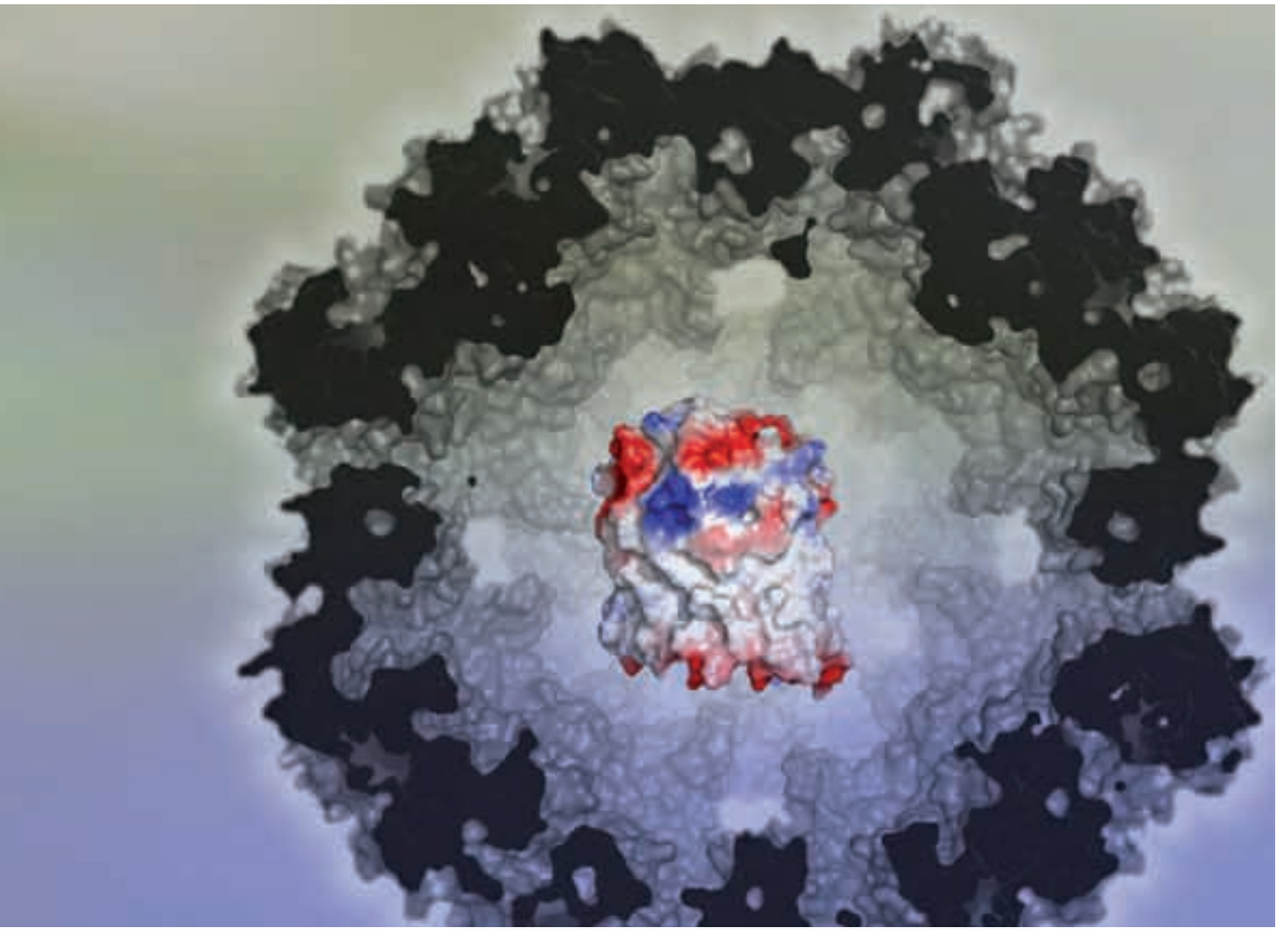
RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY
VIENNA BIOCENTER

2008



Boehringer Ingelheim

IMP
Research Institute of Molecular Pathology

CONTENTS

RESEARCH GROUPS

RESEARCH SUPPORT

SCIENTIFIC SERVICES

BARRY DICKSON
Managing Director/ Science

HARALD ISEMANN
Managing Director/ Finance and Administration

This was a year of celebration at the IMP. The highlight, of course, was our 20th anniversary in May. More than half of all the invited IMP alumni came back to Vienna for the occasion. The centerpoint of the celebrations was a two-day symposium at the Hofburg, during which many alumni had the opportunity to reflect on their time at the IMP and share their exciting science with us. It was satisfying to see how many outstanding scientific careers have been launched at the IMP. A common theme in these recollections was the intense spirit our alumni had enjoyed at the IMP: "work hard, party hard" seemed to be the motto. Needless to say, we also didn't let the 20th birthday pass without another party - or two. Just before the symposium, we held a get together at the IMP to catch up with old friends and honour the 16 IMP employees that have lasted the entire 20 years. And right after the symposium, we headed up to the penthouse of the Hofburg to wine, dine, and dance the night away.

It was a particular treat that Max Birnstiel, the founding director, shared in these celebrations and told us the unlikely story of the IMP's birth. Max also recalled the thinking behind some of the structural decisions he made at the very beginning which, in retrospect, turned out to be keys to the IMP's success. We were also pleased to be joined by Andreas Barner, chairman of the Board of Managing Directors of Boehringer Ingelheim, the mayor of Vienna Michael Häupl, and Johannes Hahn, the federal minister of science. In his opening remarks, Andreas Barner reiterated BI's commitment to the IMP's mission of basic science: "From the BI perspective, the IMP´s activities are charitable, non-profit and primarily serving the public good. No return on investment is expected other than excellent science and adding the culture of a basic research institution to the Boehringer Ingelheim R&D organization." In their addresses, Michael Häupl and Johannes Hahn acknowledged the crucial role of the IMP in the development of the scientific environment in Vienna and Austria, and expressed their gratitude to BI and the Boehringer family. We too express our deep appreciation to Boehringer Ingelheim for their continued support, and to the various local, national, and international agencies that fund specific projects at the IMP.

As always, there was plenty of good science to celebrate this year too. Some of the most exciting discoveries are covered in the short stories that are now a regular feature of our annual report. One of the most remarkable was the work by Tobias Krojer, Justyna Sawa, Tim Clausen, and their colleagues, in elucidating the structure and catalytic mechanism of the massive 24-meric DegP protease (featured on the cover of this report). Was it just coincidence that Tim, a big football fan, came out with this "soccer" structure just in time for the Euro 2008 in Vienna!? Other highlights included Kerstin Wendt and Jan-Michael Peters' work on the surprising role of cohesin in gene regulation, and Jelena Nedjic and Ludger Klein's demonstration that T-cell tolerance relies on autophagy to present self-antigens in the thymus. Our science also continues to attract various awards, giving yet more reasons to celebrate. In particular, we were delighted that Stefan Westermann and Barry Dickson were amongst the first recipients of highly-competitive research grants from the European Research Council.

Not all of our parties this year were purely joyous occasions. Two were tinged with sadness, as we said farewell to Erwin Wagner and Thomas Jenuwein. After many outstanding years at the IMP, Erwin and Thomas have moved on to leading positions elsewhere, Erwin as head of cancer cell biology at the CNIO in Madrid and Thomas as a director at the Max Planck Institute in Freiburg. Erwin has been at the IMP since the very beginning, and Thomas since 1994. Both have made tremendous contributions to the IMP and the broader scientific community, for which we are deeply grateful. We are also proud to see them moving on to such prestigious and important positions. This is exactly how Max envisioned the IMP – a place that offers the most talented young scientists the opportunity to realise their full potential, before they move on to permanent, top-level positions elsewhere. In this way, they spread the IMP spirit abroad, whilst also allowing the IMP to maintain a constant influx of new people and new ideas.

Two new colleagues setting up their labs at the IMP this year are Alex Stark, a new group leader who joins us after a postdoc at the Broad Institute, MIT, and Dave Keays, a junior fellow joining us after his graduate research at Oxford University.

There was also turnover on the Scientific Advisory Board this year. Steve McKnight and Titia de Lange rotated off, after almost record-breaking runs of 8 and 6 years, respectively. They have both been terrific SAB members, and we thank them for their tremendous support for the IMP and its scientists over the past years. Next year Angelika Amon, Tobias Bonhoeffer, Dan Littman, and Venki Ramakrishnan will all join the SAB, with Cori Bargmann taking over as chair.

One of Max's cornerstones for the IMP was the establishment of strong, independent scientific services. The IMP would not have enjoyed the success it has without the highly skilled and dedicated staff who provide these services. To cope with the continued growth of these services, in part created through the shared service agreement with IMBA, we appointed Peter Steinlein this year to the newly created position of Manager of Scientific Services. Karin Aumayr has succeeded Peter as head of the biooptics service. Peter is also overseeing the establishment of a new genomics facility that will start operation in 2009. This new service unit will bring together the microarray facility, formerly part of biooptics, and the deep sequencing facility set up by Meinrad Busslinger and his team.

On the topic of scientific services, one of our biggest causes for celebration this year was a grant from the City of Vienna and the Federal Ministry of Science to establish a new set of campus-wide service facilities. Harald Isemann coordinated a joint proposal from the four academic research institutes on the campus, as well as Intercell and the other companies. This initiative will bring in funding of about €52 M over the next 10 years. These new facilities will augment, not replace, the current IMP/IMBA services, and will be available to all members of the campus.

Last, but by no means least, we look forward to opening a new childcare facility on campus in spring 2009. This center will offer professional care for children from 3 months of age, with longer and flexible opening hours tailored to meet the needs of scientists with small children. We hope that this new childcare facility will make it even easier for our scientists – both men and women – to combine the twin joys of a career in science and raising small children.

Barry J. Dickson
Harald Isemann

WHAT YOU EAT IS WHAT YOU ARE: SELF-DIGESTION IN SELF-RECOGNITION

As long ago as 1826, Anthelme Brillat-Savarin wrote, in Physiologie du Gout, ou Meditations de Gastronomie Transcendante, "Dis-moi ce que tu manges, je te dirai ce que tu es." This translates literally as "Tell me what you eat and I will tell you what you are" and is generally interpreted as an invocation to eat healthy food. But Brillat-Savarin may unwittingly have been pointing to the solution of one of immunology's most baffling questions: how do T-cells learn to differentiate "self" from "non-self"?

T-cells protect the body from infection by recognising and destroying pathogens. The ability of our immune system to recognize and combat invading pathogens in a highly specific way, while not reacting against molecules derived from the host, depends on self-tolerance. Self-tolerance essentially means the ability to recognize (and not react to) all possible epitopes, or immunoreactive sequences, derived from the organism. Were it not for self-tolerance, the body would effectively be fighting against itself, as it does in certain autoimmune diseases such as type 1 diabetes, multiple sclerosis, lupus erythematosus and possibly also Crohn's disease.

Katharina Aschenbrenner

T-cells are called T-cells because they are produced in the thymus and by the time they leave this organ they must already be fully self-tolerant. A big clue to how self-tolerance arises was provided in 2007 by Katharina Aschenbrenner in Ludger Klein's lab at the IMP. Katharina was working with thymic epithelial cells (TECs), which have long been known to represent an anomaly in that they express the genes for class II major histocompatibility complex (MHC-II) molecules, which are otherwise transcribed only in cells of the haematopoietic lineage (which give rise to blood cells). Furthermore, TECs express a wide range of otherwise strictly tissue-specific antigens and in this respect they "mirror" all the organs in the body. In effect, then, the TECs essentially show all possible host antigens to developing T-cells. Thus, during their development in the thymus T-cells can be made tolerant not only to thymus-specific antigens but to all peripheral antigens. Katharina showed that TECs that "promiscuously" synthesize antigens are able to present antigens to developing T-cells and thereby induce tolerance in autoreactive T-cells (*Nature Immunol.* **8**: 351-358).

Katharina's work was groundbreaking but left open the issue of how the TECs present self-antigens to T-cells. A PhD student in Ludger's lab, Jelena Nedjic, has now provided strong evidence that autophagy is involved. Her results were reported in *Nature* this year (**455**: 396-400).

Jelena came to work in Vienna under the IMP's international PhD programme. She chose to apply to the programme because of the IMP's high scientific reputation and at her interviews she decided she would like to work in Ludger's group "because I was even then very interested in immunology and in the development of the immune system. Ludger's project seemed to tie in perfectly with my interests and he was an obvious first choice for me." The project itself represented "a totally new question," Jelena says. "Nobody had ever considered alternative antigen presentation pathways in the thymic epithelium so we had the chance to work on a completely novel *in vivo* question."

A good starting point seemed to be the autophagy-reporter mice recently developed by Noboru Mizushima in the Tokyo Medical and Dental University. In these mice, the green fluorescent protein, at that time not yet decorated with a Nobel Prize, was fused to the autophagy marker LC3. Autophagy could be detected in most tissues only when the mice were starved but the thymus was known to show high levels of autophagy under all conditions. Noboru was happy to collaborate on the project and made the mice available for Jelena to examine. And Jelena was able to show that autophagic activity in the thymus was concentrated in TECs and co-segregated with "promiscuous" antigen expression.

Jelena hypothesized that autophagy might be associated with antigen presentation by TECs and thus with the development of T-cells' ability to distinguish self from non-self. But testing this hypothesis turned out to be far from trivial. Mice homozygous for a mutation in the Atg5 gene, which is essential for autophagy, die shortly after birth, long before T-cell tolerance can be evaluated. This observation shows merely that autophagy is important for survival, which was no great surprise to anybody. Jelena recalls this period of the project: "At that point we had no way of creating conditional knockouts of the gene so decided to attempt thymic grafts, inserting embryonic thymuses from homozygous Atg5 mutant mice into normal adult mice. Setting up the system took up the first 6–8 months of the project and was extremely frustrating – not to say highly risky. But science is all about taking risks and luckily it finally worked."

The transplanted thymuses were significantly smaller than normal but the epithelial compartments appeared to have differentiated correctly. Furthermore, T-cells seemed to develop normally and the main lineages were present. However, the frequency of cells expressing particular MHC-II-restricted specificities was

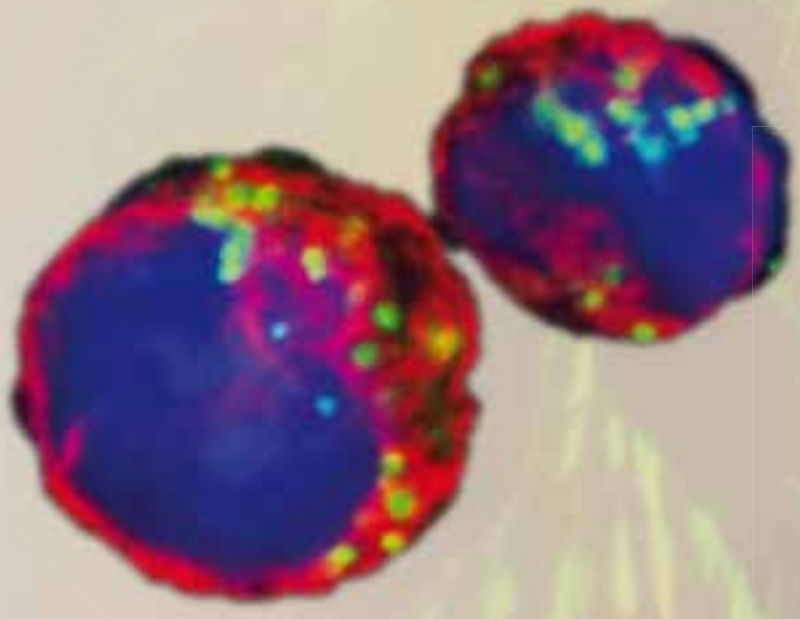
considerably lower, consistent with the idea that autophagy is involved in antigen presentation, presumably by generating combinations between MHC-II molecules and peptides from host proteins.

The most important prediction of Jelena's hypothesis, however, is that mice that receive thymuses homozygous for the Atg5 mutant should not show self-tolerance. And this turned out to be the case. Such mice contained many more "activated" T-cells than mice grafted with wild-type thymuses and started to waste within 4–6 weeks after grafting. The mice showed a number of symptoms consistent with autoimmune disease. Furthermore, T-cells purified from them gave rise to autoimmune reactions when injected into athymic mice, confirming that autoimmunity resulted from the presence of incorrectly trained T-cells.

Autophagy literally means "self eating". It is rare for animals to try to eat themselves so the term is normally used to mean breaking down of the cell's components, thus helping, for example, to reallocate nutrients to essential processes under times of energy stress. Autophagy also plays a part in the destruction of some bacteria within the cell and has been proposed to represent a mechanism for apoptosis, or programmed cell death.
Jelena's results show that autophagy is also involved in the prevention of autoimmune reactions. Her finding has highly important implications for human disease. There is no known cure for Crohn's disease, which occurs in up to about 50 cases per 100,000 people in northern Europe. Jelena's results provide a theoretical explanation for the genetic correlation between this condition and autophagy (see *Nature Genet*. **39**, 207-211 and *Nature Genet*. **39**, 596-604) and strongly indicate that there is an autoimmune component to the disease. Jelena recognizes that we are far from developing a cure but she "would be really happy if some of our basic research could be built upon – possibly by a worldwide collaboration – and one day lead to a treatment."

Jelena herself would be pleased to contribute to such a worldwide effort. She recently moved with Ludger to Munich, where she is currently writing her PhD thesis, and plans to spend another year or so in his group before looking for a post-doctoral position in the US, "preferably at the Rockefeller: there are lots of good immunology groups there and I love the idea of living in New York!" In the more distant future, she would like to return to Europe. As she says, "isn't this the clichéd idea of a scientific career? PhD in Europe, post-doc in the States and then return to closer home. But it sounds like an interesting enough career path to me! "

Text by Graham Tebb



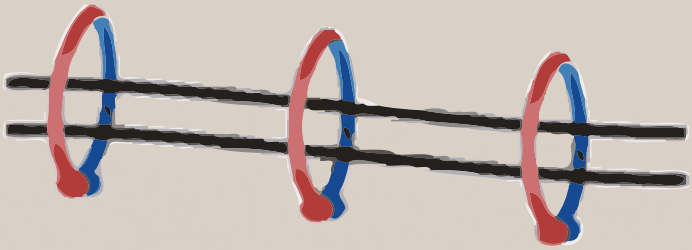
High autophagic activity in thymic epithelial cells. The image represents sorted thymic epithelial cells where the autophagic activity has been visualized by immunofluorescence staining of autophagosomes (cytoskeleton-red, autophagosome-green, nucleus-blue).

Jelena Nedjic

COHESINS: MORE THAN JUST A GLUE

Bored scientists attending the 2000 Cold Spring Harbor Laboratory Genome Meeting decided to organize a sweep-stake ("GeneSweep") to predict the number of human genes. The bets – all from respected people in the field – ranged from about 26,000 to well over 150,000. The number of genes in an organism is generally regarding as an index of genetic complexity and given that a simple organism such as the worm *Caenorhabditis elegans* has about 20,000 genes, the higher estimates did not seem widely exaggerated.

The Human Genome Project, completed in 2003, surprised many by showing that humans only have between 20,000 and 25,000 genes, an amazingly low number for a species that regards itself as so complex. This meant that GeneSweep was won by the candidate who placed the lowest bet. And it also strongly suggested that single genes in humans perform multiple functions.



The old model:
Cohesin holds sister DNA strands together by entrapping them within the ring-shaped complex.

Cohesins were initially discovered in yeast (in 1997 by Christine Michaelis in Kim Nasmyth's lab at the IMP, *Cell* **91**, 35-45) by searching for mutants that frequently lose chromosomes and are able to separate chromosomes in the absence of a functioning anaphase promoting complex. Christine's screen identified a total of three new genes that turned out to be the core components of a complex, "cohesin", which is conserved from yeast to humans, suggesting that a common cohesion mechanism might be used in all eukaryotic cells.

The function of cohesins seems to be to tie sister chromatids together following chromosome replication and to release them at the start of anaphase. But evidence has gradually been accumulating that cohesins also contribute to gene regulation and vertebrate development. Perhaps the clearest indications are provided by mutations in the human genes encoding either cohesin or proteins that regulate cohesin. The best known case is the Cornelia de Lange syndrome, a rare genetic disease characterized by a distinctive facial appearance, growth deficiency, feeding difficulties, behavioural problems and associated malformations. Cornelia de Lange syndrome was initially discovered to be caused by mutations in the cohesin regulator *NIPBL* but it has subsequently been shown that mutations in the human cohesin genes *SMC1A* and *SMC3* also lead to a milder form of the syndrome.

The mechanism underlying the regulation of gene expression by cohesins is still not fully understood but Kerstin Wendt in Jan-Michael Peter's lab, working in collaboration with the lab of Katsuhiko Shirahige at the Tokyo Institute of Technology, has provided a significant clue (Wendt *et al*. 2008, *Nature* **451**: 796-801). Kerstin came to the IMP from Martinsried, Germany – by coincidence she did her PhD in Robert Huber's structural biology lab, where Tobias Krojer and Tim Clausen (see pp. 8-9) were also working – but decided to switch her focus more towards cell biology, as she says "to enable me to test the hypotheses that come from structural studies."

Kerstin initially started to work on condensin but this project "just didn't work out at all" so she chose instead to map cohesin binding sites on the human genome. Birgit Koch, a technician in Jan's lab, had found that the mouse cohesin complex is expressed not only in proliferating cells but also in cells that never again divide and would therefore never need cohesion. This finding was consistent with a possible function for cohesin in transcription. Together with Katsuhiko Shirahige, Kerstin performed a series of chromatin immunoprecipitation experiments and analysed the results using high-density oligonucleotide tiling arrays (ChIP on chip). The analysis identified nearly 9,000 sites in the non-repetitive human genome to which cohesin preferentially bound. Interestingly, the majority of these were located in non-coding regions, either in intergenic regions or in introns.

More excitingly, Kerstin and her collaborators noticed that cohesin was enriched at several sites known to bind CTCF, suggesting that CTCF and cohesin may colocalize on the

Kerstin Wendt

human genome. CTCF was originally identified as a transcription factor involved in the regulation of the *c-myc* gene and has subsequently been shown to be an insulator protein, binding to sites such as the *H19* imprinting control region and the β-globin locus control region.

Kerstin examined other known CTCF binding sites and found that the vast majority of them were apparently identical to binding sites for cohesin. Surprisingly, when CTCF was removed from HeLa cells, cohesin no longer bound specifically to particular sites on the DNA although its overall level of DNA binding was unaffected and cohesin seemed to function correctly. CTCF thus appeared to be required for cohesin to be "correctly" positioned on DNA.

There is clearly some form of interaction between CTCF and cohesin, so Kerstin wondered whether cohesin might be involved in CTCF's function as a transcriptional insulator. To test this idea she made use of an elegant reporter system. In this system, the *H19* imprinting control region (ICR) on a reporter plasmid prevents activation of the *H19* promoter by a foreign enhancer in a CTCF-dependent manner. Remarkably, Kerstin was able to demonstrate that the inhibitory effect also depended on cohesin – and was specific, as it was not observed when the ICR was mutated so as to eliminate CTCF binding. This finding suggested for the first time that cohesin might be linked to imprinting, opening an entirely new dimension for cohesin research.

The *H19* ICR restricts transcription of the *H19* gene to the maternal allele and transcription of the neighbouring *Igf2* gene (which encodes the insulin-like growth factor 2, IGF2) to the paternal allele (earlier work at the IMP, in particular by Denise Barlow and Anton Wutz, has provided a wealth of detail on imprinting at the *Igf2r* locus, which encodes the receptor for IGF2). The imprinted expression of the *H19* and *Igf2* genes has long been known to depend on CTCF, so Kerstin investigated whether it also depended on cohesin.

Initially she addressed whether cohesin bound together with CTCF to the unmethylated maternal ICR or whether it bound instead to the methylated paternal allele, where CTCF does not bind. Using mouse cells carrying either the maternal or the paternal copy of human chromosome 11, on which the H19 is located, she was able to prove that cohesin specifically bound to the maternal (unmethylated) but not the paternal (methylated) allele. And further experiments using HeLa cells depleted for cohesin or for CTCF indicated that the expression pattern of the imprinted genes at the *H19/Igf2* locus depended not only on CTCF but also on cohesin. Importantly, the effects on transcription from the *H19/Igf2* locus were observed in cells synchronized at a stage in the cell cyle when there is no cohesion, indicating that cohesin's newly discovered role in transcriptional regulation is indeed independent of its function in cohesion.

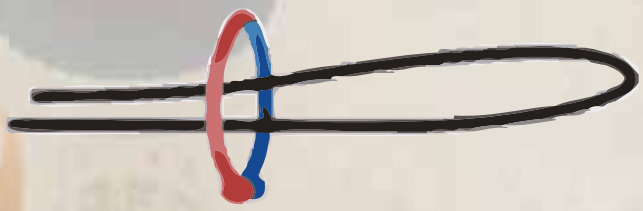
In summary, then, Kerstin has been able to show that cohesin binding sites in the human genome are associated with CTCF, a zinc-finger DNA-binding protein required for transcriptional insulation. Cohesin might enable CTCF to insulate promoters from distant enhancers; it clearly controls transcription at the *H19/Igf2* locus. Cohesin's role in transcriptional regulation seems to be independent of its function in cohesion.

Kerstin's and Jan's current working model is that "cohesin acts as a type of tool to hold DNA strands together. This is how chromosome cohesion works and the same tool may hold distant sites on the chromosomes together, e.g. in transcriptional control." The model is based on the observation that CTCF can mediate the formation of parent-specific chromatin loops at the *H19/Igf2* locus (see Murrell *et al*. 2004, *Nature Genet*. **36**: 889-893) that determine which of the genes has access to the enhancers that govern transcription from the locus. As Kerstin puts it, "our logic is that cohesin seems so important for CTCF function, so it might also be involved in stabilizing the chromatin loop – cohesin's cohesion function proves that it can hold DNA strands together. It will be exciting to address whether cohesion really does play a part in chromatin looping."

Although cohesin is extremely highly conserved among eukaryotes, CTCF is known only from vertebrates (and from the fruit fly *Drosophila*). It is thus conceivable that CTCF exists to help cohesin bind specifically to DNA and thus to enable the sophisticated regulatory mechanisms, such as genetic imprinting, that permit complex organisms to manage with so few genes.

Kerstin and Jan have been working within the framework of a so-called EuroCORES ("European COllaborative RESearch") project. Their work was embedded in a network of nine international projects, one of which was coordinated by Niels Galjart of the Erasmus University in Rotterdam, Holland. Niels works on DNA-binding proteins, especially on CTCF, and proved to be a valuable source of information on this protein. Even more importantly, he was largely instrumental in securing Kerstin a job: from January 2009 she will assume an independent group leader position at the Erasmus University, where she will continue to work on cohesin and CTCF.

Text by Graham Tebb



An alternative way to wear a ring:
The cohesin complex might also mediate the structure of a single DNA strand. By forming chromatin loops cohesin could regulate the distance between and the accessibility of DNA elements like promoters and enhancers which influence the activity of genes.

CRIMES OF PASSION – AND MORE? – AT THE MOLECULAR LEVEL

It is widely believed that violent crimes are most frequently committed by people known to the victims. This may or may not be true in human society but within cells it is certainly the case, at least when the victims are misfolded proteins. New results from Tobias Krojer, who works in Tim Clausen's lab on the bacterial DegP protein, have provided us with important information on how such "sick" proteins are broken down by a chaperone normally responsible for helping proteins assume their correct folded configurations.

DegP has formed the focus of Tobias's and Tim's work since their time in Robert Huber's group in Martinsried, Germany, when they first published its 3D structure (see *Nature* **416**: 455-459). DegP fulfills a number of functions in the cell: it can serve as a chaperone, refolding partially denatured proteins, or as a protease, degrading proteins that are too badly damaged to be refolded. DegP is extremely highly conserved in evolution and its crucial importance is indicated by the effects of defects in the human homologues, which have been associated with a number of disorders, such as Parkinson's disease and Alzheimer's disease. Both of these conditions have been shown to be associated with the buildup and aggregation of (partially) unfolded peptides. Clearly, the cell must ensure that unfolded or incorrectly folded proteins are either correctly folded or rapidly destroyed to avoid serious damage.

Tobias's and Tim's work has given rise to a number of important results over the years but even so Tobias is unlikely ever to forget the month of June 2008. Two first-author publications – in *PNAS* and in *Nature* – within the space of nine days represent a truly remarkable haul although Tobias is quick to point out that it was a real coincidence that the two papers appeared in such close succession.

Tobias's *PNAS* paper (**105**: 7702-7707) concentrates on DegP's proteolytic activity. The results show that DegP works in a processive manner, i.e. it binds target proteins and cuts them at several sites before releasing the resulting fragments. Interestingly, cutting seems to be dependent on binding to the so-called PDZ1 domain of the protein; cleavage occurs 12–17 amino-acid residues away from this domain. The obvious interpretation is that the C-terminus of an unfolded protein binds to the PDZ1 domain and thereby activates the protease, which trims 12–17 residues from the protein and allows the whole procedure to start again. Tobias refers to this as the "hold, bite and rebind model" and comments that it has the obvious advantage that potentially toxic intermediate products are not released into the cell.

