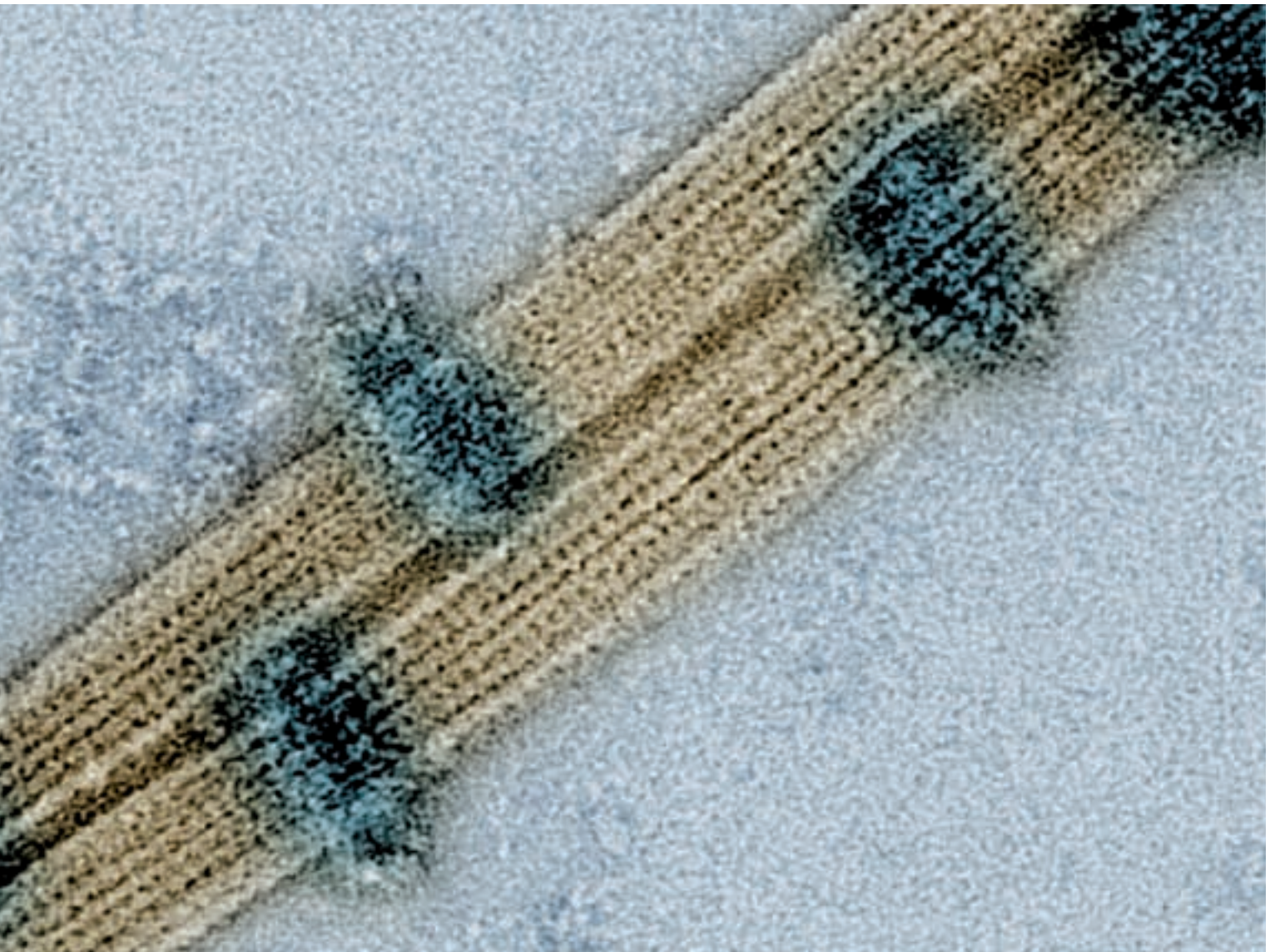


# RESEARCH INSTITUTE OF MOLECULAR PATHOLOGY

VIENNA BIOCENTER

# 2007







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BARRY DICKSON  
*Managing Director/Science*



HARALD ISEMANN  
*Director/Finance and Administration*

Birthdays are always fun, particularly the round numbers. And with the IMP entering its 20th year, there really is plenty to celebrate. It must have seemed crazy 20 years ago when Boehringer Ingelheim and Genentech decided to create a new biomedical research institute in the run-down part of a city that – despite a strong intellectual tradition – was not exactly a vibrant center of scientific innovation at the time. But with hindsight, this was an inspired decision.

In Vienna, the IMP has had an impact that it might not have had elsewhere. It has served as a role model for the neighbouring institutes of the University of Vienna and the Medical University, now restructured as the Max F. Perutz Laboratories (MFPL), as well as two new institutes of the Austrian Academy of Sciences – IMBA and GMI. And the biotech company Intercell, founded as a spin-off of the IMP and the University, has grown to become one of Europe's most successful biotech companies. The Campus Vienna Biocenter that has sprung up around the IMP is now one of the most dynamic and innovative life science centers in Europe.

There are many people to thank for this; too many to list here. Suffice it to say that this would not have been possible without the vision and generous support of Boehringer Ingelheim, the strong leadership of Max Birnstiel and Kim Nasmyth, the pioneering spirit of the “founding fathers” who staked their scientific careers on a small renovated loudspeaker factory in the 3rd district, and the efforts and spirit of a great many scientists and support staff. The City of Vienna and the Austrian Federal Government have also played a pivotal role. And it is remarkable how Vienna itself has also changed over these 20 years, becoming one of the most enjoyable, affordable, and cosmopolitan cities in Europe.

We're looking forward to the 20th birthday celebrations in May, 2008. We will host a two-day symposium at the Hofburg at which all speakers will be former or current members of the IMP, or chairs of our Scientific Advisory Board. The impressive list of speakers is itself a wonderful testament to the IMP's success in launching so many outstanding scientific careers. There will be much more time next May for reflecting on the

past. For now, let's just take a quick review of the highlights of the past year.

