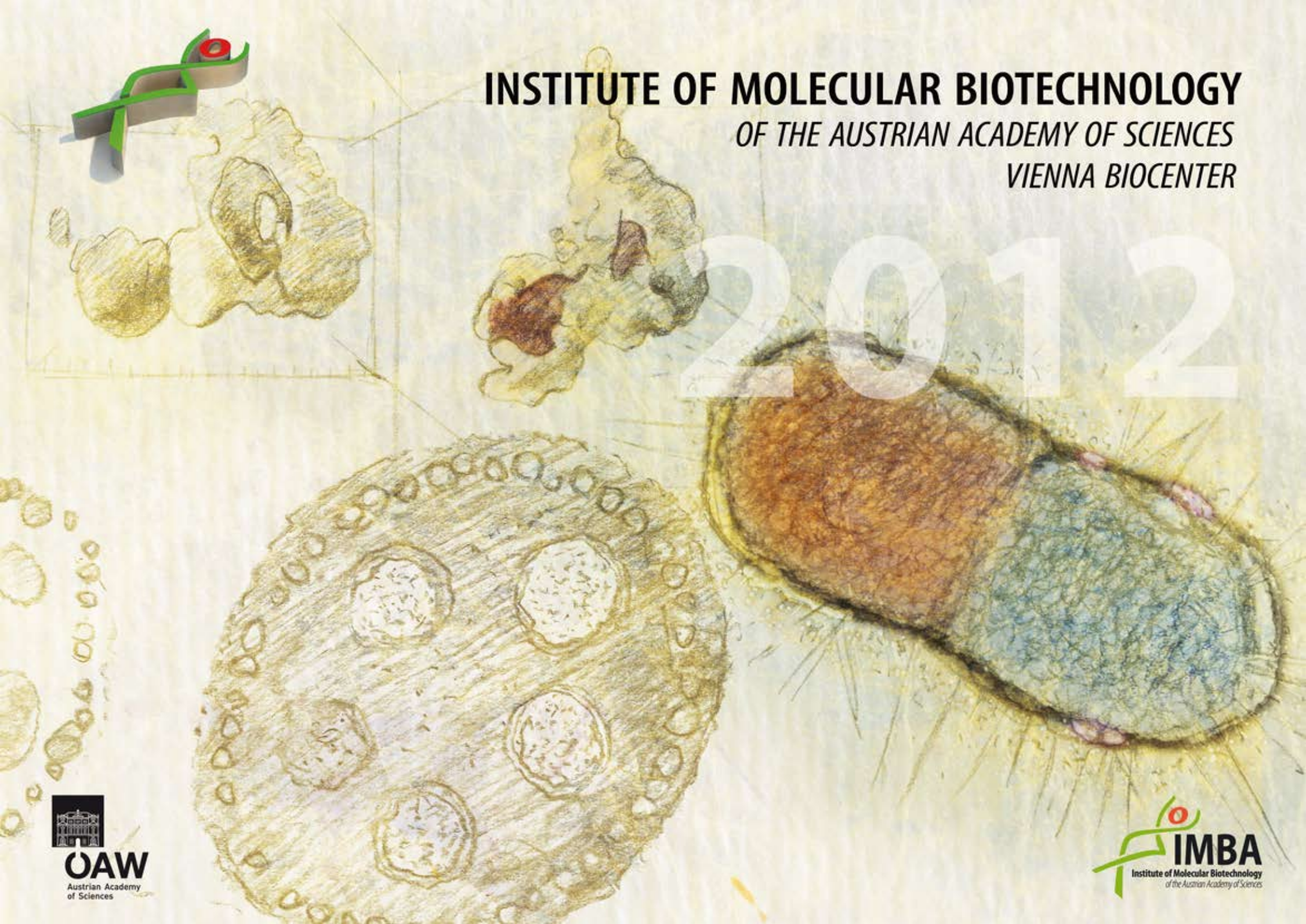


INSTITUTE OF MOLECULAR BIOTECHNOLOGY

OF THE AUSTRIAN ACADEMY OF SCIENCES

VIENNA BIOCENTER

2012

ÖAW
Austrian Academy of Sciences

IMBA
Institute of Molecular Biotechnology
of the Austrian Academy of Sciences

CONTENTS

JOSEF PENNINGER
Managing Director/Science

JÜRGEN KNOBLICH
Deputy Director/Science

MICHAEL KREBS
Managing Director/Finance & Administration

Introduction

The scientific excellence that characterizes renowned research centers involves promoting the best scientists to allow key breakthroughs that change scientific thinking. It has always been our philosophy to invest in the best minds giving them all the freedom that is necessary to succeed even in a growingly competitive world of science. Pioneering spirit not only depends on what is being investigated but also on how research is done, on the culture of an institute. Our culture is simple: to provide a place where the best ideas flourish and where the best scientists can use novel approaches to make discoveries that have the potential to change paradigms one day. The success of our research therefore builds on the shoulders of the best young minds and an infrastructure that allows such minds to shine. It is therefore great to see that IMBA has been able to grow even further during the last year – both in size and in excellence, but also in terms of a constant improvement of organization on all levels.

It is with great pleasure and pride that we present the scientific highlights of our research groups in the annual report. Among the highlights last year was a paper describing a molecular mechanism for how malnutrition and diet can affect innate immunity in the intestine and thereby alter the gut microbiome. This work showed that diet can directly affect the bacteria that live with us and was featured as the cover story in Nature. Another highlight was the first report on how PiRNAs can silence transposons in germ cells and thereby set up a cellular defense system against transposon invasion and propagation; a fundamental discovery with far reaching implications for multiple research fields that appeared in Cell during the fall. The list goes on. Essentially all research groups also presented amazing new work at our scientific recess, some of which is being highlighted in the current annual report.

IMBA has also been voted the second best place, internationally (i.e. outside the US), to work as a postdoctoral fellow, an amazing recognition by the community and testament to everybody at IMBA and the entire Vienna Biocenter Campus. As a campus, we also have hired a coordinator responsible for all PhD students and also our highly successful summer school and postdoctoral trainings. The idea is simple, we might be good and well recognized, but there is always the prospect to get even better! In addition, one of two Innovator Awards from Era of Hope/US Department of Defense went to IMBA which should markedly boost breast cancer research at the institute and Jürgen Knoblich, the Deputy Director of IMBA, just received the Erwin Schroedinger Award, the highest award of the Austrian Academy of Sciences.
One highlight of the year was certainly that all new research groups, Stefan Ameres, Kikue Tachibana-Konwalski, Fumiyo Ikeda, and Daniel Gerlich have not only safely landed in Vienna, but also have successfully started their laboratories at IMBA. These new researchers bring amazing new ideas and technologies, for instance machine

learning combined with high throughput imaging to study cell divisions, or novel methods to assay signal transduction, microRNA processing, and the first cell division after fertilization. Moreover, Oliver Bell from Stanford University will join us next year, bringing along a new technique to directly visualize epigenetic inheritance during cell division. We are proud that those outstanding young scientists have chosen IMBA as their new home. We are very grateful to all people at IMBA and our close and our trusted neighbor IMP, who have worked very hard on making those new recruitments possible – whether it is rebuilding laboratory space, equipping the new labs, administering the new groups or simply made all of them feel welcome.

We also need to acknowledge the excellent work of Andreas Tiran and his entire team who set-up the new Campus Support Facilty (CSF), a new infrastructure and support facility that became possible through a 50 Million Euro investment of the Federal Government and the City of Vienna. They have done a great job and have also integrated some of our employees into their service units. Establishing the CSF will greatly strengthen our campus and increase the technologies available to our research groups. With the implementation of the CSF, the arrival of the new group new leaders at IMBA, excellent new hirings at our neighboring institute IMP and the Max F. Perutz Laboratories (MFPL) of the Medical University of Vienna and University of Vienna, the arrival of Magnus Nordborg as new head of the Gregor Mendel Institute (GMI, our sibling for plant genetics at the Austrian Academy of Sciences), and the opening of a new biotech incubator building, our Campus is bound to continue and enhance its role as a top destination for life sciences in the world. In the next years, we will try everything to make it even better – with IMBA being a key ingredient that adds some taste and spice.

Finally we have to thank all our funding organizations, in particular our parent organization, the Austrian Academy of Sciences. We also thank our Supervisory Board that has always guided us with foresight and knowledge through sometimes stormy times. Our excellent and trusted Scientific Advisory Board has steered our research and the development of the institute with their intelligence and great wisdom. We listen to you! To strengthen the expertise in the research areas of the new IMBA groups, two new members have joined our SAB. Maria Leptin is a Professor of Genetics at Cologne University and the director of EMBO, the most prominent biology research organization in Europe and Guido Kroemer, who works at INSERM and Le Cordelliere in Paris is one of the most cited scientists of our times. Unfortunately, we also have to say Good Bye to our SAB members Susan Lindquist, Kenneth Chien, and Günter Blobel: all of you are irreplaceable and without your help we could not have done it! And, of course, you will remain on our Christmas gift list.

Science at IMBA is flourishing not just in terms of publication output but also with work that truly breaks new ground. This not only is testament to the brilliant research minds that have come together at our institute, but also to all people at all levels, from the cafeteria, to glass washing, media departments and all other service departments, house technique, reception, and of course all people in the administration! You all did an amazing job and we are privileged to work with such a splendid group of dedicated and nice colleagues!

Jürgen Knoblich, Michael Krebs and Josef Penninger

RESEARCH HIGHLIGHTS

2012 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.

1Long-term, efficient inhibition of microRNA function in mice using rAAV vectors.

Xie J, Ameres SL, Friedline R, Hung JH, Zhang Y, Xie Q, Zhong L, Su Q, He R, Li M, Li H, Mu X, Zhang H, Broderick JA, Kim JK, Weng Z, Flotte TR, Zamore PD, Gao G. Nat Methods. 2012 Mar 4;9(4):403-9.

Understanding the function of individual microRNA (miRNA) species in mice would require the production of hundreds of loss-of-function strains. To accelerate analysis of miRNA biology in mammals, we combined recombinant adeno-associated virus (rAAV) vectors with miRNA 'tough decoys' (TuDs) to inhibit specific miRNAs. Intravenous injection of rAAV9 expressing anti-miR-122 or anti-let-7 TuDs depleted the corresponding miRNA and increased its mRNA targets. rAAV producing anti-miR-122 TuD but not anti-let-7 TuD reduced serum cholesterol by >30% for 25 weeks in wild-type mice. High-throughput sequencing of liver miRNAs from the treated mice confirmed that the targeted miRNAs were depleted and revealed that TuDs induced miRNA tailing and trimming in vivo. rAAV-mediated miRNA inhibition thus provides a simple way to study miRNA function in adult mammals and a potential therapy for dyslipidemia and other diseases caused by miRNA deregulation.

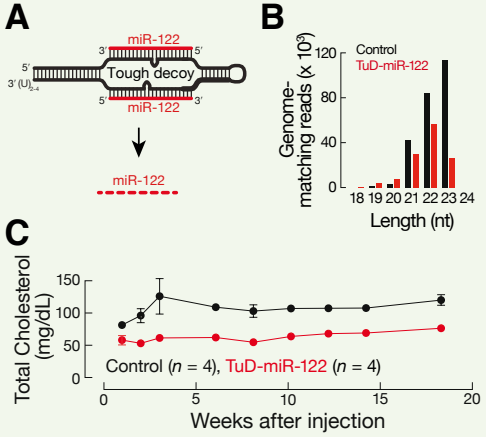
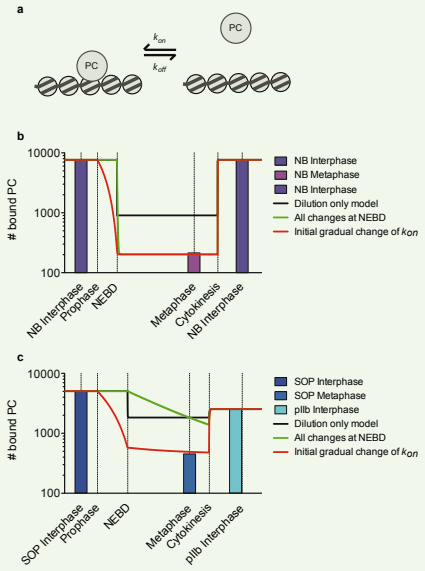


Illustration: Therapeutic inhibition of miRNAs. The expression of structured RNA polymerase III transcripts containing sites highly complementary to miR-122 (Tough Decoy, A) in adult mice induces the sequence specific decay of miR-122 (B) and long-term decrease in cholesterol levels (C) in adult mice, providing a potential therapy for dyslipidemia.



In vivo Polycomb kinetics and mitotic chromatin binding distinguish stem cells from differentiated cells.

Fonseca JP, Steffen PA, Müller S, Lu J, Sawicka A, Seiser C, Ringrose L. Genes Dev. 2012 Apr 15;26(8):857-71.

Epigenetic memory mediated by Polycomb group (PcG) proteins must be maintained during cell division, but must also be flexible to allow cell fate transitions. Here we quantify dynamic chromatin-binding properties of PH::GFP and PC::GFP in living Drosophila in two cell types that undergo defined differentiation and mitosis events. Quantitative fluorescence recovery after photobleaching (FRAP) analysis demonstrates that PcG binding has a higher plasticity in stem cells than in more determined cells and identifies a fraction of PcG proteins that binds mitotic chromatin with up to 300-fold longer residence times than in interphase. Mathematical modeling examines which parameters best distinguish stem cells from differentiated cells. We identify phosphorylation of histone H3 at Ser 28 as a potential mechanism governing the extent and rate of mitotic PC dissociation in different lineages. We propose that regulation of the kinetic properties of PcG-chromatin binding is an essential factor in the choice between stability and flexibility in the establishment of cell identities.

Illustration: We studied kinetics of Polycomb- chromatin binding (a) and used mathematical modeling to identify key differences between Drosophila stem cells (b) and differentiated cells (c).

ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation.

Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. Nature. 2012 Jul 25;487(7408):477-81.

Malnutrition affects up to one billion people in the world and is a major cause of mortality. In many cases, malnutrition is associated with diarrhoea and intestinal inflammation, further contributing to morbidity and death. The mechanisms by which unbalanced dietary nutrients affect intestinal homeostasis are largely unknown. Here we report that deficiency in murine angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (Ace2), which encodes a key regulatory enzyme of the renin-angiotensin system (RAS), results in highly increased susceptibility to intestinal inflammation induced by epithelial damage. The RAS is known to be involved in acute lung failure, cardiovascular functions and SARS infections. Mechanistically, ACE2 has a RAS-independent function, regulating intestinal amino acid homeostasis, expression of antimicrobial peptides, and the ecology of the gut microbiome. Transplantation of the altered microbiota from Ace2 mutant mice into germ-free wild-type hosts was able to transmit the increased propensity to develop severe colitis. ACE2-dependent changes in epithelial immunity and the gut microbiota can be directly regulated by the dietary amino acid tryptophan. Our results identify ACE2 as a key regulator of dietary amino acid homeostasis, innate immunity, gut microbial ecology, and transmissible susceptibility to colitis. These results provide a molecular explanation for how amino acid malnutrition can cause intestinal inflammation and diarrhoea.

Biased transcription and selective degradation of small RNAs shape the pattern of DNA elimination in Tetrahymena.

Schoeberl UE, Kurth HM, Noto T, Mochizuki K.
Genes Dev. 2012 Aug 1;26(15):1729-42.

The ciliated protozoan Tetrahymena undergoes extensive programmed DNA elimination when the germline micronucleus produces the new macronucleus during sexual reproduction. DNA elimination is epigenetically controlled by DNA sequences of the parental macronuclear genome, and this epigenetic regulation is mediated by small RNAs (scan RNAs [scnRNAs]) of~28-30 nucleotides that are produced and function by an RNAi-related mechanism. Here, we examine scnRNA production and turnover by deep sequencing. scnRNAs are produced exclusively from the micronucleus and nonhomogeneously from a variety of chromosomal locations. scnRNAs are preferentially derived from the eliminated sequences, and this preference is mainly determined at the level of transcription. Despite this bias, a significant fraction of scnRNAs is also derived from the macronuclear-destined sequences, and these scnRNAs are degraded during the course of sexual reproduction. These results indicate that the pattern of DNA elimination in the new macronucleus is shaped by the biased transcription in the micronucleus and the selective degradation of scnRNAs in the parental macronucleus.

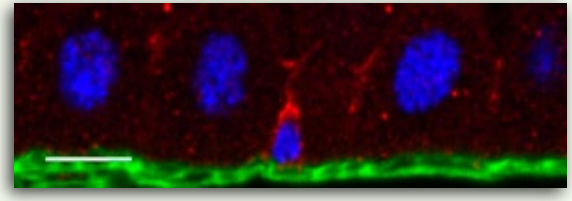
Illustration: Why you look like your parents? This is because your parents passed you not only their DNA but also their epigenetic information, which instructs how DNA should turn on and off. In this study we revealed how such epigenetic information is passed from the parents to the children using the model eukaryote Tetrahymena.

Transcriptional Silencing of Transposons by Piwi and Maelstrom and Its Impact on Chromatin State and Gene Expression.

Sienski G, Dönertas D, Brennecke J.
Cell. 2012 Nov 21;151(5):964-80.

Eukaryotic genomes are colonized by transposons whose uncontrolled activity causes genomic instability. The piRNA pathway silences transposons in animal gonads, yet how this is achieved molecularly remains controversial. Here, we show that the HMG protein Maelstrom is essential for Piwi-mediated silencing in Drosophila. Genome-wide assays revealed highly correlated changes in RNA polymerase II recruitment, nascent RNA output, and steady-state RNA levels of transposons upon loss of Piwi or Maelstrom. Our data demonstrate piRNA-mediated trans-silencing of hundreds of transposon copies at the transcriptional level. We show that Piwi is required to establish heterochromatic H3K9me3 marks on transposons and their genomic surroundings. In contrast, loss of Maelstrom affects transposon H3K9me3 patterns only mildly yet leads to increased heterochromatin spreading, suggesting that Maelstrom acts downstream of or in parallel to H3K9me3. Our work illustrates the widespread influence of transposons and the piRNA pathway on chromatin patterns and gene expression.

Illustration: Piwi and Maelstrom are essential nuclear factors of the piRNA pathway (A and B) and act by triggering transcriptional silencing of transposable elements such as gypsy or mdg1 (C) in the Drosophila ovary, which is accompanied by local H3K9me3 heterochromatin nucleation around the transposon insertion site.

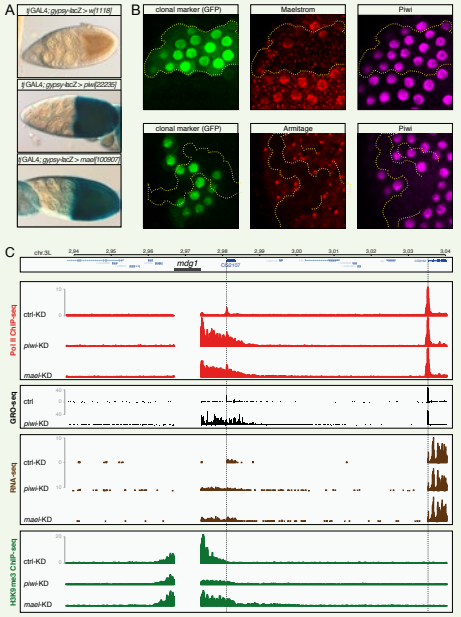


The par complex and integrins direct asymmetric cell division in adult intestinal stem cells.

Goulas S, Conder R, Knoblich JA.
Cell Stem Cell. 2012 Oct 5;11(4):529-40.

The adult Drosophila midgut is maintained by intestinal stem cells (ISCs) that generate both self-renewing and differentiating daughter cells. How this asymmetry is generated is currently unclear. Here, we demonstrate that asymmetric ISC division is established by a unique combination of extracellular and intracellular polarity mechanisms. We show that Integrin-dependent adhesion to the basement membrane induces cell-intrinsic polarity and results in the asymmetric segregation of the Par proteins Par-3, Par-6, and aPKC into the apical daughter cell. Cell-specific knockdown and overexpression experiments suggest that increased activity of aPKC enhances Delta/Notch signaling in one of the two daughter cells to induce terminal differentiation. Perturbing this mechanism or altering the orientation of ISC division results in the formation of intestinal tumors. Our data indicate that mechanisms for intrinsically asymmetric cell division can be adapted to allow for the flexibility in lineage decisions that is required in adult stem cells.

Illustration: A cross-section through a Drosophila gut where smaller stem cells sit besides larger enterocytes on a basal lamina (green) and show the asymmetric localization of the cell fate determinant Par-3 (red) that regulates the balance between proliferation and differentiation.



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 belong to the largest family of trans-acting gene regulatory molecules in multicellular organisms. In flies and mammals, they control more than one half of the protein-coding transcriptome, and act as key regulators of organismal development, physiology, and disease. We are interested in understanding molecular mechanisms that govern small RNA-mediated gene silencing in flies and mammals.

Research activities:

Since the discovery of the first microRNA (miRNA) in 1993, thousands of small non-coding RNAs have been discovered, each regulating the expression of as many as hundreds of genes. Similar 20 to 30 nucleotide-long RNA guides have been identified in protists, fungi, plants and animals, where small silencing RNAs regulate some aspect of nearly every conceivable biological process.

We focus on 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 molecular mechanisms that regulate miRNA homeostasis. To this end, we use 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.

Exonucleolytic microRNA maturation.

miRNAs are produced from longer hairpin-containing RNA transcripts by the RNase III enzymes Drosha and/or Dicer. The resulting 21- to 24-nt mature miRNAs are then loaded into an Argonaute protein, the core protein component of the RNA-induced silencing complex (RISC), to silence mRNAs with complementary sequences. We recently found that more than a quarter of all miRNAs in *Drosophila* undergo 3´ end processing after their production by Dicer, a process that is mediated by the 3´ to 5´ exoribonuclease Nibbler (Figure 1). miRNA 3´ end processing occurs after loading of the small RNAs into RISC and may be the final step in RISC assembly. Nibbler is required for normal fly development. Molecular signatures in small RNA deep sequencing libraries indicate that the process is conserved in mammals. We are currently testing our hypothesis that Nibbler converts miRNAs into isoforms that are compatible with the preferred length of small RNAs within functional RISC complexes.

Target RNA-directed small RNA decay.

Small RNAs guide Argonaute proteins to complementary sequences within mRNAs. In animals miRNAs typically exhibit only partial complementarity to the targets they regulate. We recently showed that high complementarity between miRNAs and their targets causes small RNAs to decay, 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). We aim to characterize 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, consequently, the function of miRNAs themselves.

Therapeutic miRNA inhibition.

Sequence-specific decay of miRNAs harbors a considerable therapeutic potential. The inhibition of miR-122 – a liver-specific regulator of lipid metabolism – results in a reduction of serum cholesterol levels and interferes with hepatitis C virus (HCV) replication. 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. 4) – structured RNA polymerase III transcripts with accessible and 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 to study miRNA function in adult mammals and may serve as a therapy for dyslipidemia and other miRNA-related human diseases.

Publication highlights:

Han, B.W., Hung, J.-H., Weng, Z., Zamore, P.D.&, Ameres S. L. The 3´-to-5´exoribonuclease Nibbler shapes the 3´ ends of microRNAs bound to Drosophila Argonaute 1. Current Biology, 2011 Nov 22, 21 (22), 1878-1887.

Xie, J., Ameres, S. L.*, Friedline, R., Hung, J.-H., Xie, Q., Zhong, L., Zhang, H., Su, Q., He, R., Mu, X., Li, C., Kim, J., Weng, Z., Flotte, T. R., Zamore, P. D. and Gao, G. AAV vector-mediated in vivo miRNA antagonism for studying miRNA function. Nature Methods, 2012 Apr, 9, 403. (* equal contribution)*

Ameres, S. L., Horwich, M. D., Hung, J.-H., Xu, J., Ghildiyal, M., Weng, Z. and Zamore, P. D. Target RNA-directed trimming and tailing of small silencing RNAs. Science, 2010 Jun 18, 328 (5985),1534-39.

