RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY

VIENNA BIOCENTER

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Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

CONTENTS

BARRY DICKSON
Managing Director/ Science

HARALD ISEMANN
Managing Director/ Finance and Administration

2010 has been an eventful year at the IMP. The science progresses, as ever, at an exciting pace. Some of the most important discoveries of the past year are highlighted in the news stories in this annual report, and in the research summaries from each of the groups. The year has also been marked by new recruits, new initiatives, and a fond farewell to one of the IMP's "founding fathers".

This was an exceptional year in the recruitment of new group leaders to the IMP. From 216 applications and 17 interviews, we finally appointed four new group leaders and one new IMP fellow. One of the new group leaders, Manuel Zimmer, has already opened his lab at the IMP. The other three, Wulf Haubensak, Johannes Zuber, and Alipasha Vaziri, will start early in the new year. Andrew Straw, the new IMP fellow, also just started to set up his new group at the IMP.

Manuel, Andrew, and Wulf are all neuroscientists. With their recruitment the IMP has fulfilled a recent goal to establish a strong focus in neuroscience. The brain sciences are currently undergoing something of a revolution, with molecular genetic techniques being used to probe the cellular anatomy and function of neural circuits, and the development of "optogenetics" to use light to monitor and manipulate neuronal activity. The IMP aspires to be one of the leading European institutes in this new field of circuit neuroscience, and the addition of these new groups is an important step towards this goal. The new groups each study a different model behaviour in different model organism. Manuel investigates chemosensory behaviour in the nematode *C. elegans*, Andrew studies how visual processing in the *Drosophila* brain controls its flight maneuveurs, and Wulf uses sophisticated genetic methods in the mouse to define the neural basis of innate fear.

Johannes Zuber is a mouse geneticist and oncologist, and his appointment reflects the IMP's continued commitment to research that explores the molecular mechanisms underlying human disease. Although he initially trained as a clinical oncologist, Johannes has particularly distinguished himself a research scientist. During his postdoctoral research, Johannes generated novel mouse models of leukemia. He also developed and applied clever methods for large-scale in vivo RNAi screens to identify disease-modifying genes and pathways. These models and methods hold the promise of further unraveling the molecular basis of various cancers, and in the process yielding a treasure trove of potential new drug targets.

Alipasha has a somewhat unusual background for an IMP scientist, having obtained his PhD in quantum optics. But as biomedical research becomes increasingly quantitative, and relies more and more on sophisticated experimental systems and computational methods, scientists trained in physics, engineering, mathematics or computer science are finding new challenges and opportunities in biology. Alipasha, for example, realised that his skills in laser optics could be put to good use in developing new methods for imaging and optogenetics with unprecedented spatial, temporal, and molecular resolution. These methods should have a major impact in molecular cell biology and circuit neuroscience. Alipasha is a joint appointment with one of our neighbours, the Max F. Perutz Laboratories (MFPL). With the joint backing of the IMP and MFPL, Alipasha was able to obtain a highly competitive group leader position funded by the Vienna Science and Technology Fonds (WWTF).

With these new group leader and fellow appointments, we have largely fulfilled the research strategy that we envisioned for the IMP a few years ago. Specifically, we aimed to focus our research in four inter-related themes. First, we sought to maintain and further develop the IMP's strong tradition and reputation in molecular and cell biology, including structural biology. This tradition has continued with Jan-Michael Peters and Tim Clausen, and has been further strengthened through the appointments of Carrie Cowan, Stefan Westermann, Peggy Stolt-Bergner and Thomas Marlovits, and the promotion of Tim to senior scientist. Second, we wished to expand disease-related research using mouse models. Meinrad Busslinger continues to provide leadership in this important area, while the new recruitments of Johannes Zuber and Wulf Haubensak compensate for recent turnover through external recruitment and retirement. Third, we aimed to build a critical mass in circuit neuroscience, a field previously not represented at all at the IMP. This effort began with the appointments of Simon Rumpel, Dave Keays, and Krystyna Keleman, and is now achieved with the addition of Manuel, Andrew, and Wulf. A fourth aim has been to promote interdisciplinary research, bringing in expertise and ideas from the mathematical and physical sciences. Previous appointments of Alex Stark and Katrin Heinze strengthened computational biology and optical engineering, respectively, and now Alipasha, Andrew and Manuel bring in further expertise in optical engineering, computation, and bioengineering.

This thematic organization of the IMP's research activities is now also reflected in our newly-designed website (though not yet in this report). In addition to highlighting each of the research groups, as previously, the website now also provides a short overview of our activities in each of these four areas. We hope that this makes IMP research more accessible, more recognizable, and more coherent for internet users, in particular to the undergraduate and graduate students that may be interested in pursuing research careers in these fields.

Another major initiative this year was the Vienna Biocenter Summer School (VBSS). Conceived in the minds of a few IMP group leaders, and realized in particular through the dedication and inspiration of Dave Keays (IMP) and Kristin Tessmar-Raible (MFPL), the VBSS offers an intense 10-week research experience to undergraduate students. This year 22 students from all over the world took part in the VBSS. Judging from the enthusiasm, final research seminars, and overwhelmingly positive evaluation of the students, the VBSS was a tremendous success. We'll expand the programme slightly in future, and anticipate that it will be one further measure to make the IMP more visible and more attractive to students the world over.

With our partners at the Campus Vienna Biocenter, we have now also established a campus-wide core facility platform as a new legal entity, the Campus Support Facility (CSF). Andreas Tiran, who has been heading the Unit for Research Infrastructure at the Medical University of Graz, was appointed as managing director of the CSF. Supported by a generous €52M grant from the Ministry of Science and the City of Vienna and from user fees, the CSF will bring new state-of-the-art technologies and highly qualified experts to the Campus. The CSF will also host the Vienna Drosophila RNAi Library (VDRC), which was set up as a joint IMP/IMBA initiative three years ago and has since evolved into one of the most highly recognized and valued research infrastructures for *Drosophila* research worldwide.

Along with all these exciting new developments have come, as every year, a few fond farewells. After four years as a staff scientist at the IMP, supported by BJD's Wittgenstein Prize, Katrin Heinze now moves on to take up a senior faculty position at the University of Würzburg. Katrin was the first physicist we hired, and has substantially strengthened our expertise in optical engineering.

And in March this year Hartmut Beug retired after almost 25 years shaping cancer research at the IMP. Hartmut was farewelled with a symposium featuring talks from many of his former lab members, and concluding with Harmut's own touching personal reflections on a remarkable career in cancer research. With his sharp and curious mind, broad interests and unique character, Hartmut has always been one of the IMP's leading figures - a great scientist, mentor, colleague and friend. Hartmut has now moved back to Heidelberg, but still runs a lab at the University of Veterinary Medicine in Vienna. Indefatigable as always.

We are deeply grateful to all staff members for their continued dedication and effort, making the IMP such a unique place for scientific research. And finally, we congratulate our generous sponsor Boehringer Ingelheim on its 125-year anniversary. Throughout its long and successful history, BI has shown an outstanding and lasting commitment to research and innovation. By supporting the IMP for almost 25 years, the world's largest family-owned pharmaceutical company is also Austria´s biggest private sponsor of basic research, and as such has made a tremendous contribution in establishing Vienna as a leading international location for biomedical research.

LOOKING INTO THE EYE OF THE NEEDLE: THE STRUCTURE OF A BACTERIAL SECRETION COMPLEX

What can pass through the eye of a needle? The Babylonian Talmud notes that our dreams "do not show … an elephant going through the eye of a needle," while for Christians and Moslems it is clear that camels are too large to do so. Cell biologists – of all religious persuasions – are concerned with the molecules that pass through the so-called needle complexes, huge multimolecular structures that many bacteria use to "inject" their toxins into cells under attack. Understanding what can be transported into cells by this route requires a detailed knowledge of the structure and function of needle complexes but these are unfortunately so complicated and difficult to handle that investigations have generally been limited to resolutions too low to answer the key questions. Recent work in the group of Thomas Marlovits at the IMP and the IMBA has led to a breakthrough in our understanding of the structure and function of one such complex, the type III secretion needle complex from *Salmonella*. Initial results were published in the April 2010 issue of *PloS Pathogens* and a follow-up study has just been submitted for publication.

The first author on both papers is Oliver Schraidt, a German PhD student who came to Vienna in 2006, shortly after Thomas arrived from Yale in Autumn 2005. Thomas's previous work had focused on the needle complex and he had managed to derive an initial structure and discovered the flexible behaviour of the complex during the assembly. However, the available information was insufficient to enable researchers to say for certain what components are located where in the complex, let alone how they are oriented. But it is precisely this level of information that is vital to understand how the complex functions, so he and Oliver elected to attempt to refine the structure.

Oliver Schraidt fighting for the IMP in the "Dragon Boat Cup".

Arriving when the group was being set up clearly gave Oliver the opportunity to learn a great deal about the harsh realities of research. For the first 18 months no cryo-electron microscope was available so the group had to travel to use microscopes at other institutes, which frequently turned out to be poorly maintained or hard to operate. And fresh problems waited when the experiments finally started in earnest. As Oliver recalls, "We thought that one of the components – known as PrgK – was in a very exposed part of the complex but for some reason we just couldn't label it. It took a long time before we understood why: PrgK is actually buried right in the middle of the structure." Another, perhaps unexpected, problem stemmed directly from the size of the complex. "It is simply so large that it is always falling down, so we couldn't examine it in different orientations. It was years before we were sure which parts were located at the bottom and were able to insert charged labels to make the complexes stand up properly."

Nevertheless, a combination of bacterial genetics (using *Salmonella* mutants lacking particular components) and cryo-EM finally led to results. The proteins that form the inner and outer rings and the neck region were elucidated – their identities had been suspected but not previously proven – and sophisticated labelling experiments enabled their orientations to be determined. Once the location and orientation of a few members of the complex was clear, work proceeded rapidly. Oliver makes the analogy with a jigsaw puzzle: "The start is really difficult but once the first few pieces are in place and joined up the rest becomes much easier."

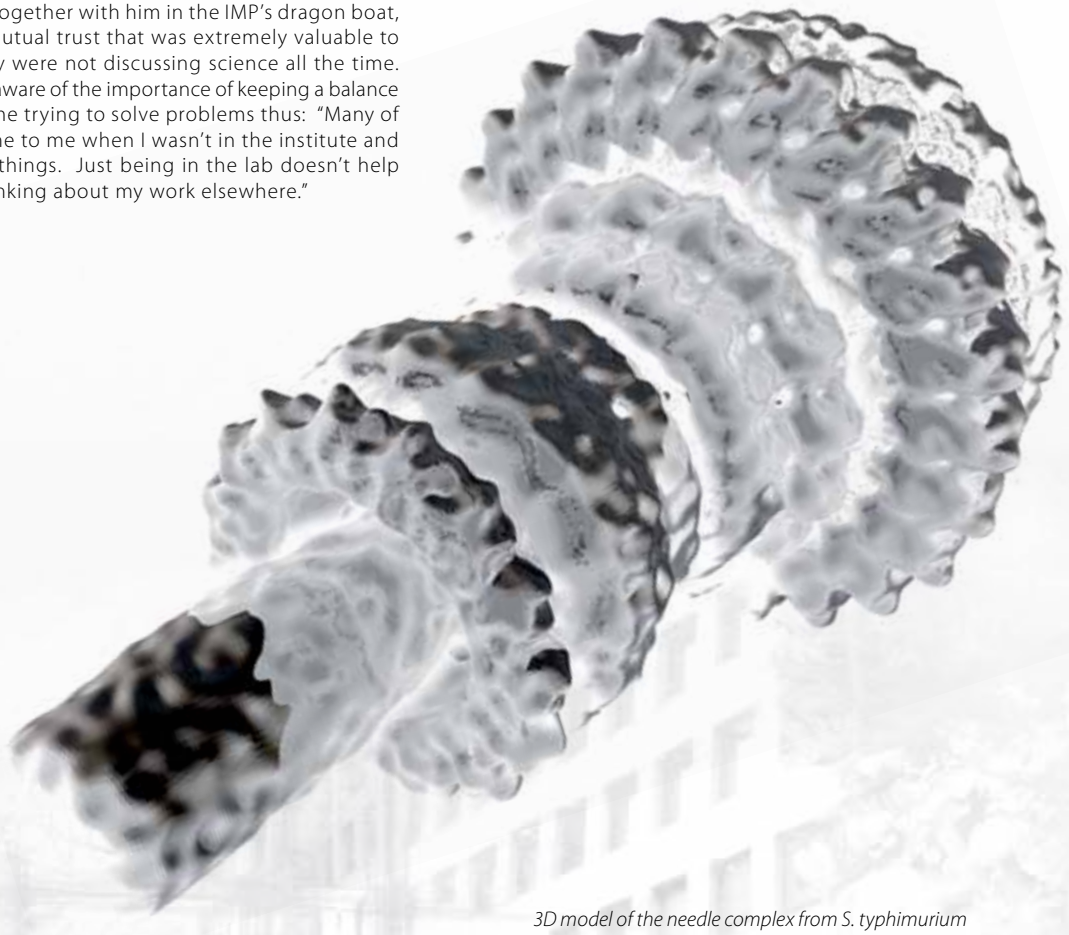
Easier, perhaps, but still far from trivial and additional information was required to give unambiguous results. A further way to examine the organization of the various proteins in the complex is to perform cross-linking experiments. By means of appropriate reagents it is possible to see which proteins are located near to each other and mass spectrometry can then be used to identify the precise residues that are in close contact. Taking all of the results into account, it was finally possible to construct a model for the arrangement of all the components. The model shows clearly which protein (InvG) forms the outer rings – the protein also makes direct contact with the inner rings, which are comprised largely of PrgH and PrgK. Indeed, the interaction between InvG and PrgH is extremely important in holding the entire complex together. And the model also shows how the various proteins are oriented in the structure, information that is vital in understanding how the overall complex functions.

The model represented a highly significant breakthrough. A number of structures had previously been proposed but as Oliver notes, "Building a model of such a huge complex with relatively little detailed information offers lots of scope for the imagination. Crystal structures of several components were available but even these didn't make it clear where the various subunits are located." The present model, in contrast, is sufficiently robust to permit the design of experiments, for example to predict the sites that interact with substrates, in this case bacterial "effector proteins" that the needle complex transfers into the cell to be infected. As the type III secretion systems are required for the virulence of a number of highly pathogenic bacteria, such as *Salmonella*, *Shigella* and *Vibrio cholerae*, the model may ultimately be useful in the design of new therapies. More immediate applications of our improved understanding of the complex's structure include the use of engineered bacteria to insert antigens into cells or to arrange for particular proteins to be exported.

Oliver has enjoyed his time at the IMP and the IMBA immensely. His pleasure stems not only from his scientific success but from his friendship with the many people who have helped him with his work. He mentions in particular the three diploma students who contributed to the project, Matthias Brunner, Wolfgang Schmied and Julia Radics, singling out Wolfgang as someone who became a close friend. Oliver spent a great deal of time together with him in the IMP's dragon boat, forging a relationship of mutual trust that was extremely valuable to their lab work, even if they were not discussing science all the time. Indeed, Oliver is very much aware of the importance of keeping a balance in his life and recalls his time trying to solve problems thus: "Many of my best ideas actually came to me when I wasn't in the institute and was thinking about other things. Just being in the lab doesn't help anyone and I far prefer thinking about my work elsewhere."

What are Oliver's plans for the future? At first he intends to take a holiday – his final manuscript is still warm from the printer – and only afterwards to look for a post-doctoral position. He hopes to work in the field of synthetic biology, as "we've recently started changing the molecules we're studying and this is a lot of fun. Designing molecules with particular functions is a very exciting challenge." And he would like to use his post-doctoral time to learn another language, such as French or even Portuguese. As he says, "You can do science all your life and it's nice to learn something else while you do so. If I can find a good institute in a country that speaks one of these languages I'd be very tempted to go there."

Text by Graham Tebb



3D model of the needle complex from S. typhimurium

SORORIN: REGULATING THE GLUE THAT HOLDS CHROMOSOMES TOGETHER

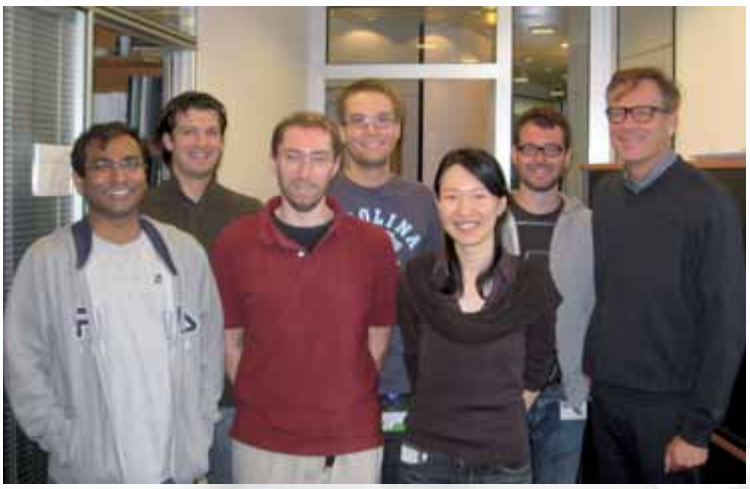
An unfortunate consequence of the fact that many women are now waiting longer to start families is that the number of children with chromosomal abnormalities is increasing. It is clear that chromosomal segregation becomes more error-prone with age but we know surprisingly little about the reasons why. In fact, we still do not really understand what regulates when chromatids are held together and when they separate. Research at the IMP has been gradually providing answers to some of the key questions and a further huge step was taken this year, when Tomoko Nishiyama in the group of Jan-Michael Peters showed that the protein Sororin is involved in bringing together sister chromatids. Her results were published in November in *Cell* (**143**, 373-349).

Perhaps somewhat unusually, Tomoko did not actively decide to come to Vienna. While working on her PhD – on the regulation of meiosis in frog oocytes – she had read several papers from Jan's group and had been impressed by the lab's ability to apply experimental techniques of the highest quality to address fundamental questions. Tomoko was keen to work on mitotic chromosomes, an interest she and Jan shared. She thus wrote to Jan enquiring about the possibility of working with him. As she says, "I only applied to Jan's lab – I didn't even know he was in Vienna at the time."

Coming to Austria represented a big change for someone who had never lived outside Japan. Tomoko does not speak German ("I haven't yet finished learning English!") and nearly everything was new to her. But the lab represented a familiar environment and she soon settled in to performing experiments. She elected to work on Sororin, a protein that previous work in Jan's group had shown to be required for holding chromatids together in the interphase of the cell cycle (see Schmitz *et al*. 2007, *Curr. Biol.* **17**, 630–636). Julia Schmitz, a former PhD student in Jan's lab, had undertaken some initial studies and had shown that Sororin binds to sister chromatids in a cohesin-dependent manner from the time DNA replication is initiated until the onset of anaphase but there were many issues to be resolved before the protein's function could be understood.

Tomoko initially attempted to set up a cell-free system to study Sororin in more detail, "an *in vitro* cohesion assay using plasmid DNA and then to test whether Sororin is required for cohesion in this system". As so often happens, though, her initial plans did not work out so she switched to using extracts of frogs' eggs: Tomoko felt that this "semi *in vivo* system" might offer a good opportunity to address some of the questions surrounding Sororin's function. A crucial point was whether Sororin binding depends on DNA replication. Tomoko's frog egg extract was ideally suited to investigate this, although the experiments turned out to be very complicated to perform. Part of the reason for the difficulty was a variation in the quality of the eggs. Tomoko recalls that "Frogs actually have good and bad seasons in terms of the quality of their eggs. Sometimes when I wanted to try a critical experiment the eggs weren't so good so I had to learn to be patient until the frogs cooperated and laid better ones."

Finally, though, she had "good eggs" and could carry out the experiments. Using a variety of inhibitors and genetically modified components of the cell-cycle control apparatus, she was able to show that although Sororin was present throughout the cell cycle it only began to associate with chromatin at the initiation of DNA replication. It is known that DNA replication is associated with a chemical modification (acetylation) to one of the cohesin components but Tomoko showed that although this modification was required for Sororin binding it was not sufficient to recruit the protein to chromatin.



Tomoko with her friends and colleagues.

There had been suggestions in the literature that Sororin and a further protein, Wap1, act in opposition. Using her frog's egg extracts, Tomoko was able to show that cohesion did not require Sororin if no Wap1 was present. Immunoprecipitation experiments had given a clue that Sororin binds to the so-called Pds5 proteins, which are also associated with cohesin. Wap1 is also known to bind to these proteins to form a Pds5A-Wap1 heterodimer and association is known to depend on a particular sequence motif in the Wap1 protein. Tomoko noticed that the Sororin sequence contained a similar motif (Jan is quick to disclaim any share of the credit for this observation!) and she changed it to give a Sororin mutant that she tested for its ability to bind to cohesin.

Surprisingly, experiments with purified proteins showed that "normal" (wild-type) Sororin was able to displace some Wap1 protein from Pds5. The mutant Sororin, however, was less able to do so. Experiments with egg extracts confirmed that the result was not an artefact and suggested that Sororin may work to remove Wap1 from Pds5. Furthermore, although addition of wild-type Sororin could restore cohesion in egg extracts lacking Sororin, addition of the mutant Sororin could not. The demonstration that Sororin's abilty to displace Wap1 from Pds5 paralleled its function in cohesion gave a further indication that Sororin operates by inactivating the cohesion inhibitor Wap1.

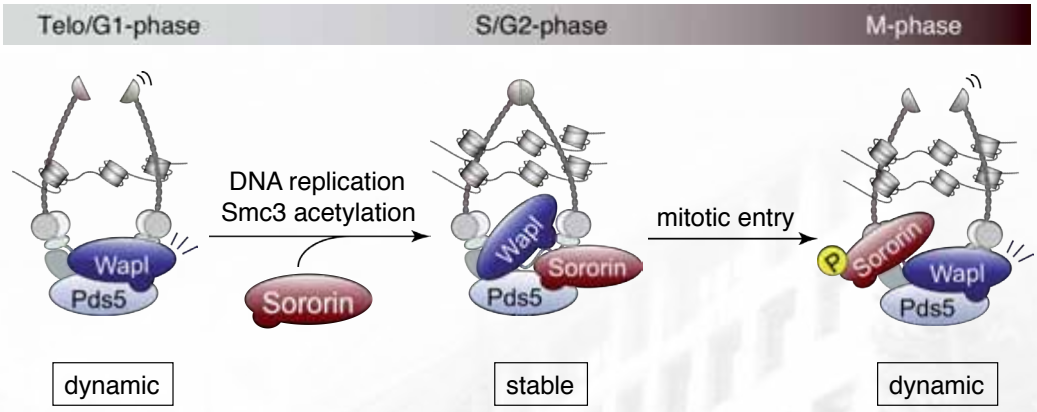
It was previously known that the removal of chromosomal cohesin at prophase requires Wap1, so it seemed conceivable that Sororin is inactivated at this point in the cell cycle. Tomoko thus tested whether Sororin could dissociate Wap1 from Pds5 in extracts prepared from eggs at different stages of the cell cycle. The experiment – which is far easier to describe than it was to perform – showed that Sororin's function in cohesion and in displacing Wap1 was lost when Sororin was incubated in an extract from mitotic cells. However, if Sororin is treated with a phosphatase its activity is restored, proving that the protein is inactivated by means of phosphorylation in a cell cycle-dependent manner.

Taken in conjunction, the data led Tomoko and Jan to propose a model for Sororin's function (see figure below). When DNA replication is underway, a number of events occur (including acetylation of a cohesin component named Smc3) that enable Sororin to bind cohesin. Sororin binds to the Psd5 proteins via a sequence motif that it shares with Wap1 and by binding it prevents Wap1 from functioning. At the appropriate point in the cell cycle, Sororin is phosphorylated and thereby inactivated, enabling Wap1 to bind to Pds5 and causing loss of cohesion.

The model is simple and elegant but one point seemed very puzzling: Wap1-like proteins are known from a huge range of species from yeast to humans but Sororin has only been found in vertebrates. If Tomoko and Jan's model is correct, Sororin should be more widely distributed throughout the animal kingdom. Alex Schleiffer in the IMP's Bioinfomatics Department thus searched a number of invertebrate genomes for sequences similar to the highly conserved C-terminal portion of the Sororin sequence. He turned up a Sororin-like protein in 18 metazoan species of a variety of taxa, showing that Sororin or at least the Sororin function is far from restricted to vertebrates. Most of the proteins he identified are still uncharacterized but the corresponding protein in the fruit fly, *Drosophila*, had been shown to be required for development of the fly's nervous system. And Rene Ladurner, a PhD student in Jan's group, was able to prove that this protein – known as "Dalmatian" – is actually required for chromosome cohesion: depleting the level of the protein led to scattered chromosomes. The model may thus be applicable over a wide range of organisms.

Tomoko freely confesses that she has been surprised by the success of her work. The initial idea for the experiments came from Jan and at first she didn't take it entirely seriously– she regarded the project as a type of back-up in case her other experiments hit problems. But from being unsure what avenues to pursue she now finds herself with a surfeit of ideas: understanding how Sororin binds to cohesin and what molecular changes are caused by the interaction will require detailed biochemical and structural work. Tomoko is looking forward to the challenge. Although she enjoys life in Vienna, especially going for walks in the many parks and gardens the city has to offer, she is happiest when she is in the lab, especially at the bench. It seems that she will have plenty of reasons to spend time at the bench for the next few years at least.

Text by Graham Tebb



The role of Sororin in chromosome cohesion (for details see text).

HARTMUT BEUG

Tumor progression: abnormal developmental plasticity/ reprogramming?

hartmut.beug@imp.ac.at

Hartmut Beug[1] / Senior Scientist

Agnes Csiszar / Postdoc
Sabine Maschler / Postdoc
Memetcan Alacakaptan / PhD Student
Boris Kovacic / PhD Student
Ulrike Mitteregger / Diploma Student
Betül Ulaca / Diploma Student
Gabi Litos / Technical Assistant
Eva Maria Wiedemann / Technical Assistant

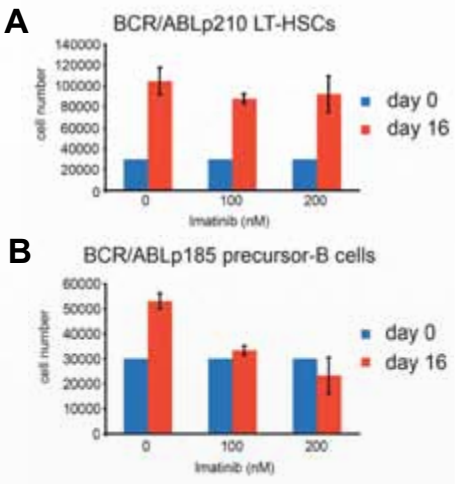
[1] *until March 2010*

During development of cancer and leukemia, various oncogenes and tumor suppressor genes cooperate to induce abnormal survival, proliferation and developmental plasticity, often reminiscent of stem cell behavior. Our aim still is to unravel molecular mechanisms in leukemia, focusing on leukemia-maintaining cells resembling hematopoietic stem cells; and breast cancer, i.e. progression of epithelial tumor cells to undifferentiated cells showing epithelial/mesenchymal transition (EMT) and aspects of mammary gland stem cells.