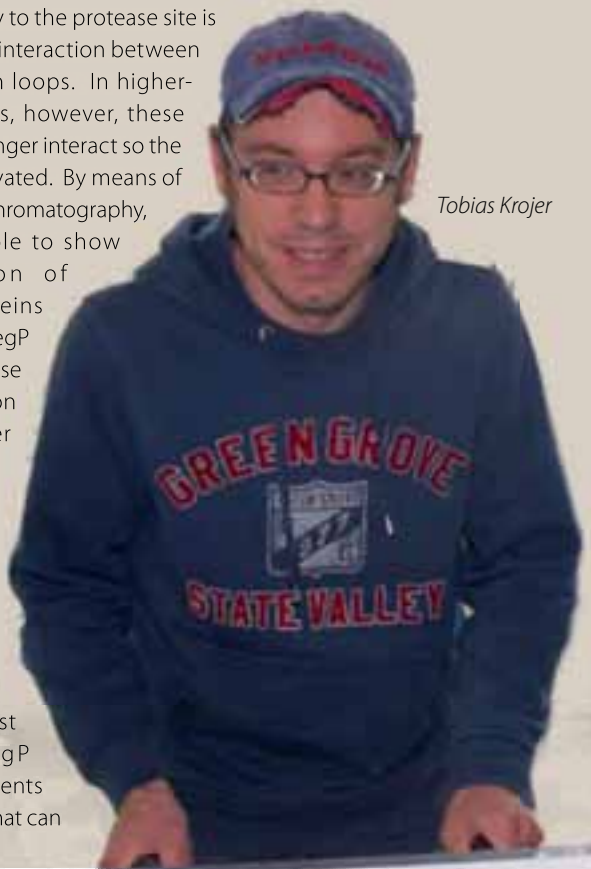
The PDZ1 domain thus functions as a "tethering" domain to prevent partially degraded proteins from escaping. But the hold, bite and rebind model is not sufficient to account for DegP's activity. Surprisingly, binding of a non-cleavable peptide to the PDZ1 was found not to block proteolysis of other substrates but to enhance it, suggesting that binding to PDZ1 causes allosteric activation of cleavage. It thus appears that the presence of misfolded proteins in the cell is able to activate the proteolytic capability of DegP, causing a switch from the "resting" state to the activated, protein-degrading conformation.

The best characterized (non-lysosomal) apparatus for breaking down cellular proteins is the proteasome. As Tim and Tobias say, "the proteasome works like a sausage machine, pulling a substrate inside and cutting bits of it off until it is fully broken down. But DegP has a totally different mechanism to accomplish the same thing." The proteasome works in an ATP-dependent manner but DegP is found in a cellular compartment where there is no free ATP. Generation of products that become substrates in the subsequent cleavage cycle thus seems to represent DegP's "trick" to help it processively cut the substrate sausage in an ATP-independent manner.

Tobias's and Tim's 2002 *Nature* paper (op. cit.) reported a structure for DegP hexamers, although as they freely concede "at that time we had no real idea what these hexamers did". Their recent *Nature* paper (**453**: 885-890) goes considerably further. A size-exclusion chromatography experiment initially suggested that DegP does not exist solely in the form of hexamers: two "puzzling bands on gels" could easily have been ignored but Tobias had a hunch that they might be important and decided to follow up on it. And to his – and everyone else's – surprise, the larger complexes turned out to be higher-level multimers of DegP, in each case bound to additional proteins.

By means of cryo-EM and X-ray crystallography, Tobias and Tim have now managed to solve the structures of the higher-level DegP complexes, the 12-mer and the 24-mer. They found that the 24-mer assembled into a giant, hollow hexahedral ball with an interior cavity of over 100 Å in diameter. The entire structure is held together by the PDZ domains, while the protease domains face inwards and so can act to break down peptides contained within the central cavity.

In the "inactive" conformation of DegP hexamers, entry to the protease site is blocked by the interaction between various protein loops. In higher-level structures, however, these loops can no longer interact so the protease is activated. By means of size-exclusion chromatography, Tobias was able to show that addition of unfolded proteins to hexameric DegP causes an increase in the proportion of higher-order multimers. Concomitantly, the level of protease activity was found to increase. These findings strongly suggest that the DegP hexamer represents a resting state that can



Tobias Krojer

be activated to form higher-level, proteolytically active structures by the presence of unfolded proteins.

The DegP hexamer thus represents a highly efficient trap for misfolded proteins. In Tim's own words, "it's like one of those traps used to catch polar bears. It's attractive to misfolded proteins because it contains a 'bait' – in this case hydrophobic residues – and once it catches them it doesn't let go." Once misfolded proteins are caught they find themselves in an extremely hostile environment thanks to the proximity of the active proteolytic sites. Only those proteins that are able to resume their correctly folded structures – and quickly – are able to escape degradation.

DegP has a further function in the cell: it is somehow involved in the transport of outer-membrane proteins (OMPs) between the inner and outer membranes. The new structure also provides a clue to DegP's role in this process. The size of the 24-mer DegP complex (195 Å) is such that it could potentially span the entire gap between the inner and outer membranes. Furthermore, the PDZ domains of the 24-mer were shown to associate with liposomes so can clearly bind to membranes. Taken together, these findings suggest that the DegP 24-mer may represent a tunnel to protect OMPs as they travel across the periplasm. Consistent with this idea, deleting DegP did not lower the total amounts of OMPs in cells but reduced the proportion of these proteins in the outer membrane. OMPs thus "run the gauntlet" of DegP protease domains on their journey to the outer membrane. To understand how this is possible, Tobias and Tim investigated the structures of complexes of an OMP with the 12-mer and 24-mer forms of DegP by means of electron microscopy. The results suggested that OMP monomers were contained within the structures in almost native conformation. DegP is thus able to sequester OMP monomers in a folded state.

Taken together, the findings enable a model for DegP's various functions to be proposed. In its resting state, the protein exists as a hexamer with a central compartment able to sequester unfolded proteins. When the level of unfolded proteins rises, higher-order DegP complexes are formed, which have greater proteolytic activity and are thus able to degrade the unfolded proteins. Nevertheless, the higher-order structures do not degrade (correctly) folded proteins, so they are able to act as pores for the transport of OMPs between the inner and outer membranes. The fate of proteins inside the DegP cavity is thus determined by their ability to adopt their fully folded conformations. Consistent with this model, *in vitro* studies have shown that OMPs spontaneously fold into their correct structures.

Attractive though the model is, it leaves several questions open. One point that Tim plans on investigating further relates to the "soft treatment" DegP appears to give to OMPs: why DegP preferentially stabilizes these proteins over others is at present completely unclear. Tim poses the question thus: "DegP may be like a bad schoolteacher who has certain favourites in the class or it may be an unbiased judge, assessing all proteins on their individual merits."

DegP may thus potentially be the first protein to show complex patterns of human behaviour. Not only does it commit crimes of passion, "murdering" other proteins that come too close to it, but it also seems capable of showing favouritism to certain proteins …

Text by Graham Tebb

Tim Clausen

Surface representation of the half-sliced DegP24 oligomer (colored white) with a modeled Outer Membrane Protein substrate (colored by electrostatic potential).

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 PR agency, 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. The collaboration is known under the name "IMP-IMBA Research Center".
More than 1000 people from 40 different nations currently work at the Campus VBC. Campus members enjoy a scientifically and socially stimulating environment and take advantage of shared facilities such as the Max Perutz Library 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.

THE IMP AND ITS SURROUNDINGS

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 Thursday seminar series invites renowned scientists to present their discoveries; everyone is encouraged to interact with these guests. Additionally, in 2008, the IMP will initiate a monthly IMP seminar series to highlight additional outstanding scientific researchers from around the world. 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 Friday 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. In 2007, Vienna hosted both the FEBS conference on Molecular Machines and the European Drosophila Research Conference, attracting numerous top researchers to the city. 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.

HARTMUT BEUG

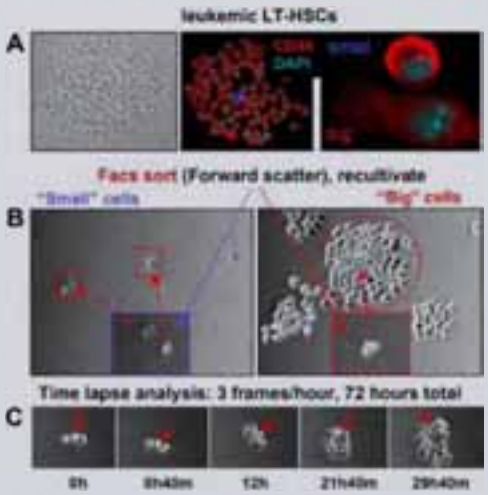
Tumor Progression: Abnormal Developmental Plasticity/ Reprogramming?

hartmut.beug@imp.ac.at

Hartmut Beug / Senior Scientist

Agnes Csiszar / Postdoc
Andrea Hoelbl / Postdoc
Sabine Maschler / Postdoc
Memetcan Alacakaptan / PhD Student
Boris Kovacic / PhD Student
Helmuth Gehart / Diploma Student
Silvia Wirth / Diploma Student
Katrin Fischhuber / Masters Student
Gabi Litos / Technical Assistant
Eva Maria Wiedemann / Technical Assistant

1



In leukaemia and carcinoma development, combinations of diverse oncogenes / tumour suppressor genes induce abnormal survival, proliferation, and developmental / behavioural plasticity in tumour cells, which may adopt stem cell properties. Using both genetically modified mice and in vivo-like cell culture models, we focus on molecular mechanisms that change haematopoietic stem cells into leukaemia-initiating cells and that reprogram epithelial cells into primitive mesenchymal-like cells during carcinoma progression / metastasis.

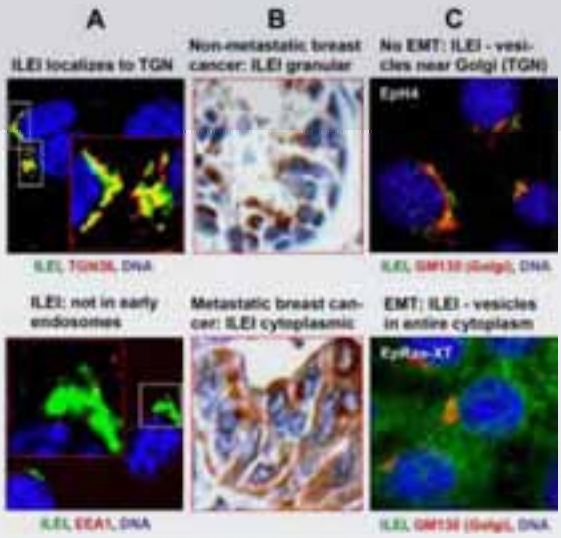
Mechanisms in haematopoietic progenitor renewal and leukaemogenesis.

To cause human leukaemia, mutated transcription factors/chromatin regulators often co-operate with mutated/overexpressed receptor tyrosine kinases/signal transducers, a paradigm established by us more than 10 years ago. In the erythroid lineage, progenitor renewal requires cooperation of the EpoR, c-Kit, and the glucocorticoid receptor (GR) – also essential for stress erythropoiesis *in vivo* – both in primary erythroblast cultures from both wt- and mutant mice, and in immortal, but diploid and *in vivo*-like murine ES cell-derived erythroblasts (ESEPs). The latter cells were almost indistinguishable from erythroid progenitors *in vivo*, both in growth factor dependence and terminal differentiation. Using these cell systems, we showed that lack of Stat5, p38- MAPK- and Flk-1 caused late- and early erythropoiesis defects, respectively. Interestingly, Stat5 also turned out to be a critical regulator of iron metabolism during erythropoiesis, leading to microcytic anaemia in Stat5-/- mice. Furthermore, we purified haematopoietic stem cells (HSC) from multi-lineage mouse leukaemias caused by the human leukaemia oncogene BCR-Abl and a constitutively active Stat5 mutant (cS5), showing that they functioned as leukaemic stem cells (LSCs). In contrast to normal HSCs, these LSCs showed unlimited expansion capacity in culture (Fig. 1), allowing their molecular characterisation. We now focus on possible molecular and cellular differences between these LSCs and normal HSCs by expression profiling and phenotypical analysis. We also study the significance of STAT1/STAT3 upregulation in STAT5-/- erythroid cells and whether Stat3 may complement the lack of the essential BCR-Abl signal transducer Stat5 (with collaborators from a joint long-term grant; SFB).

Tumour progression and metastasis: Ras plus mutated genes disrupting epithelial polarity?

Epithelial to mesenchymal transitions (EMT) are essential during normal development, carcinoma progression and metastasis, and may be a hallmark of carcinoma stem cells. Oncogenic Ras plus TGFβ cause EMT and metastasis in an *in vitro/in vivo* mammary epithelial cell model (EpH4/ EpRas). EpH4 cells expressing a Ras

2



3

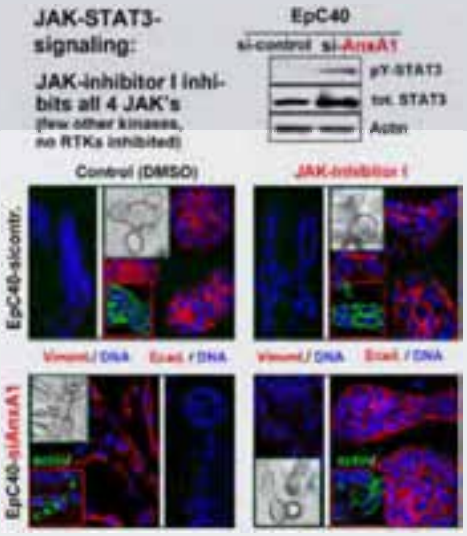


Figure 1: *In vitro culture of leukaemic stem cells: Large, CD44 low cells maintain long-term culture. A. Purified LT-HSCs from a p210-BCR-ABL induced leukaemia proliferate in serum-free medium containing FGF2, SCF, TPO and IGF2. During proliferation cultures become heterogenous, i.e. consisting of large, CD44^{lo} and smaller CD44^{hi} cells. B. Sorting of the small (blue) and large cells (red) shows that the large cells proliferate (red circle), while the small cells undergo apoptosis (red squares). To obtain long-term cultures, large cells are sorted from 1-week-old cultures, seeded at low density to form secondary cultures and sorted/recultivated after another week etc. C. Time lapse movies show that large cells divide synchronously for 4–5 generations, while one cell in the cluster remains stationary (arrows).*

Figure 2: *Delocalisation of ILEI-containing trans-Golgi-network vesicles upon EMT: predictive for metastatic breast cancer. A. Colocalisation studies of ILEI and markers for early endosomes, Golgi apparatus (see panel C, top) and trans-Golgi network (TGN) show that ILEI colocalises with the TGN. B. Human breast cancers progressing (bottom) or not progressing (top) to metastasis (15 years observation time) show granular and cytoplasmic ILEI staining by IHC ($p \approx 1x10\text{-}6$). C. EpH4 cells (nontumorigenic) and Ep-Ras-XT cells (EMT, tumours, metastases) show ILEI in Golgi-near TGF vesicles (top), which delocalise to the entire cytoplasm after EMT (EpRas-XT cells), explaining the low resolution subcellular ILEI localisations in panel B.*

Figure 3: *The JAK/STAT3 pathway is essential for EMT induction in EpC40 cells by RNAi-knockdown of AnxA1. Top panel: AnxA1-RNAi causes upregulation of activated- (pY-STAT3) and total STAT3. Middle panels: EpC40 siFFL control cells form compact, sometimes hollow structures (insets, green frame) that express plasma membrane-E-cadherin positive, cortical actin (insets, red frame) but no vimentin in the presence and absence of JAK-inhibitor 1 (see top, text on right). Bottom panels: EpC40-siAnxA1 cells without inhibitor show complete EMT (unordered structures of fibroblastoid cells; insets, green frame), that are vimentin-positive, E-cadherin-negative and express actin in stress fibres (insets, red frame). The JAK-inhibitor I reverses the EMT-phenotype of EpC40-siAnxA1 cells to an epithelial phenotype similar to the control cells (compare left middle and bottom panels).*

mutant hyperactivating the PI3K pathway (EpC40 cells), however, fail to undergo TGFβ-induced EMT and are tumorigenic but not metastatic. Out of ≈30 EMT-specific genes identified via mRNA expression profiling or protein studies we currently focus on 4 genes [interleukin-like EMT inducer (ILEI), cellular regulator of E1A gene (CREG), Annexin-A1 (AnxA1) and the mouse orthologue of Drosophila Scribble (mScrib)]. ILEI causes reversible EMT, slow tumour growth and metastasis in oncogene-free EpH4 cells, but requires oncogenic Ras to cause EMT, tumorigenicity and metastasis in cultured, *in vivo*-functional p19ARF-/- hepatocytes. ILEI thus resembles CREG, AnxA1 and m-Scrib in their requirement for oncogenic Ras to cause EMT and metastasis upon loss or gain of function. Surprisingly, ILEI could be shown to cause EMT by intracellular mechanisms, since mutation of its post-translational cleavage site – utilised by the serum protease Plasmin to produce mature, secreted ILEI – generated non-secreted forms of ILEI which still induced EMT upon overexpression. Secreted ILEI may however, also have extracellular functions since non-cleaved, secreted ILEI binds to Fibronectin – to be cleaved by plasmin? – and forms homodimers. Importantly, ILEI localises to the trans-Golgi-network in normal cells, but is redistributed to vesicles in the entire cytoplasm upon EMT-induction, explaining why the cytoplasmic localisation of ILEI detected by immunohistochemistry was highly predictive for metastasis in human breast cancer and melanoma (Fig. 2). CREG, a secreted, mannose 6P-receptor binding, lysosomal protein, also caused EMT in EpC40 cells. Finally, RNAi-mediated knockdown of AnxA1 – a Ca^{++}-binding, membrane-associated protein regulating multiple aspects of vesicle-trafficking – caused EMT and metastasis in EpC40 cells, but reversed EMT and abolished metastasis when overexpressed in dedifferentiated, metastatic human mammary carcinoma cell lines. AnxA1 RNAi-induced EMT in EpC40 cells required JAK/STAT3 signaling (Fig. 3) and Erk/ MAPK-activation. These results led to the novel idea that cooperation of Ras with loss or gain of function in proteins of molecular machines essential for epithelial polarity causes EMT and metastasis, possibly via altering intracellular organelle-associated signal transduction known to occur in MAPK-, PI3K, TGFβR- and STAT3 signaling. Encouraged by our finding that RNAi-knockdown of mScrib – a basolateral epithelial polarity complex protein – induced Ras-dependent but TGFb-independent EMT, we will employ MDCK cells (the major cell model to study epithelial polarity), undergoing complete EMT in response to an oestradiol-activated RafDD-ER protein to further investigate the above hypothesis.

MEINRAD BUSSLINGER
Stem Cell Commitment in Hematopoiesis

meinrad.busslinger@imp.ac.at

Meinrad Busslinger / Senior Scientist and Director of Academic Affairs

Anja Ebert / Postdoc
Stephen Malin / Postdoc
Ivan Bilic / Postdoc
Thorsten Decker / Postdoc
Aleksandar Dakic / Postdoc
Roger Revilla-i-Domingo / Postdoc
Heike Dorninger / PhD Student
Mareike Hoffmann / PhD Student
Jasna Medvedovic / PhD Student
Bojan Villagos / PhD Student
Anca Tigan / PhD Student
Martina Minnich / Diploma Student
Anna Azarjana / Diploma Student
Abdallah Souabni / Technical Assistant
Christian Spona [1] / Technical Assistant
Qiong Sun / Technical Assistant
Barbara Werner / Technical Assistant
Erika Kiligan / Technical Assistant

Markus Jaritz / GEN-AU Bioinformatician

[1] *until August*

Tissue-restricted stem cells give rise to the different cell types of an organ by undergoing commitment to and subsequent differentiation along distinct lineages. By using a combination of mouse transgenic, cell biological and molecular approaches, we investigate the mechanisms by which transcription factors such as Pax5, E2A, Notch1 and GATA3 control the commitment of early haematopoietic progenitors to the lymphoid lineages.

B cell commitment

A fundamental question in haematopoiesis 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 the transcription factors E2A, EBF1 and Pax5. E2A and EBF1 function as B cell specification factors by activating B-lymphoid genes. Pax5 in turn controls the commitment of lymphoid progenitors to the B cell lineage, as shown by our group. In the absence of Pax5, B cell development is arrested at an early progenitor (pro-B) cell stage. Pax5-deficient pro-B cells are uncommitted progenitor cells, as they can still develop into various haematopoietic cell types except for B cells, which are only generated upon restoration of Pax5 expression (Figure 1). Pax5 thus functions as the B-lineage commitment factor by restricting the developmental potential of progenitor cells to the B cell pathway. Surprisingly, conditional Pax5 deletion allows mature B cells from peripheral lymphoid organs to dedifferentiate in vivo back to early uncommitted progenitors in the bone marrow, which are subsequently able to develop into functional T cells. These experiments demonstrate that Pax5 functions as a critical B cell identity factor to maintain B-lineage commitment from the pro-B to the mature B cell stage. In order to identify upstream regulators of Pax5, we have identified 8 DNase I hypersensitive (HS) sites in the Pax5 promoter region and 4 HS sites in an intronic enhancer (Figure 2), which together faithfully recapitulate the Pax5 expression pattern in transgenic mice.

Molecular mechanism

Pax5 fulfils a dual role in B-lineage commitment by activating the expression of B-cell-specific genes and by repressing the transcription of lineage-inappropriate genes. This reprogramming of gene expression restricts the broad signaling capacity of uncommitted progenitors to the B cell pathway, induces V_H-DJ_H recombination, facilitates (pre)B cell receptor signaling, regulates B lymphocyte adhesion and migration and promotes development to the mature B cell stage. Chromatin profiling by ChIP-on-chip analysis demonstrated that Pax5 directly activates the chromatin at promoters or putative enhancers of Pax5 target genes.

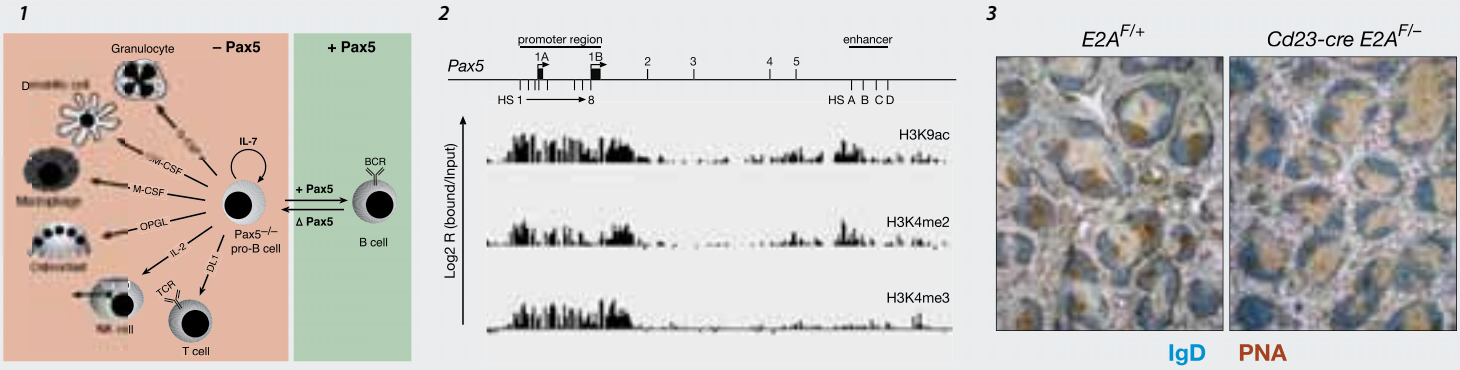


Figure 1: *B-lineage commitment by Pax5. The uncommitted Pax5$^{-/-}$ pro-B cells are able to differentiate into several haematopoietic cell types under the influence of the indicated cytokines. Conditional Pax5 deletion (ΔPax5) results in dedifferentiation of B lymphocytes to uncommitted progenitors.*

Figure 2: *Regulatory sequences of Pax5. ChIP-on-chip analysis of wild-type pro-B cells on high-density oligonucleotide arrays was used to map the active histone modifications H3K4me2, H3K4me3 and H3K9ac along the Pax5 locus (exons indicated by numbers). All three marks are present at the Pax5 promoter region containing 8 DNase I hypersensitive (HS) sites, whereas only H3K4me2 and H3K9ac are present in the intronic enhancer consisting of 4 HS sites.*

Figure 3: *Important role of E2A in germinal centre (GC) B cell development. The Cd23-cre line completely deletes the floxed (F) E2A allele in mature B cells prior to GC B cell development. At day 8 after immunisation with sheep red blood cells, PNA^{+} germinal centres (detected by immunostaining on spleen sections) were reduced in size and number in the spleen of Cd23-cre E2A$^{F/-}$ mice compared to control E2A$^{F/+}$ mice.*

Transcriptional control of B cell development

We recently studied the role of E2A and Pax5 throughout B-lymphopoiesis by using conditional mutagenesis. These analyses demonstrated that both transcription factors are essential for the development of pro-B, pre-B and immature B cells in the bone marrow and for the antigen-dependent formation of germinal centre B cells in the spleen (Figure 3).

T cell specification

Signaling through the Notch1 receptor is essential for the initiation of T cell development in the thymus. T cell specification also depends on the transcription factor GATA3. We previously demonstrated that Notch signaling induces the expression of Gata3. To investigate the molecular function of Notch1 and GATA3 in early T-lymphopoiesis, we are identifying direct target genes of these two regulators by conditional mutagenesis, gene expression profiling and ChIP analysis.

Spatial control 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 have a large size ranging from 0.67 (Tcrb) to 3 (Igh and Igk) megabases and exhibit a complex organisation with the D, J and C segments constituting the proximal (3′) domain of each locus. The largest part (> 80%) of the Ig and Tcr loci is devoted to the V gene cluster, which is separated from the proximal domain by a large distance on linear DNA. We have shown that the Igh and Igk loci undergo reversible contraction in pro-B cells and pre-B cells, respectively, whereas Tcrb and Tcra/d loci contract by looping in pro-T cells and pre-T cells, respectively. Reversible locus contraction by looping is thus a general mechanism that promotes the spatial communication between V genes and the proximal domain by juxtaposing distantly located V genes next to D or J segments, thus facilitating synapse formation and V gene recombination. We are currently elucidating the molecular mechanism controlling locus contraction.

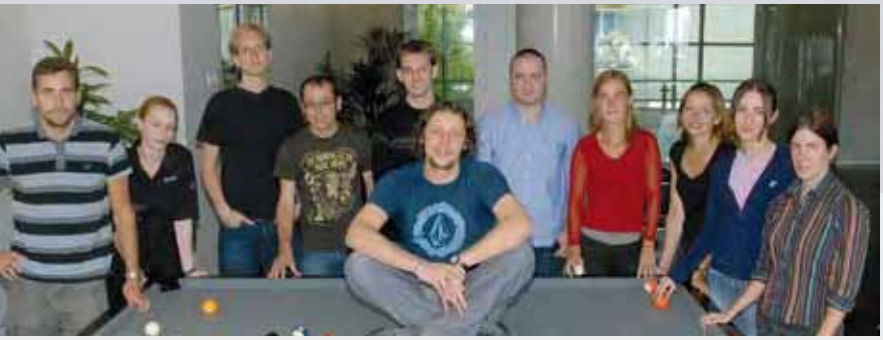
FURTHER READING

Busslinger, M. 2004. Transcriptional control of early B cell development. Annu. Rev. Immunol. 22:55-79.

Busslinger, M., and Tarakhovsky, A. (2006). Epigenetic control of lymphopoiesis. In Epigenetics, D. Allis, T. Jenuwein, D. Reinberg and Caparros, M.-L., eds. (New York, Cold Spring Harbor Laboratory Press), pp. 397-414.

Cobaleda, C., Schebesta, A., Delogu, A., and Busslinger, M. (2007). Pax5: the guardian of B cell identity and function. Nat. Immunol. 8, 463-470.

Cobaleda, C., and Busslinger, M. (2008). Developmental plasticity of lymphocytes. Curr. Opin. Immunol. 20, 139-148.



