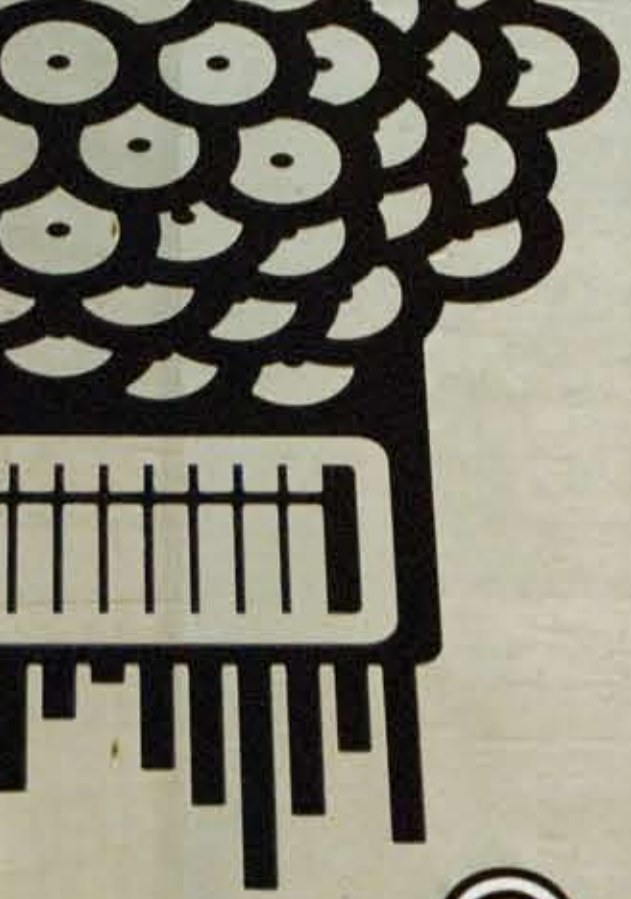
INSTITUTE OF MOLECULAR BIOTECHNOLOGY

OF THE AUSTRIAN ACADEMY OF SCIENCES

VIENNA BIOCENTER

2011



OAW
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

IMBA has had another brilliant year in research, a year of highlights that are presented in this annual report. In particular our young scientists have come into their own and have published top notch research. Successful research institutions are not known by the quantity of publications they produce but are defined by key findings that have indeed opened new fields and the international standing of their researchers. Successful research institutes are fragile palaces that need to be cultivated at all levels – from a great cafeteria to meet, faculty meetings and lecture series, young PhD students and postdocs that challenge our paradigms, open doors for discussions, to world-class administration and service infrastructures. Successful research institutes stand on multiple strong legs, nurture young talents, and live a culture of collaboration and mutual support. Many of these visions have already been implemented and the success of the young scientists is the success of everybody at IMBA – the success of a large team with a common goal.

This year we hired 4 new group leaders, the three junior PIs Fumiyo Ikeda, Kikue Tachibana-Konwalski, and Stefan Ameres, and Daniel Gerlich as a Senior Investigator. Kikue Tachibana-Konwalski joined IMBA from Kim Nasmyth's lab in Oxford in November 2011. She is working on the first cell division and meiosis in mammalian cells using gene modified mice. Fumiyo Ikeda worked with Ivan Dikic in Frankfurt and started at IMBA on December 1st, 2011.

Fumiyo is a biochemist who co-discovered linear ubiquitination as a novel intracellular signaling pathway. Stefan Ameres did his PhD with Renee Schroeder at MFPL, Vienna, and continued his postdoctoral training with Phil Zamore in the USA. Stefan is working on the biology of non-coding RNA and will start in January 2012. Daniel Gerlich will join IMBA as a permanent senior PI on March 1st, 2012. He is currently working at the ETH in Zürich and will bring high throughput imaging technologies to the Campus. His research focus is in cell biology and high throughput assays on finding genes that control mitosis. We are very happy that we continue to be able to attract world-class talents to IMBA and that these brilliant young minds have chosen IMBA as their future centre of gravity.

With the addition of our new groups, we decided to reorganize the lab spaces and focus all faculty groups on two research floors and move our excellent infrastructure groups to one floor at IMBA. With these changes we hope to strengthen the interactions and communication among the researchers and streamline our services. The rebuilding of the institute has progressed well and will be finished as planned, a testament to the great efforts made by many people, especially the groups of Alex Chlup and Michael Kratochwille.

Another key development was the initiation of the Campus Scientific Support Facility (CSF) and the hiring of Andreas Tiran as its first director. The CSF is an initiative of all research institutes and biotech companies located at the Vienna Biocenter (VBC) that brings more than 50 Million Euros in infrastructure money to our Campus. The idea of the CSF is to establish an additional service centre and expand/improve existing infrastructures that are accessible for all institutions at our Campus. According to the plan, we already transferred the deep sequencing unit and the unique Vienna Drosophila RNAi Library under the wings of the CSF. The Electron Microscopy Facility will follow early next year to develop a new centre for high resolution imaging. In addition, new facilities for mouse imaging/phenotyping, plant genetics, protein purification and protein structure, and bioinformatics are being developed under the capable guidance of Andreas Tiran and his team. We continue to maintain key services in-house in close cooperation with our partner the Research Institute of Molecular Pathology (IMP) and also the Gregor Mendel Institute of Molecular Plant Biology (GMI). The establishment of the CSF is a great example for a joint effort by all VBC members and we have to give a special "Thank you" to all people at IMBA and IMP services and administration who went the extra mile to make the CSF a reality. Our infrastructures have always been the key to our success and we strongly adhere to the principle that these services, internally at IMBA/IMP/GMI and now at the CSF, are an absolute integral part for the future and necessary for our research. We will therefore do everything possible to make sure that all of these services continue to shine.

Finally, we want to thank everybody at the IMBA family and of course our extended family at the IMP for their great work. Our world-class service units and the dedicated and excellent administration allow us – as always – to focus our efforts on doing innovative and excellent research. They are a key part of our success and deserve all the credit. A big thank you also to our Scientific Advisory Board that has done tremendous work, to our close partner Boehringer Ingelheim, our Supervisory Board, the members of the IMBA Fundraising Board, our neighbours and partners the Gregor Mendel Institute (GMI) and Max F. Perutz Laboratories (MFPL), and to the many people who have helped us to develop and continue to support IMBA. If we all stand together and all work towards the same goals, to make a little difference in this world, then we will continue to be strong.

Finally we would like to thank all the government institutions and private and institutional donors who provide the fuel for our work - our funding. All senior faculty and two of our Junior IMBA group leaders have ERC grants and more than 70% of the research personnel is funded by grants, a very high rate of third party funding. A special "Thank you" has to go to all members of the Austrian Academy of Sciences and the leadership of the Academy, who continue to believe in the future of IMBA and have made a steadfast commitment to support the development of IMBA in the next 5 years. The commitment will allow us to make the next step in the development of the institute, the step from a promising fledgling to a strong youth that stands on strong feet. Such a commitment also carries a promise from our side, a promise to create a place that attracts the top international talents and where such talents have the time and the environment to grow, and a promise that the investments in IMBA reinforce the leading role of the Austrian Academy of Sciences as a major international research institution.

IMBA has had an amazing start, has gained international standing based on the quality of it's research, and now has been given the chance to become a truly international player.

Jürgen Knoblich, Michael Krebs and Josef Penninger

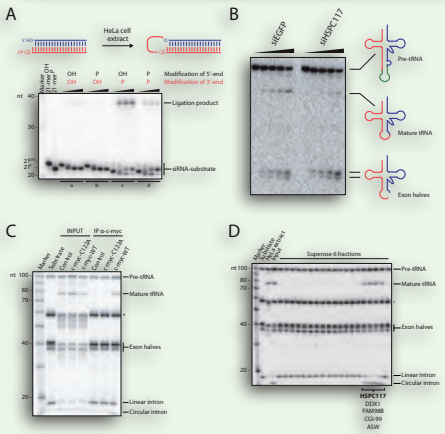
RESEARCH HIGHLIGHTS

2011 has been scientifically a very 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.

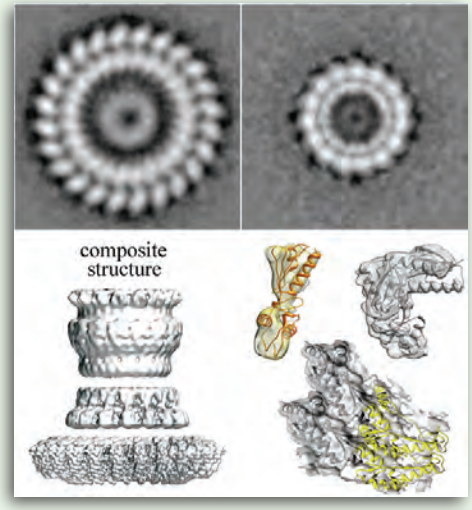
HSPC117 is the essential subunit of a human tRNA splicing ligase complex.

Popow, J., Englert, M., Weitzer, S., Schleiffer, A., Mierzwa, B., Mechtler, K., Trowitzsch, S., Will, CL., Lührmann, R., Söll, D., Martinez, J. (2011). HSPC117 is the essential subunit of a human tRNA splicing ligase complex. Science. 331(6018):760-4

Transfer RNAs (tRNAs) are absolutely necessary to translate the genetic code into proteins required for life. In humans, some tRNAs are synthesized in an immature form containing an intervening sequence that has to be removed by sequential cleavage and joining reactions. Although the factors carrying out the cleavage reactions are already known for several years, the so-called tRNA ligase joining the tRNA exon pieces together remained unknown. We applied chromatographic techniques to purify proteins required for the joining reaction from the complex cell extracts able to process tRNA precursors into their mature form. After several rounds of purification we arrived at a mixture of ca. 100 proteins which we could examine in greater detail. One of those, called HSPC117, proved to be the essential factor for the described joining reaction as we were able to demonstrate by affinity purification and RNAi-mediated inactivation experiments.



Identification of the tRNA ligase HSPC117. (A) siRNAs can be used as a substrate for human RNA ligase activity. siRNA duplexes are efficiently ligated in HeLa cell extracts only in case they display a phosphate group at their 3′ end and a hydroxyl group at their 5′ end (lanes c). Removal of the 3′-phosphate or phosphorylation of the 5′-hydroxyl ends interfere with siRNA ligation (lanes a, b and d). (B) Silencing of HSPC117 – but not EGFP as a control – impairs ligation of tRNA exon halves in tRNA splicing reactions. (C) Immunoprecipitates (IPs) of c-myc-HSPC117 ligate tRNA exons. The point mutant c-myc–HSPC117 C122A (Cys122 → Ala122) is inactive as a tRNA ligase. (D) A tRNA ligase complex is co-selected with monoclonal antibodies directed against the mRNA splicing factor SF3b. Size fractionation of the bound proteins yields a complex composed of HSPC117, DDX1, FAM98B, CGI-99 and ASW. Fractions containing the HSPC117-complex exhibit tRNA exon ligase activity.



Three-dimensional model of Salmonella's needle complex at subnanometer resolution.

Schraidt, O., Marlovits, TC. (2011). Three-dimensional model of Salmonella's needle complex at subnanometer resolution. Science. 331(6021):1192-5

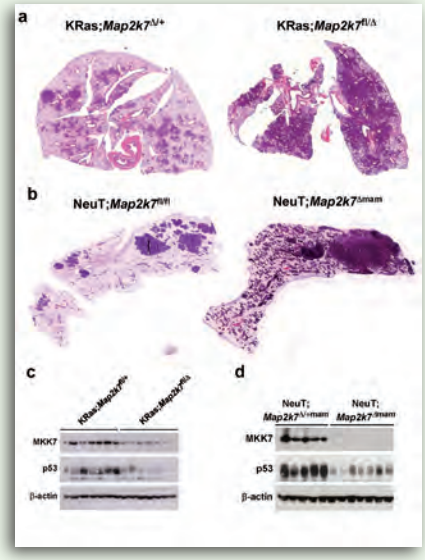
Type III secretion systems (T3SSs) are essential pathogenic factors used by many Gram-negative bacteria like EHEC, Salmonella, Shigella or Cholera to inject proteins that make eukaryotic host cells accessible to invasion. In this study we were able to generate a cryo electron microscopy density map of the core structure of the T3SS, the needle complex (NC), a ~3.5 megadalton-sized, membrane-embedded injection machine with subnanomter resolution. Showing secondary structural elements like α-helices this highly resolved density map allowed for confident docking of atomic structures. The resulting atomic model revealed insights into the NC's overall organization and into the structural requirements during assembly. We thereby provide a framework that will strongly promote further structural and functional studies of the T3SS and hopefully will also assist in generating new antibacterial strategies.

Solving the 3D-structure of the NC. Top row: Average images of NC inner ring (left) and outer ring (right) substructures obtained by selective disassembly of wild type NC. The top views allow for a direct counting of the inner and outer ring subunits and reveal a 24- and 15-fold symmetry, respectively. Bottom row: (left) Surface views of a 3D-reconstruction of the NC. With α-helical densities visible, (right) atomic structures could be placed unambiguously into the NC.

The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression

Schramek, D., Kotsinas, A., Meixner, A., Wada, T., Elling, U., Pospisilik, JA., Neely, GG., Zwick, RH., Sigl, V., Forni, G., Serrano, M., Gorgoulis, VG., Penninger, JM. (2011). The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression. Nat Genet. 43(3):212-9

This study seeks to define the molecular regulation of epithelial cells during the early steps of cellular transformation using primary cell culture and genetic ablation studies. We have focused on a signaling molecule with hitherto ill-defined functions during tumorigenesis: MKK7 and its downstream factors JNK1 and JNK2. This signaling cascade serves as an intracellular messenger receiving and allocating various cellular stimuli such as stress signals and inflammatory cues.
We could now show that MKK7 and the JNK signaling pathway also functions to suppress tumor formation in various organs such as the lung, the breast and the skin. It is well know that cells generally counteract oncogenic transformation by activating a cellular fail-safe mechanism: p53-induced cell cycle arrest or cellular suicide. This mechanism is absolutely crucial considering the trillions of cells in our body susceptible to sporadic mutations, which could ignite uncontrolled proliferation and tumor development. Failure of this mechanism leads to development of multiple, spontaneous tumors in humans and in mice. Using knock-out mice and improved primary cell culture systems I could show that MKK7 directly couples oncogenic and genotoxic stress to p53 stability required for cell cycle arrest and suppression of NeuT-induced breast and KRasG12D-induced lung tumors (Figure 1). Importantly, p53 overexpression could revert lung tumorigenesis in MKK7-deficient animals unambiguously confirming this new tumor suppressive mechanism. This study identified MKK7 as a vital molecular sensor to set a cellular anti-cancer barrier by linking oncogenic stress and the DNA damage response (DDR) to the key tumor suppressor p53. This work was recently featured as an Article in Nature Genetics.



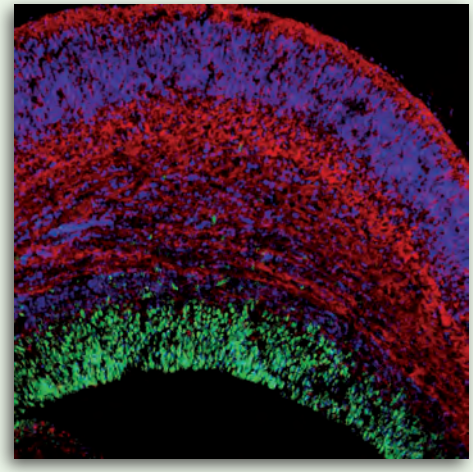
MKK7 controls KRasG12D-driven lung and NeuT-driven breast tumorigenesis through p53 expression. A, Representative H&E stainings showing accelerated progression and increased tumor burden in KRas;Map2k7fl/Δ mice. B, Representative histology of mammary cancers that developed in 16 week old NeuT;Map2k7Δ/+mam and NeuT;Map2k7Δmam littermate females. H&E stained sections are shown. C and D, Western blot analysis for p53 and MKK7 levels in KRasG12D-driven lung (C) and NeuT-driven breast tumors (D). β-actin is shown as loading control.

Genome-Wide Analysis of Self-Renewal in Drosophila Neural Stem Cells by Transgenic RNAi

Neumüller, RA., Richter, C., Fischer, A., Novatchkova, M., Neumüller, KG., Knoblich, JA. (2011). Genome-wide analysis of self-renewal in Drosophila neural stem cells by transgenic RNAi. Cell Stem Cell. 8(5):580-93

Spectacular recent advances in the field of stem cell biology have raised enormous hopes for regenerative medicine. One key property of stem cells is their ability to "self-renew", that is to generate identical copies of themselves while at the same time producing more specialized cells, which then replace damaged cells in the tissue. One of the key challenges in stem cell biology is to understand how the balance between self-renewal and specialization is regulated - with the goal to some day manipulate this balance to increase or decrease the regenerative capacity of individual tissues.
The fruitfly Drosophila has emerged as a simple key model system for stem cell biology. Neural stem cells in the developing fly brain follow a simple lineage and divide reproducibly into one self-renewing and one differentiating daughter cell. So far, all the components of the cellular machinery responsible for this asymmetric division are also present in human stem cells and many of them fulfill the same role.
Researchers in the laboratory of Juergen Knoblich at IMBA have now made a huge effort to identify essentially all the components that are important in Drosophila neural stem cells. They used a transgenic RNAi library from the Vienna Drosophila RNAi Center (VDRC) to inhibit almost all genes in the fly genome in neural stem cells. One by one, they studied the effects of inhibiting individual genes and carefully described the effects on the neural stem cells. Importantly, those experiments were done in whole living flies and not in cell culture where those effects can be very different. The results provide a unique resource for the stem cell community as most of the genes they identify are also present in human stem cells. The precise quantification of the resulting effects has allowed an unprecedented bioinformatic analysis of the data which allowed insights into stem cell biology that were not possible before. The data obtained through this study provide a unique starting point for a systems-level analysis of stem cells.

The image shows a wild type brain hemisphere on the left and a neural stem cell tumor on the right. The image resides on a background, which shows a functional network of asymmetric cell division that regulates self-renewal and differentiation of neural stem cells identified in the study.

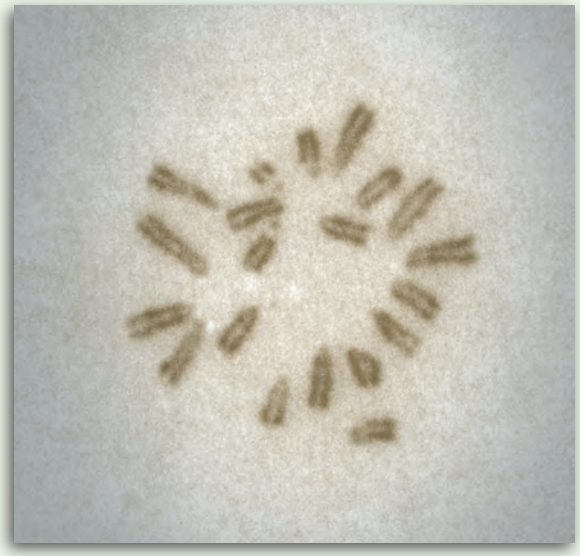


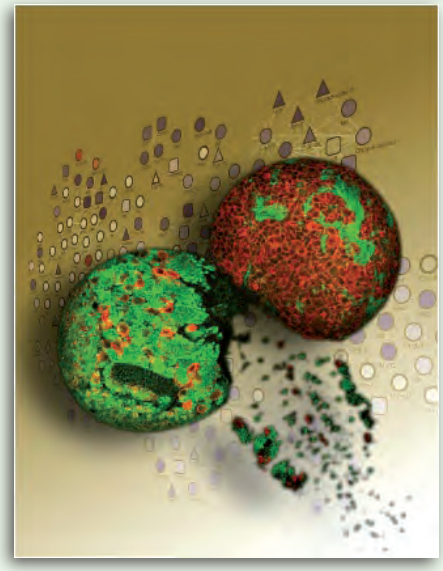
Forward and Reverse Genetics through Derivation of Haploid Mouse Embryonic Stem Cells.

Elling, U., Taubenschmid, J., Wirnsberger, G., O'Malley, R., Demers, SP., Vanhaelen, Q., Shukalyuk, AI., Schmauss, G., Schramek, D., Schnuetgen, F., von Melchner, H., Ecker, JR., Stanford, WL., Zuber, J., Stark, A. and Penninger, JM. (2011). Forward and Reverse Genetics through Derivation of Haploid Mouse Embryonic Stem Cells. Cell Stem Cell. 9(6):563-74.

All somatic mammalian cells carry two copies of chromosomes (diploidy), whereas organisms with a single copy of their genome, such as yeast, provide a basis for recessive genetics. Here we report the generation of haploid mouse ESC lines from parthenogenetic embryos. These cells carry 20 chromosomes, express stem cell markers, and develop into all germ layers in vitro and in vivo. We also developed a reversible mutagenesis protocol that allows saturated genetic recessive screens and results in homozygous alleles. This system allowed us to generate a knockout cell line for the microRNA processing enzyme Drosha. In a forward genetic screen, we identified Gpr107 as a molecule essential for killing by ricin, a toxin being used as a bioweapon. Our results open the possibility of combining the power of a haploid genome with pluripotency of embryonic stem cells to uncover fundamental biological processes in defined cell types at a genomic scale.

Single (haploid) chromosome set in a mouse stem cell.





Mouse inscuteable induces apical-Basal spindle orientation to facilitate intermediate progenitor generation in the developing neocortex.

Postiglione, MP., Jüschke, C., Xie, Y., Haas, GA., Charalambous, C., Knoblich, JA. (2011). Mouse inscuteable induces apical-Basal spindle orientation to facilitate intermediate progenitor generation in the developing neocortex. Neuron. 72(2):269-84

Neurons in the mammalian neocortex arise from asymmetric divisions of progenitors residing in the ventricular zone. While in most progenitor divisions, the mitotic spindle is parallel to the ventricular surface, some progenitors reorient the spindle and divide in oblique orientations. Here, we use conditional deletion and overexpression of mouse Inscuteable (mInsc) to analyze the relevance of spindle reorientation in cortical progenitors. Mutating mInsc almost abolishes oblique and vertical mitotic spindles, while mInsc overexpression has the opposite effect. Our data suggest that oblique divisions are essential for generating the correct numbers of neurons in all cortical layers. Using clonal analysis, we demonstrate that spindle orientation affects the rate of indirect neurogenesis, a process where progenitors give rise to basal progenitors, which in turn divide symmetrically into two differentiating neurons. Our results indicate that the orientation of progenitor cell divisions is important for correct lineage specification in the developing mammalian brain.

Section through the cerebral cortex of a mouse, stem cells can be seen glowing in green, mature nerve cells in red; cell nuclei for both types of cell are shown in blue.

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 protect the genome against the deleterious influence of selfish genetic elements such as transposons. In animals a specialized pathway centered on PIWI proteins and their interacting piRNAs silences transposons within gonads. Recent experimental and bioinformatics studies have uncovered the fascinating conceptual framework of this pathway that is conserved from invertebrates to mammals. Our group dissects the molecular and genetic makeup of this pathway and aims to understand its biological functions.

Silencing selfish genetic elements

Nearly all eukaryotic genomes contain selfish genetic elements such as transposons. Their devastating impact on the host is illustrated by the phenomenon of "hybrid dysgenesis" in *Drosophila melanogaster*: Intercrosses between laboratory strain females and males caught in the wild result in progeny with severe sterility. This is caused by the uncontrolled activity of a single transposon, which is present (and silenced) in wild populations but absent in stocks that have been kept in laboratories since ~100 years. The *Drosophila* genome, however, contains not only one, but more than one hundred transposon families, whose transposition strategies vary widely. To ensure reproductive fitness, flies (and all other organisms) have thus been under evolutionary pressure to evolve a generic transposon silencing system. Work over the past decade has demonstrated that the piRNA pathway, a specialized small RNA silencing pathway is the major silencing system that keeps transposons under control in animal gonads.

