INSTITUTE OF MOLECULAR BIOTECHNOLOGY

OF THE AUSTRIAN ACADEMY OF SCIENCES

VIENNA BIOCENTER

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of Sciences

IMBA
Institute of Molecular Biotechnology
of the Austrian Academy of Sciences

CONTENTS

JOSEF PENNINGER
Managing Director/Science

When I started IMBA as the founding director I had many dreams, one of which was to set up a place, where the best scientists can follow their visions with the outmost financial and academic freedom, irrespective of any politics. With the help and support of many people in the Austrian Academy of Sciences, the City of Vienna, the Federal Government, our scientific colleagues at the campus, our partners at the IMP and Boehringer Ingelheim, and many others who believed in this vision we have indeed managed to build a place where it is fun to work and where science comes first and foremost.

In 2007, IMBA has had a tremendous scientific year. We have now become the largest research institute of the Austrian Academy of Sciences and our scientists have published work that has had a real impact in our world. For instance, Javier Martinez's group identified the first kinase ever that specifically acts on RNA and the group of Juergen Knoblich identified key genes that control stem cells and cancer growth.

Scientific excellence and world-renowned research centers are not defined by the quantities of publication but by the promotion of the best scientists and some key breakthroughs that can change scientific thinking. We have had always a high expectation. That we have actually reached this standard within the four years of our physical existence is simply marvelous. The real delight for me is to observe how the young scientists have grown in their own careers and have started to publish outstanding research papers. I am very proud to be part of such a great team.

For the first time we have had the pleasure to welcome our own scientific advisory board (SAB). The SAB members are Guenter Blobel, Ken Chien, Tony Hyman, Eric Kandel, and Susan Lindquist. The impact of the SAB has been immediate. Their input and advice have already brought a new quality to our institute and also the responsibilities to myself and all members of IMBA to create a world-class centre of excellence. I therefore wanted to thank our SAB members for their commitment and believe that IMBA is worth their most precious possession – their time to visit, their time to talk to our scientists, and their time to advise us how we could get better.

Midway on our life's journey, I found myself
In dark words, the right road lost. To tell
About those woods is hard – so tangled and rough
Canto I, Inferno, Dante (translation by Robert Pinsky)

During the last 4 years, since IMBA exists, we have indeed experienced some rough moments. In particular, during the construction of our new research institute and development of the faculty we have had some intricate and unexpected moments. Sometimes it has been a difficult road that only recently became entirely untangled. Even if our experiments and experiences are often tangled and rough, "we must never feel disarmed: nature is immense and complex, but it is not impermeable to our intelligence; we must circle around it, pierce and probe it, look for the opening or make it." (Primo Levi – The Periodic Table).

With humility and gratitude I acknowledge the multiple contributions of some people without which we would not be even close to our current state of affairs. First, I need to thank the IMBA supervisory board, the members of the Austrian Academy of Sciences who have guided us with wisdom through the intricacies of Austrian laws, finances, and science funding and, of course, the people from the local and federal funding agencies. Finally, I wanted to give my personal thanks to the former Prime Minister of Austria Wolfgang Schuessel, the former Minister of Finances Hannes Androsch, Maximilian Eiselberg, Carl Djerassi, the Austrian-born father of the birth control pill, and Prince Max von und zu Lichtenstein for their support of our institute.

We at IMBA live and promote a culture of honest exchange and mutual respect between all employees. End results are of course important. However, ends do not necessarily justify all means to get there. Everybody at the institute and every project we decided to support is important. As scientists we have been given the unique opportunity to maybe chance the world to a better place. We live in one of the most exciting times of science with incredible opportunities to look behind the curtain of life and to maybe be able to create new medicines. Science writes incredible stories. IMBA has become and shall remain the fertile soil where such science can grow and flourish.

MICHAEL KREBS
Director/Finance and Administration

One of the major goals of the initiators of IMBA has always been to create a first class location where breakthrough science may evolve and where the most qualified people are given the opportunity to contribute to the future of biomedical research. In 2007, the first full year of operations in our novel laboratory and office environment, our vision has become true and the benefits of the new infrastructure for the working conditions and the internal communication have become fairly evident.

Probably the most critical milestone in 2007 has been the transfer of IMBA's interim animal facility at the University and the IMP mouse house. With this relocation, one of the major bottlenecks at the IMP-IMBA Research Center has finally been resolved. Sincere thanks are due to Alex Chlup, Michael Kratochwille and all the other people involved from facility management as well as Andreas Bichl and his animal house staff for their outstanding commitment and efforts during this turbulent transition period.

Last year, the IMBA and IMP management decided to join forces to establish an electron microscopy unit including the first cryo-electron microscope in Austria. Two new microscopes with a total investment of more than €2 million are now available for use by our research groups and for collaborations with other scientists in Austria and abroad. We would like to thank Günter Resch and his team for their excellent work in building up this highly sophisticated technology that will open up new ways to do research at IMBA and IMP. Thank you also to Thomas Marlovits, Vic Small and Jan-Michael Peters for their valuable scientific input and their financial contribution in the start-up phase.

The "Go Live" of the VDRC Webshop has been another very visible scientific achievement at the IMP-IMBA Research Center. The Drosophila RNAi library is now accessible to the whole scientific community either by ordering lines through a designated internet platform or by visiting as a guest scientist at IMBA to do RNAi screens in-house. We thank Krystyna Keleman, Reinhard Klug and the rest of the group for their tremendous endeavor to set up such a major initiative and for their day-to-day dedication to serve the VDRC's internal and external customers.

Some of the administrative service units such as the accounting, the purchasing and the IT departments are currently implementing new enterprise software at IMBA and IMP. Starting January, 2008, the new IT system will significantly change the way consumables and equipment is ordered at IMBA and IMP. But it will also provide timelier and much more transparent financial data for everyone. In this regard, we would like to thank Brigitte Weiser, Angela Ganglberger, Werner Kubina and Andreas Riepl for their excellent work and time commitment on top of their daily responsibilities.

In 2007, one major strategic step was the establishment of a fundraising campaign called "IMBA BioMed 2012". This campaign is targeted at raising money from individuals and private foundations in addition to our current €12 million budget which is mainly provided by public sources. One full-time employee and two part-time consultants were hired to support IMBA in the implementation of a professional fundraising process. We would like to congratulate Sabina Tandari and all the other people involved in this year's activities for the very promising start.

Last but not least, we would like to thank the Ministry of Science and Research and the Austrian Academy of Sciences, as well as all the project sponsors such as the City of Vienna, Austrian Science Fund (FWF), and the EU for their substantial financial support.

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 collaboration is known under the name of "IMP-IMBA Research Center".

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 PR agency, a non-profit scientific society and the Vienna Open Lab.

More than 1000 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.

Your Career at IMBA

IMBA offers exciting positions at all levels of your research training and career. If you consider joining IMBA, you will find first class research and state-of-the-art scientific services. As a member of one of the scientific groups, you will be part of a young, international team, using English as a working language. The atmosphere at IMBA is stimulating and focused, and a certain pioneering spirit can be felt among its staff. The brand-new state-of-the-art laboratory and office building was officially opened in 2006 and is now the inspiring home to about 150 scientists and administrative staff.

Graduate students join the IMBA through the Vienna Biocenter International PhD Program, run jointly with the Max F. Perutz Laboratories (MFPL), the 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 typically lasting 3-4 years.

IMBA's research groups are well funded to support a number of pre- and postdoctoral positions. A substantial travel budget allows scientists to take part in meetings, conferences and courses. IMBA and the IMP organize a couple of conferences, workshops and symposia every year. An intensive seminar program brings internationally renowned scientists to the institute on a regular basis.

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 parents with young children, two day-care facilities are available in the immediate neighborhood. For school-age children, Vienna offers a large range of different types of schooling, from public to private, German- or foreign language-speaking, traditional or with more experimental concepts.

Many of our new employees are accompanied by spouses who are themselves looking for a qualified position in line with their training. IMBA is certainly aware of this fact and can, in some cases, help with securing a job. We also support your efforts to learn German and sponsor language courses run by one of Vienna's best language schools as well as team sports.

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

JÜRGEN KNOBLICH

Asymmetric Cell Division and Proliferation Control in Neural Stem Cells

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Vivien Rolland [5] / PhD Student
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Elke Kleiner / Lab Manager
Noriko Nishimura / Technical Assistant
Angela Maria Peer [8] / Technical Assistant

Fellowships:
[1] FEBS, [2] HFSP, [3] EMBO, [4] Marie Curie
[5] since September, [6] since August, [7] since April,
[8] since July

Stem cells are essential for tissue maintenance and repair in our body. Although each stem cell has unique tissue specific properties, one feature is common among all stem cells: Stem cells have the unique ability to generate identical copies of themselves but at the same time can also give rise to more differentiated progeny that eventually replace cells in the target tissue. How one cell can generate two daughter cells of such dramatically different properties and how defects in this asymmetry can contribute to tumor formation, are the questions we are trying to resolve.

During *Drosophila* development, thousands of neurons arise from stem cell-like precursors called neuroblasts. Neuroblasts undergo repeated rounds of asymmetric cell division during which they form a large and a small daughter cell (Figure 1A). While the small daughter cell divides only once more into two differentiating neurons, the large cell continues to grow and proliferate in a stem cell-like manner. Why are the two daughter cells so different in their cell growth and proliferation properties ?

Stem Cell Tumors in *Drosophila*

The answer is that neuroblasts are capable of segregating key regulatory proteins into one of their two daughter cells during mitosis (Figure 1B,C). One of them is the tumor suppressor Brat (Brain tumor). We found Brat by mass-spectometry in a search for proteins regulating fly brain development. (Figure 2A). When neuroblasts divide, Brat localizes into a crescent overlying one of the two spindle poles, so that it is inherited by only one of the two daughter cells (Figure 1B). In the absence of Brat, cell growth and proliferation are no longer restricted to only one cell. As a consequence, both cells proliferate leading to an expansion of the neuroblast pool and the formation of a brain tumor that fills the whole body and kills the fly (Figure 2B). Thus, Brat is an important regulator of proliferation in *Drosophila* neural stem cells.

Brat is a member of a conserved protein family that is characterized by a similar domain structure (Figure 2A). Our results indicate that regulation of stem cell proliferation seems to be a common task of this protein family. We find that the Brat-like protein Mei-P26 regulates proliferation in stem cells of the *Drosophila* ovary. Ovarian stem cells depend on a signal coming from the surrounding stem cell niche. After division, one daughter cell looses niche contact and no longer receives the signal. After a few transit amplifying divisions, this cell upregulates Mei-P26. Like Brat, Mei-P26 inhibits cell growth and proliferation so that this cell will exit the mitotic cycle and undergo differentiation. In the absence of Mei-P26, all cells proliferate leading to the formation of an ovarian tumor. Thus, control of stem cell proliferation seems to be a common function of Brat like proteins.

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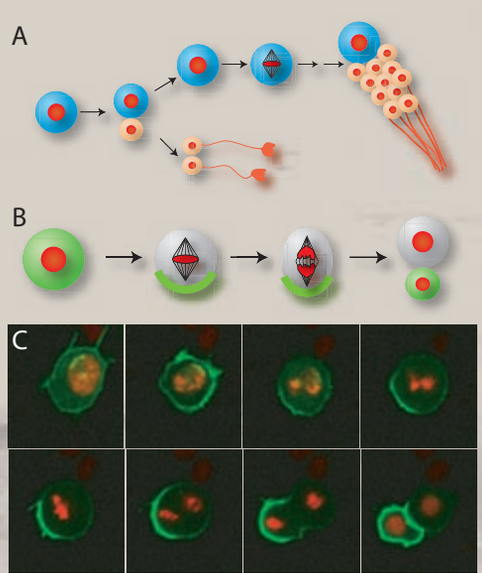

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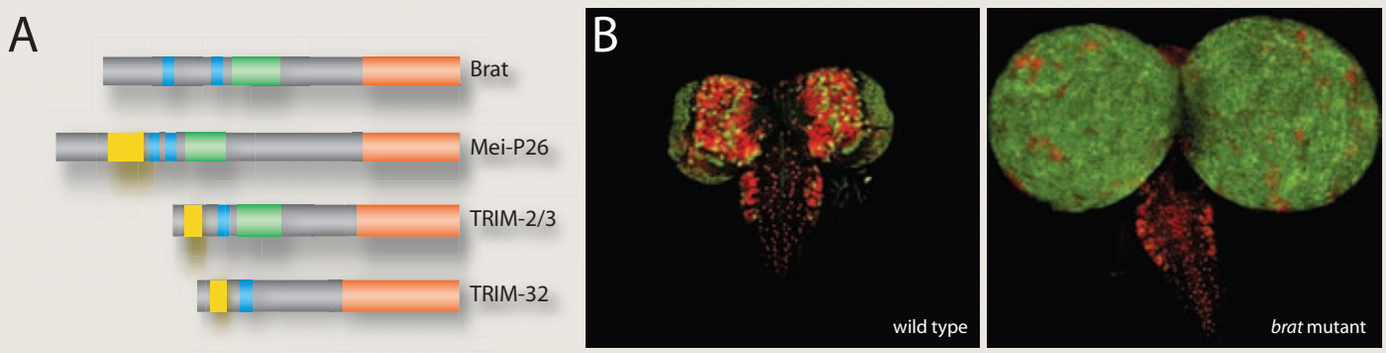

Figure 1: *How cells divide asymmetrically. (A.) Drosophila neuroblasts divide asymmetrically in a stem cell-like fashion. (B.) During each neuroblast division, Brat (green) localizes into a cortical crescent and is inherited by only one of the two daughter cells. (C.) Live imaging of asymmetric cell division. Stills from a movie (available on our website) of asymmetric cell division in the Drosophila peripheral nervous system. Histone-RFP (red, to visualize chromatin) and Pon-GFP (green, to visualize asymmetric protein segregation) are recorded.*

Figure 2: *Stem cell derived tumor formation in Drosophila. (A.) The Brat protein family. NHL domains are red, Coiled coil motifs green, B-boxes (Zn-finger-like motifs) blue and Ring fingers yellow. (B.) 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 3: *Analysis of progenitor cell proliferation in the mouse brain. Cross-section through the developing mouse neocortex (DNA in magenta) on day 15 of embryonic development. GFP (green) was introduced by in utero electroporation specifically into dividing progenitor cells one day before fixation. Cell bodies (P) and characteristic radial glia processes (R) of transfected progenitors as well as the neurons that have developed from them (N) are visible.*

Regulation of micro RNAs

How do Brat and Mei-P26 regulate proliferation? Using mass-spectrometry, we found the protein Argonaute-1 as a common binding partner of Brat and Mei-P26. Argonaute-1 is well known for its key function in the micro RNA pathway. Argonaute is part of the RNA induced silencing complex (RISC) that uses short double stranded RNA molecules to inhibit gene activity. Our genetic experiments show that Mei-P26 inhibits the activity of a large number of micro RNAs. In the ovarian stem cells, these micro RNAs are active, but when Mei-P26 is upregulated, their activity is compromised. Since micro RNAs are required for ovarian stem cell self renewal, this allows differentiation and ensures proper cell cycle control during *Drosophila* oogenesis. Thus, Brat/Mei-P26 proteins might use the micro RNA pathway to control stem cell proliferation.

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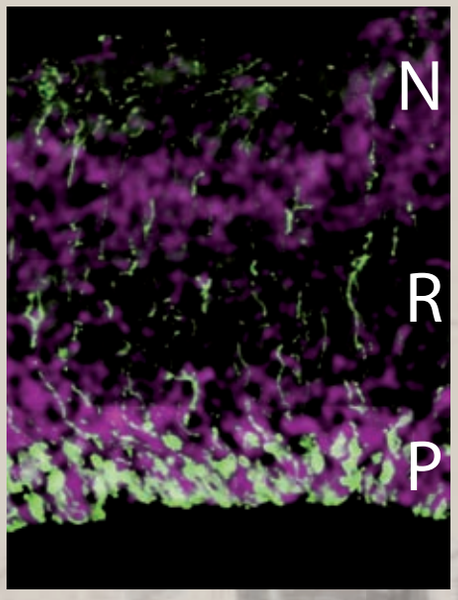
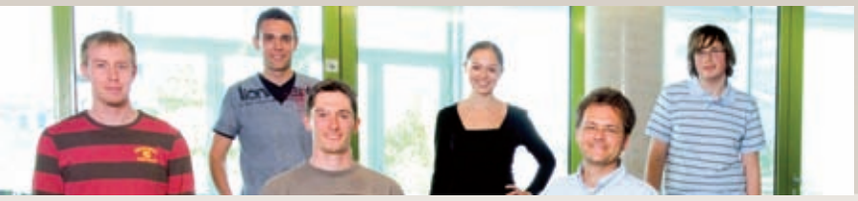


Proliferation control in *Drosophila* and mouse stem cells

To identify the genes that regulate stem cell proliferation, we use a combination of genome wide screens in *Drosophila* and transient transgenic mouse techniques. We have used a library of transgenic flies expressig hairpin RNAi constructs for essentially every gene in the fly genome (generated by Barry Dickson) to screen for genes affecting asymmetric cell division in the *Drosophila* peripheral and central nervous systems and identified a number of new genes affecting these important biological processes. Most of the genes are conserved and to characterize their mammalian homologs, we use in utero electroporation into the mouse brain. For this, DNA is injected into the ventricle of the developing brain and electroporated specifically into the dividing progenitor cells that line the ventricular surface of the brain. By using RNAi and overexpression constructs together with GFP markers, we can study the gain and loss of function effect of candicate genes on proliferation and differentiation pattern of mous neural progenitor cells. So far, these experiments have revealed a striking functional conservation of Brat-like proteins in regulating stem cell proliferation. Ultimately, we will transfer our knowledge to adult stem cells to understand, how stem cells control proliferation and lineage in our body and how these processes are deregulated in tumor development.



