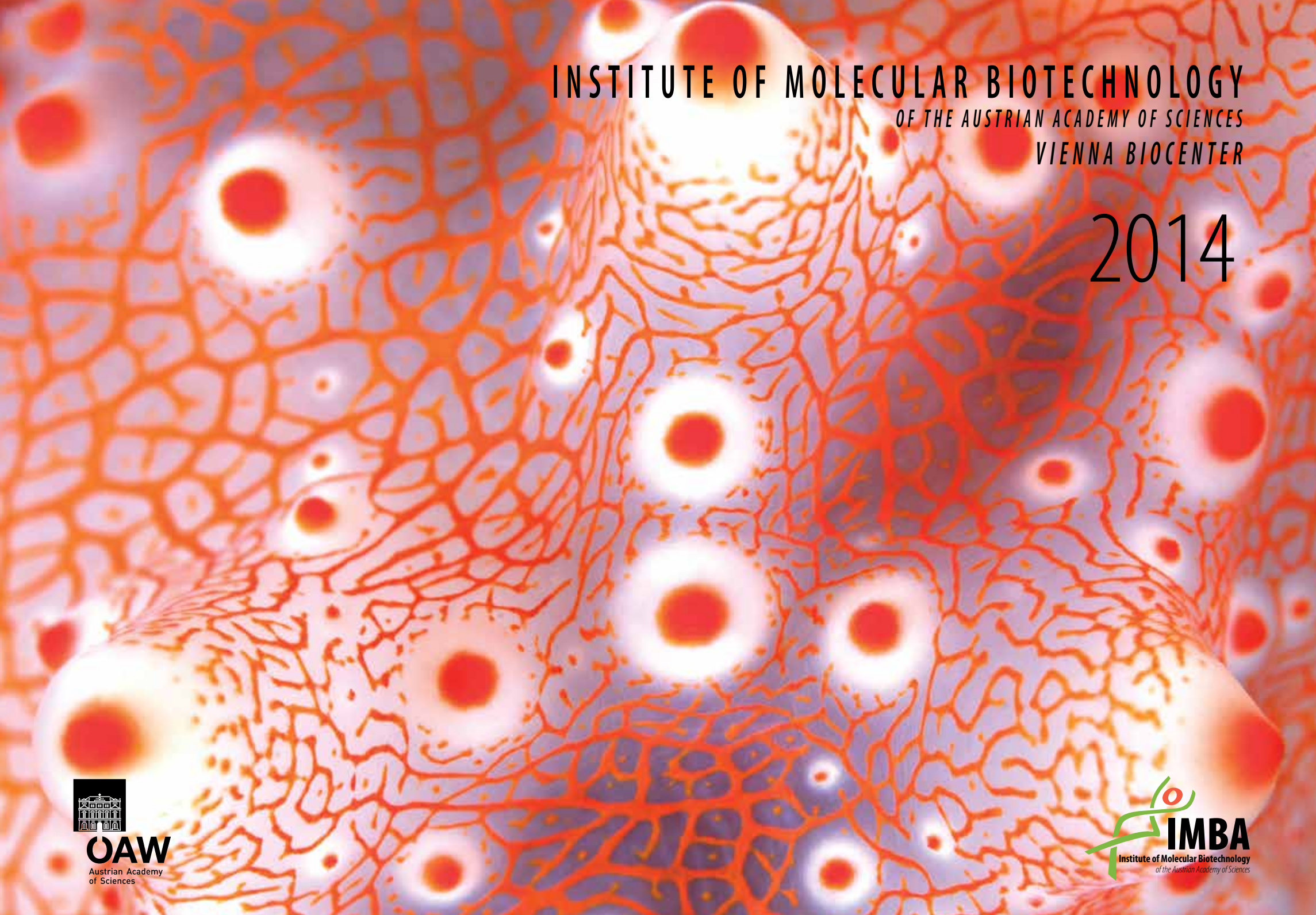


INSTITUTE OF MOLECULAR BIOTECHNOLOGY

OF THE AUSTRIAN ACADEMY OF SCIENCES

VIENNA BIOCENTER

2014

ÖAW
Austrian Academy
of Sciences

IMBA
Institute of Molecular Biotechnology
of the Austrian Academy of Sciences

CONTENTS

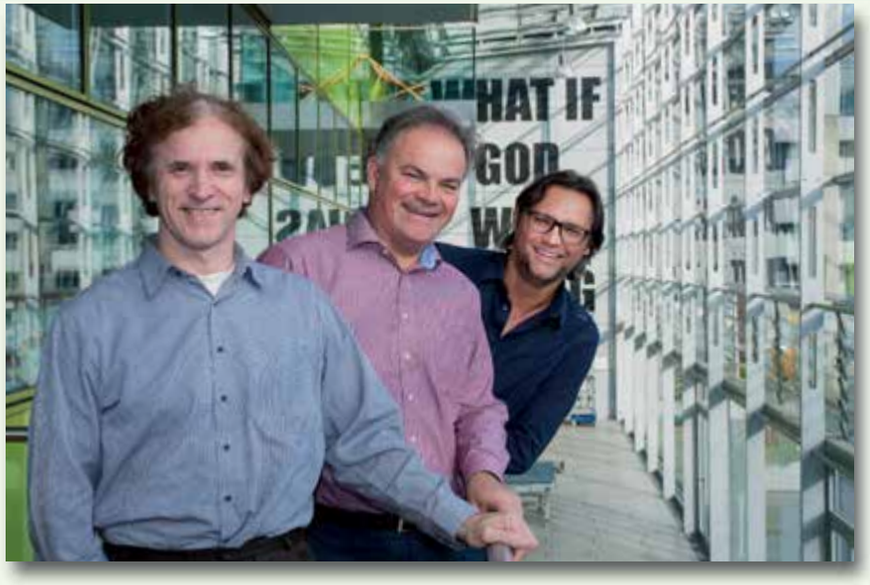
Introduction

IMBA has had another great year of science, some of which is highlighted in this annual research report. We continue our ascent to a world class institute that has true impact in the scientific community and society. This can only be achieved with a group of very gifted scientists and an effective organization ranging from skilled administration to outstanding infrastructure units – which are our little secrets to make life in science easier and in fact allow the scientists to live their visions without distractions. Also our close collaboration with our neighboring institute IMP under the new leadership of Jan-Michael Peters is on a very good path and we continue to develop a common faculty, help each other in hiring, and to set-up interlinked research arenas. Therefore a big thank you to everybody at IMP and IMBA and of course all our colleagues at the Campus Vienna BioCentre for their dedication and common quest to develop one of the top research sites in the world.

One important development in the last years was the establishment of the Campus Science Support Facility (CSF) to bring new infrastructure to our campus (e.g. a new 15.2 Tesla MRI and phenotyping units) and to maintain and expand some of the existing services, for instance the now famous Vienna Drosophila RNAi library (VDRC). The CSF is jointly funded by the Austrian Ministry of Science, Research and Economy and the City of Vienna and this financial support has now been extended to an additional 5 years after an extensive and very positive external evaluation. Congratulations to everybody at the CSF for an outstanding job!

We had to say good bye to Leonie Ringrose, whose term as Junior Group Leader has recently ended. Leonie was a key member of our faculty and she has made outstanding contributions to our institute, through her science, her intellect, and her tireless efforts to make our institute better. Her writing courses for the students and postdocs are legendary and she has also trained a great group of young scientists who will certainly continue in her spirit. We wish Leonie all the best for her new position –with lots of great science and many talented fellows.

We also had to say good-bye to Max Birnstiel, who has passed away this fall. Max was the founding director of our close partner IMP and a true legend - not only in Vienna - for his scientific work on histones and for being the "spiritus rector" of the Campus Vienna BioCentre. There is a reason why our institute IMBA is located at the Campus Vienna BioCentre, because when the Academy of Sciences founded IMBA they wanted to work closely with the top research institute in Life Sciences in Austria, namely the IMP. Max Birnstiel shaped the excellence of the IMP and hired the first group of scientists who then nucleated and inspired the entire campus. Without Max the campus would not have achieved it's excellence and international reputation as it has now. It always takes this one special person to make things happen and Max Birnstiel was this special person who balanced and integrated excellence in science with political wisdom. We will greatly miss him and he will always remain in our memory.



JOSEF PENNINGER
Managing Director/Science

JÜRGEN KNOBLICH
Deputy Director/Science

MICHAEL KREBS
Managing Director/Finance & Administration

Many IMBA scientists have been recognized with awards this years, an excellent achievement. Just to highlight some of these awardees: Everytime you open an issue of Nature you will see the picture of Madeline Lancaster, who has won this year's Eppendorf Award for developing the first human brain organoids. Madeline was the first to create brain organoids from human embryonic stem cells or iPS cells, a true breakthrough in tissue engineering that has opened up entirely new avenues into human brain research. She is also the first person to win this award as a postdoctoral fellow working in the laboratory of Juergen Knoblich. Secondly, Martin Moder – at the time a Diploma student with Juergen Knoblich - not only won the best science presentation in Austria, but he actually won the finals of the European Science slam in Copenhagen presenting genetic screens for brain cancer in flies. Please check out his extraordinary and winning performance in YouTube. Finally, Josef Penninger was awarded the 1.5 Million Euro Wittgenstein award, the top science prize in Austria. We are very proud that this is the third time within 10 years this award goes to scientists working at IMBA. The funds from this award will be used to develop new transformative technologies and to give young scientists a chance to establish their independent careers.

We welcome Uli Elling as a new team leader at IMBA. Uli is the mastermind behind the haploid stem cell technologies developed at IMBA and will continue to develop this breakthrough technology. He will also establish his own research group with a focus on stem cell and lineage commitment in early development. We wish him all the best for his endeavors.

Our annual recess in October showcased the excellent research done by all our faculty, critically evaluated by our Scientific Advisory Board, who for many years now have been guiding our progress and steered our science into the Champions league. A big Thank You to all of our past and present SAB members. We also thank Boehringer Ingelheim for supporting our great cooperation with the IMP and our parent organization, the Austrian Academy of Science, for their vision and commitment to support life sciences in Austria. We also very much indebted to all granting agencies who support our research such as the ERC, Era of Hope, FWF, WWTF, to name a few, and our private sponsors such as the Era of Hope Foundation, OMV, Lotterien Oesterreich, the Austria Vienna Soccer Club, DEBRA, and many more. Without this support we would not be able to do science that one day might make a real difference.

Jürgen Knoblich, Michael Krebs, Josef Penninger

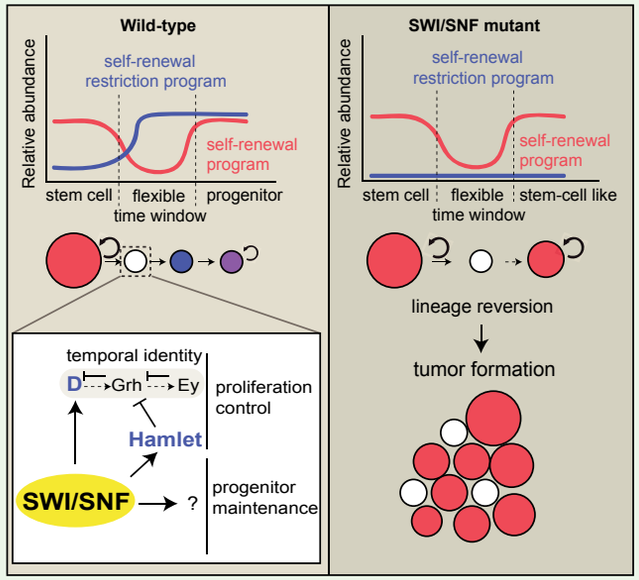
RESEARCH HIGHLIGHTS

2014 has been scientifically a successful year for IMBA. This double page shows IMBA's "Research Highlights", a selection of highly visible research articles IMBA scientists have published during this year.

SWI/SNF complex prevents lineage reversion and induces temporal patterning in neural stem cells.

Eroglu, E., Burkard, TR., Jiang, Y., Saini, N., Homem, CC., Reichert, H., Knoblich, JA. (2014). Cell. 156(6):1259-73

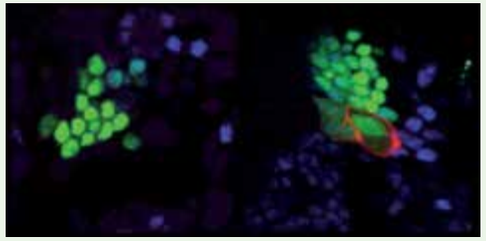
Members of the SWI/SNF chromatin-remodeling complex are among the most frequently mutated genes in human cancer, but how they suppress tumorigenesis is currently unclear. Here, we use Drosophila neuroblasts to demonstrate that the SWI/SNF component Osa (ARID1) prevents tumorigenesis by ensuring correct lineage progression in stem cell lineages. We show that Osa induces a transcriptional program in the transit-amplifying population that initiates temporal patterning, limits self-renewal, and prevents dedifferentiation. We identify the Prdm protein Hamlet as a key component of this program. Hamlet is directly induced by Osa and regulates the progression of progenitors through distinct transcriptional states to limit the number of transit-amplifying divisions. Our data provide a mechanistic explanation for the widespread tumor suppressor activity of SWI/SNF. Because the Hamlet homologs Evi1 and Prdm16 are frequently mutated in cancer, this mechanism could well be conserved in human stem cell lineages.



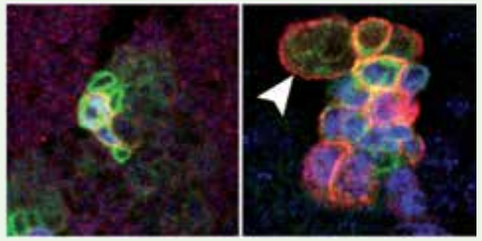
Ecdysone and mediator change energy metabolism to terminate proliferation in Drosophila neural stem cells.

Homem, CC., Steinmann, V., Burkard, TR., Jais, A., Esterbauer, H., Knoblich, JA. (2014). Cell. 158(4):874-88

Stem cells are highly abundant during early development but become a rare population in most adult organs. The molecular mechanisms causing stem cells to exit proliferation at a specific time are not well understood. Here, we show that changes in energy metabolism induced by the steroid hormone ecdysone and the Mediator initiate an irreversible cascade of events leading to cell-cycle exit in Drosophila neural stem cells. We show that the timely induction of oxidative phosphorylation and the mitochondrial respiratory chain are required in neuroblasts to uncouple the cell cycle from cell growth. This results in a progressive reduction in neuroblast cell size and ultimately in terminal differentiation. Brain tumor mutant neuroblasts fail to undergo this shrinkage process and continue to proliferate until adulthood. Our findings show that cell size control can be modified by systemic hormonal signaling and reveal a unique connection between metabolism and proliferation in stem cells.



Left side: normal brain (wild-type); Right side: "mutant" neural stem cell. (cell where metabolism was affected). In red are the neural stem cells (arrow points at stem cell), and in green and blue are the neurons.

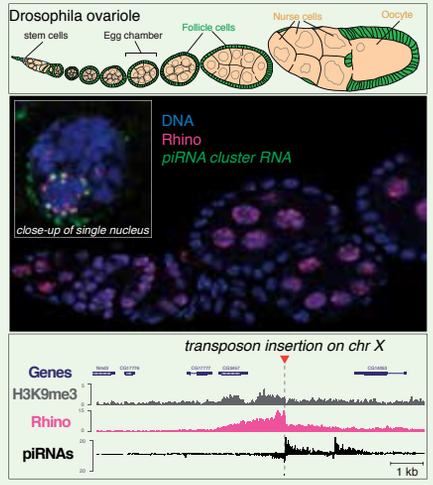


Left side: normal brain (wild-type); Right side: "mutant" neural stem cell. (cell where metabolism was affected). The big red cell is the neural stem cell (arrow points at the stem cell), and in blue are the neurons.

The rhino-deadlock-cutoff complex licenses noncanonical transcription of dual-strand piRNA clusters in Drosophila.

Mohn, F., Sienski, G., Handler, D., Brennecke, J. (2014). Cell. 157(6):1364-79

Argonaute proteins of the PIWI clade are central to transposon silencing in animal gonads. Their target specificity is defined by 23-30 nt PIWI interacting RNAs (piRNAs), which mostly originate from discrete genomic loci termed piRNA clusters. Here, we show that a complex composed of Rhino, Deadlock, and Cutoff (RDC) defines dual-strand piRNA clusters genome-wide in Drosophila ovaries. The RDC is anchored to H3K9me3-marked chromatin in part via Rhino's chromodomain. Depletion of Piwi results in loss of the RDC and small RNAs at a subset of piRNA clusters, demonstrating a feedback loop between Piwi and piRNA source loci. Intriguingly, profiles of RNA polymerase II occupancy, nascent transcription, and steady-state RNA levels reveal that the RDC licenses noncanonical transcription of dual-strand piRNA clusters. Likely, this process involves 5′ end protection of nascent RNAs and suppression of transcription termination. Our data provide key insight into the regulation and evolution of piRNA clusters.

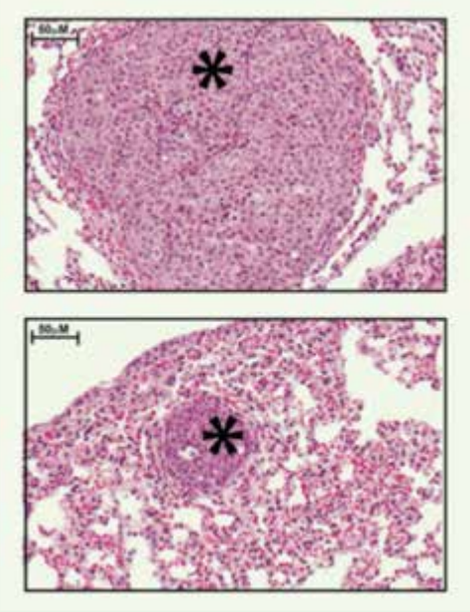


The HP1-homolg Rhino (red) regulates piRNA cluster in the Drosophila germ line (beige tissue in scheme on top). Rhino localizes to distinct nuclear foci that overlap with nascent piRNA precursor transcripts (green). The bottom panel displays a genomic region with a transposon insertion (dotted line). The transposon is silenced via the heterochromatic histone modification H3K9me3, this attracts Rhino, which induces low level transcription and piRNA production.

The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells.

Paolino, M., Choidas, A., Wallner, S., Pranjic, B., Uribesalgo, I., Loeser, S., Jamieson, AM., Langdon, WY., Ikeda, F., Fededa, JP., Cronin, SJ., Nitsch, R., Schultz-Fademrecht, C., Eickhoff, J., Menninger, S., Unger, A., Torka, R., Gruber, T., Hinterleitner, R., Baier, G., Wolf, D., Ullrich, A., Klebl, BM., Penninger, JM. (2014). Nature. 507(7493):508-12

Tumour metastasis is the primary cause of mortality in cancer patients and remains the key challenge for cancer therapy. New therapeutic approaches to block inhibitory pathways of the immune system have renewed hopes for the utility of such therapies. Here we show that genetic deletion of the E3 ubiquitin ligase Cbl-b (casitas B-lineage lymphoma-b) or targeted inactivation of its E3 ligase activity licenses natural killer (NK) cells to spontaneously reject metastatic tumours. The TAM tyrosine kinase receptors Tyro3, Axl and Mer (also known as Mertk) were identified as ubiquitylation substrates for Cbl-b. Treatment of wild-type NK cells with a newly developed small molecule TAM kinase inhibitor conferred therapeutic potential, efficiently enhancing anti-metastatic NK cell activity in vivo. Oral or intraperitoneal administration using this TAM inhibitor markedly reduced murine mammary cancer and melanoma metastases dependent on NK cells. We further report that the anticoagulant warfarin exerts anti-metastatic activity in mice via Cbl-b/TAM receptors in NK cells, providing a molecular explanation for a 50-year-old puzzle in cancer biology. This novel TAM/Cbl-b inhibitory pathway shows that it might be possible to develop a 'pill' that awakens the innate immune system to kill cancer metastases.

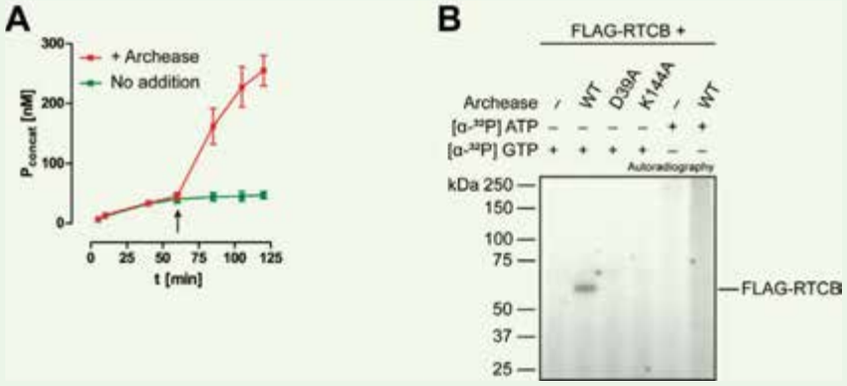


Histology of the lungs in Cbl-b sufficient (upper panel) and Cbl-b deficient (lower panel) mice showing that the absence of Cbl-b leads to fewer and smaller breast tumors.

Analysis of orthologous groups reveals archease and DDX1 as tRNA splicing factors.

Popow, J., Jurkin, J., Schleiffer, A., Martinez, J. (2014). Nature. 511(7507):104-7

RNA ligases have essential roles in many cellular processes in eukaryotes, archaea and bacteria, including in RNA repair and stress-induced splicing of messenger RNA. In archaea and eukaryotes, RNA ligases also have a role in transfer RNA splicing to generate functional tRNAs required for protein synthesis. We recently identified the human tRNA splicing ligase, a multimeric protein complex with RTCB (also known as HSPC117, C22orf28, FAAP and D10Wsu52e) as the essential subunit. The functions of the additional complex components ASW (also known as C2orf49), CGI-99 (also known as C14orf166), FAM98B and the DEAD-box helicase DDX1 in the context of RNA ligation have remained unclear. Taking advantage of clusters of eukaryotic orthologous groups, here we find that archease (ARCH; also known as ZBTB8OS), a protein of unknown function, is required for full activity of the human tRNA ligase complex and, in cooperation with DDX1, facilitates the formation of an RTCB-guanylate intermediate central to mammalian RNA ligation. Our findings define a role for DDX1 in the context of the human tRNA ligase complex and suggest that the widespread co-occurrence of archease and RtcB proteins implies evolutionary conservation of their functional interplay.



Archease acts as an RNA splicing factor in health and disease. Addition of Archease (arrow) reactivates stalled ligase complexes (A) by facilitating the formation of an essential guanylated ligase intermediate (B).

STEFAN AMERES GROUP

Mechanism and biology of RNA silencing in flies and mammals

www.imba.oeaw.ac.at/research/stefan-ameres/

Small silencing RNAs regulate gene expression in nearly all eukaryotes and have enormous biotechnological and therapeutic potential. MicroRNAs (miRNAs) belong to the largest family of trans-acting gene regulatory molecules in multicellular organisms. In flies and mammals, they control more than a half of the protein-coding transcriptome, and act as key regulators of organismal development, physiology, and disease. We are interested in understanding the molecular mechanisms that govern small RNA-mediated gene silencing in flies and mammals.

We aim to dissect the molecular processes that regulate the production of small RNAs, their assembly into ribonucleoprotein complexes, and their sequence-specific decay. Our goal is to define the principles that establish and maintain small RNA profiles in a given tissue or cell type in order to understand the molecular mechanisms that regulate miRNA homeostasis. To do so we utilize a combination of *Drosophila* genetics and biochemistry, as well as RNomics. The hypotheses emerging from our studies in flies are directly tested for their conservation in mammals.

Regulation of microRNA biogenesis

High-throughput sequencing analyses revealed that miRNAs in flies and mammals are frequently subject to posttranscriptional modifications as a consequence of non-templated nucleotide addition to their 3´ ends. We identified the first enzyme involved in the post-transcriptional modification of miRNAs in flies: a terminal nucleotidyltransferase which we named Tailor. Tailor catalyzes the addition of uridines to the 3´ end of precursor miRNAs. This process regulates the production of mature miRNAs by affecting the two-nucleotide 3´ overhang of precursor miRNAs, a feature required for the efficient processing of pre-miRNAs. Because of its intrinsic preference to recognize substrates ending in 3´ G, Tailor preferentially, but not exclusively, prevents maturation of mirtrons, a class of low-abundance miRNAs that employ the splicing machinery for their biogenesis and rapidly emerge in evolution. Therefore, Tailor regulates the biogenesis of miRNAs and may constitute a hurdle for the emergence of new miRNAs during evolution.

Target RNA-directed small RNA decay

Small RNAs guide Argonaute proteins to complementary sequences within mRNAs. In animals, miRNAs typically show just partial complementarity to the targets they regulate. We recently showed that high complementarity between miRNAs and their targets causes small RNAs to decay. This occurs in a process that involves the addition of non-templated nucleotides to the 3´ end of small RNAs (tailing) and their 3´ to 5´ exonucleolytic degradation (trimming) (Figure 2). Notably, some herpes viruses highjack the cellular miRNA decay machinery to trigger the destruction of host miRNAs in order to counteract the antiviral function of the microRNA pathway. We aim to characterize the molecular details of this novel miRNA decay pathway, identify its enzymatic components, and determine the biological function of the pathway. Our hypothesis is that mRNAs not only serve as targets for miRNA-mediated gene regulation, but also influence the abundance, and therefore the function, of miRNAs themselves.

Therapeutic miRNA inhibition

The sequence-specific decay of miRNAs harbors considerable therapeutic potential. For example, the inhibition of miR-122 – a liver-specific regulator of lipid metabolism – results in a decrease in serum cholesterol levels and interferes with replication of the hepatitis C virus (HCV). We recently developed a novel approach for efficient long-term inhibition of miRNA function *in vivo* in mice. The expression of tough decoy RNAs (TuDs, Fig. 3) – structured RNA polymerase III transcripts with accessible, highly complementary miRNA target sites – efficiently triggers miRNA decay by inducing the tailing and trimming pathway in cultured human cells and *in vivo* in mice, after recombinant adeno-associated virus (rAAV) vector delivery. rAAV-mediated miRNA inhibition provides a simple way of studying miRNA function in adult mammals, and may serve as a therapy for dyslipidemia and other miRNA-related human diseases.

Publication highlights:

Ameres, S.L. and Zamore, P.D., Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol., 2013, 14(8):475-88, doi: 10.1038/nrm3611

Figure 1

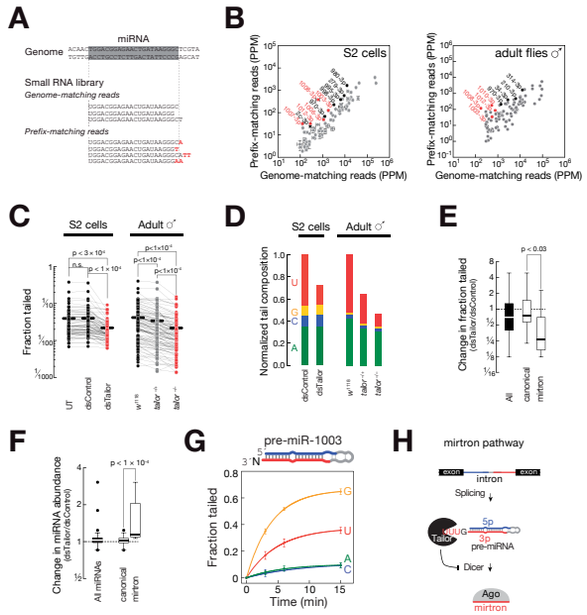
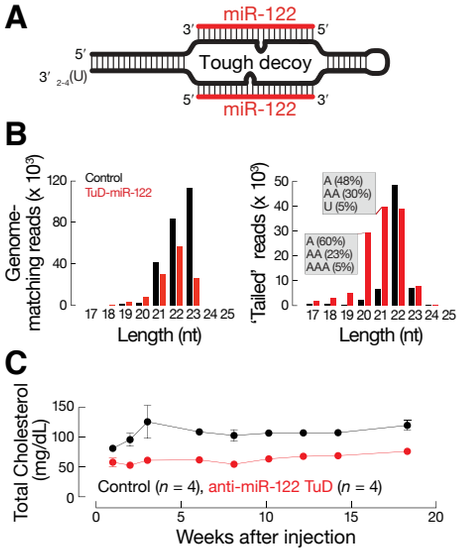


Figure 3



GROUP LEADER:
STEFAN AMERES

POSTDOC: WIEBKE WLOTZKA
PHD STUDENTS: ISABEL GRIESSHAMMER, BRIAN REICHHOLF, MADALENA REIMÃO-PINTO
DIPLOMA STUDENT: RAPHAEL MANZENREITHER
RESEARCH TECHNICIAN: SARA FARIÑA-LÓPEZ, IVICA SOWEMIMO

Figure 2

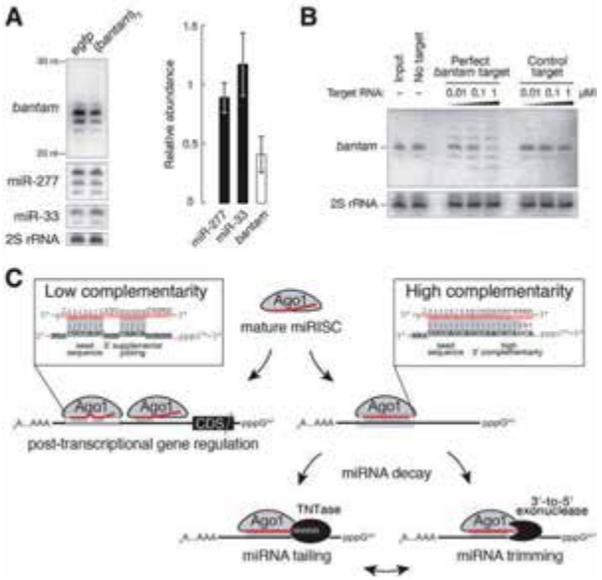


Figure 1: Regulation of microRNA biogenesis. (**A** and **B**) High-throughput sequencing datasets of miRNAs reveal prefix-matching reads, which contain non-genome matching nucleotide additions. Depletion of Tailor in Drosophila S2 cells and in vivo in flies results in a decrease in post-transcriptionally modified miRNAs (**C**), a result of the specific depletion of uridylation events (**D**). Mirtrons, a class of miRNAs that employ the splicing machinery for their biogenesis, are preferentially targeted for uridylation by Tailor (**E**) and, upon depletion of Tailor, mirtrons are upregulated (**F**). (**G**) In vitro tailing assays using immunopurified Tailor and 5′ radiolabeled pre-mirtron miR-1003 revealed a 3′ nucleotide dependency of Tailor-directed pre-miRNA modification. Model for the regulation of miRNA biogenesis by Tailor. Because the 3′ end of mirtrons is predefined by the splice acceptor site, which ends in 3′ G, mirtrons are uridylated by Tailor, a modification that prevents their accumulation.

Figure 2: Target RNA-directed microRNA decay. (**A**) Northern analysis of total RNA from a clonal S2 cell line expressing *egfp* mRNA, bearing in its 3′ UTR two target sites for *bantam* [(bantam)$_2$], and a clonal control cell line expressing sole *egfp* mRNA. Mean ± standard deviation for three biologically independent replicates of the experiment is shown (right). (**B**) Endogenous *bantam* miRNA was tailed and trimmed when *Drosophila* embryo extract was incubated overnight with a fully complementary target RNA, but not a control target. *bantam* and 2S rRNA were detected by Northern blotting. (**C**) Model for target RNA-directed tailing and trimming of miRNAs in flies. Binding of miRNAs to perfectly complementary target mRNA results in the exonucleolytic trimming and non-templated nucleotide addition (tailing) of the miRNA. miRNA tailing and trimming ultimately results in small RNA decay. The protein components of the tailing and trimming pathway are currently unknown. In contrast, miRNA binding to targets with low complementarity results in post-transcriptional gene silencing.

