ANNUAL REPORT 2020

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Institute of Molecular Biology

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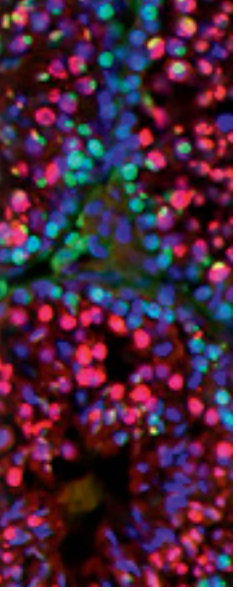
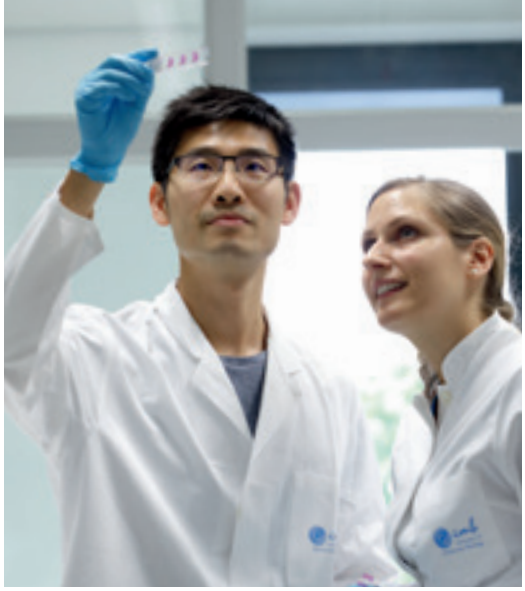
FOREWORD

Like everywhere else, the global coronavirus pandemic this year had a big impact on IMB.
Following a 3-week shutdown in spring, IMB reopened step by step, with experimental researchers engaging in shift work and everyone else staying in home office.

IMB gradually became functional again by summer, although many of us kept working from home. Despite the unique challenges of operating during a pandemic, IMB was able to hold online lectures, seminars, meetings, workshops, a retreat, and even an online PhD student symposium with more than 100 participants. Overall, IMB manoeuvred quite well through the pandemic so far, not least thanks to our employees responding with discipline and flexibility to the inevitable, and we had no major issues. The "new normality" remains challenging though, with restrictions on the number of persons per room, and online meeting formats remain a poor surrogate for real-life gatherings. The priority is, of course, to keep IMB's staff safe. Despite these constraints, 2020 has again been a year of growth for IMB, with new group leaders joining, publications going out and grants coming in.

After 10 years at IMB, we bade a fond farewell to computational biologist Stefan Legewie, who secured a Chair position as professor at the University of Stuttgart. Congratulations to Stefan, we wish him all the best for his new position! At the same time, we are pleased to welcome three new group leaders: Claudia Keller Valsecchi, Katja Luck, and Sandra Schick. Claudia joined us from the Max Planck Institute for Immunobiology and Epigenetics in Freiburg. Her research focuses on elucidating the mechanisms and physiological relevance of gene copy number for cell function in development and disease. Katja comes from the Dana-Farber Cancer Institute in Boston, USA. Her group uses integrative computational and experimental strategies to build a database of protein interactions that can be used to predict and validate genome maintenance processes that are perturbed in disease. Katja is also a recipient of the prestigious Emmy Noether Award from the German Research Foundation (DFG), which will fund her new lab. Finally, Sandra joined us from the Institute for Molecular Medicine (CeMM) in Vienna, Austria. Sandra's research investigates how chromatin modifying enzymes such as the BRG1- or BRM-associated factor (BAF) complexes regulate chromatin accessibility, and how mutations in these complexes cause neurodevelopmental disease. We were very relieved that IMB's Adjunct Director and Humboldt Professor Peter Baumann decided to decline an attractive call for a prominent position elsewhere and continue his journey together with us.


Despite the pandemic, 2020 was a productive year for IMB. IMB grew to over 240 employees and produced 60 publications. Highlights include the Ulrich group's publication in *Molecular Cell* describing a highly versatile method to map DNA strand breaks and DNA lesions, and the Butter group's publication in *Nature Communications* where they systematically linked 186 RNA structures to specific binding proteins and functions. In 2020, IMB's group leaders collectively raised more than €2.7 million in research funding. In particular, Edward Lemke was awarded a prestigious ERC Advanced Grant to engineer designer organelles that can build proteins from synthetic amino acids, opening up new ways to study protein conformational changes at high resolution.

This year, IMB adapted many of its scientific events to an online format so that we could continue to strengthen our collaborative networks despite the pandemic. IMB hosted online seminars with 16 distinguished speakers, as well as the first-ever IMB alumni career event. Other notable events in 2020 include a joint retreat of 45 group leaders from the Mainz University Medical Center and IMB in November, where clinical scientists met with basic researchers to brainstorm joint projects that bridge fundamental and translational research in ageing. Additionally, we held a joint meeting in December, where researchers at IMB and the European Research Institute for the Biology of Ageing (ERIBA) in the Netherlands met to update each other on joint initiatives and present ongoing collaborative work on ageing-related topics. Both activities reflect IMB's plan to direct its research activities more towards cellular and organismic ageing. The reason is that an increasingly ageing society poses an escalating medical and societal challenge. This came into the limelight during the coronavirus pandemic, where the risk for severe illness increases with age and the elderly are at highest risk. As IMB already conducts basic research in two major topics relating to ageing – genome stability and epigenetics – we believe that IMB can contribute to this important field.

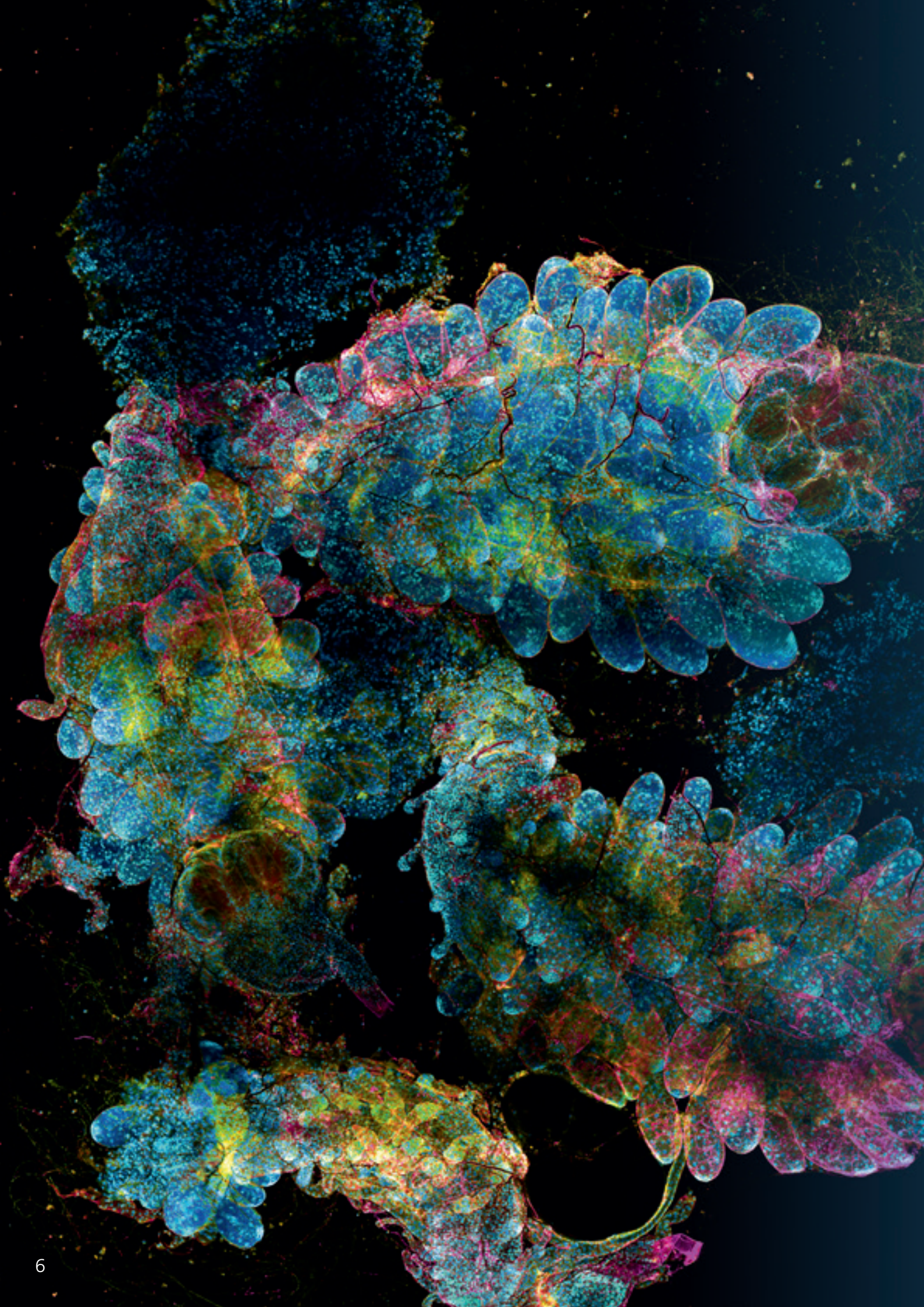
Highlighting IMB's competence in genome stability, we coordinate the SFB 1361 Collaborative Research Centre on "DNA Repair and Genome Stability", a research network funded by the DFG with €12.4 million. In September, the SFB 1361 held its first retreat online, bringing together 80 participants from IMB, Johannes Gutenberg University Mainz, the University Medical Center Mainz, the University of Technology Darmstadt, Ludwig Maximilian University Munich, and Goethe University Frankfurt to discuss their progress, as well as welcoming seven new associated members.

IMB's community continues to develop as we expand our training programme for young scientists. Our International PhD Programme (IPP) has now grown to over 150 students from 41 countries and celebrated 16 thesis defences in 2020.

Next year will be a very busy one for IMB as several events from 2020 have been rescheduled to 2021. We look forward to resuming our IMB Conference series with a conference on the "Epigenetics of Ageing: Responses to Adversity Across Scales" in June and July and an IMB/SFB 1361 Conference on "Restore, Reorganise, Repurpose: the Many Faces of DNA Repair" in September. Furthermore, the institute will officially reach its 10th anniversary milestone and undergo our 10-year international review.

I would like to thank the Boehringer Ingelheim Foundation and the State of Rhineland-Palatinate for their support and generous funding, and the members of our Scientific Advisory Board, whose advice and feedback have been instrumental in developing IMB. I also thank my colleague Helle Ulrich, from whom I took over IMB's rotating Executive Directorship, for her successful two years at the helm of the institute, passing it on in excellent shape. Last but certainly not least, I would like to thank all colleagues at IMB for defying the pandemic in this unusual year and continuing to make IMB a wonderful research institution.

Christof Niehrs
Executive Director



RESEARCH GROUPS

JOAN BARAU

> “Epigenetic changes at transposons impact genome regulation and stability”

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

Styliani Eirini Kanta PhD Student; since 04/2020
Jessica Leissmann PhD Student; since 11/2019
Violeta Morin Lab Manager; since 10/2019
Abishek Prakash PhD Student; since 05/2019

OVERVIEW

Transposable elements, or TEs, are abundant genomic repeats linked to genome instability and regulatory perturbations with 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 affecting gene regulation, genome stability, and inheritance**. In the past year, our lab has been working on three fronts aimed at discovering (i) how transposons are targeted for epigenetic silencing in mouse germ cells, (ii) how transposon sequences and their epigenetic status impact their regulatory potential in mouse germ cells, and (iii) novel regulators of the transposon 'life cycle' in pluripotent and differentiated stages of mammalian development.

RESEARCH HIGHLIGHTS

How transposons are targeted for epigenetic silencing in mouse germ cells

Germ cells have the demanding task of distinguishing 'normal' functioning genes from active TEs that should be inactivated. This is achieved by processing TE mRNAs into PIWI-interacting small RNAs (piRNAs). Production of piRNAs allows germ cells to specifically degrade TE mRNA and guide nuclear silencing factors to active TE loci, which leads to stable, life-long epigenetic silencing by DNA methylation. We now know that the final, stable step of TE transcriptional silencing in germ cells depends on the epigenetic modifier DNMT3C. In the past year, Abishek took the first steps towards understanding how DNMT3C is targeted to active TE loci as part of his PhD studies. By using a system of heterologous DNMT3C expression in mouse embryonic stem cells, we were able to understand the enzymatic kinetics of DNMT3C for different chromatin modifications. We also used this system in collaboration with the group of Petra Beli (IMB) to gain a comprehensive picture of DNMT3C's interaction partners using mass spectrometry.

How transposon sequences and their epigenetic setting impact the regulatory potential of TEs in mouse germ cells

Transposons live dual lives inside mammalian genomes: an individual transposon can act as a developmentally important enhancer of gene expression but also promote its own transposition, with potentially dire consequences for genome stability. We hypothesise that DNA methylation at TE promoters can tip the scale towards any of these opposing roles by impacting the binding of regulatory factors and interactions with readers and writers of chromatin modifications. In the past year, Jessica started her PhD to investigate proteins in mouse germ cells that interact with TE promoters when they are unmethylated or methylated. Using mass spectrometry in collaboration with Falk Butter (IMB), we now have a pioneer view on factors that may interact with TE promoters in a DNA methylation-dependent fashion.

Novel regulators of the transposon 'life cycle' in pluripotent and differentiated stages of mammalian development

The possibility of using CRISPR-Cas9 to conduct forward genetic screens opened up the opportunity to identify novel factors that regulate TEs and their life cycle in mammalian cells. However, as in any genetic screen, the devil is in the details: the phenotypic changes induced by the mutations might not be easily used as a readout to select mutants. We came up with a strategy to select mutants that accumulate endogenous mRNAs from TEs, along with the translation of these mRNAs into TE proteins. Styliani joined the lab this past year as a PhD student to tackle the challenge of setting up and using this novel readout approach to identify regulators of TE transcription, mRNA stability, and translation in pluripotent and differentiated cells.

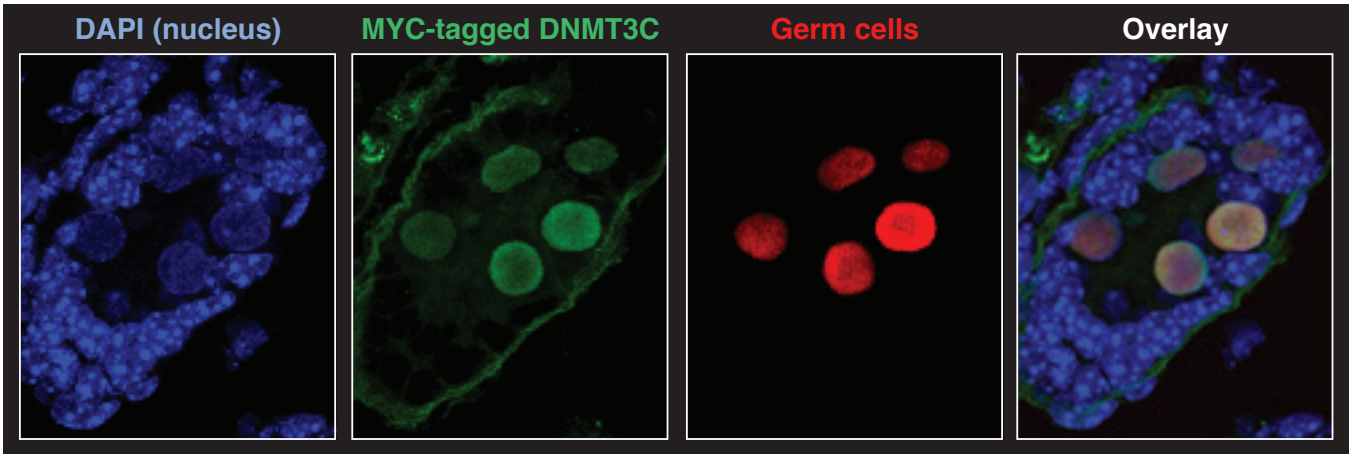


Figure 1. Immunofluorescence detection of DNMT3C in the gonads of mouse embryos at 16 days after fertilisation.
MYC-tagged DNMT3C can be found exclusively in the nucleus of male mouse embryonic germ cells (TRA98-positive in red) undergoing *de novo* DNA methylation from 14.5 days after fertilisation to 1-2 days after birth.

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 will use low input CUT&RUN and CUT&TAG to profile the identified factors in germ cells and thereby understand how DNA and histone methylation impact their distribution in the genome. We are also moving into functional genomic studies of these factors *in vivo* by developing suitable mouse models for our genetic and biochemistry studies. Finally, we are excited to start exploring the relationship between TEs and DNA damage and repair and hope to present novel exciting data about this relationship in germ cells in the upcoming year.

SELECTED PUBLICATIONS

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

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

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

PETER BAUMANN

> “Telomerase regulation plays a critical role in ageing and cancer”

EDUCATION

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

POSITIONS HELD

Since 2018	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
Since 2017	Alexander von Humboldt Professor, Johannes Gutenberg University Mainz (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
2000 – 2002	Research Associate, Howard Hughes Medical Institute, University of Colorado, Boulder
1998 – 2000	Wellcome Trust Travelling Research Fellow, University of Colorado, Boulder

GROUP MEMBERS

Lukas Ende PhD Student; since 09/2019
Thomas Faust Personal Assistant; since 05/2019
Yasmin Greiner Project Manager; since 03/2019
David Ho PhD Student; since 04/2018
Kristi Jensen Lab Manager; since 05/2018
Abinaya Manivannan PhD Student; since 08/2018
Philip McNamara Bioinformatician; since 04/2019
Aaron Odell Bioinformatician; since 01/2017
Alex Orioli PhD Student; since 01/2018
Diego Paez-Moscoso PhD Student; since 06/2014
Lili Pan Postdoc; since 02/2015
Valentine Patterson PhD Student; since 10/2019
Jayaprakash Srinivasan PhD Student; since 03/2020

OVERVIEW

Elucidating the mechanisms by which telomeric DNA sequences are replenished has far-reaching implications, as telomere lengths directly impact on the replicative potential of cells. Our research in this area is guided by the conviction that a better understanding of telomerase biogenesis and its regulation will enable us to identify compounds that modulate telomere length. Such reagents will have therapeutic uses either to limit the proliferation of tumour cells or to boost the proliferative potential of desired cell populations (e.g. in the immune system), thereby counteracting many of the detrimental phenotypes associated with ageing, such as increased susceptibility to infections and diminished response to vaccinations.

RESEARCH HIGHLIGHTS

Telomerase biogenesis and regulation

Progressive telomere shortening occurs in all dividing cells and triggers cellular senescence as they become critically short, thereby preventing further proliferation. Mechanisms that replenish telomeric sequences are a double-edged sword. They are vital for tissue homeostasis by adding back sequences lost during normal DNA replication, especially in long-lived species such as humans. On the other hand, replenishing telomeres permits the continued proliferation of malignant cells. Consequently, telomere addition must be tightly regulated. The isolation of the telomerase RNA subunit (TER1) from fission yeast by our laboratory provided a key tool for studying the biogenesis and regulation of the enzyme in a genetically tractable organism. This has led to a series of discoveries in telomere and RNA biology. We demonstrated that the RNA splicing machinery has a second, previously overlooked function in RNA 3’ end processing. Instead of removing an intronic sequence in a two-step process, the first transesterification reaction alone generates the mature 3’ end of TER1. Our work also defined roles for several RNA chaperones in telomerase biogenesis. Most interestingly, we found that the Sm proteins and a related protein complex called LSm (Like-Sm) sequentially associate with telomerase RNA and

play distinct roles in telomerase maturation. Sm and LSm proteins are members of an ancient family of RNA binding proteins that affect virtually every aspect of RNA metabolism. In contrast to the established view that they have distinct sets of RNA targets, our work revealed the first example of an RNA that requires sequential binding by the two complexes and documents specific functions for each during maturation.

While our studies on telomerase and telomeres in fission yeast have provided fundamental insights into chromosome end maintenance, they are ultimately a stepping-stone towards understanding telomere maintenance in human cells, in particular in stem cells and transiently dividing cell populations in the immune system. Recent work in our group uncovered roles for several RNA processing factors and an unusual RNA structure called a triple helix in the biogenesis of human telomerase. Importantly, our studies showed that maturation of human telomerase RNA (hTR) is in kinetic competition with its degradation (Figure 1), an observation that hints at potential treatment options for telomerase insufficiency disorders and for delaying or reversing certain degenerative processes associated with ageing.

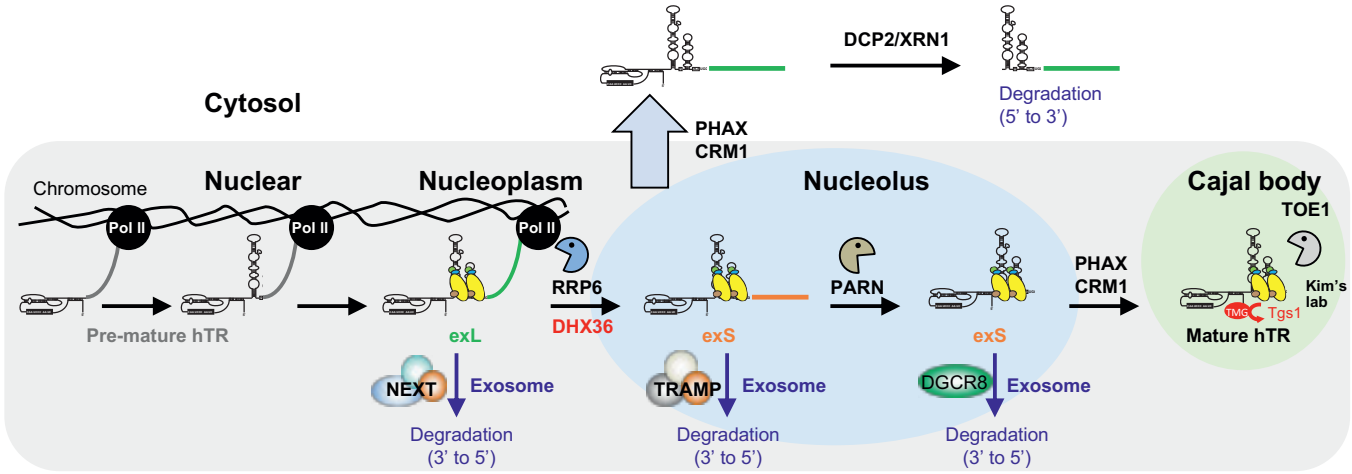


Figure 1. Telomerase assembly is a multistep process in which RNA processing and degradation are in kinetic competition (Model C.K. Tseng and P. Baumann).

