutenberg University Mainz (JGU)
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

Postdocs Lars Erichsen, Zoe Gill, Lili Pan, Valentine Patterson

PhD Students Wafa Abuhashem, Nadine Bobon, Nathaniel Deimler, David Ho, Yu-Chia Ku, Abinaya Manivannan, Alex Orioli, Jayaprakash Srinivasan

Technicians Joshua Holzapfel, Elisa Thomas

Animal Caretaker Martin Fahr

Personal Assistant Thomas Faust

OVERVIEW

Telomeres are one of the primary hallmarks of ageing as their maintenance and protection are critical for genome stability and tissue renewal. Genetic defects in the machinery that replenishes telomeric DNA are causative for a group of premature ageing diseases referred to as telomere biology disorders (TBD). At the cellular level, TBDs are characterised by premature loss of replicative capability, stem cell exhaustion, and accumulation of senescent cells in tissues. Phenotypically, TBDs are characterised by pleiotropic symptoms associated with the normal ageing process, with the age at diagnosis varying from early childhood to advanced adulthood depending in part on the severity of the mutation. Studying the mutations that underlie TBDs thus provides valuable insights into the molecular and cellular changes that lead to functional decline during ageing. Our group studies two key aspects of telomere maintenance: 1) the biogenesis and regulation of the enzyme telomerase, and 2) the mechanistic basis of chromosome end protection. We are guided by the conviction that understanding telomerase biogenesis will help us identify compounds that modulate telomere length. Telomerase inhibitors will have therapeutic uses to limit tumour cell proliferation, and compounds that stimulate telomerase can boost the proliferation of desired cell populations, such as bone marrow stem cells. The latter could not only help patients suffering from TBDs, but may also counteract manifestations of the normal ageing process. Careful targeting and regulation are critical to balance the regenerative effects with the risk of carcinogenesis. To reach these goals, we employ computational, molecular and cell biological approaches and have built a network of collaborators to examine telomere dynamics in the contexts of immune senescence, frailty and ageing.

RESEARCH HIGHLIGHTS

Telomerase biogenesis and regulation

Progressive telomere shortening is intrinsically linked to cell division via the "end replication problem" and critically short telomeres trigger cellular senescence, thus preventing further proliferation and telomere shortening. Mechanisms that replenish telomeric sequences are a double-edged sword: on one hand, they extend the replicative lifespan of a cell population and are vital for tissue renewal, but on the other hand, replenishing telomeres permits the continued proliferation of cells (including malignant cells). Consequently, telomere addition is tightly controlled in many multicellular organisms including humans. Important layers of control affect the biogenesis of telomerase from transcription and RNA processing to complex assembly and recruitment to telomeres. Our group studies these processes in fission yeast and human cells. Based on earlier work in fission yeast, we recently identified important roles for LARP3, LARP7 and MePCE during the early stages of telomerase assembly.

In collaboration with the Human Genetics Department at the University Medical Center, we are studying mutations responsible for telomere biology disorders, including dyskeratosis congenita and idiopathic pulmonary fibrosis. Combining clinical with cell biological, biochemical and bioinformatic analysis, we recently characterised a new class of telomerase mutations that affects telomere maintenance in two ways: 1) by diminishing the activity and processivity of the enzyme, and 2) by incorporation of non-canonical repeat sequences that subsequently affect the activity of wildtype telomerase even in descendants that have not inherited the causative mutation.

Although telomere length is frequently used as a biomarker in the context of ageing and stress and to predict various disease outcomes, reliably and accurately measuring telomere length has been mired by technical challenges. Classical approaches require fresh samples or large amounts of genomic DNA, and alternative approaches have suffered from issues of reproducibility and reagent availability. This has hampered the acquisition of reproducible, longitudinal datasets on telomere length dynamics. Third-generation sequencing technologies now promise to reshape the field by providing a low-cost, accurate and reliable method of determining telomere length (Figure 1). Over the past year, our group has invested considerable resources in developing telomere enrichment and sequencing protocols, as well as base calling and analysis pipelines.

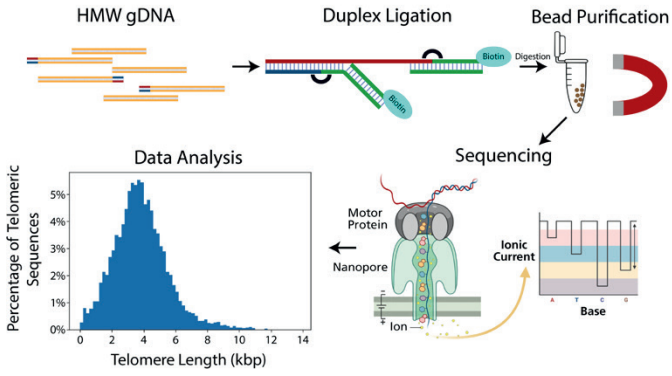


Figure 1. Telomeres are enriched from high molecular weight (HMW) genomic DNA by ligation of biotinylated capture probes and streptavidin bead purification, followed by sequencing on Oxford Nanopore Technology flow cells. Bioinformatic analysis yields information on mean telomere lengths as well as chromosome arm-specific telomere length at single nucleotide resolution.

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? Is increasing telomerase levels a genuine path toward treating 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 inquiry are projects to understand 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.

SELECTED PUBLICATIONS

Ho DV*, Tormey D*, Odell A, Newton AA, Schnittker RR, Baumann DP, Neaves WB, Schroeder MR, Sigauke RF, Barley AJ and Baumann P (2024) Post-meiotic mechanism of facultative parthenogenesis in gonochoristic whiptail lizard species. *eLife*, 13:e97035

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

*indicates joint contribution

Petra **Beli**

“We use quantitative proteomics to study cellular stress responses.”

POSITIONS HELD

Since 2020 Adjunct Director, Institute of Molecular Biology (IMB), Mainz

Professor of Quantitative Proteomics, Johannes Gutenberg University Mainz (JGU)

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

Postdocs Francesca Conte, Ivan Mikicic, Aldwin Suryo Rahmanto

PhD Students Georges Blattner, Christian Blum, Caio Almeida Batista De Oliveira, Lukas Graf, Rebecca Hobrecht*, Ekaterina Isaakova, Eric Schmitt, Nadia da Silva Fernandes Lucas*

Master Student Magdalena Schachtl-Riess

Lab Manager Katharina Mayr

Personal Assistant Ute Sideris

*indicates joint PhD students

OVERVIEW

Human cells are exposed to stress induced by pollutants from the environment, as well as compounds generated during normal metabolism, such as reactive aldehydes. Genome maintenance is essential for the fidelity of gene expression, as well as the prevention of cancer and premature ageing phenotypes. Complementary to genome maintenance mechanisms, RNA and protein quality control pathways deal with stress-induced RNA and protein damage. The research in our group focuses on identifying and characterising proteins and signalling pathways that counteract genomic instability and the pathological effects of RNA and protein damage. We develop and employ quantitative mass spectrometry-based approaches to obtain systematic insights into the proteins and signalling pathways involved in these processes.

RESEARCH HIGHLIGHTS

A complex network of proteins and signalling pathways ensures genome and proteome maintenance in response to external stressors and by-products of cellular metabolism, as well as DNA replication and transcription. Reactive aldehydes are produced by normal cellular metabolism or after alcohol consumption, and they accumulate in human tissues if aldehyde clearance mechanisms are impaired. Their toxicity has been attributed to the damage they cause to genomic DNA and the subsequent inhibition of transcription and replication. However, whether interference with other cellular processes contributes to aldehyde toxicity has not been investigated. We demonstrated that formaldehyde induces a specific type of RNA damage – RNA-protein crosslinks (RPCs) that stall the ribosome and inhibit translation in human cells. RPCs in the messenger RNA (mRNA) are recognised by translating ribosomes and marked by heterotypic K6/K48-linked ubiquitylation catalysed by the RING-in-between-RING (RBR) E3 ligase RNF14. Ubiquitylation of RPCs results in the recruitment of the ubiquitin- and ATP-dependent unfoldase VCP (also known as p97), which promotes RPC resolution. Our findings uncover an evolutionarily conserved formaldehyde-induced stress response pathway that protects cells

against RPC accumulation in the cytoplasm, and they suggest that RPCs contribute to the cellular and tissue toxicity of reactive aldehydes. Following up on our findings that reactive aldehydes induce RNA damage in the form of RPCs and ribotoxic stress, we performed a comparative phosphoproteomics screen to identify changes in cellular signalling in response to different types of DNA and RNA damage. This enabled us to distinguish stress-induced DNA or RNA damage signalling and identify potential new factors involved in the RNA damage response (RDR). We found that RNA damage-induced hyperphosphorylation occurs in intrinsically disordered regions and is enriched on proteins that localise to membraneless condensates.

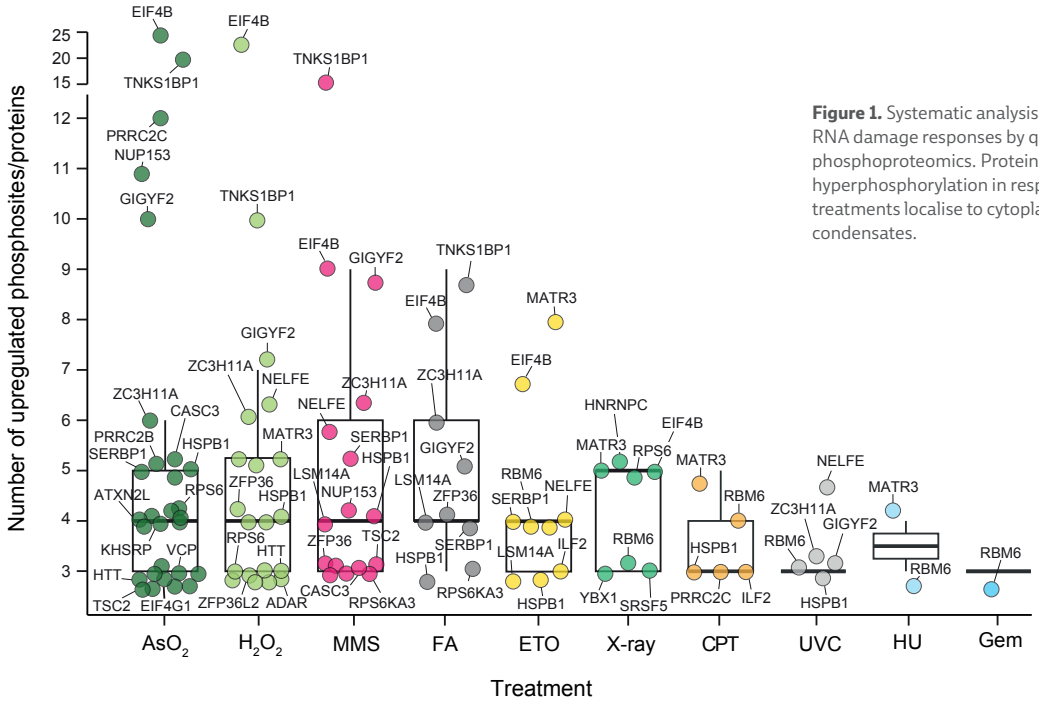


Figure 1. Systematic analysis of DNA and RNA damage responses by quantitative phosphoproteomics. Proteins showing significant hyperphosphorylation in response to different treatments localise to cytoplasmic membraneless condensates.

FUTURE DIRECTIONS

We are interested in understanding transcription- and translation-coupled quality control mechanisms that maintain the fidelity of gene expression and protein synthesis. We will use quantitative mass spectrometry-based proteomics to investigate cellular responses to stress that cause damage to DNA and RNA. Our studies will focus on components of the ubiquitin system that regulate cellular responses to stress by catalysing the modification of substrate proteins with different types of ubiquitin chains. We will characterise ubiquitin-based mechanisms and ubiquitin E3 ligases that protect human cells from the deleterious effects of transcriptional and translational stress.

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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

*indicates joint contribution

Dorothee **Dormann**

"We study molecular self-assembly processes to understand protein aggregation diseases."

POSITIONS HELD

Since 2021	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Professor of Molecular Cell Biology, Johannes Gutenberg University Mainz (JGU)
2014 – 2021	Emmy Noether Group Leader, Biomedical Center, Ludwig Maximilian University (LMU), Munich
2007 – 2014	Postdoctoral Fellow, Adolf-Butenandt Institute, Ludwig Maximilian University (LMU), Munich

EDUCATION

2007	PhD, Rockefeller University, New York
2002	Diploma in Biochemistry, Eberhard Karl University of Tübingen

GROUP MEMBERS

Staff Scientists Nicole Belo Do Couto, Bernhard Lieb, Irene Yiallouros

Postdocs Saskia Hutten, Federico Uliana

PhD Students Simone Mosna, Emre Pekbilir, Francesca Simonetti, Yongwon Suk, Sára Varga, Fatmanur Tiryaki Yildiz, Yelyzaveta Zadorozhna

Lab Manager Nora Knabe

Technician Thomas Schubert

Student Assistant Nele Kuhr

Group Administrator Andrea Rautenberg

OVERVIEW

We seek to unravel the molecular basis of age-associated neurodegenerative diseases, in particular ALS (amyotrophic lateral sclerosis), FTD (frontotemporal dementia) and Alzheimer's disease. Existing therapies treat only the symptoms of disease and there are no therapies to slow down or stop disease progression. Our main objective is to obtain a molecular understanding of the mechanisms that drive these devastating disorders. We seek to unravel how RNA-binding proteins (RBPs), in particular TDP-43 and FUS, become mislocalised and aggregated, and how their dysregulation causes a decline in cellular function and eventually neurodegeneration. We previously showed that RBP mislocalisation and aggregation 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 disease-linked RBPs are regulated, how they are misregulated in disease and how cellular proteostasis mechanisms prevent this. We are particularly interested in understanding the self-assembly behaviour of RBPs into different types of clusters and condensates, and how they relate to physiological function and disease. By understanding the molecular basis of these processes and learning how to tune them, we hope to inspire 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 self-assemble, which leads to their phase separation and partitioning into cellular condensates such as stress granules. Subsequent liquid-to-solid state transition is a molecular ageing process believed to underlie RBP aggregate formation, however such aberrant phase transitions are normally suppressed by cellular proteostasis mechanisms. We uncovered two important proteostasis mechanisms: suppression of RBP phase transitions by nuclear import receptors, and PTMs. Using *in vitro* reconstitution and cellular

experiments, we showed that nuclear import receptors (importins) suppress phase separation and stress granule recruitment of FUS, TDP-43 and toxic repeat proteins (poly-GR and -PR) that arise in the most common inherited form of ALS and FTD. This suggests that elevating importin levels or enhancing the binding of importins to aggregation-prone proteins could be used to treat protein aggregation disorders.

A second key regulator of RBP phase transitions we uncovered is disease-associated PTMs. PTMs frequently occur in IDRs and influence their self-assembly and interactions with other proteins or nucleic acids. Abnormal PTMs often arise in neurodegenerative diseases; for example, we found that FUS arginine methylation is reduced in FTD patients. We previously found that FUS hypomethylation promotes phase separation and stress granule accumulation, suggesting that loss of this PTM may promote FUS aggregation in disease. More recently, we found that the abnormal repeat protein poly-GR, which arises in *C9orf72*-linked ALS/FTD, can cause hypomethylation of RBPs: poly-GR directly binds to protein arginine methyltransferases (e.g. PRMT1) and inhibits PRMT1 activity towards several disease-linked RBPs by acting as a substrate sink.

Another disease-associated PTM is C-terminal hyperphosphorylation of TDP-43 in ALS and FTD. We found that C-terminal phosphomimetic substitutions in TDP-43 reduce phase separation and aggregation, render TDP-43 condensates more dynamic and liquid-like, and suppress TDP-43's recruitment into cellular condensates. TDP-43 phosphorylation may therefore be a protective mechanism for preventing its aggregation and a physiological mechanism for regulating its condensation.

Additionally, we found that the Tau protein, which forms abnormal fibrils in Alzheimer's disease (AD) and FTD, directly influences the condensation and aggregation of TDP-43 *in vitro*. Vice versa, TDP-43 promotes Tau condensation but inhibits Tau fibril formation. Using cellular seeding experiments with brain-derived aggregates from AD and FTD patients, we showed that TDP-43 and Tau can influence each other's seed formation in these diseases. This highlights the important pathological role that TDP-43 plays in AD and sheds the first mechanistic insights into the TDP-43/Tau co-pathology seen in up to 60% of AD patients.

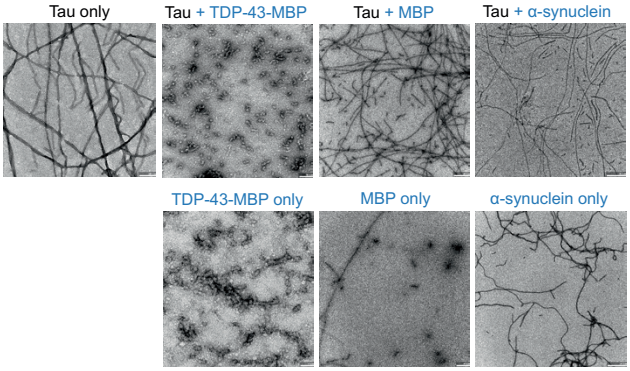

Figure 1. TDP-43 inhibits Tau fibrillization. Fibril formed from recombinant full-length Tau (50 µM) over 5 days in PBS in the presence or absence of equimolar amounts of TDP-43-MBP (or MBP or α-synuclein as control) were imaged by negative staining and transmission electron microscopy. Scale bars: 0.5 µm, except for samples where TDP-43-MBP was present, where a higher magnification was used to show the small 50 nm assemblies formed under these conditions (scale bar: 0.1 µm).

FUTURE DIRECTIONS

As molecular self-assembly into clusters or condensates is an important pathway towards RBP aggregation, we want to gain a comprehensive understanding of its drivers and regulators and find out its relevance for RBP function. Specifically, we plan to systematically decipher the intrinsic sequence features that drive the self-assembly and aggregation of TDP-43, and identify and study new regulators/modifiers of these processes. One focus will be on disease-linked TDP-43 phosphorylation and understanding when and where it is elicited in cells, whether it can dissolve TDP-43 aggregates and how it alters TDP-43's interactome and physiological functions. In addition, we will study how TDP-43 and FUS self-assembly into nanosized clusters or micron-sized condensates governs their interactions with other proteins and how this influences their functions in gene regulation, e.g. alternative splicing, transcription or translation. Finally, we will study the molecular mechanisms of how disease-linked RBPs regulate R-loops and DNA damage repair and how aberrant RBP condensates are recognised by the cellular degradation machinery.

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*indicates joint contribution, #indicates joint correspondence

Claudia **Keller Valsecchi**

“Gene dosage balance is vital for faithful development.”

POSITIONS HELD

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

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

2012 – 2013 Postdoc, Friedrich Miescher Institute (FMI), Basel

EDUCATION

2012 PhD in Biochemistry, Friedrich Miescher Institute (FMI), Basel

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

2007 BSc in Molecular Biology, Biozentrum, University of Basel

GROUP MEMBERS

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Student Assistants Joseph Andrew, Iona Bergerhoff, Anna Einsiedel, Annika Maria Fox

*indicates joint PhD students

OVERVIEW

Sexual reproduction facilitates the introduction of genetic diversity within a population. The diploid genetic state serves as a safeguarding mechanism by ensuring development when mutations occur in heterozygosity. Nevertheless, recent large-scale genome sequencing initiatives have uncovered an unexpectedly high number of human genes that exhibit intolerance to heterozygous loss-of-function mutations. Similarly, aneuploidies, characterised by the gain or loss of entire chromosomes, are a prominent cause of miscarriages and pregnancy failures. This suggests that maintaining a precisely two-fold gene dosage is of fundamental importance for the normal progression of organismal development.

Our approach is to understand these pathogenic deviations in gene dosage within the context of natural exceptions to the diploid genetic state. Notably, differentiated sex chromosomes, despite introducing heterozygosity for hundreds of genes, do not confer detrimental effects. This phenomenon can be attributed to dosage compensation (DC), a regulatory mechanism that corrects imbalances in X-chromosomal gene expression between males and females. We study this intriguing paradox surrounding natural gene dosage alterations and their potentially deleterious consequences. We investigate how cells effectively manage the interplay between advantageous elements, such as the evolution of sex chromosomes and novel genes, and adverse effects like developmental delays and malignancies.

RESEARCH HIGHLIGHTS

Dosage regulation in sex chromosomes

Sexual dimorphism is a prominent feature in the biology of various species, including the *Anopheles* mosquito, where only females require a blood meal for egg production. In comparative studies between *A. gambiae* and *D. melanogaster*, two related dipterans with similar X chromosomal gene content, we unexpectedly found entirely different molecular mechanisms for DC. To identify the new mosquito pathway, we generated a sex-specific transcriptome atlas, which revealed that DC is progressively established

in embryogenesis. This dataset also uncovered *SOA*, an uncharacterised gene that we found to be sex-specifically spliced. SOA is a DNA-binding protein that binds X-chromosomal promoters. Expressing it is sufficient to induce global X chromosome upregulation. In collaboration with Eric Marois (University of Strasbourg), we generated SOA gain- and loss-of-function mutants, which display perturbed DC. Surprisingly, this is compatible with viability but causes a developmental delay, showing that DC is non-essential in mosquitos. Based on this exciting discovery, we now aim to understand X-to-autosome specificity, focusing on DNA elements that co-evolved with sex chromosome differentiation. We have established heterologous expression systems to analyse the SOA binding pattern in non-mosquito genomes, complemented by characterisations of SOA's DNA binding domain by biophysical methods. Secondly, to explore SOA's downstream regulatory actions, we identified its interaction partners by mass spectrometry and found that it binds to splicing factors and R-loop regulators. This research angle provides key insights into how genes are collectively recognised and regulated. It also highlights the importance of gene-dosage balance and may shed light on why copy number alterations have such detrimental consequences during human development but are apparently common in, e.g. the adult nervous system or in other species.

Plasticity of gene regulation in female-heterogametic species

Spatiotemporal environmental fluctuations, like those driven by climate change, pose challenges for species. Aquatic ectotherms such as crustaceans often exhibit environmentally influenced developmental plasticity and sex determination, with largely unknown molecular mechanisms. Our recent focus has been on *A. franciscana*, a crustacean with a ZW sex determination system and two alternative developmental pathways based on environmental conditions. We established *A. franciscana* rearing in our group to generate transcriptome data for both pathways and implemented ATAC-seq and CUT&Tag for sex-biased gene expression analysis. We found that upregulation of the female Z chromosome is mediated by H4K16 acetylation, and thus occurs in a similar fashion to *Drosophila*. In addition, *A. franciscana* males (ZZ) have longer lifespans than females (ZW). We hypothesise that this relates to a change in histone acetylation, Z chromosome regulation and transposon-triggered genomic instability. This research angle provides novel insights into environmental stressors, sex determination and phenotypic plasticity.

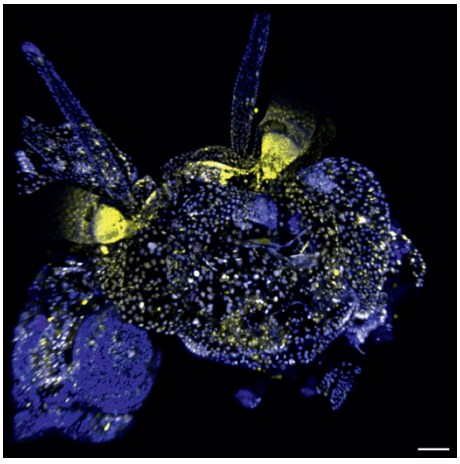

Figure 1. Immunofluorescence staining of a female *A. franciscana* head. Histone H4K16 acetylation is stained in yellow and nuclei are stained with DAPI (blue). H4K16 acetylation marks the Z chromosome for dosage compensation in this crustacean species and thus balances gene expression between females (one copy of Z-linked genes) and males (two copies of Z-linked genes). Scale bar: 100 µm.

FUTURE DIRECTIONS

Our goal is to further characterise DC mediated by SOA. These findings could ultimately inform novel strategies for fighting infectious diseases such as malaria by vector control. In addition to our work with mosquitoes, we also investigate DC in various other non-model organisms. Our research focuses on several aspects, including tissue-specific differences, regulatory dynamics throughout an organism's lifespan and adaptability in response to environmental shifts. We aim to elucidate how H4K16ac DC is controlled in *A. franciscana* – by similar writer/eraser complexes to *Drosophila* or different ones? In parallel, we develop tools to comprehensively identify dosage-sensitive genes and cellular responses in mammals. We will also expand our work on the mammalian X chromosome and study the mechanisms of re-activation during development, as well as age-related chromosomal mosaicism during ageing.

SELECTED PUBLICATIONS

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Kalita AI, Marois E*, Kozielska M, Weissing FJ, Jaouen E, Möckel MM, Rühle F, Butter F, Basilicata MF* and Keller Valsecchi CI* (2023) The sex-specific factor SOA controls dosage compensation in *Anopheles* mosquitos. *Nature*, 623:175–182

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

*indicates joint contribution, #indicates joint correspondence

René **Ketting**

"We study the effect of non-coding RNAs on germ cells & sex determination."

POSITIONS HELD

Since 2012	Scientific Director, Institute of Molecular Biology (IMB), Mainz
	Professor, Johannes Gutenberg University Mainz (JGU)
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

Senior Research Associate Nadine Wittkopp

Postdocs Walter Bronkhorst, Diego Páez Moscoso, Nadezda Podvalnya

PhD Students Fiona Carey, Joana Sofia Costa Pereirinha, Ida Josefine Isolehto, Joao Marques, Joanna Michowicz, Lizaveta Pshanichnaya, Eva Richard, Ann-Sophie Seistrup, Shamitha Shamitha

Lab Manager Yasmin El Sherif

Technicians Svena Hellmann, Shéraz Sadouki

Student Assistants Adrian Hepp, Teo Llazo, René-Maurice Pfeifer

Animal Caretakers Daniela Albore, Stefanie Schlegel, Cedric Schmitt

Personal Assistant Jutta Karn

OVERVIEW

One 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, various 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 pathways have a major role in maintaining genome integrity by controlling transposable element activity. In addition, we also study miRNAs in relation to germ cells. We use zebrafish and *C. elegans* as model systems to understand the molecular mechanisms governing these pathways and how they contribute to 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

In 2024, we made good progress on a number of fronts. In one project that I would like to mention here, we have been working on a specific type of germ granule that we previously identified in the sperm of *Caenorhabditis elegans*, the PEI (paternal epigenetic inheritance) granule. This structure is needed for the inheritance of small RNA-mediated gene regulation via the father, and it can serve as an important paradigm for how biomolecular condensates, or perhaps more appropriately named granules, can form and recruit specific proteins. For the PEI granules, we have been able to dissect two distinct domains in the PEI granule-forming proteins PEI-1 and PEI-2. First, using biochemical approaches, we could identify PEI-1:PEI-2 polymerising activities that are harboured

by the BTB and BACK domains present in both these proteins. Second, the intrinsically disordered region (IDR) of PEI-1 appears to function as an interaction site for the protein WAGO-3, which we previously found to be present in PEI granules. This distribution of tasks is the opposite of what is generally thought for granules; the IDRs are typically thought to drive granule formation and the folded domains may recruit specific factors. Dissecting this further for the PEI granule will be of great value for the general view of how protein/RNA concentrates may function in general. Other studies concerning small RNA pathways in this nematode deal with the regulation of Argonaute protein activity, transcription termination processes, and connections between small RNA pathways and other RNP homeostasis mechanisms.

In our second main model system, the zebrafish, we have gained interesting insights into how a microRNA affects sex determination. We have been able to firmly establish that miR-214 has a strong effect on sex determination, but also that the long non-coding RNA (lncRNA) in which it is embedded affects this process in a manner independent of miR-214. Our results are consistent with a role for miR-214 in controlling a gene named *gsdf*, combined with additional sex-determining activities from the lncRNA. This creates a complex sex-determination network rather than a simple linear genetic pathway. Further projects in this model system address how the germ cells are formed and function. More specifically, two lines of research are being pursued here. First, we address how a structure named germ plasm is formed. Germ plasm is a form of germ granules (see above) present in the early embryo and contains many RNAs and proteins needed to make the germ cells. Second, we study a de-ubiquitinating (DUB) enzyme that appears to play a role in oocyte growth and maturation. Here, the results suggest that the DUB enzyme is needed to stabilise RNA-protein complexes that are critical for oocyte development and germ cell formation in the embryo.

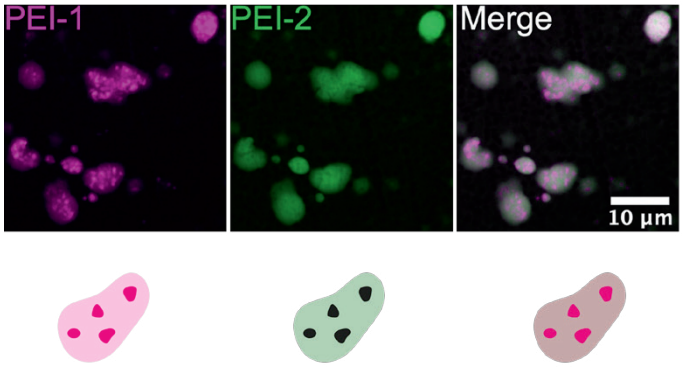

Figure 1. When mixed together, purified PEI-1 and PEI-2 proteins form PEI-granule-like structures *in vitro*, which contain sub-structures (indicated in the schematics below the images). The *in vivo* relevance of these sub-structures is not yet clear.