Figure 1

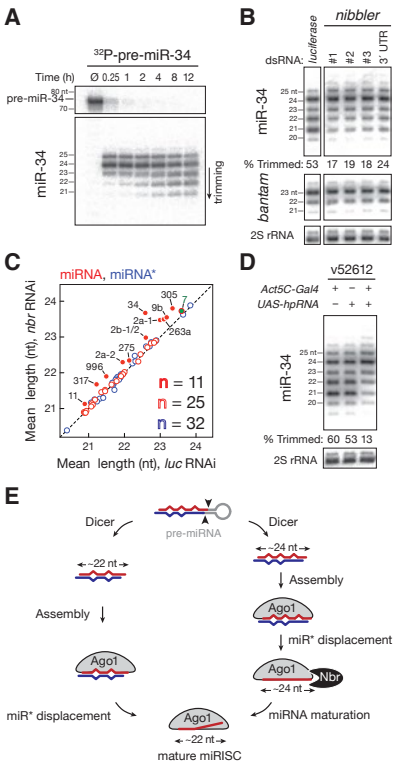


Figure 2

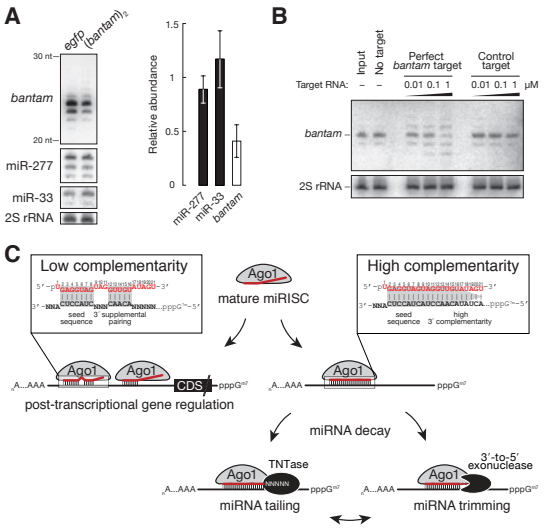
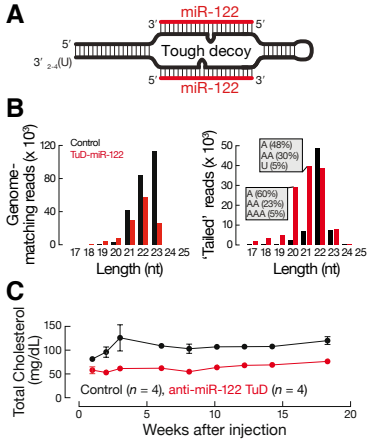


Figure 3



GROUP LEADER: AMERES, STEFAN

POSTDOC: WLOTZKA WIEBKE
PHD STUDENT: IGNATOVA VALENTINA
DIPLOMA STUDENT: BRAUKMANN FABIAN
RESEARCH TECHNICIAN: LÓPEZ SARA FARIÑA

Figure 1: Exonucleolytic maturation of microRNAs. (A) Incubation of 5′ ^{32}P-radiolabeled pre-miR-34 in *Drosophila* embryo lysate results in exonucleolytic maturation of the mature miR-34 after its production by Dicer-1. (**B**) The 3′-to-5′ exoribonuclease Nibbler trims miRNAs. Depletion of the Nibbler in *Drosophil*a S2 cells by RNA interference results in the accumulation of longer 24-nucleotide miR-34 isoforms. miR-34, bantam and 2S rRNA were detected by Northern hybridization. (**C**) More than 25% of all miRNAs in S2 cells are subjected to Nibbler-meditated trimming. The mean length of miRNAs (red) and miRNA*s (blue) in small RNA libraries generated from S2 cells after treatment with double-stranded (ds) RNA to deplete Nibbler (y-axis) or control dsRNA (luciferase, luc, x-axis). miRNAs that increase in length after depletion of Nibbler are highlighted. (**D**) Gal4-directed expression of a hairpin RNA (hp-RNA) targeting *nibbler* in vivo in flies results in the inhibition of miR-34 trimming. (**E**) Model for Nibbler-mediated miRNA maturation. A fraction of non-canonical *Drosophila* miRNAs is generated as long, ~24 nt miRNAs and requires exonucleolytic trimming by Nibbler (Nbr) to form an active silencing complex.

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 exonucleolytic trimming and non-templated nucleotide addition (tailing) of miRNA. miRNA tailing and trimming eventually causes decay of small RNA. 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 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).

JULIUS BRENNECKE GROUP

The piRNA Pathway – A Small RNA Based Genome Immune System

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 gonads. Genetic and bioinformatics studies have uncovered the fascinating conceptual framework of this pathway that is conserved from invertebrates to mammals. Our group systematically dissects the piRNA pathway regarding 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 those, the most widespread members belong to the class of mobile elements, called transposons. In humans, for example, close to 50% of the entire genome is comprised of transposons and their sequence remnants. The remarkable success of these "genome parasites" rests on 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 the reduced long-term fitness of the host.
The acute threat posed by transposable elements has triggered the evolution of powerful defense-systems in eukaryotes. Though early genetic studies pointed to the existence of such host defense-systems, their molecular nature remained mysterious for a long time. After the discovery of RNA interference (RNAi) in 1998, however, it has become increasingly evident that this regulatory mechanism is 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 the animal germline. It is the key genome surveillance system that suppresses the activity of transposons. Over the last 5-10 years a conceptual framework for this pathway emerged: The genome stores transposon sequences in defined heterochromatic loci called 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 for example only at the very beginning to understand processes such as piRNA biogenesis or PIWI mediated silencing. Moreover, the spectrum of biological processes impacted by the piRNA pathway is only 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 being used in order to control vital aspects of chromosome biology. It would not be too surprising if the piRNA pathway were also a key player in this more mutual relationship between genome and transposons.
To study this fascinating genome surveillance system, we use *Drosophila melanogaster* as a model system. For most projects, we combine genetics, biochemistry, cell biology and bioinformatics in often-unique ways. The main areas of our interest are:

1. Identifying and characterizing novel piRNA pathway members: We have established robust RNAi conditions for both, the somatic ovarian cells where a simplified piRNA pathway is active, but also for germline cells, where many piRNA pathway factors are acting specifically (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 promises a deeper understanding of essentially all levels of this pathway, from piRNA cluster biology to piRNA biogenesis and to piRNA mediated silencing.

2. Systems level analysis of gene/transposon expression in wildtype and piRNA pathway mutants: Till today, no systematic analysis on transposon activity and transposition frequency and patterns has been conducted in flies lacking the piRNA pathway. Using our established RNAi conditions we will probe the genome wide consequences of deficiencies in the somatic and germline piRNA pathways. We are taking advantage of deep sequencing technologies coupled to bioinformatics to obtain novel insight into these questions.

3. Understanding the biology of piRNA clusters: piRNA clusters are at the heart of the pathway as 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 transcription, specification, export and processing of piRNA clusters or their transcripts. Ultimately, we want to understand how the cell discriminates cluster transcripts from other RNAs in the cell.

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

GROUP LEADER :
JULIUS BRENNECKE

POSTDOCS: FABIO MOHN, KIRSTEN SENTI
PHD STUDENTS: DERYA DONERTAS, DOMINIK HANDLER, DANIEL OLIVIERI, GRZEGORZ SIENSKI, MICHAELA STEINER
FLY TECHNICIAN: KATHARINA MEIXNER
COMPUTER SCIENTIST: DANIEL JURCZAK

Figure 1

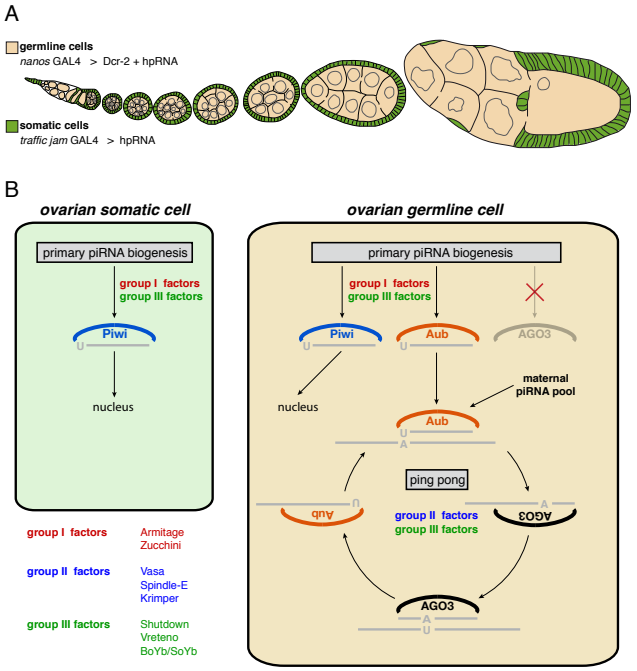


Figure 2

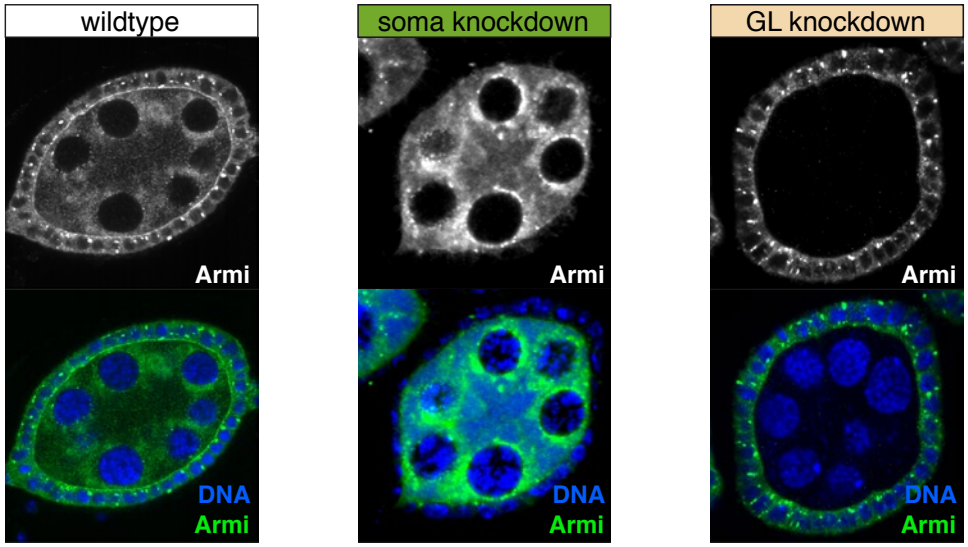


Figure 1: Scheme illustrating the piRNA pathway acting in the *Drosophila* ovary. (A) This cartoon depicts an ovariole, one of the functional units of an ovary. The two major cell types (somatic support cells and germline cells) are shown in green and beige. (B) Schematic summary of the piRNA pathway architecture in somatic (left) and germline cells (right). Depicted is the wiring of the three PIWI family proteins Piwi, Aubergine and AGO3 into piRNA biogenesis processes. Also indicated are genetically identified piRNA biogenesis factors that act at the various indicated steps.

Figure 2: Efficient RNAi approaches for somatic and germline cells in the ovary. Shown are immuno-fluorescence images from single egg chambers stained for the essential piRNA biogenesis factor Armitage. To the left, Armitage expression and localization in wildtype egg chambers is seen in somatic and germline cells. Upon *in vivo* RNAi in either somatic cells (center) or germline cells (right) Armitage protein is essentially lost in the respective cell type.

DANIEL GERLICH GROUP

Cytoskeletal and membrane dynamics in cell division

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

Our research focuses on mechanisms that underlie faithful division of human cells. To obtain large volumes of high time-resolution information on cell division, we develop technology for automated time-lapse microscopy and computational image analysis.

High-throughput microscopy and computer vision

Recent advances in microscope automation have revolutionized modern cell biology by permitting systematic high-throughput investigation of dynamic processes such as cell division. A widely used method to analyze large-scale image data involves a human annotator to train a computer algorithm by exemplary images. This approach, however, is inherently limited by the subjectivity and unreliability of user labeling during the training procedure. Therefore, we developed an image analysis method that classifies cell morphologies without prior user training (Figure 1). This "unsupervised" method of learning exploits the temporal regularity of biological processes to derive a mathematical model of underlying cell dynamics. Our method permits fully objective data quantification in high-throughput live-cell microscopy.

Kinetic framework of mitotic exit

Cell division depends on extensive cell-internal reorganization to assemble the machinery driving chromosome segregation. Exit from mitosis conversely requires the breakdown of mitotic structures such as the mitotic spindle, and reassembly of interphase organelles such as the cell nucleus. Mitotic exit is initiated by activation of the anaphase-promoting complex, which targets many mitotic regulators for destruction, resulting in the inactivation or relocalization of several mitotic kinases away from their substrates.
We aim to identify and understand the function of phosphatases, which subsequently promote the reassembly of functional interphase cells. Using a biosensor-based RNA interference screen, we recently identified PP1 phosphatases that counteract the mitotic Aurora B kinase during anaphase to stabilize chromosome segregation. These phosphatases function independently of a PP2A phosphatase, which controls many other mitotic exit events such as nuclear reassembly and Golgi reformation. Our current research aims to understand the complementary function and regulation of these different mitotic exit phosphatases. Using time-lapse imaging and intracellular laser microsurgery, we also intend to systematically quantify the reaction kinetics underlying spindle assembly checkpoint signaling and the metaphase-anaphase transition to derive a comprehensive mathematical model for this important cell cycle transition.

Mechanism of cytokinetic abscission

Once dividing cells have segregated sister chromatids to opposite spindle poles, contraction of a cortical actin-myosin filament ring partitions the cytoplasm into two domains. The ingression of a cleavage furrow leads to the formation of an intercellular bridge, which finally splits emerging sister cells by a distinct process known as abscission. Failed abscission leads to polyploidy and genomic instability, a hallmark of many solid tumors.
At our laboratory we recently discovered a new filament type that promotes secondary constriction of the intercellular bridge during abscission (Figure 3). These filaments have a diameter of 17 nm and colocalize with the Endosomal Sorting Complex Required for Transport (ESCRT-III), which is also needed for their assembly. To understand how ESCRT-III-dependent filaments contribute to cortical constriction and membrane fission, we are working to obtain further insight into their composition and structure using super-resolution fluorescence imaging and electron microscopy. We further wish to understand the mechanism that polarizes the plasma membrane of the intercellular bridge and orients the abscission filaments parallel to the cleavage plane.

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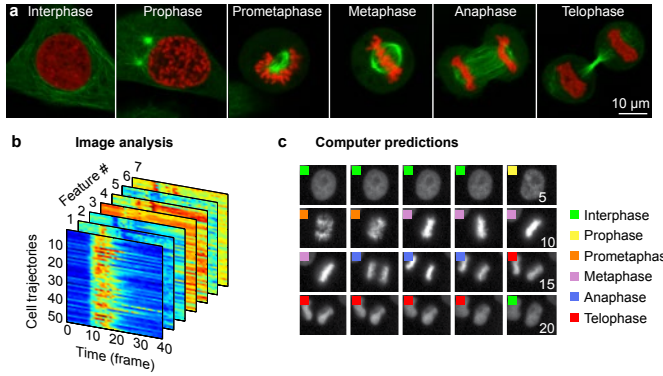
Figure 1



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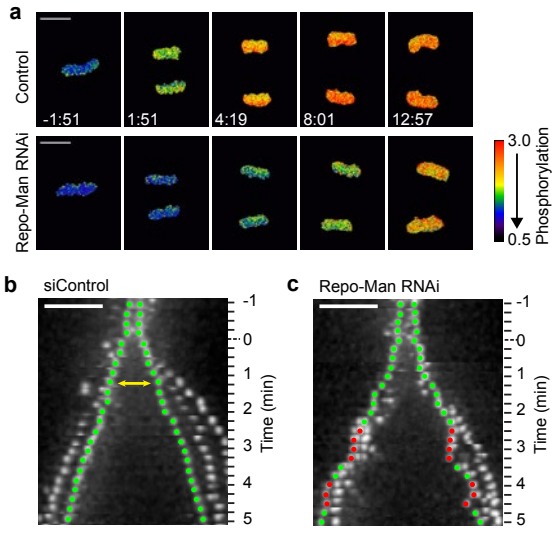

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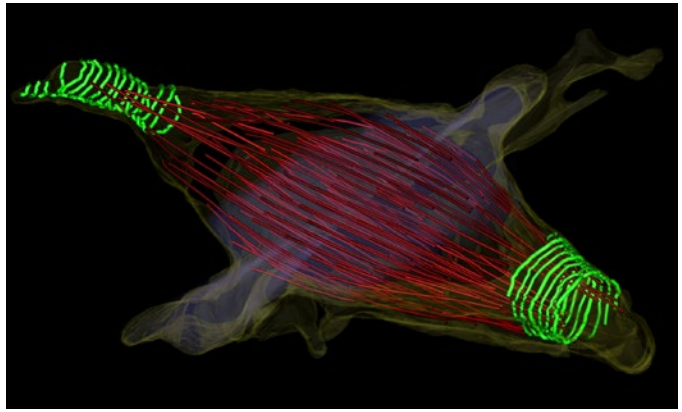

Figure 1: Unsupervised classification of mitotic morphologies. (a) Live cell images of a human tissue culture cell expressing a marker for chromatin (red) and microtubules (green). (b) Cell identification and tracking over time yields trajectories with statistical features describing texture and shape dynamics. (c) Prediction of mitotic cell morphology classes by unsupervised machine learning. Scale bars: 10 um.

Figure 2: Aurora B-counteracting phosphatase contributes to steady chromosome segregation. (a) Dephosphorylation kinetics of Aurora B substrates on chromatin measured by a Fluorescence Resonance Energy Transfer (FRET) biosensor in live human tissue culture cells. Depletion of the PP1 regulatory subunit Repo-Man by RNA interference delays biosensor dephosphorylation. (b) Depletion of Repo-Man leads to transient pausing (red dots) during chromosome segregation, visualized by a fluorescent marker for kinetochores. Scale bars: 10 um.

Figure 3: A new type of filament at the abscission site. Human tissue culture cells were high pressure-frozen for electron tomography. The figure shows a 3D graphic reconstruction of the intercellular bridge. Green: 17 nm diameter filaments; red: microtubules; yellow: plasma membrane; gray: midbody.

SCIENTIST: DANIEL GERLICH

POSTDOCS: SARA CUYLEN, JUAN PABLO FEDEDA, CHRISTOPH SOMMER, FELIX SPIRA
PHD STUDENTS: AMALIE DICK, BEATA MIERZWA, RUGILE STANYTE, QING ZHONG
TECHNICAL ASSISTANT: CLAUDIA BLAUKOPF

FUMIYO IKEDA GROUP

Linear ubiquitination signal in diseases

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

Ubiquitin is a small modifier protein that is highly conserved during evolution from yeast to human. Ubiquitin modification is one of the most sophisticated and versatile post-translational modifications that regulates numerous biological functions, including inflammation, apoptosis, cancer, cell cycle, DNA repair, Parkinson's disease and endocytosis. We are especially interested in understanding the role of a specific type of ubiquitination known as linear ubiquitination.

Generation of linear ubiquitin chain by LUBAC, an E3 ligase complex

Linear ubiquitin chain is a unique linkage type of chain which is linked via a Met residue instead of commonly used Lys residues. As this atypical type of ubiquitin chain was discovered quite recently, little is known about its regulation. The only known linear ubiquitination-specific E3 ligase is LUBAC (linear ubiquitin assembly complex), which consists of HOIP, SHARPIN and HOIL-1L (Figure 1A). We showed that the E3 ligase complex LUBAC plays a critical role in the regulation of TNF-α-induced NF-κB signaling. We aim to understand a) how LUBAC generates linear ubiquitin chains, and b) what the target substrates are. In the laboratory we established an in vitro ubiquitination assay to monitor ubiquitin chain formation by using purified proteins HOIP, SHARPIN and HOIL-1L. We identified a critical residue Cys885 in the HOIP catalytic domain in the regulation of linear ubiquitin chain formation. Cys885 is conserved in different species and is located at the second RING domain (Figure 1A). In HHARI E3 ligase, the Cys is a ubiquitin-loading residue for the intermediate status of ubiquitin transfer to the substrates, which was the first example of the 'HECT-RING hybrid' type of E3 ligase. Accordingly, the purified HOIP-C885S mutant no longer generated linear ubiquitin chains in vitro (Figure 1B) or no longer activated NF-κB (Figure 1C), suggesting HOIP as the HECT-RING type of E3 ligase. However, the manner in which HOIP is specific for generating linear ubiquitin chains is entirely unclear. By further analyzing the catalytic activity of HOIP, we aim to elucidate the enzymatic regulation of LUBAC in the generation of linear ubiquitin chains. Moreover, we are currently setting up a system to screen new targets of LUBAC E3 ligase by combining the in vitro ubiquitin assay with protein chip array.

Role of LUBAC E3 ligase complex in vivo

SHARPIN, one of the non-catalytic subunits of LUBAC, plays a critical role in the regulation of inflammatory responses in vivo. In SHARPIN-deficient (cpdm) mice, inflammation is significantly upregulated in several organs, including the skin. Histological analysis of skin tissue of cpdm mice clearly shows that a SHARPIN deficiency causes inflammation of the skin and apoptosis, as determined by immunohistochemistry using anti-cleaved caspase 3 antibody (Figure 2A). We identified FADD as a new substrate of LUBAC that regulates the TNF-induced apoptosis pathway. FADD is ubiquitinated by LUBAC E3 ligase in vitro (Figure 2B), and this ubiquitination event is required for the anti-apoptosis signaling cascade. We plan to clarify whether apoptosis in keratinocytes plays a role in the regulation of skin inflammation of cpdm, mice using genetically modified mouse models.

To further understand the linear ubiquitination signal in biological functions in vivo, we are establishing a mutant mouse line of HOIP. Depletion of HOIP in mice leads to embryonic lethality before the age of E11-E12 due to developmental defects. This is similar to the phenomena identified in many NF-κB-deficient mice, including NEMO, IKK2, and p65. We aim to understand how HOIP functions during development. Based on the results of screening, we will focus on the role of HOIP in different diseases, using genetically modified mouse lines, in order to discover new biological functions of the linear ubiquitination signal.

In summary, we will elucidate novel functions of the linear ubiquitin signal by combining the biochemical screening method to identify new targets of LUBAC and genetically modified animal models.

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Ikeda F, Deribe YL, Skanland SS, Stieglitz B, Grabbe C, Franz-Wachtel M, van Wijk SJ, Goswami P, Nagy V, Terzic J, Tokunaga F, Androulidaki A, Nakagawa T, Pasparakis M, Iwai K, Sundberg JP, Schaefer L, Rittinger K, Macek B, Dikic I (2011) SHARPIN forms a linear ubiquitin ligase complex regulating NF-kappaB activity and apoptosis. Nature 471: 637-641, 10.1038/nature09814

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Rahighi S, Ikeda F*, Kawasaki M, Akutsu M, Suzuki N, Kato R, Kensche T, Uejima T, Bloor S, Komander D, Randow F, Wakatsuki S, Dikic I (2009) Specific recognition of linear ubiquitin chains by NEMO is important for NF-kappaB activation. Cell 136: 1098-1109, 10.1016/j.cell.2009.03.007*

Figure 1

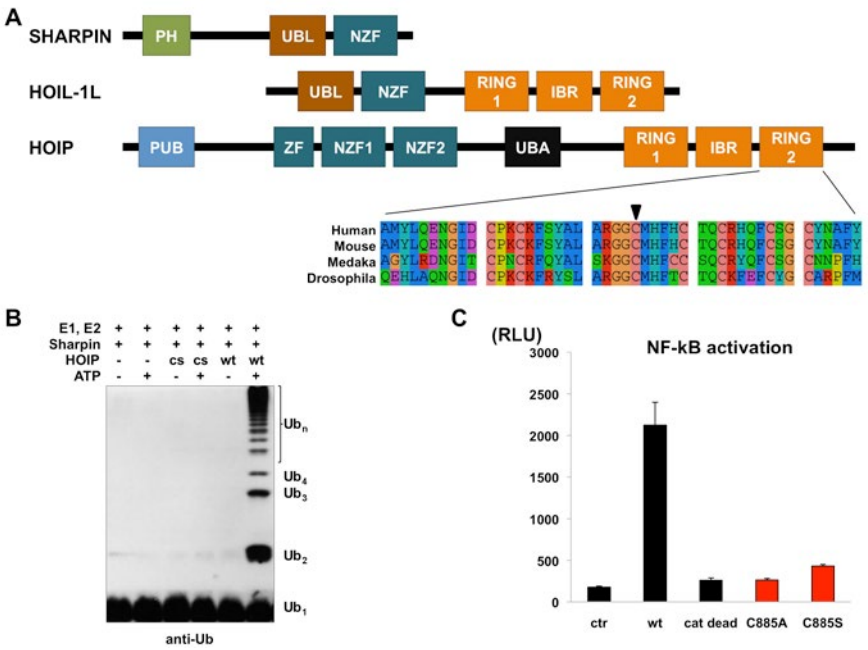
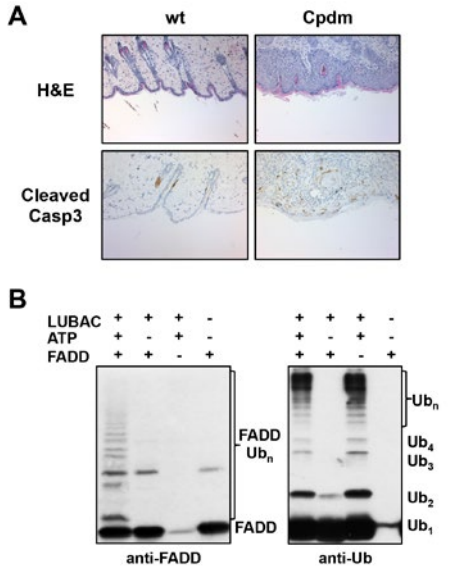
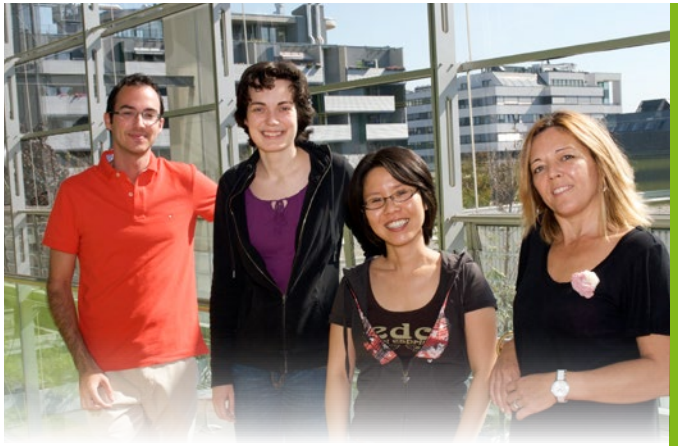


Figure 2





GROUP LEADER: FUMIYO IKEDA

PHD STUDENTS: BETTINA WANKO
DIPLOMA STUDENT: STEFAN LUTZMAYER
TECHNICAL ASSISTANT: CONCEPCION MARTINEZ

Figure 1: Linear ubiquitination is induced by the LUBAC E3 ligase complex. A) A domain structure of LUBAC components, SHARPIN, HOIL-1L and HOIP. A catalytic center is located in the second RING domain (RING2) of HOIP. Alignment of HOIP-RING2 in different species shows high conservation. The arrow indicates C885 in humans, which is perfectly conserved in various species. **B**) Mutation at HOIP-C885 fails to generate linear Ub chains. A mutation at C885 in HOIP abolishes linear ubiquitin chain formation, as seen in the in vitro ubiquitination assay. **C**) HOIP-C885 is critical for NF-κB signaling. NF-κB reporter assay was performed using C885 mutants, which abolished the ability to mediate NF-κB activation.

