INSTITUTE OF MOLECULAR BIOTECHNOLOGY

OF THE AUSTRIAN ACADEMY OF SCIENCES

VIENNA BIOCENTER

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IMBA
Institute of Molecular Biotechnology
of the Austrian Academy of Sciences

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This year was a time of incredible growth for IMBA: within a short time, we managed to turn a virtual institution into a home of world-class science where people like to work. Since IMBA was founded as a joint initiative of the Austrian Academy of Sciences and Boehringer Ingelheim, I am also very pleased that IMBA and the IMP have grown together to form a strong community. We all gain a lot from this close-knit community.

In particular, I want to thank Barry Dickson for all his help and commitment in building up IMBA. To see him stay in Vienna as the new director of our close partner, the IMP, indeed bodes well for the future of our campus. I wish him all the best for his new position. I would also like to thank Kim Nasmyth for all his support and encouragement. Kim has made an incredible personal and professional contribution to the IMP and also to the birth and years of infancy of IMBA. He has left us with an extraordinary legacy of excellence that is nearly impossible to top; if we manage to maintain a similar level of excellence, IMBA/IMP will have a great future. Dear Kim, all the best in Oxford and please keep us in your heart and mind.

The last 3 years put quite a strain on all of us working under difficult conditions and spread out in different places. This period is finally over – in early 2006 we will move to the new IMBA building and will be able to work together in close physical and intellectual proximity. The new building and infrastructure promise to be spectacular. To make this happen, many people and institutions have contributed their time, effort, resources, and unique knowledge. Despite everything, we have been able to pull it off. My sincere thanks to all of you.

At the start of the new year, we also welcome three new members to our faculty: Kazufumi Mochizuki, Leonie Ringrose, and, as the first joint appointee between IMBA and IMP, Thomas Marlovits. I am also very happy that Jürgen Knoblich accepted to become IMBA´s deputy director, with all the powers to speak for IMBA.

There are many nice places in the world. However, it is people that make places truly special. We were lucky to be able to hire great people for every position at IMBA. I am honored and privileged to work with all of you. The people working at IMBA are the future and our future looks promising. The chance has been given us to make a little difference in this world – let's fulfill the promise!

Josef Penninger
December 2005

2005 *has been another year of dynamic growth for our institute, but also a year of major scientific achievements for IMBA researchers. Since the issuance of last year's research report, our faculty has increased by 50%, with 121 people in research groups, scientific and administrative services. This number demonstrates our strong commitment to further develop IMBA into a leading research institute for molecular biology in Central Europe.*

In 2005, IMBA has been operating with an annual budget of almost € 11 million, which represents an increase by almost 10%, as compared to last year, thanks to additional funds from the Ministry of Science, Education and Culture and from different domestic and international project sponsors. As of today, we are expecting another year of budget growth in 2006, as IMBA becomes increasingly successful in setting up externally funded research groups and in commercializing its scientific results. For example, IMBA is in the process of setting up a new Ludwig Boltzmann Institute for Functional Genomics that uses our Drosophila RNAi library to study disease-related gene functions in vivo. We wish the designated director Krystyna Keleman all the best to turn this promising initiative into a major success. We are also very excited that we have started to negotiate collaboration agreements on certain target discovery programs with a number of leading industrial players such as Boehringer Ingelheim. These partnerships will secure the commercial exploitation of IMBA's research results and will finally contribute towards funding our future activities.

One of our major milestones for this year will be the completion of our new lab and office building in the immediate proximity to our partner, the Research Institute of Molecular Pathology (IMP). For the first time since starting our operations all IMBA employees will share the same location, which will definitely simplify communication and scientific collaborations between the different groups. The additional space will provide IMBA and the IMP with the opportunity to further improve the quality of our scientific services and to expand into promising new areas such as electron microscopy. In August, we also started the reconstruction of the new cafeteria at the IMP site, an important initiative to foster internal communication and of course with other organizations at the Campus Vienna Biocenter (VBC). IMBA and IMP envision developing the new cafeteria into the main meeting point for informal communication between VBC members.

In this context, I would like to thank everyone involved in the establishment of our new location for their personal contribution and sustaining commitment. In particular, I would like to mention Alex Chlup and his team from the technical department, Werner Kubina and Andreas Riepl from the IT service unit as well as Friedrich Kuntner from the Purchasing Department for their extraordinary efforts during the past months. In addition, I would like to express my gratitude to the Austrian Government, the City of Vienna and the Austrian Academy of Sciences for their substantial financial support in settingt up such a fantastic new infrastructure for molecular biology and functional genomics here in Vienna. Now it is up to us to demonstrate that their financial investment will pay off.

Michael Krebs
December 2005

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. After all, IMBA is still in the process of developing into full bloom, with a brand-new building waiting to be filled with life.

Graduate students join IMBA through the Vienna Biocenter International PhD Program, run jointly with the University, the Medical University, the IMP and the Gregor Mendel Institute of Molecular Plant Biology (GMI). A call for applications goes out twice a year, with contracts typically lasting from three to four 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 large-scale international conference every other year, with smaller workshops and symposia taking place in between. 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: a municipal kindergarten offers admission from baby-age, and a privately run nursery is also just around the corner. 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 the IMBA is available at:
www.imba.oeaw.ac.at

IMBA and Its Surroundings

The Institute of Molecular Biotechnology (IMBA) is located in Vienna, the capital of Austria. IMBA was founded in 1999 by the Austrian Academy of Sciences in cooperation with Boehringer Ingelheim, an international pharmaceutical company with its headquarters in Germany. IMBA operates in close collaboration with the Research Institute of Molecular Pathology (IMP), Boehringer's basic research center in Vienna. The two institutes are located next to each other and share large parts of their infrastructure. The 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 (University and Medical University of Vienna), the Gregor Mendel Institute (GMI) of the Austrian Academy of Sciences, a University of Applied Sciences, eight biotech companies, a PR agency and a non-profit scientific society.

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. A number of events, including seminars and lectures, are open to all.

Vienna – a City of many Facets

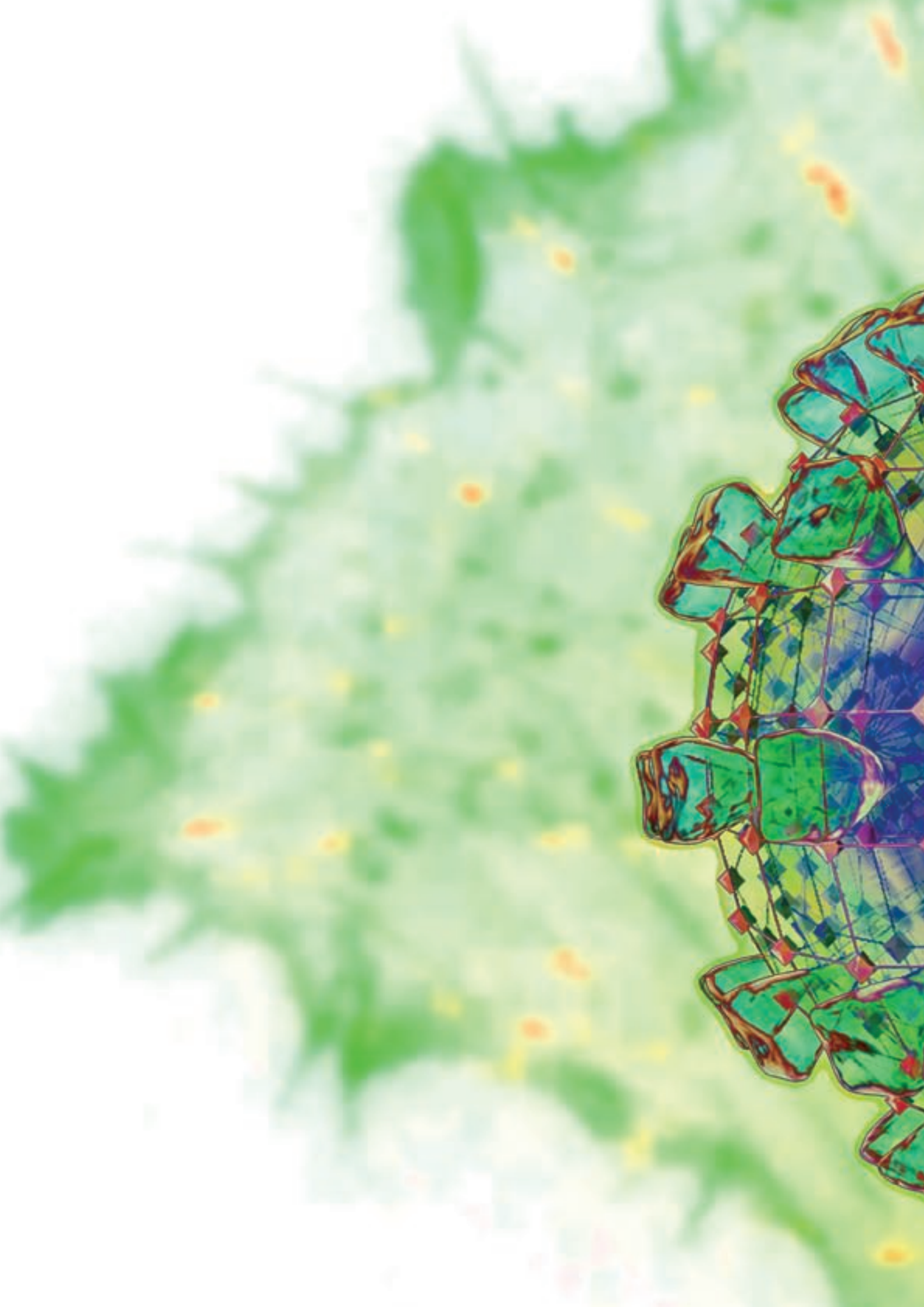
For those whose interests stretch beyond science, Vienna also has a lot to offer. Home to about 1.5 million people, the city is the political and cultural center of the country and its gateway to the east. Once the heart of the largest European empire, Vienna draws on a rich cultural heritage which is 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.

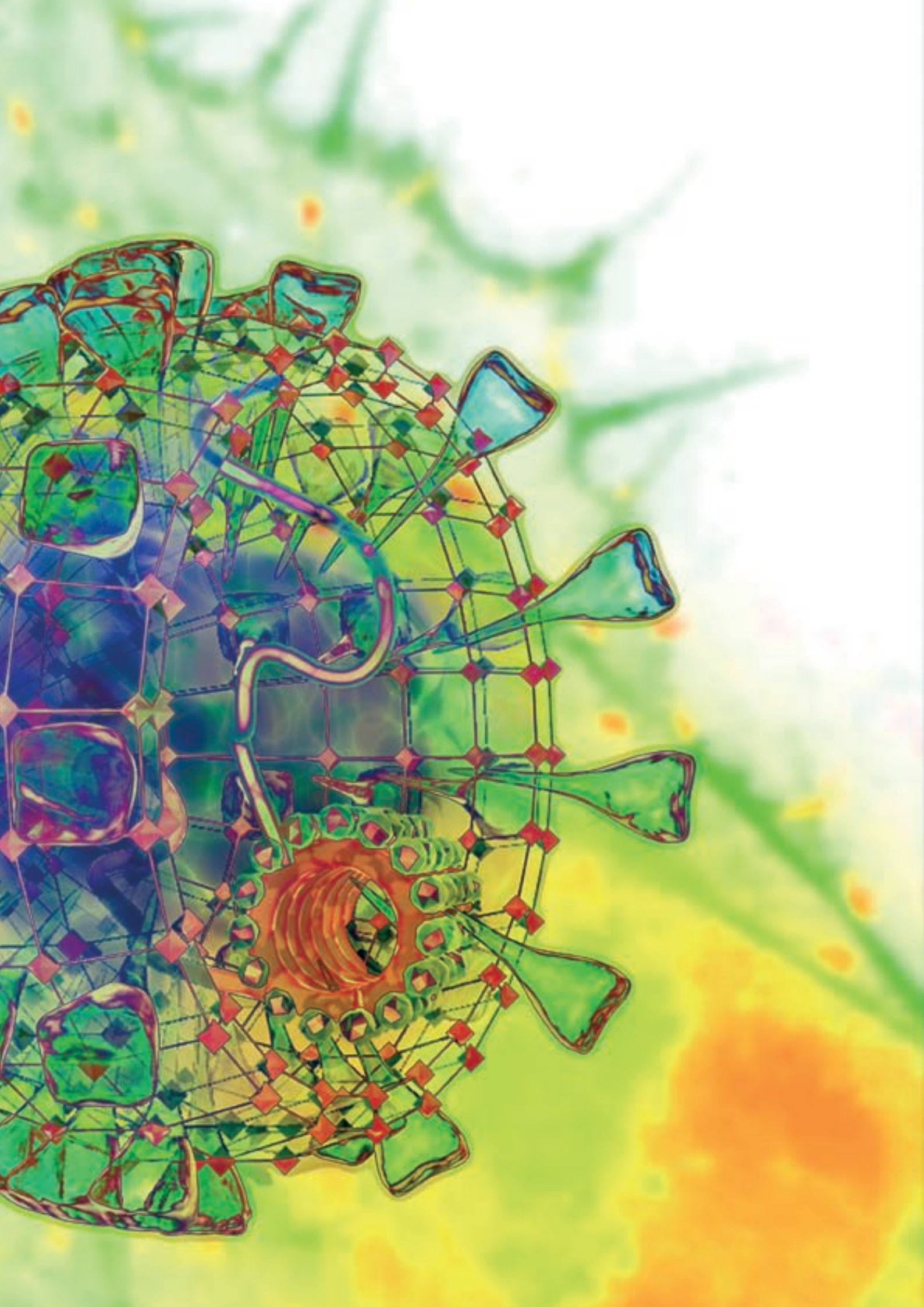
Apart from Vienna's focus on art and culture, it also has a long-standing tradition in science. Founded in 1365, the University of Vienna is the oldest university in the German-speaking world and the largest in Austria. With a student population of more than 100 000, Vienna offers not only the academic 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.