TIM CLAUSEN

Molecular Mechanisms of Protein Quality Control and Stress Response

tim.clausen@imp.ac.at

Tim Clausen / Group Leader

Rebecca Kirk / Postdoc
Tobias Krojer / Postdoc
Boril Boyanov [1] / PhD Student
Jacob Fuhrmann / PhD Student
Katharina Linn Gazda / PhD Student
Justyna Sawa-Makarska [3] / PhD Student
Bastian Schmidt / PhD Student
Doris Hellerschmied / Diploma Student
Anita Lehner / Diploma Student
Max Rössler [2] / Diploma Student
Juliane Kurt / Technical Assistant
Robert Kurzbauer / Technical Assistant
Christian Lahsnig / Technical Assistant

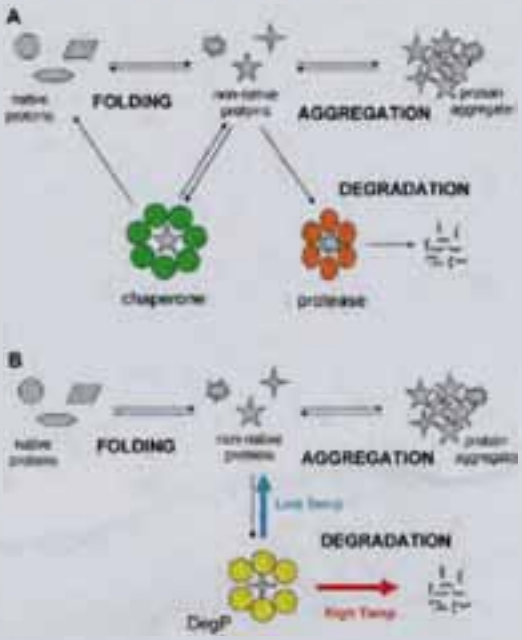
[1] *until September*
[2] *until June*
[3] *until April*

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 (Figure 1A), a process that is regulated by multiple stress response pathways. We perform a structure-function analysis of several of these factors in order to better understand how cells deal with folding stress.

"Refoldable" or "Not Refoldable" - Is that the Question?

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 (Figure 1B) and thus offers unique possibilities for investigating how cells distinguish between proteins that can be refolded and "hopeless" cases that need to be degraded. DegP consists of a protease and two PDZ domains, which are prominent protein-protein interaction motifs. It is a widely conserved protein found in most organisms. Prokaryotic DegP has been attributed to the tolerance against various folding stresses as well as to pathogenity. Human homologs are believed to be involved in arthritis, cell growth, unfolded protein response, and apoptosis.

1



As we have shown by protein crystallography, the functional DegP hexamer is formed by the staggered association of two trimeric rings and represents a novel type of cage-forming protein (Figure 2). The six protease domains construct the top and bottom of the molecular cage, whereas the twelve PDZ domains generate the mobile sidewalls. We crystallized the "low temperature" chaperone form of DegP in both open and closed conformations. The transition between these two states is mediated by the inherent *en-bloc* mobility of the PDZ domains functioning as gatekeepers of the DegP cage. Thus, the PDZ domains might permit a direct coupling of substrate binding and subsequent translocation into the inner chamber. This two-step binding process is similar to that of other cage-forming proteins, such as the proteasome or Clp proteins.
The proteolytic sites of DegP are located in the inner cavity of the cage and can only be accessed by unfolded proteins. In the chaperone form, they were present in an inactive state, in which catalysis as well as substrate-binding were abolished. Thus it still remains elusive how DegP degrades unfolded proteins and how the switch in activity is accomplished. We will screen different DegP mutants for potent protease inhibitors that could stabilize the "high temperature" protease form and allow its crystallization. Furthermore, we plan to extend the DegP approach to eukaryotic homologs (e.g. plant DegP1) and to functionally related proteases containing PDZ domains like DegQ and Tsp.

2

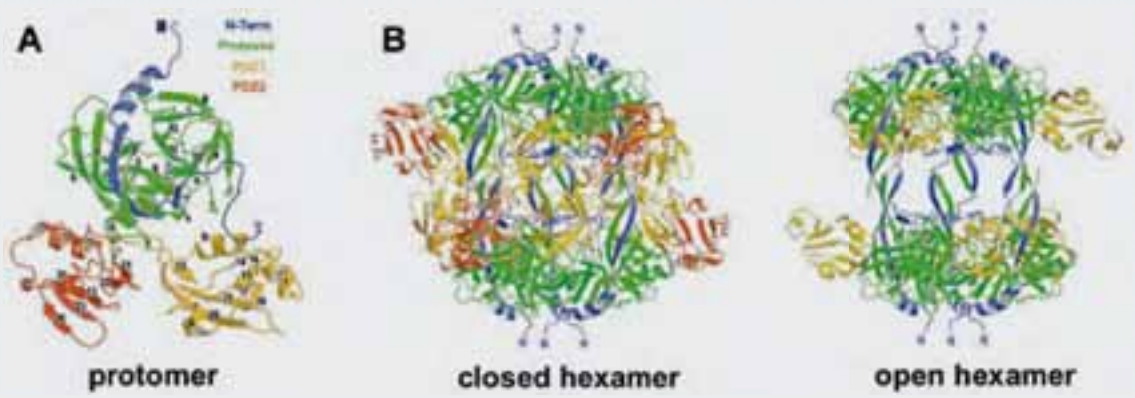


Figure 1: *Protein quality control. (A). The diagram illustrates the different fates of misfolded non-native proteins. According to the "kinetic partitioning model" of Gottesman and coworker, aggregate formation is counteracted by the action of proteases and chaperones. (B). DegP combines the antagonistic 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 elevated temperatures the protease function is dominant.*

Figure 2: *Structure of DegP. (A). Ribbon presentation of the protomer, in which the individual domains are colored differently. Residues of the catalytic triad are shown in a ball-and-stick model.*
The nomenclature of secondary structure elements and the termini of the protein and flexible regions are indicated. (B). Side views of the DegP hexamer that was observed in two different forms - in a closed form and in a remarkable open form. The transition between both states is accomplished by the mobile PDZ domains.

Figure 3: *Structure of DegS. (A). Ribbon presentation of the DegS trimer (top view) with each subunit colored differently. (B). Diagram of DegS activation. In DegS, the PDZ domain obtains a regulatory function, offering a binding site for an allosteric activator.*

Stress Response by Regulated Intramembrane Proteolysis

In the extracytoplasmic stress response, the stress signal must cross a membrane and thus sophisticated pathways are required to transduce the signal from one compartment to another. A well-studied system is the bacterial σE stress response that is triggered by excessive amounts of unfolded proteins in the periplasm, particularly unfolded outer membrane porins. The alternative σ-factor σE is a transcriptional activator that directs the expression of several stress genes. Under non-stress conditions, the activity of σE is inhibited by RseA, a membrane spanning protein, whose cytoplasmic domain captures σE, thereby preventing σE from binding to RNA polymerase. Activation of σE is controlled by a proteolytic cascade that is initiated by the DegS protease. Upon folding stress, peptides corresponding to the C-terminus of misfolded outer membrane proteins serve as stress signals, bind to the PDZ domain of DegS and activate its protease function. Activated DegS cleaves RseA, thereby triggering the σE-response.

Recently, we determined the crystal structures of three distinct states of DegS from *E. coli*. DegS alone exists in a catalytically inactive form. Binding of stress-signaling peptides to its PDZ domain induces a series of conformational changes that activates protease function. Backsoaking of crystals containing the DegS-activator complex revealed the presence of a trypsin-like activation domain and demonstrated the reversibility of activation. Taken together, the structural data illustrate in molecular detail how DegS acts as a periplasmic stress sensor (Figure 3). The central regulatory element is the PDZ domain that offers a binding site for a peptidic cofactor and thereby couples the recognition of misfolded protein with the activation of the σE response.

Future structure-function studies are aimed at validating this model. Furthermore, we will start structural work on the second protease of the σE pathway, the membrane protease YaeL.

3

FURTHER READING

Wickner, S., Maurizi, M. R. & Gottesman, S. (1999) Posttranslational quality control: Folding, refolding, and degrading proteins. Science 286, 1888-1893.

Krojer, T., Garrido-Franco, M., Huber, R., Ehrmann, M. and Clausen, T. (2002) Crystal structure of DegP (HtrA), a novel protease-chaperone machine. Nature, 416, 455-359.

Clausen, T., Southan, C. and Ehrmann, M. (2002) HtrA: High temperature requirement And more. Mol. Cell, 10, 443-455.

CARRIE COWAN
Cell polarity in development

carrie.cowan@imp.ac.at

Carrie Cowan / Group Leader

Dominika Bienkowska / PhD Student
Anne Göppert / PhD Student
Sabina Sanegre-Sans / PhD Student
Silke Reiter / Diploma Student
Sophia Millonigg / Technical Assistant
Harue Wada / Technical Assistant

Cell polarity allows an enormous variety of specialised functions during development. Despite the range of contexts in which cell polarity is essential, the general steps of polarisation are the same: establishment, transduction, and segregation. We are investigating the molecular mechanisms of cell polarisation in one-cell C. elegans embryos.

Research Activities

Cell polarity is essential for development. Polarity establishes spatial information in a cell, allowing for different functions in different places. Cell polarity allows cells to migrate, to change shape, and to transport molecules in or out. Cell polarity also mediates the segregation of fate determinants, allowing for the development of different cell types. Bacteria, fungi, plants and animals all require cell polarity for morphogenesis and functional specialisation. Without cell polarity, cells would create homogenous masses, and indeed, the loss of cell polarity is an early event in tumour formation.

Our group is investigating how cell polarity is established, transduced, and segregated in *C. elegans* embryos. We use a combination of forward and reverse genetics, in vivo biochemistry, high-resolution time-lapse microscopy, ultrastructural reconstructions, automated quantitative analysis, and mechanical manipulations to answer the following questions:

How is cell polarity established?

In one-cell *C. elegans* embryos, cell polarity is established by the centrosome. Prior to polarisation, the centrosome floats in the cytoplasm, but coincident with polarity establishment, the centrosome moves to the cell cortex. The centrosome initiates a global reorganisation of the cortical acto-myosin network, which in turn promotes an asymmetric distribution of the highly conserved PAR proteins. We are investigating how the centrosome communicates with the cortex, including the mechanism of centrosome–cortex juxtaposition and the nature of the signal that initiates polarity.

How is cell polarity transduced into the cytoplasm and nucleus?

In one-cell *C. elegans* embryos, cell polarity is defined by complementary anterior and posterior cortical domains, which comprise distinct PAR proteins. Fate determinants, such as the germline-specific transcriptional repressor PIE-1, localise to the cytoplasm and nucleus. PIE-1 is distributed asymmetrically in the one-cell embryo, ensuring it is inherited only by the future germline blastomere.

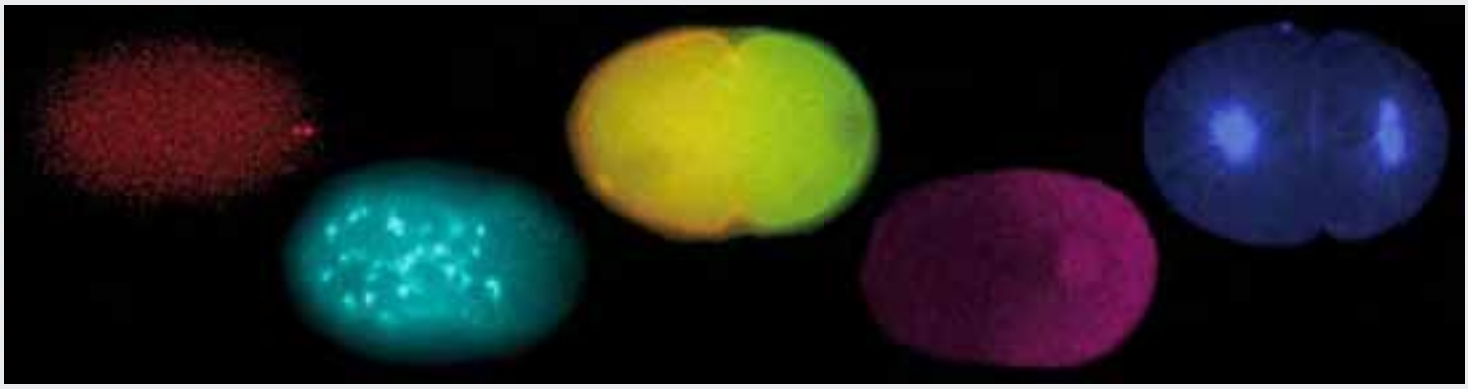


Figure: *Polarity establishment and segregation in C. elegans embryos. Centrosomes (red dots) move to the cortex to initiate polarization of the cortex. This leads to changes in the acto-myosin cytoskeleton (cyan) that underly the formation of anterior (red) and posterior (green) cortical domains, defined by PAR proteins. Cortical polarity controls the unequal segregation of fate determinants in the cytoplasm (magenta). During asymmetric division, the mitotic spindle (blue) repositions the boundary between the PAR domains, ensuring cell fate is inherited exclusively. This whole process takes approximately 25 minutes.*

We are investigating how two-dimensional polarity at the cortex can organise the three-dimensional space of the cytoplasm to achieve asymmetric PIE-1 localisation.

How is cell polarity segregated during division?

In one-cell *C. elegans* embryos, the anterior and posterior cortical cell polarity domains are inherited by either the anterior or posterior blastomere. The boundary between the PAR domains is repositioned to match the site of cell division to achieve this exclusive segregation. The process of domain boundary correction operates through a size threshold that can, in extreme cases, lead to mis-segregation of cell polarity and cell fate transformation. We are investigating how the PAR domain boundary responds to the cytokinesis furrow, including the mechanism of domain size sensing and the furrow properties that signal boundary correction.

BARRY DICKSON
Neural circuits

barry.dickson@imp.ac.at

Barry Dickson / Senior Scientist and Managing Director of Science

Young-Joon Kim / Postdoc
Carlos Ribeiro / Postdoc
Laszlo Tirian / Postdoc
Anne von Philipsborn / Postdoc
Alexandre Widmer / Postdoc
Salil Bidaye / PhD Student
Kai Feng / PhD Student
Martin Häsemeyer / PhD Student
Sabrina Jörchel / PhD Student
Christopher Masser / PhD Student
Christian Schusterreiter / PhD Student
Bettina Spitzweck / PhD Student
Nilay Yapici / PhD Student
Jai Yu / PhD Student
Simone Latkolik / Diploma Student
Katarina Bartalska / Technical Assistant
Ruth Fuchs / Technical Assistant
Katharina Schernhuber / Technical Assistant
Chuan-Ju Wang / Technical Assistant

We are using molecular genetic techniques to study the function of neural circuits in Drosophila. We want to understand how the brain works. How does information processing in neural circuits generate complex animal behaviour? As a model system, we focus on the fly's mating behaviours. These behaviours are robust, adaptive, and particularly amenable to genetic analysis.

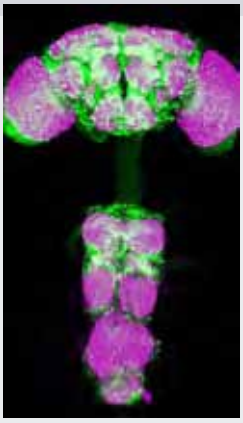
A major goal for neuroscience is to understand how information processing in neural circuits guides behavioural decisions. How, at any given moment, does an animal decide what to do? Somehow, the brain selects the best course of action by integrating information from multiple sources – sensory input, internal physiological states, and individual experience. We would like to find out how the brain does this.

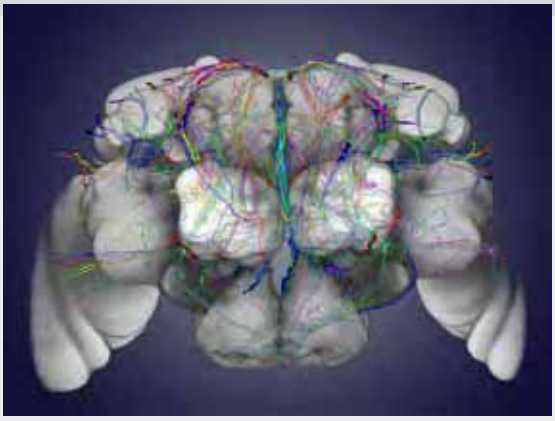
As a model, we have chosen to study the sex life of the fruit fly *Drosophila melanogaster* [1]. During mating, flies make decisions that are critical for their reproductive success, and hence their evolutionary fitness. Upon encountering another fly, the male estimates his chances of success and decides if it is worth investing time and energy in courtship. Whether or not mating actually occurs is a matter of female choice, based on how she assesses of her suitor's quality, as well as her own readiness to mate. 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 linking cellular biochemistry, circuit function, and animal behaviour.

The male brain

Remarkably, the different mating behaviour 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 are currently developing methods to gain genetic access to distinct subsets of *fru* neurons, so that we can study the anatomy and physiology of the *fru* circuit at single-cell resolution (Fig. 2). We would like to know if and how each of type of *fru* neuron contributes to courtship, what types of signal each neuron processes, and how and to what extent sexually dimorphic processing in these neurons leads to the distinct behaviours of males and females.





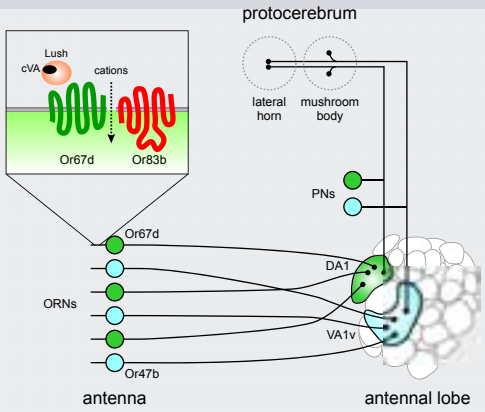


Figure 1: *fru⁺ neurons in the CNS, shown in green. Synapses are stained in magenta. This is a confocal image of the brain and ventral nerve cord of a fru^{GAL4} UAS-GFP male.*

Figure 2: *Genetic dissection of fru⁺ neurons in the central brain. Distinct neuronal cell types have been labeled, imaged, and traced in different colours. A synaptic counterstain was used to register individual samples onto a common reference brain, creating a digital atlas of the fru circuit.*

Figure 3: *cVA detection and processing in the fru⁺ Or67d⁺ ORNs and DA1 PNs.*

One important class of *fru*⁺ neurons are the olfactory receptor neurons (ORNs) that detect pheromones. We have recently found that one subclass of *fru*⁺ ORNs expresses the odorant receptor Or67d and responds to a male sex pheromone. These neurons connect to second-order olfactory neurons in the antennal lobe of the brain (called DA1 projection neurons) (Fig. 3). The neurons are also *fru*⁺, and appear to make sexually dimorphic connections in higher brain centers. This may be a critical site for sex-specific processing of pheromone signals.

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. The biggest factor in the female's decision, however, is her own mating status. Virgin females are generally receptive to courting males, whereas females that have recently mated are not. This difference can largely be attributed to a small peptide, called the sex peptide (SP), that is present in the first male's seminal fluid. If females mate to males that lack SP, they remain receptive to other males. Conversely, direct injection of SP into virgin females renders them unreceptive. But how and where does SP act in the female to modulate her mating decision?

By screening our transgenic RNAi library [2], we have recently identified a molecular receptor for SP, a G-protein coupled receptor we call SPR (sex peptide receptor) [3]. SPR is broadly expressed in the nervous system, but we find that it is specifically required in a small subset of *fru*⁺ sensory neurons that innervate the reproductive tract and project axons to various regions in the central nervous system. Our ongoing efforts are aimed at understanding how SP modulates the function of these neurons, and how this in turn impacts the functioning of circuits in the brain that assess male courtship signals and decide whether or not to allow mating.

FURTHER READING

[1] Dickson, B. J. (2008). Wired for sex: the neurobiology of Drosophila mating decisions. Science. 332: 904-909.

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

[3] Yapici, N., Kim, Y.-J., Ribeiro, C., and Dickson, B.J. (2008). A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. Nature 451: 33-37.

CHRISTINE HARTMANN

Formation and Patterning of the Vertebrate Skeleton

christine.hartmann@imp.ac.at

Christine Hartmann / Group Leader

Sayumi Fujimori / Postdoc
Daniela Kostanova-Poliakova / Postdoc
Markus Winter / Postdoc
Natalia Lyashenko / PhD Student
Mehran Rafigh / PhD Student
Chandra Sekhar Amara / PhD Student
Alexander Apschner / Diploma Student
Richard Latham / Technical Assistant
Martina Weissenböck / Technical Assistant

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 are determined as well as the positions of articulations (joints). Most skeletal elements are formed as cartilaginous templates and need to be remodeled into bony tissue to provide strength to the skeleton. This process is referred to as endochondral ossification. We use mouse and chick as model organisms to gain insight into how these different processes 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, plays an essential role in 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 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.

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)

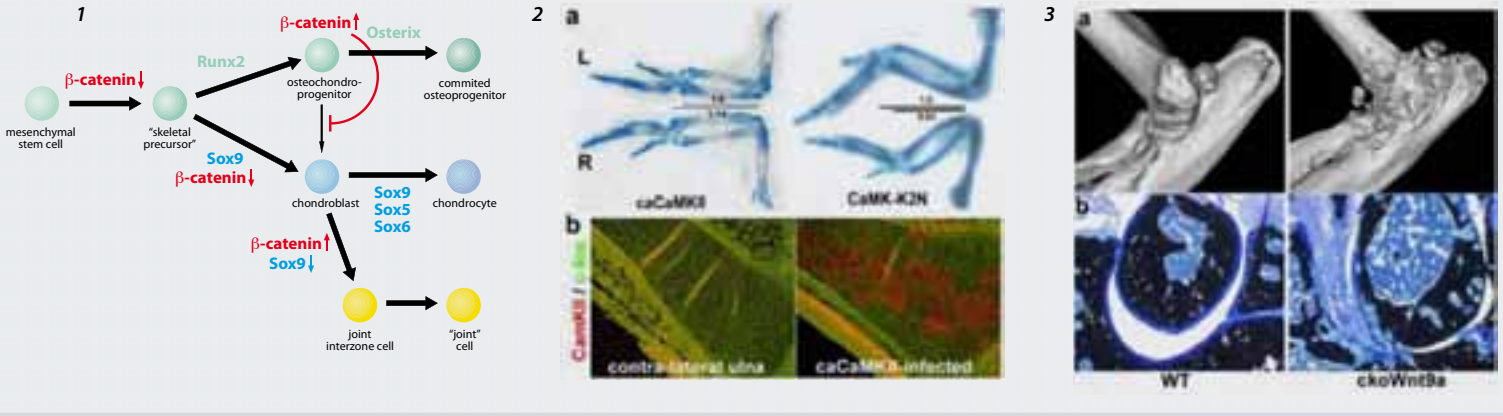


Figure 1: *β-catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of β-catenin can differentiate into skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage 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 the 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 in maintaining joint integrity. Conditional deletion of Wnt9a specifically in the limb mesenchyme results in progressive changes in various joints, which show hallmarks of osteoarthritis. In addition these mice show ectopic mineralisation within joint ligaments (Figure 3).

In summary, our analysis has show that Wnt-signaling plays an important role during skeletal development, regulating differentiation of the various cell types and maturation of chondrocytes, and is important for maintaining the integrity of the developing and mature joint.

THOMAS JENUWEIN

Epigenetic Control by Histone Methylation

thomas.jenuwein@imp.ac.at; jenuwein@immunbio.mpg.de

Thomas Jenuwein / Senior Scientist
His group moved during 2008 to the Max-Planck-Institute of Immunobiology, Freiburg, Germany

Inti Dela Rosa-Velazquez / Postdoc
Barni Fodor / Postdoc
Masato Yonezawa / Postdoc
Michael Eisold / PhD Student
Barbola Gerle / PhD Student
Valentina Perrera / PhD Student
Ines Pinheiro / PhD Student
Mario Richter / PhD Student
Manuela Scaranaro / PhD Student
Susanne Opravil / Technical Assistant
Michaela Pagani / Technical Assistant

Andras Aszodi / Head of GEN-AU Bioinformatics
Andreas Sommer / GEN-AU Bioinformatician
Ido Tamir / GEN-AU Bioinformatician

Dörthe Nickel / NoE Administrator
Ylva Linderson / NoE Research Manager

Epigenetic mechanisms, such as histone modifications control eukaryotic development beyond DNA-stored information. The characterization of histone methyltransferases (HMTases) strongly established histone lysine methylation as a central epigenetic modification for the organization of eukaryotic chromatin with far-reaching implications for proliferation, cell-type differentiation, stem cell plasticity, gene expression, genome stability and cancer.

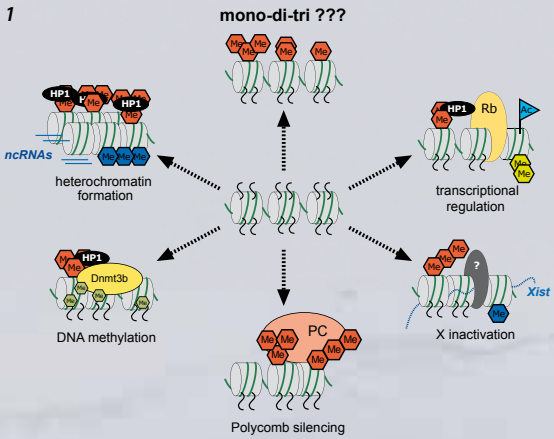
The indexing potential of histone lysine methylation.

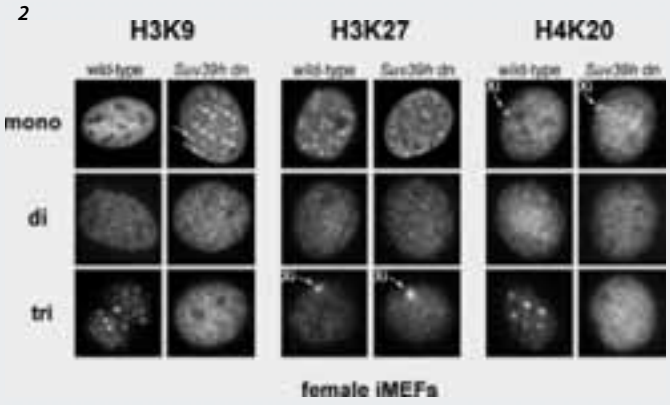
Histone lysine methylation has been linked with constitutive heterochromatin formation, X inactivation, Polycomb-group (PcG) dependent repression and epigenetic gene regulation at euchromatic positions (Figure 1). Each methylatable lysine residue in the histone N-termini can exist in a mono-, di- or tri-methylated state, thereby extending the coding potential of this particular histone modification. Using highly specific methyl-lysine histone antibodies together with quantitative mass-spectrometry, we could show that pericentric heterochromatin is selectively enriched for H3K9me3 and H4K20me3. This profile is dependent on the 'heterochromatic' Suv39h HMTases and on Suv4-20h enzymes. The Suv4-20h HMTases are nucleosomal-specific and require a pre-existing H3K9me3 mark for their recruitment to heterochromatin, thus revealing a silencing pathway for the induction of combinatorial histone lysine methylation imprints. By contrast, facultative heterochromatin at the inactive X chromosome (Xi) is characterized by a different methylation pattern (H3K27me3 and H4K20me1) (Figure 2) that is mediated by the PcG enzyme Ezh2 and another, currently unknown, HMTase. Our data underscore the combinatorial coding potential of histone lysine methylation as epigenetic landmarks in eukaryotic chromatin.

Epigenetic reprogramming by histone lysine methylation.