The piRNA pathway – a small RNA based genome immune system

The piRNA pathway is a wonderful example of how much more sophisticated nature devises a solution to a problem compared to what we would theoretically design. In essence, the piRNA pathway acts as an RNA-based genome immune system. It comprises an inheritable genetic component and an acute response system, which specifically targets active transposons. Briefly, the transcription of discrete heterochromatic loci (termed piRNA clusters) provides a template, from which primary piRNAs are produced. piRNA clusters contain vast collections of immobile and broken copies of transposons, which are or have been active in a population and therefore act as a long term storage system for transposon sequence information. If a primary piRNA encounters a target (active transposon), cleavage of the transposon RNA by the piRNA-complex leads to the synthesis of a novel, complementary piRNA. This piRNA in turn guides the production of more antisense piRNAs derived from the piRNA cluster transcript. Thus, piRNA clusters act not only as a genetically inherited memory component but also as relay stations to boost the production of silencing competent piRNAs.
Recent studies have elucidated the conceptual framework of this pathway described above. These were mostly based on the bioinformatics analysis of piRNA populations in the light of decades of genetic work on transposon control in flies. At a mechanistic level, however, our understanding of the piRNA pathway is rudimentary at best. The only thing that is clear at the moment is that this pathway is by far more complex than the related microRNA and siRNA pathways.
To further understand this fascinating silencing system, we use *Drosophila melangaster* as a model system. Here, we can combine genetics, biochemistry, cell biology and bioinformatics in unique ways. Moreover, roughly 35 years of genetic studies on transposons and host-strategies to silence them provide us with a wide range of observations, which we can now connect to this pathway.

The main areas of our interest are:

1. Identifying and characterizing novel piRNA pathway members: We have established very 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. Using these *in vivo* RNAi systems we performed genome wide screens towards the identification of novel piRNA pathway genes in *Drosophila*. The preliminary results from these screens promise 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 enigmatic piRNA clusters: piRNA clusters are typically located at telomeres or at the border between euchromatin and heterochromatin. Their transcripts are believed to traverse large (up to several hundreds of kb) heterochromatic regions. We are interested in the regulation and processing of piRNA clusters. Ultimately, we want to understand how the cell is able to discriminate cluster transcripts from other RNAs in the cell.

Publication highlights:

Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ. Discrete Small RNA-Generating Loci as Master Regulators of Transposon Activity in Drosophila. Cell. 2007 Mar 7;

Brennecke J, Malone CD, Aravin AA, Sachidanandam R, Stark A, Hannon GJ. An epigenetic role for maternally inherited piRNAs in transposon silencing. Science. 2008 Nov 28;322(5906):1387-92.

Daniel Olivieri, Martina M Sykora, Ravi Sachidanandam, Karl Mechtler and Julius Brennecke (2010) An in vivo RNAi assay identifies major genetic and cellular requirements for primary piRNA biogenesis in Drosophila EMBO Journal. 2010 Oct 6;29(19):3301-17

Figure 1

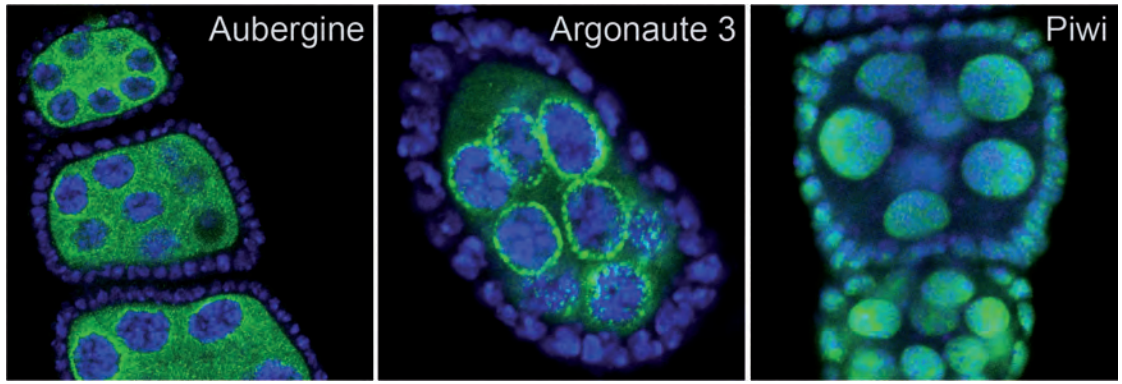


Figure 2

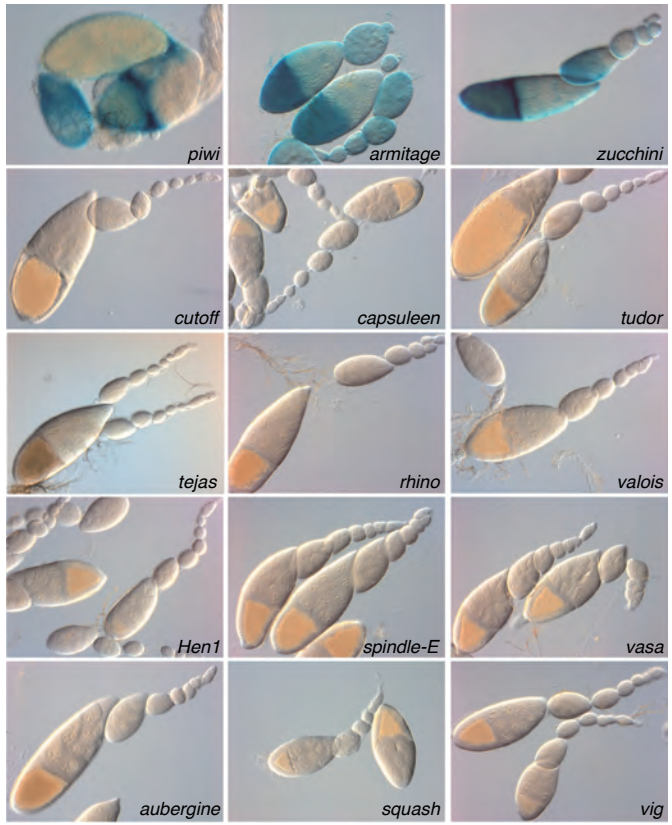


Figure 1: Immuno-fluorescence analysis of the three Argonaute proteins acting in the piRNA pathway (Aubergine, AGO3, Piwi). Shown are developing egg chambers surrounded by the follicular epithelium (DNA in blue, Argonaute proteins in green). Only Piwi is expressed in the follicular cells, whereas AGO3 and Aubergine are exclusively detected in the germline cells.

Figure 2: An *in vivo* RNAi assay identifies Piwi, Armitage and Zucchini as essential components of the somatic piRNA pathway. Shown are beta-Galactosidase stainings of ovarioles, in which the indicated genes were knocked down in somatic follicle cells by RNAi and which express a lacZ sensor for the somatic piRNA pathway (see Olivieri et al. 2010 for details).

GROUP LEADER :
JULIUS BRENNECKE

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

JÜRGEN KNOBLICH GROUP

Neural stem cells in flies and mice

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

We use Drosophila and mouse genetics to understand important aspects of neural stem cell biology. In particular, we ask how stem cells decide between self-renewal and differentiation and how defects in the underlying mechanisms can result in brain tumor formation.

Stem Cell Tumors in *Drosophila*

In the *Drosophila* brain, neural stem cells called neuroblasts undergo repeated rounds of asymmetric cell division (Figure 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 (Figure 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, only the basal daughter cell inherits the determinants. 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 to form gigantic lethal brain tumors (Figure 1C). These brain tumors can be transplanted into other flies where they become aneuploid and ultimately undergo metastasis. The precise reproducibility of these events allows us to study tumor formation from stem cells at an unprecedented level of detail. We are using transcriptomics and genome sequencing to understand the precise contribution of genetic and epigenetic events. How defects in asymmetric cell division cause the formation of stem cell derived tumors is one of the key questions we are currently investigating.

Genome-wide analysis of biological processes

We have carried out genome-wide RNAi screens to identify a large number of all genes controlling asymmetric cell division and self-renewal in neuroblasts (Figure 2). For this, we use the VDRC RNAi library, a collection of over twenty thousand transgenic *Drosophila* RNAi lines can be induced in a tissue-specific manner. Our screens have identified around 600 genes regulating *Drosophila* neuroblasts. Among those are 18 tumor suppressors that cause neuroblast overproliferation. These include Numb, Prospero and Brat and their known binding partners, but also six nuclear proteins that influence cell fate downstream of these 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. Analysis of those factors by transcriptional profiling and biochemical analysis allows us to determine how differences in protein composition lead to the stable and irreversible reprogramming of daughter cells towards terminal differentiation.

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 (Figure 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 (Figure 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.

Publication highlights:

Postiglione, M. P., Juschke, C., Xie, Y., Haas, G. A., Charalambous, C., and Knoblich, J. A. (2011). Mouse inscuteable induces apical-Basal spindle orientation to facilitate intermediate progenitor generation in the developing neocortex. Neuron 72, 269-284.

Neumuller, R. A., Richter, C., Fischer, A., Novatchkova, M., Neumuller, K. G., and Knoblich, J. A. (2011). Genome-Wide Analysis of Self-Renewal in Drosophila Neural Stem Cells by Transgenic RNAi. Cell Stem Cell 8, 580-593.

Schwamborn, J. C., Berezikov, E., and Knoblich, J. A. (2009). The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. Cell 136, 913-925.

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

Figure 1

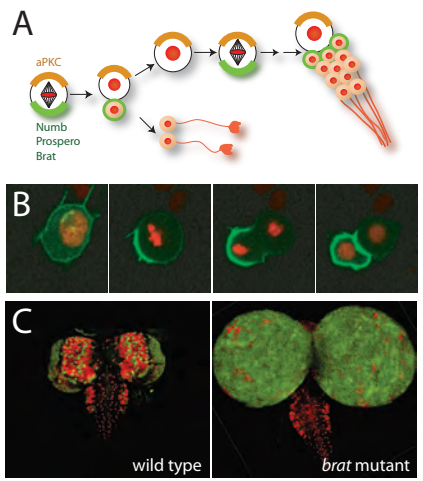


Figure 3

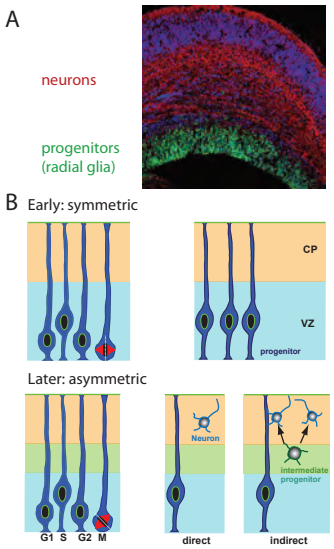


Figure 2

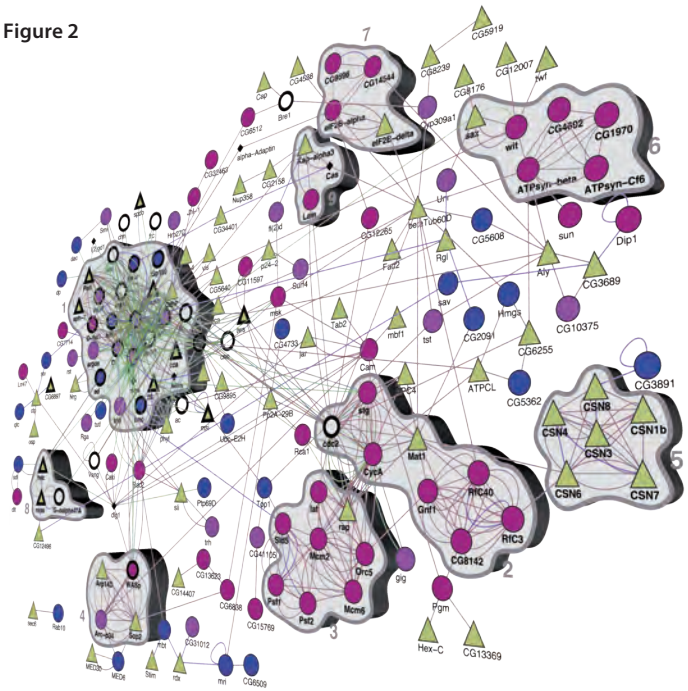


Figure 1: How cells divide asymmetrically.
A. *Drosophila* neuroblasts (white) divide asymmetrically to generate self renewing stem cells (white) and differentiating neurons (red). During each neuroblast division, aPKC (orange) guides the asymmetric segregation of Brat, Prospero and Numb (green) into 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) sebrerating 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: Genome-wide analysis of biological processes in a whole organism.
Functionally validated interaction network of the Notch signaling pathway assembled from genome-wide analysis of asymmetric cell division in *Drosophila* external sensory organ development. The network shows genes that cause phenotypes in the screen and have previously been shown to interact biochemically or genetically. The encircled groups are protein complexes identified by a clustering algorithm.

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: RYAN CONDER, NINA CORSINI, CATARINA DE CERTIMA F. HOMEM, CHRISTOPHER ESK, ANJA FISCHER, SPYROS GOULAS, MADELINE A. LANCASTER, MARIA PIA POSTIGLIONE, ILKA REICHARDT, CONSTANCE RICHTER, YUNLI XIE, TETSUO YASUGI
PHD STUDENTS: MONIKA ABRAMCZUK, ELIF EROGLU, HEIKE HARZER, ONUR KAYA, LISA LANDSKRON, FEDERICO MAURI, MARKO REPIC, VIVIEN ROLLAND, JONAS STEINMANN
RESEARCH ASSOCIATE / LAB MANAGER: ELKE KLEINER
RESEARCH ASSISTANT: ANGELA MARIA PEER
SCIENTIFIC PROGRAMMER: PEDRO SERRANO DROZDOWSKYJ
TRAINEE:SUZANNE VAN DER HORST

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

Many animal and plant pathogens share the same principles of infecting host cell organisms: they translocate specific bacterial toxins (collectively referred to as "effector proteins"), which originate from the bacterial cytoplasm, directly into the cytoplasm of a eukaryotic host cell. As a result, translocated effector proteins have the remarkable capacity to modulate various host-cell pathways, including endocytic trafficking, gene expression, programmed cell death, or cytoskeleton dynamics that induce membrane ruffling and subsequently render the host accessible to bacterial infection. At the heart of this process is the type-3 secretion system (T3SS), a protein-delivery machine that establishes intimate contact between the microorganism and the host cell, and permits safe and unidirectional passage of specific effectors. These systems are widespread among Gram-negative animal pathogens, including *Yersinia, Pseudonomas, Shigella*, enteropathogenic and enterohemorrhagic *E. coli* (EPEC and EHEC, respectively), or *Salmonella*, and the plant pathogens *Erwinia, Ralstonia* or *Xanthomonas*. They are essential for the onset of a variety of diseases ranging from diarrhea, bubonic plaque, even with fatal outcomes, to fire blight and bacterial wilt. While the task of translocating proteins from one compartment to the other has been basically solved in nature (for example the targeting and/or secretion of proteins through the Sec-system or the Tat-system), the contextual situation is complicated by the fact that the translocation must occur through a number of environments, which includes two bacterial membranes and one eukaryotic membrane, the periplasmic and the extracellular space. Consequently, the nature of a T3SS system is complex in terms of specific mechanistic details as well as the organization of all involved components. Using *Salmonella typhiumurium*, we are investigating the molecular mechanisms and structural framework required to translocate effector proteins specifically and safely into eukaryotic cells.

Architecture of the needle complex of the T3SS:

The core, and probably the most prominent structure of the T3SS (SPI-1), is the needle complex. It is a 'syringe'-like multi-component system. Overall, the needle complex is a large (approximately 30x80nm) cylindrical complex. In its native environment it is embedded in the inner as well as outer membranes, spans the periplasmic space, and protrudes into the extracellular environment with a needle filament. Its overall architecture provides a structural framework for a direct connection of bacterial and host cell cytoplasm, and delineates the secretion pathway through the needle complex. Although the needle complex is about 3.5 MDa in size, its overall shape is dictated by only five proteins. Nevertheless, mutually exclusive models of the individual protein organization have been described in the past. These models were rendered complex by a paucity of positional information, incorrect assumptions about the symmetry and stoichiometry of ring-forming base proteins, and consequent difficulties of modeling. Our laboratory 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 will serve as a basis to further understand the structural determinants required to form ring-like structures in membrane-embedded systems, and may also be used to design small molecules that interfere with the assembly pathway.

Assembly of the T3SS:

Our topological analysis revealed that additional proteins must be present. These constitute the cup/socket structure which is located in the center of the needle complex (export apparatus). Using mass spectrometry, we were able to identify five additional candidate proteins that co-fractionate in marginal quantities with purified needle complexes. Subsequent structural analysis revealed the absence of the cup/socket, suggesting that one or more of these proteins are required to build up the cup/socket (Figure 3). We were also able to show that these proteins nucleate the coordinated assembly of the needle complex (Wagner et al., 2010)

Structural Plasticity of the needle filament

Efficient effector protein translocation is known to occur only after host cell contact. Therefore, it is conceivable that the extracellular filament is a key player in the transmission of this information, probably due to small conformational changes throughout the filament. This hypothesis is supported by mutations found in the homologous Shigella needle filament, which convert the system into a constitutively "on" state. If this is true it would be justified to presume that the filament is provided with a certain degree of structural heterogeneity in order to accommodate the required conformational plasticity for signal transmission. We therefore analyzed the structure of the needle filament by cryo electron microscopy (Figure 3) and discovered that the structure is, indeed, highly variable (Galkin et al., 2010).

Although the design of the TTSS appears to be conceptually simple, many questions remain unanswered: How dynamic is the entire assembly process? How are substrates recognized by the needle complex? What is the molecular mechanism of protein translocation? We have started to address some of these questions. 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

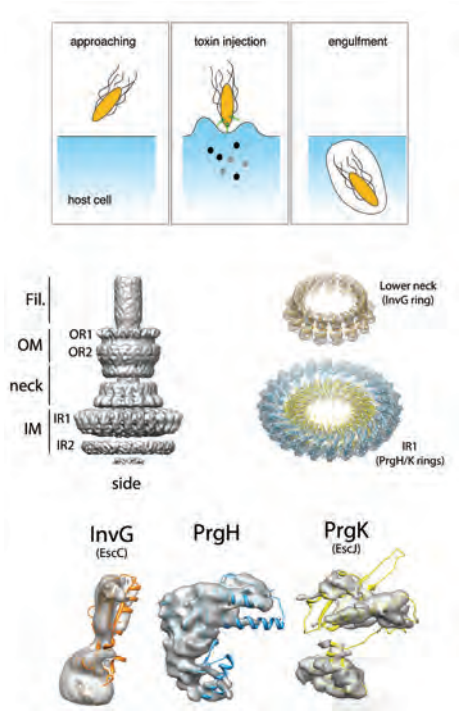
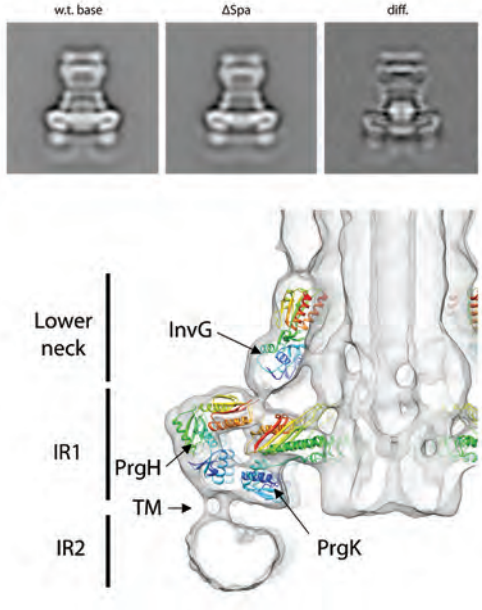


Figure 2



JOINT IMP/IMBA GROUP LEADER:
THOMAS C MARLOVITS

PHD STUDENTS: MATTHIAS BRUNNER, JESUS FERNANDEZ-RODRIGUEZ, LISA KÖNIGSMAIER, AGATA KOSAREWICZ, JULIA RADICS
DIPLOMA STUDENTS: JOANNA RADEK
SUMMER STUDENT: CECILIE OEST-JACOBSON
LAB MANAGER / RESEARCH ASSISTANT: WOLFGANG WEBER

Figure 3

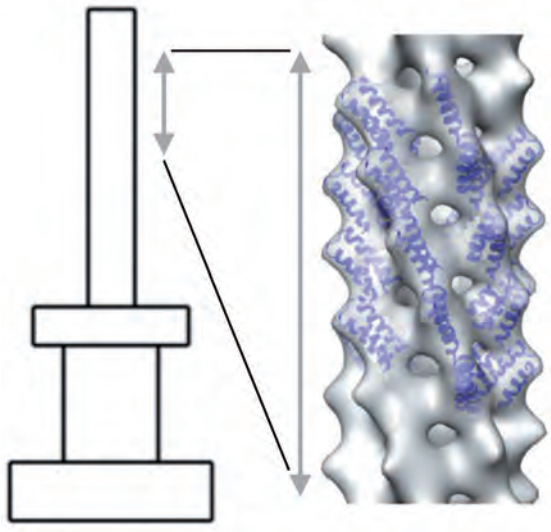


Figure 1: Three-dimensional reconstruction of the needle complex to sub-nanometer resolution and docking of atomic structures of all available protein domains.

Figure 2: Formation of the socket/cup is dependent on the presence of export apparatus proteins (SpaPQRS, InvA). Single-particle analysis of w.t. and ΔSpa bases reveal marked differences in the cup and socket region.

Figure 3: Structure of the extracellular needle filament.

JAVIER MARTINEZ GROUP

New enzymes and paradigms in RNA metabolism

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

Our laboratory follows an integrative approach to identify and characterize, in vitro and in vivo, new players in the intricate world of RNA processing. We are particularly interested in enzymes that ligate and phosphorylate RNA molecules, i.e. RNA ligases and RNA kinases. For this purpose we combine: i) Biochemistry and bioinformatics to reach a deep mechanistic understanding; ii) Cross-linking and immunoprecipitation (CLIP) followed by deep sequencing to identify RNA substrates for these enzymes and assign them to specific RNA metabolic pathways, and iii) Mouse knockout models to monitor their impact in the context of a full organism and to reveal potential connections between RNA metabolism and disease. We recently identified the human tRNA ligase complex, involved in pre-tRNA splicing and probably in the splicing of pre-mRNA molecules during the unfolded protein response. In the future we aim to reveal a general role for the tRNA ligase in mechanisms of RNA repair.

The long sought human tRNA ligase finally identified!

Similar to other RNA molecules, precursor transfer RNA (pre-tRNA) transcripts are subjected to extensive post-transcriptional processing before they are matured to fulfill their biological functions. In particular, intron-containing pre-tRNAs undergo excision of the intervening sequence in two steps: first, the tRNA endonuclease generates 5'- and 3'-exons with 2', 3'-cyclic phosphate and 5'-hydroxyl ends, respectively. In animals, the second step predominantly entails direct exon ligation in a reaction that preserves the phosphate group at the 3' end of the 5' exon (Figure 1). Yet, who ligates tRNA exons during tRNA splicing?

We purified the tRNA ligase from HeLa cytoplasmic extracts using a sequence of classic chromatographic steps. To monitor ligase activity we assayed inter-strand ligation of a particular siRNA displaying 3'-phosphate and 5'-hydroxyl termini, predicting that the "siRNA-ligase" would in fact be the tRNA ligase. After purification, mass spectrometry and bioinformatics analysis, we arrived at a very promising candidate, HSPC117, a protein of unknown function. RNAi-mediated depletion of HSPC117 inhibited maturation of intron-containing pre-tRNAs both *in vitro* and in living cells. Importantly, we could ligate exon halves with immunoprecipitates from a stable cell line expressing a myc-tagged wild type, but not a mutant, inactive form of HSPC117 (Figure 2). We were also able to assess the role of HSCP117 in living cells by monitoring the maturation of an inducible, de novo synthesized reporter pre-tRNA molecule. Therefore, we concluded that HSPC117 is the long sought catalytic component of the human tRNA ligase (Popow et al. 2011, see publication highlights).

The human tRNA ligase is a pentameric complex containing HSPC117 and four extra polypeptides, including the ATP-dependent RNA helicase DDX1 (Figure 3).

The identification of the human tRNA ligase is the group's most important recent achievement at IMBA. We value the finding itself and the enormous perspectives that it generates.

What is the plan for the tRNA ligase?

We want to address the function of the tRNA ligase *in vivo* by generating a conditional-knockout mouse and a mouse encoding an inactive tRNA ligase. We are also performing PAR-CLIP, i.e. Photoactivatable Ribonucleoside Enhanced Crosslinking and Immunoprecipitation, to identify RNA targets of the tRNA ligase in addition to tRNA exon halves. Among others, a potential candidate is the mRNA encoding Xbp1, an essential protein during the mammalian Unfolded Protein Response (UPR). This transcript undergoes non-canonical splicing requiring enzymatic cleavage by the endonuclease Ire1 and ligation by a yet unidentified RNA ligase (Figure 4). Splicing of Xbp1 leads to a frameshift giving rise to a protein that travels to the nucleus and functions as a transcription factor to orchestrate the expression of genes involved in UPR. It is known that in yeast the tRNA ligase Rlg1 splices both tRNA exon halves and the Xbp1 homolog Hac-1. Therefore it is tempting to speculate that HSPC117 could be the ligase mediating UPR in humans. We are intensively testing this hypothesis with a recently developed *in vitro* system using human cell extracts.