Quality of Life

Most Viennese citizens don't think twice about the quality of life, they just take it for granted. There is, however, a more systematic approach to the soft facts of life. In October 2005, the Economist Intelligence Unit in London published its latest 'Liveability Ranking' in which Vienna came second after Vancouver, Canada. This survey assesses living conditions in 127 cities around the world by looking at indicators such as stability, healthcare, culture and environment, education and infrastructure.





Assembly and Function of Neural Circuits

All animals are born with a set of instincts or innate behaviors. Selection has favored the evolution of genetic programs that "hard-wire" these behaviors into the nervous system. We seek to unravel these programs - to understand how genes direct the assembly of neural circuits, and how these circuits generate complex behaviors.

Assembly

Neural circuits are formed as individual neurons send out axons and dendrites to find, recognize, and connect with their appropriate target cells. We are using Drosophila genetics to investigate the molecular mechanisms that direct circuit assembly. We focus on two systems: the ventral nerve cord of the embryo, and the olfactory system of the adult.

The ventral nerve cord serves as a model system to investigate mechanisms of axon pathfinding – how do axons know which way to grow? Cells at the midline of the nerve cord secrete the guidance cues Netrin and Slit, which signal through receptors of the DCC and Robo families, respectively. Netrin is a short-range cue that helps some axons to grow across the midline. Slit is a repulsive cue that acts at short range to prevent certain axons from crossing, and may also act at long range to direct axons along specific pathways lateral to the midline. These different responses to Slit appear to depend upon the differential expression and regulation of three Robo receptors – Robo1, Robo2, and Robo3. For example, the sorting receptor Comm regulates the intracellular trafficking of Robo1 to control which axons cross the midline and which do not (Figure 1). We are currently using genetic, biochemical, and cell-biological approaches to investigate the function, expression, and regulation of the Robo family receptors. This system reveals how a relatively small number of guidance molecules can direct axons along complex and diverging pathways.

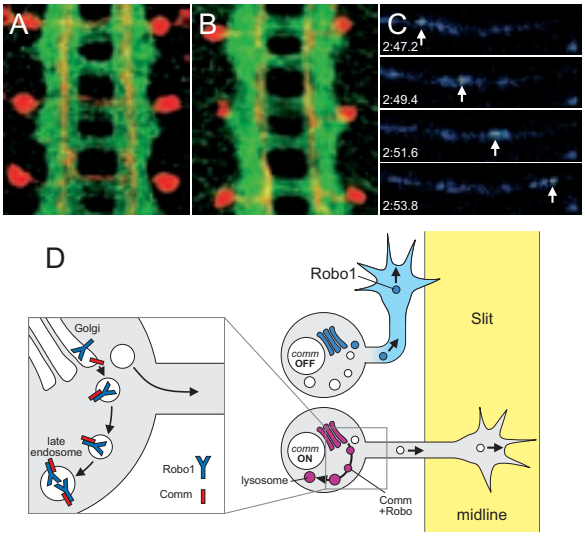


Figure 1: Crossing the midline. In the Drosophila CNS, some axons (red in A) but not others (B) cross the midline. This decision is controlled by Comm, which regulates the trafficking of Robo1 (C, D). Panel C shows a series of images from a movie in which a Robo1-GFP vesicle (red) moves towards the tip (right) of an axon in a living embryo. There is no Comm in this neuron. In neurons in which Comm is expressed, no Robo1-GFP can be seen moving down the axon.

Once axons reach their target region, how do they recognize their specific synaptic partners? For this, the olfactory system is an ideal model. The fly's sense of smell, like our own, depends on the precise wiring of olfactory neurons in the antenna (or nose) to specific target cells in the brain. We have recently determined almost the entire connectivity pattern between the ~1200 olfactory neurons (ORNs)

Barry Dickson

Barry Dickson / Senior Scientist

Mattias Alenius[1] / Postdoc
Doris Chen[2] / Postdoc
Krystyna Keleman[3] / Postdoc
Carlos Ribeiro[4] / Postdoc
Frank Schnorrer[5] / Postdoc
Laszlo Tirian[4] / Postdoc
Eleftheria Vrontou / Postdoc
Alexander Widmer[6] / Postdoc
Marko Brankatschk[7] / PhD Student
Africa Couto / PhD Student
Ebru Demir / PhD Student
Giorgio Gilestro / PhD Student
Amina Kurtovic / PhD Student
Duda Kvitsiani / PhD Student
Shay Rotkopf / PhD Student
Bettina Spitzweck[8] / PhD Student
Nilay Yapici / PhD Student
Jai Yu[9] / PhD Student
Irene Kalchhauser / Diploma Student
Katarina Bartalska[9] / Lab Manager
Karin Jäger / Technician

[1]Marie Curie, [2]EU FLYSNP, [3]until September 2005, [4]EMBO, [5]HFSP, [6]SNF, [7]until November 2005, [8]from November 2005, [9]from August 2005

in the fly's antenna and their target neurons in the ~50 synaptic "balls" (glomeruli) in the antennal lobe of the brain (Figure 2). Using our genome-wide transgenic RNAi library, we are now systematically identifying the molecules that instruct ORN axons to form synapses in specific glomeruli. Further characterization of these molecules should reveal the logic and mechanisms that control synaptic specificity in the brain.

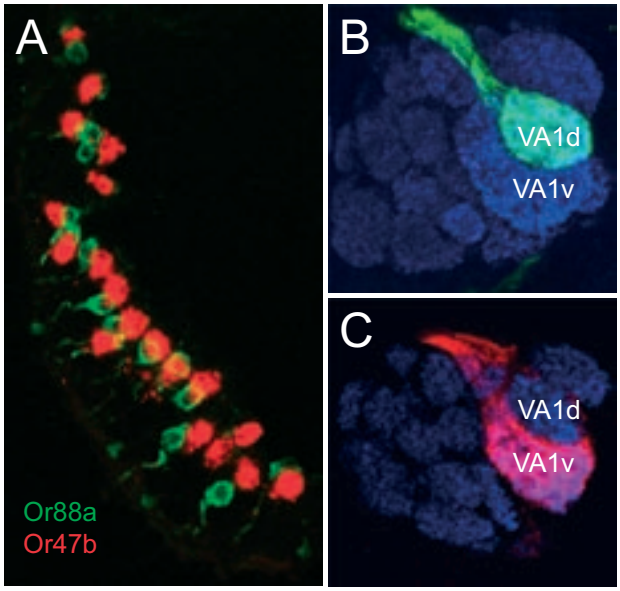


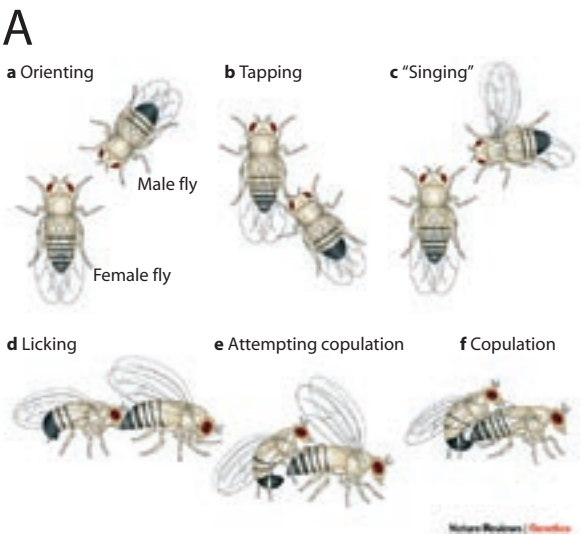
Figure 2: Olfactory wiring. (A). ORNs in the antenna expressing either of two different odorant receptors, Or88a (green) or Or47b (red). (B and C). ORNs that express the same odorant receptor project to the same glomerulus in the antennal lobe in the brain. The blue staining shows all the glomeruli in the antennal lobe. Red and green staining labels the axons of the same ORNs shown in A, which converge on glomeruli called VA1d and VA1v, respectively.

Function

How do neural circuits acquire and perform specific functions? To address this question, a major focus in the lab is the elaborate "song-and-dance" courtship ritual that Drosophila males perform to try to woo a prospective mate (Figure 3A). We have found that sex-specific splicing of the *fruitless* gene is both necessary and sufficient to "hard-wire" this behavior into the nervous system – males that splice *fruitless* in the female manner do not perform the male courtship ritual, whereas females that splice *fruitless* in the male manner do.

fruitless is expressed in about 2% of the fly's neurons, including neurons that function in sensory input, central processing, and motor output (Figure 3B). We have found that these neurons form a circuit that is dedicated to male courtship behavior. This same neural circuit is also present in females, even though they don't normally court. What these neurons do in females is still a mystery. We are currently using molecular genetics, electrophysiology and optical recording and stimulation to try to understand how this circuit functions, and why it functions differently in males and females. In parallel, we are using our RNAi library to systematically look for other genes required in the *fruitless* expressing neurons, or elsewhere in the nervous system, for male courtship behavior.

In addition to male courtship behavior, we are also beginning to study other innate behaviors, including female sexual behavior, male and female aggression, and feeding behaviors. Our goal is to use similar approaches to identify and characterize the neural circuits that mediate these behaviors. Although we aim to define both the sensory inputs and motor outputs characteristic for these behaviors, the primary long-term goal is to define the central "decision-making" pathways. Based on potentially conflicting signals from multiple sensory stimuli, as well as prior experience and current physiological status, the fly has to select the appropriate behavioral response. What are the genes and neural circuits that underlie such decision-making?



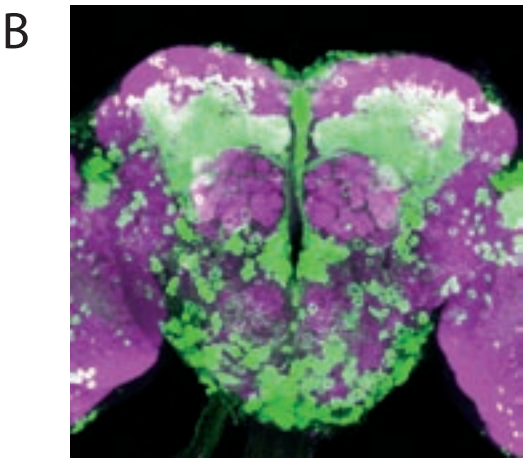
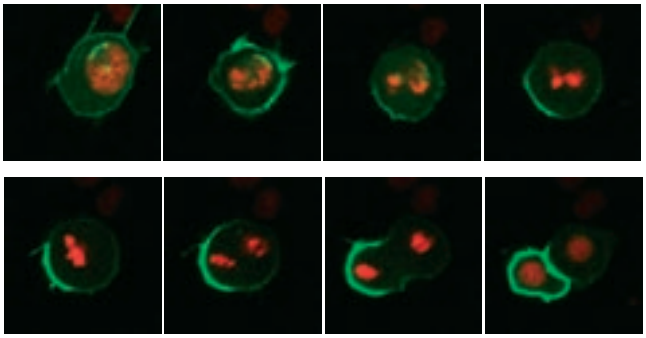


Figure 3: Fly sex. Drosophila males perform an elaborate, stereotyped courtship ritual to try to seduce a mate (A, reproduced from Sokolowski, Nature Reviews Genetics 2: 879). This requires the activity of a dedicated neural circuit (green in B).

Contact: barry.dickson@imba.oeaw.ac.at

Asymmetric Cell Division in Drosophila Neural Stem Cells

Stem cells are capable of generating two different kinds of progeny: On the one hand, they give rise to differentiating cells which exit the cell cycle and replace cells in the target tissue. On the other, they undergo self-renewal and generate other stem cells which continue to proliferate. 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.