Currently, more than 50 distinct modifications (acetylation, methylation, phosphorylation, and others) have been described in the N-termini of the four core histones. Intriguingly, there is an under-representation of repressive marks in quiescent (resting) cells and in stem and regenerating cells, but a selective accumulation of aberrant histone lysine methylation profiles in aging, 'stressed' and tumor cells (Figure 3). These data suggest that distinct histone lysine methylation profiles contribute to the epigenetic 'make-up' of stem cells vs. more committed cells. To test this hypothesis, we have generated mutant mice that lack crucial HMTases, such as the Suv39h and Suv4-20h enzymes. In addition, we have screened chemical libraries (in collaboration with Boehringer Ingelheim, Ridgefield, USA) and identified a small molecule inhibitor (BIX-01294) for the 'eukaryotic' G9a HMTase. BIX-01294 can be used to transiently modulate H3K9me2 levels in mammalian chromatin. Finally, we have been characterizing

1





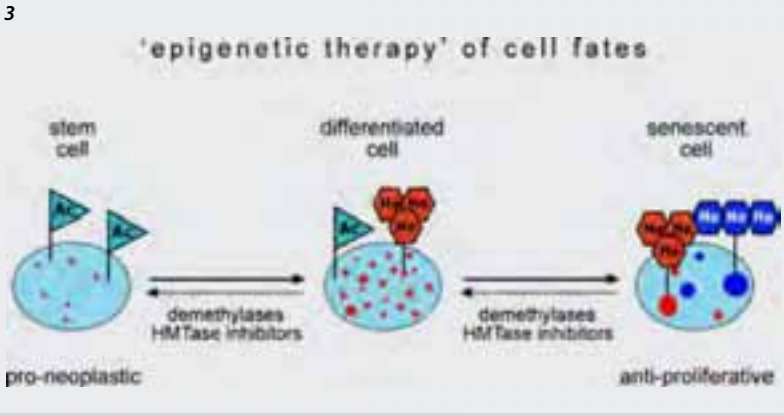


Figure 1: *The many faces of histone lysine methylation. The figure summarizes described roles of histone lysine methylation in major epigenetic paradigms. Distinct lysine positions are shown as colour-coded hexagons to indicate H3K4 (green), H3K9 (red triangular), H3K27 (red diagonal) and H4K20 (dark blue). DNA methylation is depicted by small orange hexagons.*

Figure 2: *Distinct methylation states are epigenetic landmarks in mouse interphase chromatin. Female wild-type and Suv39h dn iMEFs were stained with methyl-lysine histone antibodies that discriminate mono-, di- and tri-methylation of H3K9, H3K27 and H4K20. Foci of pericentric heterochromatin that were visualized with DAPI (not shown) are decorated by H3K9me3, H3K27me1 and H4K20me3 in wild-type nuclei. By contrast, the inactive X chromosome (Xi) is enriched for H3K27me3 and H4K20me1. The occurrence of H3K9me1 at pericentric heterochromatin in Suv39h dn nuclei is indicated by multiple arrows.*

Figure 3: *An 'epigenetic therapy' for cell fate specification. Normal, differentiated cells are characterized by a balanced representation of active (e. g. acetylation; blue flag) and repressive (e. g. lysine methylation; red and blue hexagons) histone modifications. In senescent or aged cells, repressive modifications accumulate at large blocks of 'ectopic' heterochromatin. By contrast, stem cells display a general under-representation of repressive histone modifications. These global histone modification patterns can be used as diagnostic markers for cell fate specification. In addition, they suggest a possible 'epigenetic therapy', in which demethylases and HMTase inhibitors may allow the reprogramming of senescent cells or, on the other hand, drive tumor cells into information overflow, chromatin catastrophe and apoptosis.*

jumonjiC-containing proteins that represent demethylases with the potential to remove repressive H3K9me3 marks. Together, these approaches promise to yield new insights into the plasticity of cell fate decisions and may offer novel strategies for exploratory research to modulate tissue regeneration and to revert aberrant development.

An epigenetic map of the mouse genome.

Alterations in the chromatin structure represent the key epigenetic principle to organize the information stored in the genome. In the context of the Austrian GEN-AU initiative (www.gen-au.at) and the European Network of Excellence (NoE) 'The Epigenome' (www.epigenome-noe.net), we have performed the large-scale analysis of epigenetic transitions in defined chromatin regions and along entire mouse chromosomes. In particular, we applied ChIP-chip (NimbleGen micro-arrays) and ChIP-seq (Solexa sequencing) with chromatin material from mouse ES cells, ES-derived erythroblasts and MEFs (fibroblasts). Our data indicate that very few histone lysine methylation marks (H3K4me3, H3K9me2 and H3K27me3) can be correlated with transcriptional activity of annotated promoters. For H3K9me3 and H4K20me3 we could not assign a direct function with transcriptional control. To address this paradox, we have applied mouse cells (ES and MEFs) that are mutant for each of the three major HMT pathways regulating H3K9me3 (Suv39h double-null), H3K27me3 (eed null, generated by A. Wutz) and H4K20me3 (Suv4-20h double-null). Through this wild-type vs. mutant comparison, we have derived epigenetic maps that reflect the intrinsic roles for the Suv39h and Suv4-20h enzymes and which reveal the partitioning of H3K9me3 and H4K20me3 primarily with intergenic regions that do not contain annotated genes. We propose that this context-dependent chromatin marking by distinct histone modifications will be important for the demarcation between transcription units vs. intergenic regions and contribute to the architecture and identity of mammalian chromosomes.

FURTHER READING

Allis, D., Jenuwein, T., Reinberg, D. (Editors). (2006). Epigenetics. Cold Spring Harbor Laboratory Press, New York.

DAVID KEAYS
The Molecular Basis of Migration

david.keays@imp.ac.at

David Keays / Independent Postdoctoral Fellow

Christoph Treiber / Diploma Student

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? I am investigating two very different forms of migration: (1) the migration of neurons; and (2) the migration of animals mediated by magnetic fields.

Neuronal Migration

Neuronal migration underlies the organisation of the mammalian brain. All neurons that are born in the proliferative ventricular zones (VZ) migrate to their final destination either radially or tangentially. A consequence of this precise neuronal journey is a central nervous system that is dominated by a number of laminar structures, most notably the cortex, colliculi and hippocampus. This migration is crucial as it dictates not only the final destination of a neuron, but also determines the connections that it forms, and therefore the neuronal circuits in which it operates. How do the neurons know where they are going? And how do they get there?

The study of genetic diseases in which neuronal migration is impaired has provided some answers to these questions. Lissencephaly, which means "smooth brain", is an example of such a disorder. Characterised by a lack of sulci and gyri, mutations in several genes have been shown to cause lissencephaly (DCX, reelin, LIS1, VLDLR). Most recently we have shown that mutations in a tubulin gene (TUBA1A) cause defects in neuronal migration in mice and lissencephaly in humans (Keays et al., 2007). These findings have helped investigators build a model where it is believed that nuclear migration is a key component of neuronal migration, and that both are dependent on a dynamic microtubule network. I am continuing to investigate how neurons move, and the role the tubulin genes play in this migration, primarily employing the mouse as a model organism.

Magnetoreception

It is a little known fact that life on earth relies on not five, but at the very least six, senses. Whilst hearing, touch, vision, taste and smell have been the focus of intensive investigation, the ability of certain organisms to detect and respond to magnetic fields has been largely overlooked. This facility, termed magnetoreception, is possessed by a diverse array of organisms including: bacteria, honeybees, hamsters, mice, lobsters, salmon, zebrafish, pigeons and Drosophila. The most important question in this field is: What molecular mechanism underlies magnetoreception?

1

2

3

Figure 1: *The Jenna mouse. Harbouring a S140G mutation in the TUBA1A gene, the Jenna mouse has a defect in neuronal migration. As a consequence it has anatomical abnormalities in the cortex and hippocampus, which results in hyperactivity and poor working memory.*

Figure 2: *The hippocampus of a Jenna mouse. Due the defect in neuronal migration, the the pyramidal cell of the hippocampus is fractured in the Jenna mouse (shown with an arrow). (A) shows a control mouse, and (b) a Jenna mouse.*

Figure 3: *Magnetoreceptive experiments on honeybees. Individual bees are caught from a sucrose feeding station, before being marked and trained to associate a food reward with a magnetic anomaly.*

There are two theories that attempt to explain this sense: (1) the radical pair theory of magnetoreception; and (2) the magnetite based theory of magnetoreception. The first theory holds that the earth's magnetic field influences the spin states of radical pairs in photosensitive molecules; and the second is a mechanosensitive model that relies on intracellular magnetite crystals (Fe3O4). The second theory, which I am focusing on, has sprung from observations made in magnetotactic bacteria. These aquatic bacteria use the Earth's magnetic field to direct swimming towards growth-favouring regions in natural waters. It has been discovered that magnetotatic bacteria possess organelles called magnetosomes. Magnetosomes consist of membrane-enclosed magnetite crystals (Fe3O4) 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 pigeons, fish and bees. I am investigating the genes and molecules that are required for magnetite based magnetoreception simultaneously employing behavioural, anatomical and genetic tools in the honeybee Apis mellifera.

FURTHER READING

Keays DA, Tian G, Poirier K, Huang G, Siebold S, Cleak J, Oliver P, Washbourne R, Fray M, Harvey RJ, Molnar Z, Pinon M, Dear N, Brown SD, Rawlins JP, Davies KE, Cowan NJ, Patrick Nolan P, Chelly J, Flint J. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. Cell. 2007 Jan 12;128(1):45-57.

Gegear RJ, Casselman A, Waddell S, Reppert SM. Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. Nature. 2008 Aug 21;454(7207):1014-8.

Johnsen S, Lohmann KJ. The physics and neurobiology of magnetoreception. Nature Reviews Neuroscience. 2005 Sep;6(9):703-12

THOMAS MARLOVITS
Design and Function of Molecular Machines

thomas.marlovits@imp.ac.at

Thomas C. Marlovits / joint IMP-IMBA Group Leader

Lisa Königsmaier / PhD Student
Agata Kosarewicz / PhD Student
Jesus Fernandez Rodriguez / PhD Student
Oliver Schraidt / PhD Student
Matthias Brunner / Diploma Student
Julia Radics / Diploma Student
Wolfgang Schmied / Diploma Student
Markus Höpfner / Summer Student
Wolfgang Weber / Technical Assistant

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

Host-Pathogen-Interaction

Gram negative pathogens, like *Salmonella, Yersinia, or Shigella*, use the type III secretion system (TTSS) to initiate infection in eukaryotic cells. The TTSS is a complex macromolecular system that serves as a structural platform to make physical contact between the host cells and pathogens and mediates the unidirectional transport of bacterial toxins (effector proteins) into eukaryotic cells (Figure 1A, 1B). These systems are essential for a successful infection resulting in well known clinical symptoms ranging from mild headaches and diarrhoea to even life-threatening diseases such as typhoid fever or bubonic plaque.

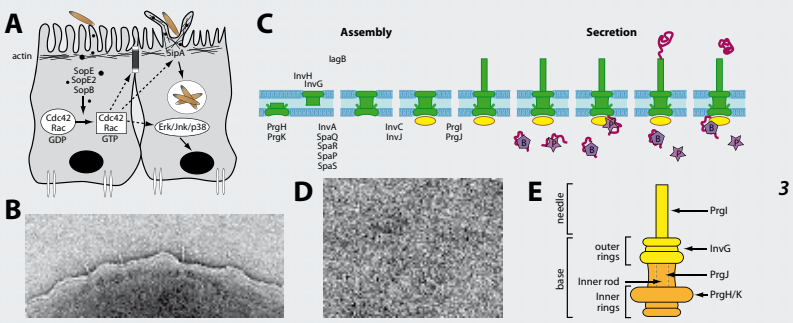
The Molecular Design

Made up of more than twenty proteins, TTSSs assemble into large "molecular nanomachines" composed of a set of soluble as well as membrane proteins. All of the structural components as well as other proteins involved in the step-wise assembly process (Figure 1C) and function are encoded on specific pathogenicity islands.

The most prominent substructure of the TTSS is known as the "needle complex", a cylindrical, needle-shaped and membrane-embedded organelle protruding from the bacterial envelope (Figure 1E). The needle complex is believed to serve as a conduit for the safe transport of virulence factors from the bacterial cytoplasm through a number of natural barriers into eukaryotic cells. In *Salmonella typhimurium*, which serves as our model for bacterial delivery systems, this complex is formed by multiple copies of only five proteins: PrgH, PrgK, and InvG build up the membrane-associated base-structure, PrgJ, the inner rod, and PrgI, the needle filament extending into the extracellular environment (Figure 1).

In order to investigate the molecular mechanism of type III secretion, we first set out to determine structural components of the TTSS (Figure 2A). We were challenged by the mega-dalton size of the complex, its natural composition (membrane and soluble proteins), and the limited availability. Nevertheless, we were able to purify sufficient amounts of the entire 'needle complex' and its precursor, the 'base', by a combination of detergent extraction and size separation by velocity gradient centrifugation. A detailed structural analysis by three-dimensional electron cryo-microscopy and single particle analysis finally revealed that several rotational symmetries or oligomeric states are present in

1



2

3

Figure 1: **The type III secretion system.** *(A) The type III secretion system is essential to deliver virulence factors such as SopE or SopB into eukaryotic cells. (B) (E) Needle-like structures (appr. 50 nm) that extend into the extracellular environment are visible on the surface of osmotically shocked S. typhimurium and can be released after detergent treatment (D). (C) Formation of intermediate substructures during assembly. Once the growth of the needle filament is terminated the type III secretion system changes substrate specificity and delivers effector proteins (reprogramming phase). (E) Schematic representation of the Salmonella needle complex and its components. PrgH, PrgK, and InvG make up the membrane-embedded base structure, whereas PrgI forms the helical filament protruding into the extracellular environment. The inner rod anchors the filament into the base.*

Figure 2: ***The structure of the needle complex.*** *Surface renderings and projections of the ~30 nm wide needle complex obtained from three-dimensional image reconstruction from vitrified needle complexes.*

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

the population of the 'needle complex' and the 'base'. Whether all of these complexes have a physiological role remains an open question.

Our analysis revealed a new structural component, the inner rod, which is located in the centre of the needle complex. It extends the secretion path from the base into the needle filament, and also serves as an anchor to stably connect the needle filament with the base (Figure 1E). During assembly the inner rod and the needle filament are added as new structural components to the base. As a consequence, it must undergo large conformational rearrangements, which demonstrates the flexible but also stable property of the base (Figure 1 and 2). While functionally, this dynamic behaviour is a crucial event during the assembly phase, in which the secretion machine is reprogrammed to become competent for the secretion of virulence factors, structurally, it underlines the importance of specific interaction epitopes critical for assembly into a functional unit. To this end we have established a method that uses high-resolution mass spectrometry of chemically derivatized complexes, to determine interaction sites and consequently topological information about individual proteins within larger macromolecular complexes (Figure 3).

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

FURTHER READING

Patel, J. C., Rossanese, O. W., and Galan, J. E. (2005). The functional interface between Salmonella and its host cell: opportunities for therapeutic intervention. Trends Pharmacol Sci. 26: 564-70

Yip, C. K., and Strynadka, N. C. (2006). New structural insights into the bacterial type III secretion system. Trends Biochem Sci. 31: 223-30.

Chen, L. M., Briones, G. , Donis, R. O., Galan, J. E. (2006). Optimization of the delivery of heterologous proteins by the Salmonella enterica serovar Typhimurium type III secretion system for vaccine development. Infect Immun. 74: 5826-33.

Nishikawa, H., Sato, E., Briones, G., Chen, L. M., Matsuo, M., Nagata, Y., Ritter, G., Jager, E., Nomura, H., Kondo, S., Tawara, I., Kato, T., Shiku, H., Old, L. J., Galan, J. E., Gnjatic, S. (2006). In vivo antigen delivery by a Salmonella typhimurium type III secretion system for therapeutic cancer vaccines. J Clin Invest. 116: 1946-54.

JAN-MICHAEL PETERS
Mitosis

jan-michael.peters@imp.ac.at

Jan-Michael Peters / Senior Scientist

Venugopal Bhaskara / Postdoc
James Hutchins / Postdoc
Tomoko Nishiyama [1] / Postdoc
Kristina Uzunova / Postdoc
Erwan Watrin [2] / Postdoc
Kerstin Wendt / Postdoc
Gordana Wutz / Postdoc
Bettina Buschhorn / PhD Student/Postdoc
Björn Hegemann / PhD Student/Postdoc
Emanuel Kreidl [3] / PhD Student
Ivana Primorac / PhD Student
Erika Schirghuber / PhD Student
Julia Schmitz / PhD Student
Hanna Schutz / PhD Student
Antonio Tedeschi / PhD Student
Marta Galova / Technical Assistant
Martina Sykora / Technical Assistant

Yan Sun / MitoCheck Project Manager

[1] *since April*
[2] *until October*
[3] *since November*

To pass the genome from one cell generation to the next, mitotic cells must package replicated DNA into chromosomes, attach the chromosomes to both poles of the mitotic spindle and then separate the chromosomes into their two sister chromatids. We are interested in understanding these processes at the molecular level.

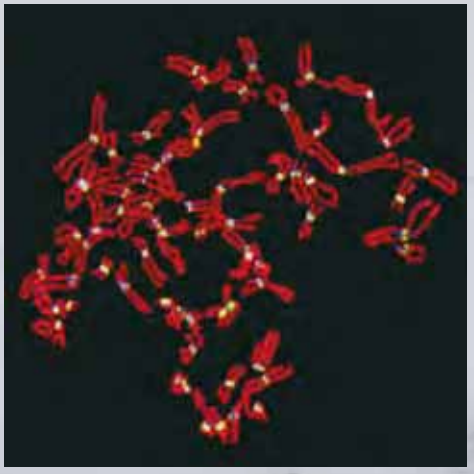
Sister chromatid cohesion

Sister chromatids remain connected from S-phase until chromosomes are segregated in the subsequent mitosis or meiosis. This cohesion is required for repair of DNA double strand breaks during G2 phase and for bi-orientation of chromosomes on the mitotic spindle in metaphase. Cohesion depends on cohesin, a multi-subunit protein complex that is loaded onto chromatin by the Scc2/Scc4 complex during telophase, and that establishes cohesion during S-phase. In vertebrates, the bulk of cohesin is removed from chromosome arms in prophase by a mechanism that depends on the cohesin-associated protein Wapl and in part on phosphorylation of the cohesin subunit SA2 by the mitotic kinase Plk1. At centromeres, cohesin is protected from this "prophase pathway" by the Sgo1 protein until all chromosomes have been attached to both poles of the spindle. At this stage, the protease separase is activated, cleaves centromeric cohesin and thereby initiates sister chromatid separation. We are interested in understanding the function of the prophase pathway of cohesin dissociation, how cohesin establishes cohesion and how the interaction between cohesin and DNA is regulated.

Roles of cohesin in gene regulation

Although cohesin is essential for sister chromatid cohesion in dividing cells, several recent observations indicate that cohesin also contributes to transcriptional regulation in both dividing and post-mitotic cells. To understand how cohesin mediates these functions, we have performed a genome-wide analysis of cohesin-binding sites in human cells and discovered that cohesin binds to the same sites in the human genome as CTCF (Figure 2). CTCF is a zinc finger protein that has been implicated in numerous aspects of transcriptional regulation. At the imprinted H19/Igf2 locus CTCF is essential for the enhancer-blocking activity of the imprinting control region. We discovered that cohesin mediates this enhancer-blocking activity of CTCF. Defects in this "insulator" function could be the cause of "cohesinopathies", human diseases such as Cornelia de Lange Syndrome that have been linked to hypomorphic mutations in cohesin and cohesin-regulatory proteins.

1



2

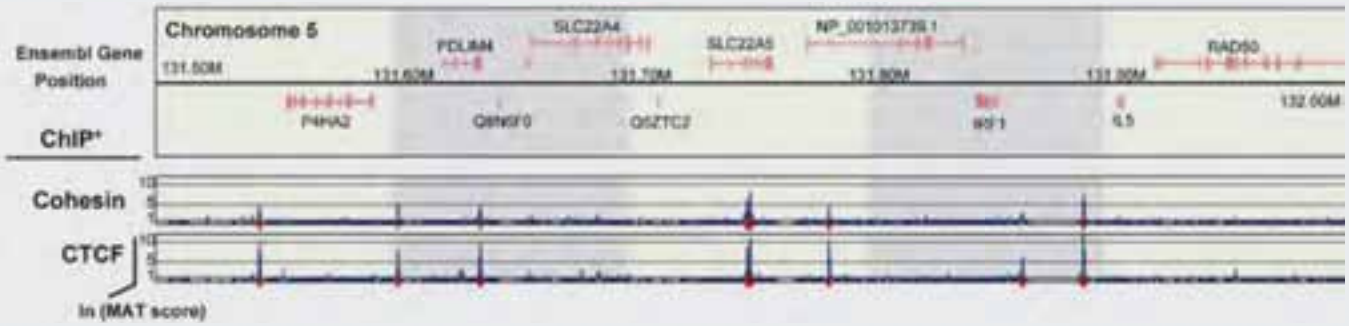


Figure 1: *Human mitotic chromosomes stained for condensin (red), cohesin (blue) and the centromere specific histone CENP-A (green). Courtesy of Peter Lénárt.*

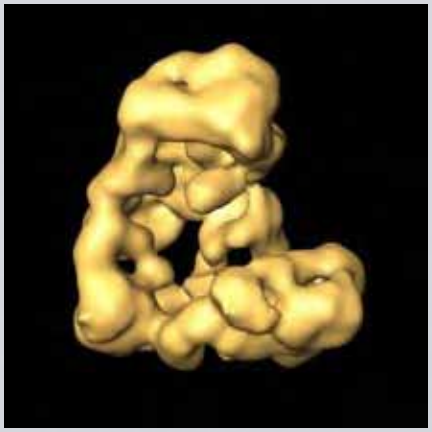
Figure 2: *Co-localization of cohesin and CTCF on a region of human chromosome 5, as detected by chromatin immunoprecipitation-chip experiments. Courtesy of Kerstin Wendt and Katsuhiko Shirahige.*

Figure 3: *A 3D model of Xenopus APC/C obtained by angular reconstitution of negatively stained cryo-elctron microscopy preparations. Courtesy of Holger Stark.*

The anaphase promoting complex/cyclosome (APC/C)

The APC/C is a 1.5 MDa ubiquitin ligase complex that targets specific proteins for destruction by the 26S proteasome. These reactions are essential for progression through mitosis, meiosis and G1-phase of the cell cycle. APC/C is activated early in mitosis by the co-activator Cdc20, but its ability to ubiquitinate substrates is inhibited by the spindle checkpoint until all chromosomes have become bi-oriented on the mitotic spindle. In metaphase, APC/C mediates the destruction of the separase inhibitor securin and of B-type cyclins and thereby allows activation of separase and inactivation of Cdk1, respectively. These reactions lead to the initiation of anaphase and to exit from mitosis. At the same time Cdc20 is replaced by the co-activator Cdh1 which keeps APC/C active throughout G1. Cdc20 and Cdh1 are required for recruitment of substrates to the APC/C, but how substrates are ubiquitinated by the APC/C and how this process is controlled by the spindle checkpoint is poorly understood. We are using biochemical approaches in human cells, Xenopus egg extracts and yeast to address these questions, and we are collaborating with crystallography and electron microscopy groups to analyse the structure of APC/C.

3



MitoCheck

Although the central importance of protein kinases in controlling mitosis has long been recognised, little is known about their substrates and how phosphorylation regulates these substrates. To be able to address these questions in a systematic fashion we have founded a consortium of eleven European institutes and companies who together have developed approaches to study mitosis in an Integrated Project that is funded through the 6th Framework Programme of the European Union. Major goals of this project, which is called MitoCheck, are to use genome-wide RNA interference screens to identify human proteins required for mitosis, to use mass spectrometry to systematically identify interaction partners and to map mitotic phosphorylation sites on these proteins, and to use small molecule inhibitors to identify substrates of the mitotic kinases Aurora B and Plk1.

FURTHER READING

Peters, J.-M. (2006). The anaphase promotig complex/cyclosome - a machine designed to destroy. Nat. Rev. Mol. Cell Biol. 7, 644-656.

Kueng, S., Hegemann, B., Peters, B.H., Lipp, J.J., Schleiffer, A., Mechtler, K. and Peters, J.-M. (2006). Wapl controls the dynamic association of cohesin with chromatin. Cell 127, 955-967.

Wendt, K.S., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., Yahata, K., Imamoto, F., Aburatani, H., Nakao, M., Imamoto, N., Maeshima, K., Shirahige, K. and Peters, J.-M. (2008). Cohesin mediates transcriptional insulation by CTCF. Nature 451, 796-801, Epub 2008 Jan 30.

SIMON RUMPEL
Synaptic and Circuit Mechanisms of Memory Formation

simon.rumpel@imp.ac.at

Simon Rumpel / Group Leader

Bruno Miguel Da Palma Pedrosa Fontinha / PhD Student
Kaja Moczulska / PhD Student
Manuel Peter / PhD Student
Juliane Tinter / PhD Student
Jiss John / Technical Assistant

Our lab is interested in the development, function and plasticity of neuronal circuits. More specifically, we would like to learn how we are able to store memories over long periods of time. This is fundamental to the understanding of our minds: memories of past experiences shape our personalities and influence our current perception.

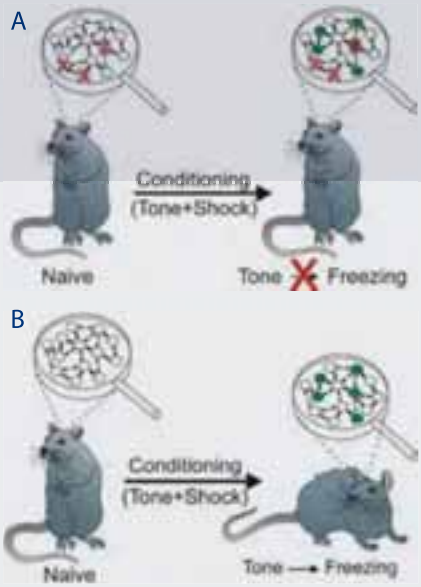
Trafficking of Synaptic Receptors during Memory Formation

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. The molecular mechanisms underlying synaptic plasticity have been mostly studied in cultured neurons. Synaptic plasticity is thought to be expressed, at least in part, through molecular changes that lead to addition or removal of synaptic AMPA receptors. In particular, synaptic addition of AMPA receptors containing the subunit GluR1 has been identified to be crucial for increases in synaptic strength. However, the processes actually happening in the brain during the formation of a memory trace are still poorly understood.

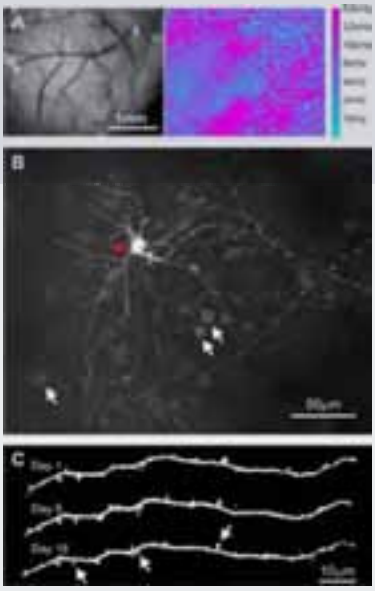
In order to delineate the role of particular candidate molecules, and to learn how memories are stored at the circuit level, we apply molecular tools in the behaving rodent in the context of memory-related behavior. Pavlovian conditioning is a simple and robust behavioral model of learning and memory formation, in which animals associate a tone with a mild foot-shock and subsequently "freeze" when presented with a tone alone. It has been previously shown that plasticity in the lateral amygdala plays an essential role in this behavior.

We recently tested the hypothesis that synaptic addition of GluR1-subunit containing AMPA-type glutamate receptors mediates an adaptation of synaptic strength in the lateral amygdala that is underlying the memory formation of the tone-shock association. We find that during Pavlovian conditioning about a third of amygdala neurons undergo synaptic plasticity by incorporation of GluR1-containing receptors. This suggests that the fear memory is encoded by many neurons and has a widely distributed nature. Surprisingly, we find that blocking synaptic delivery of GluR1-containing receptors in only 10-20% of amygdala neurons is sufficient to disrupt memory formation. This result indicates low redundancy in memory coding and suggests that successful memory retrieval requires participation of the vast majority of coding neurons.

1



2



3

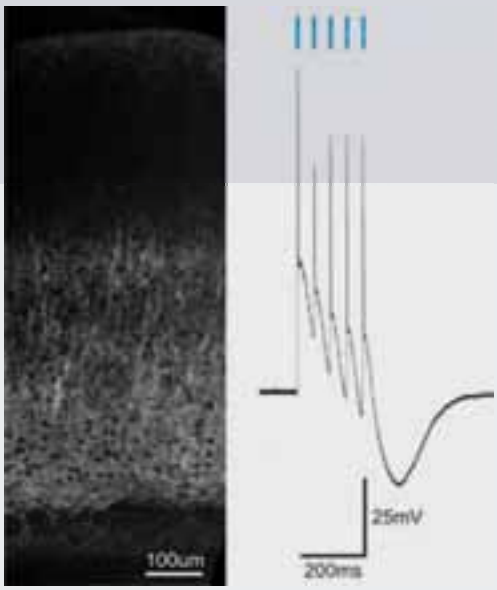


Figure 1: *Memory traces in the lateral amygdala appear to be distributed, but are highly sensitive to perturbations of synaptic plasticity. (A) Rats show freezing behaviour when presented to a tone that has previously been paired with a shock during a conditioning session. This behaviour indicates that an association between the two stimuli was formed and successfully stored. About a third of neurons in the amygdala undergo plastic changes during conditioning and show synaptic incorporation of GluR1-type AMPA receptors (green circles). (B) Blocking GluR1-type AMPA receptor-mediated plasticity in only 10–20% of neurons (red crosses) is sufficient to impair memory formation.*

Figure 2: *Imaging the auditory cortex. (A) Mapping tonotopic organisation of the auditory cortex using intrinsic imaging. (B) In vivo two-photon laser scanning microscopy of neurons in the auditory cortex expressing green fluorescent protein constitutively (red arrow) and under control of the c-fos promoter in an activity-dependent manner (white arrows). (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 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 Channel-rhodopsin. Massive expression of the construct can be 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 Channel-rhodopsin. A series of action potentials is elicited by a burst of brief light pulses (indicated by blue bars).*

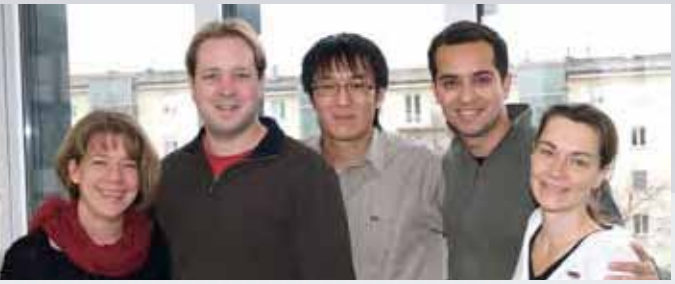
Peeking into the Brain

How can we continuously store novel memories in a given neuronal circuit without corrupting previously stored memories? In order to get a glimpse of the mechanisms how multiple memory traces are coordinated, we are currently applying chronic *in vivo* imaging techniques to the auditory cortex of mice. In addition to the amygdala, auditory areas of the cortex have been demonstrated to undergo learning-induced plasticity during auditory-cued fear conditioning. Two-photon laser scanning microscopy in transgenic animals expressing green fluorescent protein only in a small subset of cells allows revisiting the same neurons and even the same individual synapses day after day. This is truly remarkable since we estimate that the brain has about 10 trillion (10^{13}) synapses. In the future we plan to investigate the impact of auditory cued fear conditioning on the dynamics of a given set of synapses and neurons in the auditory cortex. We hope that this approach will help to open a door towards a series of novel experiments addressing information storage in living neuronal networks, a field of research that so far has been reserved mostly for theoretical neuroscientists.