FUTURE DIRECTIONS

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

SELECTED PUBLICATIONS

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

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

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

PETRA BELI

> “Ubiquitin signalling drives protein quality control”

EDUCATION

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

POSITIONS HELD

Since 2020	Adjunct Director, Institute of Molecular Biology (IMB) and Full Professor, Faculty of Biology, Johannes Gutenberg University (JGU)
Since 2013	Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz
2010 – 2013	Postdoctoral Fellow, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen

GROUP MEMBERS

Irem Baymaz Postdoc; since 11/2016
Georges Blattner PhD Student; since 07/2020
Christian Blum PhD Student; since 06/2020
Francesca Conte PhD Student; since 01/2019
Justus Gräf Bachelor Student; since 07/2020
Jan Heidelberger PhD Student; 04/2014 – 08/2020
Ekaterina Isaakova PhD Student; since 10/2020
Thomas Juretschke PhD Student; since 09/2016
Katharina Mayr Lab Manager; since 01/2020
Ivan Mikicic PhD Student; since 04/2019
Thorsten Mosler PhD Student; since 04/2017
Patrick Müller Master Student; 08/2019 – 02/2020
Matthias Ostermaier PhD Student; since 05/2015
Claudia Scalera PhD Student; since 09/2019
Aldwin Suryo Rahmanto Postdoc; since 11/2019
Andrea Voigt Lab Manager; 01/2014 – 08/2020
Juanjuan Wang PhD Student; since 08/2016

OVERVIEW

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

RESEARCH HIGHLIGHTS

The ubiquitin system is at the heart of quality control pathways that detect and degrade aberrant proteins. Human cells encode more than 500 ubiquitin E3 ligases that target substrate proteins with different types of ubiquitin chains. During gene expression, quality control pathways monitor each step to detect and degrade aberrant mRNAs and proteins. These mechanisms ensure protein homeostasis and are essential to prevent neurodegenerative diseases. A common source of aberrant mRNAs is premature polyadenylation, which can result in non-functional protein products. Therefore, mechanisms are in place that recognise such homopolymeric adenosine (poly(A)) sequences and abrogate their translation. Translating ribosomes that encounter poly(A) sequences are terminally stalled, followed by ribosome recycling and decay of the truncated nascent polypeptide via ribosome-associated quality control (RQC). Upon splitting of the 60S and 40S ribosomal subunits, the RQC complex assembles on the 60S subunit to initiate the release and rapid degradation of the truncated tRNA-bound

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

In collaboration with Julian König (IMB) and Kathi Zarnack (Goethe University Frankfurt), we demonstrated that the conserved RNA-binding E3 ubiquitin ligase makorin RING finger protein 1 (MKRN1) promotes ribosome stalling at poly(A) sequences during ribosome-associated quality control. We showed that MKRN1 directly binds to the cytoplasmic poly(A)-binding protein (PABPC1) and associates with polysomes. MKRN1 is positioned upstream of poly(A) tails in mRNAs in a PABPC1-dependent manner. Ubiquitin remnant profiling and *in vitro* ubiquitylation assays uncovered PABPC1 and ribosomal protein RPS10 as direct ubiquitylation substrates of MKRN1. We propose that MKRN1 mediates the recognition of poly(A) tails to prevent the production of erroneous proteins from prematurely polyadenylated transcripts, thereby maintaining proteome integrity. In collaboration with Jean-Yves Roignant (University of Lausanne), we also contributed insights into the functions of MKRN1 in the regulation of translation in *Drosophila melanogaster.*

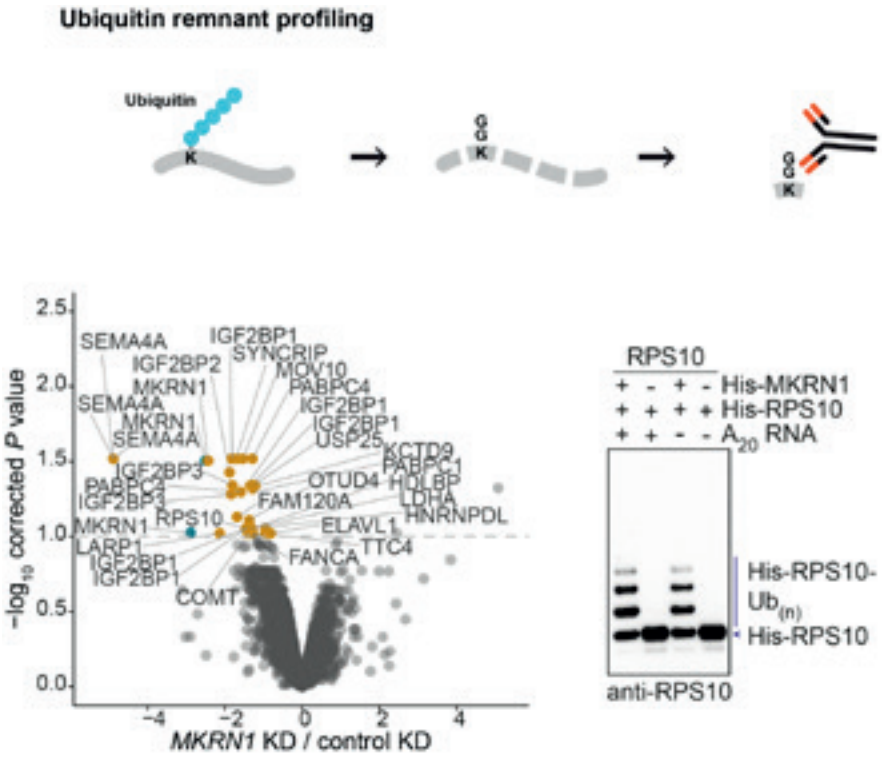


Figure 1. Ubiquitin remnant profiling for mapping ubiquitin ligase-substrate relations. Ubiquitin remnant peptides are enriched using di-glycine lysine-specific antibodies and analysed by quantitative MS. This permits the quantification of thousands of ubiquitylation sites in different conditions (upper panel). The ubiquitin-modified proteome was compared in wild type cells and upon depletion of MKRN1 to identify putative MKRN1 substrates in human cells (lower panel left). *In vitro* ubiquitylation assays confirmed the ability of MKRN1 to directly ubiquitylate the ribosome (lower panel right)

FUTURE DIRECTIONS

We will study protein quality control pathways that function in the nucleus of human cells to regulate transcription and DNA repair. The family of HECT-type ubiquitin ligases has been implicated in human disorders including neurodegeneration and cancer. We will define substrate specificity principles for nuclear HECT-type ubiquitin ligases and characterise the functional role of selected ubiquitin ligase-substrate relations in proteome dynamics and quality control. Towards these goals, we will employ proximity proteomics and ubiquitin remnant profiling, combining these approaches with quantitative mass spectrometry.

SELECTED PUBLICATIONS

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

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

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

#indicates joint correspondence

FALK BUTTER

> “Mass spectrometry advances new findings in biology”

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

Miguel Almeida Postdoc; 04/2019 – 04/2020
Hanna Braun PhD Student; since 08/2015
Alejandro Ceron PhD Student; since 10/2019
Mario Dejung Bioinformatician; since 05/2014
Emilia Rita de Caro Research Assistant; 05/2019 – 02/2020
Sabrina Dietz PhD Student; since 01/2015
Albert Fradera Sola PhD Student; since 03/2018
Carisa Goh Sho Yee PhD Student; since 10/2019
Michal Levin Postdoc; since 09/2018
Liudmyla Lototska Postdoc; since 06/2019
Katarina Luko PhD Student; since 12/2017
Emily Nischwitz PhD Student; since 09/2018
Merve Öztürk PhD Student; 11/2015 – 06/2020
Lara Perez PhD Student; 10/2015 – 01/2020
Franziska Roth Lab Technician; since 01/2019
Marion Scheibe Postdoc; since 06/2013
Marian Scherer Student Assistant; since 11/2019
Vivien Schoonenberg PhD Student; since 02/2018
Varvara Verkhova Research Assistant; since 11/2020
Maya Wilkens PhD Student; since 10/2019
Susanne Zimbelmann Master Student; 01/2020 – 12/2020

OVERVIEW

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

RESEARCH HIGHLIGHTS

Phylointeractomics reveals evolutionary changes in protein binding

We developed a new experimental workflow for comparative evolutionary biology termed “phylointeractomics”. In phylointeractomics, we interrogate a bait of interest with the proteome of evolutionarily-related species in a systematic manner to uncover similarities and differences in protein binding. In a first application, we studied the telosome of 16 different vertebrate species ranging from zebrafish to human, which span a timeframe of 450 million years of evolution. While the telomeric sequence in vertebrates is a conserved TTAGGG repeat, there are some known variations of the interacting proteins, e.g. a *Pot1* gene duplication in the rodent lineage and absence of *TIN2* in bird genomes. In our phylointeractomics screen, we recapitulated these evolutionary differences for the shelterin complex and additionally uncovered that, in contrast to predictions, not all homologues of TRF1, a direct TTAGGG-repeat binding subunit of the complex, associated with our telomeric baits. Using recombinant TRF1 DNA-binding domains of even more vertebrate species, we could locate a

gain-of-binding event at the branch point of the therian lineage, where mammals and marsupials diverged from monotremes such as the platypus. While TRF1 is present in most vertebrates, it seems to have obtained its telomeric function only later during vertebrate diversification. By exchanging selected amino acid residues in the platypus TRF1-DNA binding domain, we could recreate a gain-of-binding switch *in vitro*, recapitulating a possible evolutionary scenario. Our phylointeractomics study, therefore, underscores that sequence homologues, as determined by phylogenomics, do not necessarily need to equate to functional homology.

Characterisation of new telomeric proteins

We use quantitative interactomics to identify new telomeric proteins. Apart from HOT1, we reported that the zinc finger protein ZBTB48 is a telomeric protein in mammals. Recently, we characterised ZBTB10 as a telomeric protein that preferentially binds to telomeres elongated by the alternative lengthening pathway. The extension of our workflow to other model species resulted in the identification of novel telomere binding proteins. For example, we just identified the long-sought double-strand telomere binding proteins TEBP-1 and TEBP2 in the nematode *C. elegans*. We showed that both proteins modulate telomere length, but TEBP-1 CRISPR knockout strains have longer telomeres while TEBP-2 knockout strains have shorter telomeres, which coincides with a mortal germline phenotype. Notably, both double-strand proteins form a complex with the known *C. elegans* single-strand binder POT-1, defining the first known telomere complex in nematodes.

Systems approaches to study developmental gene regulation

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

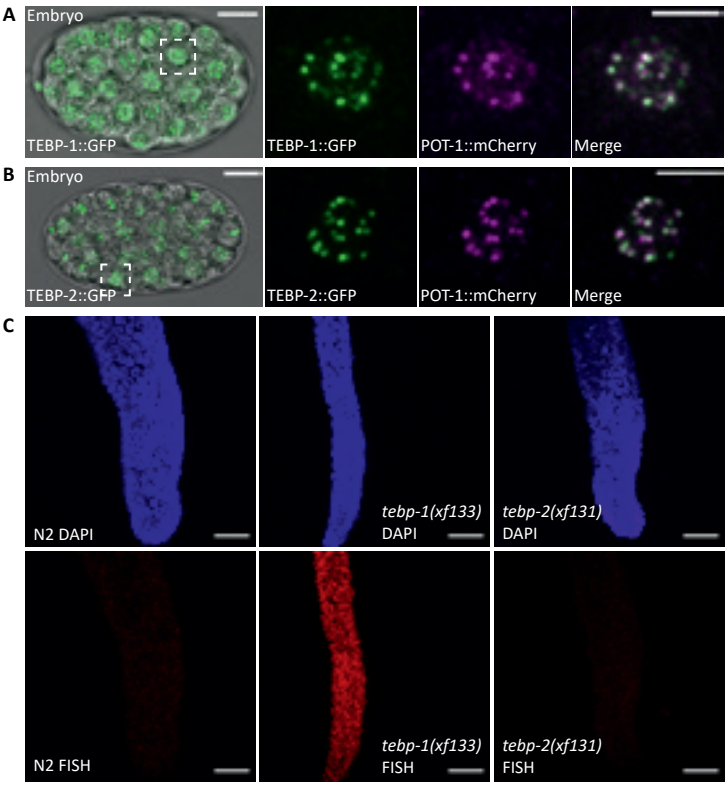


Figure 1. TEBP-1 and TEBP-2 localise to telomeres and show opposite telomere length phenotypes when mutated. (A, B) Maximum intensity projections of z-stacks of embryos expressing TEBP-1::GFP and POT-1::mCherry (A) or TEBP-2::GFP and POT-1::mCherry (B). Panels on the right show a higher magnification view of the area in the dashed box, showing co-localisation of TEBP-1 or TEBP-2 with POT-1 in nuclei. Scale bars: 10 µm (embryos), 4 µm (magnified view). (E) Maximum intensity projection z-stacks of dissected germlines used in a quantitative FISH assay. Strains used: N2, tebp-1(xf133), tebp-2(xf131). Nuclei of dissected germlines were stained with DAPI, telomeres were visualised with telomeric PNA-FISH-probe. Scale bars: 15 µm.

FUTURE DIRECTIONS

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

SELECTED PUBLICATIONS

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

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

*indicates joint contribution
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CLAUDIA KELLER VALSECCHI

"We investigate the importance of having two parental genome complements"

EDUCATION

2012	PhD in Biochemistry, Friedrich Miescher Institute (FMI)
2008	MSc in Molecular Biology, Friedrich Miescher Institute (FMI)
2007	BSc in Molecular Biology, Biozentrum, University of Basel

POSITIONS HELD

Since 2020	Group Leader, Institute of Molecular Biology (IMB), Mainz
2013 – 2020	Postdoctoral Researcher, Max Planck Institute of Immunobiology & Epigenetics, Freiburg
2012 – 2013	Postdoctoral Researcher, Friedrich Miescher Institute (FMI), Basel

GROUP MEMBERS

Agata Izabela Kalita PhD Student; since 07/2020
M. Felicia Basilicata Senior Research Associate; since 09/2020
Feyza Polat Research Assistant; since 11/2020
Daniel Mihaylov Mondeshki Student Assistant; since 11/2020

OVERVIEW

Diploid organisms contain a maternal and a paternal genome complement that is thought to provide robustness and allow developmental progression despite genetic perturbations that occur in heterozygosity. Yet, aneuploidy, a condition where chromosomes occur in different numbers than the normal two, is only rarely tolerated during embryonic development and a principal factor of miscarriage in humans. Moreover, recent advances in diagnostics by next generation sequencing revealed that haploinsufficiency is much more prevalent in humans than anticipated. Hence, the exactly two-fold gene dosage appears to be highly relevant for proper cellular and organismal function in eukaryotes. This applies particularly to cells growing in multicellular and tissue contexts that are constantly exposed to dynamic environmental signals. This creates a fascinating paradox, considering that gene and whole chromosome duplications are a major driver for the evolution of novel traits, for example in the vertebrate brain. In our laboratory, we investigate the causes and consequences of gene dosage alterations and their impact on developmental, pathogenic, and evolutionary processes. Our experimental approaches consist of genome engineering, epigenomics, biochemistry, and imaging in murine stem cells, human patient-derived cells, and non-model organisms.

RESEARCH HIGHLIGHTS

Gene regulation in sex chromosomes: a natural form of aneuploidy

Heteromorphic sex chromosomes are a natural exception to the diploid state and can induce potentially deleterious gene expression imbalances. This is frequently corrected by a cellular mechanism termed dosage compensation (DC), which ensures equal gene expression of X-linked genes between males (XY) and females (XX). Sex chromosomes and their regulatory pathways show extremely high evolutionary turnover. The reasons for the molecular differences observed in the three known DC mechanisms

operating in *Drosophila*, *C. elegans*, and the mouse remain unclear: are they a consequence of distinct genomes, functional or ecological constraints, or random initial commitment to an evolutionary trajectory? We have recently compared DC in *Anopheles* and *Drosophila*, two closely related dipterans that have similar gene content and whose X chromosomes evolved from the same ancestral autosome. Interestingly, we find that DC in *Anopheles* is achieved by an entirely different, but unknown, molecular mechanism from that in *Drosophila*. One of the projects in our lab now aims to further characterise gene expression and the epigenome in male and female mosquitos. Our goal is to get a better understanding of the factors and pathways regulating the X chromosome in dipterans, which could provide novel strategies for fighting infectious diseases by vector control.

In parallel to our work in insect models, we also aim to understand the complex biology of the mammalian sex chromosomes. Here, female individuals inactivate one of their two X chromosomes to match the male counterpart. Despite one of the X chromosomes being largely heterochromatic, some genes escape X chromosome inactivation and hence are expressed from both X chromosomes. This may become relevant during ageing, where older individuals display an increased rate of mosaic chromosome losses, which most frequently affects the inactive X and the Y chromosomes. We aim to develop a cellular system that allows us to study this poorly characterised phenomenon in the context of ageing and its implications for cellular responses to environmental cues.

Gene paralogues and dosage-sensitivity of RNA-binding proteins

Gene and whole chromosome duplications are major drivers for the evolution of novel traits, for example in the vertebrate brain. One class of duplicated gene paralogues important for neuronal functions encodes dosage-sensitive RNA binding proteins, components of the RNA spliceosome, and splicing regulatory factors. Dosage alterations of such genes can lead to neurogenetic disorders in humans that manifest with remarkably distinct phenotypes. But what are the mechanisms that drive cell type-specific functions in a situation where the paralogue "copies" encode very similar or almost identical proteins compared to the original "templates"? We aim to characterise the molecular mechanism and functional relevance of dosage-sensitive paralogues in murine and human stem cells, neurons, and tissues. We focus in particular on the cell-type specificity of RNA localisation and splicing and will use genomics, biochemistry, and imaging as experimental tools. Ultimately, we will extrapolate our findings to the disease context and study patient-derived cells.

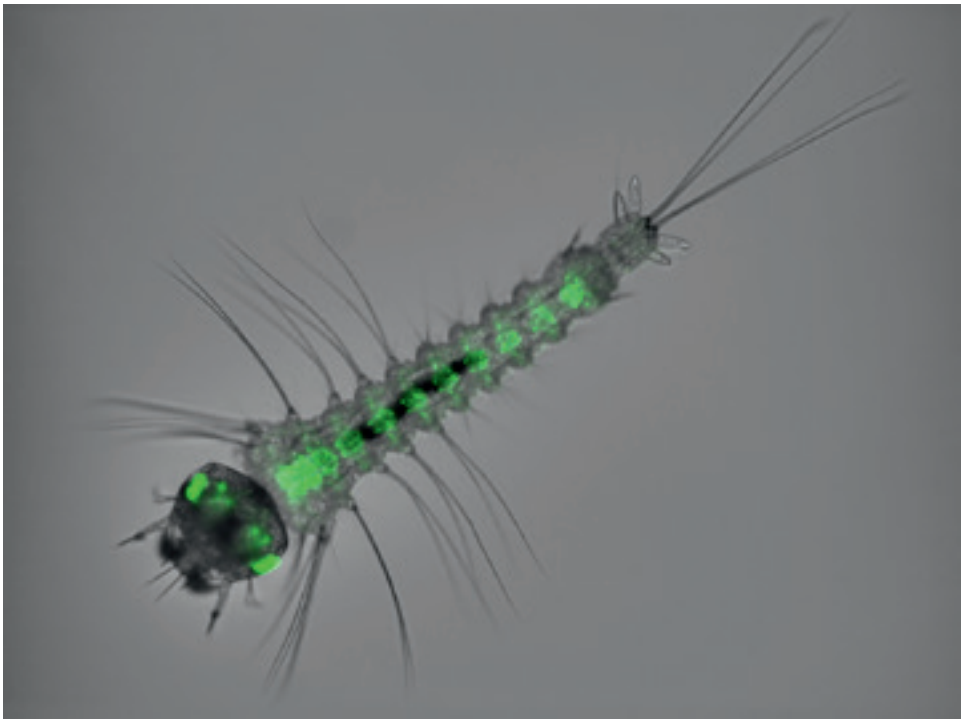

Figure 1. *Anopheles gambiae* neonate first instar larva expressing green fluorescent protein to distinguish males from females.

FUTURE DIRECTIONS

In the upcoming year, we will characterise sexually dimorphic gene expression and the epigenome of the X chromosome in malaria mosquitoes. We also plan to develop tools to comprehensively identify dosage-sensitive genes and cellular responses in mammals. Follow-up studies will then focus on dosage-sensitive RNA-binding proteins, for example with regards to phase-separated structures and the implications for cell type-specific RNA processing. We will also expand our team to generate insights into the mammalian X chromosome and chromosomal mosaicism during ageing.

SELECTED PUBLICATIONS

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

Keller Valsecchi CI, Basilicata MF, Semplicio G, Georgiev P, Gutierrez NM and Akhtar A (2018) Facultative dosage compensation of developmental genes on autosomes in Drosophila and mouse embryonic stem cells. *Nat Commun*, 9:3626

Renschler G, Richard G, Keller Valsecchi CI, Toscano S, Arrigoni L, Ramírez F# and Akhtar A# (2019) Hi-C guided assemblies reveal conserved regulatory topologies on X and autosomes despite extensive genome shuffling. *Genes Dev*, 33:1591–1612

*indicates joint contribution
#indicates joint correspondence

RENÉ KETTING

> “Our work shines new light on how cells are organised”

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

Walter Bronkhorst Postdoc; since 01/2015
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Alessandro Consorte PhD Student; since 01/2018
Yasmin El Sherif Lab Manager; since 10/2014
Svena Hellmann Technician; since 11/2016
Ida Josefine Isolehto PhD Student; since 08/2020
Monika Kornowska Animal Caretaker; since 03/2015
Saskia Krehbiel Animal Caretaker; since 05/2012
Regina Otto Personal Assistant; since 09/2015
Joana Pereirinha PhD Student; since 09/2019
René-Maurice Pfeifer Student Assistant; since 12/2018
Maria Placentino PhD Student; 11/2013 – 01/2020
Nadezda Podvalnya PhD Student; since 01/2018
Jan Schreier PhD Student; since 05/2015
Ann-Sophie Seistrup PhD Student; since 07/2019
Shamitha Shamitha PhD Student; since 11/2019
Nadine Wittkopp Postdoc; since 11/2012

OVERVIEW

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

RESEARCH HIGHLIGHTS

A novel mechanism to generate small RNAs in worms

The nematode *C. elegans* makes a special class of small RNAs that, when bound to a protein of the Piwi family, scan the genome for the presence of foreign sequences, triggering their silencing. Thus, these small RNAs act as specificity factors in a genomic immune system. These small RNAs, named 21U RNAs or piRNAs, are produced as tiny non-coding transcripts from tens of thousands of individual transcription units clustered in two large regions of the genome, resembling how other non-coding genes, e.g. tRNAs, rRNAs, and snRNAs, are organised. To mature, the precursor piRNAs of *C. elegans* first need to be processed, losing nucleotides at both the 5′ and 3′ ends. While the enzyme

responsible for processing the 3′ ends has been identified, nothing is known about 5′ end processing. In 2019, we identified a protein complex that binds to piRNA precursors and is essential for their maturation. We named this complex PETISCO–Portuguese for *tapas*. PETISCO consists of a number of subunits with different functions, one of which is to bind 5′-cap structures. Most *C. elegans* mRNAs carry a modified cap structure: a tri-methyl cap. On the other hand, piRNAs contain a 'regular cap' structure. PETISCO can bind to 'regular caps' but not tri-methylated caps, which provides the possibility of substrate selection. Furthermore, a second PETISCO subunit is predicted to bind to 5′-phosphate ends. This combination of cap and 5′-phosphate binding suggests that it is via PETISCO's binding to piRNA precursors that they lose their 5′-cap. In the past year, we have performed studies that aim to identify the enzyme that processes the 5′ end of piRNA precursor RNAs. We have identified a likely candidate for this function using genetic experiments and have been developing protein purification experiments in order to firmly establish and further analyse the enzymatic activity of this novel enzyme.