Figure 2: The linear ubiquitination signal plays a role in the regulation of apoptosis A) Histological analysis of skin tissues of wt and SHARPIN-deficient (cpdm) mice. H&E staining (upper panels) shows that the thickness of the epidermis is increased in cpdm skin tissue compared to wild type (wt), which is a sign of inflammation. In addition, apoptosis in cpdm keratinocytes is very highly induced in cpdm, as determined by cleaved-casp3 staining (lower panels). **B**) FADD is ubiquitinated by LUBAC. An in vitro ubiquitination assay using purified LUBAC and FADD shows that FADD is ubiquitinated in vitro (left panel). Free linear ubiquitin chains are generated regardless of the addition of FADD to the reaction (right panel).

JÜRGEN KNOBLICH GROUP

Neural stem cells in flies and mice

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

Stem cells are characterized by their unique ability to generate both self-renewing and differentiating daughter cells. Our group addresses the molecular mechanisms that establish these different properties within stem cell lineages. For this, we use both Drosophila and mouse genetics and focus on the developing brain where these mechanisms are essential to regulate neurogenesis and prevent tumor formation.

Neural Stem Cells in *Drosophila*

In the *Drosophila* brain, neural stem cells called neuroblasts undergo repeated rounds of asymmetric cell division (Fig. 1A). One of the resulting daughter cells continues to divide in a stem cell-like manner while the other cell terminally divides into two differentiating neurons. During each neuroblast division, the cell fate determinants Numb, Prospero and Brat segregate into the smaller, basal daughter cell where they prevent self-renewal and induce differentiation (Fig. 1A, B). This happens, because the protein kinase aPKC localizes to the opposite, apical side and removes the determinants by phosphorylating their membrane localization domains. At the same time, aPKC associates with microtubule binding proteins to ensure that the mitotic spindle is set up in an apical-basal orientation. As a result, the determinants are specifically inherited by the basal daughter cell. In the absence of Brat, Numb or Prospero, both daughter cells retain the ability to self-renew. As a consequence, stem cells expand exponentially and overgrow the brain. Eventually, they form gigantic brain tumors that can be propagated by serial transplantation into host flies, where they become aneuploid and undergo metastasis (Fig. 1C). Understanding, how the transcriptional changes induced in one daughter cell irreversibly target this cell to differentiation and how defects in this reprogramming event lead to tumorigenesis is one of our main goals.

We have used transgenic RNAi to identify over 600 genes that regulate self-renewal in *Drosophila* neuroblasts. Among those are 18 tumor suppressors that cause neuroblast overproliferation. Besides the asymmetric cell division machinery, this set includes six nuclear proteins that we believe form the transcriptional machinery acting downstream of the segregating determinants. Three of these are part of the SWI/SNF chromatin-remodeling complex, one is a known binding partner of Histone deacetylase and two are implicated in the control of transcriptional elongation. We have recently established a technology that allows us to purify neuroblasts and their differentiating daughter cells in large quantities and to determine their transcriptomes by deep sequencing technology (Fig. 2A). Together with the enormously powerful genetic tools available in *Drosophila*, this allows us to determine the transcriptional changes upon removal of any of the nuclear regulators in a time resolved manner. We have been able to establish a network of transcription factors that act in neuroblasts and establish a stable self-renewing state (Fig. 2B). Development of those resources will hopefully soon allow us to understanding, how the transcriptional network is irreversibly changed after asymmetric cell division in the differentiating daughter cell and how defects in asymmetric cell division drive the cells towards tumor formation.

Asymmetric cell division in mouse stem cells

In the mouse brain, progenitor cells called radial glia generate neurons of the cortex through lineages that are strikingly similar to *Drosophila* neuroblasts. Initially, progenitors expand through symmetric divisions but later, they divide asymmetrically giving rise to differentiating daughter cells as well (Fig. 3). In contrast to flies, however, the mechanisms that establish this asymmetry in mice are largely unknown. We are using our knowledge from *Drosophila* to understand, how those asymmetric divisions are regulated.

The machinery for asymmetric cell division is conserved between flies and mice. To test its role, we mutated the gene *inscuteable*, a specific regulator of asymmetric cell division and spindle orientation in *Drosophila*. In neuroblasts, *inscuteable* is essential for aPKC to orient the mitotic spindle. In mice, *inscuteable* is required for spindle orientation as well. In *inscuteable* knock-out mice, the characteristic re-orientation of cell division that is observed when progenitors switch from symmetric to asymmetric division (Fig. 3B,C) is not observed. Instead, progenitors continue to divide parallel to the surface even late in neurogenesis. As a consequence, lineages shift from indirect to direct neurogenesis, generating neurons instead of intermediate progenitors. Therefore, *inscuteable* mutant mice have less cortical neurons while *inscuteable* overexpression has the opposite effect. These results shed light on the role of spindle orientation during mammalian development and provide a surprising answer to a long standing question in the field of mammalian development. As the expansion of brain size from mice to humans involves intermediate progenitors that divide even more than once, *inscuteable* might play a key role in the evolution of the mammalian neocortex. We are currently extending our experiments to human model systems to test this hypothesis and to analyze the connections between spindle orientation and brain developmental disorders.

Publication highlights:

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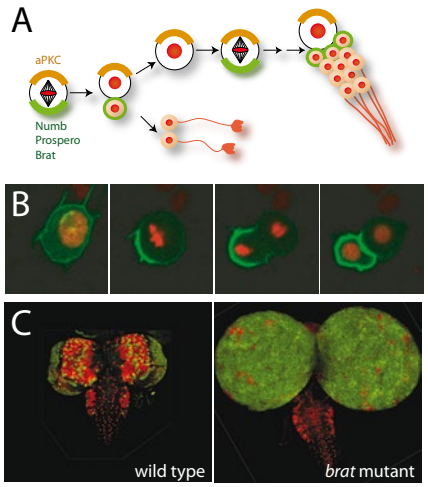
Figure 1



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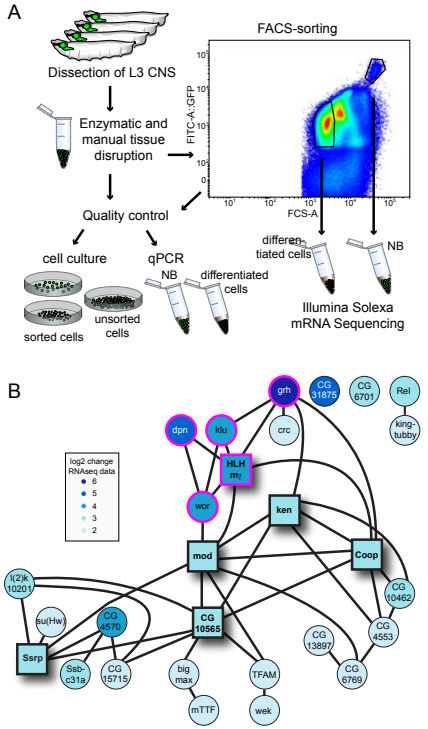

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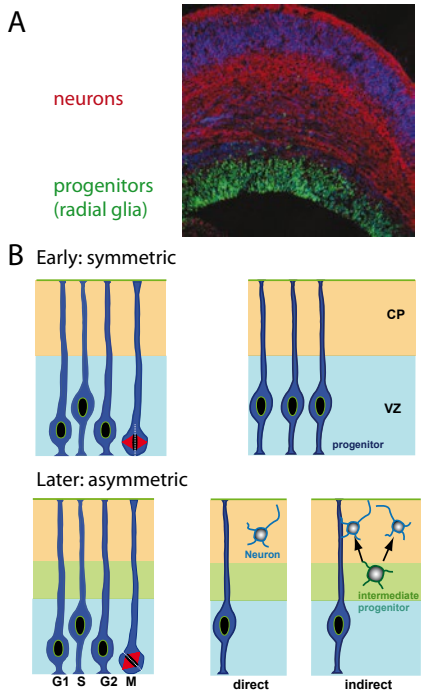


Figure 1: How cells divide asymmetrically. A. Drosophila neuroblasts (white) divide asymmetrically to generate self renewing stem cells (white) and differentiating neurons (red). During each neuroblast division, aPKC (orange) guides the asymmetric segregation of Brat, Prospero and Numb (green) into the differentiating daughter cell. B. Stills from a time-lapse movie of Histone-RFP (red, to visualize chromatin) and Pon-GFP (green, to visualize the Numb protein) separating into one of the two daughter cells during asymmetric division. C. Larval brain from a wild type (left) and *brat* mutant animal. Neuroblasts are green, differentiating neurons are red. *brat* brains show a dramatic overproliferation of neuroblasts.

Figure 2: FACS purification and transcriptional analysis of Drosophila neural stem cells. A. A method for transcriptional profiling of Drosophila neural stem cells and their differentiating sibling cells. Cells express GFP from a neuroblast specific promoter and are sorted by GFP intensity (FITC-A::GFP) and size (FCS-A). The ability to purify neural stem cells in large quantities can be combined with RNAi to determine transcriptional changes downstream of key factors and to resolve those changes in a time dependent manner. B. A transcriptional network for self renewal in Drosophila neuroblasts. Squared symbols indicate hubs, magenta indicates genes regulated by the Notch pathway. Color intensity reflects expression difference between neuroblasts and neurons.

Figure 3: 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 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 to intermediate progenitors (green) that divide once more to generate two neurons. These two modes are called direct or indirect neurogenesis and are regulated by *inscuteable* (see text).

SENIOR SCIENTIST & DEPUTY DIRECTOR/SCIENCE:
JÜRGEN KNOBLICH

POSTDOCS: CORSINI NINA STEFANIE, DE CERTIMA F. HOMEM CATARINA, ENUGUTTI BALAJI, ESK PETER-CHRISTOPHER, LANCASTER MADELINE A., MAURI FEDERICO, REICHARDT ILKA, XIE YUNLI, YASUGI TETSUO
PHD STUDENTS: ABRAMCZUK MONIKA KRISTINA, AU SIN MAN JUNE, EROGLU ELIF, HARZER HEIKE, KAYA ONUR, LANDSKRON LISA, RENNER MAGDALENA, REPIC MARKO, STEINMANN JONAS
DIPLOMA STUDENT: RITSCHKA BIRGIT
RESEARCH ASSOCIATE: KLEINER ELKE
RESEARCH ASSISTANT: PEER ANGELA MARIA

THOMAS MARLOVITS GROUP

Molecular Machines

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

Membrane-associated processes are a fundamental characteristic of all living cells. They ensure that the cells are able to effectively communicate with, and adapt to, their environment. The 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 as *Yersinia, Shigella, Pseudomonas, enteropathogenic/enterohemorrhagic E. coli (EPEC/EHEC)* and *Salmonella* in animals and *Erwinia, Ralstonia* and *Xanthomonas* in plants employ type III secretion systems (T3S systems) for the infection process and are required for persistence inside the host. Human diseases in which type III secretion (T3S) is involved range from mild, such as diarrhea, to deadly, such as bubonic plague. T3S systems are multi-component macromolecular machineries that are usually encoded on specific pathogenicity islands (Figure 1). Their function is to inject proteinacous toxins, referred to as "effectors", into the host cell upon intimate contact. 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 make the host accessible to bacterial infection.

The Injectisome

The T3S system in Gram-negative bacteria has evolved to a complex molecular machine that achieves protein translocation across three membranes – the inner and outer membrane of the bacterial cell and the plasma membrane of the eukaryotic host cell. It consists of many components, its most prominent one being the needle complex, a large hetero-oligomeric membrane protein complex with a molecular weight of about 3.5 megadalton (Figure 2). The name stems from the needle-like protrusion visible in electron micrographs of whole bacterial cells. These protrusions are protein filaments that engage with the host cell and are believed to serve as a conduit for the secretion substrate. The needle filament is linked to the membrane-embedded basal body, which in Gram-negative bacteria spans the inner and outer membrane (about 30x30nm) including the periplasmic space. The basal body has a cylindrical shape, defining a central space within which the inner rod and the socket/cup are localized. The inner rod presumably connects the socket/cup with the needle filament and may help to stably anchor the filament into the basal body. Recently, our lab was the first to provide an experimentally validated map of the topology of the proteins within the complex (Schraidt et al 2010). We subsequently determined the structure of this large organelle to sub-nanometer resolution by cryo EM and single particle analysis (Schraidt & Marlovits, 2011). The structure serves as a model to further understand the structural determinants required for protein translocation across several membranes and thus bacterial infection, and may also be used to design small molecules that interfere with the assembly pathway.

Controlled Steps during Assembly

The needle complex from *Salmonella* (SPI-1) is composed of multiple copies of approximately ten proteins (PrgH/K/I/J, InvG, SpaP/Q/R/S, InvA). A system at this level of complexity requires defined and controlled steps during the assembly. It is initially dependent on the cellular sec-machinery, in particular during the early ring-forming events of assembly. The export apparatus, a group of essential and conserved inner membrane proteins in T3S systems, plays a critical role during the initial phase of the NC assembly. It generates sub-complexes that may serve as nucleation points for the subsequent concentric ring organization of the two inner membrane rings (Wagner et al., 2010) (Figure 3) and is thus localized in the center of assembled needle complexes. In the past year, we have set out to understand the role of the individual members of the export apparatus using structural and biochemical approaches. We have learned that the very early steps of needle complex assembly require only three export apparatus proteins, all of which are essential to arrive at functional complexes. Due to its central position within complexes, we speculate that the export apparatus proteins may also play a role during protein transport. Thus, in the future we will address how substrates engage with the needle complex.
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:

Kosarewicz A, Königsmaier L, Marlovits TC. (2011) The blueprint of the Type-3 Injectisome. Phil Trans Royal Society B, in press.

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

Figure 1

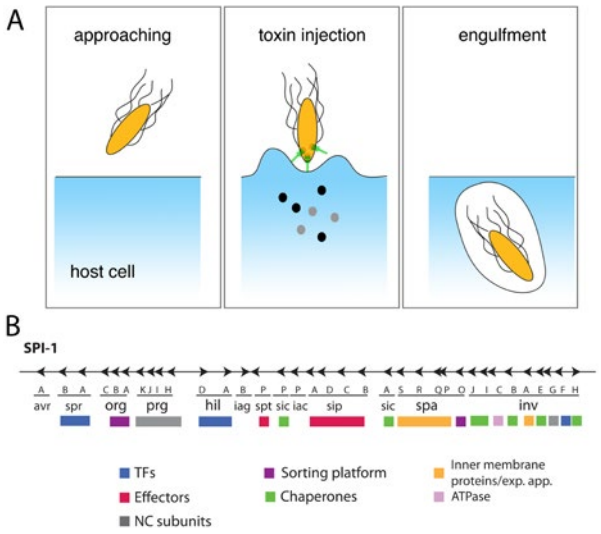
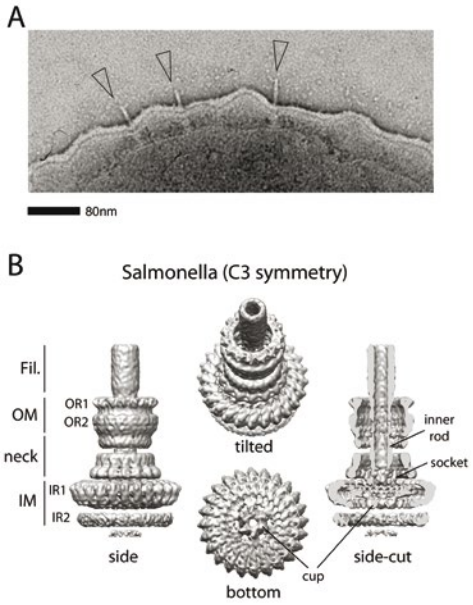


Figure 2



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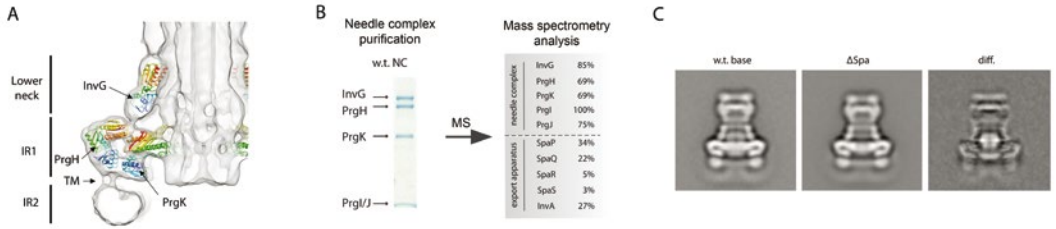


JOINT IMP/IMBA GROUP LEADER:
THOMAS C MARLOVITS

POSTDOC: LISA KÖNIGSMAIER
PHD STUDENTS: MATTHIAS BRUNNER, AGATA KOSAREWICZ, JULIA RADICS
DIPLOMA STUDENT: JULIA MAYR, DANIEL STREBINGER
SUMMER STUDENT: DAVID WARTMANN
ROTATION STUDENT: DANA ABDEEN
LAB MANAGER / RESEARCH ASSISTANT: JOANNA RADEK
RESEARCH ASSISTANT: JESUS FERNANDEZ-RODRIGUEZ

Figure 1: Infection pathway and gene organization of pathogenicity island encoding for the Type-3 secretion system form Salmonella

Figure 2: Needle complexes of the Type-3 secretion system visualized by electron microscopy and after three-dimensional reconstruction by single particle analysis (OR outer ring, IR inner ring)

Figure 3: The export apparatus is located centrally. (A) Three dimensional reconstruction of the needle complex to sub-nanometer resolution and docking of atomic structures of individual protein domains reveal the presence of additional proteins located centrally. (B) Export apparatus proteins are found in isolated wild-type complexes by mass spectrometry. (C) Formation of the socket/cup is dependent on the presence of the export apparatus proteins (SpaPQRS, InvA). Single particle analysis of w.t. and ΔSpa bases reveal strong differences within cup and socket region.

JAVIER MARTINEZ GROUP

Discovering and re-discovering enzymes and pathways in RNA metabolism

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

Enzymatic activities to phosphorylate and ligate RNA molecules were reported in the late nineteen-seventies and early nineteen-eighties, but the actual genes have remained elusive. Combining protein purification, mass spectrometry, RNAi and phyletic patterns, we identified the human RNA-kinases CLP1 and NOL9 which are able to bind ATP and phosphorylate RNA-5' termini, and the human tRNA ligase complex in charge of tRNA splicing and probably other non-canonical splicing events. We aim to dissect molecular mechanisms and identify RNA targets for all of these enzymatic activities. In addition, we are generating mouse models to observe in vivo functions and potential links to disease. Discovering new enzymes and pathways is exciting, especially when it permits renewed discoveries and introduces fresh perspectives into phenomena identified some 30 years ago.

The human tRNA ligase complex:

a) Subunit composition and molecular mechanisms: (Jennifer Jurkin and Johannes Popow).
In 2011 we identified HSPC117 (also known as RTCB) as the catalytic subunit of the long-time elusive human tRNA ligase complex. This opened new and exciting perspectives at our laboratory. In addition to RTCB, the tRNA ligase complex is composed of the DEAD-box ATP-dependent RNA helicase DDX1 and three extra subunits of unknown function, i.e. CGI-99, FAM98B and Ashwin. DEAD box proteins are capable of unwinding double-stranded RNAs as well as assembling/disassembling RNA-protein complexes and remodeling RNA molecules within ribonucleoprotein particles. They display a helical core composed of at least eleven conserved motifs. To study the function of DDX1 within the tRNA ligase complex, we generated stable cell lines that express mutant versions of DDX1 containing a FLAG-tag. We mutagenized RNA and ATP binding domains and assayed RNA ligase activity of FLAG-immunopurified tRNA ligase wild-type and mutant complexes. The results have been encouraging: we observe reduced ligation activity in those mutants when using different types of RNA substrates. In the near future we will try to reconstitute the entire tRNA ligase complex with recombinant proteins and study the catalytic activity and substrate-binding ability of recombinant DDX1.

b) A potential function in non-canonical splicing events during the Unfolded Protein Response (UPR): (Theresa Henkel, Jennifer Jurkin, Anne Nielsen, Johannes Popow and Stefan Weitzer).
We suspect that the tRNA ligase performs functions in addition to tRNA splicing. This speculation has been confirmed by PAR-CLIP experiments (Photo Activatable Ribonucleoside Enhanced Crosslinking and Immunoprecipitation), which revealed that the tRNA ligase binds to a multitude of mRNAs including Xbp1, an mRNA encoding a transcription factor required during UPR (Figure 1). In unstressed cells XBP1 encodes a cytoplasmic unstable protein. However, upon stress Xbp1-mRNA is cleaved twice by the ER-membrane endoribonuclease IRE1, leading to the removal of a 26-nucleotide intron. Joining the neighboring exons introduces a frame shift that, upon translation, generates a larger protein that moves to the nucleus and acts as a crucial transcription factor (Figure 2). The question we would like to answer is: what is the ligase that joins the exons? Interestingly, Peter Walter's group at the UCSF revealed, back in 1996, that the yeast tRNA ligase TRL1 splices Hac1-mRNA (homologous to Xbp1-mRNA) during yeast UPR. Our unpublished data strongly suggest that the human tRNA ligase complex is the long sought UPR ligase in human cells.

c) In vivo function: generation of knockout mice: (Jennifer Jurkin)
Transferring our biochemical results to mouse models, we have generated mice carrying a conditional HSPC117 allele in which exon 4 is flanked by two loxP sites. After removal of the floxed region a premature stop codon is generated, and the resulting transcript is subjected to non-sense-mediated decay. Crossing these mice to a ubiquitously expressed Cre line (i.e. β-actin-Cre, which mediates deletion in all body cells from early embryonic development onwards) leads to embryonic lethality between implantation and E10.5. We are currently trying to determine the precise time point and the reason for this early lethality. Furthermore, we are generating tissue-specific knockouts. Protein extracts obtained from these tissues lack HSPC117 and are therefore impaired in respect of tRNA ligation activity in vitro, confirming successful targeting. We are currently characterizing the phenotype of these tissue-specific knockouts. We have also replaced the wild type HSPC117 gene with a hypomorphic allele (knock-in) and will analyze mice carrying this allele in the near future.

Cellular functions of the 5'-polynucleotide kinase NOL9: (Sabrina Bandini)

Ribosome biogenesis is a finely regulated process that allows the cell to coordinate cell growth with cell proliferation. To synthesize the required number of ribosomes the cell relies on efficient and accurate generation of ribosomal RNAs (rRNAs). The 18S, 5.8S and 28S rRNAs are transcribed in the nucleolus by RNA polymerase I as one polycistronic precursor RNA, and are liberated by a complex series of endo- and exonucleolytic cleavage events. Eventually they are assembled together with the 5S rRNA and a plethora of ribosomal proteins to form the ribosomes.

We recently identified NOL9, the first human nucleolar polynucleotide kinase. The catalytically active enzyme is required for processing the 32S rRNA precursor into 28S and 5.8S rRNAs. Processing of rRNA is almost unexplored in human because of its complexity and redundancy. Our main purpose is to determine the reasons why the human rRNA pathway requires an RNA kinase, and to identify the key enzymes required for specific steps in the processing of the 32S rRNA.

We stably expressed FLAG-tagged NOL9 wild-type and ATP-binding mutants in HEK293 cells to study its nucleolar localization, its potential interaction with co-purified proteins, and reveal specific NOL9-binding sites on the rRNA by PAR-CLIP. In Figure 3 we show that NOL9 binds the 5' end of the 5.8S rRNA in vivo – a predictable result in view of the role NOL9 plays in establishing ratios between the short and long versions of the 5.8S rRNA.