Taken together, investigating the functions of the tRNA-ligase assures many years of exciting work!

Publication highlights:

Johannes Popow, Markus Englert,* Stefan Weitzer, Alexander Schleiffer, Beata Mierzwa, Karl Mechtler, Simon Trowitzsch, Cindy Will, Reinhard Lührmann, Dieter Söll† and Javier Martinez†. HSPC117 is the essential subunit of a human tRNA ligase complex. Science. 2011 Feb 11;331(6018):760-4.*co-first authors; †co-corresponding authors.*

Figure 1

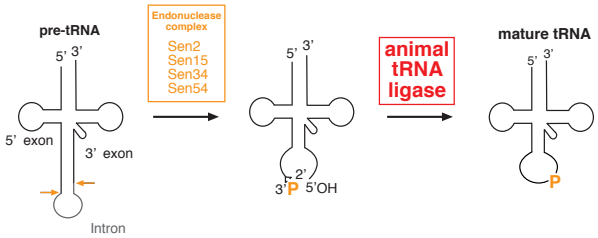


Figure 2

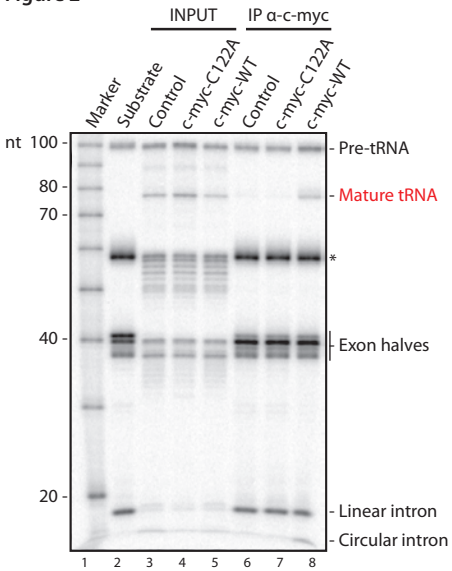
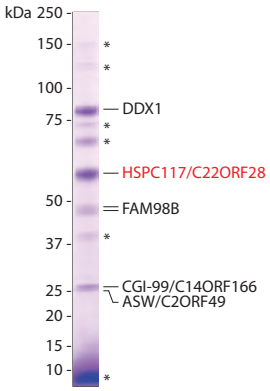


Figure 3



The human tRNA ligase complex

DDX1: DEAD-box RNA helicase

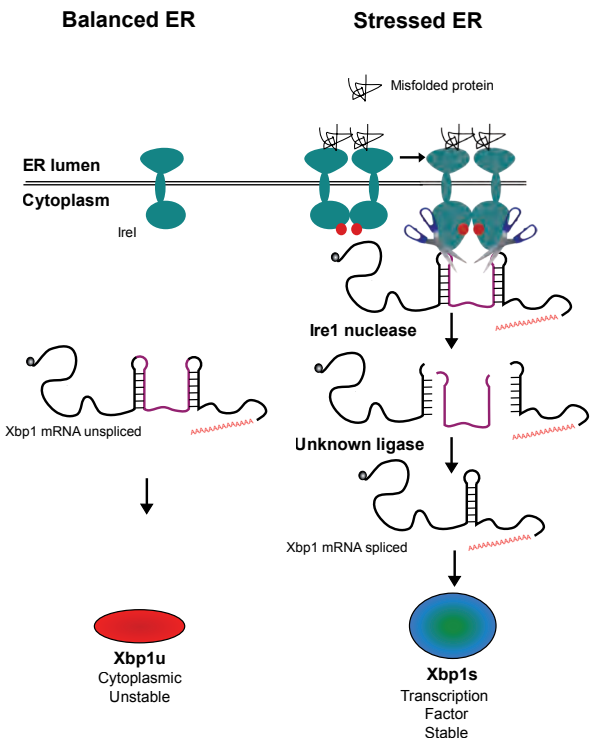
HSPC117: essential subunit

FAM98B: function unknown

CGI-99: ninein-interacting protein. Function unknown

ASW: function unknown

Figure 4



GROUP LEADER:
JAVIER MARTINEZ

POSTDOCS: JENNIFER JURKIN, ANNE NIELSEN, STEFAN WEITZER
PHD-STUDENTS: SABRINA BANDINI, JOHANNES POPOW, ARIELA REISS
RESEARCH ASSISTANT: JUTTA DAMMANN
RESEARCH TECHNICIAN: BARBARA MAIR

Figure 1: The animal tRNA splicing pathway. In humans, pre-tRNA introns are removed by the tetrameric endonuclease TSEN, composed of Sen2, Sen15, Sen34 and Sen54. Exon halves display a 2',3'-cyclic phosphate at the end of the 5' exon and a 5'-OH at the 5' end of the 3' exon. A tRNA ligase joins exon halves by direct ligation leading to a mature tRNA, with the phosphate group at the phosphodiester bond originating from the 2', 3'-cyclic phosphate.

Figure 2: HSPC117 is the essential subunit of the human tRNA ligase complex. Affinity-purification of c-myc-HSPC117 from stably transfected HeLa cell lines yields an immunoprecipitate (IP) able to ligate tRNA exon halves. IP of wild type (WT) or C122A mutant c-myc-HSPC117 were incubated with body-labeled tRNA exon halves. Mature tRNA is generated exclusively by IPs of WT c-myc-HSPC117. An IP prepared from a non-expressing clone was used as a negative control. The asterisk denotes an unrelated band.

Figure 3: The human tRNA ligase complex. SDS-PAGE analysis of Flag-tagged HSPC117 purified from HEK293 cells (left, asterisks indicate contaminant bands). Annotated functions of identified subunits constituting the tRNA ligase complex (right).

Figure 4: The "RNA side" of the unfolded protein response. The cartoon depicts events that take place upon endoplasmic reticulum stress. The endonuclease Ire1 cleaves Xbp1-mRNA with similar chemistry as the tRNA endonuclease cleaves pre-tRNAs, i.e. generating 2',3'-cyclic phosphate and 5' OH. An unknown ligase, which we are trying to identify, joins the two exons leading to a frameshift that results in a longer version of the Xbp1 protein that travels to the nucleus and functions as a transcription factor. Xbp1u, unspliced; Xbp1s, spliced.

KAZUFUMI MOCHIZUKI GROUP

Small RNA-directed programmed DNA elimination in *Tetrahymena*

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

The onion's genome is 12 times larger than the human one. Does this mean that onions are more complicated and more intelligent than we are? Perhaps yes, but it presumably means onions possess more "junk" DNA than do humans. If some DNA segments are useless, why do onions not dispense with them? Raisons d'être for junk DNA are still controversially discussed. Several creatures actually do discard such DNA during their development. In order to determine how our cells regulate junk DNA and how junk DNA regulates our genomes, we are studying a programmed DNA elimination event of Tetrahymena, a classic model eukaryote.

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

Junk DNA contains numerous transposable elements that are able to move from one genome position to another, and are therefore potentially harmful to the integrity of genomes. On the other hand, many junk DNAs are not really junk but perform important functions by way of proper chromosome segregation, recombination, and gene expression. Several organisms dispense with junk DNAs during the development of their somatic lineage. This developmentally programmed DNA elimination presumably reflects two aspects of junk DNA: its harmfulness by the action of transposons, and its usefulness in maintaining genome integrity in the germline lineage. 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 the new Mac and the new Mic, and the parental Mac is destroyed. During the development of the new Mac, ~6000 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 (Figure 1). 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 and are essential for DNA elimination. A mechanism related to RNA interference (RNAi) is also essential for DNA elimination (Figure 2). Small (~28-29 nt) RNAs 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, the formation of heterochromatin occurs downstream of the RNAi-related mechanism in the DNA elimination pathway (Figure 2). As recent studies in animals and plants have shown that transposable elements are silenced by a heterochromatin and/or RNAi-related mechanism, transposon silencing by RNAi-directed formation of heterochromatin has probably arisen in an ancestral eukaryote. Therefore, further study of the programmed DNA elimination process in *Tetrahymena* should yield basic and important data about transposon silencing by RNAi-directed formation of heterochromatin in eukaryotes.

Transnuclear comparison of whole genomes identifies transposons

Chemically, both transposable elements and the other parts of the genome are merely stretches of DNA. How is a cell able to distinguish junk from precious DNA and induce heterochromatin only on junks? In addition, how is a new transposon, which cells have never experienced, identified? *Tetrahymena* resolves these problems by transnuclear comparison of whole genomes. The idea is simple. *Tetrahymena* has a germline Mic which contains complete genome including transposons, and a somatic Mac in which transposons are removed during the last sexual reproduction. The cell is thus able to identify transposons as sequences that are in Mic but not in Mac. *Tetrahymena* utilizes scnRNAs for this transnuclear whole genome comparison (Figure 3; please see the figure legend for details). This system can perfectly sweep away not only the existing transposons but also any newly invaded transposons from the transcriptionally active Mac. The exact molecular mechanism that mediates transnuclear whole genome comparison by small RNAs is still unclear. We previously reported that nascent non-coding transcripts mediate the interaction between chromatin and scnRNA–Twi1p complexes in the parental Mac, and that this interaction is dependent on the putative RNA helicase Ema1p. Moreover, EMA1-knockout strains show defects in the selective degradation of scnRNAs (Figue 3d) and in DNA elimination. We therefore proposed that scnRNA degradation is induced by a base-pairing interaction between scnRNAs and nascent non-coding transcripts from the parental Mac, and this selective scnRNA degradation is a basis of transnuclear whole genome comparison. We are further investigating this process in order to fully understand how RNA is able to epigenetically regulate the behavior of genomes.

Publication highlights:

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.

Mochizuki, K., Fine, N. A., Fujisawa, T., and Gorovsky, M. A. (2002) Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in Tetrahymena. Cell 110, 689-699.

Figure 1

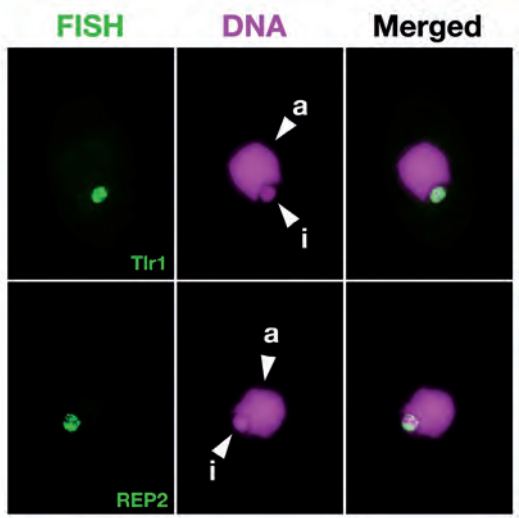
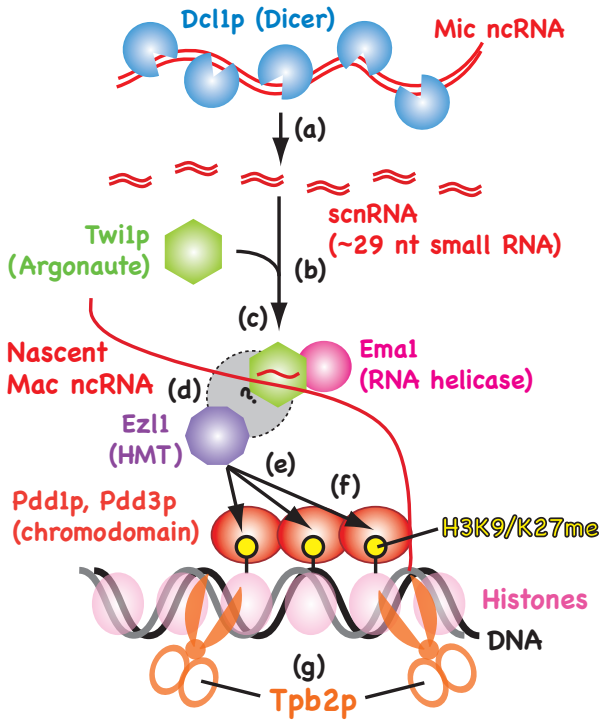
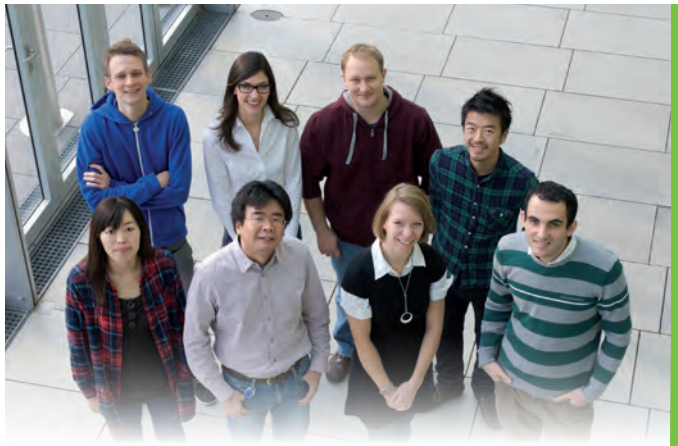


Figure 2





GROUP LEADER:
KAZUFUMI MOCHIZUIKI

POSTDOCS: KENSUKE KATAOKA, HENRIETTE KURTH
PHD STUDENTS: URSULA SCHÖBERL, ALEXANDER VOGT, SOPHIE SOYKA
RESEARCH ASSOCIATE: TOMOKO NOTO
TECHNICAL ASSISTANTS: CLARA JANA LUI BUSCH

Figure 3

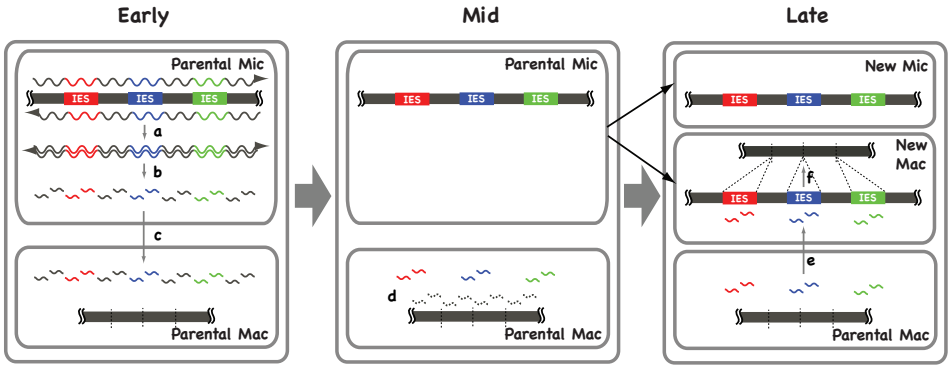


Figure 1: Transposons are eliminated from the macronucleus. *Tetrahymena* has two different nuclei (stained purple): a small micronucleus (i) and a larger macronucleus (a). Two types of transposable elements, *Tlr1* (top) and *REP2* (bottom), are found only in the micronucleus by fluorescent in situ hybridization (FISH, green).

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: A model for small RNA-directed DNA rearrangement in Tetrahymena. 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 the mid stages, scnRNAs complementary to the parental Mac genome are degraded (d). In the late stages, the scnRNAs remained are transferred to the developing new Mac (e) and target IESs to be eliminated (f).

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 have proven to be powerful tools in elucidating the essential functions of genes in normal physiology and the pathogenesis of disease. Using gene-targeted mice, my group attempts to model human disease and genetically dissect disease mechanisms.

The stress kinase MKK7 functions as a tumor suppressor in breast and lung cancer via p53 stabilization

Most pre-neoplastic lesions in our body do not progress into overt tumors. Oncogenic stress is sensed by the DNA damage response (DDR) machinery, which activates p53 and triggers cell cycle arrest or apoptosis of incipient tumor cells. Thus, it has been proposed that the cellular response to DNA damage and p53 induction constitute a vital anti-cancer barrier. However, the molecular pathways by which cells sense a premalignant state *in vivo* are largely unknown. We tested this concept using the inducible mouse model of lung cancer, a NeuT-driven breast cancer model, and mice that carry a tissue-specific mutation in MKK7, which is a specific and essential upstream regulator of the JNK signaling cascade. Activating mutations in *Kras* (10–30%) and loss-of-function point mutations in *p53* (50–70%) are frequently found in human non-small-cell lung cancer, whereas 30% of human breast tumors overexpress Her2 (Neu) and about 40% harbor p53 mutations.

Intriguingly, rather than acting as an oncogenic pathway, our results show that the stress signaling kinase MKK7 couples oncogenic stress to tumor initiation. Tissue-specific inactivation of MKK7 in $KRas^{G12D}$-driven lung carcinomas (Figure 1a) as well as in NeuT-driven mammary tumors (Figure 1b) markedly accelerated tumor onset and reduced overall survival. Mechanistically, MKK7 regulates p53 stability in response to oncogenic stress *in vivo* and genotoxic stress *in vitro*. Importantly, p53 overexpression rescued the premature onset of lung cancer, whereas loss of p53 did not further accelerate the growth of MKK7-deficient, $KRas^{G12D}$-driven lung carcinomas. These results identify the stress signaling kinase MKK7 as a novel component that senses oncogenic stress, linking the DNA damage response and p53 stability. These surprising *in vivo* data show that MKK7 functions as a tumor suppressor for breast and lung cancers downstream of oncogenes frequently mutated in humans. Our data might also reveal a molecular mechanism by which common genotoxic therapies such as doxorubicin or γ-irradiation, which are used in everyday clinical practice, function as anti-cancer therapies. They also provide support for a re-evaluation of JNK inhibitors as therapeutic strategies for inflammatory and fibrotic diseases, as inhibition of the MKK7-JNK pathway *in vivo* might lead to deregulation of the key tumor suppressor p53. The results were published in Schramek et al., Nature Genetics 2011.

Murine haploid embryonic stem cells as a novel tool for functional genomics

Some organisms such as yeast or social insects are haploid, i.e. they carry a single set of chromosomes. Haploidy in yeast has been utilized to identify fundamental mechanisms of biology. However, all somatic mammalian cells carry two copies of chromosomes (diploidy) which obscures mutational screens, whereas organisms with a single copy of their genome such as yeast provide a basis for genetic analysis. In the latter, any recessive mutation of essential genes will show a clear phenotype because of the absence of a second gene copy. Haploidy has been achieved in fish embryonic stem cells, human KBM-7 leukemia cells, or by electrofusion, to generate hybrid cells. However, since haploidy is incompatible with mammalian development with the exception of KBM-7-derived cells, no somatic haploid cell has ever been described in mammals. We now report the generation of fully haploid mouse embryonic stem (ES) cells.

Our data show that it is possible to generate mammalian haploid ES cell lines from parthenogenetic mouse blastocysts derived from activated oocytes. Detailed molecular characterization of our haploid ES cells shows that these clones express all classical markers of ES cells, carry exactly 20 chromosomes, and maintain genome integrity (Figure 2). Functionally our haploid ES cells can differentiate into all three germ layers and are capable of *in vitro* differentiation into neurons and "beating" myocytes. These haploid ES cells also contribute to chimerism in adult mice and, using teratoma assays, can differentiate into multiple cell types and tissues *in vivo*, such as muscle, neurons, chondrocytes, adipocytes, sweat glands, or ciliated and pigmented epithelium. We also performed single ES cell cloning experiments, GFP tracing, and - in cooperation with Bill Stanford in Ottawa - developed algorithms to visualize haploid versus diploid ES cells in order to directly demonstrate that haploid ES cells can initiate differentiation.

To prove the power of our approach, we developed a highly efficient mutagenesis system in cooperation with H. von Melchner and F. Schnuetgen (Cologne). Our experiments based on millions of different integrations generated in a single round of retroviral infection of haploid ES cells show that it will be indeed possible to perform large-scale genetic recessive screens. Reverse genetics is feasible using a vector system that provides immediate confirmation of gene function in the same clones. Using this system we have been able to functionally validate our approach employing clones with conditional Retinoic acid Receptor gamma and Drosha mutations. Moreover, the system was designed to "repair" mutations for immediate functional validation of target genes. No *drosha* knock-out cells have been reported in the past. Our system enabled us to generate the first mammalian knock-out cell line for Drosha, which should be of relevance for microRNA biology. We also performed a forward genetic screen for ricin, one of the most dangerous toxins, being used/investigated as a biological weapon by governments or terrorist organizations. This screen identified the GPCR Gpr107 as an essential molecule required for ricin-induced killing (Figure 3). No antitoxins are available for treatment of ricin poisoning. Thus, our screens do not only work but have already identified novel and medically relevant targets.

Our data create the option to combine the power of a haploid genome with the pluripotency of embryonic stem cells to uncover fundamental developmental and biological processes in specific cell types at the genomic level. The study also provides the experimental framework for a system that is not only of great interest to basic biologists in many fields, but will potentially revolutionize functional genomics in stem cells and differentiated mammalian cells. The results were published in Elling et al., Cell Stem Cell 2011.

Figure 1

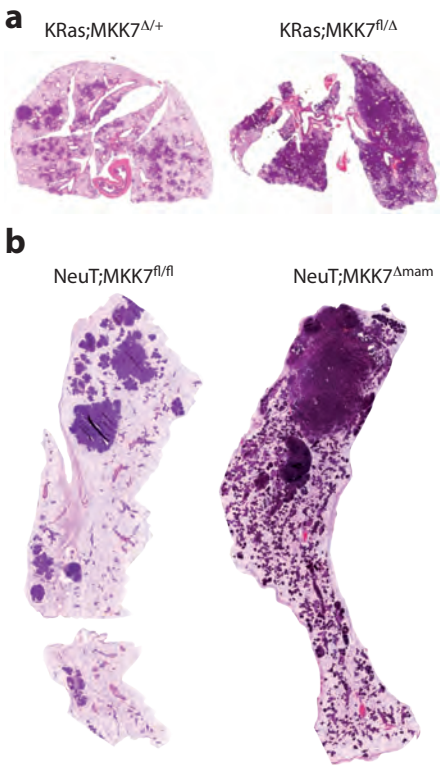
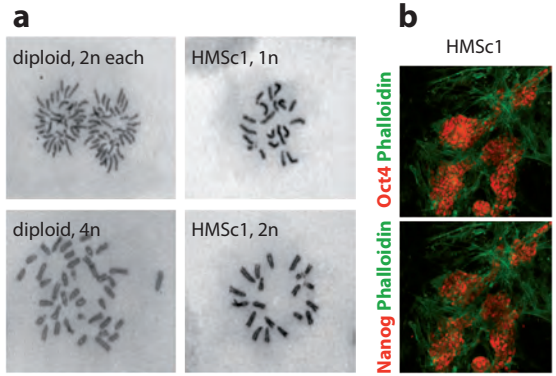


Figure 2



SENIOR SCIENTIST & MANAGING DIRECTOR SCIENCE:
JOSEF PENNINGER

POSTDOCS: SHANE CRONIN, ULRICH ELLING, TOSHIKATSU HANADA, REIKO HANADA, BERNHARD JOHANNES HAUBNER, REZAUL KARIM, VANJA NAGY, ROBERTO NITSCH, THOMAS PERLOT, SHUAN RAO, JEAN TRICHEREAU, GERALD WIRNSBERGER
PHD STUDENTS: MICHAEL ORTHOFER, MAGDALENA PAOLINO, BLANKA PRANJIC, VERENA SIGL
RESEARCH ASSISTANT: RUBINA KOGLGRUBER
RESEARCH ASSOSIATE: IVONA KOZIERADZKI, RENU SARAO

Figure 3

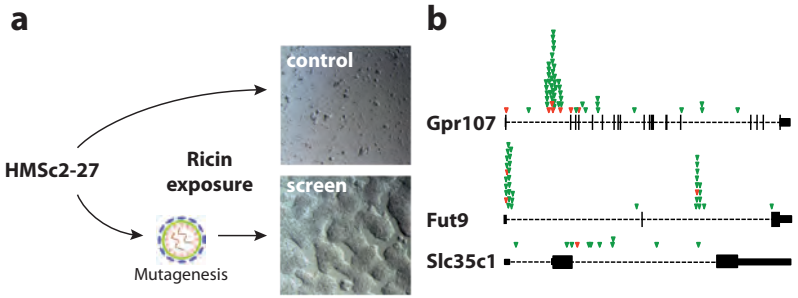


Figure 1: Suppression of lung and breast cancer by the stress kinase MKK7. (**a**) Histology of whole lungs from KRas;MKK7$^{fl/\Delta}$ and KRas;MKK7$^{\Delta/+}$ littermate control mice 9 weeks post-AdenoCre-infection. Whereas the KRas;MKK7$^{\Delta/+}$ control lung shows hyperplasia and adenomas but also a substantial fraction of normal lung tissue, the KRas;MKK7$^{fl/\Delta}$ lung is already almost completely obstructed with adenocarcinomas. (**b**) Representative histology of mammary cancers that developed in 180 day-old control NeuT;MKK7$^{fl/fl}$ (n=21) and knock-out NeuT;MKK7$^{\Delta mam}$ littermate females. H&E stained sections are shown.