Identification of novel proteins involved in piRNA-mediated gene silencing

Using a genetic screen, we identified a novel protein named PID-2, which we found to play an important role in initiating piRNA-mediated gene silencing in *C. elegans*. PID-2 was predicted to be structurally disordered and affects the liquid-liquid phase separation of a subcellular germ cell compartment known as the Z granule. Z granules are known to have a role in mediating the inheritance of gene silencing information. Indeed, when we probed how reliably epigenetic information in the form of small RNAs is transmitted across generations, we found that transmission was compromised in mutants that do not produce the PID-2 protein. We also performed experiments to find out what other proteins PID-2 binds to and identified two proteins: PID-4 and PID-5. These two proteins are needed for PID-2 to function. Interestingly, PID-5 displays strong homology to, and also binds to, a protease named APP-1, revealing that proteolysis may be involved in mediating the inheritance of small RNA molecules across generations. These results were published in the *EMBO Journal*.

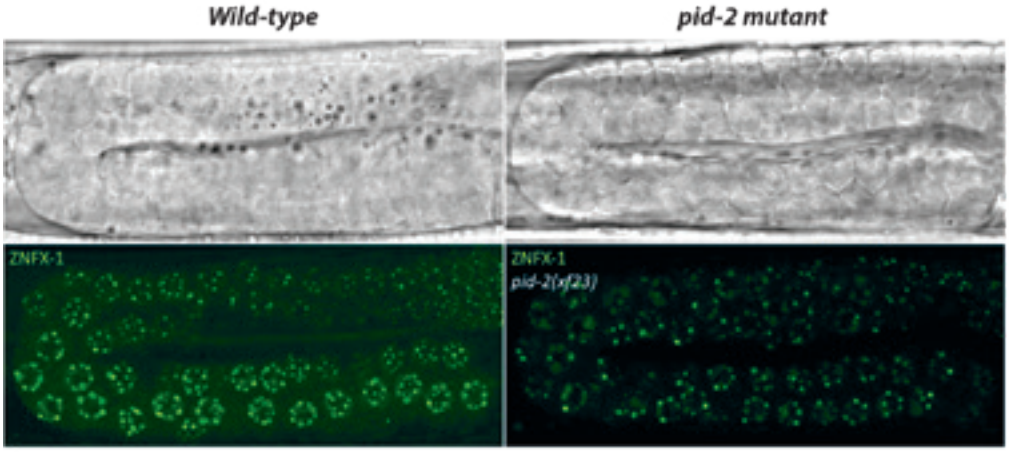

Figure 1. Top panels display brightfield images of *C. elegans* gonads. Bottom panels show the same animals under fluorescent microscopy, with Z granules labelled by GFP. In wild-type animals (left panels), more Z granules can be seen than in *pid-2* mutants (right panels), and this has an effect on how reliably small RNAs can be inherited in offspring.

FUTURE DIRECTIONS

Our future work will continue to mechanistically unravel the molecular pathways that are steered by small RNA molecules. We are performing a genetic screen to identify novel factors and are increasingly using biochemical approaches to describe their mechanisms on a more molecular level. Both *C. elegans* and zebrafish will continue to play important roles in these studies. For instance, in *C. elegans* we will focus on how the recently identified proteins PID-2, -4, and -5 affect the subcellular organisation of germ cells, and in particular how they affect interactions between phase-separated granules. We will also study how small RNAs can be inherited across generations, how piRNA precursors are processed, and start a project studying how the process of ageing affects small RNA populations. In zebrafish, we are setting up experiments aimed at understanding how liquid-liquid phase separation contributes to oocyte and embryo development and how this is coupled to small RNAs.

SELECTED PUBLICATIONS

Almeida MV, Dietz S, Redl S, Karaulanov E, Hildebrandt A, Renz C, Ulrich HD, König J, Butter F and Ketting RF (2018) GTSF-1 is required for formation of a functional RNA-dependent RNA polymerase complex in *Caenorhabditis elegans*. *EMBO J,* 37:e99325

Cordeiro Rodrigues RJ, de Jesus Domingues AM, Hellmann S, Dietz S, de Albuquerque BFM, Renz C, Ulrich HD, Sarkies P, Butter F and Ketting RF (2019) PETISCO is a novel protein complex required for 21U RNA biogenesis and embryonic viability. *Genes Dev*, 33:857–870

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

ANTON KHMELINSKII

"How are abnormal proteins recycled?"

EDUCATION

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

POSITIONS HELD

Since 2018	Group Leader, Institute of Molecular Biology (IMB), Mainz
2013	Visiting Scientist, Donnelly Centre for Cellular and Biomolecular Research, University of Toronto
2011 – 2017	Postdoc, Center for Molecular Biology (ZMBH), University of Heidelberg
2011 – 2016	Visiting Scientist, European Molecular Biology Laboratory (EMBL), Heidelberg
2010 – 2011	Postdoc, European Molecular Biology Laboratory (EMBL), Heidelberg

GROUP MEMBERS

Karla Blöcher-Juárez PhD Student; since 04/2019
Andrea Coti PhD Student; since 12/2019
Jia Jun Fung PhD Student; since 01/2018
Elena Ivanova PhD Student; since 03/2019
Ka Yiu Kong Postdoc; since 08/2019
Zhaoyan Li PhD Student; since 12/2018
Rocío Nieto-Arellano Postdoc; since 04/2018
Anke Salzer Lab Manager; since 01/2018
Susmitha Shankar PhD Student; since 06/2020
Nádia Silva Research Associate; since 11/2020
Simone Snead Technician; since 10/2018

OVERVIEW

Proteome integrity is maintained by a complex proteostasis network that controls protein synthesis, folding, transport, and degradation. Numerous protein quality control systems that operate throughout the protein lifecycle contribute to proteome homeostasis through the prevention, detection, and removal of abnormal proteins. Selective protein degradation by the ubiquitin-proteasome system plays a key role in proteome turnover and quality control. When degradation is not possible, the impact of abnormal proteins can be minimised through their asymmetric partitioning during cell division. Despite the activity of such systems, proteome homeostasis declines with ageing and in numerous diseases, resulting in 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 substrates for the various components of the ubiquitin-proteasome system and explore the functions of this system in replicative ageing and genome stability. Our goals are to understand how protein quality control is coordinated with protein biogenesis, to elucidate how cells recognise and destroy abnormal proteins, and how they adapt to challenges in proteome homeostasis.

RESEARCH HIGHLIGHTS

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

UPS in protein degradation and its association with various diseases and ageing, many UPS components remain poorly characterised and our understanding of specificity in the UPS is inadequate.

We develop and deploy proteomic approaches to gain insights into selective protein degradation. Using fluorescent timers as reporters of protein turnover (Figure 1), we established screening pipelines to identify substrates for various components of the UPS and to define signals involved in substrate recognition. To apply these methods in yeast, we developed the SWAp-Tag (SWAT) approach for high-throughput tagging of yeast open reading frames (ORFs). Genome-wide libraries of strains in which each ORF is fused at its endogenous chromosomal locus to the same tag are very useful tools to study the yeast proteome. But their construction is laborious and expensive, hindering the use of new tags in proteomic studies. Using SWAT, we are able to endogenously tag virtually every yeast ORF with any tag in approximately three weeks, greatly expanding the possibilities of proteome-wide studies.

Using our high-throughput approaches, we are undertaking systematic screens for substrates of E3 enzymes and analysing how eukaryotic cells dispose of different abnormal proteins, including misfolded, mislocalised, and orphan molecules. This work thus far led to the discovery of protein quality control pathways that recognise mislocalised proteins at the inner nuclear membrane and endoplasmic reticulum and target them for degradation.

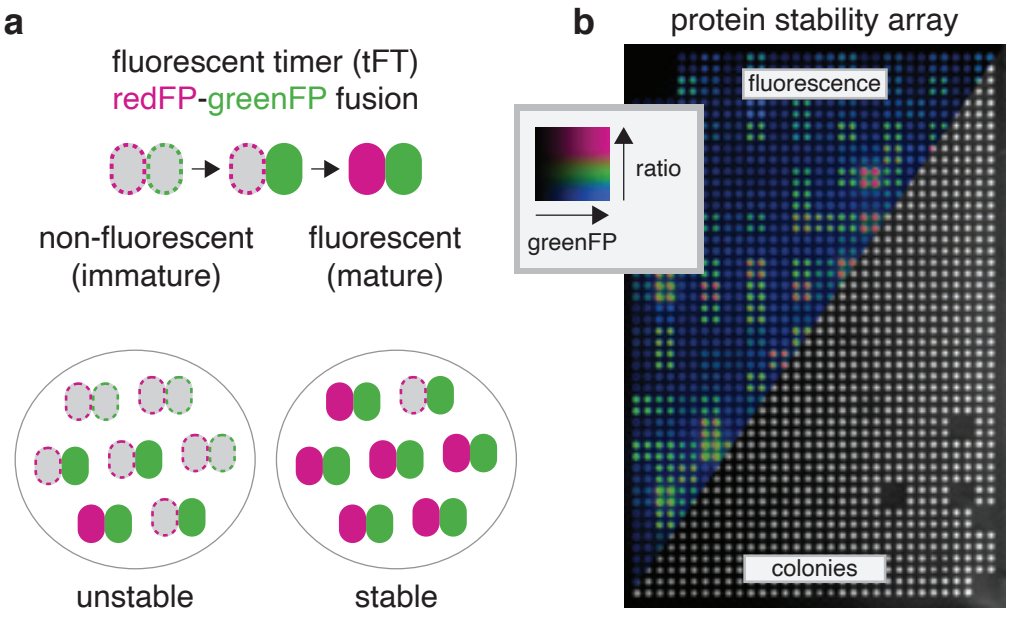

Figure 1. Analysis of protein dynamics with tandem fluorescent protein timers (tFTs).
(a) A tFT is a fusion of two conventional fluorescent proteins: a faster maturing green fluorescent protein (greenFP) and a slower maturing red fluorescent protein (redFP). Due to the difference in maturation kinetics, the redFP/greenFP ratio can be used to follow turnover of tFT-tagged proteins.
(b) Analysis of protein turnover with tFTs. Fluorescence measurements of yeast colonies expressing tFT-tagged proteins.

FUTURE DIRECTIONS

We will expand our systematic search for substrates of E3 enzymes in yeast by combining our reporter-based screens with mass spectrometry and adapt these approaches to human cells. We will also apply genetic and proteomic approaches to identify redundancies in the ubiquitin-proteasome system and, in this way, find substrates for overlapping degradation pathways. Finally, we will continue our work on mislocalised proteins to understand how such molecules are recognised as abnormal in the cell. We are eager to test the importance of such quality control pathways in aneuploidy and during the ageing process.

SELECTED PUBLICATIONS

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

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

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

# indicates joint correspondence

JULIAN KÖNIG

> “New technologies boost our understanding of RNA splicing regulation”

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

Andreas Buchbender PhD Student; 04/2017 – 06/2020
Mariela Cortes Lopez PhD Student; since 02/2017
Antonella Di Liddo Postdoc; since 07/2019
Stefanie Ebersberger Postdoc; since 04/2014
Mihaela Enculescu Postdoc; since 08/2020
Heike Hänel Lab Manager; 11/2013 – 10/2020
Andrea Hildebrandt PhD Student; 04/2014 – 03/2020
Nadine Körtel PhD Student; since 11/2018
Sofya Lipnitskaya PhD Student; since 08/2020
Mikhail Mesitov Postdoc; since 10/2019
Miriam Mulorz PhD Student; since 11/2019
Holger Mutter Student Assistant; 07/2018 – 03/2020
Anna Orekhova Lab Manager; since 07/2020
Cornelia Rückle PhD Student; since 04/2018
Laura Schulz PhD Student; since 04/2017
Kerstin Tretow PhD Student; since 12/2018
Peter Hoch-Kraft Postdoc; since 10/2020

OVERVIEW

Posttranscriptional gene regulation plays an important role not only in development and tissue identity but also in neurodegenerative diseases and cancer. The fate of mRNA is regulated by the cooperative action of RNA-binding proteins (RBPs), which recognise specific RNA sequences to form messenger ribonucleoprotein complexes (mRNPs). The information in the RNA sequence and how it is interpreted by RBPs is commonly referred to as the ‘mRNP code’. However, the molecular features that define this code remain poorly understood. My main goal is to significantly contribute to cracking the mRNP code. To this end, we focus on the molecular mechanisms of splicing regulation and ribosome-associated quality control of translation and their contribution to human physiology and disease.

RESEARCH HIGHLIGHTS

Development of *in vitro* iCLIP for high-throughput measurements of protein-RNA binding

Understanding mRNPs and mRNP-mediated regulation requires a profound knowledge about the interplay of *cis*-regulatory elements, *trans*-acting RBPs, and large cellular machineries like the spliceosome and ribosome. To build this knowledge, my group has developed a new technology: in order to study the **intrinsic RNA-binding activity of RBPs**, we established ‘***in vitro* iCLIP**’ experiments, in which recombinant RBPs are incubated with long transcripts (Sutandy *et al*, 2018). Using this technology, we addressed the RNA binding of the essential splicing factor U2AF2, which recognises the 3’ splice sites of exons. We measured U2AF2 affinities at hundreds of binding sites and compared *in vitro* and *in vivo* binding landscapes using mathematical modelling. We found that *trans*-acting RBPs extensively regulate U2AF2 binding *in vivo*, including enhanced recruitment to 3’ splice sites and clearance of introns (Figure 1). Using machine learning, we identified and experimentally validated novel *trans*-acting RBPs such as FUBP1, BRUNOL6, and PCBP1, which modulate U2AF2

binding and affect splicing outcomes. Our study offers a blueprint for the high-throughput characterisation of *in vitro* mRNP assembly and *in vivo* splicing regulation.

An autoinhibitory intramolecular interaction proof-reads RNA recognition by the essential splicing factor U2AF2

In a recent study together with the Sattler group at the Technical University of Munich, we combined our *in vitro* iCLIP approach with NMR structural biology to identify a novel molecular mechanism for U2AF2 splicing regulation (Kang *et al*, 2020). The recognition of splice sites is initiated by stable U2AF2 binding to the poly-pyrimidine tract (Py-tract) upstream of exons to assemble the spliceosome. However, it remains unclear how U2AF2 discriminates between weak and strong Py-tract RNAs. We found that the intrinsically disordered linker region connecting the two RNA recognition motif (RRM) domains of U2AF2 mediates auto-inhibitory intramolecular interactions that reduce non-productive binding to weak Py-tract RNAs. This proof-reading favours the binding of U2AF2 at stronger Py-tracts, which is required to define 3′ splice sites at early stages of spliceosome assembly. Mutations that impair the linker autoinhibition enhance U2AF2 affinity for weak Py-tracts, resulting in promiscuous binding along mRNAs and impacting splicing fidelity. Our findings highlight an important role for intrinsically disordered linkers in modulating RNA interactions of multi-domain RBPs.

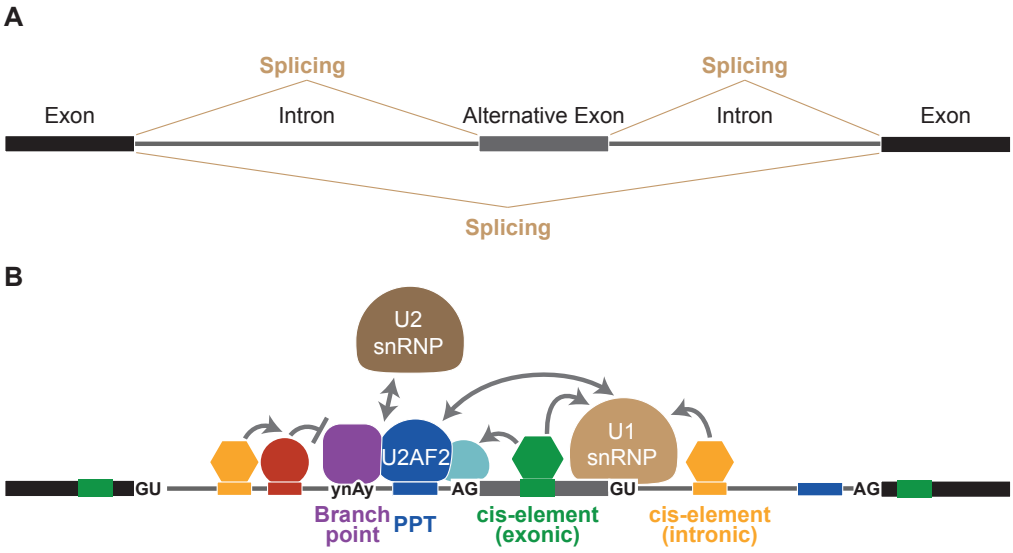

Figure 1. Regulation of alternative splicing. A) Schematic depiction of an alternative splicing event. The alternative exon can be either included or skipped in the mature RNA. B) Early spliceosome assembly at the alternative exon. U2AF2 recognises the polypyrimidine tract (PPT) and recruits the U2 subunit of the spliceosome (U2 snRNP).

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 with relevance 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. Following up on the underlying molecular mechanisms, we will analyse the contribution of multivalent interactions and phase separation to this switch-like regulation. Beyond splicing, we will investigate the functional connections between RNA-binding proteins that exhibit E3 ubiquitin ligase activity and their role in ribosome-associated quality control of poly(A) translation.

SELECTED PUBLICATIONS

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

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

*indicates joint contribution
#indicates joint correspondence

STEFAN LEGEWIE

> “We investigate the kinetic principles of alternative splicing”

EDUCATION

2008	PhD in Biophysics, Humboldt University, Berlin
2004	Diploma in Biochemistry, University of Witten/ Herdecke

POSITIONS HELD

2010 – 2020	Group Leader, Institute of Molecular Biology (IMB), Mainz
2009 – 2010	Group Leader, German Cancer Research Center (DKFZ), Heidelberg
2008 – 2009	Postdoc, Institute for Theoretical Biology, Humboldt University Berlin

GROUP MEMBERS

Alex Anyaegbunam PhD Student; since 08/2016
Mihaela Enculescu Postdoc; 10/2013 – 08/2020
Raymond Gosliga PhD Student; since 06/2019
Lorenz Hexemer PhD Student; 2019 – 08/2020
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Sofya Lipnitskaya PhD Student; 08/2017 – 08/2020
Lorenz Ripka PhD Student; since 05/2017

OVERVIEW

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

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

RESEARCH HIGHLIGHTS

Kinetic modelling of alternative splicing events

Alternative splicing increases protein diversity in eukaryotic cells and therefore plays an important role in development and tissue identity. Mutations causing aberrant splicing are frequently implicated in human diseases including cancer. Together with the groups of Julian König (IMB) and Kathy Zarnack (Goethe University Frankfurt), we established high-throughput screens of randomly mutated minigenes to decode the *cis*-regulatory landscape controlling selected disease- and therapy-relevant splicing events. We use a combination of kinetic and linear regression modelling to identify

the effects of individual point mutations from measured, combined mutations and to understand how these individual mutations interact to control splicing outcomes.

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

Single-cell dynamics of cell cycle networks

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

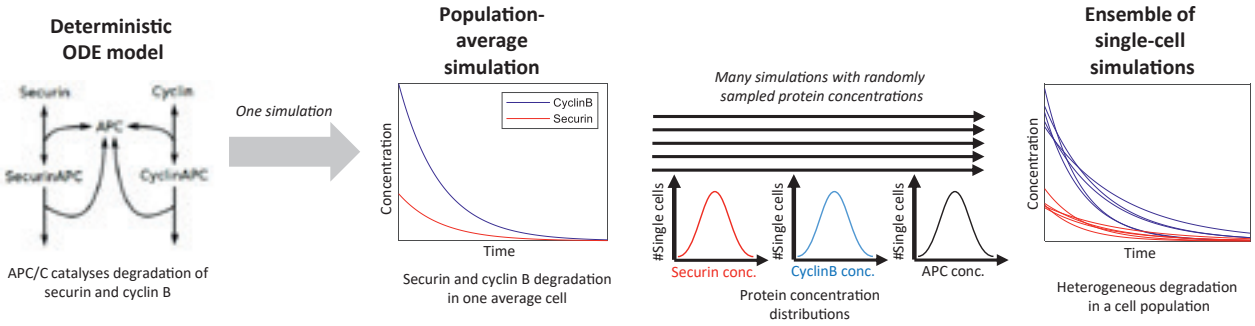

Figure 1. Deterministic modelling of cellular heterogeneity. A kinetic model of securin and cyclin B degradation by the anaphase-promoting complex (APC) during mitosis (left) is described by a deterministic ODE model and numerically integrated to simulate the protein dynamics in one average cell (middle). Cell-to-cell variability is introduced into the system by performing repeated simulations while sampling protein concentrations from log-normal distributions (right).

FUTURE DIRECTIONS

We plan to further refine and develop existing models of signalling and gene expression. For instance, we are investigating the stochastic dynamics of TGFβ/SMAD signalling and modelling downstream gene expression networks based on time-resolved RNA sequencing data. Using publicly available data from cancer patients, we will employ machine learning to predict novel RNA-binding proteins controlling selected splicing decisions and follow up on predicted candidates using knockdown approaches. Moreover, we have begun to model the dynamics of DNA methylation and de-methylation to better understand how this important epigenetic mark can be set and erased to tune gene activity.