Figure 1

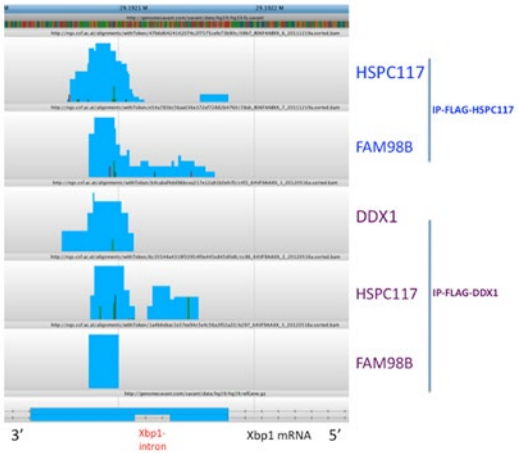
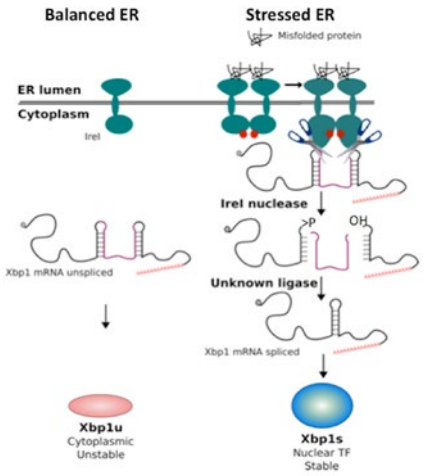


Figure 2

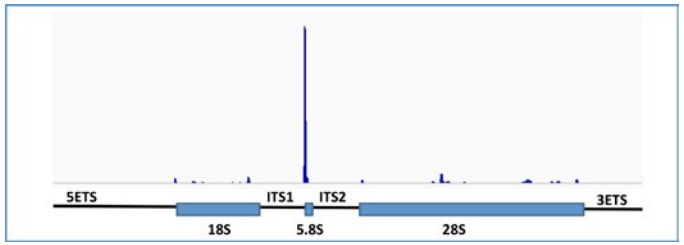


Publication highlights:

Gebeshuber, C., and Martinez, J. miR-100 suppresses IGF2 and inhibits breast tumorigenesis by interfering with proliferation and survival signaling. Oncogene, 2012 Aug 27. [Epub ahead of print]

Popow, J., Schleiffer, A., and Martinez, J. (2012). Diversity and Roles of (t) RNA Ligases. Review. Cell Mol Life Sci. 2012 Mar 17. [Epub ahead of print] Volume 69, Issue 16 (2012), Page 2657-2670.

Figure 3



GROUP LEADER:
JAVIER MARTINEZ

POSTDOCS: JENNIFER JURKIN, STEFAN WEITZER, JOHANNES POPOW
PHD-STUDENTS: SABRINA BANDINI, THERESA HENKEL
RESEARCH ASSISTANT JUTTA DAMMANN
DIPLOMA STUDENT THERESE KAUFMAN

Figure 1: Putative binding sites of tRNA ligase complex subunits at the Xbp1 intron.
The tRNA ligase complex was affinity purified from HEK293 cells by pulling down FLAG-HSPC117 (upper two panels, blue) or FLAG-DDX1 (lower three panels, purple). PAR-CLIP sequence clusters representing binding sites of the indicated tRNA ligase complex subunits at the Xbp1 intron locus are viewed in a genome browser.

Figure 2: The IRE1-branch of the Unfolded Protein Response in mammalian cells.
In unstressed cells (left) the Xbp1 mRNA is not spliced and encodes a cytoplasmic unstable protein (XBP1u). However, upon stress (right) Xbp1-mRNA is cleaved twice by the ER-membrane endonuclease IRE1, leading to the removal of a 26-nucleotide intron. Joining the neighboring exons by a still enigmatic RNA ligase introduces a frame shift which, upon translation, generates a larger protein (XBP1s) that moves to the nucleus and acts as a crucial transcription factor to re-establish homeostasis in the ER.

Figure 3: The RNA kinase NOL9 binds ribosomal RNA at the 5′ end of the 5.8S rRNA.
PAR-CLIP analysis of FLAG-NOL9 wild-type reveals specific binding to the 5.8S rRNA but not to the ITS2, supporting the role of NOL9 in the biogenesis of the 5′ end of the 5.8S rRNA.

KAZUFUMI MOCHIZUKI GROUP

Small RNA-directed transgenerational epigenetic inheritance in *Tetrahymena*

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

The phenotypes of a parent can be epigenetically transmitted to offspring through the germline in many different eukaryotes. Epigenetic information can be inherited as chromatin modifications that are incompletely reprogrammed in gametes. Alternatively, some diffusible factors can transmit epigenetic information from parents to progeny through the germline. Transgenerational epigenetic inheritance potentially allows organisms to transmit acquired characteristics to the next generation, and could thus increase the fitness of progeny in rapidly changing environments. However, the detailed molecular mechanism of transgenerational epigenetic inheritance is largely unclear. Several lines of evidence suggest that the patterns of programmed DNA elimination in ciliated protozoans are epigenetically and transgenerationally inherited. Therefore, DNA elimination in ciliated protozoans serves as a model to understand how transgenerational epigenetic inheritance can be achieved at a molecular level.

Evolutionary link between DNA elimination, heterochromatin formation, RNA interference and transposon silencing

The ciliated protozoan *Tetrahymena* possesses a somatic macronucleus (Mac) and a germline micronucleus (Mic) in each cell (Figure 1). Mac is polyploid and transcriptionally active, whereas Mic is diploid and transcriptionally inert during vegetative growth. In the sexual process of conjugation, Mic gives rise to a new Mac and a new Mic, and the parental Mac is destroyed. During the development of the new Mac, ~9000 internal eliminated sequences (IESs) are removed (DNA elimination) and the remaining Mac-destined sequences are re-ligated. Most IESs are moderately repeated in the Mic and many of them are related to transposable elements. Small RNA-directed heterochromatin formation is involved in the IES elimination process. In *Tetrahymena*, heterochromatin components, including histone H3 methylated on lysine 9 (H3K9me) and on lysine 27 (H3K27me), and the chromodomain protein Pdd1p, are specifically associated with eliminated IES sequences. Heterochromatin recruits the endonuclease Tpb2p, which catalyzes DNA elimination. Small (~28-29 nt) RNAs known as scnRNAs are produced by the Dicer protein Dcl1p, and associate with the Argonaute protein Twi1p. Dcl1p and Twi1p are required for accumulation of H3K9me/H3K27me/Pdd1p as well as for DNA elimination. Thus, heterochromatin formation occurs downstream of the RNAi-related mechanism in the DNA elimination pathway (Figure 2). As transposable elements are silenced by a heterochromatin and/or RNAi-related mechanism in many different eukaryotes, further study of the programmed DNA elimination process in *Tetrahymena* should reveal how transposons are silenced by RNAi-directed formation of heterochromatin in eukaryotes.

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 to induce DNA elimination? *Tetrahymena* solves this problem by trans-nuclear comparison of whole genomes. In a single cell, *Tetrahymena* has a germline 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 whole genome comparison (Figure 3 top). This system can 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 whole genome comparison 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 whole genome comparison (Figure 3 bottom). We also revealed 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 3, 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 3 top, g). In this way, DNA elimination in the new Mac may be epigenetically and transgenerationally controlled not only by the genome contents of the parental Mac through selective degradation of scnRNAs, but 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 can epigenetically regulate the behavior of genomes of successive generations in general eukaryotes.

Publication highlights

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

Aronica, L., Bednenko, J., Noto, T., Desouza, L.V. Siu, K.W., Loidl, J., Pearlman, R.E., Gorovsky, M.A., and Mochizuki, K. (2008) Study of an RNA helicase implicates small RNA-noncoding RNA interactions in programmed DNA elimination in Tetrahymena. Genes Dev. 22, 2228-2241.

Figure 1

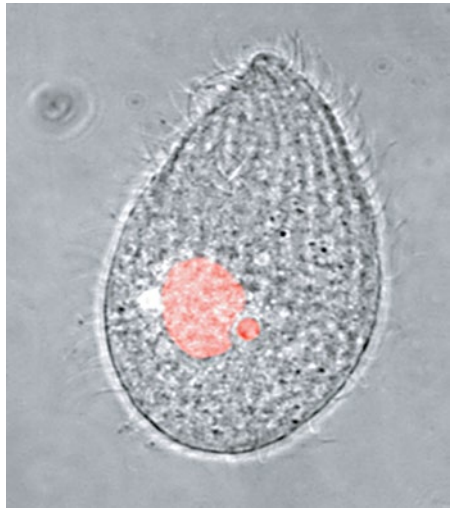


Figure 2

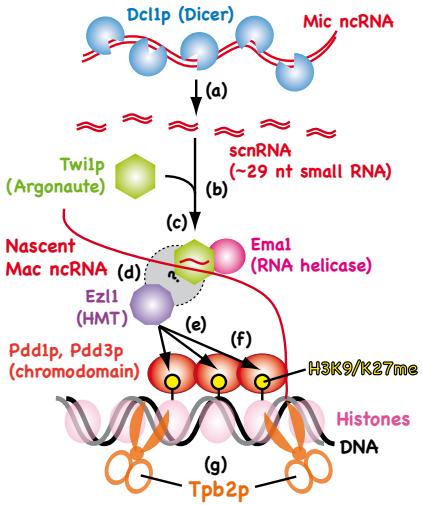
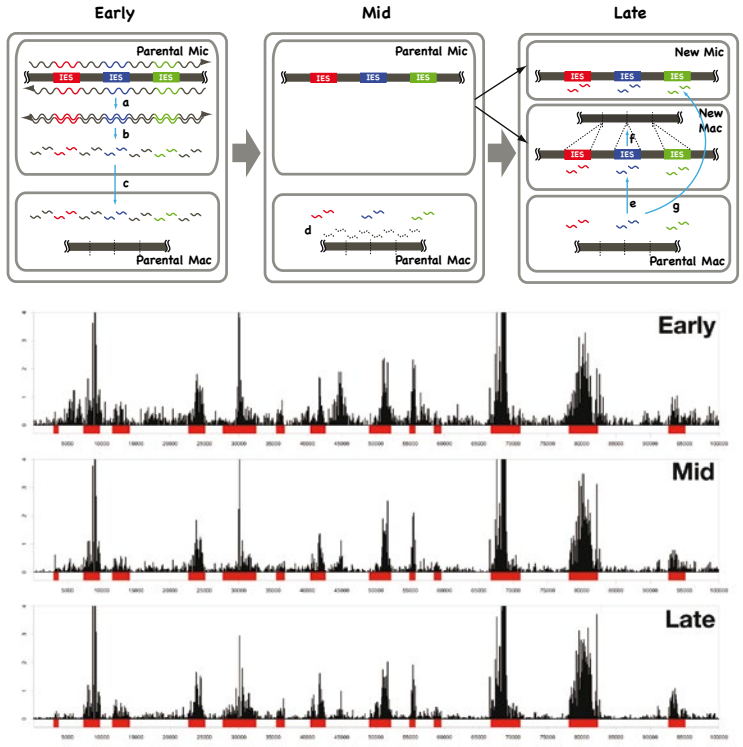
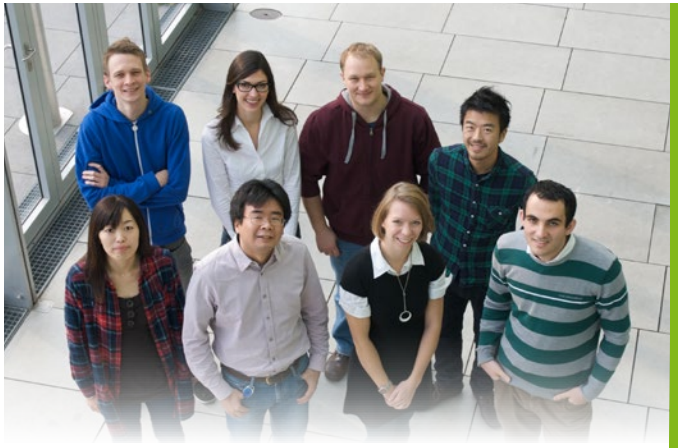


Figure 3





GROUP LEADER:
KAZUFUMI MOCHIZUKI

POSTDOC: KENSUKE KATAOKA
PHD STUDENTS: URSULA SCHÖBERL, ALEXANDER VOGT, SOPHIE SOYKA, JAN SUHREN
RESEARCH ASSOCIATE: TOMOKO NOTO

Figure 1: Two different nuclei in *Tetrahymena* *Tetrahymena* has two different nuclei (stained red): a small germline micronucleus and a larger somatic macronucleus.

Figure 2: Small RNA-directed heterochromatin formation induces DNA elimination Non-coding (nc) RNAs derived from the Mic genome, including transposons, are processed to scnRNAs by Dcl1p (a). scnRNA forms a complex with the Argonaute protein Twi1p (b). Ema1p facilitates interaction between the complex and nascent Mac ncRNA (c). This interaction recruits Ezl1p (d), which catalyzes methylations of histone H3 at lys9 and lys27 (e). Pdd1p and Pdd3p bind to the methylated histone H3 and establish heterochromatin structure (f). Tpb2p mediates the final DNA excision process (g).

Figure 3: 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, to leave some 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 different developmental stages 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

Novel sequencing technologies have led to the identification of multiple candidate genes for human diseases. 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, haploid genetics in stem cells, and fly genetics, my group tries to genetically dissect disease mechanisms.

Ace2 links dietary malnutrition to gut microbial ecology and intestinal inflammation

Malnutrition

More than one billion people are malnourished in poor countries. In fact, malnutrition is a major problem even in rich countries. The consequences of malnutrition are often severe, making it a leading cause of death in the world. For more than a hundred years now, doctors have known that a diet deficient in protein or amino acids - the building blocks of proteins - may cause disorders of the immune system, diarrhea and intestinal inflammation, which weaken the body and are potentially life threatening. However, the molecular mechanism that would explain how malnutrition causes such severe symptoms has remained largely unexplored.

ACE2 – angiotensin-converting enzyme 2

The renin-angiotensin system (RAS) has been studied for more than a century now. RAS is regulated by the opposing actions of two key carboxypeptidases known as angiotensin-converting enzyme (ACE) and ACE2. We were the first to demonstrate that ACE2 is a potent negative regulator of RAS, counterbalancing the multiple functions of ACE via catalytic cleavage of angiotensin II (Crackower et al. Nature 2002). ACE2 also functions as a key receptor for the SARS Coronavirus involved in acute lung failure (Imai et al. Nature 2005; Kuba et al. Nature Med. 2005). ACE2 is a chimeric protein that has emerged from the duplication and fusion of two genes: homology with ACE at the catalytic domain and homology with Collectrin (Tmem27) in the membrane proximal domain. We showed that the inactivation of Collectrin in mice results in nearly complete downregulation of apical amino acid transporters such as the he neutral amino acid transporter B^0AT1 in the kidney, thus regulating renal amino acid re-absorption (Danilczyk et al. Nature 2006). Variants in the *B^0AT1 (SLC6A19)* gene have been identified as a cause of Hartnup's disease, an autosomal recessive disorder associated with pellagra-like symptoms including diarrhea, which is manifested in the presence of malnutrition. However, the *in vivo* function of ACE2 in gut epithelium remained to be investigated.

ACE2 controls intestinal uptake of dietary tryptophan

In addition to its catalytic activity ACE2 associates with B^0AT1 on the luminal surface of intestinal epithelial cells, where ACE2 is required for expression of this transporter on the luminal surface of intestinal epithelial cells (Fig. 1a). Genetic inactivation of ACE2 results in impaired uptake of dietary amino acids, particularly the essential amino acid tryptophan, in the intestine. As enterocytes also express di- and tripeptide transporters, we provided tryptophan in the form of a dipeptide. Dietary dipeptidic tryptophan restored serum tryptophan levels (Fig. 1b). Thus, ACE2 exhibits a novel RAS-independent function, regulating intestinal amino acid homeostasis.

ACE2 controls intestinal inflammation

Intriguingly, when we challenged ACE2-deficient mice with dextran sodium sulfate (DSS), a chemical irritant that disrupts the intestinal epithelial barrier and results in the induction of colitis, we observed a distinctly stronger inflammatory reaction compared to wild-type littermates (Fig. 2). Thus, genetic inactivation of the key RAS enzyme ACE2 results in severe colitis after intestinal injury. Moreover, protein malnutrition may directly affect the severity of DSS-induced colitis.

Mechanistically ACE2 regulates the expression of innate antimicrobial peptides and the ecology of the gut microbiome. ACE2-dependent changes in epithelial immunity and the gut microbiome can be directly regulated by the dietary amino acid tryptophan and its metabolic product nicotinamide (Fig. 3a). Tryptophan supplementation as well as treatment with the tryptophan metabolite nicotinamide almost completely alleviated severe colitis and diarrhea to the level seen in control mice. Finally, transplantation of the altered microbiome from ACE2-mutant mice into wild-type germfree hosts was able to transmit the increased propensity to develop severe colitis (Fig. 3b).

Conclusions. Malnutrition is a major global health burden, affecting approximately a billion people in the world. Some of its consequences, such as colitis and diarrhea, can be fatal[1,2]. Chronic inflammatory conditions of the intestine, cachexia, anorexia nervosa, or amino acid mal-absorption are severe clinical problems[30]. Our data provide novel mechanistic insights into how protein malnutrition leads to colitis and diarrhea. Our data also revealed molecular crosstalk between the RAS system and intestinal amino acid homeostasis via ACE2, and demonstrated a direct link between dietary amino acid metabolism and innate immunity, the composition of the intestinal microbiota, and susceptibility to colitis (*Hashimoto, Perlot et al. Nature 2012*).

Figure 1

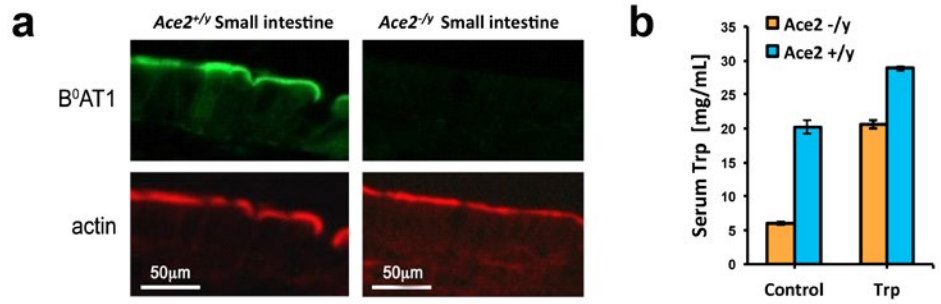


Figure 2

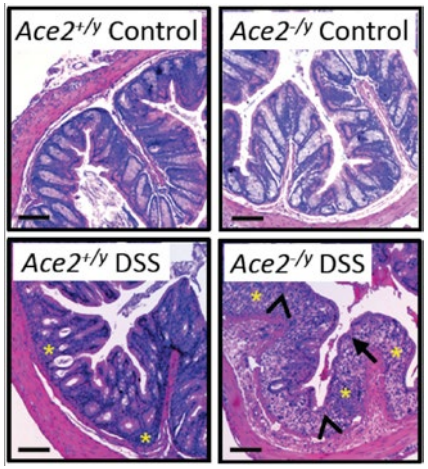
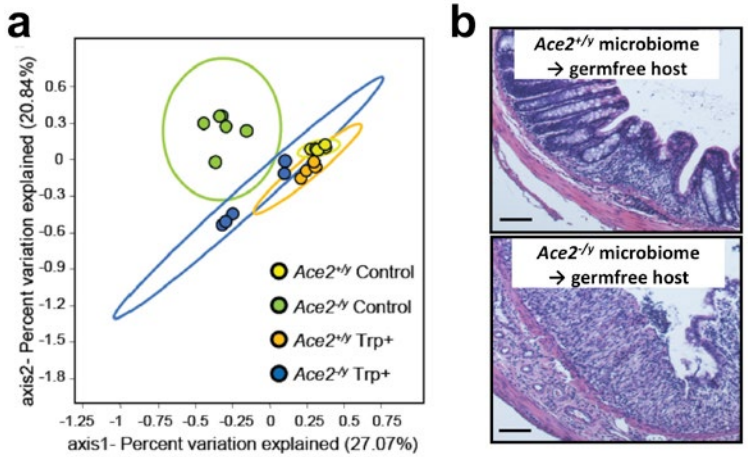


Figure 3



SENIOR SCIENTIST & MANAGING DIRECTOR SCIENCE:
JOSEF PENNINGER

POSTDOCS: CIKES DOMAGOJ, CRONIN SHANE, HAUBNER BERNHARD, KARIM REZAUL, NAGY VANJA, NITSCH ROBERTO, PAOLINO MAGDALENA, PERLOT THOMAS, RAO SHUAN, SHIRAISHI HIROSHI, TORTOLA LUIGI, URIBESALGO MICAS IRIS, WIMMER REINER ALOIS, WIRNSBERGER GERALD
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PHD STUDENTS: ORTHOFER MICHAEL, PRANJIC BLANKA, SIGL VERENA, WENZEL DANIEL

DIPLOMA STUDENT: TAUBENSCHMID JASMIN

RESEARCH ASSISTANT: KOGLGRUBER RUBINA [1]
RESEARCH ASSOSIATE: KOZIERADZKI IVONA, SARAO RENU
TRAINEE: TIEFENTHALER VIKTORIA

[1] ON MATERNITY LEAVE

Figure 1: ACE2 controls intestinal B⁰AT1 expression and dietary tryptophan uptake.
a, Immunofluorescence staining to detect B⁰AT1 and, as a control, β-actin in the small intestine of Ace2$^{+/y}$ and Ace2$^{-/y}$ mice. Note that B⁰AT1 protein is completely absent in Ace2$^{-/y}$ mice. Scale bars, 50 µm. **b**, Tryptophan levels in serum from Ace2$^{+/y}$ and Ace2$^{-/y}$ mice given a tryptophan supplemented diet (Trp) or normal chow (Control).

Figure 2: Ace2 deficiency worsens DSS-induced colitis. Colon histopathology in control and DSS-treated Ace2$^{+/y}$ and Ace2$^{-/y}$ littermates. Note the severe crypt damage (arrowheads), ulcerations (arrow), and infiltration of inflammatory cells (asterisks) in DSS-treated Ace2$^{-/y}$ mice. H&E staining of tissue taken on day 7 after DSS challenge. Scale bars, 100 µm.

Figure 3: Altered gut bacteria from Ace2 mutant mice can confer susceptibility to colitis. a, a, Principal coordinate analysis (PCoA) plot calculated by Bray-Curtis algorithm, showing similarity among ileocecal bacterial communities in Ace2$^{+/y}$ and Ace2$^{-/y}$ mice given a dipeptidic tryptophan diet (Trp+) or normal chow (Control) for 10 days. Each dot represents data from an individual animal. **b**, Colon histopathology of DSS-challenged germfree mice that received intestinal microbiota from Ace2$^{+/y}$ or Ace2$^{-/y}$ littermates. H&E staining, day 7 of DSS challenge; scale bars, 100 µm.

LEONIE RINGROSE GROUP

Epigenetic Regulation by Polycomb and Trithorax Group Proteins

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

How do different cell types remember their identities over many cell generations? Part of the answer lies in the Polycomb and Trithorax groups of proteins. The highly conserved Polycomb (PcG) and Trithorax (TrxG) group proteins constitute an epigenetic "cellular memory" system that is essential for maintaining the correct identity of both stem cells and differentiated cells. These proteins work antagonistically on several hundred developmentally important target genes, to maintain repressed (PcG) or active (TrxG) transcription states.

Current projects aim to understand the interplay of PcG and TrxG proteins with DNA sequence and non coding RNAs in PcG/TrxG regulation (Figure 1). We use a combination of quantitative live imaging, mathematical modelling, computational approaches and molecular and developmental biology to understand the interaction of the Polycomb and Trithorax proteins with their chromatin targets. We aim to unravel this fascinating epigenetic gene regulatory system in terms of the design, function and dynamic behaviour of its components. Our goal is to understand how a system whose components are in constant flux can ensure both stability and flexibility of gene expression states.

Protein: Quantitative live imaging and mathematical modeling

We have established an "*in vivo* biochemistry" approach to perform quantitative analysis of PcG and TrxG protein dynamics in living *Drosophila* in defined cell types that undergo mitosis and differentiation (Fonseca et al., 2012; Figure 2). By demonstrating clear differences between the kinetic behaviour of PcG proteins in stem cells and differentiated cells, this work brings us towards fundamental questions in epigenetics, on the nature of memory in stem cells and differentiated cells and how it survives mitosis. However it also raises several important questions about the causes and consequences of this behaviour, namely, why does it happen? and does it matter? We are currently addressing these questions by a combination of approaches, including quantitative analysis of the complete PcG/TrxG system, mathematical modeling and perturbation analysis, and structure function analysis of selected proteins. Our work so far indicates a role for mitotic chromatin attachment of TrxG proteins in the maintenance of active expression states. Current and future work aims to elucidate the molecular basis of this mitotic attachment and its role in propagating memory of cell identity through mitosis.