THOMAS MARLOVITS
Design and Function of Molecular Machines

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Molecular machines are essential for maintaining life at a cellular level. We are aiming to understand the fundamental molecular design, assembly processes, and mechanistic details of such higher-ordered structures.

Host-Pathogen-Interaction

Gram-negative pathogens, like *Salmonella, Yersinia*, or *Shigella*, use the type III secretion system (TTSS) to initiate infection in eukaryotic cells. The TTSS is a complex macromolecular system that serves as a structural platform to make physical contact between the host cells and pathogens and mediates the unidirectional transport of bacterial toxins (effector proteins) into eukaryotic cells. These systems are essential for a successful infection resulting in well-known clinical symptoms ranging from mild headaches and diarrhea to even life-threatening diseases such as typhoid fever or bubonic plaque.

The Molecular Design

Made up of more than twenty proteins, TTSSs assemble into large "molecular nanomachines" composed of a set of soluble proteins as well as membrane proteins. All of the structural components along with other proteins involved in the step-wise assembly process and function are encoded on specific pathogenicity islands (Figure 1A).
The most prominent substructure of the TTSS is known as the "needle complex", a cylindrical, needle-shaped and membrane-embedded organelle protruding from the bacterial envelope (Figure 1). The needle complex is believed to serve as a conduit for a safe transport of virulence factors from the bacterial cytoplasm through a number of natural barriers into eukaryotic cells. In *Salmonella typhimurium*, which serves as our model for bacterial delivery systems, this complex is formed by multiple copies of only five proteins: PrgH, PrgK, and InvG build up the membrane associated base-structure, PrgJ, the inner rod, and PrgI, the needle filament extending into the extracellular environment (Figure 1E and 2D).

In order to investigate the molecular mechanism of type III secretion, we first set out to determine structural components of the TTSS. We were challenged by the megadalton size of the complex, its natural composition (membrane and soluble proteins), and its limited availability. Nevertheless, we were able to purify sufficient amounts of the entire 'needle complex' and its precursor, the 'base', by a combination of detergent extraction and size separation by velocity gradient centrifugation. A detailed structural analysis by three-dimensional electron cryo microscopy and single particle analysis finally revealed that several rotational symmetries or oligomeric states are present in the population of the 'needle complex' and the 'base'. Whether all of these complexes have a physiological role remains an open question.

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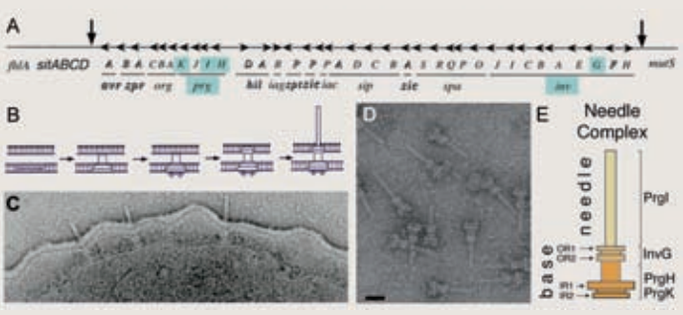

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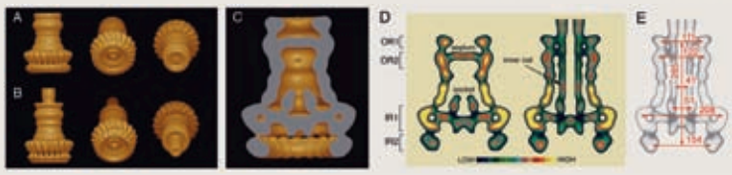

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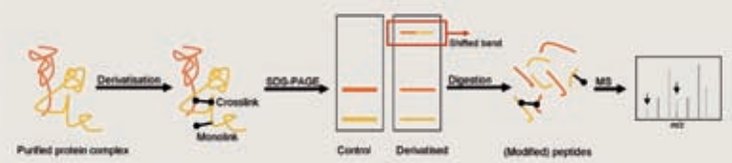


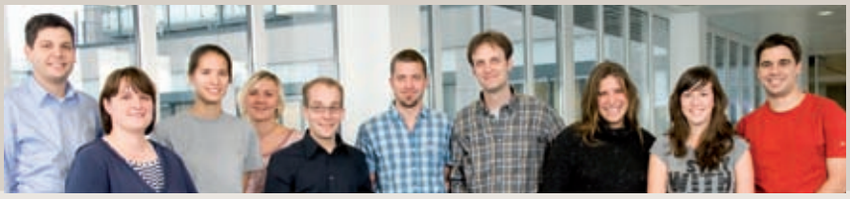
Figure 1: ***The type III secretion system*** *(A) The type III secretion system is located on the bacterial chromosome but encoded on a specific pathogenicity island. Structural proteins of the needle complex are indicated in blue color. (B) Formation of stable intermediate substructures during assembly. (C) Electron micrograph of osmotically shocked S. typhimurium showing the needle complex embedded in the bacterial envelope and released after detergent treatment (D) bar: 100 nm. (E) Schematic representation of the Salmonella needle complex and its components. PrgH, PrgK, and InvG make up the membrane embedded base structure, whereas PrgI forms the helical filament protruding into the extracellular environment. The inner rod (see Figure 2D) anchors the filament into the base.*

Figure 2 : ***The structure of the base and the needle complex****. Surface renderings of the base (A) and the needle complex (B) show that their overall shape is quite similar. However, tilted views indicate that during the assembly, individual domains must undergo large structural rearrangements. (C) The inside of the base reveals an internal structure (socket), which serves as a docking site for the inner rod. (D) Contoured longitudinal sections show the overall protein density distribution present in the base and the needle complex. (E) Key dimensions are given in Angstroms.*

Figure 3 : ***Mass spectrometry of derivatized complexes****. Isolated complexes are chemically derivatized and analyzed by SDS-PAGE and mass spectrometry. The determination of cross-linked peptides of shifted bands indicate positions near or identical to the interaction epitopes within a complex.*

Our analysis revealed a new structural component, the inner rod, which is located in the center of the needle complex (Figure 2). It (1) extends the secretion path from the base into the needle filament, and (2) serves also as an anchor to stably connect the needle filament into the base. During assembly, the inner rod and the needle filament are added as new structural components to the base. As a consequence, it must undergo large conformational rearrangements, which demonstrates the flexible but also stable property of the base. While functionally, this dynamic behavior is a crucial event during the assembly phase, in which the secretion machine is reprogrammed to become competent for the secretion of virulence factors, structurally, it underlines the importance of specific interaction epitopes critical for the assembly into a functional unit. To this end, we are currently performing high-resolution mass spectrometry of chemically derivatized complexes, which bears the potential to obtain detailed information of protein topologies within macromolecular complexes (Figure 3).

Although the design of the TTSS appears to be conceptually simple, structural characterization of the needle complex is at an early stage, leaving many questions unanswered: What nucleates the assembly of the TTSS? How are the individual proteins organized in the TTSS? How dynamic is the entire assembly process? How does the export machinery interface with the needle complex? And what determines the substrate specificity for protein secretion? We have begun to address some questions in the future, and we hope that by understanding the molecular mechanism of TTSS-mediated protein transport, we may provide the basis for the development of novel, therapeutic strategies to either inhibit its activity or modify the system for a targeted drug delivery.



JAVIER MARTINEZ

RNA Silencing and Processing in Mammalian Cells

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Andrea Salvá Giró / Diploma Student
Jutta Dammann / Technical Assistant
Anne Færch Nielsen / Visiting student (May-July)

RNA silencing phenomena keep fascinating biochemists and bioinformaticians. What is the reason for such a passionate attraction? First, RNA silencing is one of the most elegant molecular mechanisms that have evolved to regulate gene expression. We know much; literature accumulates every week, but many mysteries still remain to be solved. Secondly, tiny as they are, small RNAs are key players in the RNA silencing game where they regulate a large portion of the genome and impact a wide repertoire of cellular processes, including development and cancer. The interest in small RNAs reaches Biotech companies, reflecting the amazing potential of small RNAs as powerful gene-therapy agents. Our laboratory focuses on the molecular mechanisms underlying RNA silencing. We have revealed that microRNA (miRNA) expression can be regulated at the post-transcriptional level. We are currently analyzing mRNA targets of a panel of miRNAs that we found up- or down-regulated during tumor progression and metastasis. In addition, we are investigating the role of the RNA-kinase hClp1 in mRNA 3' end processing and in general RNA metabolism.
What follows, is a short description of three new projects that have been developed during 2007.

The impact of target site accessibility on siRNA efficiency and miRNA function

Despite their fluctuating efficacy, short interfering RNAs (siRNAs) are widely used to assess gene function. This limitation could in part be ascribed to variations in the assembly and activation of RISC, the RNA-Induced Silencing Complex. Downstream events in the RNA interference (RNAi) pathway, such as target site accessibility, have so far not been investigated extensively. We performed a comprehensive analysis of target RNA structure effects on RNAi using RNAplfold, a local folding algorithm, which recapitulates important events in RISC-mediated target recognition and cleavage. When combined with previously reported criteria for the design of potent siRNAs, the computation of target site accessibility by RNAplfold improves significantly the prediction of effective siRNAs and builds up the center of a freely accessible siRNA-design platform called RNAxs (http://rna.tbi.univie.ac.at/cgi-bin/RNAxs). We calibrated our method on two large datasets (consisting of 474 siRNAs) and tested it on an independent set of 360 siRNAs targeting four different genes (human cyclophilin B is shown in Figure 1). In addition, this approach provides adjustable parameters for targets with unusual properties and supplies the user with a sufficient number of siRNAs ranked according to their overall performance. Taken together, RNAxs represents a major advance in rational siRNA design, resulting in a significant increase of true positive candidates. We are currently investigating mRNA secondary structure effects on miRNA binding using RNAplfold combined with *in vivo* experiments in *Drosophila*.

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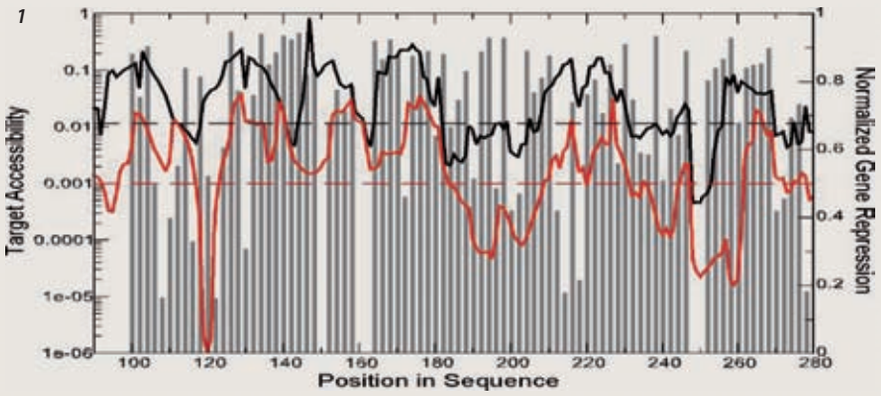

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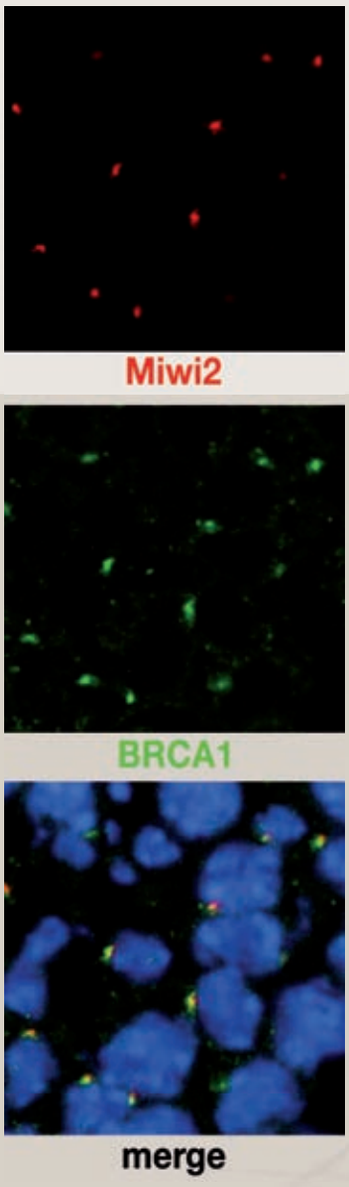

Role of Miwi2 in meiotic sex chromosome inactivation

Core components of the RNA silencing pathway, Argonaute proteins have diversified into two subfamilies, ubiquitous Argonautes and germline-specific Piwis, termed "Miwis" in mice. Miwi2-deficient mice are male-sterile, without any other apparent phenotype. Through immunolocalization studies, we found Miwi2 localized to a specific domain at the nuclear periphery of early meiotic spermatocytes, known as sex body or XY body. This structure forms during the process of meiotic sex chromosome inactivation (MSCI) and contains heterochromatinised and transcriptionally silenced sex chromosomes. Successful completion of this process is essential for progression of meiosis and male fertility. Known players in MSCI are BRCA1 (a factor involved in DNA repair) and ATR protein kinase, which is recruited to sex chromosomes by BRCA1. ATR then phosphorylates H2AX histone of unsynapsed chromatin, thereby establishing gH2AX, the first mark of heterochromatin and transcriptional silencing. We found Miwi2 co-localized at chromosome X with BRCA1, but not with gH2AX (Figure 2). Moreover, Miwi2 is expressed much prior to other sex body markers, already in late spermatogonia and pre-leptotene spermatocytes. These results suggest the involvement of Miwi2 in the initiation of meiotic silencing of unsynapsed chromatin and sex body formation, and therefore make it a potentially very important factor in male fertility.

Finding elusive enzymatic activities in the human tRNA splicing pathway

Having identified hClp1 as a key enzymatic activity of the human transfer-RNA (tRNA) splicing pathway, we now aim to isolate two still elusive components of this pathway, the cyclic phosphodiesterase (CPD) and tRNA-ligase activities. Scientists have been searching for the tRNA ligase for more than 30 years without success. We want to tackle its identification via a novel approach, i.e. identifying the CPD activity first. Having the CPD in hands, we will attempt to co-immunoprecipitate the tRNA-ligase activity if both proteins interact. If such interaction does not occur, we will fractionate HeLa extracts and assay for tRNA ligase activity in the presence of recombinant CPD, to generate a perfect substrate for the tRNA ligase. Using a bioinformatical approach, Alex Schleiffer (IMP) identified 7 human candidates with CPD domains homologous to the one displayed by the yeast tRNA ligase. Upon knocking down one of these candidates, tRNA maturation was significantly impaired. We are currently confirming this candidate as the CPD activity in the human tRNA splicing pathway.

Figure 1: Functional class distribution of siRNAs and mRNA accessibilities. A test panel of 180 siRNAs, targeting every other nucleotide of a region within the human cyclophilin B gene, is compared with target site accessibility values for the respective regions. SiRNAs are plotted such that each x-axis tick-mark represents an individual siRNA.

Figure 2: Miwi2 colocalizes with BRCA1 in the nuclei of meiotic spermatocytes. Immunohistochemistry was performed on frozen testis tissue sections. Mice were sacrificed 13 days after birth.

KAZUFUMI MOCHIZUKI

RNA-directed DNA Elimination in *Tetrahymena*

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An onion has more than 12 times as much genome as a human does. Does this mean the onion is more complicated and more intelligent than we are? Perhaps yes, but this presumably means an onion has more "junk" DNA than a human has. If some DNAs are useless, why doesn't an onion discard them? Actually, several creatures do discard such DNAs during their development. Our group is attempting to find out how one such intelligent creature, Tetrahymena, dumps useless DNA.