SELECTED PUBLICATIONS

Becker K, Bluhm A*, Casas-Vila N*, Dinges N*, Dejung M, Sayols S, Kreutz C, Roignant JY, Butter F# and Legewie S# (2018) Quantifying post-transcriptional regulation in the development of *Drosophila melanogaster*. *Nat Commun*, 9:4970

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

Enculescu M#, Braun S, Thonta Setty S, Busch A, Zarnack K, König J# and Legewie S# (2020) Exon definition facilitates reliable control of alternative splicing in the RON proto-oncogene. *Biophys J*, 118:2027–2041

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EDWARD LEMKE

> “Our work reveals how protein disorder enables precise biological function”

EDUCATION

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

POSITIONS HELD

Since 2018	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Professor of Synthetic Biophysics, Johannes Gutenberg University Mainz (JGU)
2009 – 2017	Group Leader, European Molecular Biology Laboratory (EMBL), Heidelberg (visiting since 2018)
2005 – 2008	Postdoc, The Scripps Research Institute, La Jolla

GROUP MEMBERS

Rajanya Bhattacharjee PhD Student; since 10/2020
Joana Caria Lab Manager; since 04/2017
Nike Heinss PhD Student; since 05/2019
Christine Koehler PhD Student; since 01/2017
Cathrin Lutz Group Administrator; since 05/2019
Christopher Reinkemeier PhD Student; since 09/2016
Hao Ruan Postdoc; since 09/2020
Tom Scheidt Postdoc; since 01/2020
Mikhail Sushkin PhD Student; since 09/2019
Miao Yu Postdoc; since 10/2018
Sara Mingu PhD Student; since 09/2020

OVERVIEW

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

A major technical breakthrough of my lab was the ability to engineer "click"able functionalities into any protein *in vitro* and *in vivo*. This genetic code expansion (GCE) approach has the potential to become a true GFP (fusion protein) surrogate strategy, with

the major advantages being that superior synthetic dyes can be coupled with residue-specific precision anywhere in a protein. This opens up new avenues in single-molecule fluorescence and super-resolution microscopy. More recently, we have been able to merge our understanding of protein disorder and synthetic biology into the design of new membraneless organelles dedicated to protein engineering *in situ* (Figure 1). These custom organelles do not just execute a distinct second genetic code inside the cells, but 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 wide implications for understanding gene regulatory and stress-based mechanisms carried out by distinct, naturally occurring organelles that play vital roles, from regular cellular function to ageing mechanisms.

These precision tools enable us to make even the most complex molecular machinery visible to our core methodologies, which are based on time-resolved multiparameter and nanoscopy tools. This allows innovative approaches to study the heterogeneity of IDPs. More recently, we discovered a distinct ultrafast protein-protein interaction mechanism that can explain how nuclear pore complexes (NPCs) efficiently fulfil their central role in cellular logistics, and how nuclear transport can be both fast and selective at the same time. We also used microfluidics to show how the permeability barrier of the nuclear envelope could be formed by liquid-liquid phase separation from a single disordered protein species. These findings provide a leap forward in our understanding of how IDPs maintain different functionalities through conformational changes despite the normal requirement for rigid molecular specificity.

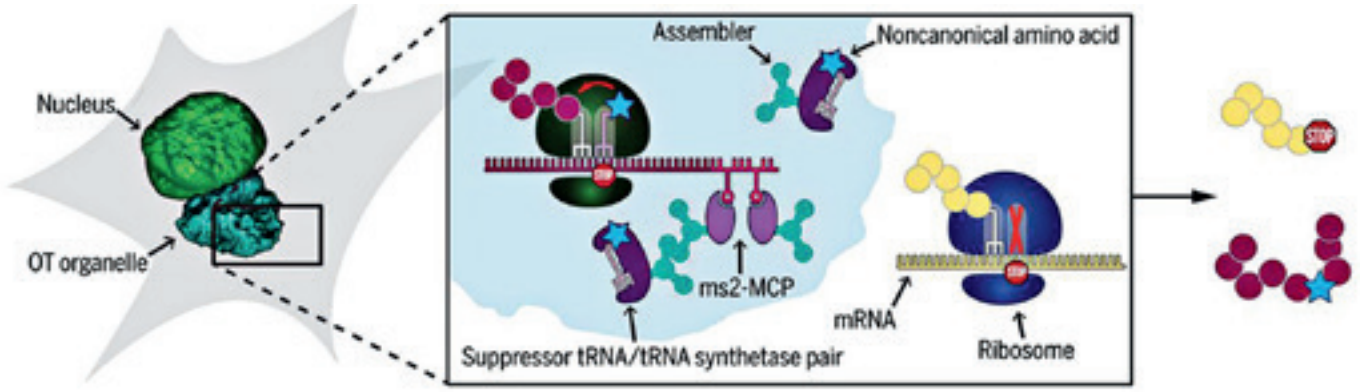

Figure 1. Membraneless orthogonally translating (OT) organelles enable mRNA-specific genetic code expansion in eukaryotes. OT organelles are designed organelles enriched in a suppressor tRNA/tRNA synthetase pair and a specific mRNA binding domain (MCP) by means of an assembler protein. A spatially distinct set of ribosomes associated with the OT organelle preferentially translates recruited mRNAs tagged with ms2 loops to yield the selected protein with the targeted site-specific noncanonical functionality.

FUTURE DIRECTIONS

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

Our work is accompanied by rigorous analysis of the physicochemical properties of IDPs and examines to what extent simple, known polymer concepts such as phase separation can be used to describe the function of IDP biopolymers *in vivo*. Vice versa, we are particularly interested in how disordered proteins play key roles in gene regulation and cellular ageing.

SELECTED PUBLICATIONS

Celetti G*, Paci G*, Caria J, VanDelinder V#, Bachand G#, Lemke EA# (2020) The liquid state of FG-nucleoporins mimics permeability barrier properties of nuclear pore complexes. *J Cell Biol* 219 (1): e201907157

Nikić I, Estrada Girona G, Kang JH, Paci G, Mikhaleva S, Koehler C, Shymanska NV, Ventura Santos C, Spitz D and Lemke EA (2016) Debugging eukaryotic genetic code expansion for site-specific click-PAINT super-resolution microscopy. *Angew Chemie Int Ed*, 55:16172–16176

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

*indicates joint contribution
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KATJA LUCK

> “We see molecular interactions as the functional unit of the cell”

EDUCATION

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

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, USA
2007 – 2008	Research Assistant, EMBL, Heidelberg

GROUP MEMBERS

Chop Yan Lee PhD Student; since 08/2020
Christian Schäfer Postdoc; since 08/2020
Mareen Welzel Lab Manager; since 08/2020

OVERVIEW

Cells function because their molecular components, i.e. DNA, RNA, proteins, etc., interact with each other. This complex network of molecular interactions in a cell mediates all cellular functions and organisation. Genetic and environmental insults perturb these interactions and can cause disease. Because of technical limitations, we still lack a comprehensive and detailed structural and functional understanding of all protein interactions in a human cell, hindering our ability to understand physiological and pathological molecular mechanisms. To tackle these limitations, my lab develops novel computational and experimental methods to identify interfaces of protein interactions, and based on that obtain information on the molecular function of the interaction. Furthermore, we explore the use of protein interaction interface information to predict the pathogenicity of variants and develop integrative systems approaches to generate more specific mechanistic hypotheses that are suitable for experimental follow-up. We apply our approaches to proteins that are associated with neurodevelopmental disorders, focusing on implicated genome integrity processes, and aim to understand the physiological role of these proteins in these processes and the mechanisms that lead to disease upon their mutation.

RESEARCH HIGHLIGHTS

Prediction of protein interaction interfaces

Considerable information has been accumulated about how proteins interact with each other. Two very common ways of interaction are those between globular domains of two proteins and those where a globular domain (hereafter referred to as domain) interacts with a short linear motif in a disordered region of a protein. Unfortunately, comprehensive and accurate information on the types of domain-domain and domain-motif interactions are not available, hindering their use in the prediction of instances of these interface types in a given protein interaction. Building upon existing information, we have manually curated a list of domain-motif types and are currently developing a predictor that uses this information

to predict domain-motif interfaces in protein interactions. We will soon complement this effort with a focus on domain-domain interfaces as well.

Validation of predicted protein interaction interfaces

Despite all efforts, predictions of protein interaction interfaces will not be 100% accurate and thus require experimental validation. With the help of collaborators, we have set up a quantitative protein interaction assay in our lab, which we will use to verify interactions between full length proteins and predicted interfaces. The latter will be achieved by designing and testing mutations that specifically disrupt the interface without perturbing the fold and stability of the protein, which can be monitored as part of the assay. Our goal is to validate predicted protein interaction interfaces at medium throughput. We are collaborating with the labs of Julian König and René Ketting (IMB) on a few protein interactions of interest to help gain more insights into the molecular characteristics of these interactions.

De novo identification of protein interaction interfaces

While it is important to have tools that identify instances of known types of interfaces in protein interactions, it is estimated that the majority of interaction interface types are still unknown. To advance the identification of new types of interfaces in protein interactions, we explore the use of crosslinking mass spectrometry (XL-MS). XL-MS has been demonstrated in case studies to provide interface information, yet we lack understanding about the sensitivity and specificity of the method and its range of applicability. We are developing a positive and negative reference set of protein interactions with which we will benchmark XL-MS protocols. In collaboration with the proteomics core facility of IMB, we are also setting up an experimental pipeline to perform XL-MS for interface mapping at medium throughput. We will then apply the method to protein interactions of interest to us and our collaborators.

Integrative systems biology

Integration of various omics data resources is a powerful strategy to derive systems properties of cells and to employ a data-driven approach towards the identification of new cellular mechanisms. We are collaborating with the Butter lab to gain a systematic and global understanding of the protein relationships that govern the eukaryotic single cell model organism *Schizosaccharomyces pombe* and explore the conservation of these protein relationships in humans.

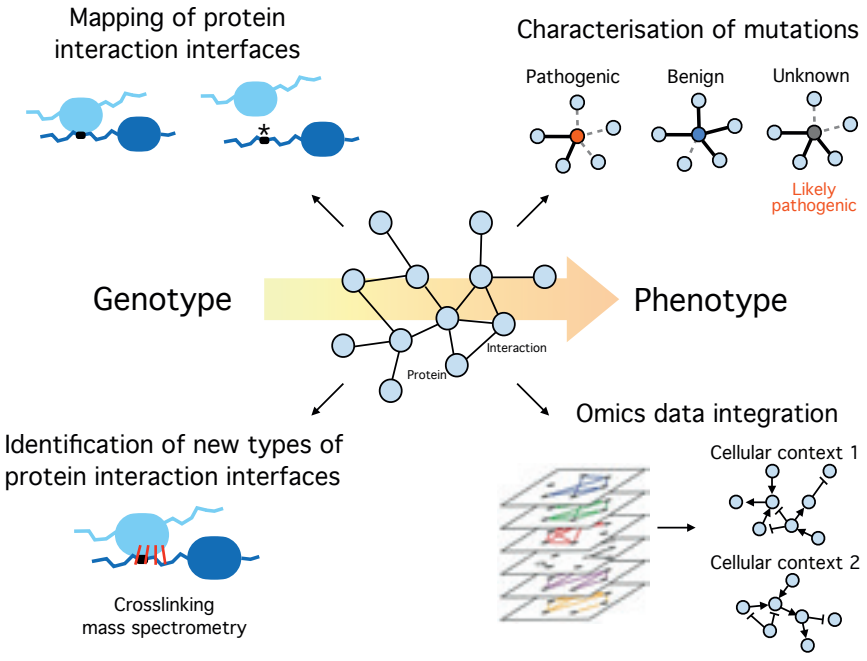

Figure 1. Interactions between proteins are central to cellular function. To improve our ability to predict phenotype from genotype, we develop computational and experimental methods to structurally and functionally characterise protein interactions, use interaction profiling to characterise mutations, and use omics data integration to predict cellular mechanisms within specific cellular contexts.

FUTURE DIRECTIONS

Our focus will be to significantly advance the above research highlights to a point where we can apply them to a set of proteins and their interactions that are implied in neurodevelopmental disorders. Phase separation is an intriguing biophysical property of biopolymers such as proteins to coordinate cellular function, often in response to stress. Protein interaction interfaces, especially those that involve disordered regions of proteins, play a critical role in the phase separation behaviour of proteins. Perturbation of these interfaces by genetic mutations has been linked to neurodegenerative disorders. We aim to apply our methods of protein interaction interface identification to study mechanisms of phase separation that control protein homeostasis in collaboration with the lab of Petra Beli (IMB). Using our expertise in omics data analysis and integration, we will also engage in a collaboration with the lab of Sandra Schick (IMB) to study the role of chromatin remodelling complexes in genome integrity.

SELECTED PUBLICATIONS

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

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

Martínez-Noël G, Luck K, Kühnle S, Desbuleux A, Szajner P, Galligan JT, Rodriguez D, Zheng L, Boyland K, Leclere F, Zhong Q, Hill DE, Vidal M and Howley PM (2018) Network analysis of UBE3A/E6AP-associated proteins provides connections to several distinct cellular processes. *J Mol Biol*, 430:1024–1050

*indicates joint contribution
#indicates joint correspondence

BRIAN LUKE

> “Controlling TERRA may allow control of senescence and ageing”

EDUCATION

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

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)

GROUP MEMBERS

Fábio Bento PhD Student; since 04/2017
Despina Giamaki Research Assistant; 11/2019 – 07/2020
Lukas Graf Bachelor Student; since 08/2020
Dennis Knorr Student Assistant; 09/2019 – 08/2020
Simon Krost Bachelor Student; since 07/2020
Arianna Lockhart Postdoc; 04/2018 – 09/2020
Nina Lohner Master Student; since 10/2020
Stefano Misino PhD Student; since 10/2016
Lara Perez Postdoc; 01/2020 – 09/2020
Vanessa Pires PhD Student; since 08/2017
Stefanie Reimann Technician; since 01/2015
Natalie Schindler Postdoc; since 12/2017
Christiane Stürzbecher Personal Assistant; since 04/2017
Matthias Tonn PhD Student; since 04/2017
Olga Vydzhak PhD Student; since 02/2016
Tina Wagner PhD Student; since 11/2015
Carolin Wagner Research Assistant; since 11/2019

OVERVIEW

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

RESEARCH HIGHLIGHTS

We used budding yeast to create alleles of the RNase H enzymes where their expression could be restricted to a specific cell cycle stage (either G1, S, or G2/M phase). We observed that restricting expression of RNase H2 to G2/M phase was sufficient to complement the phenotypic defects associated with both faulty R-loop metabolism as well as defective RER. Restricting the expression of H2 to S phase could not complement these phenotypes and, in the case of RER, even exacerbated phenotypes. We were able to

demonstrate that RNase H2 causes DNA damage in S phase that requires *RAD52* for repair. This exciting result indicated two modes for ribonucleoside monophosphate (rNMP) removal by RNase H2: the canonical RER pathway, which appears to be optimally executed in a post-replicative manner, and an alternative pathway, which occurs when RNase H2 acts in S phase. We have used genome-wide screening approaches to unravel the genetic requirements of RER in S phase. As expected, we found that the homology-directed repair (HDR) machinery becomes essential when S-RER occurs. Unexpectedly, we discovered that the E3 ubiquitin ligase (Rtt101-Mms1-Mms22) is also required. In collaboration with the lab of Petra Beli (IMB), we discovered that the E3 ubiquitin ligase Rtt101Mms1 ubiquitylates the DNA polymerase epsilon subunit Dpb2 in response to high rNMP loads. We hypothesise that this ubiquitylation may be important to allow efficient replication past nicked rNMPs. Indeed, restricting RNase H2 expression to S phase is toxic in the *rtt101* mutant background.

In the past, our lab has demonstrated that TERRA (Telomere repeat-containing RNA) plays a critical role in promoting the lengthening of short telomeres through homologous recombination. TERRA RNA:DNA hybrids become stabilised at short telomeres, which likely drives the HR reaction through the induction of replication stress. When TERRA is hyper-stabilised at telomeres, the rate of replicative senescence is strongly delayed, indicating that TERRA regulation may be a means to regulate senescence, and hence influence ageing. In order to better understand potential regulators of TERRA, we isolated RNase H and A-sensitive telomere interacting proteins in collaboration with the lab of Falk Butter (Figure 1). Besides Npl3, which we characterised in detail (Perez-Martinez *et al*, 2020), we identified multiple helicases, nucleases, and RNA binding proteins which could be potential regulators of TERRA and hence rates of senescence.

Finally, we have recently demonstrated that post-replicative senescent survivors (ALT equivalent), which maintain their telomeres through HDR, actually go through cycles of telomere shortening and lengthening. Indeed, they have senescent cycles in accordance with their telomere length. TERRA R-loops are also critical in regulating the rate of senescence in post-replicative survivors and are regulated in a similar manner as in senescent cells.

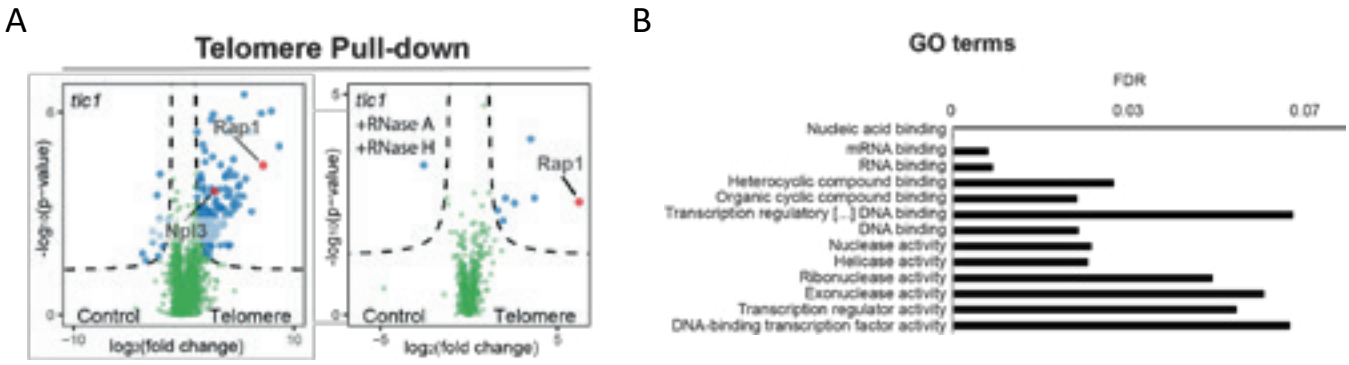

Figure 1. A) Proteins interacting with either telomeric sequences or control sequences were isolated from senescent budding yeast, where TERRA levels are increased, and analysed via mass spectrometry. Samples were then treated with RNase H and RNase A to release proteins that were associated in an RNA:DNA hybrid- or RNA -dependent manner, respectively. B) Gene ontology analysis of the respective associated proteins depicted in A (from Perez-Martinez *et al*, 2020).

FUTURE DIRECTIONS

Future work will strive to understand how TERRA is regulated in a manner that promotes homologous recombination at short telomeres. We will focus on the proteins identified from the proteomics analysis as a starting point and assess their contributions to RNA-DNA hybrids at telomeres, as well as their influence on rates of replicative senescence. These experiments will be performed in both senescent and pre-senescent cells. In addition, we will analyse the effects of RNA-DNA hybrids on the genome in terms of chromatin accessibility, gene expression, and DNA damage using an inducible system to either deplete or stabilise RNA-DNA hybrids. Finally, we will continue trying to understand how rNMPs are removed from DNA and how the DNA replication machinery deals with such lesions when the canonical RER machinery is absent.

SELECTED PUBLICATIONS

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

Lockhart A, Pires VB, Bento F, Kellner V, Luke-Glaser S, Luke B (2019) RNase H1 and RNase H2 are differentially regulated to process RNA-DNA hybrids. *Cell Rep* 29:2890–2900.e5

Graf M*, Bonetti D*, Lockhart A*, Serhal K, Kellner V, Maicher A, Jolivet P, Teixeira MT and Luke B (2017) Telomere length determines TERRA and R-loop regulation through the cell cycle. *Cell*, 170:72–85

*indicates joint contribution
#indicates joint correspondence

CHRISTOF NIEHRS

> “We study the dynamics of covalent chemical DNA modifications”

EDUCATION

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

POSITIONS HELD

Since 2020	Executive Director, Institute of Molecular Biology (IMB), 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
2010 – 2015	Executive Director, Institute of Molecular Biology (IMB), Mainz
1990 – 1993	Postdoc, University of California Los Angeles (UCLA)

GROUP MEMBERS

Khelifa Arab Postdoc; since 11/2011
Sudeshna Banerjee Postdoc; since 08/2020
Amitava Basu Postdoc; since 03/2018
Anne Baumgärtner PhD Student; since 12/2016
Tamara Dehn Animal Caretaker; since 06/2011
Annika Patricia Dohmen Master Student; since 10/2020
Gaurav Joshi PhD Student; since 07/2019
Lars Koeken Research Assistant; 04/2019 – 03/2020
Laura Krebs Technician; since 09/2015
Marcel Misak PhD Student; since 05/2019
Debasish Mukherjee Postdoc; since 03/2019
Michael Musheev Postdoc; since 07/2011
Regina Otto Personal Assistant; since 09/2015
Eleftheria Parasyraki PhD Student; since 09/2018
Ioanna Pavlaki Postdoc; since 07/2019
Mihika Pradhan PhD Student; since 12/2016
Sandra Rölle Lab Manager; since 04/2011
Carola Scholz Technician; since 05/2015
Lars Schomacher Postdoc; since 07/2011
Philipp Trnka PhD Student; since 11/2016

OVERVIEW

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

RESEARCH HIGHLIGHTS

Role of NEIL1 and NEIL2 DNA glycosylases

There is compelling evidence that 5-methylcytosine (5mC) is removed by DNA repair enzymes, which normally function in lesion control. This dual role of DNA repair enzymes raises the question as to whether phenotypic abnormalities resulting from deficiency of DNA repair factors are due to DNA damage or impaired DNA demethylation.

An example of base excision repair enzymes acting both in lesion control and in epigenetic gene regulation are the endonuclease VIII-like glycosylases 1 and 2 (NEIL1 and NEIL2). These enzymes process oxidative DNA base lesions, but recently we have implicated them in the machinery that removes 5mC from DNA during epigenetic DNA demethylation. We showed earlier that Neil2-deficient frog embryos display neural crest defects. This raised

the question of what is the relative contribution of oxidative lesion control and epigenetic DNA demethylation to neural crest defects.

To address this, we investigated Neil-deficient *Xenopus* embryos and created and characterised mouse embryonic stem cell (mESC) lines deficient for *Neil1* and *Neil2*. We described a mechanism where NEIL-deficiency elicits an oxidative stress-induced, TP53-dependent DNA damage response, which induces apoptosis and impairs early neural and neural crest specification. We showed that *Neil1* and *Neil2*-deficiency leads to accumulation of oxidative DNA damage in the mitochondria. Importantly, microinjection of a tp53 antisense morpholino into *Xenopus* embryos not only blocked induction of Tp53 target genes in Neil2-deficient embryos, but also substantially rescued phenotypic abnormalities (Figure 1).

This work demonstrated how impaired removal of oxidative lesions can lead to a selective lineage defect during embryonic development. Our study contributes to the understanding of aberrant cranial neural crest cell development, which is the root cause of congenital craniofacial malformations.