After some frustrating technical delays, we are all relieved that the mice have now finally moved into the spacious new IMP-IMBA mouse house. An undertaking of this scale is never easy, and this one was certainly not without its complications. But now, thanks to the heroic efforts of Andreas Bichl and his team, as well as Alex Chlup and the technical department, the mice are all now happily breeding in their new home. Thanks also to Erwin Wagner, who has skillfully guided the whole operation from the very beginning.

Another significant achievement of the past year was the establishment of the Vienna Drosophila RNAi Center (VDRC). The VDRC maintains the genome-wide Drosophila RNAi library and distributes lines world-wide through an online ordering system. The VDRC also supports the many RNAi screens being conducted within IMBA and the IMP. Generous funding from the City of Vienna and the Ministry of Science covers much of the bill, the rest by user fees. Krystyna Keleman, Reinhard Klug, and their team have done a terrific job in setting up the VDRC.

The Electron Microscopy facility, set up last year and headed by Günter Resch, has just added a new 300kV Polara transmission EM primarily for cryo-electron tomography to their 100kV Morgani transmission EM.

As every year, we have had to say a few sad good-byes, but also welcome new members into the IMP family. After eight years at the IMP, Frank Eisenhaber left us to take up an exciting new challenge as Head of the Bioinformatics Institute at Biopolis, Singapore. Frank established the computational biology and much of the IT infrastructure at the IMP, and contributed to several key discoveries. Almost immediately after arriving at the IMP, Frank had the insight that Thomas Jenuwein's Suv3-9 proteins were likely to be histone methyl transferases – a prediction that Steven Rea and Thomas famously confirmed.

Ludger Klein is also leaving at the end of the year to take up a professorship at the LMU in Munich. Ludger will be remembered



not only for his elegant use of transgenic mice to define the cellular mechanisms of T-cell tolerance, but also for his remarkable ability to give seminars in immunology that even a *Drosophila* geneticist could understand!

Diane Turner also left us this year, after almost 20 years of cheerful and selfless work in support of all IMP scientists. We have been very fortunate to recruit Manuela Jung to join Chris Robinson in the Scientific Secretariat.

Another new recruit is our second IMP fellow. David Keays has signed on and will join us in the new year. During his PhD studies with Jonathan Flint in Oxford, Dave studied a mouse model of mental retardation. He plans to continue some of the mouse work, but also take on an ambitious new project – investigating the molecular basis of magnetoreception. We look forward to welcoming Dave to the IMP next year.

Finally, a wonderful boost for the Campus this year has been the arrival of Graham Warren as Scientific Director of the MFPL.

With his active participation in everything from the PhD program and seminar series to lively lunchtime discussions, Graham is already a familiar figure to everyone at the Campus. One exciting new initiative now being hatched is to establish a campus-wide facility for massively parallel DNA sequencing. With a Solexa 1G and a 454 sequencer, we'll soon be able to enter a whole new era of DNA sequencing.

In all, it's been yet another exciting year at the IMP, and we also have much to look forward to in the new year. Many thanks to all of the IMP scientists and the support staff at IMP and IMBA for their fantastic efforts, as well as to Boehringer Ingelheim and our other sponsors for their generous support. We were particularly delighted that Christian Boehringer, the new chairman of the shareholder's committee, visited the IMP in August. With all the changes on Campus in the past few years, the IMP in her 20s is very different than she was in her childhood and adolescence. The 20s are good years – no less exciting, and a good age for great discoveries. Happy Birthday, IMP!



## OF BLOOD AND STONES

One of the central tenets of developmental biology is that development – like evolution – is irreversible. In other words, differentiated cells cannot be made to de-differentiate, except maybe under extreme *in vitro* conditions. Enabling differentiated cells to enter a different cell lineage under physiological conditions is one of the primary aims of regenerative medicine but experiments towards this goal have been likened to the alchemists' quest for the philosopher's stone, a mythical rock made from the transmutation of gold, silver and mercury that can heal all injuries and sicknesses of the human body and transmute any base metal into gold and silver.

The search for the philosopher's stone seems to have been discontinued, except perhaps in certain highly specialized circles, but regenerative medicine is still receiving an enormous amount of attention. And it has been boosted considerably by

recent results from César Coboleda in Meinrad Busslinger's lab (*Nature* 449, 473-477).

Since 1990, work in Meinrad's group has been largely focussed on the *Pax5* (paired box protein 5) gene, one of the nine *Pax* genes known to be present in the mammalian genome. The genes are named after their conserved "paired" domain, which binds with varying affinity to a set of distinct and loosely conserved sequences of DNA. The *Pax* genes are transcription factors and *Pax5* is the only one expressed within the mammalian hematopoietic system. It was first identified as a DNA-binding protein that interacts with the promoters of immunoglobulin genes and was thus thought to be responsible for confining expression of these genes to particular cells. Subsequent work has shown that *Pax5* has a much wider part to play in controlling the development of B cells from hematopoietic stem cells. Without *Pax5*, development of B cells is arrested at an early stage: progenitor B cells (pro-B cells) are produced but do not differentiate further.

As long ago as 2001, Markus Horcher in Meinrad's group was able to show that inactivation of *Pax5* in mature B cells caused loss of these cells, although the mechanism was unclear (*Immunity* 14, 779-790). César came as a post-doc to Meinrad's lab in the same year. In Spain he had been investigating chromosomal translocations in leukaemia and was thus working on abnormal hematopoietic differentiation. He was attracted to Meinrad's group by the possibility to study a "normal" hematopoietic transcription factor, *Pax5*. As César himself says, "it is perhaps ironic that we ended up discovering that *Pax5* can also function as a tumour suppressor."