During sexual reproduction, *Tetrahymena*, a ciliated protozoan, eliminates useless and even harmful DNAs from its nuclei, instead of continuing to silence such, as do other eukaryotes. How do they precisely recognize "useful" and "useless" DNA? In recent years, we revealed that *Tetrahymena* uses small RNA of about 28 nucleotides to identify the useless DNA for elimination. This small RNA is produced by an RNAi-related mechanism and induces formation of heterochromatin, a typical form of silenced chromatin. Thereafter, *Tetrahymena* eliminate the heterochromatins for "forever" gene silencing. Thus, the DNA elimination in *Tetrahymena* is a unique system to study two gene silencing mechanisms, RNAi and heterochromatin. Our study focuses on the molecular mechanisms linking the RNAi, heterochromatin and the DNA elimination. Links between RNAi and heterochromatin have also emerged in many other eukaryotes. Thus, this study should provide us important insights not only into the DNA elimination in a curious microbe but also into how chromatin-level silencing is epigenetically directed by tiny RNAs in general eukaryotes.

Each *Tetrahymena* cell (Figure 1) has a macronucleus (Mac) and a micronucleus (Mic). Mac is polyploid and is transcriptionally active, while Mic is diploid and is transcriptionally inert during vegetative growth. Mic has the ability to undergo meiosis during the sexual process of conjugation and to form both new Mac and Mic for the next generation. After the new Mac forms, the old, parental Mac is destroyed. During development of the new Mac, massive DNA elimination occurs. About 15% of the Mic genome, called internal eliminating sequences (IESs), is eliminated from the developing Mac. The DNA elimination sites are healed by re-ligation of the flanking macronuclear-destined sequences (MDSs). IESs range in size from ~0.5 to 20 kb and are mostly moderately repeated in Mic. Many IESs are probably derived from transposons or viruses. Thus, IES elimination could have evolved as a defense system against transposons invaded in the Mic genome by eliminating those potentially harmful DNAs.

Our recent studies revealed that an RNAi-related mechanism was involved in DNA elimination. Dcl1p, a Dicer family protein, and Twi1p (Figure 2), an Argonaute family protein, are required for DNA elimination. Both Dicer and Argonaute family proteins are known to be involved in RNAi-related gene silencing events in many eukaryotes. ~28nt siRNAs (named scnRNA) are specifically expressed during

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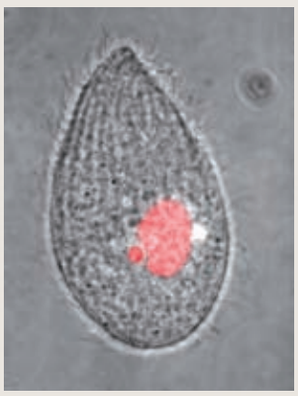

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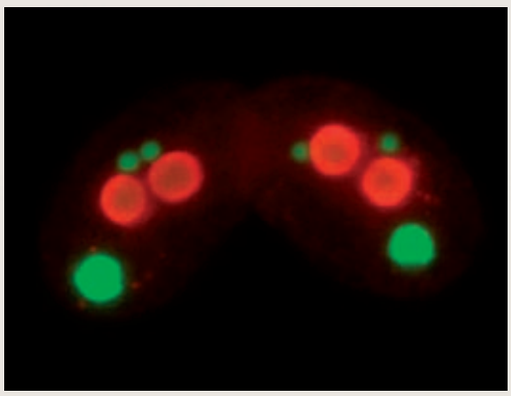

Figure 1: *Nuclear Dimorphism of Tetrahymena. Tetrahymena thermophila have two different nuclei (stained red), smaller micronucleus (Mic) and larger macronucleus (Mac), in a single cell.*

Figure 2: *Localization of Twi1p. Twi1p (stained red) and DNA (stained green) were localized in the new Mac development stage. Twi1p was observed only in new Macs.*

Figure 3: *DNA Elimination is Controlled by an RNAi-related Mechanism. Left: Localization of the proteins involved in the genome rearrangement. Middle: Putative localization of scnRNA. Right: A model that explains how DNA elimination is epigenetically controlled by RNAi-related mechanism. First, the entire Mic genome is transcribed bi-directionally in early conjugation by RNA polymerase II. These transcripts then form dsRNAs that are processed to scnRNAs by Dcl1p. The scnRNAs then accumulate in the cytoplasm and make complex with Twi1p. Next, the scnRNA-Twi1p complex transfers to (old) Mac where we propose that those having a homologous Mac DNA sequence are degraded. As a result, only scnRNAs homologous to Mic-specific (IES) sequences remain in the old Mac. Finally, these scnRNAs move (in association with Twi1p) to the developing new Mac. There, the sequences homologous to the scnRNAs are identified as IESs and are targeted for methylations of histone H3 Lys-9/Lys-27 and for accumulation of Pdd1p. Finally, these heterochromatinized sequences are eliminated.*

conjugation. Dcl1p is required for making scnRNA from the Mic transcrips. Twi1p is associated with scnRNAs and is required for their accumulation.

Other evidence suggests that heterochromatin formation is dispensable for the DNA elimination. Like the heterochromatins in other eukaryotes, methylated histone H3 on lysine-9 (H3K9me) and lysine-27 (H3K27me) residues are enriched on the eliminating IESs and the histone methyltransferase Ezl1p, which is responsible for these modifications, is required for DNA elimination. Moreover, a chromodomain containing protein Pdd1p, which specifically binds to both H3K9me and H3K27me, is also accumulated on eliminating IESs and is essential for DNA elimination. In other eukaryotes, the chromodomain protein HP1/Swi6 specifically interacts with H3K9me and is also enriched on heterochromatin. Thus, the DNA elimination in *Tetrahymena* and the heterochromatin formation in the other eukaryotes are evolutionally related.

RNAi-related mechanism is required for formation of heterochromatin-like state on IESs because disruption of *TWI1* gene causes loss of H3K9me and H3K27me. Thus, RNAi, heterochromatin and DNA elimination are linked. From these studies, we proposed a model to explain how IESs, lacking any consensus sequences, are recognized during Mac development by an RNAi-related mechanism (see Figure 3 for details).

Our long-term goal is to understand how RNAi induces heterochromatin and how heterochromatin induces DNA elimination. We are attempting to understand how the known "players" (scnRNA, Dcl1p, Twi1p, Ezl1p, H3K9me, H3K27me and Pdd1p) act individually and communicate to each other. We intend to identify novel players involved in the genome rearrangement by biochemistry, genetics and molecular biology techniques.

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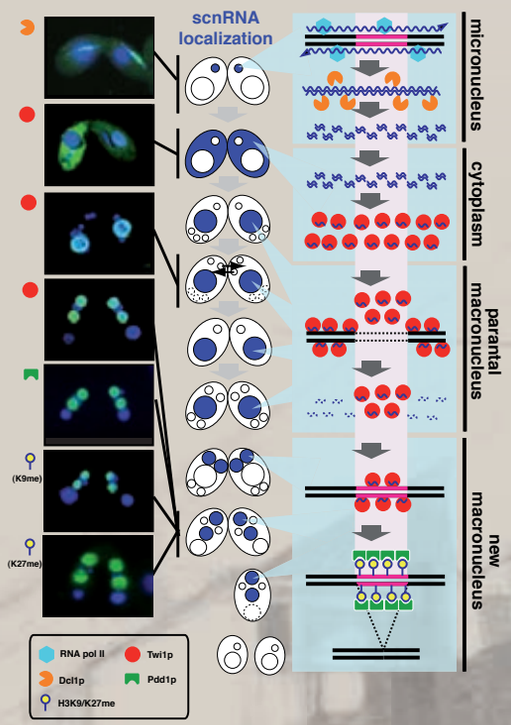

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Novel Modulators of Cancer

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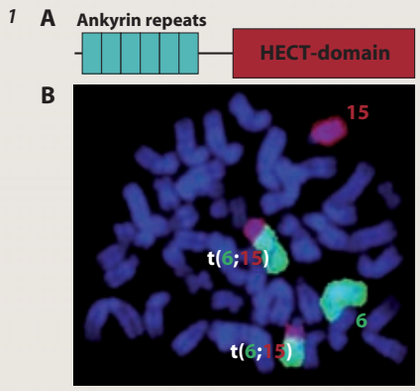
[3] FP6 EuroThymaide,
[4] JDRF fellowship,
[5] Marie-Curie RTN IMDEMI,
[6] FWF-SFB grant,
[7] Marie-Curie RTN EURO-RA,
[8] GEN-AU 2 Functional Mouse Genomics

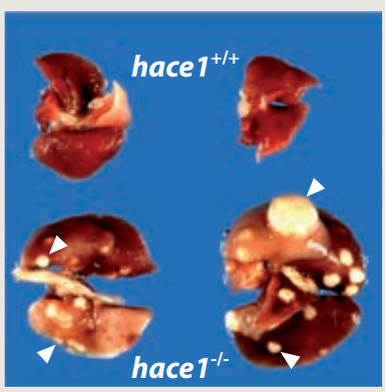
Transformation and cancer growth are regulated by the coordinate actions of oncogenes and tumor suppressors. Moreover, environmental tissue factors and immune cells can modulate cancer cell growth. Based on our previous work on RANKL/RANK as candidate genes in breast cancer and bone metastases, we have made a concerted effort to identify novel genes that control cancer cell growth and genes that regulate tumor surveillance by the immune system.

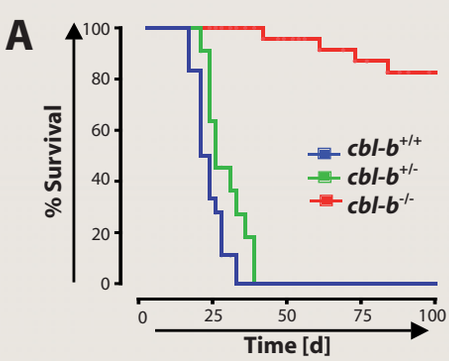
The E3 ligase HACE1 is a critical chromosome 6q21 tumor suppressor involved in multiple cancers

Deletions or LOH of human chromosome 6q21 have been widely reported in human malignancies, including carcinomas of the breast, ovary, and prostate, as well as in leukemias and lymphomas. The key tumor suppressor gene or genes on chromosome 6q21 had not been identified. We identified a t(6;15)(q21;q21) translocation in a sporadic Wilms' tumor arising in a 5-month-old child (Figure 1). Wilms' tumors account for over 90% of pediatric kidney tumors and 6% of all childhood cancers. We characterized the chromosome 6q21 breakpoint and identified a novel gene. We designated this gene HECT domain and Ankyrin repeat Containing E3 ubiquitin-protein ligase 1, or HACE1. The HACE1 gene is widely expressed and the encoded protein possesses intrinsic E3 ubiquitin ligase activity. We therefore characterized the *in vivo* function of HACE1.

HACE1 is frequently down-regulated in human tumors and was indeed mapped to a region of chromosome 6q21 implicated in multiple human cancers. Genetic inactivation of HACE1 in mice results in the development of spontaneous, late onset cancer. Radiation or inactivation of a single p53 allele on a hace1-/- background massively increases tumor incidence. hace1 mutant mice exhibit a remarkable spectrum of tumor types, including melanoma, sarcomas, breast carcinoma, hepatocellular carcinoma, lymphoma, lung carcinoma, as well as micrometastases. Moreover, loss of hace1 renders mice susceptible to lung cancer in response to alkylating agents (Figure 2). Re-expression of HACE1 in human Wilms' tumor and neuroblastoma cells directly abrogates *in vitro* and *in vivo* tumor growth in an E3 ligase dependent manner whereas downregulation of HACE1 via siRNA allows non-tumorigenic human cells to form tumors *in vivo*. Mechanistically, HACE1 controls adhesion-dependent cell growth and regulates cell-cycle progression during cell stress through degradation of cyclin D1. Thus, our data identified HACE1 as a novel chromosome 6q21 tumor suppressor gene with a potential role in the development of multiple cancers. The exact molecular pathway(s) of HACE1-regulated tumor suppression now need to be identified.







Cbl-b controls spontaneous tumor rejection by CD8+ T cells

More than 100 years ago, it was discovered that tumors regress in patients injected with bacterial extracts, suggesting that immune cells might be capable of eliminating cancer cells. However, immunotherapy is still difficult because most tumors are insufficiently recognized, do not elicit a robust immune response, or induce immunotolerance. Understanding the biochemical mechanisms of tumor immunosurveillance is of paramount significance because it might allow one to specifically modulate spontaneous anti-tumor activity. The Casitas B-cell Lymphoma-b protein, Cbl-b, is a member of the mammalian family of Cbl E3 ubiquitin ligases. Our previous studies on Cbl-b-deficient mice have revealed an essential role for this molecule in T cell tolerance, CD28 costimulation, and autoimmunity (Bachmaier et al. Nature; Jeon et al. Immunity; Krawczyk et al. Immunity; Gronski et al. Nature Med.). Thus, Cbl-b functions as a negative regulator of antigen-specific T cell activation and is a critical mediator of T cell anergy. Based on these findings, we hypothesized that Cbl-b-regulated T cell activation may hold the key to our understanding of induction and/or maintenance of T cell responses to cancer cells.

To determine whether Cbl-b contributes to anti-cancer immunity *in vivo*, we tested cancer cell growth in cbl-b-deficient mice. Intriguingly, inactivation of Cbl-b conferred spontaneous *in vivo* rejection of TC-1 tumor cells that express Human Papilloma Virus antigens (Figure 3). Moreover, cbl-b-/- mice develop significantly fewer UVB-induced skin malignancies and reject UVB-induced skin tumors. $CD8^+$ T cells were identified as key players in the spontaneous tumor rejection response. Mechanistically, cbl-b-/- $CD8^+$ T cells are resistant to Treg-mediated suppression and exhibit enhanced activation and rapid tumor infiltration. Importantly, established TC-1 tumors can be treated by the transfer of non-transgenic, "naïve" $CD8^+$ cbl-b-/- T cells that have previously never encountered the tumor antigen. Loss of Cbl-b in the $CD8^+$ compartment alone is both necessary and sufficient to induce potent anti-tumor immunity, thereby perhaps providing a direct means of targeting tumors via $CD8^+$ T-cell responses even in the context of ineffective co-stimulation, impaired $CD4^+$ T cell help, or Treg immunosuppression. Even up to one year after the first encounter with the tumor cells, cbl-b-/- mice carry an "anti-cancer memory".
Thus, we have identified a dominant "tolerogenic" factor that actively represses activation of tumor-specific T cells *in vivo*. Cbl-b is a key signaling molecule that controls spontaneous anti-tumor activity of cytotoxic T cells in different cancer models. The next steps will be to extend these data to additional genetic tumor models and to attempt RNAi shutdown of Cbl-b in T cells to confer spontaneous anti-tumor immunity. Ultimately, inhibition of Cbl-b might be a potent new strategy for anti-cancer immunotherapy on multiple levels to augment the effectiveness of tumor-specific $CD8^+$ T cells in humans.

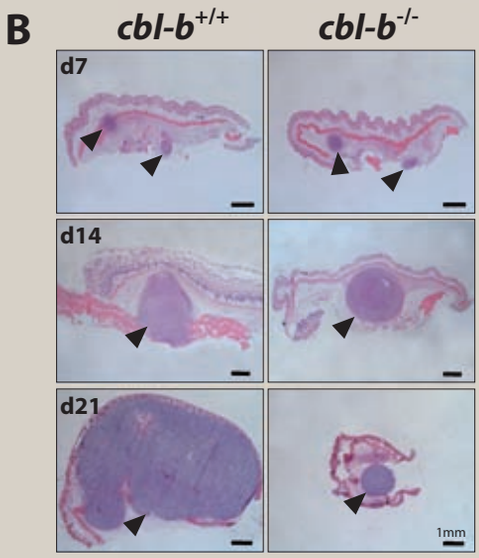


Figure 1: *(A.) HACE1 protein domain structure (http://pfam.wustl.edu). The novel architecture of Ankyrin repeats in combination with HECT ubiquitin ligase domain has not previously been described. (B.) Chromosome paint for chromosome 6 (red) and chromosome 15 (green) to illustrate the translocation in the reference patient. Chromosomes were counterstained with DAPI. (Anglesio et al. Hum. Mol. Genet. 2004; Zhang et al. Nature Med. 2007)*

Figure 2: *Lung tumors in hace1-/- mice. Two typical cases of lung tumors in hace1-/- mice are shown six months following a single injection of urethane (1000 mg/kg of body weight; i.p.) at 4 weeks of age. All hace1+/+ littermates treated with the same procedure remained tumor-free. (Zhang et al. Nature Med. 2007)*

Figure 3: *Spontaneous tumor rejection in cbl-b-/- mice. (A.) Kaplan-Meyer survival curves of cbl-b+/+ (n=18), cbl-b+/- (n=11), and cbl-b-/- (n=28) mice inoculated with 2.5x105 TC-1 tumor cells. Data are pooled from four different experiments. (B.) Representative histology of TC-1 tumors isolated on different days (d7, d14, and d21 after inoculation) from cbl-b+/+ and cbl-b-/- mice. H&E staining. Arrows point at tumor mass. (Loeser et al. J. Exp. Med. 2007)*

LEONIE RINGROSE

Epigenetic Regulation by the Polycomb and Trithorax Group Proteins

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[4] *till November*

How do different cell types remember their identities over many cell generations? Part of the answer lies in the Polycomb and Trithorax groups of proteins. We aim to understand this epigenetic regulatory system on both the molecular and the genomic levels, in terms of the design, function and evolution of its components.

