Institute of Molecular Biology

ANNUAL REPORT 2016

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Welcome to the 6th annual report of the Institute of Molecular Biology. Following our first positive 5-year evaluation late last year, 2016 has been a year of continued successes. The institute is thriving, with new publications, awards and a host of scientific and outreach events. In addition, 2016 saw IMB's 5th birthday, which we celebrated in style during our summer party, including a cake in the shape of the IMB building.

AWARDS AND RECOGNITION

The work of several IMB researchers has been recognised in 2016 with the following awards. In April, Christof Niehrs was elected as a Foreign Honorary Member of the American Academy of Arts & Sciences, one of the oldest and most prestigious honorary learned societies in the world. Brian Luke received the Walther Flemming Award in March, a recognition given to young researchers for outstanding scientific merit in cell biology. In addition, Brian was also awarded a competitive Heisenberg professorship from the DFG, and he will take up a joint position as professor within the Faculty of Biology at Mainz University and Adjunct Director at IMB.

STAFF CHANGES

On a management level, Founding Director, Christof Niehrs, handed over the baton of Executive Directorship to me in November 2015, for a period of two years. In terms of staff, one of our Group Leader, George Reid, left IMB in June 2016, and has taken up a position at EMBL in Heidelberg. Furthermore, the heads of some of our Core Facilities have recently been reappointed: The Genomics Core Facility is now headed by Maria Méndez-Lago, the Microscopy Core Facility by Sandra Ritz and the Flow Cytometry Core Facility by Stefanie Bürger. In addition, a new Core Facility for Protein Production has been established, headed by Markus Matthes, to support researchers with the expression and purification of proteins.

RESEARCH ACTIVITIES AND EVENTS

2016 has been a busy year in terms of scientific output. IMB researchers have published 77 peer-reviewed articles (11 of which are currently in print), including an article in Nature from the group of Jean-Yves Roignant. Our International PhD Programme (IPP) continues to grow and while it is still a young programme, in 2016 we saw 7 PhD defences and expect many more in 2017. In addition, the IPP was recently awarded a further €1.8 million in extramural funding.

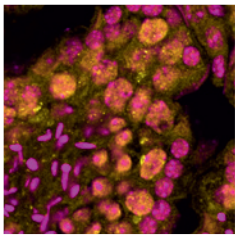
IMB has hosted 8 major scientific events in 2016, as well as a range of outreach activities. The 2016 IMB Conference on "Epigenetics in Development" was well attended, with 140 participants from 20 different countries. 2016 also saw a two-day international workshop on "Challenges in Computational Biology", which focused on the analysis of gene expression data, the international PhD student symposium on "Computational Genomics" as well as IMB's first hackathon. Apart from scientific events, IMB has also co-organised Mainz's first Science Slam focused on the life sciences and hosted a range of outreach activities, including an interdisciplinary discussion event—"Should We Live Forever? Biological and Philosophical Perspectives"—featuring prominent researchers from the life sciences, ethics, life writing, philosophy, theology and the history of science.

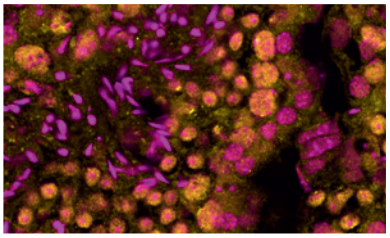
OUTLOOK

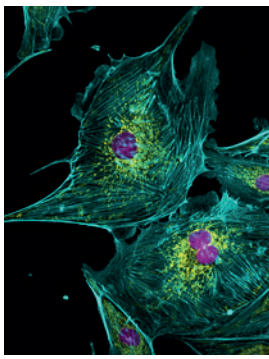
Already planned for 2017 are a number of scientific events at IMB. From 26 March to 1 April, IMB will host an EMBO Practical Course on the iCLIP method, which focuses on genomic views of protein-RNA interactions. A symposium on translational epigenetics will be held on 8 May. This event brings leaders from this field to Mainz and will explore the links between basic research and medicine in this exciting area. The 2017 IMB Conference (19-21 June) will focus on "Gene Regulation by the Numbers: Quantitative Approaches to Study Transcription", another area that is rapidly developing. And the international symposium on "Nucleic Acid Modifications" (4-6 September) will explore current research into deciphering the epigenetic code in DNA and RNA.

As always, I would like to thank the Boehringer Ingelheim Foundation for their generous funding of our institute. I would also like to thank our dedicated Scientific Advisory Board, who came to IMB again this year to provide us with very valuable advice and support. In 2016, we welcomed new members of the board, Professors Peter Becker from Ludwig Maximilians University Munich, Ruth Lehmann from the Skirball Institute at NYU School of Medicine in New York, and Jan-Michael Peters from the Research Institute of Molecular Pathology (IMP) in Vienna. Finally, I would like to thank everyone within and outside of IMB who made 2016 another exciting and eventful year and I look forward to another successful year in 2017.

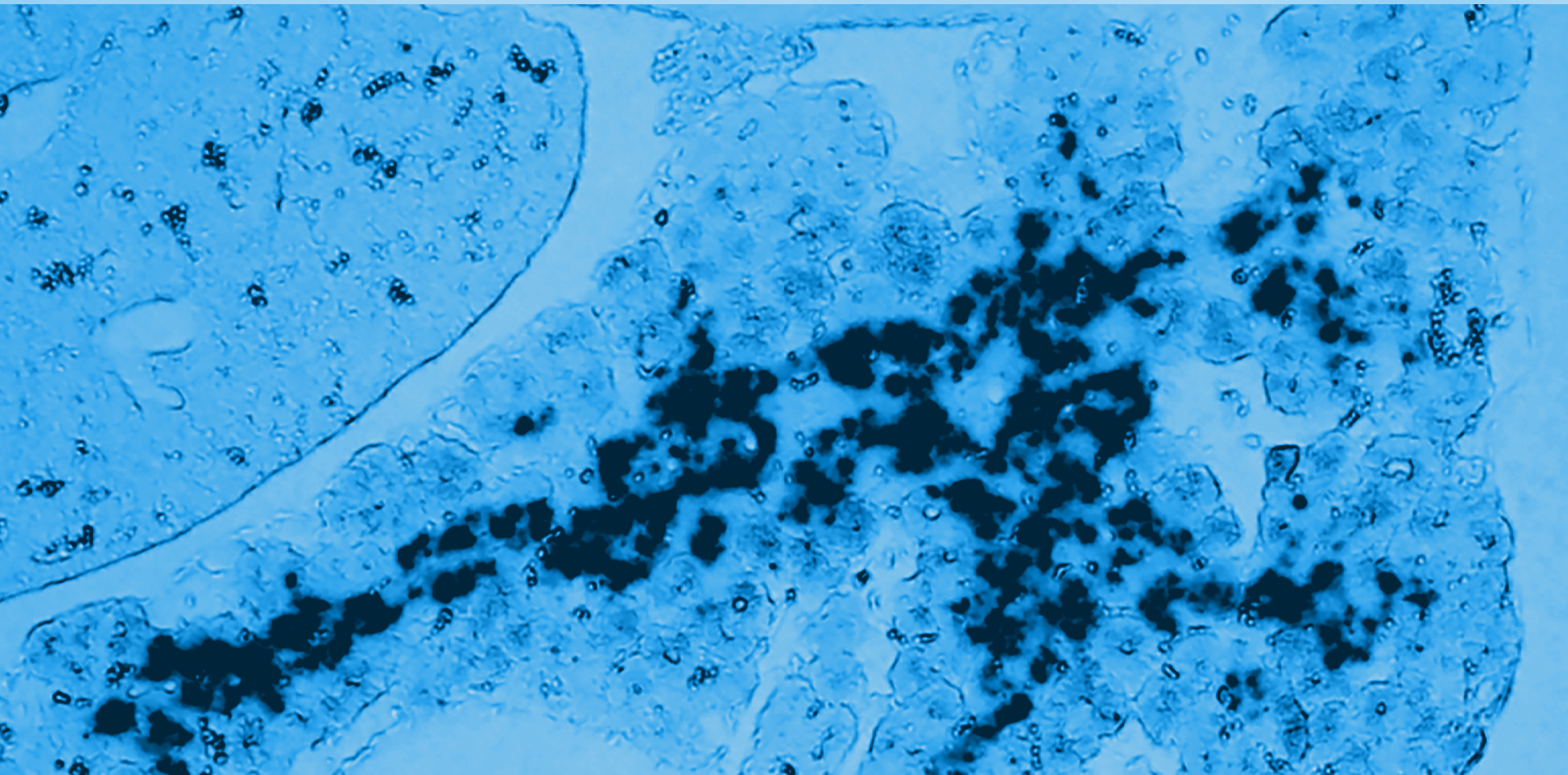
René Ketting
Executive Director

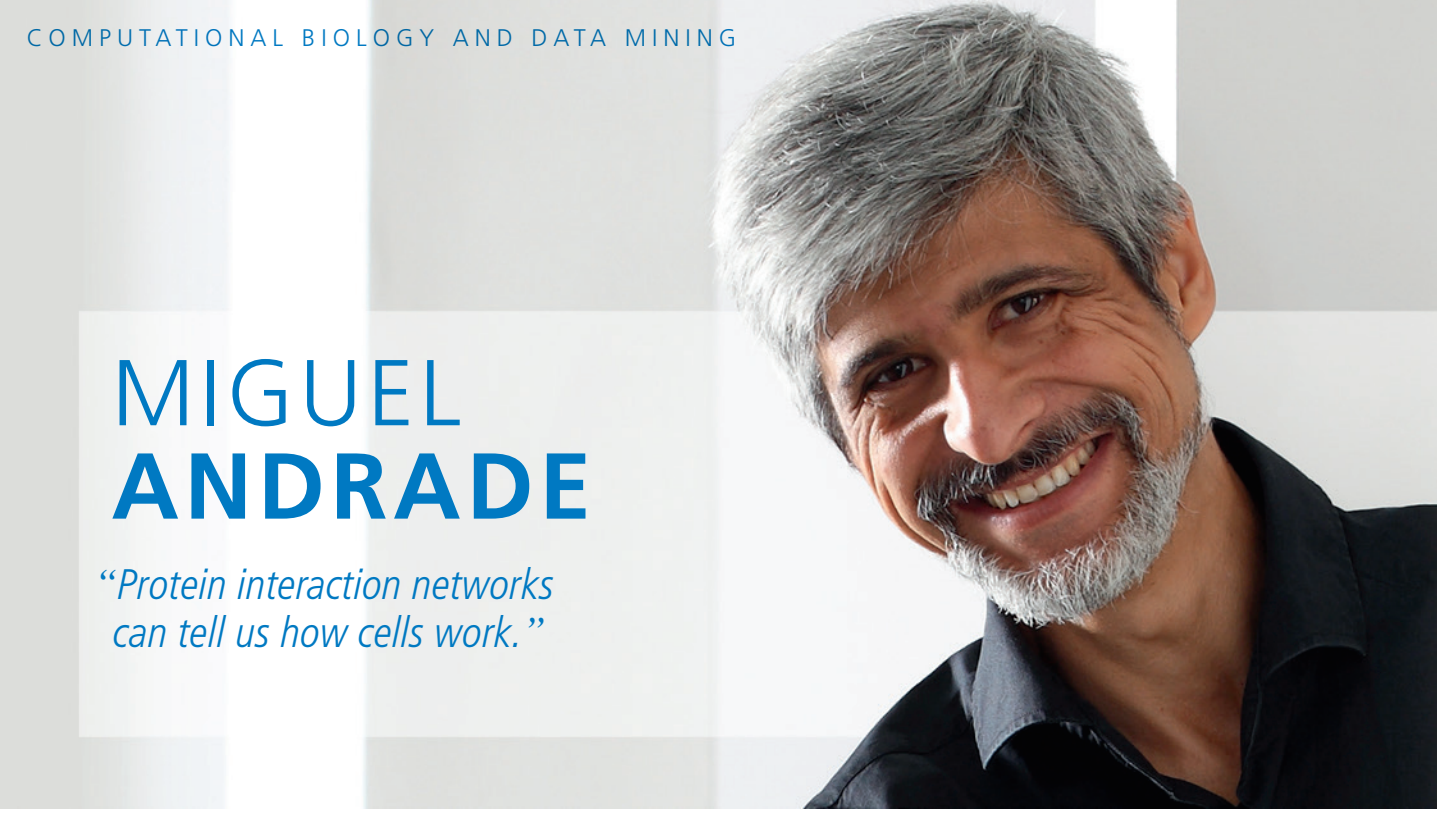






RESEARCH GROUPS





MIGUEL ANDRADE

"Protein interaction networks can tell us how cells work."

POSITIONS HELD

Since 2014	Adjunct Director, Institute of Molecular Biology (IMB), Mainz Professor of Bioinformatics, Mainz University
Since 2008	Affiliate Investigator, Ottawa Health Research Institute
2007 - 2014	Group Leader, Max Delbruck Center for Molecular Medicine, Berlin
2006 - 2008	Senior Scientist, Ottawa Health Research Institute
2003 - 2006	Scientist, Ottawa Health Research Institute
1998 - 2003	Staff Scientist, European Molecular Biology Laboratory (EMBL), Heidelberg
1996 - 1998	Postdoc, EMBL-EBI, Hinxton
1995 - 1996	Postdoc, National Centre for Biotechnology, Madrid
1994 - 1995	Postdoc, EMBL, Heidelberg

EDUCATION

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

GROUP MEMBERS

Gregorio Alanis Lobato Postdoc; since 02/2015
Tommaso Andreani PhD Student; since 01/2016
Jean-Fred Fontaine Postdoc; since 04/2014
Jonas Ibn-Salem PhD Student; since 09/2014
Pablo Mier Postdoc; since 01/2015
Enrique Muro Staff Scientist; since 04/2014
Sweta Talyan PhD Student; since 12/2014
Katerina Taškova Postdoc; since 11/2014

OVERVIEW

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

RESEARCH HIGHLIGHTS

Study of protein interaction networks

One of our recent lines of work on protein-protein interactions (PPIs) regards the study of the topological properties of PPI networks. The topologies of many networks, including the PPI network, can be modelled using hyperbolic geometry, a space whose mathematical properties naturally lead to the emergence of network scale invariance and strong clustering. Such networks can be generated by adding new nodes and connecting them according to two variables: node popularity and node similarity. To create such a network in hyperbolic space, nodes can appear sequentially at random positions in a disk with a radius proportional to the number of nodes already generated and they can then be connected if they are close (modelling similarity). Older nodes in this model are closer to the centre of the disk and to the rest of the nodes in the network, thus attracting more connections (modelling popularity). We will be using such maps for the prediction of links between proteins and to study network evolution (Figure 1).

Protein sequence and structure analysis

We studied the increasing redundancy in the new protein sequences deposited in the databases using a pragmatic clustering method that puts together sequences of similar lengths. We proposed how to use this clustering to examine taxonomic ranges that need further genome sequencing, and to direct experimental studies to proteins from large uncharacterised families. We implemented the method as a web tool (FASTA Herder). This method was further developed to account for fragments; various applications that allow

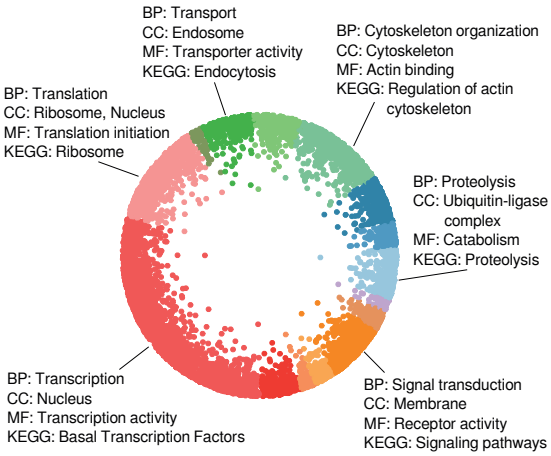


Figure 1. The protein clock. Each dot in this human protein interactome corresponds to one human protein. The proteins with many interactions have been mapped closer to the centre and proteins that interact are close to one other. Proteins were then clustered and coloured according to those clusters. Enriched functions in each cluster are indicated as Gene Ontology terms (BP: Biological Process, CC: Cellular Component, MF: Molecular Function) and KEGG pathway terms. An interactive version is accessible at http://cbdm-01.zdv.uni-mainz.de/~galanisl/pin_geom/index.html.

browsing and querying these clusters for various properties were made available (FastaHerder2) and a web service was implemented that clusters and annotates the results of a BLAST search (CABRA). A related resource that we created is orthoFind, which facilitates the interpretation of the results of a protein sequence similarity search by evaluating the homologues of the query protein in terms of orthologues and paralogues and complementing this with reports on domains and functions.

Genomic analysis and gene expression

Topologically associating domains (TADs) are genomic self-interacting regions containing multiple genes. To investigate their function and evolution, we have studied the position of pairs of paralogous genes with respect to TADs. We observed significantly more pairs within TADs than expected. Since most paraloguous gene pairs are formed by tandem duplication, we propose that there is selective pressure to keep paralogues in the same TAD. Paralogues can have related functions and might require common regulatory mechanisms. Our results support the idea that TADs may provide such mechanisms.

Data and text mining

In this topic, our current efforts are directed towards improving the functional characterisation of large lists of candidate genes derived from high-throughput biological experiments. Enrichment analysis of gene-related annotations such as Gene Ontology terms, molecular pathways or diseases, are already well established. However, methods offering disease enrichment analysis on gene sets are less developed and are based on individual gene-disease associations from manually curated or experimental data that do not cover all diseases discussed in the literature. We developed Gene Set 2 Diseases as an alternative tool to identify gene-disease associations, by deriving these directly and automatically from the biomedical literature.

FUTURE DIRECTIONS

After developing clustered datasets of proteins that can be annotated for taxonomic distribution and sequence features, we now exploit these for predictive approaches. We will study the evolution and function of homorepeats (e.g. polyQ, polyP), analysing their position within protein sequences, their relation to protein functions, their time of emergence in evolution, and their co-occurrence with other homorepeats. We are also developing computational methods to improve the predictions of pseudogenes and to identify the functions of transcripts arising from pseudogenes and from other non-protein-coding RNA sequences. Finally, we want to take advantage of the increasingly large ChIP-seq datasets that should provide information on the combinations of epigenetic marks and transcription factors that result in gene expression; however, the heterogeneity and size of the data complicates this task. Towards this goal, we are working on the extraction and integration of ChIP-seq data from different databases and we plan to create algorithms that measure similarity between ChIP-seq datasets.

SELECTED PUBLICATIONS

Alanis-Lobato G, Andrade-Navarro MA and Schaefer M (2016). HIPPIE v2.0: enhancing meaningfulness and reliability of protein-protein interaction networks. *Nucleic Acids Res*, pii: gkw985

Ibn-Salem J, Muro EM and Andrade-Navarro MA (2016). Co-regulation of human paralog genes in the three-dimensional chromatin architecture. *Nucleic Acids Res*, pii: gkw813

Andrade-Navarro MA and Fontaine JF (2016). Gene set to Diseases (GS2D): disease enrichment analysis on human gene sets with literature data. *Genomics Comput Biol*, 2, e33

PETRA **BELI**

"We employ mass spectrometry-based proteomics to study the mechanisms that maintain genome integrity."

POSITIONS HELD

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

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

EDUCATION

2011 PhD in Biology, Goethe University Frankfurt

2007 Master in Molecular Biology, University of Zagreb

GROUP MEMBERS

Irem Baymaz Postdoc; since 11/2016
Marina Borisova PhD Student; since 12/2013
Jan Heidelberger PhD Student; since 04/2014
Thomas Juretschke Research Assistant; since 09/2016
Matthias Ostermaier PhD Student; since 05/2015
Stefanie Ruf Postdoc; 05/2016 - 10/2016
Andrea Voigt Lab Manager; since 01/2014
Juanjuan Wang Research Assistant; since 08/2016

OVERVIEW

The integrity of human genomes is constantly challenged by by-products of cellular metabolism, such as reactive oxygen species (ROS), and by cellular processes, such as DNA replication, that can induce different types of DNA lesions. In addition to these endogenous factors, external factors such as ultraviolet (UV) light, ionising radiation (IR) and certain chemicals pose a threat to genome integrity. To maintain genome integrity, mammalian cells have evolved elaborate mechanisms, jointly known as the DNA damage response (DDR), that control different cellular processes in order to repair DNA or, if damage is too severe, trigger cellular senescence or apoptosis. Germline mutations in components of the DNA damage response that affect the cellular capacity to repair DNA lesions, result in the accumulation of mutations and consequently lead to syndromes that are manifested as premature ageing and development of cancer. Cells need to respond rapidly to DNA damage and thus it is not surprising that the DDR is primarily regulated by dynamic changes in posttranslational modifications (PTMs) of proteins, among which protein phosphorylation and ubiquitylation play a central role. We employ mass spectrometry (MS)-based proteomics to study how these PTMs regulate different cellular processes, including transcription and protein homeostasis, to maintain genome integrity.

RESEARCH HIGHLIGHTS

We have previously employed quantitative phosphoproteomics to analyse cellular phosphorylation patterns after different types of DNA damage in human cells. The results of these studies revealed that RNA-binding proteins (RBPs) involved in different steps of RNA metabolism are dynamically phosphorylated after DNA damage. Notably, we have shown that a major part of DNA damage-induced phosphorylation occurs independently of the canonical DNA damage kinases ATM and ATR. ATM/ATR, as well as their downstream effector kinases Chk1/Chk2, primarily phosphorylate proteins involved in DNA repair, cell cycle regulation and chromatin remodelling. Therefore, our results point to an important function of other kinases in the regulation of the RNA metabolism after DNA damage. To identify the

kinases that regulate the cellular RNA metabolism, we performed systematic analyses of cellular phosphorylation patterns after different types of DNA damage. These analyses revealed that members of the mitogen-activated protein kinase (MAPK) family are activated after DNA damage. In support of this, mitogen-activated kinase 14 (MAPK14, also known as p38) has been found to be phosphorylated in the kinase activation loop in our phosphoproteomics experiments. Further experiments have shown that p38 is rapidly and dynamically activated after DNA damage and that this occurs prominently after exposure of cells to UV light and oxidative stress. To understand the substrate spectrum and function of p38 after DNA damage, we have performed a proteome-wide analysis of phosphorylation after chemical inhibition or knockdown of p38. We were able to demonstrate that ~25% of UV light-induced phosphorylation sites are dependent on p38, thus establishing that MAPK signalling plays a hitherto underappreciated role in the cellular response to DNA damage. Intriguingly, we have shown that p38-dependent phosphorylation almost exclusively targets RBPs that are involved in the regulation of transcription, RNA splicing, stability/degradation and translation. In conclusion, our data demonstrate a broad regulatory scope of MAPK-dependent signalling in the early cellular response to DNA damage and suggest that p38 links the DDR with the regulation of the cellular RNA metabolism. Activation of p38 after UV light is independent of ATM and ATR and thus represents a parallel signalling pathway that functions after DNA damage to regulate RBPs, but not DNA repair factors. In ongoing work, we are investigating how p38-dependent phosphorylation of RBPs contributes to genome integrity maintenance in response to genotoxic stress.