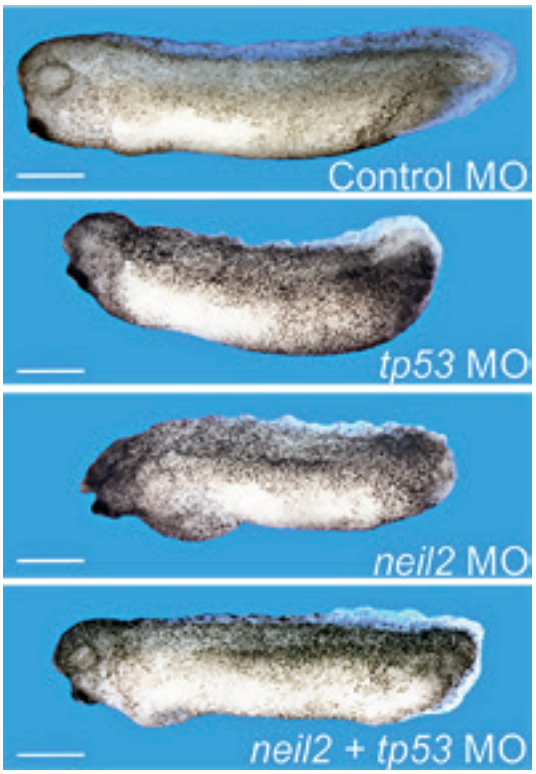

Figure 1. Neil DNA glycosylase deficiency induces a neural p53 DNA damage response which triggers neural crest defects in *Xenopus* embryos. *Xenopus* embryos were microinjected with the indicated antisense morpholino oligonucleotides, which reduce protein levels of the targeted RNA. Note that the neural defects elicited by Neil2 deficiency are substantially rescued by reducing p53, indicating that the phenotypes are due to an elevated DNA damage response (from Han *et al*, 2019).

The origin of genomic N^6-methyl-deoxyadenosine in mammalian cells

In addition to cytosine methylation, we recently studied adenine methylation (m^{6}dA; N^6-methyl-deoxyadenosine). m^{6}dA is a common modification in the nuclear DNA of multicellular organisms such as fungi and algae, and dedicated methyltransferases have been identified. Recently, genomic m^{6}dA was also reported in mammals and proposed to be a novel, heritable epigenetic mark. Yet, the origin and biological relevance of mammalian genomic m^{6}dA have remained very controversial.

We investigated the levels and origin of m^{6}dA in mammalian cells using isotopic labelling coupled with ultrasensitive mass-spectrometry. We confirmed that m^{6}dA occurs in mammalian genomic DNA, albeit at very low levels of 900-2,500 molecules per genome. However, m^{6}dA showed features clearly distinct from the bona fide epigenetic mark m^{5}dC (5-methyl-deoxycytidine). Feeding cells with isotope-labelled L-methionine as a methyl donor yielded half-maximal labelling of the established epigenetic mark m^{5}dC within one cell doubling. In contrast, half-maximal labelling of m^{6}dA required four cell doublings, which is atypical for a heritable epigenetic DNA mark. Moreover, isotope double-labelling of cells demonstrated direct methylation of cytosine but not adenine in genomic DNA. As an alternative mechanism to active DNA adenine methylation, we provided evidence that genomic m^{6}dA originates from free ribo-m^{6}A (N^6-methyladenosine), a breakdown product of methylated RNA. Ribo-m^{6}A is metabolised by a nucleotide salvage pathway to m^{6}dA and is indiscriminately (mis)-incorporated into DNA during replication. Our results constrain models of m^{6}dA acting as a heritable epigenetic DNA mark.

FUTURE DIRECTIONS

Our results indicate that the requirement of NEIL1 and NEIL2 DNA glycosylases for neural crest formation is in oxidative lesion control rather than in epigenetic DNA demethylation. However, this does not exclude other unidentified roles in reversing DNA methylation. We will therefore analyse the cytosine methylome of NEIL1,2-deficient cells. Furthermore, we will also study selected RNA modifications that we identify by mass spectrometry. Finally, we will continue studying DNA modifications. On the one hand, we will focus on the oxidation products of cytosine methylation, namely 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), and address their regulation and epigenetic function. On the other hand, we aim to identify and characterise other modified nucleobases.

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Han D*, Schomacher L*#, Schüle KM*, Mallick M, Musheev MU, Karaulanov E, Krebs L, von Seggern A and Niehrs C# (2019) NEIL1 and NEIL2 DNA glycosylases protect neural crest development against mitochondrial oxidative stress. *eLife*, e49044

Musheev MU#, Baumgärtner A, Krebs L and Niehrs C# (2020) The origin of genomic N^6-methyl-deoxyadenosine in mammalian cells. *Nat Chem Biol*, 16:630–634

*indicates joint contribution
#indicates joint correspondence

VASSILIS ROUKOS

> “We use state-of-the-art microscopy and genomics to understand genome rearrangements”

EDUCATION

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

POSITIONS HELD

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

GROUP MEMBERS

Jan Heidelberger Postdoc; since 08/2020
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Monika Kuban Lab Manager; since 01/2017
Marie Kube Research Assistant; 10/2019 – 01/2020
Gabriel Longo PhD Student; since 09/2019
Vera Minneker PhD Student; since 04/2016
Rossana Piccinno PhD Student; since 04/2015
Gabrielle Sant PhD Student; since 11/2018
Lydia Koufoudaki Research Assistant; since 10/2020

OVERVIEW

Maintaining the integrity of genetic information is essential for cell survival. Mechanisms that counteract DNA damage are important for maintaining cellular homeostasis by suppressing mutagenic events and genome rearrangements that may lead to disease, particularly cancer. One of the most severe forms of genome rearrangements are chromosome translocations. Translocations form by the illegitimate joining of chromosome breaks and often play key roles in the initial steps of tumorigenesis. Despite their prevalence and importance, our understanding of their genesis is still rudimentary. Which molecular features define recurrent chromosome breakpoints? How do the broken chromosome ends find each other within the nuclear space? What are the DNA repair mechanisms that mediate chromosome fusion and which factors favour interchromosomal fusion (translocation) over intrachromosomal repair? By using a combination of molecular biology techniques, genetics, and high-throughput imaging and sequencing approaches, we aim to shed light on the basic molecular mechanisms underlying the formation of oncogenic chromosome translocations.

RESEARCH HIGHLIGHTS

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

Modelling the formation of recurrent cancer-initiating genome rearrangements of interest requires a versatile approach that can probe rare events with high sensitivity. We have now established a method called C-Fusion 3D that uses fluorescence *in situ* hybridisation (FISH) to probe the position of individual chromosome ends of potential translocation partners in interphase cells in 3D. High-throughput microscopy and automated image analysis is then used to identify single cells with chromosome breakage and translocations. This methodology complements existing approaches and offers several advantages in detecting and quantifying translocations. It is (i) suitable for detecting translocations without the requirement to map the precise translocation breakpoints or fusion

product; (ii) compatible with both site-specific induction of breaks (mediated by endonucleases, ZNFs, CRISPR) and more physiological methods of inducing DNA damage, such as ionising radiation and chemotherapeutics (see below); and (iii) efficient in detecting translocations in interphase cells at frequencies down to 10^{-4} without the need for metaphase spread preparation. C-Fusion 3D is a powerful tool that can be used to dissect molecular and cellular mechanisms that contribute to the formation of any oncogenic chromosome translocation of interest.

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

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

We are interested in combining cutting-edge genomics and single-cell imaging methods to determine why these leukaemia-promoting translocations arise. Our current work has shown that certain sites with highly active genes tend to be close to regions of DNA folding into chromatin loops that are under more mechanical strain. This makes them susceptible to DNA breaks caused by topoisomerase poisons such as etoposide, producing translocations that drive leukaemia (Figure 1). We have also identified factors involved in the repair of these DNA breaks that actively suppress the formation of translocations. In another direction that may have clinical implications, we are performing unbiased siRNA-based screens to identify factors that suppress these types of translocations while leaving the cytotoxic effect of topoisomerase poisons intact. Our findings highlight how gene activity and the arrangement of DNA within the nucleus can have a profound impact on events that trigger genomic instability to promote cancer.

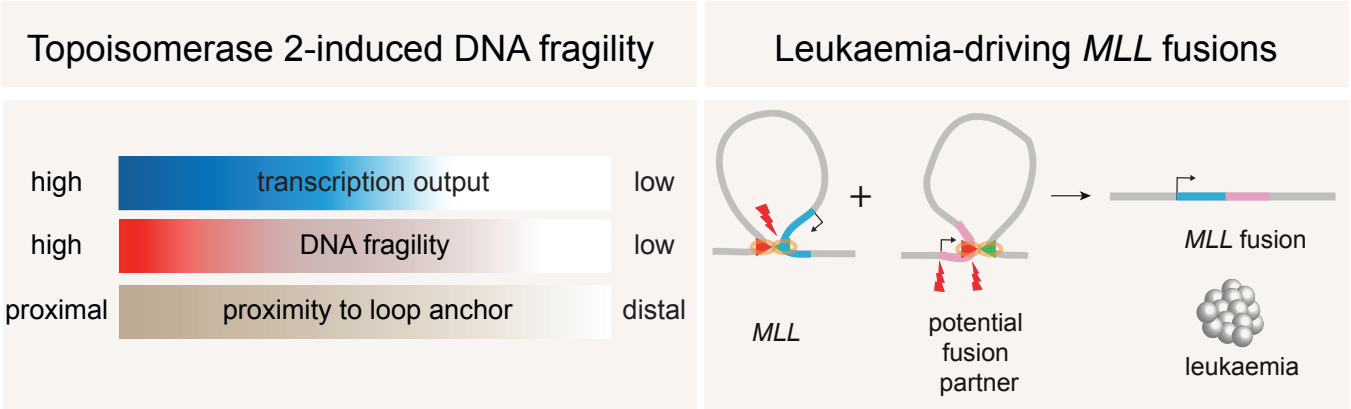

Figure 1. Etoposide-induced gene fragility (red gradient) positively correlates with transcriptional output (blue gradient) and with proximity to chromatin loop anchors (brown gradient). DNA double-strand breaks within highly transcribed *MLL* and fusion partner genes at loop boundaries promote the formation of oncogenic *MLL* translocations.

FUTURE DIRECTIONS

Central to our focus is shedding light on the events promoting genomic instability in the context of chromatin and chromosome organisation. We therefore intend to profile endogenous DNA breaks across the genome in various cell types, with the aim of identifying common or cell type-specific signatures of DNA fragility. We will then focus on identifying mechanistically how these endogenous DNA breaks form and evaluate how DNA break repair efficiency is influenced by the genomic and chromatin context. These studies will directly highlight the link between cell type-specific DNA fragility and repair in the formation of tissue-specific, recurrent oncogenic translocations. Taken together, our research will shed light on the mechanisms of cancer-initiating translocations, which will advance our knowledge of the fundamental principles of cancer aetiology.

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

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

SANDRA SCHICK

"We study chromatin regulation and the consequences of its dysregulation in disease"

EDUCATION

2016	Doctorate in Molecular Biology, Institute of Molecular Biology (IMB), Mainz
2012	Master in Biomedicine and Diploma in Biology, Johannes Gutenberg University Mainz (JGU)
2008	Bachelor in Molecular Biology, Johannes Gutenberg University Mainz (JGU)

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, Austria

GROUP MEMBERS

Marie Kube PhD Student; since 05/2020
Julia Varga Postdoc; since 07/2020

OVERVIEW

The packaging of our genome into chromatin not only enables the DNA to fit into the eukaryotic nucleus but also provides a complex platform for multi-layered regulation and maintenance of the genome. Chromatin modifying enzymes have evolved to keep chromatin flexible and adjustable to the requirements of the cell and are essential regulators for all genomic processes. Chromatin remodellers such as BRG1-/BRM-associated factor (BAF) complexes utilise the energy from ATP hydrolysis to modulate nucleosome positioning, thereby affecting the accessibility of DNA to DNA-binding proteins. Consequently, they are extremely important in various genomic processes like gene regulation or DNA repair. Their importance is underlined by the fact that mutations in genes coding for BAF complex members are frequently associated with diseases such as cancer or neurodevelopmental disorders. Our research aims to understand how chromatin remodellers integrate with other regulators to specifically modulate certain genomic regions under particular conditions to allow for proper cellular processes. Moreover, we explore how their misregulation leads to diseases to enable the identification of novel therapeutic perspectives.

RESEARCH HIGHLIGHTS

Correct regulation and maintenance of the genetic information stored in DNA is essential for proper cellular function, and this regulation is constantly adjusted in response to intra- and extracellular stimuli such as developmental signals, stress, or DNA damage. This dynamic control is mediated via alterations in chromatin, including modifications of DNA or histones, incorporation of histone variants, or changes in chromatin packaging and topology. These mechanisms function individually and in concert to regulate processes such as gene expression and DNA damage responses. Disruptions in chromatin regulation can have severe effects and lead to various diseases.

Mutations in genes coding for subunits of the BAF chromatin remodelling complexes have been found to cause developmental disorders and occur frequently in a wide range of cancers. Therefore, it is particularly important to understand the regulation and function of BAF complexes in the diverse processes they play a role in, e.g. gene regulation, DNA repair, and chromatin architecture. The diverse functions of BAF complexes are at least partly achieved through their polymorphic occurrence. In mammals, around 30 genes are known to encode for BAF subunits that can assemble in a modular, combinatorial, and sometimes cell type-specific fashion to form numerous different BAF complexes.

By utilising an isogenic cell line panel comprising individual knock-outs of most BAF-encoding genes, we obtained information about the role of specific subunits in BAF complex composition and assembly. In addition, we identified functionally important subunits, the loss of which leads to strong alterations in DNA accessibility of regulatory regions and gene expression. The most profound effects were observed after knock-out of the BAF genes that are most frequently mutated in cancer. Importantly, mutations found in malignancies are also predominantly loss-of-function mutations.

These studies also revealed dependencies between the different BAF subunits. In order to explore vulnerabilities of the mutant cells to further BAF perturbations, a viability screen was performed testing concomitant loss of different BAF complex subunits in all possible combinations. This screen revealed several new intra-complex synthetic lethalities that were further confirmed in other cancer cells and may provide a new basis for therapeutically targeting certain BAF-mutant cancers.

Although these systematic studies performed at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences provide extensive new insights into the biology of BAF complexes and related diseases, further research is needed to comprehensively understand the regulatory roles of different BAF subtypes in various cellular processes and different cellular backgrounds.

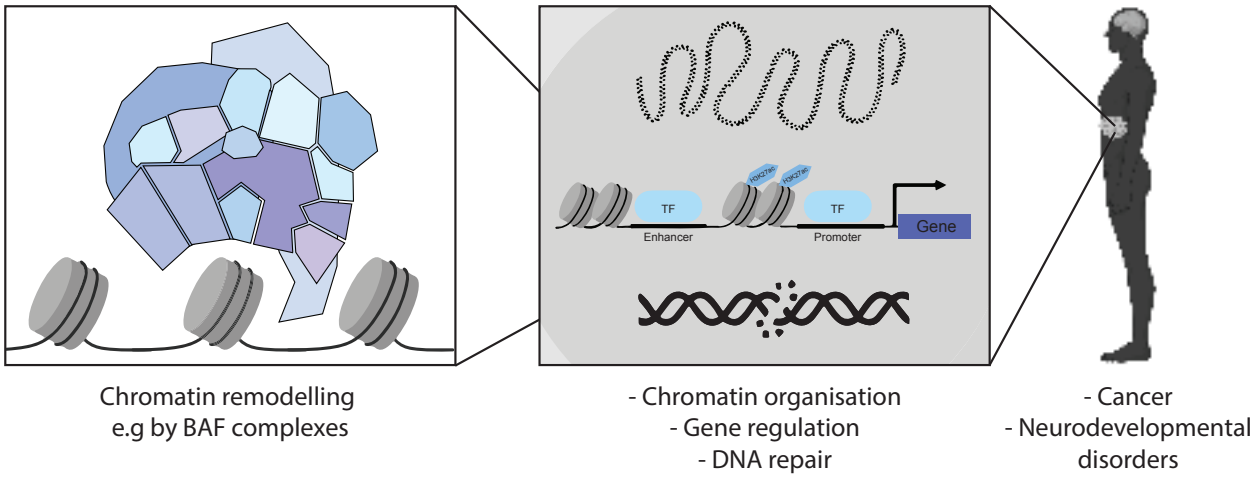

Figure 1. Chromatin remodelling, e.g. by BAF complexes, is important for genomic processes. Their deregulation can lead to various diseases, such as cancer or neurodevelopmental disorders.

This figure was created using BioRender.com.

FUTURE DIRECTIONS

To accomplish this goal, we will further explore the molecular function and regulation of individual BAF subcomplexes. We will systematically investigate the processes they are involved in and how they integrate with other regulatory mechanisms. To study the role of individual BAF subcomplexes in various cell types, developmental processes, and BAF mutation-induced diseases, we will use cell culture models closely reflecting human conditions. Ultimately, our research aims to unravel pathogenic mechanisms that can be targeted for therapy.

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Thakurela S*, Tiwari N*, Schick S, Garding A, Ivanek R, Berninger B and Tiwari VK (2016) Mapping gene regulatory circuitry of Pax6 during neurogenesis. *Cell Discov*, 2:15045

*indicates joint contribution

HELLE ULRICH

“DNA single-strand breaks: ‘neglected’ lesions with an intriguing genome-wide pattern”

EDUCATION

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

POSITIONS HELD

Since 2013	Scientific Director, Institute of Molecular Biology (IMB), Mainz
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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

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Nicola Zilio Postdoc; since 08/2014

OVERVIEW

A robust response to DNA replication stress is an important defence mechanism against genome instability and serves as a last barrier against the development of cancer. Our lab studies the regulatory mechanisms that contribute to ensuring the complete and accurate duplication of a cell’s genetic information in every cell cycle, especially as they relate to the posttranslational protein modifiers ubiquitin and SUMO. We aim to understand how cells choose between alternative processing pathways for replication-blocking lesions in the DNA template, for example between error-prone translesion synthesis and accurate recombination-mediated template switching, or between fork-associated and postreplicative modes of damage bypass. Posttranslational modifications of the replication clamp protein PCNA have proven to be critical determinants of these pathways in eukaryotes. Over the past years, our research into the temporal and spatial regulation of replicative DNA damage bypass has illustrated the need to understand the genome-wide distribution of both the lesions themselves as well as the structures associated with their processing, such as stalled replication forks, postreplicative daughter-strand gaps, and incomplete Okazaki fragments. To this end, we have started to develop next-generation sequencing (NGS)-based methods for the mapping of lesions and replication intermediates.

RESEARCH HIGHLIGHTS

NGS-based mapping tools exist for various lesion types, including abasic sites, pyrimidine dimers, and incorporated ribonucleotides. Perhaps the most extensive range of techniques is available for DNA double-strand breaks (DSBs), which have been analysed at high resolution in several cell lines and experimental conditions by multiple labs. In contrast, DNA single-strand breaks (SSBs) have so far mostly eluded systematic analysis by NGS, even though they represent by far the most frequent lesions in the genome. SSBs arise endogenously from attack by reactive oxidative species, but they are also important intermediates of DNA replication and repair.

As such, their genome-wide distribution is of immediate interest for the investigation of replication stress. We have therefore developed an NGS method based on the "Genome-wide Ligation of 3'-OH DNA Ends" (GLOE-Seq) for the mapping of such structures at nucleotide resolution (Figure 1A). The protocol relies on thermal denaturation of purified intact genomic DNA, followed by ligation of a biotinylated adaptor to exposed 3'-OH termini and their capture by means of streptavidin beads. Enzymatic pre-treatment that converts a particular lesion into a structure with an exposed 3' end allows the mapping of base modifications for which a specific endonuclease is available, thus expanding the range of potential applications. Moreover, when applied to DSBs, GLOE-Seq is capable of mapping their 3' termini, which makes the method complementary to most DSB-specific protocols that involve a blunting of the ends.

After validating the method on *in vitro*-digested DNA, we have begun to explore the power of GLOE-Seq in proof-of-principle experiments by mapping DNA lesions and base excision repair intermediates after exposure to UV irradiation and alkylating agents. In addition, we have addressed the distribution of SSBs in unperturbed cells. This analysis has revealed surprising biases in spontaneous breaks around yeast centromeres and telomeres and genomic regions of human cells relevant for transcriptional regulation, distinct from the patterns of DSBs. Importantly, we have shown that GLOE-Seq is suitable for mapping Okazaki fragments without prior size selection in yeast and human cells when combined with depletion of the replicative ligase (Cdc9 or Ligase I, respectively). Plotting the relative strand bias of SSBs – representing unligated Okazaki fragments – allows for efficient mapping of replication origins and termination zones (Figure 1B). In the course of these experiments, we identified an intriguing asymmetry of SSBs in unperturbed yeast as well as human cells, complementary to the SSB pattern in ligase-depleted cells and representing a leading-strand bias that is absent from randomly fragmented DNA. We speculate that these breaks might derive from the propensity of the leading-strand DNA polymerase ε to incorporate ribonucleotides; however, alternative explanations, such as an origin from fully or partially reversed fork structures, cannot be excluded at present.

In summary, the development of GLOE-Seq and a dedicated computational pipeline, GLOE-Pipe, has enabled us to probe the structures associated with replicative lesion processing in unprecedented detail.

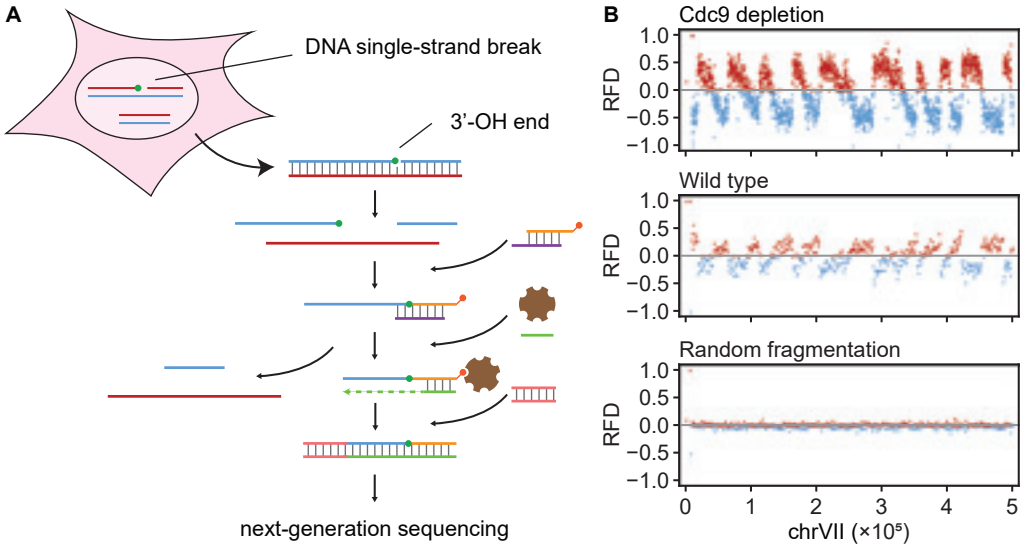

Figure 1. Genome-wide mapping of 3'-OH ends by GLOE-Seq. A) Schematic illustration of the method. Green spheres: 3'-OH groups; orange spheres: biotin label; brown shapes: streptavidin beads. **B)** Replication fork directionality (RFD) plots indicating strand bias of 3'-OH termini on chromosome VII of budding yeast after depletion of replicative ligase (Cdc9), under unperturbed conditions (wild-type), and after random fragmentation. Forward and reverse strands are depicted in blue and red, respectively.