The Polycomb (PcG) and Trithorax (TrxG) groups of proteins work antagonistically on the same target genes to maintain the repressed (PcG) or active (TrxG) transcription states that were established earlier on by transiently acting transcription factors. Both groups of proteins work as large complexes that can modify nearby chromatin. In flies and vertebrates the PcG and TrxG operate on several hundred developmentally important genes (Figure 1A) which they recognize through specialized DNA elements called PRE/TREs (Polycomb/Trithorax Response elements, Figure 1B). Our main aim is to understand the interaction between the PcG and TrxG proteins and PRE/TREs. To achieve this, we use an interdisciplinary combination of experimental biology, mathematical modeling and computational prediction.

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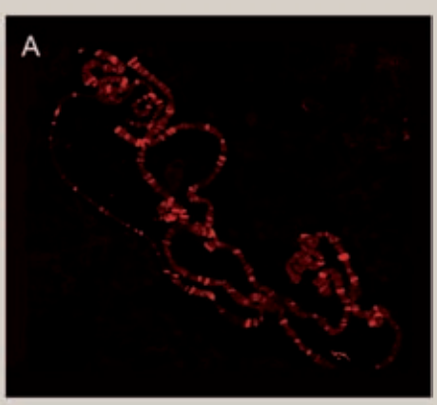

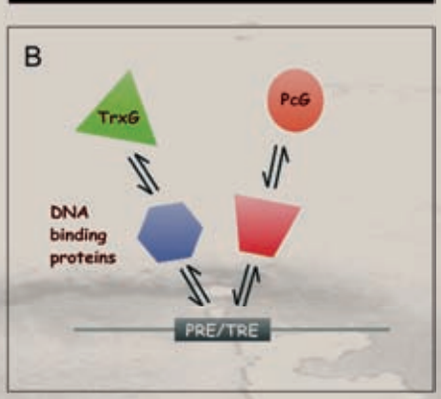

1) What makes a PRE/TRE?

PRE/TREs are fascinating pieces of DNA. Within only a few hundred base pairs, they contain information that enables them to remember and maintain the active or silenced transcriptional state of their associated genes over many cell generations. In flies, we have shown by computational analysis that PRE/TREs are complex combinatorial elements, containing clusters of binding sites for several DNA-binding proteins. All of the several hundred PRE/TREs in the fly genome have different designs (Figure 2A). Are these differences in design important for function?

Analysis of PRE/TRE function in flies has been hindered by the susceptibility of these elements to genomic position effects. To overcome this problem, we have used C31 integrase technology to compare PRE/TRE elements at the same genomic location, showing that for two elements, their different designs do indeed have profound effects on function (Figure 2B). This demonstrates that PRE/TREs have intrinsically different properties, and we propose that these elements may act not only as memory elements, but also as transcriptional amplifiers, giving added robustness to transcriptional networks (Figure 2C).

In a complementary project, we are using the recently released genome sequence and annotation for several other *Drosophila* species, in combination with *in vivo* experiments, to elucidate the principles governing the evolution of PRE/TREs. We find that PRE/TRE evolution is extraordinarily dynamic. The number, position and design of PRE/TREs changes rapidly in evolution and suggests that these regulatory elements may provide a rich source of potential phenotypic variation.

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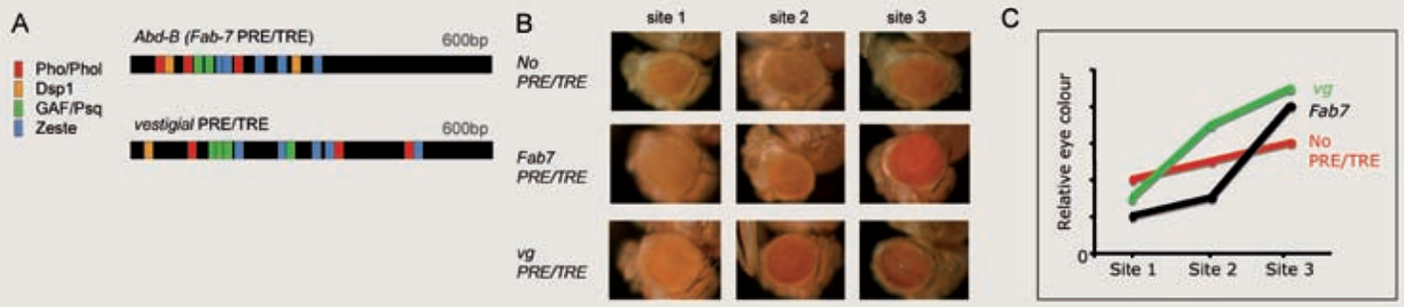

Figure 1: *(A.) Giant chromosomes from Drosophila larvae were stained with an antibody against the Polycomb protein, showing about 100 binding sites. (B.) The Polycomb and Trithorax group proteins are recruited to their targets by DNA binding proteins, that recognize DNA elements called PRE/TREs. This binding is highly dynamic.*

Figure 2: *(A.) Two PRE/TREs are shown. DNA-binding motifs important for function are shown, illustrating diversity in PRE/TRE design. (B.) Red eye color gives a readout of PRE/TRE activity. Each of the two PRE/TREs was inserted in three genomic locations using site-specific integration, and compared to the reporter gene activity at the same site with no PRE/TRE. (C.) The level of transcription given by the reporter gene alone (red line) is different at each site. This input level is modified differently by each of the two PRE/TREs (green and black lines), but in both cases, low levels are silenced further (site 1), while higher levels give a switch to activation (site 3). This indicates that PRE/TREs can behave as transcriptional amplifiers.*

2) How do active and silenced states survive mitosis?

Paradoxically, although the silenced and activated states perpetuated by the PcG and TrxG are stable over many cell generations, it is becoming clear that the proteins themselves associate with their targets as dynamic complexes, in constant flux between bound and free pools (Figure 1B). The challenge now is to obtain quantitative measurements of the kinetic processes involved, and to extend these observations to mitotically dividing cells. We aim to achieve this by studying the kinetic behavior of PcG/TrxG proteins, and of the non-coding transcription that is associated with activation of target genes, during the cell cycle. We are generating transgenic flies carrying fluorescent fusion proteins and tagged RNAs, so that we can visualize the binding of PcG, TrxG and DNA binding proteins to a single PRE/TRE locus with defined transcriptional status. We will use live imaging techniques to examine the kinetic properties of this defined system in developing *Drosophila* embryos and larval tissues. This will be combined with mathematical modeling to integrate the results into a coherent quantitative model. In this way, we hope to understand how a system in constant flux can ensure both stability and flexibility.

3) How do PRE/TREs change their states upon developmental transitions?

PcG and TrxG proteins are intimately involved in the control of stem cell identity, differentiated cell identity and cell proliferation. Aberrant expression of these proteins is associated with many types of cancer. To study these transitions in detail, we have established neural differentiation of mouse ES cells that carry Sox1 or Tau GFP-tagged loci to enable FACS sorting to obtain pure populations of ES cells, neural progenitors and neurons (Figure 3). In addition, we have designed a PcG target microarray that is suitable for the analysis of both ChIP and transcription on a single platform. This will enable us to analyze changes in the binding profiles of mouse PcG and TrxG proteins, and of non-coding RNAs at PcG/TrxG target genes upon differentiation. For this analysis we have generated antibodies against mammalian Trithorax group proteins, which are currently being characterized. The long-term aim will be to understand how the PcG and TrxG change their interactions with PRE/TREs upon changes in cell identity.

Figure 3
(A.) Monolayer differentiation of mouse ES cells to neural precursors. (B.) Differentiation is visualized in by antibodies against Oct4, Nestin and TuJ1. (C.) ES cell lines carrying Sox1-GFP (expressed in precursors) or Tau-GFP (expressed in neurons) enable sorting of cells at different stages. These pure populations will be used for analysis of dynamic chromatin changes at PcG and TrxG targets.

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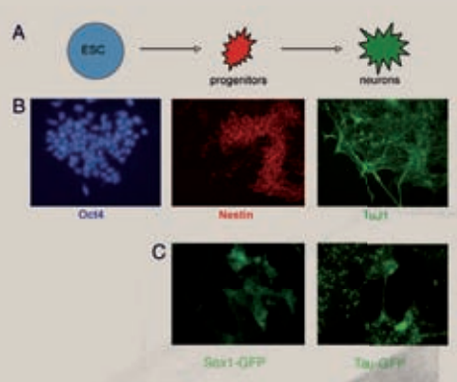

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Mechanisms Underlying Cell Migration

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The migration of cells is essential to life, as a primary feature of developmental and repair processes. It also contributes to disease states, such as in the dissemination of malignant cells during metastasis. We address questions of how the process of cell motility is driven and controlled.

Correlating structure and function in cell migration

How do tissue cells move? Studies of migrating cells by light microscopy have shown that movement occurs in two more or less coordinated steps: protrusion at the front followed by retraction at the rear (Figure 1). Both processes use actin filaments, but in different ways: at the cell front, actin filaments push by growing (polymerization) and at the rear they retract or pull by interacting with the contractile protein myosin. Much interest focuses on the mechanism of pushing at the front since it is this process that initiates movement: how are filaments organized to push and how is their polymerization and depolymerization regulated? To address the question of filament organization, we have developed new methods for correlating the movement of single cells in the light microscope with their ultrastructure in the electron microscope. Using a routine involving chemical fixation to arrest cell movement, we have already shown that the orientation of filaments at the front varies according to the rate of protrusion (Figure 2). These findings shed new light on the structural basis of protrusion and challenge current ideas about this process. The methodology we have developed also provides the springboard for studies of cell ultrastructure by cryo-electron microscopy, which opens the possibility of probing cell architecture of rapidly frozen cells, without the need of chemical fixation. Future efforts will be directed towards defining the three-dimensional organization of actin networks at close to the living state, by cryo-electron tomography.

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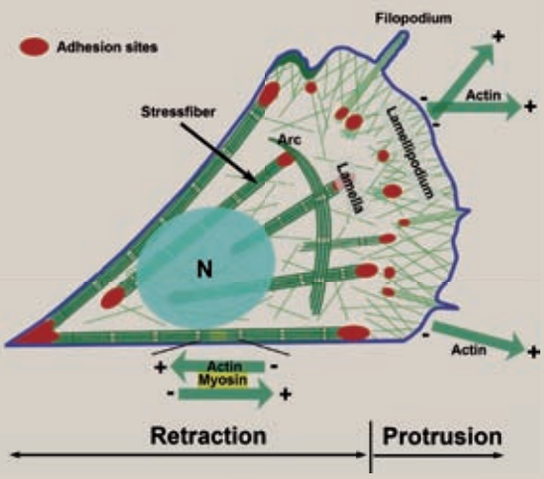

Coupling protrusion and retraction

The major site of actin filament initiation in cells is at the protruding front. This raises the question of how the actin bundles containing myosin that are used for retraction are generated. Our recent studies indicate that a sub-population of filaments initiate at the front is transported rearwards to seed the assembly of the rest of the actin cytoskeleton. Evidence for this comes from correlated video microscopy and electron microscopy of the finger-like projections at the cell front called filopodia. We have recently shown that filopodia are cycled back into the cell to provide the actin-filament building blocks for interaction with myosin in the contractile bundles used for retraction. The idea that the cell front may serve as a filament factory for the rest of the cell is being pursued by analysis of actin-filament turnover in different cell types and investigations of the role of actin-associated proteins in this process.

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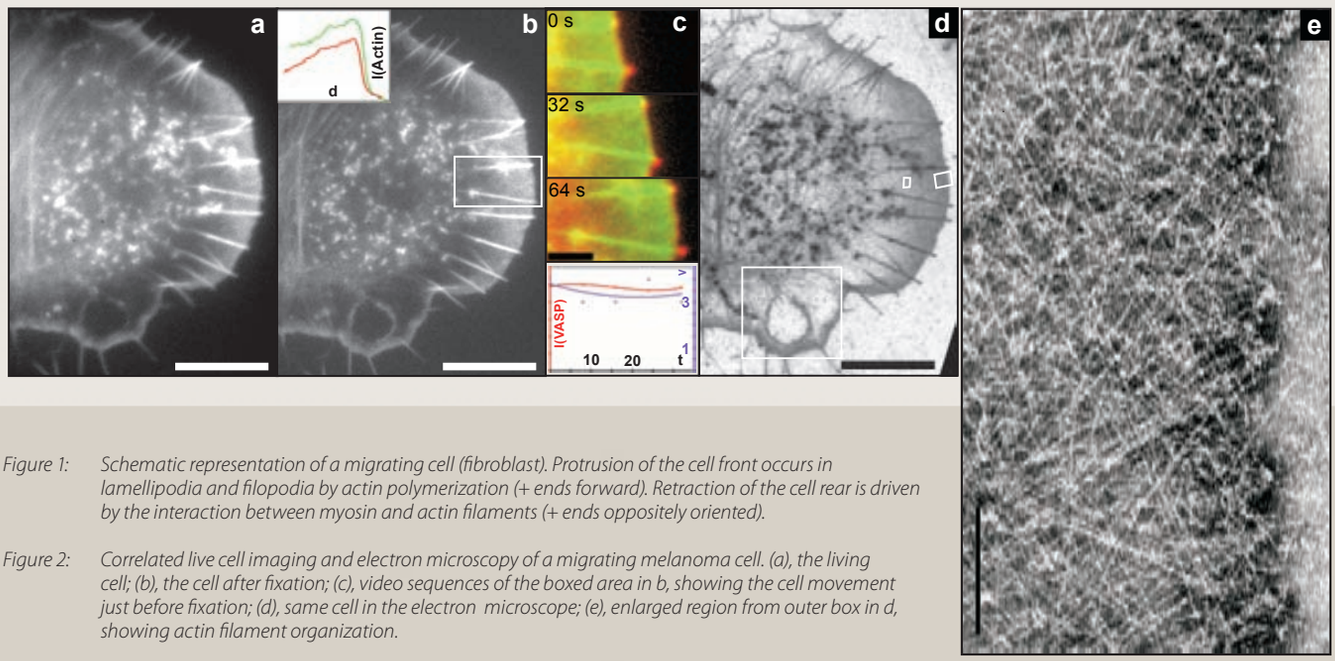

Figure 1: *Schematic representation of a migrating cell (fibroblast). Protrusion of the cell front occurs in lamellipodia and filopodia by actin polymerization (+ ends forward). Retraction of the cell rear is driven by the interaction between myosin and actin filaments (+ ends oppositely oriented).*

Figure 2: *Correlated live cell imaging and electron microscopy of a migrating melanoma cell. (a), the living cell; (b), the cell after fixation; (c), video sequences of the boxed area in b, showing the cell movement just before fixation; (d), same cell in the electron microscope; (e), enlarged region from outer box in d, showing actin filament organization.*

Figure 3: *(a), example of delayed migration of border cells (arrow) in a drosophila egg chamber caused by the suppression of one gene from the RNAi migration screen. In control egg chambers, the border cell cluster reaches the oocyte boundary, (b).*

Screening for motility genes in *Drosophila*

To what extent can we translate findings from cells migrating *in vitro* with the migration of cells in a living organism? Taking advantage of the RNAi library developed in the Dickson lab, we have undertaken a screen of genes required for the migration of border cells in *Drosophila*. Border cells move in a characteristic fashion through the *Drosophila* egg chamber (Figure 3) and defects in cell movement are readily detected by the inability to reach the oocyte boundary. Future efforts will be directed at characterizing the new genes that have emerged from this genome-wide screen.

Additional information may be obtained from our website: http://cellix.imba.oeaw.ac.at/

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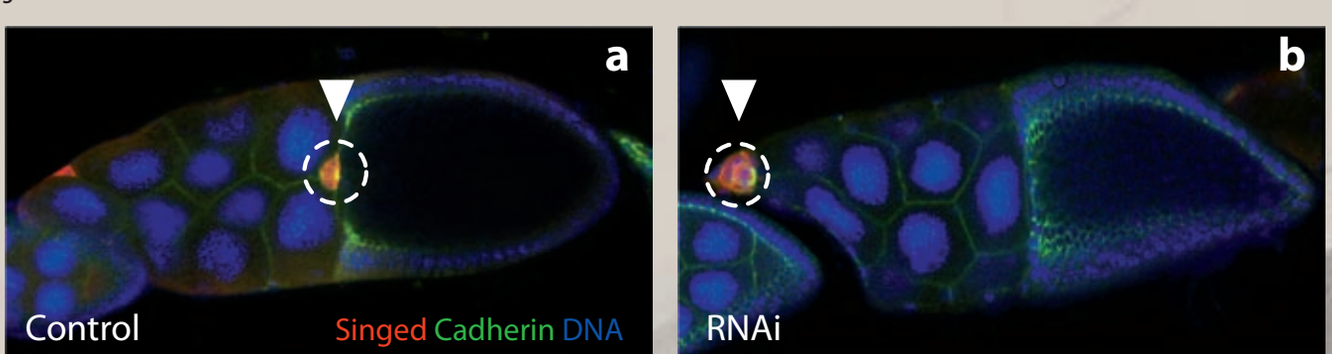

STEM CELL CENTRE – GENE TARGETING UNIT

austromouse@imp.ac.at

Mouse transgenesis has proven to be one of the most powerful approaches to study gene function both in physiology and pathological contexts. For instance, in cancer research, mouse models are an invaluable tool to identify the genetic alterations involved in the onset, progression and expansion of tumors, to define best targets for cancer treatment and to develop new, target-specific and more effective, cancer therapies. The main objective of the "Stem Cell Centre – Gene Targeting Unit" is to provide state-of-the-art technologies for the manipulation of the mouse genome and the generation of genetically modified mouse strains.