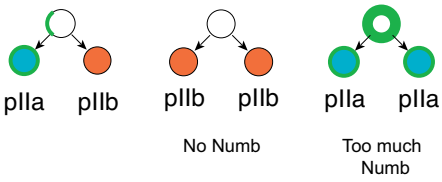


Figure 1: How cells divide asymmetrically. (A). Stills from a movie of a Drosophila neural precursor (SOP) cell undergoing asymmetric cell division. The movie was recorded from a whole living animal (a Drosophila pupa). Neural precursors are visible because they specifically express an RFP fusion to Histone (in red, to visualize DNA) and a GFP fusion to Pon (Partner of Numb, green). Pon is a Numb binding partner that colocalizes with Numb and allows indirect visualization of Numb protein localization. (B). In wildtype animals, the two daughter cells of an asymmetric cell division assume different fates. In numb mutants or when Numb is overexpressed, however, both daughter cells become identical.

In the *Drosophila* (larval) nervous system, the distinction between proliferating and differentiating daughter cells is made by protein determinants which are segregated into one of the two daughter cells during mitosis. One of these segregating determinants is the Drosophila protein Numb. Numb is a membrane-associated protein which localizes asymmetrically during mitosis and segregates into one of the two daughter cells (Figure 1). In this cell, it establishes a particular fate so that the two daughter cells differentiate into different cell types. In numb mutants or when numb is overexpressed, both daughter cells become identical. Numb is conserved in vertebrates and seems to play a similar role in asymmetric cell divisions that occur during development of the mouse brain. We are trying to understand how Numb and other cell fate determinants localize asymmetrically and how they influence the fate of one of the two daughter cells.

The asymmetric localization of Numb requires the conserved Par-protein complex. This complex contains the protein kinase aPKC and two PDZ domain proteins called Par-3 and Par-6. Before mitosis, the Par-complex localizes to the cell cortex opposite to where Numb

Jürgen Knoblich

Jürgen Knoblich / Senior Scientist

Jörg Betschinger[1] / Postdoc
Gregory Emery / Postdoc
Christoph Jüschke / Postdoc
Maria Pia Postiglione / Postdoc
Masakazu Yamazaki[2] / Postdoc
Mihaela Zigman / Postdoc
Sheetal Bhalerao / PhD Student
Sarah Bowman[3] / PhD Student
Ciara Gallagher / PhD Student
Andrea Hutterer / PhD Student
Jennifer Mummery / PhD Student
Ralph Neumüller / PhD Student
Constance Richter / PhD Student
Frederik Wirtz-Peitz[3] / PhD Student
Elke Kleiner / Technician
Stephanie Wculek / Technician

Fellowships: [1] FWF, [2] Marie Curie, *[3] BIF*

will accumulate. During mitosis, it is responsible for the asymmetric localization of several cell fate determinants – including Numb. Using preparative immunoprecipitation and mass spectroscopy, we have identified the cytoskeletal protein Lgl as an additional component of the Par-protein complex (Figure 2A). On one side of the cell, Lgl is phosphorylated by aPKC. Phosphorylation inactivates Lgl by blocking its association with the actin cytoskeleton. On the other side of the cell, however, Lgl is active and allows the recruitment of cell fate determinants to the cell cortex. A de-letion analysis of the Lgl protein told us that the C-terminus of Lgl associates with cytoplasmic myosin II. Upon phosphorylation, however, the N-terminus binds to the C-terminus and blocks myosin interaction (Figure 2B).

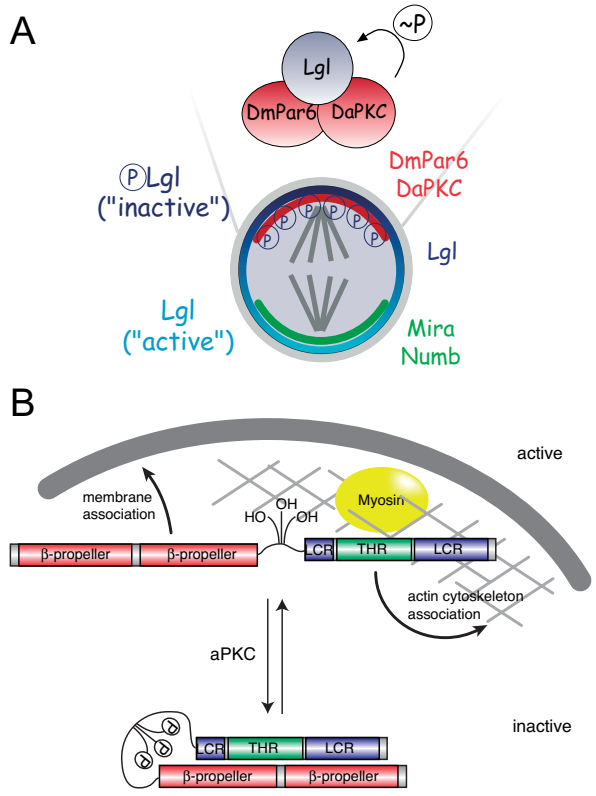


Figure 2: How Lgl directs asymmetric cell division. (A). In neural precursor cells, the Par-protein complex localizes asymmetrically and phosphorylates the cytoskeletal protein Lgl on one side of the cell. This inactivates Lgl. On the opposite side, however, Lgl is active and allows localization of cell fate determinants to the cell cortex. How Lgl does this is a major focus of our research. (B). How Lgl is inactivated. In the active conformation, the C-terminus of Lgl interacts with myosin. Upon phosphorylation, however, the N-terminus binds to the C-terminus and prevents this interaction so that Lgl becomes inactive and translocates into the cytosol.

Thus, Lgl is regulated by phosphorylation-induced auto-inhibition. We are using mass spectroscopy to identify binding partners of the various functional domains in the Lgl protein to understand how Lgl allows the localization of cell fate determinants to the cell cortex.

lgl mutants die from the overproliferation of larval tissues (hence the name: lethal giant larvae). In wild-type *Drosophila* larvae, the brain has only a few stem cells (which are called neuroblasts), while most cells exit the cell cycle and undergo differentiation into neurons (Figure 3B). In lgl mutant brains, however, many more proliferating neuroblasts are present (Figure 3C). Therefore, the brain grows to an enormous size, fills up the whole animal and ultimately kills it. Thus, *lgl* mutants can be considered a *Drosophila* tumor model.

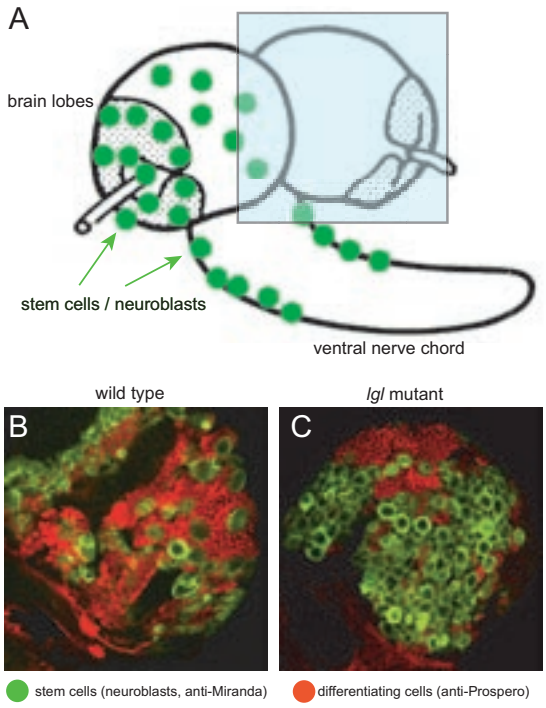


Figure 3: Tumor formation in lgl mutants. (A). The brain of a Drosophila larva consists of the ventral nerve chord (equivalent to our spinal chord) and the left and right brain lobes (equivalent to our brain hemispheres). Neuroblasts are neuronal stem cells which are present in both areas and give rise to all the neurons in the brain of an adult fly. (B). Neuroblasts can be identified by their expression of the marker Miranda while Prospero identified the differentiating daughter cells. The image shows a wild-type brain lobe which has few neuroblasts (green) and many more differentiating cells (red). (C). In lgl mutants, the number of neuroblasts is dramatically increased and almost all differentiating cells are missing. This leads to overgrowth of the brain and kills the animal.

The roles of lgl in asymmetric cell division and tumor suppression are intimately linked. lgl tumors arise because neuroblasts divide into two proliferating daughter cells. We have identified a segregating tumor suppressor called Brat (for brain tumor) that seems to be responsible for growth regulation in Drosophila neural stem cells. Normally, Brat is segregated into the differentiating daughter cells, where it inhibits cell growth and proliferation. In lgl mutants, however, Brat is not segregated and both daughter cells continue to proliferate. Brat is a conserved protein and we believe that its analysis in Drosophila will also tell us how proliferation is controlled in mammalian stem cells and how stem cells can turn into tumor stem cells to drive carcinogenesis. We have therefore begun to search for Brat binding partners using mass spectroscopy and to identify other tumor suppressor genes in Drosophila using genome-wide functional genetic screens.

Other major components of the machinery for asymmetric cell division are conserved in vertebrates as well. Mouse Numb segregates asymmetrically during mouse brain development. The Par-complex is involved in mammalian cell polarity and – as in *Drosophila* – acts by phosphorylating the Lgl homolog. To analyze the contribution of asymmetric cell divisions to mammalian development and to understand their role in stem cells, we have recently started to investigate mammalian homologs of the genes which establish this process in *Drosophila*. We analyze their subcellular distribution and study their mutant phenotype by generating knock-out mouse strains. We hope that these experiments will ultimately tell us to what extent asymmetric cell divisions contribute to the development of our own body.

Contact: juergen.knoblich@imba.oeaw.ac.at

Design and Function of Molecular Machines

What keeps cells and organisms alive are specific functions performed by highly organized macromolecular assemblies. Our research is motivated to understand the fundamental design and function of such macromolecular complexes. In particular, we are interested in systems that are involved in protein transport and signaling. At the center of our research, our laboratory takes an integrated approach and combines tools for structural and molecular biology, biochemistry and biophysics. In particular, we use three-dimensional electron cryomicroscopy and image processing as a platform to link high-resolution structural biology with cell biology in order to obtain for the first time a mechanistic view of macromolecular machines from atomic to cellular level.

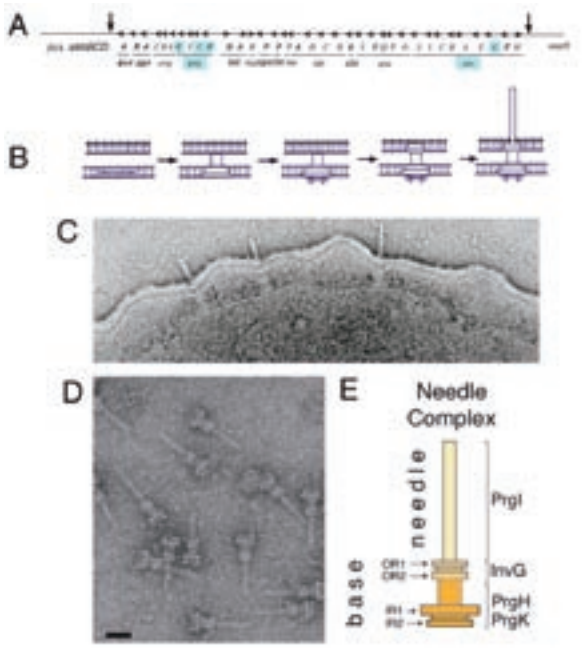


Figure 1: The type III secretion system (A) The type III secretion system is located on the bacterial chromosome but encoded on a specific pathogenicity island. Structural proteins of the needle complex are indicated in blue. (B) Formation of stable intermediate substructures during assembly. (C) Electron 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.

Host-Pathogen Interaction

A fundamental question in microbial pathogenicity is how bacterial toxins enter eukaryotic host cells. Once inside, they can trigger an entire infection cycle.
Gram negative pathogens, such as *Salmonella, Yersinia*, and *Shigella*, use the type III secretion system (TTSS), a complex macromolecular system to mediate the unidirectional transport of specific effector proteins – often also called virulence factors or toxins - between organisms. 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 plague.

The Assembly

Made up of more than twenty proteins, TTSSs assemble into large "molecular nanomachines" composed of a set of soluble as well as membrane proteins. All the structural components as well as other proteins involved in the assembly and function are encoded on specific pathogenicity islands (Figure 1A). Recently, genetic and biochemical analysis revealed that assembly is a step-wise process during which stable intermediate substructures are formed (Figure 1B).