FURTHER READING

Rumpel, S., LeDoux, J., Zador, A., Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. Science (New York, N.Y.). 308(5718):83-8

Ehrlich, I., Klein, M., Rumpel, S., Malinow, R. (2007). PSD-95 is required for activity-driven synapse stabilization. Proceedings of the National Academy of Sciences of the United States of America. 104(10):4176-81



ALEXANDER STARK

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

alexander.stark@imp.ac.at

Alexander Stark [1] / Group Leader

Antonio Mereiles-Filho [1] / Postdoc
Evgeny Kvon [1] / PhD Student
Martina Rath / Technical Assistant
Michaela Pagani / Technical Assistant

[1] *since October*

The regulation of gene expression in response to developmental or environmental stimuli is central to all organisms. It occurs at the pre- and post-transcriptional level and is mediated by trans-acting factors (e.g. transcription factors and microRNAs) that recognise cis-regulatory elements and function in a combinatorial fashion. We use both bioinformatics and molecular biology methods to gain a systematic understanding of transcription factor and microRNA targets, the combinatorial regulatory networks they define and their coordinated use in dictating cellular and developmental programs.

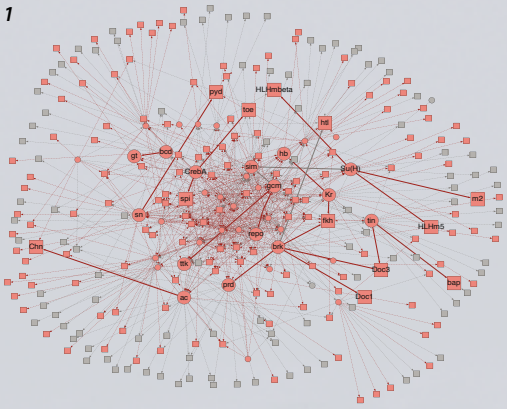
Gene regulatory motifs and networks

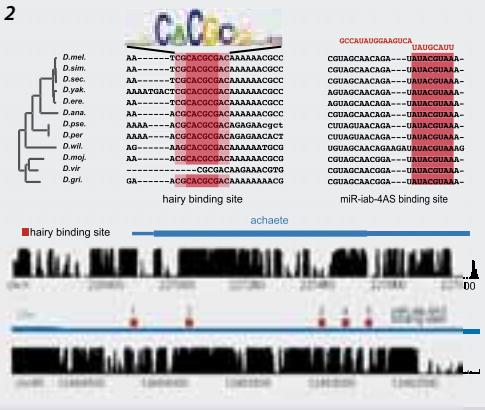
The regulation of gene expression is central to the development of all organisms. In higher eukaryotes, genes are expressed dynamically in complex spatial patterns and mis-expression often results in developmental failures and diseases such as cancer. Tissue specific gene expression is determined by regulatory programs and in turn defines the different animal cell types and their characteristics. Given their central role, core regulatory circuits or kernels have been found to be conserved between animals as divergent as flies and mammals.

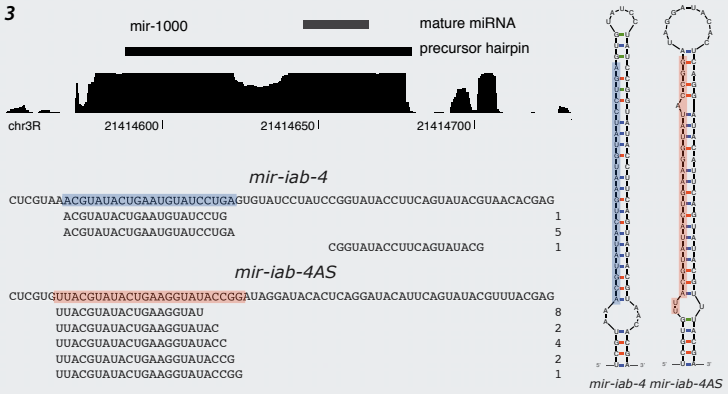
A major challenge in molecular biology is to define these circuits and to decipher how the cell utilises the regulatory information present in the DNA. Our group uses both bioinformatics and molecular biology methods to characterise tissue- and cell-type specific expression and to predict and validate regulatory targets of transcription factors and microRNAs. We focus on the different cell-types and organs in Drosophila and aim to explain their expression programs given the regulatory connections of transcription factors and microRNAs.

To identify tissue-specific targets of transcription factors we are using tissue-specific ChIP-Seq experiments of more broadly expressed transcription factors. This will also allow us to determine factors influencing the tissue-specificity of transcription factor mediated regulation, e.g. other motifs, factors and their combination. We are also working towards a quantitative description of cell-type specific gene expression in Drosophila using biosynthetic RNA-labeling, which allows the cell-type specific isolation of RNA without the need for tissue-dissection or cell sorting. Finally, we will systematically test candidate promoters and enhancers and compare candidates with different characteristics regarding transcription-factor motif density, motif conservation, and in vivo binding- or histone-modification states (as determined by ChIP-chip by us and others). We will describe bound yet inactive candidates and explore similarities and differences between functional and non-functional candidate promoters and enhancers.

1







Post-transcriptional regulation

Gene expression is also regulated post-transcriptionally, for example by RNA-binding proteins and microRNAs. microRNAs are short non-coding RNA genes processed from characteristic hairpin precursors. They guide silencing complexes to binding sites on target mRNAs, leading to translational inhibition and/or message destabilisation. Many microRNAs show highly defined organ-specific expression patterns and are highly conserved among metazoans, suggesting an ancient and important role in regulatory circuits. We have developed methods to discover novel microRNAs and predict conserved microRNA targets.

Comparative genomics

Functional elements in a genome are typically under evolutionary selection to maintain their functions in related organisms. In alignments of orthologous DNA sequences, they often stand out by their increased sequence identity and/or by specific patterns of sequence changes that reflect functional constraints (evolutionary signatures). We have developed methods to score motif conservation in 12 Drosophila genomes. We have discovered novel motif types, identified functional targets of many transcription factors and microRNAs with high confidence, and found that they can help to understand and refine experimental ChIP data.

Comparative genomics and related bioinformatics approaches will allow us to integrate our data and knowledge to predict developmental enhancers, regulatory targets for transcription factors, and the expression patterns of genes. They also allow us to integrate microRNA-mediated regulation into regulatory networks and to 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, for example, in regulating gene expression by the microRNA and siRNA pathways and in the control of mobile genetic elements through related silencing pathways involving the PIWI-clade of Argonaute proteins. We are collaborating extensively with experimental labs in the analyses of small RNA and their functional characterisation.

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

Figure 2: *Conservation of transcription factor and microRNA binding sites in 12 Drosophila genomes and their genomic context. See Kheradpour et al., 2007 and Stark et al., 2008 for details.*

Figure 3: *Comparative prediction of mir-1000 and validation of mir-iab-4 anti-sense processing by Solexa sequencing. See Stark et al., 2007 and 2008 for details.*

FURTHER READING

Davidson, E.H. (2006) The Regulatory Genome: Gene Regulatory Networks In Development and Evolution. Academic Press.

Hornstein, E. and Shomron, N. (2006). Canalization of development by microRNAs. Nature Genetics 38, S20-24.

Stark, A., Lin, M.F., Kheradpour, P., Pedersen, J.S., Parts, L., Carlson, J.W., Crosby, M.A., Rasmussen, M.D., Roy, S., Deoras, A.N., et al. (2007). Discovery of functional elements in 12 Drosophila genomes using evolutionary signatures. Nature 450, 219-232.

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
Elisabeth Malle / Diploma Student
Romana Ranftl [1] / Diploma Student
Thomas Pollak / Technical Assistant

[1] *since December*

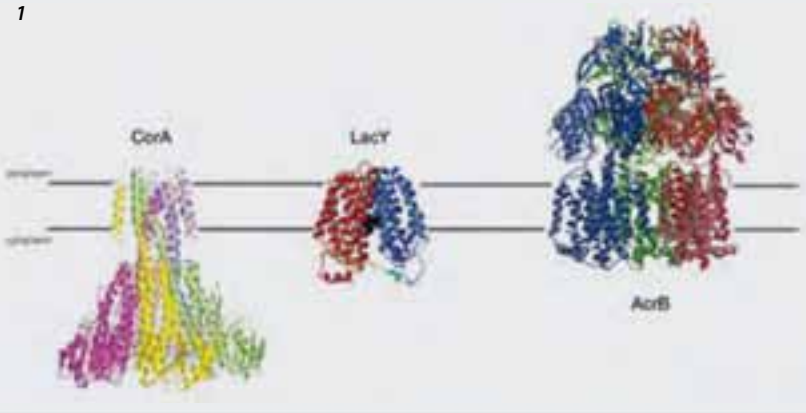
All cells are surrounded by at least one impermeable lipid bilayer, and have evolved numerous types of proteins to allow them to interact with their environment by transducing signals and transporting substrates across this membrane. We are interested in understanding the molecular mechanisms these proteins 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 characterisation of secondary active transporters, which drive the transport of substrates across the membrane by coupling this process to the energetically favourable 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, and many of these crystal structures have been determined at 3 angstrom resolution or lower. 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 uncharacterised, 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 the native membrane environment. Moreover, the conformational heterogeneity of membrane transporters can contribute to their instability. In parallel with attempts to crystallise secondary transporters, we are interested in developing methods to stabilise membrane proteins in order to facilitate their crystallisation and biophysical characterisation.



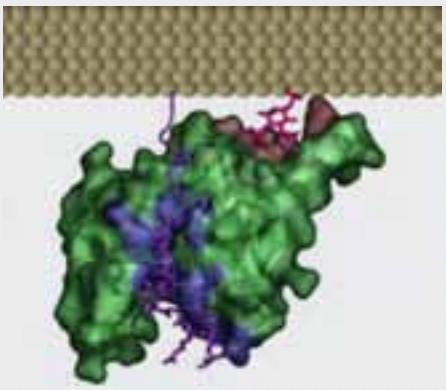


Figure 1: ***Transporter structures reveal diverse types of transport mechanisms.*** *Recent structural work has uncovered evidence for diverse transport mechanisms. The E. coli lactose permease LacY adopts the alternating access model, which most likely involves large conformational changes (figure 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 utilises 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).*

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-characterised 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 characterisation 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.

FURTHER READING

Sobczak, I. and Lolkema, JS. (2005). Structural and mechanistic diversity of secondary transporters. Current Opinion in Microbiology 8:161-167

Herz, J. and Chen, Y. (2006). Reelin, lipoprotein receptors, and synaptic plasticity. Nature Reviews Neuroscience, 7:850-859

ERWIN WAGNER

Gene Function in Mammalian Development and Disease

erwin.wagner@imp.ac.at; ewagner@cnio.es

Erwin F. Wagner [1,4] / Senior Scientist and Deputy Director
His group moved during 2008 to the CNIO in Madrid, Spain

Guillaume Beranger [2] / Postdoc
Aline Bozec [2] / Postdoc
Juan Guinea [4] / Postdoc
Lijian Hui / Postdoc
Domenico Migliorini [4] / Postdoc
Alok Mishra [3] / Postdoc
Helia Schönthaler [4] / Postdoc
Ivana Custic / PhD Student
Simon Schultze / Diploma Student
Iris Uras [4] / Diploma Student
Harald Scheuch [8] / Technical Assistant
Uta Möhle-Steinlein / Technical Assistant
Maria Helena Idarraga-Amado [4,6] / Technical Assistant
Martina Rath [5,7] / Technical Assistant
Kazem Rassi-Faghihi [2] / Trainee
Stefanie Wculek [4] / Trainee

[1] *since January leave of absence to CNIO, Madrid*
[2] *until April*
[3] *until May*
[4] *until June*
[5] *until September*
[6] *since July Lab. Wutz*
[7] *since October Lab. Stark*
[8] *since April Genomics*

The major focus of our studies is the analysis of gene function in normal and pathological conditions, e.g. in tumour development, using the mouse as a model organism. Specifically, the functions of AP-1 in regulating cell proliferation, differentiation and cell death are investigated. Our studies have revealed that the AP-1 proteins Fos and Jun play pivotal roles in bone, liver, skin and haematopoietic development.

Fos/AP-1 - Functions in bone development

Fos proteins are key regulators of bone development. Transgenic mice over-expressing c-fos develop osteoblastic bone tumours, whereas mice lacking c-fos are osteopetrotic and lack bone-resorbing osteoclasts (Figure 1). The Fos-related protein Fra-1 is essential for mouse placental development, whereas conditional deletion of Fra-1 in the embryo gives rise to viable mice which develop osteopaenia, a low bone mass disease. On the other hand, transgenic mice overexpressing Fra-1 develop an osteoblastic bone disease termed osteosclerosis. Recently, we analysed the properties of the Fos-related protein Fra-2 using loss and gain of function approaches (Figure 2). We found that Fra-2 is essential for postnatal mouse development and maintenance of proper bone mass, since mutant newborns die with severe osteopaenia and giant osteoclasts. Moreover, transgenic mice over-expressing Fra-2 have increased bone mass and develop pulmonary fibrosis. We are currently investigating the underlying molecular mechanisms leading to the bone and lung disease. We have addressed the relevance of post-translational modifications of Fos in bone development and pathogenesis. The ERK-dependent kinase RSK2 can phosphorylate Fos on serines 362 and 374 and the RSK2 kinase is essential for the development of Fos-dependent osteosarcomas in vivo (Figure 1). To further study Fos phosphorylation in vivo, Fos mutant knock-in mice were generated in which the endogenous wild-type Fos allele was replaced by a point mutant allele with serines 362 and 374 mutated to alanines (FosAA). Recent results show that Fos C-terminal phosphorylation is dispensable for skeletogenesis, but required for osteoclastogenesis in vitro and for normal bone homeostasis in adult mice.

Jun/Fos - Role in proliferation, differentiation and apoptosis

Using conditional mutagenesis, knock-in strategies and transgenic rescue experiments we have demonstrated that Jun is essential for liver regeneration and is required as a survival factor during liver tumour initiation as well as in progression (Figure 3). We were able to show that a p53/p21 and p38-MAPK-dependent pathway is essential for efficient liver regeneration. Analogous genetic strategies are being employed to investigate the functions of Jun/AP-1 and MAPK proteins in liver cancer and inflammatory disease. The upstream

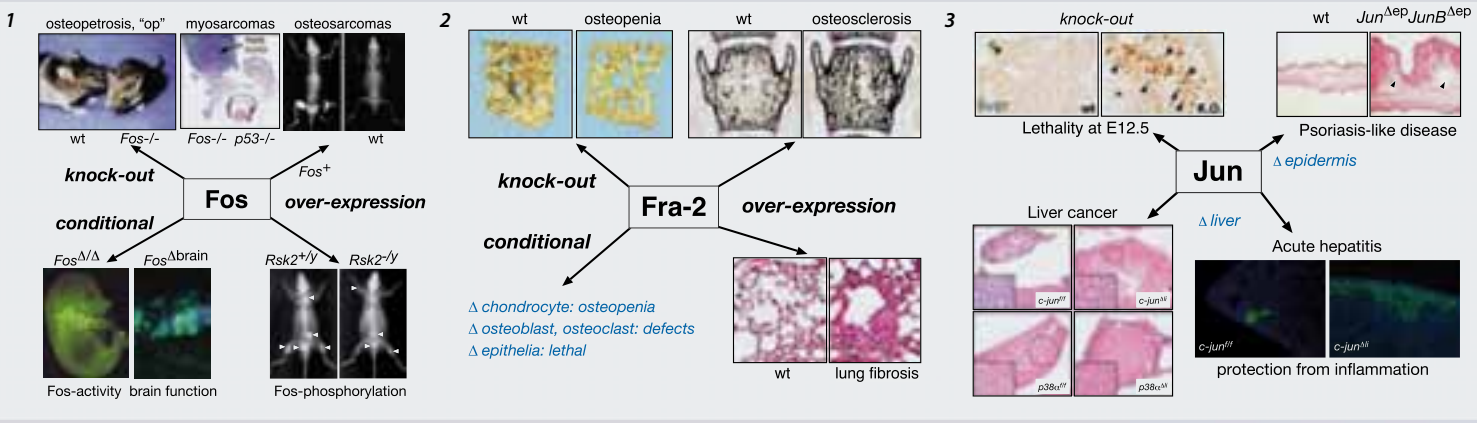


Figure 1-3: Functional analysis of Fos (1), Fra-2 (2) and Jun/p38 (3) in development and disease.

activator of Jun, JNK1, plays an oncogenic role in liver carcinogenesis, while p38a suppresses liver cancer development. During acute hepatitis, c-Jun mediates hepatocyte survival by regulating expression of iNOS, thus protecting the liver from hypoxia and oxidative stress (Figure 3); Jun's functions in hepatitis-associated carcinogenesis are currently being analysed. Moreover, c-Jun is highly expressed in colitis-associated intestinal tumours; however, the functions of AP-1 appear to be rather limited, since tumorigenesis was not affected by deletion of single AP-1 components either in epithelial or inflammatory cells. When junB is inactivated in the epidermis, mice are born healthy, but develop a multi-organ disease likely caused by deregulated cytokine expression from keratinocyte-derived G-CSF and IL-6. In addition, we have been able to demonstrate in patients' samples and by employing an inducible mouse model that downregulation of Jun and JunB proteins in keratinocytes can cause a psoriasis-like disease (Figure 3), which is a common chronic disease affecting skin and joints. Interestingly, when Jun and JunB were inactivated in the epidermis in a constitutive manner, mutant pups died due to a cytokine storm, which involves deregulation of TNF-TIMP3-TACE expression. Mice developing the psoriasis-like disease are also being studied with respect to the involvement of angiogenesis, e.g. VEGF expression, but also regarding the role of the Jun target genes S100A8 and S100A9 in disease development. Finally, deletion of Fos in keratinocytes did not induce any obvious skin phenotype under normal conditions but keratinocytes under stress conditions differentiated prematurely due to activation of the p53/Notch1 pathway.

AP-1 function in mesodermal differentiation and tissue regeneration

We are particularly interested to find out whether the lessons learned from mouse studies will apply to human cells and whether the functions of AP-1 have been conserved. To investigate this we have established the growth conditions for a human embryonic stem cell line (hES2) at the IMP. Moreover we have started to establish efficient protocols to induce differentiation of murine and human ES cells into different mesodermal lineages, including chondro-osteo and vascular progenitor cells. We will perform functional studies of the AP-1 (Fos/Jun) complex during murine and human ES cell differentiation and use in vitro differentiated genetically altered progenitor cells for tissue engineering.

STEFAN WESTERMANN
Molecular Mechanisms of Kinetochore Function

stefan.westermann@imp.ac.at

Stefan Westermann / Group Leader

Peter Hornung [1] / PhD Student
Eva Kiermaier / PhD Student
Fabienne Lampert / PhD Student
Christine Mieck [2] / PhD Student
Tomasz Zimniak / PhD Student
Sophie Woehrer [3] / Diploma Student
Susanne Opravil [2,4] / Technical Assistant
Katharina Stengl / Technical Assistant

[1] *since August*
[2] *since November*
[3] *since October*
[4] *joint appointment with Karl Mechtler*

Chromosome segregation requires a physical connection between the centromeres of chromosomes and the microtubules of the mitotic spindle. One of the most fascinating questions of mitosis is how the kinetochore, the protein complex that assembles on centromeric DNA, mediates the attachment of chromosomes to highly dynamic microtubule plus-ends and how it creates sufficient force to power sister chromatid segregation during anaphase. We are studying kinetochore function in the budding yeast Saccharomyces cerevisiae using a combination of biochemistry, yeast cell biology and in vitro assays with dynamic microtubules to gain insights into the molecular mechanisms of chromosome segregation.

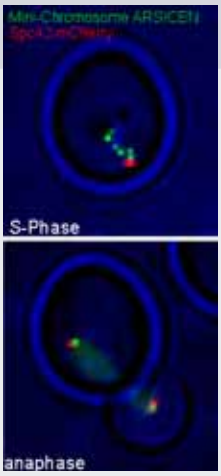
Organisational Principles of Kinetochores

Despite the small size of its centromeric DNA, the budding yeast kinetochore is a complex macromolecular structure that consists of more than 65 proteins. These proteins do not just form a simple attachment site, but they assemble into a molecular machine that controls and powers chromosome movement, monitors the state of attachment and tension across sister chromatids and signals to the mitotic checkpoint. Systematic tandem affinity purifications and mass spectrometric analysis of kinetochore proteins have identified the budding yeast kinetochore as a hierarchical assembly of multi-protein complexes. Many of these complexes have homologues in higher eukaryotes, suggesting that fundamental aspects of kinetochore organisation are conserved throughout evolution. There are a number of basic questions that need to be addressed in the future. What is, for example, the minimal set of proteins that can form a functional kinetochore? Since kinetochores are such complex structures it is desirable to identify smaller functional units and analyse their function. To address this question we have developed a system that allows us to artificially recruit proteins to DNA and ask whether they can provide kinetochore function. We have directly visualised the segregation of mini-chromosomes mediated by these artificial kinetochores and we will continue to explore their properties in the future (Figure 1).

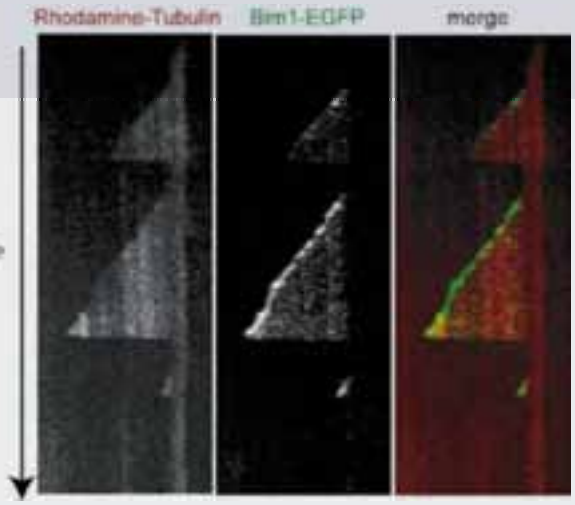
Regulation of the Kinetochore–Microtubule Interface

Kinetochore–microtubule interactions are carefully controlled to ensure that sister chromatids are connected to opposite poles of the mitotic spindle. The mitotic kinase Aurora B has a conserved role in correcting erroneous attachments to ensure kinetochore bi-orientation. We have identified the budding yeast member of the conserved EB1 family of microtubule plus-end binding proteins as a key target of the budding yeast Aurora B kinase Ipl1p. We have reconstituted the interaction of budding yeast EB1 with dynamic microtubules in vitro, using total internal reflection fluorescence (TIRF) microscopy (Figure 2). This technique allows us to study the interaction of individual molecules with dynamic microtubules

1



2



3

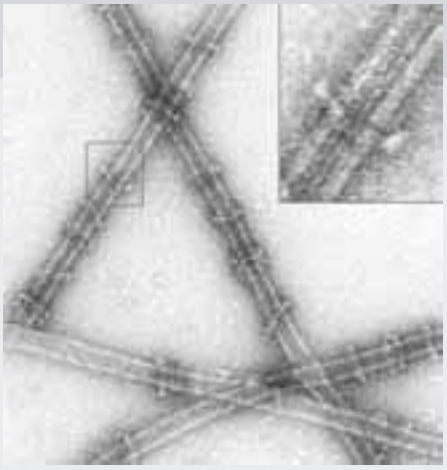


Figure 1: *Deltavision deconvolution microscopy of mini-chromosome segregation in budding yeast. Mini-chromosomes (green) appear in pairs in unbudded cells (left panel) and are segregated to spindle poles (red) as the cell enters anaphase (right panel). Courtesy of Eva Kiermaier.*

Figure 2: *Reconstitution of microtubule plus-end tracking in vitro using TIRF microscopy. Time-space plot (kymograph) of Bim1-EGFP (green) specifically decorating the plus-end of a growing microtubule (red). Courtesy of Tomasz Zimniak.*

Figure 3: *The 10-protein Dam1 complex oligomerises into a ring around the microtubule in vitro. Negative stain electron microscopy of Dam1 rings decorating taxol-stabilised microtubules; the insert shows an individual Dam1 ring on the microtubule lattice. Courtesy of Fabienne Lampert.*

in a purified system. In the future we would like to understand how kinetochore proteins recognise and interact with the microtubule plus-end and how this interaction is regulated. We would also like to reconstitute the collective behaviour of multiple proteins that work together to ensure kinetochore bi-orientation.

Mechanisms of Force Generation at Kinetochores

Electron microscopy has revealed that the 10-protein Dam1 complex, a key microtubule-binding element of the budding yeast kinetochore, oligomerises into a 16-fold ring around a microtubule in vitro (Figure 3). Importantly, this protein ring does not have a fixed binding site on the microtubule, but is able to slide laterally along the lattice, allowing the formation of a dynamic attachment site for the kinetochore.

Many questions about this ring-forming kinetochore-complex remain unanswered: How is the assembly and dynamics of the ring regulated? How does it connect to the other multi-protein complexes of the kinetochore? How does it co-operate with motors and plus-end tracking proteins to form a functional kinetochore attachment site at the microtubule plus-end? One of the key features of mitosis is that the chromosomes are able to hold on to the ends of disassembling kinetochore microtubules during Anaphase A and harvest the force generated by microtubule depolymerisation for their transport towards the spindle poles. The assembly of the Dam1 ring complex can provide an elegant mechanical solution to the attachment problem. Using Dam1 rings labeled with a fluorescent dye and and an in vitro microscopy assay, we could directly visualise that the ring stays attached to the end of a disassembling microtubule and slides over the lattice towards the minus-end of the shrinking polymer. We will continue to develop microscopy assays with dynamic microtubules to uncover further aspects of kinetochore motility.

FURTHER READING

Sandall, S., Severin, F, McLeod, IX., Yates, JR, Oegema, K, Hyman, A. and Desai, A. A Bir1-Sli15 complex connects centromeres to microtubules and is required to sense kinetochore tension. Cell (2006) 127:1179-91

Tanaka, K., Mukae, N., Dewar, H., van Breugel, M., James, E.K., Prescott, A.R., Antony, C., and Tanaka, T.U. Molecular mechanisms of kinetochore capture by spindle microtubules. Nature. 2005 Apr 21;434(7036):987-94

Cheeseman, I. M., Anderson, S., Jwa, M., Green, E. M., Kang, J., Yates, J. R., 3rd, Chan, C. S., Drubin, D. G., and Barnes, G. (2002). Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. Cell 111, 163-172

ANTON WUTZ
Mammalian X-chromosome inactivation

anton.wutz@imp.ac.at

Anton Wutz / Group Leader

Ruben Agrelo / Postdoc
Hiroyuki Kishimoto / Postdoc
Tatsuya Ohata / Postdoc
Aditya Sengupta [1] / Postdoc
Martin Leeb / PhD Student
Philipp Steffen [2] / PhD Student
Maria Helena Idarrago [3] / Technical Assistant
Mediyha Saltik / Technical Assistant

[1] *until April*
[2] *from January*
[3] *from August*

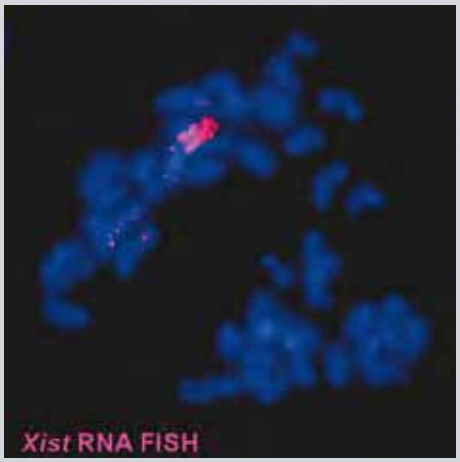
For successful development, the information stored in the genome needs to be precisely regulated. During differentiation, each individual cell uses an ever-changing repertoire of epigenetic mechanisms to achieve proper control of gene expression. Our research focuses on the regulated formation of heterochromatin during the process of X inactivation.