Figure 2: Generation of haploid murine ES cell lines
(**a**) Representative chromosome spreads of control diploid ES cells and haploid HMSc1 cells. Spreads from anaphase (1n) and prophase (2n) of mitosis are shown for haploid cells. As a control, anaphase (2n) and prophase (4n) spreads are shown for diploid ES cells. (**b**) Expression of Oct4 and Nanog, prototypical markers for murine embryonic stem cells. Phalloidin staining (green) indicates the feeder cell layer.

Figure 3: Forward genetic screen for ricin toxicity in haploid ES cells (**a**) Haploid HMSc2-27 with/without gene-trap mutagenesis were exposed to ricin from Ricinus communis for 3 weeks. Colonies only appeared in the mutagenized batch and were processed for deep sequencing. (**b**) Top hits identified in the ricin toxicity screen. Sense (green) and antisense (red) insertions in Gpr107, Fut9, and Slc35c1 genomic loci. The vertical lines indicate the respective exons for each gene. Insertions in antisense might disrupt gene function; sense integrations will do so in almost all cases.

LEONIE RINGROSE GROUP

Epigenetic Regulation by Polycomb and Trithorax Group Proteins

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

A single stem cell, with a single genomic DNA sequence, can give rise to an extraordinary diversity of cell identities and functions. 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. We aim to understand how this dynamic system can ensure both flexibility and stability of cell identities.

DNA: Polycomb/Trithorax Response elements in flies and mammals

The PcG and TrxG proteins regulate several hundred developmentally important genes in flies and mammals. These proteins act through Polycomb/Trithorax response elements (PRE/TREs) (Figure 1). 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. This year we have further investigated the role of individual motifs that were predicted computationally. Our findings point towards a structural role both at the DNA and RNA levels, of a previously uncharacterised PRE/TRE motif (Okulski et al., 2011). In addition, to examine the role of PRE/TREs in a dynamically developing tissue, we have studied the *eyes absent* (*eya*) gene in the developing *Drosophila* eye. We show that the *eya* PRE/TRE is essential for converting the output of the *eya* enhancer into spatially different expression profiles of the two alternative promoters across the eye disc. Replacement of the *eya* PRE/TRE with other PRE/TREs reveals that different PRE/TREs have profoundly different properties in this assay. This work provides unexpected insights into the rich diversity of different PRE/TRE properties, and defines a novel function of PRE/TREs in fine-tuning enhancer output during differentiation. Elucidating the molecular mechanisms by which PRE/TRE sequence, DNA and RNA structure contribute to gene activation, silencing and switching will be important tasks for the future.

In contrast to fly PRE/TREs, the corresponding mammalian elements have so far proved highly elusive. During the past year we have use experimental and bioinformatic analysis to identify mammalian PRE elements, and to extract sequence principles of mammalian PRE/TREs (Figure 2). Our data reveal unexpected similarities between fly and vertebrate elements at the DNA sequence level. In future we will continue to combine experimental and computational analyses to tackle the question of what makes a mammalian PRE/TRE.

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, and suggests an essential role for these RNAs in PRE/TRE regulation during development and differentiation. In mouse, we have shown by profiling of purified cell populations from different stages of a defined *in vitro* neural differentiation system that over 50% of regulated intergenic noncoding transcripts precisely correspond to PcG target sites. We designate these PcG recruiting elements as Transcribed Intergenic Polycomb (TIP) sites. Reporter assays show that transcribed TIP sites can repress a flanking gene. Knockdown experiments demonstrate that TIP noncoding RNAs are themselves required for repression of target genes both in *cis* and in *trans*. We propose that TIP transcription may ensure coordinated regulation of gene networks via dynamic switching and recruitment of PcG proteins both in *cis* and in *trans* during lineage commitment. Our future work in fly and mouse will address the molecular mechanisms by which selected transcripts recruit PcG proteins in specific cell types and at specific developmental stages.

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. Mathematical modeling examines which parameters best distinguish stem cells from differentiated cells. We identify phosphorylation of histone H3 at serine 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. This year we have extended our analysis to several other proteins including the TrxG protein Ash1, which shows a remarkably robust attachment to chromatin throughout mitosis (Figure 3). Future work on Ash1 aims to elucidate the molecular basis of this mitotic attachment and its role in propagating memory of cell identity through mitosis.

Publication highlights:

Okulski, H., Druck, B. Bhalerao, S., and Ringrose, L. (2011). Quantitative Analysis of PRE/TRE elements at identical genomic locations distinguishes contributions of DNA sequence and genomic environment. Epigenetics and Chromatin, 4:4.

Strübbe, G., Popp, C., Schmidt, A., Pauli, A., Ringrose, L., Beisel, C., and Paro, R. (2011). Polycomb purification by in vivo biotinylation tagging reveals cohesin and Trithorax group proteins as interaction partners. Proc Natl Acad Sci U S A. 108:5572-7.

Ringrose, L. (2010). How do RNA sequence, DNA sequence, and chromatin properties regulate splicing? F1000 Biol Rep. 12;2:74.

Figure 1

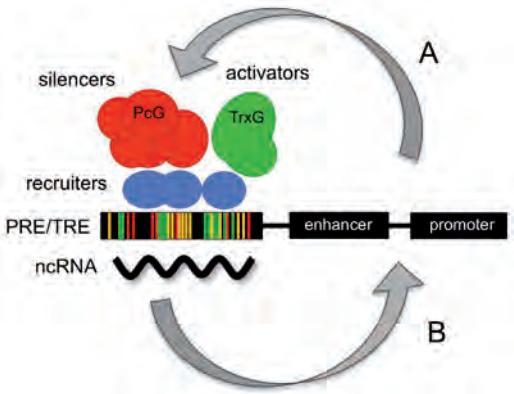


Figure 2

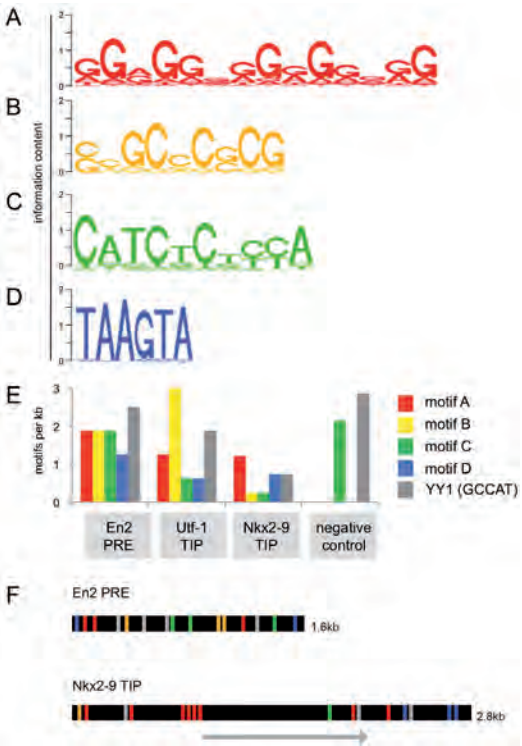


Figure 3

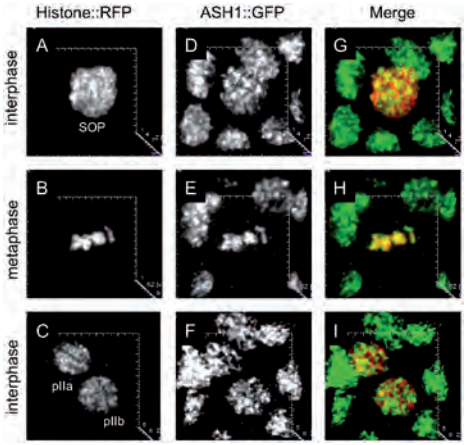


Figure 1: Polycomb/Trithorax response elements (PRE/TREs). PRE/TREs are cis- regulatory elements that can recruit both the silencing Polycomb group (PcG) proteins (red), and activating Trithorax group (TrxG) proteins (green), via a platform of sequence specific DNA binding proteins (blue). Fly PRE/TRE 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: Motifs enriched in mammalian TIPs and PRE/TREs. (A-D) Motif logos for four motifs found by motif discovery in murine Transcribed Intergenic Polycomb binding sites (TIP sites). (E) occurrence per kb of motifs A-D and the YY1 motif (GCCAT) in selected PRE/TREs identified by experimental analysis, and in two representative TIP sites. Motifs were detected using regular expressions. Negative control in (E) is an equivalent length of sequence taken from randomly chosen intergenic sites. (F) motif distributions of motifs A-D and YY1 (colour code as in E) in the En2 PRE/TRE and the Nkx2-9 TIP site. Grey arrow shows TIP noncoding RNA transcript.

Figure 3: The TrxG protein ASH1 binds to mitotic chromatin. A GFP fusion to the *Drosophila* TrxG protein ASH1 was imaged in living pupae in the sensory organ precursor (SOP) lineage, in which the SOP cell (A) undergoes mitotic division (B), giving rise to two daughter cells with different identities, pIIa and pIIb (C). (A-C) Chromatin was marked with Histone H2B:RFP specifically in the SOP lineage. (D-F) ASH1::GFP was expressed in all cells. (G-H) merge of Histone::RFP and ASH1::GFP, showing robust localisation of ASH1::GFP to mitotic chromatin (B, E, H).

GROUP LEADER:
LEONIE RINGROSE

POSTDOCS: ROBERT HEINEN, BETÜL HEKIMOGLU-BALKAN [1]
PHD STUDENTS: JOAO FONSECA, VERONIKA HERZOG, HELENA OKULSKI, FRANK RUGE, PHILIPP STEFFEN, JOHANNA TRUPKE
TECHNICAL ASSISTANTS: CHRISTINA ALTMUTTER, CHRISTINE EHRHARDT, EVA DWORSCHAK

[1] POST DOC UNTIL JUNE

VIC SMALL GROUP

The actin nanomachinery of migrating cells

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. Our studies focus on unraveling the structural basis of cell movement.

Cells move by recycling the protein polymers that make up the so-called cytoskeleton, primarily the actin filaments and the microtubules. The turnover of actin filaments, together with associated proteins provides the forces required for movement, whereas microtubules serve a role in polarity determination and guidance. We currently focus on elucidating the structural reorganizations of actin filaments required for cells to move. Actin filaments are used in two ways to create movement. As in muscle cells, actin filaments in non-muscle cells can slide over myosin filaments to produce contraction and this property is exploited during the later stages of cell migration, when the cell body catches up with the advancing front. To initiate movement in the first place a cell must protrude in the direction it wants to go, which it does by extending thin sheets of cytoplasm called lamellipodia. This protrusion is achieved by exploiting the other ability of actin filaments to push, by polymerization. Our current studies focus on determining the structural basis of this protrusive activity. Towards this aim we have developed procedures to correlate the movement of living cells in the light microscope with the structure of the same cells in the electron microscope. An important advance in this approach has been the introduction of electron tomography that allows visualization of cellular architecture at nanometer resolution (Figure 1).

A current debate in the field centers on the question of how actin networks in lamellipodia are established and maintained. To study lamellipodia initiation, we have exploited an experimental model in which a microneedle is used to produce a hole in the cytoplasm that is then repaired by lamellipodia. By capturing the earliest stages of repair we have shown by electron tomography that lamellipodia initiation involves the branching of actin filaments from the sides of filaments aligned parallel to the periphery of the membrane bordering the hole (Figure 2). We have also provided the first 3D maps of actin filament organization in established lamellipodia, which show that the actin networks are constructed from actin filaments of variable length, linked into subsets by branch junctions (Figure 3). The heptameric actin related protein complex, the "Arp2/3 complex" promotes actin branching *in vitro* and the structure of the *in vitro* branch junction has been determined by electron microscopy. From image analysis in collaboration with Akihiro Narita in Nagoya we have recently obtained the first model of branch junctions in lamellipodia *in vivo*, at 3.5nm resolution. Our data reveal a close structural homology of *in vivo* branches with those formed *in vitro* from actin and the Arp2/3 complex. In ongoing studies we are probing the dependence of lamellipodia protrusion on Arp2/3 complex activity and the roles of other actin regulators in the reorganization and turnover of actin networks necessary for movement and are developing mathematical models of protrusion.

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

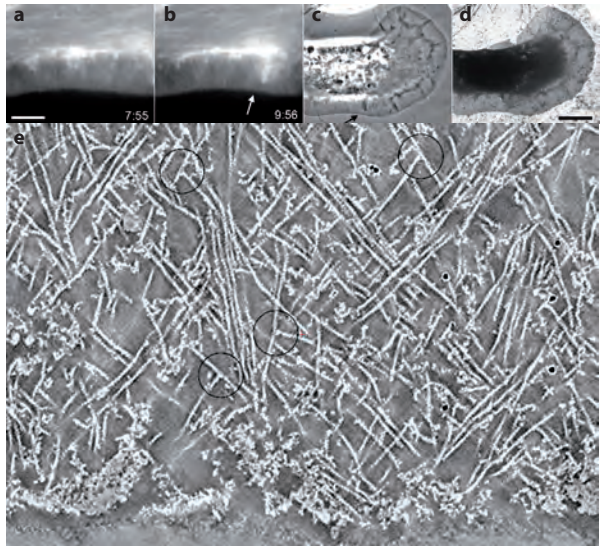


Figure 2

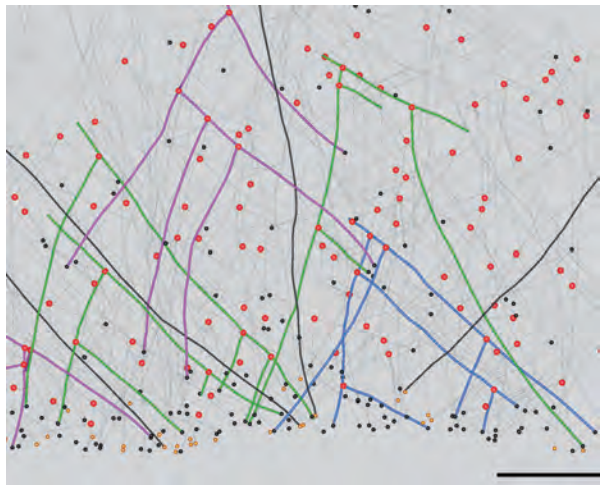


Figure 3

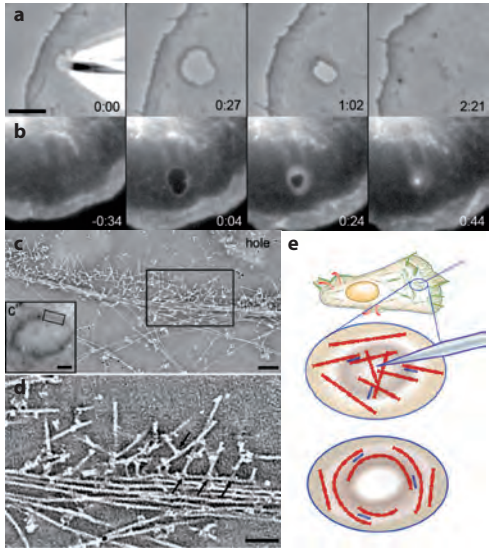


Figure 1: Structural organization of established lamellipodia. a,b Fluorescence microscope images of lamellipodium in a living NIH3T3 cell expressing lifeact-GFP to visualize actin. The cell was fixed immediately after video frame in b, corresponding to phase contrast image of cell overview in c. d, overview image of fixed and negatively stained cell in EM. e, one section (0.75nm) of electron tomogram taken from region indicated by arrows in b, c. Black circles highlight two branch junctions in the tomogram section. Bars: a, 5µm; d, 10µm; e, 100nm.

Figure 2: Established lamellipopdia are constructed from filament subsets linked by branch junctions. Figure shows projection of a 3D model of filament trajectories (grey and colored lines) in the anterior 1µm of a protruding lamellipodium, derived from an electron tomogram (like Fig.2). Selected filament subsets are highlighted in different colors. Red spots indicate branch junctions; yellow and black spots mark filament ends. The cell front is at the bottom. Bar, 200nm.

Figure 3: Initiation of lamellipodia formation. a, video sequence in phase contrast showing lesion produced by a microneedle in a B16 melanoma cell and the subsequent repair of the induced hole. b, video sequence of hole repair in a Lifeact-GFP transfected B16 melanoma cell showing the generation in the hole of an actin rich lamellipodium like that on the cell periphery. c, negative stain electron tomogram section of the edge of a hole fixed a few seconds after induction and corresponding to region boxed in the inset (c'). d, boxed region from c: arrows indicate side branches of actin filaments from filaments parallel to the edge of the hole. Bars: a,b, 5µm ; c, 100nm; c', 1µm.

SENIOR SCIENTIST:
VIC SMALL

STAFF SCIENTIST: MARIA NEMETHOVA
POSTDOC: EDIT URBAN
DIPLOMA STUDENTS: JAN MÜLLER , FLORIAN SCHUR
TECHNICAL ASSISTANTS: JAN MÜLLER, MARLENE VINZENZ

Figure 1

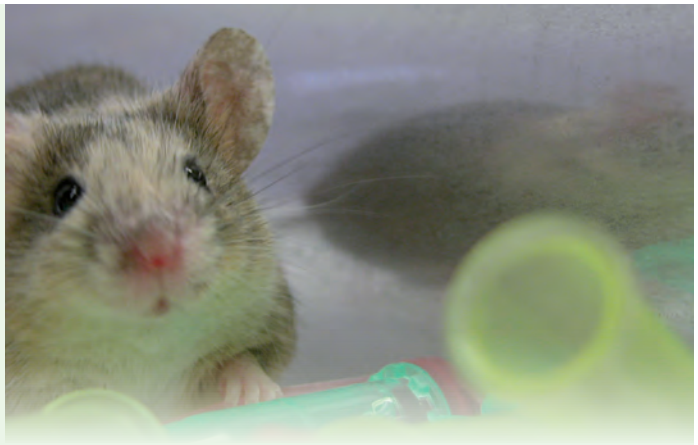


Figure 1: *www.austromouse.at*
Figure 2: Human iPS cells generated from adult dermal fibroblasts express the embryonic cell surface marker Tra-1-81

STEM CELL CENTER - MOUSE GENE TARGETING

austromouse@imp.ac.at

The main objective of the Stem Cell Center – Gene Targeting Unit is to provide state-of-the-art technologies for manipulation of the mouse genome. Ideal human disease models can be engineered by combining homologous recombination, site-specific recombination, and transgenesis using mouse ES cells. Our most recent addition is the establishment of mouse and human-induced pluripotent stem cell (iPSC) technology and efficient differentiation protocols for tissue regeneration.

ES cells and gene targeting

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 and try to generate new transgenic recombinase mouse models.

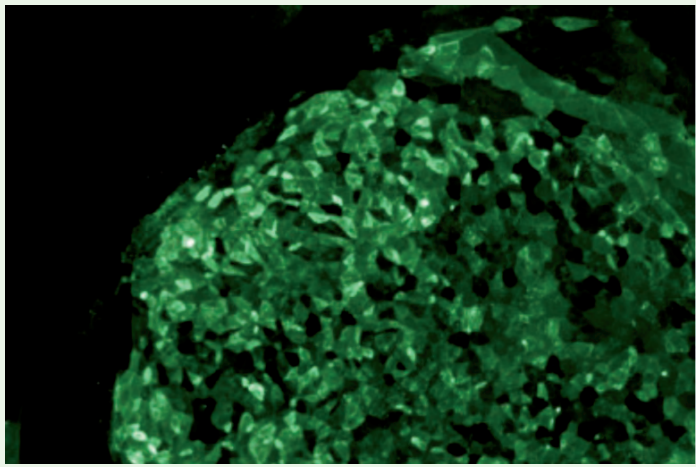
Gene targeting tool box

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.

Generation and genetic repair of induced pluripotent stem cells

iPSCs have the potential to revolutionize future medicine and could be used for patient-specific tissue repair. Genetic mouse models that mimic certain aspects of a disease allow us to test various 'repair' strategies, as well as how and under what conditions such repaired iPSCs could be used in a clinical setting. However, homologous recombination, a straightforward approach in mouse iPSCs, is very limited in human iPSCs. We urgently require alternative strategies for gene repair. Recently a new technology for modifying the human genome has emerged using transcription activator-like effector nucleases (TALENs). Based on a remarkably simple and programmable DNA-binding code, TALENs with new specificities can be easily designed and readily engineered to bind to virtually any DNA sequence. In order to extrapolate this technology to the clinical setting, we have started to establish iPSCs employing synthetic mRNAs to generate virus-free iPSCs. We are also generating *in vitro* differentiated repaired progenitor cells to test their therapeutic potential.

Figure 2



HEAD OF FACILITY:
PETER DUCHEK

TECHNICAL ASSISTANTS:
SARA FARINA LOPEZ, IVICA SOWEMIMO, VICTORIA STEINMANN[1], TAMARA THEIL[2], CLAUDIA VALENTA

[1]SINCE JULY
[2]UNTIL JUNE

FLY HOUSE

Peter Duchek

peter.duchek@imba.oeaw.ac.at

The Fly House provides research support for a growing number of scientists at IMBA working with the fruit fly 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 cornerstones of 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 subsequently 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 recently developed. Given the need to confirm RNAi knock-down phenotypes with classical loss-of-function alleles or to tag genes at the endogenous locus, we have set up a *Drosophila* gene targeting service at the institute. Currently we use an ends-out strategy of homologous recombination to create defined deletions or add tag sequences to 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 and those performing subsequent follow-up experiments to validate their hits.

Fly stock maintenance and plasmid collection

In addition to the care of 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 work towards improving these tools.

HEAD OF BIOOPTICS: KARIN AUMAYR

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

BIOOPTICS

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 in planning experiments and implementing 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 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

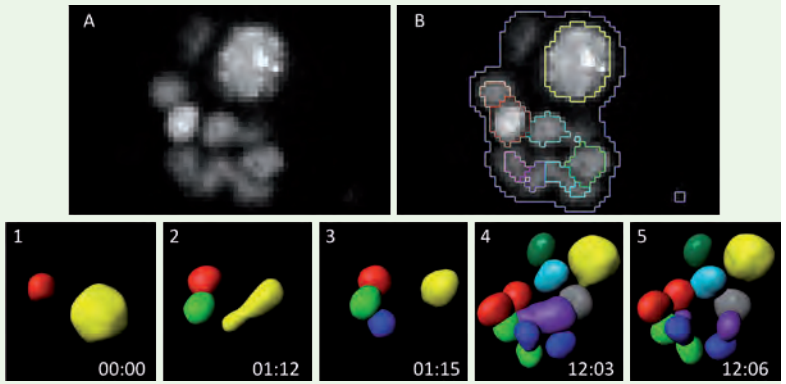
The BioOptics Facility currently manages more than twenty microcopy systems, including wide-field microscopy, confocal laser scanning microscopy (CLSM), two-photon (2P) microscopy, total internal reflection (TIRF) microscopy techniques, and automated slide scanners for samples with or without fluorescence. 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 intensive Advanced Practical Microscopy Course is organized on a yearly basis, including hands-on sessions and lectures by internal and external faculty members.