César's results from his time in Vienna – despite having an independent position in Spain for the past two years he has been commuting regularly to the IMP "to finish what I had started" – build considerably on this earlier finding. César showed that loss of *Pax5* in mature B cells in mice leads to malignant lymphoma, a type of cancer in which blood cells show characteristics of



*"De-differentiation of mature cells to plastic progenitors; or how one lineage can be converted into another by first "going backwards" to a more primitive state. In contrast to the view that lineage commitment is unidirectional and irreversible, deletion of Pax5 in mature B cells allows their de-differentiation into progenitors that can then give rise to T cells."*

progenitor cells. The tumour cells were found to contain rearranged immunoglobulin genes, suggesting that they had arisen not from progenitor cells but from mature B cells. This provided an indication that at least some de-differentiation of the mature B cells had taken place in the Pax5-deficient mice.

To test the interpretation that mature B cells had been reverting to an earlier stage in their developmental pathway, César purified the remaining mature B cells (the procedure was considerably more difficult than it looks on paper) and, after deleting the *Pax5* gene, transplanted them into mice that were unable to produce lymphocytes (B cells, T cells or NK cells) as the result of a mutation. The purified, Pax5-deleted mature B cells were found to be able to give rise to T cells in these mice. In other words, cells that had been committed to the B-cell lineage proved able to produce cells from a different lineage.

César showed further that the mechanism involved reversion (de-differentiation) to pro-B cells and subsequent differentiation to T cells. Thus, cellular reprogramming was accomplished by the cells taking several steps back in their developmental lineage until they reached the stage (of pro-B cells) where they could enter a different lineage. The "intermediate" pro-B cells seemed fully functional: when they were isolated from recipient mice and transplanted into other, secondary recipients lacking lymphocytes they were able to support T-cell development. T-cell development in the secondary recipient mice appeared normal: the newly generated T cells were found at all stages of development and the proportions of the various subpopulations (as assessed by the presence of CD4 and CD8 markers) were normal. The DNA of the resulting T cells carried some vestiges of its past "life" inside a mature B cell in that immunoglobulin genes had been rearranged. Nevertheless, the cells themselves were shown to function normally in immune reactions.

The mature B cells had thus been reprogrammed into mature T cells via a de-differentiation step. It would be interesting to learn whether this type of de-differentiation is an essential stage in cellular reprogramming or whether direct reprogramming might also be possible. It would also be interesting to investigate the sequence of events that lead to de-differentiation and reprogramming. The initial programming of cell lineages in eggs is highly rapid and efficient. In contrast, César's results suggest that de-differentiation is a much slower process, although the re-differentiation of the de-differentiated pro-B cells to T cells after their injection into lymphocyte-deficient mice seemed to proceed at the normal pace.

The implications of César's findings are enormous. Cellular reprogramming – from B cells to T cells – is clearly taking place in the mice. The mere loss of a single transcription factor is able to cause fully differentiated, mature B cells to revert to an earlier stage of their development and thus to come under the influence of other factors that may promote their re-differentiation to a different cell type. In contrast to the traditional view, then, commitment to a cell lineage is not irreversible. César puts it very elegantly. "The removal of Pax5 allows a cell to reveal its hidden potential: a fully differentiated B cell turns out to be able to produce multipotent progenitor cells."

This hidden potential is, of course, a double-edged sword.

Under the experimental conditions described above it is of benefit to the mice to be able to produce T cells. However, the underlying cellular plasticity carries, Janus-like, the danger that deregulated development may result. In César's work a large number of the original mice developed malignant lymphoma. The implication is that de-differentiation, possibly arising from the loss or inactivation of tumour suppressor genes, may underlie tumorigenesis in other instances. It is well established that the growth of many types of human tumour requires the presence of "cancer stem cells" but the origin of these cells remains a matter of debate. César's findings suggest for the first time that they may come not from pluripotent cells in the body but from fully differentiated cells that have been induced to de-differentiate. This naturally has highly important implications for studies on the origins of cancer.

César is now in Spain, where he continues to work on the role of cellular plasticity in normal and abnormal hematopoietic differentiation. His findings in Vienna will be extremely important in shaping not only his future research but also that of numerous other groups working on many aspects of development and cancer biology and on human medicine. Perhaps the single most important conclusion of his paper is that things currently held to be impossible, such as de-differentiation, may be not only possible but perfectly normal, provided that we can find the correct conditions. As he puts it, "what was held to be carved in stone [cell fate] might actually be far more flexible." This development, together with those of other research groups around the world (see, for example, Takahashi *et al.* 2007, *Cell* 131, 861-872) have enormous potential for use in human medicine.







## THE SCENT OF A MAN

An old saying has it that “the way to a man’s heart is through his stomach”. This may well be true, at least as far as humans are concerned, but in the fruit fly *Drosophila melanogaster* the sense of smell seems to be far more important. Perfume manufacturers of the world take note: at least in flies, the reaction between a pheromone and its receptor seems sufficient to initiate mating behaviour.



This discovery stems from work by Amina Kurtovic and Alexandre Widmer in Barry Dickson’s lab and was published earlier this year in *Nature* (446, 542-546), with commentaries appearing not only in the scientific press but also in newspapers and other popular media worldwide. Interestingly, two papers from other groups working on the same problem were published about the same time in *Current Biology* (Ejima *et al.* 2007, *Curr. Biol.* 17, 599-605 and Van der Goes van Naters & Carlson 2007, *Curr. Biol.* 17, 606-612).

The sexual behaviour of *Drosophila* may seem a fairly obscure area of study to attract attention from so many groups but it does represent an example of a behaviour pattern that is relatively amenable to study. As Amina says, “sex is clearly important and flies are extremely good at it.” The fly’s courtship ritual involves males touching females, “singing” to them by vibrating their wings and even licking the females’ sexual organs. Only after this extensive foreplay are females willing to mate. Barry feels that this is a highly complex behaviour pattern – it is hard to suppress memories of schoolboy jokes relating to the “courtship rituals” of Australian males while he is saying this – and a better understanding of the process may help by analogy to provide insights into aspects of behaviour in higher organisms, including humans.

The foundations for Amina and Alexandre’s work were laid two years ago, when Ebru Demir in Barry’s lab showed that alternative splicing of the *Drosophila fruitless* gene product was responsible for controlling the different behaviours of the two sexes. Males in which the *fruitless* gene was spliced in a “female” way behaved like females: they failed to court females and were receptive to courtship by other males. Conversely, females in which the *fruitless* gene was spliced in a “male” way behaved like males. Taken together, these results showed that a single gene – *fruitless* – was necessary and sufficient to specify male courtship behaviour. This spectacular result was reported in *Cell* (121, 785-794) and attracted enormous attention.