Mechanisms involved in hematopoietic progenitor renewal and leukemogenesis.

Human leukemia is caused by the cooperation of mutated transcriptional regulators with overexpressed, activated receptor tyrosine kinases or downstream signal transducers. Leukemic renewal of murine/human erythroid progenitors, including ES-cell-derived erythroblasts, requires the cooperation of EpoR, c-Kit, and the glucocorticoid receptor (GR). Genes involved in erythroblast renewal include Stat5, p38-kinase, MAPK and Flk-1, as shown using the respective genetically modified mice. Purified hematopoietic stem cells (HSC) from multilineage mouse leukemia caused by the human chronic myelogenous leukemia (CML) oncogene BCR-ABL p210 and constitutively active Stat5 were identified as drug-resistant leukemic stem cells (LSC's) in the initiation as well as maintenance of leukemia (Figure 1). In acute lymphoid leukemia induced by BCR-ABL p185, the leukemia-initiating HSCs differentiated into committed, immature, drug-sensitive B-cell progenitors, which constitute the bulk of leukemic cells, but also function as LSCs in the maintenance of leukemia. In cooperation with other investigators, we will focus on potential molecular and cellular differences between these two LSC types and normal HSCs by expression profiling and functional analysis.

1



Tumor progression and metastasis: due to deregulation of epithelial polarity?

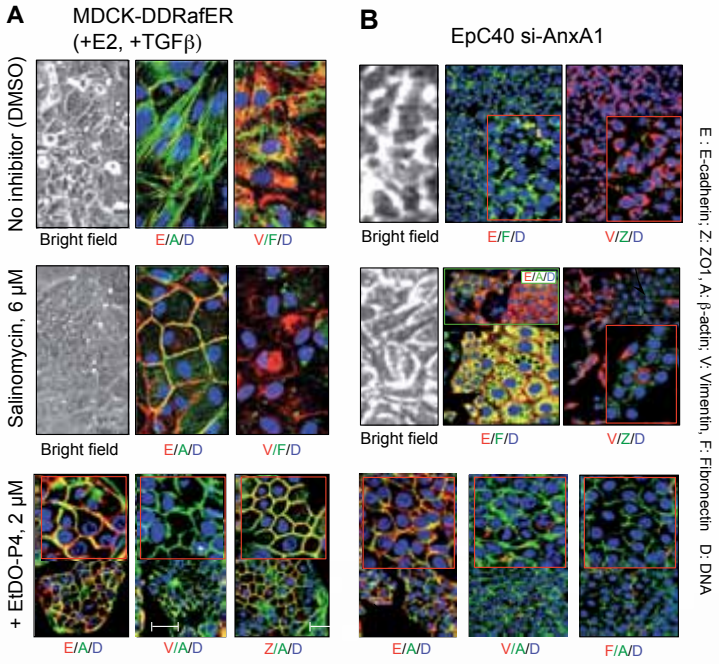
Epithelial to mesenchymal transitions (EMT) are essential during normal embryonic development, tissue fibrosis, carcinoma progression, and metastasis. Recently, EMT has also been identified as a hallmark of normal mammary and breast cancer stem cells. Oncogenic Ras plus TGFβ cause EMT and metastasis in the mammary epithelial EpRas cell model, while cells expressing a Ras mutant hyperactivating only the PI3K pathway (EpC40 cells) plus TGFβ show a partial EMT phenotype ("scattering") and are tumorigenic, but not metastatic. Using polysome-bound mRNA expression profiling and candidate gene approaches, we identified ≈ 30 EMT-specific genes, three of which have been characterized in detail. Overexpression of ILEI (interleukin-like EMT inducer) and CREG (cellular repressor of E1A regulated genes), as well as RNAi-induced knockdown of AnxA1 (Annexin-A1) required oncogenic Ras to cause EMT and metastasis, but acted independent of TGFβR signaling, as shown using EpC40 cells and pharmacological TGFβRI inhibitors.

Recently, EMT has been frequently described as a shift from apical/basal polarity of epithelial cells to anterior/posterior polarity of motile, fibroblastoid cells, both controlled by multiple, cooperating multiprotein complexes governing membrane-domain-specific vesicle trafficking, membrane domain identity, and response of polarity machines to external cues. EMT and metastasis caused by deregulation of proteins from such polarity complexes is rapidly becoming a hot topic in cancer progression. We showed that RNAi-induced loss of the basolateral polarity protein scribble causes complete EMT. We also found that ILEI acts through both intracellular and extracellular mechanisms. In normal epithelial cells, ILEI is localized to vesicles of the trans-Golgi network (TGN), which were redistributed to

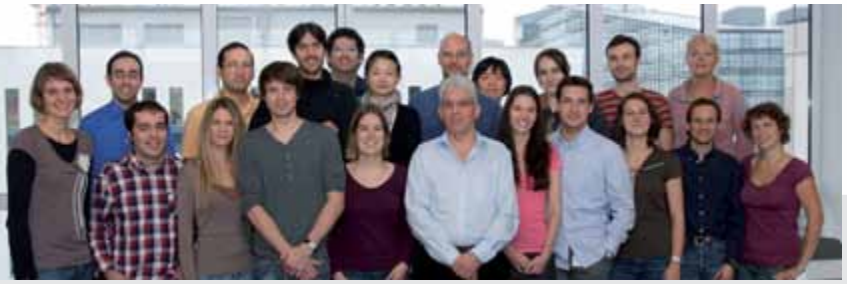
Figure 1: ***Cultivated leukemic cells from p210-BCR-ABL and p185BCR-ABL-infected mice differ in their resistance to the BCR-Abl-inhibitor imatinib.*** ***A****. p210-BCR-ABL-infected LT-HSCs grown in special growth-factor-containing media (see Research Report 2008) are not inhibited in their proliferation by imatinib. In contrast, B-cell progenitors cultivated from P185-BCR-ABL-infected mice under standard conditions.* ***B****. are highly sensitive to the inhibition of growth by imatinib.*

Figure 2: ***Are different mechanisms involved in EMT induced by (1) hyperactive Raf plus TGFβ in MDCK cells or by (2) Ras plus RNAi-induced knockdown of AnxA1 in EpC40 cells?*** ***A****. DDRafER-MDCK cells were treated or not treated with the potassium ionophore salinomycin (inhibiting breast cancer stem cell expansion) or the glucosylceramide-glycosphingolipid-synthase inhibitor EtDO-P4 during induction of complete EMT by estradiol plus TGFβ for 5 to 6 days, and analyzed by staining for EMT-markers. Both inhibitors completely prevented EMT induction.* ***B****. EpC40 cells induced to undergo EMT by RNAi-mediated knockdown of AnxA1 were treated with salinomycin and EtDO-P4 and analyzed for EMT markers, as described in panel A. While EtDO-P4 completely reversed EMT in these cells, salinomycin only induced re-expression of cytoplasmic E-cadherin and low ZO-1 levels, but did not abolish expression of the mesenchymal markers vimentin and fibronectin. EMT marker staining is indicated by colored capitals below panels, which are explained in the right section of the figure.*

2



the entire cytoplasm after EMT, strongly predicting metastasis and poor survival in human breast cancer tissue arrays. Most importantly, RNAi-mediated AnxA1 knockdown required cooperation with oncogenic Ras to induce TGFβ-independent EMT and metastasis in non-metastatic cells, and stimulated the expression of E-cadherin repressors such as snail and delta EF-1. Interestingly, forced AnxA1 expression in metastatic human mammary carcinoma cells with an EMT phenotype reversed this EMT, down-regulated E-cadherin repressors and abolished metastasis, supporting a function of AnxA1 as a gatekeeper of epithelial polarity. AnxA1 knockdown stimulated multiple signaling pathways, but only Tyk2/Stat3 and to a lesser extent Erk1/2 signaling were essential for EMT. AnxA1-RNAi-induced EMT may require the synthesis of complex glycolipids, since a pharmacological inhibitor of respective synthases reversed EMT in EMT induced by AnxA1-RNAi or hyperactive MAPK signaling plus TGFβ. In contrast, a specific potassium ionophore (salinomycin) claimed to specifically target breast cancer stem cells inhibited only Ras- plus TGFβ-induced EMT, suggesting that AnxA1-RNAi-induced EMT is based on different molecular mechanisms (Figure 2).



MEINRAD BUSSLINGER

Stem cell commitment in hematopoiesis

meinrad.busslinger@imp.ac.at

Meinrad Busslinger / Senior Scientist

Ivan Bilic / Postdoc
Roger Revilla-i-Domingo / Postdoc
Anja Ebert / Postdoc
Stephen Malin / Postdoc
Tanja Schwickert[1] / Postdoc
Leonie Smeenk / Postdoc
Sinan Gültekin / PhD Student
Mareike Hoffmann / PhD Student
Jasna Medvedovic / PhD Student
Martina Minnich / PhD Student
Markus Schäfer / PhD Student
Anca Tigan / PhD Student
Bojan Vilagos / PhD Student
Abdallah Souabni / Research Associate
Hiromi Tagoh / Research Associate
Qiong Sun / Research Assistant
Erika Kiligan / Research Technician
Barbara Werner / Research Technician

[1] *since November 2010*

Markus Jaritz / Head of GEN-AU Bioinformatics
Andras Aszodi / GEN-AU Bioinformatician
Ido Tamir / GEN-AU Bioinformatician

Acquired immunity to pathogens depends on the differentiation of B and T lymphocytes from hematopoietic stem cells, which is controlled by a multitude of transcription factors. We are interested in understanding the process by which transcription factors regulate the commitment of early hematopoietic progenitors to lymphoid lineages and control their subsequent differentiation to B and T cells. We investigate the underlying transcriptional control mechanisms by using mouse transgenic, cell biological, and genome-wide molecular approaches.

B cell development

A fundamental question in hematopoiesis is how stem cells and early progenitors become committed to a single developmental pathway and then differentiate into mature cell types of the selected lineage. The entry of lymphoid progenitors into the B cell lineage depends on several transcription factors, including STAT5, E2A, EBF1 and Pax5. STAT5, a downstream mediator of IL-7 signaling fulfills a permissive role by controlling cell survival in the early development of B cells. E2A and EBF1 function as B cell specification factors by activating B-lymphoid genes. Pax5, in turn, controls B cell commitment by restricting the developmental potential of hematopoietic progenitor cells to the B cell pathway.The function of Pax5 is required throughout the development of B cells, as it is also essential for the generation of all mature B cell types, including germinal center B cells (Figure 1). Surprisingly, conditional *Pax5* deletion enables mature B cells from peripheral lymphoid organs to dedifferentiate back to early uncommitted progenitors in the bone marrow, which subsequently develop into functional T cells. These experiments demonstrate that Pax5 functions as a critical B cell identity factor in maintaining B-lineage commitment from the pro-B to the mature B-cell stage.

T cell development

Signaling through the Notch1 receptor is essential for initiating the development of T cells in the thymus. Early T cell specification also depends on other transcription factors, such as GATA3 and E2A. However, little is known about target genes that mediate the effects of these transcriptional regulators in early T cell development. We therefore want to elucidate the molecular functions of these transcription factors in pro-T cells by conditional mutagenesis, gene expression profiling, and ChIP sequencing.

Transcriptional networks

Global genomic approaches are ideal for elucidating the transcriptional network controlling early development of B cells and T cells. To achieve this aim, we define the regulatory landscape of pro-B and pro-T cells by genome-wide mapping of DNase I hypersensitive sites, transcription start sites and chromatin modifications, in order to delineate active enhancers and promoters (Figure 2). By ChIP sequencing, we identify the binding sites of the different transcription factors at these regulatory elements (Figure 2). Conditional mutagenesis combined with mRNA sequencing is used to study the dependence of target gene expression on the different transcription factors. These genome-wide approaches have already provided important insights into the transcriptional network controlling early B cell development.

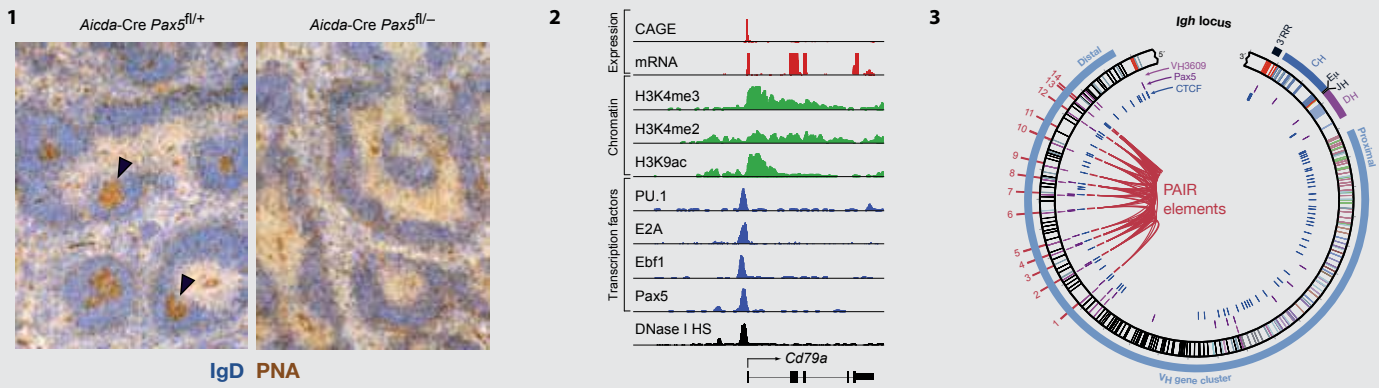


Figure 1: ***Essential role of Pax5 in germinal center (GC) B cell development****. The Aicda-Cre line deletes the floxed (fl) Pax5 allele in GC B cells of control Aicda-Cre Pax5$^{fl/+}$ mice eight days after immunization with sheep red blood cells. PNA^{+} GC B cells (arrows), which are detected by immunostaining in spleen sections, are formed in the presence of Pax5 in these control mice, but not in its absence in Aicda-Cre Pax5$^{fl/-}$ mice.*

Figure 2: ***Genome-wide identification of transcription factor target genes.*** *The Cd79a gene is shown as a representative example to indicate the different parameters that were determined by global genomic analyses. Transcriptional start sites were mapped by the Cap analysis gene expression (CAGE) assay, gene expression profiles by mRNA sequencing, DNase I hypersensitive (HS) sites by deep sequencing, and active histone modifications (H3K4me2, H3K4me3, H3K9ac) and transcription factor-binding sites (for PU.1, E2A, EBF1 and Pax5) by ChIP sequencing in pro-B cells.*

Figure 3: ***Identification of regulatory PAIR elements in the distal V_H gene cluster of the Igh locus.*** *A circular diagram indicates the positions of the V_H, D_H, J_H and C_H gene segments, iE_μ enhancer and 3' regulatory region (3'RR) of the mouse Igh locus. The different colors denote members of the distinct V_H gene families. As shown by the inner concentric tracks, Pax5-activated intergenic repeat (PAIR) elements (red) are located upstream of V_H3609 genes (pink) and contain binding sites for Pax5 (purple) and CTCF (blue).*

Spatial regulation of V(D)J recombination

The development of B cells and αβ T cells depends on functional rearrangement of the *Igh* and *Igk* or *Tcrb* and *Tcra* loci, respectively. All four loci are large in size (0.7 to 3 megabases), organized in a complex manner (Figure 3), and undergo reversible contraction by looping in rearranging lymphocytes. Locus contraction is thus a general mechanism that juxtaposes distantly located V genes of the large V gene cluster next to D or J segments, which facilitates synapse formation and V-(D)J recombination. Our previous work demonstrated that contraction of the *Igh* locus primarily depends on Pax5. Recently, we were able to show that Pax5 activates conserved intergenic repeats (PAIRs) in the distal V_H gene cluster, which most likely functions as a novel regulatory element in controlling *Igh* locus contraction (Figure 3). We are currently elucidating the molecular mechanism of *Igh* locus contraction by investigating the function of such *cis*-regulatory elements and by identifying novel *trans*-acting factors involved in this process.

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TIM CLAUSEN

Molecular mechanisms of protein quality control

tim.clausen@imp.ac.at

Tim Clausen / Senior Scientist

Jakob Fuhrmann / Postdoc
Alexander Heuck / Postdoc
Sonja Sollner / Postdoc
Katharina Linn Gazda / PhD Student
Doris Hellerschmied / PhD Student
Markus Mastny / PhD Student
Flavia Meireles / PhD Student
Bastian Schmidt / PhD Student
Juliane Kley / Research Assistant
Robert Kurzbauer / Research Associate (part time)

The misfolding and aggregation of protein molecules is a major threat to all living organisms. Cells have therefore evolved a sophisticated network of molecular chaperones and proteases to prevent protein aggregation (Fig. 1A). In addition, protein quality control and regulatory proteolysis are important mechanisms in the defense line of several bacterial pathogens. My group is performing a structure-function analysis of prokaryotic and eukaryotic factors that combat folding stress and, in parallel, ensure controlled digestion of specific target proteins. A better understanding of protein quality control might disclose novel strategies to counteract protein folding diseases and bacterial pathogenicity.

Housekeeping HtrA proteases - Guards of the extracytosolic compartment

A combination of chaperone and protease function in a single protein could provide a direct and rapid response to protein folding problems. The heat shock protein DegP (HtrA) can switch between these dual functionalities in a temperature-dependent manner (Fig. 1B), and thus offers unique possibilities to investigate how cells distinguish between proteins that can be refolded and "hopeless" cases that need to be degraded.

DegP from *E. coli* is a central component of the protein-quality-control system in the bacterial envelope that is involved in eliminating misfolded proteins and in OMP (outer membrane protein) biogenesis. To investigate the molecular basis of these dual activities, we characterized different DegP/substrate complexes. Binding of misfolded proteins transformed the resting DegP hexamer into large, catalytically active 12- and 24-meric multimers. Structural analysis of these particles revealed that DegP assembles a huge protein packaging device (Fig. 2), whose central compartment is adaptable to the size and concentration of substrate. Moreover, the inner cavity serves antagonistic functions. While encapsulation of folded OMP protomers is protective and might permit safe transit through the periplasm, misfolded proteins are eliminated in the molecular reaction chamber. Oligomer re-assembly and concomitant activation upon substrate binding may also be critical in regulating other HtrA proteases that promote diverse biological functions. Our current data provide excellent leads to address the molecular mechanisms of human DegP homologues, homologues from pathogenic bacteria, and functionally related PDZ proteases that exert housekeeping functions in mitochondria, chloroplasts and the extracellular space. Moreover, the identified activation mechanism should be helpful to obtain structural data concerning protease-substrate or protease-inhibitor complexes, which are indispensable for understanding how aberrant proteins are partitioned between refolding and degradation pathways.

The CtsR/McsB stress response

All cells have evolved highly efficient signaling pathways that sense the presence of damaged proteins and transmit the signal "folding stress" to dedicated transcription factors, which then adjust the expression of the protein-quality-control factors. One of the most intensely studied stress-response pathways is the bacterial heat-shock system. In *B. subtilis*, the transcription factor CtsR is a major component of this system that represses the *clpC* heat shock operon, the *clpE* and *clpP* genes, by binding specifically to a 7-nucleotide direct repeat sequence located upstream of the transcriptional start sites (Fig.3A). Stress-induced transcription of the *clp* genes depends on the inactivation of CtsR by McsB. To delineate the molecular basis of CtsR and McsB in the bacterial stress response, we screened the respective proteins from various Gram-positive bacteria for

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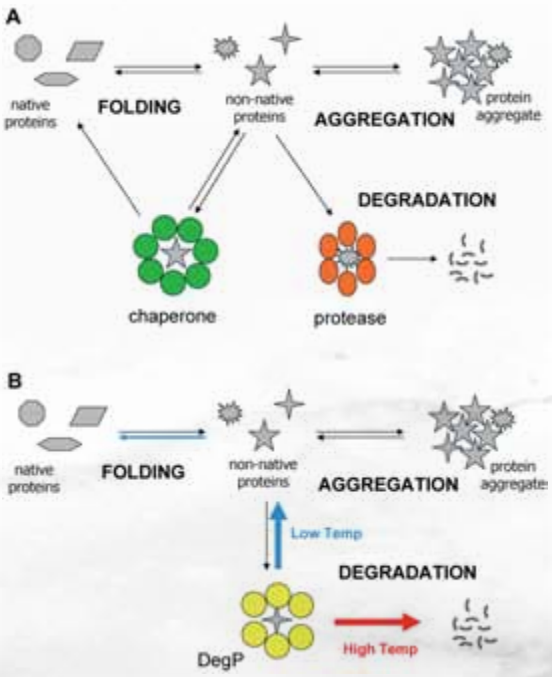

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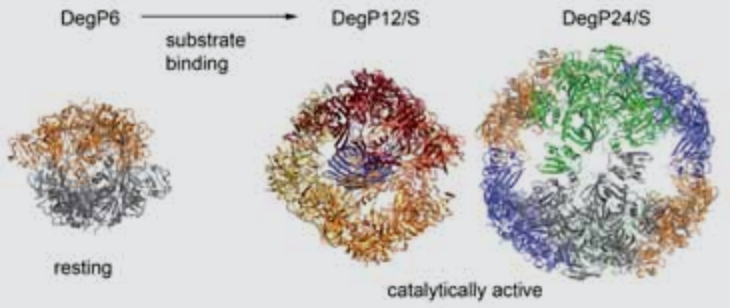

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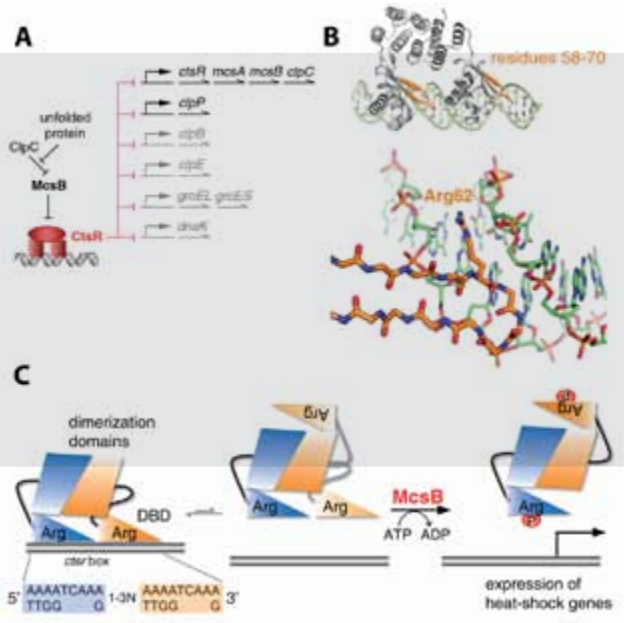

Figure 1: *Protein Quality Control (A) The scheme illustrates the different fates of misfolded non-native proteins. According to Gottesman and co-workers' "kinetic partitioning model", aggregate formation is counteracted by the action of proteases and chaperones. (B) DegP combines the antagonistic activities of a protease and a chaperone, and thus guarantees a rapid cellular response to protein folding stress. The switch in activity is regulated by temperature. At low temperatures (<25°C) the protein acts as a chaperone, whereas at higher temperatures the protease function is dominant.*

Figure 2: *Structural analysis of DegP Ribbon presentation of the resting DegP hexamer (DegP6) that is transformed upon substrate binding into the catalytically active DegP12- and DegP24-multimers. The constituting trimers are colored differently and, for DegP12, the encapsulated OMP substrate is shown in blue.*

Figure 3: *The CtsR/McsB stress-response system (A) The CtsR regulon of Gram-positive bacteria. (B) Position of the phospho-peptide (orange) which was identified by mass spectrometry is highlighted in the CtsR2/DNA structure. The binding mode of Arg62 (that also represents the main phosphorylation site) at the floor of the DNA minor groove (green) is shown in detail. (C) CtsR binds as a dimer to the highly conserved ctsr box, thereby inhibiting transcription of downstream stress genes. The phosphorylated CtsR repressor is unable to bind to DNA, thereby permitting expression of heat-shock genes. "Arg" marks critical arginine residues in the DBD, in particular Arg62, phosphorylated by McsB.*

recombinant production, and succeeded in reconstituting the *Bacillus stearothermophilus* CtsR/McsB system *in vitro*.

The crystal structure of the CtsR repressor in complex with DNA revealed how partial asymmetry in a dimeric transcription factor allows high affinity binding to tandem DNA repeats. Moreover, biochemical characterization of McsB highlighted a novel protein kinase activity. McsB specifically phosphorylates arginine residues in the DNA-binding domain of CtsR, like for example Arg62 of the beta-wing (Fig. 3B), thereby impairing its function as a repressor of stress response genes. Identification of the CtsR/McsB arginine phospho-switch (Fig. 3C) creates new perspectives to enhance our understanding of prokaryotic and eukaryotic transcriptional regulation.

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CARRIE COWAN

Symmetry breaking during cell polarization

carrie.cowan@imp.ac.at

Carrie Cowan / Group Leader

Jeroen Dobbelaere / Postdoc
Dominika Bienkowska / PhD Student
Anne Göppert [1] / PhD Student
Martin Mikl / PhD Student
Sabina Sanegre Sans / PhD Student
Harue Wada / Research Assistant
Muriel Fisser [2] / Rotation Student
Amaia Carrion Castillo [3] / Summer Student

[1] until August 2010,
[2] August 2010,
[3] July - September 2010

Cell polarity allows for spatial specialization within a cell, such as directed transport, migration, or growth. Cell polarity also controls asymmetric cell division, a dominant mechanism for dictating cell fate changes during development. The diverse roles of this fundamental organizational concept mean that understanding cell polarity is essential to understanding both normal development and diseases. We are investigating how cell polarity is established.

Research activities:

We are using one-cell *C. elegans* embryos as a model system to determine how cell polarity is established. One-cell *C. elegans* embryos polarize in response to a signal from the sperm-provided centrosomes. Centrosomes, sitting near the cell cortex, induce a local structural reorganization of the acto-myosin network: whereas most of the cortex undergoes stochastic contractions, the area around the centrosomes becomes non-contractile. This local change in cortex activity marks the functional symmetry-breaking event that allows polarization. Once cortical symmetry has been broken, mutual antagonism between antagonistic polarity proteins - the anterior and posterior PAR proteins - appears to facilitate self-organizing polarization. PAR polarity controls cell fate determinant segregation and asymmetric cell division.

Using a combination of forward and reverse genetics, in vivo biochemistry, high-resolution time-lapse microscopy, ultrastructural reconstructions, automated quantitative analysis, and mechanical manipulations, we are investigating the following questions:

How do centrosomes move to the cortex?