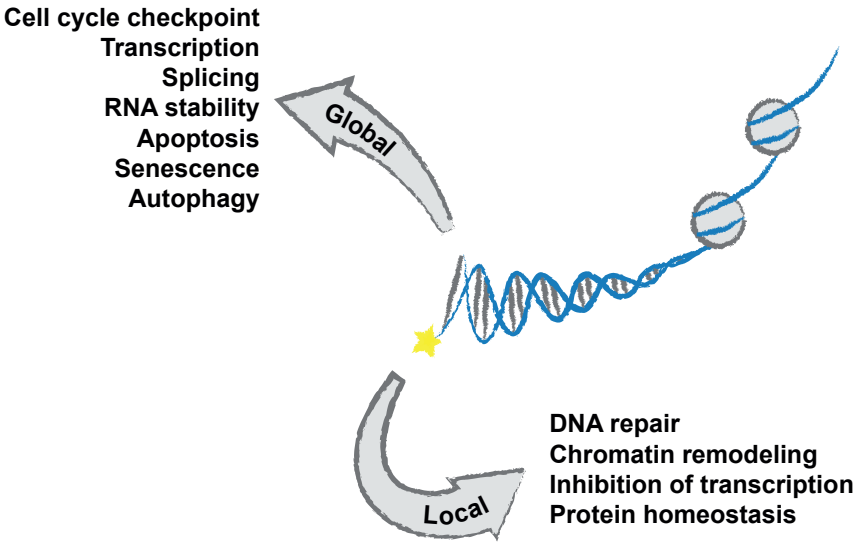


Figure 1. DNA damage response in human cells impacts on processes at sites of DNA lesions and also globally affects cellular metabolism. In addition to DNA repair and chromatin remodelling, DNA damage interplays with other cellular processes. Recent findings have suggested that the regulation of RNA metabolism, including transcription and splicing, is an integral part of the DNA damage response. However, how DNA damage impacts on RNA metabolism and which mechanisms regulate this process remain unclear.

FUTURE DIRECTIONS

Recent studies have suggested that DNA damage influences different levels of RNA metabolism, including transcription, RNA splicing, stability and translation (Figure 1). In addition, dynamic phosphorylation of RBPs has been shown to occur after different types of DNA damage. However, the causes and consequences of this regulation remain unclear. Very little is known about the effect of DNA damage on transcription, RNA splicing, stability and translation, or about the signalling pathways and molecular mechanisms that regulate these RNA metabolic processes after DNA damage. We will investigate the protein components and molecular mechanisms that regulate RNA metabolism after DNA damage, which will form a basis for further investigations concerning the role of different RNA metabolic processes and RBPs in the maintenance of cellular homeostasis after genotoxic stress.

SELECTED PUBLICATIONS

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

Schmidt CK, Galanty Y, Sczaniecka-Clift M, Coates J, Jhujh S, Demir M, Cornwell M, Beli P, Jackson SP (2015). Systematic E2 screening reveals a UBE2D-RNF138-CtIP axis promoting DNA repair. *Nat Cell Biol*, 17, 1458-70

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

*indicates joint contribution

FALK BUTTER

"We perform proteome-wide expression screens during organismal development."

POSITIONS HELD

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

EDUCATION

2010 PhD in Biochemistry, Ludwig Maximilians University, Munich
2006 Diploma in Biochemistry, University of Leipzig

GROUP MEMBERS

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

OVERVIEW

Mass spectrometry is a powerful tool to study proteins in an unbiased and global manner. The current improvements in identification accuracy, sample throughput, and data analysis allow streamlined application of proteomics to answer diverse biological questions. Our group applies quantitative approaches, such as label free quantitation (LFQ), reductive dimethylation (DML) or stable isotope labelling with amino acids in cell culture (SILAC), that enable us to directly compare thousands of proteins in complex mixtures. This lets us study changes in protein expression and we also use this approach for 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.

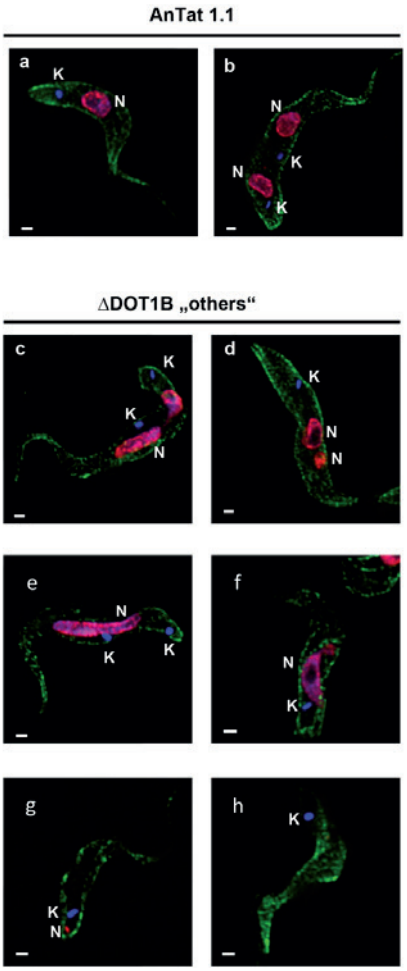
RESEARCH HIGHLIGHTS

Reading the histone code in birds

The DNA in eukaryotic cells is wrapped around small basic proteins, called histones. The tails of these proteins are highly modified by a variety of posttranslational modifications such as methylation, acetylation and phosphorylation. These modifications influence transcriptional activity: for example, methylation of lysine 4 of histone 3 is linked to active promoters, while methylation of lysine 9 of this histone marks inactive genes. Extending previous studies that focused on the elucidation of the histone methyl interactome in human and mouse, we used label-free quantitative proteomics to study the interactome in avian cells. While the overall methyl interactome is strongly conserved between mammals and birds, the binding properties of individual proteins show a few distinct differences. Notably, the mammalian N-PAC protein (also known as GLYR1) readily bound to the H3K36me3 modified peptide. In contrast, its avian orthologue showed reduced affinity for this modification using the same methylated peptide. This indicates that there are at least biochemical differences in the recognition of the mark by orthologues in different species.

Developmental differentiation in trypanosomes

Trypanosoma brucei is a protozoan pathogen, which threatens thousands of people and kills millions of farm animals. During its life-cycle it shuttles between an insect and a mammalian host, which requires specific adaptations. We used label free quantitative mass spectrometry to monitor these changes in the parasite's proteome during its differentiation from the insect to the blood-stream form. Proteomics analysis is essential to determine protein abundance in trypanosomes as mRNA is not a good predictor due to the polycistronic expression units and trans-splicing in this organism. We quantified 4,270 protein groups during stage differentiation and classified their expression profiles during development. Our label-free quantitative proteomics study revealed previously unknown components of the differentiation machinery that are involved in essential biological processes, such as signalling, posttranslational protein modifications, trafficking and nuclear transport. For example, we identified the histone methyltransferase DOT1B as a required factor in this process because a DOT1B knock-out strain showed growth defects with inaccurate karyokinesis in the first cell division during differentiation (Figure 1). This suggests that this histone methyltransferase participates in the chromatin reorganisation that is observed during differentiation. We anticipate that our dataset will serve as a valuable resource for the investigation of cell differentiation in trypanosomes and also other eukaryotes.



Characterisation of TbFlabarinL in trypanosomes

To utilise our large scale developmental proteome datasets, in collaboration with Christian Janzen (University of Wurzburg), we performed an investigation of Tb927.11.2400, a stage-specifically expressed paralogue of trypanosome flabarin. This putative flagellar host adaptation factor with a BAR domain is expressed in the mammalian form of the parasite, localises to the flagellar membrane and associates with components of the paraflagellar rod. Continued investigation of stage-specific proteins from our large scale screens will uncover further functional and mechanistic details.

Figure 1. The trypanosome screen revealed the expression levels of more than 4,000 proteins during developmental differentiation. One of these proteins, the histonemethyltransferase DOT1B was specifically expressed at 12 hours into the process. In comparison to a wild type strain (a-b), in the generated knock-out strain this coincides with major karyokinesis defects (c-h), suggesting a function of DOT1B in developmental chromatin remodeling. N indicates the nucleus, whereas K shows the kinetoplast. Scale bars are 1 µm (figure from Dejung *et al.*, PLoS Pathog 2016)

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 on several parts of the proteomics and interactomics workflow established in the last years in our group. For example, we aim to finish our first large scale interactomics screen and will further investigate the evolution of telomere end protection in diverse species.

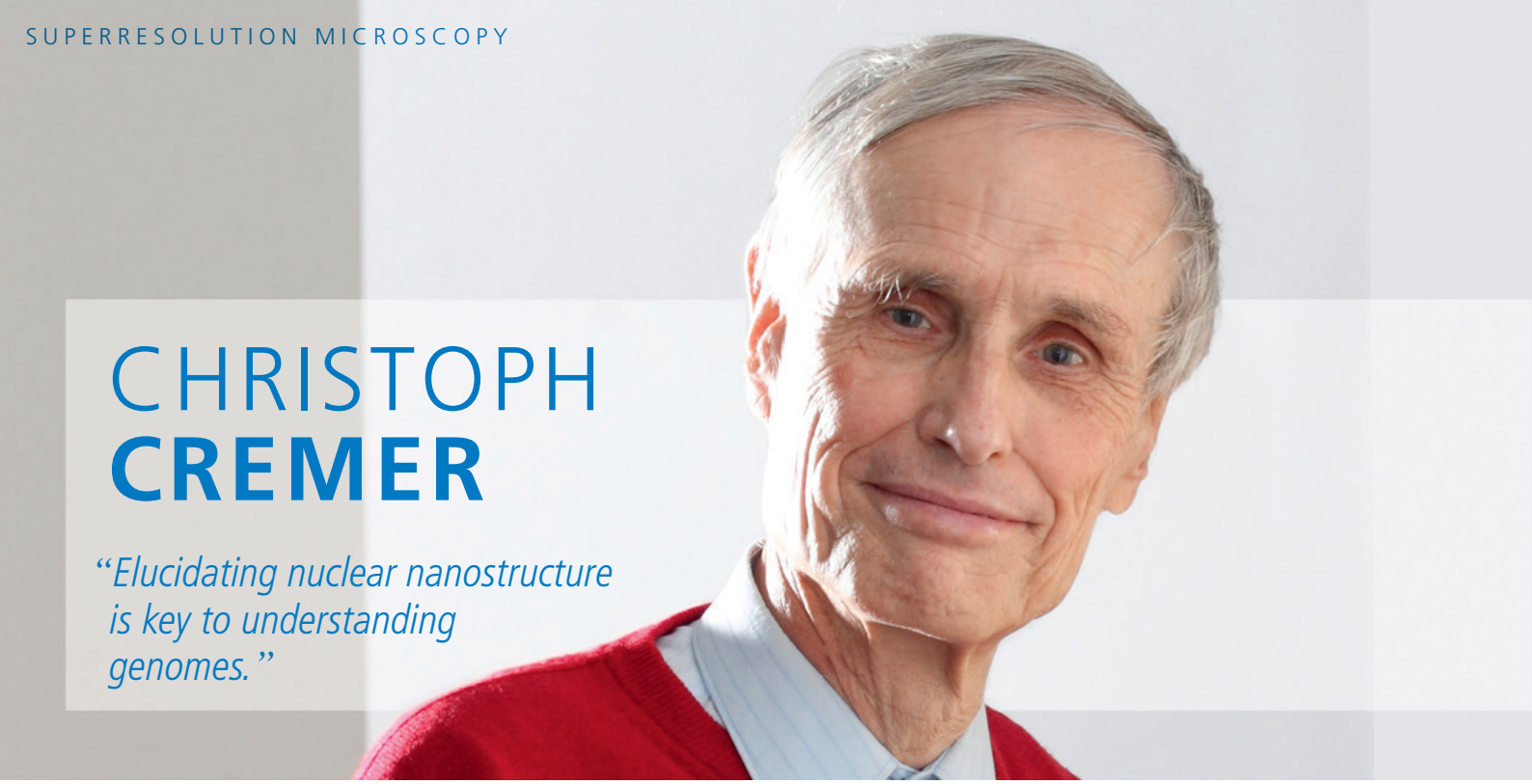
SELECTED PUBLICATIONS

Bluhm A*, Casas-Vila N*, Scheibe M and Butter F (2016). Reader interactome of epigenetic histone marks in birds. *Proteomics*, 16, 427-436

Viturawong T, Meissner F, Butter F# and Mann M# (2013). A DNA-centric protein interaction map of ultra-conserved elements reveals major contribution of transcription factor binding hubs to conservation. *Cell Rep*, 5, 531-545

Kappei D*, Butter F*, Benda C, Scheibe M, Draškovič I, Stevense M, Novo CL, Basquin C, Araki M, Araki K, Krastev DB, Kittler R, Jessberger R, Londoño-Vallejo JA, Mann M and Buchholz F (2013). HOT1 is a mammalian direct telomere repeat-binding protein contributing to telomerase recruitment. *EMBO J,* 32, 1681-1701

*indicates joint contribution; #indicates joint correspondence



CHRISTOPH **CREMER**

"Elucidating nuclear nanostructure is key to understanding genomes."

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

Udo Birk Postdoc; since 02/2012
Shih-Ya Chen PhD Student; since 03/2016
Amine Gourram Guest Researcher; since 11/2016
Jan Neumann PhD Student; since 03/2014
Kirti Prakash PhD Student; 02/2013 - 01/2016
Aleksander Szczurek PhD Student; since 09/2013

OVERVIEW

The human genome has been decoded, but we are still far from understanding the regulation of all gene activities. A largely unexplained role in these regulatory mechanisms is played by the three-dimensional arrangement of the genetic material, i.e. the spatial organisation of the genome in the cell nucleus has far-reaching functional consequences for gene regulation. Since the molecular machinery of nuclear epigenetics works on the nanoscale, it is important to further develop tools to investigate such control mechanisms using spatial analyses at enhanced resolution in single cells. Until recently, it appeared to be impossible to achieve this goal by light microscopy. However, novel developments in optical imaging technology have allowed us to radically surpass the limited resolution of conventional far-field fluorescence microscopy (ca. 200 nm). In this novel field of super-resolution microscopy (SRM), our laboratory has made special contributions to the development of highly facilitated SRM approaches that use standard fluorophores to explore the functional nuclear nanostructure. This allows us to study the general distribution of DNA and its replication, DNA and RNA oligonucleotides, epigenetic histone markers and proteins involved in replication, transcription, splicing and repair, down to the single molecule level.

RESEARCH HIGHLIGHTS

In 2016, we made further methodological improvements of the SRM technique of Spectral Precision Distance/Position Determination Microscopy (SPDM). SPDM, a variant of localisation microscopy, makes use of conventional fluorophores such as single fluorescent proteins or single standard organic fluorophores, targeting a specific cellular compound in combination with standard (or only slightly modified) specimen preparation conditions. We have extended the spectrum of fluorescence dyes for which the technique is applicable to a range of standard DNA-binding dyes. The resulting images exhibit an unprecedented light-optical structural resolution of nuclear chromatin, down to a few tenths of a nanometer. In particular, we discovered that the standard DNA-binding dye YOYO-1 can be used to

provide localisation microscopy images of DNA in the nuclei of fixed mammalian cells. This super-resolution method enabled optical isolation and localisation of large numbers of DNA-bound molecules, in excess of 10^6 single molecule signals in one cell nucleus. The technique yielded high-quality images of the signal density of nuclear DNA, revealing sub-diffraction chromatin structures as small as 40nm; the interchromatin compartment was also visualised at unprecedented optical resolution.

Using localisation microscopy, we studied unscheduled DNA synthesis (UDS) after labelling with the DNA precursor, 5-ethynyl-2'-deoxyuridine (EdU). We showed that the sub-nuclear pattern of UDS detected via incorporation of EdU is different from that when BrdU is used as a DNA precursor: whereas with BrdU, only a few small and large repair foci are detected, EdU incorporation occurs evenly throughout chromatin.

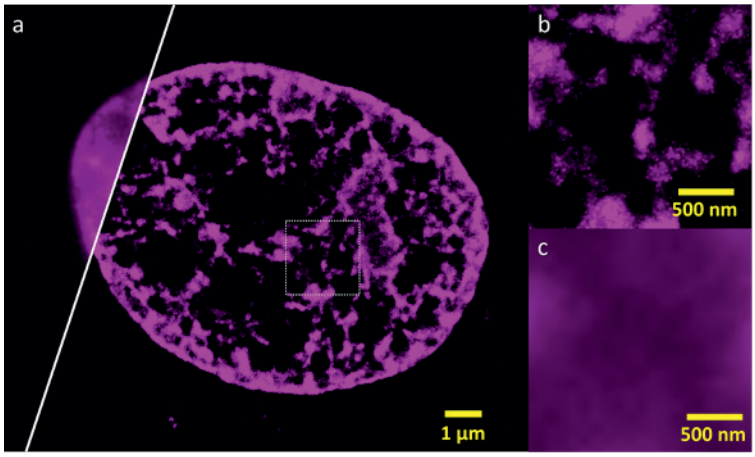


Figure 1. a) DNA-bound YOYO-1 in an HL-1 myocardial cell nucleus taken with SPDM (right of white line) or conventional widefield (left of white line) microscopy. The super-resolution SPDM technique reveals an organisation of DNA in the nucleus (b) that is not apparent with conventional microscopy techniques (c).

We also applied localisation microscopy to study the nanoscale distribution of nascent RNA within the nucleolus. RNA synthesis in U2OS cells was fluorescently targeted using the base analogue 5-ethynyl uridine (EU) for 2h prior to fixation, and EU was subsequently conjugated with Alexa 488 switchable fluorophores. The reconstruction of the single fluorophore position map exhibited a strong heterogeneity in the distribution of nascent transcripts within the cell nucleolus. The signal density attributable to the nascent RNA within the nucleoli varied locally by a factor of 3 to 4. These features indicate a number of specific sub-compartments within the nucleolus, and may be used to extract the positions of these sub-compartments. In dual colour SPDM experiments we found that the nascent RNA was confined mainly to the core part of the nucleoli, whereas the protein NPM1 was directed to the boundary.

Using localisation microscopy, we were able to show that imaging of cells at nanometer resolution can be performed together with a systematic measurement of the number of Gold Nanparticles (GNPs) incorporated into cells. In this way, it was possible to optimise GNP labelling protocols for both incubation and transfection via DNA labelling. Since the fluorescent output of the GNPs exhibits long-term blinking without the problems encountered in organic fluorophores, such as permanent photobleaching, GNPs are a promising analogue to conventional fluorophores for super-resolution single particle localisation microscopy (SPLM).

FUTURE DIRECTIONS

The methodological focus of the Cremer group will be to further improve SRM methods, in particular in terms of resolution, multicolour and *in vivo* imaging capability. In addition to localisation microscopy based approaches, this includes structured illumination microscopy and optical tomography techniques, which are particularly suited for enhanced resolution imaging of extended tissue specimens. The SRM methods will be applied to study the epigenetic nuclear landscape in various cell types and organisms, including its relevance for genome stability and repair, cancer research and other disease conditions. This will be done in the context of collaborative projects with partners from IMB, Mainz University, the Max Planck Institutes for Chemistry and for Polymer Research (Mainz), Heidelberg University, the Manitoba Cancer Center in Canada and the University of Michigan/Ann Arbor in the USA, as well as the University of Bar Ilan in Israel.

SELECTED PUBLICATIONS

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RENÉ KETTING

"We work on small RNAs, which are remarkably versatile regulators of gene activity."

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

Miguel Almeida PhD Student; since 06/2013
Walter Bronkhorst Postdoc; since 01/2015
Holger Dill Postdoc; since 02/2013
Antonio Domingues Postdoc; since 12/2014
Yasmin El Sherif Lab Manager; since 10/2014
Svena Hellmann Technician; since 11/2016
Lucas Kaaij Postdoc; 11/2013 - 10/2016
Monika Kornowska Animal Caretaker; since 03/2015
Saskia Krehbiel Animal Caretaker; since 05/2012
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Maria Placentino PhD Student; since 11/2013
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Jan Schreier PhD Student; since 05/2015
Nadine Wittkopp Postdoc; since 11/2012

OVERVIEW

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

RESEARCH HIGHLIGHTS

Maternally transmitted piRNAs are essential in zebrafish and *C. elegans*

Over the past few years, we have developed a transgenic system in zebrafish that allows us to study piRNA biology in healthy germ cells. Since piRNAs are essential for the proper development of germ cells, tampering with the proteins that interact with them leads to strong developmental defects that prohibit meaningful analyses at a mechanistic level. We have developed a system in which we can add or remove GFP-targeting piRNAs, while leaving the rest of the endogenous piRNAs untouched, permitting normal development of germ cells. We created this by randomly inserting transposons containing GFP sequences into a zebrafish strain that expresses GFP from another locus, selecting the strains in which GFP was silenced, and identifying the insertions that triggered silencing in those strains. Indeed, piRNAs targeting GFP are produced in these strains and they behave exactly

as endogenous piRNAs. We were able to show that the system responds to the amount of target RNA: the more that is present, the stronger the silencing becomes. Furthermore, this silencing appears to be largely at the post-transcriptional level. In addition, this system has made it possible to demonstrate that the maternally provided piRNAs are required to maintain silencing. This implies that during the (early) developmental phase when these maternal products are available, key steps are made to decide which sequences will be targeted for silencing and which not. Interestingly, very similar observations we made in *C. elegans*, where we could even show that maternally provided piRNAs are sufficient to silence a target in the germline for the rest of the life of the worm, again strengthening the idea that during relatively early germ cell development piRNA mediated silencing is implemented, and when it is not, it will also not establish during later development.

Coordinated deposition of mRNAs into the germ cells of zebrafish

We have been studying the effect of a protein named Tdrd6 on germ cell formation in zebrafish. Maternal loss of this protein leads to less efficient germ cell specification, but the molecular reasons behind this phenotype have remained unclear. We have now been able to show, using single-cell RNA sequencing, that Tdrd6 plays a role in establishing that every germ cell that is specified receives a certain ratio of mRNA species from different germ cell-specifying genes. The way Tdrd6 seems to do this is by helping the fusion of small, RNA-protein aggregates into bigger units, such that larger aggregates of these germ cell specifying mRNAs can be loaded into the future germ cells. Interestingly, these aggregates seem to be made out of a collection of sub-granules that carries just one, or only a limited set of, mRNA species (see Figure 1). Therefore, if future germ cells inherit fragments of such aggregates that are too small, some mRNA species may be heavily under- or overrepresented, possibly leading to the germ cell phenotype observed.