DNA: Polycomb/Trithorax Response elements in flies and mammals

The PcG and TrxG proteins act through Polycomb/Trithorax response elements (PRE/TREs). PRE/TREs are switchable bi-stable regulatory DNA elements that can preserve a memory of the activated or silenced state of their associated genes over several cell generations (Figure 1). Although fly PRE/TREs are well characterised, their mammalian counterparts have proved highly elusive. The greatest obstacle to the identification of mammalian PRE/TREs has been the lack of a computational tool for the identification of these elements using genomic sequence information. In collaboration with Marc Rehmsmeier (CBU. Bergen, Norway http://www.bccs.uni.no/units/cbu/research/rehmsmeier/), we have now established such a tool, that can accurately predict mammalian PRE elements on the basis of DNA sequence alone (Figure 3). This work represents a major step forward: the algorithm not only identifies genomic regions with the highest PRE potential, but also reveals the underlying sequence principles that these sites have in common. Our data reveal unexpected similarities between fly and vertebrate elements at the nucleic acid sequence level. Experimental testing of predicted mammalian PREs is currently underway.

RNA: Noncoding RNAs in PRE/TRE regulation

Our recent work in both fly and mouse has identified several novel long noncoding RNAs that are transcribed from Polycomb regulatory sites (Hekimoglu- Balkan et al., 2012), and suggests an essential role for these RNAs in PRE/TRE regulation during development and differentiation. We have shown that the *Drosophila vestigial* PRE/TRE undergoes a developmental switch, in which non-coding RNAs from alternate strands of the element have opposite effects on gene expression (Figure 4). Thus the *vestigial* PRE/TRE switches its function by alternating between forward and reverse strands of its non-coding RNA. We name this element a "GEARBOX" element (Gene Expression Alternating RNA). By analysis of genome wide data sets we show that a high proportion of transcribed Polycomb binding sites in flies and vertebrates show tissue specific switching of non-coding RNAs transcribed from both strands, fulfilling several hallmarks of GEARBOX function. Thus this work identifies both a novel mechanism of switching, and a novel and potentially widespread class of PRE/TREs. Key future goals will be to further elucidate molecular mechanisms by which bidirectional "GEARBOX" transcription can switch PRE/TRE elements, and to identify any common features between vertebrates and flies.

Publication highlights:

Hekimoglu- Balkan, B., Aszodi, A, Jaritz, M and Ringrose, L. Intergenic Polycomb Target sites are dynamically marked by non-coding transcription during lineage commitment. RNA Biol. 2012, 9(3)

Fonseca JP, Steffen PA, Müller S, Lu J, Sawicka A, Seiser C, Ringrose L. In vivo Polycomb kinetics and mitotic chromatin binding distinguish stem cells from differentiated cells. Genes Dev. 2012, 15:857-71

Steffen, PA, Fonseca, J, Ringrose L. Epigenetics meets mathematics: towards a quantitative understanding of chromatin biology. Bioessays, 2012, 34(10):901-13

Figure 1: Polycomb/Trithorax response elements (PRE/TREs). PRE/TREs are cis- regulatory elements that can recruit both the silencing Polycomb group (PcG) proteins (red), and activating Trithorax group (TrxG) proteins (green), via a platform of sequence specific DNA binding proteins (blue). Fly PRE/TRE elements contain multiple recognition motifs for these DNA binding proteins (coloured bars). Many fly PRE/TREs are transcribed into noncoding RNA. (A) depending on the activity of the nearby enhancer and promoter, PRE/TRE elements can switch between stably active and silent states. (B) Many fly PRE/TREs can then propagate a memory of this active or silent state through several rounds of mitosis, in the absence of the transcription factors that initially determined the state of expression of the gene. Thus they act as epigenetic memory elements.

Figure 2: Quantitative kinetics in single cells *in vivo*. (A-D) The number of chromatin bound Polycomb molecules, and the average residence time of each molecule, are shown for stem cells and determined cells in interphase and mitosis Fonseca et al., 2012. **E)** summary of mitotic chromatin binding for the PcG proteins E(Z), PHO, and PC, and the TrxG protein ASH1 (Steffen et al., submitted). ASH1 binds most abundantly to mitotic chromatin. **F)** The functional relationship between ASH1 and PC switches at mitosis.

Figure 3: Bioinformatic prediction of mammalian PRE/TREs. A) Mouse HoxD locus (40kb). Red: Score plot for prediction algorithm. Grey: ChIP-seq data for the PRC2 protein Suz12 in mouse ESCs (Peng et al., 2009, Cell. 139:1290-302). Red bar below the plots shows Hoxd11-12 PRE, one of only two functionally characterised vertebrate PREs [19]. B) Oct-4 locus (14kb) Red: Score plot for prediction algorithm. Black: ChIP-seq data for the PRC2 protein Suz12 in mouse ESCs. Grey: ChIP-seq data for H3K27me3 (Encode) in mouse megakaryocytes, showing gain of PRC2 activity.

Figure 4: GEARBOX elements: reverse and forward strand non-coding transcription switches the function of a PRE/TRE. The reverse and forward strands of GEARBOX elements are expressed in different cell lineages. **(A). The silenced state.** We have shown that the forward strand promotes silencing by facilitating pairing between PRE/TREs, possibly via additional bridging protein. E(Z) is bound at the silent vg PRE/TRE, but does not interact with the forward strand, thus it binds via a different platform. **(B) The active state.** Upon switching, transcription of the reverse PRE/TRE strand destabilises pairing by preventing forward strand transcription. We have shown that the reverse strand binds highly specifically to E(Z). Thus we propose that this interaction competes with the PRE/TRE as a binding platform, and titrates E(Z) away, allowing the TrxG proteins to gain the upper hand (Lempradl et al., submitted).

Figure 1

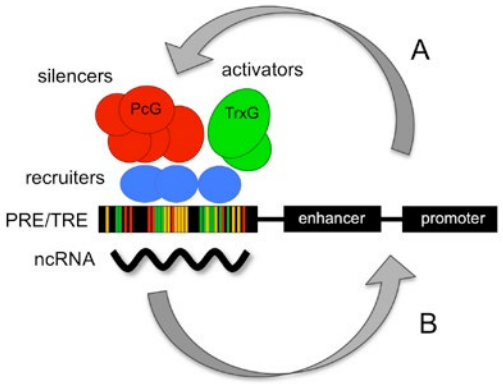
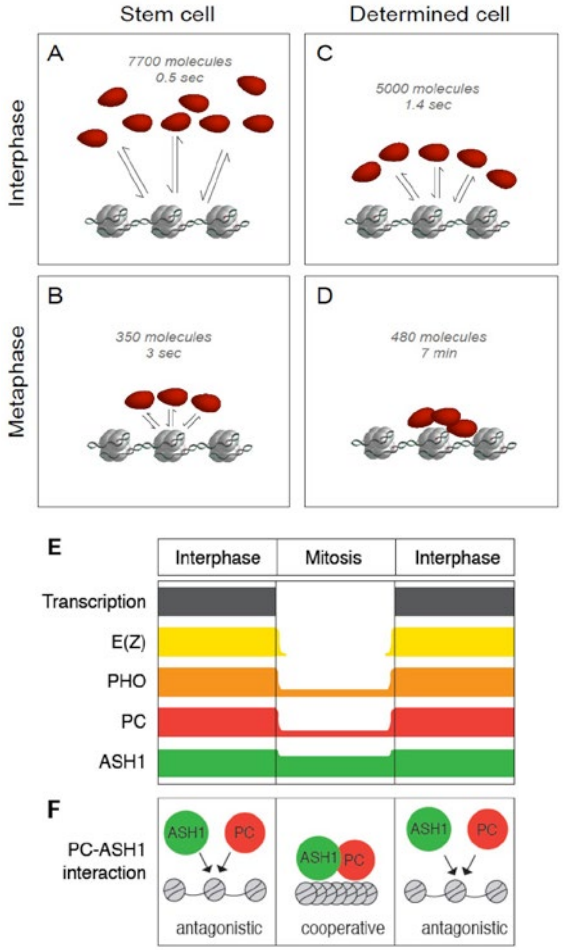
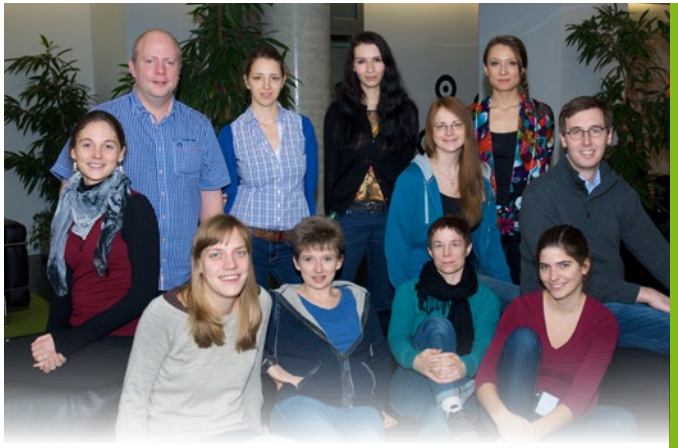


Figure 2





GROUP LEADER:
LEONIE RINGROSE

POSTDOCS: ROBERT HEINEN, JOAO FONSECA[1]
PHD STUDENTS: HELENA OKULSKI, FRANK RUGE[2], PHILIPP STEFFEN, JOHANNA TRUPKE
TECHNICAL ASSISTANTS: CHRISTINA ALTMUTTER, CHRISTINE EHRHARDT, EVA DWORSCHAK
MASTERS STUDENTS: TANJA DREXEL[3] , MELITA TICEVIC[4]

[1]PHD STUDENT UNTIL APRIL; POSTDOC MAY- AUGUST
[2]PHD STUDENT UNTIL AUGUST
[3]MASTERS STUDENT FROM JUNE
[4]MASTERS STUDENT FROM JULY

Figure 3

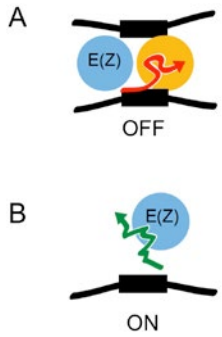
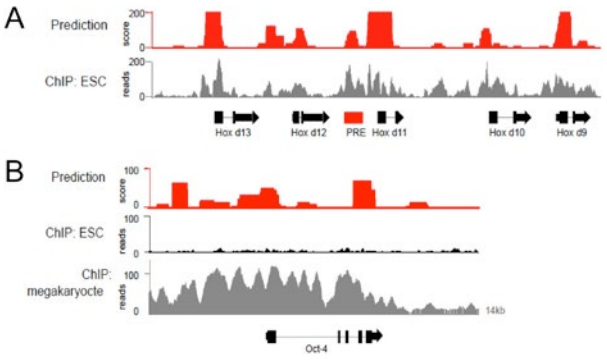


Figure 4



VIC SMALL GROUP

Moving with actin

www.imba.oeaw.ac.at/research/vic-small

There is no life without movement, at all levels of metazoan organization, from individual cells to the animal form. During development, individual cells migrate from the germ layers to lay down the body plan and in the adult organism migrating cells play key roles in immune defense and tissue repair. Pathological processes, including tumor dissemination and atherosclerosis, likewise involve cell migration. A central player in these motile processes is the protein actin and our studies focus on the mechanisms by which actin produces movement.

Actin is not however used exclusively by cells to move. Several bacterial and viral pathogens hijack the actin machinery of cells they infect to disseminate their infection. They do this by recruiting actin and co-factors to propel them in cytoplasm in a rocketing type regime, at the head of an actin comet tail, to jump from one cell to another, avoiding the surveillance of the immune system (Fig.1). Our more recent work has focused on elucidating the mechanism of pathogen propulsion. Building on previous work on the organization of actin filaments in migrating cells (Vinzenz et al., 2011) we are using the technique of electron tomography to resolve the three-dimensional structure of actin assemblies propelling pathogens in cytoplasm. Members of the baculovirus family are among the smallest pathogens propelled by actin. Owing to their small size (40x200nm), baculovirus generate actin comet tails in infected cells small enough for detailed analysis by electron tomography (Fig. 2). Using this model system we have been able to show that movement can be generated by as few as 4-5 actin filaments pushing on the virus particle, in a regime involving actin branching by the Arp2/3 complex to produce a fish tail like filament array. The structural parameters of comet tails determined by electron tomography and the trajectories of viruses observed in cytoplasm are now being used to simulate actin-dependent propulsion in a mathematical model (Fig.3).

Ongoing research includes structural analysis of actin comet tails propelling vaccinia virus to establish whether common mechanisms are at play. Electron tomography is also being applied in several collaborations on the structure and function of the actin cytoskeleton.

A Video Tour of Cell Motility
For an Introduction to the cytoskeleton and cell motility see our Video Tour website: http://cellix.imba.oeaw.ac.at/

Figure 1

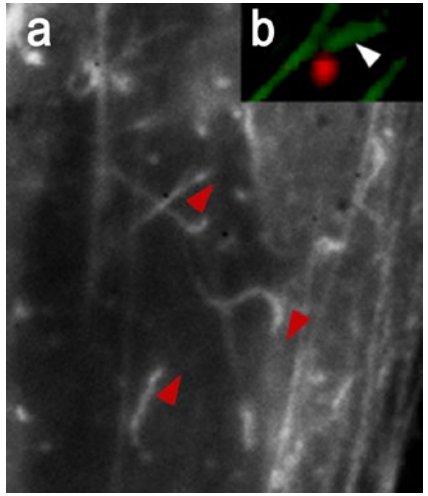
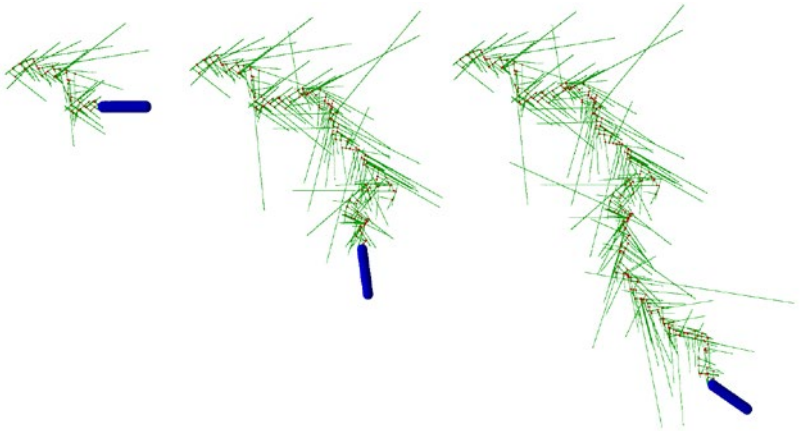
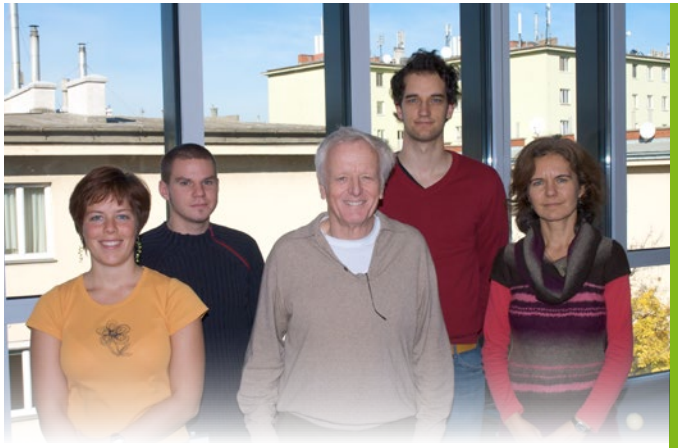


Figure 3





SENIOR SCIENTIST:
VIC SMALL

STAFF SCIENTIST: MARIA NEMETHOVA
DIPLOMA STUDENTS: JULIA PFANZELTER, FLORIAN SCHUR, DANIEL SERWAS
TECHNICAL ASSISTANT / PHD STUDENT: JAN MÜLLER

Figure 2

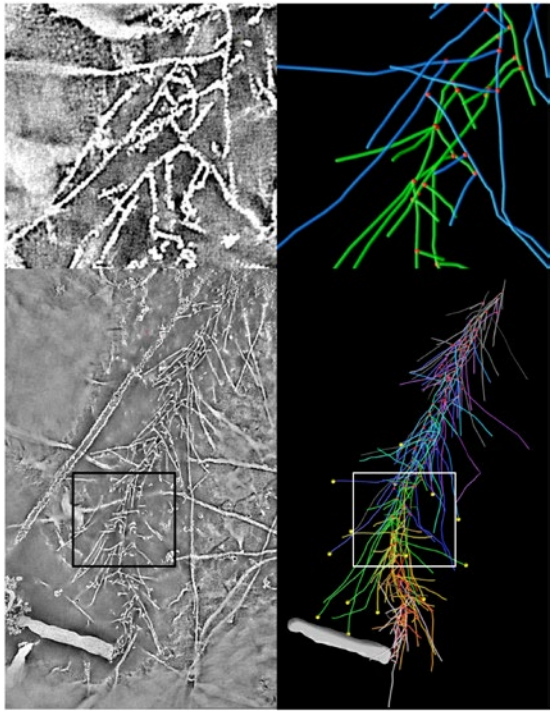


Figure 1: A. Actin comet tails in a B16 cell that was infected with baculovirus. The cell was transfected with GFP-actin. Red arrowheads indicate direction of movement of the comet tails, which have a baculovirus (not seen) at their head. B. Actin comet tail (indicated by white arrowhead) as in "A", but in a cell infected with baculovirus tagged with RFP (red), showing the virus at the head of the tail. The unlabelled green fibres are actin bundles in the host cell.

Figure 2: The fish tail like arrangement of actin filaments in baculovirus actin comet tails revealed by electron tomography. Left panels show tomogram slices of a comet tail with the region boxed enlarged in the top panel. Right panels show corresponding 3D model of the comet tail with branch junctions indicated in red and with filaments linked in subsets indicated in different colours.

Figure 3: Using a simplified set of assumptions of the rates of actin nucleation, branching, capping and polymerization the observed structural features of the comet tails and their trajectories could be closely mimicked in silico.

KIKUE TACHIBANA-KONWALSKI GROUP

Molecular control of the oocyte-to-zygote transition

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

The challenge at 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. Zygote formation involves a switch from the meiotic to the mitotic cell cycle machinery as well as sperm chromatin reorganization and natural reprogramming. How these fascinating processes are regulated at the molecular level and how their deterioration impacts fertility are key questions we aim to address in our research.

One of the most dramatic transitions in biology is the oocyte-to-zygote transition. This process refers to the maturation of the female germ cell or oocyte, which undergoes two rounds of meiotic chromosome segregation and, following fertilization, is converted to a mitotically dividing embryo. We are addressing fundamental questions concerning 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. A molecular understanding of key players is a requisite step for investigating how deterioration of these factors contributes to maternal age-dependent aneuploidy and infertility. Aneuploidy is the leading cause of mental retardation and spontaneous miscarriage. The current trend towards advanced maternal age has increased the frequency of trisomic fetuses by 71% in the past ten years. Therefore, a better understanding of mammalian meiosis is relevant to human health.

How is sister chromatid cohesion maintained during the prolonged arrest of 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 a specialized type of cell division giving rise to haploid gametes, egg and sperm. The paradigm of reproductive biology is that sister chromatid cohesion is established during meiotic DNA replication in oocytes of the embryo, recombination occurs before birth, but oocytes remain arrested until ovulation triggers the first meiotic division several months (mouse) or decades (human) later. Must cohesin therefore hold sister chromatids together for months and possibly decades? Alternatively, is cohesion regenerated during the long arrest period? We have shown that, remarkably, there is no detectable cohesin turnover for several weeks in oocytes. Whether cohesion is regenerated over months remains a crucial question. We are addressing this using TEV protease technology that we have pioneered in the mouse (Figure 1), molecular genetics, microinjection, and 4D confocal live-cell imaging. To elucidate the mechanisms maintaining cohesion in oocytes, we are complementing cell biological assays with mass spectrometric and biochemical approaches.

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

It has been known for decades that the first round of chromosome segregation in oocytes is error prone, leading to chromosomal abnormalities 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 anaphase onset until the last chromosome has bi-oriented on the spindle. The SAC also regulates the timing of chromosome segregation in oocytes, but it is less clear how it functions at the level of kinetochores. 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. Our results have implications for aging oocytes in which cohesin deterioration compromises the SAC and thereby leads to chromosome missegregation and production of aneuploid eggs.

We also discovered that zygotes with TEV-cleaved cohesin arrest in mitosis (Figure 2). Future work will focus on the molecular basis of differences in checkpoint activity of oocytes and zygotes.

How is chromatin organization regulated in zygotes?

Fertilization triggers the second meiotic division and entry into the first embryonic cell cycle. During 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, which is currently poorly understood. We are taking a conditional knockout approach combined with cell cycle kinetic studies to investigate candidate factors required for these processes. Genetic knockout zygotes have the potential to be rescued by microinjection of mRNAs encoding target proteins. We are therefore developing this powerful system for in vivo structure-function studies to dissect the mechanisms of chromatin organization and cell cycle regulation in zygotes.

Publication highlights:

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(7361):467-71. (*equal contribution)*

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(22):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(14):1880-4.

Figure 1

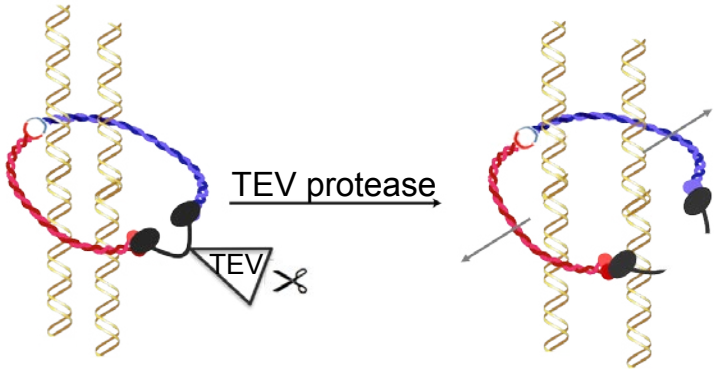
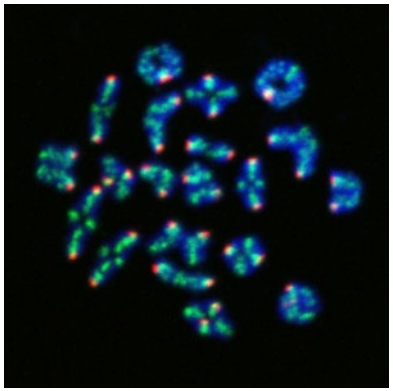
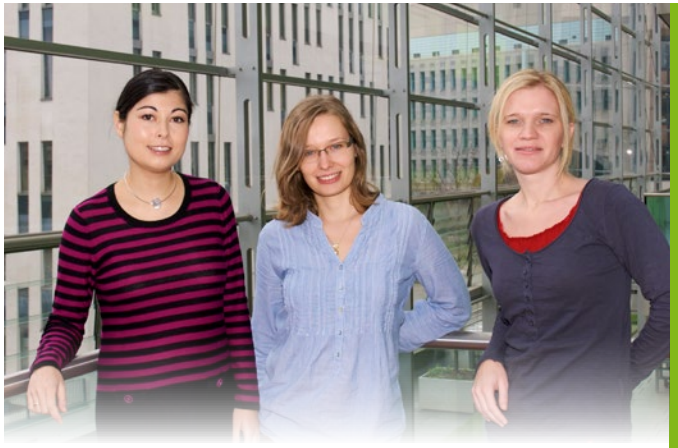


Figure 2





GROUP LEADER: KIKUE TACHIBANA-KONWALSKI

PHD STUDENT: SABRINA BURKHARDT
RESEARCH ASSISTANT: KERSTIN KLIEN

Figure 3

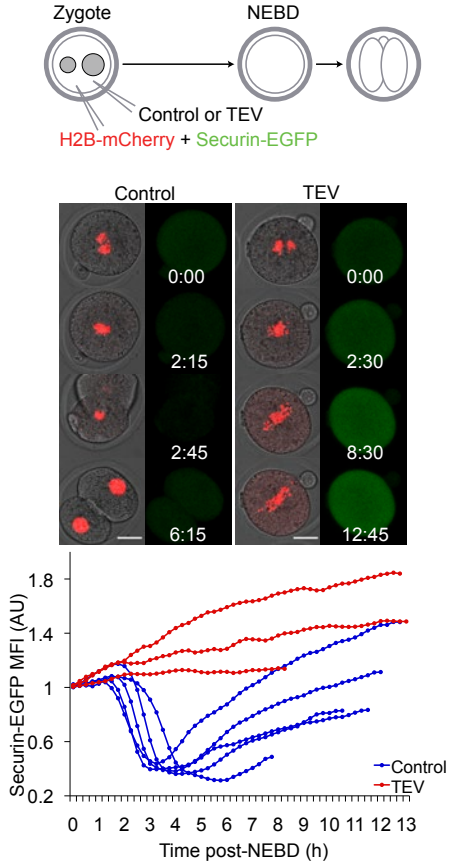
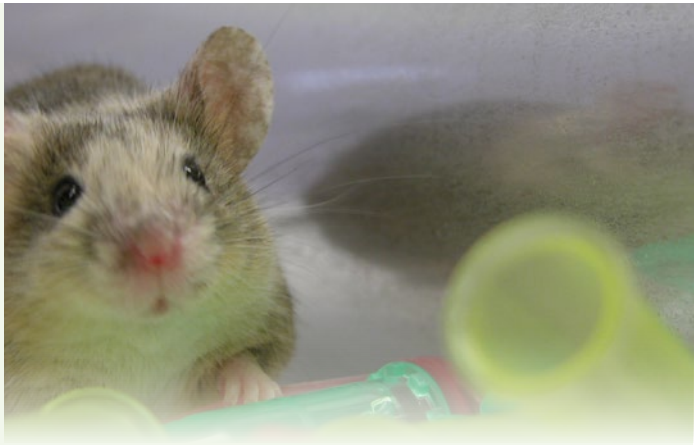


Figure 1: Application of TEV protease technology to cleave mammalian cohesin complexes. Schematic diagram of the cohesin ring consisting of an Smc1/Smc3 heterodimer (red/blue) bridged by a kleisin subunit (black), which is Rec8 in meiosis and Scc1 in mitosis. TEV protease-mediated cleavage of Rec8 or Scc1 genetically engineered to contain TEV protease recognition sites induces ring opening and destroys sister chromatid cohesion.