FUTURE DIRECTIONS

We are currently building on our initial GLOE-Seq results in unperturbed yeast and human cells by investigating the origins of the intriguing SSB patterns during replication. At the same time, we are further developing the method itself by adding features such as absolute quantification and a protocol for the simultaneous capture of 5'-ends. We are exploring ways to minimise the amount of starting material, which will give us access to very small samples, for example from specialised cell populations isolated by flow cytometry. Finally, we are starting to apply GLOE-Seq to gain insight into the impact of re-priming in the context of DNA damage bypass by examining the distribution of daughter-strand gaps under conditions of DNA replication stress.

SELECTED PUBLICATIONS

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

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

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

#indicates joint correspondence

EVA WOLF

> “We study protein interactions that control circadian gene regulation”

EDUCATION

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

POSITIONS HELD

Since 2013	Adjunct Director, Institute of Molecular Biology (IMB), Mainz
	Professor of Structural Chronobiology, Faculty of Biology, Johannes Gutenberg University Mainz (JGU)
2012 – 2013	Group Leader, Ludwig Maximilian University (LMU), Munich
2009 – 2011	Group Leader, Max Planck Institute for Biochemistry, Martinsried
2000 – 2009	Group Leader, Max Planck Institute for Molecular Physiology, Dortmund
1996 – 2000	Postdoc, Rockefeller University, New York

GROUP MEMBERS

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Marcel Conrady PhD Student; since 10/2019
Silke Helmke Technician; since 05/2014
Shruti Krishnan PhD Student; since 10/2016
Torsten Merbitz-Zahradnik Postdoc; since 01/2014
Sebastien Moniot Postdoc; since 06/2019
Ruth Schaupp Personal Assistant; since 05/2014
Maximilian Schuler Bachelor Student; since 07/2020
Til Roman Wanner Bachelor Student; since 07/2020
Jule Urschel Bachelor Student; since 11/2020

OVERVIEW

Circadian clocks – operated by cell-autonomous transcription/translation feedback loops – affect many essential cellular, physiological, and behavioural processes such as the sleep-wake cycle, hormone production, metabolism, and the immune system. In mammals, the BMAL1/CLOCK transcription factor complex activates three period (*per1,2,3*) and two cryptochrome (*cry1,2*) clock genes as well as many clock-controlled genes (ccgs) in a day-time dependent manner. Genome-wide analyses revealed three temporally separated phases of BMAL1/CLOCK-dependent circadian gene regulation (Figure 1): (1) a transcriptionally active state, where BMAL1/CLOCK recruits co-activators such as the histone acetyltransferase CREB-binding Protein (CBP) and the histone methyltransferase Mixed Lineage Leukemia 1 (MLL1), (2) an early repressive state, where BMAL1/CLOCK is repressed by a large multi-subunit CRY/PER-containing complex, which also includes CKIε, chromatin modifiers, and RNA binding proteins, and (3) a late repressive state, where CRY1 alone represses BMAL1/CLOCK.

To further our mechanistic understanding of the transcriptional and epigenetic regulation of the mammalian circadian clock and ccgs, we pursue structure-function analyses of clock protein interactions with co-activators or co-repressors of BMAL1/CLOCK.

RESEARCH HIGHLIGHTS

The transition of BMAL1/CLOCK regulation from the late repressive to the transcriptionally active state involves competitive binding between the CRY1 repressor and the KIX domain of the coactivator CBP to the C-terminal transactivation domain (TAD) of BMAL1. Using Small Angle X-ray scattering (SAXS) and biochemical and biophysical interaction studies, we structurally and mechanistically characterised the interaction between BMAL1 and the CBP-KIX domain. As CBP acetylates MLL1 and enhances its activity as a BMAL1/CLOCK co-activator, we also analysed the interaction between BMAL1 and MLL1 upon binding to CBP-KIX. We found that the MLL1 binding pocket of the CBP-KIX domain is also involved

in interacting with BMAL1, and consistent with this, BMAL1 and MLL1 cannot simultaneously bind to CBP-KIX. Notably, the active MLL1 histone methyltransferase is part of a complex including RbBP5, WD repeat-containing protein 5 (WDR5), Ash2L, and DPY30, where MLL1 and RbBP5 bind to two different binding pockets of WDR5. Interestingly, WDR5 was also identified within a large liver clock protein complex pulled down by PER proteins in the transcriptionally repressive phase. Furthermore, WDR5 was shown to interact with PER1 and PER2 in cell-based co-IP studies. Hence, WDR5 may affect the active state (via MLL1) as well as the early repressive state to regulate circadian genes.

To provide insights into the as yet unknown functions of WDR5 and the PER-WDR5 interactions in the circadian clock, we set out to structurally, biochemically, and biophysically analyse the PER1/2-WDR5 interactions. We found that the homologues PER1 and PER2 interact with WDR5 differently from each other, resulting in distinct interplays with the formation of the PER-CRY clock protein complex and with WDR5-RbBP5 interactions. This finding provides new insights into the partially non-redundant roles of PER1 and PER2 in the mammalian circadian clock. Additionally, it advances our understanding of the architecture and assembly of the early repressive BMAL1/CLOCK complex and how it crosstalks with the preceding activating complex and following late repressive complex by changing protein interactions at different stages of the circadian cycle. However, a lot of open questions remain, such as: how do the PER-WDR5 sub-complexes integrate into the dynamic interaction network of the multi-subunit early repressive complex? Do PER-WDR5 complexes also occur outside the early repressive complex? What are their functions within or outside the circadian clock? Do PER-WDR5 interactions impact MLL1 histone methyltransferase activities (e.g. upon BMAL1/CLOCK co-activation) or the activity of other WDR5-containing cellular complexes? How are PER-WDR5 interactions regulated in the cell? Answering these questions will require much more extensive studies of the interaction networks of circadian clock proteins.

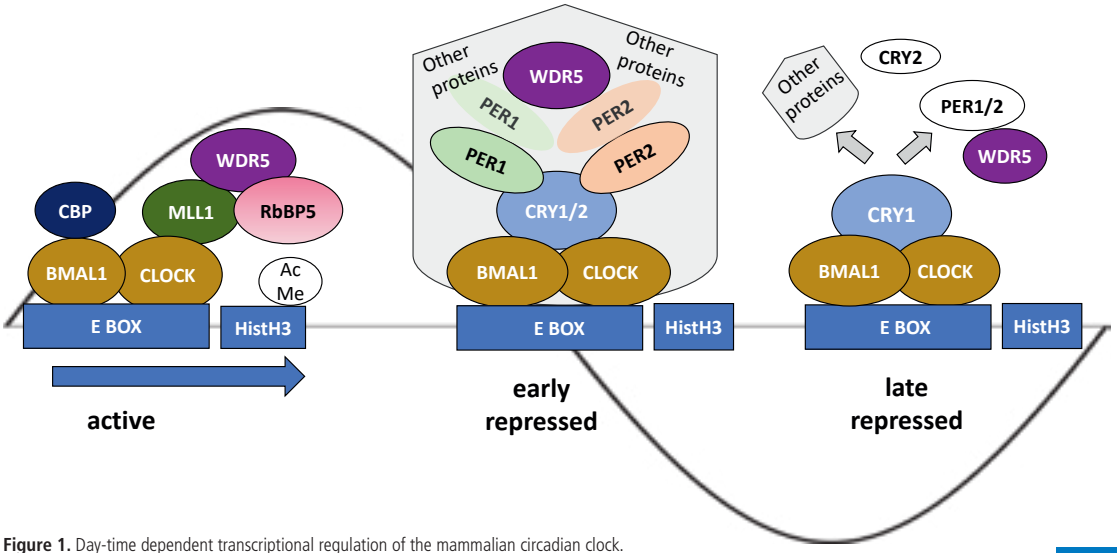


Figure 1. Day-time dependent transcriptional regulation of the mammalian circadian clock. *Left:* MLL1 (within the MLL1-WDR5-RbBP5-Ash2L-DPY30 complex) and CBP co-activate BMAL1/CLOCK. *Middle:* BMAL1/CLOCK is repressed by a multi-subunit "early repressive complex" including CRY1/2, PER1/2, WDR5, and about 30 other proteins. PER1/2 can interact with CRY1/2 or WDR5. *Right:* Late repressive CRY1/BMAL1/CLOCK complex. PER1/2-WDR5 complexes may also exist outside the early repressive complex.

FUTURE DIRECTIONS

With the structural, biochemical, and biophysical characterisation of WDR5-PER1/2 interactions, we have expanded our mechanistic understanding of the interaction network driving gene regulation in the mammalian circadian clock. Our studies identify mutants and interaction interfaces that can be used to dissect the roles of PER1/2-WDR5 interactions in the mammalian circadian clock in a targeted manner. They also provide a stepping stone to further reconstruct the architecture of multi-subunit clock protein complexes. Furthermore, the WDR5-PER interaction connects the core clock machinery to other epigenetic and gene regulatory processes in which WDR5 plays a role. In future, we will continue to structurally, biochemically, and biophysically analyse new clock protein ligands and place them in the context of our existing knowledge of clock protein interactions.

SELECTED PUBLICATIONS

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

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

Witosch J, Wolf E# and Mizuno N# (2014) Architecture and ssDNA interaction of the Timeless-Tipin-RPA complex. *Nucleic Acids Res*, 42:12912–12927

#indicates joint correspondence


imb
Institute of
Molecular Biology

CORE FACILITIES

OVERVIEW

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

There are currently seven Core Facilities (CFs) at IMB: Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics, Protein Production, and a Media Lab. The Bioinformatics, Genomics, and Proteomics CFs provide users with a "full service", from experimental design and quality control of samples to data generation, analysis, and data presentation. The Flow Cytometry and Microscopy/Histology CFs provide an "assisted service", where researchers work independently on CF equipment after introductory training by CF staff. Whether receiving full or assisted service, the CFs' staff are available for consultation and troubleshooting. Furthermore, CF staff often collaborate with researchers to provide customised or specialised services. For IMB researchers, all seven CFs are available for use. Beyond that, the Flow Cytometry, Genomics, Microscopy/Histology, and Proteomics CFs also offer their services to the larger research community in Mainz.

CF services provided are based on user demand. For each facility, a user committee gives feedback on the equipment and user experience and helps define the services that each CF provides. These committees also help shape the implementation of new applicable services within the CFs. The overall CF functions as a service axis by aligning and combining individual services within its units to create new, innovative workflows; for example, single cell sequencing, which requires a service overlap between flow cytometry and genomics. In addition to technical services, the CFs offer lectures on a variety of methods, as well as practical courses to instruct researchers in new techniques and instrumentation, data acquisition, experimental design, statistics, data processing, and analysis. These courses allow researchers to keep up-to-date with and broaden their knowledge of current and emerging technologies. Lectures are generally open to everyone.

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

Andreas Vonderheit Director of Core Facilities and Technology

BIOINFORMATICS

OVERVIEW

The Bioinformatics Core Facility (BCF) supports researchers at IMB with computing infrastructure, web services, system administration, software training, consulting on experimental design, and biostatistics. In addition, BCF members actively participate in the computational processing, analysis, visualisation, and interpretation of high-throughput "omics" data generated in the course of research projects. The BCF also provides bioinformatics expertise to the Collaborative Research Center 1361 "Regulation of DNA Repair & Genome Stability".

SERVICES OFFERED

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

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

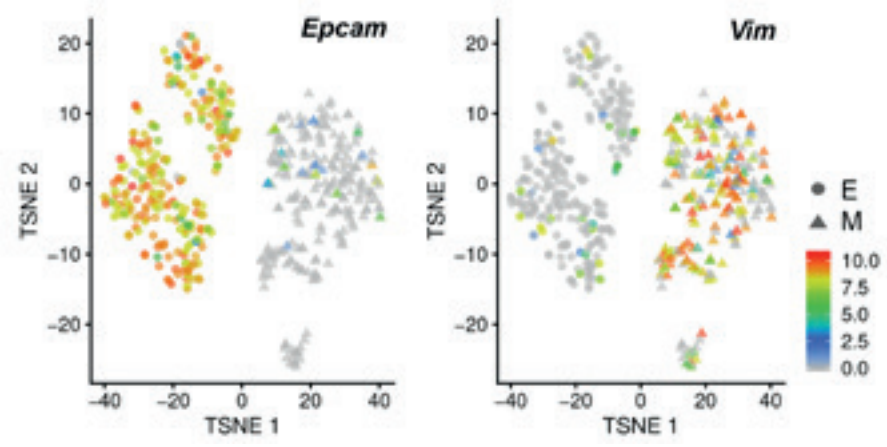


Figure 1. Dimensionality reduction t-SNE plots of scRNA-seq profiling in gastric cells from stage E13.5 mouse embryos. Single cells are coloured by the relative expression (log2[TPM+1]) of marker genes Epcam and Vim with indicated epithelial (E) and mesenchymal (M) cell populations.

The BCF operates several computing and storage servers which have been recently upgraded to support data-intensive and machine learning applications through a generous grant from the Science Ministry of Rhineland-Palatinate. The Facility maintains GitLab and GitHub repositories (https://gitlab.rlp.net/imbforge, https://github.com/imbforge) with software tools and pipelines dedicated to different types of next-generation sequencing (NGS) assays. The BCF also offers customised bioinformatics solutions and long-term analytical support for numerous omics projects on a collaborative basis.

MEMBERS

Emil Karaulanov
Head; since 10/2014
Anke Busch
Bioinformatician; since 01/2014
Christian Dietrich
System Administrator; since 04/2017
Fridolin Kielisch
Biostatistician; since 06/2020
Nastasja Kreim
Bioinformatician; since 04/2012
Martin Oti
Bioinformatician; since 12/2017
Giuseppe Petrosino
Bioinformatician; since 03/2017
Frank Rühle
Bioinformatician; since 01/2019
Sergi Sayols
Bioinformatician; since 06/2019
Pascal Silberhorn
System Administrator; since 12/2015

FLOW CYTOMETRY

OVERVIEW

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

MEMBERS

Stefanie Möckel
Head; since 10/2016
Jesús Gil Pulido
Staff Scientist; 09/2018 – 08/2020

SERVICES OFFERED

The FCCF offers a full service for sorting and an assisted service along with training for the analysers. Additionally, its staff collaborate in terms of analysing flow cytometry data and sample preparation. During the past year, the FCCF has performed various types of experiments including multicolour measurements, cell separation for next generation sequencing, sorting of isolated neuronal nuclei, classical enrichments for subsequent cell culture, qPCR analysis, mass spectrometry, and microscopy. The FCCF works with different types of material, including nuclei, stem cells, yeast, *C. elegans, Arabidopsis* seeds, autophagosomes, and lipid droplets, as well as various cultured cell lines and primary cells from humans, mice, zebrafish, and *Drosophila*. To educate and train users, the FCCF offers three different lectures and an annual practical course for basic flow cytometry analysis as well as an advanced practical course for cell sorting. However, due to restrictions caused by the COVID-19 pandemic in 2020, lectures were held online and practical courses were postponed to 2021.

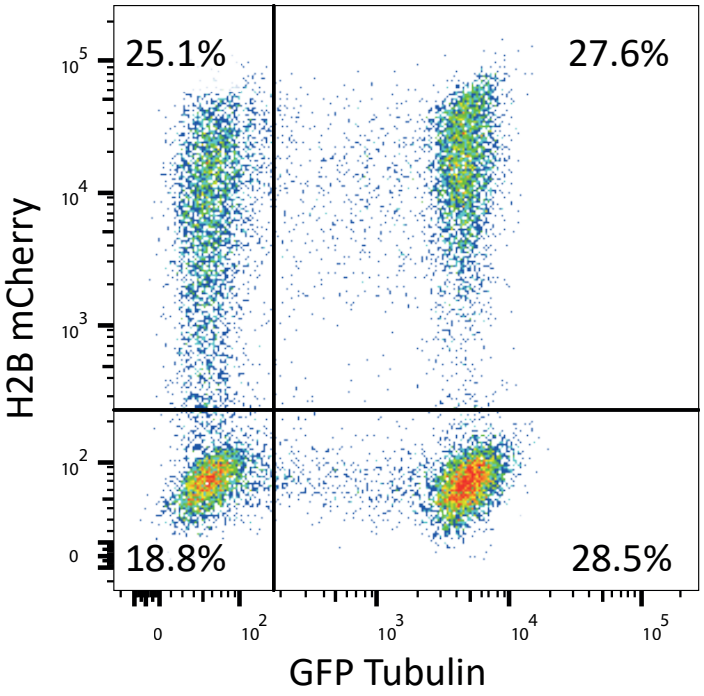


Figure 1. Analysis of HeLa cells expressing tubulin-GFP and H2B-mCherry. The measurement was performed on the BD LSRFortessa flow cytometer.

GENOMICS

OVERVIEW

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

SERVICES OFFERED

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

After submission of RNA or DNA samples, the GCF performs initial quality control of the samples, library preparation, quality control of the prepared libraries, sequencing, and raw data generation. Currently, the GCF supports library preparation for more than 20 applications as a standard service and develops new protocols to accommodate the user's needs for their specific projects.

RNA:

+ Strand-specific mRNA-Seq with poly-A selection
+ Strand-specific total RNA-Seq with rRNA depletion
+ Low input RNA-Seq
+ Small RNA-Seq
+ RIP-Seq
+ Bru-Seq
+ cDNA library preparation
+ circRNA
+ GRO-Seq
+ single-cell RNA (Smart-Seq2, 10x Genomics)
+ STARR-Seq
+ 3' Quant-seq

DNA:

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

User-prepared libraries:

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

MEMBERS

Maria Mendez-Lago
Head; since 04/2016
Annabelle Dold
Staff Scientist; since 01/2020
Hanna Lukas
Technician; since 01/2013
Regina Zimmer
Technician; since 12/2019

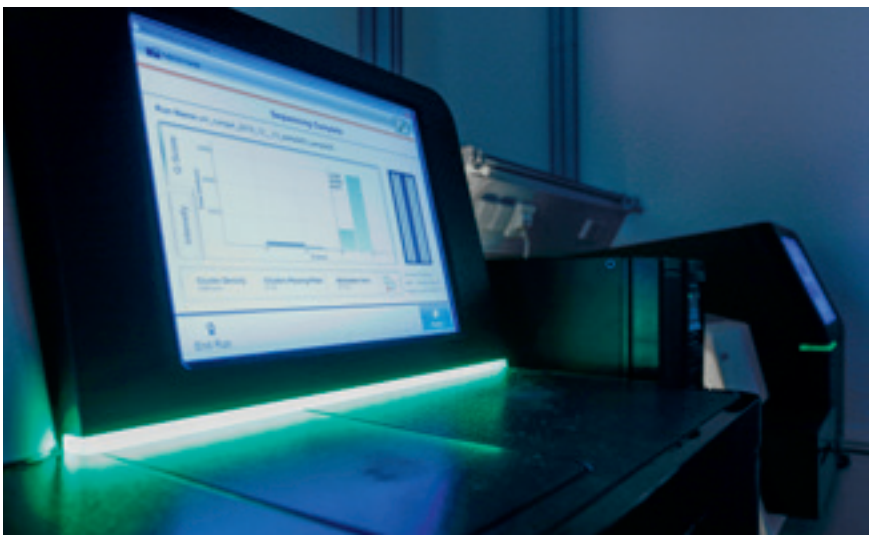


Figure 1. NextSeq 500 benchtop high throughput sequencer from Illumina

MICROSCOPY & HISTOLOGY

OVERVIEW

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

MEMBERS

Sandra Ritz
Head; since 01/2016
Márton Gelléri
Staff Scientist; since 06/2019
Petri Turunen
Staff Scientist; since 08/2019

SERVICES OFFERED

Microscopy users can choose from 10 different instruments ranging from stereo and widefield microscopes to confocal, high-content screening, and super-resolution microscopes. Four setups (one widefield, one scanning confocal, and two spinning disk confocal microscopes) are equipped for live cell imaging. Users are trained to work independently on the microscopes, although MHCF staff are always available to assist with sample preparation and image acquisition, as well as image processing and analysis (quantification).

Four high-power workstations are equipped with licensed software programs for image restoration like deconvolution (Huygens Essential, SVI) and 3D visualisation and analysis (Imaris, Harmony, Vision 4D, LAS-X, VisiView). We support the inspection of 3D images in virtual reality (Vision VR) to provide deeper insights. In addition to providing commercial software packages, we develop custom-made solutions together with users by macro programming in open source software (e.g. Fiji, ImageJ, or ilastik) or by assembling predefined building blocks in Columbus, a database and analysis software specifically designed for high-content imaging data. Most of these software tools include the option of image analysis by machine learning and artificial intelligence, which allows the classification of different cell types, cell cycle analysis, or *in silico* labelling of non-stained cells.

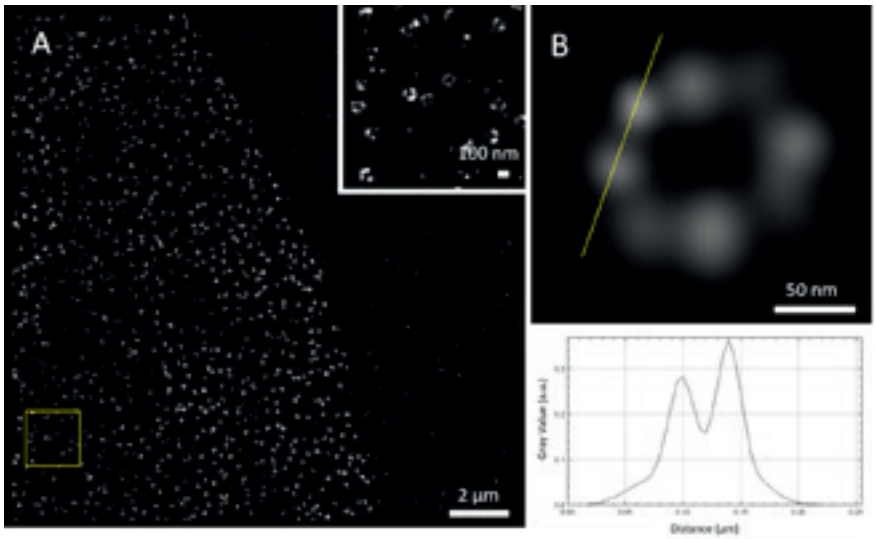


Figure 1. Fluorescence image of nuclear pore complexes (NUP96-SNAP) in the bone osteosarcoma cell line U2OS using single molecule localisation microscopy. A) Multiple nuclear pores resolved as small circles with a diameter of ca. 100 nm (inset). B) A magnified nuclear pore complex with individual subunits at a distance of ca. 40 nm. The graph below the image shows the intensity distribution along the yellow line. Sample prepared by participants of the super-resolution microscopy course, imaged with the GSDIM/Leica microscope and reconstructed under the supervision of Márton Gelléri.