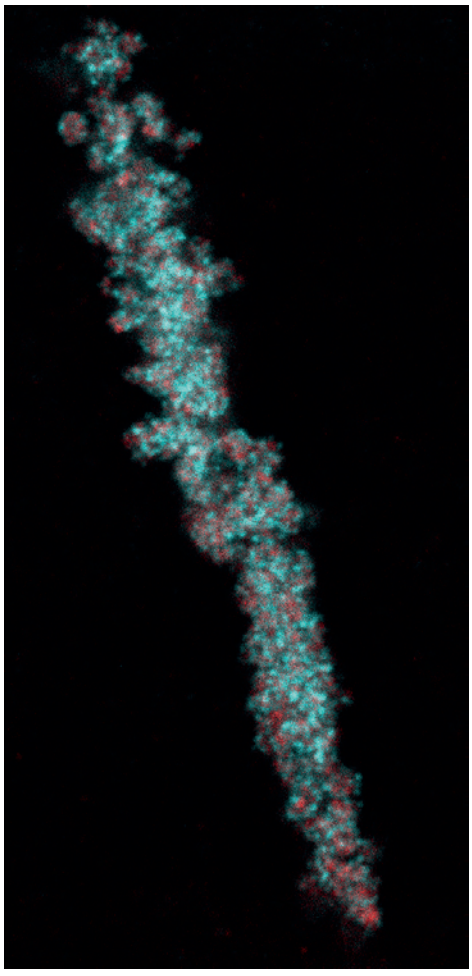

Figure 1. RNA FISH for dazl (red) and vasa (magenta) mRNA on a 4-cell stage zebrafish embryo. Depicted is a region of the embryo where germ plasm is located, an RNA-protein dense region that is required to specify germ cell fate. The signals for dazl and vasa mRNAs do not overlap, strongly suggesting that these granules contain only one, or a limited set of mRNA species. As a result, the distribution of these two mRNA species is not homogeneous.

FUTURE DIRECTIONS

Our future work will continue to mechanistically unravel the molecular pathways that are steered by small RNA guides. We are performing a genetic screen in order to identify novel factors, and are increasingly using biochemical approaches to start to describe the mechanisms on a more molecular level. We have started to extend our studies to include structural biology, in order to be able to design specific point-mutations that disrupt specific aspects of the identified mechanisms. *C. elegans* and zebrafish will continue to play important roles in these studies. We will focus on how a piRNA response can be initiated *de novo* and want to understand how and when a transient small RNA-mediated response can be transformed into a stably inherited response. In addition, we will start to work on how small RNA pathways are connecting to other aspects of the cell's gene-regulatory programmes, including those active during germ cell specification and differentiation.

SELECTED PUBLICATIONS

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

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

JULIAN KÖNIG

"Our aim is to crack the splicing code."

POSITIONS HELD

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

EDUCATION

2008 PhD in Biology, Max Planck Institute for Terrestrial Microbiology and Phillipps University, Marburg
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Reymond Sutandy PhD Student; since 12/2013
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OVERVIEW

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

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

RESEARCH HIGHLIGHTS

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

Alu elements are retrotransposons that frequently form new exons (Alu-exons) during primate evolution. Together with Jernej Ule's lab at University College London, we have assessed the interplay of splicing repression by the RBP hnRNPC and the nonsense-mediated mRNA decay (NMD) pathway in the quality control and evolution of Alu-exons. We identified 3,100 new Alu-exons and showed that NMD recognises Alu-exons more efficiently than other exons containing premature termination codons that trigger NMD. However, some Alu-exons escape NMD, especially when an adjacent intron is retained, highlighting the importance of concerted repression by the two pathways. Cross-species analysis of Alu-exons within primates showed that evolutionary progression of 3' splice sites is coupled to longer repressive U-tracts. Ancient Alu-exons have short U tracts, which decreases their splicing repression by hnRNPC, but they generally remain sensitive

to NMD. We conclude that repressive motifs are under the strongest positive selection in the *Alu* elements that contain cryptic Alu-exons, and hypothesise that gradual removal of repressive motifs might be crucial for evolutionary emergence of new exons.

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

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

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

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

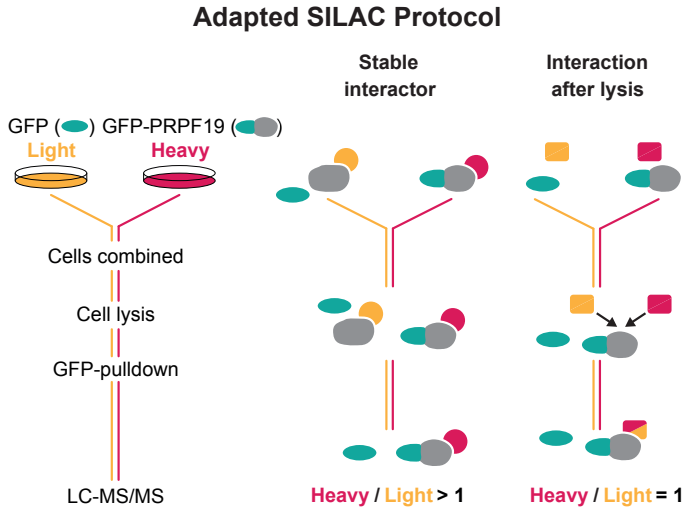

Figure 1. The adapted SILAC approach specifically enriches for stable protein-protein interactions. To this end, isotopically labelled cells expressing either GFP (Light) or a GFP-tagged protein of interest (Heavy) are mixed prior to cell lysis. Only stable interactions that persist from before lysis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

FUTURE DIRECTIONS

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

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

SELECTED PUBLICATIONS

Tajnik M, Vigilante A, Braun S, Hanel H, Luscombe NM, Ule J, Zarnack K[#] and König J[#] (2015). Intergenic *Alu* exonisation facilitates the evolution of tissue-specific transcript ends. *Nucleic Acids Res*, 43, 10492-10505

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

König J, Zarnack K, Luscombe NM and Ule J (2012). Protein-RNA interactions: new genomic technologies and perspectives. *Nat Rev Genet*, 13, 77-83

*indicates joint contribution; [#]indicates joint correspondence

STEFAN LEGEWIE

"We study how cells function robustly despite molecular fluctuations."

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

Alex Anyaegbunam PhD Student; since 08/2016
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Kolja Becker PhD Student; since 08/2013
Mihaela Enculescu Postdoc; since 10/2013
Christoph Fritzsch PhD Student; since 06/2016
Lu Huang Postdoc; 10/2012 - 10/2016
Monika Kuban Technician; 10/2012 - 12/2016
Uddipan Sarma Postdoc; 07/2013 - 09/2016

OVERVIEW

Cellular processes are coordinated by networks of interacting molecules, and many diseases are caused by perturbations in these networks. Neither can be fully understood by studying single genes or proteins. To better understand cellular function in health and disease, my group analyses the dynamic interplay of cellular components with special emphasis on how cells sense environmental changes. One recent focus was to investigate how biological signalling networks function robustly despite internal and external fluctuations. We apply systems biology approaches, and combine mathematical modelling with quantitative experimental data from collaborators to understand the wiring and dynamics of signalling and gene regulatory networks.

RESEARCH HIGHLIGHTS

Single-cell dynamics of signalling and transcription

Single-cell datasets are often multivariate and escape intuition. Quantitative mathematical models are therefore required to fully understand dynamic phenomena in single cells. We investigated the cell-to-cell variability in the TGFβ/SMAD signalling pathway which plays a key role in tumorigenesis and metastasis. Together with Alexander Loewer (University of Darmstadt), we monitored the nuclear translocation of SMAD2/4-GFP fusion proteins in thousands of living MCF10A cells. Based on time course clustering, we concluded that the phenotypic response of a cell is primarily determined by the temporal dynamics of SMAD nuclear translocation, and identified six sub-populations with qualitatively distinct signalling behaviour. We described the dynamics of these subpopulations and of the complete heterogeneous cell population using a quantitative modelling approach, and concluded that heterogeneity in signalling arises because each cell contains different amounts of signalling proteins. CRISPR/Cas9-mediated knockouts confirmed that the concentration fluctuations of certain pathway regulators are major sources of heterogeneity. In the future, we plan to further investigate the dynamics and heterogeneity of TGFβ/SMAD signalling pathway by systematically perturbing pathway components or neighbouring signalling

cascades. We hope to understand why certain cells induce a migratory response and potentially metastasis, whereas others fail to do so.

Cellular decision making is a stochastic process, as even genetically identical cells frequently respond in a very different way when subjected to the same stimulus. To better characterise the heterogeneous growth of estrogen-dependent breast cancer cells, we studied the stochastic transcription of an estrogen-sensitive gene locus. Together with George Reid, we established an imaging pipeline for visualising nascent transcripts in living MCF7 cells using the PP7-PCP system. We interpreted these time-resolved datasets using a model fitting framework, in which we simultaneously determine the topology and the kinetic parameters of a stochastic promoter model. The model and further experiments indicated that noise in transcription not only arises from stochastic bursting at each promoter, but also involves a strong extrinsic component reflecting from the state of the cell. We further investigated how varying estrogen concentrations or small-molecule inhibitors of epigenetic events control the heterogeneity of transcription.

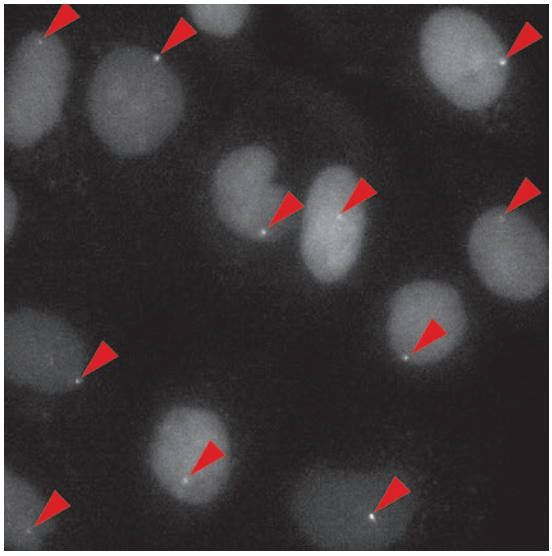

Figure 1. Imaging of estrogen-induced transcription. An estrogen-sensitive transcript was tagged using PP7 hairpins, and visualised by co-expressing the fluorescently labelled viral coat protein PCP-GFP in the nucleus (grey shaded areas). The site of ongoing transcription is visible as a bright spot (arrows), reflecting the recruitment of PCP-GFP to elongating nascent mRNAs containing PP7 stem loop structures. Estrogen-induced transcription exhibits pronounced heterogeneity even at saturated stimulus concentrations.

Regulation of systemic iron homeostasis

Systemic iron levels must be maintained in physiological concentrations to prevent diseases associated with iron deficiency or overload. A central regulatory mechanism is the binding of the hormone hepcidin to the transmembrane iron exporter ferroportin, which controls the major iron fluxes from peripheral organs into blood plasma. Together with the Muckenthaler group (Heidelberg University), we derived a mathematical model of *in vivo* iron homeostasis that is fitted and validated against experimental data to simulate the iron content in different organs following dietary changes and/or inflammatory states, or genetic perturbations of the hepcidin/ferroportin regulatory system. Surprisingly, we find that hepcidin-mediated ferroportin regulation is not sufficient to quantitatively explain several of our experimental findings, and proposed additional homeostasis mechanisms using our model. Taken together, our model quantitatively describes systemic iron metabolism and provides a better understanding of iron-related diseases such as hemochromatosis and anaemia of inflammation.

FUTURE DIRECTIONS

Cells can respond to external cues by inducing changes in gene expression. How these gene expression responses are coordinated by complex gene regulatory networks remains poorly understood. Together with the König and Tiwari groups, we are employing perturbation approaches and mathematical modelling to characterise the topology and the dynamics of transcription factor and splicing networks controlling oncogenic transformation. Using our models, we hope to contribute to better understand gene regulatory mechanisms controlling tumor growth.

SELECTED PUBLICATIONS

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

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

Casanovas G, Banerji A, d'Alessio F, Muckenthaler M# and Legewie S# (2014). A multi-scale model of hepcidin promoter regulation reveals factors controlling systemic iron homeostasis. *PLoS Comput Biol*, 10, e1003421

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BRIAN LUKE

"We try to understand how checkpoint failure drives drug resistance in chemotherapeutics."

POSITIONS HELD

Since 2014	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 in Lausanne (EPFL)
2005	Postdoc, Biochemistry, Swiss Federal Institute of Technology in Zurich

EDUCATION

2005	PhD in Biochemistry, ETH Zurich
1999	BSc in Biology, Queen's University, Ontario

GROUP MEMBERS

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Stefanie Grimm Technician; since 01/2015
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Vanessa Kellner PhD Student; since 03/2015
Julia Klermund Postdoc; 08/2015 - 06/2016
Sarah Luke-Glaser Staff Scientist; since 10/2016
Stefano Misino PhD Student; since 10/2016
René Schellhaas PhD Student; 11/2015 - 07/2016
Olgo Vydzhak PhD Student; since 02/2016
Tina Wagner PhD Student; since 11/2015

OVERVIEW

Telomeres are protective caps at the ends of linear chromosomes. When telomeres shorten, replicative senescence occurs as a result of DNA damage checkpoint activation. Cells that have undergone more divisions have a higher likelihood of becoming senescent. The accumulation of senescent cells may contribute to ageing in multi-cellular organisms. On the other hand, replicative senescence acts as a barrier to tumour development by limiting proliferative potential. Therefore, cancer cells must overcome the checkpoint barrier and furthermore, re-elongate their telomeres in order to achieve immortality. We are interested in understanding how cells transition through replicative senescence when short/dysfunctional telomeres arise and checkpoints become activated. We have observed that the long non-coding RNA, TERRA, and its ability to form RNA-DNA hybrids has an important influence on senescence dynamics and may even contribute to helping cells attain an immortal status by promoting a special type of telomere maintenance.

Checkpoint adaptation is one way that cells can overcome the DNA damage checkpoint. Our observations indicate that by altering nutrient conditions it is possible to influence the frequency of checkpoint adaptation events. We are now trying to utilise this exciting interaction to optimise the effectiveness of common chemotherapeutic agents and prevent resistance development.

RESEARCH HIGHLIGHTS

We continue to make important steps forward in the understanding of TERRA regulation and function. We have now demonstrated that TERRA and R-loops specifically accumulate at short telomeres where they trigger homologous recombination and hence promote telomere length maintenance. We have now added significant mechanistic details with regards to how this is regulated and coordinated with cell cycle timing. We show that TERRA and its R-loops are formed in the early stages of S phase. Shortly after, the ribonucleases Rat1 and RNase H2 associate with telomeres and degrade TERRA and its R-loops, respectively. This coordinated action ensures

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

When cells escape senescence they may re-elongate their telomeres via recombination, as is the case for ALT (Alternative lengthening of telomeres) cancer cells. We have now shown that when cells use the ALT mechanism they significantly up-regulate TERRA levels. We speculated that TERRA R-loops may also promote telomere recombination in these cells. Upon RNase H overexpression, we were able to reduce telomere length in cells using ALT and subsequently impair their growth capacity. Importantly, the RNase H effects were only seen to affect ALT cells and not telomerase positive cells. Taken together, our results suggest that TERRA function depends on the state of the telomere. We propose that TERRA R loops promote recombination at critically short telomeres during replicative senescence, and through a similar mechanism maintain telomere length in cells using the ALT mechanism. In cells with "normal" length telomeres, we believe that TERRA levels are suppressed because recombination is not desired when length is sufficient.

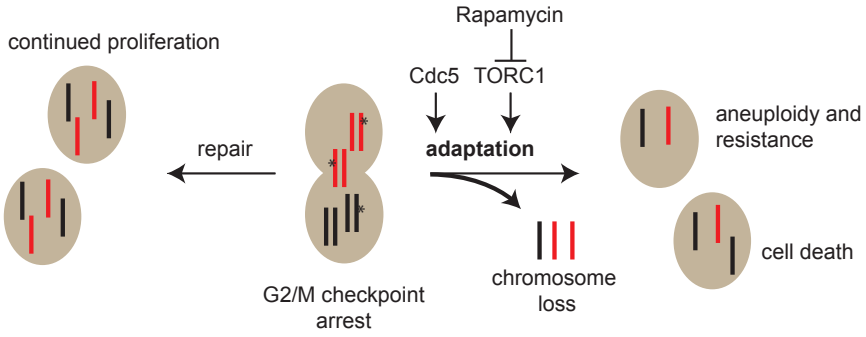

Figure 1. (A) Upon exposure to genotoxins, cells experience DNA damage (depicted as * on red and black chromosomes). When repair is not possible, the damage persists and cells eventually undergo checkpoint adaptation (proliferation in the presence of DNA damage), which is driven by TOR and Cdc5. Adapted cells become aneuploid, which leads to their eventual drug resistance. The inhibition of TOR, with rapamycin, prevents adaptation and holds cells in a permanently arrested state.

Finally, we have previously demonstrated that the inhibition of checkpoint adaptation is possible through metabolic interventions such as rapamycin treatment or caloric restriction using a dysfunctional telomere model. We have attempted to apply this knowledge to relevant chemotherapeutics and determine if the regulation of adaptation can affect their efficacies. Importantly, we have determined that in repair defective cells the combination of a chemotherapeutic with the inability to adapt leads to a synergistic increase in cytotoxicity. Importantly, repair competent cells are not affected by this combination regime, thereby demonstrating genetic specificity. Moreover, we have determined that cells that regrow following adaptation become aneuploid and moreover, resistant to future drug treatments. Therefore, the combination of chemotherapeutic agents with metabolic intervention (rapamycin treatment) may enhance cytotoxicity and reduce the development of resistance by counteracting checkpoint adaptation (Figure 1).

FUTURE DIRECTIONS

Now that we have a more complete understanding of how TERRA and R-loops are regulated at short and long telomeres we will embark on trying to understand how TERRA is regulated/deregulated in cells using the ALT telomere maintenance mechanism. These experiments will be carried out using the yeast model as well as in human cancer lines that are ALT positive. Importantly, we will focus on trying to modulate TERRA and TERRA R-loop levels with the goal of specifically affecting the viability of ALT-engaged cells. In addition, we are characterising chemical compounds that affect checkpoint adaptation with the long term goal of combining them with genotoxic chemotherapeutics to enhance cytotoxicity and prevent resistance. In addition to rapamycin, we have recently discovered two non-toxic compounds that have such effects. We will try to gain a mechanistic understanding of how these reagents act inside the cell and modulate the response to chemotherapeutic agents.

SELECTED PUBLICATIONS

Rippe K# and Luke B# (2015). TERRA and the state of the telomere. *Nat Struct Mol Biol*, 22, 853-858

Klermund J, Bender K and Luke B (2014). High nutrient levels and TORC1 activity reduce cell viability following prolonged telomere dysfunction and cell cycle arrest. *Cell Rep*, 9, 324-335

Balk B*, Maicher A*, Klermund J, Luke-Glaser S, Dees M, Bender K and Luke B (2013). Telomeric RNA-DNA hybrids affect telomere length dynamics and senescence. *Nat Struct Mol Biol*, 10, 1199-1205

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CHRISTOF NIEHRS

"We study how Gadd45 directs locus-specific DNA demethylation."

POSITIONS HELD

2010 - 2015	Executive Director, Institute of Molecular Biology (IMB), Mainz
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2000	Professor of Molecular Embryology, German Cancer Research Center (DKFZ), Heidelberg
Since 1994	Head of Division "Molecular Embryology", DKFZ, Heidelberg
1990 - 1993	Postdoc, University of California, Los Angeles (UCLA)

EDUCATION

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

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Victoria Hatch Postdoc; since 11/2014
Laura Krebs Technician; since 09/2015
Manuel Leichsenring PhD Student; 04/2011 - 08/2016
Medhavi Mallick PhD Student; since 08/2012
Bernadette Mekker PhD Student; 07/2010 - 09/2016
Svetlana Melcea PhD Student, since 07/2013
Michael Musheev Postdoc; since 07/2011
Georg Otto Technician; since 01/2016
Mihika Pradhan PhD Student; since 12/2016
Pallavi Rajput Postdoc; since 01/2016
Sandra Rölle Lab Manager; since 04/2011
Andrea Schäfer Postdoc; since 09/2010
Carola Scholz Technician; since 05/2015
Lars Schomacher Postdoc; since 07/2011
Katrin Schüle PhD Student; since 01/2016
Philipp Trnka Research Assistant; since 11/2016
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OVERVIEW

In the DNA of many multicellular organisms, DNA methylation is a common epigenetic mark associated with gene silencing. DNA methylation is a dynamic process and can be reversed by enzymatic demethylation, a process that is still incompletely understood. DNA demethylation is a widespread phenomenon and occurs in plants as well as in animals, during development, in the adult, and during somatic cell reprogramming of pluripotency genes. We have shown that growth arrest and DNA damage 45a (Gadd45a) is a key player in active DNA demethylation and acts via DNA repair. The goal of our research is to analyse the mechanism of DNA demethylation as well as the role played by Gadd45 in development. Our results indicate that Gadd45a acts as an adapter protein which directs DNA methylation machinery to specific loci. To address Gadd45 function, we use biochemical, molecular biological and cell biological approaches, employing the mouse and frog model systems.

RESEARCH HIGHLIGHTS

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

We have shown that Gadd45a mediates active DNA demethylation. Gadd45a is a member of a small gene family of stress response genes encoding histone fold proteins. Gadd45a proteins are multifunctional and regulate a range of cellular processes, including DNA repair, proliferation, apoptosis, and differentiation. Gadd45a-mediated demethylation involves recruitment of the nucleotide excision repair and/or base excision repair machineries.

One prominent mode of enzymatic DNA demethylation involves oxidation of 5mC by the Ten-eleven translocation (TET) family of dioxygenases, which

interact with Gadd45a in demethylation e.g. of the TCF21 tumor suppressor. Tet enzymes iteratively oxidise 5mC to form 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), followed by Thymidine glyosylase (Tdg) mediated-base excision repair. We found that Gadd45 binds to both Tet and TDG proteins and functionally cooperates with them in demethylation (Figure 1). Moreover, TET1 requires GADD45a for demethylation of a methylated reporter and for global mC oxidation. Synergistic gene activation by GADD45a/TET1 is accompanied by an increase in 5hmC and reduction of 5fC and 5caC. Collectively, our data support a dual role of GADD45a in TET1 mediated DNA demethylation. In a first step, GADD45a positively regulates TET activity and thereby enhances mC oxidation. This activation of TET1 might be conferred e.g. by conformational change induced by GADD45a binding, or by recruitment of cofactors. In a second step, GADD45a reduces 5fC and 5caC levels. fC/caC removal by the glycosylase TDG is well-established and GADD45a directly binds TDG. Hence, GADD45a may function as a bridging protein between TET1 and TDG, thereby physically coupling mC oxidation with repair.

We are also analysing the *in vivo* significance of the Gadd45a/Tet1 interaction. Towards this aim, we have generated *Gadd45a/Tet1* homozygous double mutant mice. While single knockout mutants for G*add45a* and *Tet1* are viable, double mutants show synthetic lethality with embryos displaying malformed heads (exencephaly). The synthetic lethality in double mutant mice corroborates the physiological relevance of the Gadd45a/Tet1 interaction. We are now characterising the double mutant embryos and studying the consequences of Gadd45a interaction with Tet1 biochemically.