Figure 3: Therapeutic miRNA inhibition. (**A**) Tough decoy RNAs are structured RNA polymerase III transcripts containing accessible sites highly complementary to miRNAs. (**B**) Size distribution of genome matching and 'tailed' miR-122 reads in the liver of mice injected with AAV control (black) or AAV expressing a tough decoy RNA targeting miR-122 (red). (**C**) Total serum cholesterol levels of mice injected with AAV control (black) or AAV expressing a tough decoy RNA targeting miR-122 (red).

OLIVER BELL GROUP

Plasticity and Memory of Chromatin Structure

www.imba.oeaw.ac.at/research/oliver-bell/

Epigenetic mechanisms are of crucial importance for faithful transmission of gene expression states through cell division, and the maintenance of cellular identities from one generation to the next. These mechanisms also need to support the plasticity of gene expression to facilitate the acquisition of new cell fates in animal development. Chromatin modifications have emerged as important regulators of transcription, and are believed to contribute to the inheritance of gene expression states.

Research activities

We investigate the dynamics and epigenetic inheritance of nucleosome modifications in the context of physiological chromatin structure in living cells. Chromatin is undergoing constant remodeling to facilitate changes in gene expression and DNA accessibility in response to cell-intrinsic and cell-extrinsic stimuli. Specifically, the antagonizing activities of histone-modifying complexes add and remove post-translational histone modifications, contributing to the dynamic organization of chromatin at regulatory regions in the mammalian genome.

Traditional genetic and biochemical analyses have yielded a largely static picture of chromatin regulation. These approaches have failed to provide a comprehensive understanding of the actual function of chromatin modifications in gene regulation. Thus, separating cause from effect will require approaches that delineate the sequence of events involved in gene induction or repression.

Chromatin in vivo assay (CiA)

We employ a novel technique using chemical inducers of proximity to dissect the sequence of events and measure histone modification kinetics at high resolution during cell fate transitions, cellular reprogramming, and signal-dependent gene regulation. This technology integrates the complex nature of chromatin with precise biochemical analysis of the sequence of events during chromatin remodeling. We have generated a murine strain that permits rapid addition and removal of chromatin regulatory activities to a genetically modified Oct4 allele in any cell type, using small-molecule-mediated recruitment (Figure 1). Chemically induced proximity (CIP) provides high temporal control, permitting the examination of the kinetics and epigenetic memory of histone modifications in single-cell resolution.

Dynamics and memory of heterochromatin

In embryonic stem (ES) cells, Oct4 expression is essential for pluripotency and self-renewal. Upon differentiation, Oct4 is silenced. This involves the HP1 heterochromatin pathway (with H3K9 trimethylation) and the Polycomb pathway (with H3K27 trimethylation). We previously investigated the kinetics of heterochromatin formation by recruiting HP1α to the modified Oct4 promoter in ES cells and fibroblasts. Tethering of HP1α induced gene repression and the formation of heterochromatic domains of up to 10 kb. Measuring H3K9me3 changes after HP1α recruitment permitted the description of *in vivo* rates of heterochromatin spread in ES cells and fibroblasts. In addition, after HP1α removal we tested epigenetic properties and found that H3K9me3 can be faithfully transmitted through cell divisions (Figure 2). Yet, we also showed that the memory and spreading of H3K9me3 may be antagonized by transcriptional activators, indicating the high plasticity of chromatin regulation. Based on the balance between the antagonizing activities of H3K9me3 addition and removal, we proposed a mathematical model which accurately expresses our empirical observation at the Oct4 locus and also predicts the dynamics of heterochromatin formation and turnover at the majority of facultative H3K9me3 domains in the mammalian genome.

The CiA system is a powerful approach to study the kinetic regulation of any chromatin-modifying activity in any murine cell type and obtaining quantitative models for testing.

Figure 1

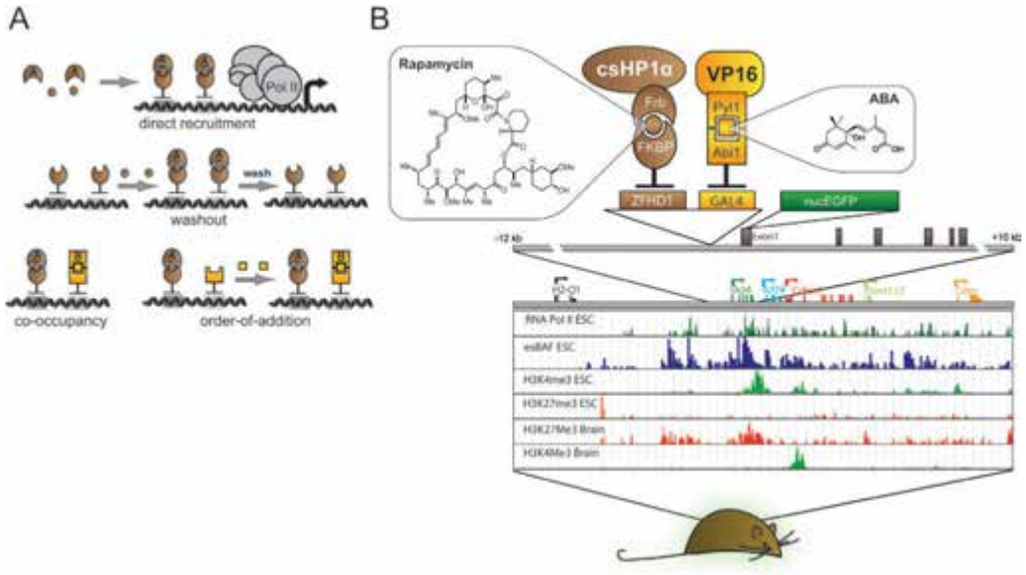
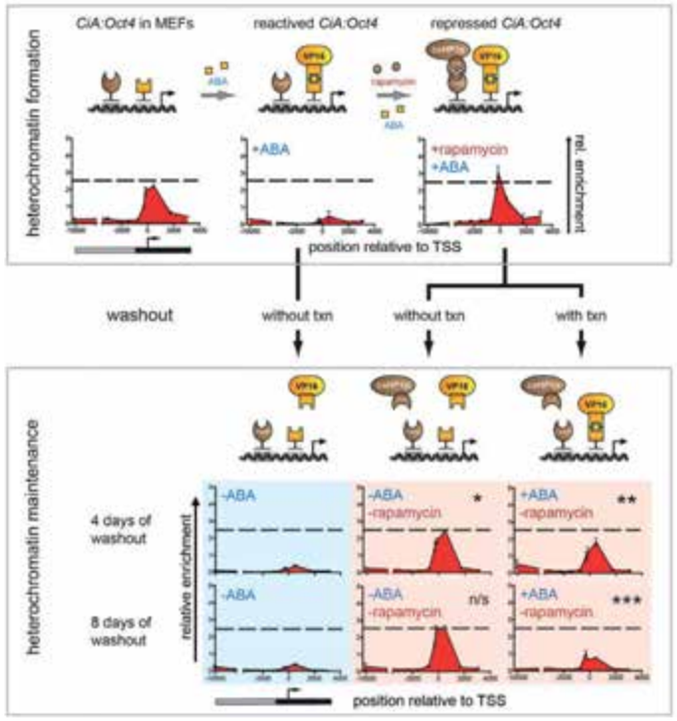


Figure 2



GROUP LEADER:
OLIVER BELL

PHD STUDENTS: HAGAR MOUSSA, JORGE ARTURO ZEPEDA MARTINEZ
RESEARCH ASSISTANT: KATARINA BARTALSKA
RESEARCH INTERN: LORENZO ORIETTI

Figure 1: Schematic diagram of the chromatin *in vivo* assay (CiA) system. The addition of small molecules mediates reversible recruitment of desired chromatin activities to modulate the local chromatin structure and gene expression of any murine cell type.

Figure 2: Initiation and maintenance of heterochromatin. Induced recruitment of HP1a leads to the formation of an H3K9me3 island, which is inherited through cell divisions. Targeting of the transcriptional activator VP16 antagonizes the epigenetic maintenance of heterochromatin.

JULIUS BRENNECKE GROUP

Genetics and Genomics of Transposon Control by Small RNAs

www.imba.oeaw.ac.at/research/julius-brennecke

Throughout the eukaryotic lineage, small RNA silencing pathways safeguard the genome against selfish genetic elements such as transposons. In animals, a specialized pathway centered on PIWI proteins and their interacting piRNAs silences transposons within the gonads. Genetic and bioinformatics studies have disclosed the fascinating conceptual framework of this pathway, which is conserved from invertebrates to mammals. Our group systematically investigates the piRNA pathway in respect of its molecular architecture as well as its biological functions in Drosophila.

The importance of silencing selfish genetic elements

Eukaryotic genomes are densely populated by selfish genetic elements. Among these, the most widespread members belong to the class of mobile elements known as transposons. Nearly 50% of the human genome is composed of transposons and their sequence remnants. The remarkable success of these "genome parasites" derives from their ability to multiply within the genome by transposition to new sites. This leads to widespread defects, ranging from insertional mutagenesis to ectopic chromosomal recombination, ultimately resulting in reduced long-term fitness of the host.
The acute threat posed by transposable elements has triggered the evolution of powerful defense systems in eukaryotes. Although early genetic studies pointed to the existence of such host defense systems, their molecular nature remained unclear for a long time. However, after the discovery of RNA interference (RNAi) in 1998, it has become increasingly evident that small RNA pathways are at the root of the eukaryotic answer to the transposon challenge.

The piRNA pathway – a small RNA based genome immune system

The piRNA pathway is an evolutionarily conserved small RNA silencing pathway acting in animal gonads. It is the central genome surveillance system that suppresses the activity of transposons. A conceptual framework for this pathway has emerged in the last five to ten years: the genome stores transposon sequences in specific heterochromatic loci known as piRNA clusters. These provide the RNA substrates for the biogenesis of 23- 29-nt-long piRNAs. Within germline cells, an intricate amplification cycle steers piRNA production predominantly to those cluster regions that are complementary to transposons being active at a given time. Finally, piRNAs guide a protein complex centered on PIWI proteins to complementary transposon RNAs in the cell, leading to their silencing (Figure 1A, B).

In stark contrast to other RNAi pathways, the mechanistic framework of the piRNA pathway is largely unknown. We are only starting to understand processes such as piRNA biogenesis or PIWI-mediated silencing. Moreover, the spectrum of biological processes influenced by the piRNA pathway is poorly understood. piRNAs are not only derived from transposon sequences, but also from various other genomic repeats that are enriched at telomeres or heterochromatin. The presence of repetitive sequences throughout the genome is probably used to control vital aspects of chromosome biology. It would not be surprising if the piRNA pathway were also a key player in the mutual relationship between genome and transposons.

We use *Drosophila melanogaster* as a model to study this fascinating genome surveillance system. For most projects we combine genetics, biochemistry, cell biology and computational biology. Our main areas of interest are the following:

1. **Identifying and characterizing novel piRNA pathway members:** We have established robust RNAi conditions for both, the somatic ovarian cells in which a simplified piRNA pathway is active, and for germline cells in which many piRNA pathway factors act in a specific manner (Figure 1, 2). Using these *in vivo* RNAi systems, we performed genome-wide screens and identified several novel piRNA pathway genes in *Drosophila*. Their genetic and molecular characterization will lead to a better understanding of the pathway at all levels - ranging from piRNA cluster biology to piRNA biogenesis and piRNA-mediated silencing.

2. **Dissecting Piwi-mediated transcriptional silencing:** Of the three *Drosophila* PIWI family proteins, Piwi is the only nuclear protein. Recent work has disclosed that Piwi is guided to nascent transposon RNAs and orchestrates highly effective transcriptional silencing, which is accompanied by heterochromatin formation. We are actively investigating the genetic and molecular basis of this process.

3. **Understanding the biology of piRNA clusters:** piRNA clusters are at the heart of the pathway because they serve as sequence repositories for transposons. They are typically located at telomeres or at the border between euchromatin and heterochromatin. Their transcripts are believed to traverse large (up to several hundreds of kb) heterochromatic regions. We are interested in the transcription, specification, export and processing of piRNA clusters or their transcripts. Ultimately we wish to understand how the cell distinguishes cluster transcripts from other RNAs in the cell, and how chromatin signatures of clusters participate in their biology.

Publication highlights

Sienski G, Dönertas D, Brennecke J. (2012) Transcriptional Silencing of Transposons by Piwi and Maelstrom and Its Impact on Chromatin State and Gene Expression Cell. 22;151(5)

Olivieri D, Senti KA, Subramanian S, Sachidanandam R, Brennecke J. (2012). The Cochaperone Shutdown Defines a Group of Biogenesis Factors Essential for All piRNA Populations in Drosophila. Mol Cell. 28;47(6):954-69.

Brennecke, J., Aravin, AA., Stark, A., Dus, M., Kellis, M., Sachidanandam, R., Hannon, GJ. (2007). Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell. 128(6):1089-103

Figure 1

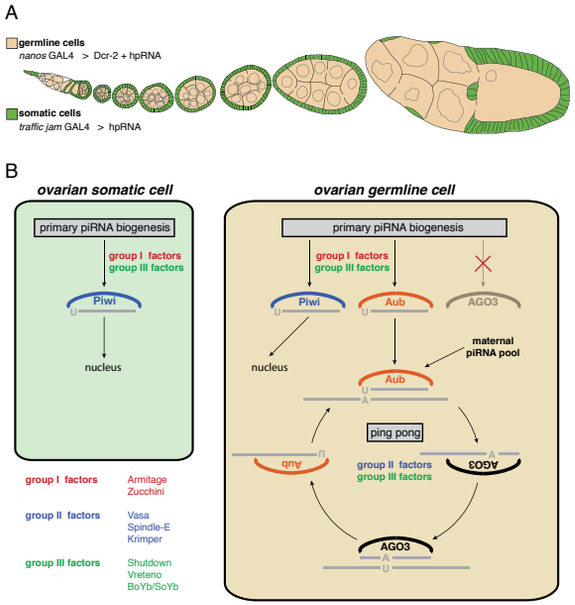
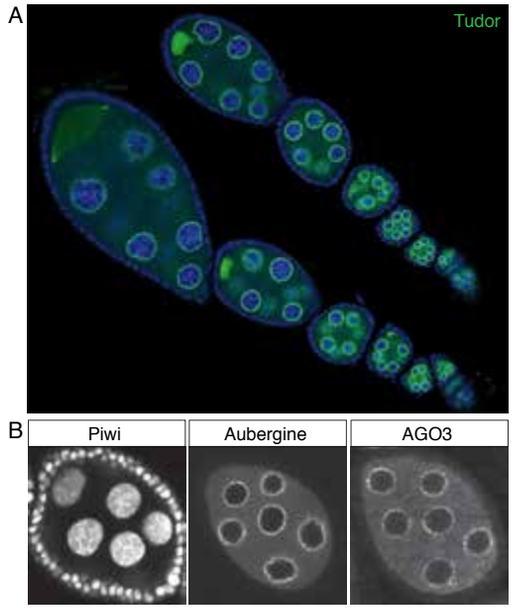


Figure 2



SENIOR SCIENTIST :
JULIUS BRENNECKE

POSTDOCS: PETER REFSING ANDERSEN, RIPPEI HAYASHI, FABIO MOHN, KIRSTEN SENTI
PHD STUDENTS: JULIA BATKI, DOMINIK HANDLER, GRZEGORZ SIENSKI, MICHAELA STEINER
DIPLOMA STUDENT: JAKOB SCHNABL
FLY TECHNICIAN: KATHARINA MEIXNER
COMPUTER SCIENTIST: DANIEL JURCZAK

Figure 1: Scheme illustrating the piRNA pathway acting in the *Drosophila* ovary. (**A**) This cartoon depicts an ovariole, one of the functional units of an ovary. The two major cell types (somatic support cells and germline cells) are shown in green and beige. (**B**) Schematic summary of the piRNA pathway architecture in somatic (left) and germline cells (right). The illustration shows the wiring of three PIWI family proteins - Piwi, Aubergine and Ago3 - into piRNA biogenesis processes. Genetically identified piRNA biogenesis factors acting at the various indicated steps are also shown.

Figure 2: Subcellular locations of PIWI family proteins. (**A**) Two ovarioles stained for DNA (blue) and the piRNA pathway protein Tudor (green). Tudor is enriched in a peri-nuclear structure known as nuage, where piRNA biogenesis is believed to occur. (**B**) Subcellular localizations of the three PIWI family proteins. Note that only Piwi is a nuclear protein; Aubergine and Ago3 are enriched in nuage.

ULRICH ELLING GROUP

Functional Genomics in Embryonic Stem Cells

http://www.imba.oeaw.ac.at/research/ulrich-elling

Embryonic stem cells (ES cells) as well as induced pluripotent stem cells (iPS cells) are promising in that they could revolutionize personalized medicine. This is because they can be expanded in vitro as well as differentiated into various patient-specific cell types Such cells can then be used to study the underlying disease mechanism or even replace patient tissue . We aim to understand the genetic cascades governing cell state as well as lineage decisions of early development by systematic genetic approaches employing genome-wide screens facilitated by the generation of haploid ES cells.

In order to allow for systematic saturating screens in ES cells, we generated haploid murine embryonic stem cells and developed genetic tools for forward and reverse genomic approaches. This setup combines the power of yeast genetics with the pluripotency of embryonic stem cells (Figure 1). In the *Omics* age, high-throughput genetic platforms are vital for the validation of genome-wide datasets as well as for performing saturated genetic screens in mammalian systems. While recent technologies such as RNAi and CRISPR have provided forward genetic screens, their saturation, off-target effects, and reproducibility still pose serious experimental challenges which have not been fully addressed yet. Our setup of haploid genetics is based on very high numbers of independent mutations per gene, thereby overcoming many challenges of genome-wide screening and thus allowing for very clean separation of signal from noise (Figure 2). Using this setup, we study the genetic pathways underlying development and disease.

Pluripotency and early development

Currently we are using haploid genetics to systematically probe the genetic framework governing early embryonic development including differentiation, dedifferentiation, reprogramming, lineage decisions, and epigenetic modifications. We ask questions such as: what genes are required for ES cell maintenance, and what genes are needed for differentiation? Or what genes are involved in lineage decisions and control of the epigenetic environment? To this end, we use reporter-driven screening approaches in haploid ES cells, or complementary systems where indicated. Analysis of screens is based on massive parallel sequencing of genomic insertion sites of mutagens.

Our goal is to identify genetic triggers that allow for more efficient lineage transition, facilitating the achievement of a better experimental regimen for ES cell dedifferentiation and differentiation.

Depletion/Dropout Screens

Dropout screens are a standing challenge for functional genomics due to the high demands of signal-to-noise ratios and the quantity of data required for analysis. Haploid genomics is promising in that it allows for genome-wide depletion screens. We intend to develop the system further to answer questions beyond what is currently achievable by classical means. These questions include the following: What mutations are lethal in synergy with an oncogenic lesion? What mutations cause hypersensitivity to compounds with unknown specificity, i.e. destabilize the drug target pathway? What genes are required for particular cell states and cellular responses? We use improved massive parallel sequencing protocols as well as our own genetic tools to address these questions.

Collaborations

The technology-driven nature of the approach fosters multiple interactions and collaborations within and outside the campus. Besides, our team interacts closely with Haplobank, an archive of mutated and sequenced conditional ES cell lines that we have jointly set up and operated over the last few years. Testing cell lines of interest harboring specific conditional mutations (of the 100,000 available cell lines) by the end of the year will permit rapid validation of hits identified in genetic screens.

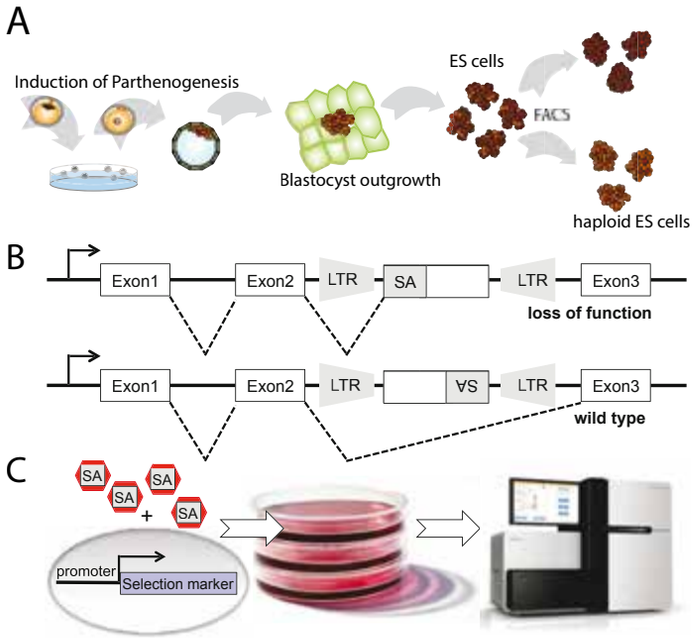
Furthermore, in close collaboration with several industrial partners we employ haploid genomics for drug target predictions.

Publication highlights:

Elling, U., and Penninger, J.M. (2014). Genome wide functional genetics in haploid cells. FEBS letters 588, 2415-2421.

Figure 1

Figure 2



TEAM LEADER:
ULRICH ELLING

TRAINEE: STEPHANIE WACHNER
PHD STUDENT: SERGEI ZHUK
STUDENT ON INTERNSHIP: MARIETTA ZINNER

Figure 1: Artistic illustration of the developmental potential of (haploid murine) embryonic stem cells: Tibor Kulcsar

Figure 2: **A**) Induction of parthenogenetic development and derivation of haploid embryonic stem cells by FACS purification of cells with haploid genome content.
B) Insertional mutagenesis disrupts gene function when splice acceptors (SA) are integrated in the orientation of gene transcription.
C) Outline of haploid genetic screens: mutagenesis, selection of desired phenotypes from pools of many million mutations, identification by deep sequencing.

DANIEL GERLICH GROUP

Assembly and function of the cell division machinery

www.imba.oeaw.ac.at/research/daniel-gerlich/

When cells enter mitosis, they undergo extensive internal reorganization to enable chromosome segregation and partitioning of cell organelles into nascent daughter cells. Our goal is to understand how cells assemble mitotic chromosomes, the mitotic spindle, and the cytokinesis machinery. We also aim to elucidate how signaling networks coordinate the different steps of cell division, and how cells reassemble interphase organelles after mitosis.

Mitotic chromosome formation

During the interphase, a set of chromosomes fills the cell nucleus as a single mass of chromatin, serving as a template for regulated gene expression. Upon mitotic entry, each chromosome forms a separate rod-shaped body that moves independently on the mitotic spindle (Figure 1). Despite the fundamental importance of this phenomenon for genome segregation, we know very little about the three-dimensional organization of mitotic chromosomes and their biophysical properties. Our laboratory has developed methods to investigate the biomechanical properties of mitotic chromosomes. We discovered that mitotic chromosomes do not adhere to each other during early stages of mitosis, whereas they cluster during mitotic exit to form a single mass of compact chromatin prior to nuclear envelope reformation. Ongoing experiments are aimed at elucidating the molecular machinery mediating biophysical changes on the chromosome surface.

Mechanism of cytokinetic abscission

After mitotic chromosome segregation, cytokinesis engages two filament systems to partition cellular organelles into nascent daughter cells. First, a contractile ring composed of actin and myosin filaments mediates ingression of the cleavage furrow between the two spindle poles. This results in the formation of a narrow membrane tube known as the intercellular bridge, which is subsequently split during abscission adjacent to a central region termed the midbody. Abscission proceeds by secondary constriction of the intercellular bridge, involving polymers of the endosomal sorting complex required for transport (ESCRT-III) (Figure 2). How ESCRT-III polymers reorganize during membrane constriction and how this contributes to abscission are poorly understood. Using photobleaching assays and fluorescently tagged subunits, we found that ESCRT-III polymers are intrinsically dynamic during all stages of abscission. Current research is focused on understanding the molecular factors regulating ESCRT-III subunit turnover and its contribution to constriction of the intercellular bridge.

Computer vision and machine learning for cell biology

Recent advances in microscope automation permit high-throughput live-cell imaging of dynamic processes such as cell division. To cope with the tremendous complexity of data generated in these experiments, our laboratory has developed computer vision and machine learning methods for automated cell phenotyping. We have established the open-source software platform *CellCognition* (*http://www.cellcognition.org*) for cellular phenotype discovery by supervised and unsupervised machine learning methods. Our current efforts are focused on integrating the in-house computer vision and machine learning methods and the microscope-controlling software to establish a fully automated experimental workflow for complex interactive perturbation experiments while cells divide on the microscope.

Publication highlights:

A. E. Dick and D. W. Gerlich. Kinetic framework of spindle assembly checkpoint signalling. Nature Cell Biology (2013).

Q. Zhong, A. G. Busetto, J. P. Fededa, J. M. Buhmann, and D. W. Gerlich. Unsupervised modeling of cell morphology dynamics for time-lapse microscopy. Nature Methods (2012) 9(7): 711-713

J. Guizetti, L. Schermelleh, J. Mäntler, S. Maar, I. Poser, H. Leonhardt, T. Müller-Reichert, and D. W. Gerlich. Helices of ESCRT-III-dependent filaments in a cortical constriction mechanism of abscission. Science (2011) 331(6024): 1616-20

P. Steigemann, M. Schmitz, C. Wurzenberger, J. Guizetti, M. Held, S. Maar, and D. W. Gerlich. Aurora B-mediated abscission checkpoint protects against tetraploidization. Cell (2009) 136(3): 473-84

Figure 1

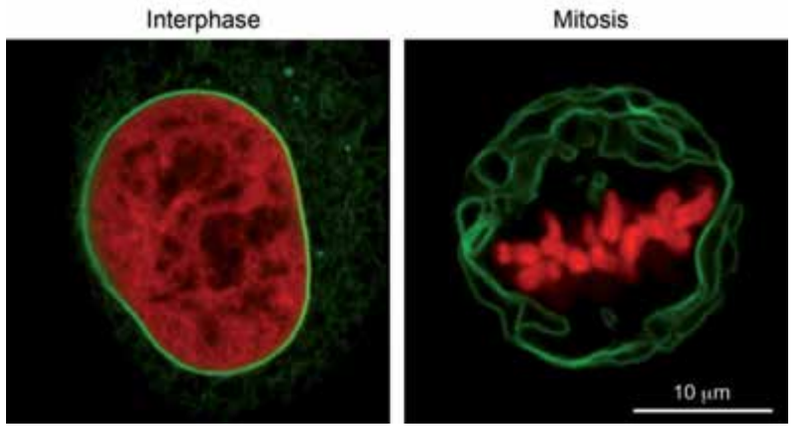
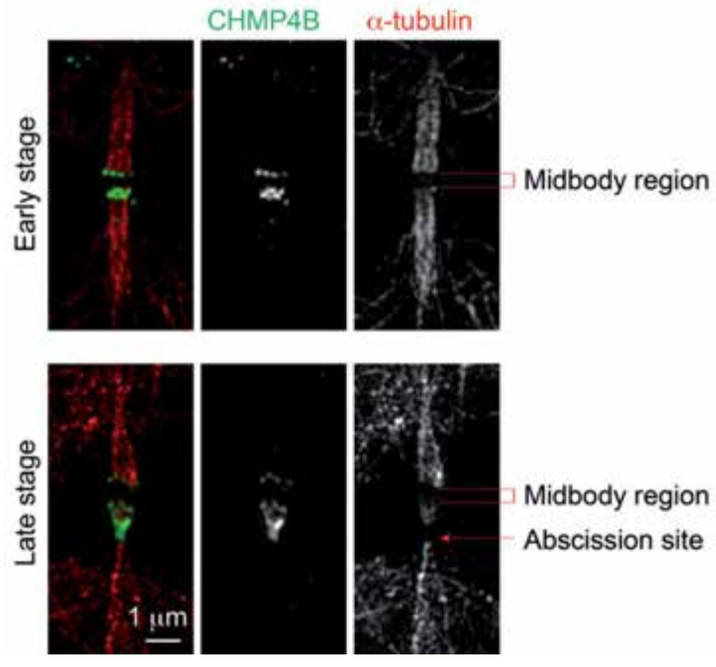


Figure 2



SENIOR SCIENTIST:
DANIEL WOLFRAM GERLICH

POSTDOCS: SARA CUYLEN, AMALIE DICK, JUAN PABLO FEDEDA, MATTHIAS SAMWER, CHRISTOPH SOMMER, FELIX SPIRA
PHD STUDENTS: ANA DAVID, BEATA MIERZWA, RUGILE STANYTE
TECHNICAL ASSISTANT: CLAUDIA BLAUKOPF
BIOINFORMATICIAN: RUDOLF HÖFLER

Figure 1: Chromosome morphology during interphase and mitosis. Confocal microscopy images of live HeLa cells expressing a marker for chromatin (histone 2B fused to mCherry, red) and the nuclear envelope (Lap2β fused to EGFP, green). The set of chromosomes fills the nuclear volume as a single mass of chromatin during the interphase. During mitosis, the nuclear envelope disassembles and Lap2β-EGFP localizes to the endoplasmic reticulum. Mitotic chromosomes appear as spatially separate rod-shaped bodies.

Figure 2: ESCRT-III localization at the abscission site. Super-resolution fluorescence microscopy of the intercellular bridge in early and late stages of abscission. A HeLa cell expressing a tagged ESCRT-III subunit (CHMP4B-EGFP, green) was stained with anti-α-tubulin antibody (red) and imaged on a 3D-structured illumination microscope. During early stages of abscission, ESCRT-III localizes at two rings adjacent to the midbody, whereas during late stages it extends as a cone-shaped structure towards the abscission site, where microtubules disassemble. Note that microtubules in the midbody are not stained by the anti-α-tubulin antibody.

FUMIYO IKEDA GROUP

Dynamic arrangement of linear ubiquitination

www.imba.oeaw.ac.at/research/fumiyo-ikeda/

Ubiquitin is a small regulatory modifier protein, which is highly conserved from yeast to humans. The modification is known as ubiquitination, and is a dynamic post-translational modification system. It is responsible for regulating numerous biological functions, including inflammation, cell death, autophagy, cancer, and the cell cycle. We are especially interested in understanding the roles of a specific type of ubiquitination - known as linear ubiquitination - in the regulation of various cellular functions.

The linear ubiquitin chain assembly complex (LUBAC)

Linear ubiquitin chain is a unique linkage type of the ubiquitin polymer, linked through the Met 1 residue instead of commonly used Lys residues, such as Lys 6, Lys 11, Lys 27, Lys 29, Lys 33, Lys 48 and Lys 63 (Figure 1A). A little is known about linear ubiquitin chains because they have been discovered just recently. The only known linear ubiquitination-specific enzymatic complex is LUBAC, which consists of HOIP, Sharpin and HOIL-1L (Figure 1B). We have previously shown that LUBAC plays a critical role in the regulation of tumor necrosis factor (TNF)-induced NF-kB signaling. However, we do not understand yet how the activity of LUBAC in generating linear ubiquitin chains is regulated upon TNF stimulation. To elucidate the molecular mechanisms how LUBAC activity is regulated, we first established an *in vitro* ubiquitination assay. This assay monitors the formation of ubiquitin chains by using the recombinant proteins of HOIP, Sharpin and HOIL-1L. Similar to HHARI E3 ligase, which was shown to be the first example of the 'HECT-RING hybrid' type of E3 ligase, we identified a conserved Cys885 residue in the HOIP catalytic domain (Figure 1C). Our current research shows that this is crucial for enzymatic activity (Figure 1D). This suggests that the Cys885 residue is used for the ubiquitin loading site at the intermediate status of ubiquitin transfer to substrates like HHARI, in line with a recent publication (Stieglitz B et al., 2013). Additionally, the HOIP Cys885 mutant no longer activated NF-kB signaling in cells (Figure 1E). Collectively these data suggest that HOIP functions as a HECT-RING type of E3 ligase, and the process of linear ubiquitination is crucial for NF-kB activation. By focusing on the formation of the specific signaling complex and protein modifications, we are analyzing how the catalytic activity of HOIP is regulated upon TNF stimulation.