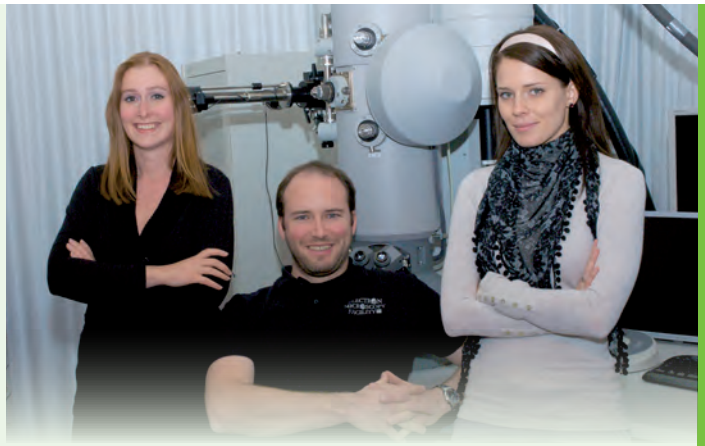
Image Processing and Analysis

Five state-of-the-art computer workstations are available at the BioOptics Facility, operating most types of common commercially available 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. Organized courses are also provided. 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: Using Fluorescence activated cell sorting (FACS) Drosophila neural stem cells, specifically labeled with a nuclear GFP, were isolated from larval brain tissue and subsequently, live-cell imaging has been performed on a spinning disk confocal to monitor cell-cycle time and cell growth of these neuroblasts. Cells were segmented in 3D and the neuroblast and its offspring were linked over time. Panel A shows a single slice of an image stack, panel B the respective segmentation and classification. The stills 1 to 5 show different surface rendered time points of the movie; the neuroblast is highlighted in yellow and its offspring is labeled by different colors. Daughter cells that itself underwent a round of division show the same color. The time is indicated in hours and minutes.





HEAD OF ELECTRON MICROSCOPY FACILITY: GUENTER RESCH

TECHNICAL ASSISTANTS: MARLENE BRANDSTETTER, NICOLE FELLNER
TRAINEE: KARIN TRIMMEL

ELECTRON MICROSCOPY

guenter.resch@imba.oeaw.ac.at

The Electron Microscopy Facility provides a wide range of preparation techniques for tissues, cells, and purified molecules for transmission electron microscopy, as well as facilities for microscopy, data management and image processing. Access to scanning electron microscopy is provided via external collaboration.

Preparation of Specimens

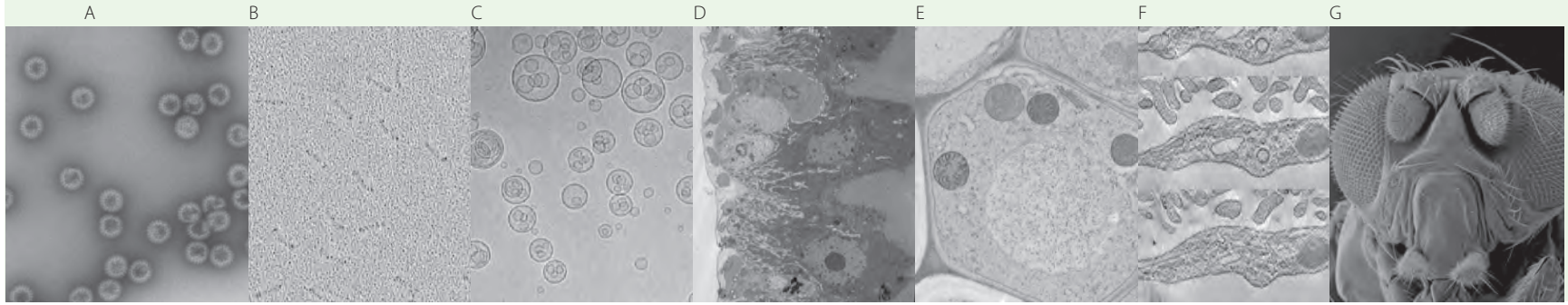
Know-how, training and instrumentation for a wide variety of preparation techniques for visualization of ultrastructure in tissues or cells and of biomolecules by transmission electron microscopy (TEM) are being offered by the Electron Microscopy Facility to researchers at IMBA, IMP and GMI. Techniques routinely used at the facility include the production of support films, negative staining, rotary shadowing of sprayed molecules, chemical fixation, immersion freezing, high pressure freezing, freeze substitution, embedding in epoxy- and acrylic resins, and ultrathin sectioning of resin-embedded or frozen samples.

Microscopy

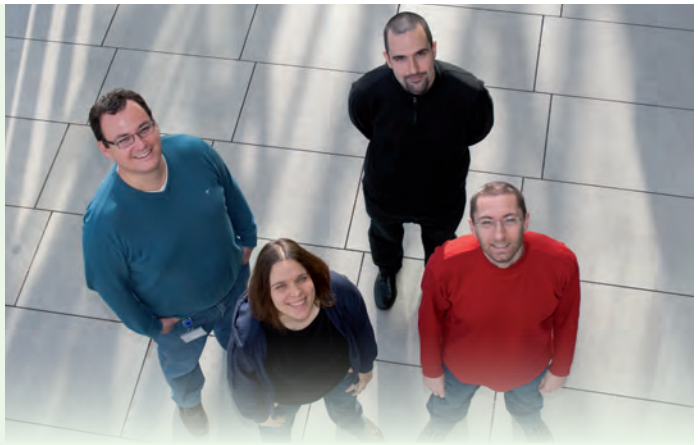
The Facility is equipped with two instruments: The FEI Morgagni is a robust and easy-to-use 100 kV TEM, equipped with an 11-megapixel CCD camera. It is tailored to meet routine requirements at the Facility's multiuser environment. Advanced applications run on the FEI TF30 Polara. This 300 kV TEM, unique in Austria and equipped with the most advanced imaging systems, was funded by a Vienna Spot of Excellence grant and became fully operational at the beginning of 2008. It is primarily used for cryo-TEM of molecules and cells, electron tomography, and electron energy loss spectroscopy.

Software Development and Image Processing

To support users with data management, a Web-based project-oriented database system named MIMAS was developed by, and is being run at, the Facility. Electron micrographs from both microscopes, including meta data, can be stored on and accessed from this database on a user-restricted basis. Furthermore, software solutions for automated image acquisition and for status monitoring of the microscope were developed on the Polara. Workstations and training are provided for image processing of EM data, especially those obtained by electron tomography.



A: Negatively stained rotavirus-like particles (Cornelia Gänger, Ringrose Group)
B: Glycerol sprayed and rotary shadowed α-actinin molecules.
C: DPPC liposomes visualized by cryo electron microscopy.
D: Drosophila melanogaster intestine also showing stem cells from a chemically fixed specimen (sample: Ryan Conder, Knoblich Group).
E: Cells from high pressure frozen Arabidopsis thaliana root (sample: Matzke Group, GMI).
F: z-Sections from a three-dimensional reconstruction by electron tomography showing the endothelial lining in a blood vessel.
G: Scanning electron micrograph of Drosophila melanogaster.



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. We engage in custom software and database development, and design computational and mathematical solutions that can cope with higher load and memory requirements. To perform complex sequence analysis tasks, we also 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, Flymine) 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, the main computing cluster has been updated to a state-of-the-art processing system using batch and parallel computing environments. The cluster is managed by the Sun Gridengine (SGE) software, which provides policy-based workload management for a large number of jobs and nodes. Software installed and maintained on the bioinformatics cluster 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.

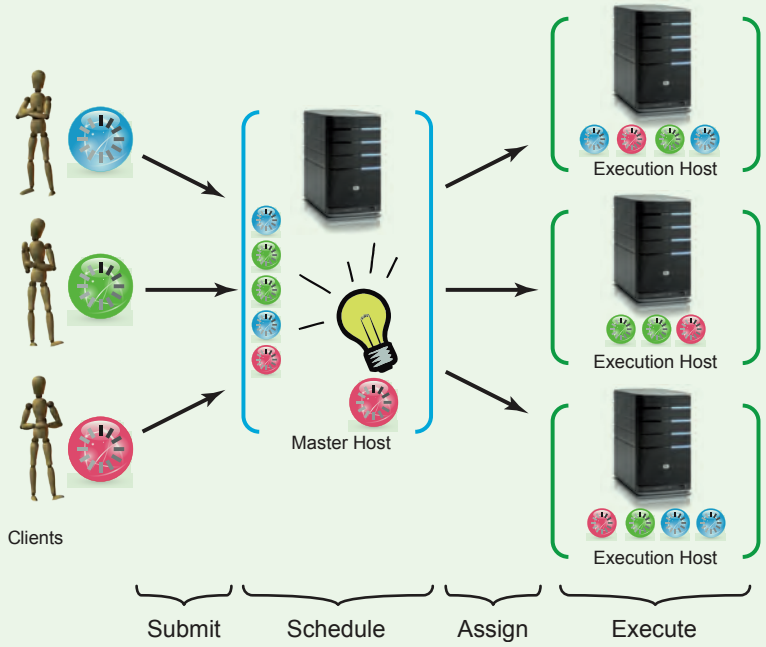
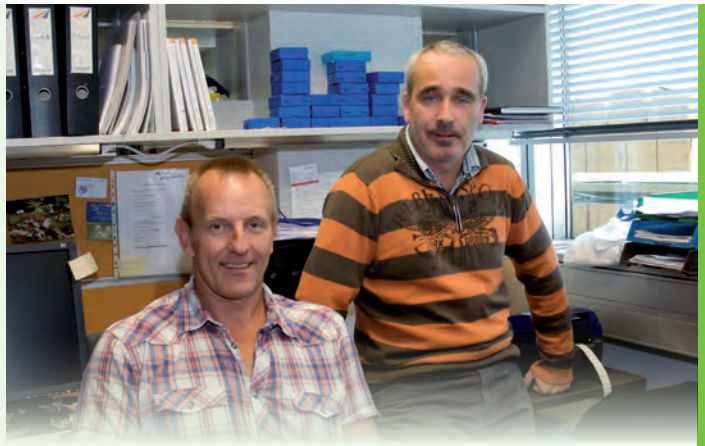


Figure: The IMP/IMBA high-performance computing (HPC) cluster. Users may submit jobs on dedicated clients to the Sun Gridengine (SGE) master, which is responsible for running the jobs on the cluster nodes.



ENGINEERS: MARTIN RADOLF, HARALD SCHEUCH

GENOMICS

genomics@imp.ac.at

After successful establishment of the Next Generation Sequencing Service, Andreas Sommer and his team joined the newly formed CSF. We routinely offer hybridization and analysis of self-spotted cDNA and Agilent Arrays for gene expression analysis for various model organisms.

Robotics:

We established a high-throughput plasmid DNA isolation platform using the Xiril 100 robot, which is also used to pipette 384 RT PCR reactions and isolate genomic DNA in a 96-well format.

In cooperation with the Zuber Lab from IMP we have established a pipeline for generating single-clone validated oligonucleotide-based libraries. Our approach combines on-chip oligonucleotide synthesis, liquid handling robotics, and a barcoding strategy for deep-sequencing-based single clone validation. In one application the pipeline will be used for rapid and cost-effective production of custom-designed shRNA libraries. The Agilent Bravo pipetting robot was mainly used to process in situ reactions together with the Stark group, PCR set-up, and purification. Like Precision XS from Biotek, the Bravo liquid handling station is used to pipette reactions for SNP genotyping.

SNP Genotyping

Together with the Nordborg Group from GMI we have designed a 384 SNP Illumina assay that can be used as a "universal" genotyping panel for Arabidopsis. We use Illumina 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.

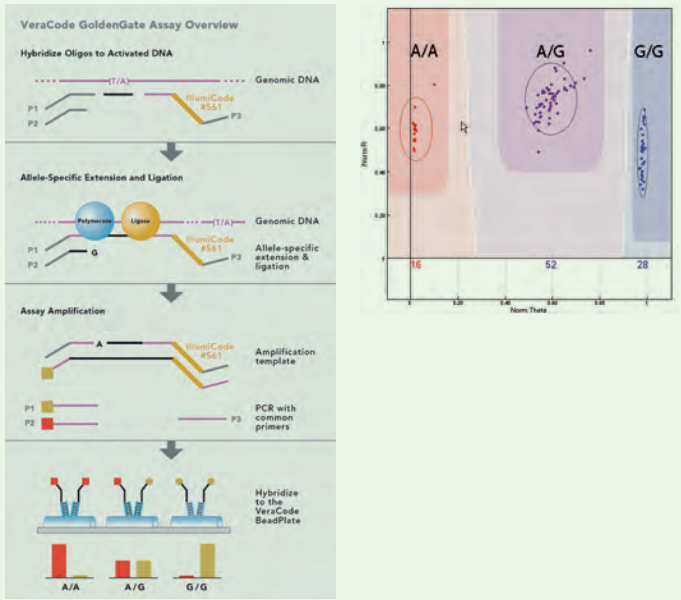


Figure: Three oligo-nucleotides are synthesized for each SNP: two allele-specific oligos that distinguish the SNP, and a locus-specific sequence just downstream of the SNP.

HEAD OF FACILITY: GOTTHOLD SCHAFFNER

TECHNICIANS: IVAN BOTTO, ZUZANA DZUPINKOVA, MARKUS HOHL, SHAHRYAR TAGHYBEEGLU
TECHNICIANS, MEDIA KITCHEN: CHRISTA DETZ-JADERNY, DAGMAR FAUSTENHAMMER, ULRIKE WINDHOLZ
TECHNICIANS, FLY FOOD PREPARATION: CHRISTINE GIESEL, JANUSZ PETRI, FRANZISKA STRANSKY
FREELANCERS, FLY FOOD PREPARATION: OLIVER BOTTO, THOMAS HAYDN, ANNA WINDHOLZ

SERVICE DEPARTMENT

gotthold.schaffner@imp.ac.at

The Service Department offers a variety of high-quality rapid services to IMP, IMBA and GMI scientists. The large part of our work involves DNA sequencing, fly food production and preparation of various media and solutions.

Gotthold Schaffner will be retiring from active service at the end of this year. The Media Kitchen (which will be an independent service group in the future) and the Fly Food unit, (which will also be an independent group in the future) prepare substantial quantities of reagent-quality solutions and media for cell cultures, flies (more than 1,800,000 tubes and bottles per year) and other organisms.

The Service Department also prepares a number of selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent *E. coli* strains; and maintains a stock of cloning vectors, sequencing primers and other reagents.

Production of antibodies

In collaboration with IMP group members, Ivan Botto, a member of the Service Department, produces and isolates many different monoclonal antibodies from hybridoma cell lines. This activity takes up a substantial part of the department's recources.

Sequencing and DNA isolation

The 48-capillary ABI 3730 DNA Genetic Analyzer was supplemented by an ABI 3730XL with 96 capillaries early this year, as the 48-capillary sequencer had clearly reached its limits. We sequenced approximately 140,000 samples during the first nine months of this year. This substantially higher demand was mainly due to screening projects, the fly library, as well as new groups at IMBA, IMP, and GMI.

The quality and concentration of DNA samples is still a problem, even when prepared by sophisticated Qiagen kits like Midi-, Maxi- or Minipreps. The same is true for incorrect primer sets or poorly documented plasmid constructs from external sources. Sequencing is performed even more rapidly now because of the tripled capacity. It has thus become much easier and faster than analyzing samples by restriction digests and running them on an agarose gel. This concurs with the new style of working with large quantities of samples.

Clean-up 96-well microtiter plates, filled automatically with a BioTek benchtop minirobot, Sephadex G50 superfine slurry, and centrifugation conditions have to be optimized further. The reason for the occasional, but sometimes quite obvious "dye blobs" one encounters when using DNA samples of low quality is still unclear. The larger quantity of contaminants as compared to PCR products, which yield a much stronger signal than double-stranded plasmid DNA, obviously plays a major role.

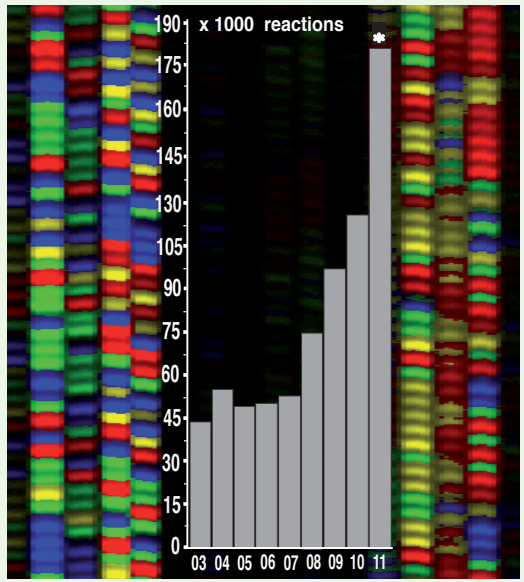


Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 3100 (since 2001) and ABI 3730 (since June 2004), performed with dye deoxy terminators (v3.0 since 2001) from 2003 to 2011 (scale 0 to 190,000).
*calculated from January 2011 to September 2011 data

HEAD OF FACILITY: KARL MECHTLER

DEPUTY LAB MANAGER: ELISABETH ROITINGER
POSTDOCS: CHRISTOPH JÜSCHKE [1], THOMAS KÖCHER, NIKOLAI MISCHERIKOW, JOHANNES STADLMANN, WERNER STRAUBE
PHD STUDENTS: DEBORA BROCH-TRENTINI [2]
DIPLOMA STUDENT: THOMAS TAUS
TECHNICAL ASSISTENTS: JOHANNES FUCHS, OTTO HUDECZ, RICHARD IMRE, GABRIELA KRSSAKOVA, MATHIAS MADALINSKI, MICHAEL MAZANEK, SUSANNE OPRAVIL [3], MICHAEL SCHUTZBIER, INES STEINMACHER

[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

Mass Spectrometry for Systems Biology

Genome-wide transcriptome analysis has provided systems-level insights into gene regulation. However, comparable analysis of post-transcriptional processes has been hampered by the limited depth of quantitative proteomics. We use chromatographic separation and iTRAQ (isobaric tag for relative and absolute quantitation) technologies to overcome these limitations. In a collaboration with the Knoblich group, we have been able to determine the brain-specific expression of more than 6000 proteins in the fruit fly *Drosophila melanogaster*, corresponding to about 70% of all potentially transcribed protein-coding genes in the brain. Our data demonstrate that quantitative proteomics yields important insights into gene regulation beyond transcriptomics, and serves as a rich source for achieving a systems-level understanding of post-transcriptional gene regulation.

Localization of Phosphorylation Sites

In addition to protein identification the detection and correct localisation of phosphorylation sites is an important task in mass spectrometry-driven proteomics. We have developed a novel software tool, phosphoRS, which permits probability-based localisation of phosphorylation sites. The algorithm assigns probabilities to all potential phosphorylation sites on phospho-peptides and works for all types of peptide fragmentation modes.

Functional Analysis of Arginine Phosphorylation

Recently, the first arginine kinase was described. This kinase, McsB, is a major regulator of stress response in Gram-positive bacteria. So far, only one substrate of McsB, the class-three transcriptional regulator (CtsR), has been studied in detail. Other targets have not been identified yet. In a collaboration with the Clausen group we develop novel methods in order to isolate and systematically detect *in vivo* arginine-phosphorylated proteins by mass spectrometry. This has enabled us to study the pivotal role of this posttranslational modification in the bacterium *Bacillus subtilis* as a response to adverse growth conditions. Furthermore, we aim to investigate the occurrence of this phosphorylation in higher organisms and thus uncover novel regulatory mechanisms.

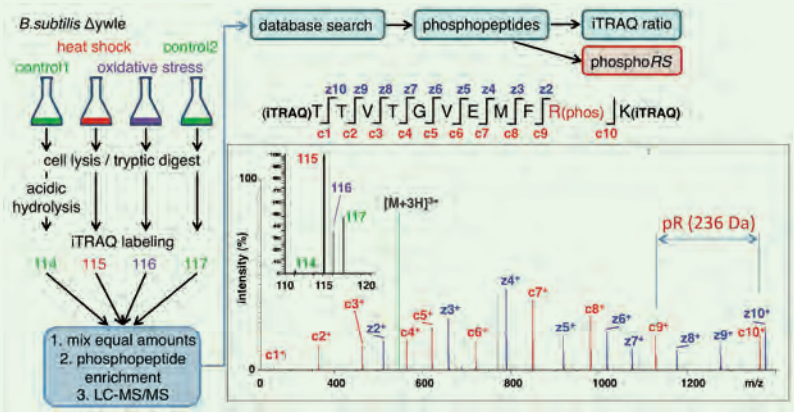


Figure: Determination of *in vivo* McsB activity by quantitative proteomics
B. subtilis arginine phosphatase-deficient cells (Δywle) were subjected to heat and oxidative stress. Phosphorylation on arginine R265 of elongation factor Tu was detected from the MS/MS spectrum (right panel). iTRAQ quantification revealed an upregulation of this phosphorylation under heat shock conditions.

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

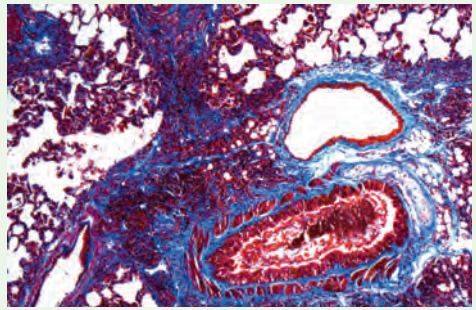
In our group we have developed a high throughput method to cut paraffin and frozen tissues. Using this method, we could increase the quality and also the quantity of services.

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*).

1



2

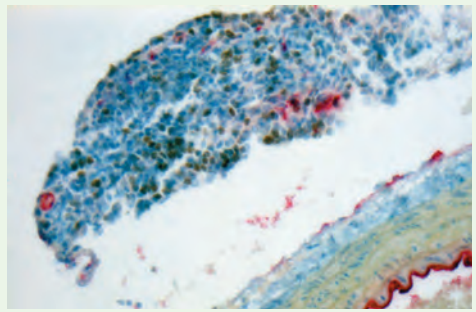
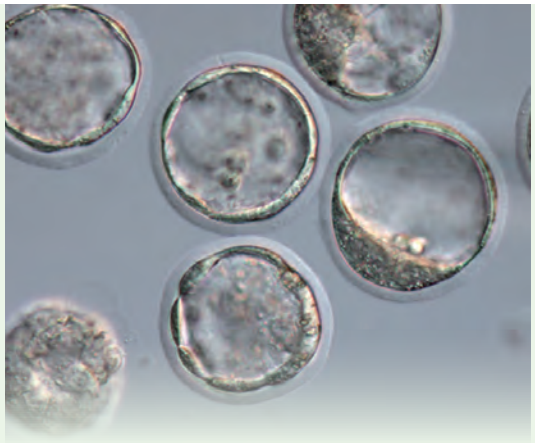


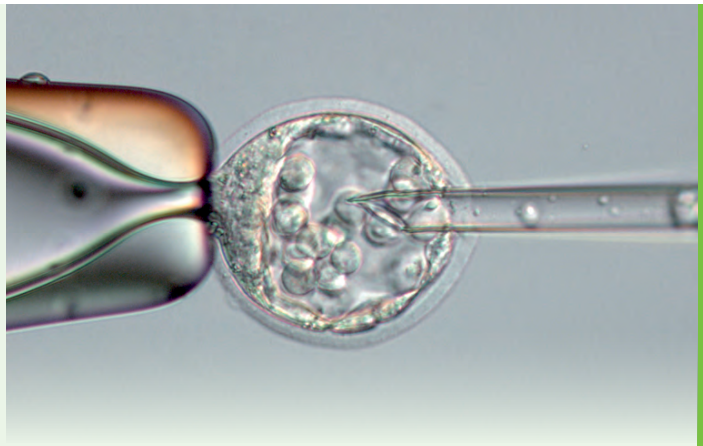
Figure 1: Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.

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).

1



2



COMPARATIVE MEDICINE

animal@imp.ac.at

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

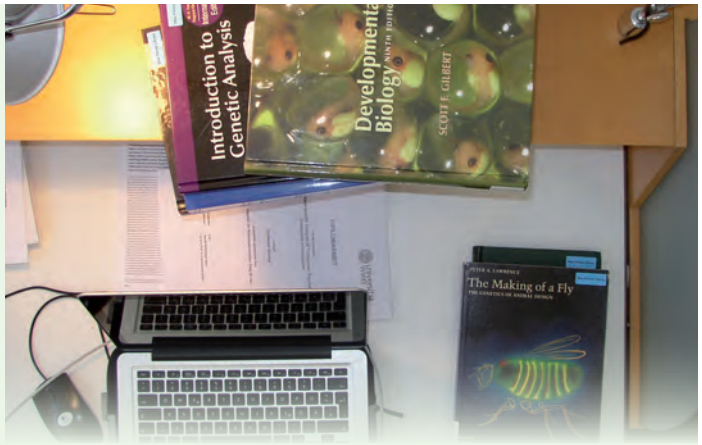
The Transgenic Service Department was set up in the beginning of 1998 to cope with the increasing demand for mouse studies and generation of transgenic mice. The Transgenic Service Department is shared by the IMP and IMBA.

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 Meinrad Busslinger.

Figure 1: Mouse blastocysts.