FUTURE DIRECTIONS

Future work will continue to mechanistically unravel the molecular pathways that are steered by small RNA molecules. One aim will be to further focus on the role of biomolecular condensates in small RNA-mediated gene silencing. Such condensates are well known to be required, but their exact functions are unclear. These studies also aim to provide a more generally applicable framework for the roles of condensates in cell biology. We will also continue to delineate how small RNAs are processed and loaded into Argonaute proteins. Finally, we are zooming into the interfaces between small RNA biology and other aspects of gene regulation. Using genetic screens and immunoprecipitation approaches, we are identifying novel factors and then implementing these into our current models of gene regulatory mechanisms.

SELECTED PUBLICATIONS

Bronkhorst AW#, Lee CY, Möckel MM, Ruegenberg S, de Jesus Domingues AM, Sadouki S, Piccinno R, Sumiyoshi T, Siomi MC, Stelzl L, Luck K# and Ketting RF# (2023) An extended Tudor domain within Vreteno interconnects Gtsf1L and Ago3 for piRNA biogenesis in *Bombyx mori*. *EMBO J*, 42:e114072

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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

*indicates joint contribution, #indicates joint correspondence

Anton **Khmelinskii**

"We use proteomic approaches to understand how cells keep their proteins healthy."

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	MSc in Biochemistry, University of Lisbon

GROUP MEMBERS

Postdoc Ka Yiu Kong

PhD Students Tatiana Aksinina, Andrea Coti, Cécile Debarnot, Ilja Gordijenko, Karla Juárez Núñez, Joep Lurvink*, Christian Ochs, Susmitha Shankar

Master Student Maurice Bouchain

Lab Manager Anke Salzer

Student Assistants Alina Jenn, Markus Zischewski

*indicates joint PhD student

OVERVIEW

The integrity of the proteome 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 reduce mistakes or remove abnormal proteins, thus contributing to proteostasis. 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, proteostasis 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 proteostasis.

RESEARCH HIGHLIGHTS

Characterisation of loss-of-function alleles is the most common approach for studying gene function. With its simple genetics and broad range of resources, the budding yeast *Saccharomyces cerevisiae* is an excellent model for functional genomics. For instance, the budding yeast knockout library was the first genome-wide collection of gene deletion strains. This library has enabled hundreds of genome-wide screens for functional profiling of the yeast genome, mapping of genetic interactions, and identification of drug targets and mechanisms of drug action. However, it has some limitations, including the masking of gene-specific phenotypes by spontaneous suppressor mutations that can arise in gene deletion strains and the need to complement the knockout library with conditional alleles of essential genes, which have their own trade-offs for complete genome-wide coverage.

To address these limitations, we constructed genome-wide libraries of conditional alleles based on the auxin-inducible degron (AID) system previously developed by the Kanemaki laboratory. In this system, proteins fused to the small AID tag can be targeted for proteasomal degradation in the presence of the auxin analog 5-Ph-IAA by expressing the OsTir1 substrate receptor of the SCF ubiquitin ligase (Figure 1). 5-Ph-IAA acts as a molecular glue between the AID tag and OsTir1, thus promoting ubiquitination and eventual destruction of the tagged protein by the SCF-OsTir1 complex. We constructed two genome-wide libraries of yeast strains, each with over 5,600 genes tagged with AID and an optional mNeonGreen fluorescent protein. We could show that almost 90% of AID-tagged proteins are degraded in the presence of 5-Ph-IAA (Figure 1), with initial protein abundance and tag accessibility as limiting factors. Thus, most AID alleles should act as loss-of-function or hypomorphs, allowing truly genome-wide screens with one resource for both essential and non-essential genes. To demonstrate how AID libraries can be exploited to uncover gene functions, we applied them in genome-wide screens for DNA damage response factors. We identified an intriguing link between membrane protein biogenesis at the endoplasmic reticulum and the toxicity of hydroxyurea, a ribonucleotide reductase inhibitor used as a cancer chemotherapeutic.

This work establishes the AID libraries as a valuable addition to the yeast toolkit for functional genomics. While the extent of protein degradation in the AID libraries can be assessed in a high-throughput manner with mNeonGreen fluorescence, the optional nature of the mNeonGreen moiety expands the scope of potential applications, for example, to include high-content fluorescence microscopy screens, where the mNeonGreen moiety could otherwise limit screen design.

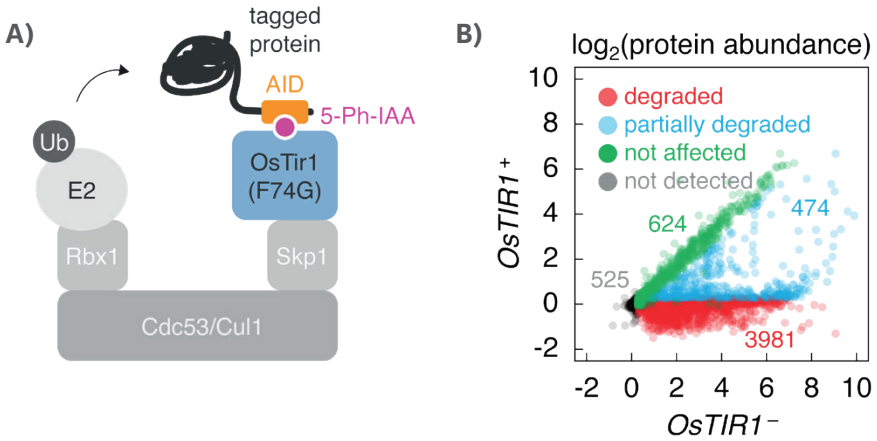


Figure 1. Efficient protein degradation with genome-wide AID libraries. A) Features of the auxin-inducible degron system in the AID libraries. An AID-tagged protein can be ubiquitinated and degraded in the presence of 5-Ph-IAA upon expression of the F-box protein OsTir1. B) Levels of AID-tagged proteins in AID libraries with and without expression of OsTir1. Detectable proteins were classified by the extent of *OsTIR1*-dependent degradation into degraded, partially degraded or not affected.

FUTURE DIRECTIONS

We are eager to dissect the connection between membrane protein biogenesis and replication stress induced by hydroxyurea and to better understand the mode of action of this chemotherapeutic. We are also excited about exploiting the AID libraries in genetic screens to identify the machinery involved in the degradation of abnormal proteins that accumulate in response to stressors and other pathological conditions.

SELECTED PUBLICATIONS

Gameiro E*, Juárez-Núñez KA*, Fung JJ, Shankar S, Luke B and Khmelinskii A (2024) Genome-wide conditional degron libraries for functional genomics. *bioRxiv*, doi: 10.1101/2024.05.29.596381

Kong KYE*#, Shankar S*, Rühle F and Khmelinskii A# (2023) Orphan quality control by an SCF ubiquitin ligase directed to pervasive C-degrons. *Nat Commun*, 14:8363

Kong KYE*, Fischer B*, Meurer M*, Kats I, Li Z, Rühle F, Barry JD, Kirrmaier D, Chevyreva V, San Luis BJ, Costanzo M, Huber W, Andrews BJ, Boone C, Knop M# and Khmelinskii A# (2021) Timer-based proteomic profiling of the ubiquitin-proteasome system reveals a substrate receptor of the GID ubiquitin ligase. *Mol Cell*, 81:2460–2476

*indicates joint contribution , #indicates joint correspondence

Julian **König**

“We merge biochemistry & transcriptomics to decipher the biology of RNAs.”

POSITIONS HELD

Since 2024 Chair for Biochemistry and RNA Biology, Julius Maximilian University of Würzburg

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

Postdocs Peter Hoch-Kraft, Mikhail Mesitov

PhD Students Miona Corovic, Jasmin Sarah Hallstein, Tim Preißendörfer

Master Student Janine Cathrin Brück

Bachelor Student Simon Klüßendorf

Lab Manager Anna Orekhova

OVERVIEW

Posttranscriptional gene regulation plays an important role 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). In addition, epitranscriptomic marks in the form of RNA modifications control mRNA fate. The information in the RNA sequence, RNA modifications and how they are 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.

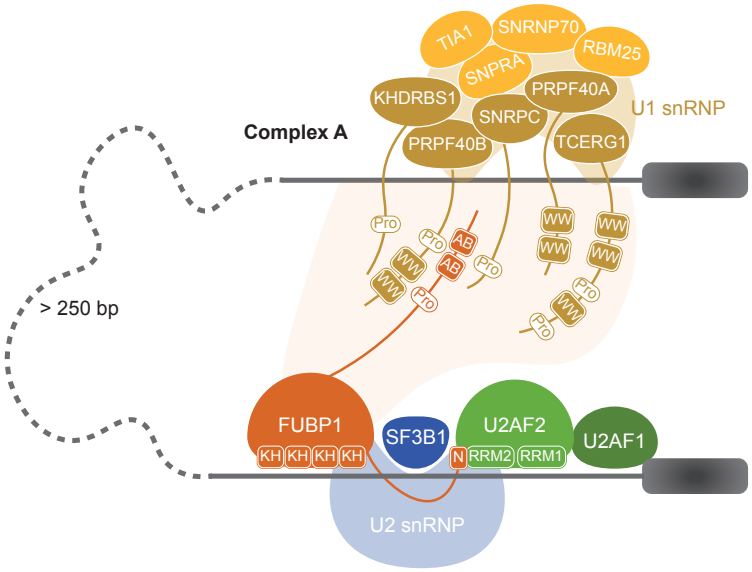
RESEARCH HIGHLIGHTS

FUBP1 is a general splicing factor facilitating 3′ splice site recognition and splicing of long introns

Splicing of pre-mRNAs critically contributes to gene regulation and proteome expansion in eukaryotes, but our understanding of the recognition and pairing of splice sites during spliceosome assembly lacks detail. We recently identified the multidomain RNA-binding protein FUBP1 as a key splicing factor that binds a hitherto unknown cis-regulatory motif. By collecting NMR, structural and *in vivo* interaction data, we demonstrated that FUBP1 stabilises U2AF2 and SF1, which are key components at the 3′ splice site, through multivalent binding interfaces located within its disordered regions. Transcriptional profiling and kinetic modelling revealed that FUBP1 is required for efficient splicing of long introns, which is impaired in cancer patients harbouring *FUBP1* mutations. Notably, FUBP1 interacts with numerous U1 snRNP-associated proteins, suggesting a unique role for FUBP1 in splice site bridging at long introns. We propose a compelling model for 3′ splice site recognition of long introns, which represent 80% of all human introns.

In a related joint study with the Sattler group (Institute of Structural Biology and Technical University of Munich), we analysed the factor PRPF40A, which mediates protein-protein interactions

in the early steps of spliceosome assembly. By binding to proteins at the 5´ and 3´ splice sites, PRPF40A promotes spliceosome assembly by bridging the recognition of the splices. Unexpectedly, a proline-rich motif in the N-terminal region of PRPF40A mediates intramolecular interactions with the WW tandem domain. We show that the intramolecular interaction acts as an autoinhibitory filter for proof-reading high-affinity, proline-rich motifs in bonafide PRPF40A-binding partners.



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. We showed that dosage compensation is achieved via differential N^6-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 stabilised 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.

Figure 1. Model for FUBP1-mediated intron bridging during splicing 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 will investigate the role of m6A modifications in splicing regulation. The aim is to compile a full catalogue of m6A-dependent splicing events in the transcriptome and reliably map all m6A sites that may impact these events.

SELECTED PUBLICATIONS

Ebersberger S*, Hipp C*, Mulorz MM*, Buchbender A, Hubrich D, Kang HS, Martínez-Lumbreras S, Kristofori P, Sutandy FXR, Llacsahuanga Allcca L, Schönfeld J, Bakisoglu C, Busch A, Hänel H, Tretow K, Welzel M, Di Liddo A, Möckel MM, Zarnack K, Ebersberger I, Legewie S, Luck K#, Sattler M# and König J# (2023) FUBP1 is a general splicing factor facilitating 3′ splice site recognition and splicing of long introns. *Mol Cell*, 83:2653-2672.e15

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Cortés-López M*, Schulz L*, Enculescu M*, Paret C, Spiekermann B, Quesnel-Vallières M, Torres-Diz M, Unic S, Busch A, Orekhova A, Kuban M, Mesitov M, Mulorz MM, Shraim R, Kielisch F, Faber J, Barash Y, Thomas-Tikhonenko A, Zarnack K#, Legewie S# and König J# (2022) High-throughput mutagenesis identifies mutations and RNA-binding proteins controlling CD19 splicing and CART-19 therapy resistance. *Nat Commun*, 13:5570

*indicates joint contribution #indicates joint correspondence

Nard **Kubben**

"Our research aims to discover therapeutic targets that improve healthspan."

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	BSc in Health Sciences, Maastricht University

GROUP MEMBERS

PhD Students Luisa Hastenplug, Lukas Mann, Felix van der Walt

Master Students Yousef Al-Sha´ar, Tim Müller

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. We focus on uncovering fundamental biological mechanisms of ageing that can be manipulated to slow down the progression of ageing-related diseases, including the rare and lethal premature ageing disease Hutchinson-Gilford Progeria Syndrome (HGPS). Our group employs unbiased genomics, proteomics and high-throughput microscopy-based screening 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 that can help 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 directly compare 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. As such, it is 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 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, suggesting 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 generated a cellular system in which we can inducibly express progerin and study the formation of many cellular ageing defects within a time frame of only four days, using a semi-automated high-throughput microscopy 'QuantitAgeing' pipeline to visualise and quantify ageing defects. This system enables us to investigate if any genetic interventions can prevent ageing upon progerin expression, thereby identifying pathways that are directly involved in driving cellular ageing.

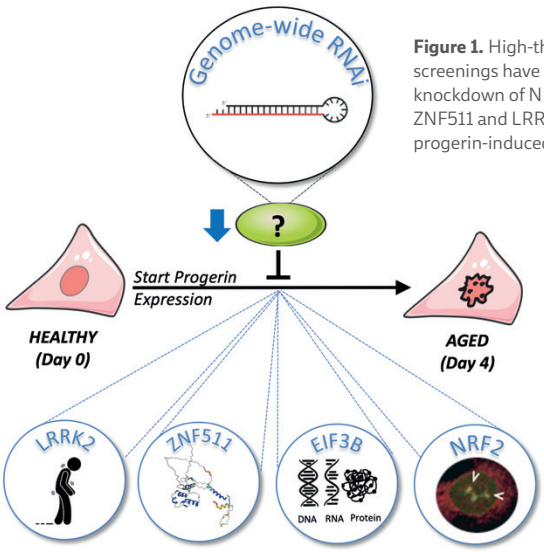

Figure 1. High-throughput siRNA screenings have revealed that knockdown of NRF2, EIF3B, ZNF511 and LRRK2 reduce progerin-induced cellular ageing.

High-throughput screening identifies LRRK2 and ZNF511 as new anti-ageing targets

We previously provided proof-of-principle that we can use our progerin-inducible cell system for high-throughput identification of pathways that drive progerin-induced ageing (Figure 1). Screening a library targeting 320 human ubiquitin ligases for their capacity to prevent cellular ageing, we identified that progerin entrapment of the proteostatic master regulator NRF2 partially drives cellular ageing. We similarly performed a kinome (~2000 targets) and genome-wide RNAi screen. The kinome screen revealed Leucine-rich repeat kinase 2 (LRRK2) as a novel regulator of progerin-induced ageing. LRRK2 knockdown prevents progerin from inducing ageing and reverses established ageing defects in primary HGPS patient cells. Interestingly, *LRRK2* mutations are a well-known cause of Parkinson's disease (PD). These data suggest that the accelerated ageing in HGPS and PD may have shared pathological roots.

Anti-ageing candidates identified from the genome-wide RNAi screen include a previously uncharacterised zinc finger protein (ZNF511), which, upon knockdown, not only prevents progerin-induced ageing, but also reverses ageing defects in cells from physiologically aged individuals. We observed that during ageing, there is an increased formation of nuclear foci that contain both ZNF511 and DNA damage repair proteins. These results suggest a potential role for ZNF511 in premature and physiological ageing through the modulation of DNA damage repair.

FUTURE DIRECTIONS

Our future work will continue to mechanistically unravel the molecular pathways that regulate ageing. LRRK2 is a major regulator of the endolysosomal system. We will apply molecular reporter assays to evaluate how the endolysosomal system is affected in HGPS and physiological ageing in the context of LRRK2 activity. We will further use CRISPR-editing to endogenously tag LRRK2 in order to perform pulldown studies and determine how the LRRK2 interactome alters with ageing. We will also investigate the role of ZNF511 in regulating genomic stability by determining whether ZNF511 nuclear foci formation is specific to certain types of DNA damage and whether ZNF511 levels affect specific types of DNA repair. Additionally, we will determine the protein and DNA interactome of ZNF511. Lastly, we will expand our molecular toolbox by creating additional inducible cellular ageing models and determine to what extent the anti-ageing drivers we identified are capable of preventing stressors that drive cellular ageing.

SELECTED PUBLICATIONS

Adriaens C, Serebryannyy LA, Feric M, Schibler A, Meaburn KJ, Kubben N, Trzaskoma P, Shachar S, Vidak S, Finn EH, Sood V, Pegoraro G and Misteli T (2018) Blank spots on the map: some current questions on nuclear organization and genome architecture. *Histochem Cell Biol*, 150:579-592

Kreienkamp R*, Graziano S*, Coll-Bonfill N*, Bedia-Diaz G, Cybulla E, Vindigni A, Dorsett D, Kubben N, Batista LFZ and Gonzalo S (2018) A cell-intrinsic interferon-like response links replication stress to cellular aging caused by progerin. *Cell Rep*, 22:2006-2015

Shen Y, Kubben N, Candia J, Morozov AV, Misteli T and Losert W (2018) RefCell: multi-dimensional analysis of image-based high-throughput screens based on "typical cells." *BMC Bioinformatics*, 19:427

*indicates joint contribution

Edward **Lemke**

"We develop tools to study the role of intrinsically disordered proteins in gene regulation & ageing."

POSITIONS HELD

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

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

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

Postdocs Sabrina Giofrè, Cosimo Jann, Anastasia Lopatina, Hao Ruan, Tom Scheidt

PhD Students Rajanya Bhattacharjee, Marius Jung, Sara Mingu, Lukas Schartel

Lab Manager Joana Caria

Technology Manager Nike Heinss

Group Administrator Kallie Küßner

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 studying 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 biotechnology with 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 and RNA editing *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 that are carried out by distinct, naturally-occurring organelles and play vital roles in regular cell function, as well as in ageing. 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.

Most recently, all the seemingly different efforts of my lab in chemical/synthetic biology and fluorescence biophysics concluded in a single study that visualised for the first time the permeability barrier of the functional nuclear pore complex *in situ*, which is responsible for regulating all the traffic between the cytoplasm and the nucleoplasm. The key finding that IDPs in this machinery can become a solvent for themselves and that this is accompanied by a giant conformational change in the protein, showcases a genuine example of how knowledge from polymer science can improve our understanding of biological systems.

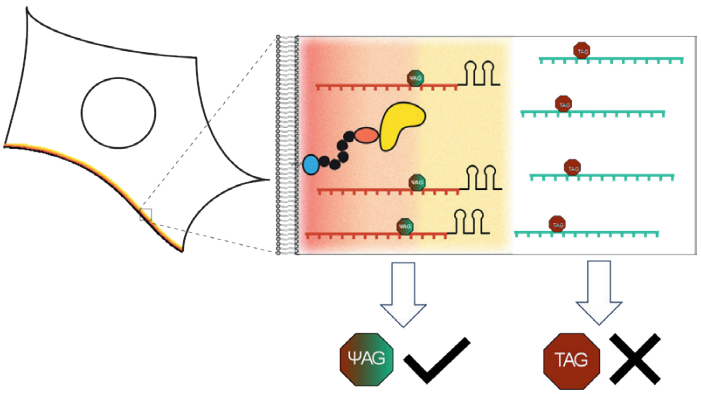

Figure 1. We have designed an Orthogonally RNA Editing Organelle (OREO) that can specifically introduce pseudouridine into an RNA.

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 beginning to understand 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*. We aim to expand our RNA editing and protein engineering approaches to develop new tools for biotechnology and basic research to assist our mechanistic studies of how disordered proteins play key roles in gene regulation and cellular ageing.

SELECTED PUBLICATIONS

Dormann D# and Lemke EA# (2024) Adding intrinsically disordered proteins to biological ageing clocks. *Nat Cell Biol*, 26:851–858

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# (2023) Visualizing the disordered nuclear transport machinery *in situ*. *Nature*, 617:162–169

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

*indicates joint contribution #indicates joint correspondence

Katja **Luck**

“Structurally-resolved protein interactomes are essential for studying genotype-to-phenotype relationships.”

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, Boston

2007 – 2008 Research Assistant, EMBL, Heidelberg

EDUCATION

2012 PhD in Bioinformatics, University of Strasbourg

2007 Diploma in Bioinformatics, Friedrich Schiller University Jena

GROUP MEMBERS

Postdocs Christian Schäfer, Jesus Alvarado Valverde

PhD Students Caroline Barry, Milena Djokic, Johanna Lena Geist, Dalmira Hubrich, Chop Yan Lee, Jonas Schönfeld*, Joelle Morgan Strom

Master Students Eleni Aretaki, Julian Ben Hey, Laura Jager, Jann Rusch

Lab Manager Mareen Welzel

*indicates joint PhD student

OVERVIEW

Cells function because their molecular components (DNA, RNA, proteins) 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 lack a comprehensive structural and functional understanding of all the protein interactions in human cells, hindering our ability to understand physiological and pathological molecular mechanisms. To tackle this, my lab develops novel computational and experimental methods to identify protein interaction interfaces and, based on this, obtains information on their molecular functions. 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 study proteins associated with neurodevelopmental disorders (NDDs) and proteins functioning in protein quality control and mRNA splicing, as well as chromatin remodelling together with our collaborators.

RESEARCH HIGHLIGHTS

Identification of protein interaction interfaces

Proteins exhibit a modular architecture consisting of folded domains and disordered regions, which can carry short linear motifs. Proteins commonly mediate interactions with each other via domain-domain or domain-linear motif interaction interfaces. We build tools to predict the interfaces in known protein interactions. To this end, we benchmarked the ability of AlphaFold Multimer to accurately predict the structures of interacting proteins. We found that AlphaFold (AF) predictions are not very specific and decrease dramatically in sensitivity when using longer protein fragments or full-length sequences. This is especially true for domain-motif interfaces and, to a lesser extent, for domain-domain interfaces (Geist *et al*, 2024, *Bioinformatics*). We therefore developed a prediction pipeline to optimise AF’s sensitivity and specificity. Using this pipeline, we predicted interfaces for 62

protein interactions that link NDD-associated proteins (Luck *et al*, 2020, *Nature*). We obtained highly confident predictions for 18 of these interactions, seven of which we experimentally validated and found to involve two novel types of domain-motif interfaces (Lee *et al*, 2024, *Mol Syst Biol*). We also successfully used this prediction pipeline to discover, in collaboration with others, a novel interface type that mediates binding between proteins in the piRNA biogenesis pathway (Bronkhorst *et al*, 2023, *EMBO J*) and in DNA replication (Arroyo *et al*, 2024, *Nucleic Acids Res*). We are combining sequence pattern-based predictions of known interface types with AlphaFold to accelerate interface prediction and have used these interfaces to characterise disease-associated variants that occur in predicted and experimentally validated linear motifs (unpublished).

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 have selected ~30 interactions with structurally-resolved interfaces to develop and benchmark our XL-MS pipeline. We have crosslinked all pairs with two different homo-bifunctional crosslinkers and explored crosslinking with hetero-bifunctional crosslinkers, as well as zero-length crosslinkers. Results indicate that crosslinks generally do not occur at interfaces, but information on interfaces might be obtained indirectly from mapping surface areas on protein structures that are depleted of mono-, intra- and interlinks.

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 to predict the molecular mechanisms that mediate brain-specific phenotypes in NDDs. We also collaborate with the Schick lab (IMB) to gain a systematic understanding of the role of BAF chromatin remodelling complexes in genome stability as part of the Collaborative Research Centre (CRC) 1361.

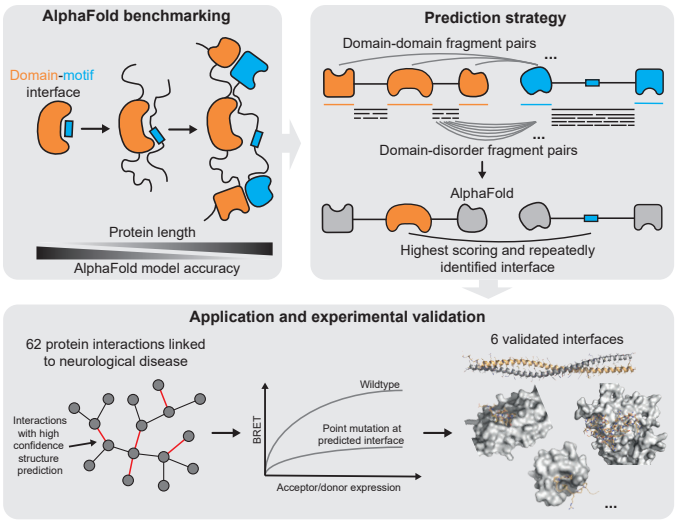

Figure 1. Schematic illustrating AlphaFold's drop in accuracy in predicting protein interaction interfaces when using longer protein fragments, our *in silico* fragmentation approach to using AlphaFold for predicting modes of protein binding, and our approach to experimentally corroborate predicted protein interaction interfaces. This work is published in Lee *et al*, 2024, *Mol Syst Biol.*

FUTURE DIRECTIONS

We will continue developing tools to predict and experimentally characterise protein interaction interfaces, with the goal of studying interactions involving disordered regions of proteins. Such interactions are often involved in the formation of liquid-like condensates, which we aim to study in the context of mRNA splicing and protein homeostasis; the latter is funded as part of the CRC 1551 in collaboration with the Beli lab (IMB), as well as the Kukharenko and Kremer labs (Max Planck Institute for Polymer Research). We are furthermore working towards a systematic resource of clustered protein interaction interfaces to explore the diversity and evolutionary aspects of modes of protein binding.

SELECTED PUBLICATIONS

Geist J*, Lee CY*, Strom JM, Naveja JJ#, Luck K# (2024) Generation of a high confidence set of domain-domain interface types to guide protein complex structure predictions by AlphaFold. *Bioinformatics*, 40:btae482

*indicates joint contribution #indicates joint correspondence

Lee CY*, Hubrich D*, Varga JK*, Schäfer C, Welzel M, Schumbera E, Djokic M, Strom JM, Schönfeld J, Geist JL, Polat F, Gibson TJ, Keller Valsecchi CI, Kumar M, Schueler-Furman O#, Luck K# (2024) Systematic discovery of protein interaction interfaces using AlphaFold and experimental validation. *Mol Syst Biol*, 20:75-97

Ebersberger S*, Hipp C*, Mulorz MM*, Buchbender A, Hubrich D, Kang HS, Martínez-Lumbreras S, Kristofori P, Sutandy FXR, Llacsahuanga Allcca L, Schönfeld J, Bakisoglu C, Busch A, Hänel H, Tretow K, Welzel M, Di Liddo A, Möckel MM, Zarnack K, Ebersberger I, Legewie S, Luck K#, Sattler M# and König J# (2023) FUBP1 is a general splicing factor facilitating 3′ splice site recognition and splicing of long introns. *Mol Cell*, 83:2653-2672.e15

Brian **Luke**

"When RNA accidentally gets incorporated into DNA, it promotes mutations in old cells."

POSITIONS HELD

Since 2017	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Heisenberg Professor, Johannes Gutenberg University Mainz (JGU)
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

Postdocs Fabio Bento, Sacha Heerschop, Natalie Schindler

PhD Students Rodolfos Danalatos, Eduardo Gameiro*, Kristi Jensen, Sana'a Khraisat*, Nina Lohner, Matteo Longaretti, Varvara Verkhova*, Carolin Wagner, Maya Wilkens*

Master Students Tobias Frank, Luca Kindinger, Sophia Sergi

Bachelor Students Jasmin Baumann, Florian Hippe, Su Ful Jung, Pauline Raifschnaider, Franziska Roithner, Ann-Kathrin Schlotterbeck

Student Assistant Linus Nuppnau

Technicians Dennis Knorr, Stefanie Reimann

Personal Assistant Christiane Stürzbecher

*indicates joint PhD students

OVERVIEW

Ageing is associated with impaired organ, tissue and cellular function as well as the increased occurrence of diseases such as cancer, Alzheimer's and osteoporosis, to name a few. The loss of organ function, or disease progression, eventually leads to death. In response, we are frequently prescribed myriad medications to treat age-related symptoms, including hypertension, arthritis, inflammation, etc. Although the above description of ageing appears rather pessimistic and even complicated, recent research has indicated that the ageing process may be easier to deal with than previously thought.