The "Stem Cell Centre - Gene Targeting Unit" initiated by GEN-AU is now established at the IMP-IMBA Research Center to support researchers in the field of embryonic stem (ES) cells and gene targeting. Following targeting construct synthesis and validation, the service provides ES cells and feeder cell dishes, and electroporation of ES cells with the targeting construct. Following successful electroporation, selection and clonal expansion we will contact the "Mouse Service Unit" for blastocyst injection request.
The Unit also maintains a collection of "tool strains" – transgenic mouse lines used for the genetic manipulation of the mouse genome. This collection includes Cre and Flp recombinase-expressing transgenic lines and Cre and Flp activity-reported lines that are essential for the generation and characterization of conditional, inducible, and/or tissue specific mutant mice.
The Unit will also maintain a collection of "ES and vector tools", feeders (neomycin resistant or neo/hygro/puro resistant), ES cells, targeting vectors, overexpression vectors, and RNAi vectors that can be distributed on demand.
One major interest of our Unit is the development and implementation of technologies to improve and accelerate the generation of genetically modified mice. We are expanding this effort to establish a collection of ES cell lines with different genetic modifications from the mouse models already available at IMP-IMBA.

We are also currently developing new tools for high-throughput mouse generation, such as QUICK-Knock-ins, tissue-specific and inducible expression of transgenes, and the "generation of homozygous ES cell clones" as well as establishing feeder-free, serum- free ES cells.

Figure : www.austromouse.at



VIENNA DROSOPHILA RNAi CENTER (VDRC)

Krystyna Keleman, Dirk Holste, Peter Duchek

office@vdrc.at

Krystyna Keleman / Head of the VDRC

Maintenance and Distribution of the RNAi Library
Reinhard Klug / Maintenance Supervisor
Thomas Micheler / Software Developer
Virginia Salva Millan / Administration
Katharina Jandrasits / Technician
Martin Kinberg / Technician
Zsuzsanna Portik Dobos / Technician
Judith Utner / Technician
Elsa Muehlbacher / Technician
Andreas Gansch / Technician
Yasmin Gravogl / Technician
Silke Perner / Technician
Alsher Tashpulatov / Technician
Michaela Fellner / Technician

Research
Silvia Oppel / Technician
Laszlo Hunor / Technician
Angela Graf / Technician
Gabi Kuenzl / Technician
Amina Kadum / Technician
Svetlana Zorinyants / Technician

Nadege Minois / Postdoc
Luiza Deszcz / Technician

Keleman Group
Krystyna Keleman / Staff Scientist
Sebastian Krüttner / PhD Student
Reinhard Hämmerle / Diploma Student
Katarina Steinleitner / Technician

Holste Group
Dirk Holste / Staff Scientist
Ralf Bortfeldt[1] / PhD Student
Martin Pohl[1] / Diploma Student
Doris Chen[2] / Postdoc

Duchek Group
Peter Duchek / Staff Scientist
Sabine Brynda / Technician
Ciara Gallagher[3] / Technician

[1] co-supervised with Prof. Stefan Schuster, Friedrich-Schiller University Jena
[2] co-supervised with Prof. Renee Schröder (2006-2007)
[3] January to June 2007

Genome-wide RNAi in *Drosophila*

A Drosophila genome-wide transgenic RNAi library has been generated, allowing researchers to systematically study gene functions in specific tissues at specific developmental stages. The VDRC maintains and distributes this library to Drosophila researchers world-wide.

The creation of a genome-wide transgenic RNAi library has revolutionized *Drosophila* genetics. The VDRC, a joint IMP-IMBA initiative, maintains and distributes this library. In just its first seven months of operation, the VDRC has delivered over 25,000 lines to more than 800 registered users world-wide. This has been made possible in part through core funding provided by the city of Vienna and the federal government, with the rest of the costs covered by user fees. We continue to further develop both the library and the service, and should soon also be able to host external researchers who wish to conduct their RNAi screens on-site. The VDRC already provides such support for researchers in-house, having delivered over 145,000 lines to IMBA and IMP groups in 2007. The VDRC is also home to the Keleman, Holste and Duchek Research Groups, supported entirely by external funds.

Krystyna Keleman

Learning and Memory

The evolutionary mission of a male fly is to father as many offspring as possible. With an almost unlimited supply of sperm, his success depends almost entirely on his ability to discriminate receptive virgin females from unreceptive females. If he is too promiscuous, he may waste a lot of time and energy in futile courtship; if he is too choosy, he will miss out on excellent mating opportunities. The right balance appears not to be hard-wired into the fly's brain, but is rather something he learns by trial-and-error during his first few courtship experiences. This memory of these first sexual encounters can shape the male's mating strategy for several days – a long time in the life of a fly.

We are trying to understand the molecular and cellular mechanisms that underlie this robust and powerful form of long-term memory. We have recently demonstrated that the CPEB protein Orb2 – a regulator of mRNA translation – plays a critical role in this process. We found that Orb2 function is required in a specific set of mushroom body neurons during or shortly after training (Figure 1). Without Orb2, or more specifically without its intriguing glutamine-rich domain, a memory initially forms but decays within just a few hours. We are now trying to identify target mRNAs that are regulated by Orb2 in these neurons, and to better define the cues – probably pheromones – that the male is learning to discriminate. Meanwhile, we are also using the transgenic RNAi library in unbiased approaches to find other factors involved in long-term memory formation.
We do also commercially exploit the *Drosophila* transgenic RNAi library in collaborative project with the biotech company to identify novel drug targets.

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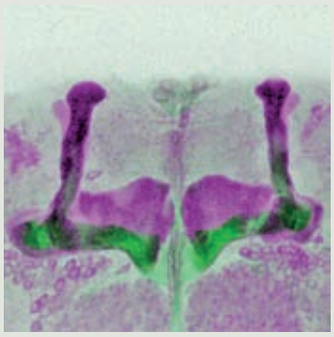

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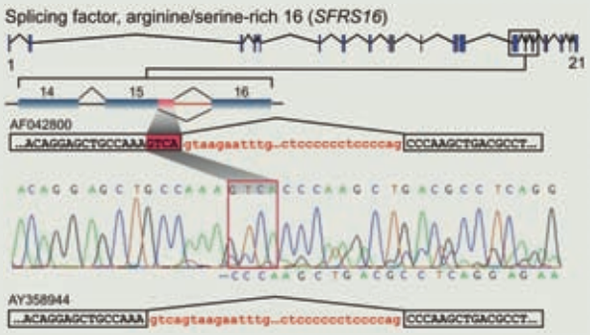

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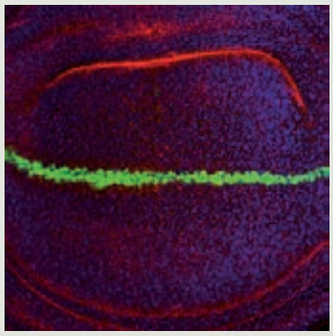


Figure 1. *Mushroom body neurons in the fly's brain – Orb2 is required in these neurons for courtship conditioning.*

Figure 2. *Tandem donor activation of exon E15 of the SFRS16 gene for proximal (TCA/ gtaaga) and distal (AAA/ GTCAgt) splicing.*

Figure 3: *A Drosophila wing imaginal disc showing expression of Cut protein (green) in response to high levels of Wingless signalling along the dorsal-ventral boundary.*

Dirk Holste

Computational Biology of post-transcriptional Gene Expression

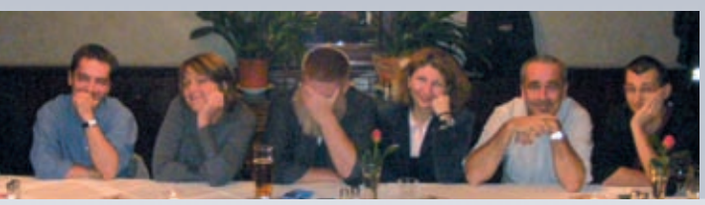
Gene expression is ubiquitous and controlled on different several cellular levels under diverse contexts. In metazoans, where the majority of genes are transcribed as pre-mRNAs, the splicing of precursors to mRNAs constitutes an essential step for the genetic regulation of gene expression at the level of RNA processing. Many pre-mRNAs show variable splicing patterns: different splice sites may be used as alternatives, giving rise to multiple alternatively spliced isoforms, and thus producing mature mRNAs and ultimately polypeptides (highly similar or markedly different) originating from the same locus (Figure 2). We use computational approaches to investigate observed patterns and underlying mechanisms of splicing, using genome analysis and mathematical-statistical models incorporating various types of data, which can be experimentally verified. A unifying goal of our research is to shift biology from case studies toward a global approach, by the dissection of circuits between functional cis-regulatory sequence elements and trans-factors governing the regulation of gene expression ("RNA code") during the development of organisms.

Peter Duchek

Cell Signaling and Morphogenesis

The movement of epithelial tissues and the directed migration of cells perform essential functions throughout an animal's lifespan. These processes are tightly regulated in time and space and can lead to birth defects or metastatic cancer if such controls go awry. Using *Drosophila* as a model system, we would like to understand how cells communicate with each other in order to coordinate their behaviour and aim to understand how an instructive extracellular signal is relayed to the cytoskeleton in order to generate the forces that bring about these movements.

Our main focus over the past two years has been the commercial exploitation of the transgenic *Drosophila* RNAi library. In collaboration with a biotech company, we performed an *in vivo* RNAi screen aimed at isolating novel components of the Wingless signalling pathway (Figure 3). The human homologues of identified genes could then serve as potential targets for the development of drugs that interfere with Wingless/Wnt activity in patients.



BIOOPTICS DEPARTMENT

Peter Steinlein / Head of Facility

Karin Aumayr / Microscopy and Image Analysis
Carmen Czepe [1] / Micro Arrays
Andreas Mairhofer [2] / Micro Arrays
Pawel Pasierbek / Microscopy
Martin Radolf / Micro Arrays
Gabriele Stengl / Flow Cytometry

[1] *until June,*
[2] *since August*

The services offered to the researchers at the IMP and IMBA by our department cover analytical flow cytometry and cell sorting, a wide variety of microscopical techniques, image analysis and processing, as well as cDNA-micro array production and analysis.

Current activities

A major area of the service is the training of scientists for various image acquisition systems, image analysis software and analytical flow cytometers. Especially the increasing complexity of advanced microscopes like confocal, deconvolution microscopes and live-cell imaging stations requires intensive training tailored to fit the applications and the knowledge of the user. The training is performed on an individual basis and span, depending on the application, up to three sessions. On average, more than 60 training courses for confocal microscopes and more than 100 training courses for conventional microscopes are carried out annually.

As already pointed out in the 2005 Research Report, the demand for truly quantitative image analysis is constantly increasing. Until recently, it was almost impossible to acquire high-resolution, digitized data of entire histological specimens as conventional microscopy only allows the examination of individual fields (limited by the camera properties) of these specimens. To circumvent this problem, a Mirax Scan slide scanner was purchased allowing the automated acquisition of high-resolution images from whole samples stained conventionally and/or fluorescently. The illumination and acquisition optics of the system deliver images of excellent quality optimally suited for automated image analysis using the Definiens eCognition software framework.

Together with the superior quality of the images and the large numbers of samples (up to 300 slides can be acquired in one batch) the image analysis of those samples yields a large volume of statistically relevant data and a much higher statistical certainty compared to conventional microscopy.

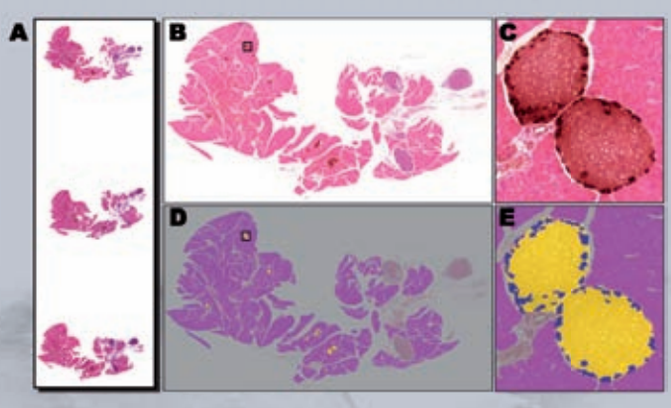


Figure: *(A.) shows the scan of a slide containing 3 sections of a mouse pancreas immunohistochemically-labelled with an anti-Glucagon antibody and counterstained with Eosin. (B.) and (D.) show one of the sections with the blow up of two of the islets of Langerhans containing the Glucagon-labeled cell (C and E). Using the Definiens eCognition software (D and E) background (dark gray), relevant tissue (purple), Islets (yellow) and Glucagon-labeled cells within the islets (blue) are differentiated and the change in ratio between these groups can be determined in wildtype and knockout mice. (Data provided by Andrew Pospisilik and Michael Orthofer)*

ELECTRON MICROSCOPY

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Günter Resch / Head of Electron Microscopy Facility

Marlene Brandstetter / Technical Assistant
Pedro Serrano Drozdowskij / Programmer

The Electron Microscopy Facility provides a wide variety of preparation techniques for visualization of ultrastructure in tissues and cells, and of purified molecules by transmission electron microscopy, as well as support with microscopy, data management and image processing.

Sample Preparation

Know-how, training and instrumentation for a wide variety of preparation techniques for visualization of ultrastructure in tissues and cells, and of purified biomolecules by transmission electron microscopy (TEM) are being provided by the Electron Microscopy Facility. Techniques routinely used by both IMP and IMBA researchers are chemical and physical fixation, resin embedding in epoxy- and acrylic resins, freeze substitution, ultrathin sectioning of resin embedded and frozen samples, production of support films, negative staining of molecules and organelles, rotary shadowing of sprayed molecules, and others. Depending on future developments and the focus of the demand from the institutes, additional preparative approaches will be introduced.

Microscopy

One cornerstone of the facility is the FEI Morgagni. This robust and easy to use 100 kV TEM equipped with an 11-megapixel CCD camera is tailored for routine needs in the multiuser environment of a facility. The high utilization reflects the demand for EM in both institutes.

While the Morgagni is an excellent tool for conventional TEM, more advanced applications will be the domain of 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. The installation (Figure) was started in March 2007 and by Autumn, the microscope was up and running except for its most advanced functions. Primarily, it will be used for cryo-TEM of molecules and cells, electron tomography, as well as analytical applications.

Data Management and Image Processing

To support users with data management, the facility is running a web-based project-oriented database system called MIMAS: electron micrographs from both microscopes including meta data can be stored on and accessed from this database on a user-restricted basis. It is continuously being developed into a more stable and versatile platform, in close collaboration with users to meet their needs. Workstations and training for image processing of EM data, especially from electron tomography, are being provided as well.

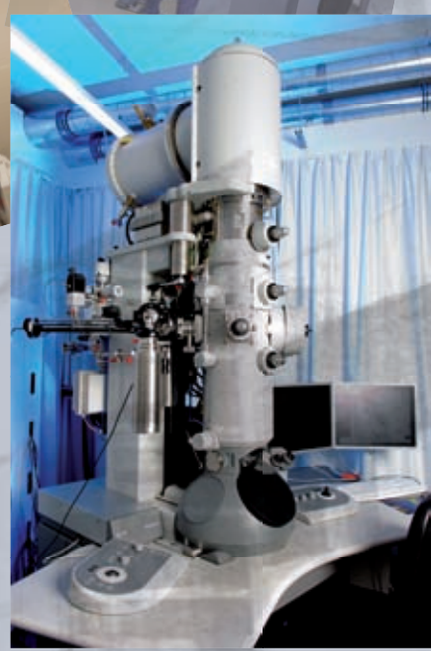

Figure: The TF30 Polara coming to life.