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



KARLO PAVLOVIC / LIBRARIAN

JÜRGEN SALLACHNER / TRAINEE

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 library is to provide comprehensive scientific literature pertaining to the areas of research pursued at the institutes. The Max Perutz Library holds approximately 3500 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. All book holdings can be searched for systematically in the online catalog, where each item is described in respect of its availability and accessibility. The most heavily used kind of literature resource are the licensed online journals: Approximately one full text view per scientific affiliate per day strikingly illustrates its value.
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 licensed by the library.

Study environment

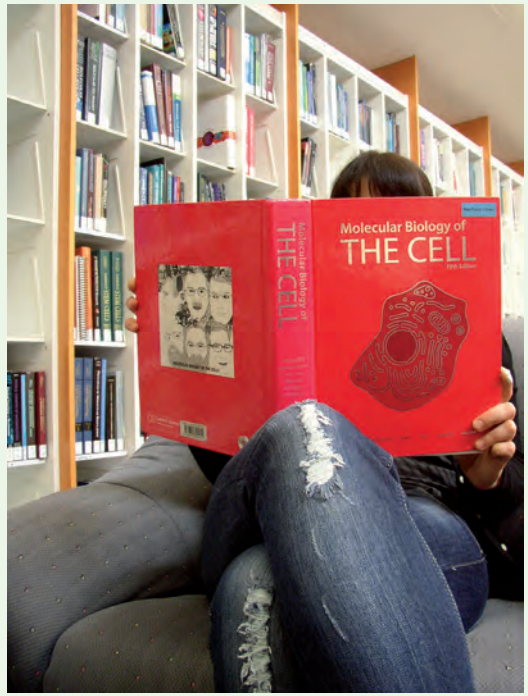
The reading room serves as a quiet and well-equipped place for reading, writing or just relaxing. Twenty-four study desks and a cozy lounge as well as two public computers, wireless LAN and a printing facility are provided.

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.



"please leave quietly" by Kay Walkowiak (see p. 49)

CAMPUS SCIENTIFIC SUPPORT FACILITY GMBH

www.csf.ac.at

The Campus Sciences Support Facilities GmbH (CSF) is the most recent member to join the wide range of research institutions and companies that make up the Campus Vienna Biocenter. The CSF was founded in the beginning of 2011 as the first ever publicly funded provider of scientific infrastructure in Austria. The CSF funding was granted jointly by the City of Vienna and the Federal Ministry for Science and Research in response to the Vision 2020 application, and comprises 52 million euros over 10 years, divided between eight core facilities.

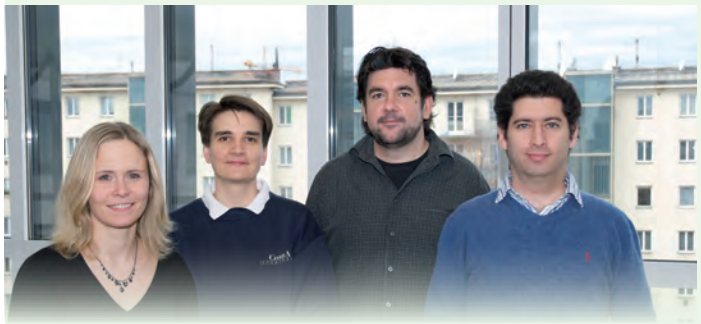
During the first year the CSF has established an administrative team and incorporated the first two Core Facilities, the **Vienna Drosophila RNAi Center (VDRC)** and the IMP-IMBA Deep Sequencing Unit (now known as **Next Generation Sequencing, NGS**), who started providing services through CSF in March 2011.

Additionally, the core facility concepts foreseen in Vision 2020 were revised, in close cooperation with the users, and the next three units are scheduled to start their Campus-wide services at the beginning of 2012:

The **Structural Biology Facility (SBF)** will offer three different services: Protein Expression in eukaryotic systems, Protein Crystallisation and Large Scale Screening. Peggy Stolt-Bergner has been recruited as the head of this service.

Andras Aszodi, head of the **Scientific Computing Core (SCC)**, will support core facilities and researchers with a streamlined collection of data analysis and visualisation tools.

Electron Microscopy (EM) will be integrated into the Ultra-Resolution Imaging unit. Günter Resch, head of the Electron Microscopy, is bringing his expertise to the start-up process.



HEAD OF NGS:
ANDREAS SOMMER

BENJAMIN ALMEIDA, CARMEN CZEPE, RENATE LANDWEHR, IDO TAMIR

NEXT GENERATION SEQUENCING (NGS)

andreas.sommer@viennabiocenter.org

Next Generation Sequencing, also known as Massive Parallel Sequencing, has become a key analysis method for a large number of biological research areas. The capacity to expand analysis from more or less defined genomic regions to genome-wide studies has boosted the pace of research discovery and has enabled researchers to obtain a global view on biological processes.

As of March 2011, sequencing resources were shifted from the joint Genomics facility to the newly founded CSF Next Generation Sequencing Unit. A team was built up consisting of a head of the unit, two bioinformaticians and two lab technicians. Also, billing was introduced to cover reagent costs and hands-on personnel time.

A HiSeq instrument, Illumina's sequencing platform with maximal throughput, was installed and is currently used for most long read applications, while the three GAIIx Systems cover short and single read sequencing.
Opening the gates to the whole VBC campus brought in new users and applications as well as an increased demand for sequencing. By the beginning of November, 950 samples from 39 scientific groups had been submitted and a total of 650 lanes sequenced.

The NGS Unit expanded its bioinformatic tool repertoire adding TopHat and Cufflinks for RNA-seq analysis as well as a local installation of Galaxy, a web-based analysis platform. Galaxy workshops and a Sequencing Symposium with invited speakers from all Viennese sequencing institutions were organized in addition to regular User Meetings.

STAFF SCIENTIST/HEAD OF THE VDRC: KRYSTYNA KELEMAN

STOCK MAINTENANCE SUPERVISOR: REINHARD KLUG
SOFTWARE DEVELOPER: THOMAS MICHELER
ADMINISTRATION: L. ZEMANN/V. SALVA[1]
TECHNICAL ASSISTANT: BARBARA MÜLLNER, ANDREAS GANSCH, JUDITH UTNER[1], ALISHER TASHPULATOV, RENÉ ZÖRNIG[2], IRINA KOLAROV, MICHAELA ECKMANN, KRISTINA BELOGRADOVA, IRENE PENZENAUER, SONJA LANG, FLORENCE MAXWELL, RAINER KECK[1], YULIA BARINOVA[1], ANGELA GRAF[1], SVETLANA ZORINYANTS, SANDOR URMOSI-INCZE, ELENA POPOWICH

[1]50%
[2]80%

VIENNA DROSOPHILA RNAi CENTER

VIENNA *DROSOPHILA* RNAI CENTER (VDRC)

office@vdrc.at

Genome-wide RNAi

RNAi can be effectively triggered in Drosophila by spatially and temporally controlled expression of a dsRNA from a transgene that contains a long inverted repeat under control of a genetic promoter, the GAL4-responsive element (Figure). The initial creation of a genome-wide transgenic RNAi library [1] has revolutionized Drosophila genetics. We have generated two independent genome-wide transgenic RNAi libraries for Drosophila, allowing researchers to systematically study gene functions in specific tissues at specific developmental stages. The VDRC maintains and further develops these libraries, and distributes RNAi lines to Drosophila researchers world-wide.

In addition to the original library based on P-element transgenesis, we have generated a second genome-wide collection by phiC31-mediated transgenesis and made it available to the *Drosophila* community in April 2009. The VDRC currently has 1821 registered users world-wide and has delivered a total of 704,926 RNAi lines to the *Drosophila* community.

Currently, the VDRC maintains and makes available 31,879 *Drosophila* lines, consisting of:
• 21,152 lines in the GD RNAi collection, constructed by P element mediated transgenesis
• 10,727 lines in the KK RNAi collection, constructed by phiC31-mediated transgenesis into preselected single genomic locus, VIE260b, on chromosome II.
• 33 miscellaneous stocks used for the construction of both collections

Additionally, the VDRC provides:
• 13,841 DNA constructs used for the generation of the GD collection

Collectively, the GD and KK libraries cover a total 13,264 *Drosophila* genes (93.1%), with the GD collection covering 11,972 genes (84.6%) and the KK collection covering 9502 genes (71.49%). For most of the genes, more than one independent RNAi line is available through the VDRC.

Administratively, the VDRC was initially operated jointly by the IMP and IMBA. From March 1, 2011, the VDRC formally became independent from IMP and IMBA and joined the Campus Science Support Facilities (CSF). This administrative move does not affect any of the operating procedures of the VDRC, but will provide for more secure long-term funding.

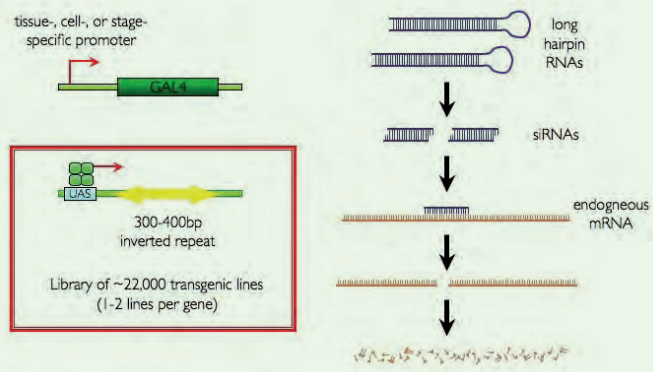


Figure: *RNAi in Drosophila.*

[1]
Dietzl, G., Chen, D., Schnorrer, F., Su, KC., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., Couto, A., Marra, V., Keleman, K., Dickson, BJ. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 448(7150):151-156

Publications

BRENNECKE GROUP

Handler, D., Olivieri, D., Novatchkova, M., Gruber, FS., Meixner, K., Mechtler, K., Stark, A., Sachidanandam, R., Brennecke, J. (2011). A systematic analysis of Drosophila TUDOR domain-containing proteins identifies Vreteno and the Tdrd12 family as essential primary piRNA pathway factors. EMBO J. 30(19):3977-93

Ni, JQ., Zhou, R., Czech, B., Liu, LP., Holderbaum, L., Yang-Zhou, D., Shim, HS., Tao, R., Handler, D., Karpowicz, P., Binari, R., Booker, M., Brennecke, J., Perkins, LA., Hannon, GJ., Perrimon, N. (2011). A genome-scale shRNA resource for transgenic RNAi in Drosophila. Nat Methods. 8(5):405-7

KNOBLICH GROUP

Khazaei, MR., Bunk, EC., Hillje, AL., Jahn, HM., Riegler, EM., Knoblich, JA., Young, P., Schwamborn, JC. (2011). The E3-ubiquitin ligase TRIM2 regulates neuronal polarization. J Neurochem. 117(1):29-37

Neumüller, RA., Richter, C., Fischer, A., Novatchkova, M., Neumüller, KG., Knoblich, JA. (2011). Genome-wide analysis of self-renewal in Drosophila neural stem cells by transgenic RNAi. Cell Stem Cell. 8(5):580-93

Postiglione, MP., Jüschke, C., Xie, Y., Haas, GA., Charalambous, C., Knoblich, JA. (2011). Mouse inscuteable induces apical-Basal spindle orientation to facilitate intermediate progenitor generation in the developing neocortex. Neuron. 72(2):269-84

Richter, C., Oktaba, K., Steinmann, J., Müller, J., Knoblich, JA. (2011). The tumour suppressor L(3)mbt inhibits neuroepithelial proliferation and acts on insulator elements. Nat Cell Biol. 13(9):1029-39

MARLOVITS GROUP

Khan, AG., Pickl-Herk, A., Gajdzik, L., Marlovits, TC., Fuchs, R., Blaas, D. (2011). Entry of a heparan sulphate-binding HRV8 variant strictly depends on dynamin but not on clathrin, caveolin, and flotillin. Virology. 412(1):55-67

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

Resch, GP., Brandstetter, M., Pickl-Herk, AM., Königsmaier, L., Wonesch, VI., Urban, E. (2011). Immersion freezing of biological specimens: rationale, principles, and instrumentation. Cold Spring Harb Protoc. 2011(7):778-82

Schraidt, O., Marlovits, TC. (2011). Three-dimensional model of Salmonella's needle complex at subnanometer resolution. Science. 331(6021):1192-5

MARTINEZ GROUP

Popow, J., Englert, M., Weitzer, S., Schleiffer, A., Mierzwa, B., Mechtler, K., Trowitzsch, S., Will, CL., Lührmann, R., Söll, D., Martinez, J. (2011). HSPC117 is the essential subunit of a human tRNA splicing ligase complex. Science. 331(6018):760-4

MOCHIZUKI GROUP

Mochizuki, K. (2011) Developmentally Programmed, RNA-directed Genome Rearrangement in Tetrahymena. Dev Growth Differ, in press.

Kataoka, K., Mochizuki, K. (2011). Programmed DNA elimination in Tetrahymena: a small RNA-mediated genome surveillance mechanism. Adv Exp Med Biol. 722:156-73

Schoeberl, UE., Mochizuki, K. (2011). Keeping the soma free of transposons: programmed DNA elimination in ciliates. J Biol Chem. 286(43):37045-52

PENNINGER GROUP

Bodiga, S., Zhong, JC., Wang, W., Basu, R., Lo, J., Liu, GC., Guo, D., Holland, SM., Scholey, JW., Penninger, JM., Kassiri, Z., Oudit, GY. (2011). Enhanced susceptibility to biomechanical stress in ACE2 null mice is prevented by loss of the p47(phox) NADPH oxidase subunit. Cardiovasc Res. 91(1):151-61

Douni, E., Rinotas, V., Makrinou, E., Zwerina, J., Penninger, J.M., Eliopoulos, E., Schett, G., and G. Kollias. (2011). A RANKL G278R mutation causing osteopetrosis identifies a functional amino acid essential for trimer assembly in RANKL and TNF. Hum. Mol. Genet., in press.

Duheron, V., Hess, E., Duval, M., Decossas, M., Castaneda, B., Klöpper, JE., Amoasii, L., Barbaroux, JB., Williams, IR., Yagita, H., Penninger, J., Choi, Y., Lézot, F., Groves, R., Paus, R., Mueller, CG. (2011). Receptor activator of NF-kappaB (RANK) stimulates the proliferation of epithelial cells of the epidermo-pilosebaceous unit. Proc Natl Acad Sci U S A. 108(13):5342-7

Elling, U., Taubenschmid, J., Wirnsberger, G., O'Malley, R., Demers, SP., Vanhaelen, Q., Shukalyuk, AI., Schmauss, G., Schramek, D., Schnuetgen, F., von Melchner, H., Ecker, JR., Stanford, WL., Zuber, J., Stark, A. and Penninger, JM. (2011). Forward and Reverse Genetics through Derivation of Haploid Mouse Embryonic Stem Cells. Cell Stem Cell. 9(6):563-74.

Gieger, C. et al., and J. M. Penninger, Prokopenko, I., Stemple, D., Toniolo, D., Wernisch, L., Sanna, S., Hicks, A. A., Rendon , A., Ferreira, M. A., Ouwehand W. H., and N. Soranzo. (2011). New gene functions in megakaryopoiesis and platelet formation. Nature, in press.

Hanada, R., Hanada, T., Sigl, V., Schramek, D., Penninger, JM. (2011). RANKL/RANK-beyond bones. J Mol Med (Berl). 89(7):647-56

Hanada, R., Penninger, JM. (2011). Central regulation of body temperature by RANKL/RANK pathway. Clin Calcium. 21(8):1201-8

Jin, R., Song, Z., Yu, S., Piazza, A., Nanda, A., Penninger, JM., Granger, DN., Li, G. (2011). Phosphatidylinositol-3-kinase gamma plays a central role in blood-brain barrier dysfunction in acute experimental stroke. Stroke. 42(7):2033-44

Kaminski, S., Hermann-Kleiter, N., Meisel, M., Thuille, N., Cronin, S., Hara, H., Fresser, F., Penninger, JM., Baier, G. (2011). Coronin 1A is an essential regulator of the TGFβ receptor/SMAD3 signaling pathway in Th17 CD4(+) T cells. J Autoimmun. 37(3):198-208

Koletar, MM., Cheng, HY., Penninger, JM., Ralph, MR. (2011). Loss of dexras1 alters nonphotic circadian phase shifts and reveals a role for the intergeniculate leaflet (IGL) in gene-targeted mice. Chronobiol Int. 28(7):553-62

Kornfeld, JW., Isaacs, A., Vitart, V., Pospisilik, JA., Meitinger, T., Gyllensten, U., Wilson, JF., Rudan, I., Campbell, H., Penninger, JM., Sexl, V., Moriggl, R., van Duijn, C., Pramstaller, PP., Hicks, AA. (2011). Variants in STAT5B associate with serum TC and LDL-C levels. J Clin Endocrinol Metab. 96(9):E1496-501

Lee, AW., Mao, Y., Penninger, JM., Yu, S. (2011). Gab2 Promotes Colony-Stimulating Factor 1-Regulated Macrophage Expansion via Alternate Effectors at Different Stages of Development. Mol Cell Biol. 31(22):4563-81

Leibbrandt, A., Penninger, JM. (2011). TNF conference 2009: beyond bones - RANKL/RANK in the immune system. Adv Exp Med Biol. 691:5-22

Leibbrandt, A., and J. M. Penninger. RANK-RANKL Signaling. in Enzyclopedia of Cancer. Springer Verlag. 2011.

Lutz-Nicoladoni, C., Wallner, S., Stoitzner, P., Pircher, M., Gruber, T., Wolf, AM., Gastl, G., Penninger, JM., Baier, G., Wolf, D. (2011). Reinforcement of cancer immunotherapy by adoptive transfer of cblb-deficient CD8(+) T cells combined with a DC vaccine. Immunol Cell Biol., in press.

Meng, X., Arocena, M., Penninger, J., Gage, FH., Zhao, M., Song, B. (2011). PI3K mediated electrotaxis of embryonic and adult neural progenitor cells in the presence of growth factors. Exp Neurol. 227(1):210-7

Miyamoto, K., Yoshida, S., Kawasumi, M., Hashimoto, K., Kimura, T., Sato, Y., Kobayashi, T., Miyauchi, Y., Hoshi, H., Iwasaki, R., Miyamoto, H., Hao, W., Morioka, H., Chiba, K., Kobayashi, T., Yasuda, H., Penninger, JM., Toyama, Y., Suda, T., Miyamoto, T. (2011). Osteoclasts are dispensable for hematopoietic stem cell maintenance and mobilization. J Exp Med. 208(11):2175-81

Mouri, Y., Yano, M., Shinzawa, M., Shimo, Y., Hirota, F., Nishikawa, Y., Nii, T., Kiyonari, H., Abe, T., Uehara, H., Izumi, K., Tamada, K., Chen, L., Penninger, JM., Inoue, J., Akiyama, T., Matsumoto, M. (2011). Lymphotoxin signal promotes thymic organogenesis by eliciting RANK expression in the embryonic thymic stroma. J Immunol. 186(9):5047-57

Muto, A., Mizoguchi, T., Udagawa, N., Ito, S., Kawahara, I., Abiko, Y., Arai, A., Harada, S., Kobayashi, Y., Nakamichi, Y., Penninger, JM., Noguchi, T., Takahashi, N. (2011). Lineage-committed osteoclast precursors circulate in blood and settle down into bone. J Bone Miner Res. 26(12):2978-90

Nakashima, T., Hayashi, M., Fukunaga, T., Kurata, K., Oh-Hora, M., Feng, JQ., Bonewald, LF., Kodama, T., Wutz, A., Wagner, EF., Penninger, JM., Takayanagi, H. (2011). Evidence for osteocyte regulation of bone homeostasis through RANKL expression. Nat Med. 17(10):1231-4

Neely, GG., Keene, AC., Duchek, P., Chang, EC., Wang, QP., Aksoy, YA., Rosenzweig, M., Costigan, M., Woolf, CJ., Garrity, PA., Penninger, JM. (2011). TrpA1 regulates thermal nociception in Drosophila. PLoS One. 6(8):e24343

Oh, SY., Park, JU., Zheng, T., Kim, YK., Wu, F., Cho, SH., Barber, D., Penninger, J., Zhu, Z. (2011). Cbl-b regulates airway mucosal tolerance to aeroallergen. Clin Exp Allergy. 41(3):434-42

Oudit, GY., Penninger, JM. (2011). Recombinant human angiotensin-converting enzyme 2 as a new renin-angiotensin system peptidase for heart failure therapy. Curr Heart Fail Rep. 8(3):176-83

Paolino, M., Thien, CB., Gruber, T., Hinterleitner, R., Baier, G., Langdon, WY., Penninger, JM. (2011). Essential role of E3 ubiquitin ligase activity in Cbl-b-regulated T cell functions. J Immunol. 186(4):2138-47

Schramek, D., Leibbrandt, A., and J. M. Penninger. (2011). The many roles of RANKL-RANK signaling in bone, breast and cancer. IBMS BoneKey. 8, 237-256.

Schramek, D., Kotsinas, A., Meixner, A., Wada, T., Elling, U., Pospisilik, JA., Neely, GG., Zwick, RH., Sigl, V., Forni, G., Serrano, M., Gorgoulis, VG., Penninger, JM. (2011). The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression. Nat Genet. 43(3):212-9

Schramek, D., Sigl, V., Penninger, JM. (2011). RANKL and RANK in sex hormone-induced breast cancer and breast cancer metastasis. Trends Endocrinol Metab. 22(5):188-94

Taschler, U., Radner, FP., Heier, C., Schreiber, R., Schweiger, M., Schoiswohl, G., Preiss-Landl, K., Jaeger, D., Reiter, B., Koefeler, HC., Wojciechowski, J., Theussl, C., Penninger, JM., Lass, A., Haemmerle, G., Zechner, R., Zimmermann, R. (2011). Monoglyceride lipase deficiency in mice impairs lipolysis and attenuates diet-induced insulin resistance. J Biol Chem. 286(20):17467-77

Yamasaki, T., Kawasaki, H., Arakawa, S., Shimizu, K., Shimizu, S., Reiner, O., Okano, H., Nishina, S., Azuma, N., Penninger, J.M., Katada, T., and H. Nishina. (2011). Stress-Activated Protein Kinase MKK7 Regulates Axon Elongation in the Developing Cerebral Cortex. J. Neurosci. 31, 16872-16883.

Zhong, JC., Ye, JY., Jin, HY., Yu, X., Yu, HM., Zhu, DL., Gao, PJ., Huang, DY., Shuster, M., Loibner, H., Guo, JM., Yu, XY., Xiao, BX., Gong, ZH., Penninger, JM., Oudit, GY. (2011). Telmisartan attenuates aortic hypertrophy in hypertensive rats by the modulation of ACE2 and profilin-1 expression. Regul Pept. 166(1-3):90-7

Zhong, J., Guo, D., Chen, CB., Wang, W., Schuster, M., Loibner, H., Penninger, JM., Scholey, JW., Kassiri, Z., Oudit, GY. (2011). Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. Hypertension. 57(2):314-22

RINGROSE GROUP

Hekimoglu- Balkan, B., Aszodi, A., Heinen, R., Jaritz, M. and Ringrose, L. Intergenic Polycomb Target sites are dynamically marked by non-coding transcription during lineage commitment. RNA Biology, in press

Okulski, H., Druck, B., Bhalerao, S., Ringrose, L. (2011). Quantitative analysis of polycomb response elements (PREs) at identical genomic locations distinguishes contributions of PRE sequence and genomic environment. Epigenetics Chromatin. 4:4

Strübbe, G., Popp, C., Schmidt, A., Pauli, A., Ringrose, L., Beisel, C., Paro, R. (2011). Polycomb purification by in vivo biotinylation tagging reveals cohesin and Trithorax group proteins as interaction partners. Proc Natl Acad Sci U S A. 108(14):5572-7

Ringrose, L. (2010). How do RNA sequence, DNA sequence, and chromatin properties regulate splicing? F1000 Biol Rep. 12;2:74.