Although the tissues, organs and cells that are affected by ageing may differ, the molecular machineries within the cells are identical. We have learned that neurodegeneration and cancer development, both age-related diseases, are likely due to defective maintenance of chromosomal DNA. We have now identified that four cellular processes are consistently dysfunctional in ageing cells: genome maintenance, epigenetic regulation, the preservation of telomeres, and the upkeep of protein function. Hence, rather than treating diseases in an organ/tissue-specific manner, which can quickly accumulate, we should be treating the molecular dysfunctions, as there are only four of them. Such an approach will help to both alleviate and prevent age-related symptoms.

RESEARCH HIGHLIGHTS

We are using a model system, *S. cerevisiae*, to assess how genomic mutations accumulate during ageing and investigate how this may be regulated or ameliorated. It is well established that rates of DNA mutation increase with age. Furthermore, organisms with low mutation rates tend to live longer than organisms with high mutation rates. It is not clear whether mutational frequency is a cause or consequence of ageing. The most frequent DNA mutation that occurs on a daily basis is the erroneous misincorporation of ribonucleotides instead of deoxyribonucleotides into the genome during DNA replication. The inserted

ribonucleotide monophosphates (rNMPs) are normally removed by ribonucleotide excision repair (RER) proteins, which are orchestrated by the ribonuclease RNase H2. When RNase H2 is mutated or deleted, rNMPs accumulate and lead to 2-5 base pair deletions throughout the genome. The deletions are due to the activity of topoisomerase I (Top1).

Using a specific reporter, we were able to show that old cells have more Top1 mutations than young cells (Figure 1). Although increased Top1 levels can lead to more mutations, we did not see that Top1 levels were increased in old compared to young cells. We used a well-characterised DNA polymerase mutant that incorporates fewer rNMPs into the genome than a wild-type copy. We found that cells with less rNMPs have an increased lifespan. This suggests that rNMPs in the genome may contribute to ageing. Multiple interventions that are known to extend lifespan and healthspan have been discovered. Caloric restriction (CR) has consistently been demonstrated to improve longevity and healthspan in eukaryotic organisms ranging from yeast to humans. We demonstrated that CR is also able to reduce Top1-related mutations, even in young cells. This suggests that, in part, CR may extend lifespan by decreasing the mutagenic load of aged cells.

By understanding how rNMP insertion and excision are controlled, we may be able to better understand and control mutation rates during the ageing process. Leveraging these processes may eventually result in the prevention of age-related disease and hence healthy ageing.

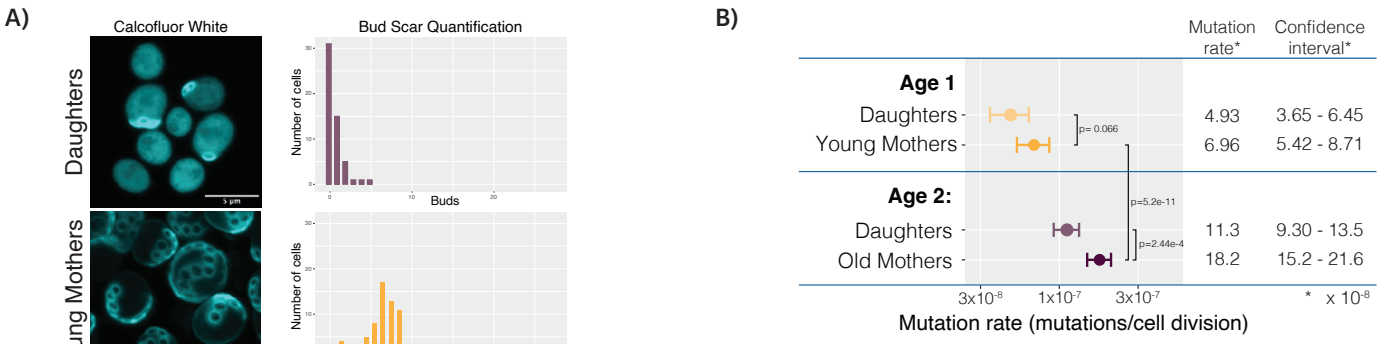

Figure 1. Figure 1. A) When yeast cells become old, their number of bud scars increases. We were able to isolate young (top), middle-aged (middle) and old (bottom) cells and confirm their ages by counting bud scars (see graphs). B) Old mothers had an increased rate of mutation compared to young mothers, and their corresponding daughters had lower mutation frequencies.

FUTURE DIRECTIONS

Although we have identified an increase in the Top1 mutation signature at rNMPs, we do not have a good understanding of the mechanistic details involved. It is possible that RNase H2 is less efficient or less expressed in old cells. We are currently generating RNase H2 antibodies to test these ideas. It is also possible that NTP to dNTP ratios are altered in old compared to young cells, another hypothesis that is being actively pursued. We are collaborating with the Padaken lab (IMB) to determine if these relationships are conserved in *C. elegans*. Preliminary data has indicated that the loss of RNase H2 activity shortens the lifespan of adult worms. Finally, we will employ genetic and chemical genetics to probe pathways that extend and shorten lifespan and assess their effects on Top1 mutagenesis at rNMPs.

SELECTED PUBLICATIONS

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Wagner CB and Luke B (2022) DNA-RNA hybrids at telomeres in budding yeast. *Methods Mol Biol*, 2528:145-157

*indicates joint contribution

Christof **Niehrs**

"We explore how DNA modifications act as molecular switches in early development."

POSITIONS HELD

Since 2021	Director, Centre for Healthy Ageing (CHA), Mainz
Since 2010	Founding & Scientific Director, Institute of Molecular Biology (IMB), Mainz
	Professor, Johannes Gutenberg University Mainz (JGU)
Since 2000	Professor of Molecular Embryology, German Cancer Research Center (DKFZ), Heidelberg
Since 1994	Head of Division "Molecular Embryology", German Cancer Research Center (DKFZ), Heidelberg
1990 – 1993	Postdoc, University of California Los Angeles (UCLA)

EDUCATION

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

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Senior Research Associate Lars Schomacher

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PhD Students Jasmin Dehnen, Deepa Jayaprakashappa, Gaurav Joshi, Marcel Misak, Eleftheria Parasyraki, Zukhra Stamgaliyeva, Umut Taşdelen

Lab Manager Sandra Rölle

Technicians Laura Frosch, Carola Scholz, Johanna Melanie Schott

Personal Assistant Jutta Karn

OVERVIEW

Although DNA is commonly perceived as a static molecule, 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 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 also characterise the roles of proteins involved in depositing, reading and removing modifications in embryonic stem cells, *Xenopus* embryos and mice.

RESEARCH HIGHLIGHTS

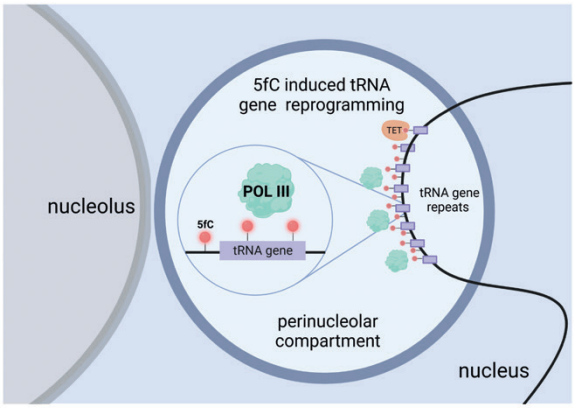
The study of oxidised 5-methylcytosine (5mC) derivatives has long been central to understanding DNA methylation and gene regulation. Among these derivatives, we have focused on 5-formylcytosine (5fC) and asked whether it acts solely as an intermediate in active DNA demethylation or functions independently as an instructive, regulatory mark. We studied 5fC's potential role as an epigenetic regulator in *Xenopus* embryos during zygotic genome activation (ZGA), a key developmental phase marked by a transition from maternal to embryonic control over gene expression. During early embryonic development, ZGA is a critical period when the zygotic genome becomes transcriptionally active, replacing maternal RNA with its own genetic programme. We discovered that 5fC forms distinct chromocenters in the nuclei of *Xenopus* embryos during ZGA, which are associated with the perinucleolar compartment (PNC). These 5fC chromocenters are transient and colocalise with RNA polymerase III (Pol III) components in the PNC. Genomic profiling further corroborated that 5fC is enriched at Pol

III target loci, notably *tRNA* genes where 5fC correlates with active chromatin marks, indicating a role in stimulating transcription.

To confirm the functional requirement of 5fC in gene regulation, we manipulated the levels of enzymes responsible for its synthesis and removal. Knockdown of Tet2 and Tet3, which produce 5fC, decreased Pol III binding and *tRNA* levels, directly implicating 5fC in the activation of these genes. Conversely, overexpression of thymine DNA glycosylase, an enzyme that removes 5fC, led to a decrease in Pol III recruitment, further supporting the hypothesis that 5fC is a crucial activating mark for *tRNA* transcription during ZGA. Further, by employing a transgene approach, we introduced *tRNA* genes with 5fC-modified cytosines into *Xenopus* embryos. We found that these modified transgenes exhibited higher *tRNA* transcription levels compared to unmodified controls, providing direct evidence that 5fC actively promotes gene expression.

By extending our study to mouse zygotes, we identified a similar pattern of 5fC accumulation during early zygotic genome activation. In mouse preimplantation embryos, 5fC chromocenters were also observed colocalising with Pol III, suggesting a conserved mechanism across vertebrates. This conservation emphasises the fundamental nature of 5fC's role in early development and provides a foundation for future studies to explore its broader implications across different organisms and developmental stages.

In conclusion, our study shifts the understanding of 5fC from being a passive byproduct of DNA demethylation to an active, instructive epigenetic mark that drives gene expression. Thus, 5fC is the second epigenetic DNA mark besides 5mC in vertebrates. Unlike 5mC, which is typically associated with gene silencing, 5fC stimulates gene expression, promoting the binding and function of Pol III during ZGA for the rapid synthesis of essential RNAs during a critical developmental transition.



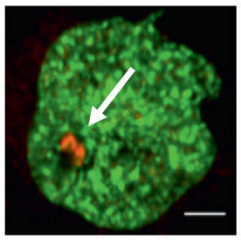


Figure 1. Left: Model for how 5-formylcytosine acts as an epigenetic DNA mark to stimulate transcription of *tRNA* tandem repeat genes by Pol III. 5-formylcytosine accumulates in the perinuclear compartment (PNC), where Pol III target gene transcription is concentrated. Right: immunofluorescence staining of a nucleus (DNA in green), highlighting a 5-formylcytosine chromocenter (red) in the PNC, which surrounds the nucleolus (black). Scale bar: 5 µm.

FUTURE DIRECTIONS

A key question for future research is whether 5fC functions as an active regulatory mark in mammalian cells, similar to its role in *Xenopus*. In mouse embryonic stem cells, 5fC is enriched at active enhancers and regions undergoing dynamic changes, suggesting a role in gene activation. Interestingly, Pol III not only regulates classical targets like *tRNA* and *5S rRNA* genes, but can also be recruited to 'non-classical' Pol III target sites. These 'extra-TFIIIC' (ETC) sites were observed in association with the chromatin architectural protein CTCF and SINEs. ETC sites may play a role in genome organisation by mediating long-range chromatin interactions. Given that CTCF and SINE elements are often found near regions of active transcription, it is plausible that 5fC might also play a role in facilitating or stabilising the binding of Pol III at ETC sites. Future studies will therefore explore whether 5fC colocalises with ETCs, potentially influencing Pol III activity beyond classical targets.

SELECTED PUBLICATIONS

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

*indicates joint contribution #indicates joint correspondence

Jan **Padeken**

"We explore the interplay between stress & the epigenome during ageing."

POSITIONS HELD

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

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EDUCATION

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

2009 Diploma in Biology, Albert-Ludwigs University, Freiburg

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Student Assistant Luisa Dietz

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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 and prevent toxic R-loops; second, it maintains the silencing of genes during and post development to ensure a stable differentiated state. Thus, it is not surprising that the loss of appropriately targeted heterochromatin is associated with cancer and ageing. In our lab, we explore how stress alters epigenetic silencing, resulting in the transient or long-term establishment of heterochromatin, impacting ageing and transgenerational adaptation.

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 simple 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 that are essential for establishing the H3K9me domain. We will use this to screen for factors essential for the establishment vs. maintenance of heterochromatin and link these pathways to the stress response and premature ageing.

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

We previously showed that loss of H3K9me results in cell-type-specific gene derepression (Methot *et al*, 2021, *Nat Cell Biol*). 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.

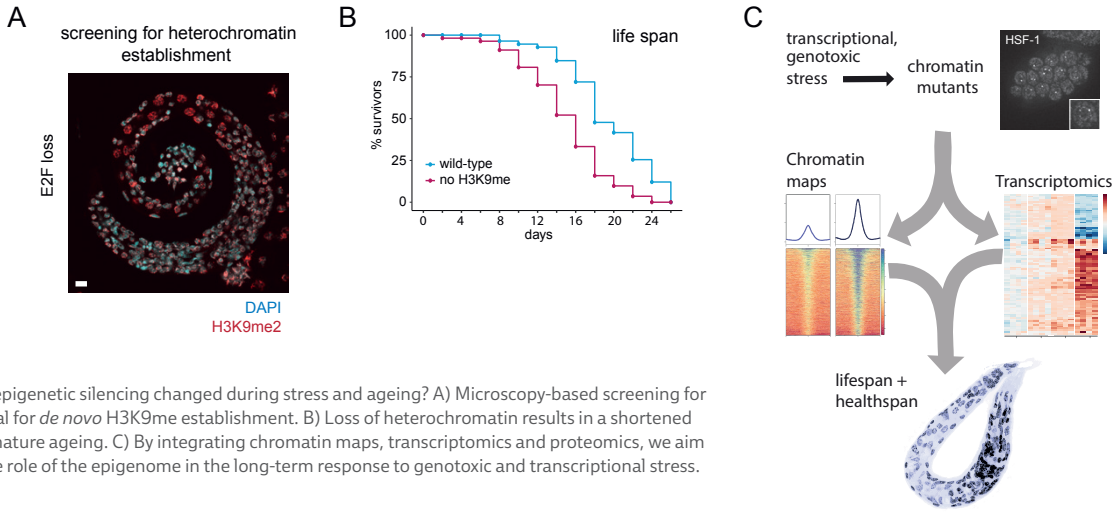

Figure 1. How is epigenetic silencing changed during stress and ageing? A) Microscopy-based screening for pathways essential for *de novo* H3K9me establishment. B) Loss of heterochromatin results in a shortened lifespan and premature ageing. C) By integrating chromatin maps, transcriptomics and proteomics, we aim to understand the role of the epigenome in the long-term response to genotoxic and transcriptional stress.

FUTURE DIRECTIONS

Ultimately, the projects above will give us a better understanding of how heterochromatin and its loss during ageing impact the processes that are thought to drive ageing, like the DNA damage response and loss of protein homeostasis. It will also give us a basis to further explore how, in general, epigenetic memory is shaped during organismal life and how it impacts normal ageing or progeria models such as CS and Hutchinson-Gilford progeria.

SELECTED PUBLICATIONS

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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

Stamatis **Papathanasiou**

"We aim to discover the mechanisms driving genetic & epigenetic instability in cancer."

POSITIONS HELD

Since 2023 Group Leader, Institute of Molecular Biology (IMB), Mainz

2015 – 2023 Postdoc, Harvard Medical School and Dana Farber Cancer Institute, Boston

EDUCATION

2015 PhD in Molecular Medicine, National and Kapodistrian University of Athens

2008 MSc in Molecular Medicine, National and Kapodistrian University of Athens

2005 BSc in Molecular Biology and Biotechnology, University of Crete

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Lab Manager Evlampia Parcharidou

Research Assistants Konstantinos Kydonakis, Nikoleta Pateraki

OVERVIEW

Proper division of the genomic material is fundamental for cell homeostasis. Although cells have many mechanisms to ensure error-free division, mistakes are common and a hallmark of disease. One consequence of mitotic errors is abnormal nuclear structures such as micronuclei and chromosome bridges – common features of nuclear atypia with a central role in the development of cancer. Micronuclei ("MN", Figure 1A) are miniature, extra nuclei that form when a chromosome lags during mitosis and recruits its own nuclear envelope. Micronuclei nuclear envelope rupture exposes DNA to the cytoplasm, leading to massive DNA damage. The lesions in the micronucleated chromosome can lead to complex chromosomal rearrangements ("chromothripsis") and ongoing genome instability. Intriguingly, cells with chromothriptic signatures are extremely penetrant in cancer, showing that they can confer a gain of fitness and even drive tumorigenesis. Although the self-amplifying genetic instability and clinical importance of these nuclear abnormalities are well-recognised, we are missing a detailed understanding of the immediate and long-term non-genetic, functional consequences of these mitotic errors.

RESEARCH HIGHLIGHTS

Transgenerational inheritance of chromatin defects from mis-segregated chromosomes

We recently proposed a new model of transgenerational epigenetic instability caused by chromosome mis-segregation in mitosis. Specifically, we discovered a phenomenon of heritable chromatin and transcriptional defects mediated by micronuclei (Papathanasiou *et al*, 2023, *Nature*). The transcriptional and chromatin states of micronucleated chromosomes are extensively altered; these can be inherited by daughter cells, even after the chromosomes are reincorporated into the normal nuclear environment. Finally, we discovered that persistent transcriptional repression is strongly associated with long-lived DNA damage to these abnormal chromosomes. Taking advantage of this finding, we generated cellular systems to detect and track damaged chromosomes from

micronuclei, e.g. fluorescently labelled-MDC1-expressing cells (Figure 1A). We called these structures of reincorporated MN chromosomes with altered chromatin "MN-bodies". The exact circumstances under which cell division errors lead to massive DNA lesions and other chromatin alterations, how they are inherited in progeny cells, and their functional significance all remain a mystery, partly due to a lack of appropriate tools. We develop novel technologies and advanced cellular systems, allowing us to approach these questions from a unique angle. For example, we developed a method that combines long-term live-cell imaging and same-cell, direct single-cell RNA sequencing of the whole family of cells after two generations, named "Look-Seq2". We are building on this by developing an advanced experimental and computational framework (Simultaneous IMAging and Direct Isolation for sequencing, "SIMADI-Seq"), which will allow us to directly link observed phenotypes to function by combining imaging and "omics" at the single-cell level. Unravelling the functional properties of abnormal genomes at the single-cell level is fundamental for understanding cellular heterogeneity in disease and developing new therapeutic strategies. Another technology bottleneck was the inability to target specific chromosomes for mis-segregation. We overcame this by developing a novel method to generate "targeted" mis-segregations and micronuclei with *a priori* knowledge of the micronucleated chromosome. While doing this, we discovered that micronuclei are a prominent on-target side effect of genome editing, unravelling a previously unknown universal action of CRISPR-Cas9 (Leibowitz*, Papathanasiou* *et al*, 2021, *Nat Genetics*). These findings were the basis of one of the first described side effects of genome editing, with fundamental implications for therapeutic applications in clinical trials. We are now further developing these "targeted" approaches and combining them with our cellular systems for tracking mis-segregated chromosomes. Our discovery that micronuclei are a source of transcriptional heterogeneity and epigenetic instability established a new paradigm for how mitotic errors may be inherently coupled to the poorly understood non-genetic cell-to-cell epigenetic variability in disease (e.g. cancer). Our research follows this new perspective on the functional consequences of mitotic errors and abnormal nuclei, which may impact tumour evolution.

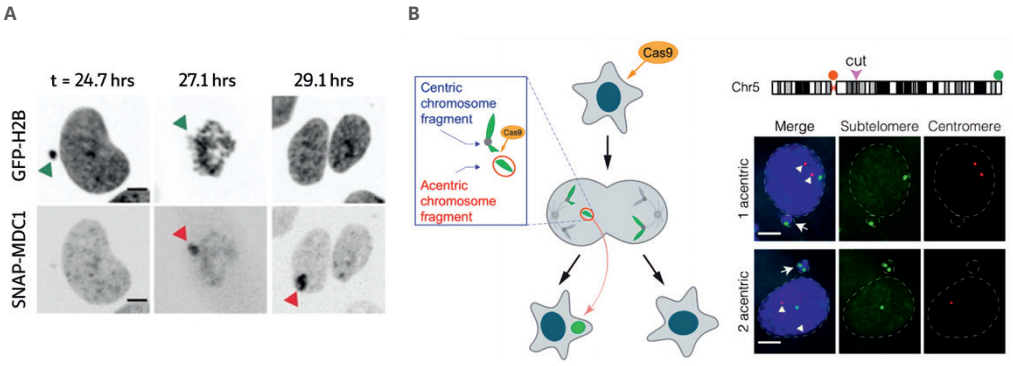

Figure 1. A) Formation of an MN-body (red arrowheads), a type of Mit-body, by reincorporation of a damaged (MDC1) micronucleus (green) into the primary nucleus of a daughter RPE-1 cell ("t" indicates time after release in mitosis, scale bar = 5µm). B) Method to generate MN-bodies from a predefined chromosome. Left, an acentric chromosome arm is generated by a Cas9-mediated cut and forms a specific micronucleus (green). Right, FISH validation with centromeric (red) and sub-telomeric (green) probes of Chr.5q targeting in a micronucleus in RPE-1 cells (scale bars 5µm, adapted from Leibowitz, Papathanasiou *et al*, 2021).

FUTURE DIRECTIONS

We will further develop and combine cutting-edge methodologies with advanced systems to track mis-segregated chromosomes over multiple generations. We aim to identify sources of inherited abnormal nuclear structures and characterise their DNA damage/repair dynamics and epigenetic alterations. We will also focus on understanding how transcription dynamics are perturbed in daughter cells upon abnormal mitosis and define chromatin architecture and the higher-order genome organisation of mis-segregated chromosomes. Finally, we will investigate long-term cellular adaptations and assess the tumorigenic potential of abnormal chromosomes. Together, these studies will offer the first comprehensive assessment of non-genetic mechanisms by which errors in mitosis may drive cellular adaptation and tumorigenesis.

SELECTED PUBLICATIONS

Papathanasiou S#, Mynhier NA, Liu S, Brunette G, Stokasimov E, Jacob E, Li L, Comenho C, van Steensel B, Buenrostro JD, Zhang CZ# and Pellman D# (2023) Heritable transcriptional defects from aberrations of nuclear architecture. *Nature*, 619:184-192

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Katharina **Papsdorf**

"We study how specific lipids extend lifespan."

POSITIONS HELD

Since 2024	Group Leader, Institute of Molecular Biology (IMB), Mainz
2016 – 2023	Postdoctoral Fellow, Stanford University

EDUCATION

2016	PhD in Biochemistry, Technical University of Munich
2011	MSc in Biochemistry, Technical University of Munich
2008	BSc in Biochemistry, Technical University of Munich

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Bachelor Student Marie Sixel

Technician Keshav Gajendra Babu

Student Assistant Luisa Dietz

OVERVIEW

The overarching goal of my lab is to decipher how specific lipids drive cellular changes that induce longevity. Lipids are attractive candidates to study in the context of lifespan regulation as they are a ubiquitous component of the human diet. Age progression can be slowed by increasing the abundance of specific lipids in invertebrates and mice. The best example is monounsaturated fatty acids (MUFAs). They are the main component of olive oil in the Mediterranean diet, correlate with human longevity and extend lifespan in several species. However, it remains largely unknown how specific lipids, such as MUFAs, protect from the cellular changes underlying ageing and if this can be leveraged to promote longevity.

To study the connection between MUFAs and lifespan regulation, we use the nematode *C. elegans*. The nematode worm is uniquely positioned because many of the pathways and organelles involved in lipid processing are conserved with mammals and its short lifespan allows us to perform ageing studies in a laboratory setting. We use a combination of cell biology methods and mass spectrometry, as well as genetics and screening techniques to dissect the functions of specific lipids and the organelles that process them for ageing and longevity.

RESEARCH HIGHLIGHTS

Lipid storage organelles and lifespan

The major lipid reservoirs in cells are lipid droplets. These conserved cellular organelles are central in lipid metabolism as they are specialised for storing and hydrolysing lipids to meet cellular energy needs. But their role extends beyond classical lipid storage as they also capture lipids that otherwise become detrimental to the cell. Lipid droplets were thought to be inert storage organelles, but it is now clear that they are dynamic organelles with specialised cellular machinery regulating their biology. Lipid droplets and the lipids within are differentially regulated during ageing and are important for longevity. For example, lipid droplet number increases in aged muscles in *C. elegans* and in microglia in the brains of

old mice. However, lipid droplet accumulation is not always detrimental to health. Notably, lipid droplets protect stem cell niches in *Drosophila* and act as innate immune hubs that kill pathogens in mice. This indicates a potential tissue-specific effect of lipid droplet accumulation. During my postdoctoral training, I discovered that an increase in lipid droplets is critical for longevity mediated by MUFAs. Importantly, these lipid droplets only accumulate in designated lipid storage tissue (the *C. elegans* intestine) and not in other tissues. We study the mechanism of how lipid droplets protect tissue and cellular homeostasis to drive longevity. To do this, we use a combination of tissue-specific and dietary manipulations, mass spectrometry and cell biology to understand how lipid droplets drive longevity in a tissue-specific manner.

Organelle contact of lipid droplets during lifespan

Lipid droplets are in frequent contact with other organelles in the cell. They often form contacts with other organelles, a characteristic found across all eukaryotic cell types. These interactions facilitate the transfer of lipids. For example, lipid droplets receive lipids from the ER, the primary site for lipid synthesis, through direct membrane contact sites. A recent study discovered the importance of multi-organelle interactions, including lipid droplets, in the metabolic adaptation of the inflammatory response in macrophages. While lipid droplet-organelle contacts are key in many physiological processes, it remains unknown how organelle interactions, including those around lipid droplets, change with age or longevity – especially in the context of a whole living organism. Importantly, it is not known how specific lipids such as MUFAs regulate the ageing organelle landscape. We are interested in identifying changes in the organelle interaction landscape with ageing and how they differ in long-lived individuals, as well as in exploring whether targeting organelle interactions could extend lifespan. To do this, we will use a combination of genome-wide screening tools, fluorescence microscopy, electron microscopy and protein engineering to find key organelle interactions for lifespan.

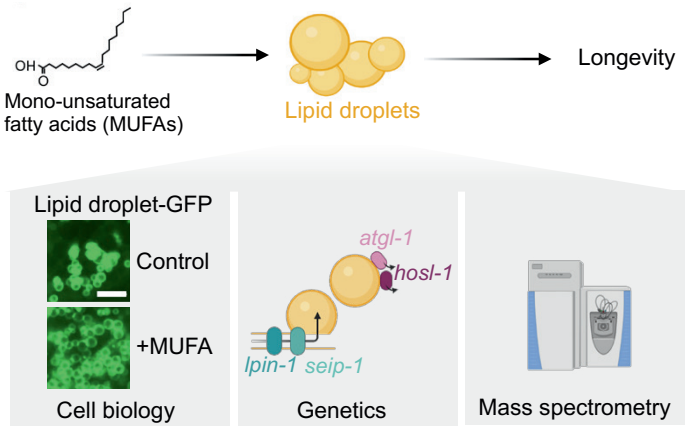

Figure 1. Tools used to determine how mono-unsaturated fatty acids and lipid droplets extend lifespan. Adapted from Papsdorf *et al*, 2023, *Nat Cell Biol* and Biorender.com.

FUTURE DIRECTIONS

Future work will continue to mechanistically unravel the molecular pathways that are driven by beneficial lipids including MUFAs and lipid storage organelles. We will use metabolomics to test how metabolism is supported by lipid droplets in long-lived worms and probe their role in lifespan regulation. In addition, we will expand our toolset for analysing and manipulating organelle interactions to unravel their role in ageing and longevity. In conclusion, we are aiming to enhance our understanding of lipid-driven processes during ageing to potentially open new avenues to use lipids as ageing interventions.

SELECTED PUBLICATIONS

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Silva-García CG, Láscarez-Lagunas LI, Papsdorf K, Heintz C, Prabhakar A, Morrow CS, Pajuelo Torres L, Sharma A, Liu J, Colaiácovo MP, Brunet A and Mair WB (2023) The CRTC-1 transcriptional domain is required for COMPASS complex-mediated longevity in *C. elegans*. *Nat Aging*, 3:1358-1371

Vassilis **Roukos**

"We develop imaging & sequencing methods to study when, where & why chromosomes break."