Linear ubiquitination in the regulation of inflammation and cell death

We previously showed that Sharpin, one of the non-catalytic components of LUBAC, plays a critical role in the regulation of the immune system *in vivo*. Inflammation in several organs, including skin tissue, is significantly upregulated in Sharpin-deficient *Sharpin*$^{cpdm/cpdm}$ mice (Figure 2A). Based on histological analysis using the apoptosis marker cleaved caspase 3, we found that apoptosis is significantly upregulated in *Sharpin*$^{cpdm/cpdm}$ skin tissue (Figure 2A). To understand how Sharpin regulates apoptosis, we generated a Sharpin-knockdown keratinocyte line (HaCaT) by stably expressing shRNA against Sharpin, and treated them with TNF (Figure 2B). Identical to the *in vivo* situation, Sharpin knockdown in HaCaT enhanced apoptosis induced by TNF, as determined by the caspase-8 activity assay. Upregulation of the apoptosis signal (caspase-8 activity) in Sharpin-knockdown HaCaT was rescued by additional depletion of FADD (Figure 2B), which is a critical factor for the induction of apoptosis. This suggests that Sharpin regulates apoptosis signaling through an FADD-containing complex known as the TNF-receptor complex II. Interestingly, we found that the knockdown of other LUBAC component, HOIP in HaCaT, also sensitized cells to apoptosis (Figure 2C). This strongly suggests that the LUBAC complex plays a role in the regulation of apoptosis. Currently we are trying to identify the targets of LUBAC-induced linear ubiquitination in the apoptosis signaling pathway, and understand how linear ubiquitination regulates apoptosis at the molecular level.

Publication highlights:

Snehlata Kumari , Younes Redouane , Jaime Lopez-Mosqueda , Ryoko Shiraishi , Malgorzata Romanowska , Stefan Lutzmayer , Jan Kuiper , Conception Martinez , Ivan Dikic , Manolis Pasparakis, Fumiyo Ikeda (2014) Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. eLife, DOI: 10.7554/eLife.03422

Figure 1

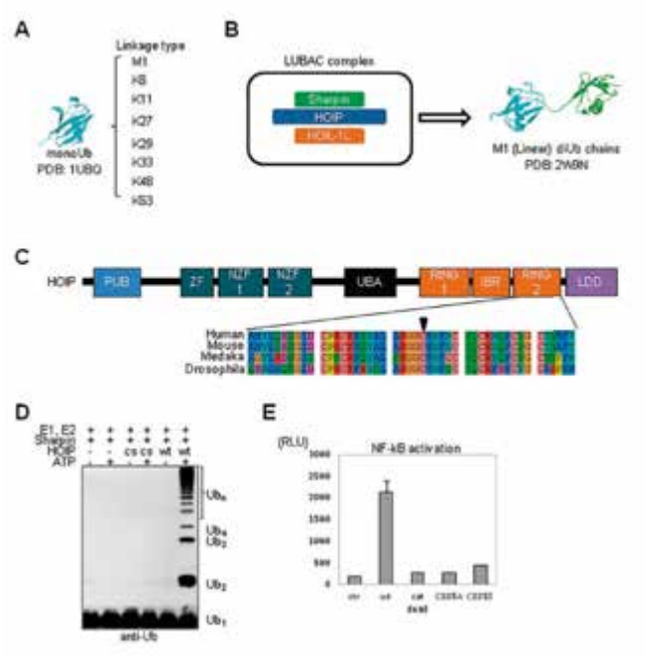
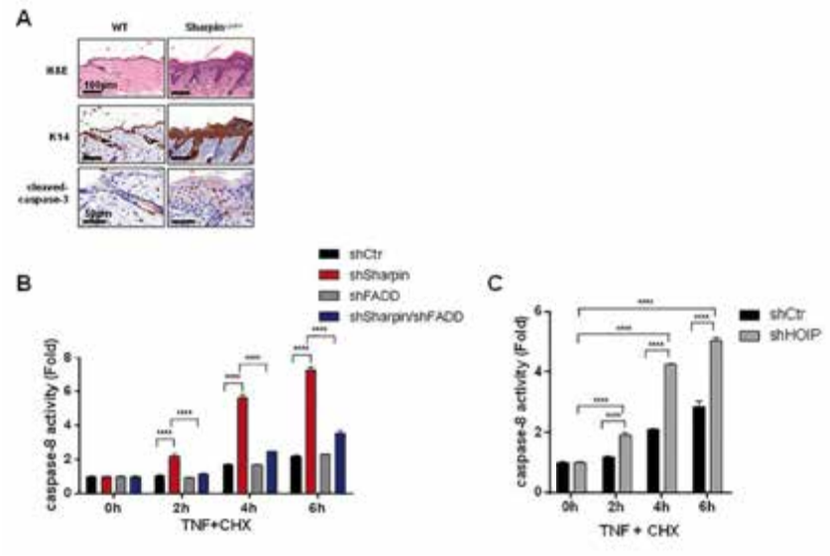


Figure 2



GROUP LEADER:
FUMIYO IKEDA

POSTDOCTORAL FELLOW: TOMOKO ASAOKA
PHD STUDENTS: PETRA EBNER, YOUNES REDOUANE, [1] LILIAN FENNELL
DIPLOMA STUDENT: [2] CHRISTINE EHRHARDT
RESEARCH ASSOCIATE: [3] CONCEPTION MARTINEZ
RESEARCH ASSISTANT: RYOKO SHIRAISHI

[1] FROM OCTOBER, [2] UNTIL AUGUST, [3] UNTIL JULY

Figure 1: Linear ubiquitination is induced by the LUBAC E3 ligase complex. A) Monoubiquitin molecule and amino acid residues used for the ubiquitin polymer formation. **B**) LUBAC components Sharpin, HOIL-1L and HOIP. **C**) A catalytic center located in the 2nd RING domain (RING2) of HOIP. The amino acid sequence of HOIP-RING2 is highly conserved in a variety of species. The arrow indicates C885 in human HOIP, which is also conserved in various species. **D**) *In vitro* ubiquitination assay using recombinant LUBAC. In comparison to HOIP wild type (wt) (lane 6), the HOIP-C885 mutant (lane 4) fails to generate linear Ub chains. **E**) The critical role of HOIP-C885 in NF-kB signaling. The NF-kB reporter assay was performed using C885 mutants (CA and CS), which abolished the ability to mediate NF-kB activation as a catalytic dead (cat dead) mutant.

Figure 2: The linear ubiquitination signal plays a role in the regulation of apoptosis. A) Histological analysis of skin tissue of wild type (wt) and Sharpin-deficient (*Sharpin*$^{cpdm/cpdm}$) mice. Apoptosis is drastically induced in *Sharpin*$^{cpdm/cpdm}$ keratinocytes (cleaved caspase 3). H&E and K14 staining of skin shows a thicker layer of epidermis in *Sharpin*$^{cpdm/cpdm}$. **B**) Caspase-8 activity assay in control, Sharpin-knockdown (shSharpin), and FADD-knockdown (shFADD) human keratinocytes with or without TNF stimulation. In comparison to control cells, shSharpin cells stimulated by TNF showed higher caspase-8 activity. Additional knockdown of FADD in shShapin cells (shSharpin/shFADD) significantly inhibited the activity. **C**) Caspase-8 assay in HOIP knockdown (shHOIP) cells. shHOIP increases the activity induced by TNF.

JÜRGEN KNOBLICH GROUP

Neural stem cells and brain development

www.imba.oeaw.ac.at/research/juergen-knoblich

The human brain is the most complex and fascinating of all organs. Our interest is focused on how a relatively small number of stem and progenitor cells are able to generate the complex structure of the human brain during development. Our group uses Drosophila, mouse, and human genetics to understand how neural stem cells generate the right neurons at the right time, and how defects in neurogenesis lead to the formation of brain tumors or inherited brain disorders.

Neural Stem Cells in *Drosophila*

In the fruit fly *Drosophila*, about 400 neural stem cells known as neuroblasts create all neurons and glia cells in the adult brain (Figure 1A). For this purpose they undergo repeated asymmetric cell divisions, which give rise to a large daughter cell that remains a dividing stem cell, and a smaller daughter that differentiates after a limited number of transit amplifying divisions. During each neuroblast division, the cell fate determinants Numb, Prospero and Brat segregate into the smaller daughter cell, where they prevent self-renewal and induce differentiation (Figure 1A, B). When these are missing, both daughter cells become stem cells, leading to exponential proliferation and the formation of a lethal and transplantable brain tumor.

Eventually, neuroblasts exit the cell cycle and differentiate. Thus, no proliferation occurs in the adult brain. We found that this is caused by a cell-intrinsic mechanism which uncouples cell cycle progression from cell growth at a precisely defined period during development, resulting in cell shrinkage and differentiation. In a transgenic RNAi screen for factors responsible for neuroblast shrinkage, we identified several components of the mitochondrial respiratory chain. Our genetic and biochemical experiments support a model in which a change in energy metabolism induced by ecdysone is responsible for the reduction in cell growth. We propose that the induction of oxidative phosphorylation deprives cells of building blocks for lipid and amino acid biosynthesis, which then accumulate as end products of glycolysis.

Our data show that changes in energy metabolism may be a cause rather than a consequence of cell fate changes. Furthermore, they reveal a surprising connection between energy metabolism and stem cell self-renewal, which has not been described previously in the *in vivo* setting.

Asymmetric cell division in mouse stem cells

In the mouse brain, progenitor cells known as radial glia generate neurons of the cortex through lineages that are strikingly similar to *Drosophila* neuroblasts (Figure 2A). Neural progenitors are located in a neuroepithelium. Initially they expand through symmetric divisions, but later they divide asymmetrically, giving rise to differentiating daughter cells as well (Figure 2B). While the early symmetric divisions are parallel to the surface of the neuroepithelium, later divisions may be oblique or – in rare cases – even vertical. We are using our knowledge derived from Drosophila to understand how cortical progenitor divisions become asymmetric and how lineages are established in the developing cortex.

A *Drosophila* RNAi screen in our laboratory revealed the protein phosphatase PP4 as a key regulator of asymmetric cell division and mitotic spindle orientation. We used a conditional knockout strategy to characterize the single mouse PP4 homolog. We found that PP4 regulates the Lis1/Ndel1 complex and is essential for correct orientation of the mitotic spindle in cortical progenitor divisions in the mouse.

This raised the question as to whether spindle orientation is important for neurogenesis. Surprisingly, we found that spindle orientation is not relevant during the peak stages of neurogenesis, but regulates the transition from symmetric to asymmetric modes of division. As this transition determines the number of progenitors, our data suggest a potential connection between spindle orientation and brain size in mammals (see Xie, et al., Neuron 2013, for details).

Modeling human cortical development

To determine whether mechanisms regulating asymmetric cell division and stem cell lineages are conserved in humans as well, we established a 3D culture model for human forebrain development (Lancaster et al., Nature 2013). We use established human ES cell lines or iPS cells to create cerebral organoids that recapitulate the development of the human brain in culture with remarkable detail (Figure 3). Cerebral organoids contain the human dorsal and ventral cortex, the choroid plexus, the retina, and sometimes the hippocampus. They recapitulate human cortical features such as the presence of an outer subventricular zone or an inner fiber layer. We demonstrated the usefulness of our organoid system by modeling microcephaly, a human disorder resulting in a severe reduction of cortical size. Our data reveal spindle orientation defects leading to premature neural differentiation at the expense of the progenitor population, which could be responsible for the disease. The establishment of a 3D culture system that recapitulates the key aspects of human forebrain development will allow us to efficiently transfer our knowledge from *Drosophila* to humans, and to determine the cellular basis of developmental disease in the human brain.

Figure 1

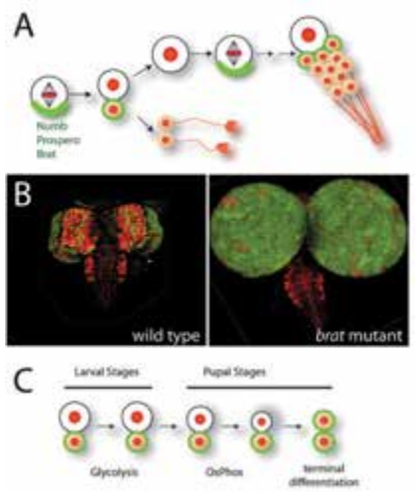


Figure 2

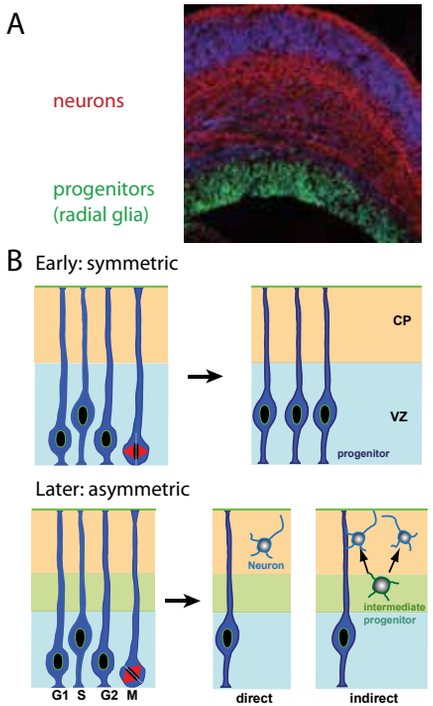


Figure 3

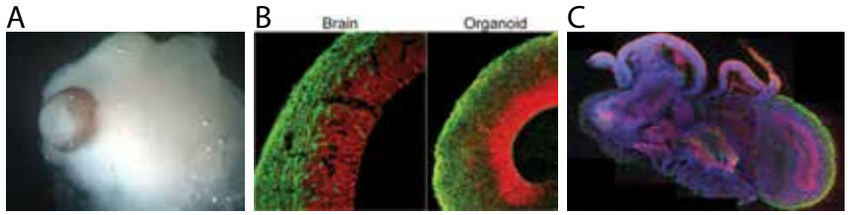


Figure 1: How cells divide asymmetrically. A. Drosophila neuroblasts (white) divide asymmetrically to generate self-renewing stem cells (white) and differentiating neurons (red). During each neuroblast division, Brat, Prospero and Numb (green) segregate into the differentiating daughter cell **B.** Larval brain from a wild type (left) and *brat* mutant animal. Neuroblasts are green and differentiating neurons are red. *Brat* brains show a dramatic over-proliferation of neuroblasts. **C.** neuroblasts regrow after each division during the larval proliferative stages. After pupariation they switch to oxidative phosphorylation. As a result their growth is reduced, they become smaller, and ultimately undergo a terminal symmetric division.

Figure 2: Analysis of progenitor cell proliferation in the mouse brain. A. Cross-section through the developing mouse neocortex (DNA in blue) on day 15 of embryonic development. Anti-TuJ1 labels early differentiating neurons (red) while radial glia progenitors are marked by anti-Pax-6 (green). **B.** Cortical progenitors (blue) in the ventricular zone (VZ, light blue) divide symmetrically during early stages of cortical development and switch to an asymmetric division mode during neurogenesis. While symmetric divisions are strictly parallel to the epithelial surface (mitotic spindles are shown in red), asymmetric divisions occur at oblique or even vertical angles. Asymmetric divisions give rise either to differentiating neurons that migrate into the cortical plate (CP, orange), or intermediate progenitors (green) that divide again to generate two neurons. These two modes are known as direct or indirect neurogenesis, and are regulated by *inscuteable* (see text).

Figure 3: Analysis of progenitor cell proliferation in the mouse brain. A. Cerebral organoids can be derived from human ES or iPS cells. The pigmented area in this brightfield image is a developing human eye. **B.** Comparison of a developing mouse cortex and the corresponding stage in a human cerebral organoid; red: progenitor cells; green: differentiating neurons. **C.** Cross-section through a cerebral organoid. Proliferating progenitor cells are red (labeled by anti Sox2), differentiating neurons are green (marked by anti TuJ1), and DNA is blue. See Lancaster et al., Nature 2013, for details.

SENIOR SCIENTIST & DEPUTY DIRECTOR/SCIENCE:
JÜRGEN KNOBLICH

POSTDOCS: BIAN SHAN, CORSINI NINA STEFANIE, DE CERTIMA F. HOMEM CATARINA, ESK PETER-CHRISTOPHER, LANCASTER MADELINE, NEUMÜLLER RALPH, REICHARDT ILKA, REPIC MARKO
PHD STUDENTS: ABDUSSELAMOGLU MERVE DENIZ, ABRAMCZUK MONIKA KRISTINA, LANDSKRON LISA, RENNER MAGDALENA, SINITCYN IVAN, WISSEL SEBASTIAN
RESEARCH ASSOCIATE: KLEINER ELKE
RESEARCH ASSISTANT: PEER ANGELA MARIA

Publication highlights:

Lancaster, M.A., Renner, M., Martin, C.-A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger J.M., Jackson, A.P. and Knoblich, J. A. (2013). Cerebral organoids model human brain development and microcephaly. Nature, 501, 373-9.

Xie, Y., Jüschke, C., Esk, C., Hirotsune, S., Knoblich, JA. (2013). The Phosphatase PP4c Controls Spindle Orientation to Maintain Proliferative Symmetric Divisions in the Developing Neocortex. Neuron. 79, 254-65
Neumüller R.A., Constance Richter, C., Fischer, A., Novatchkova, M., Neumüller, K.G. and Knoblich, J.A. (2011) Genome wide analysis of self-renewal in Drosophila neural stem cells by transgenic RNAi. Cell Stem Cell 8, 580-593.

Mummery-Widmer, J.L., Yamazaki, M., Stoeger, T., Novatchkova, M., Chen, D., Dietzl, G., Dickson, B.J., and Knoblich, J.A. (2009) Genome-wide analysis of Drosophila external sensory organ development by transgenic RNAi, Nature, 458, 987-992.

Wirtz-Peitz, F., Nishimura, T., and Knoblich, J.A. (2008). Linking cell cycle to asymmetric division: Aurora-A phosphorylates the Par complex to regulate Numb localization, Cell, 135, 161-173.

THOMAS MARLOVITS GROUP

Molecular Machines

www.imba.oeaw.ac.at/research/thomas-marlovits

Membrane-associated processes are a fundamental characteristic of all living cells because they ensure that the cells are able to effectively communicate with, and adapt to, their environment. Cells achieve this by either physically translocating molecules to the opposite site of a membrane, or by receiving, transmitting, and amplifying incoming signals. Our laboratory is interested in understanding the molecular mechanism underlying such processes. Specifically, we focus on machineries capable of translocating bacterial toxins into eukaryotic cells.

Microbial Pathogenesis

Gram-negative pathogens such *Yersinia, Shigella, Pseudomonas, enteropathogenic/enterohemorrhagic E. coli* (*EPEC/EHEC*) and *Salmonella* in animals are causative agents for many diseases in animals and humans. The diseases have mild to deadly outcomes, and often originate as food-borne diseases. A crucial element of their pathogenicity are bacterial toxins ('effectors'), which are delivered via the type III secretion system (a large membrane-embedded machinery) from the bacterium to its host cell. As a consequence, translocated effector proteins have the remarkable capacity to modulate various host-cell pathways, including endocytic trafficking, gene expression, programmed cell death, or cytoskeleton dynamics that induce membrane ruffling and subsequently render the host accessible to bacterial infection.

Unfolded protein transport across membranes?

Safe and directional transport of effector proteins across membranes is the hallmark function of all type III secretion. Our recent structural analysis (Schraidt & Marlovits, Science, 2010) of the injectisome, the most prominent cylindrical structure of the type III secretion system, revealed a potential secretion path through the central part of the membrane-embedded complex. However, the inner diameter of this path is too small to accommodate a fully folded effector protein, suggesting that either the injectisome must undergo large conformational changes during transport, or effector proteins must be unfolded.

To investigate type III secretion of human pathogens, we focused on (1) determining the secretion path of injectisomes, (2) understanding the mechanism of transport, and (3) visualizing protein transport *in situ*. In order to address these questions, we first analyzed the requirements for substrate association with, transport through, and exit through the injectisome. Surprisingly, we found that the size and length of novel substrates does not exert a major impact on their secretability. We learned that the fusion of thermodynamically stable protein domains to otherwise secreted substrates does not influence successful engagement to the injectisome, but prevents complete transport across membranes. Such designed and trapped substrates are commonly associated with injectisomes.

We discovered that the substrates are inserted into the secretion path in polar fashion (N-terminal regions first), suggesting that other substrates with a similar domain organization follow the same principle. Our structural analysis of trapped substrates revealed that they are in an unfolded state during transport, suggesting that the type III-specific ATPase acts as an unfoldase. In contrast, injectisomes stay largely invariant during protein transport. To understand whether such behavior is in fact observed *in situ*, we performed cryo-electron tomography. This method permits the investigation of molecular structures within cells in a spatiotemporal manner and in a near-native state. For the first time we were able to visualize pathogenic type III secretion systems from *Salmonella* in action and - more generally - protein transport across several membranes.

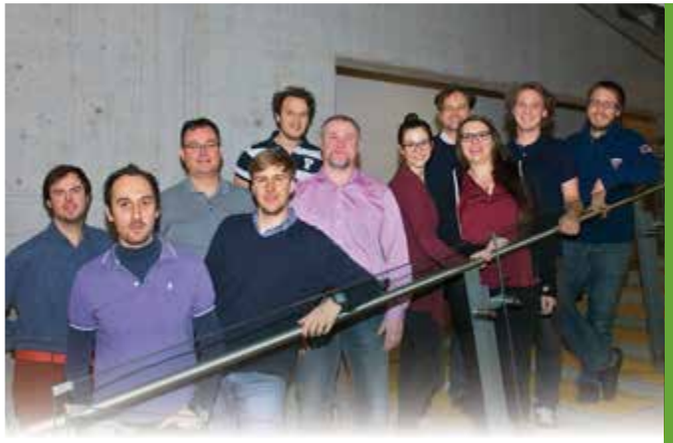
By understanding the molecular mechanism of TTSS-mediated protein transport, we hope to provide a basis for the development of novel therapeutic strategies that will either inhibit its activity or modify the system for targeted drug delivery.

Publication highlights:

Galan JE, Lara-Tejero M, Marlovits TC, Wagner S (2014). Bacterial type III secretion systems: specialized nanomachines for protein delivery into target cells. Ann Rev Microbiology 8;68:415-38

Radics J, Königsmaier L, Marlovits TC (2014). Structure of a pathogenic type 3 secretion system in action. Nature Structural & Molecular Biology 21(1):82-7

Schraidt O., Marlovits TC (2011). Three-dimensional model of Salmonella's Needle Complex at Subnanometer Resolution. Science 331:1192-95



JOINT IMP/IMBA GROUP LEADER:
THOMAS C MARLOVITS

POSTDOCS: LUCIANO CICCARELLI, NIKOLAUS GÖSSWEINER-MOHR, OLIVER VESPER
PHD STUDENTS: MATTHIAS BRUNNER, ANTOINE CHABLOZ, JIRI WALD, JAN ZALESAK
MASTER STUDENTS: PHILIPP AUSSERHOFER, TOBIAS THÖNI
RESEARCH TECHNICIAN: JULIA MAYR
ROTATION STUDENTS: BARBARA BACHMANN, DOMINIK LINDENHOFER, NICOLE NUSSBAUM, GREGOR WEISS
COMPUTATIONAL EXPERT: WOLFGANG LUGMAYR

Figure 1

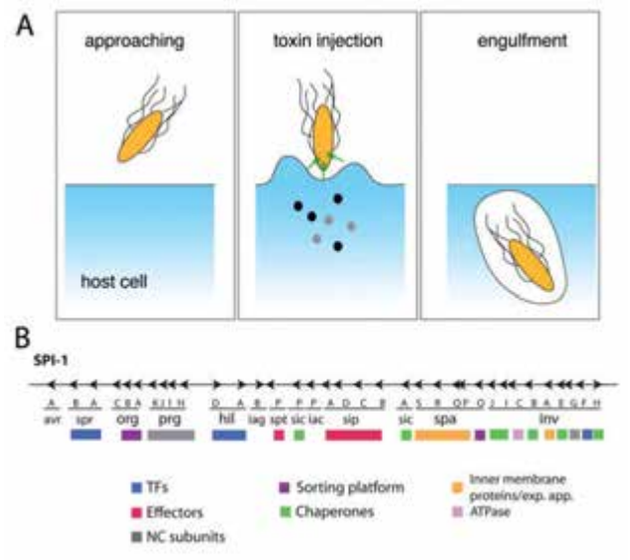


Figure 2

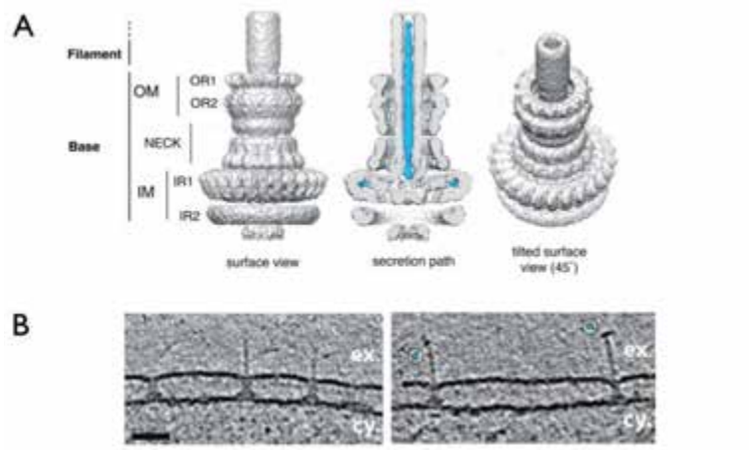


Figure 1: (**A**) Initial steps of the infection cycle mediated by type III secretion-specific delivery of bacterial toxins into host cells (**B**) Organization of Salmonella pathogenicity island 1

Figure 2: (**A**) Structure of the membrane-embedded (grey) and substrate-trapped (blue) injectisome (**B**) *In situ* visualization of substrate-free and substrate-trapped injectisomes by cryo-electron tomography

JAVIER MARTINEZ GROUP

Novel RNA metabolic Enzymes: Mechanisms, Biology, and Disease.

www.imba.oeaw.ac.at/research/javier-martinez

RNA molecules need to be processed and modified in order to become functional. Mutations in the responsible enzymes have been linked to disease. At IMBA we discovered CLP1 as an RNA-kinase activity associated with the tRNA splicing endonuclease (S. Weitzer and J. Martinez, 2007) and the long-sought human tRNA ligase, a pentameric complex with RTCB, as the catalytic subunit that joins tRNA exon halves (J. Popow et al., 2011 and 2012). More recently, combining bioinformatics and biochemistry, we identified Archease as an essential co-factor of the tRNA ligase complex (J. Popow et al., 2014). The binding of RTCB to non-tRNA targets suggests its involvement in other biological processes. In this annual report we describe the role of the tRNA ligase and Archease in the non-canonical splicing of Xbp1 mRNA during the unfolded protein response. We also outline the study of the mammalian RNA 3' phosphate cyclase, RTCD1.

The tRNA ligase complex and Archease constitute the elusive RNA ligation activity in the IRE1 axis of the unfolded protein response.

The tRNA ligase complex, together with its cofactor Archease, executes tRNA splicing in mammalian cells. The equivalent yeast tRNA ligase Trl1 joins tRNA exon halves and, surprisingly, also splices the *HAC1* mRNA during the unfolded protein response (UPR) - a stress-signaling pathway activated by the accumulation of unfolded proteins in the endoplasmic reticulum (ER). The identification of the tRNA ligase complex in mammalian cells prompted us to test its potential role in UPR, through the cytoplasmic splicing of the *XBP1* mRNA and leading to the synthesis of the transcription factor XBP1s (s for spliced), in order to re-establish homeostasis in the ER. Thus, we depleted RTCB and/or Archease by doxycycline(Dox)-inducible expression of small hairpin RNAs (shRNAs) using the miR-E backbone developed by Johannes Zuber's laboratory at the IMP. Following UPR induction with thapsigargin (Tg), an inhibitor of ER Ca^{+2}-ATPases, we observed reduced XBP1s expression mainly in Archease-depleted cells. RT-PCR experiments also revealed a reduced quantity of *XBP1s* mRNA only upon Archease knockdown, resulting in a decreased ratio of spliced (*XBP1s*) to un-spliced (*XBP1u*) mRNA. To fully abrogate the generation of XBP1s, we simultaneously depleted RTCB and Archease in HeLa cells. After Tg treatment, XBP1s was no longer detectable at the protein level (Figure 1A), and greatly reduced at the mRNA level (Figure 1B). Thus, sufficient inhibition of tRNA ligase activity can only be achieved by simultaneously targeting Archease.

Accumulation of XBP1s leads to the transcriptional activation of downstream target genes, such as the ERAD component *EDEM1* and the co-chaperone DNAJB9, which serve to reduce the load of unfolded proteins within the ER. We observed increased expression of *EDEM1* and *DNAJB9* mRNA in control cells after 8-16 hours of Tg treatment. This response was abolished in cells depleted of RTCB and Archease.

Antibody-secreting plasma cells feature a constitutively active UPR. B cells deficient in XBP1s are unable to expand ER structures, and mice lacking XBP1 show reduced serum immunoglobulin levels and impaired immunoglobulin response to immunization. We studied the *in vivo* function of RTCB during plasma cell differentiation by establishing a B cell-specific mouse knockout model – $Rtcb^{fl/fl}$ *Cd23*-Cre – that initiates Cre-mediated deletion in immature B cells of the spleen and leads to efficient gene deletion in all mature B cell types. We stimulated splenic B cells from control ($Rtcb^{fl/fl}$ or $Rtcb^{fl/+}$), heterozygous ($Rtcb^{fl/+}$ *Cd23*-Cre) or homozygous ($Rtcb^{fl/fl}$ *Cd23*-Cre) mice with lipopolysaccharide (LPS) for 4 days to induce differentiation via activated B cells and pre-plasmablasts to plasmablasts. RTCB was absent in $Rtcb^{fl/fl}$ *Cd23*-Cre cells during the entire culture period. We further analyzed the induction of XBP1 and found that, in wild-type and heterozygous B cells, both XBP1u and XBP1s were induced by day 2, whereas RTCB-deficient cells transiently up-regulated XBP1u and failed to produce XBP1s (Figure 2). Both *Xbp1s* mRNA and total *Xbp1* mRNA were significantly reduced in unfractionated RTCB-deficient cells on day 4 of LPS stimulation. After *in vitro* stimulation with LPS, IgM levels measured by ELISA were significantly reduced in culture supernatants of RTCB-deficient pre-plasmablasts and plasmablasts. ELISPOT analysis of sorted plasmablasts revealed similar numbers of IgM-secreting cells for both, the control and experimental genotypes. However, $Rtcb^{fl/fl}$ *Cd23*-Cre plasmablasts gave rise to significantly smaller spots than control plasmablasts, indicating that these cells secrete lower levels of immunoglobulins. We finally examined plasma cell differentiation during a T cell-independent immune response by immunizing mice with trinitrophenyl(TNP)-coupled LPS. On day 14 after immunization, we observed equal numbers of plasma cells in the spleen of immunized control $Rtcb^{fl/fl}$ and $Rtcb^{fl/fl}$ *Cd23*-Cre mice. However, the number of TNP-specific antibody-secreting cells was reduced in $Rtcb^{fl/fl}$ *Cd23*-Cre mice by ELISPOT assay and the size of the spots was also smaller, indicating low immunoglobulin production by antibody-secreting cells deficient in RTCB (Figure 3A). Likewise, TNP-specific serum immunoglobulin levels measured by ELISA were significantly reduced in $Rtcb^{fl/fl}$ *Cd23*-Cre mice (Figure 3B). Together, these data strengthen the *in vitro* results and confirm the notion that the ability of RTCB-deficient B cells to generate plasma cells remains largely intact while their capacity to secrete immunoglobulins is strongly affected.