Thomas Marlovits

Thomas Marlovits[1,2] / Group Leader

[1] *since September 2005*
[2] *joint IMP/IMBA Group Leader*

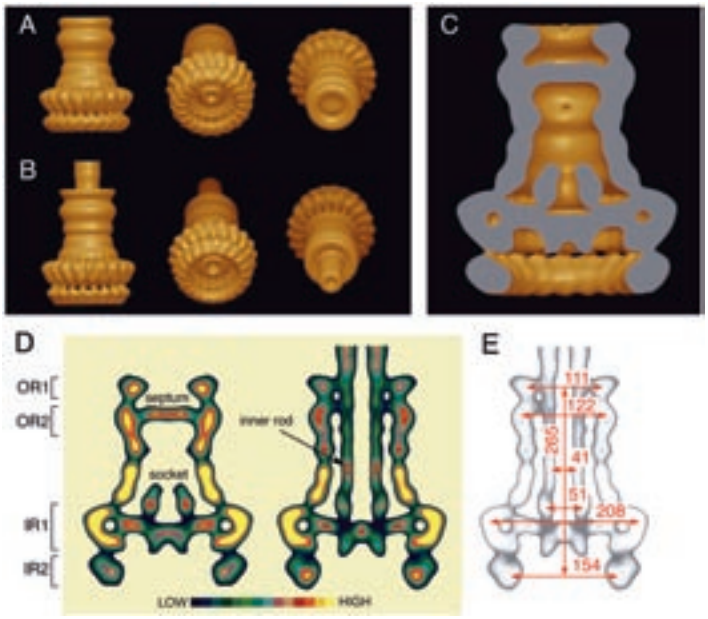


Figure 2: The structure of the base and the needle complex surface renderings of the base (A) and the needle complex (B) show that their overall shape is quite similar. However, tilted views indicate that during the assembly individual domains must undergo large structural rearrangements. (C) The inside 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 Angstroems.

The Molecular Design

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 1CD). The needle complex is believed to serve as a conduit for the 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 mechansism of type III secretion, we first set out to determine structural components of the TTSS. We were challenged by the mega-dalton size of the complex, its natural composition (membrane and soluble proteins), and its limited availability. Taken together, such factors usually put these macromolecular machines out of reach of what can be achieved by conventional approaches toward structure. However, the exceptions are the rapidly developing approaches in the field of molecular microscopy, which allow structural analysis to be carried out on scales ranging from tomographic reconstruction of cellular compartments to the near atomic detail revealed by electron crystallography on two-dimensioal ordered arrays or single particle analysis of isolated molecular complexes.

Recently, we were able to purifiy 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. Our biochemical analysis using quantitative amino acid analysis revealed that the membrane-associated base proteins are present in equimolar amounts (PrgH:PrgK:InvG = 1:1:1). On a structural level, this suggests that the three proteins are likely to share the same rotational symmetry of the cylindrically shaped base. We were, however, intrigued to discover that the membrane-anchored base can adopt different sizes. 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.

In addition, our analysis identified a new structural component, the inner rod, present right in the center of the needle complex (Figure 2). It not only extends the secretion path from the base into the needle filament, but also serves 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. Functionally, this dynamic behavior is a crucial event during the assembly process, which reprograms the secretion machine so that it becomes competent for the secretion of virulence factors only after the growth of the needle filament is complete.

The Future

Although the design of the TTSS is conceptually simple, structural characterization of the needle complex is at an early stage, leaving many questions unanswered. 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 just begun to address such questions for the future and 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.

Contact: marlovits@imp.univie.ac.at

Mechanism of RNA Interference in Human Cells

RNA interference (RNAi) has revolutionized biology in such a way that it is now easy to silence the expression of any gene with great specificity. Strikingly, RNAi is also becoming a tool for disease treatment, with major improvements in terms of the systemic delivery of modified siRNAs. The goal of our laboratory is to dissect the RNAi pathway using biochemistry. We are currently focusing on the assembly of RISC, the RNA-Induced Silencing Complex, and the identification of the kinase activity that phosphorylates synthetic siRNAs upon transfection. Furthermore, we have recently discovered a new post-transcriptional regulatory step during miRNA biogenesis.

RNA interference (RNAi) is a post-transcriptional gene silencing mechanism triggered by double-stranded RNA, which operates in most eukaryotic organisms. The use of RNAi as a tool to silence gene expression has spread worldwide since the pioneering work of Thomas Tuschl. The introduction of synthetic short RNA duplexes (short interfering, or siRNAs), consisting of 19 base-pairs and 2-nt overhangs at the 3′ end, into cells triggers degradation of homologous mRNAs with exquisite specificity. The "knock-down" phenotype generated by siRNA-directed mRNA degradation is the result of partial or total loss of the targeted protein. Our laboratory is taking a biochemical approach to identify new enzymatic activities and molecular mechanisms along the RNA pathway.

The RISC Complex: from Assembly to Activation.

A crucial step in the RNAi pathway involves the assembly of RISC, the RNA-Induced Silencing Complex. RISC initially recognizes a double-stranded siRNA, but only one strand, the "guide" strand, is finally retained in the functional ribonucleoprotein complex. We have found the non-incorporated, or "passenger" strand, to be cleaved during the course of RISC assembly prior to its removal (Figure 1A). Chemical modifications that impair cleavage of the passenger strand also impair its removal and, as a consequence, cleavage of target RNA (Figure 1B), suggesting that the cleavage of the passenger strand facilitates the assembly of active RISC. Interestingly, target RNA cleavage can be rescued, if an otherwise non-cleavable passenger strand displays a nick at the scissile phosphodiester bond. This further indicates that the cleavage event *per se* is not essential for the generation of functional RISC. We are currently testing the *in vivo* silencing performance of siRNAs containing non-cleavable passenger strands.

Identification and Characterization of a Human "siRNA Kinase"

It is well established that for the guide strand to enter the RNAi pathway, it must contain a phosphate group at the 5′ terminus. Synthetic siRNAs bearing a 5′-hydroxyl end become rapidly phosphorylated in *Drosophila* embryonic and HeLa cell extracts by an elusive kinase activity. By using a classical biochemical approach, we have successfully purified and identified the kinase that is responsible for this phenomenon. The enzyme phosphorylates double-stranded (ds) RNA, single-stranded (ss) RNA but not ssDNA. Moreover, the kinase does not discriminate between blunt ends, 5′- or 3′-overhangs of RNA duplexes. Future studies will address and evaluate its role in the RNAi pathway *in vivo* and its potential functions in cellular RNA metabolism.

Javier Martinez

Javier Martinez / Group Leader

Stefan Weitzer / Postdoc
Sebastian Ehm / PhD Student
Philipp Leuschner[1] / PhD Student
Gregor Obernosterer / PhD Student
Dubravka Pezic / PhD Student
Annerose Kuras / Diploma Student

[1] *Boehringer Ingelheim Fonds*

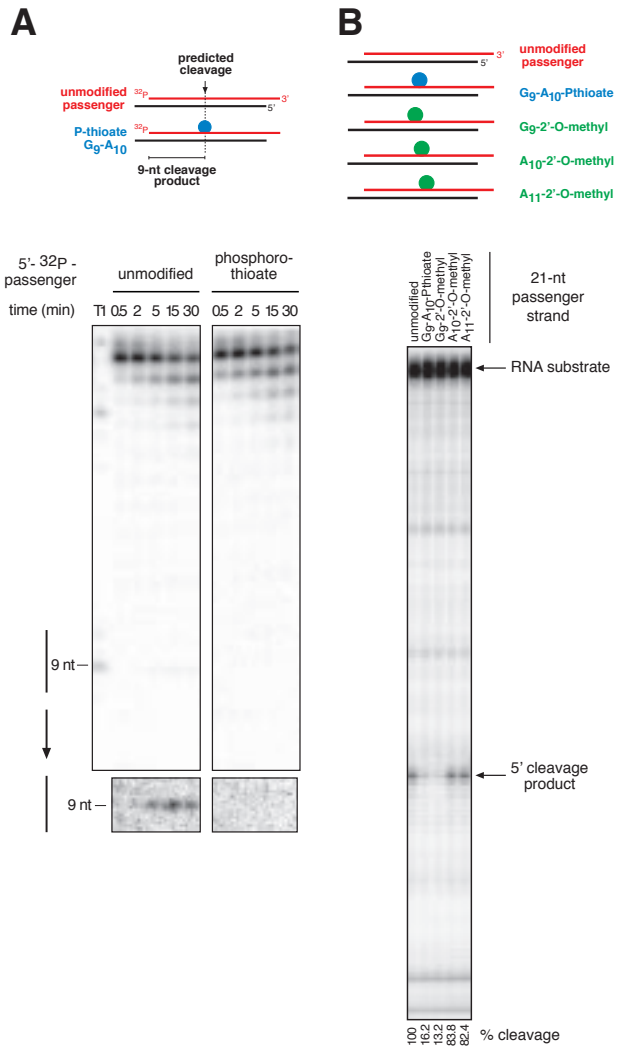


Figure 1: The passenger strand is cleaved during RISC assembly. (A) Top: Graphic representation of siRNAs containing unmodified or chemically modified passenger strands. Guide strands are depicted in black, passenger strands in red. (A) Bottom: Phosphorimaging analysis of a time course cleavage reaction resolved in a 15% denaturing polyacrylamide gel. The region of the gel corresponding to sizes between ~8 and 10 nt has been enhanced in the lower panel for optimal visualization of the 9-nt cleavage product. (B) Top: as (A). (B) Bottom: Phosphorimaging of cleavage reactions resolved in a 6% denaturing polyacrylamide gel. Arrows point to the RNA substrate and the labeled 5´-cleavage product. The percentage of cleaved target RNA, normalized to the unmodified siRNA, is indicated at the bottom of the gel.

Post-transcriptional Regulation of microRNA Expression

MicroRNAs (miRNAs) are endogenous, non-coding ~22 nucleotide (nt) RNA molecules that have recently emerged as post-transcriptional regulators of cognate target gene expression. Many mammalian miRNAs are expressed in a tissue-specific manner, a phenomenon that so far has been attributed to transcriptional regulation. We have recently found that, in addition to regulation at the transcriptional level, differential maturation of precursor miRNAs (pre-miRNAs) can also confer tissue-specific expression of mammalian miRNAs. Northern blot and *in-situ* hybridization experiments show the gene coding for miR-138 ubiquitously transcribed, but its processing into an active ~23-24 nucleotide miRNA molecule restricted to distinct areas of the adult brain as well as to the fetal brain and liver (Figure 2A). The pre-miRNA is normally translocated to the cytoplasm, thus ruling out a block of export from the nucleus as an explanation for the lack of processing (Figure 2B). We propose that differential maturation of miRNAs is an alternative mechanism to achieving tissue-specific miRNA expression.

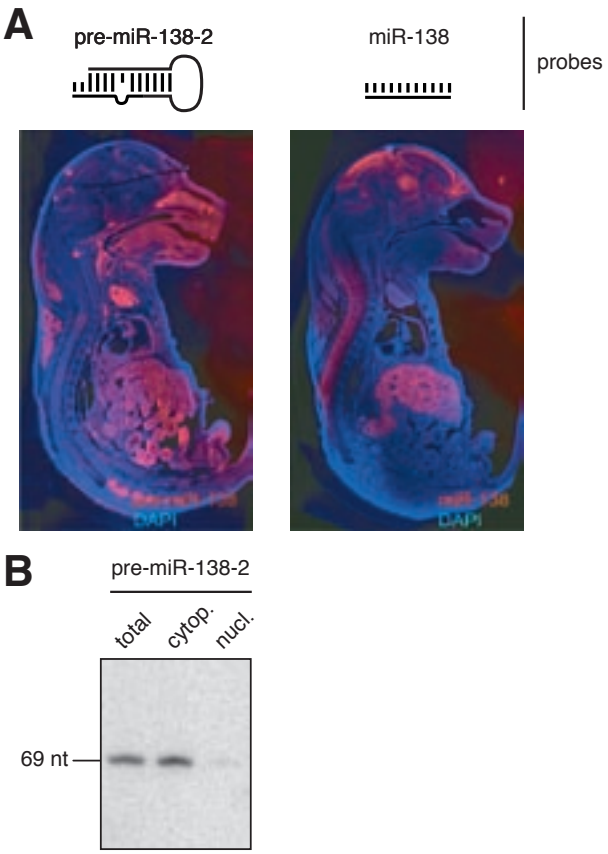


Figure 2: miR-138 expression is regulated at the post-transcriptional level. (A). In-situ hybridization reveals the ubiquitous expression of pre-miR-138-2 in mouse embryo, whereas mature miR-138 can only be detected in the fetal brain and liver, as well as the adult brain. (B). Northern blot shows that pre-miR-138-2 is faithfully exported to the cytoplasm.