*fruitless* had thus been identified as the “switch” controlling sexual behaviour in flies but the question of how it acted remained open. A first clue was provided by Petra Stockinger and Duda Kvitsiani, again in Barry’s lab, who demonstrated that *fruitless* was expressed in several types of neuron known to be involved in the fly’s sense of smell (*Cell* 121, 795-807). It seemed likely that these neurons could play a part in the detection of sex pheromones.

Around this time, Amina finished her undergraduate studies in the US and was looking for somewhere “closer to home” (she is Bosnian) to carry out a PhD. She was familiar with Barry’s research and was delighted to have the opportunity to come to the IMP and work on the role of *fruitless* in controlling sexual behaviour. She and Barry hypothesized that the male sex pheromone cVA (11-cis-vaccenyl acetate) was in part responsible for the process, as some of the neurons implicated in Petra and Duda’s studies also expressed a receptor, OR67d, thought to be involved in the response to cVA.

As Amina says, “This all seemed to make sense but there was no hard proof.” To attempt to provide proof, Amina and Alexandre generated flies lacking OR67d and examined their sexual behaviour.

Amina recalls with horror, "The worst part of the whole work was scoring the movies, sitting there watching fly porn for hours at a time and for months on end. Believe me, it's much less exciting than it sounds – it was really very tedious!"

The normal situation, at least in flies, is that males court females rather than other males. In Amina's mutant flies, however, males were often seen to court other males and they also courted females who had been rubbed with cVA, which normally causes male flies to avoid them. Furthermore, mutated females were much less willing to pair than were normal females. So mutating OR67d seemed to stimulate courtship behaviour in males while inhibiting it in females – an effect analogous to that reported for alcohol in humans.

Support for this result came from experiments in which OR67d was substituted with a moth receptor under the indirect control of the OR67d promoter, such that the moth receptor was expressed in much the same way as OR67d in normal flies. As Amina says, "This was my favourite part of the work because so many people thought it wouldn't work. And they said I wouldn't need to publish it even if it did." Rubbing the corresponding moth pheromone onto the stomach of female flies was found to discourage male flies expressing the moth receptor from mating with them, while "normal" flies were happy to do so. The conclusion was that activation of the neurons that normally express OR67d was sufficient to discourage males from their courtship behaviour. (In the fly's olfactory system, each neuron expresses only one type of receptor and all neurons expressing the same type of receptor go to the same point in the brain, which makes it much easier for us to link receptor to function.)

It should be noted that Van der Goes van Naters and Carlson (*op. cit.*) found that not only OR67d but also OR65a, a further receptor, responds to cVA. Ejima *et al.* (*op. cit.*) even have results suggesting that it is OR65a and not OR67d that is responsible for suppressing courtship behaviour in male flies. The apparent discrepancy between these findings and Amina and Alexandre's results from their studies of the Or67d mutant may be an artefact of the different experimental systems used, as the sensitivities of the two receptors to cVA are known to differ. Barry's group is currently constructing a similar mutant in the Or65a gene to see whether this receptor is also relevant under physiological conditions.

It seems remarkable that the fly's entire courtship ritual can be under the control of a single pheromone – cVA – and a single receptor – OR67d. It is also remarkable that this pheromone seems to have opposite effects on males and females. As Barry says, "The sensory processing appears to be the same in both sexes." He believes that the difference results from different pathways in the brains of the two sexes – males and females do think differently, at least in flies! – and future work will be directed towards testing whether this is the case. One exciting idea being explored is that it is the *fruitless* gene that determines whether a particular fly responds to cVA like a male or a female.

Another surprise is that in flies it is the males that produce the perfume: cVA is a male sex pheromone. The underlying rationale is highly profound. cVA is contained in the seminal fluid of male flies and its presence on a female fly is a sign that she has

recently mated. Male flies have a clear preference for mating with "virgin" females and it seems as though the inhibitory action of cVA on male courtship behaviour is a mechanism for preventing multiple matings with a single female as well, possibly, as helping to prevent males from courting other males.

Feminists may be dismayed at the implication that males choose to court other flies simply because these do not smell like males (i.e. "female" is apparently defined as the absence of "male"). Amina is quick to reject this notion: "If two flies are locked up together, males only spend 35% of their time courting other males when OR67d is mutated. If one of the flies is female, the male courts her for more than 80% of the time. So there is a lot more to being a woman than that!"

Amina finished her PhD studies in April 2007 and has returned to her Bosnian homeland, where she is working in the pathology department of a local hospital and attempting to establish FISH as a procedure for pre-natal diagnosis. "This may be routine in west European countries but we are still not able to perform the technique, although it is really needed." She is excited by the challenge of attempting to set up something new and if she is successful she hopes to stay in Bosnia and continue her work. At the same time, she has started teaching general molecular biology and genetics at a small private university. "This is great: I really love teaching and the idea that I can help future students to enjoy the opportunities I have had is very exciting."



## 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.



## 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 outstanding 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?

hartmut.beug@imp.ac.at

Hartmut Beug / Senior Scientist

Agnes Csiszar / Postdoc

Sabine Maschler / Postdoc

Memetcan Alacakaptan / PhD Student

Florian Grebien <sup>2</sup>/ PhD Student

Marc Kerenyi <sup>2</sup>/ PhD Student

Boris Kovacic / PhD Student

Susanne Soelch / Diploma Student

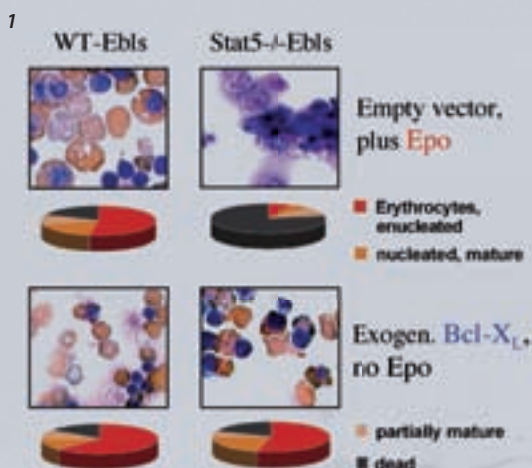
Gabi Litos / Technician

Eva Maria Wiedemann / Technician

*In leukemia and carcinoma development, multiple overexpressed, mutated and/or oncogenic receptors/signal transducers and transcriptional regulators cooperate to cause abnormal proliferation, survival and developmental / behavioral plasticity in tumor cells, including formation of tumor stem cells. We try to elucidate the cellular and molecular mechanisms by which such genes and their combinations act during tumor formation and progression/metastasis in both genetically modified mice and in vivo-like cell culture models.*