In addition to the annual "Image Analysis and Processing Course", the MHCF offered a new practical course in "Super-resolution Microscopy" (3.5 days), which covered sample preparation, several super-resolution techniques (STED, GSDIM, expansion microscopy), and data analysis.

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

PROTEIN PRODUCTION

OVERVIEW

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

SERVICES OFFERED

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

Another key function of the PPCF is the generation and functional quality control of routine laboratory enzymes and affinity probes for IMB researchers. It currently offers 24 products to IMB scientists, matching the most frequently used protein tools at the institute. With an increase in staff, the PPCF is now also able to support a significantly higher number of IMB's research projects with protein purification and assay development.

MEMBERS

Martin Möckel
Head; since 03/2018
Claire Mestdagh
Technical Assistant; since 03/2020

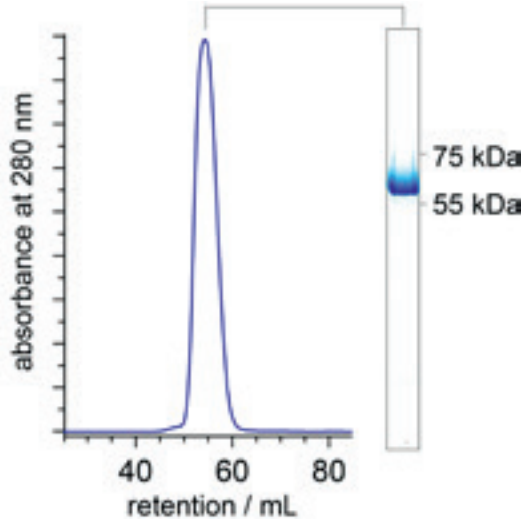


Figure 1. Left: Various chromatography columns used by the PPCF for protein purification. Right: Elution profile of a recombinant protein during the final gel filtration step using a fast liquid protein chromatography system (FPLC). The purity of the recombinant protein is indicated by a Coomassie-stained SDS-PAGE gel on the right.

PROTEOMICS

OVERVIEW

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

MEMBERS

Falk Butter
Head; since 05/2013
Jasmin Cartano
Technician; since 02/2014
Jiaxuan Chen
Staff Scientist; since 03/2019
Mario Dejung
Bioinformatician; since 05/2014
Amitkumar Fulzele
Staff Scientist; since 06/2020
Ramona Schmitt
Technician; since 12/2018

SERVICES OFFERED

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

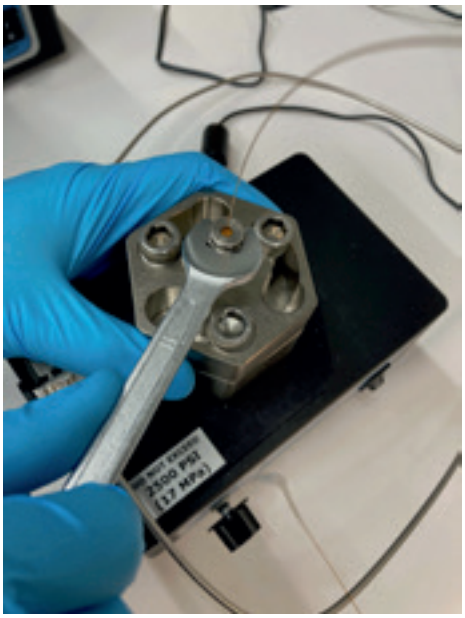


Figure 1. Analytical column packing with C18 material under high helium gas pressure.

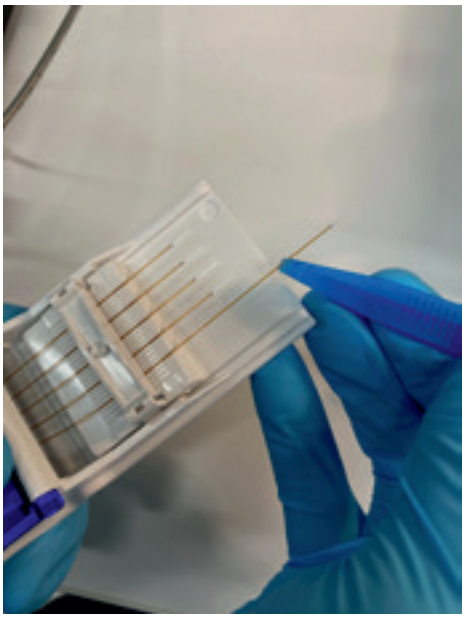


Figure 2. Analytical column selection for proteomics analysis.

MEDIA LAB

OVERVIEW

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

SERVICES OFFERED

The Media Lab provides the following services:

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

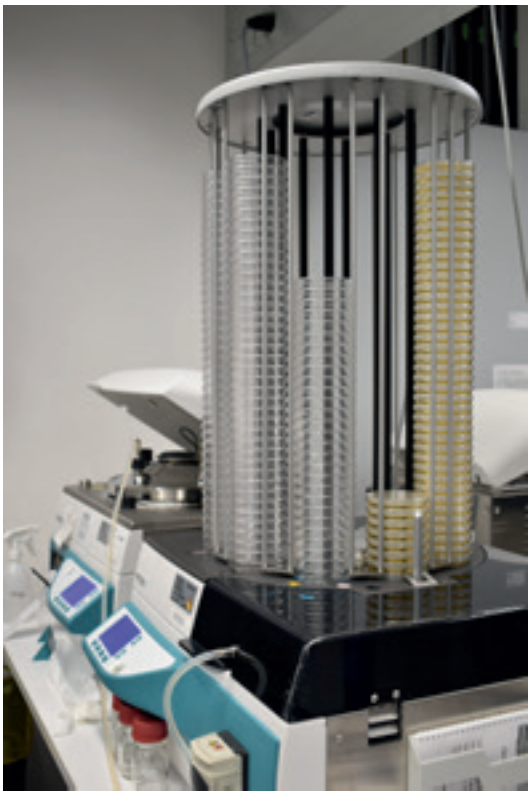
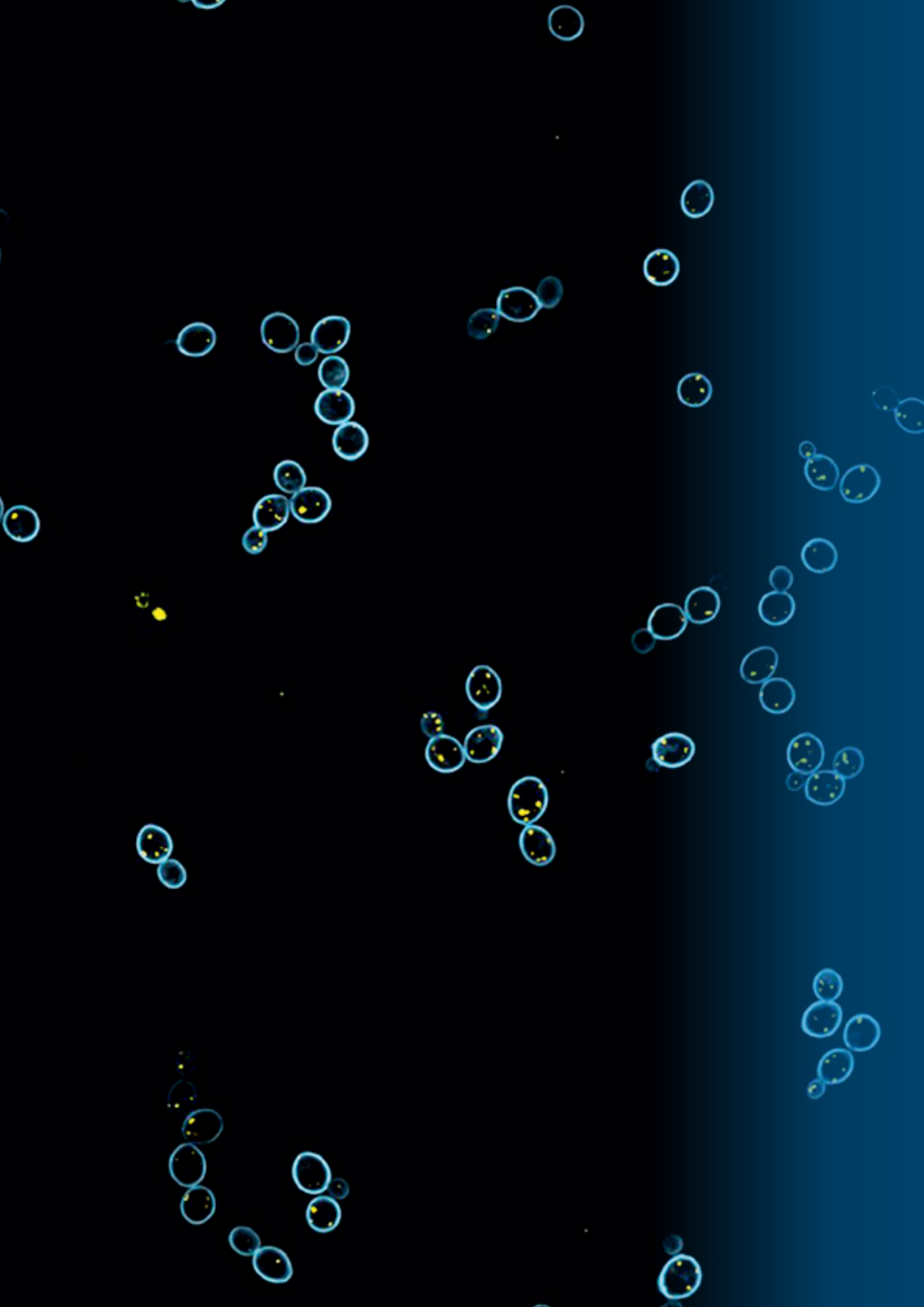


Figure 1. The media lab produces agar plates for different experimental setups with an output of about 1,700 plates per week.

MEMBERS

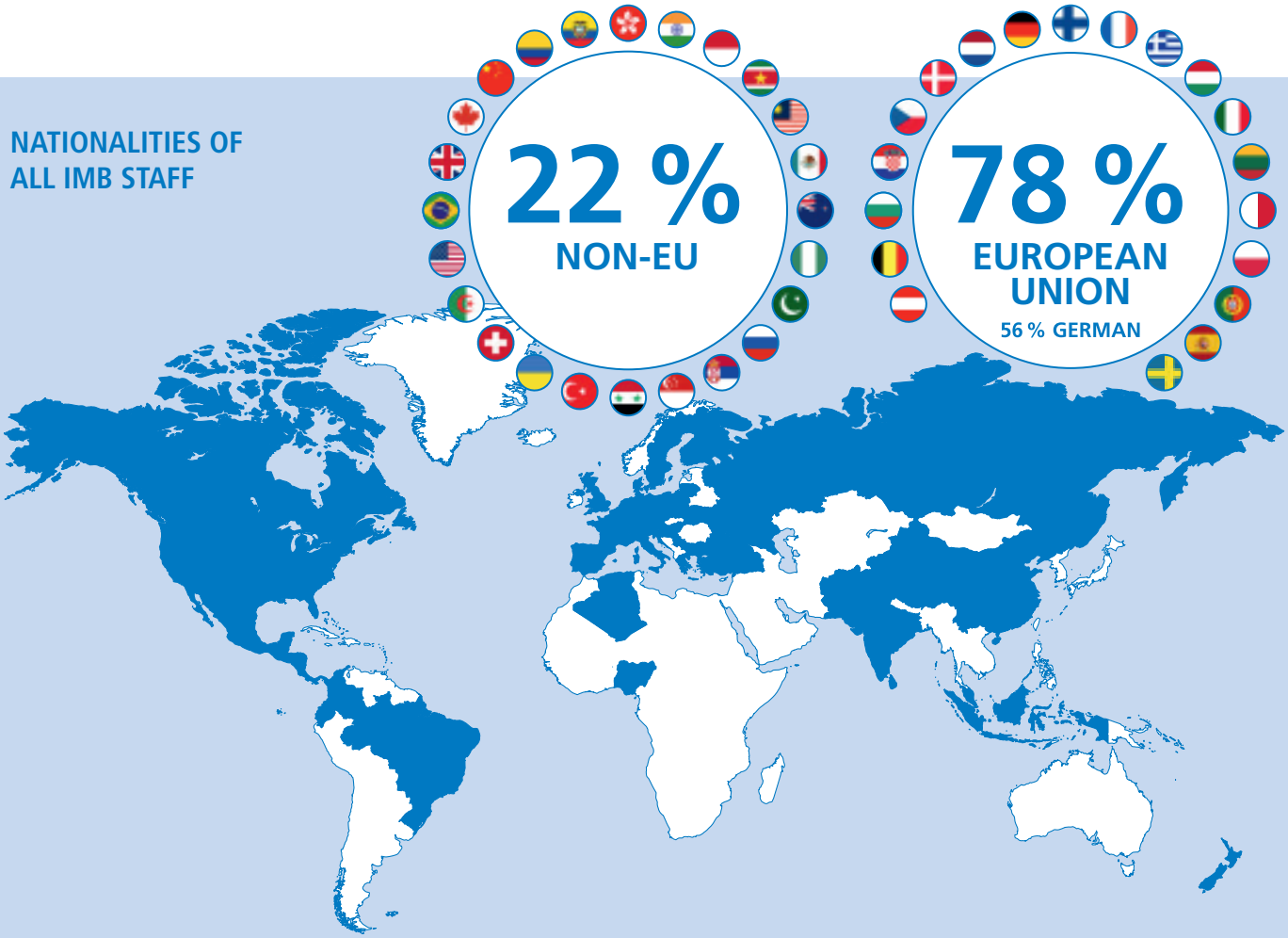
Andrea Haese-Corbit
Head; since 01/2018
Doris Beckhaus
Assistant; since 05/2011
Alwina Eirich
Assistant; since 07/2013
Pascal Hagebölling
Assistant; since 01/2015
Annette Holstein
Assistant; since 04/2012
Marion Kay
Assistant; since 04/2016
Johann Suss
Assistant; since 04/2011



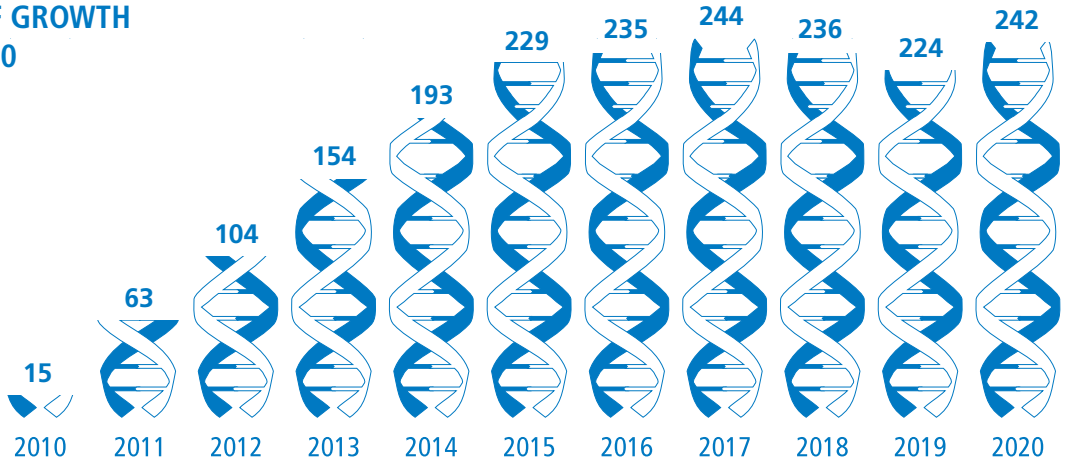
FACTS & FIGURES

IMB STAFF

NATIONALITIES OF ALL IMB STAFF



IMB STAFF GROWTH SINCE 2010



IMB STAFF BY CATEGORY

64 Technical Staff

44 Postdocs

36 Administration & Scientific Management

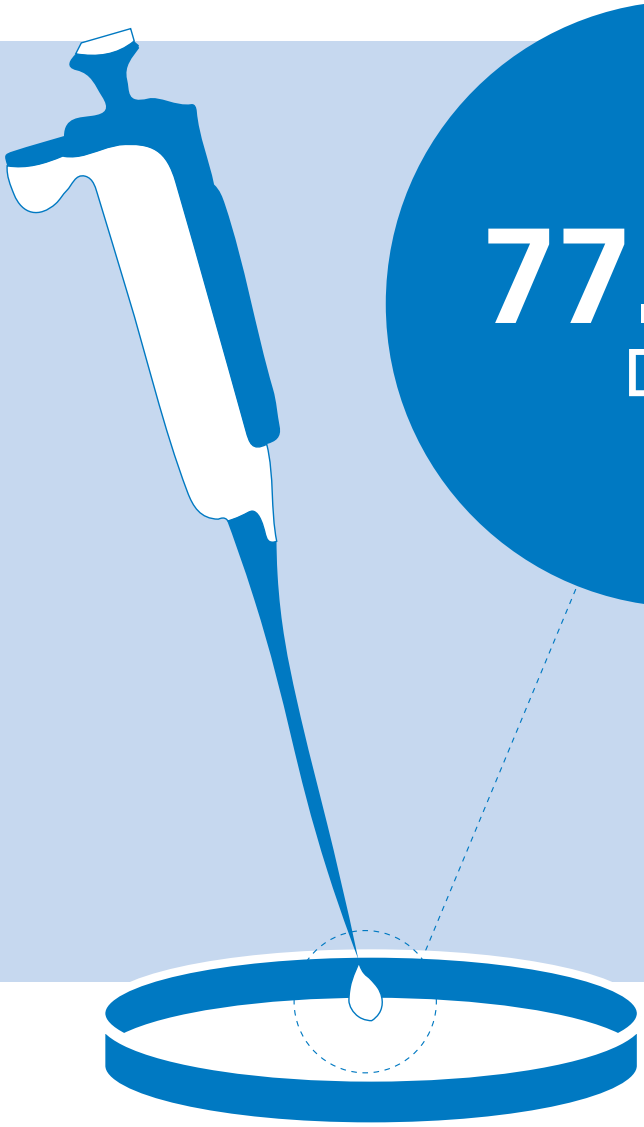
7 Masters & Undergraduate students

70 PhD Students

17 Group Leaders

EXTRAMURAL FUNDING

in addition to Boehringer Ingelheim Stiftung core funding



77.8 %
DFG

German Research Foundation (DFG)

European Research Council (ERC)

6.7 %
EU
(ERC+EC)

Marie Skłodowska-Curie Actions

2.5 %
BIS

Boehringer Ingelheim Foundation (BIS) for IMB coordinated International PhD Programme

13 %
FURTHER
SUPPORT

KLAUS TSCHIRA BOOST FUND

Klaus Tschira Stiftung

German Scholars Organization (GSO)

German Academic Exchange Service (DAAD)

Peter and Traudl Engelhorn Foundation

Wilhelm Sander Foundation

RESEARCH AND TRAINING

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

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

FACTS & NUMBERS

IMB's scientists produced over **350** publications in the last 5 years, with **60** in 2020 (of which **27 %** had an IF of 10 or higher)

IMB has

3 SPECIALISED TRAINING PROGRAMMES

for scientists at each stage of their career:

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

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

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

IMB POSTDOC PROGRAMME

FACTS & NUMBERS

Currently **41** postdocs from **18** countries

21 % Europe — **41 % Germany**

38 % Rest of world

Alumni work in industry, academia and beyond as:
› Lab heads › Senior research scientists › Managers
› Policy & governance officers › Consultants

INTERNATIONAL PHD PROGRAMME

FACTS & NUMBERS

Includes students from 3 institutions (IMB, JGU & UMC)
plus 2 affiliated research networks

500 applications per call (2 calls per year)

Currently **154** PhD students from **41** countries
in **59** research groups

35 % Europe — **36 % Germany**

29 % Rest of world

79 graduates since 2011

INTERNATIONAL SUMMER SCHOOL

FACTS & NUMBERS

350 applicants for **20** positions each year
132 participants from **35** countries since 2012

Rated as **"excellent"** or **"very good"**
by **98 %** of participants

65 % "excellent" — **33 % "very good"**

2 % "as expected"

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

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

+ Advanced training in **scientific methods** and **professional skills** through a range of lectures, focused workshops, and tailored events
+ **Guidance** from leading scientists and **mentoring** from IMB's directors
+ Networking at **career events** with leading external scientists from industry and academia

In a 2019 survey 93 % of IMB postdocs felt supported by the IPPro and 67 % were satisfied or very satisfied with their training.

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

www.imb.de/postdocs

IMB POSTDOC PROGRAMME PRO

PhD students are key to our research at IMB. To provide the structure, training, and supervision necessary to excel during a PhD, IMB created the International PhD Programme (IPP) in 2011 with funding from the Boehringer Ingelheim Foundation.

Within this programme, our students tackle ambitious research projects, receive a broad and diverse education, and have easy access to the expertise and equipment needed to drive their projects forward.

This training includes:

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

In 2015, the IPP was rated as **"on par with the five most esteemed doctoral programmes in the life sciences in Germany"** by independent experts representing leading PhD programmes in Europe.

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

www.imb.de/PhD

INTERNATIONAL PhD PROGRAMME MAINZ

IMB's International Summer School (ISS) is a 6-week programme on "Gene Regulation, Epigenetics & Genome Stability" that brings talented undergraduate and Masters' students from around the world to Mainz every summer. Through the ISS, enthusiastic students get the chance to work hands-on with their own project at the forefront of biological research.