SMALL GROUP

Breitsprecher, D., Kiesewetter, AK., Linkner, J., Vinzenz, M., Stradal, TE., Small, JV., Curth, U., Dickinson, RB., Faix, J. (2011). Molecular mechanism of Ena/VASP-mediated actin-filament elongation. EMBO J. 30(3):456-67

Breitsprecher, D., Koestler, SA., Chizhov, I., Nemethova, M., Mueller, J., Goode, BL., Small, JV., Rottner, K., Faix, J. (2011). Cofilin cooperates with fascin to disassemble filopodial actin filaments. J Cell Sci. 124(Pt 19):3305-18

Oelkers, JM., Vinzenz, M., Nemethova, M., Jacob, S., Lai, FP., Block, J., Szczodrak, M., Kerkhoff, E., Backert, S., Schlüter, K., Stradal, TE., Small, JV., Koestler, SA., Rottner, K. (2011). Microtubules as platforms for assaying actin polymerization in vivo. PLoS One. 6(5):e19931

Resch, GP., Brandstetter, M., Pickl-Herk, AM., Königsmaier, L., Wonesch, VI., Urban, E. (2011). Immersion freezing of biological specimens: rationale, principles, and instrumentation. Cold Spring Harb Protoc. 2011(7):778-82

Small, JV., Winkler, C., Vinzenz, M. and Schmeiser, S. (2011). Reply: Visualizing branched actin filaments in lamellipodia by electron tomography. Nature Cell Biol. 13(9):1012-3

STEM CELL CENTER

Schramek, D., Kotsinas, A., Meixner, A., Wada, T., Elling, U., Pospisilik, JA., Neely, GG., Zwick, RH., Sigl, V., Forni, G., Serrano, M., Gorgoulis, VG., Penninger, JM. (2011). The stress kinase MKK7 couples oncogenic stress to p53 stability and tumor suppression. Nat Genet. 43(3):212-9

FLY HOUSE

Neely, GG., Keene, AC., Duchek, P., Chang, EC., Wang, QP., Aksoy, YA., Rosenzweig, M., Costigan, M., Woolf, CJ., Garrity, PA., Penninger, JM. (2011). TrpA1 regulates thermal nociception in Drosophila. PLoS One. 6(8):e24343

BIOINFORMATICS

Böhmdorfer, G., Schleiffer, A., Brunmeir, R., Ferscha, S., Nizhynska, V., Kozák, J., Angelis, KJ., Kreil, DP., Schweizer, D. (2011). GMI1, a structural-maintenance-of-chromosomes-hinge domain-containing protein, is involved in somatic homologous recombination in Arabidopsis. Plant J 67(3):420-33.

Ebert, A., McManus, S., Tagoh, H., Medvedovic, J., Salvagiotto, G., Novatchkova, M., Tamir, I., Sommer, A., Jaritz, M., Busslinger, M. (2011). The distal V(H) gene cluster of the Igh locus contains distinct regulatory elements with Pax5 transcription factor-dependent activity in pro-B cells. Immunity 34(2):175-87

Grosstessner-Hain, K., Hegemann, B., Novatchkova, M., Rameseder, J., Joughin, BA., Hudecz, O., Roitinger, E., Pichler, P., Kraut, N., Yaffe, MB., Peters, JM., Mechtler, K. (2011). Quantitative Phospho-proteomics to Investigate the Polo-like Kinase 1-Dependent Phospho-proteome. Mol Cell Proteomics 10(11):M111.008540

Handler, D., Olivieri, D., Novatchkova, M., Gruber, FS., Meixner, K., Mechtler, K., Stark, A., Sachidanandam, R., Brennecke, J. (2011). A systematic analysis of Drosophila TUDOR domain-containing proteins identifies Vreteno and the Tdrd12 family as essential primary piRNA pathway factors. EMBO J 30(19):3977-93

Hegemann, B., Hutchins, JR., Hudecz, O., Novatchkova, M., Rameseder, J., Sykora, MM., Liu, S., Mazanek, M., Lénárt, P., Hériché, JK., Poser, I., Kraut, N., Hyman, AA., Yaffe, MB., Mechtler, K., Peters, JM. (2011). Systematic phosphorylation analysis of human mitotic protein complexes. Sci Signal 4(198):rs12

Heuck, A., Schleiffer, A., Clausen, T. (2011). Augmenting β-augmentation: structural basis of how BamB binds BamA and may support folding of outer membrane proteins. J Mol Biol 406(5):659-66

Neumüller, RA., Richter, C., Fischer, A., Novatchkova, M., Neumüller, KG., Knoblich, JA. (2011). Genome-wide analysis of self-renewal in Drosophila neural stem cells by transgenic RNAi. Cell Stem Cell 8(5):580-93

Popow, J., Englert, M., Weitzer, S., Schleiffer, A., Mierzwa, B., Mechtler, K., Trowitzsch, S., Will, CL., Lührmann, R., Söll, D., Martinez, J. (2011). HSPC117 is the essential subunit of a human tRNA splicing ligase complex. Science 331(6018):760-4

BIOOPTICS

Adlassnig, W., Peroutka, M., Lendl, T. (2011). Traps of carnivorous pitcher plants as a habitat: composition of the fluid, biodiversity and mutualistic activities. Ann Bot. 107(2):181-94.

Elling, U., Taubenschmid, J., Wirnsberger, G., O'Malley, R., Demers, SP., Vanhaelen, Q., Shukalyuk, AI., Schmauss, G., Schramek, D., Schnuetgen, F., von Melchner, H., Ecker, JR., Stanford, WL., Zuber, J., Stark, A. and Penninger, JM. (2011). Forward and Reverse Genetics through Derivation of Haploid Mouse Embryonic Stem Cells. Cell Stem Cell. 9(6):563-74.

ELECTRON MICROSCOPY

Hasanovic, A., Winkler, R., Resch, G.P., Valenta, C. (2011): Modification of the conformational skin structure by treatment with liposomal formulations and its correlation to the penetration depth of aciclovir. Eur J Pharm Biopharm. 79(1):76-81.

Schwarz, J.C., Klang, V., Karall, S., Mahrhauser, D., Resch, G. P., and Valenta, C. (2011): Optimisation of multiple W/O/W nanoemulsions for dermal delivery of aciclovir. International Journal of Pharmaceutics, in press.

Stöger-Pollach, M., Treiber, C.D., Resch, G.P., Keays, D.A., Ennen, I. (2011): EMCD real space maps of Magnetospirillum magnetotacticum. Micron. 42(5):456-60

Resch, G.P., Brandstetter, M., Pickl-Herk, A.M., Königsmaier, L., Wonesch, V.I., Urban, E. (2011). Immersion freezing of biological specimens: rationale, principles, and instrumentation. Cold Spring Harb Protoc. 2011(7):778-82

Resch, G.P., Brandstetter, M., Königsmaier, L., Urban, E. , Pickl-Herk, A.M. (2011): Immersion Freezing of Suspended Particles and Cells for Cryo-Electron Microscopy. Cold Spring Harb Protoc. 2011(7):803-14

Resch, G.P., Brandstetter M., Wonesch, V.I., Urban, E. (2011): Immersion Freezing of Cell Monolayers for Cryo-Electron Tomography. Cold Spring Harb Protoc. 2011(7):815-23

Fuest, M., Willim, K., Macnelly, S., Fellner, N., Resch, G.P., Blum, H.E., Hasselblatt, P. (2011): The transcription factor c-Jun protects against sustained hepatic endoplasmic reticulum stress thereby promoting hepatocyte survival. Hepatology, Sep 27. doi: 10.1002/hep.24699

GENOMICS

Schönbauer C, Distler J, Jährling N, Radolf M, Dodt HU, Frasch M, Schnorrer F. (2011). Spalt mediates an evolutionarily conserved switch to fibrillar muscle fate in insects. Nature. 479(7373):406-9

PROTEIN CHEMISTRY

Alexander, J., Lim, D., Joughin, BA., Hegemann, B., Hutchins, JR., Ehrenberger, T., Ivins, F., Sessa, F., Hudecz, O., Nigg, EA., Fry, AM., Musacchio, A., Stukenberg, PT., Mechtler, K., Peters, JM., Smerdon, SJ., Yaffe, MB. (2011). Spatial exclusivity combined with positive and negative selection of phosphorylation motifs is the basis for context-dependent mitotic signaling. Sci Signal. 4(179):ra42

Breitwieser, FP., Müller, A., Dayon, L., Köcher, T., Hainard, A., Pichler, P., Schmidt-Erfurth, U., Superti-Furga, G., Sanchez, JC., Mechtler, K., Bennett, KL., Colinge, J. (2011). General statistical modeling of data from protein relative expression isobaric tags. J Proteome Res. 10(6):2758-66

Grosstessner-Hain, K., Hegemann, B., Novatchkova, M., Rameseder, J., Joughin, BA., Hudecz, O., Roitinger, E., Pichler, P., Kraut, N., Yaffe, MB., Peters, JM., Mechtler, K. (2011). Quantitative Phospho-proteomics to Investigate the Polo-like Kinase 1-Dependent Phospho-proteome. Mol Cell Proteomics. 10(11):M111.008540

Handler, D., Olivieri, D., Novatchkova, M., Gruber, FS., Meixner, K., Mechtler, K., Stark, A., Sachidanandam, R., Brennecke, J. (2011). A systematic analysis of Drosophila TUDOR domain-containing proteins identifies Vreteno and the Tdrd12 family as essential primary piRNA pathway factors. EMBO J. 30(19):3977-93

Hegemann, B., Hutchins, JR., Hudecz, O., Novatchkova, M., Rameseder, J., Sykora, MM., Liu, S., Mazanek, M., Lénárt, P., Hériché, JK., Poser, I., Kraut, N., Hyman, AA., Yaffe, MB., Mechtler, K., Peters, JM. (2011). Systematic phosphorylation analysis of human mitotic protein complexes. Sci Signal. 4(198):rs12

Holzmann, J., Fuchs, J., Pichler, P., Peters, JM., Mechtler, K. (2011). Lesson from the stoichiometry determination of the cohesin complex: a short protease mediated elution increases the recovery from cross-linked antibody-conjugated beads. J Proteome Res. 10(2):780-9

Köcher, T., Pichler, P., Mazanek, M., Swart, R., Mechtler, K. (2011). Altered Mascot search results by changing the m/z range of MS/MS spectra: analysis and potential applications. Anal Bioanal Chem. 400(8):2339-47

Köcher, T., Pichler, P., Swart, R., Mechtler, K. (2011). Quality control in LC-MS/MS. Proteomics. 11(6):1026-30

Köcher, T., Swart, R., Mechtler, K. (2011). Ultra-high-pressure RPLC hyphenated to an LTQ-Orbitrap Velos reveals a linear relation between peak capacity and number of identified peptides. Anal Chem. 83(7):2699-704

Pichler, P., Köcher, T., Holzmann, J., Möhring, T., Ammerer, G., Mechtler, K. (2011). Improved precision of iTRAQ and TMT quantification by an axial extraction field in an Orbitrap HCD cell. Anal Chem. 83(4):1469-74

Popow, J., Englert, M., Weitzer, S., Schleiffer, A., Mierzwa, B., Mechtler, K., Trowitzsch, S., Will, CL., Lührmann, R., Söll, D., Martinez, J. (2011). HSPC117 is the essential subunit of a human tRNA splicing ligase complex. Science. 331(6018):760-4

Sixt, BS., Heinz, C., Pichler, P., Heinz, E., Montanaro, J., Op den Camp, HJ., Ammerer, G., Mechtler, K., Wagner, M., Horn, M. (2011). Proteomic analysis reveals a virtually complete set of proteins for translation and energy generation in elementary bodies of the amoeba symbiont Protochlamydia amoebophila. Proteomics. 11(10):1868-92

Taus, T., Köcher, T., Pichler, P., Paschke, C., Schmidt, A., Henrich, C., Mechtler, K. (2011). Universal and Confident Phosphorylation Site Localization Using phosphoRS. J Proteome Res. 10(12):5354-62

TRANSGENIC SERVICE

Fischer H, Szabo S, Scherz J, Jaeger K, Rossiter H, Buchberger M, Ghannadan M, Hermann M, Theussl HC, Tobin DJ, Wagner EF, Tschachler E, Eckhart L. (2011). Essential role of the keratinocyte-specific endonuclease DNase1L2 in the removal of nuclear DNA from hair and nails. J Invest Dermatol. 2011 Jun;131(6):1208-15

Taschler, U., Radner, FP., Heier, C., Schreiber, R., Schweiger, M., Schoiswohl, G., Preiss-Landl, K., Jaeger, D., Reiter, B., Koefeler, HC., Wojciechowski, J., Theussl, C., Penninger, JM., Lass, A., Haemmerle, G., Zechner, R., Zimmermann, R. (2011). Monoglyceride lipase deficiency in mice impairs lipolysis and attenuates diet-induced insulin resistance. J Biol Chem. 286(20):17467-77

NEXT GENERATION SEQUENCING (CSF)

Ebert, A., McManus, S., Tagoh, H., Medvedovic, J., Salvagiotto, G., Novatchkova, M., Tamir, I., Sommer, A., Jaritz, M., Busslinger, M. (2011). The distal V(H) gene cluster of the Igh locus contains distinct regulatory elements with Pax5 transcription factor-dependent activity in pro-B cells. Immunity 34(2):175-87

Huang R, Jaritz M, Guenzl P, Vlatkovic I, Sommer A, Tamir IM, Marks H, Klampfl T, Kralovics R, Stunnenberg HG, Barlow DP, Pauler FM. (2011). An RNA-Seq Strategy to Detect the Complete Coding and Non-Coding Transcriptome Including Full-Length Imprinted Macro ncRNAs. PLoS One. 2011;6(11):e27288

McManus, S., Ebert, A., Salvagiotto, G., Medvedovic, J., Sun, Q., Tamir, I., Jaritz, M., Tagoh, H., Busslinger, M. (2011). The transcription factor Pax5 regulates its target genes by recruiting chromatin-modifying proteins in committed B cells. EMBO J. 30(12):2388-404

Awards & Honors 2011

Darko Barisic
(Martinez Group)
- *VBC Summer School Prize by BD Biosciences*

Julius Brennecke
- *Elected member of the Young Academy of the Austrian Academy of Sciences*

Fiona Grimm
(Brennecke Group)
- *VBC Summer School Prize by Canon Austria*

Jürgen Knoblich
- *"Jenkinson Memorial lecture", University of Oxford*
- *Karl Friedrich Bonhoeffer Lecture, Max Planck Institute, Goettingen*

Petra Loidolt
(Penninger Group)
- *GEN-AU Summer School Award 2011*

Thomas Marlovits
- *Monash Lecture, Australian Academy of Sciences*

Karl Mechtler
- *Elected president of the Austrian Proteomics Society*

Cecilie Oest-Jacobsen
(Marlovits Group)
- *VBC Summer School Prize by VWR*

Josef Penninger
- *Elected Fellow of the American Association for the Advancement of Science (AAAS)*

Constance Richter
(Knoblich Group)
- *Vienna Biocenter PhD Award*
- *Kirsten Peter Rabitsch Award*

Oliver Schraidt
(Marlovits Group)
- *Vienna Biocenter PhD Award*

Daniel Schramek
(Penninger Group)
- *Alois Sonnleitner-Preis by the Austrian Academy of Sciences*
- *ÖGMBT Award by the Austrian Association of Molecular Life Sciences and Biotechnology*

Greg Sienski
(Brennecke Group)
- *PhD Fellowship Boehringer Ingelheim*

JANUARY

14.01.11 Manolis Pasparakis
Institute for Genetics, University of Cologne
Epithelial TNF receptor signalling in chronic inflammation

20.01.11 Mitsuhiro Yanagida
Kyoto University, Okinawa Institute of Science and Technology
Metabolic control of chromosome dynamics and cell division

27.01.11 Lumir Krejci
Masaryk University
Homologous recombination and its quality control

FEBRUARY

03.02.11 Daniel Gerlich
ETH Zurich
Bridging spatial and temporal resolution gaps in the study of cell division

14.02.11 Karl-Lenhard Rudolph
Ulm University
Genetic and Environmental Factors influencing Stem Cell Function

14.02.11 Makoto Asashima
Tokyo University
Control of organogenesis and cell differentiation in vertebrate development

24.02.11 Michael Rosbash
Brandeis University
Circadian Rhythms and Gene Expression: Molecules, Neurons and Circuits

MARCH

03.03.11 Walter Schaffner
University of Zurich
A career in gene regulation - what went right, what went wrong

10.03.11 Nipam Patel
University of California, Berkeley
Developmental and Evolutionary Insights from Newly Emerging Model Organisms

14.03.11 Fumiyo Ikeda
Goethe University Medical School
Regulation of inflammatory signaling by linear ubiquitin chains

18.03.11 Michael Wilson
Cancer Research UK
Functional insights into mammalian tissue function through comparative evolutionary genomics

24.03.11 Jerzy Paszkowski
University of Geneva
Epigenetic control of retrotransposition

28.03.11 Rudolf Oehler
Anna Spiegel Center of Translational Research
Advances, Challenges, and Limitations in Clinical Proteomics

28.03.11 Alexandros Vegiopoulos
DKFZ Heidelberg
Switching from storing fat to burning fat: Mesenchymal stem cells and adipose tissue plasticity

29.03.11 Kikue Tachibana
Department of Biochemistry, University of Oxford
Cohesin rings at the mouse oocyte-to-zygote transition

30.03.11 Ruth Birner-Grünberger
Center of Medical Research, Medical University of Graz
Lipo-proteomics: functional proteomics in lipid research

30.03.11 Eileen Furlong
EMBL
cis-regulatory networks during development: Is there a code?

APRIL

04.04.11 Baris Tursun
Columbia University
Direct reprogamming of cellular identities

05.04.11 Rui Benedito
MPI for Molecular Biomedicine
Understanding the regulation of angiogenesis by Notch and VEGF through inducible mouse genetics

06.04.11 Stephanie Blandin
Louis Pasteur University
Immune responses and resistance to malaria parasites in the mosquito Anopheles gambiae

08.04.11 Mikel Zaratiegui
Cold Spring Harbor Laboratory
Dissecting Heterochromatin inheritance

11.04.11 Stefan Ameres
University of Massachusetts Medical School
Target RNA-directed Tailing and Trimming of Small Silencing RNAs – Mechanism, Biology and Application of a small RNA Decay Pathway

13.04.11 Liisa Kauppi
Memorial Sloan-Kettering Cancer Center
The tricky path to recombining X and Y chromosomes in meiosis

14.04.11 Yang Shi
Harvard Medical School
Histone demethylases: mechanisms and link to mental retardation

14.04.11 Luisa Di Stefano
Massachusetts General Hospital
Functional antagonism between histone H3K4 demethylases in vivo

18.04.11 Martin Jinek
University of California, Berkeley
Molecular recognition in genome expression and defence: Structural insights into microRNA-mediated silencing, mRNA decay, and CRISPR-guided immunity

21.04.11 William Martin
Heinrich-Heine-University, Duesseldorf
Eukaryote origin, endosymbiosis, and the energetics of genome complexity

26.04.11 Peter Reddien
Howard Hughes Medical Institute
Title to be announced

28.04.11 John Tainer
Scripps Research Institute
An allosteric paradigm for DNA damage response networks: A dawn for mechanistic systems biology in disease prediction and intervention

29.04.11 Werner L. Straube
Max Planck Institute of Biochemistry
A practical approach towards high-throughput absolute quantitation using high resolution mass spectrometry

MAY

11.05.11 Suresh K. Alahari
Stanley S. Scott Cancer Center
Nischarin, a novel integrin alpha5 binding protein regulates breast cancer progression

19.05.11 John Doebley
University of Wisconsin
Evolution under Domestication: An example from Maize

20.05.11 R. Mark Henkelman
Mouse Imaging Centre, University of Toronto
Genes into Geometry: Imaging for Mouse Phenotyping

JUNE

09.06.11 Gero Miesenboeck
University of Oxford
Lighting Up the Brain

16.06.11 Nikolai Mischeriko
University Medical Centre Utrecht
Characterization of post-translational modifications of the transcription-related protein complexes TFIID and SAGA from Saccharomyces cerevisiae

16.06.11 Alexander Tomasz
The Rockefeller University
Accelerated Evolution: Tracking Genes and Phenotypes in Antibiotic Resistant S. aureus

17.06.11 Christian Huber
University of Salzburg
High-resolution liquid chromatography-mass spectrometry of intact proteins: challenges and perspectives for proteome analysis

22.06.11 Julie Cooper
Cancer Research UK, London Research Institute
Telomeres and the challenges to chromosome integrity

30.06.11 Neil Hunter
University of California
Making A Connection Between Chromosomes During Meiosis

JULY

07.07.11 Lewis Cantley
Beth Israel Deaconess Medical Center
PI 3-Kinase and Disease

14.07.11 Eric Selker
University of Oregon, Institute of Molecular Biology
Control of DNA methylation and heterochromatin formation in Neurospora

15.07.11 Joseph Schlessinger
Yale University School of Medicine
Cell Signaling by Receptor Tyrosine Kinases: from basic principles to cancer therapy

SEPTEMBER

01.09.11 Alberto Kornblihtt
University of Buenos Aires
Chromatin and transcription regulate alternative splicing

06.09.11 Wolfgang Weninger
University of Sydney
Functional imaging of immune responses and tumour formation

08.09.11 Joanna Wysocka
Stanford School of Medicine
Stem cells, enhancers and emergence of epigenomes in development

15.09.11 Maarten Fornerod
Erasmus University Medical Center
Chromatin interactions with the nuclear periphery

19.09.11 Karen Adelman
NIEHS
Potentiating Signal-responsive Transcription: A Dynamic Dance Between Paused Polymerase and Chromatin

21.09.11 Bas Tolhuis
Netherlands Cancer Institute
Epigenetics and nuclear organization: towards mechanistic insights into chromosome folding and genome function

22.09.11 Peter Donnelly
Oxford University
The Evolution of Recombination Hotspots

29.09.11 Frank Schnorrer
Max Planck Institute of Biochemistry, Martinsried
The Making of Flight Muscles

OCTOBER

14.10.11 Manu Rangachari
Brigham & Women's Hospital/Harvard Medical School
Modulating tissue inflammation and autoimmunity by inducing T cell exhaustion: role of the Tim-3 signaling pathway

19.10.11 Björn Brembs
Freie Universität Berlin
Action – outcome evaluation: the genetics of Drosophila self-learning

20.10.11 Enrico Coen
John Innes Centre
From Genes to Shape

27.10.11 Sir Philip Cohen
MRC Protein Phosphorylation Unit, University of Dundee
Novel and unexpected roles for the IKK-related kinases in the regulation of innate immunity

NOVEMBER

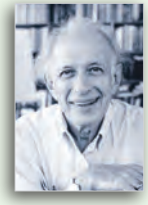
24.11.11 Nikolaus Pfanner
University of Freiburg
Mitochondrial protein import: from proteomics to functional mechanisms

DECEMBER

02.12.11 Melina Schuh
MRC Laboratory of Molecular Biology
Oocyte + Actin: Old Love - New Affairs

16.12.11 Pierre Leopold
Institute of Developmental Biology & Cancer
Genetics and Physiology of growth and size determination in Drosophila

Scientific Advisory Board



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

Prof. Günter Blobel
Rockefeller University New York, USA

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

Prof. Kenneth Chien
Cardiovascular Research Center, Massachusetts, USA

Prof. Susan Lindquist
Whitehead Institute for Biomedical Research, Cambridge, MA, USA

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

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

Supervisory Board

Prof. Erich Streissler
Department of Economics, University of Vienna, Austria (chairman)

Mag. Gottfried Schellmann
Müller-Koch & Schellmann Steuerberatungs GmbH, Austria

Prof. Josef Smolen
Department of Rheumatology, Internal Medicine III, University of Vienna, Austria

Prof. Georg Wick
Division of Experimental Pathophysiology & Immunology, Dept. Biocenter, Innsbruck Medical University, Austria

Prof. Josef Aicher
Institut für Unternehmens- und Wirtschaftsrecht, Faculty of Law, University of Vienna, Austria (deputy chairman)

Prof. Oskar Grün
Institute of Business Organisation and Supply Management, Vienna University of Economics and Business Administration, Austria

REPRESENTATIVES OF THE AUSTRIAN ACADEMY OF SCIENCES

Prof. Helmut Denk
President of the Austrian Academy of Sciences, Vienna, Austria

Prof. Georg Stingl
President of the Section for Mathematics and the Natural Sciences of the Austrian Academy of Sciences, Vienna, Austria

Prof. Bernhard Keppler
Department of Inorganic Chemistry, University of Vienna, Austria

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

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.