After the sperm centrosomes are delivered to the egg during fertilization, they wander randomly in the cytoplasm for approximately thirty minutes. The acto-myosin cortex confines centrosome movement so they remain within ~10 µm of the cortex. Upon a cell cycle signal, centrosomes start to move directly toward the cortex in a highly processive migration. We are investigating both the mechanism and significance of centrosome migration, and if centrosomes use predetermined spatial information to guide their movement.

How do centrosomes signal a change in acto-myosin activity?

Symmetry breaking is normally coordinated with centrosome position. The Aurora kinase family member AIR-1 is essential for this coordination. Embryos depleted of AIR-1 often fail to break cortical symmetry in response to the centrosomes. AIR-1 is required for centrosome assembly, which could contribute to its role in polarity establishment. However, it appears to have an additional role during polarization, acting independent of centrosomes to suppress ectopic symmetry breaking. We are trying to understand how AIR-1 achieves its different functions by identifying temporal and spatial regulators and effectors of AIR-1 kinase activity.

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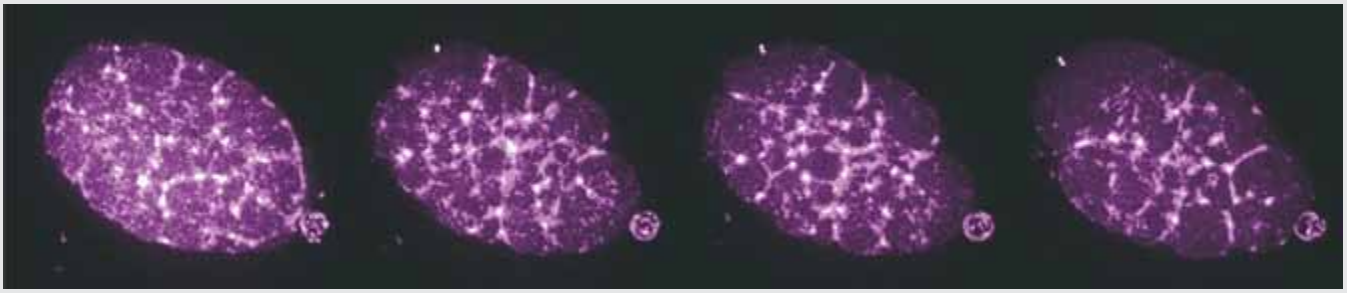

Figure 1: *Centrosomes and the contractile acto-myosin cortex during symmetry breaking. Centrosomes (dots in the upper left end of the embryo) and myosin during the first five minutes of polarity establishment.*

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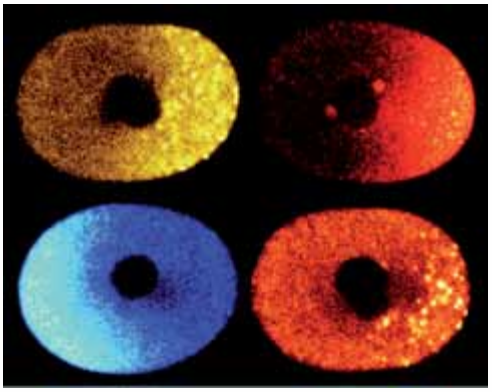


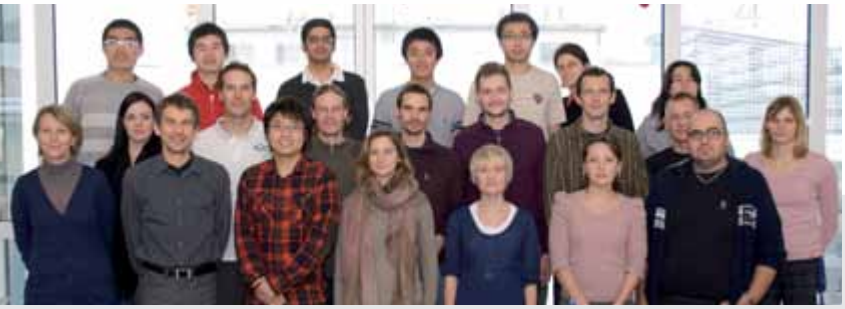
Figure 2: *Asymmetric distribution of fate determinants. The RNA binding proteins MEX-1 (yellow), PIE-1 (red), and MEX-5 (blue) and P granules (orange) upon entry into mitosis.*

What regulates PAR polarity in response to symmetry breaking?

After the initial symmetry-breaking event in the cortex, the mutual exclusivity of PAR protein domains facilitates the subsequent establishment of cell polarity. The balance between the amounts of anterior and posterior PAR domain components appears essential for normal polarization. Controlling PAR protein amounts - both absolute and at the cortex - is an important regulatory point. Total PAR protein levels appear to depend on processing of relevant mRNAs, while the proportion of cortical PAR proteins appears to be influenced by membrane trafficking. We would like to determine the molecular mechanisms by which these pathways control cortical PAR protein localization.

How does cortical polarity control cytoplasmic asymmetry?

The establishment of polarity at the cortex provides spatial information to polarize the entire cell, ultimately allowing asymmetric changes in gene expression and cell fate. The cytoplasmic fate determinant PIE-1 is restricted to the posterior half of one-cell embryos through interactions among multiple RNA binding proteins coupled with a general destabilizing activity. PIE-1 forms a concentration gradient that appears to be facilitated by association of a fraction of PIE-1 with RNA processing bodies, the P granules. We are studying how the PIE-1 gradient is established in response to PAR polarity and how robustness is provided in the fate determinant network.



BARRY DICKSON

Neural circuits

barry.dickson@imp.ac.at

Barry Dickson / Senior Scientist

Kai Feng / Postdoc
Martin Häsemeyer / Postdoc
Mark Palfreyman / Postdoc
Laszlo Tirian / Postdoc
Hirofumi Toda / Postdoc
Sonja Vernes / Postdoc
Anne von Philipsborn / Postdoc
Tracy Yamawaki / Postdoc
Vera Belyaeva / PhD Student
Salil Bidaye / PhD student
Sabrina Jörchel / PhD Student
Tianxiao Liu / PhD Student
Christopher Masser / PhD Student
Yang Wu / PhD Student
Katarina Bartalska / Research Assistant
Attila Gyorgy / Research Technician
Martin Kinberg / Research Technician
Thomas Peterbauer / Research Assistant
Christian Machacek / Software Developer

We use molecular genetic techniques to study the function of neural circuits in Drosophila. Our goal is to understand how information processing in defined neural circuits generates complex animal behaviours. As a model system, we focus on the fly's mating behaviours. These behaviours are robust, adaptive, and particularly amenable to genetic analysis.

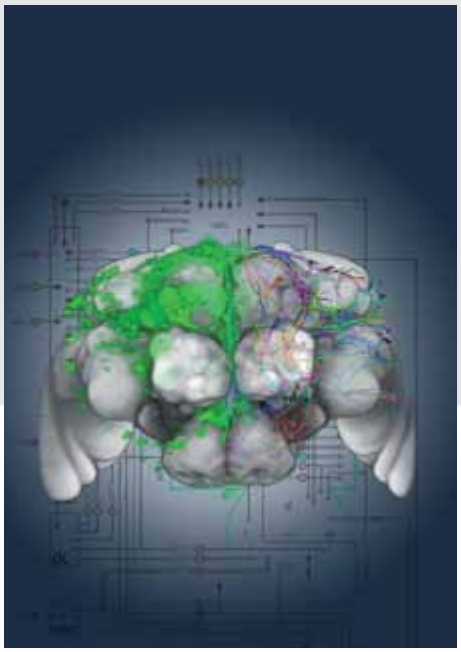
A major goal for neuroscience is to understand how information processing in neural circuits guides animal behaviour. At any given moment, the brain receives a rich set of sensory inputs, from both external and internal sources. This information must be integrated, interpreted in the light of prior experience, and a specific behavioural action selected. These processes of sensory integration, learning and memory, decision making, and action selection are the essence of animal cognition and behaviour. How are such processes implemented in the brain?

As a model, we have chosen to study the sex life of the fruit fly *Drosophila melanogaster* [1]. During mating, flies make decisions that are critical for their reproductive success, and hence their evolutionary fitness. Upon encountering another fly, the male weighs his chances of success, based on sensory input and past experience, and decides if it is worth investing the time and energy to court. If he does court, the female then decides whether to accept or reject him, based on how she assesses his quality as a potential mate and her own maturity and mating status. These are complex decisions made by complex brains, but working with flies has the great advantage that genetic tools can be used to identify and manipulate the relevant neurons and circuits in the brain. With these tools, it should be possible to establish causal relationships between genes, cellular biochemistry, circuit function, and animal behaviour.

The male brain

Remarkably, the different behaviours of male and female flies can largely be explained by the sex-specific splicing of a single gene – *fruitless* (*fru*). If females are forced to express male-specific fru^{M} transcripts, they behave like males. Conversely, males that lack fru^{M} behave like females.

fru is expressed in some 2000 neurons, distributed in clusters throughout the nervous system (Fig. 1). The activity of these neurons is essential for courtship behaviour. We have recently developed genetic tools that provide specific access to distinct subsets of *fru* neurons. With these tools we can selectively express visible markers in these cells to trace out their anatomy and connectivity (Fig. 2), or various proteins that measure or modulate neuronal activity and allow us to study circuit function. Using this genetic toolkit, we have constructed a cellular resolution wiring diagram of the entire *fru* circuit [2] (Fig. 3) Now, we can begin to examine what each of type of *fru* neuron contributes to courtship, the nature of the signals it processes, and how and to what extent sexually dimorphic processing leads to the distinct behaviours of males and females.



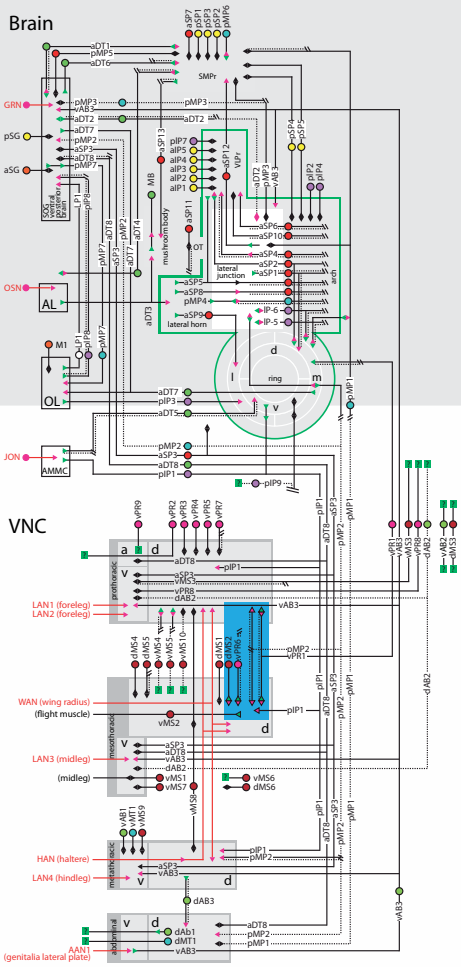


Figure 1: ***fru⁺ neurons in the CNS.*** *fru neurons are shown in green, synapses are stained in magenta. This is a confocal image of the brain and ventral nerve cord of a fru^{GAL4} UAS-GFP male.*

Figure 2: ***Genetic dissection of fru⁺ neurons in the central brain.*** *The image shows processes of fru neurons in the male brain, visualized with GFP (green, left), and their digitial reconstruction into a 3D cellular resolution atlas (right).*

Figure 3: ***The fru circuit.***

The female brain

The female decides whether to accept or reject the male based in part on her perception of his courtship song and pheromones. We are currently examining the sensory pathways that process and integrate these two external signals. A third important factor in the female's decision is her own mating status. Females that have recently mated are usually reluctant to do so again. This is due to a small peptide, called the sex peptide (SP), that is present in the first male's seminal fluid. We have recently identified a molecular receptor for SP, a G-protein coupled receptor we call SPR (sex peptide receptor), and shown that it acts in a small subset of *fru⁺* sensory neurons that innervate the reproductive tract and project axons into the central nervous system [3]. Our ongoing efforts are aimed at understanding what SP does to these neurons, and to characterise the neural pathways in the brain that further process this signal – ultimately integrating it with the song and pheromone signals to guide the female's mating decision.

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CHRISTINE HARTMANN

Formation and Patterning of the Vertebrate Skeleton

christine.hartmann@imp.ac.at

Christine Hartmann / Group Leader

Sayumi Fujimori[2] / Postdoc
Natalia Lyashenko[1] / Postdoc
Hermann Novak[2] / Postdoc
Daniela Kostanova-Poliakova / Postdoc
Markus Winter / Postdoc
Chandra Sekhar Amara / PhD Student
Richard Latham / Research Technician
Martina Weissenböck / Research Technician

[1]since January 2010
[2]until April 2010

The skeleton is essential for vertebrates; it supports the body, provides the mechanical framework for physical movements, and protects internal organs. During embryonic development the sites where the future skeletal elements (bones) are formed and their size as well as the positions of articulations (joints) need to be determined. Most skeletal elements are formed as cartilaginous templates and need to be remodeled into bony tissue to strengthen the skeleton - this process is referred to as endochondral ossification. We use mouse and chick as model organisms to gain insight how different aspects of skeletogenesis are regulated by Wnt-signaling pathways during embryonic and postnatal development.

Regulation of cell lineage differentiation

The different cell types of the appendicular skeleton, the chondrocytes, osteoblasts and cells contributing to the future synovial joints are of mesenchymal origin. In recent years we have demonstrated that the canonical Wnt-signaling pathway, which is mediated through β-catenin, is playing a very essential roles for the development of the different lineages of the mouse skeleton (see Figure 1). In the absence of functional canonical β-catenin signaling, osteoblast precursors, the osteochondroprogenitor cells, differentiate into chondrocytes (Hill et al., 2005). Furthermore, we have shown that the canonical Wnt/β-catenin pathway is required to suppress the chondrogenic potential of cells in the joint interzone (Spaeter et al., 2006).

Differentiation and Maturation of Chondrocytes

The various skeletal elements of the vertebrate skeleton differ in size and shape, but little is known about the molecular mechanisms controlling these two features. Interestingly, alterations in the process of chondrocyte maturation often lead to changes in the size of skeletal elements. Studying the Wnt9a knock-out animals we uncovered a very specific requirement for one of the Wnt-ligands, Wnt9a, during long-bone development: Wnt9a via β-catenin signaling controls the expression of the central regulator of chondrocyte maturation, Indian hedgehog, in prehypertrophic chondrocytes in a spatio-temporal manner (Spaeter et al., 2006). Thus sequential activation, or temporal recruitment of regulatory molecules, which like Wnt9a control a central regulator of chondrocyte maturation, are likely to be involved in fine-tuning the size of the skeletal element. We are currently analyzing, if β-catenin plays a role in hypertrophic chondrocytes.
We also uncovered a potential role for Calcium/Calmodulin dependent kinase II (CaMKII) in the maturation process of chondrocytes. Activation of CaMKII results in premature maturation of the chondrocytes expressing the molecule and elongation of the skeletal element, while inhibition of the kinase by expression of a peptide inhibitor leads to a delay in chondrocyte maturation and shortening of the skeletal element (Taschner et al., 2008). (Figure 2)

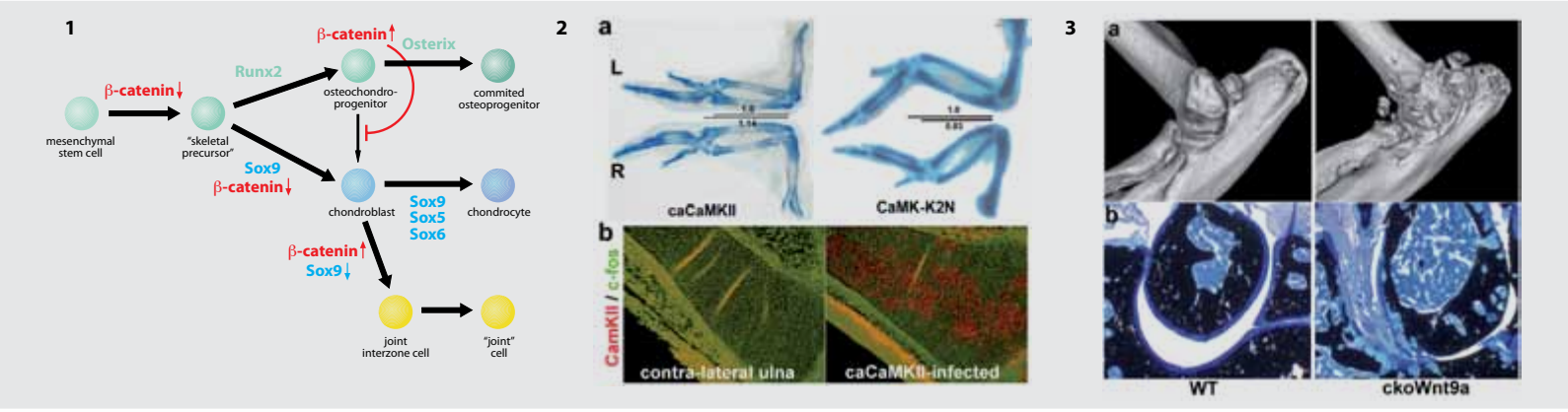

Figure 1: *β-catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of β-catenin can differentiate into skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage β-catenin levels need to be increased to enable differentiation into osteoblasts and cells of the joint, respectively. Within the chondrocyte lineage β-catenin levels need to be maintained at a low level.*

Figure 2: *a) Expression of an constitutively active form of CaMKII (caCaMKII) results in lengthening of the skeletal elements in the infected right (R) chicken limb, while expression of the inhibitor CaMK-K2N results in shortening of the infected R limb. b) The lengthening is associated with the down-regulation of cfos expression (green) in the caCaMKII infected regions (red).*

Figure 3: *a) MicroCT of a wt elbow and an elbow of a limb lacking Wnt9a (cko Wnt9a), the latter showing ectopic ossifications. b) Histology of the elbow joints, showing loss of the articular cartilage (arrow in the wt elbow joint).*

Synovial joint development

In gain-of-function analysis in the chicken, Wnt9a was identified as a major player for the induction of synovial joint development (Hartmann and Tabin, 2001). In mouse Wnt9a is expressed in the early joint interzone and, after the formation of the synovial joint, in the articular cartilage and in cells of the joint capsule. In mice lacking Wnt9a an ectopic cartilage nodule differentiates from synovial cells in the humeral-radial joint. In humans this phenotype is known as synovial chondroid metaplasia. Two other Wnt-genes are expressed in the joint regions, Wnt4 and Wnt16. Wnt4;Wnt9a double-mutant mice show synovial chondroid metaplasia in two additional joints and fusions of carpal and tarsal elements (Später et al., 2006a, 2006b), but not to a complete fusion of all joints. Thus, the embryonic phenotypes point to a role for Wnts maintaining joint integrity.
Conditional deletion of Wnt9a specifically in the limb mesenchyme results in progressive changes of various joints, which show hallmarks of osteoarthritis. In addition, these mice show ectopic mineralization within joint ligaments (Figure 3).

In summary, our analysis has show that Wnt-signaling is playing important roles during skeletal development, regulating differentiation of the various cell types, maturation of chondrocytes and is important to maintain the integrity of the developing and mature joint.

DAVID KEAYS

The Molecular Basis of Migration

david.keays@imp.ac.at

David Keays / Independent Postdoctoral Fellow

Martin Breuss / PhD Student
Andreas Braun / Diploma Student
Paul Pichler / Diploma Student
Christoph Treiber / Technical Assistant
Marion Salzer / Trainee

One of the most remarkable aspects of life, whether it be a single cell or a multicellular organism, is the ability to move. This is even more astounding when one considers that this movement is frequently equipped with directionality. Whether it be a migratory bird that crosses continents, or a cell that inches forward a micrometer at a time, both have a drive, a direction and a destination. What molecular mechanisms underlie this migration? The Keays lab is investigating two very different forms of migration: (1) the migration of neurons; and (2) the migration of animals mediated by magnetic information.

Tubulins in Neuronal Migration and Disease

Neuronal migration underlies the organisation of the mammalian brain. All neurons that are born in the proliferative ventricular zones migrate to their final destination by extending their primary neurites and translocating their nuclei. This migration is crucial as it determines the destination of a given neuron as well as the circuit in which it operates. Moreover, a host of neurodevelopmental diseases are known to be linked to defective neuronal migration. An example is lissencephaly, a disease that is characterised by a "smooth brain", epilepsy and mental retardation.

We have shown that mutations in an alpha tubulin gene (TUBA1A) cause lissencephaly in humans and neuronal migration abnormalities in mice (Keays et al, 2007) (Figure 1). The importance of the tubulin gene family in neuronal migration is further evidenced by our finding that mutations in a beta tubulin gene (TUBB2B) (Jalgin et al, 2009), cause another rare neurdevelopmental disorder known as asymmetric polymicrogyria. To gain insight into the role of different tubulin genes, how they cause disease, and the molecular mechanisms underlying the migration of neurons the Keays lab is employing the mouse as a model system (Figure 2). In addition to the generation of novel mouse mutants by transgenic means, we make wide use of histological, cellular, and molecular techniques.

Circuits, Cells and Molecules in Magnetoreception

Many species on the planet, whether they be birds, fish or insects rely on the earths magnetic field to guide migration or assist navigation. This remarkable sense is known as magnetoreception. One idea that aims to explain how animals detect magnetic fields is known as the magnetite based theory of magnetoreception. This theory holds that mechanosensitive ion channels coupled to an intracellular compass made of an iron oxide called magnetite (Fe3O4) transduce local magnetic information into a neuronal impulse.

This hypothesis originates from the discovery of magnetotactic bacteria. These aquatic bacteria use the Earth's magnetic field to direct swimming towards growth-favouring regions in natural waters. It has been shown that magnetotatic bacteria possess organelles called magnetosomes. Magnetosomes consist of membrane-enclosed magnetite crystals that twist into alignment with the Earth's magnetic field – thereby directing bacterial movement. The theory of magnetite based magnetoreception has been supported by the discovery of magnetite in a range of other organisms that detect and respond to magnetic fields; most notably birds, fish and bees.

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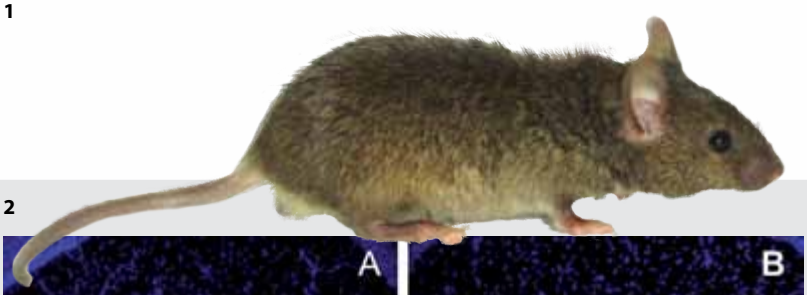

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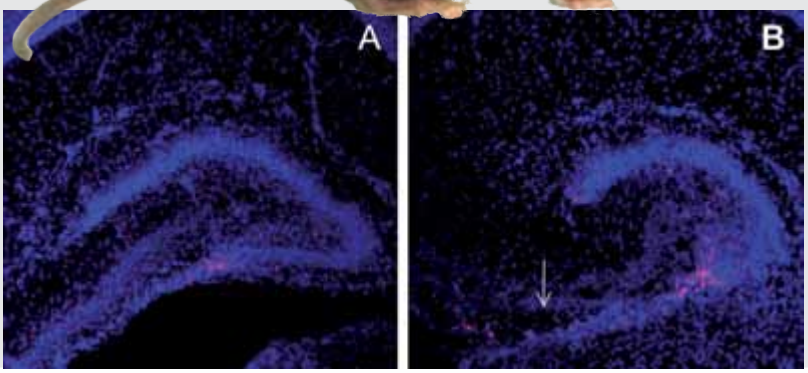

Figure 1: *The Jenna mutant mouse. This mouse harbours a S140G mutation in the Tuba1a gene which results in defect neuronal migration during development. As a consequence the Jenna mouse is characterised by abnormal lamination of the hippocampus that is accompanied by hyperactivity and deficits in cognitive tasks.*

Figure 2: *The developing hippocampus in the Jenna mutant mouse. TBR2 staining of dentate progenitor cells in wild type mice (A) and the Jenna mutant mouse (B) reveals a trail of neuronal progenitors (arrowed) along the subpial route in mutant animals, indicative of a defect in migration.*

Figure 3: *Iron rich cells stained with Prussian Blue in the beak of Columbia livia. We are investigating whether these cells are magnetoreceptive.*

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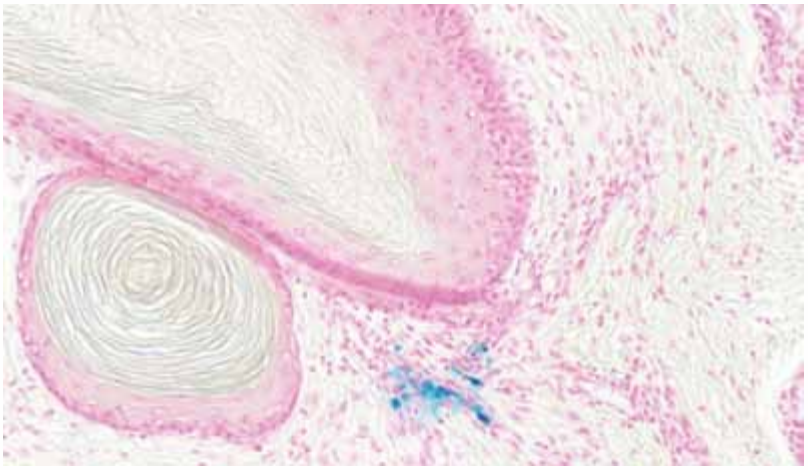

The Keays lab is investigating the magnetite based theory of magnetoreception employing the pigeon *Columbia livia* as a model system. Our current efforts are focused on the trigeminal system as it has been shown that the ophthalmic branch of the trigeminal nerve is required for magnetoreception in pigeons (Mora et al, 2004). Employing Prussian blue iron staining (Figure 3), immunohistochemistry, live cell imaging, transmission electron microscopy (TEM) and energy-filtered element mapping we aim to identify and characterise the circuits and cells required for magnetoreception. Our ultimate objective is to identify the molecules nature employs to construct a magnetoreceptor.