X inactivation is the process that mammals use to compensate for the dosage difference in X-linked genes between the sexes. This is achieved by transcriptional silencing of one of the two X-chromosomes in female cells (Wutz and Gribnau, 2007). The long non-coding *Xist* RNA is critical for initiation of this silencing process and associates physically along the entire chromosome (Figure 1).

The function of Polycomb group proteins at the initiation of X inactivation

Recruitment of Polycomb group (PcG) complexes by *Xist* can be observed at an early stage in X inactivation. Polycomb repressor complex 1 (PRC1) and PRC2 mediate chromosome-wide modifications of histones. We have shown that recruitment of both PRCs is strictly dependent on *Xist* RNA at all stages of differentiation. Nonetheless, the function of PcG complexes remains elusive. *Xist* can initiate chromosome-wide silencing in ES cells lacking PRC2 activity by a mutation in *Eed*. Notably, in *Eed*-deficient ES cells PRC1 can be recruited by *Xist* and mediates ubiquitination of histone H2A. In order to investigate the function of PRC1 in X inactivation we have disrupted *Ring1B*. In *Ring1B*-deficient ES cells several PRC1 proteins are lost. We observe derepression of developmental control genes and aberrant differentiation of ES cells lacking PRC1 function (Figure 2). Notably, *Xist* can still recruit PRC2, which mediates chromosome-wide tri-methylation of histone H3 lysine 27 (Figure 3). Despite the disruption of the Polycomb system we find that chromosome-wide silencing by *Xist* is unaffected. Thus, neither PRC1 nor PRC2 are essential for X inactivation in embryonic cells. Since PRC1 and PRC2 both have essential roles in embryogenesis and can be recruited by *Xist* independently of each other, we propose that they might act redundantly during X inactivation. To address this question we have established ES cell lines which are deficient in both PRC1 and PRC2. Current work aims to characterise these cells to define Polycomb function in X inactivation and stem cell self-renewal. Recent work indicates that PRCs can act together or individually on distinct classes of genes (Shibata et al., 2008). It will be important to identify the mode of Polycomb regulation genome wide by analysing gene expression patterns.

1



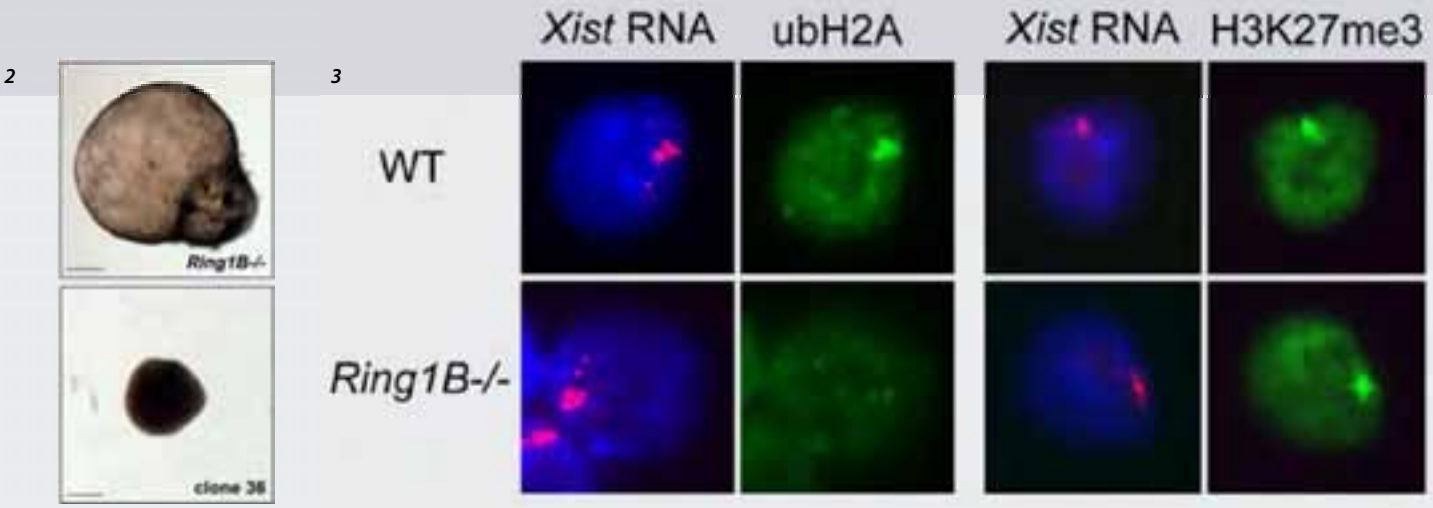


Figure 1: *Xist RNA association with chromatin revealed by RNA FISH. Mouse chromosome spreads were prepared from ES cells expressing Xist from a transgene on chromosome 11 and hybridised with a fluorescent probe detecting Xist RNA (red). DNA was counter-stained with DAPI (blue).*

Figure 2: *Aberrant differentiation of ES cells lacking Ring1B. Embryoid bodies derived from Ring1B-deficient ES cells show striking hollow sphere morphology compared to the compact spherical structures formed by control ES cells (WT).*

Figure 3: *Recruitment of Polycomb group proteins and histone modifications by Xist. Xist expression recruits PRC1 and PRC2, which mediate chromosome-wide ubiquitination of histone H2A (ubH2A) and tri-methylation of histone H3 lysine 27 (H3K27me3). In Ring1B-deficient ES cells ubH2A is not triggered by Xist expression, whilst H3K27me3 can still be observed.*

Developmental control of the gene silencing function of *Xist*

Xist expression is the trigger for the inactivation of one of the two female X chromosomes early in embryogenesis. As differentiation progresses, *Xist* becomes dispensable for the maintenance of the inactive X. Conversely, its function for initiation of silencing is lost in differentiated cells. Thus, initiation of silencing is restricted to a specific time interval at the onset of cellular differentiation. How *Xist* mediates gene repression remains an open question. Using an inducible *Xist* allele in mice we defined the potential for *Xist* to initiate silencing during embryogenesis. In adult male mice, ectopic expression of *Xist* causes an anaemia that can eventually lead to lethality. *Xist* has the ability to initiate silencing in immature blood precursor cells. In contrast, stem cells and mature blood cells are unable to initiate ectopic X inactivation. This indicates that critical pathways for silencing are transiently activated in hematopoietic differentiation. We have identified a mouse tumour model, which contains cells that have the appropriate context for gene silencing by *Xist*. Current approaches are directed towards understanding the molecular basis for this epigenetic difference and the identification of pathways that are crucial for *Xist*-mediated silencing.

Functional studies of X inactivation will provide insight into the epigenetic regulation of gene expression in mammals. It appears that *Xist*-mediated silencing is a paradigm for a powerful epigenetic system that is capable of hetero-chromatinising an entire chromosome and determining its specific nuclear localisation. It is expected that similar interactions underlie the regulation of other genes – however, with less dramatic consequences. Regulation of chromatin by non-coding RNAs is the focus of contemporary epigenetic research, and might pinpoint a general process by which transcription feeds back onto the chromatin structure.

KATRIN HEINZE

Fluorescence Imaging and Spectroscopy on a Single Molecule Scale

katrin.heinze@imp.ac.at

Katrin Heinze / Staff Scientist

Kareem Elsayad / Postdoc
Klaus Gerald Neumüller / Diploma Student

Discoveries in biosciences are often stimulated by the invention of new scientific tools. We like to push fluorescence techniques beyond the classic spatial and typical temporal resolution limits. Such low-invasive approaches offer the fascinating prospect of observing biomolecules in their native environment and understanding how they act in concert.

Single molecule techniques resolve important phenomena that are otherwise "averaged out" by ensemble measurements, such as information about subpopulations in heterogeneous samples. Our current research focuses on the development and adaptive modification of single molecule sensitive imaging techniques, particularly for answering questions concerning cell signaling. A second branch of our research addresses the question of how protein binding may (unintentionally) be affected by the light illumination itself used for fluorescence imaging and spectroscopy.

Super-resolution concepts for imaging live cells and tissue

Do specific proteins define subsets of synapses? In collaboration with the group of Simon Rumpel, we plan to pinpoint the localisation of various synaptic proteins (e.g. AMPA-type glutamate receptors, NMDA receptors) in a set of synapses by using a 3-D super-resolution microscope that we have designed. We are currently completing the assembly of this microscope which is based on multi-axis imaging and selective photo-activation of fluorescent proteins.

A different super-resolution approach is based on a novel design of a so-called far-field superlens which makes use of the negative index of refraction of certain structures and materials. Our models show that using appropriate material combinations (Metamaterials) it is feasible to perform surface imaging with sub-diffractional resolution. Our novel superlens could be implemented into any standard wide-field microscope setup similar to Total Internal Reflection Microscopy (TIRFM), however providing a super-resolution feature. In collaboration with the laboratory of Vic Small, we will image fixed and migrating RFP- and YFP-expressing fibroblasts and melanoma cells to study the dynamics of signaling molecules in an adhesion complex and its recently discovered substructure.

Beyond photobleaching, laser light unbinds fluorescent proteins

Recently, we have found that light can cause the reversible dissociation of antibodies from their ligands if they carry a fluorescent label. Thus, light can be used to selectively 'unbind' fluorescently labeled proteins in a non-invasive fashion.
This photo-unbinding phenomenon may have a big impact on studies using photobleaching as a tool (e.g. FRAP Fluorescence Recovery after Photobleaching) as the binding equilibrium may be changed unintentionally by light exposure. Unfortunately, the underlying molecular mechanisms which control, amplify or even inhibit photo-unbinding are largely unknown.

1

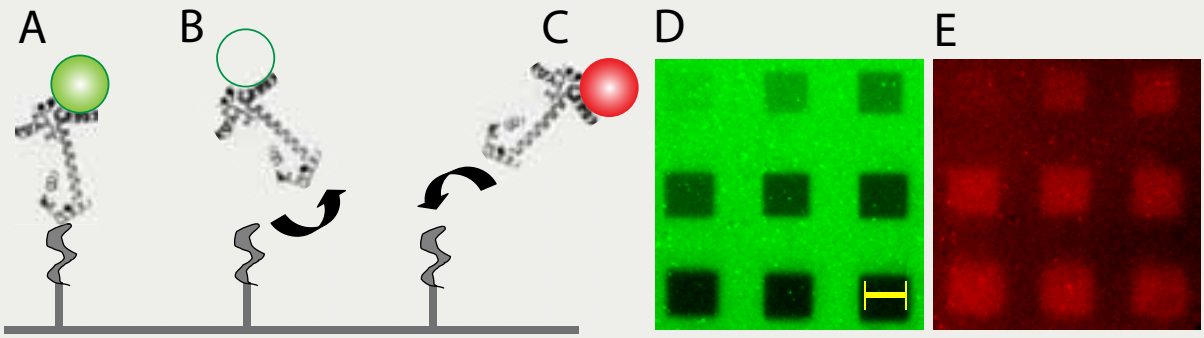


Figure: *Photo-unbinding of Alexa488-CaM from its target (CaM binding peptide). Laser illumination (488 nm) of the green labeled CaM (A) creates photobleaching and/or photo-unbinding from its target peptide (B) which can be visualised and quantified by subsequent re-incubation of the sample with a red labeled CaM (C) followed by fluorescence imaging (D (green channel, scale bar: 15 µm), E (red channel)). The Alexa488-CaM/peptide complex was scanned with a blue laser in square patches using different laser intensities (lowest intensity: upper left corner, highest intensity: lower right corner) and subsequently incubated with Alexa647-CaM, and finally imaged in green (D) and red (E) detection channels. The square patches in D show the decrease in Alexa488-CaM fluorescence upon bleaching and/or photo-unbinding; the patches in E show the corresponding increase in fluorescence upon rebinding of Alexa647-CaM.*

So far, we have found that photo-unbinding a) occurs after both one- and two-photon excitation, b) is strongly dependent on the absorption cross-section of the protein system, c) is reversible, d) is (almost) indistinguishable from photobleaching.

Most importantly, photo-unbinding is not restricted to antibody-antigen binding. We are currently extending our studies to a Calmodulin (CaM) system and various CaM binding peptides which exhibit a wide range of binding constants. Here, we found that photo-unbinding is more likely if the initial binding is stronger.

FURTHER READING

1. Manley, S., Gillette, JM., Patterson, GH., Shroff, H., Hess, HF., Betzig, E. and Lippincott-Schwartz, J. (2008). High-density mapping of single-molecule trajectories with photoactivated localization microscopy. Nat Methods. 5(2):155-7.

2. Pendry, JB., Schurig, D. and Smith, DR. (2006). Controlling electromagnetic fields. Science. 312(5781):1780-2.

VIENNA DROSOPHILA RNAi CENTER (VDRC)

Krystyna Keleman

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

Krystyna Keleman / Staff Scientist/Head of the VDRC

RNAi library maintenance and development

Reinhard Klug / Stocks Maintenance Supervisor
Thomas Micheler / Software Developer
Virginia Salva Millan / Administration
Kristina Belogradova / Technical Assistant
Imen Ben Dridi / Technical Assistant
Michaela Eckmann / Technical Assistant
Michaela Fellner / Technical Assistant
Andreas Gansch / Technical Assistant
Angela Graf / Technical Assistant
Yasmin Gravogl / Technical Assistant
Attila Gyorgy / Technical Assistant
Laszlo Hunor / Technical Assistant
Selen Irez / Technical Assistant
Katharina Jandrasits / Technical Assistant
Amina Kadum / Technical Assistant
René Kaiser / Technical Assistant
Martin Kinberg / Technical Assistant
Irina Kolarov / Technical Assistant
Gabi Kuenzl / Technical Assistant
Zsuzsanna Portik Dobos[1] / Technical Assistant
Alsher Tashpulatov / Technical Assistant
Sandor Urmosi-Incze / Technical Assistant
Judith Utner / Technical Assistant
Stefanie Wandl / Technical Assistant
Svetlana Zorinyants / Technical Assistant

Keleman research group members

Sebastian Krüttner / PhD Student
Barbara Stepien[2] / PhD Student
Reinhard Hämmerle / Diploma Student

[1]from August
[2]from September

Genome-wide RNAi and memory formation

A Drosophila genome-wide transgenic RNAi library has been generated, allowing researchers to systematically study gene functions in specific tissues at specific developmental stages. The VDRC maintains, further develops and distributes this library to Drosophila researchers world-wide. In our own research group, we use this and other methods to understand how the fly forms memories that shape its mating behaviour.

Genome-wide RNAi

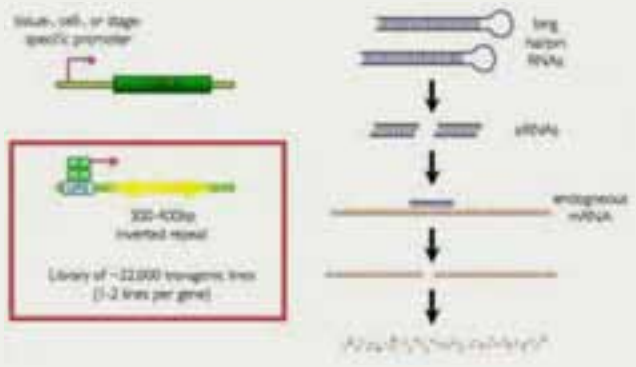
RNAi can be effectively triggered in Drosophila by spatially and temporally controlled expression of a dsRNA from a transgene that contains a long inverted repeat under control of a genetic promoter, the Gal4-responsive element (Figure 1). The creation of a genome-wide transgenic RNAi library [1] has revolutionised Drosophila genetics. The VDRC, a joint IMP-IMBA initiative, maintains, further develops and distributes this library. In its first year and a half, the VDRC has already delivered over 100,000 lines to more than 1,000 registered users world-wide. This has been made possible in part through core funding provided by the city of Vienna and the federal government, with the rest of the costs covered by user fees. We continue to further develop both the library and the service. Since summer 2008, we are also able to host external researchers who wish to conduct their RNAi screens in our on-site screening centre. The VDRC also provides such support for researchers in-house, having delivered over 50,000 lines to IMBA and IMP groups in 2008.

Learning and memory

The evolutionary mission of a male fly is to father as many offspring as possible. With an almost unlimited supply of sperm, his success depends almost entirely on his ability to discriminate receptive virgin females from unreceptive females. If he is too promiscuous, he may waste a lot of time and energy in futile courtship; if he is too choosy, he will miss out on excellent mating opportunities. The right balance appears not to be hard-wired into the fly's brain, but is rather something he learns by trial-and-error during his first few courtship experiences (Figure 2). This memory of these first sexual encounters can shape the male's mating strategy for several days – a long time in the life of a fly.

We are trying to understand the molecular and cellular mechanisms that underlie this robust and powerful form of long-term memory. We have recently demonstrated that the CPEB protein Orb2 – a regulator of mRNA translation – plays a critical role in this process [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.

1



2

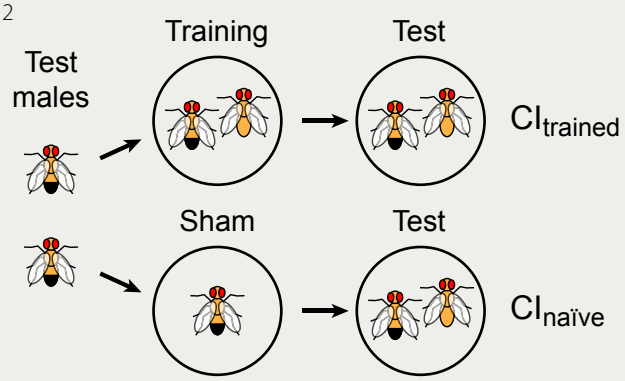


Figure 1: *RNAi in Drosophila.*

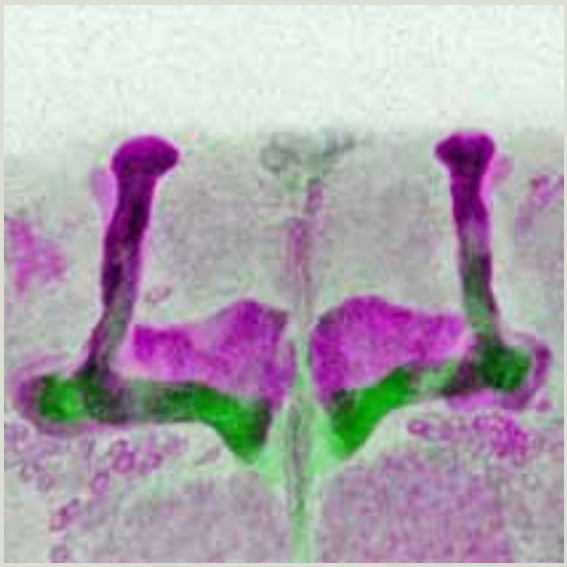
Figure 2: *Courtship conditioning. When tested with unreceptive mated females, males previously exposed to mated females court less than naïve (sham-trained) males (CI = courtship index; CItrained < CInaïve).*

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

To learn more about 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?

3



FURTHER READING

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

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

BIOOPTICS DEPARTMENT

aumayr@imp.ac.at

Karin Aumayr / Head of Facility

Pawel Pasierbek / Microscopy
Gabriele Stengl / Flow Cytometry

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

Current activities

Our newest addition is a Total Internal Reflection Fluorescence (TIRF) microscope, which substantially broadens the range of cutting-edge imaging tools available to the researchers' community at the IMP and IMBA. A second aspect of our recent activities is the use of deconvolution image analysis, especially for low light imaging.

TIRF microscopy enables selective visualisation of surface regions such as the basal plasma membrane or the growth of microtubules bound to the coverslip in a sea of fluorescence. The underlying optical principle is the generation of so-called evanescent waves if the incident light is totally reflected at a glass–water interface to selectively excite the sample in an approximately 100–200 nm thin layer above the cover slip surface. Thus, TIRFM provides crisp images of surfaces and can highlight cell membrane features without creating background light.

Deconvolution is the process of reversing the optical distortion imposed by an optical microscope by software algorithms and has been demonstrated to dramatically improve the final image quality. In the last couple of years, this software-based image processing technique has been preferentially used by users of the Deltavision Microscope System. We are now able to extend it to all suitable images taken on both widefield as well as on the various confocal systems because of additional improvements of the algorithm itself, the possibility to batch-process many images with a minimal amount of user interaction using the Huygens deconvolution software as well as increased computer power and therefore increased speed. Beside the general enhancement of the analysed images this technique also allows the amount of light for imaging to be reduced without impairing data quality. Minimising light exposure is especially important in live cell imaging to avoid both bleaching and damage to the cell caused by phototoxicity.

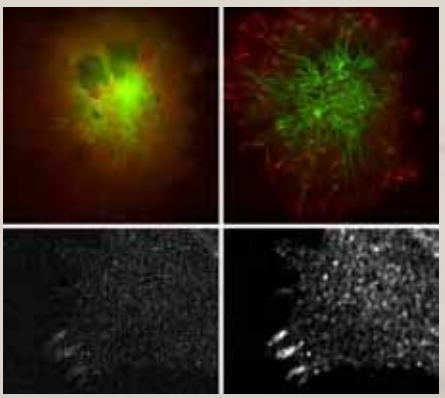


Figure: *The upper panel shows the comparison of a widefield (left) and a TIRF image (right) of a CAR cell stained with EB1 in red and alpha-Tubulin in green (Maria Nemethova). The lower panel shows an example for deconvolution of a low light image (left: image before and right: image after deconvolution) of a HeLaCell with Paxillin-GFP (Martin Breuss).*

ELECTRON MICROSCOPY

guenter.resch@imba.oeaw.ac.at

Günter Resch / Head of Electron Microscopy Facility

Marlene Brandstetter / Technical Assistant
Matthias Brunner / Programmer

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

Specimen Preparation

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

Microscopy

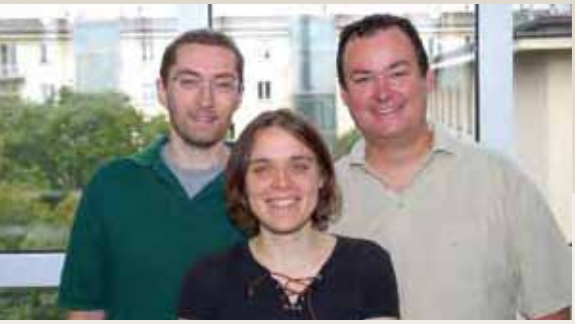
One cornerstone of the facility is the FEI Morgagni. This robust and easy to use 100 kV TEM equipped with a 11 megapixel CCD camera is tailored for routine needs in the multiuser environment of the facility.

While the Morgagni is an excellent tool for conventional TEM, advanced applications are the domain of the FEI TF30 Polara. This 300 kV TEM, unique in Austria and equipped with the most advanced imaging systems, was funded by a Vienna Spot of Excellence grant and became fully operational at the beginning of 2008. It is primarily being used for cryo-TEM of molecules and cells, and electron tomography.

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 in 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 for automated image acquisition (predominantly for single particle EM; collaboration with the Marlovits Group) and for status monitoring of the microscope was developed on the Polara. For image processing of EM data, especially from electron tomography, workstations and training are being provided.

A: *z-Sections from a three-dimensional reconstruction of the endothelial lining in a blood vessel by electron tomography.*
B: *Negatively stained rotavirus-like particles (micrograph courtesy of Cornelia Gänger, Ringrose Group, IMBA)*
C: *Glycerol sprayed and rotary shadowed α-actinin molecules*
D. *Dam1-decorated microtubules visualised by cryo-electron microscopy (sample: Fabienne Lampert, Westermann Group)*
E+F: *Cross-section of myelinated neurons (sample: Toshikatsu Hanada, Penninger Group, IMBA)*



BIOINFORMATICS

bioinfo.grp@imp.ac.at

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

Large Scale Data Analysis

Additional demands arise from the investigation of large functional genomics or high-throughput biological datasets. We engage in custom software and database development, and design computational and mathematical solutions that can cope with the higher load and memory requirements. To perform complex sequence analysis tasks we also maintain the IMP ANNOTATOR, a user-friendly web application and 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 now managed by the Sun Grid Engine (SGE) software, which provides policy-based workload management for a large number of jobs and nodes.

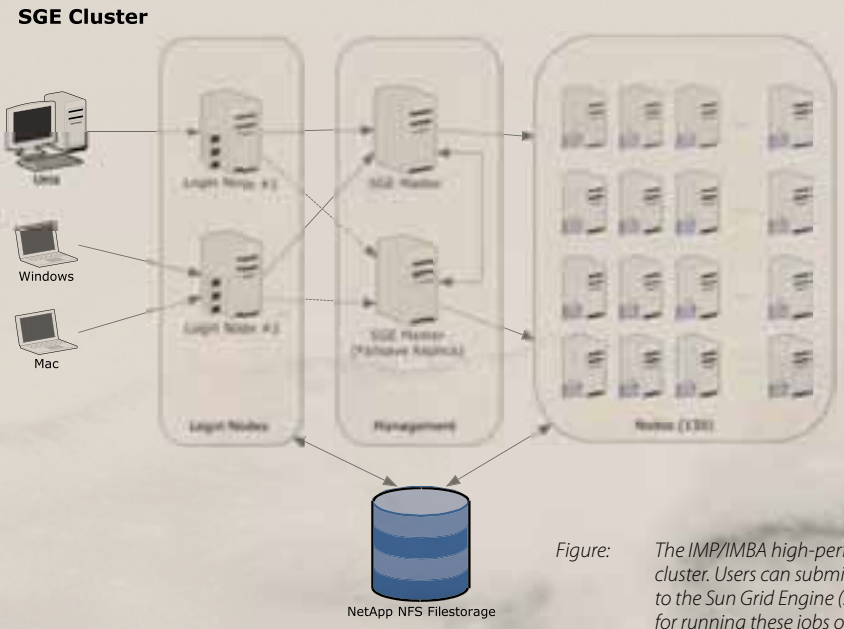


Figure: The IMP/IMBA high-performance computing (HPC) cluster. Users can submit jobs on dedicated login nodes to the Sun Grid Engine (SGE) master, which is responsible for running these jobs on the cluster nodes.

Training

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

GENOMICS

genomics@imp.ac.at

The newly founded Genomics Department currently comprises the microarray services, the cDNA clone repository and liquid handling robotics, formerly part of the Biooptics Department. Next Generation (Solexa) Sequencing will be offered as a new service to IMP and IMBA researchers in 2009.

Harald Scheuch / Engineer
Martin Radolf / Engineer
Markus Sonntagbauer / Trainee

Current activities

A major effort in 2008 was the re-arraying of RIKEN (FANTOM I to III) clones to provide a single chip representing more than 22,500 murine genes. To date, almost 100,000 RIKEN clones have been processed and spotted on a set of four chips (200 chips per batch). Hybridisation information from more than 500 hybridised samples was used to identify clones that reproducibly yielded good hybridisation signals in at least three independent experiments. This information was used to re-array a non-redundant set of 22,500 clones that were subsequently processed for printing.

The reduction in the number of slides used for hybridisation will not only reduce the cost per experiment but also the time for analysis by a factor of three, allowing the processing of more samples. Currently we are trying to establish hybridisation of labeled RNA instead of cDNA; this will also substantially shorten the handling time and decrease the cost per experiment.

Processing of microarray data has been completely automated by combining several packages from the BioConductor project. We can now provide users with fully annotated lists of differentially regulated genes.

In the past six months, together with scientists from the Busslinger and Jenuwein labs we have set up the infrastructure for Next Generation Sequencing. Sample preparation and in-process quality control have been established and are currently being optimised.
We expect a throughput of up to two runs per week yielding up to 2.7 Gigabases per run depending on sample quality. By the end of this year, deep sequencing applications like ChIP-seq and sequencing of small RNAs will be offered as a service to scientists of both institutes.

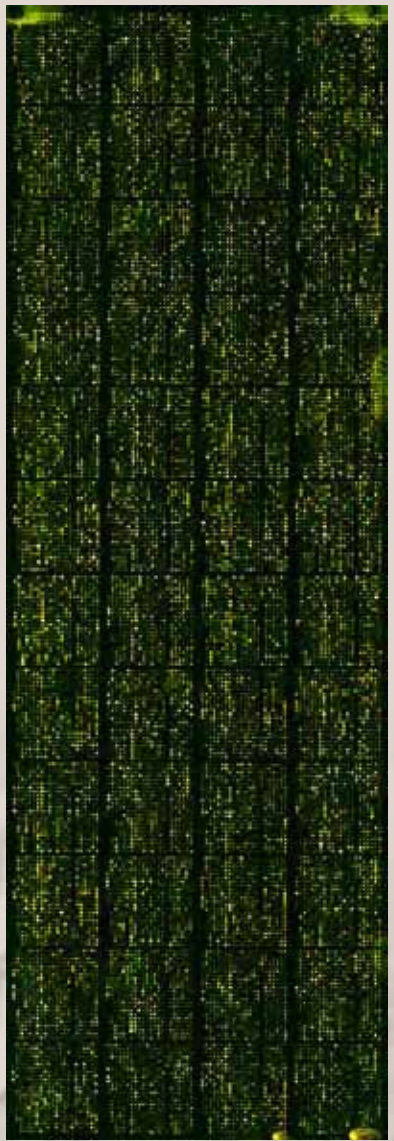


Figure: The new 23 K microarray comprising a non redundant set of cDNAs.