Figure 2: Cohesin complexes maintain sister chromatid cohesion in oocytes. Chromosome spread prepared from a mouse oocyte and stained with c-Myc antibody to visualize Rec8-Myc (green) expressed from a constitutive BAC transgene, CREST to mark centromeres (red) and Hoechst to visualize DNA (blue).

Figure 3: Cohesin complexes maintain sister chromatid cohesion in zygotes. Zygotes expressing TEV-cleavable cohesin were injected with mRNA encoding H2B-mCherry to mark chromosomes (red) and Securin-EGFP to monitor cell cycle progression (green), control buffer or TEV protease, and imaged for 24 hours. Still images from representative movies of zygotes are shown, starting with nuclear envelope breakdown (NEBD), which marks entry into mitosis (h:mm). The mean fluorescence intensity of Securin-EGFP was calculated relative to NEBD.



STEM CELL CENTER - GENE TARGETING UNIT

austromouse@imp.ac.at

The main objective of the Stem Cell Center is to broaden and strengthen stem cell research. Ideal human disease models can be engineered by combining homologous recombination, site-specific recombination, and transgenesis using mouse ES (embryonic stem) cells. Our iPS (induced pluripotent stem) cells core facility was created to accelerate research in the stem cell field by facilitating the derivation of iPSCs cell lines. The study of human iPSCs offers a potentially unlimited source of numerous cell types to study regulation of pluripotency and differentiation, drug screening and cell-based therapies.

ES core facility

The several missions of the ES cell core facility include the production of quality-controlled ES cell lines with mutations introduced by homologous recombination, the creation and handling of quality-controlled ES cell lines, and enhancing knowledge of mouse genetics, ES cell culture, and manipulation. Targeted ES cells can be used to generate germline ES cell-mouse chimeras. The latter can be bred to generate mouse lines or for *in vitro* assays. 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. This provides essential tools for the analysis of mutations, especially when the phenotype is embryonic and lethal in the early stages of development. 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 iPS cell lines. In order to extrapolate this technology to the clinical setting, we have established the generation of human iPS cells using 4F or microRNAi lentiviral, sendaiviral or synthetic mRNAs for virus-free iPS cells from dermal fibroblasts or keratinocytes. The motivation underlying our core facility is to exploite the unique possibilties for generating patient-specific pluripotent stem cell lines for preclinical research, to provide research groups access to this technique, and to broaden and strengthen further stem cell research.

iPS cells open new ways to treat human diseases, such as heritable skin disease recessive dystrophic epidermolysis bullosa (RDEB), for which no effective treatments are available. EB is a group of inherited disorders defined by fragile skin and mucous membranes that blister easily in response to minor injury or no apparent trauma. We have already successfully developed human iPS cells from EB patients and mice that develop EB-like syndromes. In such cells, we will utilize a novel, rapid, and highly efficient novel technology (called transcription activator-like effector nucleases, TALEN) to repair the underlying genetic defects. Next, we will establish iPSCs from other accessible resources, such as human hair follicle dermal pupilla or nasal epithelia for making iPS cells for patient-specific personalized medicine. Our singular vision is to develop efficient and safe technologies to repair causative gene mutations in EB patient-derived iPS cells.

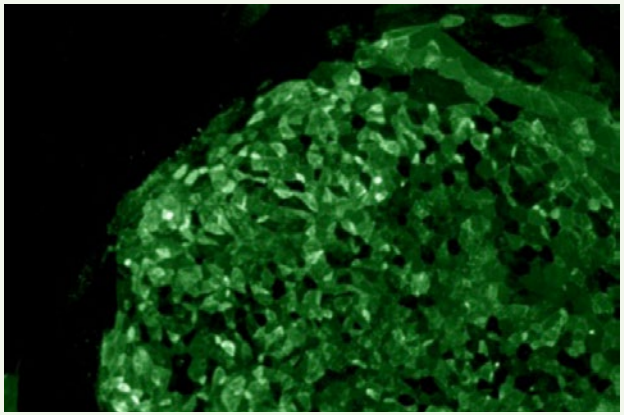


Figure: Human iPS cells generated from adult dermal fibroblasts express the embryonic cell surface marker Tra-1-81

HEAD OF FACILITY:
PETER DUCHEK

TECHNICAL ASSISTANTS:
SARA FARINA LOPEZ, VICTORIA STEINMANN, CLAUDIA VALENTA

FLY HOUSE

Peter Duchek

peter.duchek@imba.oeaw.ac.at

The Fly House provides research support for scientists at IMBA who work with the fruitfly Drosophila melanogaster as a genetic model system. Our service includes the generation of transgenic fly lines, gene targeting via homologous recombination to generate knock-out or knock-in mutants, large-scale in vivo RNAi screens, and the maintenance of stock collections.

Embryo injections

One of the principal activities at the Fly House is the generation of transgenic fly lines. We routinely inject DNA constructs into a range of commonly used host strains, including various landing site stocks for phiC31-mediated targeted integration, and then perform all crosses to establish mapped and balanced transgenic stocks.

Gene targeting

Although the use of homologous recombination to generate defined mutations is a well-established technique in several genetic model organisms, gene targeting in Drosophila has been developed quite recently. Given the need to confirm RNAi knock-down phenotypes with classical loss-of-function alleles or to tag genes at their endogenous locus, we established a Drosophila gene targeting service at the institute. Currently we use an ends-out strategy of homologous recombination to create defined deletions or modify endogenous genes.

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 subsequent follow-up experiments to validate their hits.

Fly stock maintenance and plasmid collection

In addition to maintaining lab stock collections, we keep various commonly used fly stocks such as balancers and virginizer lines. We also have a growing plasmid collection consisting mainly of vectors used for targeted integration or homologous recombination, and are working to improve these tools.

HEAD OF BIOOPTICS: KARIN AUMAYR

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

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 and implementation of new fluorophore combinations. 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 processing and analysis. Two 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

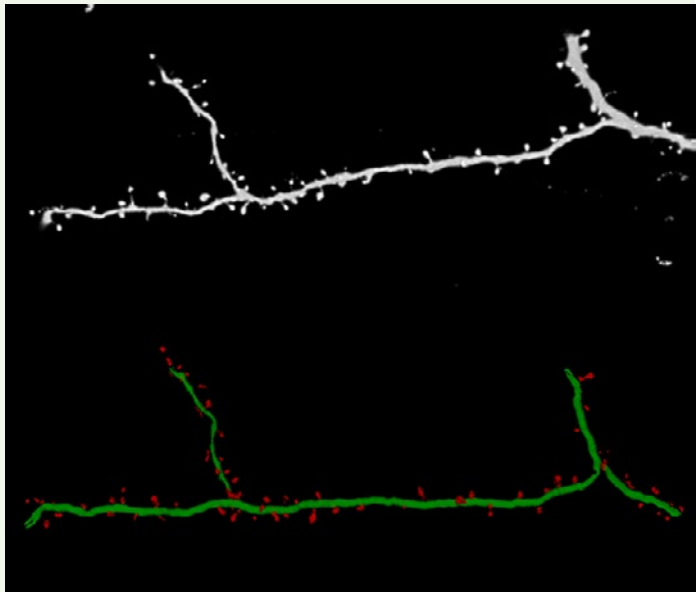
The BioOptics Facility currently manages more than twenty microcopy systems, including wide-field microscopy, confocal laser scanning microscopy (CLSM), two-photon (2P) microscopy, total internal reflection (TIRF) microscopy techniques, and automated slide scanner for samples with or without fluorescence. 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 an intense "Advanced Practical Microscopy Course" is organized on a bi-annual base including hands-on sessions as well as lectures by internal and external faculty.

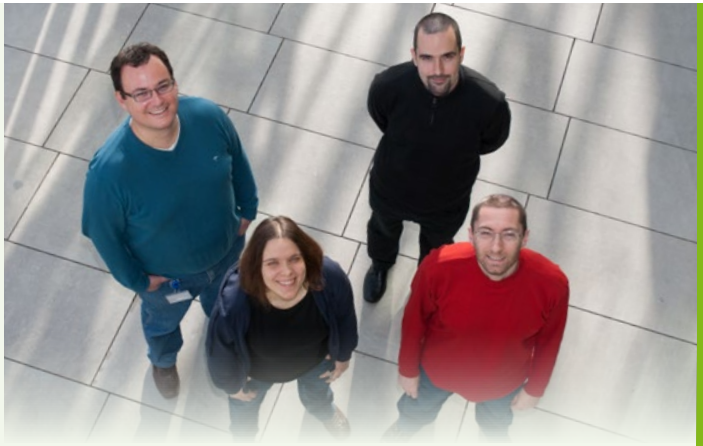
Image Processing and Analysis

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. Several image analysis algorithms are available, such as object tracking and tracing, determination of measurement parameters like intensity, distance, area, volume and co-localization. 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: Samples of a transgenic mouse brain containing sparse GFP expressing neurons were immunohistochemically stained for synaptic proteins. 3D image stacks were acquired using a confocal microscope. The purpose of the image analysis was to automatically detect GFP labeled dendrites and its spines within multiple 3D volumes and extract morphological parameters from them, including volume, size, distance to dendrite, as well as the amount of labeling for the synaptic proteins. The top shows a maximum intensity projection of the original data, the bottom a 3D rendering of the classified neuron in green and the spines in red.





COMPUTATIONAL BIOLOGIST: THOMAS BURKARD, MARIA NOVATCHKOVA, ALEXANDER SCHLEIFFER
SOFTWARE ENGINEER: WOLFGANG LUGMAYR

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, hardware and 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. We also engage in custom software and database development, and design computational and mathematical solutions that can cope with higher loads and memory requirements. To perform complex sequence analysis tasks we maintain the IMP ANNOTATOR, which is a user-friendly Web application and a high-throughput protein annotation system. 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 heterogeneous computational tasks, we maintain a high-performance computing cluster in which dedicated software is adapted to run in a batch and parallel computing environment. This includes tools for statistical computing (e.g. R, Bioconductor), motif discovery and analysis (e.g. AlignAce, MDscan, MEME, Weeder), structural biology (e.g. VMD, pyMOL, HKL2000), image processing (e.g. Xmipp), a wide range of sequence analysis, assembly, mapping and classification tasks (e.g. RNAhybrid, phylip, HMMer), and others.

Training

We provide hands-on training courses on the ANNOTATOR, at which attendees learn the basic principles and limitations of sequence analysis and data integration. 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.

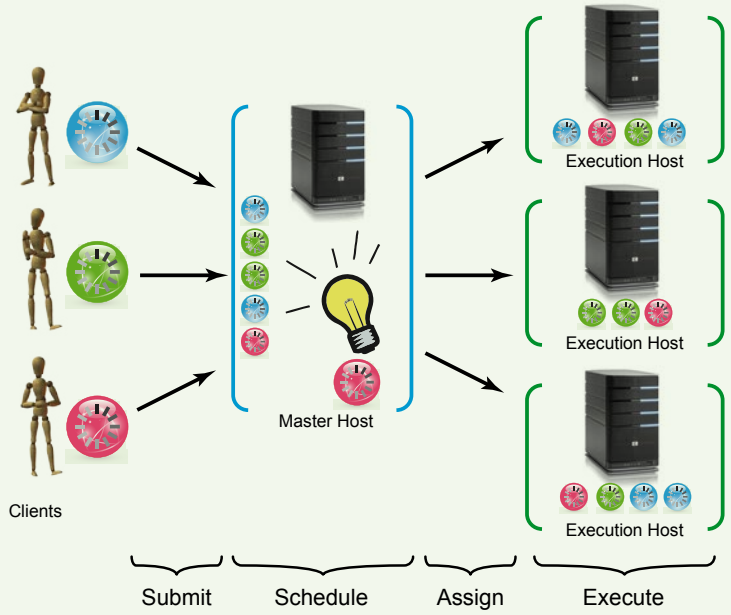


Figure: The IMP/IMBA high-performance computing (HPC) cluster. Users may submit jobs for dedicated clients to the Sun Gridengine (SGE) master, which is responsible for running the jobs on the cluster nodes.

HEAD OF FACILITY: KARL MECHTLER

DEPUTY LAB MANAGER: ELISABETH ROITINGER
POSTDOCS: CHRISTOPH JÜSCHKE [1] , THOMAS KÖCHER, NIKOLAI MISCHERIKOW, PETER PICHLER, JOHANNES STADLMANN, WERNER STRAUBE
BIOINFORMATICIAN: GERHARD DÜRNBERGER
PHD STUDENT: DEBORA BROCH-TRENTINI [2]
MASTER STUDENTS: FREDERICO DUSBERGER, THOMAS TAUS
TECHNICAL ASSISTANTS: JOHANNES FUCHS, OTTO HUDECZ, RICHARD IMRE, GABRIELA KRSSAKOVA, MATHIAS MADALINSKI, MICHAEL MAZANEK, SUSANNE OPRAVIL [3] , MICHAEL SCHUTZBIER, INES STEINMACHER
TRAINEES: ANNA ELISA GATTINGER, DOMINIK MAYER

[1] IN COOPERATION WITH KNOBLICH LAB, [2] IN COOPERATION WITH CLAUSEN LAB, [3] IN COOPERATION WITH WESTERMANN LAB

PROTEIN CHEMISTRY

karl.mechtler@imp.ac.at

Stoichiometry of Protein Complexes

Quantitative mass spectrometry using stable isotope labeling has been extensively used in biology to quantify relative changes in proteomes. In conjunction with internal standard peptides or proteins, quantitative mass spectrometry can be used to determine absolute quantities of selected proteins in biological matrices. We have established a spectroscopic method for the production of LC-MS-compatible internal standard peptides that permits their accurate quantification in a fast and highly parallelized fashion.

In collaboration with the Peters and the Westermann groups, we will apply this technique to determine cellular copy numbers of a range of mitotic proteins and determine the stoichiometries of protein complexes involved in mitosis.

Localization of post-translational modifications

Post-translational modifications (PTM) extend the functional range of a protein by attaching different chemical groups. In addition to the identification of a protein, localization of its modifications is an important and challenging task in proteomics. We developed an algorithm which permits localization of *any PTM*. It can be used for all types of peptide fragmentation modes. The algorithm assigns to each possible modification site its modification probability. This, we believe, will provide a better understanding of the functional value of a protein.

Glycoproteomics of mouse embryonic stem cells:

Glycosylation of proteins by the covalent attachment of carbohydrate structures onto amino-acid side chains is an abundant, yet functionally less characterized group of post-translational modifications. Due to the structural complexity and unfavorable fragmentation behavior of glycopeptides, current mass-spectrometric (MS) approaches to glycoprotein identification typically remove the carbohydrate portions prior to analysis. As a result, these commonly employed MS-based glycoproteomic strategies do not contain information on glycan structures. In order to provide high-confidence glycoprotein identifications as well as site-specific glycan structure information from complex biological samples, we developed a new glycoproteomic workflow. For automated identification of glycopeptides from such large scale high-mass accuracy MS/MS data sets we developed novel software tools, including species-specific glycan structure databases and spectral interpretation algorithms.
In collaboration with the Penninger group we apply this approach to ricin-resistant mouse embryonic stem cell lines and aim to identify cell surface proteins potentially mediating ricin binding and uptake by these cells.

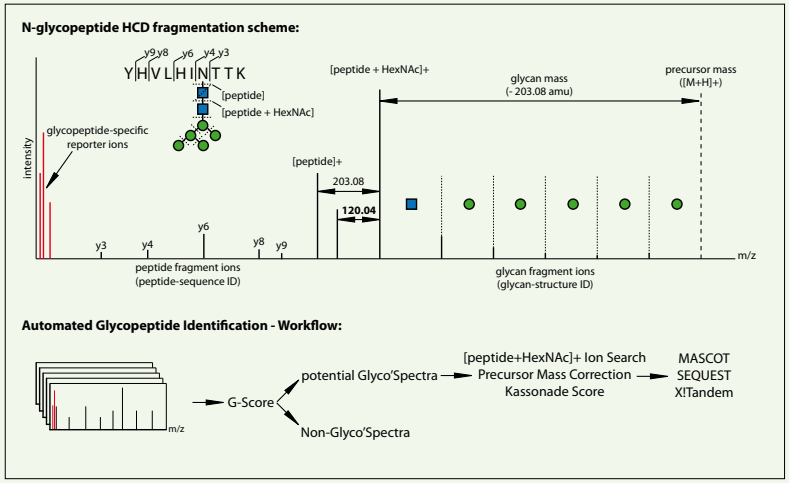
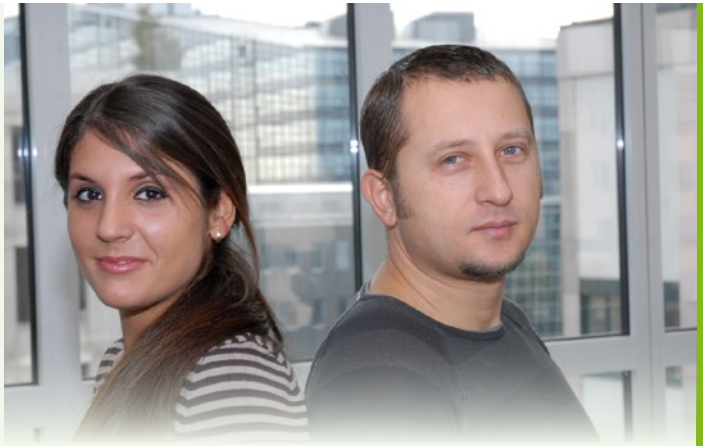


Figure: Glycoproteomic workflow. Based on the fragmentation behavior of glycopeptides in HCD, our software tools automatically identify and process MS/MS spectra of glycopeptides from large-scale datasets and provide both, peptide sequence identification using standard MS/MS Ion Search Engines (i.e. MASCOT, X!Tandem, SEQUEST) as well as glycan structure information.



HEAD OF FACILITY: VUKOSLAV KOMNENOVIC

TECHNICAL ASSISTANT: MIHAELA ZEBA[1]

[1]*ON MATERNITY LEAVE*

HISTOLOGY

vukoslav.komnenovic@imba.oeaw.ac.at

The Histology Service Department offers state-of-the art sectioning, histology and immuno-histochemistry services to all IMBA and IMP scientists. In addition, we offer training and support for researchers in new technologies.

Histology Services

The histology services include the embedding of tissues, assistance with the sectioning of paraffin and cryo-preserved tissues, and preparation of slides for standard H&E stains, as well as specialized stainings such as PAS, Alcian blue, Cab, Gomeri, MayGruenwald-Giemsa and van Kossa stains for human, mouse, *Xenopus* and *Drosophila* studies. With these services, we are able to offer support to get quick results.

Sectioning of Paraffin and Frozen Tissues

In our group we have developed a high throughput method to cut paraffin and frozen tissues. Using this method, we could increase the quality and also the quantity of services.

Immunohistochemistry

The Histology Service Department also provides automated preparation and processing facilities for standardized immuno-histochemistry, in situ hybridization, FISH analysis, DNA microarray and tissues microarray applications. Various antibodies have been characterized for optimized in situ signaling studies. Standard antibodies such as apc, cd (several markers), l-ad, gfp, gfap, c-fos, c-jun, junB, fra1,2, jun-D, ki67, smad3, brdu, egf, egfr, H3K9me tri meth, H4K20me3 tri meth, cl. caspase3, caspase7, procatepsiK are available.

In addition, the Histology Service Department administrates legal regulatory affairs such as the record-keeping and documentation of experiments in accordance with the Austrian Histolaboratories guidelines (*www.mta-labor.info*).

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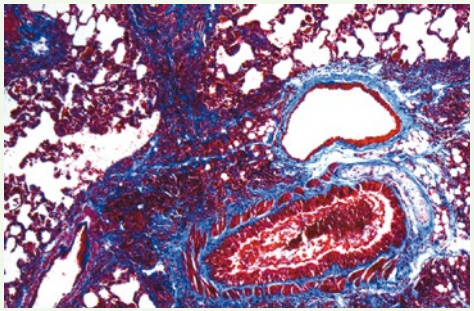

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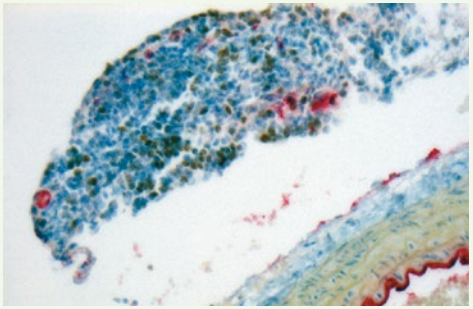

Figure 1: Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.

Figure 2: Double Staining with Ki67 and von Willebrand Factor. Ki67 turnes out to be brown whereas vWF shows a red signal. The counter staining was done with Hematoxylin (blue).

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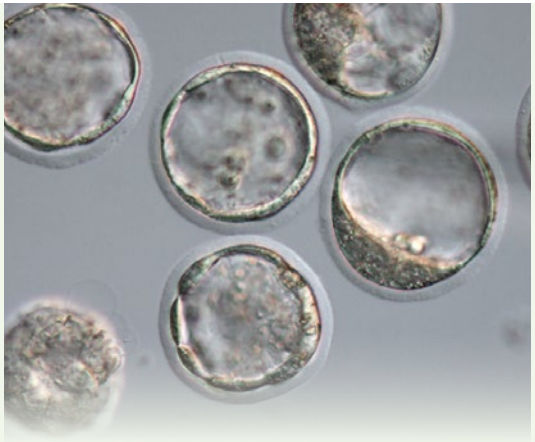

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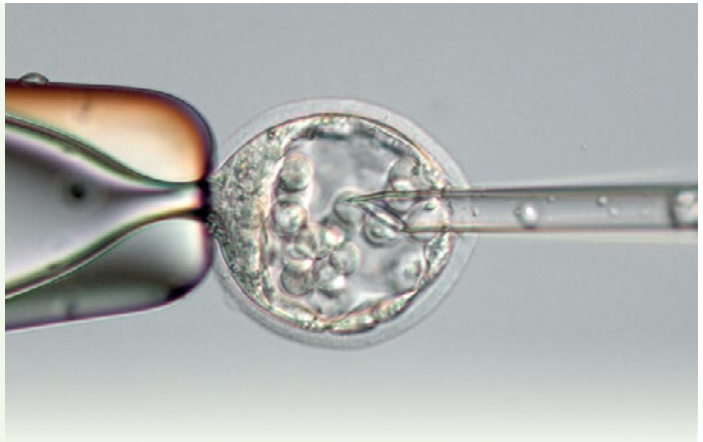


COMPARATIVE MEDICINE

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 attandants - 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: MARTIN RADOLF

ENGINEER: HARALD SCHEUCH
TECHNICAL ASSISTANTS: IVAN BOTTO, ELISA HAHN, MARKUS HOHL, SABINA KULA, CAROLINE SCHUSTER
SANGER SEQUENCING: ZUZANA DZUPINKOVA

MOLECULAR BIOLOGY SERVICE

martin.radolf@imba.oeaw.ac.at

Sanger Sequencing Laboratory, formerly the General Service Department, is a part of newly formed Molecular Biology Service Facility. Sanger Sequencing Lab was shifted from the ground floor to the plaza floor during the summer, and joined the rest of the facility at this site.
The fly food unit and the media kitchen are now independent units. The facility offers a variety of high-quality rapid services to IMP, IMBA and GMI scientists. DNA sequencing, preparation of competent cells strains, production of antibodies, lab automation, and various machines for molecular biology analysis are some of the tasks performed at our facility. We keep a stock of commonly used sequencing primers and competent *E. coli* strains.