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THOMAS MARLOVITS

Molecular Machines

thomas.marlovits@imp.ac.at

Thomas C Marlovits / Joint IMP/IMBA Group Leader

Matthias Brunner / PhD Student
Agata Kosarewicz / PhD Student
Lisa Königsmaier / PhD Student
Julia Radics / PhD Student
Jesus Fernandez Rodriguez / PhD Student
Oliver Schraidt / PhD Student
Joanna Radek / Diploma Student
Wolfgang Schmied / Diploma Student
Kimberly McManus / Summer Student (MIT - Boston, USA)
Wolfgang Weber / Lab Manager / Technical Assistant

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

Microbial Pathogenesis

One of the most exciting discoveries in the last few years is that supramolecular assemblies are one of the key elements involved in the infection of eukaryotic cells by bacterial pathogens. These systems give rise to intimate contact between cells, deliver specific toxins - which are collectively known as effectors proteins - into host cells, and possess the remarkable ability to modulate diverse regulatory networks. In particular, many Gram-negative pathogens such as *Salmonella*, *Yersinia*, *Pseudomonas*, or *Shigella* utilize the type III secretion system (TTSS) to initiate infection in eukaryotic cells. TTSS is a multicomponent system comprising more than 20 different proteins. A mere fraction of these assemble into the so-called needle complex, which is the most prominent core structure of the system. It is a membrane-associated complex with > 3.5 MDa, and is composed of a set of soluble and membrane proteins. Although it is essential for microbial infection in many animal as well as plant pathogens, the assembly of the needle complex and how the needle complex identifies and triggers efficient translocation of substrates are still poorly understood. Using *Salmonella typhiumurium*, we are investigating the molecular mechanisms and structural framework required to translocate effector proteins specifically and safely into eukaryotic cells.

Recent crystallographic analyses of individually separate domains, which are predicted to be located within the plasma, revealed a common structural motif organized in repeating modules. Attempts have been made to "dock" these protein domains into the needle complex structure, which resulted in several mutually incompatible locations. We have used a combination of methods including bacterial genetics, biochemistry, mass spectrometry and cryo electron microscopy/single particle analysis to experimentally determine the position of specific protein domains within the needle complex. In addition, we have identified specific sites of interaction among components of the needle complex, which are critical for stable assembly and the subsequent functional complex. Jointly, this analysis provides the first experimentally validated topographic map of different components of the needle complex of the *S. typhimurium* TTSS (Figures 1 and 2) (Schraidt et al., 2010)

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

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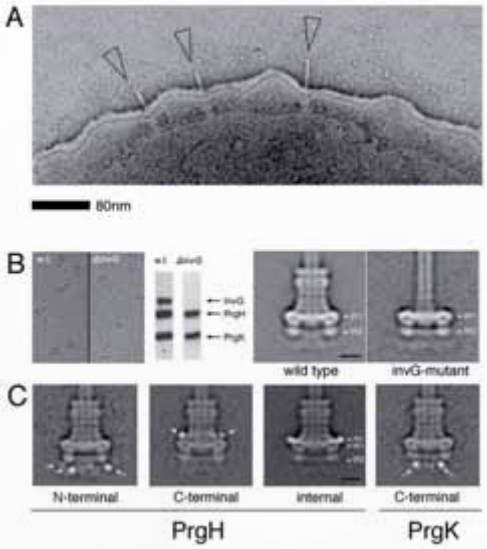

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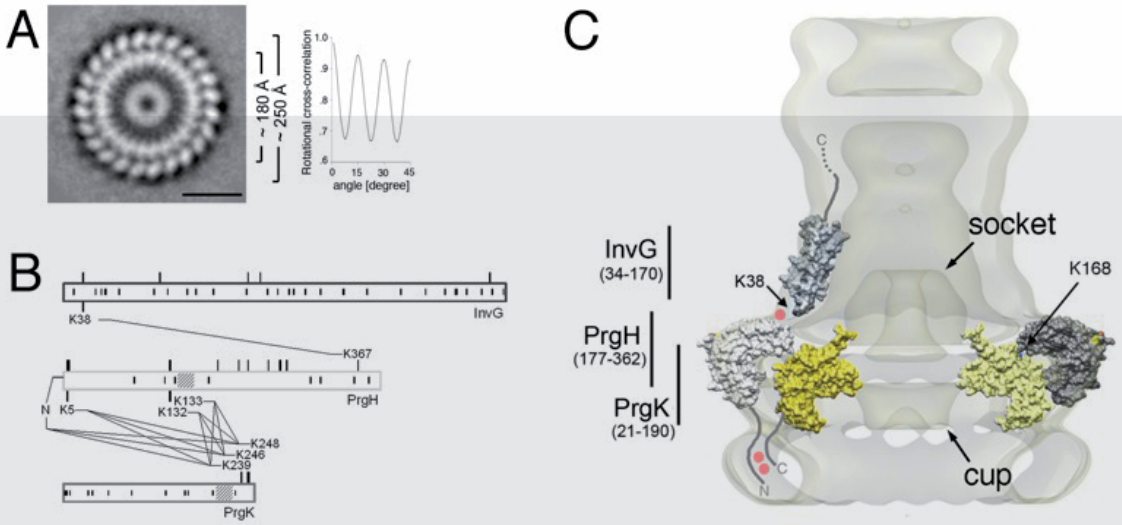

Figure 1: ***The needle complex is the core structure of the type III secretion system.*** *(A) Needle-like structures (approx. 50 nm) that extend into the extracellular environment are visible on the surface of osmotically shocked S. typhimurium. (B) Isolated complexes from wild-type and a invG mutant strain are structurally different. (C) Isolated needle complexes harboring poly-histidine tags at various positions in PrgH or PrgK can be specifically labeled using nanogold.*

Figure 2: ***Organization of PrgH, PrgK and InvG within the needle complex*** *(A) End view class average derived from single particle analysis from negatively stained electron microscopy images of sub-structures of the inner rings. The sub-structures were obtained by selective disassembly of needle complexes isolated from a mutant strain encoding for a C-terminally, four amino acid truncated PrgH. Rotational cross-correlation analysis revealed that the maximum of the cross-correlation peak is repeatedly obtained every 15°, demonstrating that the larger concentric rings of the inner ring structure exhibit 24-fold symmetry. (B) Proximity of specific domains of the base proteins, InvG, PrgH, and PrgK within the needle complex. The block diagrams shows the three major base proteins, InvG, PrgH, and PrgK, and covalent cross-links of peptides obtained from chemically derivatized needle complexes at primary amino groups. (C) Topographic model of the needle complex: Localization of domains of InvG, PrgH, and PrgH within the base of the needle complex. The N-terminal domain of InvG (blue-grey) reaches far down into the neck region and is in close contact with the C-terminal domain of PrgH (white and grey). Sites of interaction found by cross-linking and mass spectrometry are labeled as red dots. The N-terminal domain of PrgH is pointing to the cytoplasmic side of the complex, and interacts with the C-terminal domain of PrgK. For both, no high-resolution structure is available as of now. The N-terminal domain of PrgK is located within the complex and is therefore packed into its position by PrgH from the side and InvG from the top.*

Figure 3: ***Sub-structures of the needle complex.*** *(A) Structural analysis of complexes from strains lacking the export apparatus proteins (SpaSPQR, InvA) reveal marked differences in the central portion of the complex (socket/cup) compared to the wild type. (B) The needle filament protein PrgI is the building block of the extracellular needle filament.*

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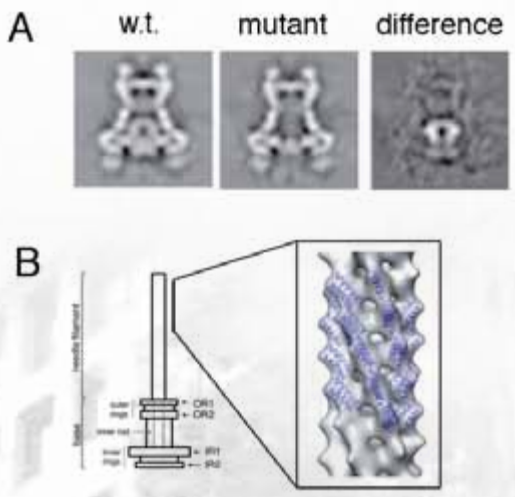

Efficient effector protein translocation is known to occur only after host cell contact. Therefore, it is conceivable that the extracellular filament is a key player in the transmission of this information, probably due to small conformational changes throughout the filament. This hypothesis is supported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

Although the design of the TTSS appears to be conceptually simple, many questions remain unanswered: How dynamic is the entire assembly process? How are substrates recognized by the needle complex? What is the molecular mechanism of protein translocation? We have begun to address some of these questions. By understanding the molecular mechanism of TTSS-mediated protein transport, we hope to provide a basis for the development of novel therapeutic strategies that will either inhibit its activity or modify the system for targeted drug delivery.

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JAN-MICHAEL PETERS

Mitosis and chromosome biology

jan-michael.peters@imp.ac.at

Jan-Michael Peters / Senior Scientist

Venugopal Bhaskara / Postdoc
James Hutchins [1] / Postdoc
Petra van der Lelij [5] / Postdoc
Tomoko Nishiyama / Postdoc
Ivana Primorac [2] / Postdoc
Antonio Tedeschi / Postdoc
Kristina Uzunova / Postdoc
Georg Adrian Busslinger / PhD Student
Pim Huis In't Veld / PhD Student
Miroslav Ivanov / PhD Student
Marc Jarvis [4] / PhD Student
Emanuel Kreidl / PhD Student
Rene Ladurner / PhD Student
Georg Petzold / PhD Student
Erika Schirghuber / PhD Student
Hanna Schutz / PhD Student
Marta Galova / Research Associate
Maria-Helena Idarraga-Amada / Research Assistant
Uta Moehle-Steinlein / Research Associate
Wen Tang [3] / Research Technician
Gordana Wutz / Research Associate
Yan Sun / MitoCheck / MitoSys Project Manager

[1]until June, [2]until July, [3]since April, [4]since September, [5]since December

To pass the genome from one generation to the next, eukaryotic cells first replicate their DNA, then biorient chromosomes on the mitotic spindle and finally separate their sister chromatids, enabling division of one cell into two genetically identical daugther cells. We are interested in understanding these processes at the molecular level.

How is sister chromatid cohesion established and maintained?

Numerous sites in the genome are bound by cohesin complexes. During DNA replication these complexes establish physical connections between the newly synthesized sister chromatids. It is well established that the resulting cohesion is essential for chromosome segregation and for DNA damage repair, but how cohesion is established and maintained for many hours, or in the case of mammalian oocytes even for years, is poorly understood. We discovered recently that cohesin is converted into a 'cohesive' form that binds to DNA very stably by the protein Sororin, which associates with cohesin during DNA replication. Our data indicate that Sororin stabilizes cohesin on DNA by antagonizing a protein that can dissociate cohesin from DNA, called Wapl.

How does cohesin control chromatin structure and gene regulation?

Although cohesin is best known for its role in mediating cohesion, we and others discovered that cohesin has also important roles in gene regulation. We suspect that these functions are the reason why cohesin binds to chromatin already before cohesion is established and why cohesin associates with DNA even in postmitotic cells, which will never establish cohesion. We found that cohesin co-localizes in mammalian genomes with the transcriptional insulator protein CTCF and showed that cohesin is required for gene regulation at the imprinted *H19-IGF2* locus. Gene expression at this locus is believed to be controlled by formation of a chromatin loop which forms between CTCF sites specifically on the maternal allele. Our recent work indicates that cohesin is required for this chromatin interaction. Our future aims are to test if cohesin has a general role in forming chromatin loops, and to understand the mechanistic basis of this function.

How is sister chromatid cohesion dissolved during mitosis?

Sister chromatid separation in anaphase depends on the removal of cohesin from chromosomes. We discovered a number of years ago that this process depends on two mechanisms in vertebrate cells, the dissociation of cohesin from chromosome arms in prophase and the proteolytic cleavage of cohesin at centromeres in metaphase. The prophase pathway depends on the cohesin associated protein Wapl, whereas the metaphase pathway is mediated by the protease separase. Although the prophase pathway has been identified many years ago, its function and importance for chromosome segregation are still unknown. We have therefore generated a conditional Wapl "knockout" mouse to study the role of the prophase pathway *in vivo*.

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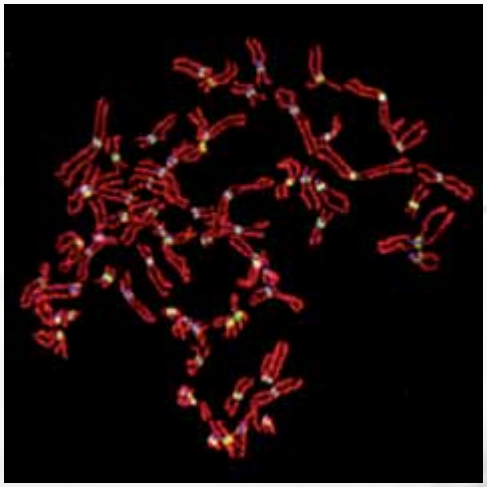

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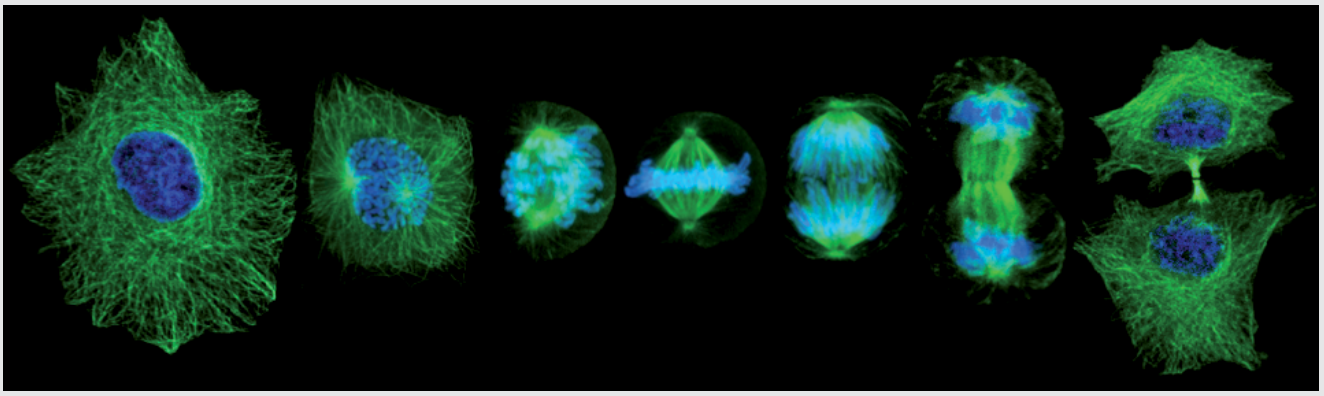

Figure 1: *Human mitotic chromosomes stained for condensin (red), cohesin (blue) and the centromere-specific histone Cenp-A (green). Courtesy of Peter Lenart.*

Figure 2: *Human cultured cells in different stages of mitosis, stained for chromosomes (blue) and microtubules (green). Courtesy of Toru Hirota.*

Figure 3: *Structures of human APC/C in different states. 'Inhibited', bound to the mitotic checkpoint complex (red); 'primed', bound to the coactivator Cdh1 (red); 'active', bound to substrate (blue) and Cdh1. Courtesy of Georg Petzold, Franz Herzog and Holger Stark.*

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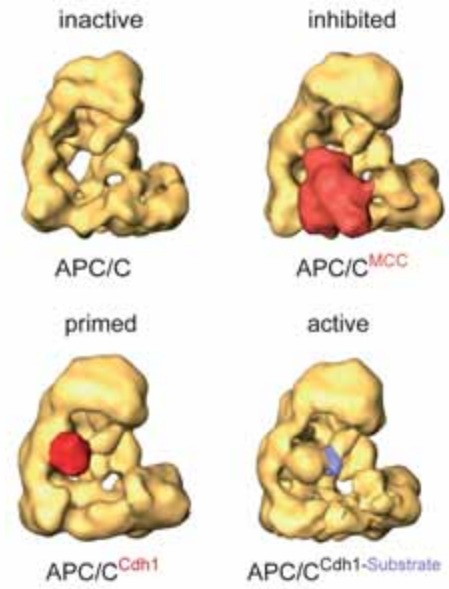

How does the APC/C initiate anaphase?

In metaphase, when all chromosomes have been bioriented, the anaphase promoting complex/cyclosome (APC/C) is activated. The APC/C is a 1.5 MDa complex which assembles ubiquitin chains on securin and cyclin B. The subsequent destruction of these proteins by the 26S proteasome allows activation of separase, cleavage of centromeric cohesin and sister chromatid separation. Until chromosome biorientation is complete, the APC/C is inhibited by the spindle assembly checkpoint (SAC). The SAC ensures that sister chromatids are only separated once chromosomes have been attached to both spindle poles. Despite the crucial importance of the APC/C, it is poorly understood how this complex is inhibited by the SAC, how this inhibition is relieved in metaphase, and how active APC/C recruits and ubiquitylates its substrates. We are using biochemical assays and electron microscopic analyses of the APC/C in different functional states to address these questions.

MitoCheck / MitoSys

Although mitosis has been studied for more than a century, our molecular understanding of this complicated process is far from complete. During the past five years, the MitoCheck consortium, funded by the European Union, has therefore developed and applied genomic and proteomic approaches to study mitosis. The consortium has used RNA interference screens to identify proteins required for mitosis in human cells, tagging of genes in bacterial artifical chromosomes (BACs) to enable the intracellular localization and affinity purification of these proteins, and mass spectrometry to identify protein complexes and mitosis-specific phosphorylation sites on these. This work has identified about 100 human protein complexes, many of which had previously not or only incompletely been characterized. Importantly, the approaches developed by MitoCheck will generally be applicable to high throughput analyses of other processes in mammalian cells. In the future, we will develop quantitative assays for mitosis in a new project funded by the European Union, called MitoSys.

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SIMON RUMPEL

Synaptic and circuit mechanisms of memory formation

simon.rumpel@imp.ac.at

Simon Rumpel / Group leader

Brice Bathellier / PostDoc
Manuel Peter / PhD Student
Bruno Fontinha / PhD Student
Konstantina Rowald / PhD Student
Kaja Moczulska / PhD Student
Juliane Tinter / PhD Student
Tanja Wernle [1] / Diploma Student
Jiss John / Technician
Eva-Maria Wiedemann / Technician
Dorin Gugonatu / Software developer

[1] *until April 2010*

Our lab is focused on the development, function and plasticity of neuronal circuits. More specifically, we would like to know how memory is stored over long periods of time. This is fundamental to understanding the mind: Memories of past experiences shape our personalities and influence our current perception.

A look at the brain

Long-term storage of information about relevant experiences is essential for successful adaptation of human and animal behavior in a changing environment. A current model of memory formation suggests plastic adaptations in neuronal connections (synapses) caused by relevant experiences. Yet, how such changes in synaptic connectivity lead to the formation of a memory trace remains elusive. How is the processing of external stimuli altered after memory formation? How can we continuously store novel memories in a given neuronal circuit without corrupting previously stored memories? In order to understand the mechanisms by which multiple memory traces are coordinated, we are currently applying *in vivo* imaging techniques to the auditory cortex of mice. The auditory cortex mediates processing of sounds and is involved in the formation of memories of sounds.

Two-photon laser scanning microscopy in transgenic animals expressing green fluorescent protein in just a small subset of cells permits the same neurons, and even the same individual synapses, to be revisited day after day. This is truly remarkable because we estimate that the brain comprises about 10 trillion (10^{13}) synapses. We find that neocortical circuits are highly dynamic: remodeling occurs by the formation/elimination of synaptic connections as well as adaptations in the strength of existing connections. We are currently investigating the impact of auditory learning paradigms on the dynamics of a given set of synapses in the auditory cortex.

In vivo imaging not only permits analysis of synaptic connections, but also monitoring of neuronal activity in tens of neurons simultaneously. Action potential-mediated increases in calcium are reported as changes in fluorescence by calcium indicators. We are investigating the activity patterns elicited by various sounds in neuronal populations of the auditory cortex in order to learn about the principles how sounds are encoded and recognized in the brain. In the future we will investigate how far auditory learning paradigms lead to changes in the neuronal representation of memorized sounds.

Jointly, these approaches will pave the way for a series of novel experiments addressing the storage of information in living neuronal networks, a field of research that has been almost exclusively the domain of theoretical neuroscientists thus far.

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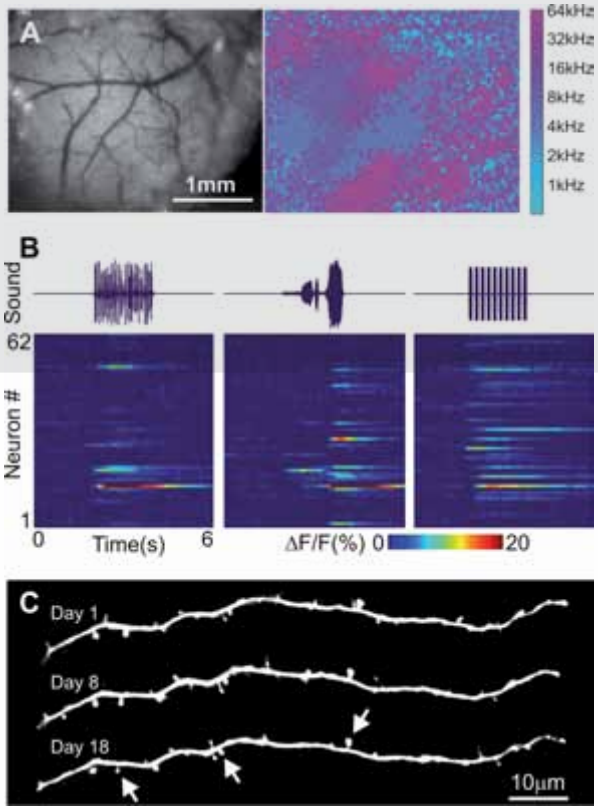

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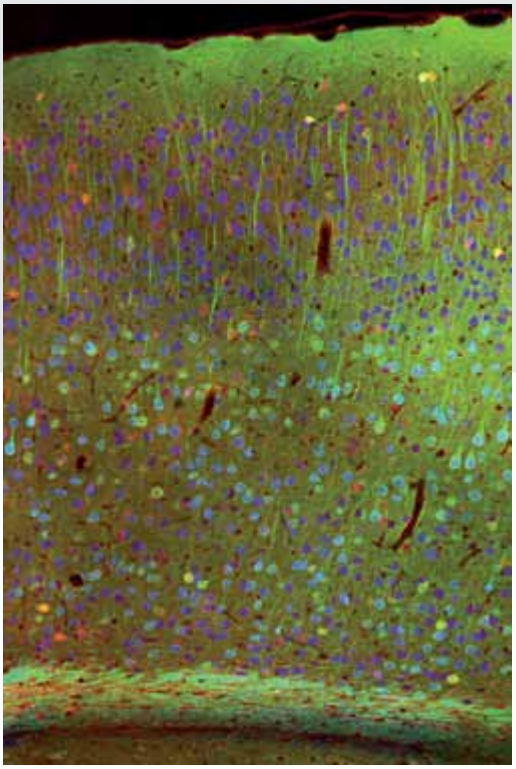

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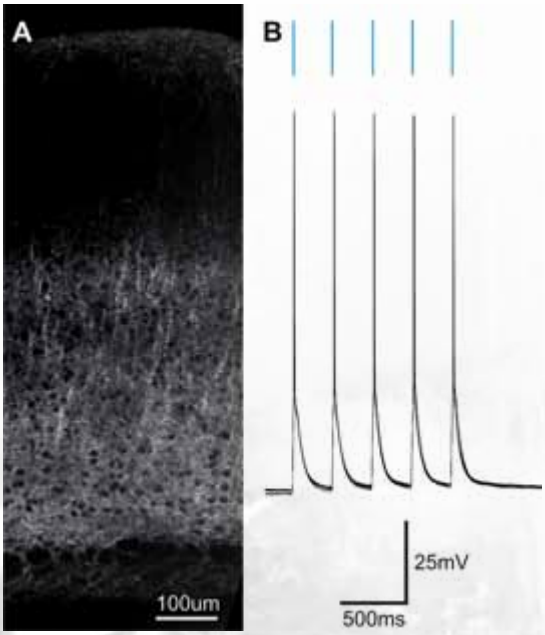

Figure 1: ***Imaging the auditory cortex.*** *(A) Mapping tonotopic organization of the auditory cortex using intrinsic imaging. (B) Using calcium imaging to monitor sound-evoked responses in multiple neurons simultaneously. Average population activity of 62 neurons in response to three different sounds. (C) Two-photon images of an individual neuronal process with synaptic contacts (so called spines, indicated by arrows) taken on different days. The connectivity of the neuron can be monitored by following the appearance, disappearance and size of synaptic spines.*

Figure 2: ***Genetic approaches to photolabel individual neurons.*** *Expression of photoactivatable GFP (PA-GFP) permits photolabeling of neurons after functional characterization using calcium imaging. The figure shows a coronal section of the auditory cortex taken from a transgenic mouse expressing PA-GFP immunostained for a neuronal marker (NeuN, blue), inhibitory neurons (GABA, red) and PA-GFP (green).*

Figure 3: ***Using light to control neurons.*** *(A) Cross-section of the auditory cortex after infection with a virus coding for the light-activated ion channel Channelrhodopsin. Massive expression of the construct is seen in layers V and VI. (B) Patch-clamp whole-cell recording of the membrane potential of a hippocampal neuron in an acute brain slice taken from an infected animal expressing Channelrhodopsin. A series of action potentials is elicited by a burst of brief light pulses (indicated by blue bars; overlay of five repetitions).*

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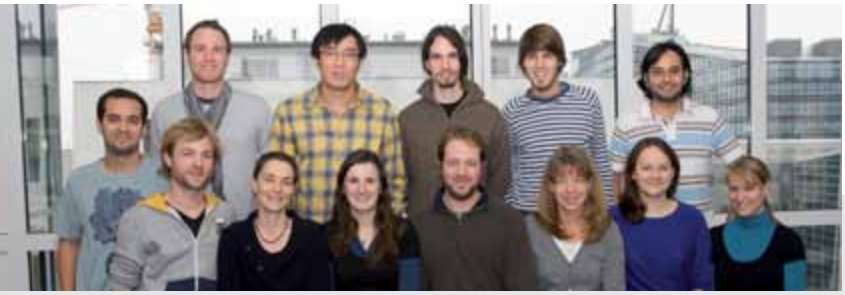

ALEXANDER STARK

Systems biology of regulatory motifs and networks – towards understanding gene expression from the DNA sequence

alexander.stark@imp.ac.at

Alexander Stark / Group Leader

Daniel Gerlach / Postdoc
Antonio Mereiles-Filho / Postdoc
Cosmas Arnold / PhD Student
Anaïs Bardet / PhD Student
Evgeny Kvon / PhD Student
Daria Shlyueva / PhD Student
J. Omar Yanez Cuna / PhD Student
Robert Maier / Trainee
Gerald Stampfel / Trainee
Michaela Pagani / Lab manager, Technical Assistant
Martina Rath / Lab manager, Technical Assistant
Katharina Schernhuber / Technical Assistant

The regulation of gene expression in response to developmental or environmental stimuli is a crucial process in all organisms. Transcription is regulated by trans-acting transcription factors that recognize cis-regulatory DNA elements (CRMs or enhancers) and function in a combinatorial fashion. Enhancers retain their activity even when placed in artificial contexts (e.g. in reporter gene assays), but the exact requirements for enhancer function, i.e. a regulatory code, remains unknown and enhancer activity cannot be predicted from the DNA sequence.