BIOINFORMATICS

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Wolfgang Lugmayr/ Software Engineer
Maria Novatchkova/ Computational Biologist
Alexander Schleiffer/ Computational Biologist

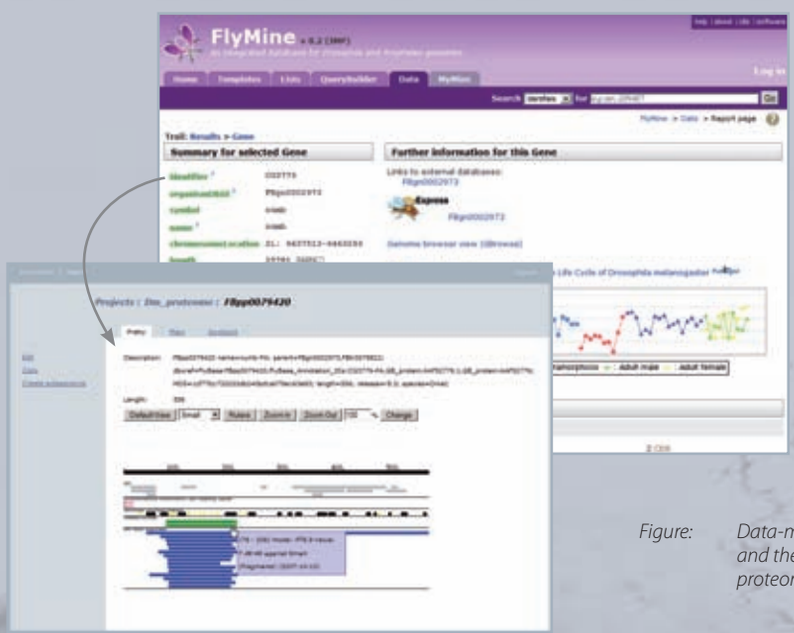
The Bioinformatics unit assists research groups in molecular biology-related fields, by providing sequence analytic services, scientific data mining, hardware and software infrastructure, and training in bioinformatics.

Sequence Analysis

The IMP-IMBA Bioinformatics unit has its main expertise in the field of sequence analysis. Typical tasks include the functional and structural characterization of proteins and genomic regions using methods such as pattern matching, complexity analysis and homology searches. As bioinformatics conclusions are a synthesis of results from multiple algorithms, we maintain and develop a set of specialized software tools to support this type of meta-analysis.

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 the higher load and memory requirements. In the past year, we have set up a data warehouse system based on the InterMine project to integrate data from various biological databases and formats. The IMP-IMBA FlyMine cross-links extensive public genome information with in-house fly screen results and custom annotations. To perform complex sequence analytic tasks we also maintain the IMP ANNOTATOR, a user-friendly web application and high throughput protein annotation system.



Training

We provide hands-on training courses on the use of both the ANNOTATOR and the FlyMine system, where participants learn the basis and limitations of sequence analysis and data integration.

Figure: Data-mining using our in-house FLYMINE installation and the annotation of the Drosophila melanogaster proteome with the IMP ANNOTATOR.

PROTEIN CHEMISTRY FACILITY

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The IMP-IMBA Protein Chemistry Facility performs a large variety of mass spectrometry experiments, including identification of proteins by peptide sequencing and characterization of post-translational modifications (PTMs), such as phosphorylation. In addition, we are developing new methods for the quantification of PTMs. Finally, our facility specializes in peptide synthesis and antibody purification.

Karl Mechtler / Head of Facility

Karin Grosstessner-Hain / PhD Student
Gabriela Krssakova / Technician
Goran Mitulovic / Technician
Mathias Madalinski / Technician
Elisabeth Roitinger / Technician
Ines Steinmacher / Technician
Christoph Stingl / Technician

Improvement in Phosphorylation Analysis

Protein phosphorylation is the most important reversible post-translational modification. Thus, analysis of phosphorylated proteins and identification of the phosphorylation sites help us to understand their biological functions.

Analysis of protein complexes and Enrichment of phosphopeptides from complex mixtures

We have developed a new offline chromatographic approach for the selective enrichment of phosphorylated peptides that is directly compatible with subsequent analysis by online nano electrospray ionization tandem mass spectrometry (ESI-MS/MS). A titanium dioxide-packed pipette tip is used as a phosphopeptide trap for an offline first-dimension separation step. This is followed by online nano reversed-phase high-performance liquid chromatography. The method developed allows the identification of very low abundant peptides from protein complexes.

The Christian Doppler Laboratory for Proteome Analysis

Together with Prof. Gustav Ammerer from the Max F. Perutz Laboratories, a project for the quantitative analysis of proteins based on mass spectrometry was established. Our goal is to study the composition of multi-protein complexes and their associated partners.

Peptide Synthesis and Antibody Purification

We synthesize about 350 peptides per year, including an increasing number of peptides containing acetylated, phosphorylated or methylated amino acid residues. We have developed procedures for affinity-purification of antibodies, including optimized elution under mild conditions.

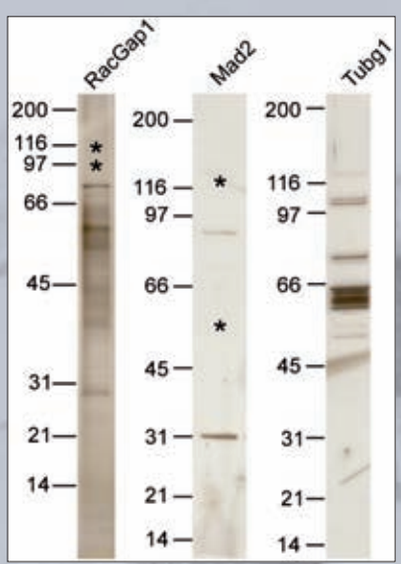
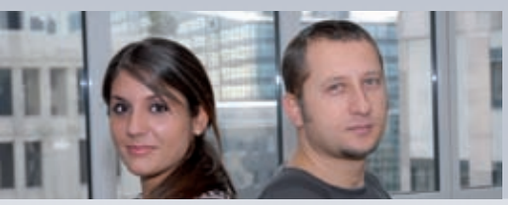


Figure: Silver-stained gels of protein complexes subjected to phospho-site mapping.Following tryptic digestion and enrichment of phosphopeptides on TiO2 tips, it was possible to isolate and identify a significant number of phosphorylation sites. A number of these originated from proteins in gel regions where silver staining indicated no proteins were present (marked with asterisk).



HISTOLOGY DEPARTMENT

vukoslav.komnenovic@imba.oeaw.ac.at

Vukoslav Komnenovic / Head of Facility

Mihaela Grivej / Technician

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

Histology Services

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

Sectioning of Paraffin and Frozen Tissues

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

Immunohistochemistry

The Histology Service Department also provides automated preparation and processing facilities for standardized immuno-histochemistry, in situ hybridization, FISH analysis, DNA microarray and tissues microarray applications. Various antibodies have been characterized for optimized in situ signaling studies. Standard antibodies such as apc, cd (several markers), l-ad, gfp, gfap, c-fos, c-jun, junB, fra1,2, jun-D, ki67, smad3, brdu, egf, egfr, H3K9me tri meth, H4K20me3 tri meth, cl. caspase3, caspase7, procatepsiK are available.
In addition, the Histology Service Department administrates legal regulatory affairs such as the record-keeping and documentation of experiments in accordance with the Austrian Histolaboratories guidelines (www.mta-labor.info).

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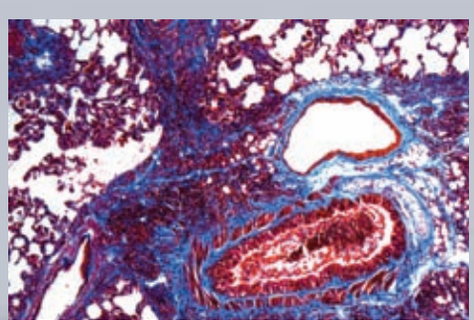

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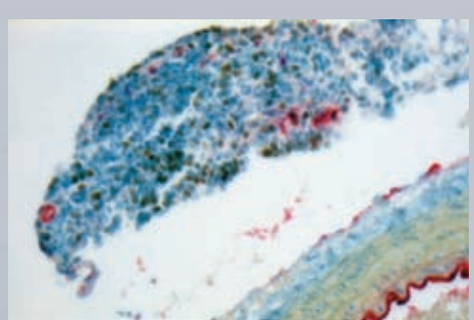

Figure 1: *Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.*

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

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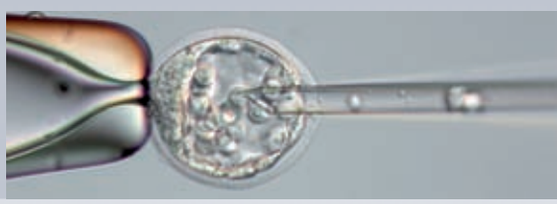

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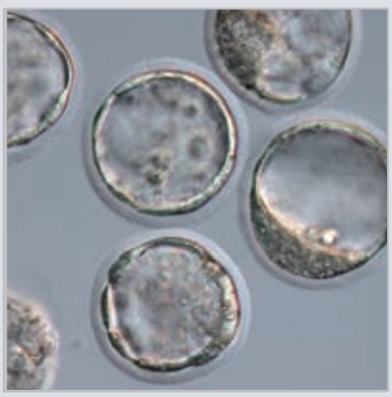

ANIMAL HOUSE

animal@imp.ac.at

MOUSE SERVICE

transgenic@imp.ac.at

Animal House

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.

Animal House 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.

The Mouse Service Department *was set up at the beginning of 1998 to cope with the increasing demand for mouse studies and generation of transgenics. The Mouse Service Department services are shared by the IMP and IMBA.*

The main duties of this service unit are the injection of ES cells into blastocysts (also tetraploid and 8-cell) and of DNA into the pronucleus of fertilized mouse eggs. This service also provides for the transfer of 'clean' embryos into our Animal House, the freezing of embryos for the preservation of specified mouse strains and the teaching of basic embryological techniques to the IMP and IMBA staff.
Many different ES cell clones and DNA/BAC constructs are being injected per year. The activities of this department are overseen by an Animal User Committee, which meets bimonthly to set priorities and to coordinate the duties. At present, it is chaired by Erwin F. Wagner.

Figure 1: *Injection of embryonic stem cells into mouse blastocyst.*

Figure 2: *Mouse blastocysts.*

SERVICE DEPARTMENT

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Gotthold Schaffner / Scientist

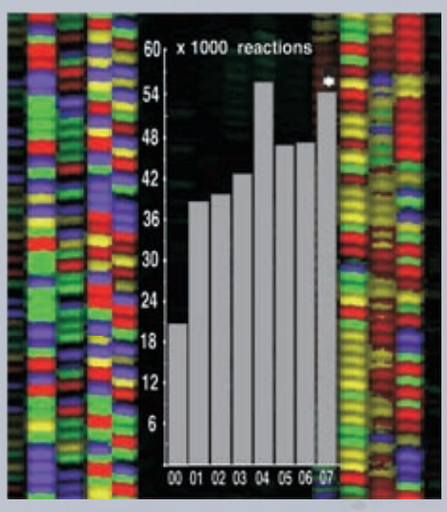
Ivan Botto / Technician
Markus Hohl / Technician
Shahryar Taghybeeglu / Technician
Gabriele Botto / Technician Media Kitchen
Christa Detz-Jaderny / Technician Media Kitchen
Ulrike Windholz / Technician Media Kitchen
Sabine Jungwirth / Technician Fly Food Preparation
Franziska Stransky / Technician Fly Food Preparation
Anna Windholz / Technician Fly Food Preparation
Oliver Botto / Help Fly Food Preparation
Thomas Haydn / Help Fly Food Preparation

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

Our Media Kitchen and Fly Food staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies (more than 1,200,000 bottles and tubes per year) and worms. The Fly Food staff will soon move back to the IMP building where we have more space to create better and more convenient working conditions for preparing fly food.
We also prepare many selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent *E.coli* strains and we maintain a stock of cloning vectors, sequencing primers and other cloning reagents.

Production of antibodies

The production and isolation of many different monoclonal antibodies in hybridomas in collaboration with IMP group members, and organizing the antibody production in rabbits with an outside company requires a portion of our work capacity.



Sequencing and DNA isolation

The 16 capillary ABI 3100 Genetic Analyzer is rarely used at the moment. The 48 capillary ABI 3730 DNA Analyzer is our workhorse. We sequenced approximately 45,000 samples in the first 10 months of this year. This increased demand due to a number of new customers, but also due to many screening projects and to new groups at the IMBA as well as at the IMP.
We primarily use the 3730 DNA Analyzer because of its sensitivity and lower running costs. The average read-length is 700-900 bases for standard DNA samples, with both Genetic Analyzers equipped either with 80 cm capillaries of ABI 3100 or 50 cm capillaries of ABI 3730.
DNA sample quality even from sophisticated Kits like Qiagen Midi- or Maxipreps is still a problem, as are wrong primersets and not sufficiently documented plasmid constructs from outside sources. The clean-up protocol with Sephadex G50 superfine columns on 96-well microtiter plate format with optimized sephadex consistency and centrifugation conditions is still working fine and does not produce "dye blobs" with good quality DNA samples.

Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (- 2001), on ABI 3100 (since 2001) and on ABI 3730 (since June, 2004) done with dye deoxy terminators (v3.0 since 2001) in the years 2000 to 2007 (scale 0 to 60,000).
**calculated from January 2006 to October 2007 data*

Publications

KNOBLICH

Betschinger, J., Mechtler, K., Knoblich, JA. (2006). Asymmetric segregation of the tumor suppressor brat regulates self-renewal in Drosophila neural stem cells. Cell. 124(6):1241-53

Bowman, SK., Neumüller, RA., Novatchkova, M., Du, Q., Knoblich, JA. (2006). The Drosophila NuMA Homolog Mud regulates spindle orientation in asymmetric cell division. Developmental Cell. 10(6):731-42

Gallagher, CM., Knoblich, JA. (2006). The conserved c2 domain protein lethal (2) giant discs regulates protein trafficking in Drosophila. Developmental Cell. 11(5):641-53

Hutterer, A., Berdnik, D., Wirtz-Peitz, F., Zigman, M., Schleiffer, A., Knoblich, JA. (2006). Mitotic activation of the kinase Aurora-A requires its binding partner Bora. Developmental Cell. 11(2):147-57

Jüschke, C. and Knoblich, J.A. (2007) Purification of Drosophila Protein Complexes for Mass Spectrometry. Drosophila: Methods and Protocols. in press

Knoblich J. (2007). On the backroads to cellular asymmetry. Development. 134(24):4311-3

MARTINEZ

Ameres, SL., Martinez, J., Schroeder, R. (2007). Molecular basis for target RNA recognition and cleavage by human RISC. Cell. 130(1):101-12

Leuschner, PJ., Martinez, J. (2007). In vitro analysis of microRNA processing using recombinant Dicer and cytoplasmic extracts of HeLa cells. Methods. 43(2):105-9

Martinez, J., Busslinger, M. (2007). Life beyond cleavage: the case of Ago2 and hematopoiesis. Genes & Development. 21(16):1983-8

Nielsen, AF., Leuschner, PJ., Martinez, J. (2007). Not miR-ly a splicing factor: hnRNP A1 succumbs to microRNA temptation. Nature Structural & Molecular Biology. 14(7):572-3

Obernosterer, G., Martinez, J., Alenius, M. (2007). Locked nucleic acid-based in situ detection of microRNAs in mouse tissue sections. Nature Protocols. 2(6):1508-14

Obernosterer, G., Meister, G., Poy, MN., Kuras, A. (2007). The impact of small RNAs. Microsymposium on small RNAs. EMBO Reports. 8(1):23-7

Weitzer, S., Martinez, J. (2007). The human RNA kinase hClp1 is active on 3' transfer RNA exons and short interfering RNAs. Nature. 447(7141):222-6

Weitzer, S., Martinez, J. (2007). hClp1: a novel kinase revitalizes RNA metabolism. Cell Cycle. 6(17):2133-7

PENNINGER

Alnaeeli, M., Park, J., Mahamed, D., Penninger, JM., Teng, YT. (2007). Dendritic cells at the osteo-immune interface: implications for inflammation-induced bone loss. Journal of Bone and Mineral Research. 22(6):775-80

Cheng, HY., Dziema, H., Papp, J., Mathur, DP., Koletar, M., Ralph, MR., Penninger, JM., Obrietan, K. (2006). The molecular gatekeeper Dexras1 sculpts the photic responsiveness of the mammalian circadian clock. The Journal of Neuroscience. 26(50):12984-95

Cheng, H-Y.M, Penninger, JM. (2006). Dream/KchIP/ Calsenilin: a multifunctional player in health and disease. In Neuronal Calcium Sensor Proteins (Philippov, Koch, eds.); Nova Science Publishers. :245-270

Cronin, SJ., Penninger, JM. (2007). From T-cell activation signals to signaling control of anti-cancer immunity. Immunological Reviews. 220(1):151-68