What is the function of RTCD1, the mammalian RNA 3'- phosphate cyclase?

The RNA 3'-phosphate cyclase (RtcA in bacteria; RTCD1 in mammalian cells) generates 2', 3'-cyclic phosphates from 3' phosphate-ended RNAs. The *in vivo* function of this enzyme has remained unknown. Both in subcellular fractionation experiments and in immunofluorescence staining, RTCD1 was mainly found in the cytoplasm (Figure 4). Mass spectrometry will be performed on FLAG immunoprecipitates from HEK293-stable cell lines expressing wild-type and mutant FLAG-RTCD1 to identify interacting factors. We are also searching for RNA targets through PAR-CLIP. We have already generated a *Rtcd1* conditional knockout mouse, where ubiquitous deletion of RTCB was observed in all tested tissues upon crossing with mice expressing β-actin-Cre.

Figure 1

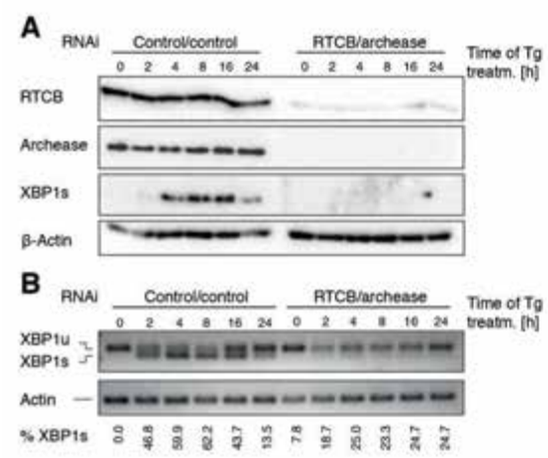


Figure 2

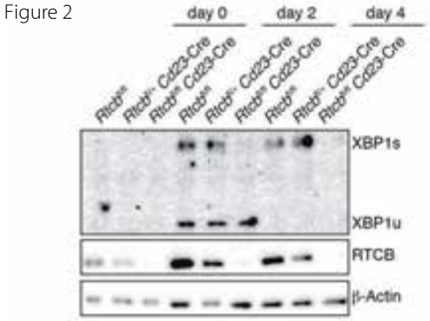


Figure 3

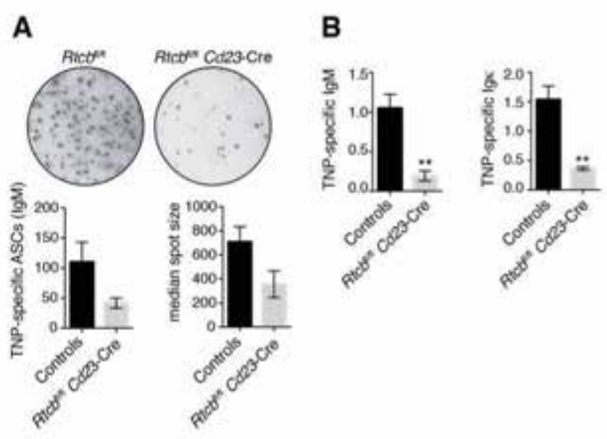
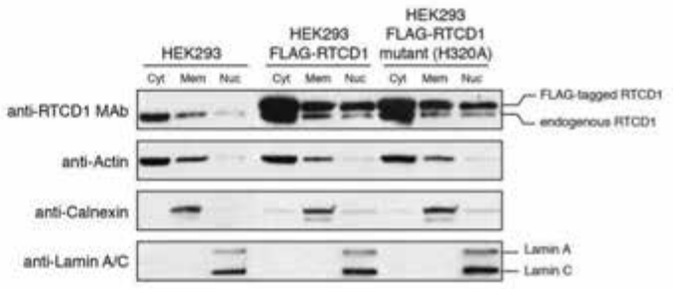


Figure 4



Publication highlights:

Jennifer Jurkin[§], Theresa Henkel[§], Anne Færch Nielsen, Martina Minnich, Johannes Popow, Therese Kaufmann, Katrin Heindl, Thomas Hoffmann, Meinrad Busslinger and Javier Martinez. The mammalian tRNA ligase complex mediates splicing of XBP1 mRNA and controls antibody secretion in plasma cells. [§]Co-first authors. EMBO J. Epub 2014, Nov. 6.

Toshikatsu Hanada, Stefan Weitzer, Josef M Penninger and Javier Martinez. Killing the RNA kinase CLP1: consequences on tRNA metabolism and motor neuron maintenance. Wiley Interdiscip Rev RNA. Epub 2014 Aug 20.

Johannes Popow, Jennifer Jurkin, Alexander Schleiffer and Javier Martinez. Analysis of orthologous groups reveals Archease and DDX1 as tRNA splicing factors. Nature. 2014 Jul 3;511(7507):104-7.

Ender Karaca[#], Stefan Weitzer[#], Davut Pehlivan[#], Hiroshi Shiraishi, Toshikatsu Hanada, Wojciech Wiszniewski, Marjorie Withers, Ian M. Campbell, Serkan Erdin, Sedat Isikay, Luis M. Franco Claudia Gonzaga-Jauregui, Violet Gelowani, Jill V. Hunter, Erkan Koparir, Sarenur Yilmaz, Christian Bernreuther, Markus Glatzel, Siegfried Trattnig, Joachim Friske, Claudia Kronnerwetter, Matthew N. Bainbridge, Alper Gezdirici, Mehmet Seven, Donna M. Muzny, Eric Boerwinkle, Mustafa Ozen[7], Centers for Mendelian Genomics, Tim Clausen[13], Adnan Yuksel[7], Richard A. Gibbs, Javier Martinez, Josef M. Penninger*, James R. Lupski*. [#] co-first authors, *co-corresponding authors. Identification of a novel human neurological syndrome defined by CLP1 mutations that impair tRNA splicing. Cell. 2014 Apr 24;157(3):636-50.*

GROUP LEADER:
JAVIER MARTINEZ

POSTDOCS: JENNIFER JURKIN, SILVIA PANIZZA, STEFAN WEITZER
PHD-STUDENTS: THERESA HENKEL, PAOLA HENTGES PINTO
DIPLOMA STUDENT: INES STURMLECHNER
LAB MANAGER: JUTTA DAMMANN
TECHNICAL ASSISTANT: THERESE KAUFMANN

Figure 1: **Simultaneous depletion of RTCB and Archease abolishes XBP1s expression in cell culture. A and B:** Tetracyclin-inducible (Tet-ON) HeLa cells were incubated with 1 µg/ml doxycycline (Dox) for six consecutive days to stimulate expression of shRNAs targeting RTCB, Archease or a control cell line expressing two copies of the control shRNA followed by treatment with 300 nM Tg for the indicated times. Induction of expression of XBP1s was monitored by Western blot (A) and by RT-PCR (B). The relative contribution of *XBP1s* mRNA to total levels of *XBP1* mRNA was analyzed by densitometry (n=5).

Figure 2: **RTCB is required for induction of XBP1s during plasma cell differentiation.** Splenocytes of control (*Rtcb*$^{fl/fl}$ or *Rtcb*$^{fl/+}$), *Rtcb*$^{fl/+}$ *Cd23*-Cre or *Rtcb*$^{fl/fl}$ *Cd23*-Cre mice were stimulated with 20 mg/ml LPS for 4 days. Protein levels of RTCB and XBP1 were monitored by Western blot analysis.

Figure 3: **B cells deficient in RTCB show an impaired capacity to secrete immunoglobulins *in vivo*.** Control (*Rtcb*$^{fl/fl}$ or *Rtcb*$^{fl/+}$) and *Rtcb*$^{fl/fl}$ *Cd23*-Cre mice were injected intra-peritoneally with 50 mg of TNP- (0.5)-LPS and analyzed two weeks after immunization. **A**: IgM ELISPOT analysis of MACS-enriched CD138^{+} cells after plating identical numbers for 16 - 18 h. A representative assay is shown in the upper panel. Bar diagrams (lower panel) show the average number of ASCs (Antibody Secreting Cells) (IgM) and their median spot size (measured in pixels) (n=3 mean and SEM are displayed). **B**: Serum titers of TNP-specific IgM and Igk were determined by ELISA (n=4 mean and SEM are displayed).

Figure 4. **RTCD1 localizes primarily to the cytoplasm.** RTCD1 localization was analyzed by Western blotting after subcellular fractionation of HEK293 cells and HEK293 cells stably expressing FLAG-RTCD1 or FLAG-RTCD1 H320A catalytically inactive mutant using a monoclonal antibody against RTCD1. Actin, Calnexin and Lamin A/C were used as markers for cytoplasmic (Cyt), membrane (Mem) and nuclear (Nuc) fractions, respectively.

KAZUFUMI MOCHIZUKI GROUP

Small RNA-directed DNA elimination in *Tetrahymena*

www.imba.oeaw.ac.at/research/kazufumi-mochizuki

Two discoveries which were awarded the Nobel Prize have shown that Tetrahymena is a useful eukaryotic model for RNA and chromatin biochemistry. Using this marvelous unicellular eukaryote as a model, we are trying to elucidate how small non-coding RNAs epigenetically regulate genome activities. For this purpose we are studying a process known as programmed DNA elimination, which is a gold mine of examples for basic biological processes, including small non-coding RNA biogenesis, selective nuclear transports, RNA and protein degradation, small and long non-coding RNA-mediated regulation of chromatin activity, chromatin organization in the nucleus, transposon silencing, and transposon-driven genome evolution.

A *Tetrahymena* Hsp90 co-chaperone promotes siRNA loading by ATP-dependent and ATP-independent mechanisms

The loading of small interfering RNAs (siRNAs) and microRNAs into Argonaute proteins is enhanced by Hsp90 and ATP in diverse eukaryotes. However, whether this loading also occurs independent of Hsp90 and ATP remains unclear. We found that the *Tetrahymena* Hsp90 co-chaperone Coi12p promotes siRNA loading into the Argonaute protein Twi1p in both, ATP-dependent and ATP-independent manners *in vitro*. ATP-dependent activity requires Hsp90 and the tetratricopeptide repeat (TPR) domain of Coi12p, whereas these factors are dispensable for ATP-independent activity. Both activities facilitate siRNA loading by counteracting the Twi1p-binding protein Giw1p, which is important to specifically sort the 26- to 32-nt siRNAs to Twi1p. Although Coi12p lacking its TPR domain does not bind to Hsp90, it can partially restore the siRNA loading and DNA elimination defects of *COI12* knockout cells, suggesting that Hsp90- and ATP-independent loading of siRNA occurs *in vivo* and is an integral part of the DNA elimination process in *Tetrahymena*.

DNA elimination is epigenetically regulated by trans-nuclear genome comparison

The fact that IESs do not share any common sequence motifs raises the following question: how is *Tetrahymena* able to identify IESs in order to induce DNA elimination? *Tetrahymena* solves this problem by trans-nuclear comparison of whole genomes. In a single cell, *Tetrahymena* has a germline known as Mic which contains complete genome including IESs, and a somatic Mac in which IESs are removed during the last sexual reproduction. Thus, the cell is able to identify IESs as sequences existing in Mic but not in Mac. *Tetrahymena* utilizes scnRNAs for this trans-nuclear comparison of whole genomes (Figure 2 top). This system is able to perfectly sweep away not only the existing transposons, but also any newly invaded transposons from the transcriptionally active Mac. We are trying to understand the exact molecular mechanism regulating this trans-nuclear comparison of whole genomes by small RNAs. We recently reported that only scnRNAs complementary to IESs escape degradation during conjugation, and this selective turnover of scnRNAs mediates trans-nuclear comparison of whole genomes (Figure 2, bottom). We also showed that the selective turnover of scnRNAs alone does not fully explain the observed sequence specificity of scnRNAs to IESs, because scnRNAs are produced to a greater extent from IESs than from the rest of the genome (Figure 2, lower section).

We proposed that scnRNAs target not only IESs in the new Mac for DNA elimination, but also IESs in the germline Mic to mark sites for future biased production of scnRNAs (Figure 2 top, g). Thus, DNA elimination in the new Mac may be epigenetically and transgenerationally controlled by the genome contents of the parental Mac through selective degradation of scnRNAs, and also by those of the grandparental Mac through transcriptional regulation of Mic. We believe that understanding the mechanism of DNA elimination in *Tetrahymena* will shed light on how ancestral genomes are able to epigenetically regulate the behavior of genomes of successive generations in general eukaryotes.

Publication highlights

Woehrer, S. L, Aronica, L., Suhren, J. H., Busch, C. J., Noto, T & Mochizuki, K. (2014) A Tetrahymena Hsp90 co-chaperone promotes siRNA loading by ATP-dependent and ATP-independent mechanisms. EMBO Journal, in press

Schoeberl, U. E., Kurth, H. M., Noto, T. and Mochizuki, K. (2012) Biased transcription and selective degradation of small RNAs shape the pattern of DNA elimination in Tetrahymena. Genes Dev 26, 1729-1742.

Noto, T., Kurth H.M., Kataoka, K. Aronica, L., Desouza, L.V. Siu, K.W., Pearlman, R.E., Gorovsky, M.A., and Mochizuki, K. (2010) The Tetrahymena Argonaute-binding protein Giw1p directs a mature Argonaute-siRNA complex to the nucleus. Cell 140, 692-703

Figure 1

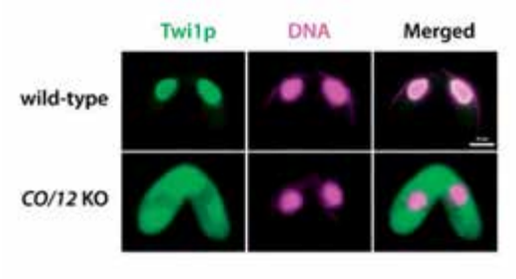
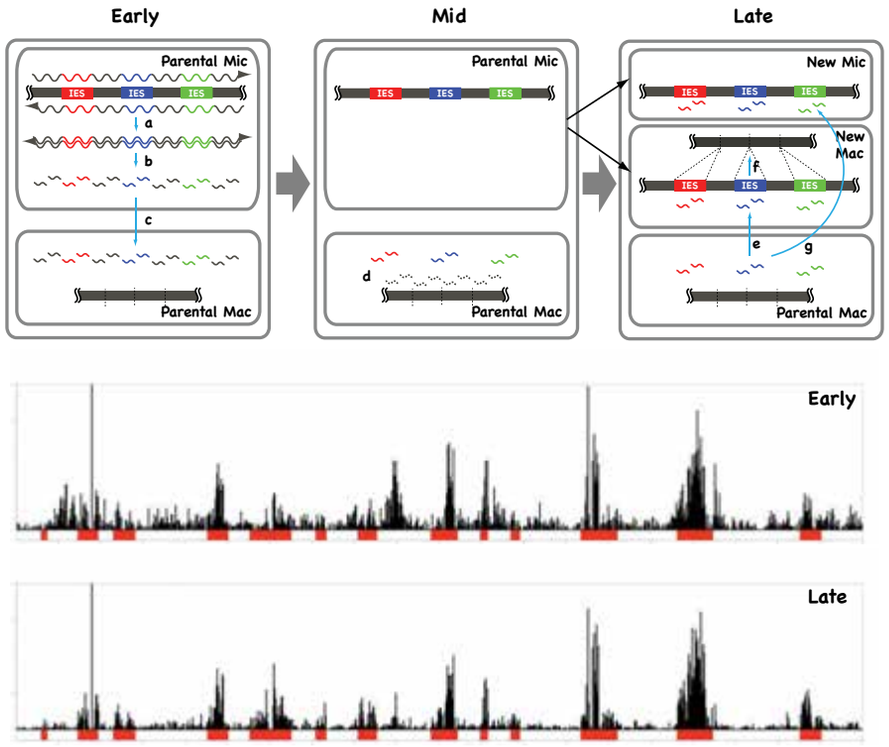
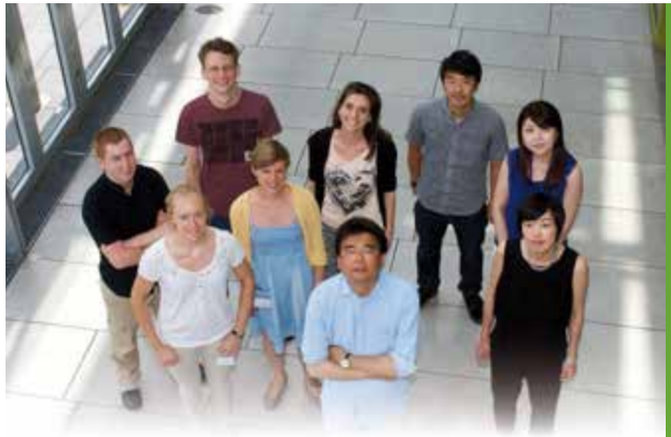


Figure 2





GROUP LEADER:
KAZUFUMI MOCHIZUKI

POSTDOCS: KENSUKE KATAOKA, KATRINA WOOLCOCK, [1]AGNIESZKA LUKASZEWICZ
PHD STUDENTS: JAN SUHREN, [2]QUENTIN RAHMS
RESEARCH ASSOCIATE: TOMOKO NOTO
RESEARCH ASSISTANT: AZUSA HAYASHI
TRAINEE: [3]DELYANA STOEVA

[1]FROM MAY, [2]FROM OCTOBER, [3]TILL JUNE

Figure1: The Hsp90 co-chaperone Coi12p is required for loading scnRNAs into Twi1p. In the absence of Coi12p, siRNAs are not loaded into Twi1p and thus Twi1p does not localize to the macronucleus (bottom)

Figure 2: Biased transcription and selective degradation of small RNAs shape the pattern of DNA elimination. (Top) A model for small RNA-directed DNA elimination. In the early developmental stages, the Mic genome is transcribed bi-directionally and the transcripts form double-stranded RNAs (a), which are processed into scnRNAs (b). scnRNAs are transferred to the parental Mac (c). In mid stages, scnRNAs complementary to the parental Mac genome are degraded (d). In late stages, the remaining scnRNAs are transferred to the developing new Mac (e) and target IESs to be eliminated (f). It has also been proposed that scnRNAs may move to the new Mic, leaving a signature on IESs for biased production of scnRNAs in the next sexual reproduction (g). (Below) Comparison of scnRNAs from different conjugation stages. Sequences of scnRNAs from various stages of development were obtained by deep sequencing, and were mapped to a Mic locus. IESs are marked in red.

JOSEF PENNINGER GROUP

Genetic dissection of disease mechanisms

www.imba.oeaw.ac.at/research/josef-penninger

Gene targeting and mutagenesis using stem cell technologies are powerful tools to elucidate essential functions of genes in normal physiology and the pathogenesis of disease. Using gene-targeted mice, my group tries to genetically dissect disease mechanisms and establish new principles of immunoregulation.

Licensing NK cells to kill cancer metastases.

Tumor metastasis is the primary cause of mortality in cancer patients, and constitutes a major challenge in cancer therapy. The immune system is not only responsible for controlling infection, but also for recognizing and destroying cancer cells. We showed that deletion or targeted inactivation of the E3 ligase Cbl-b efficiently enhances the anti-tumor function of NK cells. As a result, the progression of metastases in melanoma (**Figure 1**) and breast cancer was significantly inhibited. We then identified a molecular pathway by which Cbl-b blocks NK cell activity towards metastatic tumors, namely via a family of molecules known as TAM receptors, and developed an inhibitor directed against TAM receptors. Blocking TAM receptors through different routes of application, including administering it as an oral 'pill', markedly reduced metastatic spread in two model systems. We further showed that the widely used anticoagulant warfarin exerts anti-metastatic cancer activity in mice via Cbl-b/TAM receptors in NK cells, thus providing a molecular explanation for the enigmatic role of warfarin in cancer metastases reported more than 50 years ago. This novel TAM/Cbl-b inhibitory pathway holds promise for targeted immunotherapy of cancer metastases. ***Paolino et al. Nature 2014.***

Identification of a new neurological syndrome in children.

CLP1 is a RNA kinase involved in tRNA splicing, initially identified by Javier Martinez's lab (Weitzer et al. Nature 2007). In 2013 we showed that Clp1 kinase-dead mice develop progressive loss of motor neurons, resulting in muscle paralysis and the eventual death of the mutant mice (Hanada et al. Nature). In collaboration with Jim Lupski's lab (Texas), genome analyses identified a CLP1 homozygous missense mutation (R140H) in five unrelated families. The affected individuals develop severe motor and sensory defects as well as cerebral dysgenesis, and exhibit microcephaly. Importantly, using 15.2 Tesla MRI imaging, microcephaly and reduced cortical brain volumes were confirmed in mice that carry a kinase-dead CLP1 (**Figure 2**). The CLP1 mutation in patients causes loss of interaction with the tRNA splicing endonuclease (TSEN) complex and largely reduced pre-tRNA cleavage activity, resulting in the accumulation of linear tRNA introns. These data elucidated a novel neurological syndrome affecting both, the PNS and CNS defined by *CLP1* mutations that impair tRNA splicing. ***Kacara, Weitzer et al. Cell 2014.***

Jagn1 is a novel regulator of neutrophil functions.

Neutrophils are key innate immune effector cells, essential for combating bacterial and fungal pathogens. Our close collaborator Christoph Klein in Munich identified mutations in Jagunal homolog 1 (Jagn1) in patients with neutropenia and recurrent infection. We generated mice carrying a hematopoietic lineage-specific deletion of Jagn1. These mutant mice are unable to mount an efficient neutrophil-dependent immune response to the prototypic human fungal pathogen *Candida albicans*. Global glycobiome analysis, which enabled us to directly map glycosylation patterns to proteins, revealed marked alterations in specific glycoproteins involved in cell adhesion and the cytotoxicity of *Jagn1*-deficient neutrophils. Functional analysis confirmed marked defects in neutrophil migration in response to intraperitoneal or systemic *Candida albicans* infection, impaired formation of cytotoxic granules (**Figure 3**), as well as defective MPO release and killing of *Candida albicans*.

GM-CSF treatment protected mutant mice from weight loss and accelerated mortality after *Candida albicans* challenge. Importantly, GM-CSF restored the defective fungicidal activity of bone marrow cells in patients with *JAGN1* mutations. These mouse and human data show Jagn1/JAGN1 to be a key regulator of neutrophil functions in microbial pathogenesis, and uncover a treatment option for human patients. ***Boztug et al. Nature Gen. 2014; and Wirnsberger et al. Nature Gen. 2014.***

Figure 1

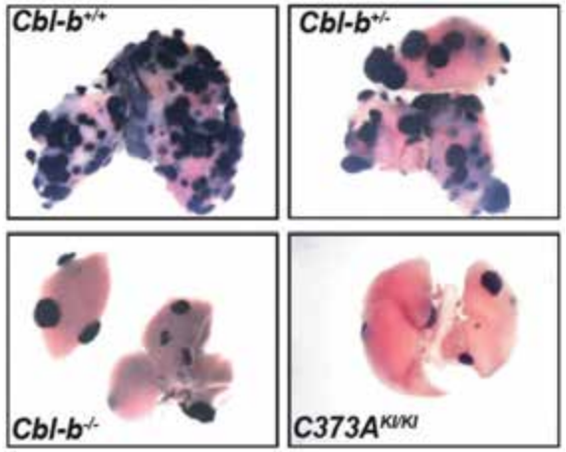


Figure 2

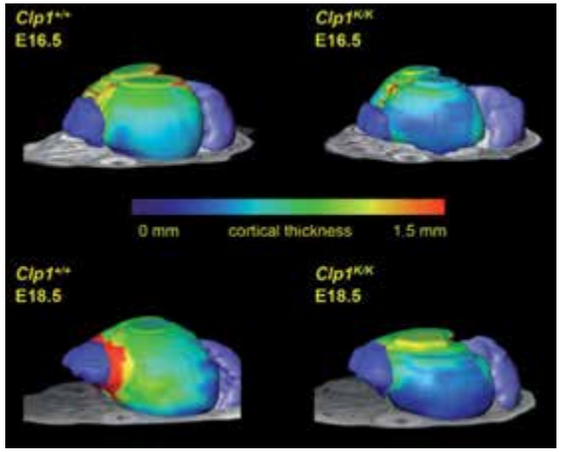
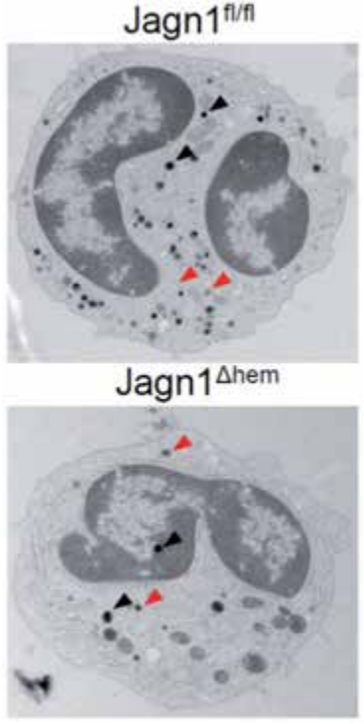


Figure 3



SENIOR SCIENTIST & MANAGING DIRECTOR SCIENCE:
JOSEF PENNINGER

POSTDOCS: DOMAGOJ CIKES, SHANE CRONIN, BERNHARD JOHANNES HAUBNER, REZAUL KARIM, VANJA NAGY, YONATAN NISSENBAUM, MICHAEL ORTHOFER, TSUNG-PIN PAI, BLANKA PRANJIC, SHUAN RAO, HIROSHI SHIRAISHI, LUIGI TORTOLA, IRIS URIBESALGO MICAS, REINER ALOIS WIMMER, GERALD WIRNSBERGER, ROBERTO NITSCH, THOMAS PERLOT

PHD STUDENTS: RENGIN ERDEM, VERENA SIGL, JASMIN TAUBENSCHMID, DANIEL WENZEL

DIPLOMA STUDENT: DANA ABDEEN, BARBARA HOHENSINN

RESEARCH ASSISTANT: RUBINA KOGLGRUBER, MELITA TICEVIC

RESEARCH ASSOCIATE: IVONA KOZIERADZKI, RENU SARAO

Figure 1: Cbl-b mutant mice efficiently control metastatic melanoma. Representative photographs showing melanoma metastases in the lungs of individual *Cbl-b*$^{+/+}$, *Cbl-b*$^{+/-}$, *Cbl-b*$^{-/-}$, and E3 ligase-dead *C373A*$^{KI/KI}$ mice, 21 days after intravenously inoculated B16F10 tumor cells.

Figure 2: Microcephaly in kinase-defective *Clp1* mice. Representative views of individual E16.5 and E18.5 *Clp1*$^{+/+}$ and *Clp1*$^{K/K}$ mouse embryos on the B6 background. 3D renderings of the olfactory bulb (dark blue), the cerebellum (light blue), and the cortex on an MRI slice isosurface. The cortex is rainbow color-coded to illustrate cortical thickness from 0 mm (blue) to 1.5 mm (red, thicker).

Figure 3: Jagn1 controls neutrophil granules. Representative electron micrographs of segmented neutrophils isolated from the bone marrow of *Jagn1*$^{fl/fl}$ and *Jagn1*$^{\Delta hem}$ mice. 36000 x magnification. Black arrowheads indicate primary granules while red arrowheads indicate secondary granules.

LEONIE RINGROSE GROUP

Epigenetic Regulation by Polycomb and Trithorax Group Proteins

www.imba.oeaw.ac.at/research/leonie-ringrose

Epigenetics research aims to understand how a single cell, with a single genomic DNA sequence, can give rise to and maintain the extraordinary diversity of cell identities and functions that constitute the adult organism. Modifications in chromatin and the binding of other chromatin proteins and non-coding RNAs provide a regulatory layer that modulates genome function, so that one genome gives rise to several 'epigenomes'. The highly conserved Polycomb (PcG) and Trithorax (TrxG) group proteins are essential components of the epigenome in every cell type studied so far (Fig.1).

PcG and TrxG proteins constitute an epigenetic "cellular memory" system that is essential for maintaining the correct identity of stem cells and differentiated cells, and for orchestrating transitions between them. Aberrant expression of these proteins leads to developmental defects and cancer. PcG and TrxG proteins work antagonistically on several hundred developmentally important target genes, to maintain repressed (PcG) or active (TrxG) transcription states. We focus on three key questions.

1) How does the system maintain memory? PcG and TrxG proteins bind mitotic chromatin.
In any biological system with memory, the state of the system depends on its history. Epigenetic memory maintains gene expression states through cell generations without a change in DNA sequence and in the absence of initiating signals. It is immensely powerful in biological systems; it adds long-term stability to gene expression states and increases the robustness of gene regulatory networks. PcG and TrxG proteins can confer long-term, mitotically heritable memory by sustaining silent and active gene expression states, respectively. In mechanistic terms, the two stages of the cell cycle at which the epigenetic memory of the transcrip¬tional status is most likely to be erased are replication and mitosis. Each of these stages poses distinct molecular challenges for the maintenance of memory (Steffen and Ringrose, 2014). We showed that both, PcG and TrxG proteins bind to chromatin during mitosis, with different quantitative kinetic properties.

Furthermore, we showed that mitotic chromatin attachment of the TrxG protein ASH1 is essential for survival to adulthood and for the maintenance of correct cell identity in living animals. Genome-wide transcriptome analysis identifies key genes that are deregulated upon loss of mitotic chromatin binding (Fig 2; Steffen et al., 2013; Steffen et al., submitted).

2) How does the system switch between active and silent states?
Non-coding RNA strand switching defines PRE/TRE status. PcG and TrxG proteins work through specialized DNA elements known as Polycomb/Trithorax response elements (PRE/TREs). We discovered that, in vertebrates as well as flies, specific developmentally regulated non-coding RNAs transcribed from these elements are involved in both silencing *and* activation by the PcG/TrxG proteins. Remarkably, a *Drosophila* PRE/TRE switches its function by alternating between forward and reverse strands of its non-coding RNA. This work identifies a novel and potentially widespread class of PRE/TREs that switch their function by alternating between forward- and reverse-strand non-coding RNA transcription. Surprisingly, the two strands act by very different mechanisms to switch the function of the PRE/TRE (Fig. 3; Herzog, Lempradl et al., 2014).

3) What makes a PRE/TRE? DNA sequence principles in flies and mammals. We use quantitative assays to identify novel motifs required for PRE/TRE function in flies (Okulski et al., in preparation). However, the analogous elements in mammals have proved to be highly elusive. The race to understand the sequence principles of mammalian PRE/TREs is currently one of the most active and controversial areas in the PcG/TrxG field. Building on previous work in flies, we established a computational tool that can accurately identify candidate PRE/TRE elements on the basis of DNA sequence alone (collaboration with Marc Rehmsmeier, University of Bergen, Norway). We are currently testing these predictions in quantitative experimental assays. Future work will be focused on identifying mammalian PRE/TREs on a genome-wide basis and understanding the relationship between DNA sequence and function in quantitative assays (Trupke et al., in preparation).

Publication highlights:

Steffen P.A., and Ringrose, L. What are memories made of? How Polycomb and Trithorax proteins mediate epigenetic memory. Nat Rev Mol Cell Biol 2014, 15, 340-56.