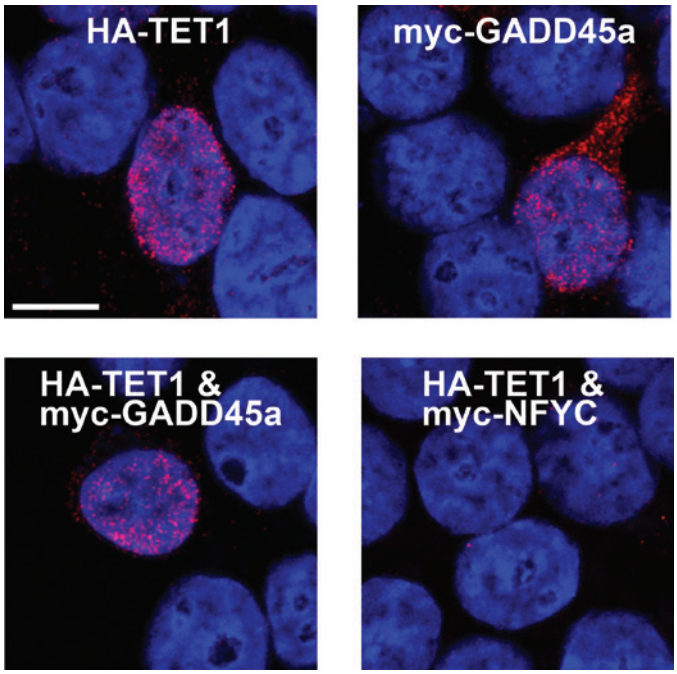

Figure 1. Gadd45a and Tet1 physically interact. *In-situ* analysis of TET1-GADD45a interaction by Proximity Ligation Assay (PLA) in HEK293T cells transiently transfected as indicated. Single transfected "HA-TET1" and "myc-Gadd45a" represent self-PLA assays as positive controls. "HA-TET1 & myc-GADD45a" shows PLA interaction signal between both proteins in nuclei. Scale bar, 10 µm. (from Kienhöfer *et al.*, *Differentiation*, 2015).

FUTURE DIRECTIONS

Our discovery of the interaction between TET in Gadd45a in demethylation raises new questions. Which target genes are regulated by Gadd45a? What are the sequence determinants that recruit Gadd45a to specific genes during demethylation? What is the role of Gadd45a-mediated demethylation in mammals? To investigate these questions, we will analyse mouse embryonic stem cells (mESC) that are mutant for Gadd45a,b and g. We plan to monitor gene expression and DNA methylation in mESCs and analyse the differentiation capacity of Gadd45-mutant cells, as well as identifying Gadd45 target genes. We will also continue to characterise the Gadd45a/Tet1 double mutant mice.

SELECTED PUBLICATIONS

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Arab K, Park YJ, Lindroth AM, Schäfer A, Oakes C, Weichenhan D, Lukanova A, Lundin E, Risch A, Meister M, Dienemann H, Dyckhoff G, Herold-Mende C, Grummt I#, Niehrs C# and Plass C# (2014). Long noncoding RNA TARID directs demethylation and activation of the tumor suppressor TCF21 via GADD45A. *Mol Cell*, 55, 604-614

#indicates joint correspondence

HOLGER **RICHLY**

"We aim to decipher chromatin signalling pathways."

POSITIONS HELD

Since 2011	Group Leader, Institute of Molecular Biology (IMB), Mainz
2005 - 2011	Postdoc, Centre for Genomic Regulation (CRG), Barcelona

EDUCATION

2005	PhD in Biology, Ludwig Maximilian University, Munich
2000	Diploma in Biochemistry, Ruhr University, Bochum

GROUP MEMBERS

Jonathan Byrne PhD Student; since 08/2011
Shalaka Chitale PhD Student; since 02/2013
Ekaterina Gracheva PhD Student; 08/2011 - 07/2016
Aysegül Kaymak PhD Student; since 02/2013
Rebeca Medina Technician; since 08/2013
Thaleia Papadopoulou PhD Student; 10/2011 - 09/2016
Jens Stadler PhD Student; since 09/2014
Thomas Wilhelm PhD Student; since 11/2011

OVERVIEW

The research undertaken in my laboratory aims at deciphering molecular pathways that underlie chromatin signalling networks that regulate physiological processes such as cellular differentiation, DNA repair and organismal ageing. Our scientific approach relies largely on dissecting the functions of diverse chromatin components, as for example epigenetic players, in the cell culture system biochemically and by applying high resolution microscopy. We complement our *in vitro* approach by employing genetics and RNAi screening techniques in *C. elegans*.

RESEARCH HIGHLIGHTS

Epigenetic networks govern most cellular processes that take place in a chromatin environment, for example differentiation, DNA repair and replication. Our research provides evidence for how epigenetic factors act in concert with DNA repair factors. In our investigations studying DNA repair we have largely concentrated on one particular histone mark, the mono-ubiquitylation of histone H2A at lysine 119 (H2A-ubiquitin). H2A-ubiquitylation is a hallmark of signalling cascades as part of the DNA damage response. We have recently demonstrated that timing of DNA repair specific E3 ligases is an important feature of nucleotide excision repair (NER) and we have discussed a new concept of remodelling E3 ligase complexes at chromatin during DNA lesion recognition. In brief, we have discovered that H2A-ubiquitin is catalysed predominantly by a novel E3 ligase complex (UV-RING1B complex) that operates early during lesion recognition (Figure 1). ZRF1 tethers to the H2A-ubiquitin mark at the damage site and mediates the remodelling of the UV-RING1B complex, a process that we have coined *on-site remodelling*. In analogy, ZRF1 also remodels multiprotein complexes during differentiation of mouse embryonic stem cells. During cellular differentiation, ZRF1 is recruited to regulatory regions of genes that are silenced by PRC1 and Mediator complexes. Upon tethering, ZRF1 abrogates PRC1-MED12 binding, facilitating the association of CDK8 with Mediator. This remodelling of Mediator-associated protein complexes converts Mediator from a transcriptional repressor to a transcriptional enhancer, which then

mediates ncRNA-dependent activation of PRC1-MED12 target genes. To complement our mechanistic approach, we have further investigated the function of ZRF1 *in vivo* showing its importance for proper mesoderm development and the generation of cardiomyocytes.

Another interest of my lab is the sub-nuclear localisation of NER, which is controlled through DNA damage-dependent setting of histone marks. We have characterised a DNA repair specific multi-protein complex, which catalyses the specific modification of histones at damaged chromatin upon UV irradiation. We have demonstrated that this histone mark is read by a specific anchor protein that tethers damaged chromatin at sub-nuclear structures to generate DNA repair foci. Thus, we have provided the first evidence for compartmentalisation of DNA repair in the NER pathway.

Further, we are interested in understanding gene regulation during organismal ageing. To this end we investigate ageing in the nematode *C. elegans*, employing sophisticated RNAi screening techniques, genetics and high resolution microscopy. We have generated a semi-automated RNAi screening technique to isolate novel factors involved in the ageing of the worms. We have analysed in detail the function and molecular mechanism of one factor identified by the screen and we have started to investigate its impact on ageing in the fruit fly. Our research shows that the timing of gene regulation is an important determinant of longevity. In the near future, we will characterise the function of other epigenetic factors using physiological assays and, more importantly, we will start to investigate how they reprogramme the epigenome in the course of ageing.

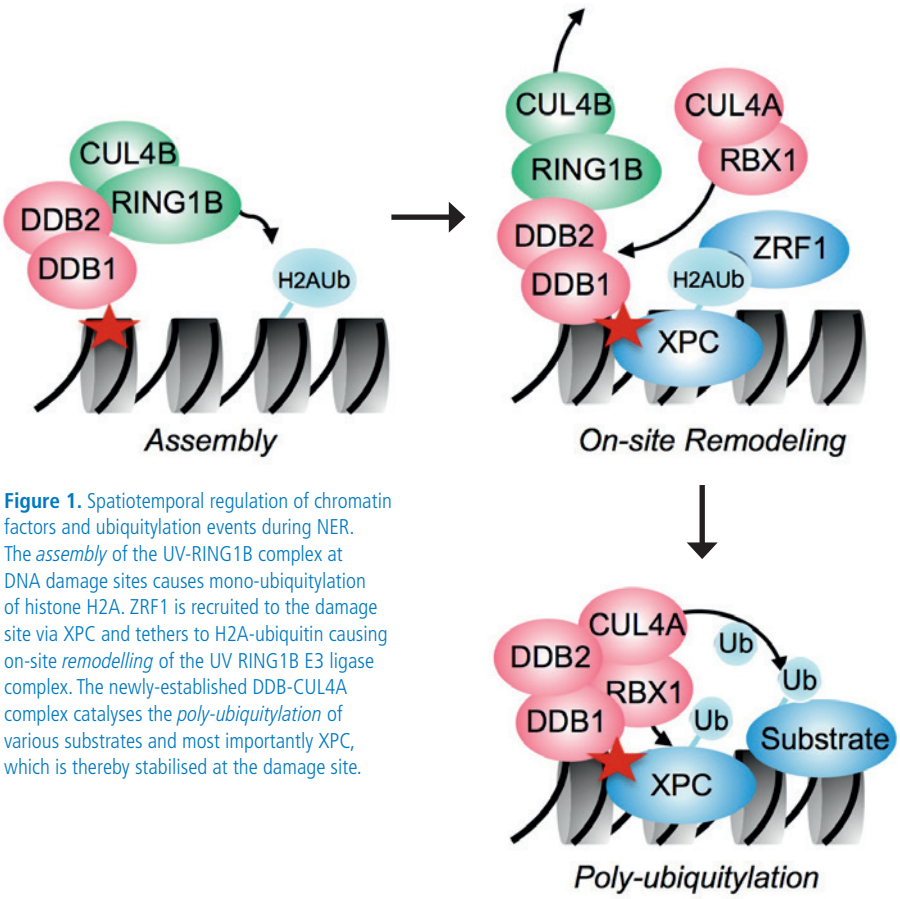

Figure 1. Spatiotemporal regulation of chromatin factors and ubiquitylation events during NER. The *assembly* of the UV-RING1B complex at DNA damage sites causes mono-ubiquitylation of histone H2A. ZRF1 is recruited to the damage site via XPC and tethers to H2A-ubiquitin causing on-site *remodelling* of the UV RING1B E3 ligase complex. The newly-established DDB-CUL4A complex catalyses the *poly-ubiquitylation* of various substrates and most importantly XPC, which is thereby stabilised at the damage site.

FUTURE DIRECTIONS

In the future, we will prioritise the research on DNA repair in the NER pathway and the investigation of organismal ageing. One of our main aims is to understand the chromatin signalling network underlying DNA damage recognition in the NER pathway and the transition from recognition to the verification of DNA damage. In particular, we will analyse how ubiquitin signalling cascades crosstalk to other chromatin factors and histone marks. Furthermore, we will investigate the function of K63-linked polyubiquitylation in NER, which presumably provides a means of recruiting repair factors. To extend our studies in organismal ageing, we plan to study how epigenetic factors and environmental cues extend the lifespan and the health span of *C. elegans*. In particular, we will study how the identified late life factors crosstalk with other ageing-related pathways. We will also extend our studies employing the fruit fly *D. melanogaster* to identify conserved epigenetic mechanisms of ageing.

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JEAN-YVES ROIGNANT

"RNA modifications are an exciting unexplored layer of post-transcriptional gene regulation."

POSITIONS HELD

Since 2012	Group Leader, Institute of Molecular Biology (IMB), Mainz
2008 - 2011	Research Associate, Skirball Institute of Biomolecular Medicine, New York University
2003 - 2008	Postdoc, Skirball Institute of Biomolecular Medicine, New York University

EDUCATION

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

GROUP MEMBERS

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Annabelle Dold PhD Student; since 01/2015
Giriram Kumar PhD Student; since 04/2014
Tina Lence PhD Student; since 07/2013
Violeta Morin Technician; since 05/2015

OVERVIEW

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

RESEARCH HIGHLIGHTS

Mechanisms of the exon junction complex in pre-mRNA splicing

The exon junction complex (EJC) is a ribonucleoprotein complex that binds RNAs during splicing in order to influence their cytoplasmic fate. In addition to this function, we previously demonstrated two important roles in pre-mRNA splicing, one in intron definition and a second in exon definition. While we uncovered the role of the EJC in intron definition two years ago, the role of the EJC in exon definition has remained unclear. We now provide insights into this mechanism. We found that the depletion of EJC components, but not of the EJC splicing subunits, led to genome-wide changes in the phosphorylation state of Pol II and to a global decrease in promoter proximal pausing. Most of these changes correlate with a modification in chromatin accessibility. In addition we found that the EJC associates directly with promoter regions, providing a link between the EJC and the transcription machinery. The EJC associates with a specific phosphorylated form of Pol II in order to restrict binding of the positive transcription elong-

ation factor complex (P-TEFb) at promoter regions. Remarkably, reducing P-TEFb levels is sufficient to partially rescue exon skipping and the eye phenotype associated with the absence of the EJC, indicating that the effect of the EJC on transcription indirectly impacts exon definition. This work presents for the first time a connection between the EJC and promoter proximal pausing of RNA Pol II, which increases the functional repertoire of this versatile ribonucleoprotein complex.

Functions of m^{6}A RNA modification in *Drosophila*

The widespread roles of m^{6}A RNA modification in the regulation of post-transcriptional gene expression have recently been brought to light. From yeast to mammals, m^{6}A has been shown to regulate pre-mRNA splicing, translation and mRNA decay. However, the precise mechanisms of this modification in these processes still remain to be determined. Furthermore, the physiological functions of m^{6}A in multi-cellular organisms have not yet been fully investigated. To address these questions, we have started to characterise m^{6}A in *Drosophila*. We found that a conserved m^{6}A methyltransferase complex composed of four proteins controls alternative splicing in *Drosophila* cells and *in vivo*. Furthermore, we identified the split ends (SPEN) family protein, Spenito (Nito), as an additional bona fide subunit of the complex. Like in mammals, components of the complex are ubiquitously expressed but show significant enrichment in the nervous system, which is consistent with the high level of m^{6}A in this tissue. We find that flies mutant for *Ime4* and for the second catalytic subunit are viable but suffer from severe locomotion defects due to impaired neuronal functions. Components of the m^{6}A methyltransferase complex also control the female-specific splicing of *Sex lethal (Sxl)* transcript and of its downstream targets, revealing a role for this modification in sex determination and dosage compensation. Members of the YTH domain-containing protein family were previously shown to specifically recognise m^{6}A and mediate its posttranscriptional functions. We identified two members of the YTH family in *Drosophila*, the nuclear YT521-B and the cytoplasmic CG6422. We confirmed that *Drosophila* YT521-B specifically binds m^{6}A *in vitro* and demonstrated that its loss *in vivo* resembles the absence of the methyltransferase complex, suggesting that this protein is a primary effector of m^{6}A in *Drosophila*. Our study provides for the first time a comprehensive *in vivo* characterisation of m^{6}A biogenesis and function in *Drosophila*, demonstrating the crucial importance of the methyltransferase complex in neurogenesis and sex determination via the activities of YT521-B.

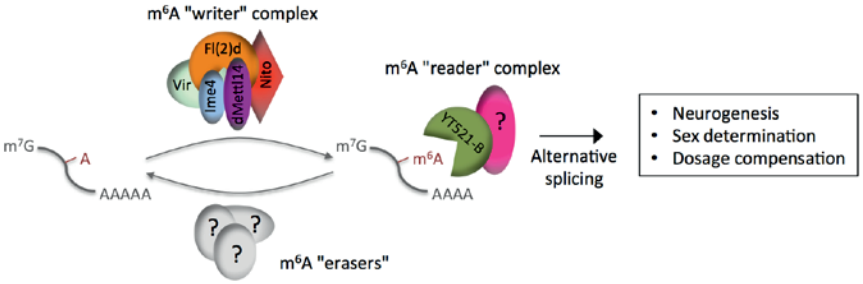

Figure 1. m^{6}A is required for neural development and sex determination in *Drosophila*. The *Drosophila* m^{6}A methyltransferase complex is composed of five factors, including the novel SPEN-domain containing protein Spenito. The methyltransferase complex controls neural development, sex determination and dosage compensation via its nuclear reader YT521-B.

FUTURE DIRECTIONS

Our long-term goal is to address the impact of promoter proximal pausing and RNA modifications on alternative splicing. How these elements integrate to specifically fine-tune RNA splicing in the context of developing organisms is poorly understood and remains an important challenge. We have initiated genetic screens to address these questions. Altogether, our studies have the potential to provide fundamental insight into the contribution of epigenetics and epitranscriptomics in pre-mRNA processing and will impact on our general understanding of gene regulation and diseases.

SELECTED PUBLICATIONS

Lence T, Akhtar J, Bayer M, Schmid K, Spindler L, Ho CH, Kreim N, Andrade-Navarro MA, Poeck B, Helm M and Roignant JY (2016). m^{6}A controls neurogenesis and sex determination in *Drosophila* via its nuclear reader protein YT521-B. *Nature* 540, 242-247

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

VASSILIS ROUKOS

"We shed light on the formation of translocations that can lead to cancer."

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 Institutes of Health (NIH), Bethesda

EDUCATION

2005 - 2008 PhD in Molecular Biology and Cytogenetics, Medical School, University of Patras
2002 - 2005 MSc in Applications in Medical Sciences, Medical School, University of Patras
1998 - 2002 BSc in Biology, University of Patras

GROUP MEMBERS

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Henrike Gothe PhD Student; since 05/2016
Vera Minneker PhD Student; since 04/2016
Rossana Piccinno PhD Student; since 04/2015
Ulrike Stapf Technician; 07/2015 - 07/2016

OVERVIEW

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

RESEARCH HIGHLIGHTS

Spatial dynamics of chromosome translocations in living cells

The lack of a direct assessment on the formation of chromosome translocations in living cells has led to contradicting ideas regarding how individual double-strand breaks (DSBs) find each other within the mammalian nuclear space and illegitimately repair to form translocations. To shed light on the timing and sequence of the events leading to translocation formation, we have established the first cell-based experimental system to visualise the formation of translocations in real time. We have employed high-throughput microscopy and automated image analysis that has enabled us to track individual chromosomes during the formation of these highly rare translocations. Using this approach, we have demonstrated that the rate of the formed DSBs, their motion properties, their illegitimate pairing and their joining efficiency are important determinants of the observed translocation

frequency. In combination with chemical inhibition or loss-of-function experiments for key players of the DNA damage response, we identified distinct DSB-repair factors acting as promoters or suppressors of chromosome translocations. Our findings provide a comprehensive spatial and temporal framework to understand the formation of chromosome translocations in living cells.

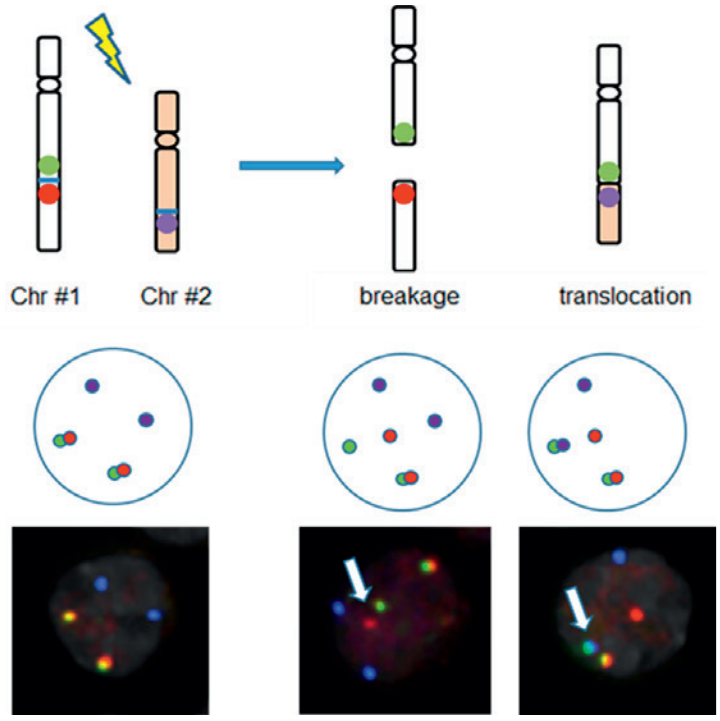

Figure 1. Quantification of intra-chromosomal breakage and translocations by high-throughput imaging. Fluorescence *in situ* hybridisation (FISH) is used to probe the position of chromosome ends of potential translocation partners. Intra-chromosomal breakage is detected by the physical separation of different fluorescent probes (green and red) and translocations by the additional co-localisation of the chromosome end of the potential translocation partner (blue probe).

Modelling of the formation of recurrent cancer-initiating translocations of interest requires a versatile approach. We have now established a methodology that uses fluorescence *in situ* hybridisation (FISH) to probe the position of individual chromosome ends of potential translocation partners in interphase cells in 3D (Figure 1). Using this approach in combination with high-throughput imaging and automated image analysis, we are able to probe and quantify individual cells with intra-chromosomal separation and rare cancer-initiating translocations with high sensitivity. We are currently using these tools in combination with molecular techniques and sequencing approaches to help us: 1) understand which are the molecular features that define recurrent breakpoint sites on recurrent translocation partners, 2) assess whether the chromatin environment predisposes to chromosome breakage and translocation formation, 3) identify molecular players of the DNA damage response and novel factors that mediate synapsis of chromosome breaks and their illegitimate fusion.

FUTURE DIRECTIONS

Our goal is now to use our established tools in combination with novel recombineering approaches to identify molecular mechanisms underlying the formation of recurrent cancer-initiating translocations. We are particularly interested in the mechanisms governing the formation of a distinct type of translocations, which form as a direct response to chemotherapy and lead to secondary malignancies (mainly leukaemias) that exhibit very short latency and poor prognosis. Despite the fact that the therapy-related malignancies are a major problem in chemotherapy today, the lack of experimental systems to model and quantify directly the formation of these fusions *in vivo*, has limited our understanding of the molecular pathways that contribute to their occurrence.

We also plan to develop tools that allow us to perform siRNA and CRISPR-based, unbiased and targeted, screens to identify novel factors that govern key steps of the translocation process. Taken together, our research will shed light on the mechanisms of cancer-initiating translocations, which will advance our understanding of fundamental principles of cancer aetiology.

SELECTED PUBLICATIONS

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

Roukos V, Voss T, Lee S, Meaburn K, Wangsa D and Misteli T (2013). Spatial dynamics of chromosome translocations in living cells. *Science,* 341, 660-664

NATALIA SOSHNIKOVA

"Understanding the mechanisms of embryonic development is key to understanding adult stem cell biology."

POSITIONS HELD

From 2012 Group Leader, Institute of Molecular Biology (IMB), Mainz
2004 - 2011 Postdoc, University of Geneva

EDUCATION

2004 PhD in Molecular Biology, Humboldt University, Berlin
1998 Diploma in Molecular Biology, Novosibirsk State University

GROUP MEMBERS

Margarita Dzama PhD Student; 09/2014 - 10/2016
Juri Kazakevych PhD Student; 09/2012 - 08/2016
Jakub Klassek Technician; since 08/2016
Berith Messner Technician; 03/2015 - 12/2016
Lira Nigmatullina PhD Student; since 04/2013
Maxim Norkin PhD Student; since 08/2015

OVERVIEW

Developmental and stem cell biology are two tightly linked disciplines. Stem cells are found in adult tissues, yet their progenitors are specified during embryogenesis. Gene expression and lineage tracing studies revealed *Lgr5* as a marker of the adult intestinal stem cells (ISCs) and defined their position at the bottom of the crypts. Despite tremendous progress in the adult ISC field, very little is known about the specification of Lgr5$^+$ cells during embryogenesis. We are elucidating the mechanisms of ISC specification during mouse development by addressing the following questions: When are embryonic Lgr5$^+$ progenitors specified? How abundant is this population? What triggers the specification of Lgr5$^+$ progenitors during embryogenesis? How does patterning of the small intestine during embryogenesis influence Lgr5$^+$ cell formation? Do Lgr5$^+$ cells influence patterning of the embryonic intestine? To answer these questions, we apply tools of mouse genetics, cellular (*ex vivo* 3D organoids) and molecular biology, genomics, proteomics and computational biology.