Contact: javier.martinez@imba.oeaw.ac.at

ACE2: Molecular Control of Acute Lung Failure by a Killer Virus

Angiotensin converting enzyme 2 (ACE2) is a critical negative regulatory component of the renin angiotensin system (RAS). ACE2 has also been identified as a receptor for the Severe Acute Respiratory Syndrome (SARS) Coronavirus. Our group is trying to genetically dissect the role of ACE2 in acute lung failure and SARS infections.

Angiotensin Converting Enzyme 2 (ACE2)

ACE2 is a homolog of ACE, both of which are central enzymes in the renin-angiotensin-system (RAS) involved in blood pressure regulation, as well as in fluid and salt balance. Whereas ACE cleaves the decapeptide Angiotensin I (AngI) into an octapeptide Angiotensin II (AngII), ACE2 functions as a carboxypeptidase, cleaving a single residue from AngI, generating Ang1-9, and a single residue from AngII to generate Ang1-7. Our group made the first *ace2* mutant mice. Targeted disruption of murine ACE2 resulted in increased AngII levels, impaired cardiac contractility in older mice, and upregulation of hypoxia-induced genes in the heart. Loss of ACE on an ACE2 background reversed this heart phenotype. Thus, ACE2 is a negative regulator of the RAS and counterbalances the function of ACE (Crackower et al. Nature).

ACE2 is the Critical SARS Receptor

For several months during 2003, a newly identified illness termed severe acute respiratory syndrome (SARS) spread rapidly through the world, disrupting travel and the economy. A novel coronavirus was identified as the SARS pathogen which triggered atypical pneumonia characterized by high fever and severe dyspnea. The death rate following infection approached ~ 10% due to the development of acute severe lung failure. Moreover, influenza such as the Spanish flu and the emergence of new respiratory disease viruses caused high lethality among infected individuals due to acute lung failure. The high lethality of SARS infections, their enormous economic and social impact, fears of renewed outbreaks of SARS as well as the feared misuse of such viruses as biological weapons make it paramount to understand the disease pathogenesis of SARS and acute severe lung failure.

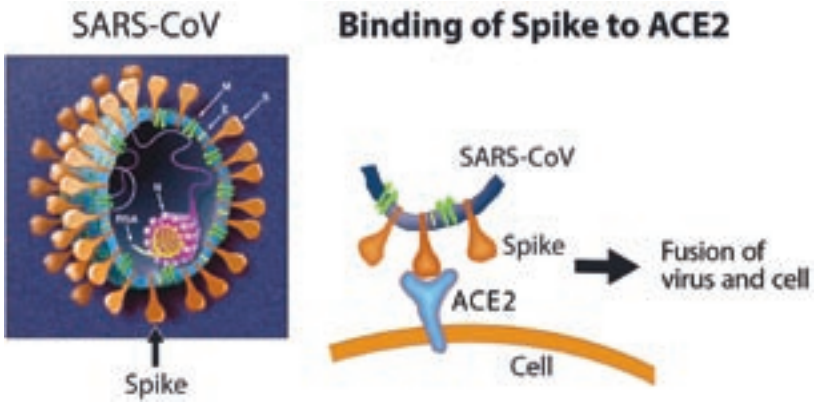


Figure 1: Scheme of SARS-Spike binding to ACE2 and SARS entry into cells via ACE2 (Kuba et al. Nat. Med. 2005).

Josef Penninger

Josef Penninger / Senior Scientist and Director

Ursula Danilczyk[1] / Postdoc
Reiko Hanada[1] / Postdoc
Toshi Hanada[1] / Postdoc
Yumiko Imai[2] / Postdoc
Keiji Kuba[3] / Postdoc
Arabella Meixner[4] / Postdoc
Tomoki Nakashima[3] / Postdoc
Greg Neely[3] / Postdoc
Andrew Pospisilik[3] / Postdoc
Claudia Vorbach[3] / Postdoc
Shane Cronin[5] / PhD Student
Nick Joza[2] / PhD Student
Stefanie Löser[6] / PhD Student
Manu Rangachari[7] / PhD Student
Tamara Zoranovic[1] / PhD Student
Andreas Leibbrandt[5] / Research Associate
Renu Sarao[6] / Research Associate
Rubina Yaghubian[4] / Research Associate

[1] Marie Curie Excellence grant, [2] Austrian National Bank grant, [3] Fellowship Marie Curie, [4] GEN-AU grant, [5] SFB grant (FWF), [6] EuroThymaide EU grant , [7] University of Toronto,

In cell lines, ACE2 was identified as a potential SARS receptor. We therefore studied whether ACE2 is indeed important for SARS infections and could provide the first genetic proof that ACE2 is a critical SARS receptor *in vivo*. No infectious SARS virus can be recovered in *ace2* mutant mice. SARS infections and the Spike protein of the SARS corona virus reduce ACE2 expression. Importantly, SARS Spike injection into mice worsens acute lung failure *in vivo*. The condition can be attenuated by blocking the renin-angiotensin pathway. These results provide a molecular explanation as to why SARS infections cause severe and often lethal lung failure and suggest a rational therapy for SARS and possibly other respiratory viral diseases.

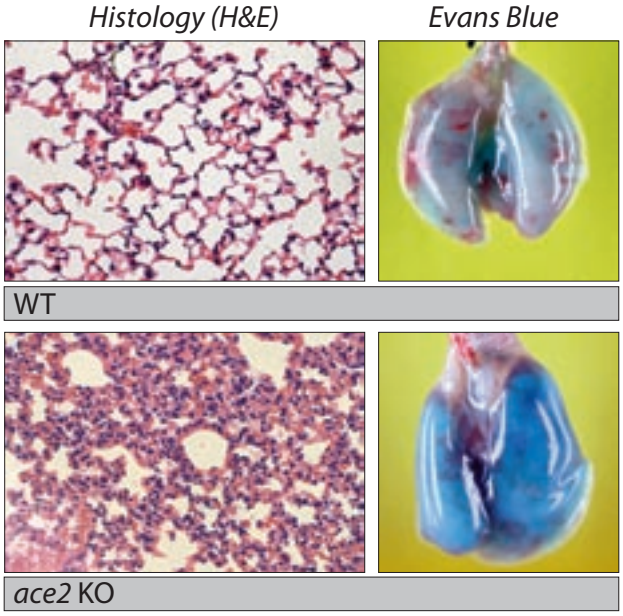


Figure 2: Lung histopathology. Note enhanced hyaline membranes, cell infiltrates, and lung edema in acid-treated wild-type (WT) and ace2 mutant mice (H&E staining, ×200). Pulmonary vascular permeability was determined by the intravenous injection of Evans Blue. Representative images of Evans Blue-injected lungs of WT and Ace2 KO mice 3 h after acid aspiration are shown above (Imai et al. Nature 2005).

ACE2 Controls Acute Lung Failure

Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury, is a devastating clinical syndrome with a high mortality rate of 30–60%. Predisposing factors for ARDS are diverse and include sepsis, aspiration, pneumonia, and infections with SARS or avian flu. At present, there are no effective drugs for improving the clinical outcome of ARDS. Since we identified ACE2 as the critical SARS receptor *in vivo* and ACE2 is expressed in lungs, we examined the effect of *ace2* gene deficiency in mouse experimental models that mimic the common lung failure pathology in humans. Indeed, ACE2 protects mice from acute lung injury induced by acid aspiration and sepsis. Disease pathogenesis was mapped to the ACE-angiotensin II-angiotensin receptor 1a (AT1a) pathway, while ACE2 and the angiotensin 2 receptor (AT2) negatively regulate lung function and lung edemas. Importantly, recombinant human ACE2 protects mice from severe acute lung failure. Our data thus identified a critical function for ACE2 in acute lung injury, pointing to a possible therapy for a syndrome affecting millions of people worldwide every year.

In conclusion, our data provide a molecular link between SARS pathogenesis and the role of the renin-angiotensin system (RAS) in lung failure. Recombinant ACE2 protein could therefore not only be a treatment to block the spreading of SARS but modulation of the RAS could be also utilized to protect SARS patients, and possibly patients infected with other viruses such as avian influenza strains, from developing acute lung failure and ARDS.

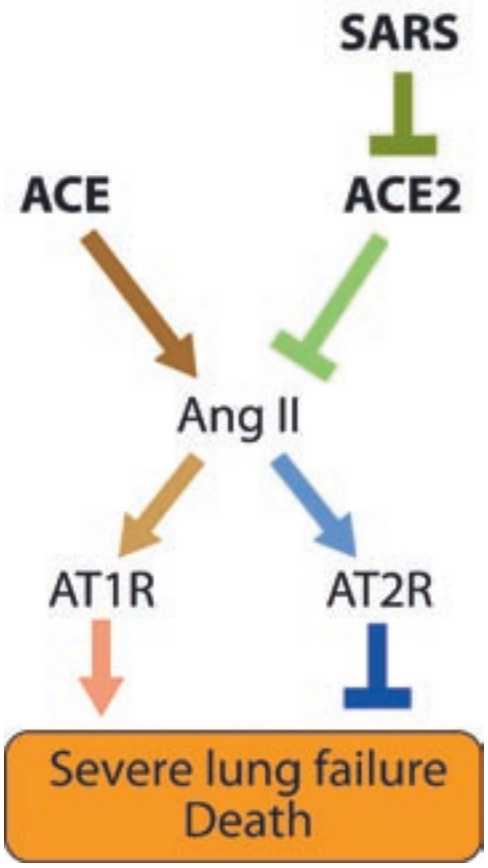


Figure 3: Schematic diagram of the renin-angiotensin system in lung edema and ARDS pathogenesis. SARS-CoV appears to trigger lung failure via ACE2 downregulation which then results in increased vascular permeability and severe acute lung failure.

Contact: josef.penninger@imba.oeaw.ac.at

Mechanisms Underlying Cell Motility and Guidance

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.

Guiding the Way with Microtubules

One area of our research program addresses the question of how a cell polarizes to move in a given direction. We now know that cell motility relies on the dynamic formation and reorganization of actin filaments that form the "actin cytoskeleton". But in many cells, polarization requires the "microtubule cytoskeleton" and our investigations are aimed at reveaing how microtubules exert their influence on the turnover of the actin cytoskeleton to confer this polarization. The dependence on microtubules for polarization generally parallels the degree of anchorage of a cell with the substrate, namely with the extent of formation of "focal adhesions". Our recent work on fibroblasts has provided evidence for the involvement of microtubules in focal adhesion turnover. Thus, we have shown that the growing ends of microtubules specifically target focal adhesions and that multiple targeting events lead to focal adhesion disassembly, or their release from the substrate. Focal adhesion targeting by microtubules occurs also in rapidly moving cells, such as neutrophils, suggesting it has a more general role in cell guidance.

The idea that interactions of microtubules with the "cell cortex" are involved in morphogenetic processes has been substantiated in studies of diverse biological systems, from yeast to eukaryotes. A striking development in the field is the realization that microtubules accumulate at their growing tips a complex of protein components that appears to influence microtubule dynamics and mediate cortical interactions. Our current studies focus on characterizing the nature of the crosstalk between microtubules and the cell cortex and the mechanisms underlying the guidance of microtubules into cortical adhesion sites.

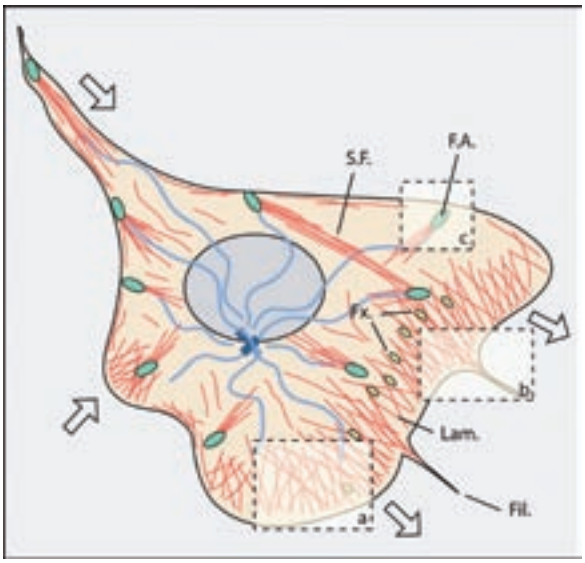


Figure 1: Schematic illustration of a migrating cell, indicating the general organization of the actin (red fibres) and microtubule (blue fibres) cytoskeletons. The actin cytoskeleton is indirectly coupled to the extra-cellular matrix through focal points of adhesion (green). Microtubules influence cell polarity by targeting adhesion sites to promote their turnover.