### Mechanisms of hematopoietic progenitor renewal and leukemogenesis.

To cause human leukemia, mutated transcription factor/chromatin regulators (e.g. bcrABL, RAR/PML, AML-ETO) often co-operate with mutated/overexpressed receptors/signal transducers (Flt3/c-Kit/Jak2). We established this paradigm (i.e. the cooperation of normal or abnormal signal transduction and transcriptional regulation) already over 10 years ago, first in avian leukemia (v-ErbB/v-ErbA; v-Raf/v-Myc) and later in mutant mouse hematopoietic models and respective culture systems. In the (best studied) erythroid lineage, progenitor renewal requires co-operation of the EpoR, c-Kit, and the glucocorticoid receptor (GR). A similar cooperation is also necessary for stress erythropoiesis *in vivo*. Our model systems derived from both wt- and mutant mice are primary erythroblast cultures and immortal, but diploid murine ES cell-derived erythroblasts (ESEPs). These cells show *in vivo*-like growth factor dependence (apoptosis) and normal terminal differentiation. Using these systems, we showed that lack of Stat5 (Figure1), p38 kinase and Flk-1 caused late- and early erythropoiesis defects, respectively. Interestingly, both the human leukemia oncogene BCR-Abl and overexpressed, constitutively active, mutant Stat5 (cStat5) caused multilineage leukemia and rescued erythro- and myelopoiesis of EpoR<sup>-/-</sup> and Jak2<sup>-/-</sup> mice, both *in vivo* and in primary culture. We are now using cStat5- and BCR-Abl-expressing, purified hematopoietic stem cells from leukemic mice to elucidate whether such cells can act as leukemic stem cells and whether they show altered self-renewal/differentiation behavior in cell culture as a consequence of leukemic transformation. We also study the significance of Stat1/Stat3 upregulation (probably complementing Stat5) in erythroid cells completely lacking Stat5 (with collaborators from a joint long-term grant; SFB).



### New genes essential for epithelial plasticity, tumor progression and metastasis.

Epithelial to mesenchymal transition (EMT) and its reversal (MET) represent important epithelial plasticity changes during progression and metastasis of epithelial cancer cells, increasingly recognized as crucial processes during



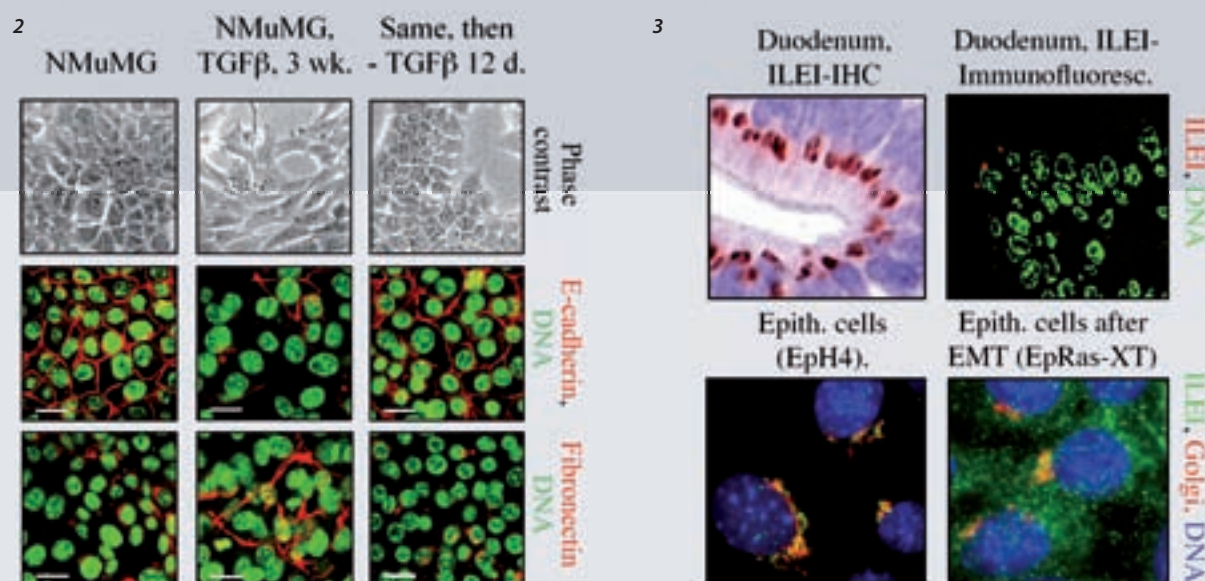


Figure 1: *Bcl-XL* substitutes for *Epo* signalling via *Stat5* in terminal erythroid differentiation. Top panels: Primary erythroblasts (Ebls) from WT mice terminally differentiate upon differentiation induction in defined medium plus *Epo*, insulin and Dex-antagonists (left) Cells bearing a hypomorphic *Stat5* allele (*Stat5*<sup>-/-</sup>) undergo apoptosis under the same conditions (dead, right). Bottom panels: Wt and *Stat5*<sup>-/-</sup> cells exogenously overexpressing the anti-apoptotic protein *Bcl-XL* both differentiate into enucleated erythrocytes even in the absence of *Epo*.