RESEARCH HIGHLIGHTS

We elucidated the mechanisms regulating transcriptional programmes accompanying important processes during the life cycle of ISCs, such as specification, maintenance and differentiation. For this purpose, we have generated transcriptome profiles, as well as chromatin state maps, from various embryonic and adult intestinal epithelial cell populations. We have studied the distribution of methylated DNA (5meC), methylated histones H3K4 and H3K27, acetylated H3K27, and histone variant H2A.Z at different developmental stages (embryonic versus adult) and in different cell types (stem cells versus differentiated absorptive and secretory cells). As a result, we defined changes in transcriptional programs during specification, maintenance and differentiation of the adult ISCs. By correlating the changes in transcriptome with changes in chromatin marks, we were able to define functions for each DNA or histone modification during ISC specification and differentiation. Our data demonstrate that dramatic redistribution of active marks takes place during the transition between embryonic epithelium and

the adult ISCs, illustrating strong differences between these cells at the chromatin level. Furthermore, we have assessed the dynamics of chromatin marks at potential distal and intragenic regulatory elements and correlated them with the transcriptional activity of the neighbouring genes. Our analysis revealed that functionally distinct classes of regulatory elements could be defined based on the presence of different chromatin modifications.

Based on chromatin profiles we found that embryonic epithelium is heterogeneous. Using lineage tracing analyses and intestinal organoid cultures, we characterised, at the molecular and phenotypic levels, distinct populations of embryonic intestinal epithelial progenitors that contribute to the adult ISC pool. We have identified embryonic precursors of the adult ISCs and defined the time of their specification (Figure 1). Moreover, we have characterised their molecular signature compared to the rest of the embryonic epithelium. Based on these transcriptome data, we have identified a factor responsible for the correct specification of the embryonic progenitors. Using both mouse genetics and *in vitro* biochemical assays, we have elucidated the mechanisms of embryonic progenitor specification on the molecular level.

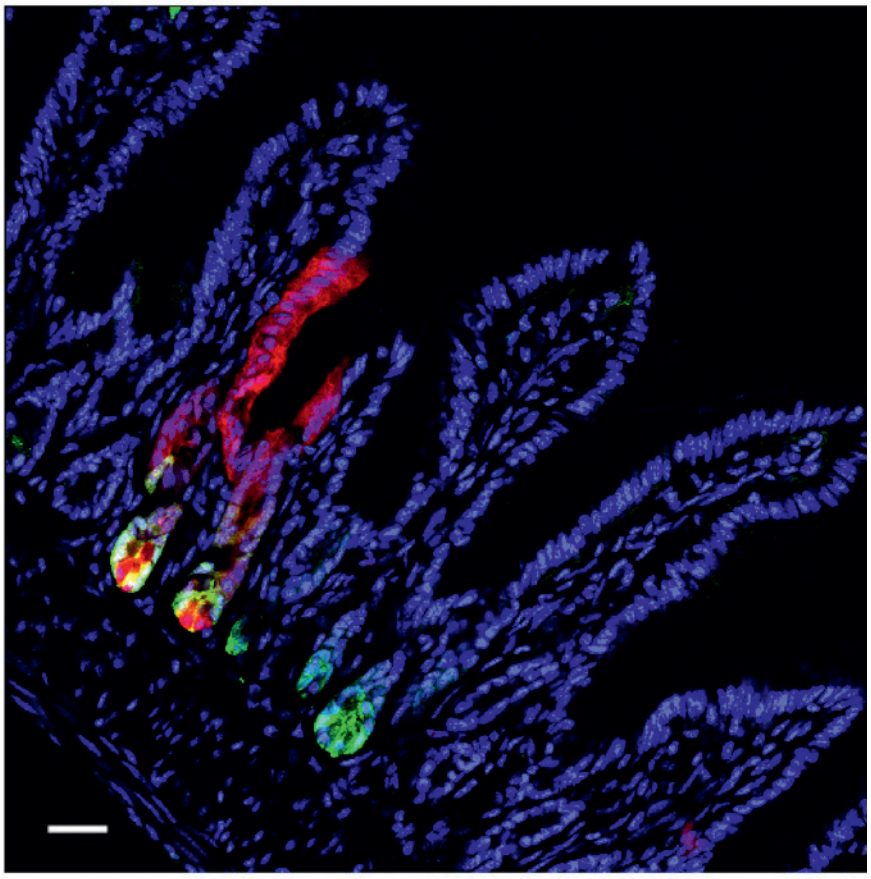

Figure 1. A section from adult small intestine of tamoxifen-treated *Lgr5*$^{EGFP\text{-}Cre\text{-}ERT}$:*Rosa26*^{tdTomato} mice showing distribution of Lgr5-EGFP^{+} ISCs (green) and their differentiated progenies expressing tdTomato (red) 2 weeks after treatment. Nuclei stained with DAPI are shown in blue. Scale bar: 100 µm.

FUTURE DIRECTIONS

We are continuing our studies on cellular and molecular mechanisms of intestinal patterning and stem cell specification during embryogenesis. To identify the factors that are responsible for the specification of ISCs, and elucidate their mechanisms of action, we are performing phenotypic screens using organoid cultures. For this purpose, we either overexpress candidate genes or ablate them using the CRISPR/CAS9 technique.

SELECTED PUBLICATIONS

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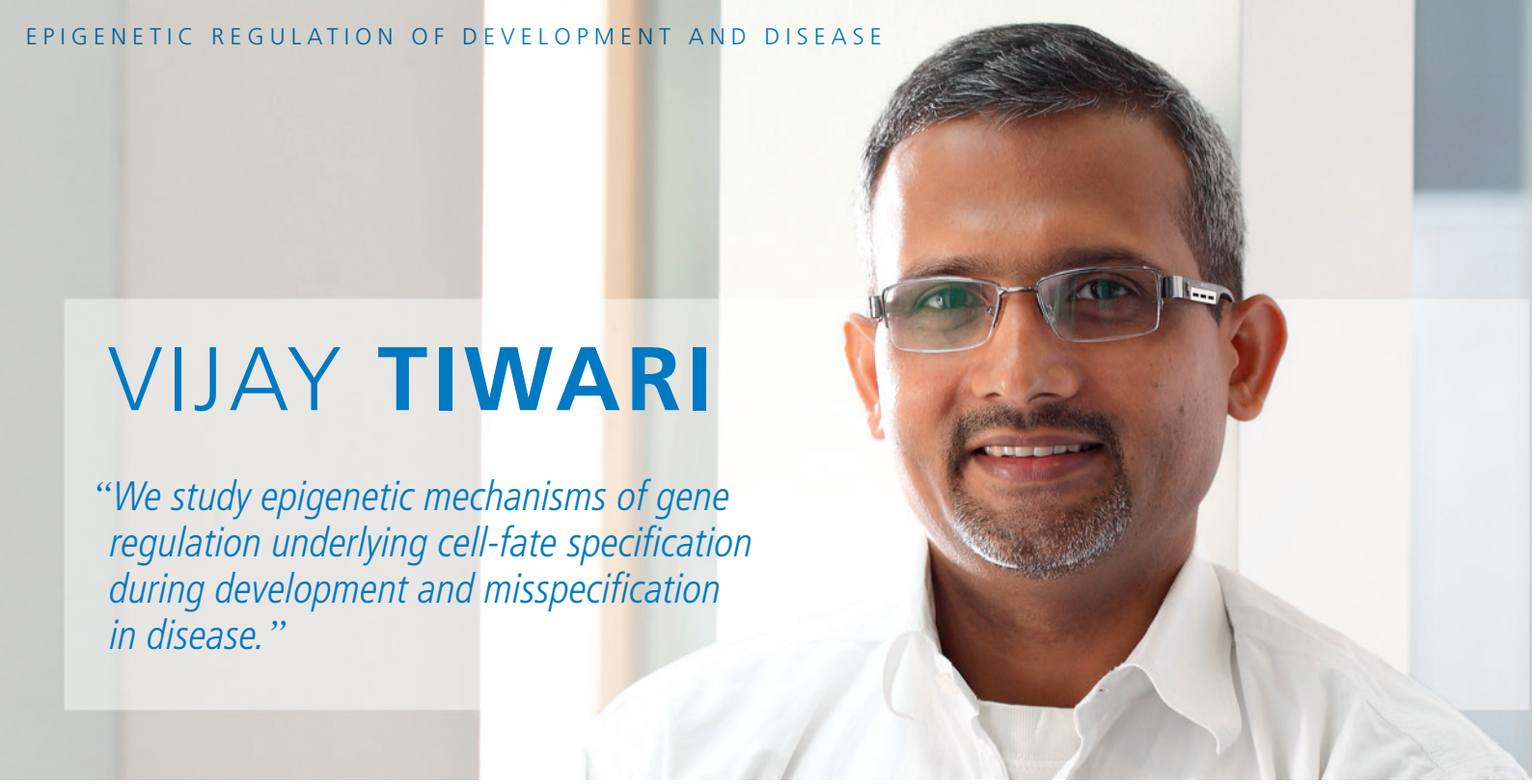
Soshnikova N, Montavon T, Leleu M, Galjart N and Duboule D (2010). Functional analysis of CTCF during mammalian limb development. *Dev Cell*, 19, 819-830



VIJAY **TIWARI**

"We study epigenetic mechanisms of gene regulation underlying cell-fate specification during development and misspecification in disease."

POSITIONS HELD

Since 2012 Group Leader, Institute of Molecular Biology (IMB), Mainz
2008 - 2011 Postdoc, Friedrich Miescher Institute for Biomedical Research (FMI), Basel
2006 - 2008 Postdoc, Johns Hopkins University School of Medicine, Baltimore

EDUCATION

2016 Habilitation in Molecular Biology, Mainz University
2006 PhD in Developmental Biology, Uppsala University
2002 MSc in Molecular and Human Genetics, Banaras University, Varanasi

GROUP MEMBERS

Amitava Basu Postdoc; since 05/2016
Tamer Butto PhD Student; since 09/2016
Angela Garding Postdoc; since 04/2012
Hyobin Jeong Postdoc; since 02/2016
Johannes Jung PhD Student; since 05/2013
Markus Muckenhuber PhD Student; 01/2015 - 09/2016
Mohit Navander PhD Student; since 10/2016
Attila Nemeth Staff Scientist; since 09/2016
Abhijeet Pataskar PhD Student; since 11/2014
Sanjeeb Kumar Sahu PhD Student; since 05/2013
Anke Salzer Lab Manager; since 07/2012
Mariangela Spagnuolo PhD Student; since 10/2016

OVERVIEW

Cell-fate specification during mammalian development involves extensive remodelling of gene expression programmes. The role of epigenetic mechanisms and transcription factors in this process has been increasingly appreciated. The research in our lab is aimed at understanding the mechanisms by which epigenetic regulators and transcription factors contribute to the transcriptional reprogramming that defines cell fate during development and how this communication is altered in diseases such as cancer. We employ a multidisciplinary approach combining cutting-edge epigenetics and genomics, together with computational biology tools in sophisticated and defined models of cellular differentiation and carcinogenesis. Our primary research interests include:

- Signalling to chromatin crosstalk in gene regulation
- Transcription factors and lineage specification
- Epigenomics of cell-fate specification
- Epigenetic regulation of neurogenesis
- Chromatin and cancer
- Systems biology of gene regulatory networks

RESEARCH HIGHLIGHTS

Epigenetic regulation of neuronal development and function

The nervous system is the most complex organ in all mammalian organisms. The last decade has seen extensive research into how this complexity is generated during neuronal development. Despite exciting progress, very little is known about the function of epigenetic mechanisms in neurogenesis and neuronal activity. Using epigenetics, genomics and molecular biology tools in combination with extensive computational biology approaches, we have been investigating the crosstalk of transcription factors with chromatin during specification of neuronal fate and the dynamics of epigenetic state during neurogenesis and neuronal activity. We recently discovered the gene regulatory programme through which a critical proneural transcription factor, NeuroD1, specifies neuronal fate. We further revealed how this function

involves reprogramming the transcription factor and chromatin landscapes at its target sites (Figure 1). Our data further show that a transient activity of NeuroD1 during development is sufficient to induce a permanent neuronal gene expression programme that is stably maintained by epigenetic memory. In another study, we investigated the dynamics of the distal regulatory landscape during neurogenesis. Here we uncovered novel regulatory elements that function in concert with epigenetic mechanisms and transcription factors to generate the transcriptome-dynamics underlying neuronal development and activity.

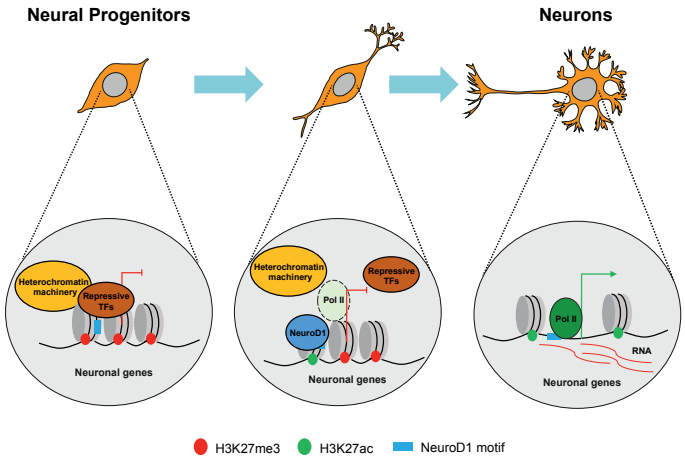


Figure 1. NeuroD1 reprogrammes chromatin and transcription factor landscapes to induce the neuronal programme. Upon onset of neurogenesis, NeuroD1 directly binds regulatory elements of neuronal genes that are developmentally silenced by epigenetic mechanisms. This targeting is sufficient to initiate events that confer transcriptional competence, including reprogramming of transcription factor landscape, conversion of heterochromatin to euchromatin and increased chromatin accessibility, indicating potential pioneer factor ability of NeuroD1. The transcriptional induction of neuronal fate genes is maintained via epigenetic memory despite a transient NeuroD1 induction during neurogenesis. From Pataskar *et al.*, 2016.

Gene regulatory mechanisms underlying epithelial to mesenchymal transition (EMT)

The epithelial to mesenchymal transition (EMT) is a process by which epithelial cells lose their cell polarity and cell-cell adhesion and gain migratory and invasive properties to become mesenchymal cells. EMT plays a crucial role in generating the body plan by contributing to the morphogenesis of multiple tissues and organs during embryonic development. It further contributes to wound healing and tissue regeneration in adults. However, its aberrant activation is known to cause organ fibrosis and promote carcinoma progression. We find that the induction and progression of EMT involves massive transcriptional and epigenetic reprogramming. Interestingly, a number of transcription factors and epigenetic regulators are also upregulated during EMT. We also find that while the Smad pathway is required for initiation of EMT, the JNK pathway is required for the progression of phenotypic changes associated with EMT. Such dependency is the result of JNK-driven transcriptional reprogramming of critical EMT genes and changes in their chromatin state. Furthermore, we identified eight novel JNK-induced transcription factors that were required for proper EMT. Three of these factors were also highly expressed in invasive cancer cells, where they function in gene regulation to maintain mesenchymal identity. These factors were also induced during neuronal development and function in neuronal migration *in vivo*. These comprehensive findings uncovered kinetically distinct roles for various signalling pathways in defining the transcriptome that underlies mesenchymal identity and revealed novel transcription factors that mediate these responses during development and disease.

FUTURE DIRECTIONS

Using extensive computational biology tools for prediction, in combination with analysis of multiple tissues from all three lineages during embryonic development, we have identified new candidate epigenetic regulators of neurogenesis. Preliminary analysis suggests that a set of these factors are critically required for neuronal development. By employing a multidisciplinary approach, we next aim to generate a mechanistic insight into how these potential novel epigenetic regulators function in the gene regulation programme underlying neurogenesis. Furthermore, our recent findings have also uncovered a role for distinct signalling pathways in defining the transcriptome that specifies mesenchymal fate and revealed epigenetic mechanisms and a new repertoire of transcription factors that mediate these responses. We next plan on gaining a molecular understanding of the crosstalk between transcription factors and epigenetic machinery in organising the gene expression programme that drives cell-fate changes during EMT.

SELECTED PUBLICATIONS

Pataskar A*, Jung J*, Smialowski P, Noack F, Calegari F, Straub T and Tiwari VK (2016). NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program. *EMBO J*, 35, 24-45

Thakurela S*, Sahu SK*, Garding A and Tiwari VK (2015). Dynamics and function of distal regulatory elements during neurogenesis and neuroplasticity. *Genome Res*, 25, 1309-1324

Sahu SK, Garding A, Tiwari N, Thakurela S, Toedling J, Gebhard S, Ortega F, Schmarowski N, Berninger B, Nitsch R, Schmidt M and Tiwari VK (2015). JNK-dependent gene regulatory circuitry governs mesenchymal fate. *EMBO J*, 34, 2162-2181

*indicates joint contribution

HELLE ULRICH

"Checkpoint signalling is essential for postreplicative processing of DNA damage."

POSITIONS HELD

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

EDUCATION

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

GROUP MEMBERS

Liliana Batista Postdoc; since 02/2013
Sonja Braun Research Assistant; since 02/2016
Karolin Eifler-Olivi Postdoc; since 10/2015
Stephanie Freiß PhD Student; since 11/2014
Néstor García-Rodríguez Postdoc; since 05/2013
Nataliia Gralievska PhD Student; since 01/2014
Martin Möckel Postdoc; since 11/2016
Christian Renz Postdoc; since 08/2014
Annie Sriramachandran Postdoc; since 03/2015
Laura Tomini Technician; since 01/2015
Sabrina Wegmann PhD Student; since 11/2013
Hanna Windecker Postdoc; since 05/2013
Hans-Peter Wollscheid Postdoc; since 09/2014
Ronald Wong Postdoc; since 04/2013
George Yakoub PhD Student; since 01/2015
Nicola Zilio Postdoc; since 08/2014

OVERVIEW

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

RESEARCH HIGHLIGHTS

DNA damage is perceived by so-called checkpoint pathways, which react to a variety of insults and facilitate an appropriate defensive response. Locally, i.e. at the site of damage, they initiate the first steps towards DNA repair, but they also modulate the overall state of the cell by affecting replication pattern, cell cycle progression and transcriptional activity. In human cells, strand breaks and other replication-independent DNA damage activates the ATM kinase, while replication fork problems are largely perceived by the related ATR kinase. In budding yeast, the dominant sensor kinase is Mec1, which mediates damage-dependent signalling via the mediator protein Rad9 and monitors replication stress via the replisome component Mrc1. Both signals converge in the activation of the effector kinase Rad53. Coordination of damage bypass with the cellular checkpoint response appears to be essential for genome maintenance, as simultaneous defects in both

systems result in an extreme sensitivity towards genotoxic agents. However, the basis of this synergism is not well understood.

Although ATR is the predominant kinase mediating the response to replication stress in human cells, a second signalling pathway has been described involving ATM in conjunction with a cofactor, ATMIN. In collaboration with the group of Axel Behrens (The Francis Crick Institute, London) we found that PCNA ubiquitylation actually contributes to the activation of this ATM-dependent pathway via the DNA-dependent ATPase WRNIP1, which serves as a bridging factor between ATMIN and ubiquitylated PCNA. These observations are consistent with our previous observations that the yeast homolog of WRNIP1, Mgs1, preferentially binds to the ubiquitylated forms of PCNA. Such an activation mechanism for ATM signalling may in part explain the synergy between defects in PCNA ubiquitylation and the ATR pathway.

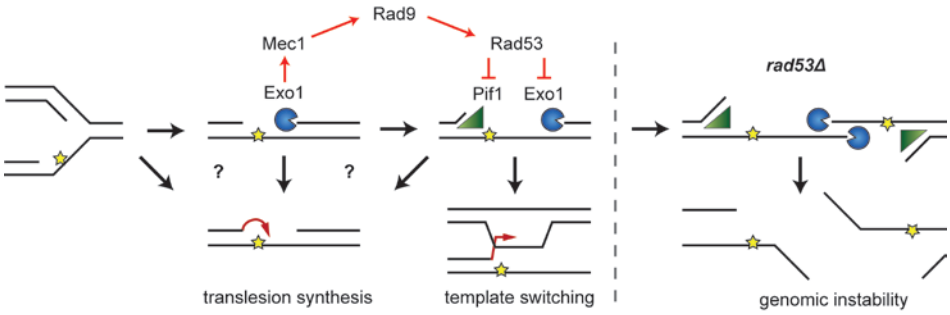


Figure 1. Model for the role of checkpoint signaling in DNA damage bypass. Exonucleolytic activity of Exo1 mediates expansion of daughter-strand gaps left behind replication forks under conditions of DNA damage. This allows robust damage checkpoint activation, which in turn leads to Exo1 and Pif1 inhibition by Rad53-mediated phosphorylation. Damage bypass by TS might require gap expansion, whereas TLS is likely to proceed independently of gap size or even at stalled forks. Unrestrained Exo1 and Pif1 activity in the absence of checkpoint signaling leads to genome instability and irreversible loss of bypass competence.

In budding yeast, we had previously found that damage bypass can be separated in time from bulk genome replication without adverse effects: in the absence of the PCNA-specific ubiquitin ligase Rad18, daughter-strand gaps accumulate during replication and can be filled postreplicatively by ubiquitin-dependent damage bypass upon induction of Rad18 in the G2/M phase. Combining such an inducible damage bypass assay with an auxin-inducible degron system for the reversible ablation of individual proteins, we now found that DNA damage signalling is absolutely required to maintain viability and damage bypass competence during S phase. Consistent with a fork-independent action of the pathway, a functional Rad9- and Rad53-dependent damage checkpoint, but not the Mrc1-dependent replication checkpoint, was found to be essential. Among the many possible downstream factors controlled by Rad53, we identified an exonuclease, Exo1, as being mainly responsible for the effect. While Rad53-dependent phosphorylation normally limits Exo1 activity in DNA damage conditions, excessive resection of postreplicative daughter-strand gaps upon checkpoint inactivation leads to irreversible chromosome fragmentation and catastrophic recombination (Figure 1). Our results thus indicate a possible mechanism by which checkpoint signalling not only contributes to the stabilisation of stalled replication forks, but also prevents inappropriate processing of postreplicative gaps associated with damage bypass.

FUTURE DIRECTIONS

Having demonstrated the importance of checkpoint signalling for the stabilisation of single-stranded DNA that accumulates when damage bypass is intentionally delayed, it will now be essential to obtain a good understanding of how DNA replication and damage processing are temporally coordinated in a physiological setting. To this end, our efforts are currently aimed at establishing assays for the real-time analysis of DNA damage bypass in live cells. A generally applicable strategy involves monitoring a GFP-labelled single-strand binding protein, RPA, in synchronised cultures after exposure to damaging agents. We have observed that RPA forms obvious foci distinct from the damage-independent replication pattern that culminate in G2/M and are resolved in a Rad18-dependent manner. Hence, they likely represent the substrates of damage bypass, and analysis of their formation and resolution by live-cell imaging is expected to give insight into the dynamics of the pathway in the course of a cell cycle.