POSITIONS HELD

Since 2022	Affiliated Group Leader, Institute of Molecular Biology (IMB), Mainz
	Assistant Professor, Medical School, University of Patras
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
2002	BSc in Biology, University of Patras

GROUP MEMBER

PhD Student	Gabriel Longo

OVERVIEW

The focus of our lab is to understand how cells maintain the integrity of their genome in the context of 3D genome organisation. We are particularly interested in the life cycle of DNA double-strand breaks (DSBs), a very dangerous lesion for cells, which if not faithfully repaired, can lead to cell death or the formation of tumorigenic genome rearrangements. DSBs can be evoked exogenously upon cancer treatment or the use of programmed nucleases such as CRISPR/Cas9, which both have important clinical implications, or upon perturbation of intrinsic fundamental cellular processes such as DNA replication and transcription. A central focus of our work is to understand when, where, why and how chromosomes break across the 3D genome, and to understand how these fragile DNA sites can be turned into persistent breaks that promote the formation of genomic rearrangements. Moreover, we are interested in understanding how programmed nucleases, such as Cas9 and Cas12, generate specific cleavage patterns at different locations across the genome and harness this information to increase the fidelity, precision and predictability of genome editing.

RESEARCH HIGHLIGHTS

Linking CRISPR-Cas9 double-strand break profiles to gene editing precision with BreakTag

CRISPR/Cas9 is a powerful genome-editing platform with immense potential for facilitating gene therapy to treat various diseases. However, Cas9 has a flexible scission profile, which might impact repair outcomes, and it is largely unknown what dictates the type of Cas9 incision that is made. We have developed a sensitive, fast, scalable and cleavage pattern-aware methodology to profile CRISPR/Cas9 on and off-target DSBs, which can be used to identify the determinants of Cas9 incisions. We have found that the target sequence determines how Cas9 cleaves DNA and that the type of incisions made is strongly associated with the repair outcome. Moreover, we identified Cas9 variants with altered scission profiles and demonstrated that human genetic variation influences Cas9 cleavage and editing outcome, suggesting that patients'

genetic backgrounds must be taken into consideration before clinically relevant efforts. Our work illuminates the fundamental characteristics of the Cas9 nuclease and lays the foundation for harnessing the flexible cutting profiles of Cas9 and engineered variants for template-free, precise and personalised genome editing (Figure 1).

Type II topoisomerases shape multi-scale 3D chromatin folding in regions of positive supercoils.

Type II topoisomerases (TOP2s) resolve torsional stress accumulated during various cellular processes and are enriched at chromatin loop anchors and TAD boundaries, where they, when trapped, can lead to genomic instability, promoting the formation of oncogenic fusions (Gothe *et al*, 2019, *Mol Cell*). Whether TOP2s relieve topological constraints at these positions and/or participate in 3D chromosome folding remains unclear. To address this question, we have combined 3D genomics, imaging and GapRUN, a method for genome-wide profiling of positive supercoiling, to assess the role of TOP2s in shaping chromosome organisation in human cells. Our work showed that acute TOP2 depletion led to the emergence of new, large-scale contacts at the boundaries between active, positively supercoiled and lamina-associated domains. TOP2-dependent changes at the higher-order chromatin folding were accompanied by remodelling of chromatin-nuclear lamina interactions and gene expression changes, while at the chromatin loop level, TOP2 depletion predominantly remodelled transcriptionally-anchored, positively supercoiled loops. We propose that TOP2s act as a fine-regulator of chromosome folding at multiple scales.

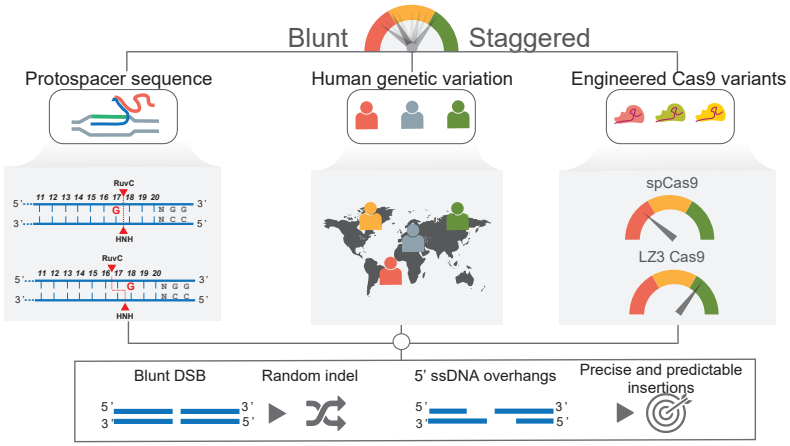

Figure 1. The protospacer sequence, human genetic variation and engineering Cas9 variants can dictate Cas9 scission profile, which is strongly associated with precise and predictable genome editing.

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. 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. In a different direction, we will perform directed evolution and saturation mutagenesis experiments to engineer novel Cas9 variants with higher specificity and predictable editing and will identify the determinants of other Cas9 nucleases, such as Cas12.

SELECTED PUBLICATIONS

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Longo GMC*, Sayols S*, Kotini AG, Heinen S, Möckel MM, Beli P and Roukos V (2024) Linking CRISPR-Cas9 double-strand break profiles to gene editing precision with BreakTag. *Nat Biotechnol*, doi: 10.1038/s41587-024-02238-8

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

*indicates joint contribution , #indicates joint correspondence

Sandra **Schick**

“We decipher how chromatin regulation influences development & diseases.”

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 PhD in Molecular Biology, Institute of Molecular Biology (IMB), Mainz

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

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

GROUP MEMBERS

Postdoc Julia Varga

PhD Students Sam Ezrael Dela Cruz, Marie Kube, Thi Tinh Nguyen*, Christina Ntasiou, Karolina Romaniuk*, Alina Schaaf, Samuel Shoup, Katharina Spang

Master Students Thérèsa Koppel, Mara Wolf, Julia Ziegler

Student Assistants Amy-Sue Sattler, Mara Wolf

*indicates joint PhD students

OVERVIEW

The condensation of the genome into higher-order chromatin structures requires various dynamic regulatory mechanisms that control the spatiotemporal organisation of genomic processes. These regulatory mechanisms ensure proper gene expression and as such the appropriate execution of all cellular processes. To achieve this, 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. To uncover how these regulators integrate and contribute to gene regulation, genome stability and other genomic processes, we employ human cellular model systems and mouse models 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 to unravel the mechanisms underlying diseases and to 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, also known as the mammalian SWI/SNF complexes. These remodellers are polymorphic complexes comprised of multiple subunits that are encoded by around 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. They are also crucial for 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 further highlights the importance of these remodellers. Therefore, it is of great relevance to elucidate the functions of the diverse BAF complexes and the molecular consequences of mutations in genes encoding BAF complex subunits. These insights will likely enable the development of new targeted therapeutics for BAF-associated diseases.

To achieve this, we systematically investigate the role of distinct BAF complexes in different cellular processes in conventional cell lines using a wide variety of experimental approaches, ranging from live-cell and super-resolution microscopy to genomics and proteomics. Using these approaches, we observe BAF subtype-specific regulatory mechanisms, sometimes with opposing effects. In addition, we have established human organoid cultures that closely reflect the development and cellular heterogeneity of organs. These models allow us to investigate the role of BAF complexes in more physiological settings and to unravel their cell type-specific roles. For example, it has been shown that their composition and function can differ by cell type and changes during development. In addition, these models offer a great opportunity 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. Apart from this, we use mouse models to study the role of BAF complexes in specific cell types and explore alterations that occur during ageing and may promote age-related disorders.

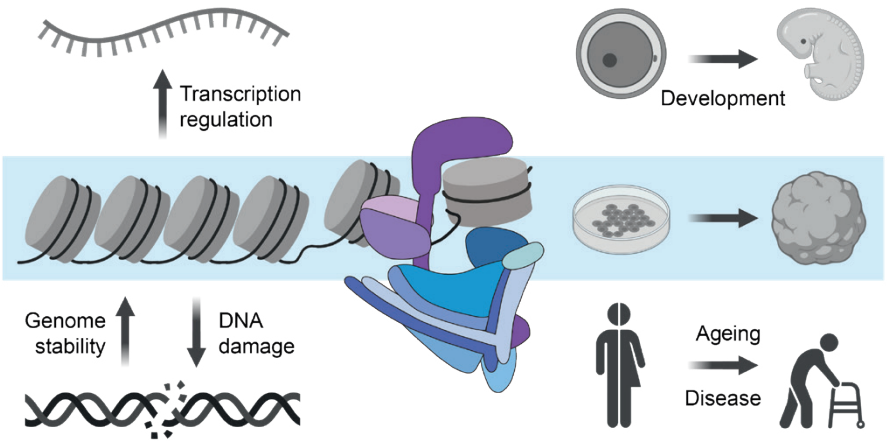


Figure 1. Multi-level investigation of BAF complexes under physiological and pathological conditions. BAF chromatin remodellers are key regulators in many biological contexts. Our research investigates BAF complexes from multiple perspectives, from the molecular to the organismal level. At the molecular level, we study how BAF complexes control transcription and genome stability. In developmental or tissue contexts, we investigate their function mainly using organoid models. With this multi-perspective approach, we aim to elucidate the role of dysregulation of BAF complexes in various diseases such as cancer, neurodevelopmental disorders and ageing – conditions that are the focus of our research.
Figure was created using BioRender. Schick S (2024) and Adobe Illustrator.

FUTURE DIRECTIONS

We will further explore the molecular function and regulation of 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, disease and ageing. For example, we will systematically explore the role of different BAF complex subunits in brain development and how their disruption by mutations results in neurodevelopmental disorders. Ultimately, our research aims to unravel pathogenic mechanisms that can be targeted for therapy.

SELECTED PUBLICATIONS

Nguyen TT, Baumann P, Tüscher O, Schick S and Endres K (2023) The aging enteric nervous system. *Int J Mol Sci*, 24:9471

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

*indicates joint contribution , #indicates joint correspondence

Lukas **Stelzl**

"We study how dynamic self-organisation gives rise to specific molecular recognition in cells."

POSITIONS HELD

2024	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Professor of Biomolecular Simulations, Johannes Gutenberg University Mainz (JGU)
2020 – 2024	Adjunct Group Leader, Institute of Molecular Biology (IMB), Mainz
	ReALity Junior Group Leader, Johannes Gutenberg University Mainz
2015 – 2020	Postdoctoral Fellow, Max Planck Institute of Biophysics, Frankfurt am Main

EDUCATION

2015	PhD in Biochemistry, University of Oxford
2010	MSc in Molecular and Cellular Biochemistry, University of Oxford

GROUP MEMBERS

PhD Students Ritika Aggarwal, Lucia Baltz, Denís Arribas Blanco, Arya Changiarath Sivadasan, Kumar Gaurav, Cyrille Ngueldjou, Jonas Paulus, Xiaofei Ping, Vasilis Xenidis, Mahesh Yadav, Emanuele Zippo

Master Students Maximilian Mager, Leon Persch

Student Assistants Aayush Ayra, Yehor Tuchkov, Rebecca Ziora

OVERVIEW

We aim to elucidate how liquid-liquid phase separation and phase-separated condensates of proteins and nucleic acids provide specific regulation and how this is lost in pathologies. We are a computational group that uses chemically detailed multi-scale simulations of biomolecules in our research (Stelzl *et al*, 2020, *eLife*; Stelzl* & Pietrek*, 2022, *JACS Au*), bridging atomic-resolution simulations to phase-separated condensates (Grujis da Silva *et al*, 2022, *EMBO J*). The discovery that liquid-liquid phase separation and phase-separated condensates of proteins and nucleic acids are important regulators is revolutionising our understanding of cell biology. Phase separation organises biological functions in time and space. Thus, it not only plays an important role in regulating genes at the transcriptional level, but also at the post-transcriptional level. Dysregulation of liquid-liquid phase separation is hypothesised to be an important driver of ageing and age-related diseases.

RESEARCH HIGHLIGHTS

With Jan Padken (IMB), we showed that RNA polymerase II CTD forms distinct condensates to regulate transcription initiation and elongation (Changiarath *et al*, 2024, *bioRxiv*). In simulations, phosphorylation of RNA polymerase II CTD triggers the formation of two distinct condensates for transcription initiation and elongation, respectively, which could underpin differential recruitment of transcription machinery components. We identified Pro-Tyr interactions (Flores-Solis *et al*, 2023, *Nat Comm*), which may be important for recruiting the Mediator complex to the CTD phase for transcription initiation. Intriguingly, the condensate phase of unphosphorylated CTD is fully and partially engulfed by condensates of phosphorylated CTD and elongation factors. Super-resolution microscopy in *C. elegans* by Jan Padeken confirmed the existence of these multi-phasic condensates. We trained a neural network on simulations of CTD condensates with different proteins (Changiarath *et al*, 2024, *Faraday Discuss*) to identify residues that can interact with the CTD. Based on this, we inverted the morphology of CTD condensates. We asked the neural network to design

suitable peptides and investigated them in simulations. The results are in turn fed into the neural network, which also predicted that heterochromatin-associated protein sequences identified by the Padeken lab interact poorly with CTD. This shows how combining molecular dynamics and neural networks can provide biologically meaningful insights.

Our simulations predicted that CTD condensates would be more stable at higher temperatures, contrary to most protein condensates. *In vivo* experiments by the Padeken group agreed with our simulations and showed gene expression is modulated as a consequence. Taken together, we are starting to explain how CTD condensates can regulate different biological processes.

Together with the Ketting lab (in CRC 1551), we are elucidating how different phase-separated condensates specifically recruit proteins. Mutator foci are essential for small RNA biology in *C. elegans* and for suppressing transposable elements. To do this, they recruit proteins with the scaffold protein Mut-16. Mut-16 recruits Rde-2/Mut-7, but how Rde-2 binds to Mut-16 is not understood. Using multi-scale simulations, we resolved how the disordered prion-like domain of Rde-2 binds Mut-16 condensates at atomic resolution (Gaurav *et al*, 2024, *bioRxiv*). Simulations highlighted specific Arg residues which were also found to be important *in vitro* experiments. Subsequent experiments showed that our simulations correctly predicted the phase separation propensities of different MUT-16 regions.

With Dorothee Dormann (IMB/JGU) we study TDP-43 phosphorylation, which is a hallmark of neurodegenerative disease. We are elucidating how interactions between different parts of TDP-43 shape its behaviour and how they are influenced by disease-linked phosphorylation. Recently, we developed a new simulation method to study ATP-driven processes in cells (in the CRC/TRR 146; Zippo *et al*, 2024, *bioRxiv*) and are using this to simulate how TDP-43 is enzymatically phosphorylated by casein kinase 1δ (Ck1d). In the simulations, TDP-43 condensates dissolve as they become phosphorylated (Figure 1), suggesting that sequence patterning rather than sequence position determines which residues are the most readily phosphorylated.

Figure 1. Snapshots from a simulation of enzymatic phosphorylation of TDP-43 by Ck1d. The low complexity domain (LCD) of TDP-43 is shown in turquoise. Ck1d is shown in blue. Phosphorylated Ser residues are highlighted in red. Adapted from Zippo *et al*, 2024, *bioRxiv*.

FUTURE DIRECTIONS

We will continue to further develop simulation methods to improve our models and better match experimental complexity. The group is also part of the CRC 1552 "Molecular defects in soft matter" and GRK 2516 "Control of structure formation in soft matter at and through interfaces", where we focus on the recognition of PTMs and small molecules by proteins and the control of dynamic self-organisation as applied to artificial DNA-based transmembrane receptors, respectively. We are also part of the new Carl Zeiss Centre MAINCE, where we are combining simulations with neural networks to understand on- and off-target interactions of small molecules with complex mixtures of proteins. Longer term, our simulation methods will be vital for understanding cellular homeostasis, including the proper functioning of phase-separated condensates. In the future, we will combine molecular dynamics simulations and neural networks to understand the principles of dynamic self-organisation and how these underpin biological function.

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

Stelzl LS*, Pietrek LM*, Holla A, Oroz J, Sikora M, Köfinger J, Schuler B, Zweckstetter M and Hummer G (2022) Global structure of the intrinsically disordered protein Tau emerges from its local structure. *JACS Au*, 2:673–686

Zippo E, Dormann D, Speck T and Stelzl LS (2024) Molecular simulations of enzymatic phosphorylation of disordered proteins and their condensates. *bioRxiv* , doi: 10.1101/2024.08.15.607948

*indicates joint contribution

Helle **Ulrich**

"We use custom enzymes to explore the functions of polyubiquitin linkage."

POSITIONS HELD

Since 2013	Scientific Director, Institute of Molecular Biology (IMB), Mainz
	Professor, Johannes Gutenberg University Mainz (JGU)
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	Diplom in Biology, Georg August University Göttingen

GROUP MEMBERS

Team Leaders Maximilian Reuter, Hans-Peter Wollscheid

Postdocs Katarzyna Maslowska, Cindy Meister, Kirill Petriukov, Christian Renz, Virender Kumar Sharma, Jie Shi, Ronald Wong, Nicola Zilio

PhD Students Kezia Ann, Nadia Da Silva Fernandes*, Yael Hartig, Wiktoria Kabza, Yogita Mallu Kattimani*, Nils Krapoth, Aina Mas Sanchez*, Philipp Schönberger, Markus Schraft, Tina Strauch, Abhik Thapa

Master Student Vanessa Rauthe

Bachelor Students Majd Hadji, Christian Jabs, Julia Reidel

Lab Managers Julia Jager, Valerie Madsen, Violeta Morin, Ulrike Seeburg

Student Assistant Lea Helfrich

Personal Assistant Jutta Karn

*indicates joint PhD students

OVERVIEW

Our lab studies the mechanisms that contribute to the regulation of DNA repair and the management of DNA replication stress, especially as they relate to the posttranslational protein modifier ubiquitin. By modulating the activities, interactions or the stability of its target proteins, ubiquitin participates in the regulation of virtually all cellular pathways. The structural diversity of polyubiquitin chains, collectively called the 'ubiquitin code', is thought to determine the fate of the modified proteins. Although many analytical and inhibitory reagents exist to manipulate the ubiquitin system, we lack the tools to create polyubiquitin chains of defined linkage on a protein of interest in cells. We have now established a method to induce substrate-specific polyubiquitylation via three major linkages *in vitro* and in cells. This allows us to investigate the functional consequences of a specific ubiquitylation event in isolation from its native signal and probe the relevance of polyubiquitin chain linkage.

RESEARCH HIGHLIGHTS

Numerous tools exist to decipher the ubiquitin code, including antibodies, affinity probes and proteomic methods to analyse polyubiquitin chains, and linkage-selective deubiquitylation enzymes or dominant-negative ubiquitin mutants to inhibit chain formation. Compared to these analytical and inhibitory tools, our ability to create defined ubiquitylation patterns is much more limited. Notably, up until now it has been impossible to enforce the polyubiquitylation of a protein of interest with the desired linkage in cells. Yet, being able to do so would be essential to separate the consequences of a ubiquitylation event from the signal that normally induces it. Moreover, it would allow for the targeted polyubiquitylation of proteins that are not normally subject to such modification. Finally, fundamental questions about the relevance of a given linkage for a particular biological function cannot be addressed without selectively altering the linkage of an individual ubiquitylation event – which has been impossible due to the inherent preferences of the enzymes responsible for the modification.

Our newly established 'Ubiquiton' tool combines tailor-made, linkage-specific ubiquitin protein ligases (E3s) with a generalised substrate targeting strategy that is based on split ubiquitin fused to a rapamycin-inducible pair of dimerisation domains (Figure 1A-C). We have developed enzymes for M1-, K48-, and K63-specific polyubiquitylation of substrates carrying a matching ubiquitin acceptor tag, thus representing the three most abundant linkages detectable in cells. Following *in vitro* validation (Figure 1D), we demonstrated functionality in budding yeast and mammalian cells for a range of soluble cytoplasmic and nuclear as well as membrane- and chromatin-associated proteins (Figure 1E). We found that the K48-specific Ubiquiton can serve as an efficient rapamycin-inducible degradation signal, comparable but orthogonal to other established degron systems such as the auxin-inducible AID-tag (Figure 1F). We also applied the Ubiquiton tool to induce endocytosis and subsequent lysosomal degradation of model plasma membrane proteins in yeast and human cells. In this manner, we confirmed that a K63-linked ubiquitin chain is necessary and sufficient to drive the internalisation of a cargo protein. A linear (M1-linked) polyubiquitin chain is able to replace the physiological K63-chain in yeast, whereas induction of K48-linked polyubiquitylation of the same protein results in extraction from the membrane and proteasomal degradation. Finally, we have implemented an alternative substrate recruitment system based on a regulable GFP-specific nanobody, which avoids unwanted side effects resulting from the use of rapamycin and at the same time affords improved reversibility. Thus, our proof-of-concept applications of the Ubiquiton tool illustrate its versatility and demonstrate how it can be used to elucidate the signalling mechanisms of polyubiquitin chains.

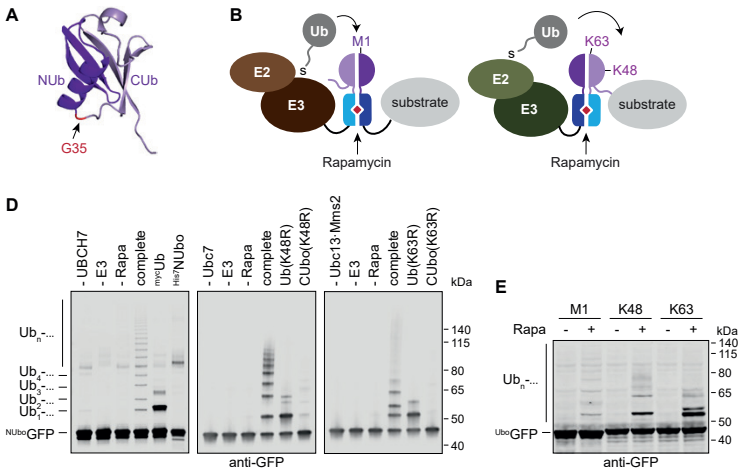


Figure 1. Design and application of the Ubiquiton system. A) Structure of split ubiquitin. B) Schematics of the Ubiquiton system, illustrating substrate recruitment and formation of the acceptor ubiquitin via rapamycin-inducible dimerisation domains (blue) fused to split ubiquitin (purple). Relevant acceptor lysine (K) residues are indicated. C) Use of the Ubiquiton system for M1-, K48- and K63-polyubiquitylation. D) Ubiquiton-mediated polyubiquitylation of GFP, detected by western blotting. E) Ubiquiton-mediated polyubiquitylation of GFP in budding yeast. F) Inducible degradation of GFP tagged with an auxin-inducible degron and the rapamycin-inducible K48-specific Ubiquiton in yeast.

FUTURE DIRECTIONS

Based on the Ubiquiton technology, we plan to develop a new generation of research tools for the scientific community. This will include the identification and design of custom enzymes for assembling the rarer, still poorly characterised non-canonical linkages and a functionalisation with modules for chain editing, branching and the identification of effectors. We will apply these tools to major genome maintenance pathways with prominent roles in the defence against disorders such as cancer and premature ageing. We envision that our research will not only provide new insight into ubiquitin signalling in genome maintenance, but that the tools developed in this manner will facilitate future investigations of polyubiquitin chains, and their readers and writers in other signalling pathways.

SELECTED PUBLICATIONS

Renz C, Asimaki E, Meister C, Albanèse V, Petriukov K, Krapoth NC, Wegmann S, Wollscheid HP, Wong RP, Fulzele A, Chen JX, Léon S and Ulrich HD (2024) Ubiquiton – an inducible, linkage-specific polyubiquitylation tool. *Mol Cell*, 84:386-400

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

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

Sara **Vieira-Silva**

"We examine human gut microbial populations to assess their impact on disease & therapeutic outcomes."

POSITIONS HELD

Since 2022	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Professor, University Medical Center, Johannes Gutenberg University Mainz (JGU)
2022	Group Leader, University Medical Center, Johannes Gutenberg University Mainz (JGU)
2015 – 2022	Postdoc, Catholic University of Leuven (KU Leuven)
2011 – 2015	Postdoc, Free University of Brussels

EDUCATION

2007 – 2010	PhD in Genomics, Pierre and Marie Curie University/Institut Pasteur, Paris
2005 – 2006	Postgraduate studies in Computational Biology, Gulbenkian Institute of Science, Oeiras
2003	Diploma in Biology, University of Lisbon (FCUL)

GROUP MEMBERS

Staff Scientist Gwen Falony

PhD Students Javier Centelles-Lodeiro*, Bharat Joshi, Laura Peschke

*indicates joint PhD student

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 and/or to therapeutic success or failure. 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 gut microbiome perturbations as a risk factor for disease development and help develop strategies for microbiota remediation in 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 inflammatory bowel disease

Inflammatory bowel disease (IBD) comprises a range of complex multifactorial disorders of the gastrointestinal tract triggered by both innate and adaptive immune responses to environmental factors, often in genetically predisposed individuals. The gut microbiota plays a role both as an immune modulator and a triggering environmental factor. Using a large cohort of patients with IBD followed longitudinally while ongoing treatment with various biologicals (immune modulators), we explored – using quantitative microbiota profiling – how the microbiota is differentially altered depending on IBD presentation and different medications. First, we found that disease location (ileum vs colon) impacted microbiota composition more than IBD presentation, separating

patients with Crohn's disease (CD, patchy inflammation with ulcers) from those with ulcerative colitis (UC, uniform colonic inflammation). Second, we identified specific opportunistic bacteria whose abundance quantitatively correlated with inflammation and its dampening after treatment, suggesting a potential role in unsuccessful therapeutic outcomes.

How to predict the best therapy choice for patients with inflammatory bowel disease

Using a large prospective cohort of patients with IBD followed longitudinally while undergoing treatment with various biologicals (immune modulators), we build machine learning models to predict therapeutic outcomes. These models were built using patient clinical data and microbiota-derived features to evaluate which features (prior to therapy) would best classify their response to therapy. A model based on clinical data, stool features and classification of dysbiosis allowed us to predict the treatment outcomes of different biologicals with 73.9% accuracy. This model further allowed us to predict the best alternative therapy for non-responders, the accuracy of which could be evaluated in 26 patients with posterior interventions (65% accuracy). A refined version of such a model, built from data obtained from a randomised intervention trial, would be of value to aid in prioritising biological choice for optimal therapeutic outcomes in inflammatory bowel disease.

Quantitative approach for better assessment of fecal microbiota transplant efficacy in inflammatory diseases

The transplant of healthy donor stools into patients – fecal microbiota transplant (FMT) – has proven to be highly successful in the competitive exclusion of dangerous, antibiotic-resistant pathogens such as *Clostridioides difficile*. As a therapeutical approach in inflammatory diseases however, it remains of minimal efficacy. Design limitations have made it difficult to learn lessons from past trials, notably the lack of quantification of the transfers in terms of microbial load. With our clinical partners (UZ Leuven, Belgium), we organised a multi-centric, double-blind, sham-controlled, randomised trial with quantified FMTs. While it was halted for futility (not meeting the primary endpoint), its strict design provided the opportunity for constructive lessons for the next trial, which will address FMT density and viability of cells prior to administration, and frequency of administration.

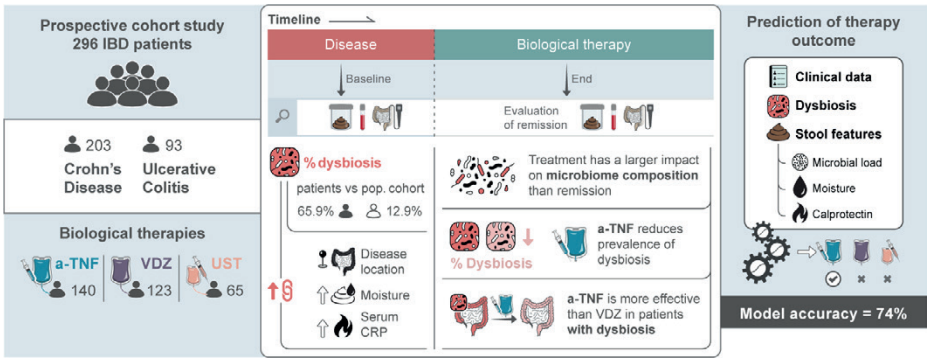


Figure 1. Graphical abstract (adapted from Caenepeel *et al*, 2024). Building machine learning models for the prediction of response to biological therapy in inflammatory bowel disease (based on patients' clinical status and microbiome composition).

FUTURE DIRECTIONS

Our group will continue to devise new approaches to study the role of human gut microbiota in health and its perturbation as a potential risk factor for increased disease susceptibility throughout life.

We aim to uncover how gut microbiota composition modulates therapeutic efficacy and patient outcomes to devise new remediation approaches for personalised medicine.