Employing an interdisciplinary approach, we use both bioinformatics- and molecular biology-based methods to achieve a systematic understanding of the structure and functions of enhancers. Our goal is to "crack" the *regulatory code*, predict enhancer activity from the DNA sequence, and understand how transcriptional networks define cellular and developmental programs.

In vivo and *in vitro* enhancer screens

Collections of enhancers that function in the same cell-types would be an invaluable resource to study the sequence basis of enhancer activity. We are screening for active enhancers and promoters in the *Drosophila melanogaster* embryo and in specific cell types, and will analyze the sequences using bioinformatics and machine-learning tools.

Gene expression analysis by automatic image processing

We are developing computational tools to automatically find and extract embryos from *in situ* images (Figure 1) in order to compare gene expression patterns obtained from BDGP (Tomancak et al., 2007). Clustering genes by their spatio-temporal co-expression and intersecting transcription factor expression patterns will enable us to suggest regulatory interactions and integrate these data with sequence analyses.

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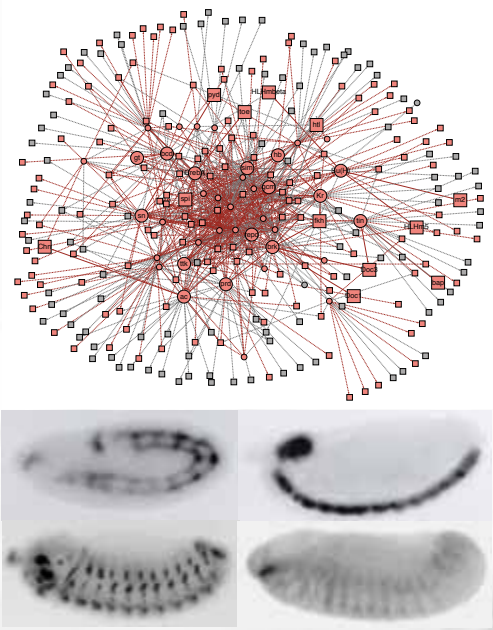

The regulatory code of context-specific transcription factor binding

Transcription factors are employed in different contexts, i.e. in various tissues or at different stages of development. Typically, they bind to and regulate context-specific targets that are determined by the respective enhancer sequences and transcription factor combinatorics. We use tissue-specific ChIP-Seq, bioinformatics, and machine-learning to determine the sequence determinants of context-specific transcription factor binding in *Drosophila*. We focus on transcription factor binding during embryonic mesoderm and muscle development (Figure 2; Zinzen et al., 2009), tissue-specific targets of the circadian clock factors, and homeobox (Hox) transcription factors.

Comparative genomics and the evolution of transcriptional regulation

Functional elements in a genome are typically under evolutionary selection to maintain their functions in related organisms. In collaboration with the Zeitlinger group (Stowers Institute), we study in vivo transcription factor binding sites in 6 *Drosophila* species at various evolutionary distances from *D. melanogaster* (Figure 3). We find that transcription factor binding is highly conserved in species as distant from *D. melanogaster* as platypus or chicken from human. Conserved binding correlates with sequence motifs for Twist and its partners, permitting the *de novo* discovery of their combinatorial binding.

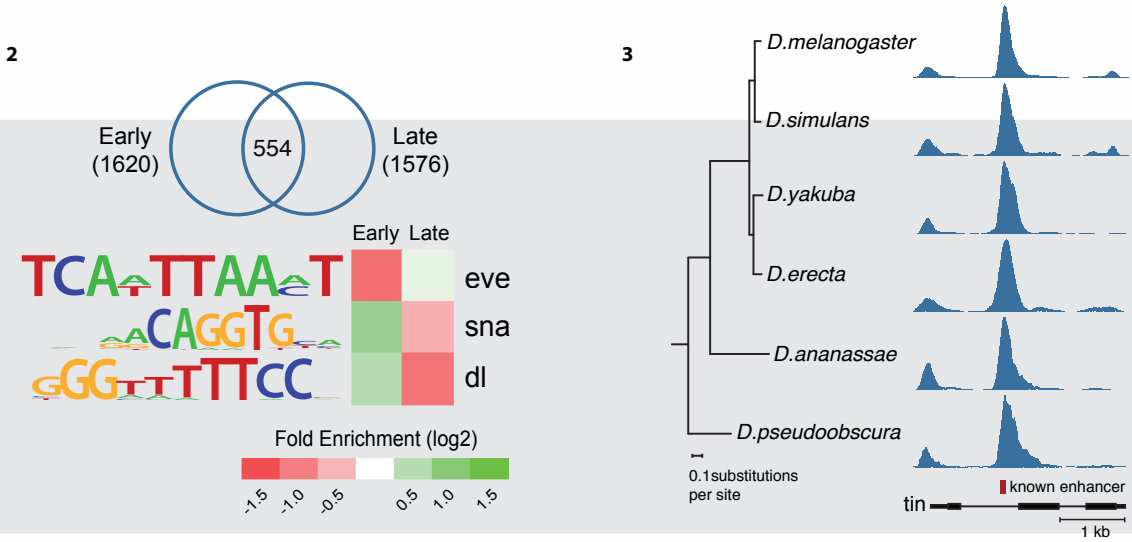

Figure 1: *Regulatory network and tissue-specific expression patterns in Drosophila embryos. See Stark et al., 2005 and Tomancak et al., 2007 for details.*

Figure 2: *Twist binding sites in 2-4 hours old D. melanogaster embryos (early) differ from those in 6-8 hours old embryos (late; top). Motifs of Twist partner transcription factors even-skipped (eve), snail (sna), and dorsal (dl) are differentially enriched near these early or late Twist binding sites, and might explain the temporal dynamics of Twist binding (bottom; ChIP-chip data from Zinzen et al., 2009).*

Figure 3: *Conserved Twist binding at a functional enhancer in the tinman locus. D. melanogaster and 5 other Drosophila species at increasing phylogenetic distances show highly significant ChIP-Seq tag enrichment, indicative of positionally conserved binding (He & Bardet et al., in revision).*

It also includes more than 10,000 low-occupancy sites near the detection limit, which tend to mark enhancers of later developmental stages.

We have developed computational methods to score motif conservation in 12 *Drosophila* genomes. These enabled us to discover novel motif types, as well as identify functional targets of many transcription factors and microRNAs with a high degree of certainty. Comparative genomics and related bioinformatics approaches will permit us to integrate our data and knowledge to predict developmental enhancers, regulatory targets for transcription factors, and the expression patterns of genes. They will also allow us to integrate microRNA-mediated regulation into regulatory networks and understand their role in tissue-specific expression programs.

Regulation of gene expression and genome stability by novel classes of small RNAs

Novel high-throughput sequencing technology reveals a myriad of novel small RNAs from different functional classes. These are involved in regulating gene expression by the microRNA and siRNA pathways as well as in controlling mobile genetic elements through related silencing pathways involving the PIWI clade of Argonaute proteins. We are collaborating with experimental labs in analyzing small RNA and characterizing their functions.

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PEGGY STOLT-BERGNER

Transfer of Signals and Substrates Across the Cell Membrane

peggy.stolt-bergner@imp.ac.at

Peggy Stolt-Bergner / Independent Postdoctoral Fellow

Barbara Nussbaumer / PhD Student
Bettina Spitzenberger / PhD Student
Hongwen Zhou / Research Assistant

All cells are surrounded by at least one impermeable lipid bilayer, and have evolved numerous types of proteins to allow them to interact with their environment by transducing signals and transporting substrates across this membrane. We are interested in understanding the molecular mechanisms these proteins use to accomplish their tasks.

Substrate Transport

Integral membrane proteins perform many vital cellular functions including energy metabolism, nutrient uptake, and signal transduction, yet comparatively little is known about their structure and the molecular mechanisms these proteins use to carry out their functions due to the challenges inherent in working with such hydrophobic proteins.

Currently, we are focusing on the three-dimensional structural characterization of secondary active transporters, which drive the transport of substrates across the membrane by coupling this process to the energetically favorable transport of a proton or ion. While recent work has begun to uncover some of the details underlying secondary transport mechanisms, there are still few structures available, especially when considering proton-coupled transporters. Therefore, in many cases important questions remain unresolved, such as how substrate and ion translocation are coupled, how substrate specificity is determined, and what conformational changes occur during transport. In addition, due to the small number of available structures, it is likely that some transport mechanisms remain uncharacterized, as the available structures do show diversity (Fig. 1). Additional structures at higher resolution and structures of different conformational states of the transport cycle will help to address these questions.

Structural studies on membrane proteins are challenging primarily due to the instability of these proteins when outside of the native membrane environment. Moreover, the conformational heterogeneity of membrane transporters can contribute to their instability. In parallel with attempts to crystallize secondary transporters, we are interested in developing methods to stabilize membrane proteins in order to facilitate their crystallization and biophysical characterization.

Signal Transduction

Signal transduction from the external environment to the interior of the cell is essential in controlling diverse processes such as cell growth and proliferation, metabolism, and cell migration. Many transmembrane receptors transduce signals by clustering upon binding of an extracellular ligand. However, the mechanisms by which receptor clustering leads to downstream signal transduction are in many cases unclear. Reelin signaling is a pathway that regulates the migration of newly formed neurons during brain development, and also plays a less well-characterized role in learning and memory formation in the adult brain. Binding of the extracellular ligand Reelin to the lipoprotein receptors ApoER2 and VLDLR triggers receptor clustering, and this leads to phosphorylation of the Disabled-1 (Dab1) adaptor protein by Src family kinases, which in turn leads to further downstream signaling. My previous work in this field focused on structural and biochemical characterization of the Dab1 adaptor protein and its interaction with the Reelin receptors and phosphoinositides in the cell membrane (Fig. 2). We are now using the Reelin signaling pathway as a model system to understand the molecular mechanisms by which receptor clustering triggers downstream signaling, using both biochemical and structural approaches.

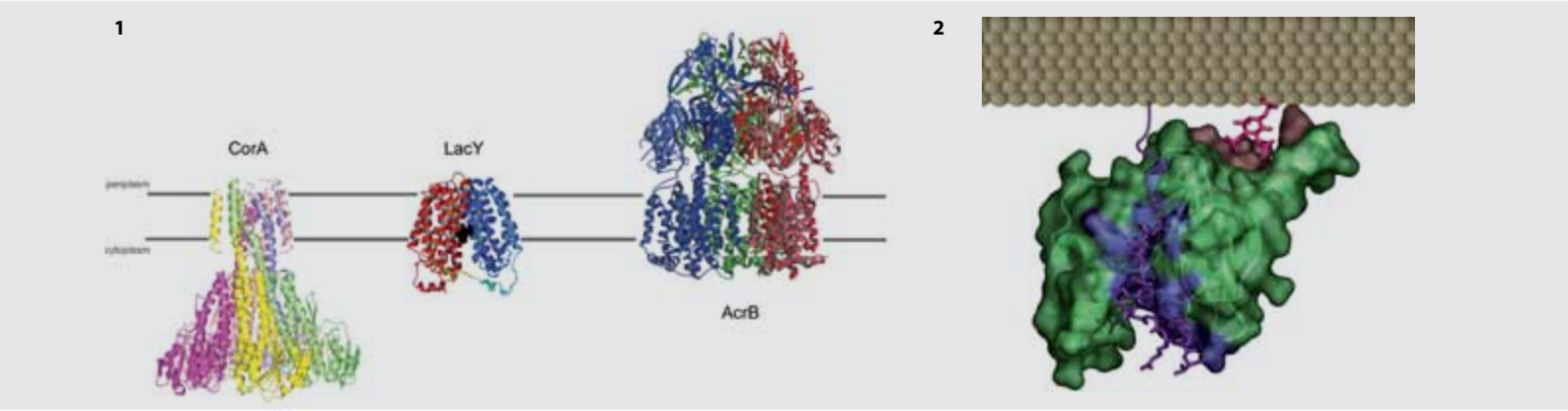

Figure 1: ***Transporter structures reveal diverse types of transport mechanisms***
Recent structural work has uncovered evidence for diverse transport mechanisms. The E. coli lactose permease LacY adopts the alternating access model, which most likely involves large conformational changes (figure adapted from Abramson et al, 2004 Curr Opin Struc Biol 14:413). In contrast, the pentameric structure of the CorA divalent metal transporter from T. maritima suggests that its mechanism of transport is similar to that of a gated channel (figure adapted from Eshaghi et al, 2006 Science 313:354). The trimeric E. coli multidrug efflux pump AcrB utilizes an alternating binding site mechanism, which involves more subtle conformational changes (Seeger et al, 2006 Science 313:1295; figure adapted from Murakami et al, 2002 Nature 419:587).

Figure 2: ***Crystal structure of the Disabled-1 PTB domain bound to peptide and phosphoinositide ligands***
The Dab1 phosphotyrosine binding (PTB) domain interacts with the NPxY motif of ApoER2 through a peptide binding groove on one face of the domain, and with the phosphatidylinositol-4,5-bisphosphate headgroup through a group of positively charged residues on the opposite side of the domain. Interaction with both ligands is necessary for transduction of the Reelin signal. The Dab1 molecular surface is shown in green, while the ligands are shown in ball-and-stick representation (Stolt et al, 2003 Structure 11:569).

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ANDREW STRAW

The neural basis of locomotor visual guidance in *Drosophila*

andrew.straw@imp.ac.at

Andrew Straw / Independent Postdoctoral Fellow

The brain of a fly is capable of steering the animal through a complex environment at high relative speeds, avoiding stationary obstacles and moving predators. Because it is relatively easy to study how flies do this at several levels from the behavioral to the cellular, fly vision has long been recognized as an ideal system to address a fundamental question in neuroscience -- how does the distributed activity of neurons orchestrate animal-environment interactions to result in successful coordinated behavior? We work on this basic question using techniques including automated realtime 3D fly tracking, virtual-reality displays, molecular genetic tools, and neuroanatomy.

A powerful toolkit to study neural basis of visual behavior

To enable our experiments, we developed a high-throughput, virtual-reality free flight arena. Flies are tracked in realtime by a multi-camera computer vision system. This unique technology permits rapid testing of physically un-manipulated and unrestrained flies to repeated presentations of arbitrary visual stimuli projected on the arena walls and floor. This apparatus is used to perform experiments that allow the fly to exhibit a large part of its natural behavioral repertoire while simultaneously measuring and limiting the effect of behavioral variability. Thousands of digitized 3D flight trajectories are collected in virtual environments, with the capability of experimentally triggered events such as the disappearance of objects. Such behavioral experiments are combined with targeted genetic manipulation of the nervous system and analyzed to reveal the magnitude and reliability of effects. The activity of individually identified neurons is perturbed by transgenic expression of exogenous ion channels or altered synaptic machinery. The effects of these genetic manipulations on flight control are quantified using statistical tests derived from the field of machine learning. These techniques to control stimulus conditions and measure behavioral responses in detail permit us to demonstrate the contribution of individual neurons to behavior.

Do flies build a neural representation of nearby objects?

A basic, unanswered question is whether flies construct an internal model of the layout of their world. Alternatively, a direct coupling may exist between visual and motor neurons, causing steering to result from feed-forward neural activity without sophisticated use of visual information stored from previous moments. Have flies evolved the ability to create a neural representation of nearby objects (the internal model hypothesis)? Or does a set of visual motor reflexes govern steering that, when executed during normal flight, result in trajectories that avoid undesired collisions (the direct coupling hypothesis)? A key prediction that distinguishes these possibilities is that a reconstruction of the instantaneous visual stimuli just prior to any steering command is sufficient to predict responses of the direct coupling case, but not of the internal model hypothesis. In the extreme, particular sequences of behaviorally-generated visual stimulation predict turns in opposite directions for the two hypotheses. To discriminate between these hypotheses, we measure steering behavior of freely flying flies in response to such specially designed stimuli using our unique virtual-reality flight arena. Additionally, machine learning techniques are used to distinguish between computer models of fly behavior, allowing us to exclude specific behavioral algorithms as being unlikely and to predict behavioral responses that can be tested in new experiments.

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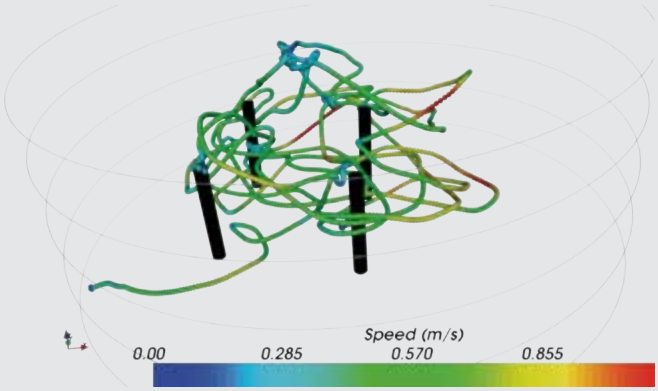

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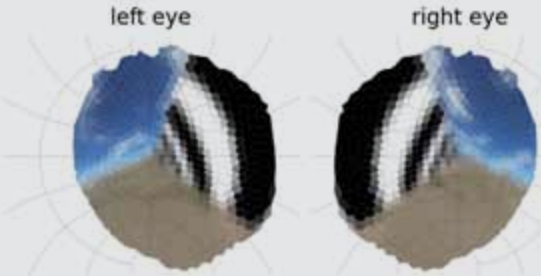

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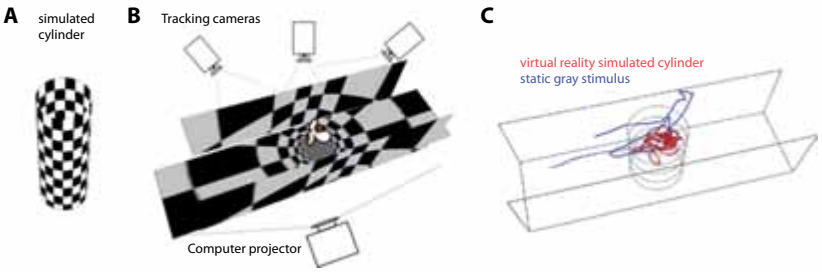

Figure 1: *A 3D fly trajectory captured as a fly explored its environment, which consisted of a cylinder measuring 2 meters in diameter and four vertical posts.*

Figure 2: *A simulated fly's eye view of a virtual world consisting of a black-and-white pattern in a desert landscape with a partly cloudy blue sky.*

Figure 3: *To perform virtual-reality simulations, a simulated environment - in this case a cylinder made of checkerboard wallpaper (A) - is projected onto the walls and floor of a flight arena (B) such that, from the perspective of a freely flying fly, it is within this virtual object. The simulation is compelling enough to confine the flies within this virtual cylinder (C).*

Behavioral characterization of neural function using genetically targeted lesions.

Neurons in the fly visual system, especially the lobula plate tangential cells, have been used for decades as a model to study cellular neurophysiology, including the basis of visual motion detection and spike timing precision. Nevertheless, the behavioral role of most of these cells remains unclear. To link our knowledge of cellular properties with natural behavior, we are performing genetic ablations on small numbers of neurons and measuring behavioral deficits in response to precisely defined visual stimuli. To attain greater cellular specificity than that achieved with the standard Drosophila GAL4-UAS system, we are evaluating recent intersectional techniques and stochastic mosaic techniques to target small numbers of visual neurons for inactivation. Flies with such manipulations are tested behaviorally in our virtual-reality free flight arena, and the affected neurons are identified using immunohistochemistry and confocal microscopy. The aim of this work is to establish a causative link between identified cells and their function in visual guidance. One of our explicit goals is to achieve sufficient quantitative anatomical and behavioral descriptions to serve as a basis for future investigations of network connectivity, cellular physiology, and molecular mechanisms.

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STEFAN WESTERMANN

Molecular Mechanisms of Kinetochore Function

stefan.westermann@imp.ac.at

Stefan Westermann / Group Leader

Tomasz Zimniak / Postdoc
Peter Hornung / PhD Student
Eva Kiermaier[1] / PhD Student
Fabienne Lampert / PhD Student
Christine Mieck / PhD Student
Michael Maier / Diploma Student
Veronika Fitz[2] / Diploma Student
Gabriele Litos / Lab Manager, Research Assistant
Susanne Opravil[3] / Research Assistant

[1]*until August 2010*
[2]*from November 2010*
[3]*joint appointment with Karl Mechtler*

Eukaryotic cells have evolved complex and extremely precise chromosome segregation mechanisms which ensure that genetic information is passed correctly from one generation to the next. In order to understand how the cell moves chromosomes during mitosis, our group is studying the structure, function and regulation of the kinetochore, a complex multi-protein assembly that connects chromosomes to dynamic microtubules. In a reductional biochemical approach, we aim to investigate and assemble functional kinetochores from their constituent parts in vitro. In addition, we use yeast genetics to manipulate and engineer simple chromosome segregation systems in vivo.

Building a kinetochore in the test tube

Understanding kinetochore function requires the study of its constituent parts, the assembly of these parts into higher-order structures, and ultimately the reconstitution of kinetochore function *in vitro*. The kinetochore is a complex macromolecular machine that hierarchically assembles from a set of conserved multi-protein complexes. We have reconstituted a number of these complexes by co-expressing multiple subunits in bacteria, and studied their biochemical properties. This analysis has already yielded some important insights: For instance, the Dam1 complex, which is a specialized microtubule-binding component of the budding yeast kinetochore (Figure 1), oligomerizes to form a ring around microtubules *in vitro*. This ring slides along the microtubule lattice and remains attached to the plus-end even during microtubule disassembly. These properties make the Dam1 ring a very efficient force coupler at the kinetochore. A challenge for the future is to understand how the Dam1 ring is connected to the rest of the kinetochore, visualize the structure of the fully assembled interface, and analyze how it is regulated, for example by mitotic kinases.

Building a kinetochore in the cell

To define functional modules within the complex kinetochore architecture, we adopted a reductional approach to design simple kinetochores *in vivo*. By artificially recruiting individual kinetochore components to engineered binding sites on circular plasmids and on native yeast chromosomes, we were able to demonstrate that the Dam1 complex is not only necessary but also sufficient to generate an interface that supports chromosome segregation (Figure 2). In the future, we will further characterize the protein composition and precise function of these "artificial" kinetochores.
A further challenge for the future is to understand how kinetochore structure and function are modulated through the course of the cell cycle. The basic signals that couple cell cycle progression with the regulation of kinetochore function have remained elusive thus far. Combining time-resolved analysis of post-translational modifications with yeast genetics will permit us to identify general principles of regulation.

Analyzing the interaction of kinetochores with dynamic microtubules

A defining feature of kinetochores is their ability to interact with microtubule plus-ends through multiple rounds of polymerization and depolymerization. How does the kinetochore achieve this remarkable task? What are the features that enable it to follow a polymerizing microtubule end, but also stay connected during disassembly? How does the kinetochore modulate microtubule dynamics? To analyze this process, we have reconstituted dynamic microtubules *in vitro* and visualized the interaction of individual kinetochore components

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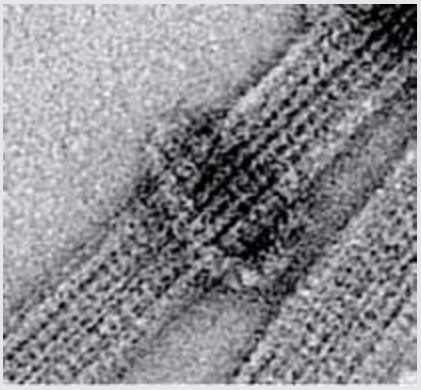

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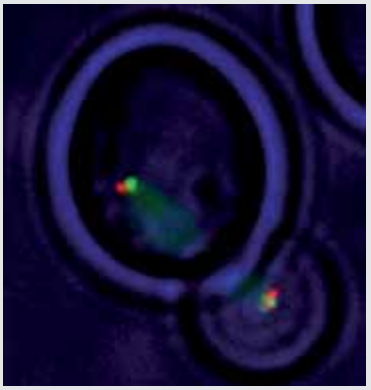

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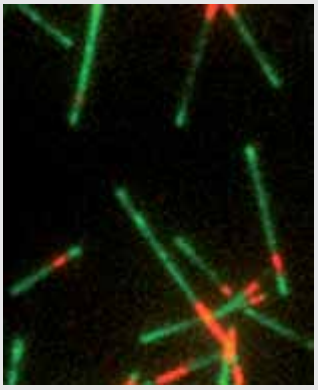

Figure 1: ***The 10-protein Dam1 complex oligomerizes into a ring around the microtubule in vitro.*** *Negative stain electron microscopy of a Dam1 ring around a taxol-stabilized microtubule.*

Figure 2: ***Segregation of mini-chromosomes (green) during anaphase in a yeast cell.*** *The spindle pole bodies are labeled with a red fluorescent protein (Spc42-mCherry). A bright field image was pseudo-colored in blue to reveal the outline of the yeast cell.*

Figure 3: ***Total internal reflection fluorescence (TIRF) microscopy of dynamic microtubules growing from stable microtubule seeds (red).*** *The growing segments are decorated with the yeast EB1 protein Bim1p (green), which accumulates at the growing ends of the microtubules.*

using total internal reflection fluorescence (TIRF) microscopy. This technique permits the observation of individual kinetochore complexes and microtubule-binding proteins with single-molecule sensitivity to reveal their mode of interaction with dynamic plus-ends. Our initial analysis was focused on the yeast EB1 protein Bim1p (Figure 3). We demonstrated that this protein uses a microtubule-binding interface composed of a calponin-homology domain and a flexible basic linker to autonomously track growing microtubule ends *in vitro*. Multi-site phosphorylation of the linker domain by the Aurora kinase Ipl1p regulates the interaction of Bim1p with microtubules and critically regulates the amount of Bim1p on the mitotic spindle *in vivo*. In the future we will reconstitute additional kinetochore plus-end tracking systems in order to define functional dependencies.

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MANUEL ZIMMER

The neural basis of behavior

zimmer@imp.ac.at

Manuel Zimmer / Group Leader

Ingrid Hums / PhD Student

One of the prime goals pursued by current neuroscientists is to gain a comprehensive understanding of how networks of neurons operate as a single brain to produce sensations, thoughts and behavior. This is a challenging endeavor because of the sheer complexity of mammalian nervous systems. To address this problem, at our lab we study the nematode C. elegans, which is equipped with a simple and anatomically well defined nervous system of just 302 neurons. Specifically, we combine powerful worm genetics, quantitative behavioral assays, and functional neuronal imaging techniques to elucidate the precise neural circuits that control oxygen chemosensory behavior.