Herzog V.A., Lempradl A*., Trupke J., Okulski H., Altmutter C., Ruge F., Boidol B., Kubicek S., Schmauss G., Aumayr K., Ruf M., Pospisilik A., Dimond A., Senergin H.B., Vargas M.L., Simon J.A., Ringrose L. A strand-specific switch in noncoding transcription switches the function of a Polycomb/Trithorax response element. Nat Genet. 2014, 46, 973-81.*
** Equal contributions.*

Figure 1

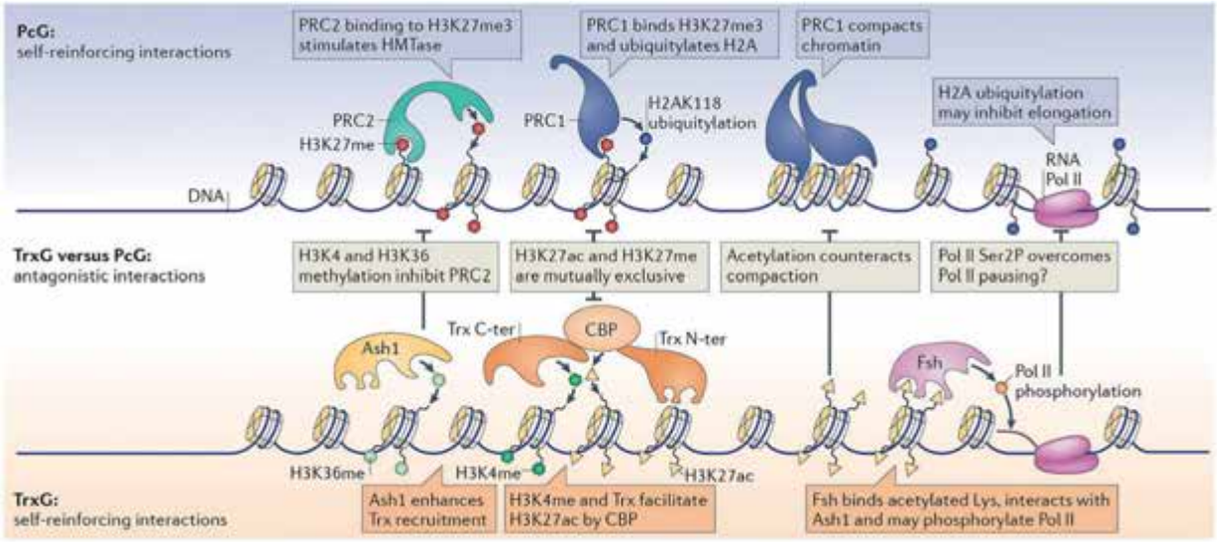


Figure 2

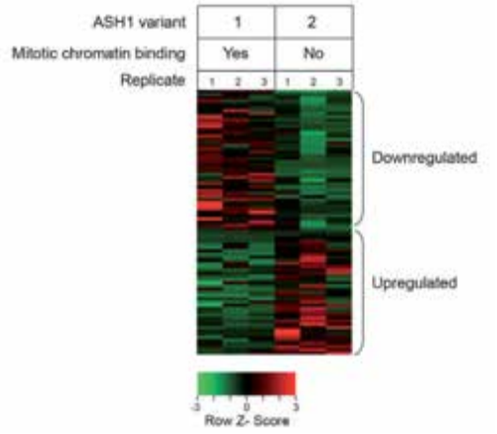
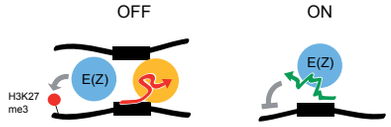


Figure 3



GROUP LEADER:
[1]LEONIE RINGROSE

PHD STUDENTS: [1]HELENA OKULSKI, [2]JOHANNA TRUPKE, [1]VERONIKA HERZOG
TECHNICAL ASSISTANTS: [3]MELITA TICEVIC, [5]CHRISTINA ALTMUTTER, [5]EVA DWORSCHAK
MASTERS STUDENTS: [4]MORITZ BAUER

[1]JAN- JULY, [2]JAN- DECEMBER, [3]JAN; MAY - JUNE, [4]JAN - MARCH, [5]JAN - JUNE

Figure 1: Polycomb/Trithorax group proteins act on chromatin. Self-reinforcing (top and bottom) and antagonistic (middle) interactions of PcG and TrxG with chromatin. Top: Polycomb-mediated silencing. PRC2: Polycomb repressive complex 2 trimethylates histone H3 at Lys27 (H3K27me3). PRC2 can bind H3K27me3, which propagates and stimulates its activity. The PRC1 complex can bind to H3K27me3 histone tails. PRC1 catalyzes monoubiquitylation of H2AK118 (Lys119 in vertebrates), and also mediates compaction of chromatin, Bottom: TRX-mediated activation. The TrxG proteins ASH1 and TRX are histone methyltransferases that cooperate to counteract PcG-mediated silencing. ASH1 methylates H3K36. The TRX C ter domain methylates H3K4. TRX and H3K4me facilitate CBP-mediated H3K27 acetylation (H3K27ac) (adapted from Steffen and Ringrose, 2014).

Figure 2: Loss of ASH1 mitotic chromatin binding causes widespread gene deregulation. RNAs-Seq analysis of 3rd instar larval wing discs from animals in which wt ASH1 is replaced by different variants. Variant 1 binds mitotic chromatin, whereas variant 2 does not. The two variants differ by only 2 amino acids. (Steffen et al., Submitted.)

Figure 3: Non-coding strand switching switches the function of a Polycomb/Trithorax response element. Left: The silent state is induced by forward-strand non-coding transcription. Forward-strand non-coding RNA promotes silencing by facilitating pairing between PRE/TREs. The PRC2 protein E(Z) does not interact with the forward strand *in vivo*, and binds at the silenced PRE/TRE independent of RNA. However, E(Z) interacts promiscuously with any RNA *in vitro*, and this interaction inhibits its enzymatic activity. Thus, other proteins (yellow) may prevent binding and inhibition of E(Z) by the RNA. Right: The active state is induced by forward-strand non-coding transcription. Transcription of the reverse PRE/TRE strand is incompatible with forward-strand transcription because the reverse transcript runs through the forward-strand promoter. Reverse-strand transcription would thus destabilize pairing, enabling the activation of PRE/TRE. In addition, the reverse strand binds E(Z) *in vivo* and, upon binding, inhibits E(Z) histone methyltransferase activity. Thus, multiple self-reinforcing events may contribute to the stable switching of PRE/TRE into an active state (adapted from Herzog, Lempradl et al., 2014).

KIKUE TACHIBANA-KONWALSKI GROUP

Molecular control of the oocyte-to-zygote transition

www.imba.oeaw.ac.at/research/kikue-tachibana-konwalski/

The challenge in fertilization is to transform two highly differentiated cells, egg and sperm, into a single totipotent cell - the zygote - with vastly different properties compared to its parental cells. To generate a zygote, the meiotic cell cycle machinery switches to the mitotic form while sperm chromatin is reorganized and reprogrammed. How these fascinating processes are regulated at the molecular level and how their deterioration influences fertility are key questions we aim to address in our research.

The oocyte-to-zygote transition is one of the most dramatic cell conversions in biology. It refers to the female germ cell or oocyte, which undergoes two rounds of meiotic chromosome segregation and, following fertilization, is converted into a mitotically dividing embryo. We are addressing fundamental questions relating to the processes that ensure the inheritance of genomes from one generation to the next by combining germ cell and chromosome biology with cell cycle and epigenetic studies. Achieving a molecular understanding of key players such as cohesin is a requisite step for investigating how the deterioration of these factors contributes to maternal age-dependent aneuploidy and infertility. The trend towards advanced maternal age has increased the frequency of trisomic fetuses by 71% in the last ten years. Therefore, a better understanding of mammalian meiosis is relevant to human health.

How is sister chromatid cohesion maintained for months and decades in oocytes?

The inheritance of chromosomes from mother to daughter cell and from one generation to the next depends on sister chromatid cohesion mediated by the cohesin complex. Cohesin is especially important in meiosis, which is the specialized cell division giving rise to haploid gametes, egg and sperm. The paradigm of reproductive biology is that all oocytes are generated before birth. Cohesion is established during meiotic DNA replication, recombination occurs before birth, and oocytes remain arrested until ovulation triggers the first meiotic division several months (mouse) or decades (human) later. Does cohesin hold sister chromatids together for months and possibly decades without reinforcement? Alternatively, is cohesion reinforced during the long arrest? Using TEV protease technology which we pioneered in the mouse, molecular genetics, and 4D confocal live-cell imaging, we showed that no detectable cohesin turnover occurs in oocytes for several weeks (Figure 1). Our current work addresses the crucial question as to whether cohesion is reinforced during the months of arrest or, indeed, whether it is at all possible to generate cohesion after DNA replication (Figure 2). Future work will address the mechanisms that protect long-lived proteins such as cohesin, and what might go awry with age.

How does the spindle assembly checkpoint function at the oocyte-to-zygote transition?

It has been known for decades that the first meiotic division of oocytes is error prone, leading to aneuploidies such as trisomy 21 or Down's syndrome, but the molecular basis has remained elusive. In mitotic cells the spindle assembly checkpoint (SAC) monitors kinetochore-microtubule attachments, and delays the onset of anaphase until the last chromosome has bi-oriented on the spindle. The SAC also regulates the timing of chromosome segregation in oocytes, but how it functions at the level of kinetochores is less clear. By specifically targeting TEV protease to kinetochores in oocytes expressing TEV-cleavable cohesin, we demonstrated that cohesin is necessary for sister kinetochore mono-orientation and robust SAC activity, which has implications for aging oocytes with cohesin deterioration. We also discovered that zygotes with TEV-cleaved cohesin arrest in mitosis (Figure 3). Future work will focus on the molecular basis of differences in the checkpoint activity of oocytes and zygotes.

How is chromatin reprogramming coordinated with cell cycle progression in zygotes?

Fertilization triggers the second meiotic division and entry into the first embryonic cell cycle. In the zygote stage, maternal and paternal genomes remain as separate entities with distinct chromatin signatures. Maternal factors control sperm chromatin reorganization as protamines are replaced by histones, and chromatin remodeling erases cell type-specific epigenetic marks. We are specifically interested in how chromatin organization, epigenetic reprogramming, and cell cycle progression are coordinated; these aspects are poorly understood at the present time. We combine detailed cell cycle kinetic studies by live-cell imaging with conditional knockout approaches to investigate candidate factors required for these processes. Knockout zygotes have the potential to be rescued by microinjection of mRNAs encoding target proteins. We are developing this powerful system for *in vivo* structure-function studies in order to determine the mechanisms of chromatin organization and cell cycle regulation in zygotes.

Publication highlights:

Tachibana-Konwalski, K. Cell division: hold on and let go. Nature, in press.

Tachibana-Konwalski, K., Godwin, J., Borsos, M., Rattani, A., Adams, D.J., Nasmyth, K. (2013). Spindle assembly checkpoint of oocytes depends on a kinetochore structure determined by cohesin in meiosis I. Curr. Biol. 23: 2534-2539.

Seitan, VC., Hao, B.*, Tachibana-Konwalski, K.*, Lavagnolli, T., Mira-Bontenbal, H., Brown, KE., Teng, G., Carroll, T., Terry, A., Horan, K., Marks, H., Adams, DJ., Schatz, DG., Aragon, L., Fisher, AG., Krangel, MS., Nasmyth, K., Merkenschlager, M. (2011). A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation. Nature 476: 467-71.*

Tachibana-Konwalski, K., Godwin, J., van der Weyden, L., Champion, L., Kudo, NR., Adams, DJ., Nasmyth, K. (2010). Rec8-containing cohesin maintains bivalents without turnover during the growing phase of mouse oocytes. Genes Dev. 24: 2505-16.

Gonzalez, MA., Tachibana, KE.*, Adams*, DJ., van der Weyden, L., Hemberger, M., Coleman, N., Bradley, A., Laskey, RA. (2006). Geminin is essential to prevent endoreduplication and to form pluripotent cells during mammalian development. Genes Dev. 20:1880-4.*
*(*equal contribution)*

Figure 1

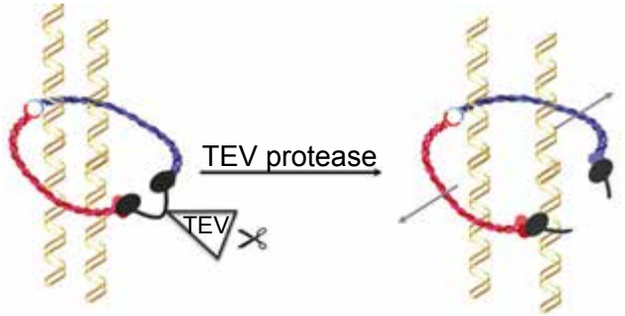


Figure 2

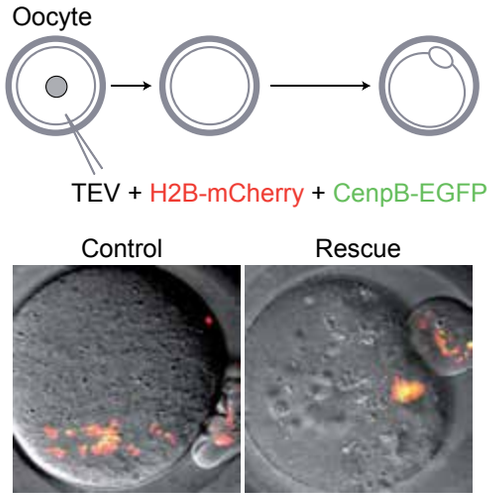


Figure 3

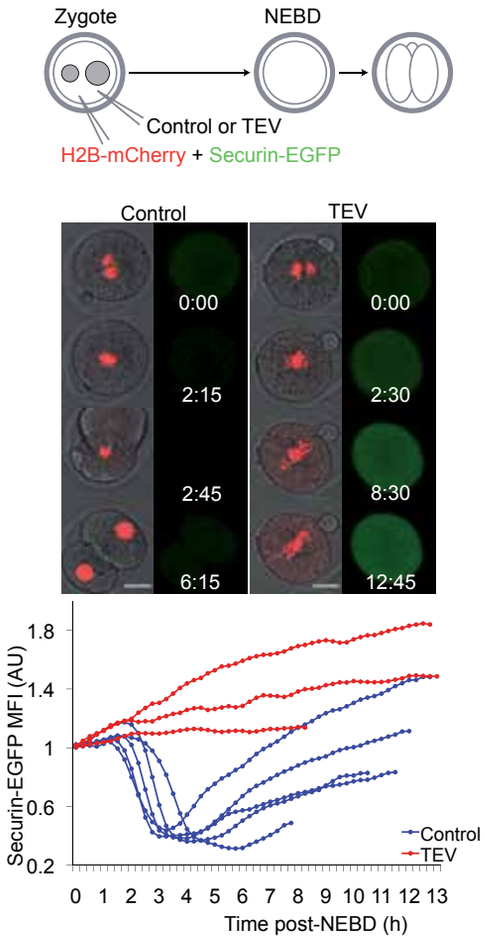


Figure 1: A rapid protein inactivation method based on TEV protease-mediated cleavage of the cohesin complex. Cohesin consists of an Smc1/Smc3 heterodimer (red/blue) bridged by an α-kleisin subunit (black). The kleisin is genetically engineered to contain TEV protease recognition sites. TEV protease-mediated cleavage of cohesin induces ring opening and destroys sister chromatid cohesion.

Figure 2: Cohesion rescue assay in live mouse oocytes. Oocytes were injected with mRNA encoding TEV protease, H2B-mCherry to mark chromosomes (red), and CenpB-EGFP to mark kinetochores (green). Oocytes with TEV-cleavable cohesin lose cohesion (left), whereas cohesion remains intact when additional non-TEV-cleavable cohesin is expressed during meiotic DNA replication (right).

Figure 3: Cell cycle kinetics of zygotes with or without intact cohesion. Zygotes expressing TEV-cleavable cohesin were injected with mRNA encoding H2B-mCherry (red), securin-EGFP to monitor cell cycle progression (green), and buffer or TEV protease. Still images are shown, starting with nuclear envelope breakdown (NEBD), which marks entry into mitosis (h:mm).

GROUP LEADER:
KIKUE TACHIBANA-KONWALSKI

POSTDOC: EMMANOUELLA CHATZIDAKI
PHD STUDENTS: SABRINA BURKHARDT, NICO LAUMANN-LIPP, ANNA SZYDLOWSKA
DIPLOMA STUDENTS: ILYA FLYAMER, JOHANNA GASSLER
RESEARCH ASSISTANT: KERSTIN KLIEN
TECHNICAL ASSISTANT: MARKUS FABITS

HEAD OF FACILITY:
ARABELLA MEIXNER

TECHNICAL ASSISTANTS: MICHELLE FOONG, PAUL MÖSENEDER, ESTHER RAUSCHER, ROMAN FERDINAND STEMBERGER
DIPLOMA STUDENT: JONATHAN BAYERL

STEM CELL CENTER - GENE TARGETING UNIT

The main objective of the Stem Cell Center is to broaden and strengthen stem cell research. Ideal human disease models can be engineered by genetic modification using pluripotent stem cells. iPSCs (induced pluripotent stem cells) technology allows to generate unlimited useful iPSCs-derived cell population. iPSCs provide a potential avenue to study regulation of pluripotency and differentiation, drug screening and cell-based therapies for the treatment of a variety of disorders.

ES Core Facility

The several missions of the ES (embryonic stem) cell core facility include the production of quality-controlled ES cell lines with genetic mutations, the creation and handling of quality-controlled ES cell lines, and enhancing knowledge of mouse genetics, ES cell culture, and manipulation. In addition, highly germline-competent ES cells derived from inbred strains, which are preferentially used in biomedical research, would greatly facilitate the generation of targeted mutations in appropriate genetic backgrounds, such as FVB/N and C57BL6/N ES cell lines. We also develop *in vivo* inducible gene targeting systems new transgenic recombinase mouse models.

We maintain a shared "gene targeting tool box" in addition to providing expertise, cell lines, efficient exchange of information, and stem cell assays. The collection includes Cre and Flp recombinase-expressing transgenic lines and activity-reporter lines, which are essential for the generation and characterization of conditional, inducible, and/or tissue-specific mutant mice. The unit also maintains a collection of "ES and vectors tools", reagents, cell lines, and plasmid vectors.

iPS Core Facility

Our iPS core facility was created to accelerate research in the stem cell field by facilitating the derivation of iPSC cell lines. We are working on integration free reprogramming using also urinary cells or plucked hair as source for reprogramming – as a means for easier sampling. We also envision the development of patient-specific iPSCs followed by treatment with autologous repaired cells. To overcome the limitations of conventional gene targeting in human pluripotent cells, TALEN or CRISPR/Cas9 technology has been adapted to repair disease-associated mutations.

Genetically corrected iPSCs as cell therapy for recessive dystrophic epidermolysis bullosa

Epidermolysis bullosa (EB) is one of the most sever rare inherited skin disorders affecting children and adults. Currently, at least 18 causative genes with defined DNA mutations have been identified. All of these mutations affect proteins involved in epithelial cell-cell and cell-matrix adherence or attachment of the epidermis to the underlying extracellular matrix. To provide proof-of-principle on the applicability of iPSCs for the treatment of RDEB, we developed iPSCs from *type VII collagen* (*Col7a1*) mutant mice that exhibited skin fragility and blistering resembling human RDEB. Intradermal injection of fibroblasts derived from genetically repaired iPSCs resulted in faithful restoration of type VII collagen and long-term increase in skin integrity and resistance to mechanical stress. Thus, genetically corrected iPSCs appear to be a viable and well tolerated therapeutic strategy for the treatment of EB. Our next approaches suggest to increase fibroblast survival upon transplantation. Another aspect would be the use of fibroblast or epidermal progenitor cells with the potential to differentiate *in vivo*.

HEAD OF FACILITY:
PETER DUCHEK

TECHNICAL ASSISTANTS:
SHEETAL BHALERAO, JOSEPH GOKCEZADE, VICTORIA STEINMANN, CLAUDIA VALENTA

FLY HOUSE

Peter Duchek

peter.duchek@imba.oeaw.ac.at

The Fly House provides research support for those scientists at IMBA using the fruit fly Drosophila melanogaster as a genetic model system. Our services include the generation of transgenic fly lines, gene targeting to generate knock-out or knock-in mutants, and large-scale in vivo RNAi screens.

Embryo injections

A fundamental service of the Fly House is the generation of transgenic fly lines. We routinely inject DNA constructs, including large BAC clones, into a range of commonly used host strains as well as attP landing site stocks for phiC31-mediated targeted integration. Over the course of a year, more than 400 different constructs get injected at the facility.

Gene targeting

Genome engineering in *Drosophila* via traditional homologous recombination is a time-consuming and labour-intensive process. Recent advances in the development and application of programmable nucleases significantly enhanced gene targeting rates. After setting up a versatile and efficient genome engineering platform using the CRISPR/Cas9 technology in 2013, we applied this strategy in the current year to generate more than 60 knock-out and knock-in mutations. Rather than screening $5x10^5$ flies required with the conventional method, we are now able to isolate targeted mutations from 100 flies in a process that takes two months.

Research support

The first genome-wide collection of inducible RNAi lines, available through the Vienna Drosophila RNAi Center (VDRC), has revolutionized loss-of-function genetic screens in *Drosophila*. It is now possible to systematically investigate the function of each gene in a tissue of interest. We support scientists conducting large-scale *in vivo* RNAi screens as well as with subsequent follow up experiments to validate their hits.

HEAD OF FACILITY:
ANDREAS LEIBBRANDT

SCIENTIFIC COORDINATOR OF HAPLOBANK
ULRICH ELLING

RESEARCH ASSISTANTS:
MARIA HUBMANN, JULIA LIEBERGESELL,
ELLEN WETZEL, MANUELA KINZER

HAPLOBANK

Genome-wide recessive genetics in ES cells

haplobank@imba.oeaw.ac.at

In the post-genomic era, science is very rapidly accumulating extensive high-throughput datasets, which in many cases still require genetic testing and independent validation, respectively. Over the last years, Haplobank produced a genome-wide library of murine embryonic stem cell lines with defined genetic mutations, in order to allow scientists to functionally validate hits from high-throughput screens, but also to study phenotypic effects of gene loss in various developmental pathways, thereby helping the scientific community to functionally annotate the mouse genome.

Some organisms such as yeast are haploid, i.e. they carry a single set of chromosomes, and thus provide a basis for genetic analyses where recessive mutations of genes will show a clear phenotype due to the absence of a second gene copy. However, all somatic mammalian cells carry two copies of chromosomes (diploidy) that complicate mutational screens. We have previously described the generation of mammalian haploid embryonic stem cells from parthenogenetic mouse embryos. Haploid mESCs show stable growth over multiple passages, can be efficiently subcloned, and differentiate in vitro and in vivo. Importantly, haploid mESCs can be readily mutagenized at the haploid state to generate complete, homozygous mutants for virtually any gene allowing for reverse and high-throughput forward genetic screens.

Over the last year, we have further developed and optimized various conditional transposon-, lentiviral-, and retroviral-based mutagenic gene trap vectors in order to account for insertional biases and to hit as many genes as possible, respectively. In addition, each introduced single mutation carries a unique internal DNA barcode which can be employed e.g. for haploinsufficiency profiling.

Using these optimized mutagenesis systems, the Haplobank team is generating an archive of homozygously mutated ES cell lines for functional genomics. We have streamlined and automatized cell culture, DNA preparation, and deep sequencing protocols using a Hamilton robotic platform. Haplobank already comprises 61'000 clones with fully annotated gene trap integration sites translating to 13'000 unique genes with either single or multiple gene trap insertions. Taking only those clones carrying a single gene trap, we hit 9192 unique genes. Currently, we distribute clones only on campus and to a few collaborators around the world, and have since its start last year already provided over 500 clones from our collection for such diverse projects as e.g. cardiomyocyte differentiation or sprouting angiogenesis. In addition to selected clones, we supply protocols for cell culture, confirmation of mutations, reversion of conditional mutations, etc. on our homepage at:

http://www.haplobank.at (only from internal IP addresses).

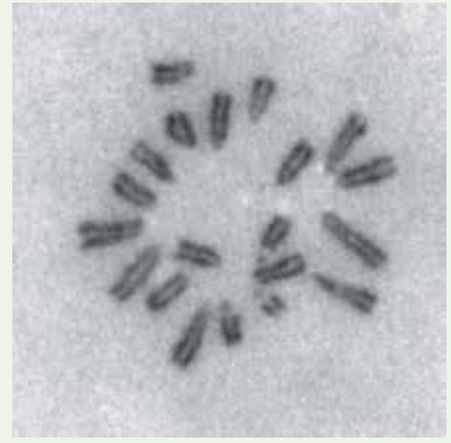
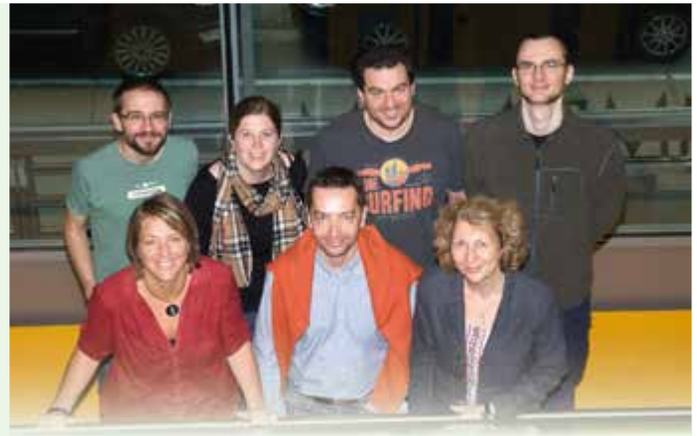


Figure: Chromosome spread of haploid ES cell line

corallimorph detail © J.A.Alvarez



HEAD OF BIOOPTICS: KARIN AUMAYR

MICROSCOPY: TOBIAS MÜLLER, PAWEL PASIERBEK
MICROSCOPY/FLOW CYTOMETRY: GABRIELE STENGL
FLOW CYTOMETRY: MARIETTA WENINGER
FLOW CYTOMETRY/IMAGE ANALYSIS: THOMAS LENDL, GERALD SCHMAUSS

BIOOPTICS FACILITY

aumayr@imp.ac.at

The services offered by the BioOptics Facility to researchers at IMP, IMBA and GMI encompass analytical flow cytometry and cell sorting, as well as a large variety of microscopy techniques, image processing and analysis.

Flow Cytometry

We provide instrumentation, education and expertise for all flow cytometry requiring experiments, and offer high-speed cell sorting, as well as advanced and general flow cytometry analysis. Users are given guidance and support with the planning of experiments, they are trained in the use of hardware and software for all of the three available state-of-the-art flow cytometers, as well as in data analysis. Three cell sorters are operated by the staff of the facility. Four cell populations can be sorted simultaneously in bulk, or single cell sorting can be performed.

Microscopy

The BioOptics Facility currently manages more than twenty microcopy systems, including wide-field microscopy, confocal laser scanning microscopy (CLSM), two-photon (2P) microscopy, total internal reflection (TIRF) microscopy, structured illumination microscopy (SIM) techniques and automated slide scanning. Most of the systems are motorized - thus providing automation for higher throughput - and are suitable for fixed samples as well as live cell experiments. The facility provides assisted use and training on instrumentation and consultation concerning all microscopy-related subjects, including project planning, staining, microscope selection, etc. Additionally intense basic as well as advanced practical microscopy courses are organized including hands-on sessions as well as lectures by internal and external faculty.

Image Processing and Analysis

Five state-of-the-art computer workstations are available at the BioOptics Facility, operating most of the common commercial image processing and visualization software. A server solution with a Web-based interface has been set up to enable deconvolution of microscopy images. The server permits efficient, multi-user, parallel, batch deconvolution that can easily be started from the individual scientist's computer. Users are trained in the use of specific software, depending on their demands or are trained in an annual course on image processing and analysis with lectures and hands-on sessions by the BioOptics staff. Customized classification and measuring algorithms are developed at the facility for advanced image analysis and automated object recognition.

For more information please visit http://cores.imp.ac.at/biooptics/

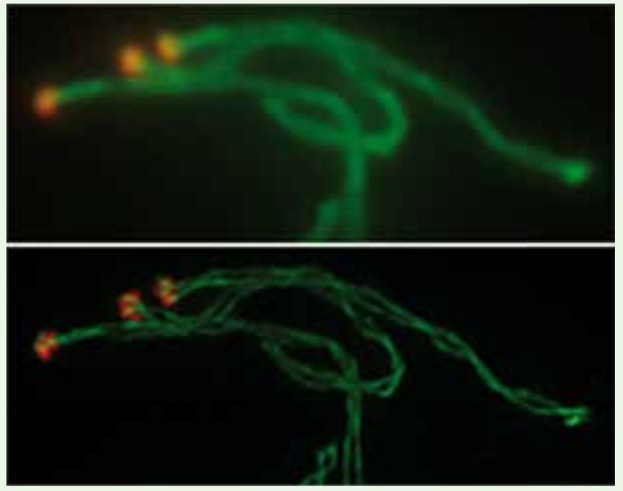


Figure: Chromosome spreads of pachytene oocytes in widefield (upper image) and structured illumination (lower image) microscopy. green: synaptonemal complex; red: centromeres.
Sample from Mariana C.C. Silva, Peters Lab

COMPUTATIONAL BIOLOGISTS: THOMAS BURKARD, MARIA NOVATCHKOVA, ALEXANDER SCHLEIFFER
SOFTWARE ENGINEER: HANNES SCHABAUER

BIOINFORMATICS

bioinfo.grp@imp.ac.at

The Bioinformatics unit assists research groups in molecular-biology-related fields by providing sequence analysis services, scientific data mining, software infrastructure, and training in bioinformatics.

Sequence analysis

One of the principal areas of expertise at IMP-IMBA Bioinformatics is sequence analysis. Typical tasks include functional and structural characterization of proteins and genomic regions using methods such as pattern matching, complexity analysis, and homology searches. As conclusions in bioinformatics are achieved by synthesizing the results of multiple algorithms, we maintain and develop a set of specialized software tools to support this type of meta-analysis. Web access is provided for widely used scientific applications related to protein motif analysis (in-house development), similarity searching (BLAST, PSI-BLAST, FASTA), whole-genome viewing (GBrowse, UCSC browser), and various sequence manipulation and exploration tasks (EMBOSS).

Large-scale data analysis

Additional demands arise from the investigation of large functional genomics or high-throughput biological datasets. Assistance is provided in experimental design and subsequent analysis of next-generation sequencing, microarray, and mass-spectrometry-based proteomics experiments. The current main focus is on data analysis of small RNA-Seq, mRNA-Seq and haploid ES cell screens. We also engage in custom software and database development, and design computational and mathematical solutions that can cope with higher loads and memory requirements. Local instances of integrated model organism databases (Wormbase) and genome annotation portals permit visualization and analysis of in-house data with dedicated resources and additional privacy. User-driven data exploration is supported by the Ingenuity Pathway Analysis System. For scientists interested in computational biology, we offer hands-on training courses on basic principles and limitations of sequence analysis and data integration.

For heterogeneous computational tasks, we maintain a high-performance computing cluster (HPC) in which dedicated software is adapted to run in a batch and parallel computing environment. To enable researchers to use our server environment in an optimal way, we also provide training in Bash and Unix command line tools specific to the IMP/IMBA infrastructure.