SELECTED PUBLICATIONS

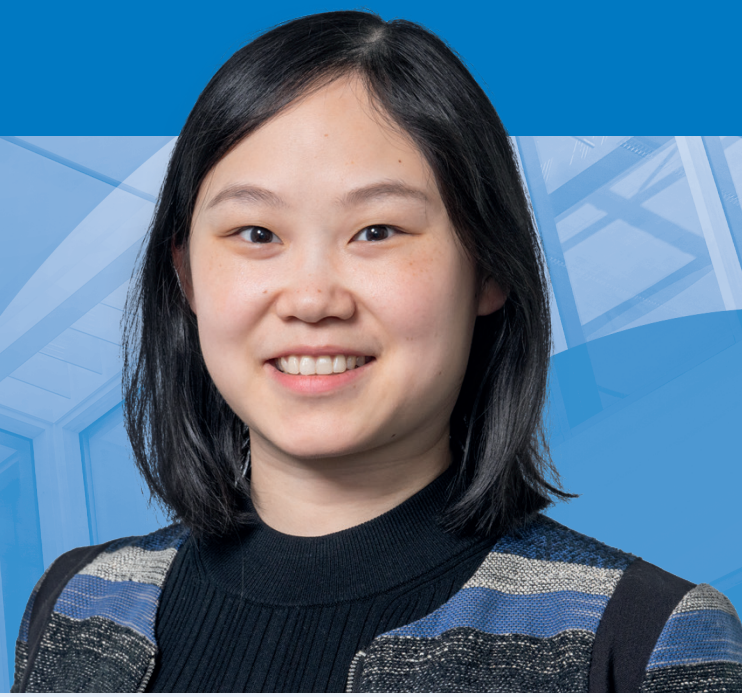
Caenepeel C*, Falony G*, Machiels K, Verstockt B, Goncalves PJ, Ferrante M, Sabino J, Raes J*, Vieira-Silva* S and Vermeire S* (2024) Dysbiosis and associated stool features improve prediction of response to biological therapy in inflammatory bowel disease. *Gastroenterology*, 166:483-495

Valles-Colomer M*, Bacigalupe R*, Vieira-Silva S*, Suzuki S, Darzi Y, Tito RY, Yamada T, Segata N, Raes J* and Falony G* (2022) Variation and transmission of the human gut microbiota across multiple familial generations. *Nat Microbiol*, 7:87-96

Vieira-Silva S*, Falony G*, Belda E*, Nielsen T, Aron-Wisnewsky J, Chakaroun R, Forslund SK, Ass-mann 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

*indicates joint contribution

Siyao **Wang**



"We study the transgenerational implications of DNA damage."

POSITIONS HELD

Since 2023 Group Leader, Institute of Molecular Biology (IMB), Mainz

Since 2022 Group Leader, Institute for Genome Stability in Ageing and Disease (IGSAD), University Hospital of Cologne

2015 – 2022 Postdoc, CECAD, University Hospital of Cologne

EDUCATION

2015 PhD in Molecular Cancer, University of Manchester

2010 BMed in Preclinical Medicine, Southern Medical University, Guangzhou

GROUP MEMBERS

PhD Students Rose Mary Roshan, Jóhann Örn Thorarensen

Lab Technician Neda Bakhshandeh

Student Assistant Nadine Spiegler

OVERVIEW

DNA damage poses a major threat to genome stability, chromosomal integrity and cellular function. Defects in transcription-coupled nucleotide excision repair (TC-NER) cause growth and mental retardation, photosensitivity and premature ageing in Cockayne syndrome (CS) patients. To ensure the success of DNA repair, chromatin serves as a platform and is dynamically changed during the DNA damage response (DDR), as described by the Access-Repair-Restore model. As a crucial part of chromatin, histones are post-translationally modified via methylation, ubiquitination and acetylation to regulate DDR-related chromatin functions. Importantly, in contrast to the transient process of DNA repair, many histone modifications can leave a long-term epigenetic memory in cells and be passed down to further generations, raising the question of whether DNA damage could reshape the epigenome in damaged cells and even affect their descendants. My lab uses *C. elegans* as a model to study the role of histone modifications on genome stability, longevity and transgenerational inheritance.

RESEARCH HIGHLIGHTS

Transgenerational inheritance of paternal DNA damage via histone-mediated DNA repair restriction

Epigenetic modifications are well-known for their role in the transgenerational inheritance of several traits, including longevity. However, whether DNA damage-induced epigenetic alterations can lead to a transgenerational effect is still unknown. The transgenerational effect of DNA damage was previously studied mainly via epidemiological and genetic approaches, but contradictory observations were obtained. Interestingly, many studies pointed to the hypothesis that the transgenerational effect is attributed to paternal, but not maternal, DNA damage, although the mechanism underlying this phenomenon was unclear.

Previously, I identified a novel mechanism underlying the transgenerational genetic and epigenetic effect of paternal DNA damage. Using sex-separated *C. elegans* strains, we found that paternal, but not maternal, ionising radiation (IR) exposure leads

to transgenerational embryonic lethality. We also determined that IR-induced paternal DNA double-strand breaks (DSBs) are mainly repaired via maternally-provided error-prone polymerase-theta mediated end joining (TMEJ), while on the other hand, maternal DNA DSBs mainly engage in error-free homologous recombination repair (HRR). Consequently, offspring of irradiated males display various genome instability phenotypes, including chromosomal bridging, chromosomal lagging and DNA fragmentation. This persistent paternal DNA damage in the offspring of irradiated males triggers an alteration in the epigenome during gametogenesis, as increased linker histone H1 levels and excessive heterochromatic regions were detected in the F1 generation (Figure 1). Knockdown of histone H1 or heterochromatin protein HPL-1 can significantly reduce heterochromatin formation in the germline of the F1 generation, activate error-free HRR and consequently rescue the high embryonic lethality observed in the F2 generation. This work identified a novel mechanism for transgenerational inheritance of paternal DNA damage and provided a potential therapeutic target for improving the progeny viability of radiotherapy-treated patients.

H3K4me2 regulates the recovery of protein biosynthesis and homeostasis following DNA damage

How DNA damage reshapes the epigenome and influences ageing is a fascinating question. I previously identified a specific role for histone 3 lysine 4 (H3K4me2) di-methylation in the recovery of protein biosynthesis and homeostasis following UV-induced TC-NER. Upon UV treatment, H3K4m2 deposition is transiently increased in the somatic tissues of *C. elegans*. Blocking the deposition of H3K4me2 by removing the H3K4 methyltransferase complex MLL/COMPASS results in developmental arrest and lifespan shortening after UV treatment. In contrast, elevating H3K4me2 by depleting the histone demethylase SPR-5 can accelerate development and extend lifespan upon UV damage. Specifically, we have shown that UV-induced H3K4me2 facilitates the transcriptional recovery of protein biosynthesis and homeostasis genes. Repressing protein biosynthesis by treating worms with the translational inhibitor cycloheximide can reverse the beneficial effect of elevating H3K4me2 deposition upon UV treatment. This study highlights the importance of H3K4me2 in the regulation of development and ageing in somatic tissues following transcription-blocking DNA damage.

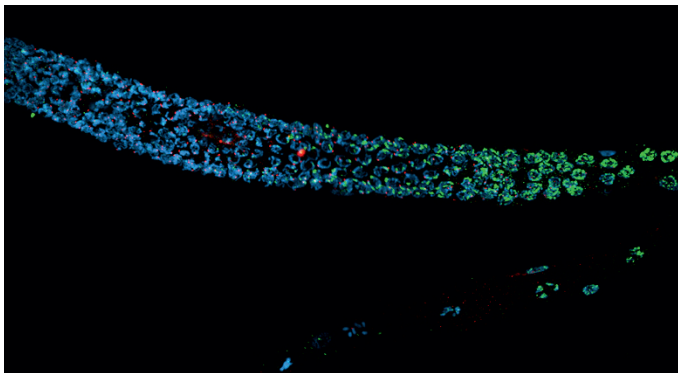


Figure 1. Heterochromatin formation in the germline of the F1 generation. H3K9me2 (green), HIM-8 (red) and DAPI (blue).

FUTURE DIRECTIONS

Our future work will explore the long-term and transgenerational effects of DNA damage on the epigenome and protein homeostasis. We will use the well-established ChIP-seq technique and SILAC proteomics analysis to monitor the deposition of epigenetic modifications and proteome alterations at different time points and generations following DNA damage. Meanwhile, to understand the transgenerational effect of paternal DNA damage, we will use RNA-seq, ATAC-seq and Hi-C techniques to examine transcriptional regulation and chromatin conformation in the subsequent generations following paternal radiation exposure. In addition, we will measure *de novo* mutations, DNA fragmentation and chromosomal rearrangements in the subsequent generations through whole-genome sequencing, karyotype analysis and comet assay. Importantly, by screening mutants deficient in different epigenetic modifications, we will be able to identify the role of epigenetic regulation in paternally inherited genome instability and find potential therapeutic targets for paternal hereditary disorders.

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Soltanmohammadi N*, Wang S* and Schumacher B (2022) Somatic PMK-1/p38 signaling links environmental stress to germ cell apoptosis and heritable euploidy. *Nat Commun*, 13:701

Wang S, Meyer DH and Schumacher B (2020) H3K4me2 regulates the recovery of protein biosynthesis and homeostasis following DNA damage. *Nat Struct Mol Biol*, 27:1165–1177

*indicates joint contribution

Sina **Wittmann**

"We study how intrinsically disordered regions contribute to transcriptional condensate formation."

POSITIONS HELD

Since 2023 Group Leader, Institute of Molecular Biology (IMB), Mainz

2017 – 2023 Postdoc, Max Planck Institute of Molecular Cell Biology & Genetics, Dresden

EDUCATION

2017 PhD in Biochemistry, University of Oxford

2012 MSc in Biochemistry, University of Regensburg

2009 BSc in Biochemistry, University of Regensburg

GROUP MEMBERS

PhD Students Radhika Khatter, Felizitas Stiehler

Bachelor Student Mia Behrensmeyer

Lab Technician Franziska Roth

Research Assistant Mahdi Narimani

OVERVIEW

In my group, we are trying to understand the molecular mechanisms by which gene activation is regulated. Many decades of intricate work have identified hundreds of proteins that participate in the gene activation process, as well as their roles. Traditionally, transcriptional research focussed on structured protein regions, which elucidated in the highest molecular detail how RNA is synthesised. However, the development of structure predictors and the emergence of AlphaFold have shown us that structured regions constitute only a small part of the transcriptional proteome. DNA-binding transcription factors were shown to be particularly disordered; these are the main focus of my research group. Here, we are trying to characterise how transcription factors use their intrinsically disordered regions to communicate with each other and with other transcriptional proteins. This communication involves the formation of small liquid-like droplets directly on DNA. We can rebuild such transcription factor droplets on single DNA molecules *in vitro*, which we use to study their formation and to biophysically characterise their properties. The knowledge gained helps us to design cellular experiments that are aimed at understanding why transcriptional droplets form in the first place.

RESEARCH HIGHLIGHTS

Formation of transcription factor droplets on DNA

Gene activation plays a major role during development and needs to be precisely regulated for different cell types to form. If this regulation breaks down, the cell loses control of its transcriptional programme, which can result in cancerous transitions.

One of the first steps in gene activation is the binding of transcription factors (TFs) to regulatory DNA regions. For this, TFs use DNA-binding domains that allow them to recognise short target sequences. However, computational prediction of TF binding in the genome is very poor, demonstrating that the presence of the motif alone is insufficient to determine localisation.

In addition to the DNA-binding domain, TFs typically contain large, disordered regions. Previous work indicates that

transcriptional proteins can use these regions to interact with each other, thereby forming little condensates on the genome. However, the function of these condensates remains elusive. The hope is that by understanding how TF condensates are regulated, we will be able to control their activity in diseases such as cancer, where they are unusually big and drive the expression of oncogenes.

In my past research, we visualised the interaction of the TF Klf4 (Krüppel-like factor 4) with individual DNA molecules *in vitro*. This protein plays a major role in keeping cells in a pluripotent state during embryonic development. My research showed that Klf4 is able to form little condensates on its own but only at high protein concentrations. Interestingly, DNA enables this condensation to occur at much lower – physiological – concentrations and specifically at sites that contain Klf4 recognition motifs.

Using a variant of Klf4 that is unable to condense, we were able to demonstrate that sequence-specific binding in the absence of condensation can only be obtained at extremely low protein concentrations. However, sequence-specific localisation in the physiological concentration range required the Klf4 variant that is able to condense. These data are very intriguing as they show a potential new role of condensation for finding regulatory DNA regions in the genome. Our results were surprising because until now, only the DNA-binding domain was thought to determine DNA localisation. We speculate this can explain why the computational prediction of TF binding is so poor. In contrast, we could show that condensation via the disordered region is also important. While the exact motif is indeed identified by the DNA-binding domain, the larger sequence context in which the motif occurs is read out by the cooperative action of hundreds of Klf4 molecules acting together inside a condensate. This mechanism allows regulatory regions to be identified and distinguished from randomly-occurring recognition sites elsewhere in the genome.

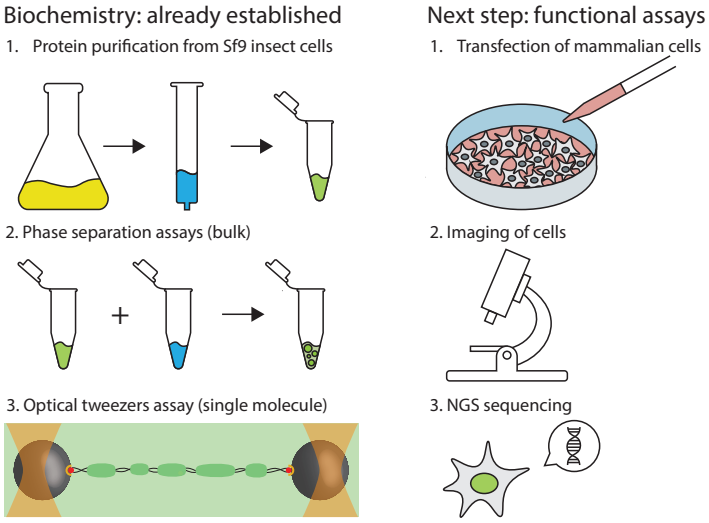

Figure 1. Methods used in the lab. Left: Schematic depiction of the biochemical methods that have been established over the last year. Right: In the future, we plan to expand our research to include *in vivo* methods. For this, we have started to culture mammalian cells that will be used for genetic manipulation and assessment of functional consequences by microscopy and different NGS sequencing techniques.

FUTURE DIRECTIONS

Going forward, we are currently establishing a cellular model to test our *in vitro* findings in living cells. On the one hand, we are looking for evidence of Klf4 condensation on DNA in a specific concentration range. At very low concentrations, we expect Klf4 to merely adsorb to DNA. At very high concentrations, the protein is predicted to phase separate in the nucleoplasm. On the other hand, we want to connect condensation to function by testing the transcriptional responses of cells with different Klf4 concentrations and (hence condensation states).

At the same time, we are studying how general TF condensation is by characterising the behaviour of different TFs on DNA. Within the CRC 1551, we also want to understand how protein sequence dictates TF condensation. This is being done in collaboration with the group of Edward Lemke (JGU/IMB), who is performing additional biophysical assays, and Martin Girard (Max Plank Institute for Polymer Research), who is simulating different protein variants.

SELECTED PUBLICATIONS

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Wittmann S, Renner M, Watts BR, Adams O, Huseyin M, Baejen C, El Omari K, Kilchert C, Heo DH, Kecman T, Cramer P, Grimes JM and Vasiljeva L (2017) The conserved termination factor Seb1 bridges RNA polymerase II and nascent RNA. *Nat Commun*, 8:14861

*indicates joint contribution

2 ADJUNCT CLINICIANS

Stephan **Grabbe**

“We study the role of β2 integrins in skin ageing & cancer immunity.”

POSITIONS HELD

Since 2022	Adjunct Clinician, Institute of Molecular Biology (IMB), Mainz
Since 2007	Director, Department of Dermatology, University Medical Center (UMC), Mainz
2003 – 2007	Director, Department of Dermatology, University of Essen Medical Center
2000 – 2003	Professor of Dermatology & Dermato-Oncology, University of Münster
1998 – 1999	Heisenberg Scholarship Visiting Scientist, Skin Disease Research Center, Brigham and Women’s Hospital, Harvard University, Boston
1992 – 1998	Research Associate, 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	MD, University of Münster
1987	Medical School, 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 addition, DCs that phagocytose a pathogen or pathogen-infected cell play a role in activating antigen-specific effector T cells. Activated cytotoxic T cells (CTL) can directly kill infected cells and tumour cells, while other activated T cells exerts helper functions (Th cells) and promote CTL activation.

Due to their versatile role, DCs are interesting targets for immunotherapeutic strategies to treat autoimmune and allergic diseases, or to mount profound and sustained anti-tumour responses. We work to test multi-functionalised nano-vaccines for their ability to activate DC and stimulate DC-mediated T cells, as well as testing 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.

β2 integrins: leukocyte adhesion molecules with multiple immune functions

β2 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 β2 integrins play in maintaining tolerance and how their dysregulation contributes to autoimmune disease, with the goal of discovering 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 β2 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 β2 integrins.

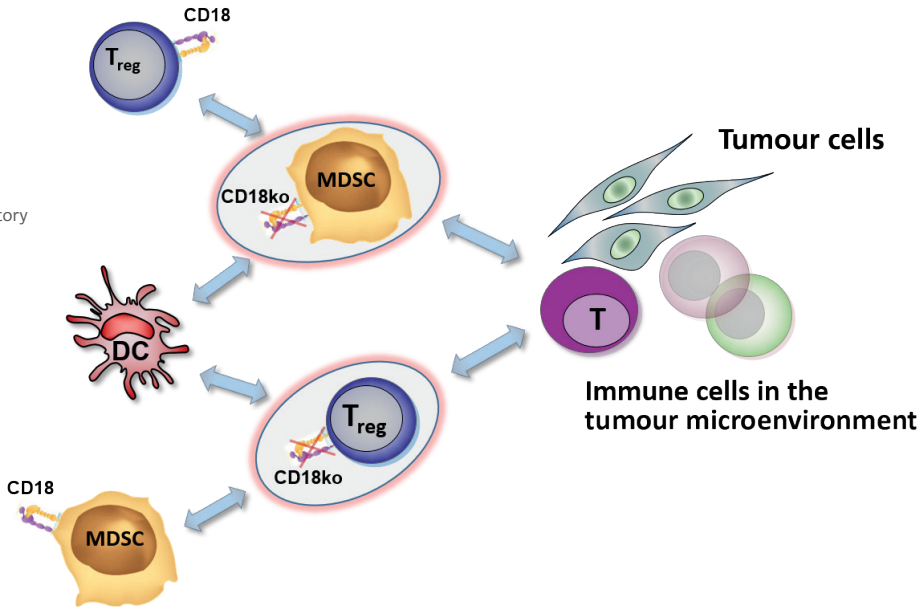

Figure 1. Relevance of β2 integrins for regulatory cell functions in tumours.

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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 work to understand the mechanisms of neuropsychiatric diseases & neurodiversity.”

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 of 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, Max Planck Institute 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 particular 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 that underlie variability in 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; these were associated with subtle behavioural abnormalities. We found that the synthesis of disease-causing protein in HD is driven by a protein complex that contains the mTOR kinase (mammalian target of rapamycin). Metformin inhibits the formation of this complex and, as we can show, substantially reduces the production of disease-causing protein in an animal model of HD. Currently, we are following the hypothesis that Huntingtin RNA and proteins assemble in a condensate with the mTOR kinase and protein phosphatase 2A through phase separation and that metformin and other small compounds might interfere with this. Furthermore, we are 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.

Patients with telomeropathies

In our rare disease clinic, we have identified a three-generation family with dyskeratosis congenita. The patients are characterised by, among other traits, early greying of hair, hyperpigmentation of the neck, pulmonary fibrosis and bone marrow failure. We have found a target mutation in the *TERC* RNA in three members of the family. We have further searched for reasons for significant anticipation of disease symptoms in the third generation and identified an *RTEL1* mutation on top of the *TERC* mutation coming from the paternal part of the family. Telomeres of *TERC* mutation carriers were short, while telomeres of *TERC* and *RTEL1* mutation carriers as well as only *RTEL1* mutation carriers were very short. Together with the Baumann group (IMB/JGU), we are currently establishing Nanopore technology to analyse the telomeres of the affected patients base by base. Furthermore, we are establishing telomerase-negative fibroblasts and telomerase-positive induced pluripotent stem cells and lymphoblastoid cells to observe how telomeres behave in the proliferating cells of affected patients.

We are using the fruitful collaboration with the Baumann lab and the Department of Pneumonology (Michael Kreuter) to establish Nanopore technology as a tool for telomere diagnostics in Mainz.

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. Using induced pluripotent stem cells, neural precursor cells, neurons and brain organoids as models, we have found that expression of X-chromosomal genes can be dynamically reactivated from the inactive X chromosome during neurodevelopment, thereby allowing facultative escape of selected genes. This substantially influences the phenotype and development of X-linked neurodevelopmental diseases in females, adding an extra layer of resilience in the female brain.

In collaboration with the ReALity community and with Claudia Keller Valsecchi (IMB), Felicia Basilicata (UMC), Joan Barau (IMB), Peter Baumann (IMB/JGU) and collaboration partners in Erlangen, we analysed a single-cell RNA sequencing dataset from human embryos and, in support of the *in vitro* system, found a highly dynamic usage of alleles from the inactive X chromosome in the developing human nervous system. We also plan to study X chromosomal gene reactivation in the developing immune system and during ageing and work to understand the molecular mechanisms underlying X chromosomal reactivation.

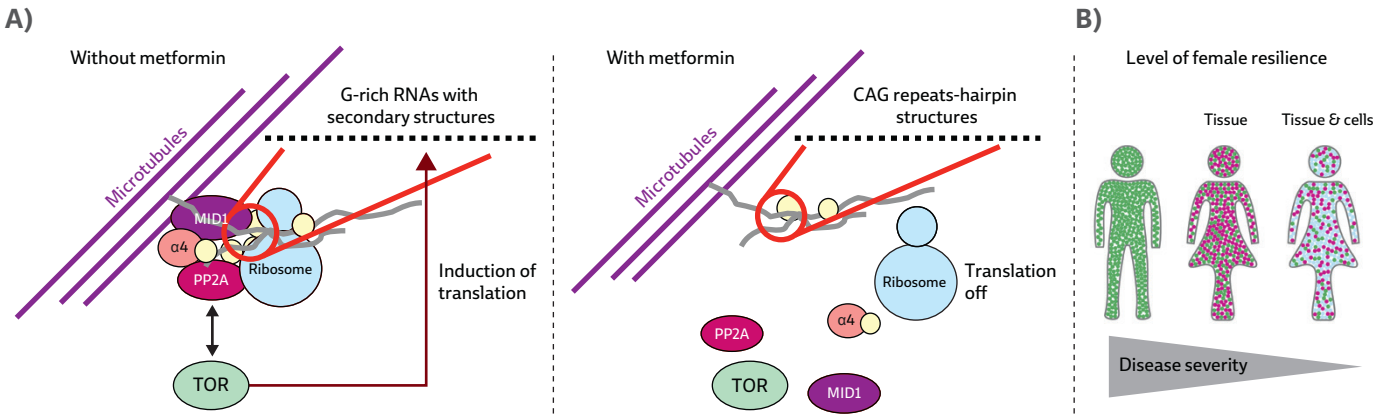

Figure 1. A) Synthesis of aberrant protein in Huntington's disease is induced by an mTOR-containing protein complex that binds to a hairpin made by the RNA containing the expanded CAG repeat. Metformin destroys this complex and thereby inhibits the synthesis of aberrant proteins in Huntington's disease. B) In females with random X-inactivation, mutations in X-chromosomal genes are expressed in 50% of cells (resilience on the tissue level). Through re-activation of the wild-type allele on the inactive X-chromosome, the phenotype in those cells expressing the mutant allele becomes milder (second level of resilience in females).

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Käseberg S*, Bertin M*, Menon R*, Gabassi E*, Todorov H*, Frank S, Brennenstuhl H, Lohrer B, Winter J, Krummeich J, Winkler J, Winner B, Weis E, Hartwich D, Diederich S, Luck K, Gerber S, Lunt P, Berninger B, Falk S*, Schweiger S* and Karow M* (2023) Dynamic X-chromosomal reactivation enhances female brain resilience. *biorXiv*, doi: 10.1101/2023.06.17.545424

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*indicates joint contribution , #indicates joint correspondence

Oliver **Tüscher**

“We discover the molecular mechanisms of resilience in ageing.”

POSITIONS HELD

Since 2024	Chair, Department of Psychiatry, Psychotherapy & Psychosomatics, University Medicine Halle (Saale)
Since 2022	Adjunct Clinician, Institute for Molecular Biology (IMB), Mainz
Since 2020	Founding Member, Research Group Leader & Head of the Clinical Investigation Center (CIC), Leibniz Institute for Resilience Research (LIR), Mainz
Since 2016	Professor of Mental Health & Cognitive Resilience in Old Age, University Medical Center (UMC) & German Resilience Center (DRZ), Mainz
Since 2015	Vice-chair, Department of Psychiatry, UMC, Mainz
Since 2013	Attending in Psychiatry & Psychotherapy, UMC, Mainz
2010 – 2013	Residency in Psychiatry & Psychotherapy, UMC, Mainz
2009 – 2010	Residency in Psychiatry & Psychotherapy, University of Freiburg
2006 – 2010	Head of the Emotion Regulation & Impulse Control Imaging Group (ERIC), Freiburg Brain Imaging, University of Freiburg
2006 – 2009	Residency in Neurology, University of Freiburg
2003 – 2006	Postdoc, Weill Medical College Cornell University, New York
2001 – 2003	Residency in Neurology, University Medical Center Hamburg-Eppendorf, University of Hamburg

EDUCATION

2013	Board Certification for Psychiatry and Psychotherapy
2010	Board Certification for Neurology
2011	Habilitation in 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

Our research focus is on resilience mechanisms in “Healthy ageing, neurodegeneration and neuropsychiatry” at the Department of Psychiatry and Psychotherapy. The group is co-led by Kristina Endres, Katharina Geschke/Isabel Heinrich and myself. We use a broad spectrum of methods applied in preclinical lab work up to clinical studies to investigate the mechanisms of healthy ageing and resilient ageing in particular. Based on our findings, we aim to develop preventive and disease-modifying therapeutic interventions. Our interdisciplinary research group includes biologists, chemists, computer scientists, psychologists and physicians, enabling us to implement findings from research on 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) to identify and investigate biomarkers and mechanisms of (resilient) 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 the following areas:

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

We are investigating the mechanism(s) conveying resilience to body and brain ageing by comprehensively assessing the (epi)genomic, proteomic, cellular-immunologic and cardiovascular phenotypes of participants in the AgeGain study (with the Bopp Lab, FZI/UMC and the Wild Lab, CTH/UMC & IMB). On the methylome level, we have been able to show that resilient ageing is associated with having a significantly younger biological age (PhenoAge epigenetic clock)

compared to "normal agers" (in collaboration with the Wild/Niehrs ReALity Project EpiHF). Intriguingly, PhenoAge correlates with the volume and the connectivity of memory-related brain structures (see Figure 1). We will further uncover the genetic and cellular senescence mechanisms related to this by comparing resilient and non-resilient participants (in collaboration with the Baumann Lab, JGU/IMB) (Fischer *et al*, 2024, *iScience*).

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 (together with the Baumann (IMB/JGU) 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 (Nguyen *et al*, 2023, *Int J Mol Sci*).

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 in humans to unravel novel biomarkers of cognitively healthy ageing (Brahmer *et al*, 2023, *Cell Commun Signal*).

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. We have just shown that 5xFAD mice (which are used as models of neurodegeneration) had significantly lower quantities of *Bacteroides* spp. in their gut microbiota when only considering frailty, and lower levels of *Bacteroidetes* when considering both frailty and chronological age compared to their wild-type littermates. Thus, the quality of ageing—as assessed by frailty measures—should be taken into account to unravel potential changes in the gut microbial community in Alzheimer's disease (Kapphan *et al*, 2023, *Microorganisms*).

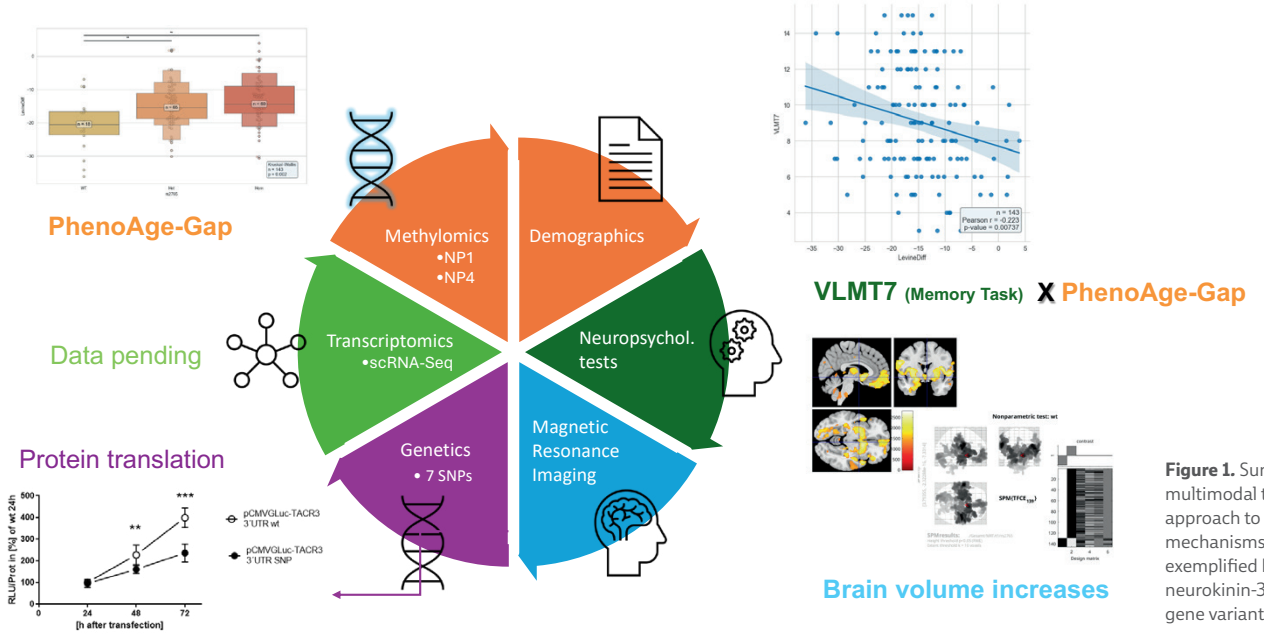


Figure 1. Summary of our multimodal translational approach to identify molecular mechanisms of resilient ageing, exemplified here with the neurokinin-3 receptor *TACR3* gene variant (rs2765).