MANAGEMENT

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

MANAGEMENT ASSISTANCE

Sandra PRASSEL	Head of Scientific Secretariat
Denise LANGER	Assist. to the Admin. Director
Sonja PAULICK	Assist. to the Deputy Scientific Director
Erika WOOD (until 02/11)	Assist. to the Scientific Director

ACCOUNTING/CONTROLLING

Brigitte WEISER	Head of Accounting/Controlling
Renate ZETL	Accounting Clerk
Anita SOMMER	Accounting Clerk
Hanna VOGL	Accounting Clerk
Gesine WALNSCH (until 09/11)	Controller
Romana BOHNENSTINGL	Controller

HUMAN RESOURCES

Werner LEITNER	Personnel Officer
Daniela PILLER	Personnel Officer
Sabine STEURER	Recruiting & Development Manager

GRANTS MANAGEMENT

Tanja WINKLER	Head of Grants Management
Uta POHN	Assistant to the Grants Manager

IT SERVICE DEPARTMENT

Werner KUBINA	Head of IT-Service Dept.
Andreas RIEPL	Deputy Head of IT-Service Dept.
Benjamin ALMEIDA (until 02/11)	IT System Manager
Christian BRANDSTÄTTER	IT System Manager
Gert BRÜNNER	IT System Manager
Kirk BUTUK	IT System Manager
Sebastian RUF	IT System Manager
Johannes TRUSCHNIGG (until 04/11)	IT System Manager
Herlind ECKHARDT	IT System Manager

GRAPHICS DEPARTMENT

Hannes TKADLETZ	Graphics Artist
Tibor KULCSAR	Graphics Artist

RECEPTION

Christian LAUFER	Receptionist
Christine SCALISI	Receptionist
Nadja MAYR	Receptionist

PURCHASING DEPARTMENT / STORE

Erwin KUNZ	Head of Purchasing Dept./Store
Brigitte LENZ	Deputy Head of Purchasing Dept.
Angela GANGLBERGER	Purchasing Agent
Caroline FORMANN	Purchasing Agent
Eva SCHARNER-KÖCK	Purchasing Agent
Jürgen Gerhard RUSCHER	Store Manager
Robert BLÜMEL	Store Clerk

COMMUNICATIONS

Evelyn DEVUYST	Communications Manager
Heidemarie HURTL (IMP)	Communications Manager

LIBRARY

Karlo PAVLOVIC	Head Librarian

PRIVATE FUNDING

Sabina TANDARI	Head of Private Funding
Sylvia WEINZETTL	Fundraising Assistant
Renate OTTO	Team Assistant

CAFETERIA

Michael ENZBERGER	Chef de Cuisine
Taoufik BAHMOU	Chef
Wolfgang BRAUN (until 06/11)	Chef
Mohamed MOUELHI	Chef
Joachim LINS (until 08/11)	Pastry Chef
Shirli SEKER	Pastry Chef
Katrin HUPF	Buffet
Helga MEHOFER	Buffet
Sabine SMALCL	Buffet
Hanna AHARODNIKAVA	Kitchen Assistant
Nataija BERTA	Kitchen Assistant
Asuman CAKAR	Kitchen Assistant
Güler CICOS	Kitchen Assistant
Lugano MWAKILEMBE	Kitchen Assistant
Selma YILDIRIM (until 08/11)	Kitchen Assistant
Semra YILDIRIM	Kitchen Assistant

FACILITY MANAGEMENT

Alexander CHLUP	Head of Facility Management
Michael KRATOCHWILLE	Deputy Head of Facility M.
Nadir AYKUT	Maintenance Technician
Christian DAVID	Maintenance Technician
Patrick GUST	Maintenance Technician
Gerhard MÜLLER	Maintenance Technician
Wolfgang RACHOLZ	Maintenance Technician
Martin ROJDL	Maintenance Technician
Alexander TRIEB	Maintenance Technician
Stefan TRINKER-GRONAU	Maintenance Technician

WORKSHOP

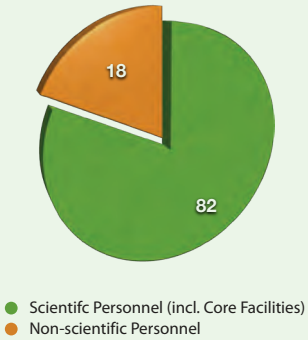
Martin COLOMBINI	Head of Workshop
David KUMMERER	Deputy Head of Workshop
Vladimir KOLCAR	Workshop Technician
Michael ZIEGLER	Workshop Technician

DISH WASHING

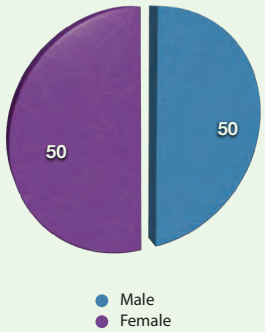
Renate WÖHRER	Head of Dish Washing
Aysel AYKUT	Technical Assistant
Sultan CAN	Technical Assistant
Vesna DJORDJEVIC (until 06/11)	Technical Assistant
Nuray KILIC	Technical Assistant
Bettina RADICH	Technical Assistant
Renate STIX	Technical Assistant
Norbert DEMETER	Technical Assistant

IMBA Key Facts *(Data as of November 2011)*

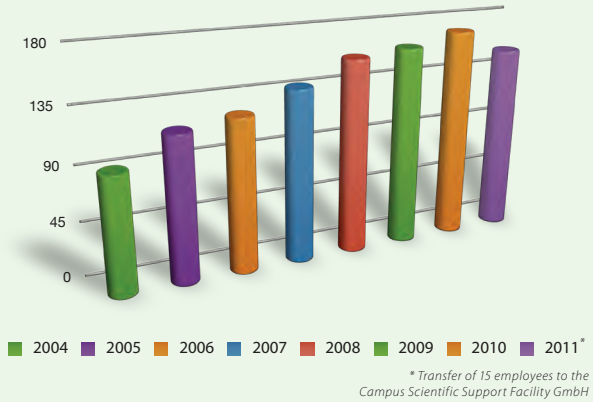
IMBA Staff (in %)



IMBA Staff - Gender Distribution (in %)

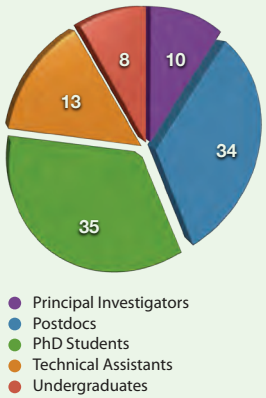


IMBA Staff - Development (Headcount)

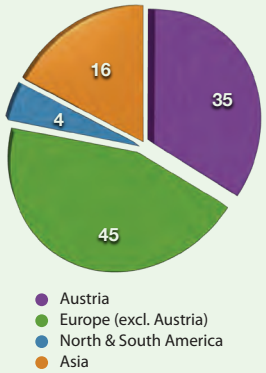


** Transfer of 15 employees to the Campus Scientific Support Facility GmbH*

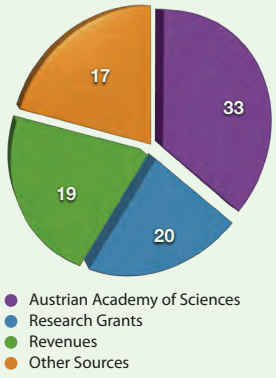
Scientific Staff - Functions (in %)



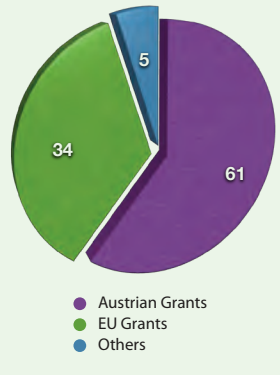
Scientific Staff - Nationalities (in %)



IMBA Budget 2011 (in %)



Public Research Grants 2011 (in %)



Sponsors & Partners

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

Funding Partners and Public Sponsors

BMWFJ
BMWF
Boehringer Ingelheim
EMBO *
ESF
European Research Council
European Union
FEBS *
FFG
Fondation Leducq
FWF
GEN-AU
HFSP
LISA
Marie Curie
ÖAW
OeNB
Stadt Wien
Wirtschaftsagentur Wien
WWTF
ZIT

** individual fellowships*

Major Private Sponsors *(in alphabetical order)*

We thank all our donors and sponsors for their valuable contribution, especially for the major donations from:

Special Thanks to DEBRA Austria

debra
Hilfe für die Schmetterlingskinder.

for their generous support of the Research Project "Development and genetic repair of induced pluripotent stem cells in RDEB".

Special Thanks to Dr. Ludwig Scharinger

Raiffeisen Landesbank Oberösterreich

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*

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.

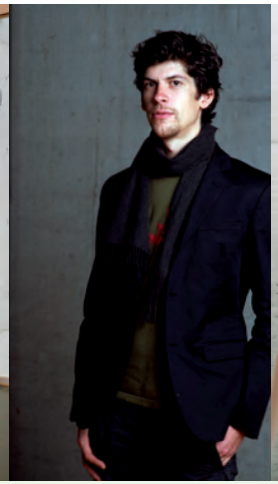
Vienna – a City of many Facets

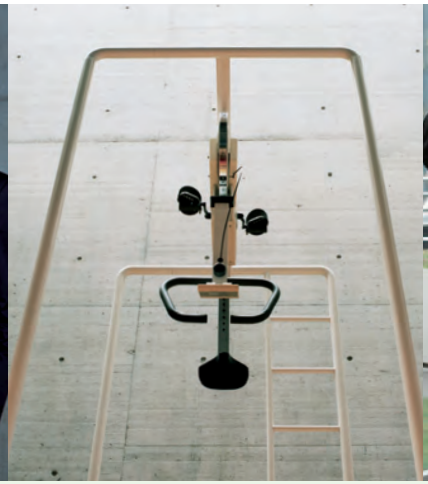
For those whose interests stretch beyond science, Vienna also has a lot to offer. Home to about 1.7 million people, the city is the political and cultural center of the country and its gateway to the east. Once the heart of the largest European empire, Vienna draws on a rich cultural heritage which is 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.

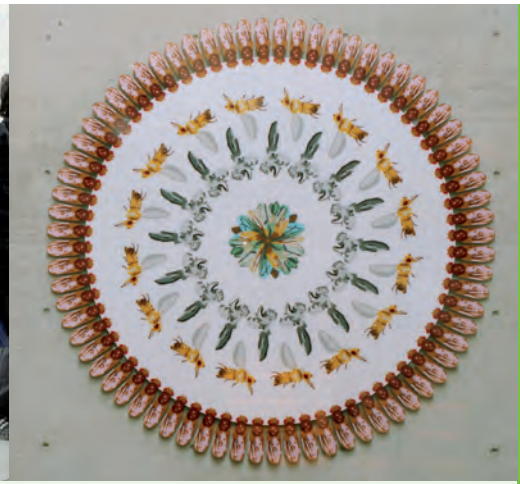
And if your brain needs to be refreshed, there is always the call of nature. A day away from the lab may take you skiing to the nearby Alps, windsurfing at Lake Neusiedl or watching rare birds in the Seewinkel. Even within the borders of Vienna you can enjoy a hike through dense woods, go canoeing in 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.

"The unexpected result of an euphoric relationship" Ian Deschka





"please leave quietly" Lukas Troberg & Erwin Wurm



Mandalas

"Genetics & Art - A Symbiosis"

End of 2010 IMBA and the University of Applied Arts in Vienna realized a joint project. Inspired by science topics and impressions gained in discussions with researchers from IMBA, young art students, including some from the Erwin Wurm master class, designed a total of eighteen projects as part of a competition. Four of these projects have been realized at IMBA. The competition was jointly organized by IMBA and the University of Applied Arts in Vienna, the project aiming to promote the interaction of science and art. Internationally renowned Austrian artist Erwin Wurm was also on the jury; "Science can serve as a source of inspiration, my concern is to extend perceptions and foster interest in discovery on both sides," he says.
The art works were created in direct response to impressions left on the artists by the topic of genetics and the IMBA institute and its researchers, an extended visit to IMBA granting students an initial insight into the work of a research institute. Individual topics were discussed and expanded in personal conversations with scientists. Rector of the University of Applied Arts in Vienna, Gerald Bast was one of the two initiators of the project. His aim is to bring science and art closer together and he was pleased that private sponsors were found to implement some works of art. He says: "It is only through the realization of the project that these works become public, where as if they remain drafts, it's just art for the archive."
The four works of art are a twelve-meter high installation, three giant mandelas with plant and animal motifs, vector graphics developed from 'found' organisms and a provocative logo which can be read in the winter garden at IMBA. Implementation directly in the research building means art has become part of the daily working environment of the IMBA staff, the scientists seeing the new works as an inspiration and impetus for discussion. IMBA's director Josef Penninger who was the second initiator and committed supporter of the project thinks: "We researchers are intensively searching for meaning behind the results of our research and attempt to critically question any claims of absolute truth. I therefore believe it is important to bring art to an institute like this. I want people to occasionally stop and consider what the objectives of our actions actually are."
As in the arts, so too in science and particularly in fundamental research, the path to knowledge is not linear and the objective can often not be reached directly. Josef Penninger thus sees a symbiosis between the two disciplines of genetics and art; "Good science is like good art, it's about raising unexpected questions, identifying relationships beyond the obvious, and developing completely new ideas."

Kay Walkowiak
please leave quietly, 2010
Mixed Media Installation, 1200 x 150 x 120 cm
(see also p. 33)
Leander Schönweger
Mandalas, 2008/10
Diasec
Ian Deschka
The unexpected result of an euphoric relationship, 2008/10
36 vector graphics
Lukas Troberg
WHAT IF GOD WAS WRONG, 2008/10

All four works of art were donated by Liqun Zhang and family in memory of Limin.

VBC SPRING CONCERT

This years' VBC Spring Concert took place on March 23. The first half of the concert featured three piano duets, several solo performances on cello and piano, and was rounded off with a Neapolitan love song, two Turkish folk songs and two jazz pieces. The second half featured the "MolBio Orchestra" which performed classical pieces, including Mozart's Clarinet Concerto in A major and the Waltz No. 2 by Shostakovich, followed by modern pieces including the Overture from "Phantom of the Opera" and a medley from "Indiana Jones".

AN EVENING OF SHORT PLAYS

The Vienna Biocenter Amateur Dramatic Club invited all VBC employees to attend the show, "Smorgasbord", on May 4 and 5. The theatrical banquet of four short plays included "The Proposal" and "The Boor" by the Russian playwright Anton Chekhov, "Press Cuttings" by the Irish playwright George Bernard Shaw and "Die Panne" by the Swiss playwright Friedrich Dürrenmatt.

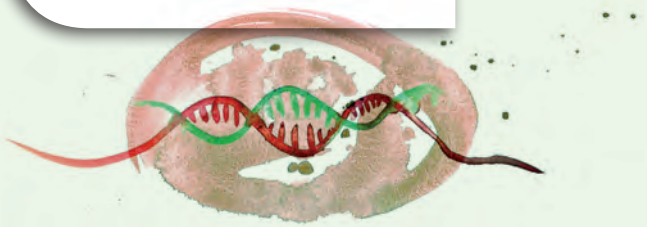
VBC SUMMER SCHOOL

For the second time this year, the VBC Summer School provided the opportunity for undergraduate students to spend ten weeks at one of Europe's leading research centers. From June 23 to August 29, twenty-six students representing nineteen different countries worked on their assigned research projects in one of the labs at IMP, IMBA, GMI and MFPL. The program was accompanied by a series of lectures and many social activities. Again, the Summer School was a tremendous success and concluded with a scientific mini-symposium in which each student presented his or her results and competed for an award.

March 11 **May 11** **June 11** **July 11** **August 11**

MICROSYMPOSIUM ON SMALL RNAS

The Microsymposium on Small RNAs has become a tradition: three days in mid-May that showcase the latest research and newcomers to the field. Since the Symposium was launched in 2006 by Javier Martinez, it has developed into one of the key meetings of the field, each year attracting hundreds of participants. The 6th edition, from May 16-18, 2011, featured keynote speakers David Bartel from MIT and Thomas Tuschl from Rockefeller, and numerous student and postdoc speakers, presenting their outstanding work. The 2012 edition of the Microsymposium will be held on May 21-23 in Basel, Switzerland.



VBC PHD RETREAT

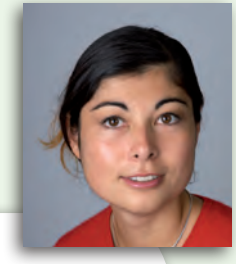
In the last week of June, 75 PhD students and three invited speakers met for a common/joint PhD Retreat in the southeast of Austria, near the Hungarian and Slovenian borders. The students presented their projects in a poster session and the speakers gave three entertaining, versatile and very interesting talks. Julie Cooper talked about her life as a group leader in the US and the UK and about her postdoctoral experiences in the labs of Tom Cech and Paul Nurse, two Nobel laureates. Thomas Höger explained how he ended up as the CEO and CFO of Apogenix, a small company that took a novel drug from the research lab through to clinical trials. Barbara Pauly who works as an editor for EMBO-reports gave an insight into the journals' side of publishing and into a typical day at the office of an editor.

"TWELFTH NIGHT"

On 24th and 25th August 2011, the Vienna Biocenter Amateur Dramatic Club performed its annual outdoor Shakespeare production, which was this year "Twelfth Night". Regarded by many as the finest of the Bard's comedies, this piece involves slapstick humor, wordplay, music and a large dose of anarchy to create a matchless atmosphere of mirth. The play took place on the lawn of the Campus Vienna Biocenter and thanks to the beautiful weather both the show and the barbecue afterwards were very well-attended. More than 100 people turned out for each performance.

IMBA TRIP

After a brilliant autumn with hot and sunny days well into October, IMBA was not so lucky with the weather for the annual trip this year. When the employees set out to climb Schneeberg on October 10, they were greeted by a fresh cover of snow on the mountain. The "Salamander"-train took the group as far up as the snow permitted. Next destination was the "Buchtelhütte", famous for their jam-filled sweet dumplings. Despite the rain, quite a few hikers set off for their descent on foot. The rest followed later by train. Following this adventure, the busses took the group to the castle of Gloggnitz. Fittingly to the merry atmosphere, Josef Penninger delivered an address that was very positive and optimistic. He thanked all members of IMBA for their work and gave an overview of the students who had finished their theses in the past year. He briefly recalled the recess and summarized the opinion of the SAB-members. The speech was followed by a "knight-style" meal served with rustic charm.



NEW IMBA GROUP LEADERS

Kikue Tachibana-Konwalski started her new group on 1 November. Since 2006 she worked as a postdoctoral fellow on cohesin in mammalian meiosis in the laboratory of Kim Nasmyth, University of Oxford, UK. At IMBA her lab aims to define the molecular events driving the mammalian oocyte-to-zygote transition and to understand how age-dependent deterioration of these mechanisms impacts fertility.

Fumiyo Ikeda started at IMBA on 1 December. She is a biochemist from Japan who co-discovered linear ubiquitination as a key intracellular signaling pathway. Fumiyo worked as a postdoctoral fellow in the laboratory of Ivan Dikic at the Goethe-University in Frankfurt, Germany. At IMBA she aims to dissect how ubiquitin networks control inflammatory responses by using various approaches from biochemistry techniques to genetically modified animal models.

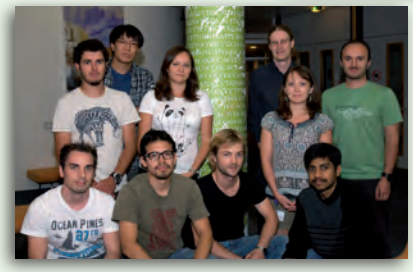
September 11 **October 11** **November 11** **December 11**

RECESS

From October 5-7 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, were once more impressed by the scientific performance and high standards of the research presented. IMBA would like to thank all its SAB members and the representatives of the Austrian Academy of Sciences for their tremendous support.
IMBA SAB members: page 42 in this booklet.

THINK ALTERNATIVE!

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 9th VBC International PhD Symposium was held on November 3-4, 2011. It was entitled "Think Alternative! Insights from Unconventional Model Organisms". The idea was to discover the opportunities and benefits that are offered by systems other than the classical genetic models, such as fruit flies, Arabidopsis or mice. The symposium covered a broad range of research topics, including regeneration, epigenetic inheritance, germline specification, development, evo-devo, neurobiology, behavior, and even paleogenomics.



Impressum

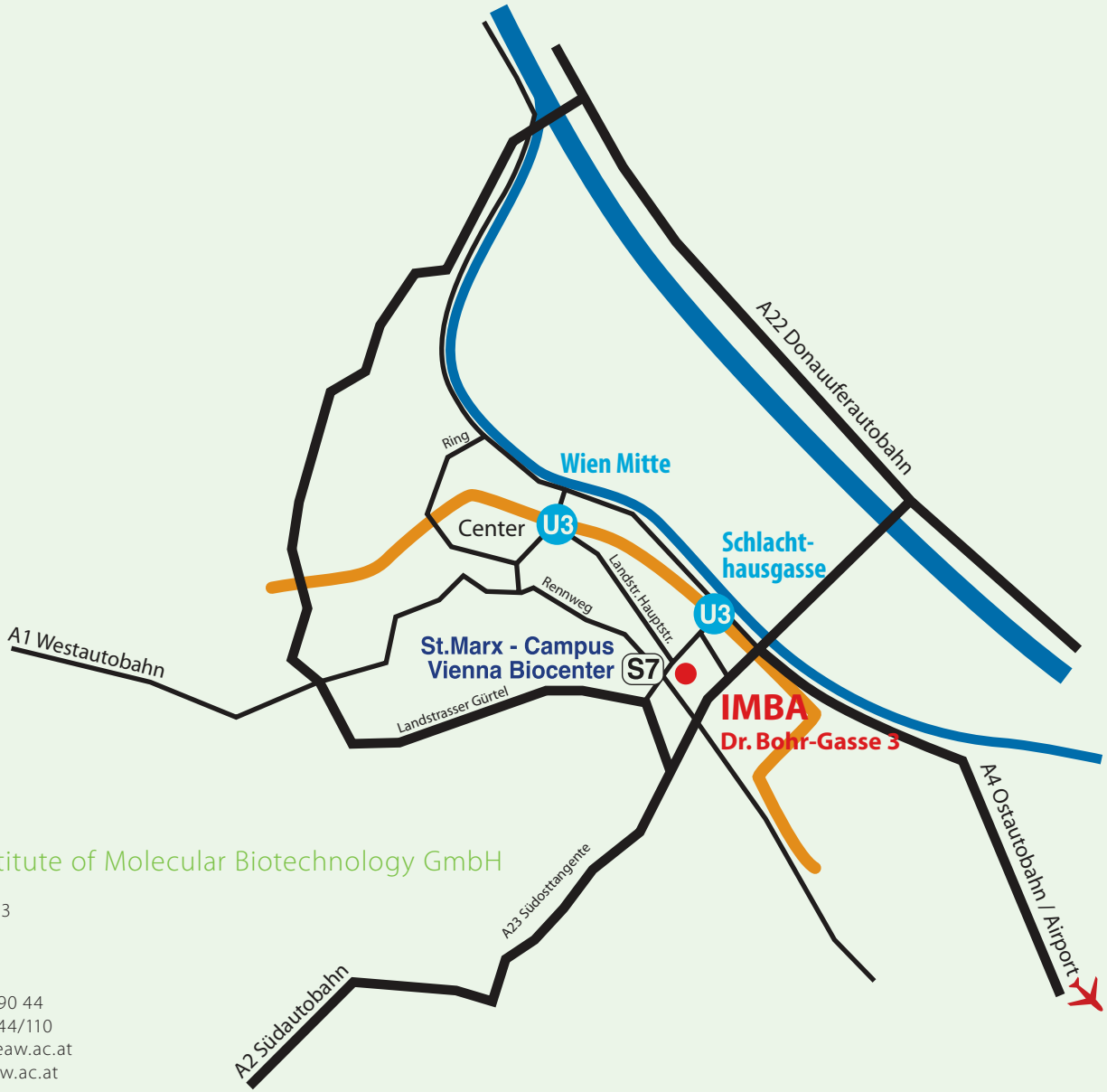
Published by
IMP-IMBA Communications Department

Project Management
Evelyn Devuyst

Layout & Design
IMP-IMBA Graphics Department

Pictures, Artwork & Credits
IMP/IMBA
IMP-IMBA Graphics Department
Karl Mayr (p: 49)
Hans Krist (pp: 2, 46)
Wolfgang Simlinger/MQ (p: 48)
VBC ADC (p: 50)

Printed by
Ueberreuter Print GmbH
Industriestraße 1
A-2100 Korneuburg



IMBA - Institute of Molecular Biotechnology GmbH

Dr. Bohr-Gasse 3
1030 Vienna
Austria

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

The Institute for Molecular Biotechnology (IMBA) is a basic research institute of the Austrian Academy of Sciences (Österreichische Akademie der Wissenschaften) and a member of the Campus Vienna Biocenter. IMBA and IMP operate in partnership as a joint initiative of the Austrian Academy of Sciences and Boehringer Ingelheim.