Danilczyk, U., Sarao, R., Remy, C., Benabbas, C., Stange, G., Richter, A., Arya, S., Pospisilik, JA., Singer, D., Camargo, SM., Makrides, V., Ramadan, T., Verrey, F., Wagner, CA., Penninger, JM. (2006). Essential role for collectrin in renal amino acid transport. Nature. 444(7122):1088-91

Feraud, O., Debili, N., Penninger, JM., Kroemer, G. (2007). Cavitation of embryoid bodies requires optimal oxidative phosphorylation and AIF. Cell Death and Differentiation. 14(2):385-7

Fischer, L., Gukovskaya, AS., Penninger, JM., Mareninova, OA., Friess, H., Gukovsky, I., Pandol, SJ. (2007). Phosphatidylinositol 3-kinase facilitates bile acid-induced Ca(2+) responses in pancreatic acinar cells. AmericanJournal of Physiology. Gastrointestinal and Liver Physiology. 292(3):G875-86

Hara, H., Ishihara, C., Takeuchi, A., Imanishi, T., Xue, L., Morris, SW., Inui, M., Takai, T., Shibuya, A., Saijo, S., Iwakura, Y., Ohno, N., Koseki, H., Yoshida, H., Penninger, JM., Saito, T. (2007). The adaptor protein CARD9 is essential for the activation of myeloid cells through ITAM-associated and Toll-like receptors. Nature Immunology. 8(6):619-29

Imai, Y., Kuba, K., Penninger, JM. (2007). Angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Cellular and Molecular Life Sciences. 64(15):2006-12

Jones, HD., Nakashima, T., Sanchez, O., Kozieradzki, I., Komarova, SV., Sarosi, I., Morony, S., Rubin, E., Sarao, R., Hojilla, CV., Komnenovic, V., Kong, Y-Y., Schreiber, M., Dixon, FJ., Sims, SM., Khokha, R., Wada, T., and Penninger, JM. (2007). Can osteoclasts be excluded – a reply. Nature 445, E19-E20

Joza, N., Pospisilik, JA., Benit, P., Rangachari, M., Nakashima, Y., Rustin, P., Abrams, JM., Kroemer, G., Penninger, JM. (2007). The molecular archaeology of a caspase-independent death effector: AIF in Drosophila. Cell Death and Differentiation. in press

Kuba, K., Zhang, L., Imai, Y., Arab, S., Chen, M., Maekawa, Y., Leschnik, M., Leibbrandt, A., Makovic, M., Schwaighofer, J., Beetz, N., Musialek, R., Neely, GG., Komnenovic, V., Kolm, U., Metzler, B., Ricci, R., Hara, H., Meixner, A., Nghiem, M., Chen, X., Dawood, F., Wong, KM., Sarao, R., Cukerman, E., Kimura, A., Hein, L., Thalhammer, J., Liu, PP., Penninger, JM. (2007). Impaired heart contractility in Apelin gene-deficient mice associated with aging and pressure overload. Circulation Research. 101(4):e32-42

Leibbrandt, A., Penninger, JM. (2007). RANK(L) as a key target for controlling bone loss. In Therapeutic Targets of the TNF Superfamily; (Grewal, ed.). Landes Bioscience/Eurekah.com. in press

Leibbrandt, A., Penninger, JM. (2007). The role of RANKL in cancer. Encyclopedia of Cancer. in press

Liu, L., Puri, KD., Penninger, JM., Kubes, P. (2007). Leukocyte PI3Kgamma and PI3Kdelta have temporally distinct roles for leukocyte recruitment in vivo. Blood. 110(4):1191-8

Loeser, S., Loser, K., Bijker, MS., Rangachari, M., van der Burg, SH., Wada, T., Beissert, S., Melief, CJ., Penninger, JM. (2007). Spontaneous tumor rejection by cbl-b-deficient CD8+ T cells. The Journal of Experimental Medicine. 204(4):879-91

Loeser, S., Penninger, JM. (2007). Regulation of peripheral T cell tolerance by the E3 ubiquitin ligase Cbl-b. Seminars in Immunology. 19(3):206-14

Loeser, S., Penninger, JM. (2007). The ubiquitin E3 ligase Cbl-b in T cells tolerance and tumor immunity. Cell Cycle. 6(20):2478-85

Loeser, S., Penninger, JM. (2008). The Role of the E3 ubiquitin ligase Cbl-b in T-cells signalling and tolerance. In Cbl Proteins (Tsygankov, ed.) Nova Science Publishers. in press

Nakamichi, Y., Udagawa, N., Kobayashi, Y., Nakamura, M., Yamamoto, Y., Yamashita, T., Mizoguchi, T., Sato, M., Mogi, M., Penninger, JM., Takahashi, N. (2007). Osteoprotegerin reduces the serum level of receptor activator of NF-kappaB ligand derived from osteoblasts. Journal of Immunology. 178(1):192-200

Nishio, M., Watanabe, K., Sasaki, J., Taya, C., Takasuga, S., Iizuka, R., Balla, T., Yamazaki, M., Watanabe, H., Itoh, R., Kuroda, S., Horie, Y., Förster, I., Mak, TW., Yonekawa, H., Penninger, JM., Kanaho, Y., Suzuki, A., Sasaki, T. (2007). Control of cell polarity and motility by the PtdIns(3,4,5)P3 phosphatase SHIP1. Nature Cell Biology. 9(1):36-44

Oudit, GY., Kassiri, Z., Patel, MP., Chappell, M., Butany, J., Backx, PH., Tsushima, RG., Scholey, JW., Khokha, R., Penninger, JM. (2007). Angiotensin II-mediated oxidative stress and inflammation mediate the age-dependent cardiomyopathy in ACE2 null mice. Cardiovascular Research. 75(1):29-39

Penninger, JM. (2007). ACE2 und SARS Infektionen. BioSpektrum. 13, 571

Pospisilik, JA., Knauf, C., Joza, N., Benit, P., Orthofer, M., Cani, PD., Ebersberger, I., Nakashima, T., Sarao, R., Neely, G., Esterbauer, H., Kozlov, A., Kahn, CR., Kroemer, G., Rustin, P., Burcelin, R., Penninger, JM. (2007). Targeted Deletion of AIF Decreases Mitochondrial Oxidative Phosphorylation and Protects from Obesity and Diabetes. Cell. 131(3):476-91

Rossi, SW., Kim, MY., Leibbrandt, A., Parnell, SM., Jenkinson, WE., Glanville, SH., McConnell, FM., Scott, HS., Penninger, JM., Jenkinson, EJ., Lane, PJ., Anderson, G. (2007). RANK signals from CD4(+)3(-) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. The Journal of Experimental Medicine. 204(6):1267-72

Séverin, S., Gratacap, MP., Lenain, N., Alvarez, L., Hollande, E., Penninger, JM., Gachet, C., Plantavid, M., Payrastre, B. (2007). Deficiency of Src homology 2 domain-containing inositol 5-phosphatase 1 affects platelet responses and thrombus growth. The Journal of Clinical Investigation. 117(4):944-52

Shambharkar, PB., Blonska, M., Pappu, BP., Li, H., You, Y., Sakurai, H., Darnay, BG., Hara, H., Penninger, J., Lin, X. (2007). Phosphorylation and ubiquitination of the IkappaB kinase complex by two distinct signaling pathways. The EMBO Journal. 26(7):1794-805

Tassi, I., Cella, M., Gilfillan, S., Turnbull, I., Diacovo, TG., Penninger, JM., Colonna, M. (2007). p110gamma and p110delta phosphoinositide 3-kinase signaling pathways synergize to control development and functions of murine NK cells. Immunity. 27(2):214-27

Wada, T., Stepniak, E., Hui, L., Leibbrandt, A., Katada, T., Nishina, H., Wagner, EF., Penninger, JM. (2007). Antagonistic control of cell fates by JNK and p38-MAPK signaling. Cell Death and Differentiation. in press

Wong, DW., Oudit, GY., Reich, H., Kassiri, Z., Zhou, J., Liu, QC., Backx, PH., Penninger, JM., Herzenberg, AM., Scholey, JW. (2007). Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury. The American Journal of Pathology. 171(2):438-51

Zhang, L., Anglesio, MS., O'Sullivan, M., Zhang, F., Yang, G., Sarao, R., Mai, PN., Cronin, S., Hara, H., Melnyk, N., Li, L., Wada, T., Liu, PP., Farrar, J., Arceci, RJ., Sorensen, PH., Penninger, JM. (2007). The E3 ligase HACE1 is a critical chromosome 6q21 tumor suppressor involved in multiple cancers. Nature Medicine. 13(9):1060-9

Zuber, AM., Singer, D., Penninger, JM., Rossier, BC., Firsov, D. (2007). Increased Renal Responsiveness to Vasopressin and Enhanced V2 Receptor Signaling in RGS2-/- Mice. Journal of the American Society of Nephrology. 18(6):1672-8

RINGROSE

Lempradl, H., Ringrose, L. (2007). How do noncoding RNAs regulate Hox Genes? BioEssays, Review. in press

Ringrose, L. (2007). Polycomb comes of age: genome-wide profiling of target sites. Current Opinion in Cell Biology. 19(3):290-7

Ringrose, L., Paro, R. (2007). Polycomb/Trithorax response elements and epigenetic memory of cell identity. Development. 134(2):223-32

SMALL

Auinger, S. and Small, JV. (2007). Correlated Light and Electron Microscopy of the Cytoskeleton. In Methods in Cell Biology, Basic Biological Electron Microscopy; (Allen, ed.) Academic Press. in press

Efimov, A., Kharitonov, A., Efimova, N., Loncarek, J., Miller, PM., Andreyeva, N., Gleeson, P., Galjart, N., Maia, AR., McLeod, IX., Yates, JR., Maiato, H., Khodjakov, A., Akhmanova, A., Kaverina, I. (2007). Asymmetric CLASP-dependent nucleation of noncentrosomal microtubules at the trans-Golgi network. Developmental Cell. 12(6):917-30

Koestler, S., Auinger, S. and Small, JV. (2007). Correlative live cell imaging and negative stain electron microscopy of migrating cells. In Procedings of Microscopy Conference 2007, Microscopy and Microanalysis. Vol13, Suppl3, 184-185

Small, JV., Auinger, S., Nemethova, M. Koestler, S., Goldie, KN., Hoenger, A., and Resch, GP. (2007). Unraveling the Structure of the Lamellipodium. J Microscopy. in press

KELEMAN

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., and Dickson, BJ. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature. 448(7150):151-156

Keleman, K., Krüttner, S., Alenius, M., and Dickson, B.J. (2007). Function of the Drosophila CPEB protein Orb2 in long-term courtship memory. Nature Neuroscience. 10(12):1587-93

HOLSTE

Holste, D., Ohler, U. (2007). Strategies for identifying RNA splicing regulatory motifs and making alternative splice site predictions. PLoS Computational Biology. in press

BIOOPTICS

Bakiri, L., Takada, Y., Radolf, M., Eferl, R., Yaniv, M., Wagner, EF., Matsuo, K. (2007). Role of heterodimerization of c-Fos and Fra1 proteins in osteoclast differentiation. Bone. 40(4):867-75

Bakker, WJ., van Dijk, TB., Parren-van Amelsvoort, M., Kolbus, A., Yamamoto, K., Steinlein, P., Verhaak, RG., Mak, TW., Beug, H., Löwenberg, B., von Lindern, M. (2007). Differential regulation of Foxo3a target genes in erythropoiesis. Molecular and Cellular Biology. 27(10):3839-3854

Jantsch, V., Tang, L., Pasierbek, P., Penkner, A., Nayak, S., Baudrimont, A., Schedl, T., Gartner, A., Loidl, J. (2007). Caenorhabditis elegans prom-1 Is Required for Meiotic Prophase Progression and Homologous Chromosome Pairing. Mol. Biol. Cell. 18(12):4911-20

Regha, K., Sloane, MA., Huang, R., Pauler, FM., Warczok, KE., Melikant, B., Radolf, M., Martens, JH., Schotta, G., Jenuwein, T., Barlow, DP. (2007). Active and repressive chromatin are interspersed without spreading in an imprinted gene cluster in the mammalian genome. Molecular Cell. 27(3):353-66

ELECTRON MICROSCOPY

Small, JV., Auinger, S., Nemethova, M. Koestler, S., Goldie, KN., Hoenger, A., and Resch, GP. (2007). Unraveling the Structure of the Lamellipodium. J Microscopy. in press

BIOINFORMATICS

Burkard, T. R., Trajanoski, Z., Novatchkova, M., Hackl, H., Eisenhaber, F. (2007). Identification of new targets using expression profiling. In Antiangiogenic cancer therapy (Davis, D.R., Herbst, R.S., & Abbruzzese, J.L., eds.); CRC Press Taylor & Francis Group, Boca Raton London New York. 415-41

Gregan, J., Rabitsch, PK., Rumpf, C., Novatchkova, M., Schleiffer, A., Nasmyth, K. (2006). High-throughput knockout screen in fission yeast. Nature Protocols. 1(5):2457-64

Gregan, J., Riedel, CG., Pidoux, AL., Katou, Y., Rumpf, C., Schleiffer, A., Kearsey, SE., Shirahige, K., Allshire, RC., Nasmyth, K. (2007). The kinetochore proteins Pcs1 and Mde4 and heterochromatin are required to prevent merotelic orientation. Current Biology. 17(14):1190-200

Herzig, M., Savarese, F., Novatchkova, M., Semb, H., Christofori, G. (2007). Tumor progression induced by the loss of E-cadherin independent of beta-catenin/ Tcf-mediated Wnt signaling. Oncogene. 26(16):2290-8

Penkner, A., Tang, L., Novatchkova, M., Ladurner, M., Fridkin, A., Gruenbaum, Y., Schweizer, D., Loidl, J., Jantsch, V. (2007). The nuclear envelope protein Matefin/SUN-1 is required for homologous pairing in C. elegans meiosis. Developmental Cell. 12(6):873-85

Skok, JA., Gisler, R., Novatchkova, M., Farmer, D., de Laat, W., Busslinger, M. (2007). Reversible contraction by looping of the Tcra and Tcrb loci in rearranging thymocytes. Nature Immunology. 8(4):378-87

Wiesenberger, G., Speer, F., Haller, G., Bonnefoy, N., Schleiffer, A., Schafer, B. (2007). RNA degradation in fission yeast mitochondria is stimulated by a member of a new family of proteins that are conserved in lower eukaryotes. Journal of Molecular Biology. 367(3):681-91

PROTEIN CHEMISTRY FACILITY

Gillardon, F., Kloss, A., Berg, M., Neumann, M., Mechtler, K., Hengerer, B., Dahlmann, B. (2007). The 20S proteasome isolated from Alzheimer's disease brain shows post-translational modifications but unchanged proteolytic activity. Journal of Neurochemistry. 101(6):1483-90

Gregan, J., Riedel, CG., Petronczki, M., Cipak, L., Rumpf, C., Poser, I., Buchholz, F., Mechtler, K., Nasmyth, K. (2007). Tandem affinity purification of functional TAP-tagged proteins from human cells. Nature Protocols. 2(5):1145-51

Hartler, J., Thallinger, GG., Stocker, G., Sturn, A., Burkard, TR., Körner, E., Rader, R., Schmidt, A., Mechtler, K., Trajanoski, Z. (2007). MASPECTRAS: a platform for management and analysis of proteomics LC-MS/MS data. BMC Bioinformatics. 8:197

Kubicek, S., O'Sullivan, RJ., August, EM., Hickey, ER., Zhang, Q., Teodoro, ML., Rea, S., Mechtler, K., Kowalski, JA., Homon, CA., Kelly, TA., Jenuwein, T. (2007). Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Molecular Cell. 25(3):473-81

Mazanek, M., Mituloviae, G., Herzog, F., Stingl, C., Hutchins, JR., Peters, JM., Mechtler, K. (2007). Titanium dioxide as a chemo-affinity solid phase in offline phosphopeptide chromatography prior to HPLC-MS/MS analysis. Nature Protocols. 2(5):1059-69

Mitulovic, G., Mechtler, K. (2006). HPLC techniques for proteomics analysis--a short overview of latest developments. Briefings in Functional Genomics & Proteomics. 5(4):249-60

Pasquali, C., Bertschy-Meier, D., Chabert, C., Curchod, ML., Arod, C., Booth, R., Mechtler, K., Vilbois, F., Xenoarios, I., Ferguson, CG., Prestwich, GD., Camps, M., Rommel, C. (2007). A chemical proteomics approach to pi3k signaling in macrophages. Mol. Cell Proteomics. 6(11):1829-41

Schittenhelm, RB., Heeger, S., Althoff, F., Walter, A., Heidmann, S., Mechtler, K., Lehner, CF. (2007). Spatial organization of a ubiquitous eukaryotic kinetochore protein network in Drosophila chromosomes. Chromosoma. 116(4):385-402

Schmitz, J., Watrin, E., Lénárt, P., Mechtler, K., Peters, JM. (2007). Sororin is required for stable binding of cohesin to chromatin and for sister chromatid cohesion in interphase. Current Biology. 17(7):630-6