Pushing Forward

The first stage of cell movement involves the protrusion of a thin layer of cytoplasm, termed the lamellipodium, which is driven by the polymerization of actin. The lamellipodium, together with integrated bundles called filopodia, serve in turn to initiate adhesion

Vic Small

Vic Small / Senior Scientist

Stefanie Benesch / Postdoc
Katrien Neirynck / Postdoc
Natalia Andreyeva / PhD Student
Stephan Köstler / PhD Student
Hannah Neumeier / PhD Student
Günter Resch / PhD Student
Herbert Polzhofer / Diploma Student
Sonja Auinger / Technician
Maria Nemethova /Technician

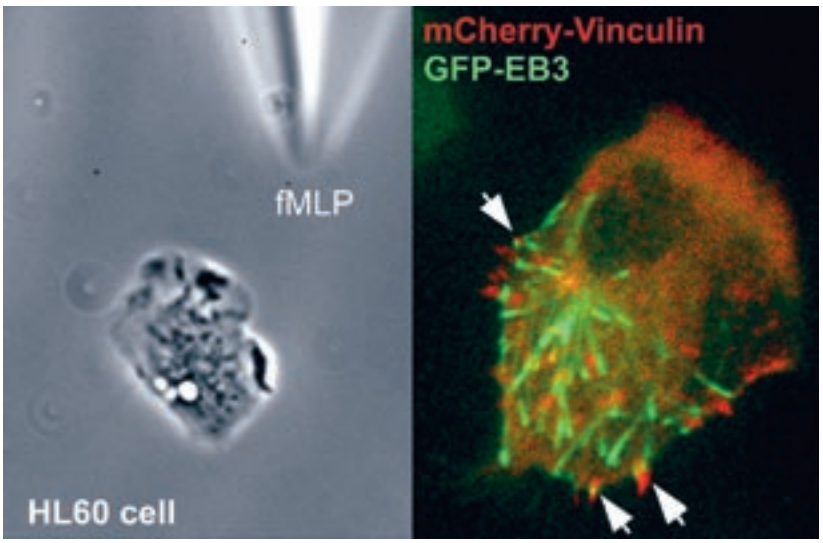


Figure 2: Microtubules interact with adhesion sites in rapidly migrating neutrophil-like cells (HL-60) to promote retraction of the rear. The left panel shows a frame from a phase contrast movie of an HL-60 cell moving towards the chemo-attractant fMLP in a micro-needle. The right panel shows the combined frames of a dual color fluorescence video of a motile HL-60 cell recorded in a spinning disc confocal microscope. The cell expresses mCherry vinculin (red) to mark substrate adhesions and EB3-GFP (green) to mark the tips of microtubules. Arrows mark microtubule-adhesion interactions.

with the substrate. Understanding the structural basis of motility requires knowledge of the organization of the actin networks that make up the protrusive lamellipodia of migrating cells. Divided opinions about the mode of generation and assembly of actin filament networks in lamellipodia have, however, arisen through discrepancies in results obtained by different preparative techniques used for electron microscopy. To help resolve current controversies we have initiated the application of cryo-electron microscopy for investigations of cytoskeleton architecture. In parallel studies we are developing techniques for the correlation of the motile activity of the living cell, in the light microscope, with the ultrastructure in the EM. Future aims include the characterization of actin reorganization leading to adhesion formation and defining the organizations of actin in mimetic models of actin driven motility.

Screening for Cell Motility Genes in *Drosophila*

Fertilization in the *Drosophila* oocyte requires the migration of border cells through the egg chamber; if migration is defective, the eggs are sterile. In collaboration with the Dickson Lab, we have initiated an RNAi screen for genes conferring sterility. In the longer term, we aim to characterize the role of candidate genes in the motility of selected tissue cells involved in developmental and repair processes in *Drosophila*.

Additional information may be obtained from our website:
http://cellix.imba.oeaw.ac.at/Videotour/video_tour_1.html

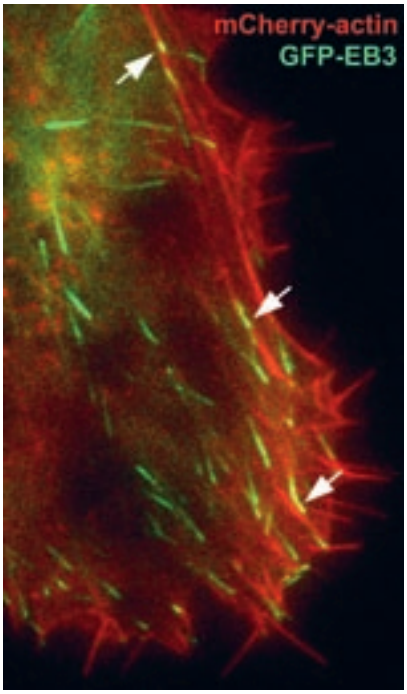


Figure 3: Microtubules track along fibres of the actin cytoskeleton to the adhesions at cell edge. The panel shows one frame of a dual color video recorded using total internal reflection fluorescence microscopy to illuminate only the base of the cell. Arrows indicate where microtubule tips (green, EB3-GFP) follow actin fibre tracks (red, cherry actin).

Contact: vic.small@imba.oeaw.ac.at

Drosophila RNAi Group

In Drosophila, RNAi can be triggered in vivo from transgenes that produce "hairpin" RNAs. With this approach, it is possible to perturb the function of almost any gene in any cell or tissue at any time. We are constructing a genome-wide library of such transgenes, to enable systematic surveys of gene function in vivo.

The sequencing of the complete genomes of humans and several model organisms has revealed the entire set of genes that direct all the complex biological processes underlying their development and physiology. The major challenge now is to assign functions to each of these genes – this is the goal of functional genomics. The success of functional genomics relies on a set of reagents and procedures for systematically perturbing the function of each and every gene in an organism's genome. Significant new insights into complex multicellular processes will require that such gene perturbation studies be performed in the intact, living organism. A further requirement, necessitated by the pleiotropy of gene function, is that gene perturbation be restricted as far as possible to the tissues or cells of interest.

Genome-wide, tissue-specific gene perturbation studies are currently feasible only in a single multicellular organism: *Drosophila melanogaster*. RNAi can be triggered in *Drosophila* by the spatially and temporally controlled expression of an RNAi transgene, which produces a potent and tissue-specific RNAi effect (Figure 1). In the *Drosophila* RNAi group, we are constructing a library of transgenic RNAi strains, comprising over 20,000 lines – 1 or 2 lines for each of the 13,681 genes in the *Drosophila* genome. This library should be completed during 2006.

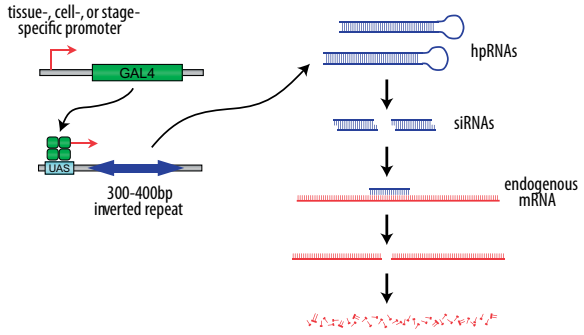


Figure 1: Transgenic RNAi in Drosophila. The generic GAL4/UAS system is used to drive the expression of a hairpin RNA (hpRNAs). These double-stranded RNAs are processed by Dicer into siRNAs which direct sequence-specific degradation of the target mRNA.

From preliminary tests, we estimate that over 70% of the lines are effective. And by testing RNAi lines for genes for which classical loss-of-function mutations are available, we know that the effect is both potent and specific (Figure 2).

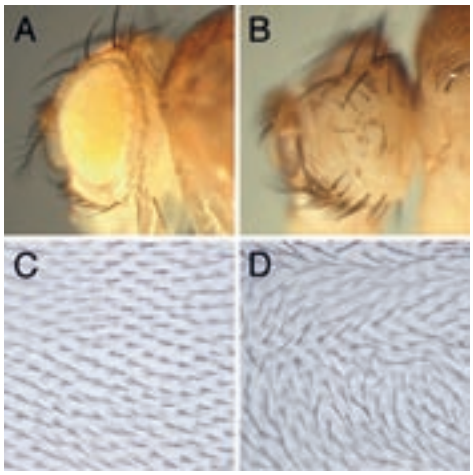


Figure 2: RNAi phenotypes. (A). Control with GAL4 driver only. (B). GAL4 driver + UAS-eyRNAi, targeting the eyeless gene. The eye is missing, as in the eyeless mutant. (C). Wing hairs in a wild-type fly all point in the same direction. (D). GAL4 driver + UAS-fmiRNAi, targeting flamingo, a gene required for planar cell polarity. The wing hairs are misorientated, as in the flamingo mutant.

We will exploit this library in academic research at IMBA, and strive to make it available to the academic research community worldwide. For commercial exploitation, the library will be licensed to the newly-founded Ludwig Boltzmann Institute of Functional Genomics, which will be housed within the IMBA building and commence operation early in 2006.

Contact: krystyna.keleman@imba.oeaw.ac.at

Drosophila RNAi Group

Barry Dickson / Senior Scientist
Krystyna Keleman / Project Manager
Georg Dietzl /PhD Student

Kaolin Kinsey / Research Associate
Johanna Aigner[1] / Technical Assistant
Ursula Aigner / Technical Assistant
Yulia Barinova / Technical Assistant
Michaela Fellner / Technical Assistant
Ruth Fuchs / Technical Assistant
Martha Garstkiewicz[1] / Technical Assistant
Beate Gasser / Technical Assistant
Daniel Hofmann Rodrigues[1] / Technical Assistant

Katharina Jandrasits / Technical Assistant
Gabriela Künzl / Technical Assistantl
Juliane Mayerhofer[1] / Technical Assistant)
Silvia Oppel / Technical Assistant
Sandra Sagmeister / Technical Assistant
Susanne Scheiblauer / Technical Assistant
Katharina Schernhuber[1] / Technical Assistant
Judith Schluder[1] / Technical Assistant
Alexandra Schmatz[1] / Technical Assistant
Caterina Sturtzel[1] / Technical Assistant
Christian Schusterreiter / Software Developer

[1]*part-time*

Biooptics Department

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

Current Research Activities

The demand for true quantitative image analysis such as the determination of distances, the area or volume of objects and the quantification of structurally or spatially defined objects, ranging from subcellular structures like chromosomes to large, multicellular objects like tumors is constantly increasing.

The manual recognition of objects and classification is not only limited to rather small numbers of images but is also prone to a user-related bias. However, reproducible and reliable analyses require (i) consistent acquisition of high-quality images and (ii) an automated object recognition system capable of dealing with a variety of parameters.

To meet these requirements, we have set up several microscopes for automated image acquisition. As these images are of consistent quality, they are suitable for unbiased automated object recognition, classification and quantification by using the Definiens eCognition framework. This software enables the implementation of algorithms for the recognition, classification and measurement of image objects. Furthermore, it supports the automated application of these algorithms to large numbers of images and the extraction of the results.

A typical example for such a quantitative analysis of multivariate image data is the automated determination of the "Differential Mitotic Index", i.e. the quantification not only of the number of cells in mitosis but also of the percentage of cells in the different phases of mitosis.

To achieve this, the spatial localization of dsDNA, Phospho(Ser10)-Histone H3 (a marker for all cells from Pro- to Anaphase), and Aurora-B (a marker for centromers from Pro- to Metaphase, for the midspindle in Anaphase and the midbody in Telophase) of statistically significant amounts of cells was determined by wide-field fluorescence microscopy.

The spatial localization, size, shape and texture of nuclei and /or chromosomes during mitosis was used to develop an algorithm for the classification and quantification of cells in the different phases. A representative example is shown in the Figure.

Contact: steinlein@imp.univie.ac.at

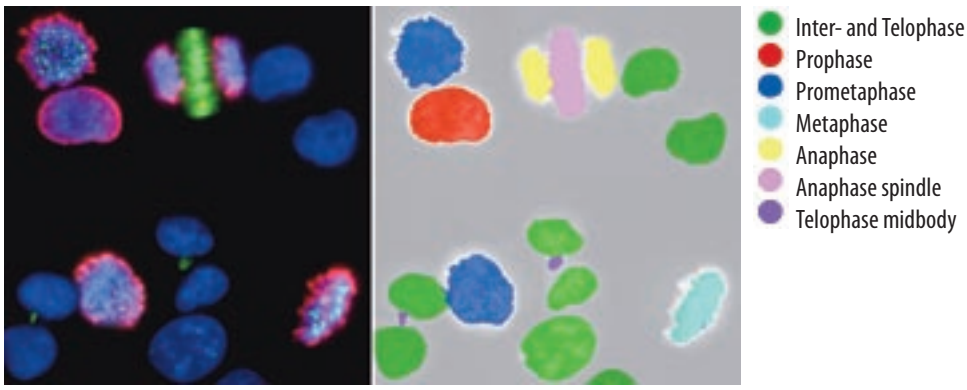


Figure: Left: Input image of cells in various phases of mitosis stained with DAPI (dsDNA) in blue, Anti-phospho(Ser10) Histone H3 in red and anti-Aurora-B in green (40x magnification; data provided by Jan-Michel Peters Group). Right: Output image after fully automated classification.