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

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

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

www.imb.de/ISS

IMB INTERNATIONAL SUMMER SCHOOL

TRAINING COURSES

IMB provides our scientists with comprehensive training spanning both scientific and non-scientific skills. This ensures they have the expertise to perform top-quality research and succeed in their careers. In 2020, IMB offered the following training courses in scientific and transferable skills:

LECTURES

CORE FACILITY	DATES	TITLE	
GENERAL	20 Apr	Molecular & Biochemistry Techniques	●
BIOINFORMATICS	25 May	Databases in Bioinformatics	●
	06 Jul	Design and Analysis of NGS Experiments	●
FLOW CYTOMETRY	09 Mar	Advanced Flow Cytometry: Principles of Cell Sorting	●
	29 Jun	Flow Cytometry	●
	19 Oct	Flow Cytometry: Introduction I	●
	26 Oct	Flow Cytometry: Introduction II	●
GENOMICS	15 Jun	Genomics (NGS)	●
MICROSCOPY & HISTOLOGY	27 Apr	Introduction to Microscopy	●
	04 May	Microscopy: F-Techniques & Super-Resolution	●
	11 May	Histology and Fluorescent Labeling	●
	22 Jun	Electron Microscopy	●
	23 Nov	Image Manipulation: The Slippery Slope to Misconduct	●
PROTEOMICS	08 Jun	Proteomics	●
PROTEIN PRODUCTION	18 May	Protein Production & Crystallography	●

PRACTICAL COURSES

CORE FACILITY	DATE	TITLE	
BIOINFORMATICS	02 Mar	Bioinformatics: Introduction to Base R for Data Analysis and Visualisation (Part I)	
	05 Mar	Bioinformatics: Introduction to Base R for Data Analysis and Visualisation (Part II)	
	09 Mar	Bioinformatics: Introduction to Base R for Data Analysis and Visualisation (Part III)	
	12 Mar	Bioinformatics: Introduction to Base R for Data Analysis and Visualisation (Part IV)	
	05 Oct	Bioinformatics: Introduction to RNA–seq Analysis with Galaxy and R (Part I)	●
	12 Oct	Bioinformatics: Introduction to RNA–seq Analysis with Galaxy and R (Part II)	●
	19 Oct	Bioinformatics: Introduction to ChIP–seq Analysis with Galaxy and R (Part I)	●
	26 Oct	Bioinformatics: Introduction to ChIP–seq Analysis with Galaxy and R (Part II)	●
MICROSCOPY	27 – 29 Jan	Image Processing & Analysis Microscopy Course	
	13 – 16 Oct	Super-Resolution Microscopy Course	
PROTEOMICS	12 – 13 Feb	Proteomics Data Analysis Course	

● Courses were offered online

LECTURES

DATES	TITLE	
11 Feb	Stress and Health in Academia: When you cannot see clearly now, cause of obstacles in your way?	
16 – 30 Apr	GenEvo Advanced Lecture Series	●
17 Apr – 29 May	Advanced Lectures on "Gene Regulation, Epigenetics and Genome Stability"	●
21 Apr – 30 Jun	SFB 1361 Lecture Series on "DNA Repair and Genome Stability"	●
18 Jun	The Reproducibility Crisis: Can you trust your data?	●
25 Jun	Essential Data Analysis and Reporting for Quality Manuscript Submissions	●
29 – 30 Sep	GenEvo Advanced Lecture on "Protein Evolution"	●
10 Nov	All You Need to Know About Patenting	●

PROFESSIONAL COURSES

DATE	TITLE	
23 – 24 Jan	Convincing Scientific Presentations	
23 Feb & 23 – 26 Mar	Time Management for PhD students	●
03 – 11 Mar	Project Management	
31 Mar & 01 – 23 Apr	Global Leadership	●
5 – 6 May	Conflict Management	●
26 – 27 May & 23 Nov	Stress Competence	●
03 – 12 Jun	Scientific Writing	●
16 & 29 Jun	Scientific Presentations	
06 – 09 Jul	Scientific Writing	●
31 Aug – 01 Sep	Presentation Skills	
02 – 03 Sep	Presentation Skills	
04 Nov	Data Management	●
09 Nov	Proposal Writing	●
18 Nov	Adobe Illustrator (Intermediate Level)	●
19 Nov	Adobe Illustrator (Beginner Level)	●
17 Dec	Self-Initiated Job Application	●

● Courses were offered online ● Courses were offered partly online

INVITED SPEAKERS

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

DATE	EVENT	SPEAKER	INSTITUTION	TITLE	
23 Jan	IMB Seminar*	MATTHIAS PETER	ETH Zürich, CH	Regulation of cell growth and division	
27 Feb	IMB Seminar*	GRANT STEWART	University of Birmingham, UK	Understanding how genome instability contributes to human disease	
12 Mar	Alumni Career Seminar	MANUEL LEICHSENRING & OSCAR WERNER	Allolio&Konrad Consulting GmbH, Bonn, DE	From cell culture to consulting – how scientists fit into the world of IT transformations	
15 Apr	Ad Hoc Seminar	JOHN WEIR	Friedrich Miescher Laboratory (FML), Tübingen, DE	Structural biochemistry of meiosis	●
09 Jun	Ad Hoc Seminar	ANDREW BERGLUND	The RNA Institute, University at Albany, USA	Targeting a toxic RNA that causes a common form of muscular dystrophy	●
02 Jul	Ad Hoc Seminar	ALEJANDRO SANCHEZ ALVARADO	Stowers Institute for Medical Research, Kansas City, USA	Understanding the source of regenerative potential in animals	●
04 Aug	IMB Seminar#*	BJÖRN SCHUMACHER	University of Cologne, DE	DNA damage responses in ageing and cancer	●
03 Sep	IMB Seminar	MATTHIAS SELBACH	Max Delbrück Center for Molecular Medicine (MDC), Berlin, DE	Proteome dynamics of influenza A virus and SARS-CoV-2 infection	●
22 Sep	IMB Seminar#	ERIC MISKA	University of Cambridge, UK	New biology and therapeutics from RNA structure and modification	●
14 Oct	IMB Seminar*	KARIM LABIB	University of Dundee, UK	Destroying the eukaryotic replisome	●
15 Oct	IMB Green Seminar	JAMES CONNELLY	My Green Lab, San Diego, USA	My Green Lab and the future of sustainable science	●
20 Oct	IMB Seminar#	HINRICH SCHULENBURG	University of Kiel, DE	Evolutionary interactions between the nematode *C.elegans* and its naturally associated microbes	●
05 Nov	IMB Seminar*	ANDREW JACKSON	University of Edinburgh, UK	Being the right size: a human story of cell number	●
24 Nov	IMB Seminar#	MICHAEL HILLER	Max Planck Institute, Dresden and Senckenberg Society, Frankfurt, DE	Linking phenotypic differences between species to differences in genes and cis-regulatory elements	●
10 Dec	IMB Seminar	EDDA SCHULZ	Max Planck Institute for Molecular Genetics, Berlin, DE	Input decoding by the cis-regulatory landscape controlling Xist at the onset of random X-chromosome inactivation	●

*These seminars are part of the SFB 1361 Seminar series on "Regulation of DNA repair and genome stability"

These seminars are part of the GenEvo Seminar series on "Gene regulation in evolution"

● Seminars were offered online

RESEARCH INITIATIVES

IMB is a major driver in two major research initiatives launched in 2019, both of which bring together scientists from multiple research centres across Germany.

SFB 1361 REGULATION OF DNA REPAIR & GENOME STABILITY

Spokesperson: Helle Ulrich (IMB)

The SFB 1361 was launched in January 2019 and is funded by the DFG with €12.4 million until December 2022. This initiative consolidates 18 projects from investigators across 6 institutions (IMB, JGU, UMC, Darmstadt University, Munich University, and Frankfurt University) with the goal of understanding the molecular mechanisms modulating the activities of genome maintenance in the cell.

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

In 2020, researchers in the SFB 1361 published a total of 22 papers that were directly connected to projects in this initiative, and 7 new associated members joined. A highlight was the SFB 1361 Retreat, which took place online in September and offered researchers an opportunity to present their work and network with other groups in the initiative.

www.sfb1361.de

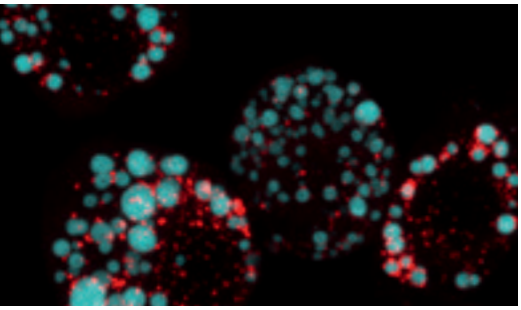
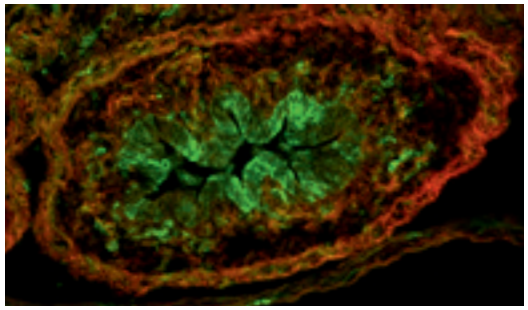
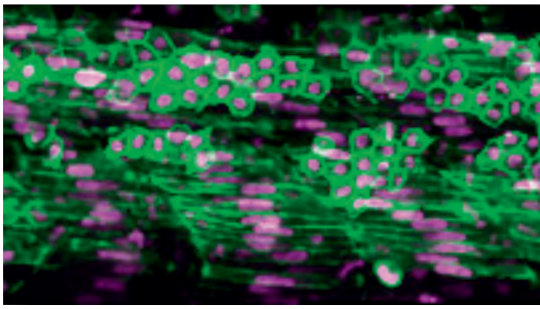
GenEvo GENE REGULATION IN EVOLUTION

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

GenEvo is a DFG-funded Research Training Group organised in a collaboration between IMB and JGU's Faculty of Biology. The initiative is centred around the core question of how complex and multi-layered gene regulatory systems have both evolved and driven evolution. Mixing both junior and senior researchers, GenEvo brings together outstanding scientists in 14 projects, fusing expertise in evolutionary and molecular biology. The programme focuses on training a new generation of PhD students to work on ambitious research projects at the interface of these two themes, while also receiving a broad, interdisciplinary education.

GenEvo launched in June 2019 and is funded by the DFG with €5.8 million until December 2023. In 2020, 6 new group leaders joined the initiative as contributing members.

www.imb.de/genevo

SCIENTIFIC EVENTS organised by IMB in 2020

28 September

SFB 1361 RETREAT

VIRTUAL EVENT ON "REGULATION OF DNA REPAIR AND GENOME STABILITY"

Scientific organiser: Helle Ulrich (IMB)

17 November

UMC-IMB GROUP LEADER RETREAT

VIRTUAL EVENT TO ESTABLISH NEW COLLABORATION PROJECTS

Scientific organisers: Peter Baumann (IMB/JGU), Ulrich Förstermann (UMC), Stephan Grabbe (UMC), Christof Niehrs (IMB), Norbert Pfeiffer (UMC) and Hansjörg Schild (UMC)

14 December

ERIBA-IMB MEETING

VIRTUAL MEETING ON PROGRESS UPDATE OF COLLABORATIVE PROJECTS

Scientific organisers: Gerald de Haan (ERIBA) and Christof Niehrs (IMB)

AWARDS

WALTER **BRONKHORST**
Postdoc, Ketting Group
Klaus Tschira Boost Fund

CHRISTOPHER **REINKEMEIER**
PhD Student, Lemke Group
International Birnstiel Award for Doctoral Research in Molecular Life Sciences

GAURAV **JOSHI**
PhD Student, Niehrs Group
Boehringer Ingelheim PhD Fellowship

BRIAN **LUKE**
Gutenberg Lehrkolleg teaching award for the Faculty of Biology (JGU)

VIVIEN **SCHOONENBERG**
PhD Student, Butter Group
Add-on Fellowship for Interdisciplinary Life Science (Joachim Herz Foundation)

EDWARD **LEMKE**
ERC Advanced Grant

MIAO **YU**
Postdoc, Lemke Group
Biophysical Society IDP Subgroup Postdoctoral award

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.

GENEVIÈVE **ALMOUZNI**

Institut Curie, PSL University, Sorbonne University, UPMC University, CNRS, UMR3664, Paris, France

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Research Institute of Molecular Pathology (IMP), Vienna, Austria

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RESEARCH ENVIRONMENT

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

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

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

Mainz is also surrounded by a number of towns and cities with extensive research activities. For instance, Frankfurt is only 35 km away and is home to Goethe University, which has over 46,000 students and 10 research institutes within the Biochemistry, Chemistry and Pharmacy Department alone. Furthermore, there are several Max Planck Institutes in Frankfurt (including the Max Planck Institute for Biophysics, the Max Planck Institute for Brain Research and the Ernst Strungmann Institute for Cognitive Brain Research). In addition to Frankfurt, nearby Darmstadt is home to both a Technical University, whose Department of Biology has a focus on synthetic biology and the biology of stress responses, and a University of Applied Sciences that includes a focus on biotechnology.

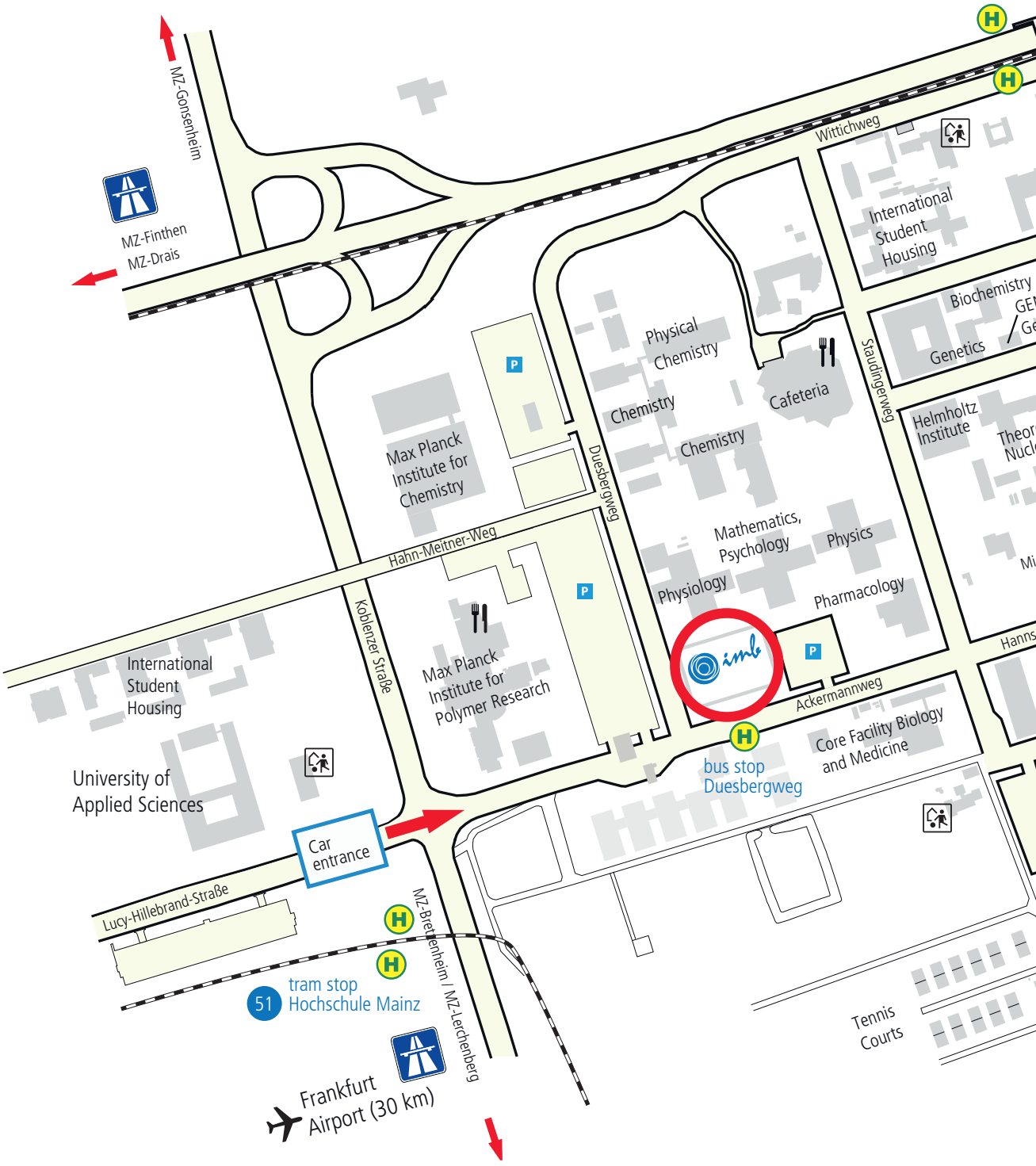
In addition, there is an extensive industry R&D presence, with, for example, the headquarters of Boehringer Ingelheim and the Merck Group both in close vicinity.

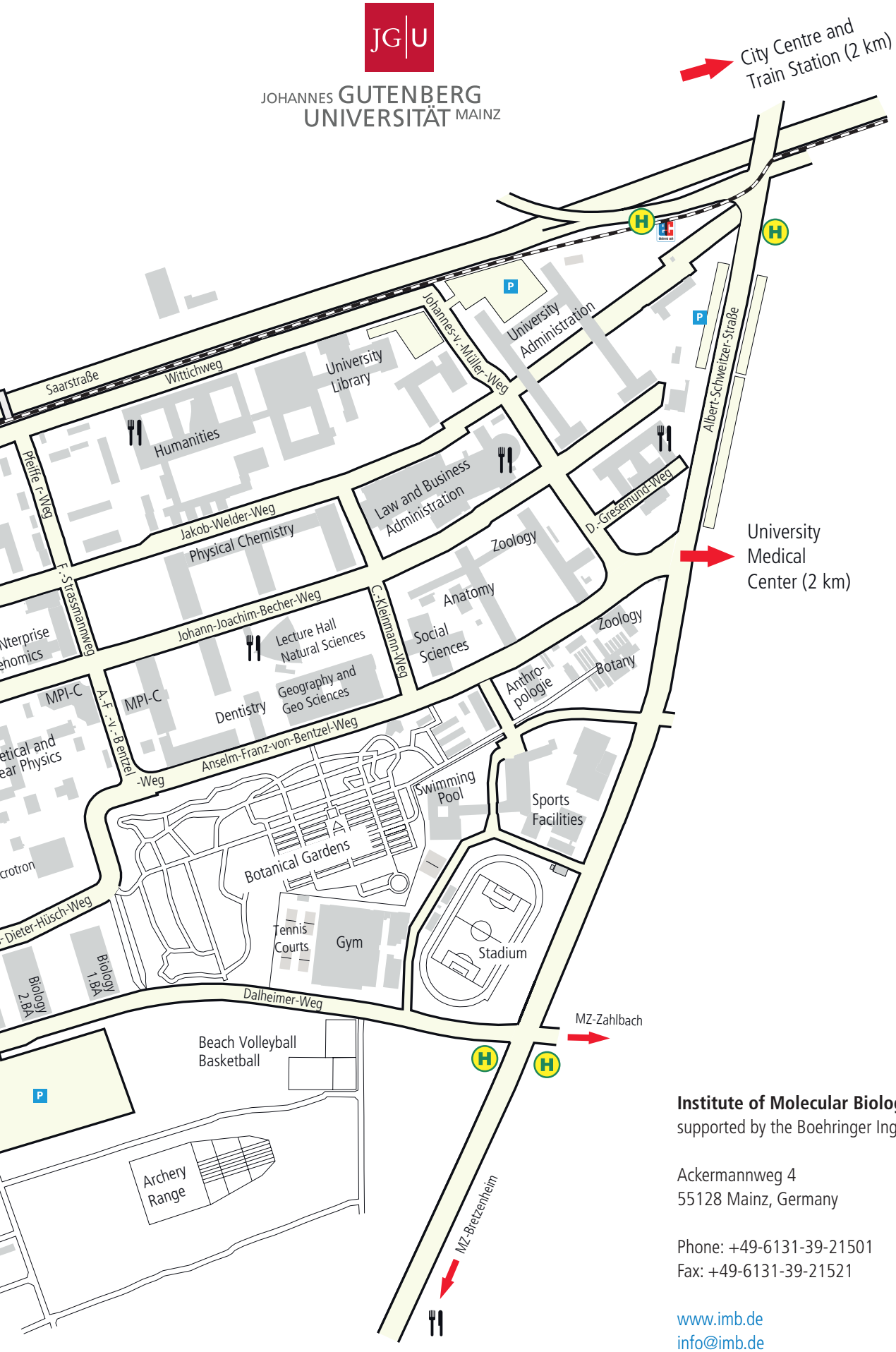
WHERE WE ARE

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

Mainz is within easy reach of both cosmopolitan Frankfurt, with its famous opera house, avant-garde museums and glass-and-steel banking district, and the Rhine valley region with its castles, vineyards and nature reserves that offer great outdoor activities. With Frankfurt airport – one of the largest airports in Europe – only 25 minutes away, countless European and overseas destinations are within easy reach.

CAMPUS MAP
OF JOHANNES GUTENBERG UNIVERSITY MAINZ





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Cover: Fluorescence microscopy image of ovaries in Mkrn1 knockout *Drosophila*. Sample is stained for DNA (DAPI, blue), cytoskeleton (red) and Oskar (green). Image credit: Annabelle Dold (Roignant group).

Portraits of group leaders and core facility heads (p4, 8-40, 44-51), pictures of IMB researchers (p5, 42, 56-61): Thomas Hartmann.

Microscopy images: (p3) DNA repair in yeast cells. The outline of the cells is indicated in white and DNA repair activity is labelled in green. The nuclear periphery is indicated in red, and an unrelated repair compartment at the nuclear periphery in blue. Image credit: Ronald Wong (Ulrich group).

(p4) Immunofluorescence image of a mouse testis cross-section showing complete spermatogenesis. DNA is labelled in blue (DAPI), germ cells are shown in red (GCNA), and spermatogonial stem cells in green (PLZF). Image credit: Joan Barau.

(p6) False-coloured fluorescence image showing midgut caeca of the grain weevil *Sitphilus oryzae*, stained for nuclei (cyan), *Sodalis pierantonius* endosymbionts (yellow) and wheat germ agglutinin (magenta). Image credit: Petri Turunen (Microscopy Core Facility), Tobias Engl (Institute of Organismic and Molecular Evolution, JGU).

(p52) Budding yeast *Saccharomyces cerevisiae* expressing mNeonGreen fused with plasma membrane localisation signal (light blue) and peroxisomal marker Pex3 tagged with mScarletI at C-terminus (yellow). Image credit: Elena Ivanova (Khmelinskii group).

(p63) Left: Phase-separated structure affinity in the cytoplasm: small granules (mCherry-Tdrd6cABCpld) surrounding GFP-DCP1 large droplets. Image credit: Alessandro Consorte (Ketting group). Middle: Immunostaining showing the distribution of Lgr5-GFP+ cells and EPCAM-RFP+ cells in the intestinal tube of a mouse embryo at embryonic stage E15.5. Image credit: Margarita Dzama (Kühn group, UMC). Right: *Xenopus tropicalis* embryo at tail-bud stage. The membranes are stained green and the nuclei (H2B) are shown in magenta. Image credit: Eleftheria Parasyraki (Niehrs Group).

SAB (p65): All images courtesy of SAB members.

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