Schulz, DM., Kalkhof, S., Schmidt, A., Ihling, C., Stingl, C., Mechtler, K., Zschörnig, O., Sinz, A. (2007). Annexin A2/P11 interaction: new insights into annexin A2 tetramer structure by chemical crosslinking, high-resolution mass spectrometry, and computational modeling. Proteins. 69(2):254-69

Winter, S., Simboeck, E., Fischle, W., Zupkovitz, G., Dohnal, I., Mechtler, K., Ammerer, G., Seiser, C. (2007). 14-3-3 Proteins recognize a histone code at histone H3 and are required for transcriptional activation. EMBO J. in press

HISTOLOGY

Andrade, N., Komnenovic, V., Blake, SM., Jossin, Y., Howell, B., Goffinet, A., Schneider, WJ., Nimpf, J. (2007). ApoER2/VLDL receptor and Dab1 in the rostral migratory stream function in postnatal neuronal migration independently of Reelin. P.N.A.S. 104(20):8508-13

Hasselblatt, P., Rath, M., Komnenovic, V., Zatloukal, K., Wagner, EF. (2007). Hepatocyte survival in acute hepatitis is due to c-Jun/AP-1-dependent expression of inducible nitric oxide synthase. P.N.A.S. 104(43):17105-10

Hui, L., Bakiri, L., Mairhorfer, A., Schweifer, N., Haslinger, C., Kenner, L., Komnenovic, V., Scheuch, H., Beug, H., Wagner, EF. (2007). p38alpha suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. Nature Genetics. 39(6):741-9

Kuba, K., Zhang, L., Imai, Y., Arab, S., Chen, M., Maekawa, Y., Leschnik, M., Leibbrandt, A., Makovic, M., Schwaighofer, J., Beetz, N., Musialek, R., Neely, GG., Komnenovic, V., Kolm, U., Metzler, B., Ricci, R., Hara, H., Meixner, A., Nghiem, M., Chen, X., Dawood, F., Wong, KM., Sarao, R., Cukerman, E., Kimura, A., Hein, L., Thalhammer, J., Liu, PP., Penninger, JM. (2007). Impaired heart contractility in Apelin gene-deficient mice associated with aging and pressure overload. Circulation Research. 101(4):e32-42

MOUSE SERVICE

Eferl, R., Zenz, R., Theussl, HC., Wagner, EF. (2007). Simultaneous generation of fra-2 conditional and fra-2 knock-out mice. Genesis. 45(7):447-51

Awards

Sarah Bowman

VBC PhD Award
(November 2007)

Javier Martinez

BioRad European RNAi Research Award
(September 2007)

Elected into the EMBO Young Investigator Programme
(October 2007)

Ralph Neumüller

Kirsten Peter Rabitsch Award
(October 2007)

Josef Penninger

Descartes Prize for Research by the European Commission.
(March 2007)

Ernst Jung Prize for Medicine by the Jung-Stiftung for Science and Research
(May 2007)

Carus-Medal by the German Academy of Sciences Leopoldina
(October 2007)

Elected to the Austrian Academy of Sciences as Full Member
(April 2007)

Wellenreiter Prize by the Austrian Management Club
(September 2007)

Stefan Weitzer

BioRad European RNAi Research Award
(September 2007)

Seminar Speakers

JANUARY

08.01.07 Thomas Sternsdorf
Salk Institute San Diego
Artificial oncogenes for understanding leukemogenesis in Acute Promyelocytic Leukaemia (APL)

11.01.07 Richard A. Rachubinski
Department of Cell Biology, University of Alberta, Edmonton
Global Analysis of Kinase and Phosphatase Action in Peroxisome Biogenesis in Saccharomyces cerevisiae

12.01.07 Georg Schett
Dept. of Internal Medicine, University of Erlangen
Wnt signaling in murine and human joint disease

17.01.07 Christopher Gerner
Medical University of Vienna
Sekretome analysis by a combination of 2D-PAGE with shotgun proteomics of human dendritic cells treated with LPS, oxidised phospholipids or infected with rhinovirus

18.01.07 Julian Downward
Signal Transduction Laboratory, ICRF, London
Use of functional genomics to study Ras signaling networks in oncogenesis

24.01.07 Wolfgang Zachariae
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
How sisters stay together in meiosis

FEBRUARY 2007

01.02.07 Philipp Khaitovich
CAS-MPG Partner Institute for Computational Biology, Shanghai
Gene Expression in Human Evolution

02.02.07 Conrad Bleul
Max-Planck-Institute for Immunobiology, Freiburg
Multipotent hematopoietic precursors in the mouse thymus

07.02.07 Yusuke Nakamura
Human Genome Center, Institute of Medical Science, University of Tokyo
From cancer genomics to cancer therapy

13.02.07 Walter Lerchner
Division of Biology, California Institute of Technology
Neurons Turning Silent - Mice Turning Quietly

15.02.07 Christine Mézard
Institut Jean-Pierre Bourgin, Institut National de la Recherche Agronomique (INRA), Versailles
Meiotic recombination in Arabidopsis thaliana : sex and interference

16.02.07 Andrea Musacchio
European Institute of Oncology, Milan
Kinetochore-microtubule attachment and the spindle assembly checkpoint

16.02.07 Bill Dougall
Department of Cancer Biology, Amgen, Washington
RANK and RANKL: functionality in bone metastasis and breast tumors and development of a pharmacologic inhibitor of RANKL

22.02.07 Susan Gasser
Friedrich Miescher Institute for Biomedical Research
A novel function for Orc in cohesion

27.02.07 Karin Garber
Karin Garber Vienna Open Lab - dialog<>gentechnik
Hands on - Minds on". The Vienna Open Lab and its Strategy to Improve the Relationship between Science and the Public

28.02.07 Steve Blacklow
Department of Pathology, Harvard Medical School
Structural and biochemical insights into Notch restraint and activation

MARCH 2007

02.03.07 Wouter de Laat
Dept. of Cell Biology and Genetics, Erasmus MC, Rotterdam
Chromatin architecture uncovered by 4C technology

08.03.07 Edith Heard
Mammalian Developmental Epigenetics Group, Curie Institute, Paris
The nuclear dynamics and epigenetic plasticity of X-chromosome inactivation

14.03.07 Jean-Christophe Marine
University of Ghent (DMBR)
Studying the p53 tumor suppressor pathway using the mouse as a model

15.03.07 Yves Barral
Institute for Biochemistry, ETH-Hoenggerberg, Zurich
Temporal coordination of late mitotic events

20.03.07 Michel Seidelin
CLC Bio Arhus (Denmark)
How can bioinformatics help you?

22.03.07 Tada Taniguchi
Medical University Tokyo
Nucleic acid sensing and activation of IRFs in the immune system

22.03.07 Allan Mowat
Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre
Regulation of intestinal immunity and tolerance by dendritic cells

APRIL 2007

02.04.07 Elizabeth Winzeler
The Scripps Research Institute, La Jolla, California
Systems biology of malaria

04.04.07 Gian Paolo Dotto
University of Lausanne
Notch1 is a p53 target gene involved in human keratinocyte tumor suppression

04.04.07 Stefan Hoppler
Institute of Medical Sciences, Foresterhill, University of Aberdeen
Wnt signalling in Xenopus development: mechanisms of tissue-specific Wnt signalling and Wnt6 function in heart organogenesis

12.04.07 Brian Johnstone
Oregon Health and Science University, Portland
Skeletal tissue regeneration with mesenchymal stem cells

18.04.07 Thomas Mueller-Reichert
Max Planck Institute for Molecular Cell Biology & Genetics, Dresden
Spindle organization in three dimensions

20.04.07 Philipp Selenko
Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology
High-resolution in-cell NMR spectroscopy

26.04.07 Phillip Karpovicz
University of Toronto
Support for the Immortal Strand Hypothesis: Mouse Neural Stem Cells and Drosophila Germline Stem Cells Partition DNA Asymmetrically

26.04.07 Pernille Rørth
Cell Biology and Biophysics Unit, EMBL, Heidelberg
Guiding migration: one cell cluster, multiple signaling mechanisms

MAY 2007

03.05.07 Mike Farzan
Department of Microbiology and Molecular Genetics, Harvard Medical School
Viral zoonosis and disease: insights from viral receptors and entry proteins

04.05.07 Robert D. Goldman
Feinberg School of Medicine, Northwestern University, Chicago
Mutations in The Human Lamin A Gene are Revealing Remarkable Functions for Intermediate Filament Proteins in the Nucleus

09.05.07 Ka Wan Li
Research Institute of Neurosciences, Amsterdam
Quantitative proteomics analysis of brain synapses

09.05.07 Joshua Mendell
Institute of Genetic Medicine, Johns Hopkins University School of Medicine
Dynamic Regulation of microRNA expression during the cell cycle and in cancer

10.05.07 Thomas E. Willnow
Max-Delbrueck-Center for Molecular Medicine, Berlin
LR11/SorLA, a Novel Risk Factor for Sporadic Alzheimer's Disease

16.05.07 Danny Reinberg
Howard Hughes Medical Institute, Department of Biochemistry, Piscataway
Chromatin and Its Impact on Gene Expression and Cellular Memory

21.05.07 Alexandra Goll
Core Unit for Medical Statistics and Informatics, Medical University of Vienna
Two-stage designs applying methods differing in costs

24.05.07 Abby Dernburg
Lawrence Berkeley National Labs, Berkeley, California
Chromosome choreography during meiosis: It takes two to tango

25.05.07 David Tuveson
CRUK Cambridge Institute
Modeling Malignancy in Mice

31.05.07 Bas Van Steensel
Netherlands Cancer Institute, Amsterdam
Chromatin genomics in flies and humans

JUNE 2007

06.06.07 Martin W. Hetzer
The Salk Institute, La Jolla
Biogenesis of the cell nucleus

08.06.07 Constanze Bonifer
University of Leeds, Leeds Institute of Molecular Medicine
Mechanistic insights into priming and early gene activation processes in the hematopoietic system

13.06.07 Karin Loser
Westfälische Wilhelms University, Münster
Effects of regulatory T cells on cutaneous immune responses

14.06.07 Rene Bernards
The Netherlands Cancer Institute, Division of Molecular Carcinogenesis
Using functional genomic approaches to identify biomarkers of therapy resistance in cancer

21.06.07 Hamilton Smith
J. Craig Venter Institute, Rockville, USA
Synthetic Biology: Present and Future

21.06.07 Wendell A. Lim
UCSF
The Modular Logic of Cell Signaling Systems

27.06.07 Kim Nasmyth
University of Oxford Department of Biochemistry
Does the cohesin ring really trap sister DNAs?

28.06.07 Chi-Chung Hui
Hospital for Sick Children, Toronto
Conserved and divergent features of Hedgehog signal transduction

JULY 2007

05.07.07 Jian-Kang Zhu
Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, University of California
Stress, small RNAs and epigenetic regulation in Arabidopsis

10.07.07 Stefan Strack
University of Iowa, Carver College of Medicine
Kinases and phosphatases in mitochondrial morphogenesis and neuronal death

11.07.07 Peter Murray
St. Jude Children´s Research Hospital, Memphis
Signal transduction from the IL-10 receptor to the anti-inflammatory response

12.07.07 John Mattick
Institute of Molecular Biosciences, University of Queensland
The human genome as an RNA machine

17.07.07 Rainer Pepperkok
EMBL Heidelberg, Advanced Light Microscopy Core Facility
Illuminating the secretory pathway

19.07.07 Bernd Bukau
ZMBH, Heidelberg
Mechanisms of molecular chaperones

SEPTEMBER 2007

13.09.07 Narry V. Kim
Institute of Molecular Biology and Genetics, Seoul National University
MicroRNA biogenesis

18.09.07 Jeremy Green
Department of Craniofacial Development, Kings College London
PAR polarity, Wnt signalling and neurogenesis in Xenopus development

20.09.07 Christopher E. Rudd
Head of the Cell Signalling Section, Department of Pathology, Cambridge University
Co-receptors, adaptors and T-cell adhesion

20.09.07 Peter Becker
Adolf-Butenandt-Institute, Munich
Dosage compensation in Drosophila: fine tuning transcription through chromatin structure

27.09.07 So Iwata
Imperial College London
Towards structural determination of membrane proteins

OCTOBER 2007

18.10.07 Rodney Phillips
Nuffield Department of Clinical Medicine, Oxford
HIV Evolution

NOVEMBER 2007

05.11.07 Judy Lieberman
CBR Institute for Biomedical Research, Boston
let-7 regulates self-renewal and tumorigenicity of breast cancer stem cells

06.11.07 Oliver Muehlemann
Institute of Cell Biology, University of Berne
Quality control of gene expression: mechanisms to recognize and eliminate expression of nonsense mRNA

09.11.07 Rainer Leitgeb
Center of Biomedical Engineering and Physics, Medical University of Vienna
Mouse Imaging

19.11.07 Maria Leptin
Insitut für Genetik, Universität zu Köln
A gene hierarchy from morphogen to morphogenesis: functions of conserved and fast evolving genes in the Drosophila embryo

29.11.07 Craig S. Pikaard
Biology Department, Washington University
Roles of the Arabidopsis RNA Polymerase IV-dependent siRNA pathway in chromatin organization and repetitive gene silencing

30.11.07 Irmgard Irminger
University Hospitals Geneva
BARD1 isoforms: the Trojan horse in gynecological cancers

DECEMBER 2007

06.12.07 Michael Dickinson
California Institute of Technology, Pasadena
How Flies Fly

Spotlight on 2007

IMP-IMBA Mini Recess

The IMP-IMBA Mini Recess took place at "Burg Schlaining", Burgenland, from April 27-28. All group leaders were invited to participate and discuss new scientific ideas with their colleagues in an informal setting. The main focus was, quite naturally, on the areas of biology represented at the two institutes: behavioral, molecular, structural, and developmental biology, as well as immunobiology and neurobiology.

Microsymposium on Small RNAs

The Second Microsymposium on Small RNAs which took place at IMBA from May 21-23 assembled 40 young and motivated Junior Group Leaders and PhD students, together with company representatives, working in the field of RNA Silencing. The sessions on "Mechanism, Function, Technology and Bioinformatics of Small RNAs" were followed with great enthusiasm by an audience dominated by scientists and students from the Vienna Biocenter Campus and from other European countries. In total, more than 250 participants enjoyed the Microsymposium.

Joint Retreat of Viennese and German PhD Students

A new experience for the IMBA PhD students was the joint retreat of the Göttingen Molecular Biology and Vienna Biocenter PhD programs, which took place in Tulln, Lower Austria from July 26-29. The main idea was to introduce the young scientists to topics like "Project Management for Scientists" and "Job Hunting, Interview Skills and Assessment Center", which may not play a role in their daily working routine but could be important for getting organized and selling one's craft. In addition, there was also a scientific poster session included as the students came from very different fields such as structural biology, developmental biology, biochemistry and bioinformatics.

Vienna Biocenter PhD Retreat

One of the most important events in the PhD calendar is the annual VBC PhD Retreat. Now in its second year, many students from the program took part in this year's Retreat in Litschau, Lower Austria. The PhD Retreat provides a great opportunity for students from different institutes and at different stages of their PhDs to get to know each other and their science, away from the lab.

The program for the Retreat included poster sessions and talks from two scientists: prominent British stem cell researcher Fiona Watt, who talked about women in science, as well as IMP alumnus Hartmut Vodermaier, who gave a talk about being a journal editor.

EDRC 2007 - 20th European Drosophila Research Conference Vienna

The European fly meeting has become a highlight in the calendar of every Drosophilist. This year, the meeting took place at the Vienna Reed Exhibitions & Congress Center from September 12 to 14. The conference organizers managed to attract a spectacular list of speakers to represent the areas of cell and developmental biology, neurobiology, population genetics, evolution, growth control and cell division, chromatin & gene expression, physiology, genomics and immunity.

IMP-IMBA Recess

From October 3-5, IMBA scientists met with members of the Scientific Advisory Board (SAB) to present their work and discuss their research. The SAB members, consisting of internationally recognized scientists, were once more impressed by the scientific performance and high standards of the research presented. For the first time this year, IMBA has its own SAB. We would like to welcome the board members Guenter Blobel, Ken Chien, Tony Hyman, Eric Kandel and Susan Lindquist, and sincerely thank them and the representatives of the Austrian Academy of Sciences for their commitment and advice.
IMBA SAB members: page 44 in this booklet.

Symposium "Molecules to Mind"

During the last few years, it has become a tradition for the students in the VBC PhD Program to organize a scientific symposium. From November 15-16, this year's meeting, "Molecules to Mind", covered both the complexity of the mind and the molecular basis of the brain. Various scientific sessions addressed questions like "How does our mind work?", "In which way do molecular details play a role in diseases, for example schizophrenia?" and "How do neural networks influence our memory?" To discuss this multi-faceted topic from a different angle, speakers from the field of ethics were invited to provide insight on the impact of this kind of research on society.

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

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