Peter Steinlein / Head of Facility

Sahra Derkits / Diploma Student
Volker Leidl[1] / Software Architect
Karin Paiha / Microscopy and Image Analysis
Pawel Pasierbek / Microscopy
Martin Radolf / Microarrays
Gabriele Stengl / Flow Cytometry

[1]*until April 2005*

Service Department

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 and the preparation of various media and solutions.

Preparation of Media and Other Cloning Reagents

Our Media Kitchen staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies (approximately 1,000,000 bottles and tubes per year) and worms. At the end of this year, we will move to the new IMBA building, where we will have more convenient working conditions for preparing fly food and all other products.
We also prepare many selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent *E.coli* strains and maintain a stock of cloning vectors, primers and other cloning reagents.

Production of Antibodies

Part of our capacity goes into the production and isolation of increasing amounts of many different monoclonal antibodies in hybridomas in collaboration with IMP and IMBA group members, and into organizing the antibody production in rabbits with an outside company.

Sequencing and DNA Isolation

With the ABI 3100 Gentic Analyzer, and the ABI 3730 DNA Analyzer 16 and 48 capillary sequencers, we sequenced approximately 38,000 samples in the first 10 months of this year. This is a decrease as compared to 2004 because most of the screens in Barry Dickson's FLYSNP project were finished last year. We expect a steady increase in the future, not only because of an increasing number of "new customers" from the IMBA but also due to new groups at the IMP.
The average read-length on the 3100 Gentic Analyzers equipped with the 80 cm capillaries is 700-900 bases, as it is on the 50 cm capillaries of the 3730 DNA Analyzer for standard DNA samples. The 3730 DNA Analyzer is more sensitive and we need smaller amounts of expensive reagents and less DNA. Very importantly, we speeded up the "return time" (bringing the sample and getting results) quite substantially (less than 12 hours on average). We are using the same easy and fast clean-up protocol with Sephadex G50 superfine columns on 96-well microtiter plate format for both platforms, and could reduce the so-called "dye-blobs" byoptimizing the sephadex consistency and the centrifugation conditions.

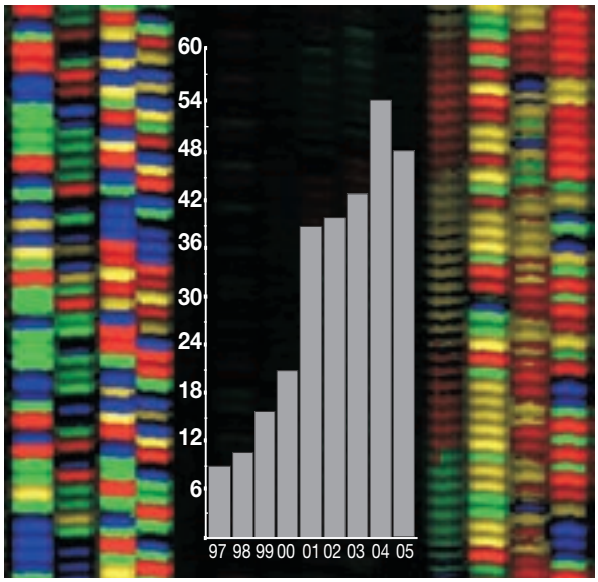


Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (1997 - 2001), on ABI 3100 (2001 - 2005) and on ABI 3730 (2004 - 2005) done with dye deoxy terminators (v3.0 since 2001) from 1998 to 2005 (scale 0 to 60'000).
**calculated from data for January 2005 to October 2005*

Contact: schaffner@imp.univie.ac.at

Gotthold Schaffner / Head of Facility

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
Franziska Stransky / Technician Fly Food Preparation
Oliver Botto / Part-time Help Fly Food Preparation
Anna Windholz / Part-time Help Fly Food Preparation

Protein Chemistry Facility

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

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.

A. Analysis of protein complexes

In our group we have developed a high-throughput method to analyze phosphorylation sites using our new mass spectrometer (Qtrap 4000 from Applied Biosystems). Using newly-adapted protocol and hardware changes, we could increase the sensitivity by a factor of 10. Quantification is performed using an MRM scan option (multiple reaction monitoring). The calibration curve is linear in a range of several orders of magnitude with a sensitivity limit at approx. 1 fmol phosphopeptide.

B. Enrichment of phosphopeptides from complex mixtures

We have established Immobilized Metal-ion Affinity Chromatography (IMAC), which is based on the affinity of negatively-charged phosphate groups for positively-charged metal ions (Fe^{3+}, Ga^{3+}), immobilized on a chromatographic support. We use methylesterified peptides to reduce non-specific binding of unphosphorylated peptides containing residues with acidic side chains.In collaboration with Prof. Lukas Huber´s Group, we already applied this method with the aim of identifying the targets of the MAPK signaling cascade. Several interesting candidates, which could be targets of Erk1, were found.

Doppler Lab for Proteome Analysis

Together with Prof. Gustav Ammerer from the Max F. Perutz Laboratories we set up a project for the quantitative analysis of proteins based on mass spectrometry to study the composition of multi-protein complexes and their associated partners. To obtain information on the dynamic nature of these complexes, we specifically want to follow temporal and conditional changes in the abundance and modification of the relevant proteins.

Proteome Bioinformatics

We have designed a novel algorithm to enhance the quality of MS/MS spectra, and hence improve the confidence of correct protein identification. The algorithm transforms multiply-charged peaks into singly-charged monoisotopic peaks and removes heavy isotope replicates as well as random noise. Non-interpretable spectra can be detected and eliminated prior to analysis. The algorithm has been implemented in a program called IMP MS Cleaner, which runs either on a stand-alone computer, or on the IMP Bioinformatics cluster. The program is now routinely being used in-house before MS/MS spectra interpretation.

	Sequence coverage	Peptides found	Mascot score
UNCLEANED	45 %	58	1331
CLEANED	55 %	68	1710

Table: Influence of background removal on the recovery of BSA in MS/MS spectra of 100 fmol test sample 100 fmol tryptic digest solution were separated by Nano-HPLC chromatography (LC-Packings, Netherland) on a PepMap C 18 column. The eluate of the column was applied online to a LCQ-XP ion trap mass spectrometer (Thermo Finnigan). MS/MS spectra were interpreted with Mascot directly ("raw spectra") and after processing with the background removal tool "MS-Cleaner" ("cleaned spectra").

Peptide Synthesis and Antibody Purification

We synthesize about 300 peptides per year, including an increasing number of branched peptides containing acetylated, phosphorylated or methylated amino acid residues, and isotopically labeled peptides for protein quantification. The affinity purification of antibodies is performed under mild conditions.

Contact: mechtler@imp.univie.ac.at
http://mendel.imp.univie.ac.at/mass-spectrometry/

Karl Mechtler / Head of Facility

Goran Mitulovic / Postdoc
Elisabeth Roitinger / Postdoc
Karin Grosstessner-Hain / PhD Student
Nedim Mujezinovic / PhD Student
Otto Hudecz / Technician
Richard Imre / Technician
Gabriela Krssakova / Technician
Mathias Madalinski / Technician
Michael Mazanek / Technician
Michael Schutzbier / Technician
Ines Steinmacher / Technician
Christoph Stingl / Technician

Animal House

The Animal House group provides husbandry of animals and services for the various research groups at the IMP and IMBA.

Husbandry

The husbandry is divided into two main areas containing the following species: mice and *Xenopus*. The larger area is the mouse section, where more than 10,000 mice are kept. These comprise 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, 20 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 the collection of blood, implantation of tumor cells and administration of substances by iv, ip or sc injections. All procedures are performed to a high standard under appropriate anaesthetic regimes and in conjunction with the necessary project licenses.

Animal procurement, such as the ordering of mice from external breeding companies, and the organizing and handling of approximately 50 incoming and outgoing mouse shipments per year.

Administration of regulatory affairs in accordance with the Austrian laboratory animal law, which include record-keeping and updating of laboratory animal statistics, and specific documentation of laboratory animal experiments.

Contact: bichl@imp.univie.ac.at

Mouse Service

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 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. *In vitro* fertilization experiments (IVF) are performed and the mouse strain database is kept up-to-date. About 30 different ES cell clones and several DNA constructs are being successfully 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.

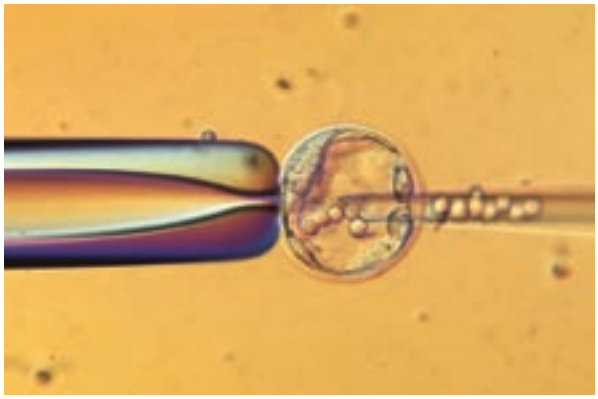


Figure: Injection of embryonic stem cells into mouse blastocytes.

Contact: theussl@imp.univie.ac.at

Animal House

Andreas Bichl / Head, Veterinarian
Erwin F. Wagner / Scientific Coordinator
Norma Howells / Consultant
Mijo Dezic / Technician
Katja Flahndorfer-Stepanek / Technician
Sabine Häckl / Technician
Anita Helm / Technician
Sabine Jungwirth / Technician
Erika Kiligan / Technician
Milan Lazic / Technician
Elisabeth Pölzlbauer / Technician
Esther Rauscher / Technician
Alexandra Stepanek / Technician
Manuela Telsnig / Technician
Sandra Vicen / Technician

Mouse Service Department

Hans-Christian Theussl / Head of Facility, Technician
Jacek Wojciechowski [1]/Technician

[1] *since October 2005*

Histology Service Department

The Histology Service Department offers state-of-the art sectioning, histology and immunohistochemistry 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.

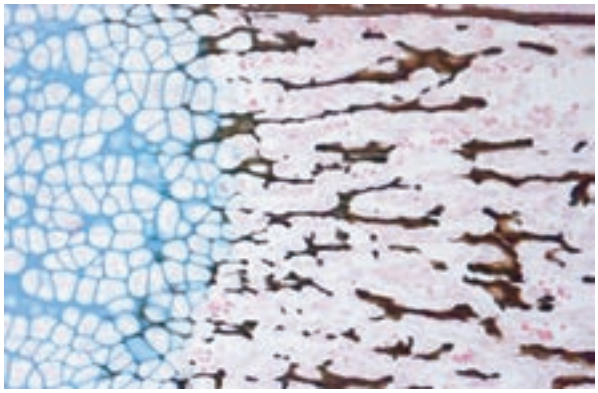


Figure 1: Van Kossa staining. This picture shows 3 different signals: dark for bone mineralization, blue for bone tissues and red for red blood cells (erythrocytes).

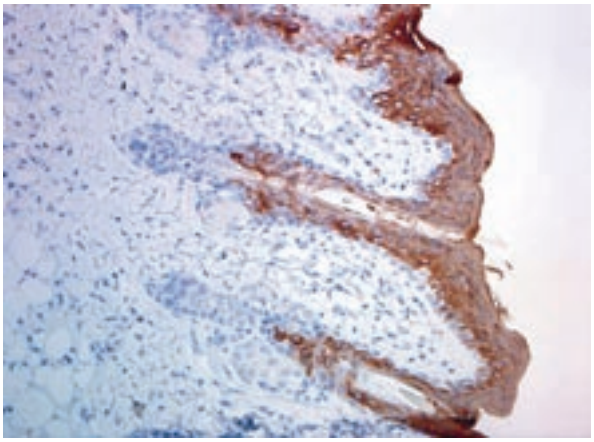


Figure 2: Keratin 10 Staining. The brown signal shows the suprabasal, cornified and granular layers of the epidermis. (skin from c-fos f/f mouse after TPA treatment), Courtsey of Juan Guinea.

Immunohistochemistry

The Histology Service Department also provides automated preparation and processing facilities for standardized immunohistochemistry, 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), I-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*).