carcinoma progression. Multiple signal transduction pathways and transcriptional regulators can cooperate to cause EMT and metastasis, including oncogenic Ras plus TGF $\beta$  in an *in vitro/in vivo* mammary epithelial cell model (EpH4/ EpRas). In another cell model (NMuMG), chronic exposure to TGF $\beta$  was sufficient to induce reversible EMT (Figure 2) and pre-tumorous lesions. Expression profiling and protein expression/interaction experiments identified  $\approx 30$  new and known EMT-specific genes, e.g. interleukin-like EMT inducer (ILEI), cellular regulator of E1A gene (CREG), AnnexinA1, delta-EF-1/SIP1, as well as genes from the NF $\kappa$ B- and PDGF receptor-signaling pathways. Stably over-expressed ILEI was necessary and sufficient to induce TGF $\beta$ -independent, reversible EMT, slow tumor growth and metastasis in mammary and hepatocyte-derived epithelial models but still cooperated with v-Ras-signaling for maximal effects. In contrast, both CREG overexpression and knockdown of annexin-A1 required hyperactive ERK- and/or PI3K signaling pathways to induce EMT and metastasis, involving activation of autocrine TGF $\beta$  signaling. ILEI strongly induced PDGFR- and chemokine signaling, the latter currently being tested for their ability to substitute for ILEI in EMT induction. Cells after Ras/TGF $\beta$ - or ILEI-induced EMT showed a hyperactive PDGF-R pathway which activated  $\beta$ -catenin signaling. By RNAi, we found that loss of basolateral epithelial polarity genes (mLgl, m-scribble) could induce Ras-dependent EMT in the absence of TGF $\beta$ R signaling. Finally, we showed that ILEI is localized to intracellular organelles including Golgi vesicles in normal and benign epithelial tumor cells, but redistributed to a general, cytoplasmic – probably vesicular – localization in cells after EMT and in malignant tumors/metastases (Figure 3). Thus, ILEI (and CREG) are both secreted and localized to organelles of the biosynthetic and/or endocytic pathways. These pathways clearly represent major targets of Annexins (including Anxa A1) and seem to be profoundly altered in EMT, which could grossly change signaling from intracellular receptor/adaptor complexes, a concept emerging for e.g. Ras-, TGF $\beta$ - and Stat3 signaling. Therefore, we currently analyze whether ILEI acts through such intracellular signaling complexes, using ILEI mutants predicted to be defective in proteolytic cleavage and/or secretion. This also might clarify how the above misregulation of intracellular ILEI localization found in both ILEI-overexpressing cells and in tumors correlates with metastasis and reduced patient survival. Since migratory cells expressing both stem cell- and EMT-markers were recently detected at malignant colon carcinoma invasion fronts, we also intend to study the possible relationships between EMT and breast carcinoma stem-initiating cells.

Figure 2: Sustained TGF $\beta$ -stimulation induces reversible EMT in murine mammary epithelial cells lacking exogenous oncogenes (NMuMG). Left panels: NMuMG control cells show an epithelial morphology (phase, top) and express E-cadherin (middle) but not fibronectin (bottom). Middle panels: The same cells after exposure to TGF $\beta$  for 3 weeks (middle panels) show EMT (fibroblastoid morphology, little E-cadherin, abundant fibronectin). Further cultivation of these cells in the absence of TGF $\beta$  for 6-12 days leads to a complete reversion of EMT (right panels). Green, nuclei (DNA)

Figure 3: Delocalization of ILEI from vesicular structures adjacent to Golgi to small cytoplasmic vesicles correlates with EMT. Top panels: Typical ILEI localization in structures apical of the nucleus in normal duodenal epithelium (left, immunohistochemistry – IHC; brown) and immunofluorescence using ILEI-antibodies (on 1  $\mu$ M EM sections, red dots; green, DNA). Bottom panels: Normal EpH4 mammary epithelial cells (left) and Ras-transformed EpH4 cells after EMT induction (EpRas-XT) were stained for ILEI (green) and Golgi structures (red) by immunofluorescence and evaluated by confocal microscopy. Blue, nuclei (DNA)

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# MEINRAD BUSSLINGER

## Stem Cell Commitment in Hematopoiesis

meinrad.busslinger@imp.ac.at

Meinrad Busslinger / Senior Scientist

Cesar Cobaleda <sup>1</sup> / Postdoc

Anja Ebert / Postdoc

Stephen Malin / Postdoc

Shane McManus / Postdoc

Beatriz Cebolla / Postdoc

Ivan Bilic / Postdoc

Thorsten Decker / PhD Student

Heike Dörninger / PhD Student

Mareike Berning / PhD Student

Pia Viviani / PhD Student

Jasna Medvedovic <sup>2</sup> / PhD Student

Abdallah Souabni / Research Assistant

Sonja Höflinger / Technician

Qiong Sun / Technician

Barbara Werner / Technician

Erika Kiligan / Technician

<sup>1</sup> until February,

<sup>2</sup> since June

*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 and Notch1 control the commitment of early hematopoietic progenitors to the lymphoid lineages.*

### B cell commitment

A fundamental question in hematopoiesis is how stem cells and early progenitors become committed to a single developmental pathway and then differentiate into mature cell types of the selected lineage. By analyzing the transcription factor Pax5, we have gained insight into the commitment process of the B-lymphoid lineage. Pax5 is essential for the progression of B cell development beyond an early progenitor (pro-B) cell stage. Pax5-deficient pro-B cells can be cultured *ex vivo* on stromal cells in the presence of IL-7. However, these pro-B cells are uncommitted progenitor cells, as they can develop *in vitro* and *in vivo* into various hematopoietic cell types except for B cells, which are only generated upon retroviral restoration of Pax5 expression (Figure 1). Pax5 was thus identified as the B-lineage commitment factor, which restricts 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 rescue T-lymphopoiesis in T cell-deficient mice. B cell-derived T-lymphocytes participate as functional T cells in immune reactions (Figure 2). These experiments uncovered an extraordinary plasticity of mature B cells and demonstrated 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.