SELECTED PUBLICATIONS

Tüscher O*, Muthuraman M*, Horstmann JP*, Horta G, Radyushkin K, Baumgart J, Sigurdsson T, Endle H, Ji H, Kuhnhäuser P, Götz J, Kepser LJ, Lotze M, Grabe HJ, Völzke H, Leehr EJ, Meinert S, Opel N, Richers S, Stroh A, Daun S, Tittgemeyer M, Uphaus T, Steffen F, Zipp F, Groß J, Groppa S, Dannlowski U, Nitsch R and Vogt J (2024) Altered cortical synaptic lipid signaling leads to intermediate phenotypes of mental disorders. *Mol Psychiatry*, 29:3537-3552

Stroh A, Schweiger S, Ramirez JM and Tüscher O (2024) The selfish network: how the brain preserves behavioral function through shifts in neuronal network state. *Trends Neurosci*, 47:246-258

Fischer FU, Gerber S and Tüscher O; Alzheimer's Disease Neuroimaging Initiative (2024) Mathematical model of the Alzheimer's disease biomarker cascade demonstrates statistical pitfall in identifying surrogates of cognitive reserve. *iScience*, 27:111188

*indicates joint contribution

Philipp **Wild**

"We use systems medicine to understand the pathomechanisms of age-related disease."

POSITIONS HELD

Since 2022	Adjunct Clinician, Institute of Molecular Biology (IMB), Mainz
Since 2020	Rhine-Main Deputy Site Speaker, German Center for Cardiovascular Research (DZHK)
Since 2015	Head of Liquid Biobank, University Medical Center (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	Professor of Clinical Epidemiology, Center for Thrombosis & Hemostasis Mainz (CTH), UMC, Mainz
Since 2012	Head of Clinical Epidemiology & Systems Medicine, Center for Thrombosis & Hemostasis (CTH), UMC, Mainz
Since 2011	Coordinating Principal Investigator & Steering Committee Member of the Gutenberg Health Study (GHS), UMC, Mainz
2010 – 2012	Senior Physician, UMC, Mainz

EDUCATION

2022	Board certification in Cardiology
2012	MSc in Epidemiology, IMBEI, 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. We focus on investigating complex common diseases, which are strongly driven by the ageing process. Our research themes range from cardiovascular diseases to cardiometabolic conditions as well as infectious diseases (e.g. SARS-CoV-2) and cancers. The study of how the ageing process induces pathological changes is a key priority for our group.

Developing tailor-made therapeutic treatments for disease

Using artificial intelligence (AI) methods and state-of-the-art high-throughput omics profiling, we holistically integrate multi-omics data with environmental exposures, (sub)clinical parameters and advanced imaging data to discover new biomarkers and biosignatures, detect diseases earlier and predict their further progression. This is the basis for the development of tailor-made therapies, diagnostics, prognostics and therapy monitoring tools to determine a patient's response to therapy.

Exemplary highlights

In 2024, the Federal Ministry of Education and Research (BMBF)-funded cluster of excellence *curATime* (funding for the first 3 of 9 years: €15 million), in which our group has multiple projects, opened several promising avenues for joint academic-industrial exploration of new therapeutic targets in cardiovascular medicine. Through the unique combination of extensive human and murine multi-omics and other multimodal data and advanced AI methods, the *curATime* cluster is on track to produce high-ranking academic output, while continuing to honour its focus of contributing to precision cardiovascular medicine through intensive academic-industrial collaboration.

As part of the BMBF-funded project MSCoreSys (Research cores for mass spectrometry in systems medicine), the multidisciplinary

Mainz research core DIASyM co-headed by myself is developing and optimising innovative methods and workflows to improve our understanding of the complex pathomechanisms underlying the development and progression of heart failure.

In 2024, the group received funding from the Carl-Zeiss Foundation for *Multi-dimensionAI,* a multi-site initiative to advance the diagnosis and treatment of heart failure with preserved ejection fraction (HFpEF) through cutting-edge AI. The project will utilise novel AI algorithms to analyse diverse patient data across multiple scales, from molecular-level omics data to broader medical imaging, enabling a more comprehensive understanding of HFpEF.

This year, we expanded our scope to lung cancer, taking part in the LUCAS (Lung cancer screening) consortium funded by the BMBF. This project investigates DNA methylation and proteomic profiles of individuals with diagnosed lung cancer in large population-based cohorts in collaboration with UCT Mainz (Thomas Kindler), DKFZ Heidelberg (Hermann Brenner) and UMIT Tirol (Uwe Siebert). The goal is to develop a non-invasive method of early patient identification that can contribute to the early triage of individuals at risk of lung cancer, so as to ultimately diagnose lung cancer in an earlier and more treatable stage.

Two new projects were launched this year in the BMBF-funded research network *EPIC-AI*, where we are investigating the complex and heterogeneous pathomechanisms of post-COVID syndrome. Using AI techniques, endotypes will be identified based on highly granular (sub-)clinical and molecular data. The team will evaluate (off-label) therapies used to treat post-COVID patients (*TheraSurv Post-COVID*) in a project funded by the Ministry of Science and Health of Rhineland-Palatinate. Using an app-based surveillance system, physicians treating post-COVID patients will enter medical treatment data into a digital registry, and the health status of the patients will be monitored weekly via smartphone-based assessments.

In the context of IMI SOPHIA, our group contributed to developing a breakthrough clinical risk prediction algorithm that categorises obesity into five distinct diagnostic profiles, each with different health consequences and treatment needs (Coral *et al*, 2024, *Nat Med*). About 20% of the population had health markers that did not match what was expected for their body weight. For example, 8% of women had elevated blood pressure, while their cholesterol levels and body fat distribution were healthier than expected, a pattern not observed in men.

This year, the Systems Medicine group significantly expanded its multi-omics resources: following the successful establishment and certification of the high-plex proteomics platform Olink Explore, based on affinity-NGS and the Proximity Extension Assay (PEA) technology, this platform was expanded to the new Explore HT system. Explore HT enables the simultaneous quantification of 5,416 proteins, making it one of the two most advanced high-plex proteomics platforms. Our laboratory was one of the first in the world to be certified for the Explore platform. To date, almost 4,000 samples from large-scale internal human studies and external cooperation partners have been analysed. The resulting comprehensive system-wide protein profiles in several biobanks have enabled unprecedented in-depth proteomic phenotyping and the identification of disease-related pathomechanisms.

In addition, the group has further expanded its clinical epigenetics resources by obtaining more DNA methylation measurements (Illumina MethylationEPIC 850k array). With a combined sample size of more than 5,000 individuals, this dataset is one of the five largest cohorts with epigenetic data on cardiovascular disease. The project builds on the existing EpiHF project, a collaboration of the ReALity/SHARP network.

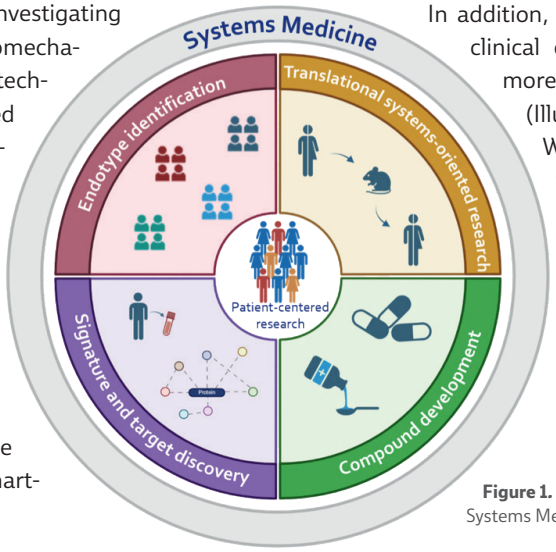


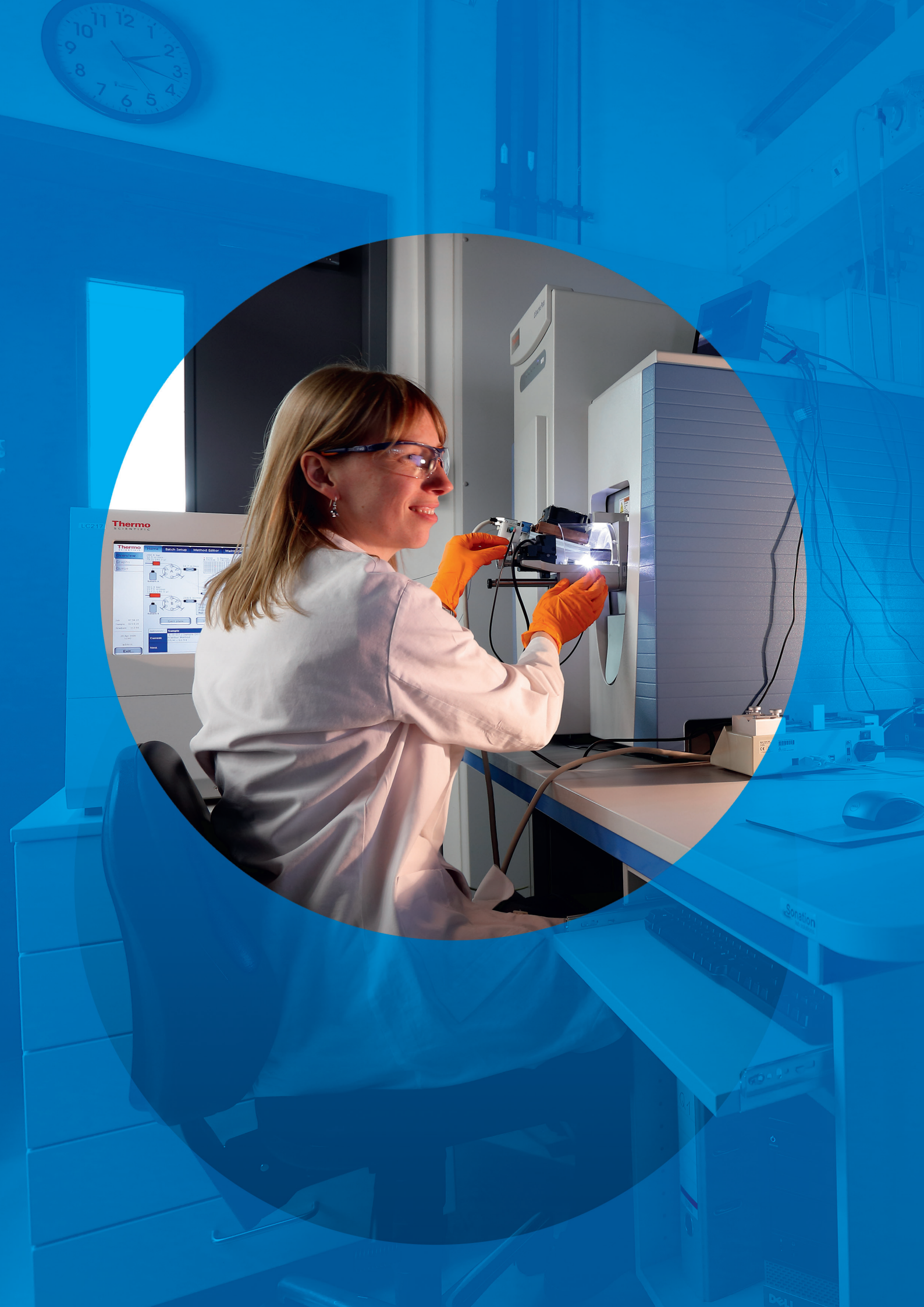
Figure 1. Areas of clinical medicine studied by the Systems Medicine Group.

SELECTED PUBLICATIONS

Müller FS, Aherrahrou Z, Grasshoff H, Heidorn MW, Humrich JY, Johanson L, Aherrahrou R, Reinberger T, Schulz A, Ten Cate V, Robles AP, Koeck T, Rapp S, Lange T, Brachaczek L, Luebber F, Erdmann J, Heidecke H, Schulze-Forster K, Dechend R, Lackner KJ, Pfeiffer N, Ghaemi Kerahrodi J, Tüscher O, Schwarting A, Strauch K, Münzel T, Prochaska JH, Riemekasten G, and Wild PS (2023) Autoantibodies against the chemokine receptor 3 predict cardiovascular risk. *Eur Heart J*, 44:4935-4949

Tröbs SO, Prochaska JH, Schwuchow-Thonke S, Schulz A, Müller F, Heidorn MW, Göbel S, Diestelmeier S, Lerma Monteverde J, Lackner KJ, Gori T, Münzel T and Wild PS (2021) Association of global longitudinal strain with clinical status and mortality in patients with chronic heart failure. *JAMA Cardiol*, 6:448-456

Ten Cate V, Prochaska JH, Schulz A, Koeck T, Pallares Robles A, Lenz M, Eggebrecht L, Rapp S, Panova-Noeva M, Ghofrani HA, Meyer FJ, Espinola-Klein C, Lackner KJ, Michal M, Schuster AK, Strauch K, Zink AM, Laux V, Heitmeier S, Konstantinides SV, Münzel T, Andrade-Navarro MA, Leineweber K and Wild PS (2021) Protein expression profiling suggests relevance of noncanonical pathways in isolated pulmonary embolism. *Blood*, 137:2681-2693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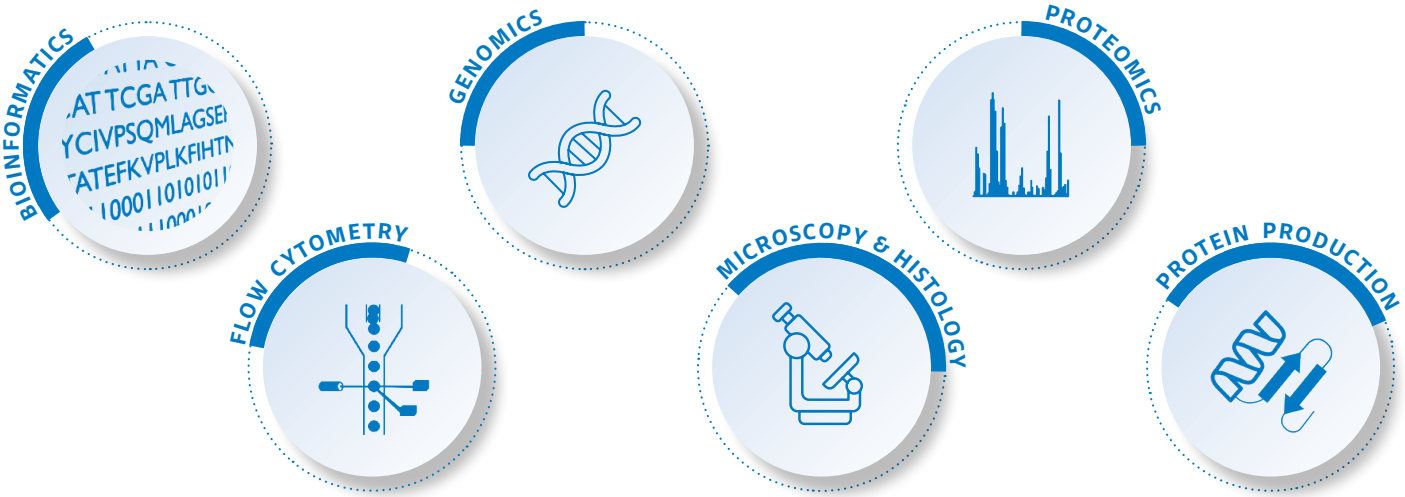
3 CORE FACILITIES

Overview

"The Core Facilities provide access to key technologies, as well as support & training by experts."

IMB has six Core Facilities (CFs):



The Bioinformatics, Genomics and Proteomics CFs provide a "full service", covering experimental design and quality control to the generation, analysis and presentation of data. The Flow Cytometry and Microscopy/Histology CFs provide an "assisted service", where researchers work independently on CF equipment after training. CF staff are available for consultation and troubleshooting for all users. Furthermore, we offer collaborations for customised or specialised services. IMB researchers can access all CFs, while external users in Mainz can access the Flow Cytometry, Genomics, Microscopy/Histology and Proteomics CFs. In addition, many CFs are involved in supporting IMB's collaborative research initiatives.

We adjust CF services based on user demand. Each facility has a user committee to provide feedback on the equipment and user experience. This also helps determine the implementation of new CF services.

Innovation is a pivotal aspect of the facilities. One example is the recent formation of the "Competence Hub for Single-Cell Genomics" and the "Competence Hub for Spatial Transcriptomics". These combine the expertise and service of two core facilities to develop new methods that go beyond the standard services of any individual facility.

We offer lectures and practical courses on new techniques and instruments, 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 IMB.

The CFs also run "Core Support Units" (CSU). The Core Equipment unit maintains a broad range of standard lab equipment and offers training as well as troubleshooting for them. The Media Lab unit supplies internal researchers with a variety of buffers, solutions and agar plates. Additional CSUs include the radionuclide lab, the S2 lab, in-house animal facilities for mice, zebrafish and *Xenopus*, and IT support.

To offer users the best and most modern research equipment, this year we purchased new state-of-the-art instrumentation, such as the C-Trap from Lumicks; the DFG provided partial funding for some of these larger equipment purchases.

Andreas Vonderheit
Director of Core Facilities and Technology

Bioinformatics

"The Bioinformatics Core Facility supports the analysis, interpretation & publication of NGS & other complex datasets."

SERVICES OFFERED

The Bioinformatics Core Facility supports researchers with computing infrastructure, software training, experimental design, biostatistics and data analysis. Our staff offer different levels of assistance depending on project needs:

- 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
- Data mining of published datasets, correlation and integration of results
- Assistance with preparing manuscripts, presentations and grant proposals
- Testing, implementation and customisation of software tools and services
- Bioinformatics and biostatistics courses

The facility maintains GitLab and GitHub repositories with software tools and pipelines for comprehensive NGS data analysis, which are also used by many computational biologists in the research groups. An ongoing major modernisation includes transitioning from Bpipe to Nextflow workflow management, containerising the entire software stack, enhancing NGS pipeline accessibility and usability, and implementing best practices using GitLab.

In addition to standard tools and pipelines, the Bioinformatics Core Facility offers customised bioinformatics solutions and long-term analytical support for numerous data-intensive projects that require expert handling for optimal results. The facility also provides bioinformatics and biostatistics expertise to the CRC 1361 on "Regulation of DNA Repair & Genome Stability" and the "Science of Healthy Ageing Research Programme" (SHARP). This year, competence hubs for single-cell genomics and spatial transcriptomics were established in collaboration with the Genomics Core Facility, together with the Flow Cytometry or Microscopy Core Facilities, respectively.

MEMBERS

Head Emil Karaulanov

Bioinformaticians Anke Busch, Antonella di Liddo, Patrick Hüther, Sivarajan Karunanithi, Nastasja Kreim, Michal Levin, Giuseppe Petrosino, Frank Rühle, Sergi Sayols Puig

Biostatistician Fridolin Kielisch

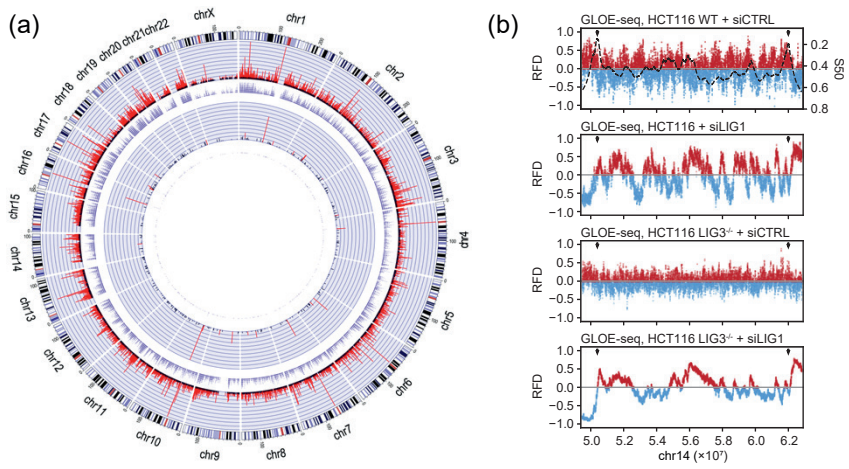


Figure 1. Genome-wide profiling of DNA strand breaks in human cells using the recently developed sBLISS (A) and GLOE-seq (B) NGS methods.

Flow Cytometry

"The Flow Cytometry Core Facility offers high-throughput measurements, analysis & separation of biological units."

SERVICES OFFERED

We offer a full service for sorting and an assisted service with training for the analysers. Additionally, staff members are available for collaborations to analyse flow cytometry data and prepare samples. During the past year, the FCCF has performed multicolour measurements, sorted isolated neuronal nuclei and performed classical enrichments for subsequent cell culture, qPCR analysis, mass spectrometry and microscopy. Together with the Genomics and Bioinformatics Core Facilities, the Flow Cytometry Core Facility established the Competence Hub for Single-Cell Genomics to perform cell separation for next-generation sequencing. We work with many different materials, including nuclei, stem cells, yeast, *C. elegans* and autophagosomes, as well as various cultured cell lines and primary cells from humans, mice, zebrafish and *Drosophila*. To educate and train users, the facility offers three different lectures, an annual practical course for basic flow cytometry analysis and an advanced practical course for cell sorting.

In 2024, we co-organised and hosted the 1st German Flow Core Summit. The facility also participated in a nationwide trial in Germany focused on UV light-based inactivation of flow cytometry waste. The objective was to develop a large-volume liquid waste disposal process that allows efficient management of waste liquids generated by flow cytometers and similar equipment. The results were also presented at several cytometry congresses, and the findings will be published and presented to the local authorities.

MEMBERS

Head Stefanie Möckel

Staff Scientist Stephanie Nick

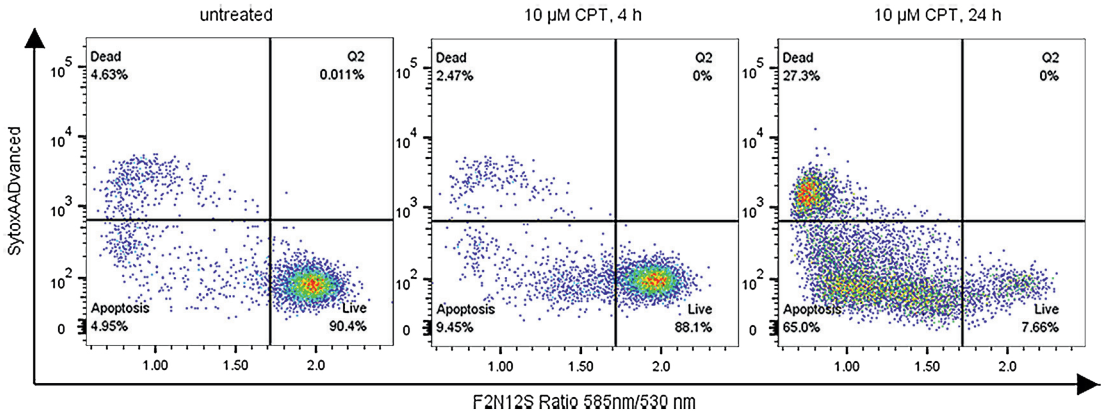


Figure 1. Apoptosis of adherent cells as measured by membrane asymmetry. HeLa cells were left untreated or treated with 10 µM camptothecin (CPT) for 4 and 24 hours. Cells were labelled with Violet Ratiometric Membrane Asymmetry Probe (F2N12S) and SytoxAADvanced for labelling of dead cells and analysed by flow cytometry using the BD LSRFortessa. Apoptotic cells can be identified by a decreased ratio of the F2N12S probe.

Genomics

"The Genomics Core Facility offers a full NGS service, from sample quality control & library preparation to sequencing."

SERVICES OFFERED

We provide a full service for NGS, beginning with the experimental design of the project and continuing all the way to the generation of sequencing data. In addition, the facility also sequences self-prepared libraries from researchers at IMB, Mainz University, the University Medical Center and other scientists outside Mainz. Services are based on the Illumina NextSeq 2000 and MiniSeq platforms. Oxford Nanopore Technologies are also available.

After submission of RNA or DNA samples, the facility performs initial quality control of the samples, library preparation, quality control of the prepared libraries, sequencing and raw data generation. Currently, we support library preparation for 20 applications as a standard service.

In 2024, the facility successfully implemented two commercial solutions for single-cell RNA sequencing based on SPLiT-seq (split-pool ligation-based transcriptome sequencing) from Parse Biosciences and Scale Biosciences. In collaboration with the Flow Cytometry and Bioinformatics Core Facilities, we established a Competence Hub for Single-Cell Genomics to optimally coordinate single-cell projects, as well as an additional Competence Hub for Spatial Transcriptomics together with the Microscopy and Bioinformatics Core Facilities to study the spatial transcriptome profile across mouse brain tissue sections.

Figure 1. The NextSeq 2000.

MEMBERS

Head Maria Mendez-Lago

Staff Scientists Annabelle Dold, Pablo Llavona, Maria Camila Fetiva Mora, Robert Pyne

Technicians Hanna Lukas, Ramona Rohde, Joshua Wachlin

RNA:

- Strand-specific mRNA-Seq with poly-A selection
- Strand-specific total RNA-Seq with rRNA depletion
- Small RNA-Seq
- RIP-Seq
- STARR-Seq
- QuantSeq
- eTAM-Seq

Single-cell sequencing:

- SmartSeq2 scRNA-Seq
- 10x Genomics scRNA 3′ gene expression
- 10 x Genomics multiplex RNA-Seq (Fixed RNA profiling)
- 10x Genomics sc Multiome (3′ gene expression & ATAC)
- 10x Genomics scRNA gene expression 5′ & CRISPR screening
- Parse Biosciences Evercode whole transcriptome scRNA-Seq
- Scale Biosciences single-cell RNA-Seq

DNA:

- Whole genome sequencing
- Single-stranded DNA-Seq
- ChIP-Seq
- DIP-Seq
- DRIP-Seq
- Amplicon-Seq
- GLOE-Seq
- dI-Seq

User-prepared libraries:

- Amplicon-Seq
- ATAC-Seq
- ATAC-DIP
- CUT&Tag
- CUT&RUN
- GLOE-Seq v1 & v2
- iCLIP-Seq v1 & v2
- miCLIP
- multi-CUT&Tag
- sBLISS
- RAD-Seq
- Targeted-capture bisulfite sequencing
- TTchem-Seq

Microscopy & Histology

"The Microscopy & Histology Core Facility offers a comprehensive array of high-performance microscopes & expert support to ensure top-notch & reliable imaging."

SERVICES OFFERED

The Microscopy and Histology Core Facility provides state-of-the-art microscopes and histology instruments, as well as expertise and training in sample preparation and data post-processing. Users can choose from an independent, assisted or full service.

The facility has 16 instruments, ranging from stereo and wide-field microscopes to confocal, high-content screening and super-resolution microscopes. Eight are equipped for live cell imaging. Image analysis is performed on five high-performance workstations with open source, licensed software for deconvolution, 3D visualisation and analysis or fluorescence lifetime analysis. Most of these software tools can analyse images with the help of artificial intelligence.

For histology users, we provide a comprehensive range of techniques, including semi-automated tissue fixation and paraffin embedding. There is also specialised equipment for sectioning, such as a microtome for paraffin-embedded tissues, a cryotome for frozen samples, and a vibratome for gelatin/agarose-embedded or fresh tissues. The facility can furthermore assist with optimised protocols for immunodetection and tissue clearing, along with solutions for traditional tissue staining.

In 2024, we acquired a confocal microscope equipped with optical tweezers (traps) and five-channel microfluidics (C-Trap from Lumicks) through a major DFG grant. The optical tweezers can capture objects such as polystyrene beads and precisely measure minute forces acting on molecules, making the system ideal for studying DNA/RNA-protein interactions, protein folding, cellular transport, phase separation and mechanobiology.