Research activities:

An animal's decision as to how it should respond to changes in the environment is based not only on the available sensory information, but also on internal factors such as stress, sleep/wakefulness, hunger/satiety and experience. Small molecule neurotransmitters and neuropeptides in the brain modulate neural circuits according to these conditions, so that appropriate behaviors are generated. Aberrant neuromodulation is implicated in conditions such as insomnia, obesity, or anorexia. Given the complexity of most neural systems that have been studied thus far, we lack good models to investigate how neuromodulatory alterations systemically affect the activities of networks that generate behavioral outcomes. We use the simple model organism *C. elegans* as a tool to solve these questions.

Wild *C. elegans* live in soil, which is a very heterogeneous environment. As worms navigate, they are constantly challenged to evaluate their environment in order to determine the best survival tactic. The ability to locate food sources (bacteria) while avoiding pathogens, predators or other noxious conditions is far from simple. To optimize this search, nematodes have evolved a highly sophisticated repertoire of behavioral strategies. Oxygen chemotaxis is one such strategy. Local oxygen concentrations in soil range from atmospheric levels (21% O_2) to toxic hypoxia (<1% O_2), with soil bacteria creating a milieu of intermediate oxygen levels. When we present *C. elegans* with a range of oxygen concentrations in the laboratory, they show a homeostatic preference for these intermediate oxygen concentrations while avoiding both atmospheric and hypoxic conditions. Oxygen chemotaxis is regulated by various factors such as experience, nutritional status, and the genetic background of different strain isolates. It therefore serves as a tractable paradigm to study the modulation of the underlying circuits.

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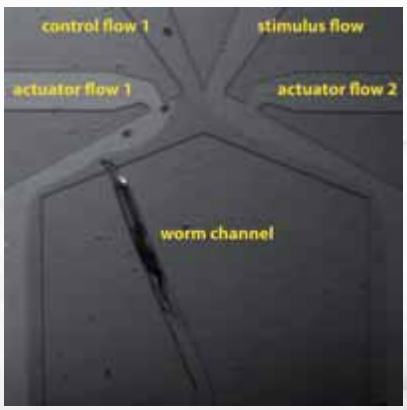

To study behavior, we are filming worm populations that experience downshifts and upshifts in environmental oxygen levels. Image processing and further computer analysis is used to quantify simple locomotion responses. To measure the neuronal activity of individual neurons, we use real-time fluorescence imaging of intracellular calcium levels. We employ microfabrication technologies to generate small microfluidic devices that allow the immobilization of worms onto microscope stages while the animals are being stimulated by chemical or gaseous stimuli (Figure 1).

Surprisingly, all behaviors observed in the assay can be explained by the action of just two sensory neuron classes in the entire worm brain: The BAG neurons cause deceleration of locomotion rate, which may persist for several minutes after oxygen concentrations drop to preferable levels. Conversely, when oxygen concentrations rise to undesirable levels, the URX neurons trigger very brief slowing responses that last for no more than a few seconds (Figure 2). Calcium imaging demonstrated that decreasing oxygen concentrations activate BAG neurons (Figure 3) while increasing oxygen concentrations activate URX neurons (Figure 3). A genetic analysis of behavioral responses and neuronal calcium signals

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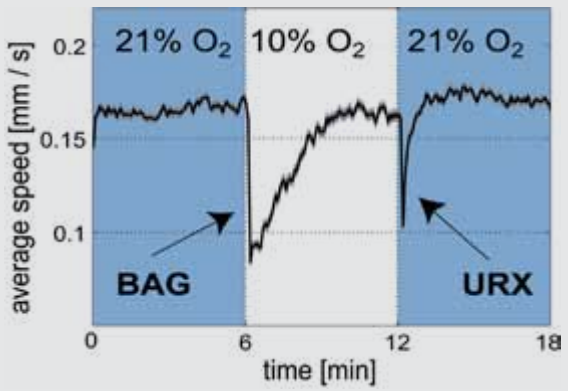

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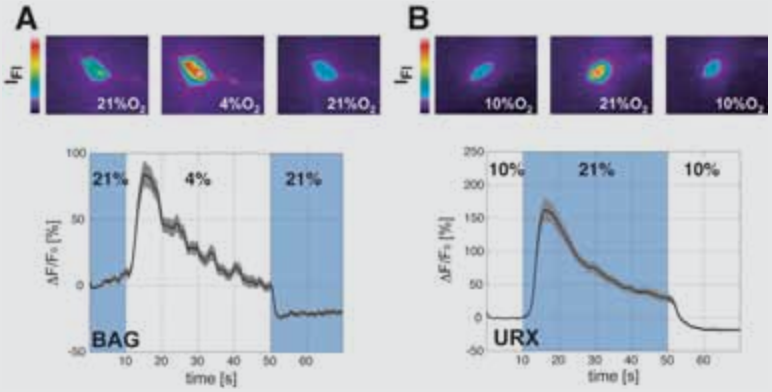

Figure 1: ***A microfluidic device for worm immobilization and stimulus delivery.*** *We designed a worm immobilization channel from an optically transparent silicon polymer. The channel is connected to a microfluidic delivery network. A worm is pushed into the immobilization channel so that its nose contacts the delivery network. Two actuator flows, which are labeled with a fluorescent dye, direct either a control flow or a stimulus flow (weak fluorescent label) to the worm's nose. The device allows for precise spatiotemporal control of chemosensory stimuli delivered to the worm's nose, while the animals can be imaged by microscopy.*

Figure 2: ***Analysis of oxygen chemotaxis behaviors.*** *Plotted is the average locomotion speed of animals exposed to changes in ambient oxygen concentrations, which shift between aversive atmospheric levels (21% O_2, blue shaded bars) and preferred intermediate levels (10% O_2). Following an oxygen downshift to 10% O_2, animals respond with sustained slowing. This behavior lasts for about 3 minutes. The response specifically requires BAG oxygen sensory neurons. Following an oxygen upshift to 21% O_2, the animals respond with transient slowing for a few seconds. This behavior specifically requires URX oxygen sensory neurons. (Zimmer et. al., 2009).*

Figure 3: ***Calcium responses in BAG and URX neurons. A.*** *The upper panels show fluorescence images of the calcium indicator G-CaMP expressed in BAG. Oxygen concentrations are indicated. The trace shows the average calcium response in BAG to O_2 downshift (21% O_2 to 4% O_2).* ***B****. The upper panels show fluorescence images of the calcium indicator G-CaMP expressed in URX. Oxygen concentrations are indicated. The trace shows the average calcium response in URX to O_2 upshift (10% O_2 to 21% O_2).*

showed that the reciprocal chemosensory properties of BAG and URX are the result of differentially expressed molecular oxygen sensors of the soluble guanylate cyclase family (Zimmer et. al. 2009).

These behavioral paradigms and imaging technologies are ideal tools to study the neuromodulation of circuits and behavior. Oxygen downshift and upshift elicit two distinct slowing responses that can be assigned to the activities of just one sensory neuron in each case. Thus, behavior can be studied in a robustly quantifiable manner at single cell resolution. Moreover, the imaging technologies we developed are tailor-made to mimic conditions under which behavioral experiments are performed. Thus, behavioral responses and neural activity can be directly correlated. BAG and URX share a small neural circuit of postsynaptic interneurons. The simplicity of this system, powerful worm genetics, and tractable behavioral and physiological assays, enable us to study the neuromodulation of circuits at all levels; i.e. at the level of networks, single cells, as well as single genes and molecules.

We are currently focusing on the following goals:

- To elucidate the precise functions of interneurons that connect to BAG and URX.
- To determine the mechanism by which neuropeptides mediate experience-dependent modulation of behavior.
- To investigate the mechanisms by which neural circuits integrate sensory information with other external and internal conditions, such as the availability of food and the nutritional status.

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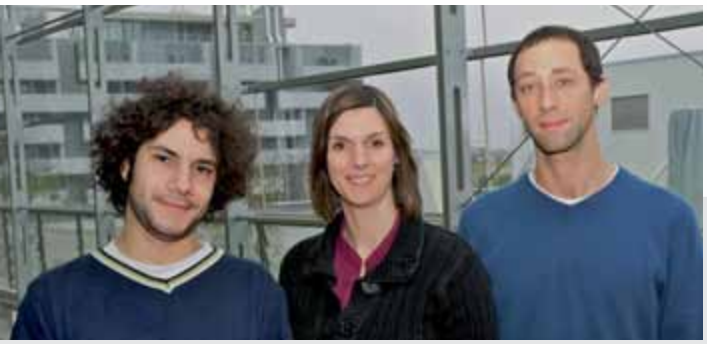


KATRIN HEINZE

Fluorescence tools operating on a single-molecule scale

katrin.heinze@imp.ac.at

Katrin Heinze / Staff Scientist

Kareem Elsayad / Postdoc
Johannes Reisecker / Diploma Student

Discoveries in bioscience are frequently stimulated by the invention of new scientific tools. We have focused on pushing fluorescence techniques beyond their usual limits of spatial and temporal resolution. Low-invasive approaches such as these offer a fascinating prospect of observing biomolecules in their native environment and understanding how they act in concert.

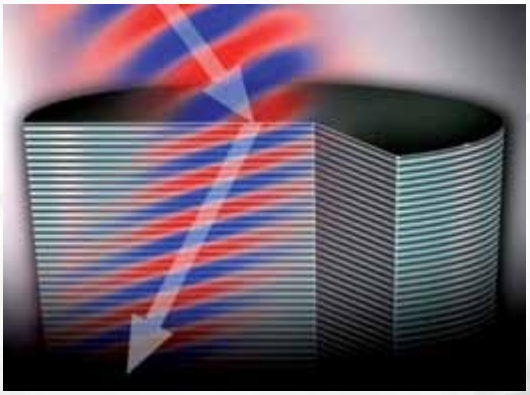
Our group focuses on the measurement and manipulation of inter- and intramolecular dynamics in a cellular setting. The tools we would like to highlight this year are based on nanostructured materials that permit sub-diffraction limit imaging near surfaces.

Nanostructured materials for sub-diffraction limit imaging near surfaces

A superlens is a 'planar lens' constructed by the use of metamaterials (i.e. sub-wavelength scale metal-dielectric structures, see Figure 1), whose counterintuitive interactions with light permit imaging beyond the diffraction limit. Certain nanometer-thin metal based structures are known to exhibit negative refractive properties at certain wavelengths and may therefore be used for super-resolution imaging near interfaces.
However, the use of previous designs in bio-imaging was limited by the required fabrication accuracies of the structures, as well as impractical and tedious readout techniques.
Recently we proposed a means of reading out super-resolution information from a set of simultaneous far-field measurements, employing a spatial resolution that is nearly an order of magnitude better than that achieved using previous far-field readout techniques. This new technique relies on the use of a superlens design that exploits the finite frequency bandwidth of a fluorophore's emission. We have shown, in theory [Elsayad & Heinze 2010] as well as in experiment [Elsayad et. al. 2010], that a metal dielectric stacked metamaterial structure can be designed to amplify different discrete spatial Fourier components of an incident field at different frequencies.
By fabricating a scattering structure on the exit face of such a superlens, far-field measurements can be made to contain a wide range of the evanescent field information of a source near the superlens surface. In the past, such scattering structures permitted the investigator to obtain far-field super-resolution in a single lateral dimension. We have now developed a technique that provides super-resolution in both lateral dimensions.

We refined a technique for fabricating superlens structures with unprecedented metal/dielectric interface smoothness, which is critical for obtaining optimal imaging results. Furthermore, we confirmed the biocompatibility of these structures in live cell experiments. Most recently, we developed a technique for directly fabricating the scattering structures with the required accuracy on the superlenses. The proposed setup now enables us to perform 'near-field' imaging in the far field with a lateral resolution and frame rate limited only by photon statistics.

1



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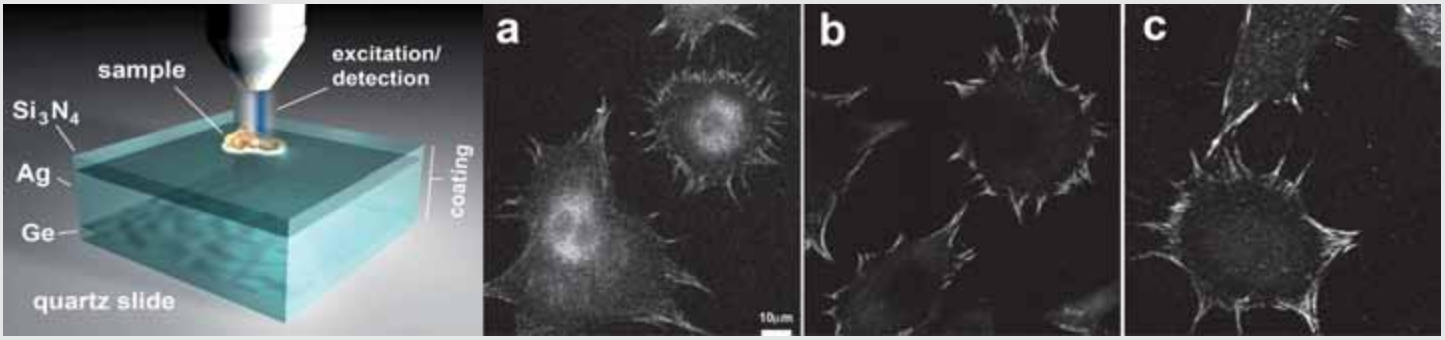

Figure 1: *Sketch of a layered metal/dielectric stack that can exhibit negative refractive properties and thus be used for super-resolution surface imaging.*

Figure 2: *Sketch of the PASI setup used for imaging near the surfaces of the coated substrates. Panels (a) and (b) show paxillin-stained 3T3 cells in aqueous solution, imaged using a conventional confocal microscopy setup and PASI. (c) is the image obtained using PASI for the same cells mounted in n=1.56 optical medium, showing that PASI – unlike TIRFM – permits high contrast surface imaging when cells are immersed in a medium with a higher refractive index.*

Plasmon Assisted Surface Imaging (PASI): Applications in fluorescent high- and super-resolution imaging

In our efforts to develop a superlens-based fluorescence microscope, we found that certain nanoscale metal-dielectric coatings on conventional microscopy slides or coverslips allow for flexible high-resolution surface imaging (see Figure 2). Based on the deduced underlying mechanism, we have named the discovered technique "PASI" (Plasmon Assisted Surface Imaging). The results obtained with PASI are comparable to those produced by Total Internal Reflection Fluorescence Microscopy (TIRFM), but with several additional advantages. These include the following:
(1) The technique can be used with an unmodified widefield or confocal microscope with the only addition of using coated (biocompatible and very affordable) coverslips.
(2) The axial penetration depth of the illumination into the sample can be rendered significantly smaller than is possible using TIRFM, i.e. 15-50 nm is easily achieved, as compared to 100-200nm with TIRFM.
(3) The technique is compatible with samples mounted using most standard mounting media and optical adhesives. In contrast, TIRFM requires that samples be immersed in a medium with a refractive index comparable to that of an aqueous solution.
(4) Due to the unique interactions of the metallic structure with the nearby fluorophores, it is possible to construct a "z-stack" with an axial resolution of about 5 nm from a single spectral measurement. This is due to the peak in the emission wavelength of the fluorophores becoming blueshifted when they are closer to the interface.

To illustrate the effect, we present PASI versus confocal and widefield images of Alexa488-labeled paxillin in NIH 3T3 mouse fibroblasts which were grown directly on optimized coated slides (see Figure 2a-2c).
While this technique is of much interest in itself, PASI could also be employed to improve the axial resolution and reduce the background when using other super-resolution fluorescence imaging techniques. We may be able to report on these the next year.

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KRYSTYNA KELEMAN

Vienna Drosophila RNAi Center (VDRC)

keleman@imp.ac.at; office@vdrc.at

Krystyna Keleman / Staff scientist/Head of the VDRC

RNAi library maintenance and development

Reinhard Klug / Stock Maintenance Supervisor
Thomas Micheler / Software Developer
Selen Irez Alacaptan [2] / Administration

Kristina Belogradova / Technical Assistant
Luiza Deszcz [2] / Technical Assistant
Michaela Eckmann / Technical Assistant
Michaela Fellner / Technical Assistant
Andreas Gansch / Technical Assistant
Angela Graf [2] / Technical Assistant
Laszlo Hunor / Technical Assistant
Amina Kadum / Technical Assistant
Rainer Keck [2] / Technical Assistant
Irina Kolarov / Technical Assistant
Sonja Lang / Technical Assistant
Florence Maxwell / Technical Assistant
Barbara Müllner / Technical Assistant
Irene Penzenauer / Technical Assistant
Elena Popowich / Technical Assistant
Alisher Tashpulatov / Technical Assistant
Sandor Urmosi – Incze / Technical Assistant
Judith Utner [2] / Technical Assistant
Stefanie Wandl / Technical Assistant
Svetlana Zorinyants / Technical Assistant
René Zörnig[1] / Technical Assistant

Keleman research group members

Sebastian Krüttner / PhD Student
Daniela Lenek / PhD Student
Barbara Stepien / PhD Student
Pei Sun / PhD Student
Katharina Jandrasits / Technical Assistant
Zsuzsanna Portik Dobos / Technical Assistant

[1] 80%, [2] 50%

Genome-wide RNAi and memory formation

We have generated two independent genome-wide transgenic RNAi libraries for Drosophila, allowing researchers to systematically study gene functions in specific tissues at specific developmental stages. The VDRC maintains and further develops these libraries, and distributes RNAi lines to Drosophila researchers world-wide. In our own research group, we use transgenic RNAi and other methods to understand how the fly forms memories that shape its mating behaviour.

Genome-wide RNAi

RNAi can be effectively triggered in Drosophila by spatially and temporally controlled expression of a dsRNA from a transgene that contains a long inverted repeat under control of a genetic promoter the GAL4-responsive element (Figure1). The initial creation of a genome-wide transgenic RNAi library [1] has revolutionized Drosophila genetics. The VDRC, maintains, further develops and distributes this library.

Recently, we have embarked on the construction of a new RNAi library (the KK library) that exploits site-specific transgene integration and to overcome some of the problems associated with random insertion of the P element transgenes of the original collection (GD library). In the new KK library, all RNAi transgenes are targeted to the VIE-260b site, selected on the basis of its low basal expression and high levels of GAL4-induced expression. An additional attractive feature of our new collection is that it targets a different gene fragments than our first library. We have completed 10,000 RNAi lines, which were made available to researchers world-wide in March last year.

Since the VDRC opened two and a half years ago, we have already delivered over 340,000 lines to more than 1,500 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. The VDRC also provides such support for researchers in house, having delivered over 200,000 lines to IMBA and IMP groups.

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 largely on his ability to discriminate receptive virgins 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 choosey, he will miss out on excellent mating opportunities. The right balance appears not to be hard-wired into the fly's brain, but is something he learns by trial-and-error during his first few courtship experiences (Figure 2). His 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 demonstrated that the CPEB protein Orb2 – a regulator of mRNA translation – plays a critical role in this process [2]. We found that Orb2 function is required in a specific set of mushroom body neurons during or shortly after training (Figure 3). Without Orb2, or more specifically without its intriguing glutamine-rich domain, a memory initially forms but decays within just a few hours.

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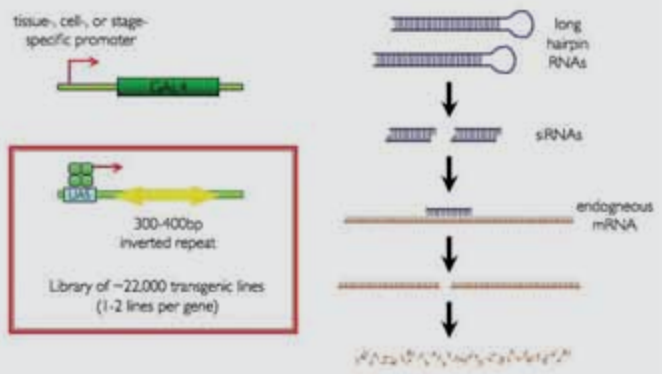

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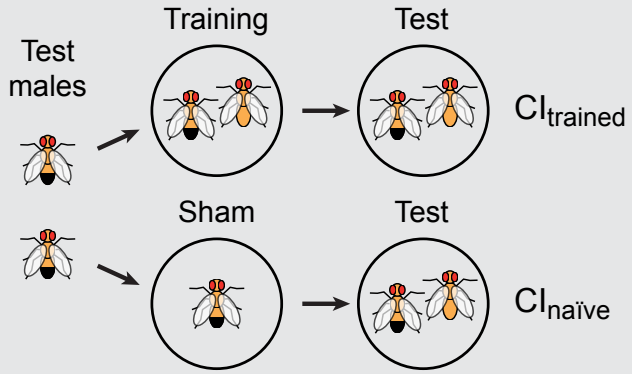


Figure 1: ***RNAi in Drosophila.***

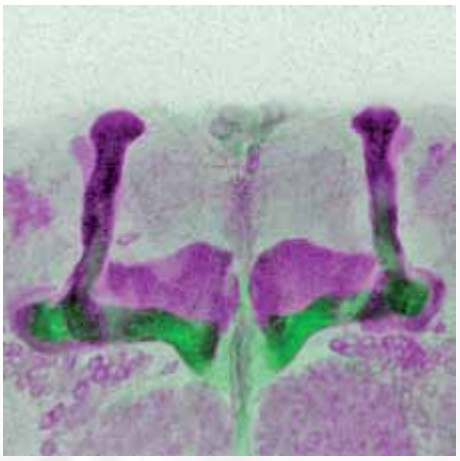
Figure 2: ***Courtship conditioning****. When tested with unreceptive mated females, males previously exposed to mated females court less than naïve (sham-trained) males (CI = courtship index; CItrained < CInaïve)*

Figure 3: ***Mushroom body neurons in the fly's brain*** *– Orb2 is required in these neurons for courtship conditioning.*

To learn more how Orb2 functions in Drosophila long-term memory, we are dissecting its structural and functional requirements using both genetic and biochemical approaches. We are also planning to identify Orb2-interacting proteins and target mRNAs, and to assess their roles in long-term memory. Meanwhile, we are also using the transgenic RNAi library in unbiased approaches to find other factors involved in long-term memory function and dysfunction.

We are also trying to find out exactly what the Drosophila male learns during courtship conditioning. What are the specific cues – probably pheromones – that the male learns to discriminate? And, as the ultimate goal, we would like to define the specific molecular and cellular changes in the brain that underlie this form of learning.

3



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BIOOPTICS

aumayr@imp.ac.at

Karin Aumayr / Head of BioOptics

Pawel Pasierbek / Microscopy
Gabriele Stengl / Microscopy
Thomas Lendl / Flow Cytometry/Image Analysis
Gerald Schmauss / Flow Cytometry/Image Analysis

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

Flow Cytometry

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

Microscopy

The BioOptics Facility currently manages more than twenty microcopy systems, including wide-field microscopy, confocal laser scanning microscopy (CLSM), two-photon (2P) microscopy, total internal reflection (TIRF) microscopy techniques, and an automated slide scanner for samples with or without fluorescence. Most of the systems are motorized - thus providing automation for higher throughput - and are suitable for fixed samples as well as live cell experiments. The facility provides assisted use and training on instrumentation and consultation concerning all microscopy-related subjects, including project planning, staining, microscope selection, etc.

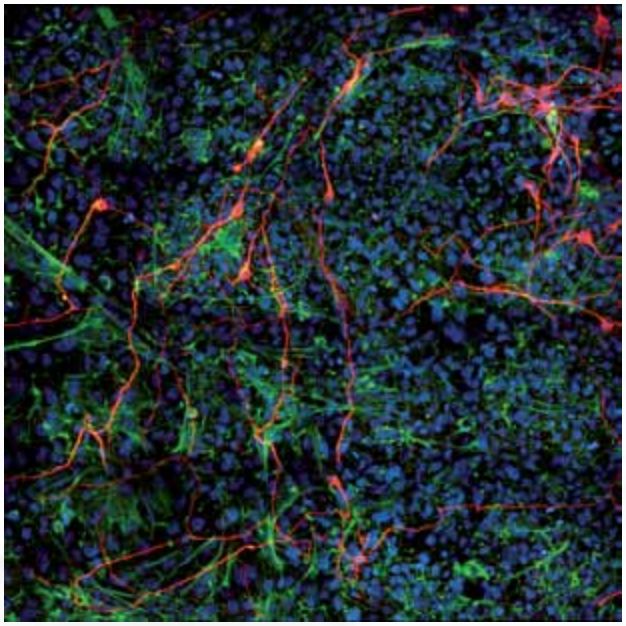


Image Processing and Analysis

Five state-of-the-art computer workstations are available at the BioOptics Facility, operating most of the common commercial image processing and visualization software. A server solution with a Web-based interface has been set up to enable deconvolution of microscopy images. The server permits efficient, multi-user, parallel, batch deconvolution that can easily be started from the individual scientist's computer. Users are trained in the use of specific software, depending on their demands. Several image analysis algorithms are available, such as object tracking and tracing, determination of measurement parameters like intensity, distance, area, volume and co-localization. Customized classification and measuring algorithms are developed at the facility for advanced image analysis and automated object recognition.

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

Figure: *In vitro differentiation of a murine embryonic stem cell line established by multiple rounds of FACS sorting. Differentiation was induced by embryoid body formation and retinoic acid treatment. Stitched image of 5x5 fields of view (FOV) acquired with the spinning disk (SD) confocal microscope. Blue: DAPI, green: phalloidine, red: TuJ1(neuron specific beta tubulin)*

ELECTRON MICROSCOPY

guenter.resch@imba.oeaw.ac.at

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

Preparation of Specimens

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

New instrumentation for specimen preparation is also being developed. The Leica EM GP immersion freezer, developed jointly by the EM Facility and Leica Microsystems, is commercially available from 2010 onward.