SELECTED PUBLICATIONS

Kanu N, Zhang T, Burrell RA, Chakraborty A, Cronshaw J, DaCosta C, Grönroos E, Pemberton HN, Anderton E, Gonzalez L, Sabbioneda S, Ulrich HD, Swanton C and Behrens A (2016). RAD18, WRNIP1 and ATMIN promote ATM signalling in response to replication stress. *Oncogene*, 35, 4009-4019

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

Saugar I, Parker JL, Zhao S and Ulrich HD (2012). The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Res*, 40, 245-257

EVA WOLF

"We study molecular links between circadian clocks and genome maintenance."

POSITIONS HELD

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

2012 - 2013 Group Leader, Ludwig Maximilan University, Munich

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

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

1996 - 2000 Postdoc, Rockefeller University, New York

EDUCATION

2007 Habilitation in Biochemistry, Ruhr University, Bochum

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

1991 Diploma in Biology, University of Heidelberg

GROUP MEMBERS

Arne Börgel PhD Student; since 01/2016
Archit Garg PhD Student; since 11/2014
Tim Grimmelsmann PhD Student; since 01/2015
Silke Helmke Technician; since 05/2014
Shruti Krishnan PhD student; since 10/2016
Torsten Merbitz-Zahradnik Postdoc; since 01/2014
Roberto Orru Postdoc; since 10/2015

OVERVIEW

Many essential cellular, physiological and behavioral processes are regulated in a daily (circadian) manner. Molecular connections between circadian clocks, cell cycle regulation and the control of genome stability have been described and are becoming a focus of attention, partly due to their potential significance for chronotherapy in cancer. The mammalian circadian clock is operated by a gene regulatory feedback loop with the transcription factors BMAL1/CLOCK as activators and the clock proteins PERIOD1/2 (PER1/2) and Cryptochrome 1/2 (CRY1/2) as repressors of BMAL1/CLOCK. The mammalian TIMELESS (TIM) protein plays a less well defined role in the mammalian circadian clock and has additional functions in DNA replication, checkpoint signalling and DNA repair. Hence, TIM is thought to link the circadian clock with genome maintenance pathways, potentially via interactions with the clock protein CRY1. To further our mechanistic understanding of the molecular connections between the circadian oscillator and genome maintenance, we pursue biochemical, biophysical and high resolution 3D structural studies of the mammalian TIM protein and its complexes with replication fork components and clock proteins.

RESEARCH HIGHLIGHTS

Mammalian TIMELESS as a linker between circadian clocks and genome maintenance

In contrast to the *Drosophila* TIMELESS protein, which constitutes a central component of the fruit fly's circadian oscillator, the mammalian TIMELESS (TIM) protein plays a less well defined role in the circadian clock. However, knockout of mammalian TIM is embryonically lethal, presumably due to its additional roles in genome maintenance including for example the coordination of DNA unwinding and DNA synthesis during DNA replication, ATR-Chk1 dependent checkpoint signalling and DNA repair. Mammalian TIM forms a tight complex with the Timeless interacting protein Tipin. TIM or the TIM-Tipin complex interact with diverse proteins in the replication fork, including helicase and polymerase subunits, checkpoint proteins and Replication protein A (RPA). TIM also interacts with the central clock protein

Cryptochrome 1 (CRY1) and thereby potentially influences the mammalian circadian clock by affecting CRY1's activity as a repressor of the BMAL1/CLOCK transcription factor complex (Figure 1). CRY1 on the other hand is reported to modulate ATR-mediated DNA damage checkpoint responses in a daily manner by interacting with TIM. Hence TIM and CRY1 are able to link the circadian clock and genome maintenance mechanistically.

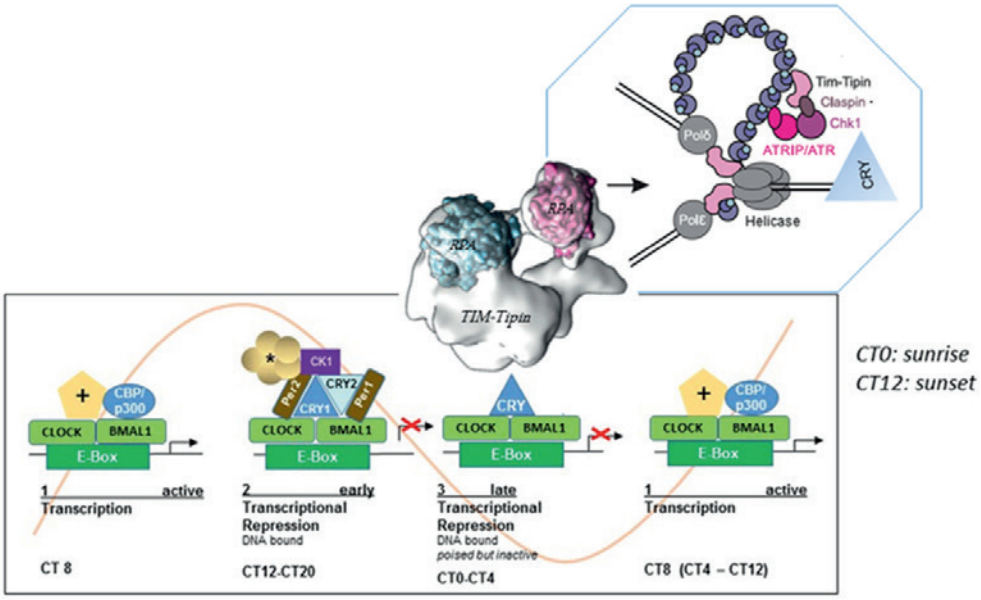


Figure 1. *Black box (lower)*: Transcriptional regulation of the circadian clock in mouse liver. "*****" corepressor complex. "**+**" coactivators of Bmal1/Clock. *Blue box (upper)*: TIM-Tipin (pink) in DNA replication and checkpoint signalling. *Centre:* Cryo-EM structure of the TIM-Tipin-RPA complex with docked RPA crystal structures. TIM/TIM-Tipin interacts with CRY1 (clock) or with RPA, helicase, polymerase and claspin (replication fork). (Adapted from Merbitz-Zahradnik and Wolf, *FEBS Letters*, 2015 and Witosch *et al*., *Nuc Acids Res*, 2014)

To date, very limited structural data are available of the mammalian TIMELESS protein and its biologically important complexes. To fill this gap and to provide mechanistic insights into the diverse roles of mammalian TIM and Tipin in genome maintenance and circadian clock regulation, we are pursuing the determination of high resolution 3D structures of TIM and TIM-Tipin complexes using X-ray crystallography and advanced single particle cryo-EM (electron microscopy) techniques. So far, we have determined a medium resolution cryo-EM structure of mouse TIM in complex with Tipin and RPA (Figure 1, centre) and biochemically analysed the stability and ssDNA binding of TIM-Tipin-RPA and TIM-Tipin complexes. The results of this work suggested that RPA conformational changes may regulate the recruitment of TIM-Tipin to the replication fork. In the past year, we have purified a number of TIM containing complexes for high resolution 3D structural analyses. We also biophysically analyse TIM protein interactions to determine their affinities and to establish binding site competition between different ligands that mediate different TIM functions. These studies will substantiate the emerging picture of mammalian TIMELESS as a multitasking protein that fulfils its diverse functions in the circadian clock or in cell cycle regulation dependent on distinct molecular interactions, day-time and cellular localisation.

FUTURE DIRECTIONS

A deeper mechanistic understanding of mammalian TIMELESS as a component of circadian clock, DNA replication, DNA repair or checkpoint signalling complexes will help us understand the interconnection between the circadian clock, the cell cycle and genome maintenance at a molecular level. Our high resolution 3D structures of TIM complexes will inspire new directions for *in vivo* studies. These studies will allow us to dissect at which time of day and cellular locations TIM interacts with its diverse binding partners to control the circadian clock on one hand and the cell cycle on the other. Understanding this networked interconnection of essential cellular processes in molecular and atomic detail will also facilitate the development of cancer chronotherapy, a novel and promising approach to enhance therapeutic efficacy while minimising toxicity of cancer drugs by exploiting the results of modern molecular chronobiology research.

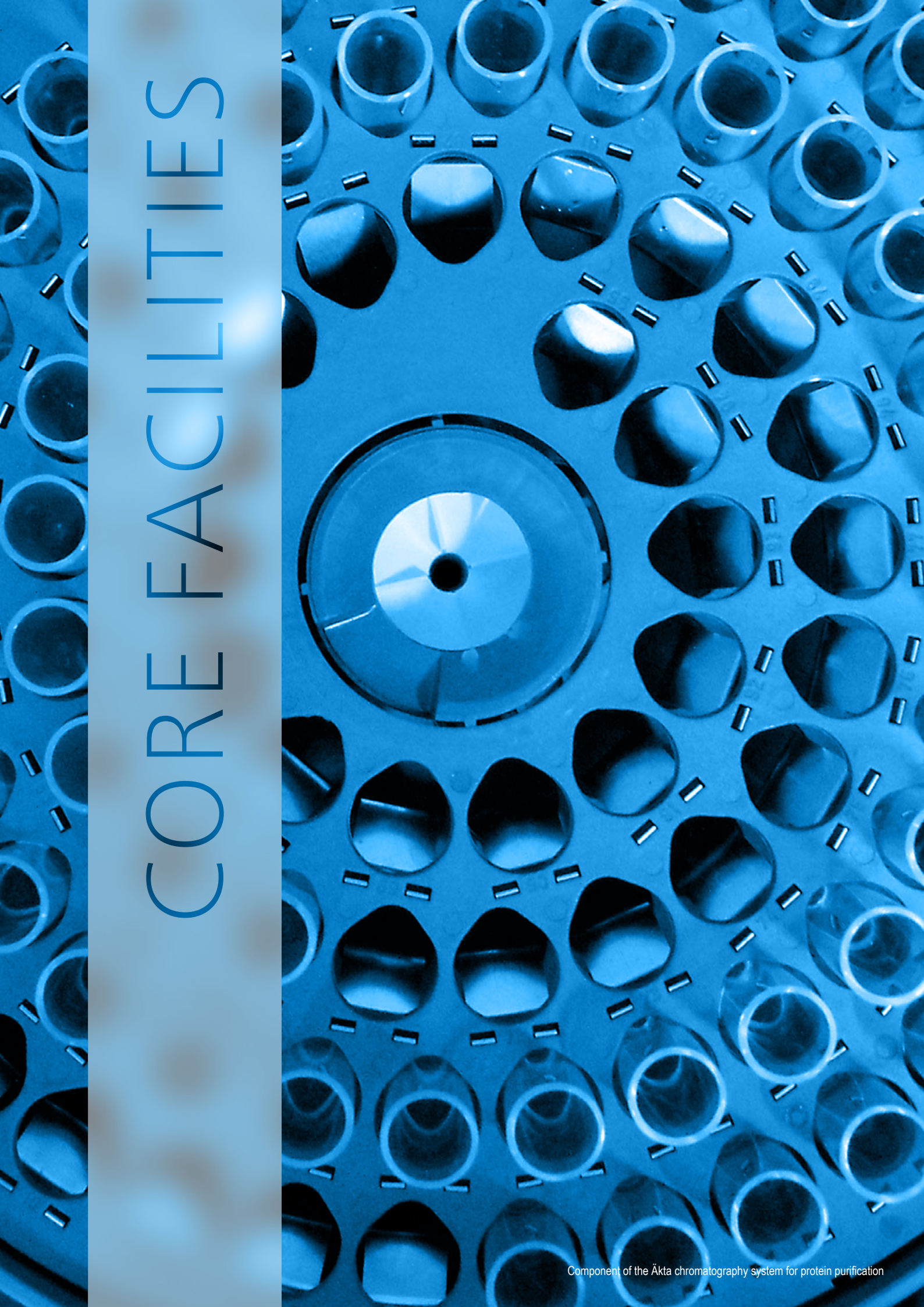
SELECTED PUBLICATIONS

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

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

Czarna A, Berndt A, Singh HR, Grudziecki A, Ladurner A, Timinszky G, Kramer A and Wolf E (2013). Structures of *Drosophila* Cryptochrome and mouse Cryptochrome1 provide insight into circadian function. *Cell*, 153, 1394-405

CORE FACILITIES



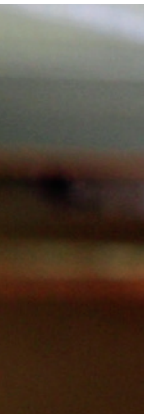
Component of the Äkta chromatography system for protein purification

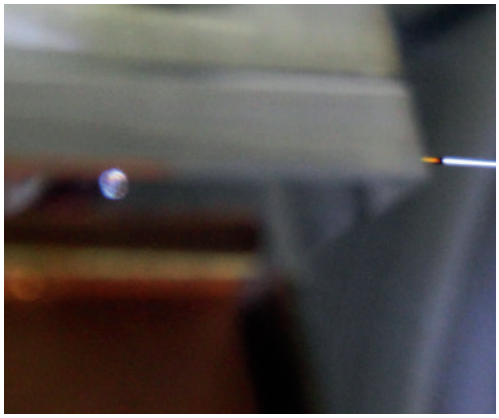
OVERVIEW
CORE FACILITIES

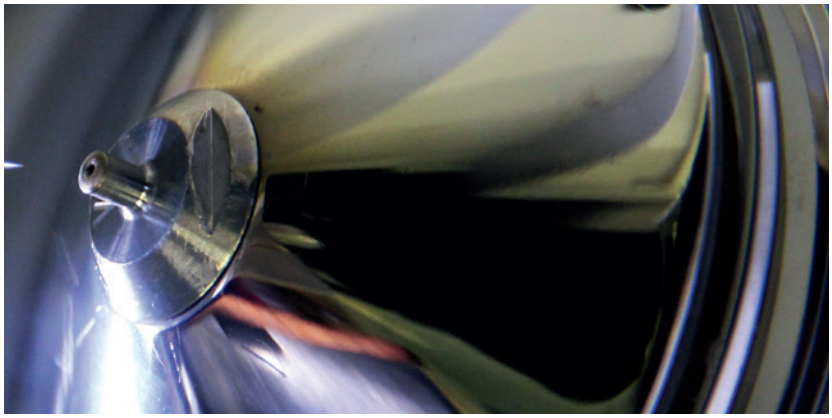
The Core Facilities (CFs) at IMB provide access to state-of-the-art technology, and offer services and training from expert staff. In addition, the CFs organise lectures and courses to tutor researchers in new techniques and instrumentation, as well as experimental design and data processing. There are currently seven Core Facilities at IMB: Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics, a Media Lab, and the new Protein Production Core Facility, which was established together with Mainz University this year. The Bioinformatics, Genomics and Proteomics CFs provide users with a "full service", from quality control of samples to data production and analysis. The Flow Cytometry and Microscopy/Histology CFs provide an "assisted service", where researchers work independently on CF equipment after introductory training by CF staff. Whether receiving full or assisted service, the CFs' staff are available for consultation and troubleshooting. Furthermore, CF staff often collaborate with researchers to provide customised or specialised services. The CFs are open to all IMB researchers, and the Flow Cytometry, Microscopy/Histology and Proteomics CFs also offer services to external users at Mainz University and further afield.

The services provided are based on user demand. For each CF, a user committee gives feedback on the equipment and user experience and helps to define the services that a CF provides. In addition to technical services, the CFs offer lectures on a variety of methods, as well as practical courses on data acquisition and analysis. These allow researchers to keep up-to-date with and broaden their knowledge of current and emerging technologies. Lectures are generally open to everyone, including the wider scientific community in Mainz. IMB's CFs are also responsible for maintaining and providing training on core equipment that is available at IMB, as well as the radioactivity lab, the S2 lab, and IMB's in-house animal facilities (mouse, zebrafish, *Xenopus* and *Drosophila*).

Andreas Vonderheit
Director of Core Facilities and Technology







BIOINFORMATICS CORE FACILITY

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

SERVICES OFFERED

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

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

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

CORE FACILITY MEMBERS

Emil Karaulanov Head
since 10/2014
Jan Bockelmann System Administrator
since 08/2015
Anke Busch Bioinformatician
since 01/2014
Oliver Drechsel Bioinformatician
04/2015 - 11/2016
Nastasja Kreim Bioinformatician
since 04/2012
Sergi Sayols Puig Bioinformatician
since 10/2013
Pascal Silberhorn System Administrator
since 12/2015
Sri Dewi Student Assistant
since 10/2016

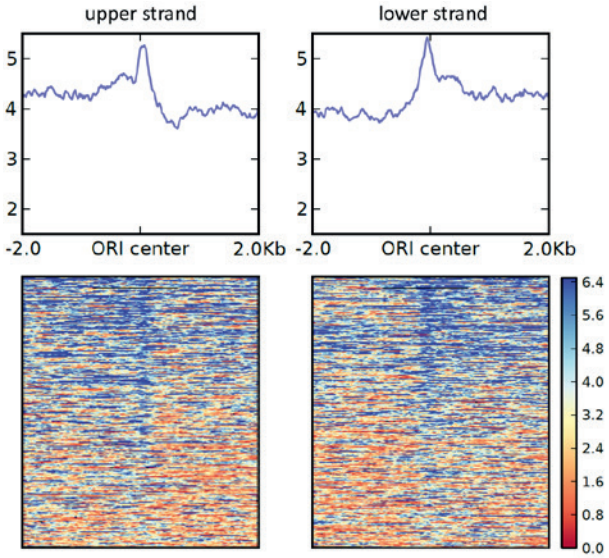


Figure 1. Read density profiles around yeast origins of replication (ORI) from a strand-specific IP-seq experiment.

FLOW CYTOMETRY
CORE FACILITY

The Flow Cytometry Core Facility (FCCF) offers high-throughput measurements, analysis and separation of biological units through three different systems: a large particle sorter, a cell sorter, and an analyser. With this equipment, the FCCF can analyse and sort particles of 0.5 µm to 1,500 µm in diameter under S1 conditions, covering nearly the whole target material spectrum.

CORE FACILITY MEMBERS

Stefanie Bürger Head
since 10/2016

Jens Hartwig Head
10/2013 - 07/2016

Ina Schäfer Biotechnologist
since 08/2011

SERVICES OFFERED

The FCCF offers a full service for sorting and assisted service for the analyser. Additionally, its staff collaborates in terms of analysing flow cytometrical data and sample preparation. During the past year, the FCCF has performed various types of experiments, including for example, multicolour measurements, single cell separation for sequencing RNA content, sorting of isolated neuronal nuclei, classical enrichments for subsequent cell culture or qPCR analysis, and cell sorting for microscopic investigations.

The FCCF works with different types of material: nuclei, stem cells, *C. elegans*, as well as various cultured cell lines and primary cells from humans, mice, zebrafish, and *Drosophila*. To educate and train users, the FCCF offers three different lectures per year, as well as an annual practical course.

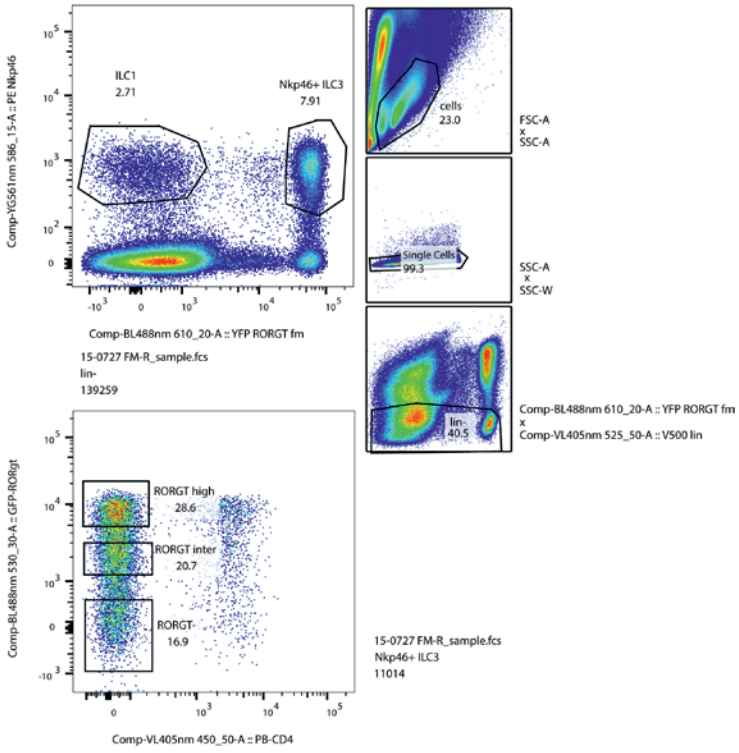


Figure 1. A 9-colour-panel for studying group 3 innate lymphoid cells (ILC3) based on two transgenic markers: GFP and YFP. ILC3s are developmentally dependent on the expression of the transcription factor RORγt. The establishment of a RORγt-YFP fatemap x RORγt-GFP reporter mouse enables isolation of ILC3 by FACS and interrogation of the functional consequences of the RORγt loss that is seen in some of the natural killer receptor (NKR) positive ILC3s. Data courtesy of Michael Kofoed-Nielsen, Mainz University Medical Center.

GENOMICS CORE FACILITY

The Genomics Core Facility (GCF) offers next-generation sequencing (NGS) services based on the Illumina HiSeq 2500 and MiSeq platforms.

CORE FACILITY MEMBERS

Maria Mendez-Lago Head
since 04/2016
Chung-Ting "Tina" Han Head
04/2013 - 01/2016
Hanna Lukas Technician
since 01/2013
Clara Werner Technician
since 07/2015

SERVICES OFFERED

The GCF provides a full service for NGS, starting at the project planning stages up to sequencing data generation.

After submission of RNA or DNA samples, the GCF performs an 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 15 different applications as standard services (Figure 1), and develops new protocols to accommodate user needs for their specific sample type. In 2016, this included the successful testing of new methods for low input strand-specific mRNA-seq and low input small RNA library prep, which are now on offer in the GCF. The GCF also offers training on genomics techniques, including on the principles of NGS and the application of NGS in translational epigenetics.

Input material	Application
RNA	Strand specific mRNA-Seq, with poly-A selection
	Strand specific total RNA-Seq, with rRNA depletion
	Low input strand-specific RNA-Seq
	Small RNA-Seq
	RIP-seq
	Bru-seq
	cDNA library preparation
DNA	ChIP-Seq
	MBD-Seq
	ATAC-seq
	Single-stranded DNA library preparation
	Whole genome sequencing
	Whole genome bisulfite sequencing
	5fC DIPseq
	Hi-C
	Capture-3C
User-prepared libraries	iCLIP-Seq
	Amplicon-seq (low diversity)

Figure 1. Services offered by the GCF. In order to accommodate user needs for their specific sample type, the GCF continuously develops new workflows for library preparation and sequencing. The GCF fully supports various types of library preparation as standard operating procedures (SOPs). For experimental applications, the GCF provides advice for user-made libraries so that the highest quality of sequencing output can be achieved.

MICROSCOPY & HISTOLOGY
CORE FACILITY

The Microscopy and Histology Core Facility (MHCF) offers supervised access to microscopy equipment, ranging from stereo macroscopes and widefield microscopes to confocal (Figure 1) and super-resolution microscopes.