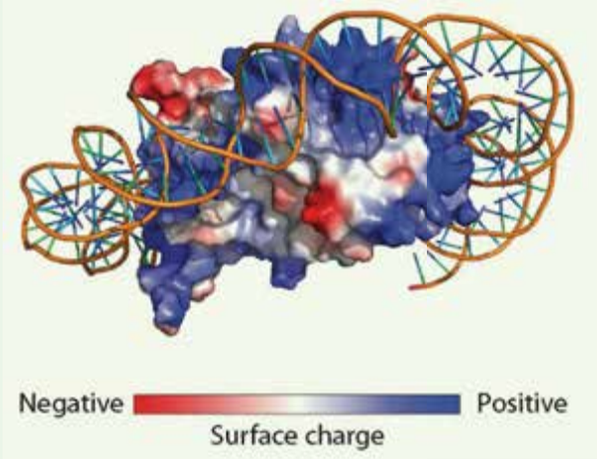


Figure: Hypothetical model of the non-canonical histone fold proteins CENP-T/W/S/X enclosed by a 73bp DNA fragment. The model is based on crystal structures of the CENP-T/W/S/X heterotetramer (PDB: 3VH5) superimposed on one half of the nucleosome core particle (only the DNA fragment is shown, PDB: 1KX5). The CENP-T/W/S/X heterotetramer is plotted as electrostatic surface potentials, illustrating the high density of positively charged surface residues (blue) that form the putative DNA interface.

HEAD OF FACILITY: Karl Mechtler

POSTDOCS: Zuzana Demianova, Thomas Köcher,
BIOINFORMATICIAN: Gerhard Dürnberger, Sergey Maltsev, Julia Hafenscher, Aline Dörrbaum
TECHNICAL ASSISTANTS: Johannes Fuchs, Otto Hudecz, Richard Imre, Gabriela Krssakova, Mathias Madalinski, [1]Michael Mazanek, Susanne Opravil, [1]Elisabeth Roitinger, Michael Schutzbier, Ines Steinmacher, Nadine Blaha
TRAINEE: Dominik Mayer, Florian Stanek, Johannes Dobelmann

[1]part time

PROTEIN CHEMISTRY

karl.mechtler@imp.ac.at

At the Mechtler laboratory we identify, quantify and further characterize proteins with mass spectrometry. We also develop novel methods required by the research groups at IMP-IMBA for addressing their biological questions.

The sweet side of proteins…

Glycosylation is an abundant - yet functionally not very well characterized - post-translational modification of proteins with both high structural complexity and diversity. As a consequence, the exact structure of glycoproteins is difficult to analyze. Amongst many important topics, research in the Penninger group is also directed towards the elucidation of mechanisms in the immune response. Proteins of the immune system and plasma membrane proteins are frequently and extensively glycosylated. In order to successfully assess the proteome-wide abundance of N-glycosylations sites in neutrophils and to map glycosylated residues on the respective proteins, we had to design a novel method. The workflow combines a novel in-house developed software with an analytical strategy, highly optimized for the analysis of glycoproteins.

…and their social properties.

Proteins almost never act alone. Instead, proteins assemble into proteins complexes of defined stoichiometries and structure, fulfilling biological functions. Some of these complexes are very large molecular machines such as the ribosome. We apply different targeted proteomics approaches to quantify the stoichiometry of protein complexes. In collaboration with the Westermann group, we employ these methods to study the cell cycle-dependent changes in the composition of the kinetochore. Usually, proteomics is not used for directly studying structure; however, in cases were other techniques fail to deliver a model of protein complexes at atomic resolution, mass spectrometry might deliver structural information at a low resolution. Proteomic approaches do so by converting proximity of specific amino acids present in the three-dimensional structure into chemical links. These cross-links can be detected by mass spectrometry, allowing to construct low resolution models of the complex from which they originate. We recently adapted, optimized and down-scaled available protocols and started to apply them to biological questions addressed by several biological groups at IMP-IMBA.

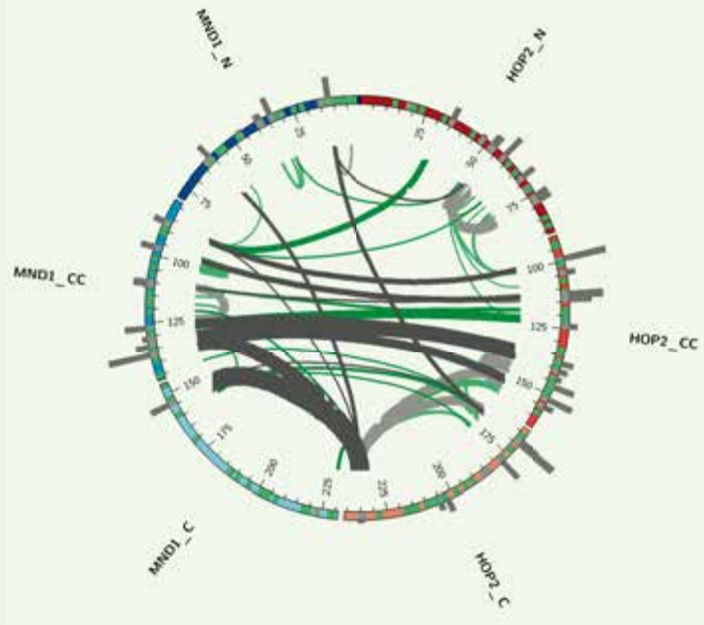
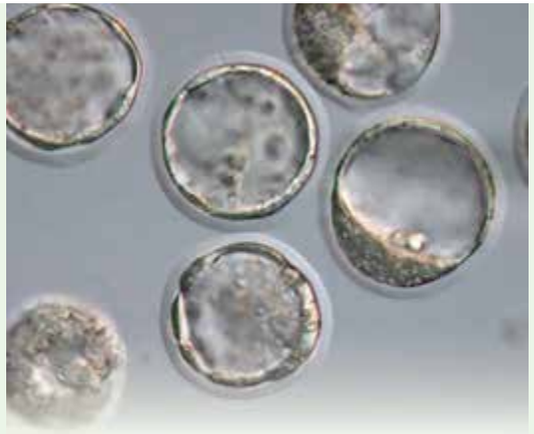
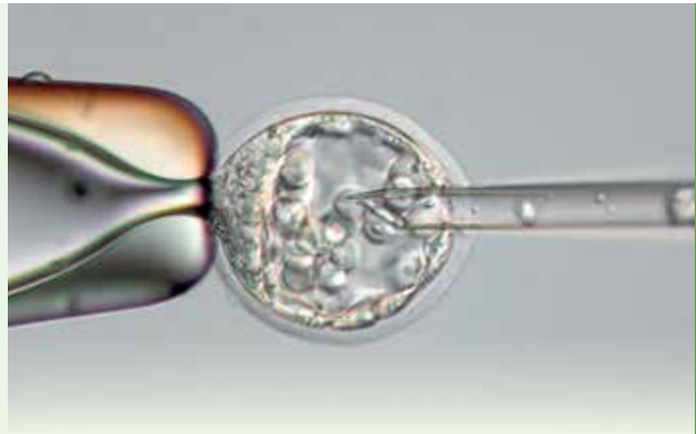


Figure: Circos Plot of crosslinks within the 3 domains of HOP2 (red) and MND1 (blue). The grey bars pointing outwards represent monolink abundance derived from amine-reactive linker.

1



2



COMPARATIVE MEDICINE

animal@imp.ac.at

Scientific work at the IMP and IMBA depends to a high degree on the use of model organisms. IMP and IMBA acknowledge and accept responsibility for the care and use of those animals according to the highest ethical standards. The institute ensures that all employees dealing with the animals understand their individual and collective responsibilities for compliance with Austrian laboratory animal law as well as all relevant regulations and rules concerning laboratory animal husbandries. In accordance with this institutional policy the animal house group - trained and highly qualified animal attendants - provides husbandry of animals and services for the various research groups.

Husbandry:
The largest area of the animal house is the mouse section, which comprises breeding colonies, stock and experimental animals including many transgenic and knock-out mouse lines. To provide a constant supply of mice for the various projects, commonly used standard strains are routinely bred in-house.

Comparative Medicine Services:
Veterinary services, such as monitoring of the facility's health-status (sentinel-program etc.), experimental procedures in animals such as collection of blood, implantation of tumor cells and administration of substances. All procedures are performed to a high standard under appropriate anaesthetic regimes and in conjunction with the necessary project licenses.
Animal procurement, such as ordering of mice from external breeding companies, organizing and handling of incoming and outgoing mouse-shipments per year.
Administration of regulatory affairs in accordance with the Austrian laboratory animal law, which includes record-keeping and updating of laboratory animal statistics, specific documentation of laboratory animal experiments.

TRANSGENIC SERVICE

transgenic@imp.ac.at

The Transgenic Service Department was set up in the beginning of 1998 to cope with the increasing demand for mouse studies and generation of transgenic mice. The Transgenic Service Department is shared by the IMP and IMBA.

The main tasks of this service unit are the injection of ES cells into blastocysts [also tetraploid] and 8-cell morulae, and DNA into the pronucleus of fertilized mouse eggs. The service also provides for the transfer of 'clean' embryos into our Animal House, freezing of embryos for the preservation of specified mouse strains, and teaching basic embryological techniques to the staff of IMP and IMBA.

Many different ES cell clones and DNA/BAC constructs are being injected every year. The activities of the department are supervised by an Animal User Committee, which meets on a regular basis to set priorities and coordinate tasks. Currently it is chaired by Meinrad Busslinger.

Figure 1: Mouse blastocysts.

Figure 2: Injection of embryonic stem cells into mouse blastocyst.

HEAD OF FACILITY: HARALD SCHEUCH

SEQUENCING SPECIALISTS: ZUZANA DZUPINKOVA, MARKUS HOHL , CAROLINE SCHUSTER
PROTEIN EXPRESSION SPECIALIST : KRISTINA MARINOVA UZUNOVA
MOLECULAR BIOLOGY SPECIALIST AND ANTIBODY SPECIALIST : ROBERT HEINEN
TECHNICAL ASSISTANT: ZSUZSANNA MUHARI-PORTIK
TRAINEE: ELISA HAHN, ANNA HAYDN

TEAM MEDIAKITCHEN:
CHRISTA DETZ-JADERNY, GABRIELE BOTTO, ULRIKE WINDHOLZ, JENS SCHAICH

MOLECULAR BIOLOGY SERVICE

harald.scheuch@imba.oeaw.ac.at

The unit offers a wide variety of standard services to all scientists at IMP/IMBA and GMI. These include Sanger Sequencing, a "Speed Congenics Service", the preparation of competent cells of various E. coli strains, production of monoclonal antibodies and a routine mycoplasma testing service for tissue culture cells to mention the most important ones. In addition, we provide instrumentation and expertise for lab automation and high-throughput methods.

In the past year, expression of recombinant proteins became a rapidly growing task for our facility. We produce a wide range of proteins ranging from enzymes to cytokines providing our researchers with low cost, high quality material for their research.

Besides the use of E. coli, we have developed and established protocols for eukaryotic expression systems (Komagataella pastoris). This system helped us to overcome the common problems especially encountered expressing cytokines in prokaryotic systems, e.g. production of inclusion bodies. It not only allows fast and easy purification of proteins from supernatant, we also observe a far higher biological activity of these proteins compared to the material produced in E. coli. Currently, our current batch size lies in the range of 100 mg.

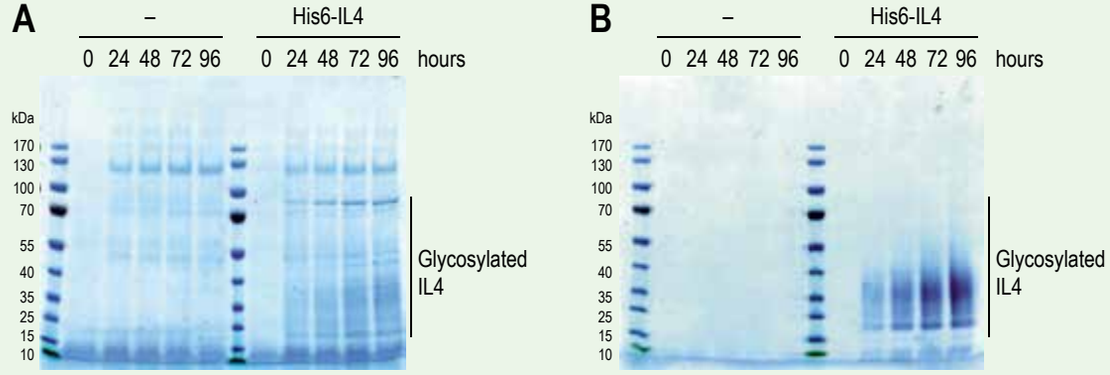


Figure: Example of a cytokine produced in K. Pastoris
Panel A shows the protein composition of wildtype (-) and murine Il4 (His6-Il4) expressing cells; Panel B the protein eluted from a Ni-NTA column after binding and washing of the respective supernatants. (SDS gel stained with Coomassie brilliant blue). Biological Activity was measured by E. Wiedemann, Pavri group.

KARLO PAVLOVIC / LIBRARIAN

MAX PERUTZ LIBRARY

library@imp.ac.at

The Max Perutz Library is a specialized reference library located at the Vienna Biocenter (VBC). Its mission is to develop and maintain collections and services that support research at the IMP, IMBA and GMI.

The main task of the library is to provide comprehensive scientific literature pertaining to the areas of research pursued at the institutes. The Max Perutz Library holds approximately 3000 titles in print. These are partly available in the library's premises itself, being labeled and shelved according to a specific classification system. A part of the titles are shelved in the group laboratories and offices. Increasingly, online books are being licensed in order to provide access for many readers simultaneously and from every workstation. Those several hundred individually selected online books along with the print books can be searched for systematically in the online catalog, where each title is described in respect of its availability and accessibility. The most heavily used kind of literature resource are the licensed online journals. Several bibliographic and full-text databases can be searched for relevant literature on a given topic. This also applies to literature on methods and protocols, including Springer Protocols, Current Protocols or the Journal of Visualized Experiments. A document delivery option is offered for the literature not held by the library, which is delivered within some hours for online content and one week for printed matter. Management as well as researchers are supported with bibliometric services.

Study environment

The reading room serves as a quiet and well-equipped place for reading, writing or just relaxing. Six study desks and a cozy lounge as well as two public computers, wireless LAN, a printer, a book scanner and a spiral binding machine are provided.

Teaching

The library offers special individually oriented training on literature search tools such as catalogs and bibliographic databases for all library users. This ranges from a comprehensive hands-on course on Pubmed searching, to a specific consultation concerning a single challenge in retrieving literature. Assistance is also provided for a variety of client- and web-based literature management tools, such as Endnote or Mendeley. The more, teaching is also provided during the VBC PhD Curriculum twice a year.

Users

The core user group consists of affiliates of the Research Institute of Molecular Pathology (IMP), the Institute of Molecular Biotechnology (IMBA), and the Gregor Mendel Institute of Molecular Plant Biology (GMI). External users from the Max F. Perutz Laboratories (MFPL), the FH Campus Vienna and other readers from the Vienna Biocenter (VBC) are welcome to visit the library premises.

CAMPUS SCIENCE SUPPORT FACILITIES

The Campus Science Support Facilities GmbH (CSF) was established in 2011 at the Vienna Biocenter (VBC) to provide top scientific infrastructure operated and constantly further developed by highly qualified experts. The CSF supports IMP, IMBA and other institutions and companies situated on the VBC at the forefront of science. Besides scientific infrastructure, the CSF also offers social infrastructure such as the Child Care Center. For more information visit the CSF website: www.csf.ac.at

Preclinical Phenotyping (pcPHENO)

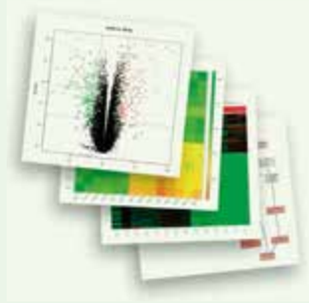
The Preclinical Phenotyping Facility (pcPHENO) provides state of the art equipment and services to test mouse behavior, motor functions, physiology, and metabolism. Phenotypic screening is becoming an increasingly important step in the characterization of genetically modified mice, aiming to link molecular mechanisms to whole-body effects. After training under expert supervision, researchers can perform their experiments alone or make use of our services, ranging from the planning and performing of the experiments to final data analysis and interpretation.

Next Generation Sequencing (NGS)

The goal of the Next Generation Sequencing Facility (NGS) is to provide cutting edge next generation sequencing technology to its users. Next Generation Sequencing has become a key analysis method for biological research. The capacity to expand analysis from more or less defined genomic regions to genome wide studies has boosted the pace of research discovery and enabled researchers to obtain a global view on biological processes. Advice and guidance of sequencing projects are offered by our team that relies on years of experience with sequencing systems and sequencing data analysis. All common sequencing applications are supported and the development of novel methods and protocols encouraged.

Bioinformatics & Scientific Computing (BioComp)

The Bioinformatics & Scientific Computing Facility (BioComp) offers data analysis services and scientific software development for academic research groups and industrial research laboratories. Our wide range of services provides help to understand and exploit the large-scale data sets generated in modern biological and biomedical research. As a "knowledge hub" our facility also offers training, consultation and help-desk for all Vienna Biocenter (VBC) researchers in the fields of biostatistics, programming and bioinformatics.

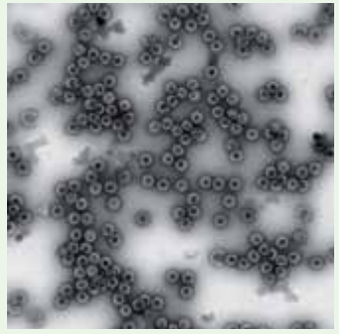


Protein Technologies Facility (ProTECH)

The mission of the Protein Technologies Facility (ProTech) is to help researchers at the Vienna Biocenter overcome two major experimental bottlenecks: protein production and purification. In addition we offer services upstream and downstream of these areas, including molecular cloning and protein characterization, and can provide expertise in most protein-related technologies. Our customers use the proteins and other reagents we generate for antibody generation, biochemical and cell biological assays, structural analysis, study of biomolecular interactions, CRISPR/Cas9 genome engineering experiments.

Electron Microscopy Facility (EM)

The EM Facility (EM) offers a large range of instruments, techniques and expertise to visualize the ultrastructure of biological samples - from molecules to cells & tissues. We have scanning (SEM) and transmission electron microscopes (TEM) and apply numerous techniques (from negative staining to cutting edge cryo-EM) to deliver quick sample screening and high resolution 2D or 3D imaging. Users chose whether they wish to get trained on how to use our equipment themselves or if they prefer us to do everything for them.



Vienna Drosophila Resource Center (VDRC)

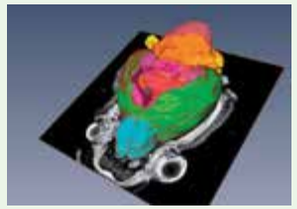
The Vienna Drosophila Resource Center (VDRC), established in 2007, is a professionally organized bio-resource center of international significance. Our primary aim is to facilitate systematic analysis of gene function in Drosophila using in vivo transgenic technology. We maintain and distribute over 30,000 unique transgenic Drosophila stocks, including a genome-wide collection of RNAi lines, and to date have delivered more than 1,000,000 lines to over 2200 registered customers worldwide. We aim to further develop and expand our resources according to emerging technologies and community needs.

Plant Sciences (PlantS)

Many of the groundbreaking findings of molecular mechanisms of life were first described in plants. The Plant Sciences Facility (PlantS) provides 22 state-of-the-art plant growth chambers that allow precise control of environmental conditions. Our services include automated phenotyping for the objective, reproducible and high-throughput assessment of plant phenotypic traits and environmental simulation. Various plant stress conditions such as frost, drought and diverse light conditions can be realized.We help plant researchers to answer their most complicated questions.

Preclinical Imaging Facility (pclMAG)

Preclinical Imaging Facility (pclMAG) offers state of the art ultra-high-field magnetic resonance imaging on a 15.2 T Bruker magnet. We are continuously increasing our range of services to stay current with emerging applications relevant for biological systems. We are currently offering anatomical characterization of organ systems, axonal track tracing, quantitative perfusion measurement, angiography and proton magnetic resonance spectroscopy (1H MRS). Special focus is laid on top quality image analysis, data processing and 3D visualization. Our future outlook includes setting up combination of functional MRI (fMRI) and optogenetics.



Advanced Microscopy (advMICRO)

The Advanced Microscopy Facility (advMICRO) offers users access to a selection of cutting-edge optical microscopy and spectroscopy techniques, along with assistance in their implementation and data analysis. They also offer the development or customization of microscopes for applications where commercial solutions are not available.

Whether one is interested in measuring the dynamics of single molecules in live cells, the morphology and mechanical properties of entire embryos, or something in between – the Advanced Microscopy Facility has an expanding inventory of techniques at your disposal.

Histo Pathology (HP)

The HistoPathology Facility (HP) aims to combine expertise in histological techniques with scientific input from certified veterinary pathologist to provide customers with means for complete analysis of tissues. We offer top quality of standard services such as tissue processing, sectioning and most of the common histological stains for both paraffin and cryoblocks. In addition, customers can benefit from automatic immunostaining protocols, a continuously growing list of optimized antibodies and advanced pathological evaluations. Finally, our service includes consultation before and during the course of the experiment, interpretation of the results and pathology reporting.

Publications

BRENNECKE GROUP

Hayashi, R., Handler, D., Ish-Horowicz, D., Brennecke, J. (2014). The exon junction complex is required for definition and excision of neighboring introns in Drosophila. Genes Dev. 28(16):1772-85

Mohn, F., Sienski, G., Handler, D., Brennecke, J. (2014). The rhino-deadlock-cutoff complex licenses noncanonical transcription of dual-strand piRNA clusters in Drosophila. Cell. 157(6):1364-79

ELLING GROUP

Boztug, K., Järvinen, PM., Salzer, E., Racek, T., Mönch, S., Garncarz, W., Gertz, EM., Schäffer, AA., Antonopoulos, A., Haslam, SM., Schieck, L., Puchałka, J., Diestelhorst, J., Appaswamy, G., Lescoeur, B., Giambruno, R., Bigenzahn, JW., Elling, U., Pfeifer, D., Conde, CD., Albert, MH., Welte, K., Brandes, G., Sherkat, R., van der Werff Ten Bosch, J., Rezaei, N., Etzioni, A., Bellanné-Chantelot, C., Superti-Furga, G., Penninger, JM., Bennett, KL., von Blume, J., Dell, A., Donadieu, J., Klein, C. (2014). JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nat Genet. 46(9):1021-7

Elling, U., Penninger, JM. (2014). Genome wide functional genetics in haploid cells. FEBS Lett. 588(15):2415-21

Paul DS1, Albers CA, Rendon A, Voss K, Stephens J; HaemGen Consortium, van der Harst P, Chambers JC, Soranzo N, Ouwehand WH, Deloukas P. (2014) Maps of open chromatin highlight cell type-restricted patterns of regulatory sequence variation at hematological trait loci. Genome Res. Jul;23(7):1130-41. doi: 10.1101/gr.155127.113. Epub 2013 Apr 9.*
* Elling U is part of HaemGen Consortium*

Wirnsberger, G., Zwolanek, F., Stadlmann, J., Tortola, L., Liu, SW., Perlot, T., Järvinen, P., Dürnberger, G., Kozieradzki, I., Sarao, R., De Martino, A., Boztug, K., Mechtler, K., Kuchler, K., Klein, C., Elling, U., Penninger, JM. (2014). Jagunal homolog 1 is a critical regulator of neutrophil function in fungal host defense. Nat Genet. 46(9):1028-33

GERLICH GROUP

Lukinavičius, G., Reymond, L., D'Este, E., Masharina, A., Göttfert, F., Ta, H., Güther, A., Fournier, M., Rizzo, S., Waldmann, H., Blaukopf, C., Sommer, C., Gerlich, DW., Arndt, HD., Hell, SW., Johnsson, K. (2014). Fluorogenic probes for live-cell imaging of the cytoskeleton. Nat Methods. 11(7):731-3
Mierzwa B.and Gerlich DW. Cytokinetic abscission: molecular mechanisms and temporal control. Dev Cell. (in press)

Turgay, Y., Champion, L., Balazs, C., Held, M., Toso, A., Gerlich, DW., Meraldi, P., Kutay, U. (2014). SUN proteins facilitate the removal of membranes from chromatin during nuclear envelope breakdown. J Cell Biol. 204(7):1099-109

Vázquez-Novelle, MD., Sansregret, L., Dick, AE., Smith, CA., McAinsh, AD., Gerlich, DW., Petronczki, M. (2014). Cdk1 inactivation terminates mitotic checkpoint surveillance and stabilizes kinetochore attachments in anaphase. Curr Biol. 24(6):638-45

IKEDA GROUP

Paolino M, Choidas A, Wallner S, Pranjic B, Uribesalgo I, Loeser S, Jamieson AM, Langdon WY, Ikeda F, Fededa JP, Cronin SJ, Nitsch R, Schultz-Fademrecht C, Eickhoff J, Menninger S, Unger A, Torka R, Gruber T, Hinterleitner R, Baier G, Wolf D, Ullrich A, Klebl BM, Penninger JM.
The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. Nature. 2014 Mar 27;507(7493):508-12.

Snehlata Kumari , Younes Redouane , Jaime Lopez-Mosqueda , Ryoko Shiraishi , Malgorzata Romanowska , Stefan Lutzmayer , Jan Kuiper , Conception Martinez , Ivan Dikic , Manolis Pasparakis, Fumiyo Ikeda Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. In press, eLife, DOI: 10.7554/eLife.03422

KNOBLICH GROUP

Ayukawa, T., Akiyama, M., Mummery-Widmer, JL., Stoeger, T., Sasaki, J., Knoblich, JA., Senoo, H., Sasaki, T., Yamazaki, M. (2014). Dachsous-dependent asymmetric localization of spiny-legs determines planar cell polarity orientation in Drosophila. Cell Rep. 8(2):610-21

Eroglu, E., Burkard, TR., Jiang, Y., Saini, N., Homem, CC., Reichert, H., Knoblich, JA. (2014). SWI/SNF complex prevents lineage reversion and induces temporal patterning in neural stem cells. Cell. 156(6):1259-73

Hagelkruys, A., Lagger, S., Krahmer, J., Leopoldi, A., Artaker, M., Pusch, O., Zezula, J., Weissmann, S., Xie, Y., Schöfer, C., Schlederer, M., Brosch, G., Matthias, P., Selfridge, J., Lassmann, H., Knoblich, JA., Seiser, C. (2014). A single allele of Hdac2 but not Hdac1 is sufficient for normal mouse brain development in the absence of its paralog. Development. 141(3):604-16

Homem, CC., Steinmann, V., Burkard, TR., Jais, A., Esterbauer, H., Knoblich, JA. (2014). Ecdysone and mediator change energy metabolism to terminate proliferation in Drosophila neural stem cells. Cell. 158(4):874-88

Jüschke, C., Xie, Y., Postiglione, MP., Knoblich, JA. (2014). Analysis and modeling of mitotic spindle orientations in three dimensions. Proc Natl Acad Sci U S A. 111(3):1014-9

Lancaster, MA., Knoblich, JA. (2014). Generation of cerebral organoids from human pluripotent stem cells. Nat Protoc. 9(10):2329-40

Lancaster, MA., Knoblich, JA. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. Science. 345(6194):1247125

Marchetti, G., Reichardt, I., Knoblich, JA., Besse, F. (2014). The TRIM-NHL Protein Brat Promotes Axon Maintenance by Repressing src64B Expression. J Neurosci. 34(41):13855-64

Mauri, F., Reichardt, I., Mummery-Widmer, JL., Yamazaki, M., Knoblich, JA. (2014). The conserved discs-large binding partner Banderuola regulates asymmetric cell division in Drosophila. Curr Biol. 24(16):1811-25

Williams, SE., Ratliff, LA., Postiglione, MP., Knoblich, JA., Fuchs, E. (2014). Par3-mInsc and Gai3 cooperate to promote oriented epidermal cell divisions through LGN. Nat Cell Biol. 16(8):758-69

Yasugi, T., Fischer, A., Jiang, Y., Reichert, H., Knoblich, JA. (2014). A regulatory transcriptional loop controls proliferation and differentiation in Drosophila neural stem cells. PLoS One. 9(5):e97034

MARLOVITS GROUP

Galán, JE., Lara-Tejero, M., Marlovits, TC., Wagner, S. (2014). Bacterial type III secretion systems: specialized nanomachines for protein delivery into target cells. Annu Rev Microbiol. 68:415-38

Hornung, P., Troc, P., Malvezzi, F., Maier, M., Demianova, Z., Zimniak, T., Litos, G., Lampert, F., Schleiffer, A., Brunner, M., Mechtler, K., Herzog, F., Marlovits, TC., Westermann, S. (2014). A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. J Cell Biol. 206(4):509-24

Radics, J., Königsmaier, L., Marlovits, TC. (2014). Structure of a pathogenic type 3 secretion system in action. Nat Struct Mol Biol. 21(1):82-7

MARTINEZ GROUP

Hanada T., Weitzer S., Penninger J.M. and Martinez J. Killing the RNA kinase CLP1: consequences on tRNA metabolism and motor neuron maintenance. Wiley Interdiscip Rev RNA. Epub 2014 Aug 20.

Jurkin, J., Henkel, T., Nielsen, AF., Minnich, M., Popow, J., Kaufmann, T., Heindl, K., Hoffmann, T., Busslinger, M., Martinez, J. (2014). The mammalian tRNA ligase complex mediates splicing of XBP1 mRNA and controls antibody secretion in plasma cells. EMBO J.

Karaca E.#, Weitzer S.#, Pehlivan D.#, Shiraishi H., Hanada T., Wiszniewski W., Withers M., Campbell I.M., Erdin S., Isikay S., Franco L.M., Gonzaga-Jauregui C., Gelowani V., Hunter J.V., Koparir E., Yilmaz S., Bernreuther C., Glatzel M., Trattnig S., Friske J., Kronnerwetter C., Bainbridge M.N., Gezdirici A., Seven M., Muzny D.M., Boerwinkle E., Ozen M., Centers for Mendelian Genomics, Clausen T., Yuksel A., Gibbs R.A., Martinez J., Penninger J.M.*, Lupski J.R.*. # co-first authors, *co-corresponding authors. Identification of a novel human neurological syndrome defined by CLP1 mutations that impair tRNA splicing. Cell. 2014 Apr 24;157(3):636-50.*

Popow, J., Jurkin, J., Schleiffer, A., Martinez, J. (2014). Analysis of orthologous groups reveals archease and DDX1 as tRNA splicing factors. Nature. 511(7507):104-7

MOCHIZUKI GROUP

Chalker, D., Meyer, E., and Mochizuki, K. (2014). Epigenetics of Ciliates. In Epigenetics 2nd edition, C. D. Allis, M.L. Caparros, T. Jenuwein and D.Reinberg ed. (Cold Spring Harbor Laboratory Press), in press.

Noto, T., Kurth, HM., Mochizuki, K. (2014). Analysis of Piwi-loaded small RNAs in Terahymena. Methods Mol Biol. 1093:209-24

Vogt, A., Mochizuki, K. (2014). The taming of the shrew: Regulation of a catalytically active domesticated transposase. Mob Genet Elements. 4:e29383

Woehrer, S. L, Aronica, L., Suhren, J. H., Busch, C. J., Noto, T & Mochizuki, K. (2014) A Tetrahymena Hsp90 co-chaperone promotes siRNA loading by ATP-dependent and ATP-independent mechanisms. EMBO Journal, in press.