Microscopy

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

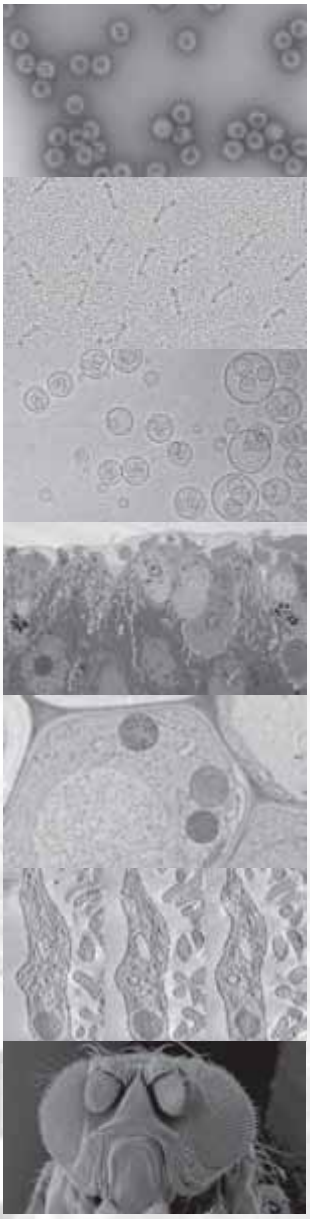
Software Development and Image Processing

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

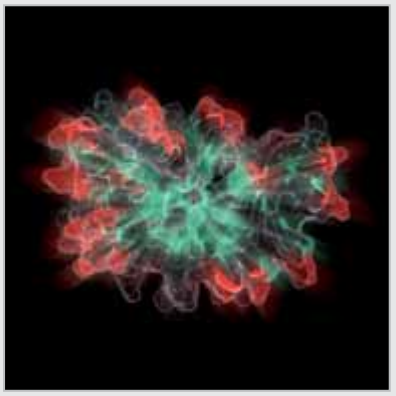
More and up-to-date information about the work of the EM Facility can be found at *http://cores.imp.ac.at/em*

Guenter Resch / Head of Electron Microscopy Facility

Marlene Brandstetter / Technical Assistant
Nicole Fellner / Technical Assistant
Veronika Wonesch / Trainee



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



BIOINFORMATICS

bioinfo.grp@imp.ac.at

Thomas Burkard / Computational Biologist
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 analysis services, scientific data mining, hardware and software infrastructure, and training in bioinformatics.

Sequence analysis

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

Large-scale data analysis

Additional demands arise from the investigation of large functional genomics or high-throughput biological datasets. We engage in custom software and database development, and design computational and mathematical solutions that can cope with the higher load and memory requirements. To perform complex sequence analysis tasks, we also maintain the IMP ANNOTATOR, which is a user-friendly web application and a high-throughput protein annotation system.
For heterogeneous computational tasks, the main computing cluster has been updated to a state-of-the-art processing system using batch and parallel computing environments. The cluster is managed by the Sun Grid Engine (SGE) software, which provides policy-based workload management for a large number of jobs and nodes.
Software installed and maintained on the bioinformatics cluster includes tools for statistical computing (e.g. R, Bioconductor), motif discovery and analysis (e.g. AlignAce, MDscan, MEME, Weeder), structural biology (e.g. VMD, pyMOL, NAMD), a wide range of sequence analysis, assembly, mapping and classification tasks (e.g. RNAhybrid, phylip, HMMer), and others.

Training

We provide hands-on training courses on the ANNOTATOR. Attendees of the course learn the basic principles and limitations of sequence analysis and data integration.

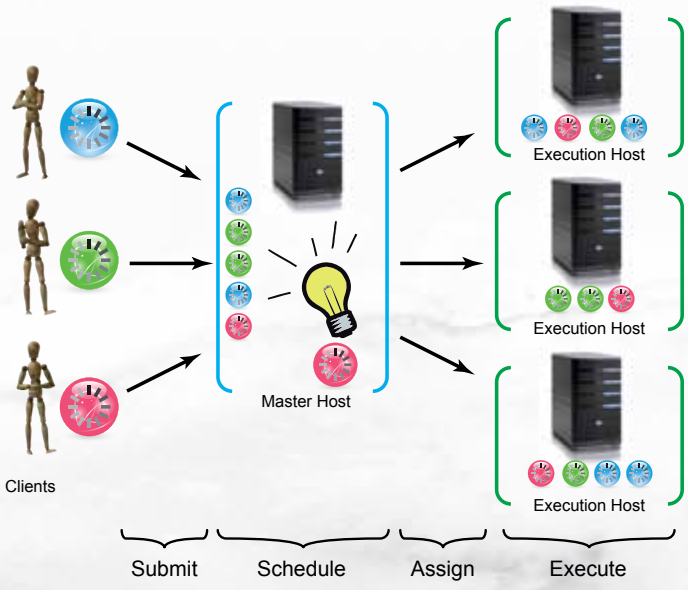
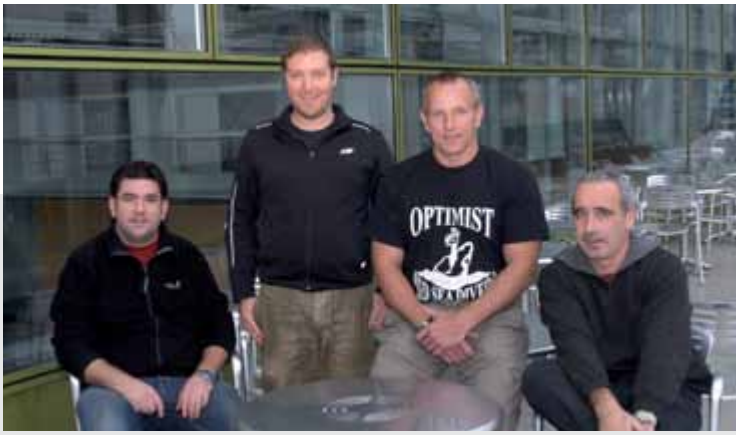


Figure:
The IMP/IMBA high-performance computing (HPC) cluster. Users may submit jobs on dedicated clients to the Sun Gridengine (SGE) master, which is responsible for running the jobs on the cluster nodes.



GENOMICS

genomics@imp.ac.at

A new high-throughput technology, the BeadXpress Reader (Illumina), was established in 2010. Using VeraCode® holographic microbead technology, we are able to perform SNP genotyping, gene expression, and protein-based assays.

We routinely hybridized more than 200 microarrays on self-spotted cDNA arrays containing RIKEN 3 clones. Over 22,500 genes per array were analyzed and processed using an internally programmed, fully automated analysis software tool. We offer Spotfire from the summer of 2009 onward.
As an additional platform we offer hybridization on Agilent arrays. Currently we use the 4x44K and 8x44K designs for different model organisms. With Agilent, we can also offer microarrays to analyze DNA methylation, microRNAs, and custom-made microarrays

A further noteworthy aspect of our work at the genomics department is the use of robotics in the lab. We are currently working with a Tecan TeMO provided with 96 channels, and two Biotek Precison XS for 8-channel and single pipetting. In early 2010 we purchased a XIRIL 100 which helps in automated genomic DNA isolation of mouse tails and single fly. The second main task of this robot is automated plasmid mini-preparation. Given the increasing demand and complexity of liquid handling processes, we decided to establish another robot platform: an Agilent Bravo robot was purchased in October 2010. Its main task will be automated in situ hybridization for fly embryos.

The demand for next-generation sequencing continued to grow in 2010: more than 600 samples were processed this year. We acquired a third paired-end module, which increased flexibility and throughput by permitting paired-end runs on all three existing genome analyzers. The read length for standard paired-end runs was increased to 76 base pairs and the maximal offered read length to 100 base pairs. As in the last year, constant upgrades led to higher sequencing yields: more than 25 million raw reads per lane were consistently achieved. In order to deal with next year's workload, a Hi-Seq 2000 (Illumina's most advanced sequencing instrument) was purchased and is currently being installed.

Martin Radolf / Engineer
Harald Scheuch / Engineer
Andreas Sommer / Engineer
Markus Sonntagbauer / Technical Assistant (part time)

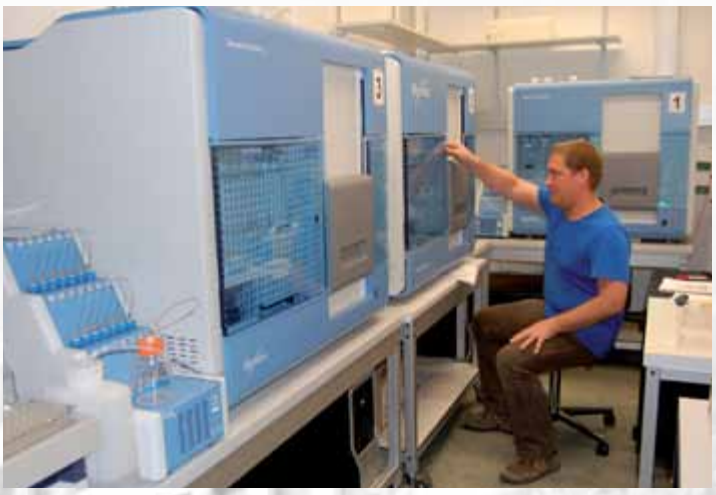


Figure: *Illumina Genome Analyzer II*

PROTEIN CHEMISTRY

karl.mechtler@imp.ac.at

Karl Mechtler / Head of Facility

Johann Holzmann / Postdoc
Thomas Köcher / Postdoc
Rositsa Jordanova / Postdoc
Christoph Jüschke[1] / Postdoc
Debora Broch Trentini / PhD Student
Thomas Taus / Bachelor Student
Johannes Fuchs / Technical Assistant
Otto Hudecz / Technical Assistant
Gabriela Krssakova / Technical Assistant
Mathias Madalinski / Technical Assistant
Michael Mazanek[2] / Technical Assistant
Susanne Opravil[3] / Technical Assistant
Elisabeth Roitinger / Technical Assistant
Michael Schutzbier / Technical Assistant
Ines Steinmacher / Technical Assistant

[1] *joint appointment with Jürgen Knoblich, IMBA*
[2] *maternity leave*
[3] *joint appointment with Stefan Westermann, IMP*

Studying the Abundance and Functions of Protein Arginine Phosphorylation

The discovery of arginine phosphorylation as a post-translational modification of proteins in bacteria, which performs an important function in stress adaptation of cells, demonstrates that this acid labile post-translational modification is now accessible to investigation by proteomic methods. To detect this modification and also elucidate its function in bacteria and mammalian cells types, we established specific methods which improve the stability of the modification through sample preparation, including specific enrichment techniques and reliable methods for detection by LC-MS/MS, and permit improved data analysis by the use of a probability-based scoring algorithm.

Methodological Advances in LC-MS/MS

LC-MS/MS (liquid chromatography-tandem mass spectrometry) experiments in expression-based proteomics aim for extremely high numbers of identified proteins which, however, is actually achieved only by two-dimensional LC-MS/MS. Given the fact that 2D-LC-MS/MS is very time-consuming, we investigate and design methods to increase the numbers of peptides and proteins identified in a single LC-MS/MS experiment. We succeeded in raising this number to about 2,500 proteins, based on approximately 14,000 peptides, which is sufficient to analyze samples of moderate complexity.

Measuring Protein Complexes with Mass Spectrometry

Proteins assemble into macromolecular protein complexes that regulate fundamental cellular processes such as cell cycle progression and mitosis. Using our previously described EtEP strategy to generate an equimolar set of internal reference peptides, we are determining protein complex stoichiometries and protein copy numbers per cell using selected reaction monitoring (SRM)- based absolute quantification. Applying this workflow to affinity-purified cohesin complexes, we determined the stoichiometries of the four core cohesin subunits throughout the cell cycle. Furthermore, using isoform-specific reference peptides, we determined the relative abundance of the two populations of cohesin complexes in human somatic cells (Figure).

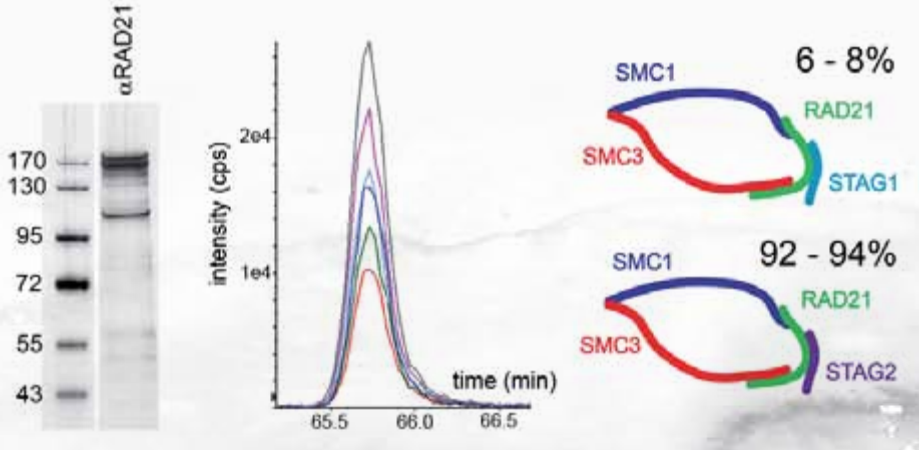


Figure: Absolute quantification of cohesin populations by SRM. (Holzmann et al., JPR, 2010)

SERVICE DEPARTMENT

gotthold.schaffner@imp.ac.at

The Service Department offers IMP, IMBA and GMI scientists a wide range of rapid and high-quality services. Our efforts primarily consist of DNA sequencing, fly food production, and the 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 1'700'000 tubes and bottles per year) and other organisms. The Fly Food facilities are located in the IMP building, which is provided with sufficient daylight and space to create better and more convenient working conditions for preparing fly food and storing the goods we need.
We also prepare several selected reagents such as DNA molecular weight markers, enzymes, and a variety of transformation-competent E. coli strains. We maintain a stock of cloning vectors, sequencing primers and other reagents.

Production of antibodies

A part of our working hours are devoted to the production and isolation of many different monoclonal antibodies in hybridomas in collaboration with IMP group members, and the organization of antibody production in rabbits in collaboration with an external company.

Sequencing and DNA isolation

The 48-capillary ABI 3730 DNA Genetic Analyzer is the only workhorse, but clearly at its limit. An additional sequencer will be needed as soon as possible to keep the return time within reasonable limits. We sequenced approximately 100'000 samples during the first ten months of this year. This implies a substantially higher demand due to screening projects, the new fly library, as well as new groups at IMBA, IMP, GMI and, last but not least, SUMMER STUDENTS.

The quality and concentration of DNA samples, even when prepared by sophisticated Qiagen kits such as Midi-, Maxi- or Minipreps, is still a problem. The same is true for incorrect primer sets or insufficiently documented plasmid constructs from external sources. Sequencing is performed faster and more easily than analyzing the samples by restriction digests or running them on an agarose gel!
The clean-up 96-well microtiter plates are no longer filled manually but with a BioTek benchtop minirobot with an optimized Sephadex G50 superfine slurry. The centrifugation conditions have also been optimized.

Occasionally, we still see "dye blobs" in cases of poor quality or with low yield DNA from standard procedures. The greater proportion of contaminants as compared to PCR products obviously plays a major role.

Gotthold Schaffner / Head of Facility

Ivan Botto / Technical Assistant
Markus Hohl / Technical Assistant
Shahryar Taghybeeglu / Technical Assistant
Christa Detz-Jaderny / Media Kitchen
Dagmar Faustenhammer / Media Kitchen
Ulrike Windholz / Media Kitchen
Christine Giesel / Fly Food Preparation
Franziska Stransky / Fly Food Preparation
Oliver Botto / Help Fly Food Preparation
Thomas Haydn / Help Fly Food Preparation
Anna Windholz / Help Fly Food Preparation

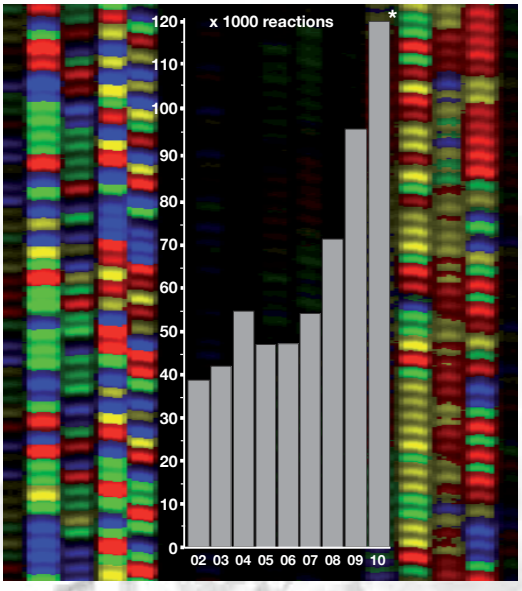


Figure: *A sequencing run on an ABI 377 PRISM and numbers of reactions analyzed on ABI 3100 (since 2001) and ABI 3730 (from June 2004 onward) using dye deoxy terminators (v3.0 from 2001 onward) from 2002 to 2010 (scale 0 to 120,000).*
**calculated from data obtained between January 2010 and October 2010*

HISTOLOGY

vukoslav.komnenovic@imba.oeaw.ac.at

Vukoslav Komnenovic / Head of Facility

Mihaela Zeba / Technical Assistant

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

Histology Services

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

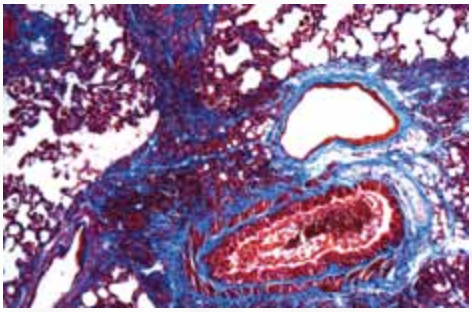
Sectioning of Paraffin and Frozen Tissues

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

Immunohistochemistry

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

1



2

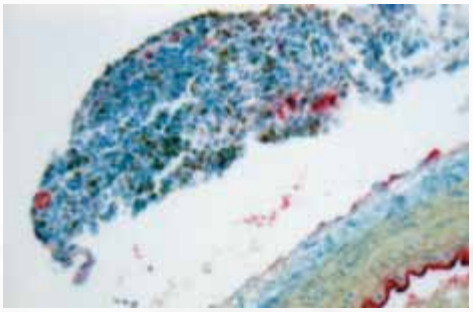
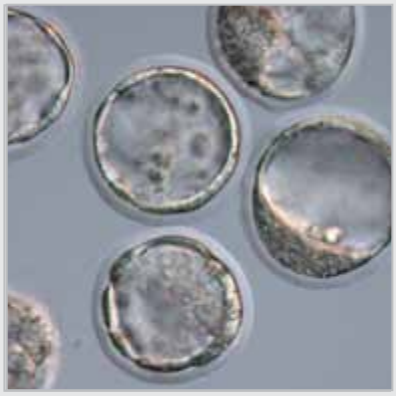


Figure 1: *Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.*

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

1

2



COMPARATIVE MEDICINE

animal@imp.ac.at

TRANSGENIC SERVICE

transgenic@imp.ac.at

Comparative Medicine

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

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

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

Transgenic Service

The Transgenic Service Department was set up at the beginning of 1998 to cope with the increasing demand for mouse studies and the generation of transgenic mice. Both IMP and IMBA avail themselves of the services of the Transgenic Service Department.

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 to our animal house, as well as the freezing of embryos for preservation of specified mouse strains and for teaching basic embryological techniques to the staff of IMP and IMBA. Many different ES cell clones and DNA/BAC constructs are being injected every year. The activities of this department are overseen by an Animal User Committee, which meets on a regular basis to set priorities and coordinate tasks. The committee is currently chaired by Meinrad Busslinger.

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

Figure 2: *Mouse blastocysts.*

MAX PERUTZ LIBRARY

library@imp.ac.at

Karlo Pavlovic / Head Librarian

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

The main task of the library is to provide comprehensive scientific literature pertaining to the areas of research pursued at the institutes. The Max Perutz Library holds more than 3500 titles in print. These are partly available in the library's premises itself, being labeled and shelved according to a specific classification system. A part of the titles are shelved in the group laboratories and offices. All book holdings can be searched for systematically in the online catalog, where each item is described in respect of its location and lending status.
Special book collections are available online on the platform of the relevant publishers.
Several bibliographic and full-text databases can be searched for relevant literature on a given topic. This also applies to literature on methods and protocols, including Springer Protocols, Current Protocols, Cold Spring Harbor Protocols and the recently licensed Journal of Visualized Experiments. A document delivery option is offered for the literature not licensed by the library.

Study environment

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

Teaching

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

Users

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

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GENOMICS

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Seminar Speakers

JANUARY

07.01.10 Stanley A. Plotkin
Sanofi Pasteur
Correlates of Immunity After Vaccination

12.01.10 Scott W. Lowe
Cold Spring Harbor Laboratory
Dissecting tumor suppressor gene networks in vivo

13.01.10 Ed Lein
Allen Institute for Brain Science
Neurodevelopmental gene expression atlasing from mice to humans

18.01.10 Botond Roska
Friedrich Miescher Institute, Basel
Insights into the busy life of neural circuits

18.01.10 Hongjuan Dong
Institute of Chemical Technologies and Analytics, Vienna
Intact cell mass spectrometry of mycotoxin-producing fungi Fusarium for differentiation and identification

20.01.10 Claire Wyart
University of California, Berkeley
Optogenetic manipulation of sensory inputs modulating locomotion

21.01.10 Ron Hay
University of Dundee
Role of SUMO modification in ubiquitin mediated proteolysis

22.01.10 Frank Uhlmann
Cancer Research UK, London Research Institute
The irreversibility of cell cycle transitions: Why the clock never turns back

28.01.10 Paolo Norio
Albert Einstein College of Medicine
Roles of Pax5 and the intronic enhancer in the developmental regulation of DNA replication initiation within the mouse immunoglobulin heavy chain locus

FEBRUARY

02.02.10 Nick Cowan
New York University
Tubulin Folding Defects in Neurological Diseases

04.02.10 Phillip Olk
TU Dresden
Metal Nanoparticles as Nano-Antennas — Applications in Optical and Scanning Probe Microscopy

09.02.10 Doug Hilton
Walter and Eliza Hall Institute of Medical Research
Pathways regulating blood cell production

10.02.10 Tom Rapoport
Harvard Medical School
Mechanisms of protein transport across membranes

11.02.10 Reinhard Luehrmann
Max Planck Institute for Biophysical Chemistry
Assembly, structural dynamics and function of the spliceosome

15.02.10 Todd Stukenberg
University of Virginia, School of Medicine
Mechanisms of the mitotic motors that move chromosomes–building strong motors from weak forces

16.02.10 Sui Huang
University of Calgary
Systems Biology of Cell fate decision: Transcriptome fluctuations and the Epigenetic Landscape

22.02.10 Manuel Zimmer
Rockefeller University
Aerotaxis in C. elegans: A Model to Study Chemosensation and Neural Circuit Function

22.02.10 Minna-Liisa Änkö
MPI, Dresden
How splicing factors regulate gene expression programs in stem cells and differentiation

24.02.10 Niels Gehring
EMBL, Heidelberg
How pre-mRNA processing tunes the efficiency and accuracy of gene expression

25.02.10 Gaby Maimon
Caltech
Active vision and higher brain function in Drosophila melanogaster

25.02.10 Andrew Murray
Harvard University
A manipulative approach to understanding chromosome behavior in mitosis and meiosis

MARCH

01.03.10 Wulf Haubensak
Caltech
Fear control by inhibitory gating in the amygdala

01.03.10 Axel Nimmerjahn
Stanford University
Imaging reveals fundamental glial and neuronal actions in the brain

03.03.10 Kevin Corbit
UCSF
High-throughput genetic manipulation of ES cells: modeling ciliopathies in mice

03.03.10 Dirk Remus
Cancer Research UK, London Research Institute
Reconstitution of early events in Saccharomyces cerevisiae DNA replication

04.03.10 Alipasha Vaziri
Janelia Farm
New frontiers in super-resolution biological imaging and optogenetic probing of neuronal circuits

08.03.10 Simon Hippenmeyer
Stanford University
Genetic Dissection of Cerebral Cortex Development using Mosaic Analysis with Double Markers (MADM)

08.03.10 Zhenyue Hao
Campbell Family Institute for Cancer Research, Ontario
Genetic Dissection of Apoptotic Pathway in Lymphocyte Homeostasis and Immune Disorders

10.03.10 Sevinc Ercan
University of North Carolina
Regulation of X-chromosome transcription by a condensin-like complex

11.03.10 Philipp Niethammer
Harvard Medical School
Spatio-temporal control of wound detection

11.03.10 Cathie Martin
John Innes Centre, Department of Metabolic Biology
Engineering phenylpropanoid metabolism for healthy foods

15.03.10 Andrew Jackson
MRC Human Genetics Unit
Cellular pathways determining human brain size

16.03.10 Oliver Hobert
Columbia University
Making and breaking neurons in the nematode C.elegans

18.03.10 George Coupland
Max Planck Institute for Plant Breeding
Seasonal flowering in annual and perennial plants

22.03.10 Johannes Zuber
Cold Spring Harbour
An in-vivo RNAi approach to drug target discovery in chemoresistant AML

23.03.10 Paul Nurse
Rockefeller University
Controlling the fission yeast cell cycle

24.03.10 Karl-Peter Hopfner
Gene Center, University of Munich
Mechanism of signaling and repair of DNA double-strand breaks

29.03.10 Andrew Straw
Caltech
Dissecting a fly's autopilot: novel approaches for linking neural mechanisms to behavior

30.03.10 Duncan Odom
Cambridge Research Institute (CRI)
Five vertebrate ChIP-seq reveals the evolutionary dynamics of transcription

APRIL

01.04.10 Joachim Lingner
Swiss Institute for Experimental Cancer Research
Telomeres and telomerase: RNA-dependent machines at chromosome ends

06.04.10 Len Pennacchio
DOE Joint Genome Institute
Large-Scale Identification of Tissue-Specific Enhancers In Vivo

07.04.10 David Anderson
Howard Hughes Medical Institute
Neural Circuits for innate defensive Behaviors in flies and mice

08.04.10 Detlef Weigel
Max Planck Institute for Developmental Biology
Next-generation genetics in plants: Evolutionary tradeoffs, immunity and speciation

12.04.10 Carolin Kutzki
Thermo Fisher Scientific
Protein Interactions – The way proteins are communicating

19.04.10 Thomas Klausberger
Medical University Vienna
Neuronal timing in cortical circuits

26.04.10 Thorsten Hoppe
CECAD Cologne
Ubiquitin chain editing couples longevity and proteostasis

28.04.10 Anna Akhmanova
Erasmus Medical Center
Friends at the ends: a dynamic protein network at the tips of growing microtubules

MAY

03.05.10 Gonzalo Otazu
Ludwig-Maximilians-University Munich
A recursive cortical circuit model for invariant sound source identification in auditory scenes

05.05.10 Bill Newsome
Stanford University School of Medicine
Value, decision-making, and the brain

06.05.10 Paul Anderson
Harvard Medical School
Polysomes, P-bodies and Stress Granules: Spatial Control of mRNA Translation/Decay

11.05.10 Ana Losada
CNIO
Cohesin Functions in Cohesion and Beyond

11.05.10 Detlev Arendt
EMBL
Evolution of brains and eyes in animals: a cell type perspective

12.05.10 Andreas Lüthi
Friedrich Miescher Institute
Defining the neuronal circuitry of fear

14.05.10 Roland Strauss
Johannes Gutenberg University, Mainz
Multiple Memories in the Oriented Locomotion of Drosophila

18.05.10 Mike Snyder
Stanford University
Personal and Nonpersonnel genomes: their analysis and variation

18.05.10 Mathias Mueller
University Halle
Collision-Induced Dissociative Chemical Cross-Linkers for Protein Structure Characterization