### Molecular mechanism

At the molecular level, Pax5 fulfils a dual role by activating the expression of B-cell-specific genes and by repressing the transcription of lineage-inappropriate genes. By cDNA microarray screening, we have identified 110 Pax5-repressed and 170 Pax5-activated genes. This reprogramming of gene expression restricts the broad signaling capacity of uncommitted progenitors to the B cell pathway, induces V<sub>H</sub>-DJ<sub>H</sub> recombination, facilitates (pre)B cell receptor signaling, regulates B lymphocyte adhesion and migration and promotes development to the mature B cell stage. Chromatin immunoprecipitation (ChIP) of Pax5 together with chromatin profiling by ChIP-on-chip analysis demonstrated that Pax5 directly activates the chromatin at promoters or putative enhancers of Pax5 target genes, as shown for the *Blnk* gene in Figure 3.

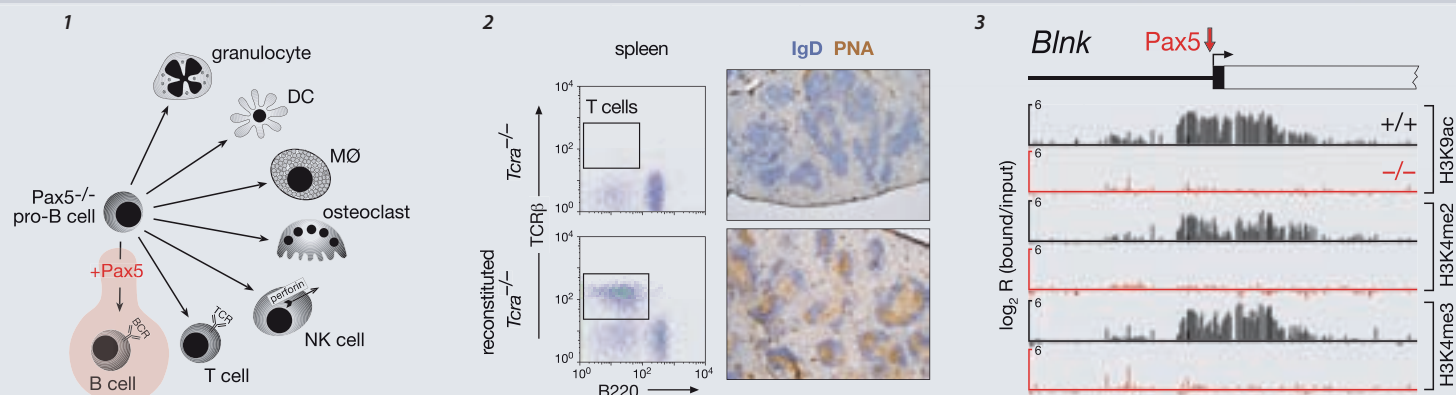


Figure 1: B-lineage commitment by Pax5. Pax5<sup>-/-</sup> pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.

Figure 2: Conversion of mature B cells into functional T cells. Mature B cells were purified from the spleen of CreED-30 Pax5<sup>f/f</sup> mice, and the floxed (F) Pax5 alleles were deleted *in vitro* by activation of the Cre-estrogen receptor protein (CreED-30). The Pax5-deleted B cells were subsequently injected into Tcrα<sup>-/-</sup> mice (lacking T cells) followed by immunization 2 months later. FACS analysis demonstrated that the mature Pax5-deleted B cells gave rise to peripheral TCRβ<sup>+</sup> T cells, which induced the differentiation of PNA<sup>+</sup> germinal center B cells (detected by immunostaining on spleen sections).

Figure 3: Chromatin regulation by Pax5. ChIP-on-chip analysis of Pax5<sup>+/+</sup> (+/+) and Pax5<sup>-/-</sup> (-/-) pro-B cells on high-density oligonucleotide arrays was used to map the histone modifications H3K4me2, H3K4me3 and H3K9ac along Pax5 target genes. These active chromatin marks are enriched in the 5' region of the Pax5-activated gene Blnk only in Pax5<sup>+/+</sup> pro-B cells consistent with the presence of a Pax5-binding site in its promoter.

## 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 organization with the D, J and C segments constituting the 3' domain of each locus. The largest part (> 80%) of the *Ig* and *Tcr* loci is devoted to the V gene cluster consisting of an array of 31 (*Tcrb*) to 195 (*Igh*) V genes. Antigen receptor loci can therefore be viewed as consisting of two distinct entities, the proximal domain and the V gene region, which are separated by a large distance on linear DNA. We previously showed that the *Igh* and *Igk* loci undergo reversible contraction in pro-B cells and pre-B cells, respectively. Recently, we have demonstrated that the *Tcrb* and *Tcra/d* loci also 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. Locus contraction therefore enables V genes to participate in V(D)J recombination.

## T cell specification by Notch1

Notch1 is the T cell commitment factor, as signaling through the Notch1 receptor is essential for the initiation of T cell development in the thymus. Stromal cells ectopically expressing the Notch ligand Delta-like-1 mimic the thymic environment by inducing uncommitted Pax5-deficient progenitor cells to undergo efficient *in vitro* differentiation to immature CD4<sup>+</sup>CD8<sup>+</sup> T cells. Notch-stimulated Pax5<sup>-/-</sup> progenitors rapidly down-regulate the expression of B-cell-specific genes, consistent with a role of Notch1 in preventing B-lymphopoiesis in the thymus. At the same time, Notch signaling induces expression of the transcription factor genes *GATA3* and *Tcf1*, indicating that Notch1 acts upstream of *GATA3* and *Tcf1* in early T cell development.