In collaboration with the Genomics and Bioinformatics Core Facilities, we established a competence hub for spatial transcriptomics that supports the generation of spatial transcriptome profiles across entire tissue sections, including fixed samples (Figure 1).

As part of the CRC 1551, the facility launched a new workshop on "Quantitative Microscopy for (Bio)Polymers", offering advanced microscopy techniques to study the dynamics of (bio)polymers, phase behaviour, molecular environment and spatial proximity.

MEMBERS

Head Sandra Ritz

Staff Scientists Márton Gelléri, Anusha Bargavi Gopalan, Rossana Piccinno, Petri Turunen

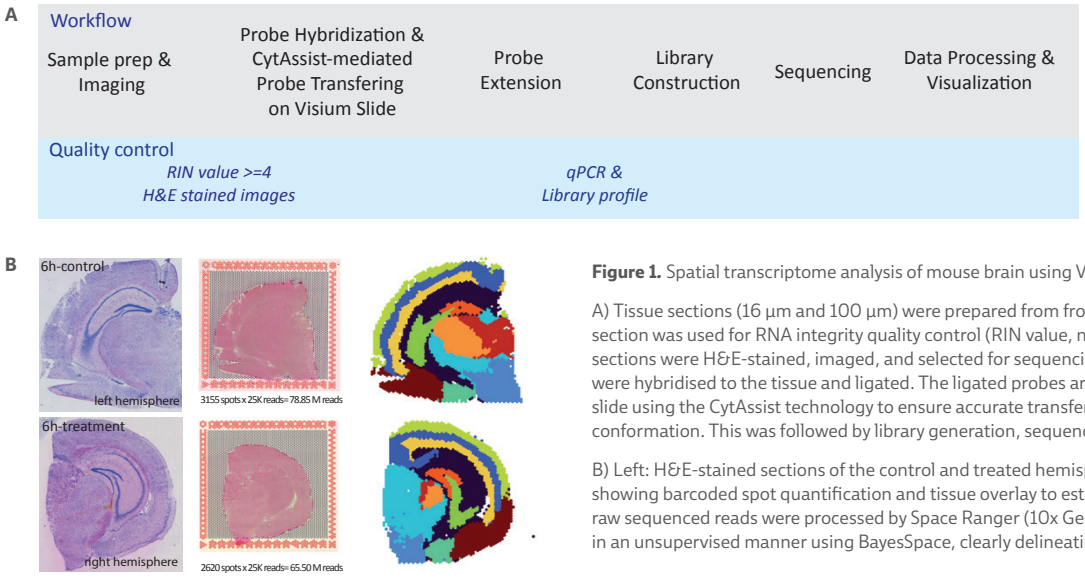


Figure 1. Spatial transcriptome analysis of mouse brain using Visium (10x Genomics).

A) Tissue sections (16 µm and 100 µm) were prepared from frozen hemispheres. The 100 µm section was used for RNA integrity quality control (RIN value, not shown), while the 16 µm sections were H&E-stained, imaged, and selected for sequencing. Mouse genome-specific probes were hybridised to the tissue and ligated. The ligated probes are then transferred to a barcoded slide using the CytAssist technology to ensure accurate transfer, retaining the original tissue conformation. This was followed by library generation, sequencing and data processing.

B) Left: H&E-stained sections of the control and treated hemispheres. Center: CytAssist images showing barcoded spot quantification and tissue overlay to estimate sequencing depth. Right: The raw sequenced reads were processed by Space Ranger (10x Genomics). Spots were further clustered in an unsupervised manner using BayesSpace, clearly delineating the sub-structures of the brain.

Protein Production

"The Protein Production Core Facility assists during all stages of producing & purifying recombinant proteins."

SERVICES OFFERED

The Protein Production Core Facility specialises in the design, expression and purification of recombinant proteins used at IMB. The facility also assists in the development of *in vitro* assays involving purified proteins.

We support 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 five automated chromatography systems, which enable the use of the latest chromatographic methods for state-of-the-art protein purification strategies.

Another of our key tasks is to generate and perform functional quality control of routine laboratory enzymes and affinity probes for IMB researchers. We currently offer 32 products to IMB scientists, matching the most frequently used protein tools at the institute.

The facility consists of a Head and a full-time staff scientist who assist researchers with their project needs and offer services tailored to specific user requests. Since January 2023, the facility has been part of the CRC 1551 on "Polymer Concepts in Cellular Function" and manages a support project alongside a group from the Max Planck Institute for Polymer Research. The project objective is to aid researchers in the production of intrinsically disordered proteins. To meet these additional demands, the CRC 1551 funds one technical assistant position in the facility.

In 2024, the facility purified approximately 120 recombinant proteins and antibodies. In most cases, molecular cloning of candidate constructs and expression screenings were also conducted by the facility.

MEMBERS

Head Martin Möckel
Staff Scientists Eugenio Ferrario, Sabine Heinen
Technical Assistant Kanish Siddarth Ravi Chandran

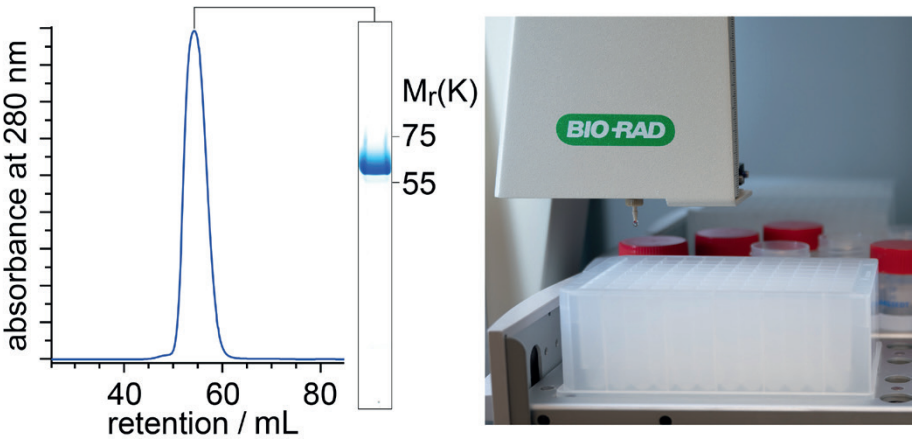


Figure 1. Left: Elution profile of a DNA-editing enzyme during the final purification step by gel filtration. Plotted is the absorbance at 280 nm over the column volume (retention). The enzyme is further visualised by Coomassie-stained SDS-PAGE next to the elution profile. Right: Sample fractionation of proteins for liquid chromatography.

Proteomics

"The Proteomics Core Facility provides advanced mass spectrometry techniques & flexible, tailored solutions to meet diverse research needs."

SERVICES OFFERED

Equipped with multiple high-resolution mass spectrometers and ultra-high-performance liquid chromatography systems, the Proteomics team supports and collaborates with research groups at IMB, Mainz University and beyond. The facility is staffed by wet- and dry-lab scientists with a broad range of research experience and technical expertise.

The facility actively participates in the experimental design of each user project and offers tailored solutions ranging from simple gel band identification to quantitative analysis of complex samples. We support multiple quantitation strategies (label-free, SILAC, dimethyl, TMT), PTM mappings (acetylation, phosphorylation, ubiquitylation) and structural studies using crosslinking mass spectrometry. The Proteomics Core Facility also works closely with users on downstream bioinformatic data analysis to support them in making discoveries from the data. As part of IMB's annual Modern Techniques in Life Sciences lecture series, the facility gives a theoretical lecture on proteomics technologies. Additionally, we offer an annual practical training course on proteomics sample preparation and related bioinformatic data analysis.

MEMBERS

Head Jiaxuan Chen
Staff Scientist Amitkumar Fulzele
Technician Jasmin Cartano
Bioinformatician Mario Dejung

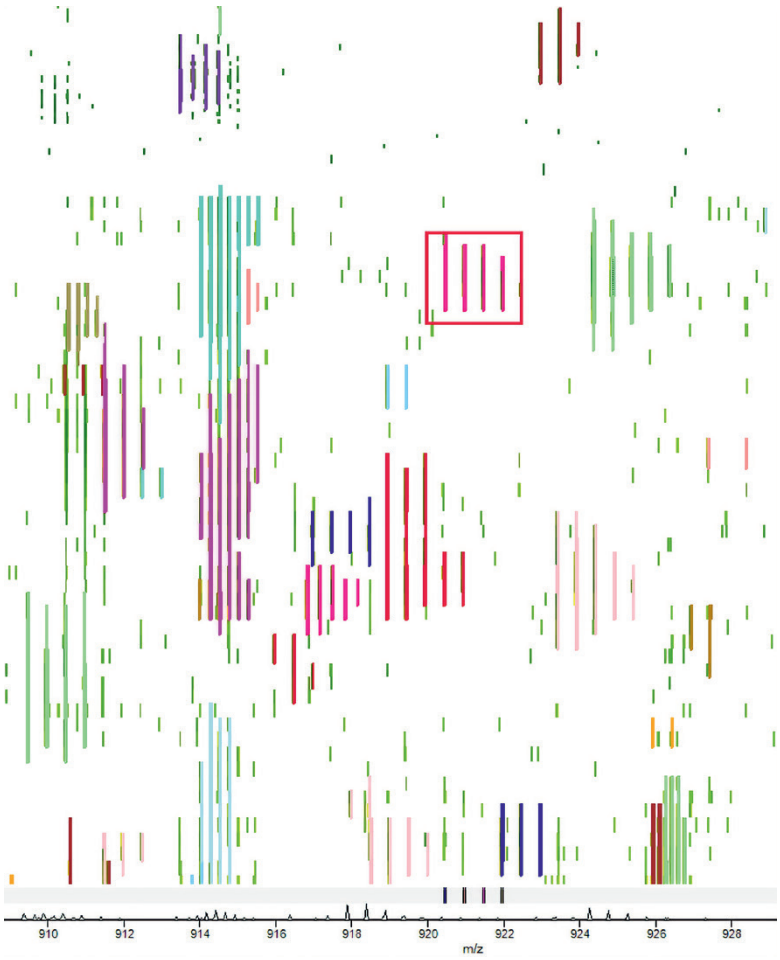


Figure 1. Peptide elution profiles acquired by liquid chromatography-mass spectrometry. Highlighted in the red rectangle is the isotopic cluster of the peptide NLESISQLISSDGSYAR, derived from protein RBAK (RB-associated KRAB zinc finger protein).

Core Support Units

In addition to the Core Facilities, further infrastructure and support services are provided by smaller Core Support Units (CSUs), which include the following:

→ MEDIA LAB

The Media Lab supports IMB's scientific groups by producing media, buffers and agar plates. It administers three supply centres, S1/S2 waste management and the cleaning and sterilising of glassware. In 2024, the Media Lab expanded our lineup of in-house media and solutions to over 60 products, which are available to researchers 24/7 via a self-service store.

MEMBERS

Head Andrea Haese-Corbit
Assistants Alwina Eirich, Pascal Hagebölling, Annette Holstein, Marion Kay, Monika Kornowska, Abraham Welday Gebre

→ CORE EQUIPMENT

The Core Equipment unit maintains a broad range of standard lab equipment and offers comprehensive training as well as troubleshooting for around 50 instruments. It also manages a consumables self-service store that provides researchers with 24/7 access to common lab items and central services for dry ice and liquid nitrogen supplies. In addition, the Core Equipment unit maintains all IMB workbenches, centrifuges/multifuges and equipment within the CSU.

MEMBERS

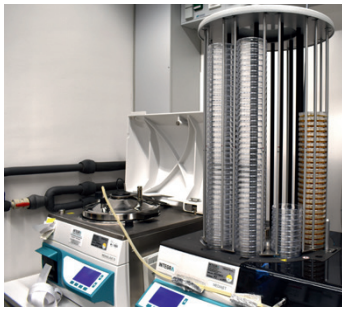
Head Ashley Westerback
Student Assistant Annika Pins

→ IT SUPPORT

The IT Support unit provides comprehensive support for Windows, macOS, hardware, telephones and computer networking. Additionally, two dedicated Linux administrators manage Linux-based servers and oversee the maintenance of IMB's high-performance computing (HPC) cluster, featuring state-of-the-art CPUs and GPUs.

MEMBERS

IT Admins Erias Buxbaum, Pascal Silberhorn, Mike Wendel
Linux Admins Christian Dietrich, Mike Wendel




→ S2 LAB

The S2 Lab provides bookable workplaces, sterile hoods and incubators in a dedicated S2 area, where IMB groups can conduct their registered S2 work.

→ RADIONUCLIDE LAB

The Radionuclide Lab provides bookable workspaces for working with ^{32}P and ^{35}S.

MEMBERS

Radiation Protection Officers Laura Frosch, Heike Hänel, Svenja Hellmann

→ ANIMAL FACILITIES

The Animal Facilities supports and equips a fish facility with 1,500 tanks, a mouse facility with 330 cages, and a *Xenopus* facility with up to 150 tanks.

MEMBERS

Animal Caretaker Tamara Dehn

4 FACTS & FIGURES

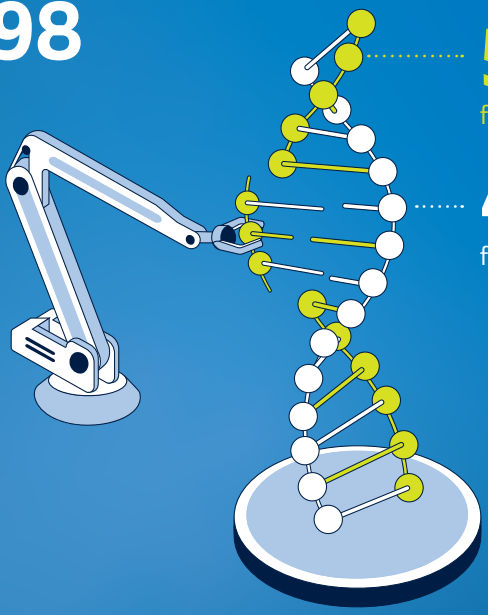
IMB Staff

No. employees by category

13 Master/ Bachelor Students
83 PhD Students
68 Postdocs
28 Group Leaders
68 Technical staff
48 Admin & SciMan

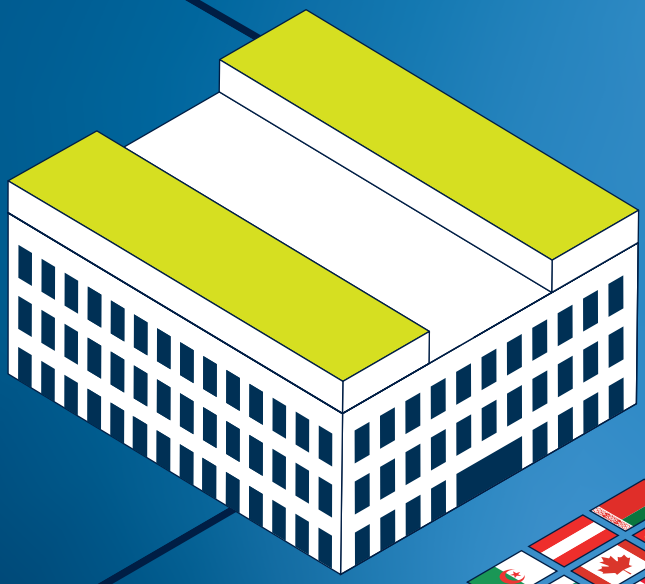
No. scientific staff

198



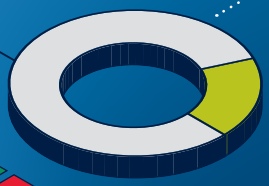
53% from abroad

47% from Germany



No. staff nationalities

50



82% European

18% Rest of the world

Staff growth

2010: **15**

2015: **229**

2020: **242**

2024: **308**

Scientific Advisory Board

IMB is grateful to the members of our Scientific Advisory Board for the insight, guidance and advice that they have provided in order to help us continue to be a leading research centre.

Peter Becker (Chair)
Biomedical Center Munich, Ludwig Maximilian University (LMU), Munich, Germany

Marina Rodnina
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

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

SPECIAL THANKS TO

Ruth Lehmann
The Whitehead Institute for Biomedical Research, Cambridge, USA

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

Ian Hickson
Center for Chromosome Stability and Center for Healthy Aging, University of Copenhagen, Denmark

Malene Hansen
Buck Institute for Research on Aging, Novato, USA

Scientific Management

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

IMB's Scientific Management team takes on administrative tasks, so that our scientists have more time for research.

We foster our researchers' success by promoting a friendly atmosphere where they can enjoy working with their colleagues, sparking innovative ideas, and build a strong community spirit. We also organise regular scientific events for them to engage with outstanding leaders in research from around the world.

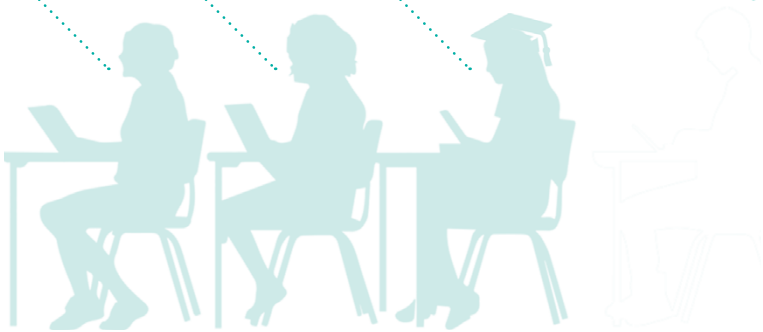
Ralf Dahm Director of Scientific Management

35
POSTDOCS

203
PHD STUDENTS

172
PHD GRADUATES SINCE 2015

2-3
PHD RECRUITMENT CALLS PER YEAR



RECRUITMENT, TRAINING & CAREER DEVELOPMENT

ANNUAL CAREER DEVELOPMENT WORKSHOPS, EVENTS & TALKS

IMB MENTORING PROGRAMME

SUMMER SCHOOLS & INTERNSHIPS

COURSES & LECTURES IN SCIENTIFIC & TRANSFERABLE SKILLS

"We attract talented young researchers, manage IMB's training programmes & organise events to advance their careers."

"We help our researchers find, obtain & manage extramural funding to advance their careers."

FUNDRAISING & GRANT MANAGEMENT

3RD PARTY FUNDING
OBTAINED IN 2024

€10.3 MILLION

10 ERC GRANTS
SINCE 2011
4 ADVANCED GRANTS
1 CONSOLIDATOR GRANT
4 STARTING GRANTS
1 PROOF OF CONCEPT GRANT

2 RESEARCH TRAINING GROUPS & INNOVATIVE TRAINING NETWORKS

2 HEISENBERG MEMBERS
2 EMMY NOETHER GROUP LEADERS

2 COLLABORATIVE RESEARCH CENTRES

COORDINATION OF RESEARCH INITIATIVES

R-LOOP REGULATION IN ROBUSTNESS & RESILIENCE
15 PRINCIPAL INVESTIGATORS
14 PHD STUDENTS + 1 POSTDOC
€8.3M (2023-2027)

GENE REGULATION IN EVOLUTION
14 PRINCIPAL INVESTIGATORS
34 PHD STUDENTS + 1 POSTDOC
€5.8M (2019-2023)
€7.0M (2024-2028)

SCIENCE OF HEALTHY AGEING RESEARCH PROGRAMME
25 PRINCIPAL INVESTIGATORS
11 PHD STUDENTS
€1.8M (2021-2024)

COHORTS FOR HEALTHY AGEING
7 PRINCIPAL INVESTIGATORS
7 PHD STUDENTS
€1.3M (2024-2027)

REGULATION OF DNA REPAIR & GENOME STABILITY
23 PRINCIPAL INVESTIGATORS
18 PHD STUDENTS + 9 POSTDOCS
€12.4M (2019-2022)
€13.1M (2023-2026)

"We support research initiatives by organising recruitment, training, events, reporting & finances to help them run smoothly."

Scientific Management

SOCIAL EVENTS & RETREATS

ALUMNI EVENTS

IMB'S PUBLIC OUTREACH & SOCIAL MEDIA
NUMBER OF FOLLOWERS:

>3,500

>9,700

>10,400

25
SEMINARS
WITH NATIONAL & INTERNATIONAL SPEAKERS PER YEAR

COMMUNICATIONS & EVENTS

5
WORKSHOPS & CONFERENCES
PER YEAR

"We organise events where our scientists can meet leaders in their field, build collaborations & showcase their research."

152
PRESS RELEASES
SINCE 2011

ANNUAL REPORTS

INSTITUTIONAL GRANT APPLICATIONS

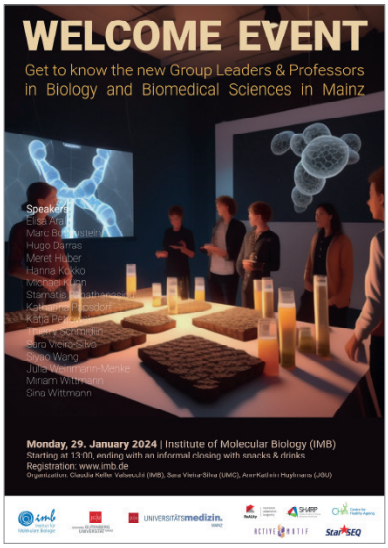
"We increase the visibility of our scientists' work by producing & distributing news & reports about their research."

SCIENCE WRITING

IMB'S WEBSITE

Scientific Events

Scientific events organised by IMB in 2024:



29 JANUARY

Welcome Event for New Group Leaders & Professors in Biology & Biomedical Sciences in Mainz

Scientific organisers: Claudia Keller Valsecchi (IMB), Sara Vieira-Silva (IMB/University Medical Center Mainz), Ann-Kathrin Huylmans (Mainz University)

12-13 MARCH

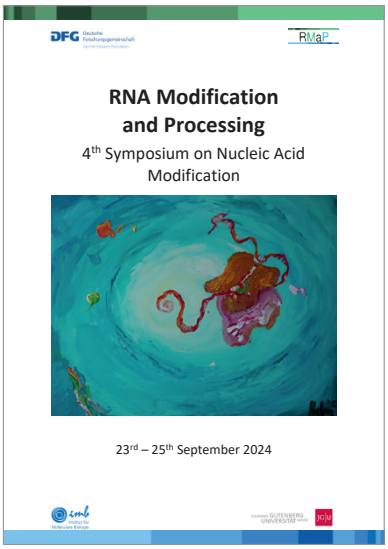
1st German Flow Core Summit 2024

Scientific organisers: Stefanie Möckel (IMB) and a committee of nine other experts from flow cytometry core facilities all over Germany

27-28 JUNE

Annual Meeting of the German Association for Aging Research

Scientific organisers: Peter Baumann (IMB/Mainz University), Christof Niehrs (IMB), Wolfram Ruf (University Medical Center Mainz), Oliver Tüscher (Leibniz Institute for Resilience Research/IMB)



23-25 SEPTEMBER

TRR 319 RNA Modification and Processing: 4th Symposium on Nucleic Acid Modification

Scientific organisers: Mark Helm (Mainz University), Julian König (IMB)

21-22 NOVEMBER

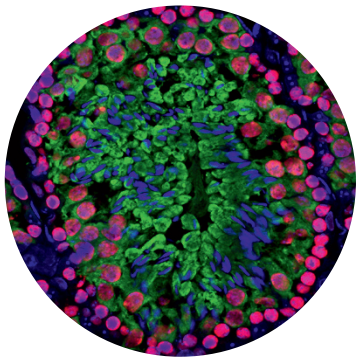
Centre for Healthy Ageing Workshop

Scientific organisers: Peter Baumann (IMB/Mainz University), Christof Niehrs (IMB)

Extramural Funding in 2024

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

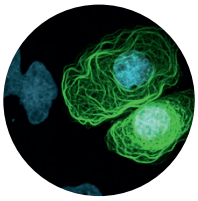
Awards & Patents



AWARDS

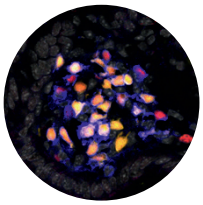
CLAUDIA KELLER VALSECCHI
Sibylle Kalkhof-Rose Prize
(Johannes Gutenberg University Mainz) & selected to join the EMBO Young Investigator Programme

JULIAN KÖNIG
Selected to join the Heisenberg Programme

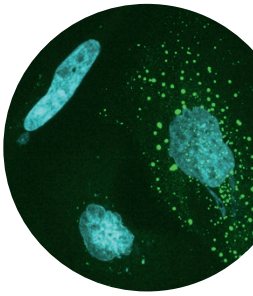


RUXANDRA LAMBUTA
Postdoc (Papathanasiou lab)
Angelika Amon Young Scientist Award & Swiss National Foundation fellowship

EDWARD LEMKE
Fellow of the Biophysical Society



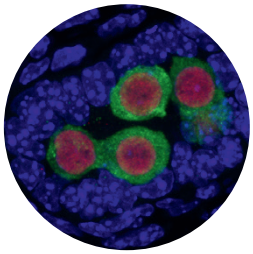
STAMATIS PAPATHANASIOU
ERC Starting Grant (€1.5 million)



SANDRA SCHICK
ERC Starting Grant (€2.3 million)

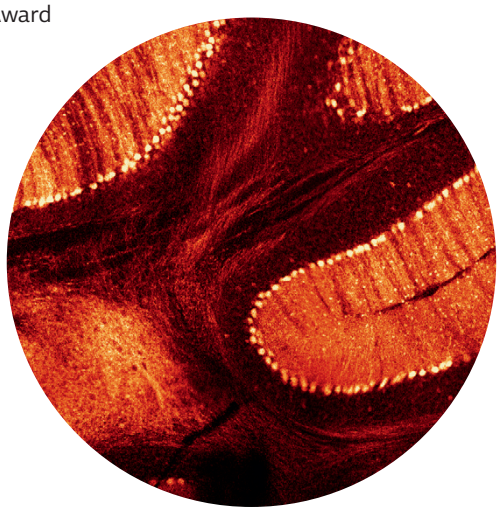
HELLE ULRICH
ERC Advanced Grant (€2.5 million)

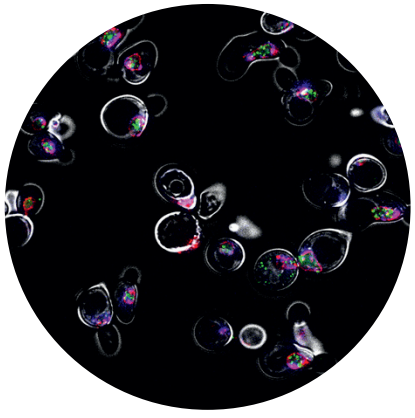
SARA VIEIRA-SILVA
Highly Cited Researchers List
(Clarivate Analytics, 2022 & 2023)



SIYAO WANG
FEBS Excellence Award
(€100,000)

SINA WITTMANN
'Win a Labbot' (€49,000)





PATENTS

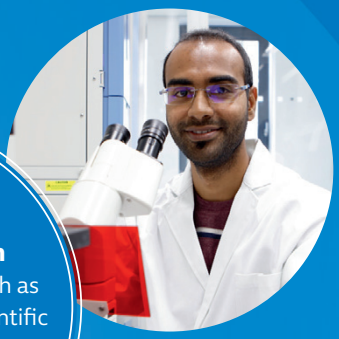
EDWARD LEMKE
Lemke EA, Schartel L (2024),
Nucleic acid molecule complex for targeted pseudouridylation in mammalian cells,
EP 24 183 463.9

Research & Training

In our training programme, we support our students and postdocs so they can succeed in their research projects and advance in their careers.

We provide comprehensive training in scientific, technical and complementary skills, so the PhD students and postdocs at IMB:

Get **scientific & technical training** in using state-of-the-art equipment, as well as support from experts in our Core Facilities for obtaining quality results



Get **trained by qualified experts in professional skills** such as giving presentations, scientific writing, project management & leadership

Meet with leaders in industry & academia at **career events, seminars & symposia** to advance their career prospects

Bond with peers at regular **social events & annual retreats** to form a supportive community

IMB Postdoc Programme (IPPro) & International PhD Programme (IPP)

IMB POSTDOC PROGRAMME

The **IMB Postdoc Programme (IPPro)** helps postdocs develop the skills and independence to manage their own projects and develop into scientific leaders.

We offer:

- **Guidance** from a supervisor and **mentoring** from leading scientists through the Mentoring Programme to support career development
- **Training** in skills for higher-level scientists, such as leadership, negotiation, writing grant proposals and management
- Support in raising **funds** for research, to help them become more independent

→ **www.imb.de/postdocs**

35
postdocs from
14
countries

INTERNATIONAL PHD PROGRAMME MAINZ

IMB's **International PhD Programme (IPP)** prepares our PhD students for a successful scientific career by providing structured training and supervision, so they can excel at tackling ambitious research projects.