PENNINGER GROUP

Boztug, K., Järvinen, PM., Salzer, E., Racek, T., Mönch, S., Garncarz, W., Gertz, EM., Schäffer, AA., Antonopoulos, A., Haslam, SM., Schieck, L., Puchałka, J., Diestelhorst, J., Appaswamy, G., Lescoeur, B., Giambruno, R., Bigenzahn, JW., Elling, U., Pfeifer, D., Conde, CD., Albert, MH., Welte, K., Brandes, G., Sherkat, R., van der Werff Ten Bosch, J., Rezaei, N., Etzioni, A., Bellanné-Chantelot, C., Superti-Furga, G., Penninger, JM., Bennett, KL., von Blume, J., Dell, A., Donadieu, J., Klein, C. (2014). JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nat Genet. 46(9):1021-7

Elling, U., Penninger, JM. (2014). Genome wide functional genetics in haploid cells. FEBS Lett. 588(15):2415-21

Galluzzi, L., Bravo-San Pedro, JM., Vitale, I., Aaronson, SA., Abrams, JM., Adam, D., Alnemri, ES., Altucci, L., Andrews, D., Annicchiarico-Petruzzelli, M., Baehrecke, EH., Bazan, NG., Bertrand, MJ., Bianchi, K., Blagosklonny, MV., Blomgren, K., Borner, C., Bredesen, DE., Brenner, C., Campanella, M., Candi, E., Cecconi, F., Chan, FK., Chandel, NS., Cheng, EH., Chipuk, JE., Cidlowski, JA., Ciechanover, A., Dawson, TM., Dawson, VL., De Laurenzi, V., De Maria, R., Debatin, KM., Di Daniele, N., Dixit, VM., Dynlacht, BD., El-Deiry, WS., Fimia, GM., Flavell, RA., Fulda, S., Garrido, C., Gougeon, ML., Green, DR., Gronemeyer, H., Hajnoczky, G., Hardwick, JM., Hengartner, MO., Ichijo, H., Joseph, B., Jost, PJ., Kaufmann, T., Kepp, O., Klionsky, DJ., Knight, RA., Kumar, S., Lemasters, JJ., Levine, B., Linkermann, A., Lipton, SA., Lockshin, RA., López-Otín, C., Lugli, E., Madeo, F., Malorni, W., Marine, JC., Martin, SJ., Martinou, JC., Medema, JP., Meier, P., Melino, S., Mizushima, N., Moll, U., Muñoz-Pinedo, C., Nuñez, G., Oberst, A., Panaretakis, T., Penninger, JM., Peter, ME., Piacentini, M., Pinton, P., Prehn, JH., Puthalakath, H., Rabinovich, GA., Ravichandran, KS., Rizzuto, R., Rodrigues, CM., Rubinsztein, DC., Rudel, T., Shi, Y., Simon, HU., Stockwell, BR., Szabadkai, G., Tait, SW., Tang, HL., Tavernarakis, N., Tsujimoto, Y., Vanden Berghe, T., Vandenabeele, P., Villunger, A., Wagner, EF., Walczak, H., White, E., Wood, WG., Yuan, J., Zakeri, Z., Zhivotovsky, B., Melino, G., Kroemer, G. (2014). Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ.

Huang, F., Guo, J., Zou, Z., Liu, J., Cao, B., Zhang, S., Li, H., Wang, W., Sheng, M., Liu, S., Pan, J., Bao, C., Zeng, M., Xiao, H., Qian, G., Hu, X., Chen, Y., Chen, Y., Zhao, Y., Liu, Q., Zhou, H., Zhu, J., Gao, H., Yang, S., Liu, X., Zheng, S., Yang, J., Diao, H., Cao, H., Wu, Y., Zhao, M., Tan, S., Guo, D., Zhao, X., Ye, Y., Wu, W., Xu, Y., Penninger, JM., Li, D., Gao, GF., Jiang, C., Li, L. (2014). Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients. Nat Commun. 5:3595

Itoh, T., Horiuchi, M., Ikeda, RH., Xu, J., Bannerman, P., Pleasure, D., Penninger, JM., Tournier, C., Itoh, A. (2014). ZPK/DLK and MKK4 form the critical gateway to axotomy-induced motoneuron death in neonates. J Neurosci. 34(32):10729-42

Karaca, E., Weitzer, S., Pehlivan, D., Shiraishi, H., Gogakos, T., Hanada, T., Jhangiani, SN., Wiszniewski, W., Withers, M., Campbell, IM., Erdin, S., Isikay, S., Franco, LM., Gonzaga-Jauregui, C., Gambin, T., Gelowani, V., Hunter, JV., Yesil, G., Koparir, E., Yilmaz, S., Brown, M., Briskin, D., Hafner, M., Morozov, P., Farazi, TA., Bernreuther, C., Glatzel, M., Trattnig, S., Friske, J., Kronnerwetter, C., Bainbridge, MN., Gezdirici, A., Seven, M., Muzny, DM., Boerwinkle, E., Ozen, M., Clausen, T., Tuschl, T., Yuksel, A., Hess, A., Gibbs, RA., Martinez, J., Penninger, JM., Lupski, JR. (2014). Human CLP1 mutations alter tRNA biogenesis, affecting both peripheral and central nervous system function. Cell. 157(3):636-50

Kotsinas, A., Papanagnou, P., Galanos, P., Schramek, D., Townsend, P., Penninger, JM., Bartek, J., Gorgoulis, VG. (2014). MKK7 and ARF: new players in the DNA damage response scenery. Cell Cycle. 13(8):1227-36

Paolino, M., Choidas, A., Wallner, S., Pranjic, B., Uribesalgo, I., Loeser, S., Jamieson, AM., Langdon, WY., Ikeda, F., Fededa, JP., Cronin, SJ., Nitsch, R., Schultz-Fademrecht, C., Eickhoff, J., Menninger, S., Unger, A., Torka, R., Gruber, T., Hinterleitner, R., Baier, G., Wolf, D., Ullrich, A., Klebl, BM., Penninger, JM. (2014). The E3 ligase Cbl-b and TAM receptors regulate cancer metastasis via natural killer cells. Nature. 507(7493):508-12

Qiao, G., Ying, H., Zhao, Y., Liang, Y., Guo, H., Shen, H., Li, Z., Solway, J., Tao, E., Chiang, YJ., Lipkowitz, S., Penninger, JM., Langdon, WY., Zhang, J. (2014). E3 ubiquitin ligase Cbl-b suppresses proallergic T cell development and allergic airway inflammation. Cell Rep. 6(4):709-23

Rao, S., Tortola, L., Perlot, T., Wirnsberger, G., Novatchkova, M., Nitsch, R., Sykacek, P., Frank, L., Schramek, D., Komnenovic, V., Sigl, V., Aumayr, K., Schmauss, G., Fellner, N., Handschuh, S., Glösmann, M., Pasierbek, P., Schlederer, M., Resch, GP., Ma, Y., Yang, H., Popper, H., Kenner, L., Kroemer, G., Penninger, JM. (2014). A dual role for autophagy in a murine model of lung cancer. Nat Commun. 5:3056

Rao, S., Yang, H., Penninger, JM., Kroemer, G. (2014). Autophagy in non-small cell lung carcinogenesis: A positive regulator of antitumor immunosurveillance. Autophagy. 10(3):529-31

Rotblat, B., Southwell, AL., Ehrnhoefer, DE., Skotte, NH., Metzler, M., Franciosi, S., Leprivier, G., Somasekharan, SP., Barokas, A., Deng, Y., Tang, T., Mathers, J., Cetinbas, N., Daugaard, M., Kwok, B., Li, L., Carnie, CJ., Fink, D., Nitsch, R., Galpin, JD., Ahern, CA., Melino, G., Penninger, JM., Hayden, MR., Sorensen, PH. (2014). HACE1 reduces oxidative stress and mutant Huntingtin toxicity by promoting the NRF2 response. Proc Natl Acad Sci U S A. 111(8):3032-7

Sigl, V., Penninger, JM. (2014). RANKL/RANK - from bone physiology to breast cancer. Cytokine Growth Factor Rev. 25(2):205-14

Wang, W., Patel, VB., Parajuli, N., Fan, D., Basu, R., Wang, Z., Ramprasath, T., Kassiri, Z., Penninger, JM., Oudit, GY. (2014). Heterozygote loss of ACE2 is sufficient to increase the susceptibility to heart disease. J Mol Med (Berl). 92(8):847-58
Weitzer, S., Hanada, T., Penninger, J.M., and J. Martinez. CLP1 as a novel player in linking tRNA splicing to neurodegenerative disorders. Wiley Interdiscip Rev RNA. 2014 Aug 20. doi: 10.1002/wrna.1255. PMID:25142875. 2014.

Wirnsberger, G., Zwolanek, F., Stadlmann, J., Tortola, L., Liu, SW., Perlot, T., Järvinen, P., Dürnberger, G., Kozieradzki, I., Sarao, R., De Martino, A., Boztug, K., Mechtler, K., Kuchler, K., Klein, C., Elling, U., Penninger, JM. (2014). Jagunal homolog 1 is a critical regulator of neutrophil function in fungal host defense. Nat Genet. 46(9):1028-33

Yang, P., Gu, H., Zhao, Z., Wang, W., Cao, B., Lai, C., Yang, X., Zhang, L., Duan, Y., Zhang, S., Chen, W., Zhen, W., Cai, M., Penninger, JM., Jiang, C., Wang, X. (2014). Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. Sci Rep. 4:7027

Zhang, L., Chen, X., Sharma, P., Moon, M., Sheftel, AD., Dawood, F., Nghiem, MP., Wu, J., Li, RK., Gramolini, AO., Sorensen, PH., Penninger, JM., Brumell, JH., Liu, PP. (2014). HACE1-dependent protein degradation provides cardiac protection in response to haemodynamic stress. Nat Commun. 5:3430

Zou, Z., Yan, Y., Shu, Y., Gao, R., Sun, Y., Li, X., Ju, X., Liang, Z., Liu, Q., Zhao, Y., Guo, F., Bai, T., Han, Z., Zhu, J., Zhou, H., Huang, F., Li, C., Lu, H., Li, N., Li, D., Jin, N., Penninger, JM., Jiang, C. (2014). Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. Nat Commun. 5:3594

RINGROSE GROUP

Herzog, VA., Lempradl, A.*, Trupke, J., Okulski, H., Altmutter, C., Ruge, F., Boidol, B., Kubicek, S., Schmauss, G., Aumayr, K., Ruf, M., Pospisilik, A., Dimond, A., Senergin, HB., Vargas, ML., Simon, JA., Ringrose, L. (2014). A strand-specific switch in noncoding transcription switches the function of a Polycomb/Trithorax response element. Nat Genet. 46(9):973-81*
**equal contributions*
Steffen, PA., Ringrose, L. (2014). What are memories made of? How Polycomb and Trithorax proteins mediate epigenetic memory. Nat Rev Mol Cell Biol. 15(5):340-56

TACHIBANA-KONWALSKI GROUP

Rattani, A., Vinod, PK., Godwin, J., Tachibana-Konwalski, K., Wolna, M., Malumbres, M., Novák, B., Nasmyth, K. (2014). Dependency of the spindle assembly checkpoint on Cdk1 renders the anaphase transition irreversible. Curr Biol. 24(6):630-7

Tachibana-Konwalski, K. Chromosomes skate together or apart. Nature, in press.

BIOINFORMATICS

Shodhan A., Lukaszewicz A., Novatchkova M., Loidl J. Msh4 and Msh5 Function in SC-Independent Chiasma Formation During the Streamlined Meiosis of Tetrahymena. Genetics. 2014 Nov; 198(3):983-93.

Rao S., Tortola L., Perlot T., Wirnsberger G., Novatchkova M., Nitsch R., Sykacek P., Frank L., Schramek D., Komnenovic V., Sigl V., Aumayr K., Schmauss G., Fellner N., Handschuh S., Glösmann M., Pasierbek P., Schlederer M., Resch G.P., Ma Y., Yang H., Popper H., Kenner L., Kroemer G., Penninger .JM. A dual role for autophagy in a murine model of lung cancer. Nat Commun. 2014;5:3056. doi: 10.1038/ncomms4056.

Homem C.C., Steinmann V., Burkard T.R., Jais A., Esterbauer H., Knoblich J.A.. Ecdysone and mediator change energy metabolism to terminate proliferation in Drosophila neural stem cells. Cell. 2014 Aug 14;158(4):874-88. doi: 10.1016/j.cell.2014.06.024.

Eroglu E., Burkard T.R., Jiang Y., Saini N., Homem C.C., Reichert H., Knoblich J.A. SWI/SNF complex prevents lineage reversion and induces temporal patterning in neural stem cells. Cell. 2014 Mar 13;156(6):1259-73. doi: 10.1016/j.cell.2014.01.053.

Moczulska K.E., Pichler P., Schutzbier M., Schleiffer A., Rumpel S., Mechtler K. Deep and Precise Quantification of the Mouse Synaptosomal Proteome Reveals Substantial Remodeling during Postnatal Maturation. J Proteome Res. 2014 Oct 3;13(10):4310-24. doi: 10.1021/pr500456t.

Hornung P., Troc P., Malvezzi F., Maier M., Demianova Z., Zimniak T., Litos G., Lampert F., Schleiffer A., Brunner M., Mechtler K., Herzog F., Marlovits T.C., Westermann S. A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. J Cell Biol. 2014 Aug 18;206(4):509-24. doi: 10.1083/jcb.201403081.

Popow J., Jurkin J., Schleiffer A., Martinez J. Analysis of orthologous groups reveals archease and DDX1 as tRNA splicing factors. Nature. 2014 Jul 3;511(7507):104-7. doi: 10.1038/nature13284.

BIOOPTICS

Herzog V.A:, Lempradl A., Trupke J., Okulski H., Altmutter C., Ruge F., Boidol B., Kubicek S., Schmauss G., Aumayr K., Ruf M., Pospisilik A., Dimond A., Senergin H.B., Vargas M.L., Simon J.A. & Ringrose L.. A strand-specific switch in noncoding transcription switches the function of a Polycomb/Trithorax response element. Nature Genetics 2014 August 10 doi:10.1038/ng.3058

Rao S., Tortola L., Perlot T., Wirnsberger G., Novatchkova M., Nitsch R., Sykacek P., Frank L., Schramek D., Komnenovic V., Sigl V., Aumayr K., Schmauss G., Fellner N., Handschuh S., Glösmann M., Pasierbek P., Schlederer M., Resch G.P., Ma Y., Yang H., Popper H., Kenner L., Kroemer G., Penninger J.M. A dual role for autophagy in a murine model of lung cancer. Nat Commun. 2014 Jan 20;5:3056. doi: 10.1038/ncomms4056.

Csiszar A., Kutay B., Wirth S., Schmidt U., Macho-Maschler S., Schreiber M., Alacakaptan M., Vogel G.F., Aumayr K., Huber L.A., Beug H. Interleukin-like epithelial-to-mesenchymal transition inducer activity is controlled by proteolytic processing and plasminogen ¿ urokinase plasminogen activator receptor system-regulated secretion during breast cancer progression. Breast Cancer Res. 2014 Sep 9;16(5):433.

PROTEIN CHEMISTRY

Dorfer, V., Pichler, P., Stranzl, T., Stadlmann, J., Taus, T., Winkler, S., Mechtler, K. (2014). MS Amanda, a universal identification algorithm optimized for high accuracy tandem mass spectra. J Proteome Res. 13(8):3679-84

Dürnberger, G., Camurdanoglu, BZ., Tomschik, M., Schutzbier, M., Roitinger, E., Hudecz, O., Mechtler, K., Herbst, R. (2014). Global analysis of muscle-specific kinase signaling by quantitative phosphoproteomics. Mol Cell Proteomics. 13(8):1993-2003

Hofbauer, HF., Schopf, FH., Schleifer, H., Knittelfelder, OL., Pieber, B., Rechberger, GN., Wolinski, H., Gaspar, ML., Kappe, CO., Stadlmann, J., Mechtler, K., Zenz, A., Lohner, K., Tehlivets, O., Henry, SA., Kohlwein, SD. (2014). Regulation of gene expression through a transcriptional repressor that senses acyl-chain length in membrane phospholipids. Dev Cell. 29(6):729-39

Hornung, P., Troc, P., Malvezzi, F., Maier, M., Demianova, Z., Zimniak, T., Litos, G., Lampert, F., Schleiffer, A., Brunner, M., Mechtler, K., Herzog, F., Marlovits, TC., Westermann, S. (2014). A cooperative mechanism drives budding yeast kinetochore assembly downstream of CENP-A. J Cell Biol. 206(4):509-24

Köcher, T., Pichler, P., De Pra, M., Rieux, L., Swart, R., Mechtler, K. (2014). Development and performance evaluation of an ultralow flow nanoliquid chromatography-tandem mass spectrometry set-up. Proteomics. 14(17-18):1999-2007

Mérai, Z., Chumak, N., García-Aguilar, M., Hsieh, TF., Nishimura, T., Schoft, VK., Bindics, J., Slusarz, L., Arnoux, S., Opravil, S., Mechtler, K., Zilberman, D., Fischer, RL., Tamaru, H. (2014). The AAA-ATPase molecular chaperone Cdc48/p97 disassembles sumoylated centromeres, decondenses heterochromatin, and activates ribosomal RNA genes. Proc Natl Acad Sci U S A. 111(45):16166-71

Moczulska, KE., Pichler, P., Schutzbier, M., Schleiffer, A., Rumpel, S., Mechtler, K. (2014). Deep and precise quantification of the mouse synaptosomal proteome reveals substantial remodeling during postnatal maturation. J Proteome Res. 13(10):4310-24

Nasso, S., Hartler, J., Trajanoski, Z., Di Camillo, B., Mechtler, K., Toffolo, GM. (2014). 3DSpectra: A 3-dimensional quantification algorithm for LC-MS labeled profile data. J Proteomics. 112C:156-165

Sawicka, A., Hartl, D., Goiser, M., Pusch, O., Stocsits, RR., Tamir, IM., Mechtler, K., Seiser, C. (2014). H3S28 phosphorylation is a hallmark of the transcriptional response to cellular stress. Genome Res. 24(11):1808-20

Schmidt, A., Trentini, DB., Spiess, S., Fuhrmann, J., Ammerer, G., Mechtler, K., Clausen, T. (2014). Quantitative phosphoproteomics reveals the role of protein arginine phosphorylation in the bacterial stress response. Mol Cell Proteomics. 13(2):537-50

Trentini, DB., Fuhrmann, J., Mechtler, K., Clausen, T. (2014). Chasing phosphoarginine proteins: development of a selective enrichment method using a phosphatase trap. Mol Cell Proteomics.

Waidmann, S., Kusenda, B., Mayerhofer, J., Mechtler, K., Jonak, C. (2014). A DEK Domain-Containing Protein Modulates Chromatin Structure and Function in Arabidopsis. Plant Cell.

Walzer, M., Pernas, LE., Nasso, S., Bittremieux, W., Nahnsen, S., Kelchtermans, P., Pichler, P., van den Toorn, HW., Staes, A., Vandenbussche, J., Mazanek, M., Taus, T., Scheltema, RA., Kelstrup, CD., Gatto, L., van Breukelen, B., Aiche, S., Valkenborg, D., Laukens, K., Lilley, KS., Olsen, JV., Heck, AJ., Mechtler, K., Aebersold, R., Gevaert, K., Vizcaíno, JA., Hermjakob, H., Kohlbacher, O., Martens, L. (2014). qcML: an exchange format for quality control metrics from mass spectrometry experiments. Mol Cell Proteomics. 13(8):1905-13

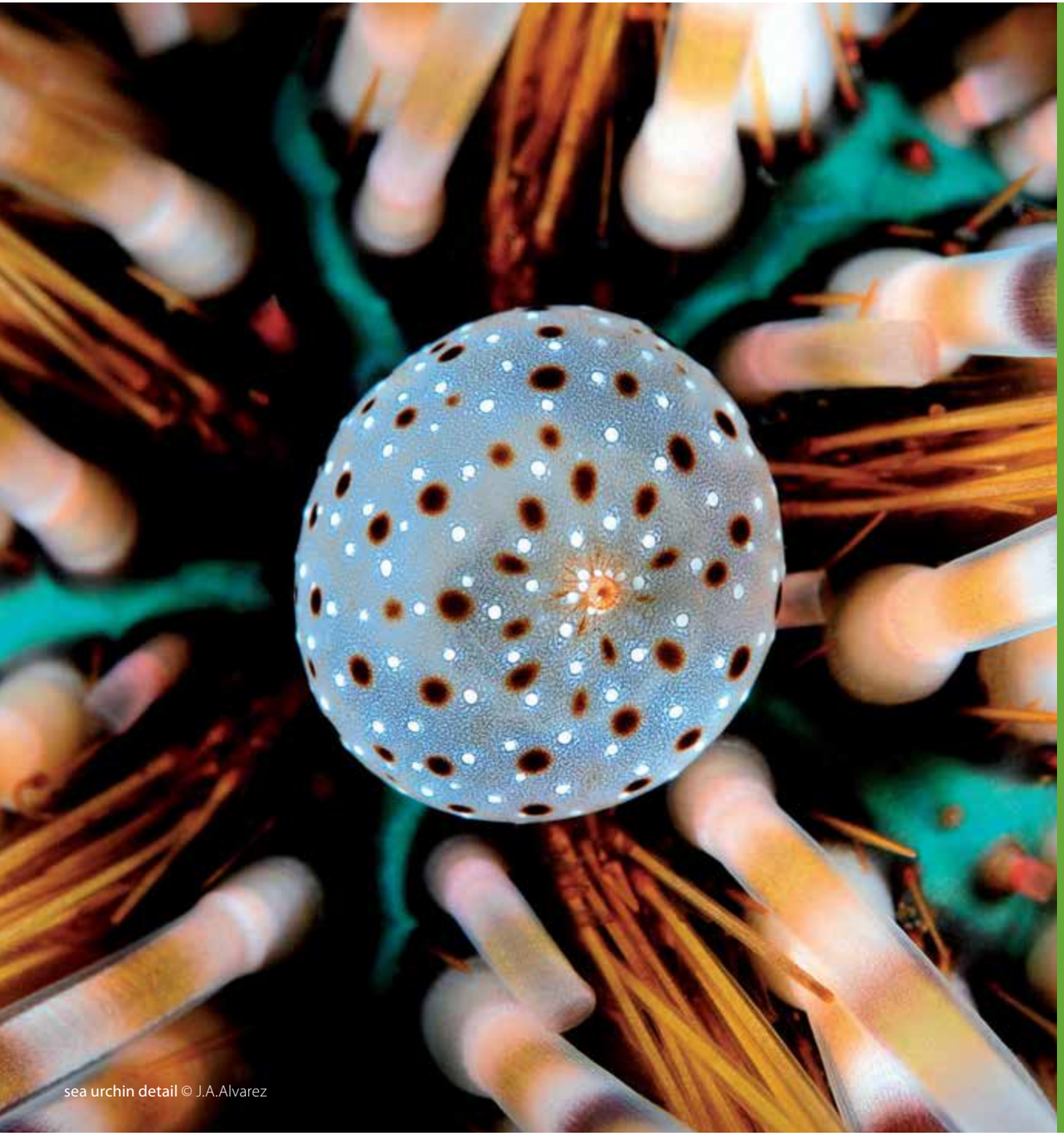
Wirnsberger, G., Zwolanek, F., Stadlmann, J., Tortola, L., Liu, SW., Perlot, T., Järvinen, P., Dürnberger, G., Kozieradzki, I., Sarao, R., De Martino, A., Boztug, K., Mechtler, K., Kuchler, K., Klein, C., Elling, U., Penninger, JM. (2014). Jagunal homolog 1 is a critical regulator of neutrophil function in fungal host defense. Nat Genet. 46(9):1028-33

STEM CELL CENTER

Wenzel D., Bayerl J., Nyström A., Bruckner-Tuderman L., Meixner A., Penninger J.M.. Genetically corrected iPSCs as cell therapy for recessive dystrophic epidermolysis bullosa. Sci. Transl.Med. 6, 264ra165 (2014)

FLY HOUSE

Gokcezade, J., Sienski, G., Duchek, P. (2014). Efficient CRISPR/Cas9 Plasmids for Rapid and Versatile Genome Editing in Drosophila. G3 (Bethesda) 4(11):2279-82



sea urchin detail © J.A.Alvarez

Awards & Honors 2014

Oliver Bell

- *New Frontiers Group – OeAW Impulse Program*
- *Award Human Frontiers Science Program (HFSP) – Career Development Award*
- *Boehringer Ingelheim PhD Fellowship*

Julius Brennecke

- *Elected as EMBO member*

Elif Eroglu (Knoblich Group)

- *VBC PhD Award*
- *Kirsten Peter Rabitsch Award*

Fumiyo Ikeda

- *Consolidator Grant from the European Research Council (ERC)*

Jürgen Knoblich

- *Elected Member of EMBO Council*

Madeline Lancaster (Knoblich Group)

- *Eppendorf Award*
- *Best Paper Award of the Austrian Academy of Sciences*

Martin Moder (Knoblich Group)

- *Wins the Austrian Science Slam Championship*
- *Wins the European Science Slam Finale in Copenhagen*

Josef Penninger

- *Wittgenstein Award by the Austrian Government*
- *Honorary Award for Basic Science by the German Society for Cardiology*

Madalena Reimão-Pinto (Ameres Group)

- *Boehringer-Ingelheim PhD Fellowship*

Kikue Tachibana-Konwalski

- *ERC Starting Grant*
- *Elected member of the "Young Academy" of the Austrian Academy of Sciences (OeAW)*

JANUARY

09.01.14 Susan Gasser
Friedrich Miescher Institute for Biomedical Research
Probing roles for heterochromatin in development and genome stability in C elegans

10.01.14 Chantal Lucini
University of Natural Resources and Life Sciences, Vienna
Not all sugars are sweet: some insights into (gastropod) glycobiology

23.01.14 Patrick Cramer
Gene Center Munich
Molecular systems biology of genome transcription

30.01.14 Keith Slotkin
The Ohio State University
The Regulation of Plant Transposable Elements - The Initiation of Epigenetic Silencing and How the Transposable Elements Fight Back

FEBRUARY

12.02.14 David Ish-Horowicz
MRC Lab for Molecular Cell Biology
Time, space and transcription during development

13.02.14 Benny Shilo
Weizmann Institute of Science
Variability and robustness in embryonic patterning

28.02.14 Katja Wassmann
University of Paris 6
The spindle checkpoint and chromosome segregation in mouse oocytes

MARCH

05.03.14 Gaia Novarino
Institute of Science and Technology Austria
Whole Exome Sequencing and functional analysis identify novel pathways and treatments for cognitive disorders

13.03.14 Craig Pikaard
Indiana University
Tales of repression: mechanisms of selective gene silencing and epigenetic inheritance

APRIL

03.04.14 Emmanuelle Charpentier
Helmholtz Center for Infection Research
CRISPR-Cas9: from bacterial adaptive immunity to RNA-programmable genome engineering

10.04.14 Lynne Maquat
University of Rochester Medical Center
"Alu"strious Effects on Human RNA Metabolism

17.04.14 Dan Finley
Harvard Medical School
Regulation of proteasome activity by ubiquitin chain editing

24.04.14 Pierre Bruhns
Institut Pasteur
Roles of IgG receptors and myeloid cells in antibody-induced cancer immunotherapy

30.04.14 Duncan Smith
New York University
Eukaryotic DNA replication: the view from the lagging strand

MAY

08.05.14 Nikolaus Rajewsky
MDC Berlin
Regulatory RNAs

14.05.14 Martin Rossel Larsen
University of Southern Denmark
Comprehensive quantitative proteomics and PTMomics applied to studying signal transduction pathways

14.05.14 Edward Ballister
University of Pennsylvania
Inducible protein dimerization: new tools and applications to understanding the mitotic checkpoint

15.05.14 Scott Keeney
Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center
A dangerous game: DNA breaks and meiotic chromosome dynamics

22.05.14 Iain Cheeseman
Whitehead Institute for Biomedical Research
Generating a Dynamic Kinetochore-Microtubule Interface

JUNE

12.06.14 Marc Veldhoen
The Babraham Institute
The role of the AhR in epithelial immunology

17.06.14 Ivan Oransky
Retraction Watch
Can We Still Trust Science?

JULY

03.07.14 Tim Hughes
University of Toronto
C2H2 zinc fingers greatly expand the human regulatory lexicon

09.07.14 Lothar Schermelleh
University of Oxford
New insights into X chromosome inactivation by 3D superresolution microscopy

10.07.14 Nicolas Tonks
Cold Spring Harbor Laboratories
Drugging the undruggable: new approaches to exploiting the protein tyrosine phosphatase PTP1B as a therapeutic target

17.07.14 Iannis Aifantis
NYU Cancer Institute
Coming out of the shadows: epigenetic regulation and non-coding RNAs in acute leukemia

22.07.14 Catarina Vicente
The Node Community Manager
Joining the online conversation: how to use social media to communicate your science

23.07.14 Rob Klose
Oxford University
A new logic for polycomb domain formation

24.07.14 Robert Roeder
The Rockefeller University
Transcriptional Regulatory Mechanisms in Animal Cells

25.07.14 Jan Huisken
Max Planck Institute of Molecular Cell Biology and Genetics
Visualizing zebrafish development with high-speed light sheet microscopy

31.07.14 Neil Hunter
HHMI, University of California, Davis
Regulation of Meiotic Prophase by Post-Translational Protein Modification

SEPTEMBER

04.09.14 Tony Hyman
MPI of Molecular Biology and Genetics, Dresden
Liquid like compartments in cells: Implications for polarity and disease

09.09.14 Noboru Mizushima
Tokyo University
Physiological role and molecular mechanisms of autophagy

18.09.14 David Julius
UCSF
TRP channels of the pain pathway: Connecting physiology to atomic structure

24.09.14 Nicole Föger
Austrian Agency for Research Integrity
Research integrity, questionable research practices and research misconduct – a narrow windy road?

25.09.14 Svante Pääbo
Max Planck Institute for Evolutionary Anthropology
Archaic Genomics

OCTOBER

09.10.14 Jonathan Weissman
UCSF
Globally monitoring translation in space and time with ribosome profiling

16.10.14 Wendy Bickmore
MRC Human Genetics Unit
Gene regulation in the context of nuclear space

23.10.14 Andre Nussenzweig
NIH, Center for Cancer Research
Mechanisms that maintain genome stability

30.10.14 John T. Lis
Cornell University
Probing Mechanisms of Transcription Regulation In Cells and Across Genomes

NOVEMBER

13.11.14 Herwig Baier
MPI of Neurobiology
Neural circuits for zebrafish behavior

20.11.14 James Hurley
NIH Bethesda
Structural Choreography of Cellular Self-Cannibalism

27.11.14 Adele Marston
Wellcome Trust Centre for Cell Biology
Orienting chromosomes in mitosis and meiosis

DECEMBER

01.12.14 Robin Allshire
Wellcome Trust Centre for Cell Biology, University of Edinburgh
Establishment and maintenance of specialised chromatin states

02.12.14 Fiona Doetsch
Biozentrum, Uni Basel
Stem Cells in the Adult Brain: Identity and Regulation

04.12.14 Takehiko Kobayashi
National Institute of Genetics
Stability of ribosomal RNA gene cluster and cellular senescence

11.12.14 Stirling Churchman
Genetics Department, Harvard Medical School
Global dynamics of nuclear and mitochondrial gene expression at nucleotide resolution

11.12.14 Manuel Mayr
King's College London
Proteomics and Lipidomics Combined for Cardiovascular Biomarker Discovery

Scientific Advisory Board

Eric R. Kandel
Columbia University,
New York, USA
(co-chair)

Maria Leptin
Institute of Genetics,
University Cologne,
Germany

Elaine Fuchs
Rockefeller University
/ Howard Hughes
Medical Institute
New York, USA

Anthony Hyman
Max Planck Institute of Molecular
Cell Biology and Genetics,
Dresden, Germany
(co-chair)

Joseph Schlessinger
Yale School of Medicine
New Haven, USA

Gregory J. Hannon
Cold Spring Harbor Laboratory/
Howard Hughes Medical Institute
Cold Spring Harbor, USA
Cancer Research UK Cambridge Institute
University of Cambridge

Fiona Watt
Centre for Stem Cells
and Regenerative
Medicine, King´s
College London, UK

Guido Kroemer
Cordeliers Research
Center
University of Paris
Descartes
Paris, France

Gary Ruvkun
Department of Genetics/
Harvard Medical School,
Boston, USA

REPRESENTATIVES OF THE AUSTRIAN ACADEMY OF SCIENCES

Anton Zeilinger
President of the Austrian
Academy of Sciences
Vienna, Austria

Michael Alram
Vice president of the Austrian
Academy of Sciences
Vienna, Austria

In order to maintain the highest standard of research, IMBA has installed a process of review and feedback: the Scientific Advisory Board (SAB), consisting of internationally recognized scientists. The Board meets yearly at IMBA, and, together with IMBA researchers, discusses the quality, significance, and main focus of research conducted at IMBA.