PROTEIN CHEMISTRY FACILITY

karl.mechtler@imp.ac.at

Karl Mechtler / Head of Facility

Johann Holzmann / Postdoc
Thomas Köcher / Postdoc
Karin Großteßner-Hain / PhD Student
Otto Hudecz / Technical Assistant
Gabriela Krssakova / Technical Assistant
Mathias Madalinski / Technical Assistant
Michael Mazanek / Technical Assistant
Goran Mitulovic / Technical Assistant
Susanne Opravil [1] / Technical Assistant
Elisabeth Roitinger / Technical Assistant
Michael Schutzbier / Technical Assistant
Ines Steinmacher / Technical Assistant

[1]since November 2008,
joint appointment with Stefan Westermann

The IMP-IMBA Protein Chemistry Facility performs a large variety of mass spectrometry experiments, including identification of proteins, characterisation of post-translational modifications and their quantification. Finally, our facility specialises in peptide synthesis and antibody purification.

Establishing and improving iTRAQ-based protein quantification

During the last decade there has been a growing interest in the description of biological systems in a quantitative and systematic manner. In parallel, methods have been developed for quantifying and identifying proteins in an unbiased way. One of these methods, termed isobaric tags for relative and absolute quantitation (iTRAQ), has become increasingly popular (Fig.1). We have applied iTRAQ to the analysis of protein complexes, which we analysed under different conditions in order to elucidate their dynamic behaviour. The methodology can be used in conjunction with many mass spectrometers and different fragmentation techniques. We evaluated the different approaches and developed a novel analytical strategy.

Quantitative analysis of knock-out mice

Many proteins regulated in disease may serve as candidates for clinically useful biomarkers and may also provide insight into the molecular mechanisms of disease processes. We successfully applied iTRAQ methodology to a mouse liver hepatitis model utilising c-jun knock-out mice.

Continuous improvements in the qualitative and quantitative analysis of phosphorylation sites

Protein phosphorylation is one of the most important post-translational modifications. We have developed several approaches for the selective enrichment of phosphorylated peptides based on the different metal-based enrichment methods such as immobilised metal affinity chromatography (IMAC) or affinity chromatography with titanium oxide. During the last few years we have also started to combine large-scale phosphorylation site analysis with relative quantification. We have applied these techniques to the large-scale analysis of kinase networks under different biological conditions.

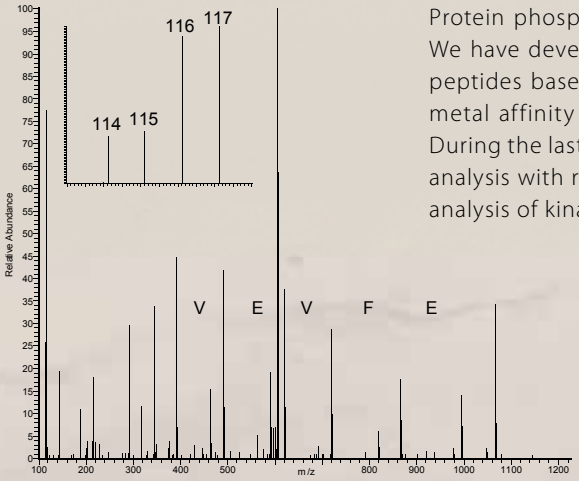


Figure: *The spectrum of the iTRAQ-labeled peptide AEFVEVTK is shown. A sufficient number of fragment ions allows its identification with high confidence. Part of the sequence is indicated. The insert shows the four iTRAQ reporter ions allowing the relative quantification of the samples. The ratio of the reporter ions reflects the original protein ratios.*

SERVICE DEPARTMENT

gotthold.schaffner@imp.ac.at

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

Our Media Kitchen and Fly Food staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies (more than 1,700,000 tubes and bottles per year) and other organisms. The Fly Food staff have moved back to the IMP building. There is now more space to create better and more convenient working conditions for preparing fly food and for storing all the goods we need. We also prepare many selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent E. coli strains and we maintain a stock of cloning vectors, sequencing primers and other reagents.

Gotthold Schaffner / Scientist

Ivan Botto / Technical Assistant
Markus Hohl / Technical Assistant
Shahryar Taghybeeglu / Technical Assistant
Gabriele Botto / Media Kitchen
Christa Detz-Jaderny / Media Kitchen
Ulrike Windholz / Media Kitchen
Sabine Jungwirth / 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

Production of antibodies

The production and isolation of many different monoclonal antibodies in hybridomas in collaboration with IMP group members, and organising antibody production in rabbits with an outside company, takes some of our working time capacity.

Sequencing and DNA isolation

The 48 capillary ABI 3730 DNA Genetic Analyser is the only work horse. The 16 capillary ABI 3100 Genetic Analyser is solely used as a back-up sequencer for emergencies.
We sequenced approximately 55,000 samples in the first 9 months of this year. This substantially increased demand is due to screening projects and the new fly library, but also to new groups at IMBA as well as at the IMP.
We are primarily using the 3730 DNA Analyser because of its sensitivity and lower consumables running costs. The average read-length is 700–900 bases for standard DNA samples with 50 cm ABI 3730 capillaries.
DNA sample quality and concentration are a problem even when prepared by sophisticated Qiagen Kits like Midi-, Maxi- or Minipreps, as are wrong primer sets or insufficiently documented plasmid constructs from outside sources. Compared to analysis by restriction digest, sequencing is faster and easier. The clean-up protocol with Sephadex G50 superfine columns on a 96-well microtiter plate format with optimised Sephadex consistency and centrifugation conditions has been transferred to a BioTek benchtop minirobot. The results tell us that there is no difference between the plates prepared by the robot and those prepared manually and they do not show "dye blobs" with good quality DNA samples.

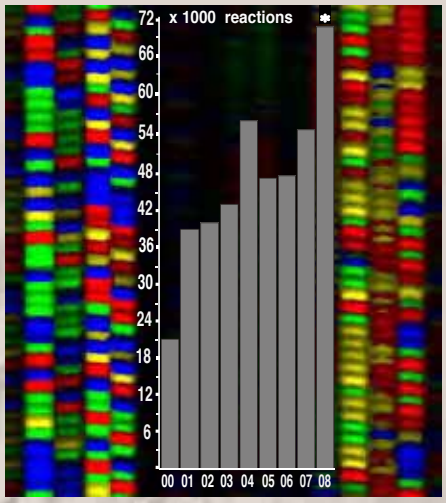


Figure: *A sequencing run on an ABI 377 PRISM and number of reactions analysed on ABI 377 (- 2001), on ABI 3100 (since 2001) and on ABI 3730 (since June 2004) with dye deoxy terminators (v3.1 since 2001) in the years 2000 to 2008 (scale 0 to 72,000).*
**calculated from January 2008 to September 2008 data*

HISTOLOGY DEPARTMENT

vukoslav.komnenovic@imba.oeaw.ac.at

Vukoslav Komnenovic / Head of Facility

Mihaela Grivej / 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.

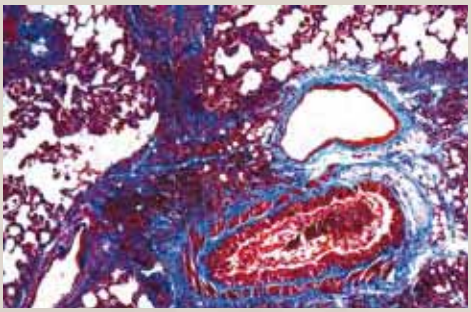
Sectioning of Paraffin and Frozen Tissues

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

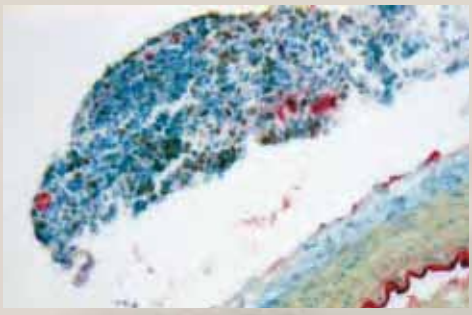
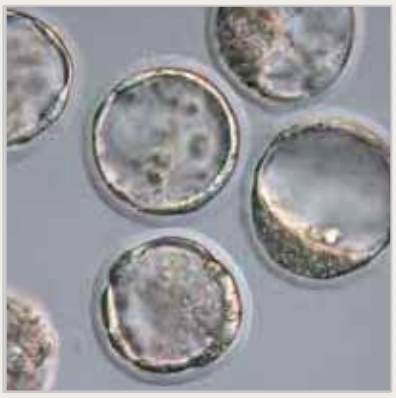


Figure 1: *Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloiud cells and dark blue for the Nuclei.*

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



ANIMAL HOUSE

animal@imp.ac.at

MOUSE SERVICE

transgenic@imp.ac.at

Animal House

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

Husbandry:

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

Animal House Services:

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

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

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

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

Figure 2: *Mouse blastocysts.*

Publications

BEUG

Gal, A., Sjöblom, T., Fedorova, L., Imreh, S., Beug, H., Moustakas, A. (2008). Sustained TGF beta exposure suppresses Smad and non-Smad signalling in mammary epithelial cells, leading to EMT and inhibition of growth arrest and apoptosis. Oncogene. 27(9):1218-30

Grebien, F., Kerenyi, MA., Kovacic, B., Kolbe, T., Becker, V., Dolznig, H., Pfeffer, K., Klingmüller, U., Müller, M., Beug, H., Müllner, EW., Moriggl, R. (2008). Stat5 activation enables erythropoiesis in the absence of EpoR and Jak2. Blood. 111(9):4511-22

Grech, G., Blázquez-Domingo, M., Kolbus, A., Bakker, W.J., Müllner, EW., Beug, H., von Lindern, M. (2008). Igbp1 is part of a positive feedback loop in stem cell factor-dependent, selective mRNA translation initiation inhibiting erythroid differentiation. Blood. 112(7):2750-60

Kerenyi, MA., Grebien, F., Gehart, H., Schifrer, M., Artaker, M., Kovacic, B., Beug, H., Moriggl, R., Müllner, EW. (2008). Stat5 regulates cellular iron uptake of erythroid cells via IRP-2 and TfR-1. Blood. 112(9):3878-88

Kornfeld, JW., Grebien, F., Kerenyi, MA., Friedbichler, K., Kovacic, B., Zankl, B., Hoelbl, A., Nivarti, H., Beug, H., Sexl, V., Muller, M., Kenner, L., Mullner, EW., Gouilleux, F., Moriggl, R. (2008). The different functions of Stat5 and chromatin alteration through Stat5 proteins. Front Biosci. 13:6237-54

Lahsnig, C., Mikula M., Petz, M., Zulehner, D., Schneller, D., Huber, H., Csiszar, A., Beug, H. and Mikulits, W. (2008). ILEI requires oncogenic Ras for the epithelial to mesenchymal transition of hepatocytes and liver carcinoma progression. Oncogene advance online publication, Nov. 17th doi: 10.1038/onc.2008.418.

Schähs, P., Weidinger, P., Probst, OC., Svoboda, B., Stadlmann, J., Beug, H., Waerner, T., Mach, L. (2008). Cellular repressor of E1A-stimulated genes is a bona fide lysosomal protein which undergoes proteolytic maturation during its biosynthesis. Exp Cell Res. 314(16):3036-47

BUSSLINGER

Cobaleda, C., Busslinger, M. (2008). Developmental plasticity of lymphocytes. Curr Opin Immunol. 20(2):139-48

Graf, T., Busslinger, M. (2008). B young again. Immunity. 28(5):606-8

Guo, F., Tänzer, S., Busslinger, M., Weih, F. (2008). Lack of nuclear factor-kappa B2/p100 causes a RelB-dependent block in early B lymphopoiesis. Blood. 112(3):551-9

Kwon, K., Hutter, C., Sun, Q., Bilic, I., Cobaleda, C., Malin, S., Busslinger, M. (2008). Instructive role of the transcription factor E2A in early B lymphopoiesis and germinal center B cell development. Immunity. 28(6):751-62

Schotta, G., Sengupta, R., Kubicek, S., Malin, S., Kauer, M., Callén, E., Celeste, A., Pagani, M., Opravil, S., De La Rosa-Velazquez, IA., Espejo, A., Bedford, MT., Nussenzweig, A., Busslinger, M., Jenuwein, T. (2008). A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev. 22(15):2048-61

CLAUSEN

Krojer, T., Pangerl, K., Kurt, J., Sawa, J., Stingl, C., Mechtler, K., Huber, R., Ehrmann, M., Clausen, T. (2008). Interplay of PDZ and protease domain of DegP ensures efficient elimination of misfolded proteins. Proc Natl Acad Sci U S A. 105(22):7702-7

Krojer, T., Sawa, J., Schäfer, E., Saibil, HR., Ehrmann, M., Clausen, T. (2008). Structural basis for the regulated protease and chaperone function of DegP. Nature. 453(7197):885-90

Meltzer, M., Hasenbein, S., Hauske, P., Kucz, N., Merdanovic, M., Grau, S., Beil, A., Jones, D., Krojer, T., Clausen, T., Ehrmann, M., Kaiser, M. (2008). Allosteric activation of HtrA protease DegP by stress signals during bacterial protein quality control. Angew Chem Int Ed Engl. 47(7):1332-4

DICKSON

Barkus, RV., Klyachko, O., Horiuchi, D., Dickson, BJ., Saxton, WM. (2008). Identification of an axonal kinesin-3 motor for fast anterograde vesicle transport that facilitates retrograde transport of neuropeptides. Mol Biol Cell. 19(1):274-83

Berger, J., Senti, KA., Senti, G., Newsome, TP., Asling, B., Dickson, BJ., Suzuki, T. (2008). Systematic identification of genes that regulate neuronal wiring in the Drosophila visual system. PLoS Genet. 4(5):e1000085

Chen, D., Ahlford, A., Schnorrer, F., Kalchhauser, I., Fellner, M., Viràgh, E., Kiss, I., Syvänen, AC., Dickson, BJ. (2008). High-resolution, high-throughput SNP mapping in Drosophila melanogaster. Nat Methods. 5(4):323-9

Datta, SR., Vasconcelos, ML., Ruta, V., Luo, S., Wong, A., Demir, E., Flores, J., Balonze, K., Dickson, BJ., Axel, R. (2008). The Drosophila pheromone cVA activates a sexually dimorphic neural circuit. Nature. 452(7186):473-7

Dickson, B. J. (2008). Wired for sex: the neurobiology of Drosophila mating decisions. Science. 332: 904-909.

Pauli, A., Althoff, F., Oliveira, RA., Heidmann, S., Schuldiner, O., Lehner, CF., Dickson, BJ., Nasmyth, K. (2008). Cell-type-specific TEV protease cleavage reveals cohesin functions in Drosophila neurons. Dev Cell. 14(2):239-51

Yapici, N., Kim, YJ., Ribeiro, C., Dickson, BJ. (2008). A receptor that mediates the post-mating switch in Drosophila reproductive behaviour. Nature. 451(7174):33-7

Yu, JY., Dickson, BJ. (2008). Neuroscience: hidden female talent. Nature. 453(7191):41-2

HARTMANN

Taschner, MJ., Rafigh, M., Lampert, F., Schnaiter, S., Hartmann, C. (2008). Ca2+/Calmodulin-dependent kinase II signaling causes skeletal overgrowth and premature chondrocyte maturation. Dev Biol. 317(1):132-46

Fujimori, S., Kostanova Poliakova, D., Hartmann C. (2008). Role of Growth factors in Bone development and differentiation. In: The Skeletal System. Ed. Pourquie, O. (Cold Spring Harbor Laboratory Press, New York). Chapter 8, in press.

HEINZE

Bachir, AI., Kolin, DL., Heinze, KG., Hebert, B., Wiseman, PW. (2008). A guide to accurate measurement of diffusion using fluorescence correlation techniques with blinking quantum dot nanoparticle labels. J Chem Phys. 128(22):225105

JENUWEIN

Allis CD, Berger SL, Cote J, Dent S, Jenuwein T, Kouzarides T, Pillus L, Reinberg D, Shi Y, Shiekhattar R, Shilatifard A, Workman J and Zhang Y. (2007). New nomenclature for chromatin-modifying enzymes. Cell 131, 633-636.

American Association for Cancer Research Human Epigenome Task Force; European Union, Network of Excellence, Scientific Advisory Board. (2008). Moving AHEAD with an international human epigenome project. Nature 454, 711-715.

Pannetier, M., Julien, E., Schotta, G., Tardat, M., Sardet, C., Jenuwein, T., Feil, R. (2008). PR-SET7 and SUV4-20H regulate H4 lysine-20 methylation at imprinting control regions in the mouse. EMBO Rep. 9(10):998-1005

Schotta, G., Sengupta, R., Kubicek, S., Malin, S., Kauer, M., Callén, E., Celeste, A., Pagani, M., Opravil, S., De La Rosa-Velazquez, IA., Espejo, A., Bedford, MT., Nussenzweig, A., Busslinger, M., Jenuwein, T. (2008). A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev. 22(15):2048-61

PETERS

Kastner, B., Fischer, N., Golas, MM., Sander, B., Dube, P., Boehringer, D., Hartmuth, K., Deckert, J., Hauer, F., Wolf, E., Uchtenhagen, H., Urlaub, H., Herzog, F., Peters, JM., Poerschke, D., Lührmann, R., Stark, H. (2008). GraFix: sample preparation for single-particle electron cryomicroscopy. Nat Methods. 5(1):53-5

Koch, B., Kueng, S., Ruckenbauer, C., Wendt, KS., Peters, JM. (2008). The Suv39h-HP1 histone methylation pathway is dispensable for enrichment and protection of cohesin at centromeres in mammalian cells. Chromosoma. 117(2):199-210

Peters, JM. (2008). Checkpoint control: the journey continues. Curr Biol. 18(4):R170-2

Peters, JM, Tedeschi, A and Schmitz, J (2008). The cohesin complex and its roles in chromosome biology. Genes Dev. 22, 3089-3114

Petronczki, M., Lénárt, P., Peters, JM. (2008). Polo on the Rise-from Mitotic Entry to Cytokinesis with Plk1. Dev Cell. 14(5):646-59

Poser, I., Sarov, M., Hutchins, JR., Hériché, JK., Toyoda, Y., Pozniakovsky, A., Weigl, D., Nitzsche, A., Hegemann, B., Bird, AW., Pelletier, L., Kittler, R., Hua, S., Naumann, R., Augsburg, M., Sykora, MM., Hofemeister, H., Zhang, Y., Nasmyth, K., White, KP., Dietzel, S., Mechtler, K., Durbin, R., Stewart, AF., Peters, JM., Buchholz, F., Hyman, AA. (2008). BAC TransgeneOmics: a high-throughput method for exploration of protein function in mammals. Nat Methods. 5(5):409-15

Taylor, S., Peters, JM. (2008). Polo and Aurora kinases: lessons derived from chemical biology. Curr Opin Cell Biol. 20(1):77-84

Vernos, I., Peters, JM. (2008). Twenty years of cell-cycle conferences in Roscoff. Nat Cell Biol. 10(8):877-80

Wendt, KS., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., Yahata, K., Imamoto, F., Aburatani, H., Nakao, M., Imamoto, N., Maeshima, K., Shirahige, K., Peters, JM. (2008). Cohesin mediates transcriptional insulation by CCCTC-binding factor. Nature. 451(7180):796-801

Wendt, KS. and Peters, JM. (2009). How cohesin and CTCF cooperate in regulating gene expression. Chromos. Res., in press.

WAGNER

Bozec, A., Bakiri, L., Hoebertz, A., Eferl, R., Schilling, AF., Komnenovic, V., Scheuch, H., Priemel, M., Stewart, CL., Amling, M., Wagner, EF. (2008). Osteoclast size is controlled by Fra-2 through LIF/LIF-receptor signalling and hypoxia. Nature. 454(7201):221-5

Durchdewald, M., Guinea-Viniegra, J., Haag, D., Riehl, A., Lichter, P., Hahn, M., Wagner, EF., Angel, P., Hess, J. (2008). Podoplanin is a novel fos target gene in skin carcinogenesis. Cancer Res. 68(17):6877-83

Eferl, R., Hasselblatt, P., Rath, M., Popper, H., Zenz, R., Komnenovic, V., Idarraga, MH., Kenner, L., Wagner, EF. (2008). Development of pulmonary fibrosis through a pathway involving the transcription factor Fra-2/AP-1. Proc Natl Acad Sci U S A. 105(30):10525-30

Gerlitz, O., Wagner, EF., Moreno, E. (2008). When flies and mice develop cancer. Meeting on development and cancer. EMBO Rep. 9(8):730-4

Gurzov, EN., Bakiri, L., Alfaro, JM., Wagner, EF., Izquierdo, M. (2008). Targeting c-Jun and JunB proteins as potential anticancer cell therapy. Oncogene. 27(5):641-52

Gurzov, EN., Ortis, F., Bakiri, L., Wagner, EF., Eizirik, DL. (2008). JunB Inhibits ER Stress and Apoptosis in Pancreatic Beta Cells. PLoS ONE. 3(8):e3030

Hasselblatt, P., Gresh, L., Kudo, H., Guinea-Viniegra, J., Wagner, EF. (2008). The role of the transcription factor AP-1 in colitis-associated and beta-catenin-dependent intestinal tumorigenesis in mice. Oncogene. 27(47):6102-9

Hoffmann, E., Ashouri, J., Wolter, S., Doerrie, A., Dittrich-Breiholz, O., Schneider, H., Wagner, EF., Troppmair, J., Mackman, N., Kracht, M. (2008). Transcriptional regulation of EGR-1 by the interleukin-1-JNK-MKK7-c-Jun pathway. J Biol Chem. 283(18):12120-8

Hui, L., Zatloukal, K., Scheuch, H., Stepniak, E., Wagner, EF. (2008). Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-dependent p21 downregulation. J Clin Invest; on line Nov. 6

Jiao, X., Katiyar, S., Liu, M., Mueller, SC., Lisanti, MP., Li, A., Pestell, TG., Wu, K., Ju, X., Li, Z., Wagner, EF., Takeya, T., Wang, C., Pestell, RG. (2008). Disruption of c-Jun reduces cellular migration and invasion through inhibition of c-Src and hyperactivation of ROCK II kinase. Mol Biol Cell. 19(4):1378-90

Kawamata, A., Izu, Y., Yokoyama, H., Amagasa, T., Wagner, EF., Nakashima, K., Ezura, Y., Hayata, T., Noda, M. (2008). JunD suppresses bone formation and contributes to low bone mass induced by estrogen depletion. J Cell Biochem. 103(4):1037-45

Kolev, V., Mandinova, A., Guinea-Viniegra, J., Hu, B., Lefort, K., Lambertini, C., Neel, V., Dummer, R., Wagner, EF., Dotto, GP. (2008). EGFR signalling as a negative regulator of Notch1 gene transcription and function in proliferating keratinocytes and cancer. Nat Cell Biol. 10(8):902-11

Meixner, A., Zenz, R., Schonthaler, HB., Kenner, L., Scheuch, H., Penninger, JM., Wagner, EF. (2008). Epidermal JunB represses G-CSF transcription and affects haematopoiesis and bone formation. Nat Cell Biol. 10(8):1003-11

Ruiz-Bonilla, V., Perdiguero, E., Gresh, L., Serrano, AL., Zamora, M., Sousa-Victor, P., Jardí, M., Wagner, EF., Muñoz-Cánoves, P. (2008). Efficient adult skeletal muscle regeneration in mice deficient in p38beta, p38gamma and p38delta MAP kinases. Cell Cycle. 7(14):2208-14

Wada, T., Stepniak, E., Hui, L., Leibbrandt, A., Katada, T., Nishina, H., Wagner, EF., Penninger, JM. (2008). Antagonistic control of cell fates by JNK and p38-MAPK signaling. Cell Death Differ. 15(1):89-93

Wolter, S., Doerrie, A., Weber, A., Schneider, H., Hoffmann, E., von der Ohe, J., Bakiri, L., Wagner, EF., Resch, K., Kracht, M. (2008). c-Jun controls histone modifications, NF-kappaB recruitment, and RNA polymerase II function to activate the ccl2 gene. Mol Cell Biol. 28(13):4407-23

Yotova, IY., Vlatkovic, IM., Pauler, FM., Warczok, KE., Ambros, PF., Oshimura, M., Theussl, HC., Gessler, M., Wagner, EF., Barlow, DP. (2008). Identification of the human homolog of the imprinted mouse Air non-coding RNA. Genomics. 92(6):464-73.

Zenz, R., Eferl, R., Scheinecker, C., Redlich, K., Smolen, J., Schonthaler, HB., Kenner, L., Tschachler, E., Wagner, EF. (2008). Activator protein 1 (Fos/Jun) functions in inflammatory bone and skin disease. Arthritis Res Ther. 10(1):201

WESTERMANN

Grishchuk, EL., Efremov, AK., Volkov, VA., Spiridonov, IS., Gudimchuk, N., Westermann, S., Drubin, D., Barnes, G., McIntosh, JR., Ataullakhanov, FI. (2008). The Dam1 ring binds microtubules strongly enough to be a processive as well as energy-efficient coupler for chromosome motion. Proc Natl Acad Sci U S A. 105(40):15423-8

Grishchuk, EL., Spiridonov, IS., Volkov, VA., Efremov, A., Westermann, S., Drubin, D., Barnes, G., Ataullakhanov, FI., McIntosh, JR. (2008). Different assemblies of the DAM1 complex follow shortening microtubules by distinct mechanisms. Proc Natl Acad Sci U S A. 105(19):6918-23

Wang, H.-W., Long, S., Ciferri, C., Westermann, S., Drubin, D, Barnes, G. and Nogales, E. (2008). Architecture and Flexibility of the Yeast Ndc80 kinetochore complex. J. Mol. Biol. 383(4):894-903.

WUTZ

Shibata, S., Yokota, T., Wutz, A. (2008). Synergy of Eed and Tsix in the repression of Xist gene and X-chromosome inactivation. EMBO J. 27(13):1816-26

Mietton, F., Sengupta, AK., Molla, A., Picchi, G., Barral, S., Heliot, L., Grange, T., Wutz, A. and Dimitrov S. (2008). Weak, but uniform enrichment of the histone variant macroH2A1 along the inactive X chromosome. Mol Cell Biol. 29(1):150-6.

Shibata, S. and Wutz, A. (2008). Transcript versus transcription? The regulation of X-inactivation by antisense Tsix expression. Epigenetics 3:5, 1-4

ELECTRON MICROSCOPY

Breitsprecher D., Kiesewetter AK., Linkner J., Urbanke C., Resch GP., Small JV., and Faix J. (2008). Clustering of VASP actively drives processive, WH2 domain-mediated actin filament elongation. EMBO Journal. 27(22):2943-54

Small, JV., Auinger, S., Nemethova, M., Koestler, S., Goldie, KN., Hoenger, A., Resch, GP. (2008). Unravelling the structure of the lamellipodium. J Microsc. 231(3):479-85

BIOINFORMATICS

d'Erfurth, I., Jolivet, S., Froger, N., Catrice, O., Novatchkova, M., Simon, M., Jenczewski, E. and Mercier, R. (2008). Mutations in AtPS1 (Arabidopsis thaliana parallel spindle 1) lead to the production of diploid pollen grains. PLoS Genet. 4(11):e1000274. Epub 2008 Nov 28.