Sanger Sequencing

The facility works with 2 DNA genetic analyzers: ABI 3730 (48 capillaries) and ABI 3730XL (96 capillaries). We sequenced approximately 140,000 samples during the first ten months of this year. The high demand was mainly due to screen projects, the fly library, and the new groups at IMP, IMBA and GMI.
The facility maintains approximately 30 different strains of *E. coli* competent cells. We usually prepare DH5α competent cells for all 3three institutes, and the others on request from scientists.

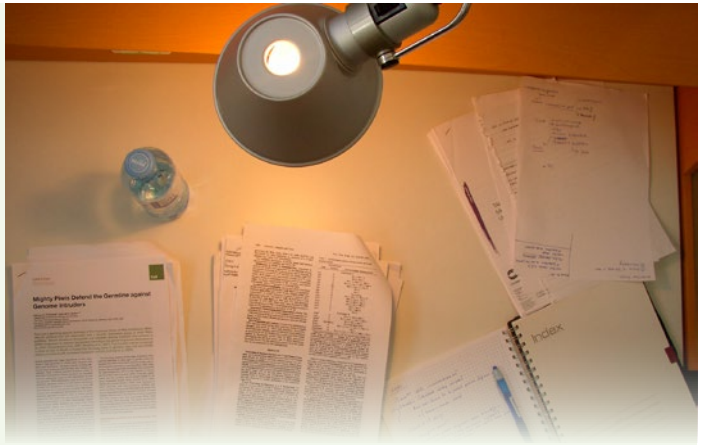
Lab Automation

We recently acquired another Agilent Bravo, which enables scientists to use three Agilent Bravo96 channel liquid handling stations. The main uses of the machines are automated in situ hybridization together with the Stark group, 384 real-time PCR set up, PCR clean up for Sanger sequencing, automated plasmid preparation, generation of single-clone validated oligonucleotide-based libraries->production of custom designed shRNA in cooperation with the Zuber Lab. This robotics-supported method utilizes a DNA barcoding strategy to sequence verify complex libraries involving thousands of clones in a single deep-sequencing experiment. We first used the method to establish a single-clone validated murine shRNA library targeting all 450 known chromatin-associated genes. Following amplification and bulk cloning of 1,500 on-chip synthesized shRNA-encoding oligos, we deep-sequenced 18,000 bacterial clones and were able to retrieve 1,300 shRNAs in a validated single clone format. Following its introduction, the method is currently being used to produce additional focused shRNA libraries which, in contrast to commercially available RNAi reagents, can be highly customized and adapted to most recent design rules and optimal expression vectors. Another robotic platform we offer our users consists of two Biotek Precision XS liquid handling machines. These are small and flexible 8-channel or single-channel robots which can be used in a cell culture hood to pipette repetitive workflows. Precision XS can also be used for hit picking projects. We use the Xiril 100 for preparation of genomic DNA from single fly, and also for plasmid preparation.

Molecular Biology Services

In cooperation with the Nordborg Group from GMI we designed a 384 SNP Illumina assay that can be used as a "universal" genotyping panel for Arabidopsis. We employ Illuminas Custom VeraCode GoldenGate Genotyping kits to generate SNP-specific PCR products that are subsequently hybridized to beads. This enables us to simultaneously genotype 384 loci in a single well of a standard 96-well microplate. In 2012 we were able to analyze about 2700 samples.

This year we started to perform mycoplasma tests on a regular basis. The tests are based on the bioluminescent reaction of luciferin, oxygen and ATP in the presence of luciferase which can be analyzed with our fluorescence plate readers Synergy2 and Synergy H1. The presence of ATP is an indicator for mycoplasma contamination. We also established a PCR protocol to detect mycoplasma contamination.



KARLO PAVLOVIC / LIBRARIAN

JÜRGEN SALLACHNER / LIBRARIAN

MAX PERUTZ LIBRARY

library@imp.ac.at

The Max Perutz Library is a specialized reference library located at the Campus Vienna Biocenter. Its mission is to develop and maintain collections and services that support research at the various institutes.

The main task of the libra ry 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 300 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.

Study environment

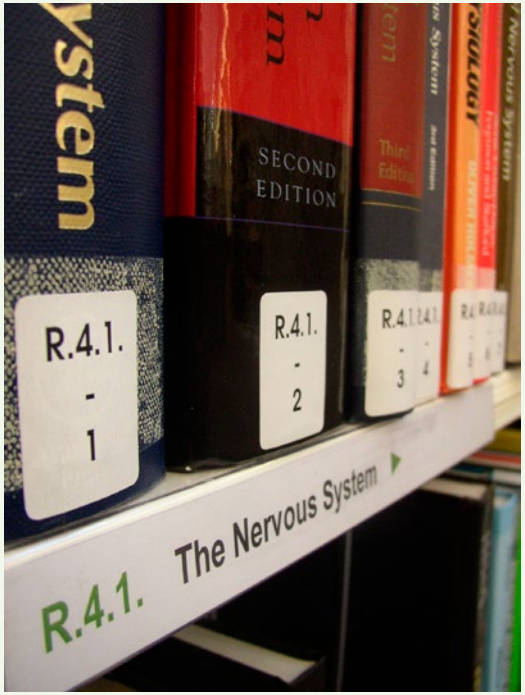
The reading room serves as a quiet and well-equipped place for reading, writing or just relaxing. Six study desks and a cozy lounge as well as two public computers, wireless LAN and a printing facility are provided.

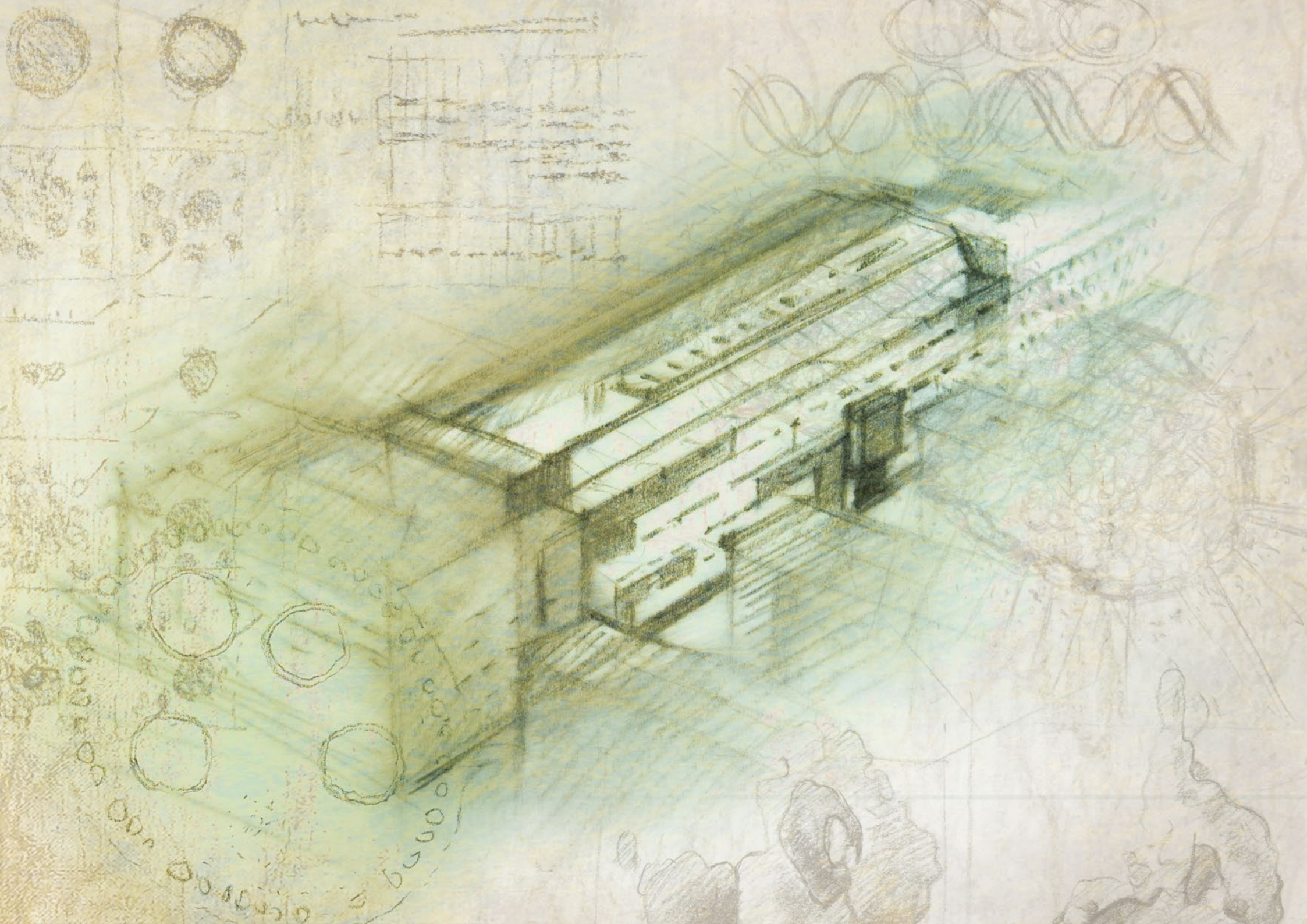
Teaching

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.

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 are welcome to visit the library premises.



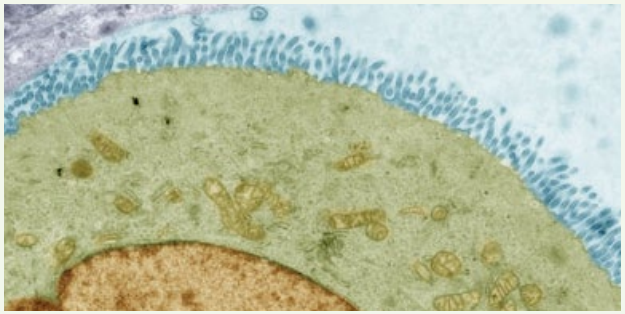


CAMPUS SCIENCE SUPPORT FACILITIES

The Campus Science Support Facilities GmbH (CSF) was established in 2011 at the Campus Vienna Biocenter 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 Campus at the forefront of science. Besides scientific infrastructure, the CSF also offers social infrastructure such as the Campus Child Care Facility. The CSF is located directly on the Vienna Biocenter Campus and is a non-profit organisation funded by a 10-year grant of the Austrian Ministry of Science and Research and the City of Vienna. For more information visit the CSF website: www.csf.ac.at

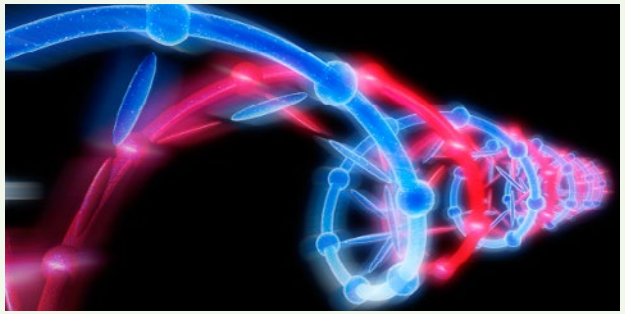
ELECTRON MICROSCOPY

The Electron Microscopy Facility offers a variety of preparation techniques for biological samples of diverse origin. The techniques reach from conventional preparation methods to cryo-preparation techniques for phenotyping the ultrastructure of tissues and cells. Furthermore, the Electron Microscopy Facility provides the processing of purified molecules for conventional and cryo-electron microscopy as well as sample preparation for scanning electron microscopy. Additionally, the facility offers trained users access to a comprehensive set of equipment for sample preparation and visualization of biological specimens at nanometer resolution.



NEXT GENERATION SEQUENCING

The NGS Facility provides cutting edge next generation DNA sequencing technology. The facility offers advice and guidance of sequencing projects as well as a set of selected bioinformatics tools. All common sequencing applications are supported, the development of novel methods and protocols encouraged.



PRECLINICAL PHENOTYPING

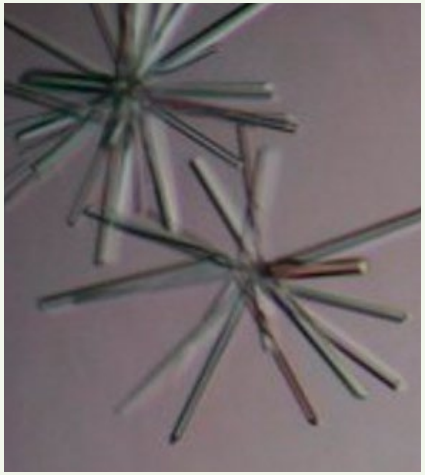
Phenotypic screening is becoming increasingly important in the complex analysis of genetically manipulated mice. Thus, the primary objective of the Preclinical Phenotyping Facility at the CSF is to provide centralized support to investigators at the Campus Vienna Biocenter and off-campus academic/Industrial companies, developing and studying genetically modified animal models relevant to human diseases.

The facility is offering a broad range of standardized tests for a high-throughput phenotypic screen in whole mouse including Metabolism, Neuro-Physiological and a range of Behavioral tests. In addition to the comprehensive panel of phenotyping tests, the facility also provides a range of other services such as consultation, tailoring protocols to the need of the investigators, guidance, training, assistance to a complete performance and analysis of the experiments.

SCIENTIFIC COMPUTING CORE

The scientific Computing Core will provide a scientific computing infrastructure externally to the CSF customers and internally to the other CSF facilities. This infrastructure will consist of a customer database and a service ordering/billing system. these tools will be accessible through a web portal. the facility also provides scientific software development services in the form of small projects or longer-term collaborations.

Another important task of the Scientific Computing Core is to build up a computational biology "knowledge hub" on campus. To this end training courses in biostatistics and programming are already offered to enable researchers to analyze their data more efficiently. The facility also plans to provide a platform where analysis tools written by computational biologist colleagues can be shared so that everyone on campus can benefit from their work.



STRUCTURAL BIOLOGY

The mission of the Structural Biology Facility is to further research in protein biochemistry and structural biology by overcoming two major bottlenecks in these fields – protein production and purification. We will provide high quality protein production in eukaryotic expression systems as well as protein purification and biophysical characterization. Benefits for customers include:

1) expertise on project design and strategy,
2) access to specialized infrastructure,
3) high quality and cost effective service.

VDRC
VIENNA DROSOPHILA RNAi CENTER

VIENNA DROSOPHILA RNAi CENTER

The Vienna Drosophila RNAi Center is a bioresource center maintaining and further developing one of the largest collections of transgenic RNAi lines for conditional in vivo gene function studies. Currently, 31,920 Drosophila lines are available to researchers world-wide. In future the VDRC will further develop and expand its resources according to emerging new technologies and community needs.

Publications

AMERES GROUP

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van der Waal, MS., Saurin, AT., Vromans, MJ., Vleugel, M., Wurzenberger, C., Gerlich, DW., Medema, RH., Kops, GJ., Lens, SM. (2012). Mps1 promotes rapid centromere accumulation of Aurora B. EMBO Rep. 13(9):847-54

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This is a multiauthor paper; featuring U. Elling (IMBA) as shared first author; M. Novatchkova (IMP-IMBA Bioinformatics); J. M. Penninger (IMBA) as shared co-last author.

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van der Harst, P. et al. (2012). 75 genetic loci influencing the human red blood cell. Nature, full article. in press.
This is a multiauthor paper; featuring U. Elling (IMBA) as shared first author; M. Novatchkova (IMP-IMBA Bioinformatics); J. M. Penninger (IMBA) as shared co-last author.

PROTEIN CHEMISTRY

Ferdous M, Higgins JD, Osman K, Lambing C, Roitinger E, Mechtler K, Armstrong SJ, Perry R, Pradillo M, Cuñado N, Franklin FC. (2012). Inter-homolog crossing-over and synapsis in Arabidopsis meiosis are dependent on the chromosome axis proteinAtASY3. PLoS Genet. 8(2):e1002507

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Uzunova K, Dye BT, Schutz H, Ladurner R, Petzold G, Toyoda Y, Jarvis MA, Brown NG, Poser I, Novatchkova M, Mechtler K, Hyman AA, Stark H, Schulman BA, Peters JM. (2012). APC15 mediates CDC20 autoubiquitylation by APC/C(MCC) and disassembly of the mitotic checkpoint complex. Nat Struct Mol Biol. 19(11):1116-23

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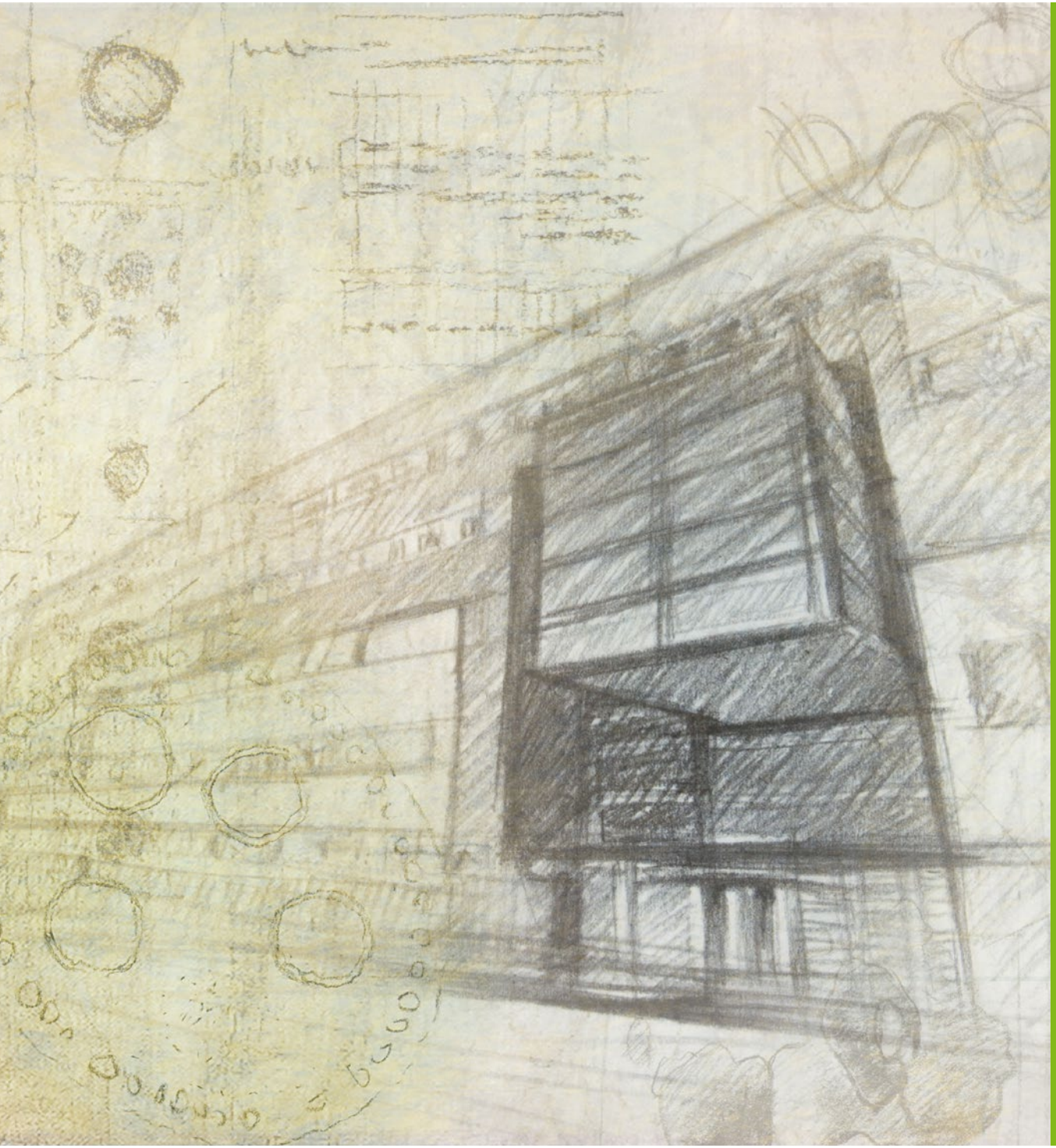

Awards & Honors 2012

IMBA

- *IMBA voted second to top international workplace for Postdocs by the US based life sciences magazine "The Scientist"*

Julius Brennecke

- *Promotion Award for Science ("Förderungspreis für Wissenschaft") by the City of Vienna*

Daniel Gerlich

- *Starting Independent Researcher Grant by the European Research Council ERC*

Fumiyo Ikeda

- *FWF Stand Alone Grant*

Jürgen Knoblich

- *Erwin Schrödinger-Award by the Austrian Academy of Sciences (ÖAW)*

Javier Martinez

- *FWF Stand Alone Grant*

Magdalena Paolino

(Penninger Group)

- *Vienna Biocenter PhD Award*

Josef Penninger

- *Innovator Award by the US Department of Defense (Congressionally Directed Medical Research Program)*
- *Honorary membership by the American Association for the Advancement of Science (AAAS)*

Johannes Popow

(Martinez Group)

- *Kirsten P. Rabitsch Award*
- *Vienna Biocenter PhD Award*

Felix Spira

(Gerlich Group)

- *EMBO Long-term Fellowship*

Iris Uribesalgo

(Penninger Group)

- *EMBO Long-term Fellowship*
- *Marie Curie IEF*

Wiebke Wlotzka

(Ameres Group)

- *EMBO Long-term Fellowship*

JANUARY

12.01.12 Alexander Rudensky
Memorial Sloan-Kettering Institute
Molecular mechanisms of differentiation and function of regulatory T cells

16.01.12 James Dowdle
Memorial Sloan-Kettering Cancer Center
Dissecting the Role of ACF1/BAZ1A in Mammalian Spermatogenesis

16.01.12 Richard Scheltema
Max Planck Institute for Biochemistry
Real-time monitoring and control of shotgun proteomics experiments

FEBRUARY

13.02.12 Titia de Lange
The Rockefeller University
How telomeres solve the end-protection problem

23.02.12 Keith Joung
Harvard Medical School
Genome Editing Using "Open-Source" Engineered Nucleases

MARCH

01.03.12 Chris Lima
Sloan-Kettering Institute
Receptor specificity and signal transduction in ubiquitin and ubiquitin-like protein modification pathways

02.03.12 Thomas Neubert
Skirball Institute, New York University School of Medicine
Using Quantitative Mass Spectrometry (SILAC) to Study Cell Signaling in Neurons

08.03.12 John Diffley
London Research Institute
A wheel within a wheel: the chromosome replication cycle in the cell cycle

12.03.12 Stephan Sigrist
FU Berlin
Shedding light on synapse assembly

12.03.12 Gerhard Dürnberger
CeMM, Vienna
Characterization of human nucleic acid-protein interactions

15.03.12 Josh Kaplan
Harvard University, Massachusetts General Hospital
Synapse and circuit plasticity in C. elegans

22.03.12 Elly Tanaka
Max Planck Institute of Molecular Cell Biology and Genetics Cellular and molecular processes controlling spinal cord and limb regeneration

APRIL

12.04.12 Gerry Rubin
Janelia Farm Research Campus
Studying the Drosophila Brain with Single Cell-Type Resolution

19.04.12 Steve Harrison
Harvard Medical School
Structural biology of kinetochores

26.04.12 Peter Reddien
MIT Biology
The cellular and molecular basis for planarian regeneration

MAY

10.05.12 Rene Medema
University Medical Center Utrecht
Phosphatases in control of checkpoint recovery

21.05.12 Markus Landthaler
MDC, Berlin
The mRNA-bound proteome and its occupancy profile on protein coding transcripts

24.05.12 Josef Jiricny
IMCR, Zurich
The human mismatch repair interactome

31.05.12 Ulla Bonas
Martin-Luther-University, Halle-Wittenberg
Plant targets of bacterial pathogen effector proteins

JUNE

13.06.12 Sebastian Carotta
The Walter & Eliza Hall Institute
iHSC - reprogramming of committed blood cells into hematopoietic stem cells

14.06.12 Thomas Schwartz
Massachusetts Institute of Technology
Taming a Monster - Toward the Structure of the Nuclear Pore Complex

20.06.12 Andrea Hutterer
EMBO
EMBO - Funding opportunities

28.06.12 Rachel Green
HHMI, Johns Hopkins University
Quality Control on the Ribosome during Translation

JULY

03.07.12 Matthias Samwer
Max Planck Institute for Biophysical Chemistry
A novel actin-bundling kinesin that is essential for meiotic cell division

05.07.12 Luis Serrano
Centre for Genomic Regulation
A quantitative systems biology study on a model bacterium

06.07.12 Scott Williams
The Rockefeller University
Divisions and decisions in development and disease

09.07.12 Carsten Janke
Institut Curie
Molecular mechanisms and biological functions of microtubule diversity

09.07.12 Oliver Bell
Stanford University School of Medicine
Dynamics and Memory of Heterochromatin in Living Cell

09.07.12 Chunguang Guo
Harvard Medical School
V(D)J recombination: New insights into long range control and end-joining mechanisms

12.07.12 Cees Dekker
Delft University of Technology
Using the toolbox of nanotechnology for single-molecule biophysics

13.07.12 Cezary Treda
Tohoku University
Secretory leukocyte protease inhibitor modulates urethane-induced lung carcinogenesis

17.07.12 Lukas Kapitein
Utrecht University
From soma to synapse: sorting out polarized transport in neurons

19.07.12 Pietro De Camilli
Yale University School of Medicine
Membrane dynamics and phosphoinositide signaling in the endocytic pathway

AUGUST

02.08.12 Gerald R. Crabtree
Stanford University
Assessing chromatin memory in vivo and unexpected instructive roles in cellular reprogramming

16.08.12 Ralph Neumüller
Harvard Medical School
Comparative Phenomics Identifies an Evolutionarily Conserved Core Set of Cell Growth Regulators

SEPTEMBER

06.09.12 Dan Gottschling
Fred Hutchinson Cancer Research Center
Organelle deterioration with age: The limits of an interconnected cellular system

07.09.12 Zsuzsanna Izsvak
Max-Delbrück-Center for Molecular Medicine
Regulation and Application of Transposable Elements in Vertebrates

13.09.12 Michael P. Rout
The Rockefeller University
The Hole Picture: the Architecture and Mechanism of the Nuclear Pore Complex

20.09.12 James Kadonaga
University of California
Peculiarities of Promoters, Prenucleosomes, and other Planetary Phenomena

25.09.12 Clemens Cabernard
Biozentrum, University of Basel
Cellular and molecular mechanisms of asymmetric cell division

OCTOBER

11.10.12 Alan Hinnebusch
NIH
The mechanism of scanning and start codon recognition in translation initiation: where to begin?