CORE FACILITY MEMBERS

Sandra Ritz Head
since 01/2016
Katharina Böse Staff Scientist
01/2013 - 12/2016
Mária Hanulová Staff Scientist
since 02/2014
Jonas Schwirz Staff Scientist
since 06/2016

SERVICES OFFERED

The MHCF combines state-of-the art microscopes and histology instruments with a broad range of lectures and hands-on training to offer users independent, assisted, or full service. For histology purposes, the MHCF provides a variety of histology techniques, comprising semi-automated fixation and paraffin embedding, as well as microtomes for paraffin, cryo- or gelatine/agarose sectioning (Vibratome). Microscopy users can select from over nine different instruments and become trained to work independently on them, although MHCF staff are always available to assist with sample preparation, image acquisition and analysis, as well as processing and deconvolution. Super-resolution microscopy is offered on a full service or collaborative basis. User training for both microscopy and histology takes place throughout the year through practical courses and lectures, ranging in emphasis from confocal, live cell microscopy and super-resolution microscopy to image processing. In 2016, the MHCF also introduced a new lecture in histology "Basics in Histology" and a "Histology Crash Course" allowing for practical training in different sectioning and staining techniques.

In 2016, the MHCF extended its services by acquiring a new high-throughput screening microscope (Opera Phenix, Perkin Elmer). MHCF users can now use spinning-disk technology along with sophisticated analysis software and a database running on a 40TB-server to facilitate the handling and storage of data. In addition, the MHCF also upgraded some of its existing instrumentation by fitting out the widefield microscope AF7000 with a new LED light source and an sCMOS camera, improving the sensitivity as well as the image field of view.

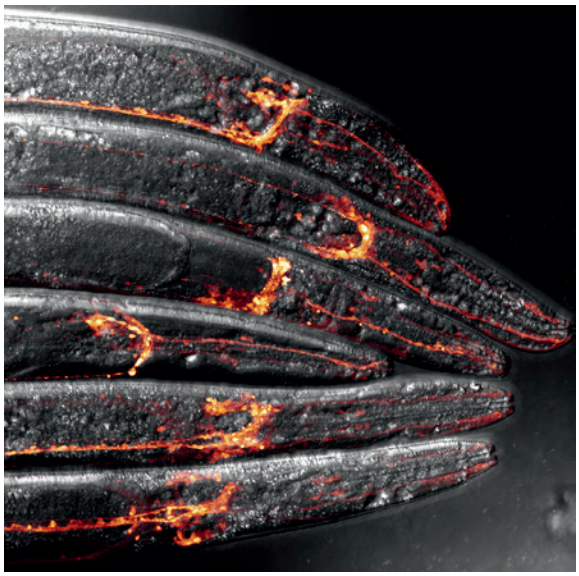


Figure 1. *C. elegans* heads imaged at the age of 20 days. Neurons are highlighted using a GFP reporter. Courtesy of Johannes Geisinger & Thomas Wilhelm (Richly group, IMB).

PROTEIN PRODUCTION
CORE FACILITY

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

CORE FACILITY MEMBERS

Markus Matthes Head
since 02/2016

SERVICES OFFERED

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

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

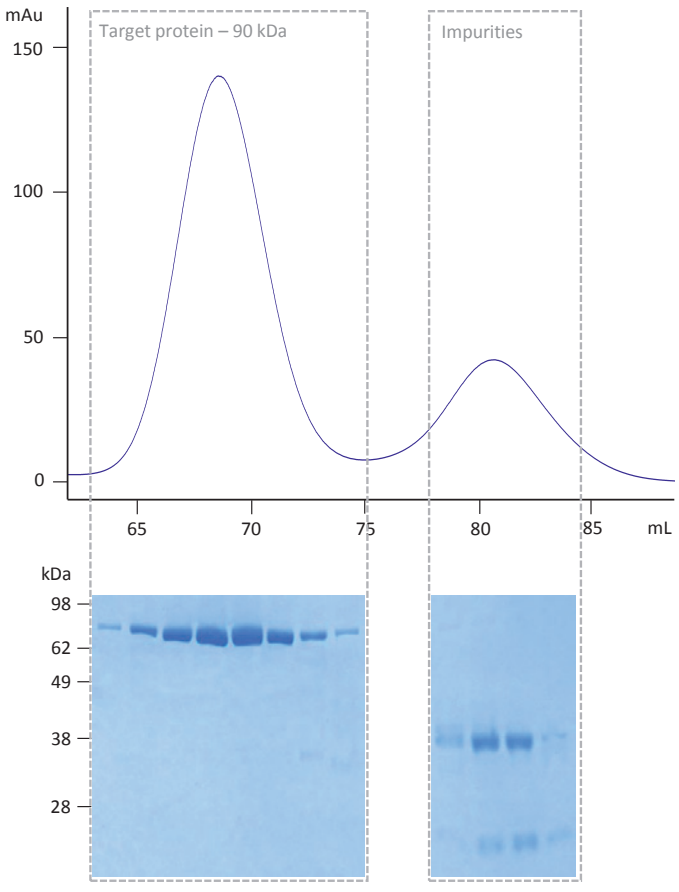


Figure 1. Purification of a 90kDa protein: Chromatogram (top) and SDS-PAGE gels (bottom) from the final step of the purification process of a 90 kDa protein (left hand side), in which the last impurities (right hand side) are removed. The SDS-PAGE gels show protein fractions corresponding to the chromatogram above.

PROTEOMICS
CORE FACILITY

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

SERVICES OFFERED

The PCF provides 1,500 measurement hours annually to IMB and the surrounding research centres in Mainz using a state-of-the-art mass spectrometry platform (Figure 1). Current services include band identification, analysis of posttranslational modifications on single proteins and measurement of SILAC (stable isotope labelling with amino acids in cell culture) experiments. Additionally, we offer reductive dimethylation for quantitative analysis. The mass spectrometry service is provided as a full service, including initial consultation, sample preparation and basic proteomics data analysis by the PCF. Advanced proteomic workflows, label-free quantitation measurements, in-depth statistical and bioinformatics analysis are available in a collaborative context. We offer lectures on proteomics and data analysis as well as to provide researchers with hands-on experience during our practical courses.

CORE FACILITY MEMBERS

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

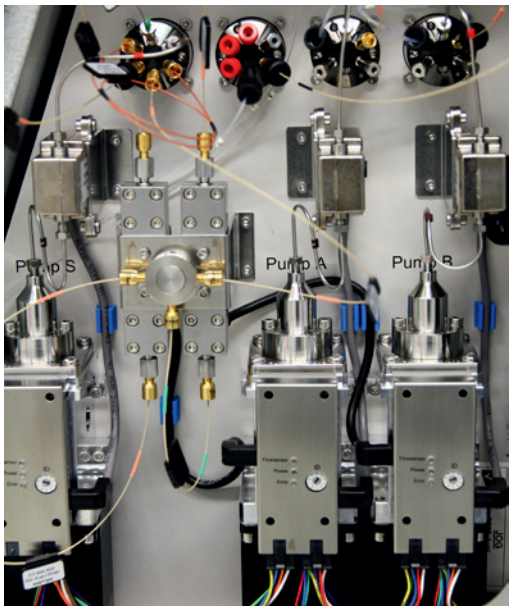


Figure 1. We use electrospray ionisation (ESI) to ionize peptides for mass spectrometry measurement. The peptides are separated on a chromatography column and sprayed directly into the mass spectrometer.

MEDIALAB
CORE FACILITY

The Media Lab primarily supports scientific groups and facilities by producing media, buffers, and agar plates. In addition, the Media Lab is responsible for the administration of two supply centres, plasmid/cell lines banks, general waste management and sterilisation of glassware.

CORE FACILITY MEMBERS

Andreas Vonderheit Head
since 05/2016
Ina Schäfer Head
03/2013 - 04/2016
Doris Beckhaus Media Lab Assistant
since 05/2011
Alwina Eirich Media Lab Assistant
since 07/2013
Andrea Haese-Corbit Operations Manager
since 05/2016
Pascal Hagebölling Media Lab Assistant
since 01/2015
Valentina Helmus Media Lab Assistant
04/2011 - 02/2016
Annette Holstein Media Lab Assistant
since 04/2012
Marion Kay Media Lab Assistant
since 04/2016
Johann Suss Media Lab Assistant
since 04/2011

SERVICES OFFERED

The Media Lab provides the following services:

- Supply of routinely-used buffers, solutions, liquid media and agar plates (Figure 1) for molecular biological research and for culturing bacterial, yeast, and insect cells, as well as *C. elegans*
- Management of a central supply for New England Biolabs and Thermo Fisher Scientific products
- Administration of a vector data bank, human ORF clone collection and cell line bank
- Overnight incubation and expansion of bacterial and cell cultures prior to plasmid purification
- Cleaning and sterilisation of glassware and lab equipment
- Autoclaving of S1/S2 waste
- Maintenance of the in-house transport system

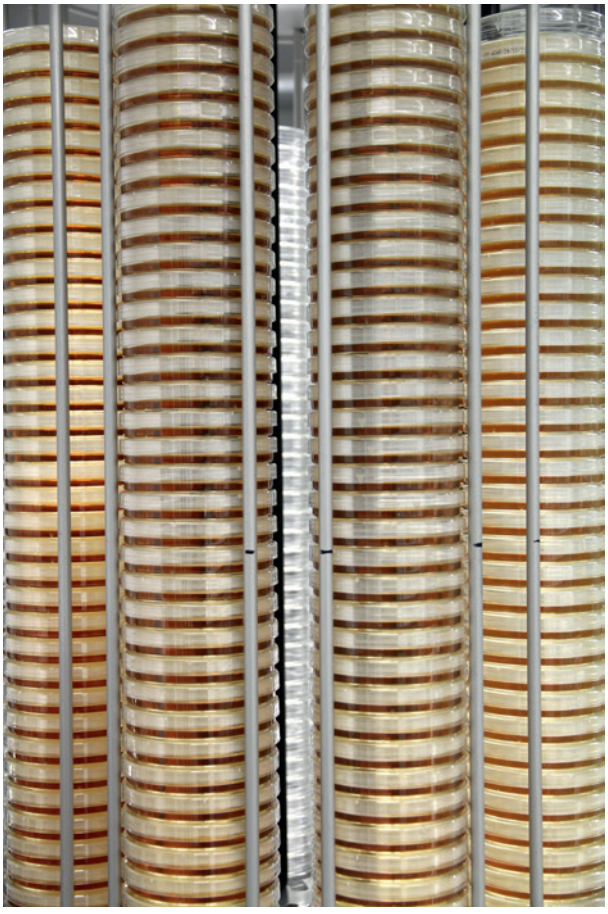
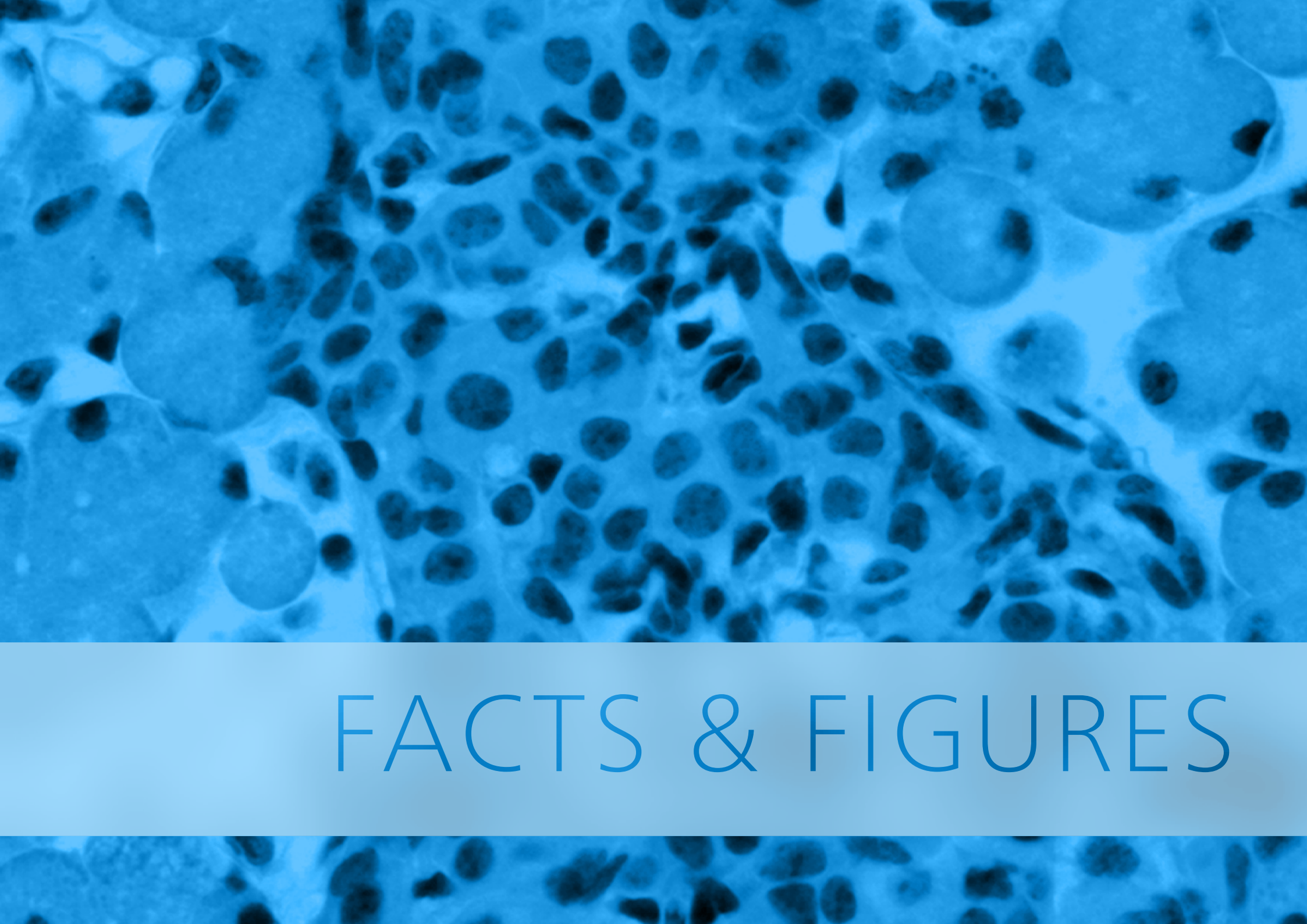
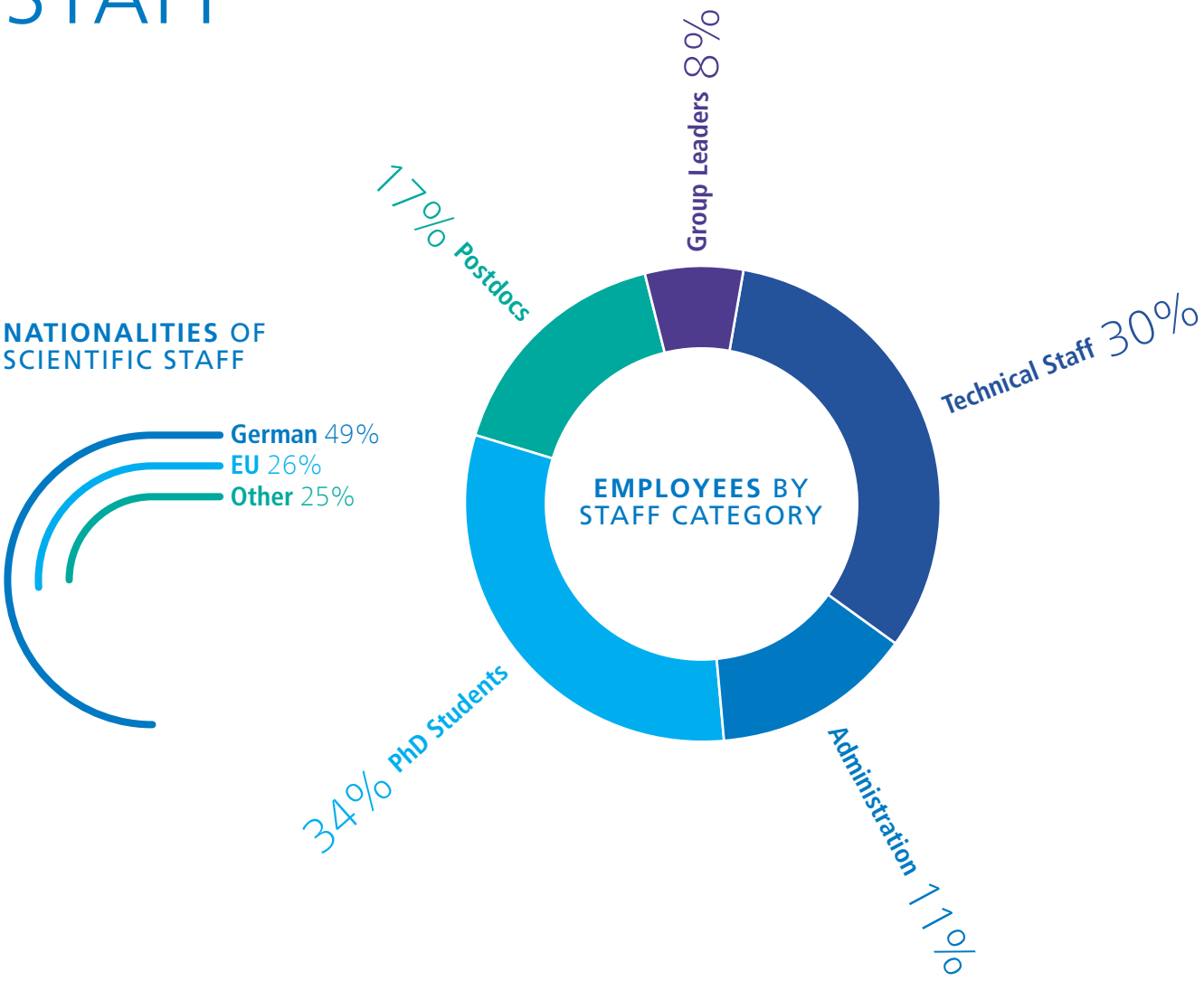


Figure 1. The media lab produces more than 1,700 plates per week.



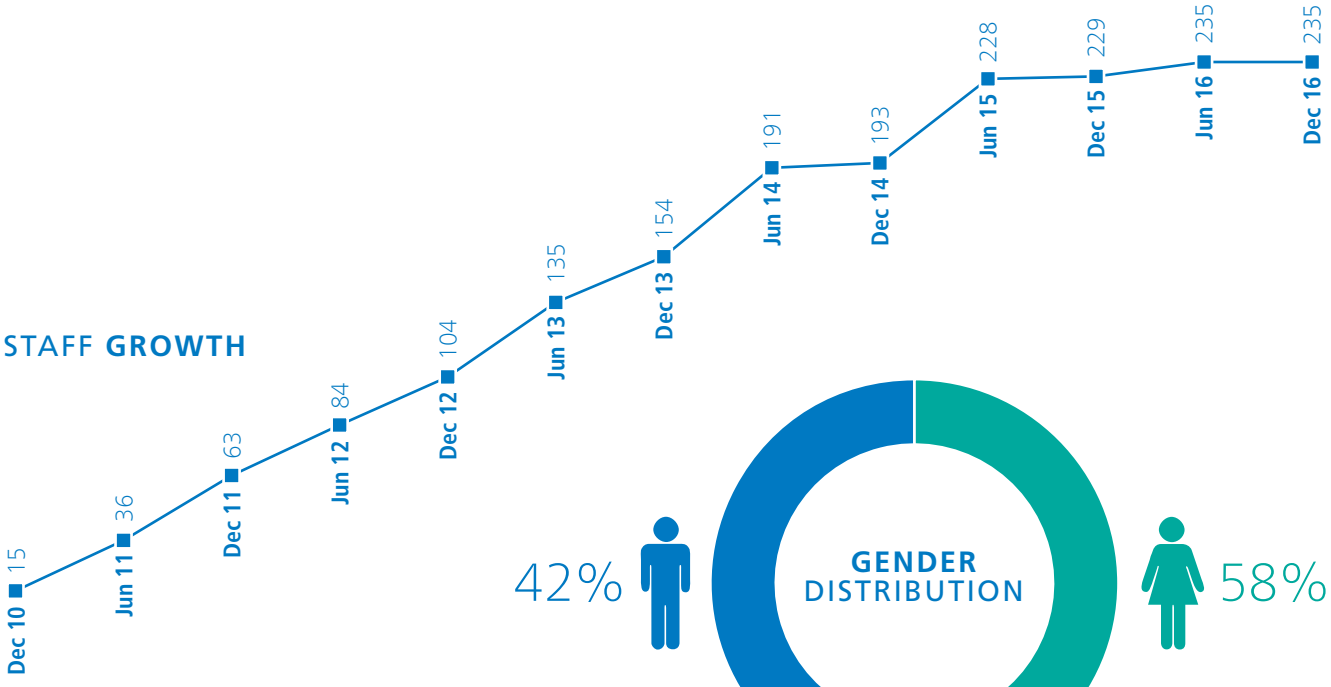
FACTS & FIGURES

STAFF

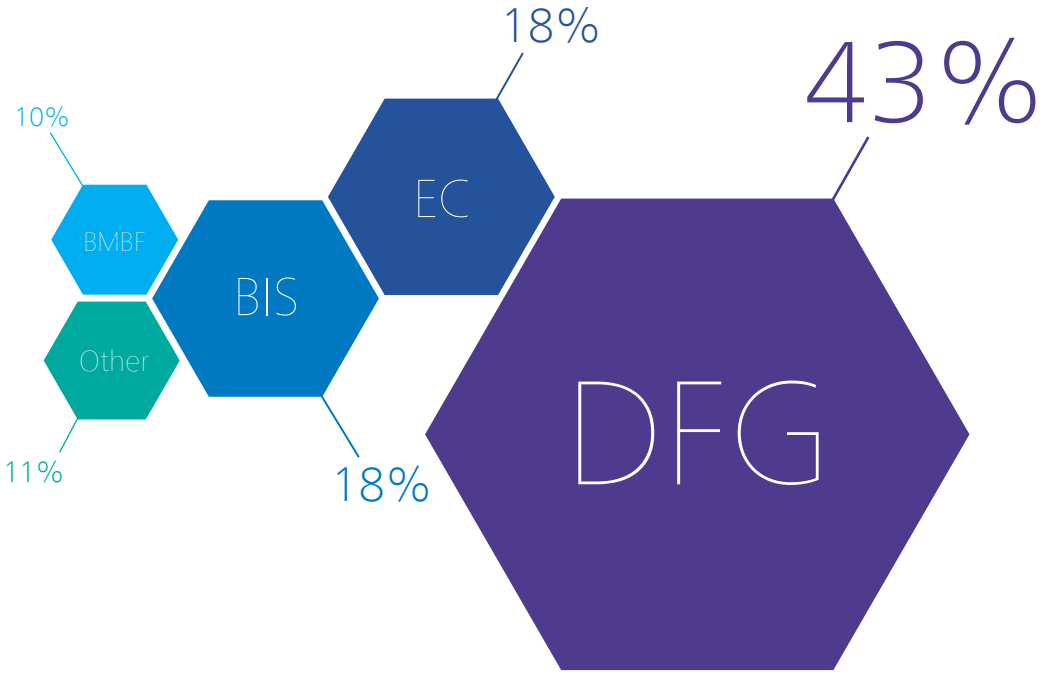


NATIONALITIES OF SCIENTIFIC STAFF

STAFF GROWTH



EXTRAMURAL FUNDS



MAJOR FUNDERS

Boehringer Ingelheim Foundation (BIS)

Federal Ministry of Education and Research (BMBF)

German Research Foundation (DFG)

EUROPEAN COMMISSION (EC)

European Research Council (ERC)

EpiGeneSys Network of Excellence

Marie Curie Actions

FURTHER SUPPORT

Boehringer Ingelheim Fonds

European Molecular Biology Organisation (EMBO)

Fundação para a ciência e a tecnologia

German Academic Exchange Service (DAAD)

German Cancer Aid

International Society for Differentiation

National Research Foundation of Korea

Naturwissenschaftlich-Medizinisches Forschungszentrum (NMFZ)

Netherlands Organisation for Scientific Research (NWO)

RNA Society

The Company of Biologists

Wilhelm Sander-Stiftung

RESEARCH AND TRAINING

The scientists at IMB carry out research in epigenetics, developmental biology, genome stability and the interfaces between these fields. In 2016, the international community of researchers at IMB included 64 PhD students, 47 postdocs and 16 group leaders, from over 25 different countries.