Macaisne, N., Novatchkova, M., Peirera, L., Vezon, D., Jolivet, S., Froger, N., Chelysheva, L., Grelon, M., Mercier, R. (2008). SHOC1, an XPF endonuclease-related protein, is essential for the formation of class I meiotic crossovers. Curr Biol. 18(18):1432-7

Mochizuki, K., Novatchkova, M., Loidl, J. (2008). DNA double-strand breaks, but not crossovers, are required for the reorganization of meiotic nuclei in Tetrahymena. J Cell Sci. 121(Pt 13):2148-58

Riehs, N., Akimcheva, S., Puizina, J., Bulankova, P., Idol, RA., Siroky, J., Schleiffer, A., Schweizer, D., Shippen, DE., Riha, K. (2008). Arabidopsis SMG7 protein is required for exit from meiosis. J Cell Sci. 121(Pt 13):2208-16

Tomasi, T., Hakeda-Suzuki, S., Ohler, S., Schleiffer, A., Suzuki, T. (2008). The transmembrane protein Golden goal regulates R8 photoreceptor axon-axon and axon-target interactions. Neuron. 57(5):691-704

PROTEIN CHEMISTRY FACILITY

Krojer, T., Pangerl, K., Kurt, J., Sawa, J., Stingl, C., Mechtler, K., Huber, R., Ehrmann, M., Clausen, T. (2008). Interplay of PDZ and protease domain of DegP ensures efficient elimination of misfolded proteins. Proc Natl Acad Sci U S A. 105(22):7702-7

Neumüller, RA., Betschinger, J., Fischer, A., Bushati, N., Poernbacher, I., Mechtler, K., Cohen, SM., Knoblich, JA. (2008). Mei-P26 regulates microRNAs and cell growth in the Drosophila ovarian stem cell lineage. Nature. 454(7201):241-5

Poser, I., Sarov, M., Hutchins, JR., Hériché, JK., Toyoda, Y., Pozniakovsky, A., Weigl, D., Nitzsche, A., Hegemann, B., Bird, AW., Pelletier, L., Kittler, R., Hua, S., Naumann, R., Augsburg, M., Sykora, MM., Hofemeister, H., Zhang, Y., Nasmyth, K., White, KP., Dietzel, S., Mechtler, K., Durbin, R., Stewart, AF., Peters, JM., Buchholz, F., Hyman, AA. (2008). BAC TransgeneOmics: a high-throughput method for exploration of protein function in mammals. Nat Methods. 5(5):409-15

Schmidt, A., Csaszar, E., Ammerer, G., Mechtler, K. (2008). Enhanced detection and identification of multiply phosphorylated peptides using TiO2 enrichment in combination with MALDI TOF/TOF MS. Proteomics. 8(21):4577-92

Winter, S., Simboeck, E., Fischle, W., Zupkovitz, G., Dohnal, I., Mechtler, K., Ammerer, G., Seiser, C. (2008). 14-3-3 proteins recognize a histone code at histone H3 and are required for transcriptional activation. EMBO J. 27(1):88-99

HISTOLOGY DEPARTMENT

Bozec, A., Bakiri, L., Hoebertz, A., Eferl, R., Schilling, AF., Komnenovic, V., Scheuch, H., Priemel, M., Stewart, CL., Amling, M., Wagner, EF. (2008). Osteoclast size is controlled by Fra-2 through LIF/LIF-receptor signalling and hypoxia. Nature. 454(7201):221-5

Eferl, R., Hasselblatt, P., Rath, M., Popper, H., Zenz, R., Komnenovic, V., Idarraga, MH., Kenner, L., Wagner, EF. (2008). Development of pulmonary fibrosis through a pathway involving the transcription factor Fra-2/AP-1. Proc Natl Acad Sci U S A. 105(30):10525-30

MOUSE SERVICE

Yotova, IY., Vlatkovic, IM., Pauler, FM., Warczok, KE., Ambros, PF., Oshimura, M., Theussl, HC., Gessler, M., Wagner, EF., Barlow, DP. (2008). Identification of the human homolog of the imprinted mouse Air non-coding RNA. Genomics. 92(6):464-73.

GENOMICS

Bozec, A., Bakiri, L., Hoebertz, A., Eferl, R., Schilling, AF., Komnenovic, V., Scheuch, H., Priemel, M., Stewart, CL., Amling, M., Wagner, EF. (2008). Osteoclast size is controlled by Fra-2 through LIF/LIF-receptor signalling and hypoxia. Nature. 454(7201):221-5

Hui, L., Zatloukal, K., Scheuch, H., Stepniak, E., Wagner, EF. (2008). Proliferation of human HCC cells and chemically induced mouse liver cancers requires JNK1-dependent p21 downregulation. J Clin Invest; on line Nov. 6

Meixner, A., Zenz, R., Schonthaler, HB., Kenner, L., Scheuch, H., Penninger, JM., Wagner, EF. (2008). Epidermal JunB represses G-CSF transcription and affects haematopoiesis and bone formation. Nat Cell Biol. 10(8):1003-11

IMP Awards

Barry Dickson

Advanced Investigator Grant by the European Research Council (October)

Jakob Fuhrmann

Kirsten Peter Rabitsch Award (October)

Karl Mechtler

Outstanding ABRF Poster Award 2008, ABRF Conference at Salt Lake City, USA (February)

Jan-Michael Peters

Mendel Lecture Brno (October)

Justyna Sawa-Makarska

VBC PhD Award (November)

Stefan Westermann

ERC Starting grant by the European Research Council (January)

Seminar Speakers

JANUARY

10. January — Ulrike Gaul
Rockefeller University, New York
Glial function in nervous system development

10. January — Anne Ridley
Ludwig Institute for Cancer Research, London
Rho GTPases: signalling in cell adhesion and migration

21. January — Daryl Gohl
Dept. of Molecular Biology, Princeton University
Boundary elements and the formation of regulatory domains

23. January — Takanobu Nakazawa
Institute of Medical Science, University of Tokyo
Roles of tyrosine-phosphorylation of NMDA receptors in brain

24. January — George Klein
Karolinska Institutet, MTC
Why do we not all die of cancer at an early age?

FEBRUARY

01. February — Sylvia Synowsky
Department of Biomolecular Mass Spectrometry, Utrecht University
Macromolecular mass spectrometry of RNA regulating protein machineries

04. February — Wael Tadros
University of Toronto, Canada
Posttranscriptional regulation of maternal transcripts by the PAN GU kinase in the early Drosophila embryo

05. February — Christopher D. Lima
Sloan-Kettering Institute, New York
Structure and function in the SUMO pathway

07. February — Pascale Cossart
Pasteur Institute, Paris
The fascinating strategies used by Listeria monocytogenes during infection: new concepts in infection biology and cell biology

18. February — Magnus Nordborg
Molecular & Computational Biology, University of Southern California
How genomics is helping us understand adaptive variation

21. February — Jürgen Soll
Ludwig-Maximilians-University Munich
Protein and metabolite transport in chloroplasts

MARCH

03. March — Jonathan Comeau
McGill University, Montreal
Advances in image cross-correlation spectroscopy to measure protein interactions in cells

06. March — Scott Keeney
Memorial Sloan-Kettering Cancer Center
Mechanism and regulation of meiotic recombination

06. March — Ichiro Taniuchi
Research Center for Allergy and Immunology, RIKEN
Mechanism of Th-POK gene regulation during differntiation of helper-lineage T cells

07. March — Paul De Koninck
Centre de Recherche Université Laval Robert-Giffard
Ins and outs of CaM kinase II at synapses

26. March — Oliver Griesbeck
Max-Planck-Institute of Neurobiology
Engineering and imaging Green Fluorescent Protein biosensors

27. March — Michel Tremblay
McGill Cancer Centre
Tyrosine phosphatases in signaling and diseases

27. March — Philipp Staber
Division of Hematology, Medical University, Graz
Functions of AP-1 in ALC(L)-lymphoma formation

APRIL

07. April — Clemens Schmitt
Charité - Universitätsmedizin Berlin
Control and complexities of oncogene-induced senescence

08. April — Jerome Dejardin
Department of Molecular Biology, Massachussetts General Hospital
Telomere composition in human cancer cells

10. April — Wendy Bickmore
MRC Human Genetics Unit, Edinburgh
Alteration of gene expression at the nuclear periphery of human cells

11. April — Bryan Turner
University of Birmingham
The ups and downs of X-linked gene expression in embryonic stem cells

11. April — Stephen West
Cancer Research UK
Defective DNA strand break repair and human disease

14. April — Arthur Houweling
Bernstein Center for Computational Neuroscience, Berlin
Two-photon imaging of cortical networks and single neuron stimulation in the awake behaving animal

17. April — James Poulet
Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne
Cortical processing during behaviour

17. April — John Hammer
Lab of Cell Biology, NHLBI, Bethesda, USA
Design principles for myosin V-dependent organelle transport

18. April — Jan Siemens
University of San Francisco
Molecular mechanisms of pain and temperature sensation

21. April — Thomas McHugh
Picower Institute, MIT, Cambridge
A circuit genetics approach to understanding hippocampal function

24. April — Craig Mello
Howard Hughes Medical Institute, University of Massachusetts
Return to the RNAi world: Rethinking gene expression, evolution and medicine

25. April — Jody Puglisi
Stanford University School of Medicine
Dynamics of translation

MAY

08. May — Rudolf Grosschedl
Max-Planck-Institute of Immunobiology
Regulation of B lymphopoiesis and hematopoiesis by EBF transcription factors

20. May — Anthony Hyman
Max-Planck-Institute for Cell Biology and Genetics, Dresden
Systems approaches to cell division

29. May — Thomas Cremer
LMU Biozentrum
Chromosome territories and nuclear organization: structural, functional and evolutionary aspects

30. May — Antonius Rolink
University Basel
Involvement of Notch signaling in T and dendritic cell development

JUNE

03. June — Christof Niehrs
German Cancer Research Center, Heidelberg
Wnt regulation in early vertebrate development: From molecules to mice

05. June — Sebastian Carotta
WEHI, Melbourne
Important roles of PU.1 and IRF-8 in plasma cell development and leukemia

10. June — Julien Mouysset
University Clinic Hamburg Eppendorf
A novel role for the C. elegans CDC-48/UFD-1/NPL-4 complex in DNA replication

12. June — Masanori Hatakeyama
Institute for Genetic Medicine, Hokkaido University
Oncogenic mechanism of Helicobacter pylori

13. June — Ethan Shevach
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda
Regulatory T cells in medicine – mostly facts and some fantasies

19. June — Steve Reiner
Abramson Family Cancer Research Institute, University of Pennsylvania
Asymmetric cell division during mammalian immunity

20. June — Hiromi Tagoh
Leeds Institute of Molecular Medicine, St. James's University Hospital
Stem cell specific epigenetic priming and B cell specific transcriptional activation at the murine Cd19 locus

24. June — Thomas Hummel
Institute for Neurobiology, University of Muenster
Patterning the Drosophila olfactory system

26. June — Miguel C. Seabra
Imperial College London, Faculty of Medicine
Rab GTPases, membrane traffic and disease

JULY

02. July — Karel Svoboda
HHMI, Janelia Farm Research Campus
Illuminating cortical synapses and circuits

03. July — Antonio Cassone
Instituto Superiore di Sanita, Rome
Beta glucan based fungal vaccines and anti-virulence antibodies

04. July — David Tuveson
Cancer Research UK, Cambridge
Modeling and manipulating cancer in mice

09. July — Barbora Maralikova
Royal Holloway University of London
Characterisation of Entamoeba histolytica proteome and search for mitosomes

10. July — Johann Holzmann
Medical University of Vienna, Center of Anatomy and Cell Biology
RNase P without RNA: Identification and functional reconstitution of the human mitochondrial tRNA processing enzyme

14. July — Marc Kirschner
Harvard Medical School, Boston
Control of protein level: Effects of gene dosage in yeast and response to Wnt signaling in mammalian cells

15. July — Oliver Bell
Friedrich Miescher Institute for Biomedical Research, Basel
Localization and function of histone methylation at active genes in Drosophila

16. July — Antonio Meireles-Filho
Institute of Zoology, University of Regensburg
A chimeric cycle gene reveals ancestral features of the Drosophila melanogaster circadian clock

17. July — Mark Hochstrasser
Department of Molecular Biophysics & Biochemistry, Yale University
Regulation of the ubiquitin-proteasome system

23. July — Brice Bathellier
University of Bern, Switzerland
Dynamics and coding in the mammalian olfactory system

24. July — Magdalena Götz
GSF-Institut für Stammzellforschung
Glial cells generate neurons: new views on reactive gliosis and neural repair

AUGUST

05. August Yonatan Loewenstein
Hebrew University, Jerusalem
A neuroeconomics approach to the matching law

25. August Alexander Tarakhovsky
Rockefeller University, New York
Late summer epigenetics

29. August Florian Engert
Harvard University
Visual processing and motor control in the larval zebrafish

SEPTEMBER

03. September Hitoshi Niwa
RIKEN Center for Developmental Biology, Kobe
Molecular mechanism to maintain pluripotency

04. September Rachel Wilson
Harvard Medical School
Lessons from a tiny brain: using electrophysiology and genetics to understand sensory processing in Drosophila

18. September Kristian Helin
Biotech Research & Innovation Centre, Copenhagen
Epigenetics, stem cells and cancer

24. September David Allis
The Rockefeller University
Beyond the double helix: Writing and reading the "Histone Code"

25. September Elisa Izaurralde
Max-Planck-Institute for Developmental Biology, Tuebingen
Mechanisms of miRNA-mediated gene silencing

OCTOBER

06. October Woj Wojtowicz
University of California, Los Angeles (UCLA)
A role for molecular diversity and specificity in wiring the brain

09. October Louis Staudt
National Cancer Institute, Bethesda
RNA interference genetic screening meets cancer gene resequencing

13. October Zachary F. Mainen
Instituto Gulbenkian de Ciência
Neural substrates of olfactory decisions in the rat

17. October Adele Murrell
Cancer Research UK Cambridge Research Institute
Long-term and short-term epigenetic memory in cancer

23. October Paul Sharp
The University of Edinburgh
The origins and evolution of AIDS viruses

30. October Dietmar Schmitz
Neuroscience Research Center (NWFZ) - Charité Berlin
Temporal compression mediated by short-term synaptic plasticity

30. October Pierre Vanderhaeghen
University of Brussels
Generating neuronal diversity from pluripotent stem cells

NOVEMBER

05. November Jonathan Weissman
Howard Hughes Medical Institute
Biology without bias: functional insights from high-resolution genetic interaction maps

06. November Vicki Chandler
University of Arizona
Interchromosomal mediated epigenetic silencing across generations

07. November Tom Mrsic-Flogel
Dept. of Physiology, University College London
Imaging functional organization and plasticity in mouse visual cortex

21. November Andrea Musacchio
European Institute of Oncology, Milan
Molecular bases of chromosome segregation

27. November Caroline Dean
John Innes Centre, Norwich
Epigenetic regulation in the cold-induced switch to flowering

28. November Magdalena Zernicka-Götz
Wellcome Trust/Cancer Research Institute, University of Cambridge
Cellular and molecular mechanisms underlying cell fate decisions in the early mouse embryo

DECEMBER

11. December Elena Conti
Max Planck Institute of Biochemistry
Molecular mechanisms of nonsense-mediated mRNA decay

Spotlight on 2008

Science Dance Party

On 18th January 2008 the world's first 'Dance your PhD' contest took place at the IMP, organized by IMP-IMBA and John Bohannon, correspondent from Science magazine. In three categories (students, postdocs and group leaders) researchers where challenged to express their PhD theses using the medium of dance. Twelve dance entries were handed in and each performance was judged by a four-member panel, which included a professional dancer.

One dance entry came from the IMP: Anton Wutz, expressing his thesis "Functional studies of the imprinting box of the mouse Igf2r gene" - flanked by two murine assistants - elegantly negotiated a box placed onstage.

In addition to the dance contest, five musical tracks using sounds recorded in the lab like a DNA sequencer, or courtship song of a fruit fly, were premiered at the event accompanied by vivid graphics. Also shown was Cell Dance: a compilation of movies depicting beauty on the microscopic scale like cultured human cells undergoing synchronous mitosis and rotating 3D protein structures.

20 years IMP anniversary

This year the IMP celebrated its 20th anniversary. The first groups were established and the present IMP building renovated in 1988 under the leadership of Max Birnstiel who was appointed as the first Scientific Director, and the IMP has since then played an important international role in the field of molecular biology.

To celebrate the IMP's 20th birthday in a worthy way, a scientific conference was organized in the Hofburg Conference Centre on the 15th and 16th of May 2008 with an exciting programme including outstanding speakers and personalities from the IMP both past and present. More than 220 alumni and former IMP colleagues from all over the world joined the rest of the altogether 500 guests to reestablish old friendships and make new contacts.

The IMP's parent company Boehringer Ingelheim, represented by Andreas Barner, member of the management board, also delivered congratulatory wishes in his speech, confirming the ongoing commitment of the sponsors expecting no return on investment other than excellent science and the addition of the culture of a basic research institution to the Boehringer Ingelheim R&D Organization.

After two days of outstanding science, the conference ended with a great party in the impressive Hofburg Penthouse.

To see some pictures visit the anniversary website at: http://www.imp.ac.at/20yearsimp

Dragon Boat Cup

This year the IMP entered the Austrian Dragon Boat Cup for the second time and with a new crew of paddlers, the "IMPerfectos" competed at a regional contest on May 31st at the Arbeiterstrandbad in Vienna. The team won a thrilling head-to-head race and therefore entry to the all-Austrian final in Velden, Carinthia on July 5th as one of only eight teams out of the more than 120 competing nationally.

In the Velden competition the IMP team ended up in the grand final, but lost the play-off and finished fourth overall; still a great triumph and hopefully enough motivation to represent the IMP in the Dragon Boat Cup next year too.

VBC PhD Retreat

On June 5 and 6, a record number of 55 students from the VBC PhD program headed to the Hotel Wende at Neusiedl am See for the 3rd Campus Vienna Biocenter PhD retreat. It was two days full of science, career perspectives, discussions, food, and lots of fun. Like last year, poster sessions gave the students a chance to get to know what their colleagues are working on and explain their own PhD projects in a relaxed and informal atmosphere.

The external speakers invited this year were Gottfried Himmler, CEO of F-Star, a biotech company based in Vienna and Alper Romano who did his PhD thesis at the IMP in the group of Michael Glotzer and is now patents examiner at the European Patent Office (EPO).

IMP summer party

On Friday, August 1st, the IMP management invited its staff to the first IMP Summer Party at the Strandbad "Kleines Gänsehäufel" in Vienna. Many people followed the invitation in order to enjoy a relaxing day, take a break from work and get a little bit of holiday atmosphere. While some preferred taking sunbaths, swimming or just chilling out, others indulged their sports passion. Following hard fights for goals and a nerve-wracking penalty kick, Meinrad Busslinger's team "Bussi Galore" celebrated its victory in a thrilling soccer tournament. An air mattress race was just the right thing to cool down after kicking the ball in the heat of day. Barbecue and a beach bar providing free drinks rounded off an at the same time hot and cool day for the IMP people.

Postdoc Retreat

The third Postdoc Retreat took place in Bratislava on the 18th and 19th of September. The program started with a scientific lecture presented by Prof. Dontscho Kerjaschki of the Medical University of Vienna, followed by a talk by Claudia Fila sharing her experiences of being a postdoc at Roche with the audience. Finally, Brigitte Gschmeidler from the non-profit-organisation "Dialog Gentechnik" spoke about her efforts in trying to educate the public about DNA, gene technology and related topics. The official part was followed by a get together in the evening and a walk around the old town the next morning, the final highlight on the way back to Vienna being a visit to the Roman Ruins at Carnuntum.

Recess

From October 1-3, IMP scientists met with members of the Scientific Advisory Board (SAB) to present their work and discuss their research. The SAB, consisting of internationally recognized scientists, were once more impressed by the scientific performance and high standards of the research presented. The IMP would like to thank all its SAB members for their commitment and advice. Sincere thanks go to Titia de Lange and Steve McKnight who will leave the board this year.
IMP SAB members: page 67 in this booklet

VBC PhD Symposium

The PhD Symposium can be considered a fixed appointment of the scientific year at the Vienna Biocenter. This year, it was dedicated to the cutting edge topic of Synthetic Biology. On the 13th and 14th of November an audience of about 150 participants gathered in the IMP lecture hall for the symposium "Life under (re)contruction" to attend the talks of 16 well renowned scientists coming from various countries. The speakers presented the multi-faceted aspects of this scientific area to give an overview of the possibilities and challenges offered by the emerging field of Synthetic Biology. Among the speakers were well known experts such as Sven Panke, who talked about the possibility of engineering bological pathways, Ehud Shapiro, who described the new frontiers of DNA editing, and Steven Benner, who gave the closing lecture on the progress of molecular evolution and its applications to personalized medicine. The event was not only very successful, but also represented the first comprehensive symposium dedicated to Synthetic Biology in Austria.

Administration and Other Services

Management

Barry DICKSON	*Managing Director/ Science*
Harald ISEMANN	*Managing Director/ Finance and Administration*
Meinrad BUSSLINGER	*Director/ Academic Affairs*
Peter STEINLEIN	*Manager Scientific Services*

Management Assistance/ Scientific Secretariat

Chris ROBINSON	*Assistant to the Scientific Director/ PhD Affairs*
Manuela JUNG	*Assistant to the Senior Scientists/ Seminar Coordinator*

Administrative Assistance

Gertrude KÖLBL	*Assistant to the Managing Director/Administration*

IT Service Department

Werner KUBINA	*Head of IT-Service Dept.*
Andreas RIEPL	*Deputy/IT-Service Dept.*
Herlind WURTH	*IT System Manager*
Christian BRANDSTÄTTER	*IT System Manager*
Sebastian RUF	*IT System Manager*

Accounting/Controlling

Brigitte WEISER	*Head of Accounting/Controlling*
Renate ZETL	*Accounting Clerk*
Anita SOMMER	*Accounting Clerk*
Hanna VOGL	*Accounting Clerk*
Gesine WALNSCH	*Controller*

Graphics Department

Hannes TKADLETZ	*Graphics Artist*
Tibor KULCSAR	*Graphics Artist*

Grants Management

Tanja WINKLER	*Grants Manager*

Personnel Department

Werner LEITNER	*Head of Personnel Department*
Susanne KASTENBERGER	*Personnel Officer*
Sabine STEURER	*Recruiting & Development Manager*

Reception

Robert LASETZKY	*Head of Reception/Driver*
Doris JADIDI-KHOI NIGISCHER	*Receptionist*

Library

Kerstin STIEG	*Chief Librarian*
Rudolf MANNHARD	*Assistant to Librarian*

Public Relations

Heidemarie HURTL	*Public Relations Officer*
Evelyn MISSBACH	*Public Relations Officer*

Purchasing Department

Angela GANGLBERGER	*Head of Purchasing Dept.*
Kashinath MITRA	*Store Manager*
Brigitte LENZ	*Purchasing Agent*
Nikolaus KUNTNER	*Purchasing Agent*
Caroline FORMANN	*Purchasing Agent*
Eva SCHARNER-KÖCK	*Purchasing Agent*
Sabine SPILKA	*Purchasing Agent*
Jürgen Gerhard RUSCHER	*Purchasing Agent*

Technical Department

Alexander CHLUP	*Head of Technical Dept.*
Michael KRATOCHWILLE	*Deputy / Technical Dept.*
Martin COLOMBINI	*Mechanical Workshop*
Nadir AYKUT	*House Maintenance*
Christian DAVID	*House Maintenance*
Gerhard MÜLLER	*House Maintenance*
Wolfgang RACHOLZ	*House Maintenance*
Martin ROJDL	*House Maintenance*
Stefan TRINKER-GRONAU	*House Maintenance*
Vladimir KOLCAR	*Technical Maintenance*
David KUMMERER	*Technical Maintenance*
Michael ZIEGLER	*Technical Maintenance*

Washing Kitchen

Renate WÖHRER	*Head of Washing Kitchen*
Sultan CAN	*Technical Assistant*
Nuray KILIC	*Technical Assistant*
Erika KLEIN	*Technical Assistant*
Bettina SOMMER	*Technical Assistant*
Renate STIX	*Technical Assistant*

Cafeteria

Michael ENZBERGER	*Chef de Cuisine*
Taoufik BAHMOU	*Chef*
Markus GIGLER	*Chef*
Helga MEHOFER	*Buffet*
Sabine SMALCL	*Buffet*
Farzana UDDIN	*Buffet*
Güler CICOS	*Kitchen Helper*
Selma YILDIRIM	*Kitchen Helper*

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

BMWF
Boehringer Ingelheim
CNIO
Dionex
* EMBO
ESF
European Research Council
European Union
* FEBS
FFG
FWF
GEN-AU
HFSP
LISA
Marie Curie
Stadt Wien
WWFF
WWTF
ZIT

** individual fellowships*

SPONSORS & PARTNERS

Scientific Advisory Board

PROF. FREDERICK W. ALT
Harvard Medical School, Boston, USA (Chair)

DR. DR. ANDREAS BARNER
Boehringer Ingelheim GmbH, Ingelheim, Germany

PROF. CORNELIA BARGMANN
Rockefeller University, New York, USA

PROF. TITIA DE LANGE
Rockefeller University, New York, USA

PROF. VENKI RAMAKRISHNAN
MRC Laboratory of Molecular Biology, Cambridge, GB

PROF. STEVE MCKNIGHT
Southwestern Medical Center, Dallas, USA

PROF. RUDOLF JAENISCH
The Whitehead Institute, Cambridge, USA

DOZ. DR. WOLFGANG RETTIG
Boehringer Ingelheim Austria, Vienna, Austria

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.

Impressum

Published by

IMP-IMBA Communications Department

Project Management

Evelyn Missbach

Layout & Design

Tibor Kulcsar, Hannes Tkadletz

Pictures, Artwork & Credits

IMP/IMBA
IMP-IMBA Graphics Department
Robert Herbst/point of view
Mauritius Images (p 10)

Cover Illustration

Surface representation of the half-sliced DegP24 oligomer (colored white) with a modeled Outer Membrane Protein substrate (colored by electrostatic potential).
Courtesy of Clausen Group

Contributions pp 62-63

Anne Göppert, Heidemarie Hurtl, Jim Hutchins, Stephen Malin, Federico Mauri, Evelyn Missbach, Jennifer Mummery-Widmer, Chris Robinson, Kerstin Stieg, Nilay Yapici

Printed by

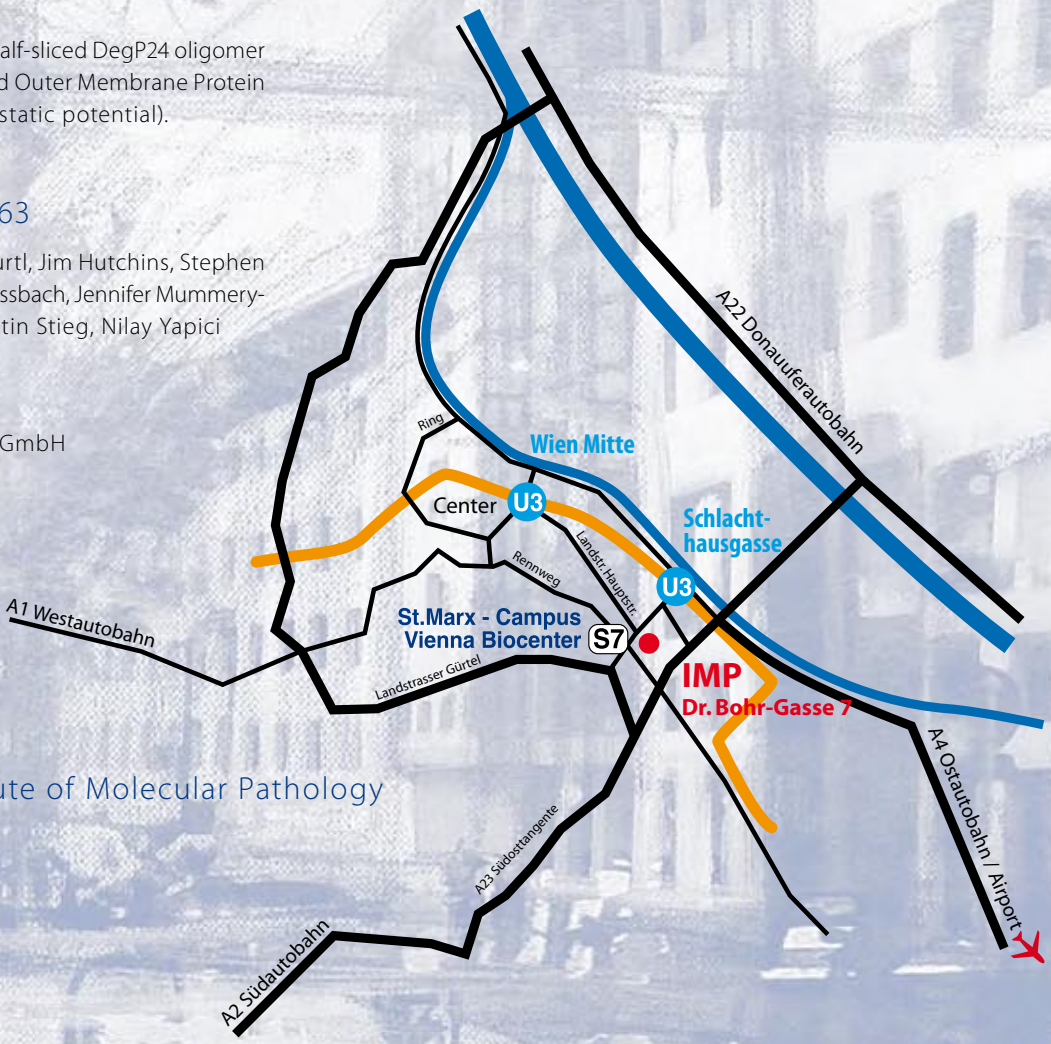
Holzhausen Druck & Medien GmbH

Where We Are

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



The IMP is a basic research institute within the Boehringer Ingelheim group of companies.
IMP is a member of the Campus Vienna Biocenter.