NOVEMBER

16.11.12 Sofia Guimarães
Institute for Molecular and Cell Biology, Porto
Identification of dominant modifiers of bubR1-mutant meiotic phenotype

21.11.12 Ina Poser
Max Planck Institute of Molecular Cell Biology and Genetics
Endogenous protein tagging for fluorescence studies and identification of protein-protein interactions using BAC transgenesis

22.11.12 Pierre Gönczy
ISREC, EPFL
SAS-6 proteins and the mechanisms of centriole assembly

27.11.12 Sonja Bäumel

Hidden Connections

DECEMBER

06.12.12 Matthew van der Heiden
MIT, Koch Institute
Regulation of Cell Metabolism to Support Proliferation

13.12.12 Bo Huang
University of California, San Francisco
Molecular complexes under the light: super-resolution microscopy

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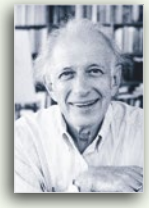

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In order to maintain the highest standard of research, IMBA has installed a process of review and feedback: the Scientific Advisory Board (SAB), consisting of internationally recognized scientists. The Board meets yearly at IMBA, and, together with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

The Supervisory Board of IMBA serves as advisor to and monitors the actions of the management team on a regular basis. It consists of persons with a strong background in academic science and medicine, legal and tax affairs, auditing and other areas of business administration.

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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.

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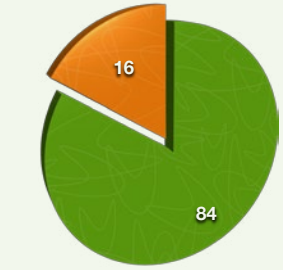
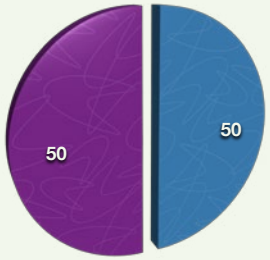
IMBA Key Facts *(Data as of November 2012)*

IMBA Staff (in %)



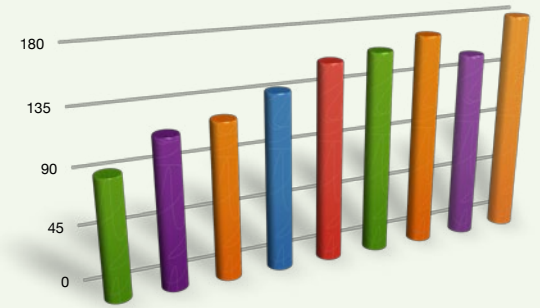
- Scientifc Personnel (incl. Core Facilities)
- Non-scientific Personnel

IMBA Staff - Gender Distribution (in %)



- Male
- Female

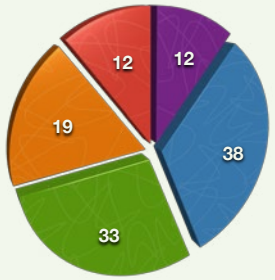
IMBA Staff - Development (Headcount)



2004 2005 2006 2007 2008 2009 2010 2011* 2012

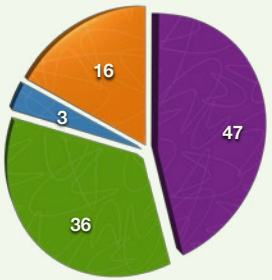
** Transfer of IMBA service units into Campus Science Support Facility GmbH*

Scientific Staff - Functions (in %)



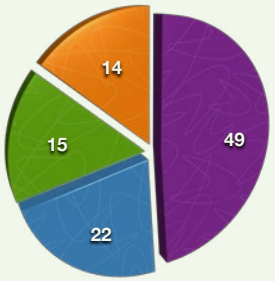
- Principal Investigators
- Postdocs
- PhD Students
- Technical Assistants
- Undergraduates

Scientific Staff - Nationalities (in %)



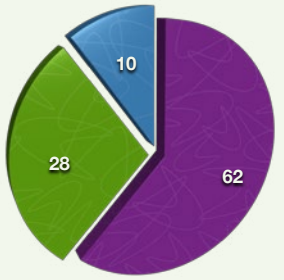
- Austria
- Europe (excl. Austria)
- North & South America
- Asia

IMBA Budget 2012 (in %)



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

Public Research Grants 2012 (in %)



- Austrian Grants
- EU Grants
- Others

Your Career at IMBA!

At IMBA we believe that science and education are inseparable. We offer an excellent research environment for undergraduates, PhD students, postdocs and principal investigators to experience the intellectual freedom that goes hand in hand with the superb infrastructure, generous funding and passion for science that are the hallmarks of IMBA. In addition we offer specific training at each level, for example the international summer school, training courses for PhD students and for post docs, to help prepare each generation for the next step.

PhD students

The "Vienna Biocenter PhD Programme" is among the best postgraduate courses in European life sciences. Students work on biologically relevant issues as part of their doctoral thesis. Being a member of a rather small to medium group guarantees close contacts to all team-members, intense supervision by the team leader and an intimate and stimulating environment. Further aspects are availability of intensive professional training, an internationally competitive salary, and scientific and social events and activities.

The program is run jointly with the Max F. Perutz Laboratories (MFPL), the Research Institute of Molecular Pathology (IMP) and the Gregor Mendel Institute of Molecular Plant Biology (GMI). A call for applications goes out twice a year, with contracts usually lasting 3-4 years. *www.vbcphdprogramme.at*

Postdocs

IMBA focuses on providing an excellent environment for state of the art science including education, which makes it a perfect place to develop your career. A special IMBA postdoc training program is designed to give you a competitive edge on the job market by providing a high level of soft skills' training. Currently, the program includes courses in grant writing, paper writing and leadership and all are operated by excellent and experienced coaches. Applications for postdoc positions at IMBA are made by emailing the relevant group leader directly and attaching an up-to-date CV. Funding is available from internal sources; however postdoc applicants are strongly encouraged to apply for external fellowships to support their research.

Summer School

The Vienna Biocenter Summer School is a collaboration between IMBA and its partner institutes IMP, MFPL and GMI. It provides a unique opportunity for undergraduate students to work side by side with leading researchers in a dynamic scientific environment. It is the perfect preparation for students who are interested in graduate study in the life sciences arena. Applicants who are successful are provided with accommodation, a travel allowance and a stipend for the duration of the Scholarship. *www.vbcsummerschool.at*

Outstanding scientific infrastructure

Scientific success at IMBA benefits to a significant extent from a diverse array of scientific service facilities, the variety and quality of these units being unmatched by most of the top international research institutes with support spanning over a broad range of areas including conventional and high throughput sequencing, state of the art mass spectrometry analysis, various microscopy and electron microscopy systems, and a bioinformatics unit for example. With very few exceptions, all of these facilities are provided free of charge for the scientist.

Attractive and family friendly environment

IMBA cares a lot about the quality of life for its staff. Various social activities such as weekly social hours, annual outing and ski holiday, as well as various retreats help in quickly getting to know your colleagues. A cafeteria provides lunch and refreshments.

If you come to work at IMBA, you'll obviously come in the first place for the science. We do, however, appreciate your private needs and try to make relocation as smooth as possible. For newcomers, there are several in-house apartments to bridge the time until they have found a place of their own. Our administrative staff is helpful in finding housing, and our personnel department will take care of your legal requirements including visas, registration, health insurance and family matters. For school-age children, Vienna offers a large range of different types of schooling, from public to private, German- or foreign language-speaking. For parents with young children, the campus has its own Kindergarten, offering opening hours according to the needs of scientists.

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

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 Campus Vienna Biocenter

IMBA is situated at the "Campus 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 Campus VBC. Campus 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.

New Website Online: Life@IMBA

A goldmine of information for newcomers
„Life@IMBA" prepares you with information about the life at our institute and gives you interesting hints that help you exploring the city of Vienna as well as its surroundings. It is designed primarily for those who have just started at IMBA, and those who have recently moved to Vienna.

If you're thinking about joining IMBA, the website will surely help you to find out why it is definitely worth moving to Vienna to become a part of IMBA.

We are pretty sure that not only potential new colleagues, but also the „old hats" at IMBA will discover some things they didn't know before, including some of the attractions Vienna has to offer in the way of leisure-time activities.

The website is a work in progress – it is intended to be an ongoing project that will continue to develop and grow. We'll be adding at least one new article per month, so make sure to check in regularly!

January

STEFAN AMERES: NEW IMBA GROUP LEADER

Stefan Ameres joined IMBA at the beginning of January. After studying biology at the Friedrich-Alexander University in Erlangen-Nuremberg, Germany, Stefan Ameres did his PhD in the lab of Renée Schroeder at the Max. F. Perutz Laboratories on campus. As a Postdoc he joined the lab of Phillip Zamore at the University of Massachusetts Medical School in Worcester, USA. At IMBA Stefan will continue his research on the mechanism and biology of RNA silencing. His lab is particularly interested in molecular mechanisms governing small RNA silencing pathways in flies and mammals.
www.imba.oeaw.ac.at/research/stefan-ameres

March

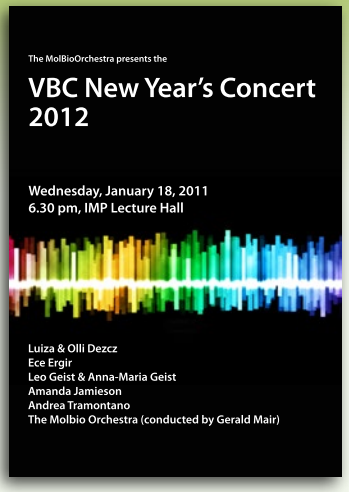
DANIEL GERLICH: NEW IMBA SENIOR SCIENTIST

Daniel Gerlich started his new group at the beginning of March. After doing his PhD at the German Cancer Research Center and the Ruprecht Karls University, Heidelberg, Germany, Daniel went on to do his postdoctoral studies in the laboratory of Jan Ellenberg at the EMBL in Heidelberg. From 2005 to 2012 he worked as an Assistant Professor at the Institute of Biochemistry at the ETH Zurich in Switzerland. In 2011 he was awarded the ERC Starting Grant which will also contribute to setting up his new lab here in Vienna. Daniel and his team of biologists and computer scientists aim to understand the complex interplay between membranes and cytoskeletal structures that mediates chromosome segregation and cytokinesis in human cells. Technology for automated live-cell microscopy and computer vision to investigate mitosis at a systems level is also developed within their multidisciplinary research projects.
www.imba.oeaw.ac.at/research/daniel-gerlich

April

IMBA 2ND BEST PLACES TO WORK FOR POSTDOCS

The US based life sciences magazine, The Scientist, has run its survey for the last ten years, asking readers to identify the top US and international institutes as chosen by research scientists. The 2012 results identify IMBA as second to top international workplace for postdoctoral researchers. The journal's readers cast their votes according to criteria like quality of training, career advancement opportunities and pay, as well as soft criteria like support in work-life balance.



VBC NEW YEAR'S CONCERT

On January 18 the MolBio Orchestra together with other artists from the VBC once more created a formidable and varied program for its New Year's concert. In the first half of the concert soloists featured a wide range of compositions spanning from movie soundtracks and pop classics to folk songs from various countries. After the intermission the MolBioOrchestra performed pieces from Sibelius, Beethoven, Lehár, Strauss and Gershwin.

LONG NIGHT OF RESEARCH

On April 27 IMP/IMBA took part in the Long Night of Research, Austria's biggest science event with more than 1,000 locations. The event is aimed at raising public acceptance of research and motivating younger generations toward a career in science. Guests were able to look into recent topics at various interactive stations, the more adventurous even being able to try out some simple experiments themselves.

VBC SUMMER CONCERT

This years' VBC Summer Concert took place on June 5. The MolBio Orchestra was formed in 2006 by a group of molecular biology and biology students. It has featured concerts since 2010 which in the meantime have become a pleasant tradition on campus. This time the joyful concert included Offenbach, Grieg and Brahms as well as for example the Star Wars main title theme. Preceding the orchestra's performance a range of soloists featured a potpourri of Bach, Sibelius and various other pieces including Jazz, Pop or TV series' themes.

„INSUFFICIENCY"

On June 26 the VBC Amateur Dramatic Club featured a rehearsed reading of the play „Insufficiency" by Carl Djerassi. This was only the third ever public performance of the script, after similar rehearsed readings at Caltech (USA) and Cambridge (UK), and ahead of its professional debut in London in September 2012. Carl Djerassi is an emeritus professor of chemistry at Stanford University (USA), inventor of the birth control pill, playwright and fiction author, and member of the IMBA fundraising committee. The performers had great fun bringing the text to life and the audience, predominantly members of the public, were enthusiastic in their participation. The event was rounded off by a Q&A session with Carl Djerassi and members of the Campus VBC, who openly discussed some of the themes raised in the text.

VIENNA BIOCENTER SUMMER SCHOOL

This year saw the third VBC Summer School, 23 undergraduates from 20 different countries joining one of the labs at IMP, IMBA, GMI and MFPL for nine weeks to work on their own research projects. The results were presented at the final Summer School Symposium in which the students also competed for the VBC Summer School Prize. The whole program was accompanied by a series of lectures and many social activities – and was once again a great success.

POSTDOC ACTIVITIES IN 2012

The Vienna Biocenter offers a great range of further education and activities for Postdocs to optimally prepare them for the next step in their scientific career. Specific grant and paper writing courses held by external experts are scheduled at regular intervals to provide all necessary skills in these important fields. In 2012, for the second year in a row, the Postdocs at the VBC campus also organized their own Postdoc Invited Speaker Series in which the invited speakers shared their experiences getting to the junior group leader stage. The annual Postdoc Retreat in 2012 took place from 13-14 of September in the area around Hollabrunn, Lower Austria, where the VBC Postdocs enjoyed two days of science discussion in a relaxed setting.

June

VBC PHD RETREAT

For this year's PhD retreat, on June 21st & 22nd, 63 VBC students, 4 IST students and 2 invited speakers headed to Schloss Krumbach, a middle-ages fortress. Topics included the results of the PhD survey and how to improve the VBC PhD program as well as the presentation of the student's individual research projects in a poster session. First of the two invited speakers was Kevin Verstrepen who talked about his studies of gene regulation in yeast and the development of his career from being a PhD student to a Harvard fellow to finally becoming a group leader back in Belgium. The second invited speaker was Andrea Hutterer, a former IMP PhD student. In her talk she told how, after doing a PhD in the Knoblich lab and a postdoc in the UK, she became the manager of the EMBO fellowships program thus providing an example of an "alternative" career.

August

"AS YOU LIKE IT"

In 2012 the Vienna Biocenter Amateur Dramatic Club again staged its traditional annual Summer Shakespeare play on August 21-22 in the VBC courtyard. This year the group performed William Shakespeare's „As You Like It". The play follows its heroine Rosalind as she flees persecution in her uncle's court, accompanied by her cousin Celia and Touchstone the court jester, to find safety and eventually love in the Forest of Arden. More than 100 people joined the charming performance which was followed by a relaxed get-together and barbecue.

September

DAYS OF MOLECULAR MEDICINE 2012

Every year, the American Association for the Advancement of Science (AAAS), and the Days of Molecular Medicine Global Foundation (DMM GF) organize the "Days of Molecular Medicine". In 2012, IMBA joined as a co-organizer to launch the three-day meeting „The Translational Science of Rare Diseases: From Rare to Care". The conference was held at the Palais Lichtenstein and featured a set of well-known and international speakers, headlined by Nobel Laureate Eric Kandel. Topics included a new targeted therapy for cystic fibrosis, exon skipping for treating muscular dystrophy, gene therapy for SCID and hemophilia, tailoring treatments with genomics, and embryonic stem cell therapy for treating retinal diseases.

IMBA RECESS

From October 3-5 IMBA scientists met with members of the Scientific Advisory Board (SAB) to present their work and discuss their research. The SAB, consisting of internationally recognized scientists, was once more impressed by the scientific performance and high standards of the research presented. In 2012 two new members joined IMBA's Scientific Advisory Board: Maria Leptin, Professor at the Institute of Genetics University Cologne and Director of the European Molecular Biology Organisation in Heidelberg, and Guido Kroemer, Founding Director European Research Institute for Integrated Cellular Pathology in Paris and "Directeur de Recherche de classe exceptionnelle" of the INSERM (French Medical Research Council) in Paris. IMBA would like to thank all SAB members and the representatives of the Austrian Academy of Sciences for their tremendous support.

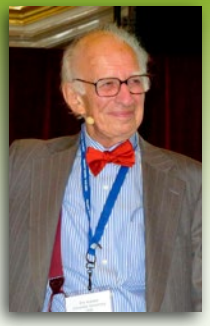
IMBA SAB members: page 48 in this booklet.

BIOMIMETICS

One of the highlights of every academic year at the Vienna Biocenter (VBC) is the PhD symposium organized by VBC graduate students for students and scientific fellows from all over the world. The 10th VBC International PhD Symposium was held on November 8-9, 2012, entitled "Biomimetics – Inspired by Nature".
A string of renowned speakers gave a broad overview of the interdisciplinary field of bionics, including strategies for energy generation, architectural models, nature inspired materials, robots, and bionic approaches in information technology. A highlight of the symposium was the presentation of the best dissertations of 2012 at the Campus Vienna Biocenter (Vienna Biocenter PhD Award).

October

November



US BREAST CANCER AWARD FOR IMBA

In October IMBA's Scientific Director Josef Penninger was awarded a $7.4 million "Innovator Award" for his project "Novel Approaches to Breast Cancer Prevention and Inhibition of Metastases" by the US Department of Defense through the USA's Congressionally Directed Medical Research Program. The grant recognises Josef Penninger's work in identifying a key molecular pathway in how hormone replacement therapies and contraceptive pills can lead to breast cancer (Nature, 2010.). Penninger now intends to develop a new diagnostic method to help in predicting the chances that any patient will develop breast cancer.

IMBA EXCURSION

This year's IMBA outing on 15 October 2012 took the IMBA staff initially to the Lower Austrian Zwettl Abbey, with refreshments in the abbey restaurant followed by a tour through the abbey buildings and stroll through the surrounding herb gardens and park. The second part of the outing came after lunch and a presentation by the IMBA management – a visit to Retz and a traditional old wine cellar. After the fascinating tour the evening was brought to a close in a wine tavern with generous buffet and wine tasting.

THINKING THE UNTHINKABLE – THE FUTURE OF BIOLOGY

10 Years of Research at IMBA

IMBA will celebrate "10 Years of Research" in 2013. To celebrate its 10 year anniversary, IMBA will organize a scientific symposium themed "Thinking the Unthinkable – The Future of Biology". In four sessions a broad range of topics will be presented by external speakers as well as several IMBA scientists. The symposium will be held in the "Aula der Wissenschaften" in the first district of Vienna on June 27-28, 2013. Save those days in your calendar as well as the evening of June 26 which is reserved for IMBA's big birthday party!

For more information and the exact program please visit: www.imba.oeaw.ac.at/symposium

Sessions:

IMAGING: FROM MOLECULES TO CELLS
Scott Fraser (Caltech, USA)
Jan Ellenberg (EMBL Heidelberg, Germany)
Werner Kuehlbrandt (MPI Goettingen, Germany)

STEM CELLS & TISSUE MODELING
Yoshiki Sasai (Riken, Japan)
Austin Smith (University of Cambridge, UK)
John Gurdon (Gurdon Institute, UK)

METABOLISM & DISEASE
Eric Olson (Southwestern Medical Center, USA)
Gokhan Hotamisligil (Harvard School of Public Health, USA)
Bruce Spiegelman (Harvard Medical School, USA)

RNA & CHROMATIN
Witek Filipowicz (FMI Basel, Switzerland)
David Bartel (Whitehead Institute, MIT, USA)
Dirk Schübeler (FMI Basel, Switzerland)

Dates:

26th June 2013
Ceremonial Act & Party
27th - 28th June 2013
Scientific Symposium (free of charge)

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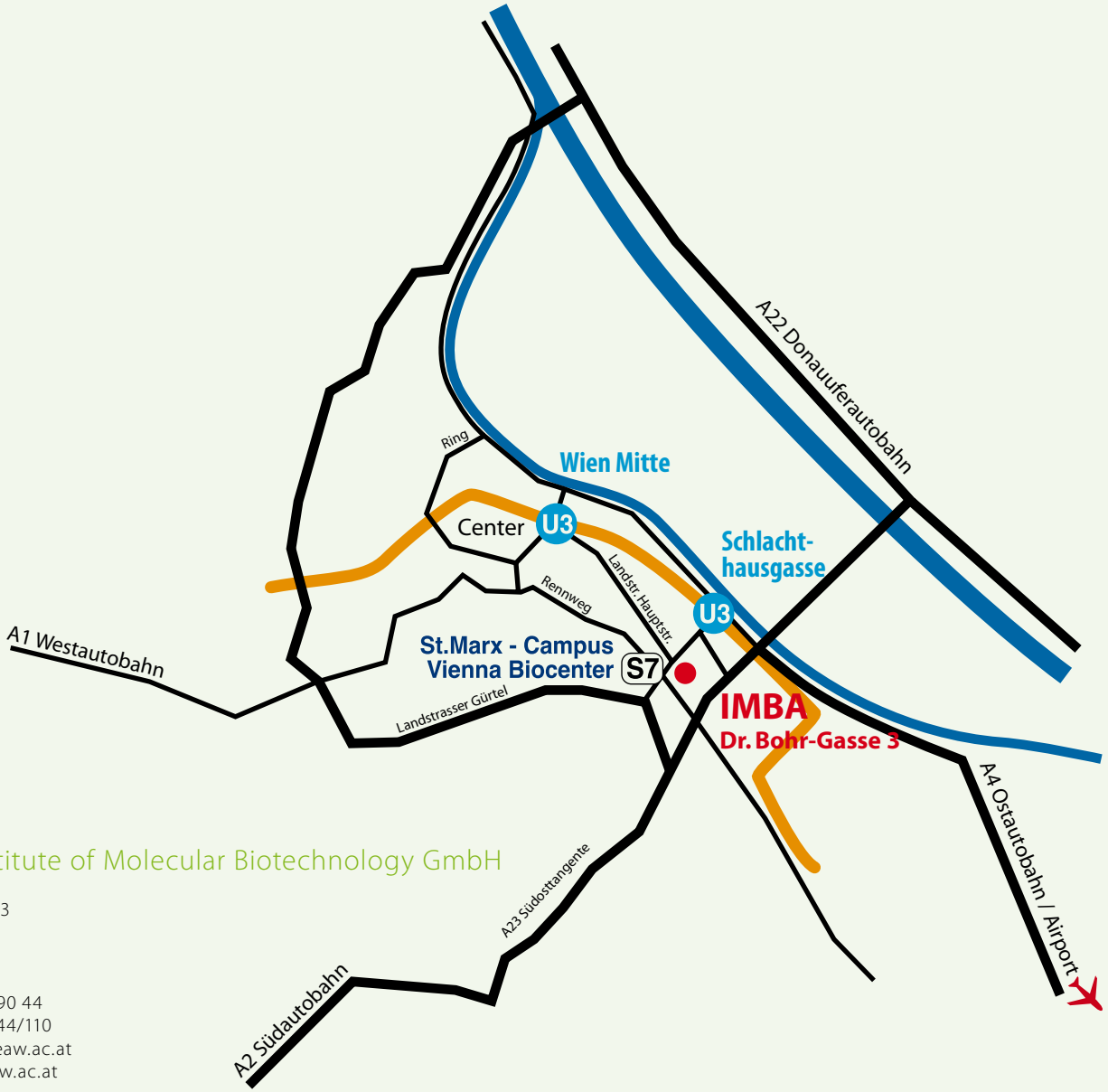

IMBA - Institute of Molecular Biotechnology GmbH

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 Campus Vienna Biocenter.