20.05.10 Peter Stadler
University of Leipzig
The RNA Zoo: Diversity and Complexity of Transcriptomes

25.05.10 Jussi Taipale
University of Helsinki
Systems biology of growth control and cancer

28.05.10 Ueli Schibler
University of Geneva
The regulation of circadian gene expression in cells and organs

JUNE

09.06.10 Gohta Goshima
Nagoya University
Mechanisms of microtubule nucleation and growth in cells

10.06.10 Sebastian Kreuz
Boehringer Ingelheim, Biberach
AAV vectors as a tool for efficient gene delivery

10.06.10 Alfred Wittinghofer
Max Planck Institute of Molecular Physiology
G protein signaling and diseases

11.06.10 Mike Levine
University of California
Whole-genome analysis of transcriptional precision in the Drosophila embryo

15.06.10 Kevin White
University of Chicago
Mining transcriptional networks in flies and humans for cancer biomarkers

15.06.10 Kenneth Harris
Imperial College London
The Neural Marketplace

17.06.10 Jeffrey Gerst
Weizmann Institute of Science
mRNA trafficking: An entire genome in transit?

23.06.10 Charlie Boone
University of Toronto
The Genetic Landscape of a Cell

28.06.10 Richard Benton
University of Lausanne
Olfactory genes, circuits and behaviours

29.06.10 Martin Singleton
London Research Institute
Structural Studies on the Budding Yeast Kinetochore

JULY

01.07.10 Bart Deplancke
EPFL
Deciphering the Drosophila regulatory code

01.07.10 Matthias Hentze
EMBL Heidelberg
Control of protein synthesis by miRNAs and regulatory proteins

15.07.10 Arturo Casadevall
Albert Einstein College of Medicine
Rethinking antibody-mediated immunity

20.07.10 Eric Davidson
Caltech
Genomic Control System for development: The sea urchin embryo gene regulatory network

20.07.10 Agnel Sfeir
Rockefeller University
How telomeres solve the end protection problem

29.07.10 Ian Krantz
The Children's Hospital of Philadelphia
Developmental Repercussions of Cohesin

AUGUST

23.08.10 David Pellman
Dana-Farber Cancer Institute
Mitotic errors, chromosome breakage, and evolution

SEPTEMBER

01.09.10 Raymond Deshaies
Howard Hughes Medical Institute
Mechanisms of ubiquitination

02.09.10 Ann-Shyn Chiang
Brain Research Center & Institute of Biotechnology, Taiwan
Three-dimensional reconstruction of brainwide wiring networks in Drosophila at single cell resolution

02.09.10 Frank McCormick
UCSF
Cancer therapy based on the Ras/MAPK pathway

08.09.10 Garrett Greenan
MPI-CBG, Dresden
Centrosome size: cause and consequence

16.09.10 Sheena Josselyn
University of Toronto
Continuing the search for the engram

23.09.10 Christian Haering
EMBL, Heidelberg
How does condensin pack mitotic chromosomes?

23.09.10 Bill Kelly
Emory University
Trans-Generational Epigenetic Regulation of the Germ Line in C. elegans

27.09.10 Keji Zhao
National Institutes of Health
Decoding T cell epigenomes

30.09.10 Peter Baumann
Stowers Institute for Medical Research
Telomerase Biogenesis and Function

OCTOBER

01.10.10 Irmgard Sinning
Biochemistry Center, Heidelberg University
Novel insights into SRP-mediated protein targeting

14.10.10 Alexey Khodjakov
Wadsworth Center
Keeping centriole duplication under control

19.10.10 Greg Hannon
Cold Spring Harbor Laboratory
Conserved roles of small RNAs in genome defense

NOVEMBER

03.11.10 David Drubin
University of California
Harnessing actin dynamics for endocytic trafficking

04.11.10 Arshad Desai
Ludwig Institute for Cancer Research
Segregating the Genome During Cell Division

05.11.10 Iain Campbell
University of Oxford
Protein-protein interactions and cell migration

09.11.10 Sebastien Huet
EMBL
Fractal crowding in the nucleus: enhanced affinity of nuclear proteins to heterochromatin

10.11.10 Helen Saibil
Birkbeck College London
Chaperone machines for protein folding. unfolding and disaggregation

11.11.10 Tomas Hromadka
Cold Spring Harbor Laboratory
From function to structure: Towards studying circuits in auditory cortex in vivo

15.11.10 Frank Uhlmann
Cancer Research UK London Research Institute
Chromosome condensation: Compacting the eukaryotic genome

17.11.10 Mark Stitt
Max Planck Institute for Molecular Plant Physiology
Balancing Metabolism and Growth with the Carbon Supply in Arabidopsis; Sensing, Budgets and Clocks

22.11.10 Benoît Zuber
MRC Laboratory of Molecular Biology
The structure of acetylcholine receptor clusters at the neuromuscular junction

23.11.10 Ewa Bednarek
FMI, Basel
Impact of hippocampal structural rearrangements on learning and memory

DECEMBER

01.12.10 Brenda Schulman
St. Jude Children's Research Hospital
Dynamic Mechanisms in Ubiquitin Conjugation Cascades

02.12.10 Matthias Merkenschläger
MRC Imperial College London
Non-canonical cohesin functions in T cell differentiation

03.12.10 Kristin Scott
HHMI/University of California, Berkeley
Taste recognition in Drosophila

10.12.10 Pavel Belov
Queen Mary University of London & St Petersburg State University
Approaches to Super-resolution Imaging: Manipulation of the Near-field by means of Metamaterials

Awards & Honors

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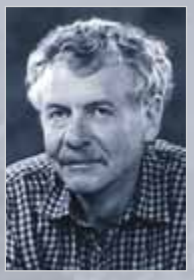

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The Whitehead Institute,
Cambridge, USA

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

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The IMP and Its Surroundings

The Research Institute of Molecular Pathology (IMP) is a basic biomedical research center in Vienna, the capital of Austria. Its major sponsor is Boehringer Ingelheim, a globally operating pharmaceutical company with its headquarters in Germany. The knowledge created at the IMP is at the disposal of Boehringer Ingelheim for the development of innovative diagnostic and therapeutic concepts.

The Campus Vienna Biocenter

Opened in 1988 close to the city center, the IMP triggered the development of its neighbourhood into a teeming biotechnology hub. Today's "Campus Vienna Biocenter" (VBC) is also home to the Max F. Perutz Laboratories (MFPL; University and Medical University of Vienna), the Institute of Molecular Biotechnology (IMBA; Austrian Academy of Sciences) and the Gregor Mendel Institute of Molecular Plant Biology (GMI; Austrian Academy of Sciences), a University of Applied Sciences, several biotech companies, a non-profit scientific society and the Vienna Open Lab.

Close ties have been knit between the IMP and IMBA: the institutes are not only linked physically by an attractive skywalk, but cooperate closely on all levels and share large parts of their infrastructure.
More than 1400 people from 40 different nations currently work at the Campus VBC. Campus members enjoy a scientifically and socially stimulating environment and take advantage of shared facilities such as the Max Perutz Library at the IMP 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 the IMP

The IMP offers a dynamic scientific environment for students and scientists at all levels of training. IMP scientists have a passion for research, which extends not only to their own work but also to work of colleagues and neighbors. Research at the IMP is multidisciplinary, combining biologists, computer scientists, and physicists, for example, in one place. Regardless of one's background, the IMP is a place to learn. The concentration of top class research at the IMP generates energy among the scientists. This intensity and excitement of discovery provide the foundations of the outstanding research at the Institute.

Researchers of all backgrounds will find intellectual stimulation at the IMP. The weekly VBC seminar series invites renowned scientists to present their discoveries; everyone is encouraged to interact with these guests. Additionally, the IMP has initiated a IMP seminar series, named after the IMP´s founding director Max Birnstiel, to highlight outstanding scientific researchers from around the world (see pages 64-67).
"Monday Seminars" provide an opportunity to find out what breakthroughs are being made within the IMP and at the VBC: doctoral students and post-doctoral researchers present their work once a year to the VBC community. Journal clubs, informal seminars, and the VBC lecture series provide opportunities to delve deeper into a subject of interest. The annual IMP recess provides an intense overview of current research from all of the IMP groups.

Vienna has a continuously expanding offering of scientific meetings and conferences. The students of the VBC International PhD program organize an annual symposium focused on an emerging field in biology, again recruiting international researchers both as speakers and as guests. Interactions with other scientific research institutions in Vienna provide opportunities to extend one's scope beyond the disciplines investigated on the VBC campus.

The IMP has a tremendous infrastructure, on par with the best research institutes in the world despite the IMP's relatively small size. The support facilities and services are substantial; they eliminate the need to worry about mundane details and allow everyone to focus on science. The IMP provides an extremely supportive environment for scientists at all levels. For instance, the grants department provides help with funding, public relations will prepare press releases, and the graphics department can advise on figures and presentations. Researchers will find they have few or no administrative duties, due to a talented and extremely helpful administrative support staff.

The excellent reputation of the IMP attracts out-standing students and post-docs from around the world. Vienna is a fantastic city, with a high quality of life and an international appeal. English is widely spoken. All of these factors contribute to the international character of the IMP and make it an exciting place to pursue scientific discovery.

More information about career opportunities at the IMP is available at: *www.imp.ac.at*

MAX BIRNSTIEL LECTURE

"Mechanisms of protein transport across membranes"
Tom Rapoport, Harvard Medical School

Tom Rapoport's lab at Harvard Medical School is studying how polypeptides are transported across - or into - the ER membrane. After starting his scientific research career by cloning insulin from carp, Tom became interested in how preproinsulin was targeted for secretion and has been investigating the molecular mechanisms underlying protein translocation ever since. During his visit to the IMP, Tom discussed his group's elegant biochemical and structural reconstitution of polypeptide movement through the translocating channel. Distinct mechanisms drive the transport of proteins across membranes in prokaryotes and eukaryotes - pushing or ratcheting - highlighting the diversity of mechanical solutions even when solving the same molecular problem.
Lecture hosted by Stefan Westermann

MAX BIRNSTIEL LECTURE

„Value, decision-making, and the brain"
Bill Newsome, Stanford University School of Medicine

William T. Newsome, a physicist by training, early on in his career became interested in how the nervous system encodes higher brain functions such as perception and cognition. He established electrophysiological recordings in awake, behaving monkeys performing a visual perception task and could show for the first time that manipulation of neurons can modulate the percept reported by the monkey. Now a professor at the Stanford University School of Medicine he has shifted focus from studying visual perception to an even more daunting question: How does the brain form decisions? William Newsome's lecture summarized the impressive progress his lab has made over the recent years in identifying neurons whose firing correlates with the decision the monkey is about to make. Identifying these neurons together with cleverly crafted behavioral tasks allowed forming a mathematical model of how the primate nervous system can form decisions.
Lecture hosted by Martin Häsemeyer

02/10

04/10

05/10

MAX BIRNSTIEL LECTURE

„Neural Circuits for innate defensive Behaviors in flies and mice"
David Anderson, Howard Hughes Medical Institute

David Anderson is a HHMI investigator and the Roger Sperry Professor of Biology at Caltech, U.S.A. Having made major contributions to the fields of neural development and stem cell biology, David has now completely switched his research efforts to study animal emotions. In his Birnstiel lecture, he showed how his team has used sophisticated genetic methods to begin to decipher the neural circuits that underlie innate fear responses in mice and flies.
Lecture hosted by Barry Dickson

VBC PHD RETREAT

The VBC PhD Retreat 2010 was held at the Hotel Kongress in Leoben from June 21 to 22. 50 PhD students from the VBC spent their time in Styria to interlink science, career perspective and simply having a good time. The 2010 PhD retreat was organized by the VBC PhD representatives Anita Kazda, Juliane Mayerhofer, Sascha Waidmann (GMI); Heike Harzer, Federico Mauri, Alexander Vogt (IMBA); Cosmas Arnold, Dominika Bienkowska (IMP); Ursula Pilat and Lanay Tierney (MFPL). During two poster sessions each student had the opportunity to present and discuss his/her project in a very relaxed atmosphere. The poster sessions were followed by presentations of Rafael Carazo Salas (The Gurdon Institute, University of Cambridge) and Ueli Grossniklaus (Institute of Plant Biology, University of Zurich). Both of these top notch researchers gave interesting insights into their sometimes more sometimes less straightforward career in science. Like the whole summer, so was also the VBC PhD Retreat 2010 marked by the FIFA world cup. Therefore everyone gathered at the local pub in the evening to watch the 2-0 win of later world champion Spain over Honduras. The retreat ended with a tour through the ancient iron mines of Erzberg. The 2011 VBC PhD retreat will be held from June 20 to 21, 2011 in "Eisenberg an der Raab".

VBC SUMMER CONCERT

It has become a nice tradition to have music as a part of the VBC's cultural program. At this year's summer concert the audience enjoyed a lively potpourri of different performances: singers, a jazz group, and a Turkish folk music group, and then of course the MolBioOrchestra - featuring students and staff from all over the campus.

THE FIRST VIENNA BIOCENTER SUMMER SCHOOL

This summer, the VBC organized its first ever Summer School for undergraduate students to attract the best and the brightest of the new generation of scientists. The program received more than 250 applications from all over the world. Eventually, 22 participants from 13 different countries qualified for the intensive ten-week course. The experience involved an independent research project in one of the scientific groups and a series of lectures, and was accompanied by a number of social and cultural activities in and around Vienna. On September 3, the Summer School was rounded off by a scientific mini-symposium where the participants presented the projects they had been working on for the past weeks. The event was entirely organised by the students themselves and was an impressive demonstration of the skills and knowledge they had acquired during those summer months.
www.vbcsummerschool.at

06/10 07/10 08/10

IMP SUMMER PARTY

This year it was really hot. Blue sky and lots of sunshine during the third IMP summer games! After Pink Panther had opened the games, the teams tried to score at the "IMP's Next Topmodel" beauty contest. Also other games like "Up in the air", "Sponge Bob", "Charlie Chaplin" and "Da Vinci Code" were taken up enthusiastically. On a hot day like this everybody was happy to jump into the water for the "Baywatch" buoy race. But the last game "James Bond" was definitely the highlight. The teams had to cover an obstacle course – using a pair of extra large skis and a toy car. It proved to be once more a hilarious afternoon for all and everybody is already looking forward to next year's party!

A MIDSUMMER NIGHT'S DREAM

On September 6th and 7th at the Campus Vienna Biocenter, a cast of 22 performed an experimental version of Shakespeare's "A Midsummer Night's Dream". The play was directed by Brooke Morriswood (MFPL). Experimental in more ways than one - the performers were all resident scientists of the Biocenter, not actors! They were all members of the VBC's newly-formed Amateur Dramatic Club, which had also produced Peter Shaffer's "Amadeus" at the IMP lecture theatre earlier in the year. Reflecting the diversity of the campus, most of the actors were speaking English as a second language, and a majority had no previous stage experience prior to their involvement in the Club. A total of roughly 200 people braved the elements over the two nights to watch the action, which utilised multiple locations in the VBC courtyard to represent the various settings for the play's action. Thus, the Intercell patio became Duke Theseus' Court in Athens, the basketball court became the Mechanicals' workplace, and the rectangular lawn the forest outside Athens, with the audience being moved between the locales to coincide with the shifting action of the play. On the second night, the weather very nearly conspired to ruin things, with Puck (Martina Doetsch, MFPL) delivering the famous "Give me your hands, if we be friends" soliloquy in the rain. The soggy crowd obliged, first by applauding and then by helping the performers haul the set and equipment out of the downpour, and back into the labs.

MAX BIRNSTIEL LECTURE

"Mechanisms of ubiquitination"
Raymond Deshaies, Howard Hughes Medical Institute

In the course of the Max Birnstiel Lecture Series we had the unique chance to welcome Ray Deshaies, an outstanding scientist in the ubiquitin field. His lab at the California Institute of Technology (Caltech) is mainly interested in unraveling the mechanisms underlying the regulated protein turnover going along with progression of the cell cycle. Ray gave an inspiring talk focusing on the essential multi protein complex SCF that mediates the ubqiuitination of key regulatory proteins thereby targeting them for degradation. He perspicuously explained the sophisticated biochemical techniques that were employed to study the molecular mechanisms underlying the reaction. Ray completed his excellent talk by touching on the topic of applications for therapeutic agents thereby linking basic biochemistry of the ubiquitin pathway to actual pharmaceutical intervention.
Lecture hosted by Doris Hellerschmied and Linn Gazda

MAX BIRNSTIEL LECTURE

"Conserved roles of small RNAs in genome defense"
Greg Hannon, Cold Spring Harbor Laboratory

Greg Hannon made an impact on many fields of biology: During his career he worked on trans-splicing in nematodes, tumor suppressor genes and cell cycle regulators in mammals, before he entered the field of RNA interference. His laboratory identified Dicer and Slicer and made key contributions to the roles of small RNAs in biology. He is also one of the leading figures in exploring how small RNA pathways can be utilized for targeted gene knockdowns in mammals and built genome wide resources to do so.
In his lecture, Greg illustrated his group's recent efforts to understand changes in DNA methylation patterns during mouse and human germline development. The remarkable findings indicate that small RNA pathways do impinge on methylation patterns, especially for active transposable elements, but that the bulk of methylation is independent of small RNAs.
Lecture hosted by Alex Stark

09/10

10/10

POSTDOC RETREAT 9TH/10TH SEPTEMBER 2010

This year the annual Postdoc Retreat, themed SCIENCE IN FICTION, took us, the IMP/IMBA/GMI Postdocs, to Lake Balaton in Hungary. After a three-hour drive and a few cookies, we arrived at Club Dobogomajor, a lovely resort close to Keszthely, the former capital of the Balaton region. Things got underway quickly as the afternoon sessions were opened by Prof Charalambos Kyriacou (Affectionately, Bambos, for short) from Leicester University/UK who told us 25 years worth of amazing stories, detailing experiences from his scientific life. We learned some very valuable lessons, particularly to watch out if Bambos has a camera. Next was Chris Mooney, a science writer from the US. He gave an interesting talk about politics and science, which sparked some stimulating discourse following the talk. We concluded that at the moment, we are all extremely happy we are working in the EU and not the US. The evening discussion and dinner were spent on lake Balaton shipping around in a rather classic looking boat where we were treated to a beautiful sunset as the night began. As people relaxed and got to know each other a little better over fruit, the dropping temperatures were ignored in exchange for some brilliant conversation and even a little late night swimming for the courageous few. For the next morning session, we invited Prof Carl Djerassi, who is most famous for the invention of the birth control pill. He spent hours (yes, hours) recanting about the ethics in modern science that he extensively described in his fascinating theatrical performances and novels such as The Cantor's dilemma. Our brains full of science in fiction, we shifted the discussion to a lovely winery atop a hill that not only offered us excellent regional wines but also a gorgeous view over the lake spanned by a pretty rainbow. After a few wines and wonderful cheese, we eventually made our way back to Vienna where the night ended this very SMart experience.

RECESS

From October 6-8, IMP scientists met with members of the Scientific Advisor y Board (SAB) to present their work and discuss their research. The SAB, consisting of internationally recognized scientists, were once more impressed by the scientific performance and high standards of the research presented. The IMP would like to thank all its SAB members for their commitment and advice.
IMP SAB members: page 59 in this booklet

MAX BIRNSTIEL LECTURE

"Harnessing actin dynamics for endocytic trafficking"
David Drubin, University of California

David Drubin's lab at UC Berkeley is interested in understanding cell morphogenesis and division: how do cells coordinate the cytoskeleton according to signals from their surroundings to generate spatially defined responses?
After commenting on the recent US elections, the San Francisco Giants baseball championship and the status of George Bush's marriage, David's talk showcased different approaches to study the endocytic machinery in both human cells and yeast. Electron microscopy studies revealed how two classes of membrane-binding proteins, F-Bar and Bar-proteins, work together to shape endocytic vesicles. Gene-editing using site-specific zinc finger nucleases allowed David's lab to analyze the dynamics of endocytosis in human cells with yeast-like precision. Finally, biochemical approaches, such as actin-tail proteomics and reconstitution of vesicle dynamics in extracts, promise a deep mechanistic understanding of actin-driven endocytosis in the future.
Lecture hosted by Carrie Cowan

MAX BIRNSTIEL LECTURE

„Dynamic Mechanisms in Ubiquitin Conjugation Cascades"
Brenda Schulman, St. Jude Children's Research Hospital

The Max Birnstiel Lecture series 2010 was perfectly rounded off with an exciting talk by Brenda Schulman. Brenda heads the Structural Biology section of the Saint Jude's Children Hospital in Memphis Tennessee and presented work on the regulation of Cullin RING type ligases, huge enzymatic complexes that promote ubiquitination reactions and target client proteins for degradation. By determining a series of crystallographic snapshots, Brenda provided fascinating insight into the dynamic action of this ubiquitin ligase deciphering the molecular anatomy of the proteinous kiss-of-death mechanism.
Lecture hosted by Tim Clausen and Linn Gazda

11/10

12/10

VBC-PHD SYMPOSIUM "ORIGIN OF LIFE"

This year's PhD Symposium from November 18th to 19th was dedicated to the very old but still hotly debated topic of the Origin of Life. The aim of the Symposium was to address this timeless question by looking at a variety of different perspectives and furthermore to draw a circle from the chemical and biological origins of life over to evolution and species diversification and finally the extinction of species.
About 280 registered students, 100 off-campus guests from all over Europe, and numerous journalists took part in this event. The scientific highlights were the 2009 Nobel laureate Ada Yonath presenting her ongoing work on the structure of the Ribosome, Antonio Lazcano who gave a fascinating overview about what is known about the molecular Origin of Life and what remains still elusive. Janet Siefert explained her work on microbial evolution and Lewis Dartnell introduced the amazing world of extremophiles. To close the circle, the symposium concluded with Arnold Miller and Stefan Lötters raising the question if there is a man-made sixth mass extinction approaching. Even though many questions on the origin of life have been addressed and partially answered during the symposium the puzzle of how life actually originated still remains open for further investigation.

MAX BIRNSTIEL LECTURE

"Chaperone machines for protein folding. unfolding and disaggregation"
Helen Saibil, Birkbeck College London

Helen Saibil is heading the Structural Biology program at Birkbeck College, London. Her main interests are in analyzing conformational changes in macromolecular machines, using cryo-electron microscopy and image processing. Her cryo-EM analysis of the structure and movements of the E. coli chaperonin GroEL has elucidated the allosteric mechanism of this protein folding machine, as she outlined in the Max-Birnstiel Lecture. Recent work identified different ATP-bound states that illustrate, for the first time, the dynamics of the chaperonine in capturing substrate and interacting with its partner GroES.
Lecture hosted by Tim Clausen

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Cover Illustration

front:
Asymmetric cell division during *C. elegans* embryogenesis. Reciprocal PAR protein domains, marked by GFP-PAR-2 (green) and mCHERRY-PAR-6 (red), establish cell polarity and dictate the asymmetric segregation of cell fate.
back:
Establishment of cell polarity in a one-cell *C. elegans* embryo. The sperm-supplied centrosomes (small dots in the upper left embryo pole) induce a localized change in contractility of the cortical acto-myosin network, establishing distinct cortical domains.
Courtesy by Carrie Cowan

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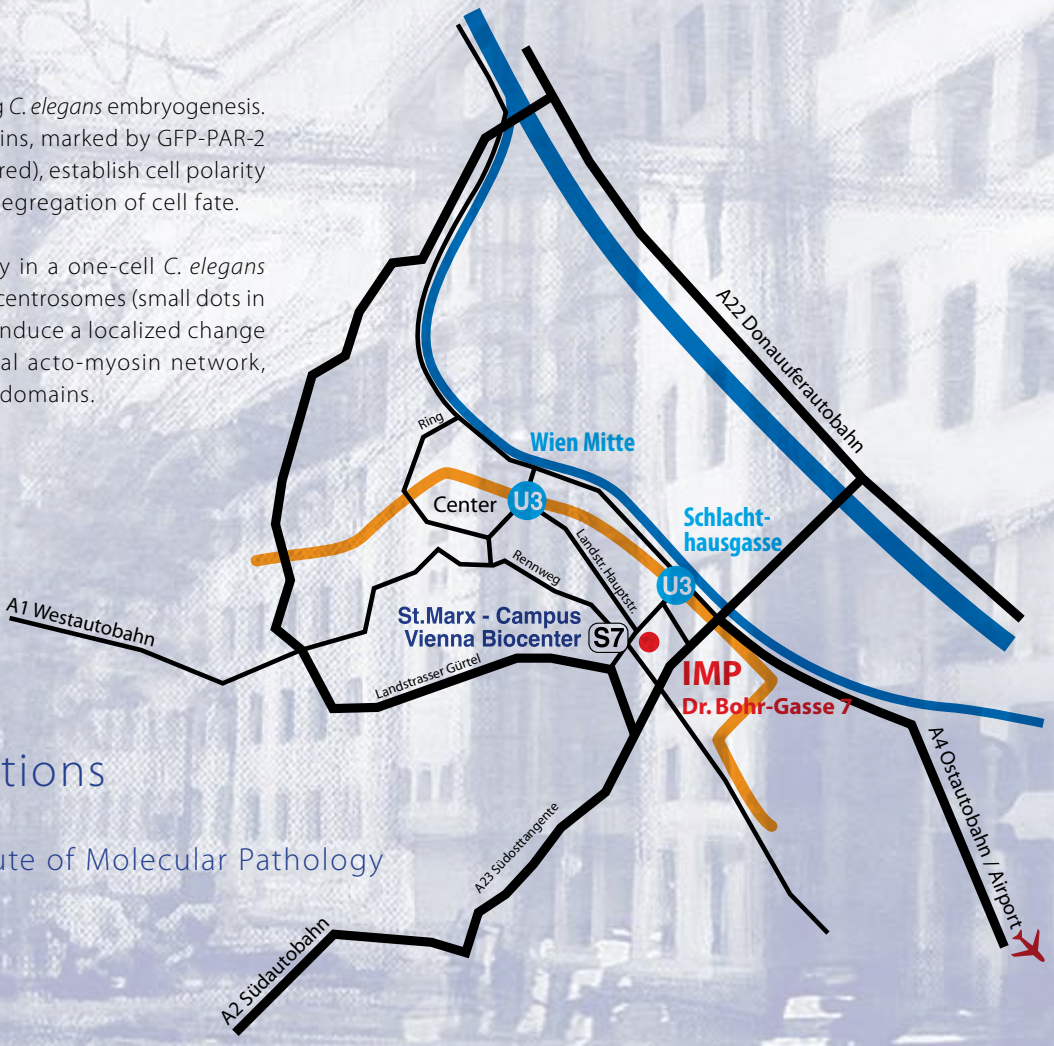

Contact/Directions

IMP - Research Institute of Molecular Pathology

Dr. Bohr-Gasse 7
1030 Vienna
Austria

Phone: +43(1)797 30
Fax: +43(1)798 71 53
research@imp.ac.at
administration@imp.ac.at
media@imp.ac.at
www.imp.ac.at

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