We offer:

- A broad and diverse **education** through lectures from leaders in the field, providing a solid foundation for their PhD projects
- **Regular supervision** from 3 or more experts to guide them at every step
- **Comprehensive training** in scientific and professional skills to ensure they gain the skills to succeed as a scientist

→ **www.imb.de/phd**

203
PhD students from
43
countries in
76
research groups
172
graduates
since 2011

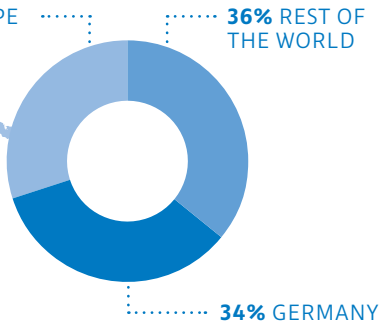
See our list of Scientific & Transferable Skills courses on page 91 for more details.

*"My postdoc at IMB **greatly broadened both my scientific & personal experience**. The scientific & technical training provided, together with the support from my supervisor, helped me develop my own line of research in a top research institute."*

Néstor García Rodríguez, 2018 IPPro Alumnus, EMERGIA Project Leader, Cabimer & University of Seville

IMB Postdoc Programme (IPPro) & International PhD Programme (IPP)

Nationalities of IPP students & IPPro postdocs:



Alumni from the IPP and IPPro work in industry, academia and beyond as:

- Assistant professors
- Lab heads
- Senior research scientists
- Managers
- Policy & governance officers
- Consultants
- Start-up founders

404
IMB publications in the last 5 years

87 in 2024
49% with an impact factor of 10 or higher

*"I loved the **vivid scientific & intercultural atmosphere** in the IPP. The array of scientific & soft skill courses **prepared me well for my next career steps** as a postdoc at the Babraham Institute in Cambridge & later when transitioning into industry."*

Juri Kazakevych, 2016 IPP Alumnus, Epigenomics Application Specialist, Diagenode

International Summer School (ISS) & IMB Internship Programme

For undergraduate and Master students, IMB offers two programmes:

IMB's International Summer School (ISS) is a 6-week programme during the summer holidays for talented university students from around the world to come to IMB and experience working in the lab of a leading scientist.

→ **www.imb.de/ISS**

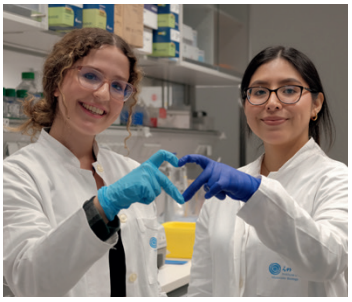
IMB INTERNATIONAL SUMMER SCHOOL

IMB's Internship Programme (IIP) is for university students from all over the world who want to do a research internship, including their thesis or an Erasmus placement (from one month to a year) at IMB.

→ **www.imb.de/internships**

Within these programmes, students can:

- Work on their **own project** at the forefront of biological research
- Get **trained by leading experts** in key scientific and transferable skills to give them a competitive edge in their studies
- Gain comprehensive insights into the latest research through **lectures** from leading scientists to prepare them for their Masters or PhD projects



198 participants from **42** countries since 2012

98% of participants rated the ISS as **"excellent"** or **"very good"**

*"The ISS more than met my expectations! It combines great education with excellent **hands-on experience**."*

Despina Giamaki, ISS 2019

*"The ISS was a **life-changing experience**, both academically & socially. The project I worked on was very interesting, as it opened my eyes to an entire area of research, & it **allowed me to enhance my coding skills**, especially in data science."*

Ahmed Hesham, ISS 2023

Core Facilities Training

IMB's Core Facilities provide lectures and hands-on courses to train researchers in key scientific techniques and a wide range of cutting-edge methodologies. Courses are open to IMB researchers, students and affiliated staff (with some limited places for external researchers). Lectures are open to everyone.

IN 2024, IMB OFFERED THE FOLLOWING LECTURES AND COURSES:

→ CORE FACILITY LECTURES

GENERAL
- Molecular & biochemistry techniques

BIOINFORMATICS
- Databases in bioinformatics
- Design & analysis of NGS experiments
- AI methods and novel LMM tools in biomedical research

FLOW CYTOMETRY
- Flow cytometry: Introduction I
- Flow cytometry: Introduction II
- Advanced flow cytometry: Principles of cell sorting
- Flow cytometry overview (for MSc students)

GENOMICS
- Genomics (NGS)

MICROSCOPY & HISTOLOGY
- Introduction to microscopy
- Microscopy: F-techniques & super-resolution
- Histology & fluorescent labelling
- Electron microscopy

PROTEIN PRODUCTION
- Protein production & crystallography

PROTEOMICS
- Proteomics

→ CORE FACILITY PRACTICAL COURSES

BIOINFORMATICS
- Introduction to biostatistics (6-day course, twice a year)
- Introduction to R (3-day course + one optional exercise session)
- Plotting with R (2-day course)
- Introduction to RNA-seq analysis (2-day course)
- Introduction to ChIP-seq and related NGS assays (2-day course)
- Data analysis using HPC and Nextflow (2-day course)

FLOW CYTOMETRY
- Basic flow cytometry practical course (2-day course)
- Advanced flow cytometry practical course: Principles of cell sorting (1-day course)

MICROSCOPY
- Image processing & analysis (5-day course)
- Quantitative microscopy for (bio)polymers (CRC 1551) (5-day course)

PROTEOMICS
- Proteomics data analysis (1-day course)
- Proteomics practical course (2-day course)

*"So far I'm having a really amazing experience here & one of the really good things is that we have **access to Core Facilities**. So, when your PhD is evolving into a topic that you didn't see it would in the beginning, you always have **super professional & amazing people who can help you**."*

Miona Ćorović, IPP student, König group

Scientific & Transferable Skills Training

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

→ LECTURES

- Advanced lectures on "Gene Regulation, Epigenetics & Genome Stability"
- CRC 1361 lecture series on "DNA Repair & Genome Stability"
- Good scientific practice
- Good scientific practice – protecting research integrity

→ PRACTICAL COURSES

- Adobe Illustrator for beginners (1-day course)
- AlphaFold workshop (1-day course)
- Critical reasoning & logic (2-day course)
- Good manufacturing practice (2-day course)
- How to make your next job application a success (2-day course)
- Negotiation skills (2-day course)
- Presentation skills (2-day course, twice a year)
- Project management* (2-day course)
- Research data management* (2-day course)
- Scientific writing (2-day course, twice a year)
- Think before you write – scientific writing (2-day course)
- True data (2-day course)
- Writing for the public* (2-day course)

* Online course

*I have enjoyed the entire journey, including all the soft **skills courses & the scientific training** that was provided to us & I am very much looking forward to applying these skills in my future career.*

Gaurav Joshi, 2024 IPP Alumnus, Head of the Molecular Genetics Diagnostics Lab, Institute for Transfusion Medicine, University Medical Center Mainz

*IMB offers **great leadership courses** & opportunities to **meet experts** from non-academic environments, which helped me target my applications to pharma companies. Getting to my current position wouldn't have been possible without my experience in leadership at IMB.*

Nikenza Viceconte, 2019 IPPro Alumna, Head of Strategic Offerings, Centogene

Invited Speakers

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

DATE	SEMINAR HOSTED BY	SPEAKER	AFFILIATION	TALK TITLE
25 Jan	CRC 1361 & RTG 4R	**Gaëlle Legube**	Center for Integrative Biology, Toulouse, FR	Chromatin and chromosome dynamics at DNA double-strand breaks
21 Feb	CRC 1361 & RTG 4R	**Stephan Hamperl**	Helmholtz Munich, DE	Transcription-replication conflicts drive R-loop-dependent chromatin alterations
22 Feb	IQCB-IMB	**Heiko Runz**	European Molecular Biology Laboratory (EMBL), Heidelberg, DE	Genomic medicine: how population-scale research incites precision medicine discovery for rare and common diseases
28 Feb	RTG GenEvo	**Beatriz Vicoso**	Institute of Science and Technology Austria, AT	Evolution and regulation of ZW sex chromosomes in sexual and asexual brine shrimp (*Artemia*)
14 Mar	RTG GenEvo	**Joachim Kurtz**	University of Münster, DE	Experimental evolution in the red flour beetle
21 Mar	International PhD Programme	**Evi Soutoglou**	University of Sussex, UK	Compromised DNA repair fidelity in embryonic stem cells
04 Apr	CRC 1361	**Johannes Walter**	Howard Hughes Medical Institute & Harvard Medical School, Boston, US	Driving mechanistic discovery with AI: lessons from genome maintenance
11 Apr	CRC 1361	**Georg Winter**	Research Center of Molecular Medicine (CeMM), Austrian Academy of Sciences, Vienna, AT	Targeted protein degradation via molecular glues
17 Apr	CRC 1361 & RTG 4R	**Puck Knipscheer**	Hubrecht Institute, Utrecht, NL	Functions and mechanisms of G-quadruplex structure regulation
25 Apr	IMB	**Claire Rougeulle**	Epigenetics & Cell Fate Center, Paris City University, FR	X chromosome inactivation in primates: when, where and why
26 Apr	RTG GenEvo & IMB	**Harmit Malik**	Fred Hutchinson Cancer Research Center, University of Washington, US	Genetic conflicts during meiosis drive the rapid evolution of essential chromatin proteins
16 May	CRC 1361 & RTG 4R	**Katrin Päschke**	University Medical Center Bonn, DE	The relevance of G-quadruplex DNA structures for genome stability and instability
22 May	RTG GenEvo	**Susana Coelho**	Max Planck Institute for Biology, Tübingen, DE	Brown algae as comparative models for investigating the evolution and regulation of sexual life cycles and reproduction
24 May	IMB	**Guoliang Xu**	Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences, Shanghai, CN	Enzymatic DNA oxidation in the regulation of development and adaptation
27 May	IMB	**Narry Kim**	Seoul National University, KR	RNA stability control: lessons from viruses and mRNA therapeutics

DATE	SEMINAR HOSTED BY	SPEAKER	AFFILIATION	TALK TITLE
13 Jun	CRC 1361	**Jonas Paulsen**	University of Oslo, NO	Modelling 3D genome organisation: implications for mutagenesis and carcinogenesis
20 Jun	CRC 1361	**Lars-Oliver Essen**	Philipps University of Marburg, DE	Kinetic structural biology of photolyase and cryptochrome function
26 Jun	CRC 1361 & RTG 4R	**Sérgio de Almeida**	Institute of Molecular Medicine João Lobo Antunes, Lisbon, PT	Live-cell imaging of R-loops and their impact on gene expression
10 Jul	RTG GenEvo	**David Baulcombe**	University of Cambridge, UK	RNA silencing in disease and disease resistance
18 Jul	CRC 1361	**Sebastian Eustermann**	European Molecular Biology Laboratory (EMBL), Heidelberg, DE	Far from equilibrium: exploring the energy-driven chromatin landscape
11 Sep	RTG GenEvo	**Arne Sahm**	Leibniz Institute for Environmental Medicine Research & Ruhr University Bochum, DE	Studying the evolution of long lifespans in alternative animal models
12 Sep	CRC 1361	**Matthias Dobbelstein**	University of Göttingen, DE	MDM2 and MDM4: regulators and effectors of the tumour suppressor p53
07 Oct	IMB	**Benjamin Towbin**	University of Bern, CH	Growth control from cells to organisms
23 Oct	RTG 4R	**Kavitha Sarma**	The Wistar Institute, Philadelphia, US	Epigenetic regulation through R-loops and G-quadruplexes
29 Oct	RTG GenEvo	**Guillem Ylla**	Jagiellonian University, Kraków, PL	E93 as a key factor in the regulation and evolution of insect metamorphosis
04 Nov	CRC 1361 & IMB Postdoc Programme	**Karim Labib**	University of Dundee, UK	Destroying the eukaryotic replisome
20 Nov	CRC 1361 & RTG 4R	**Jesper Svejstrup**	University of Copenhagen, DK	Transcription and the maintenance of genome stability
25 Nov	RTG GenEvo	**Christine Merlin**	Texas A&M University, US	Molecular basis of seasonal migratory physiology and behaviour in monarch butterflies
05 Dec	CRC 1361	**Kenji Shimada**	Friedrich Miescher Institute for Biomedical Research, Basel, CH	The strange tale of nuclear actin: TORC2 inhibition and nuclear actin drive chromosome fragmentation through base excision repair
12 Dec	IMB & CRC 1551	**Madan Babu**	St Jude Children's Research Hospital, Memphis, US	Data science approaches to GPCR signalling and implications for physiology and drug discovery

Research Initiatives

CRC 1361 "REGULATION OF DNA REPAIR & GENOME STABILITY"

Spokesperson: Helle Ulrich

SFB 1361 Regulation of DNA Repair and Genome Stability

The Collaborative Research Centre (CRC) 1361 seeks to elucidate the regulatory mechanisms governing the choice between individual genome maintenance pathways and their fidelity, interdependencies and contributions to cellular physiology.

Launched in January 2019 with €12.4 million in funding from the DFG

7 **Institutions** (IMB, Johannes Gutenberg University Mainz & its University Medical Center, Darmstadt University of Technology, Ludwig Maximilian University Munich, Goethe University Frankfurt & Julius Maximilian University Würzburg)

Evaluated as "excellent" by an external review board in 2022 & **renewed** with another €13.1 million from the DFG until December 2026

KEY FACTS

34 **PhD students** (18 funded via the CRC & 16 associated)

23 **Postdoctoral researchers** (9 funded via the CRC & 14 associated)

22 **Projects** (17 active & 5 completed)

23 **Group leaders** in structural biology, organic chemistry, biochemistry, molecular & cell biology & genetic toxicology

In 2024, CRC 1361 researchers published 22 papers from projects funded by this initiative. A highlight was a retreat at Nürnberg featuring several guest scientists. The 70 participants attended workshops on "Inclusive Leadership" and "Unconscious Bias", discussed science, exchanged ideas and networked to build collaborations. Together with the IMB Postdoc Programme, the CRC also organised a career panel as part of Postdoc Appreciation Week for postdocs to learn how the panellists navigated their careers along different paths ranging from a university professorship to a patent lawyer.

» www.sfb1361.de

CRC 1551 "POLYMER CONCEPTS IN CELLULAR FUNCTION"

Spokesperson: Edward Lemke, Vice-Spokesperson: Dorothee Dormann

The Collaborative Research Centre (CRC) 1551 brings together polymer and life scientists to understand the dynamic interplay between different biopolymers and how they govern cellular function.

KEY FACTS

Launched in January 2023 with €12 million in funding from the DFG

15 **Interdisciplinary projects** in transcription, protein aggregation, proteostasis & the formation of higher-order complexes & organelles

35 **Group leaders**, as well as 8 associate group leaders

4 **Main institutions** (Johannes Gutenberg University, its University Medical Center, IMB, the Max Planck Institute for Polymer Research) & 2 satellites (the Max Planck Institute for Biophysics & University of Stuttgart)

In 2024, the CRC 1551 gained DFG approval for a new project on "Unravelling Multivalent Interactions in the Pre-synapse", led by Carla Schmidt (JGU) and Jasper Michels (MPIP). A notable event was the CRC's interactive workshop on "Wiggly Spaghetti in the Brain" at the Curious 2024 Future Insight Conference, with presentations by Dorothee Dormann (JGU/IMB) and Tanja Weil (MPIP). The CRC also held an annual retreat in Mainz, which brought together 100 members for a vibrant scientific exchange. Educational highlights include our winter school in Bad Dürkheim, the launch of a "Polymer Concepts in Cellular Function" Master Module, and workshops in data management to teach students FAIR data principles. The CRC hosted 12 expert speakers in our Seminar Series, alongside 7 "Methods Talks" and a new Student Seminar Series.
Research funded by the CRC has appeared in several high-impact journals, including *Nature Cell Biology*, *Nature Chemical Biology* and *Molecular Cell*.

» https://crc1551.com

Research Initiatives

RTG GENEVO: "GENE REGULATION IN EVOLUTION"

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

GenEvo centres on the core question of how complex and multi-layered gene regulatory systems have both evolved and driven evolution. The initiative trains PhD students to work at the interface of these two themes while receiving a broad, interdisciplinary education.
This Research Training Group (RTG) is a collaboration between Johannes Gutenberg University's Faculty of Biology and IMB.

GenEvo
RESEARCH TRAINING GROUP

Launched in June 2019 with €5.8 million in funding from the DFG

Positively evaluated in 2023 & approved for a second funding period with an additional €7 million from the DFG until 2028

KEY FACTS

29 **Group leaders**, one postdoc & 34 PhD students, plus an additional 13 PhD students starting in January 2025

14 **Interdisciplinary projects** fusing evolutionary & molecular biology

GenEvo students have been authors on 26 papers, including in *Science*, *Molecular Cell* and *Nature Communications*. In 2024, six GenEvo students successfully defended their theses (making 11 GenEvo graduates in total) and three new group leaders joined (Jan Padeken, Katharina Papsdorf and Miya Pan). Notable events included a seminar and mentoring session with Prof. Harmit Malik (Fred Hutchinson Cancer Research Center, Washington), who is a leader on genetic conflicts that drive evolutionary change, and joint workshops with 4R, CRC 1361 and CRC 1551 on "Navigating challenging conversations" for students and "Communication in intergenerational and intercultural teams" for group leaders.

» www.genevo-rtg.de

4R RTG: "R-LOOP REGULATION IN ROBUSTNESS & RESILIENCE (4R)"

Spokespersons: Brian Luke and René Ketting

The Research Training Group (RTG) 4R delves into the impact of R-loops on the orchestration of complex cellular processes promoting robustness and resilience. The biological processes that are comprehensively explored include DNA repair, telomere maintenance, gene regulation and RNA processing.

R-loop Regulation in Robustness & Resilience

Launched in 2023 with €8.3 million in funding from the DFG

KEY FACTS

15 **Group leaders**, 14 PhD students & one bioinformatics postdoc, as well as five associated members

3 **Institutes** (IMB, Johannes Gutenberg University Mainz & the University Medical Center Mainz)

24 **Projects**

2024 marked significant growth and development for 4R. We began with a kick-off meeting in January featuring two seminars, a discussion of 4R projects, and the election of the student representatives. In total, we hosted seven speakers in our R-loop club seminar series (four joint with the CRC 1361 consortium). Furthermore, we co-organised the Gutenberg Workshop "RNase H 2024" at Kloster Wasem in Ingelheim in September, which featured 13 speakers from the UK, France, USA, Sweden and Germany.

https://4r-rtg.de

SCIENCE OF HEALTHY AGEING RESEARCH PROGRAMME (SHARP)

This joint PhD training programme combines the complementary skills of basic and clinical/translational researchers to gain new insights into the underlying causes of ageing and discover new ways to prevent age-related diseases. SHARP brings together researchers from IMB, Johannes Gutenberg University Mainz and its University Medical Center to work on projects focusing on ageing and longevity. As of 2024, six papers have been published from this programme.

SHARP

Launched in 2021 with €1.8 million in funding from Rhineland-Palatinate's Ministry of Science, Education & Culture

KEY FACTS

25 **Group leaders**

11 **PhD students**

11 **Projects** covering stem cell biology, epidemiology, immunology, cardiology, neurobiology, cancer biology, epigenetics, proteomics, telomere biology, RNA biology, DNA repair & autophagy

» https://www.cha-mainz.de/SHARP

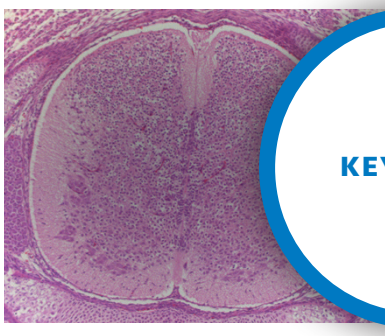
Research Initiatives

CHA PROGRAMME FOR CLINICIAN SCIENTISTS (CHANCE)

CHANCE strengthens translational ageing research within the framework of the Centre for Healthy Ageing (CHA) by fostering collaborations between IMB, Johannes Gutenberg University Mainz and its University Medical Center on key research topics in ageing and age-related diseases.

CHANCE funds three Clinician Scientists and two Advanced Clinician Scientists, allowing them to establish independent research programmes in ageing with a strong translational focus.

» www.cha-mainz.de/en/clinician-scientists



KEY FACTS

5 **Clinician Scientists & Advanced Clinician Scientists**

3 **Research institutes** (IMB, Johannes Gutenberg University & its University Medical Center)

Launched in 2023 & funded with €1.2 million by Rhineland-Palatinate's Ministry of Science & Health

COHORTS FOR HEALTHY AGEING (CoAGE)

CoAGE brings together seven experts in healthy ageing and age-related diseases from across Germany to address current issues in an interdisciplinary manner. The CoAGE experts all lead major cohort studies and will supervise a PhD student in an ageing-related project. The findings will provide valuable insights into the causes of disease and healthy ageing.

Launched in 2024 & funded with €1.3 million by Rhineland-Palatinate's Ministry of Science, Education & Culture

KEY FACTS

7 **Group leaders**

7 **PhD students**

7 **Projects** covering different aspects of epidemiological research, including cardiometabolic multimorbidity, sarcopenia, organic ageing clocks, biomarker research in heart failure, sexual dimorphism in lipid metabolism, thrombotic diseases & COVID-19 population research

6 **Research institutes** (Helmholtz Munich, the German Center for Neurodegenerative Diseases (DZNE), the University Medical Center Hamburg-Eppendorf, Leipzig University, Greifswald University Hospital, & the University Medical Center Mainz)

» https://www.cha-mainz.de/en/joint-programmes/coage

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*indicates joint contribution, #indicates joint correspondence

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*indicates joint contribution, #indicates joint correspondence

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*indicates joint contribution, #indicates joint correspondence

PREVIOUS IMB GROUP LEADERS

(published while still affiliated with IMB or with data generated at IMB)

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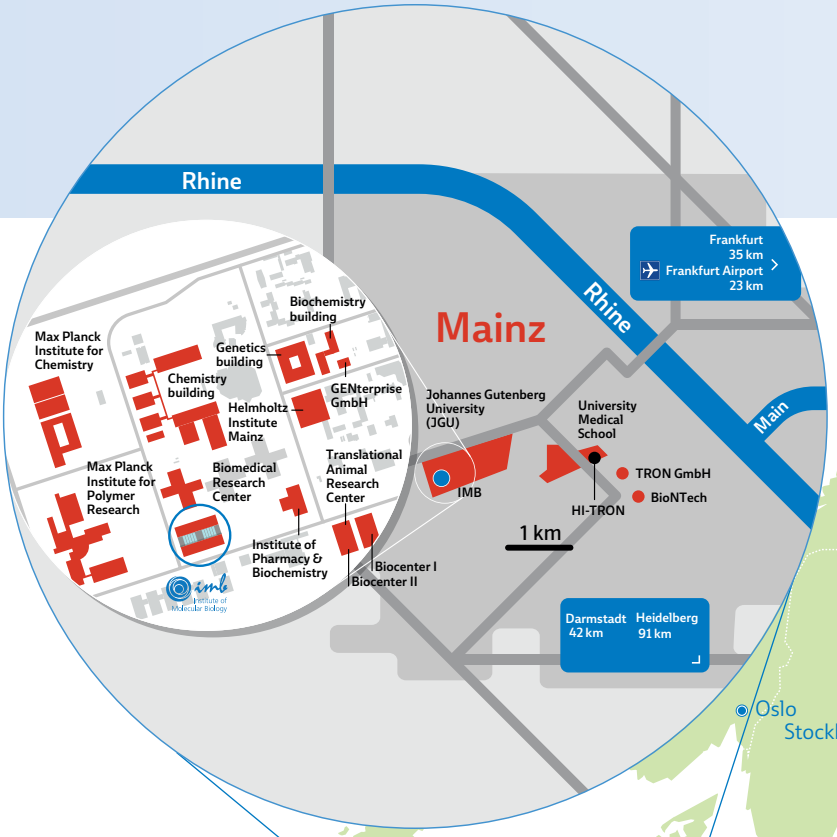
Research Environment



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

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

IMB also has strong collaborative links to the **University Medical Center**, which is located near the main campus and has a strong focus on clinical and translational research. The **Max Planck Institute for Chemistry**, **Max Planck Institute for Polymer Research**, **Leibniz Institute for Resilience Research** and **Mainz's University of Applied Sciences** are also all within easy reach.

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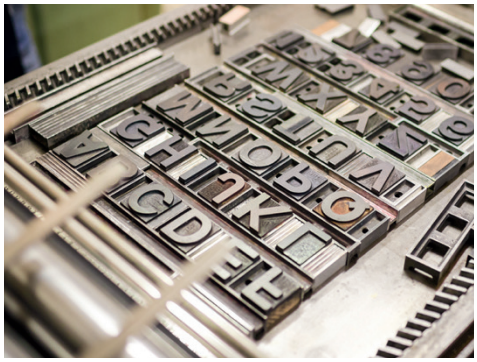
Nearby, Darmstadt is home to both a **Technical University**, whose Department of Biology has a focus on synthetic biology and the biology of stress responses, and a **University of Applied Sciences** which includes a focus on biotechnology.

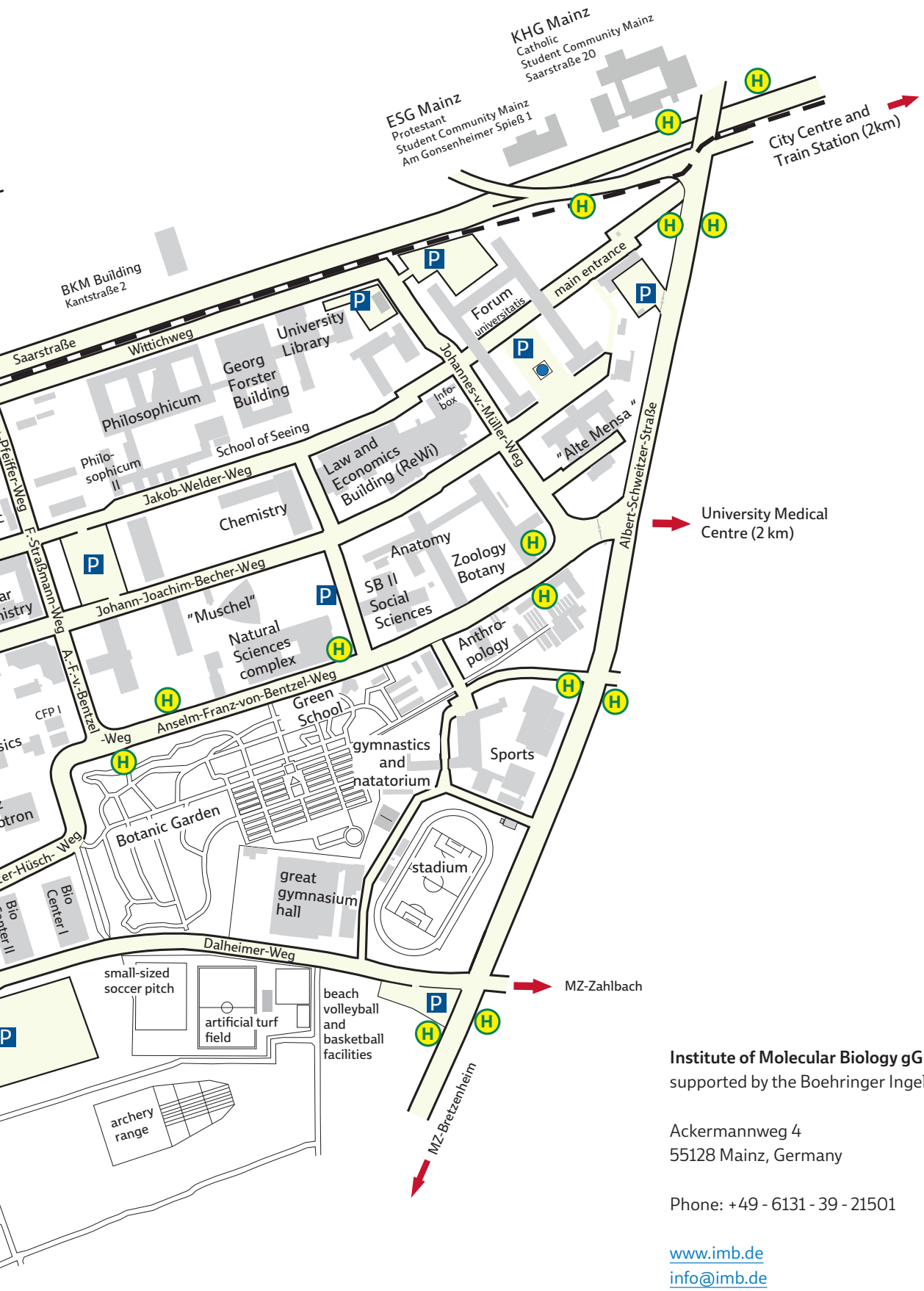
There is an **extensive industry R&D** presence in Mainz, with the headquarters of **Boehringer Ingelheim**, **BioNTech**, **Translational Oncology (TRON)** and the **Merck Group** in close vicinity.



WHERE WE ARE

Mainz is 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 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 destinations are within easy reach.

Campus Map & Contact



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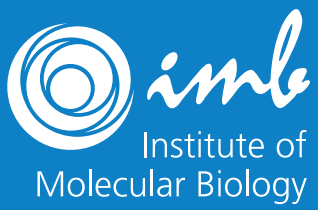
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