MANAGEMENT

Josef PENNINGER	Managing Director/Science
Jürgen KNOBLICH	Deputy Director/Science
Michael KREBS	Managing Director Finance & Administration
Peter STEINLEIN	Manager Scientific Services

MANAGEMENT ASSISTANCE

Sandra PRASSEL	Head of Scientific Secretary
Sonja Marietta PAULICK	Assist./Admin. Director
Denise LANGER	Assist./Managing Director Finance & Administration

ACCOUNTING/CONTROLLING

Brigitte WEISER	Head of Accounting/Controlling Department
Lothar HÖLZL	Senior Accountant
Angela GANGLBERGER	Accounting Clerk
Brigitte HORVATH	Accounting Clerk
Andreas LIEBHARD	Accounting Clerk
Anneliese STRELL	Accounting Clerk
Renate ZETL	Accounting Clerk
Markus BAUSBEK	Senior Controller
Romana RONGITSCH	Senior Controller

HUMAN RESOURCES & GRANTS MANAGEMENT

Tanja WINKLER	Head of HR & Grants Management
Sabine STEURER	Dep. Head of HR & Grants Management
Xiao-Hui GAO	Grant Controller
Werner LEITNER	Senior Personnel Officer
Renate OTTO	Assist./Dep. Head of HR & Grants Manager
Daniela PILLER	Senior Personnel Officer
Uta POHN	Assist./Head of HR & Grants Manager
Michael RIEDER	Grant Controller

IT SERVICE DEPARTMENT

Ronny ZIMMERMANN	Head of IT-Service Department
Andreas RIEPL	Deputy Head of IT-Service Department
Gero BEYERLE	IT-System Manager
Christian BRANDSTÄTTER	IT-System Manager
Gert BRÜNNER	IT-System Manager
Herlind ECKHARDT	IT-System Manager
Petar FORAI	IT-System Manager (extern)
Georg RATH	IT-System Manager
Sebastian RUF	IT-System Manager
Alexander WEISS	IT-System Manager (extern)
Max WOHLGEMUTH	IT-System Manager (extern)

GRAPHICS DEPARTMENT

Tibor KULCSAR	Graphic Artist
Johannes TKADLETZ	Graphic Artist

RECEPTION

Doris JADIDI-KHOI-NIGISCHER	Head of Reception
Virginia SALVA MILLAN	Receptionist
Angela WEISSINGER	Receptionist

PURCHASING/STORE

Erwin KUNZ	Head of Purchasing/Store Dep.
Brigitte LENZ	Dep. Head of Purchasing/Store Dep.
Caroline FORMANN	Purchasing Agent
Nina MASTALLER	Purchasing Agent
Eva SCHARNER-KÖCK	Purchasing Agent
Jürgen-Gerhard RUSCHER	Store Manager
Robert BLÜMEL	Store Clerk

COMMUNICATIONS

Evelyn DEVUYST	Head of Communication
Elena BERTOLINI	Communications Manager
Tanja SCHACHNER	Communications Assistant

PRIVATE FUNDING

Thomas ZLABINGER	Fundraising Officer
Sylvia WEINZETTL	Fundraising Officer

CAFETERIA

Michael ENZBERGER	Chef de Cuisine
Taoufik BAHMOU	Chef
Mohamed MOUELHI	Chef
Melanie SIMO	Buffet
Cornelia KUKOVEC	Buffet
Sabine SMALCL	Buffet
Hanna AHARODNIKAVA	Kitchen
Natalija BERTA	Kitchen
Albina HADZIHASANOVIC	Kitchen
Ana MAMUZIC	Kitchen
Nikolina MIHAJLOVIC	Kitchen
Ilona SALEGA	Kitchen
Snezana TOMIC	Kitchen

FACILITY MANAGEMENT

Alexander CHLUP	Head of Facility Management
Michael KRATOCHWILLE	Dep. Head of Fac. Management
Nadir AYKUT	Maintenance Technician
Christian DAVID	Maintenance Technician
Patrick GUST	Maintenance Technician
Franz HICKL	Maintenance Technician (extern)
Wolfgang RACHOLZ	Maintenance Technician
Martin ROJDL	Maintenance Technician
Herbert SCHMIDT	Maintenance Technician (extern)
Stefan TRINKER-GRONAU	Maintenance Technician
Pascal WENINGER	Maintenance Technician

WORKSHOP

Martin COLOMBINI	Head of Workshop
David KUMMERER	Deputy Head of Workshop
Vladimir KOLCAR	Workshop Technician
Michael ZIEGLER	Workshop Technician

as of December 2014

Funding Partners and Public Sponsors

IMBA would like to thank the following funding organizations for their valuable financial commitment and continuing support:

BMFJ
BMWFW
Boehringer Ingelheim
CDMRP, US Dept. of Defense
COST
EMBO
European Research Council
European Union
FFG
Fondation Leducq
FWF
Helmsley Charitable Trust
HFSP
LISAvienna
Novo Nordisk Fonden
ÖAW
OeNB
Stadt Wien
Wirtschaftsagentur Wien
WWTF

EMBO

European Commission

bmwfw
Bundesministerium für Wissenschaft, Forschung und Wirtschaft

erc

FWF
Der Wissenschaftsfonds.

Boehringer Ingelheim

Fondation Leducq

novo nordisk fonden

CDMRP

ÖNB
OESTERREICHISCHE NATIONALBANK

FFG

bmfj

HELMSLEY CHARITABLE TRUST

WWTF
VIENNA SCIENCE AND TECHNOLOGY FUND

wirtschaftsagentur wien
Ein Fonds der Stadt Wien

HFSP

ÖAW
Austrian Academy of Sciences

cost

LISAvienna
life science austria

StaDt Wien

IMBA Donors

Scientific excellence needs individual commitment. We would like to say THANK YOU to all of our supporters, donors and partners. Thank you for your contribution.

Fundraising Committee

Dr. Wolfgang Schüssel (Chair)

Dr. Hannes Androsch

Prof. Dr. Carl Djerassi

Dr. Maximilian Eiselsberg

S.D. Prinz Max von und zu Liechtenstein

KR Dr. Erich Hampel

KR DR. Ludwig Scharinger

Private Supporters & Major Private Donors

Martina Hofstetter

S.D. Prinz Max von und zu Liechtenstein

S.D. Fürst Hans-Adam II von und zu Liechtenstein

Prof. Dr. Josef Penninger

Dr. Hans Michel Piëch

Christian Schmiderer

Prof. Erich W. Streissler

Major Institutional & Corporate Partners

Raiffeisen Landesbank Oberösterreich

Hans Mayr-Fonds and Bank Austria UniCredit Group

OMV

Stiftung Südtiroler Sparkasse

Cooperation Partners

debra – Hilfe für die Schmetterlingskinder.

Fussballklub Austria Wien

Technology Sponsors

eppendorf

Thermo Scientific

Corporate Sponsors

AMGEN

österreichische Lotterien

LGT Private Banking

B&C Privat Stiftung

Borealis

iv Industriellenvereinigung Wien

SAG – Progress in Aluminium

PFLAUM · KARLBERGER · WIENER · OPETNIK Rechtsanwälte

Constantia Industries

RZB

ERSTE Bank – In jeder Beziehung zählen die Menschen.

vienna business agency – zit – The Technology Agency of the City of Vienna

Roche

Hotel Sacher

T-

acm Projektentwicklung GmbH

Spitz

bernhard-av audio-visual systems

FRAUENSCHUH Luxury Sportswear

FANCLUB BULLDOGS

IMBA Key Facts *(Data as of November 2014)*

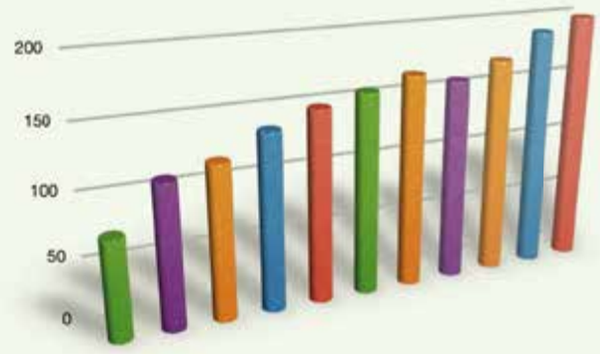
IMBA Staff (in %)

- Scientifc Personnel (incl. Core Facilities)
- Non-scientific Personnel

IMBA Staff - Gender Distribution (in %)

- Male
- Female

IMBA Staff - Development (Headcount)



2004 2005 2006 2007 2008 2009 2010 2011* 2012 2013 2014

** Transfer of IMBA service units into Campus Science Support Facility GmbH*

Scientific Staff - Functions (in %)

- Core Facilities
- Technical Assistants
- Undergraduates
- Postdocs
- PhD Students
- Principal Investigators

Scientific Staff - Nationalities (in %)

- Austria
- Europe (excl. Austria)
- North & South America
- Asia
- Africa

IMBA Budget 2014 (in %)

- Austrian Academy of Sciences
- Research Grants
- Revenues
- Other Sources

Public Research Grants 2014 (in %)

- Austrian Grants
- EU Grants
- Others

Your Career at IMBA

The IMBA focuses on providing a perfect environment for excellent science as well as education, which makes it the right place to develop your career. We offer an exciting setting for undergraduates, PhD students, postdocs, and principal investigators alike. All researchers have access to a superb infrastructure and generous funding, thus allowing for intellectual freedom.

The IMBA is part of the Vienna Biocenter (VBC), and you will be part of a large scientific community. Most of our training offers are run jointly with the other research institutes at the VBC: Research Institute of Molecular Pathology (IMP), the Gregor Mendel Institute of Molecular Plant Biology (GMI), and Max F. Perutz Laboratories (MFPL).

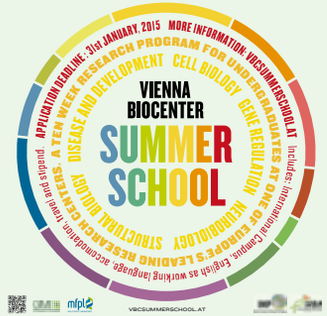
We have specific educational programs for different career stages:

Undergraduate students - Vienna Biocenter Summer School

Every year the Vienna Biocenter Summer School provides a unique opportunity for approx. 25 international undergraduate students to experience cutting-edge scientific research for a period of two months. Our summer fellows are provided with a stipend, accommodation and a travel allowance. Throughout its five editions we have received over 3000 applications from 97 nations, and have hosted 115 fellows.

"A great opportunity to learn new things, see how working in a leading European research institute looks like, meet new people from all over the world – altogether an amazing experience."
Testimonial from a student, member of the 2013 class

Every year we open a competitive call (from December 1 – January 31). For more information visit our website: *www.vbcsummerschool.at*



Graduate students – Vienna Biocenter PhD programme

As a PhD student at the IMBA you can virtually do any experiment you can think of. Our goal is to train independent, critical and creative researchers. The VBC PhD programme is focused on a 4-year research project. The research project is primed by an introductory course at the beginning of your studies and further complemented by courses, lectures, and seminars that run continuously on campus. The PhD Students organize numerous networking activities, and the programme puts great emphasis on the career development of our students.

Most importantly, our faculty includes renowned scientists from all over the world, with outstanding publication records. They are all extremely committed to the training of young researchers. All PhD students benefit from the support extended by our scientific facilities, and are employed on a full-time contract.

The VBC PhD programme is open for applications twice a year (September-November and March-April). We invite the best candidates for an interview and to visit the research institutions at the VBC for a few days. Check out our website for detailed information:
www.vbcphdprogramme.at

Postdocs

The IMBA is the ideal scientific environment for Postdocs to further develop as scientists. At the IMBA postdocs find a supportive and mentoring faculty, have access to a state-of-the-art facility infrastructure, and are part of a lively scientific community.

The IMBA postdocs are part of a larger network – the Vienna Area Postdoc Association – that organizes an annual retreat, career development Initiatives, and a training programme, specifically designed to give you a competitive edge on the job market. Currently the programme includes courses in scientific writing, project management and leadership. All of the courses are facilitated by experienced and outstanding coaches.

Applications for postdoc positions at the IMBA can be submitted directly to the relevant group leader. Funding is available from internal sources, however, postdoc applicants are strongly encouraged to apply for external fellowships to support their research.

No matter which stage of your career you are in, at the IMBA you will always benefit from an

Outstanding scientific infrastructure

Scientific success at the IMBA results, to a significant extent, from a wide array of scientific service facilities. The variety and quality of these units is unmatched by most of the top international research institutes. Support is provided for a wide range of subjects, including conventional and high-throughput sequencing, state-of-the-art mass spectrometry analysis, various microscopy and electron microscopy systems, and a bioinformatics unit. With very few exceptions, the scientist may access all of these facilities free of charge.
For further information on the facilities provided at the VBC, please refer to the pages 32-43 in this booklet.

IMBA and its Surroundings

The Institute of Molecular Biotechnology (IMBA) is located in Vienna, the capital of Austria. IMBA was founded in 1999 by the Austrian Academy of Sciences in cooperation with Boehringer Ingelheim, an international pharmaceutical company with its headquarters in Germany. IMBA operates in close collaboration with the Research Institute of Molecular Pathology (IMP), Boehringer's basic research center in Vienna. The two institutes are located next to each other and share large parts of their infrastructure.

The Vienna Biocenter

IMBA is situated at the "Vienna Biocenter" (VBC), a rapidly growing biotechnology hub located halfway between Vienna's international airport and the city center. Apart from IMBA and its partner institute IMP, the VBC is also home to the Max F. Perutz Laboratories (MFPL; University and Medical University of Vienna), the Gregor Mendel Institute of Molecular Plant Biology (GMI; Austrian Academy of Sciences), a University of Applied Sciences, several biotech companies, a non-profit scientific society and the Vienna Open Lab.
More than 1400 people from 40 different nations currently work at the VBC. Members enjoy a scientifically and socially stimulating environment and take advantage of shared facilities such as the Max Perutz Library and the Vienna Biocenter International PhD Program. A number of events, including seminars and lectures, are open to all.

Vienna–a City of many Facets

For those whose interests stretch beyond science, Vienna also has a lot to offer. Home to about 1.7 million people, the city is the political and cultural center of the country and its gateway to the east. Once the heart of the largest European empire, Vienna draws on a rich cultural heritage which is reflected in splendid buildings and unique art collections. But Vienna is a city of many facets. Modern architecture, splendid galleries and stylish shops are as much a part of everyday life as the famous concert halls, the big museums and the nostalgic little streets. As any European capital, Vienna also offers a vibrant nightlife, with a rich selection of restaurants, cozy bars, and trendy clubs.

Apart from Vienna's focus on art and culture, it also has a long-standing tradition in science. Founded in 1365, the University of Vienna is the oldest university in the German-speaking world and the largest in Austria. With a student population of more than 120,000, Vienna offers not only the academic but also the cultural and social infrastructure that comes with student life. And if your brain needs to be refreshed, there is always the call of nature. A day away from the lab may take you skiing to the nearby Alps, windsurfing at Lake Neusiedl or watching rare birds in the Seewinkel. Even within the borders of Vienna you can enjoy a hike through dense woods, go canoeing in a National Park, climb impressive limestone cliffs or stroll through rolling vineyards. In fact, more than 50% of Vienna's surface area is covered by vegetation.

Attractive and family-friendly environment

IMBA cares a lot about the quality of life of its staff. We offer competitive salaries with health and social benefits. The campus has its own kindergarten (with opening hours suited to the scientists' needs). You may also avail yourself of a number of social activities - such as weekly social hours, a ski trip, and various retreats - which help you to get acquainted with your colleagues.

We try to make relocation as smooth as possible. Several in-house apartments are available to bridge the time until a staff member has found a place of his/her own. Our administrative staff is helpful in finding housing, and our personnel department takes care of your legal requirements including visas, registration, health insurance, and family matters. For school-aged children Vienna offers different types of schooling ranging from public to private, German or foreign language based.

More information about career opportunities at IMBA is available at:
www.imba.oeaw.ac.at/career
http://life.imba.oeaw.ac.at/

Child Care Center (CCC)

Besides scientific infrastructure, the CSF is offering social infrastructure to the Vienna Biocenter, such as the Child Care Center. It is run by Wiener Kinderfreunde and hosts not only little researchers from the Vienna Biocenter but also young media kids from the Media Quarter Marx.
First and foremost the head of our Child Care Center, Dagmar Mirek, and her highly motivated team provide a loving and caring atmosphere for the children. Besides that they offer extended opening hours and the possibility to attend a crèche from 3 month on. Also important for the international surrounding of the Vienna Biocenter are the English lessons with native speaker Betsy Higgins-Pösinger.
The Child Care Center is a creative place for children where they undertake excursions into the countryside, visit kids theatre, grow vegetables, go ice skating and do everything else a children's heart desires.
For more information please visit our website (*www.csf.ac.at*) or contract Dagmar Mirek under +43 (0)1 798 56 10 or kdg.campus@speed.at

IMBA SPOTLIGHTS 2014

Additional Vienna Open Lab

On January 30th, the Vienna Open Lab officially inaugurated its second lab for visitors, more than doubling the capacity for workshops. The new room located next to the IMBA-GMI lecture hall offers 104m² of lab-space and can accommodate up to 30 people. In the course of the extension, the offices were relocated and are now situated conveniently between the two labs. The new lab was sponsored by the Austrian oil and gas company OMV and will offer a number of chemistry-related workshops on top of the current program that focuses on molecular biology.

Junior Science Club

On April 3rd, the "Junior Science Club", which is a seminar series initiated last year by the Vienna Schools Council for students attending secondary school, was held at IMBA. The series aims to inspire and motivate pupils between the age of 11-14 years to look into different fields of science. Under the title "The Future of Medicine" Josef Penninger gave an interesting and charming lecture. The students got fascinating insight into powerful research tools and state-of-the-art technologies.

IMP-IMBA Faculty Retreat

On the 9th and 10th of May, the IMP and IMBA Group Leaders met in Zwettl, Lower Austria, for the annual Faculty Retreat. The two days were devoted to science, team building and the general exchange of ideas.

Ninth Microsymposium on small RNAs

In its ninth edition, the Microsymposium on small RNAs, was once again held at IMBA from May 21st-23rd. Since the Conference was initiated in 2006 by Javier Martinez, it has developed into one of the key meetings of the field, bringing together hundreds of leading scientists. Featured keynote speakers Bill Theurkauf from the University of Massachusetts Medical School and Feng Zhang from the Broad Institute, Harvard and MIT, presented their outstanding work.
The symposium was jointly organized by Javier Martinez, Julius Brennecke and Stefan Ameres. As in previous years, the organizers put special emphasis on promoting young scientists, including a PhD workshop.

Science Soccer Cup

One of the sportive highlights of this year was certainly the Science Soccer Cup. IMBA organized and held the event for the first time on June 13th 2014. Teams from eleven different institutions from the Viennese Life Science Scene participated. The winning team was the Austrian Academy of Sciences core team itself. The overall aim of the game was not only to reinforce the network and communication between the different institutes but a huge focus was put on FairPlay with the statement "show racism the red card" as well. This initiative is meant to strengthen the awareness of kids and young adults towards racism and discrimination in a playful way, which is important in an international work environment like research.

VBC PhD Retreat

This year's VBC PhD retreat was held from June 13th to 15th in Zwettl, Lower Austria. 57 students from all four research institutes at the VBC attended. The talks focused on the often-neglected ethics of publishing in science and featured workshops, talks and stimulating discussion by and with Dr. Nicole Föger (Austrian Agency for Research Integrity) and Dr. Ivan Oransky (Retraction Watch). In addition, nine students presented their current progress in a chalk talk format, and the remaining students showcased their work in poster sessions. Furthermore, one afternoon was dedicated to social activities, especially helping new students get to know each other.

Postdoc Retreat

The postdoc retreat in september brought together 40 postdocs from all four institutes at the VBC. In addition, the Research Center for Molecular Medicine (CeMM) and the Institute of Science and Technology (IST) Austria was represented.
The retreat with the topic bones took place in Prague and was organized by the Vienna Postdoc Organisation.

The Vienna Biocenter Amateur Dramatic Club (VBC ADC)

presented Oscar Wilde's play, "The Importance of Being Earnest", on the 6th and 7th August in the Vienna Biocenter courtyard. Perfect weather in combination with BBQ & Happy Hours made the performances wonderful evening open-air events with laughter and fun. This, the club's 14th production, was also taken "on tour" to IST Austria on August 5th.

Martin Moder wins European Science Slam finale in Copenhagen

Martin Moder, former microbiologist in the group of Jürgen Knoblich, has won the European Science Slam finale held by the Euroscience Open Forum (ESOF) in Copenhagen. The challenge of the Science Slam is to present highly complex topics within 5-10 minutes to a non-scientific audience. Martin Moder explained how he tries to identify genes that can stop tumor growth in Drosophila which might lead to the development of new therapies for cancer patients. With his performance - wearing a fly-costume - he was the clear favorite of the almost 900 viewers.
His performance at the Austrian Science Slam can be found on YouTube *https://www.youtube.com/watch?v=f1TxhMixNGc*

New IMBA Team Leader (Ulrich Elling)

Ulrich Elling started out at IMBA in 2006. After completing his PhD at the EMBL in Heidelberg and his postdoctoral studies in the lab of Josef Penninger, he was promoted to become a team leader here at IMBA. Since July he is running his own lab as well as supporting the project "Haplobank" run by Andreas Leibbrandt (see page 34 in this booklet). During his postdoctoral studies his major discovery was the development of haploid stem cells, which made this "Haplobank" even possible. As for the goals of his group the aim will be to understand early lineage development as well as the establishment of depletion screens.

Vienna Biocenter Summer School

Once again the VBC Summer School, which was initiated by Barry Dickson five years ago, was hugely popular. Out of the nearly 2000 inquiries from all over the world, 21 students were invited to join one of the labs at IMP, IMBA, GMI and MFPL for nine weeks. The whole program was as usual accompanied by a series of lectures giving the opportunity for open discussions and many social activities for a little less scientific get to know each other. Each summer fellow can work on an independent research project under the close supervision of a graduate student. The results of each of their projects were presented at the final Summer School Symposium held on August 28th-29th. Jorge Almagro, who had joined the Ikeda lab at IMBA, was one of the winners of the awards for the best presentations.

IMBA Recess

From October 1st-3rd, IMBA scientists met with members of the Scientific Advisory Board (SAB) to present their work and discuss their research. The SAB, consisting of internationally renowned scientists, was impressed by the scientific performance and high standards of the research presented during the recess. IMBA would like to thank all SAB members for their tremendous support.

IMBA SAB members: please refer to page 50.

Exhibition of photographs by José Alejandro Alvarez

As part of this year's Recess Josef Penninger had invited a special guest to hold the Added Dimensions talk, which marks the end of every Recess. José Alejandro Alvarez, an accomplished underwater photographer, who is also involved in organizations supporting the conservation of the marine ecosystems of this planet, spoke about his experiences as a diver. Josef Penninger and José Alvarez met on a research expedition with Francesca von Habsburg and the tba21 group to the Galapagos Islands. The cover image chosen for this booklet is one of José's many fascinating macro shots of the underwater world giving insights into otherwise hidden worlds. A selection of his photographs is still on display at IMBA.

Come.See.Meet

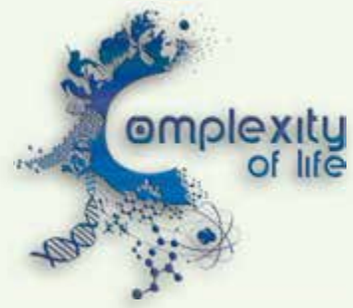
The 2014 „Come.See.Meet" Fundraising Event took place on October 21st, 2014 at IMBA Lecture Hall. Among the distinguished guests Josef Penninger and his team were pleased to welcome, were the members of the IMBA Fundraising Committee Dr. Wolfgang Schuessel, HSH Prince Max von und zu Liechtenstein, Dr. Max Eiselsberg, Dr. Erich Hampel and Dr. Ludwig Scharinger, as well as representatives of UniCredit Bank Austria, Privatbank, Capital Bank, DEBRA Austria, Estée Lauder, Roche, Amgen, Vamed, Eppendorf, Yokogawa, com charis communications, B&C Privatstiftung and FK Austria Wien. After Josef Penninger`s welcome talk, the visitors were given two scientific presentations by Dr. Arabella Meixner ("Stem Cells: New hope for Butterfly Children") and Dr. Bernhard Haubner ("Regeneration of the heart – A utopia or soon a reality"?), an outline of fundraising activities in the past and new strategies for the future by IMBA Administrative Director Michael Krebs, and as an acclaimed breaking-up the entertaining "Fly Show", performed by Martin Moder, winner of the European Science Slam 2014 and former IMBA diploma student.

IMBA director Josef Penninger receives the Wittgenstein Award 2014

At 1.5 million euros, the Wittgenstein Award is the most prestigious and highly endowed prize presented to scientists in Austria, awarded on behalf of the Federal Ministry for Science and Research. This year Josef Penninger was honored for his scientific achievements in the fields of biomedicine and disease pattern research.
In 2011 Penninger's team developed a method to breed stem cells with only one chromosome set (haploid stem cells). Part of the award money is dedicated to further develop this new haploid stem cell technology which will revolutionize modern genetics and has a vast range of potential uses, for example to study the impact of chemotherapeutics used in cancer therapy.
Josef Penninger is the third researcher at IMBA to receive the Wittgenstein Award. In 2005 the prize went to neurobiologist Barry Dickson. And in 2009, molecular biologist and IMBA deputy scientific director, Jürgen Knoblich received the award for his trailblazing findings in the field of stem cell biology.

VBC PhD Symposium Complexity of Life

While in the process of our research we aim to answer questions by reducing them to simple problems, this year's PhD symposium went big and focused on the complexity of life. More than 250 attendees from almost 20 different countries enjoyed talks from a broad array of international high profile speakers such as Mark Vidal (Dana-Farber Cancer Institute, Harvard), Uri Alon (Weizmann Institute of Science, Israel), David Baulcombe (University of Cambridge, UK) and Sean Carroll (Howard-Hughes Medical Institute/University of Wisconsin-Madison, USA). In addition, 13 international Master students were awarded travel grants to visit the symposium and went out of their way to give very positive feedback of the event.



Impressum

Published by
IMBA Communications Department

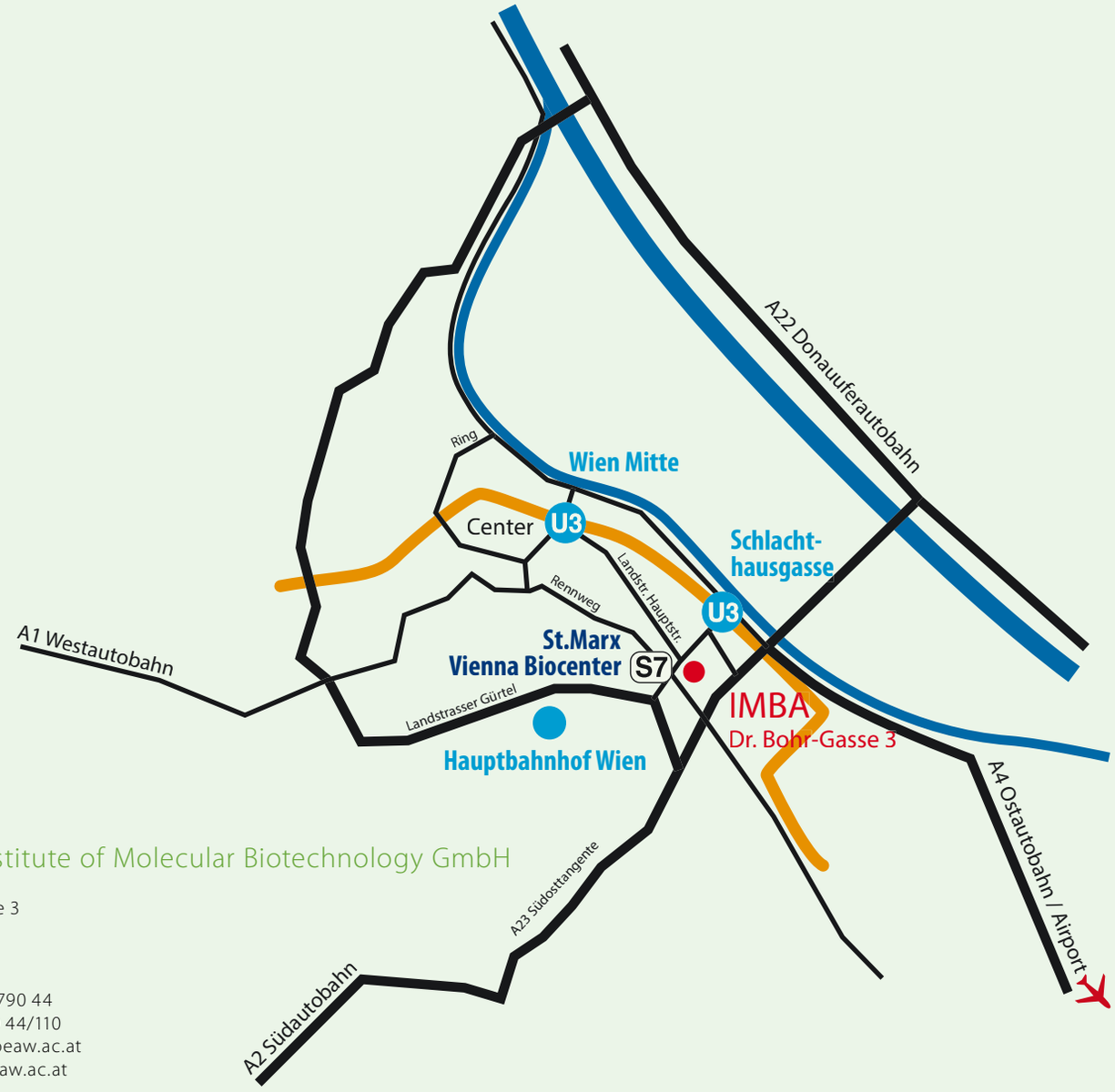
Project Management
Tanja Schachner

Layout & Design
IMP-IMBA Graphics Department

Pictures, Artwork & Credits
IMP/IMBA
IMP-IMBA Graphics Department
Vienna Open Lab/Astrid Bartl (p: 57)
FWF/Hans Schubert (p: 59)
CSF (pp: 42-43, 56)

Cover Illustration
nudibranch detail © J.A.Alvarez

Printed by
Ueberreuter Print GmbH
Industriestraße 1
A-2100 Korneuburg



IMBA - Institute of Molecular Biotechnology GmbH

Dr. Bohr-Gasse 3
1030 Vienna
Austria

Phone: +43(1)790 44
Fax: +43(1)790 44/110
office@imba.oeaw.ac.at
www.imba.oeaw.ac.at

The Institute for Molecular Biotechnology (IMBA) is a basic research institute of the Austrian Academy of Sciences (Österreichische Akademie der Wissenschaften) and a member of the Vienna Biocenter (VBC).