Contact: komnenovic@imp.univie.ac.at

Vukoslav Komnenovic / Head of Facility

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HISTOLOGY SERVICE DEPARTMENT

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05/01/05	EMAMANUELLE PASSEGUE (Stanford University School of Medicine, USA)
18/01/05	ULRICH JAEGER (Medical University of Vienna, Austria)
25/01/05	THOMAS LECUIT (IBDM/LGPD, Marseilles, France)
26/01/05	ERHARD HOHENESTER (Imperial College London, UK)
28/01/05	ANDREAS TRUMPP (ISREC, Lausanne, Switzerland)

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08/02/05	CLIFF TABIN (Harvard Medical School, Boston, USA)
08/02/05	GUNTER REUTER (Martin Luther University, Halle-Wittenberg, Germany)
10/02/05	MARIO DE BONO (MRC Cambridge, UK)
11/02/05	PURA MUNOZ CANOVES (CRG, Barcelona, Spain)

MARCH

10/03/05	JEAN-PIERRE JULIEN (University of Laval, Quebec, Canada)
18/03/05	ALFONSO MARTINEZ-ARIAS (University of Cambridge, UK)
24/03/05	TIM STEARNS (Stanford University School of Medicine, USA)
30/03/05	STEPHEN P. SCHOENBERGER (La Jolla Institute, San Diego, USA)
31/03/05	YASUNORI MACHIDA (Nagoya University, Japan)

APRIL

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08/04/05	GEORG KROHNE (University of Würzburg, Germany)
12/04/05	PETER MEYER (University of Leeds, UK)
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15/04/05	MICHEL NUSSENZWEIG (Rockefeller University, New York, USA)
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22/04/05	NOBORU MIZUSHIMA (Tokyo Metropolitan Institute of Medical Science, Japan)
25/04/05	ZHAO-QI WANG (IARC, Lyon, France)

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Spotlight on 2005

IMP-IMBA Spring Conference

The Spring Conference 2005, which took place from May 19-21, was held jointly with the Institute of Molecular Pathology. The meeting was organized by Barry Dickson and Josef Penninger and represented the Inaugural Conference for IMBA. The high standard of the work presented drew an international audience to the historic Hofburg-palace in the heart of Vienna.

Contrary to previous events, this year's conference was not devoted to a single topic. The 24 scientific talks and 40 posters covered a wide range of fields, from the biology of taste to the optical control of neuronal circuits, from stem cells to the genetic basis of innate behavior. The speakers, among them Nobel laureate Christiane Nüsslein-Volhard, also drew the attention of the local media who covered the event in a number of articles and radio-programs.

Social interaction and networking are just as much part of any conference as the presentations. The breaks and poster sessions offered ample opportunity for discussion and an evening at a local wine-cellar provided the cozy background to renew old contacts and establish new ones.

IMP-IMBA Recess

At the yearly Recess, IMP- and IMBA-members meet with the Scientific Advisory Board and with BI-collegues from different research sites. It is a time for presentation and evaluation, consultation and advice. The Recess 2005 took place from October 5-7. With more participants than ever, the meeting was held for the first time at one of Vienna's newest event-locations, the beautifully renovated "Palais Niederösterreich". After three days of intense discussions, the 'chillout' was accompanied by tea and the IMP-IMBA piano trio.

Long Night of Research

On October 1st, research-institutions in Vienna, Linz and Innsbruck opened their doors till midnight to let visitors experience science on a first-hand basis. The Austrian Council for Research and Technology development and three ministries had initiated the first "Long Night of Research" under the motto "safety". At the Campus Vienna Biocenter, about 1300 curious minds were attracted that night. A number of enthousiastic scientists from IMBA participated in the event. Using Drosophila as a model, they demonstrated how small living organisms provide clues to fundamental questions in molecular biology. The visitors used the opportunity to take a close look at the familiar fruit-fly from an unfamiliar angle and showed their interest by asking numerous questions.

Awards and Honors

Barry Dickson
Received the Wittgenstein Prize from the Austrian Government (July 2005).

Jörg Betschinger
Received the Campus Vienna Biocenter PhD Award (December 2005).


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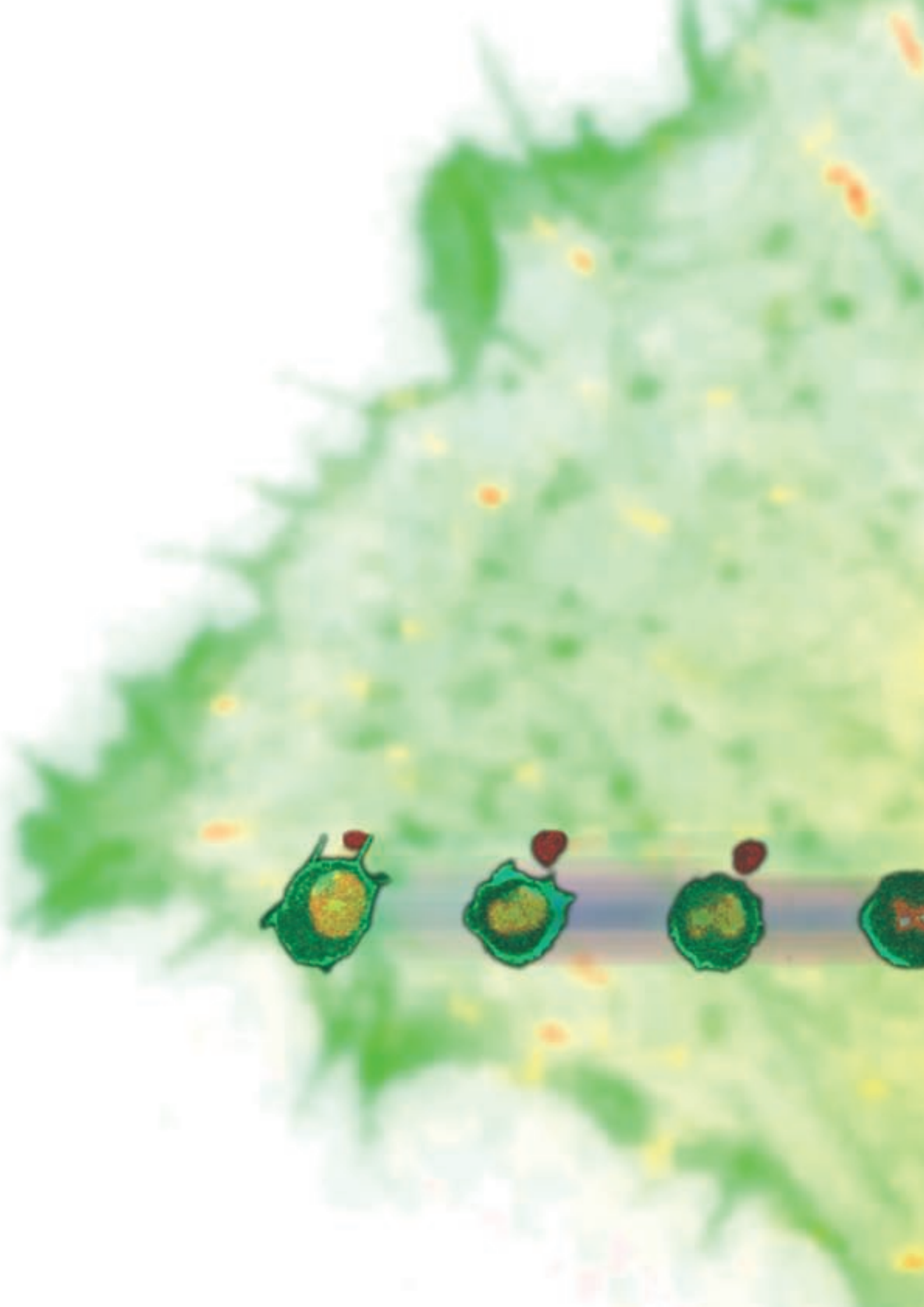

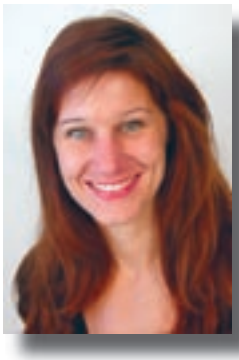

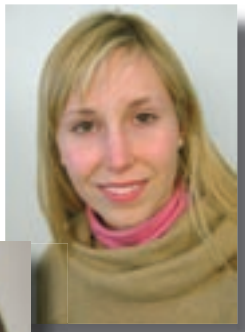

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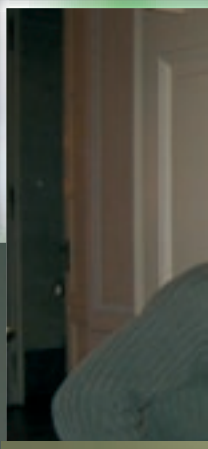




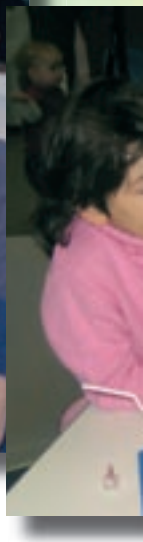











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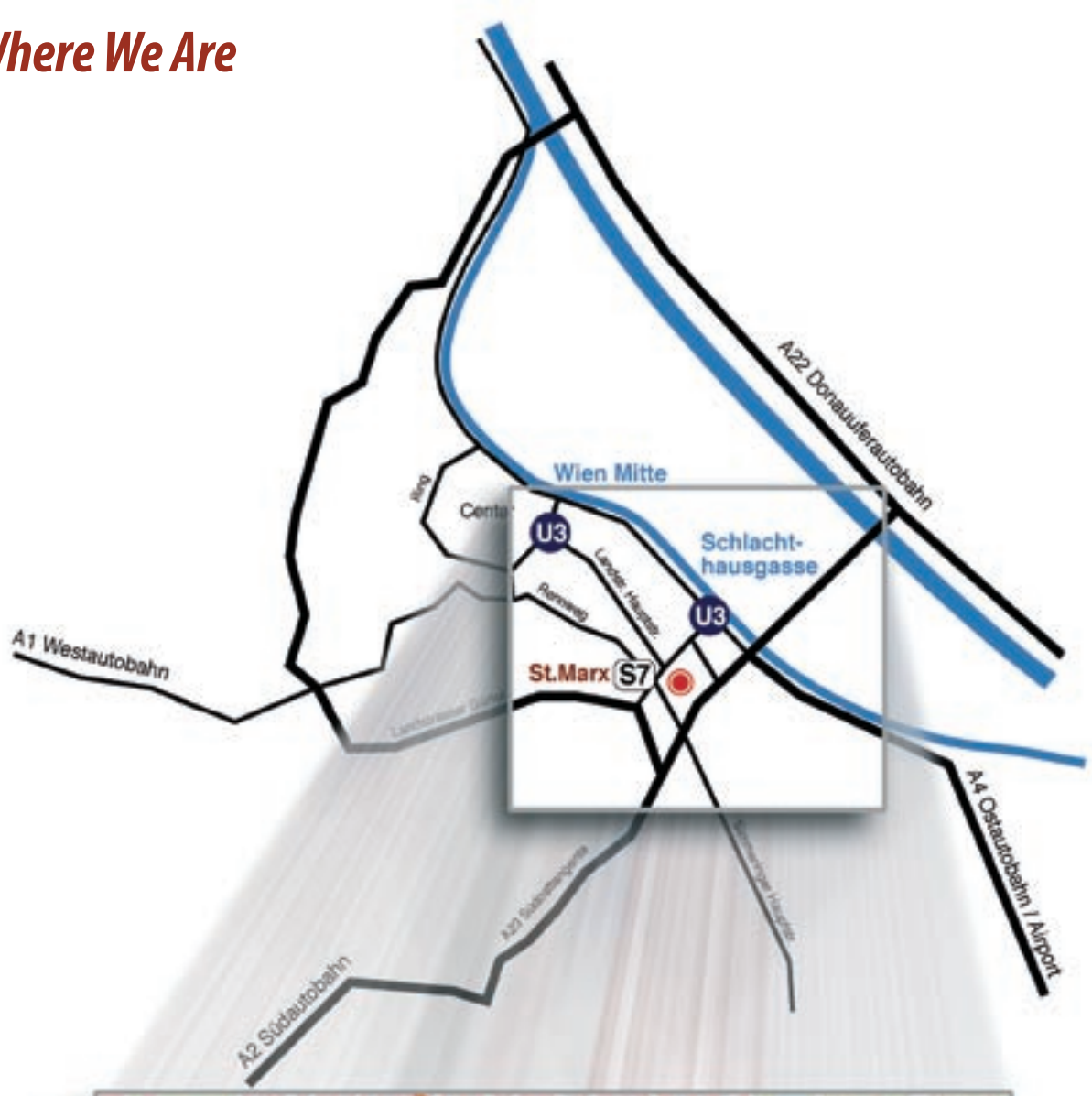

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IMBA - Institute of Molecular Biotechnology GmbH

Dr. Bohr-Gasse 3
1030 Vienna
Austria

Phone: +43(1)79044
Fax: +43(1)79044/110
office@imba.oeaw.ac.at
www.imba.oeaw.ac.at

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