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

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

INTERNATIONAL PHD PROGRAMME

PhD students are a key part of our research teams at IMB and work on projects of fundamental biological importance or of relevance to human disease. Their research is conducted in a vibrant and highly interdisciplinary environment with leaders in their respective fields.

Our International PhD Programme (IPP) on Gene Regulation, Epigenetics and Genome Stability, supported by the Boehringer Ingelheim Foundation, gives talented and enthusiastic students the opportunity to undertake PhD research at the cutting-edge of modern biology.

The IPP has a unique profile that provides students with interdisciplinary education in the following fields:

- Epigenetics
- Gene Regulation
- DNA Repair & Genome Stability
- Functional Morphology of the Nucleus
- Systems Biology & Bioinformatics

The Programme is coordinated by IMB and participating groups are located at the:

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

www.imb.de/PhD

POSTDOC PROGRAMME

The IMB Postdoc Programme has been established to meet the specific needs of postdocs, and to ensure that they are able to build the strongest possible foundation for success in their future careers. The programme ensures sound scientific training through a variety of lectures, workshops and events available at IMB, and offers postdocs full support with raising funds for their research.

IMB also recognises the need for career development. In addition to the guidance given by Group Leaders, who provide postdocs with day-to-day scientific and career advice, the Postdoc Programme also offers mentoring discussions with IMB's Scientific Directors, Career Days, and preparation for applications and interviews. To succeed in today's competitive job market, postdocs must have excellent presentation, writing, project management and time management skills. As such, IMB provides courses and lectures on these elements. The programme also organises talks by representatives from local scientific companies, and sets up company site visits and job shadowing initiatives, so that IMB postdocs have the opportunity to learn more about a range of future career opportunities.

www.imb.de/Postdocs

INTERNATIONAL SUMMER SCHOOL

IMB's International Summer School (ISS) is a six-week programme for outstanding and enthusiastic undergraduate, masters and PhD students who want to acquire excellent practical skills and hands-on training from leading scientists in molecular biology. Research groups participating in the ISS include Group Leaders at IMB, Johannes Gutenberg University and Mainz's University Medical Centre.

The ISS offers an attractive framework for training prospective scientists in an informal and international atmosphere. This includes theory modules (lectures and discussion groups) and practical research projects. The lectures give students comprehensive insights into the latest research findings and identify key open questions in gene regulation, epigenetics and genome stability. Furthermore, the ISS teaches students the complementary skills, such as presentation and communication techniques, that are required for a successful career as a scientist.

Beyond these specific events, ISS participants are also fully integrated into scientific life at IMB by participating in lab meetings and journal clubs. Furthermore, each student works on a cutting-edge research project within the lab of one of the participating research groups.

www.imb.de/ISS

TRAINING COURSES

CORE FACILITIES TRAINING

In 2016, IMB offered the following training courses in scientific and transferable skills.

LECTURES

CORE FACILITY	DATES	TITLE
BIOINFORMATICS	24 May	ChIP-seq
	31 May	RNA-seq
	7 Jun	DNA Methylomics
	14 Jun	Variant Calling in DNA-seq
	21 Jun	Experimental Design & QC
	28 Jun	Systems Biology & Modeling
FLOW CYTOMETRY	8 Mar & 11 Oct	Introduction to Flow Cytometry I
	15 Mar & 18 Oct	Introduction to Flow Cytometry II
	5 Jul	Data Analysis
GENOMICS	26 Apr	What Can NGS Do for You?
MICROSCOPY & HISTOLOGY	2 Feb & 6 Sep	Introduction to Microscopy I
	9 Feb & 13 Sep	Introduction to Microscopy II
	16 Feb & 20 Sep	Introduction to Microscopy III
	23 Feb	Pitfalls in Image Acquisition
	1 Mar	Ethics in Image Acquisition & Processing
	3 May	Image Processing
	12 Jul	Basics in Histology
PROTEOMICS	8 Nov	Proteomics

PRACTICAL COURSES

CORE FACILITY	DATES	TITLE
BIOINFORMATICS	6, 13, 20 & 27 Apr	Introduction to R (I - IV)
	4 & 11 May	ChIP-seq & RNA-seq Analysis with R (Parts I - II)
	28 - 29 Jun	ChIP-seq & RNA-seq Analysis with GALAXY
FLOW CYTOMETRY	4 - 11 Apr & 7 - 14 Nov	Flow Cytometry Practical Course
MICROSCOPY & HISTOLOGY	17 - 20 May	Image Pro Practical Course
	18 & 19 Jul	Histology Crash Course
PROTEOMICS	24 & 25 May	Proteomics Data Analysis
	7 - 9 Nov	Proteomics Practical Course

SCIENTIFIC AND TRANSFERABLE SKILLS TRAINING

LECTURES

DATE	TITLE
16 Mar	Science Communication
1 - 12 Aug	Block Lecture Weeks of IMB's International PhD Programme
25 Oct - 7 Feb 2017	Lecture Series: Introduction to Epigenetics

PRACTICAL COURSES

DATE	TITLE
20 - 21 Jan	Project Management in Science for Postdocs
25 - 26 Jan, 27 - 28 Jan & 2 - 4 Aug	Presentation Skills
22 Apr	Application Training
27 - 28 Sep	Time & Self Management
5 - 6 Oct	Introduction to Biostatistics
13 - 14 Oct	Scientific Talks
24 - 25 Oct	Scientific Writing
14 Nov	Professional Orientation for PhD students
7 - 8 Dec	How to Design Scientific Figures
8 Dec	Strategic Application
15 - 16 Dec	Reading and Memo Techniques

GRANTS OFFICE TRAINING

DATE	TITLE
9 Jun	HORIZON 2020 & DFG: Funding Opportunities for Advanced Researchers*
28 Jun	Research Careers in Europe: Funding Opportunities for Postdocs*
8 Sep	Academic Careers in Europe: Funding Opportunities for Excellent Researchers*

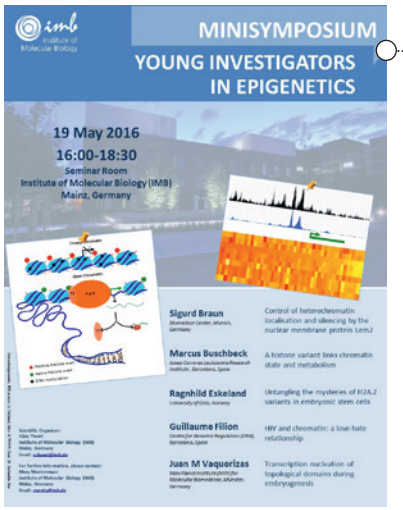
* organised in cooperation with JGU, JGU's University Medical Center and the Max Planck Institute for Polymer Research

INVITED SPEAKERS

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
14 Jan 2016	Seminar	**ANDRÉS AGUILERA**	University of Seville	RNA-mediated genome instability and replication stress
11 Feb 2016	Seminar	**DIETER HEERMANN**	Heidelberg University	Spatial organisation of the genome and the interplay with proteins
25 Feb 2016	Seminar	**CHENG-FU KAO**	Academia Sinica, Taipei	The roles of H2B ubiquitylation in DNA replication and transcription
29 Feb 2016	TechTalk	**NICOLA CROSETTO**	Karolinska Institute, Stockholm	Quantitative DNA breakome analysis in low input samples
17 Mar 2016	Seminar	**HENRIK KAESSMANN**	Heidelberg University	The evolutionary dynamics of mammalian gene expression across multiple dimensions
21 Apr 2016	Seminar	**LUIS SERRANO**	Centre for Genomic Regulation, Barcelona	Quantitative understanding of a bacterium, towards a realistic whole cell model
28 Apr 2016	Seminar	**PETER STADLER**	University of Leipzig	Catching monsters in RNA land: mapping atypical transcripts
12 May 2016	Seminar	**HEMMO MEYER**	University of Duisburg-Essen	Ubiquitin-mediated protein extraction from chromatin as a regulatory principle
19 May 2016	Seminar	**HOWARD CEDAR**	The Hebrew University of Jerusalem	Understanding the role of DNA methylation in health and disease
02 Jun 2016	Seminar	**ROLF ZELLER**	University of Basel	Limb organogenesis and evolution: from signals to gene regulatory landscapes
30 Jun 2016	Seminar	**MARKUS SAUER**	University of Würzburg	Super-resolution fluorescence imaging by dSTORM

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
28 Jul 2016	Seminar	**THORSTEN HOPPE**	University of Cologne	Ubiquitin sets the timer: coordination of aging and proteostasis
08 Sep 2016	Seminar	**IAN HICKSON**	University of Copenhagen	The effects of DNA replication stress on chromosome stability
04 Oct 2016	TechTalk	**HOLGER ERFLE**	Heidelberg University	RNAi screening and the development of novel tools for the high content analysis of the cell
13 Oct 2016	Seminar	**ACHIM KRAMER**	Charité, University Medicine Berlin	Posttranscriptional mechanisms in the mammalian circadian clock
25 Oct 2016	TechTalk	**SARANTIS CHLAMYDAS & MATTHIAS SPILLER-BECKER**	Active Motif Inc., Frankfurt	Focus on chromatin: tools & services for epigenetics and gene regulation
03 Nov 2016	Seminar	**SIMON BOULTON**	The Francis Crick Institute, London	Function and regulation of RTEL1 at vertebrate telomeres
10 Nov 2016	Seminar	**ALEXANDER STARK**	Research Institute of Molecular Pathology, Vienna	Decoding transcriptional regulation
15 Nov 2016	Seminar	**ROBERT HÄNSEL-HERTSCH**	University of Cambridge	G-quadruplex structures mark transcriptionally active regulatory chromatin
24 Nov 2016	Seminar	**ANTON GARTNER**	University of Dundee	From worm to man: studying genome maintenance in *C. elegans* - new approaches and surprising results
29 Nov 2016	TechTalk	**JONAS V SCHAEFER**	University of Zurich	Multipurpose DARPin binders for innovative applications: how to identify affinity reagents for challenging tasks

SELECTED SCIENTIFIC EVENTS ORGANISED BY IMB



19 May 2016

YOUNG INVESTIGATORS IN EPIGENETICS MINISYMPOSIUM

Scientific organiser: Vijay Tiwari (IMB)

23 - 24 May 2016

SYSTOX MEETING

Scientific organiser: Miguel Andrade (IMB)

20 - 21 JUL 2016

INTERDISCIPLINARY DISCUSSION EVENT

"SHOULD WE LIVE FOREVER? BIOLOGICAL AND ETHICAL PERSPECTIVES"

Scientific organisers: Mita Banerjee (Univ. Mainz), Ralf Dahm (IMB), Christof Niehrs (IMB) and Ruben Zimmermann (Univ. Mainz)

29 Sep 2016

COMPUTATIONAL SCIENCE IN MAINZ MEETING

Scientific organisers: Benjamin Müller (Univ. Mainz) and Miguel Andrade (IMB)

20 - 22 Oct 2016

2016 IMB CONFERENCE

"EPIGENETICS IN DEVELOPMENT"

Scientific organisers: Bradley Cairns (Huntsman Cancer Institute, Utah, USA), René Ketting (IMB), Jean-Yves Roignant (IMB) and Natalia Soshnikova (IMB)

28 - 29 Nov 2016

LOW-COMPLEXITY HACKATHON

Scientific organiser: Pablo Mier Munoz (IMB)

30 Nov 2016

STUDENT SYMPOSIUM ON COMPUTATIONAL GENOMICS

Scientific organiser: Jonas Ibn-Salem (IMB) together with a committee from the Regional Student Group Germany of the Student Council of the International Society of Computational Biology (RSG-ISCBSC)

1 - 2 Dec 2016

WORKSHOP

"CHALLENGES IN GENE EXPRESSION DATA ANALYSIS"

Scientific organisers: Miguel Andrade (IMB), Jean-Fred Fontaine (IMB) and Vijay Tiwari (IMB)

PUBLICATIONS 2016

ANDRADE

Alanis-Lobato G and Andrade-Navarro MA (2016). Distance distribution between complex network nodes in hyperbolic space. *Complex Systems*, 25, 223-236

Alanis-Lobato G, Mier P and Andrade-Navarro MA (2016). Efficient embedding of complex networks to hyperbolic space via their Laplacian. *Sci Rep*, 6, 30108

Alanis-Lobato G, Mier P and Andrade-Navarro MA (2016). Manifold learning and maximum likelihood estimation for hyperbolic network embedding. *Applied Network Science*, 1, 10

Andrade-Navarro MA and Fontaine JF (2016). Gene set to diseases (GS2D): Disease enrichment analysis on human gene sets with literature data. *Genomics and Computational Biology*, 2, e33

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

Mier P and Andrade-Navarro MA (2016). CABRA: Cluster and annotate blast results algorithm. *BMC Res Notes*, 9, 253

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de Oliveira KA, Kaergel E, Heinig M, Fontaine JF, Patone G, Muro EM, Mathas S, Hummel M, Andrade-Navarro MA, Hübner N and Scheidereit C (2016). A roadmap of constitutive NF-κB activity in Hodgkin lymphoma: Dominant roles of p50 and p52 revealed by genome-wide analyses. *Genome Med*, 8, 28

Petrakis S and Andrade-Navarro MA (2016). Editorial: Protein interaction networks in health and disease. *Front Genet*, 7, 111

Riemke P, Walter C, Ghani S, Fischer J, Zepper M, Agelopoulos K, Lettermann S, Gebhardt ML, Mah N, Weilemann A, Grau M, Haferlach T, Delwel R, Prinz M, Andrade-Navarro MA, Lenz G, Dugas M, Müller-Tidow C and Rosenbauer F (2016). Myeloid leukemia with transdifferentiation plasticity developing from T-cell progenitors. *EMBO J*, 35, 2399-2416

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Wethmar K, Schulz J, Muro EM, Talyan S, Andrade-Navarro MA and Leutz A (2016). Comprehensive translational control of tyrosine kinase expression by upstream open reading frames. *Oncogene*, 35, 1736-1742

BELI

Heidelberger JB, Wagner SA and Beli P (2016). Mass spectrometry based proteomics for investigating DNA damage-associated protein ubiquitylation. *Front Genet*, 7, 109

Richter B, Sliter DA, Herhaus L, Stolz A, Wang C, Beli P, Zaffagnini G, Wild P, Martens S, Wagner SA, Youle RJ and Dikic I (2016). Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. *Proc Natl Acad Sci U S A*, 113, 4039-4044

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

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BUTTER

Bluhm A, Casas-Vila N, Scheibe M and Butter F (2016). Reader interactome of epigenetic histone marks in birds. *Proteomics*, 16, 427-436

Cicova Z, Dejung M, Skalicky T, Eisenhuth N, Hanselmann S, Morriswood B, Figueiredo LM, Butter F[#] and Janzen CJ[#] (2016). Two flagellar BAR domain proteins in *Trypanosoma brucei* with stage-specific regulation. *Sci Rep*, 6, 35826

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Klassen R, Ciftci A, Funk J, Bruch A, Butter F and Schaffrath R (2016). tRNA anticodon loop modifications ensure protein homeostasis and cell morphogenesis in yeast. *Nucleic Acids Res*, 44, 10946-10959

CREMER

Boyd PS, Struve N, Bach M, Eberle JP, Gote M, Schock F, Cremer C, Kriegs M and Hausmann M (2016). Clustered localization of EGFR-vIII in glioblastoma cells as detected by high precision localization microscopy. *Nanoscale*, 8, 20037-20047

Cremer C and Birk U (2016). Perspectives in super-resolved fluorescence microscopy: What comes next? *Front Phys*, 4, 1-11

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Moser F, Hildenbrand G, Müller P, Al Saroori A, Biswas A, Bach M, Wenz F, Cremer C, Burger N, Veldwijk MR and Hausmann M (2016). Cellular uptake of gold nanoparticles and their behavior as labels for localization microscopy. *Biophys* J, 110, 947-953

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Żurek-Biesiada D, Szczurek AT, Prakash K, Best G, Mohana GK, Lee HK, Roignant JY, Dobrucki JW, Cremer C and Birk U (2016). Quantitative super-resolution localization microscopy of DNA in situ using Vybrant® DyeCycle™ Violet fluorescent probe. *Data in Brief*, 7, 157-171

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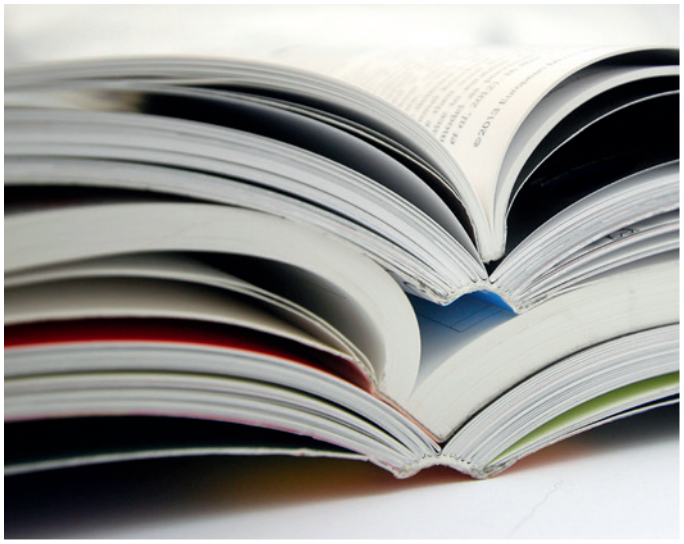

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(*indicates joint contribution, #indicates joint correspondence)

RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located within the leafy campus of the Johannes Gutenberg University, just west of Mainz city centre. With 10 departments, 150 institutes and 33,000 students, Johannes Gutenberg University is one of the largest German universities. In biomedical research, the university has built strong, interdisciplinary centres dedicated to neuroscience, cardiovascular medicine, immunology and oncology.

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

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

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

WHERE WE ARE

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

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IMB IS GRATEFUL TO THE FOLLOWING OUTSTANDING SCIENTISTS FOR THE INSIGHT, GUIDANCE AND ADVICE THAT THEY HAVE PROVIDED IN ORDER TO HELP US BECOME A WORLD-LEADING RESEARCH CENTRE.

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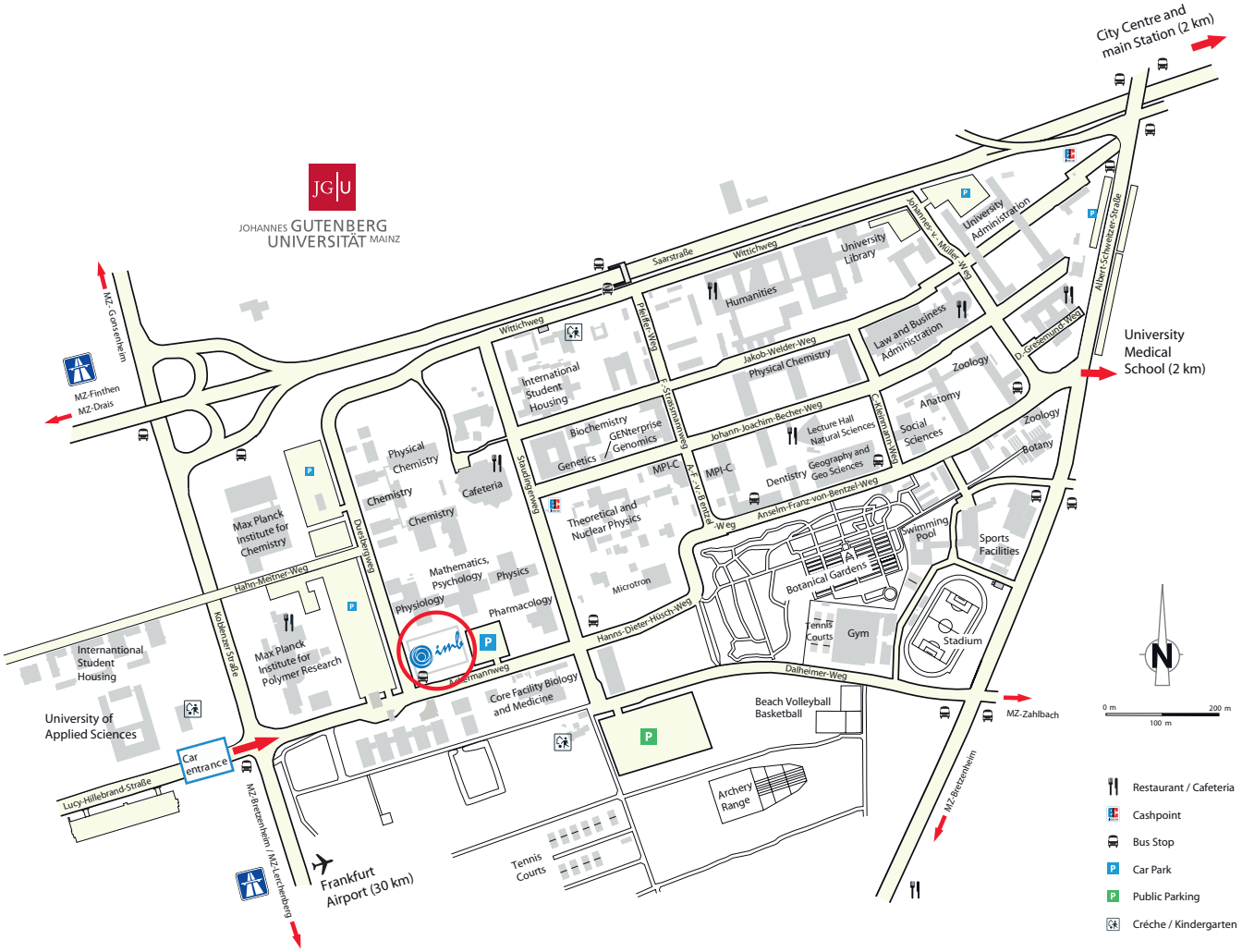

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