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# 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 / PhD Student

Jacob Fuhrmann / PhD Student

Justyna Sawa-Makarska / PhD Student

Bastian Schmidt / PhD Student

Katharina Linn Gazda / PhD Student

Max Rössler / Diploma Student

Juliane Kurt / Technician

Robert Kurzbaue / Technician

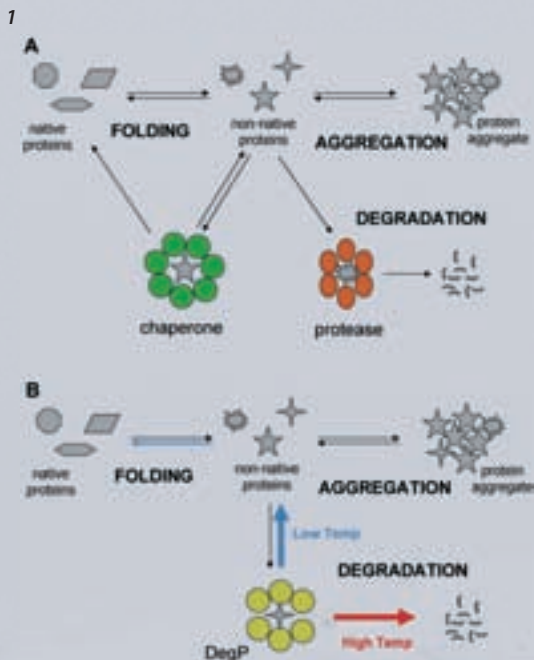
*The misfolding and aggregation of protein molecules is a major threat to all living organisms. Cells have therefore evolved a sophisticated network of molecular chaperones and proteases to prevent protein aggregation (Figure 1A), a process that is regulated by multiple stress response pathways. We perform 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 pathogenicity. Human homologs are believed to be involved in arthritis, cell growth, unfolded protein response, and apoptosis.

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.





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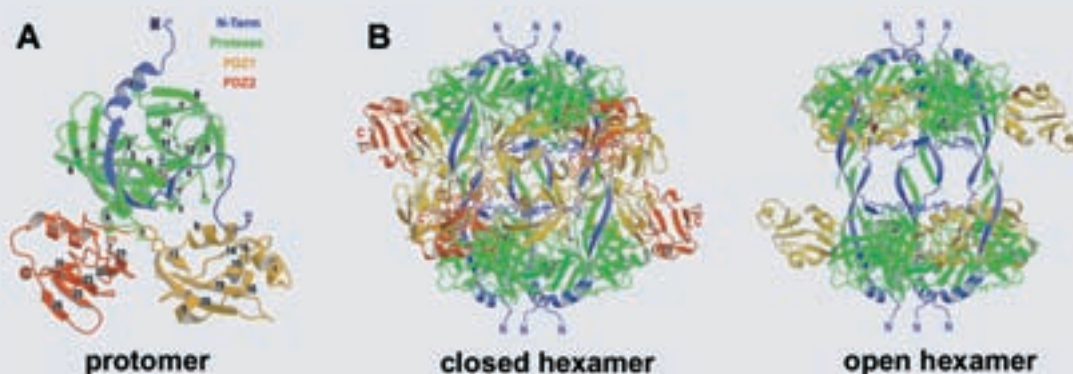


Figure 1: Protein quality control. (A). The diagram illustrates the different fates of misfolded non-native proteins. According to the "kinetic partitioning model" of Gottesman and coworker, aggregate formation is counteracted by the action of proteases and chaperones. (B). DegP combines the antagonistic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

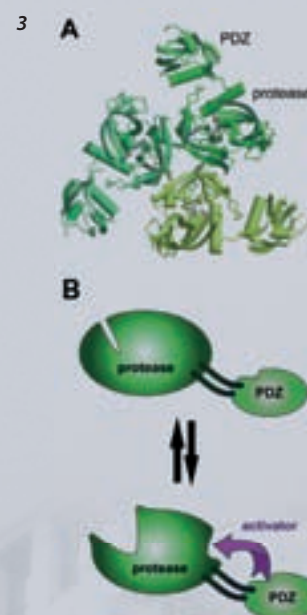
Figure 3: Structure of DegS. (A). Ribbon presentation of the DegS trimer (top view) with each subunit colored differently. (B). Diagram of DegS activation. In DegS, the PDZ domain obtains a regulatory function, offering a binding site for an allosteric activator.

## 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  $\sigma^E$  stress response that is triggered by excessive amounts of unfolded proteins in the periplasm, particularly unfolded outer membrane porins. The alternative  $\sigma$ -factor  $\sigma^E$  is a transcriptional activator that directs the expression of several stress genes. Under non-stress conditions, the activity of  $\sigma^E$  is inhibited by RseA, a membrane spanning protein, whose cytoplasmic domain captures  $\sigma^E$ , thereby preventing  $\sigma^E$  from binding to RNA polymerase. Activation of  $\sigma^E$  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  $\sigma^E$ -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  $\sigma^E$  response.

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



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## CARRIE COWAN

### Cell Polarity Establishment during Development

carrie.cowan@imp.ac.at



*Cell polarity allows for morphological and functional differentiation and is essential for all organisms. The general process of cell polarization is conserved among very different sorts of polarized cells. We use a combination of quantitative time-lapse microscopy, genetics, and micromanipulations in *C. elegans* embryos to investigate the molecular mechanisms controlling the establishment of cell polarity during development.*

### Cell Polarity in *C. elegans* Embryos

Cell polarity is the spatial asymmetry of cellular content and function. Despite the range of processes in which cell polarity is important, the general mechanism of polarity establishment is the same for most cells. First, a signal provides spatial and temporal information for polarization. Second, the signal is propagated to form distinct domains within the cell which define the polarity axis. Third, the domains communicate downstream to the cellular contents to allow for functional differences. *C. elegans* embryos begin with no polarity. Polarity is established in one-cell embryos by the formation of the anterior-posterior body axis of the worm (Figure 1). The anterior-posterior axis is defined by the segregation of PAR proteins into two distinct cortical domains, each domain occupying half the embryo. The cortical PAR domains control the unequal segregation of cell fate determinants during cell division, therefore the daughter cells differ in developmental fate. Cells arising from the posterior will become the germ cells of the adult worm, while cells arising from the anterior will form the rest of the adult tissues. *C. elegans* embryos are an excellent system for investigating cell polarity. We are studying the molecular mechanism of polarity establishment using RNAi-based mutant screens, quantitative time-lapse microscopy and mechanical manipulations, such as laser ablation and optical trapping.

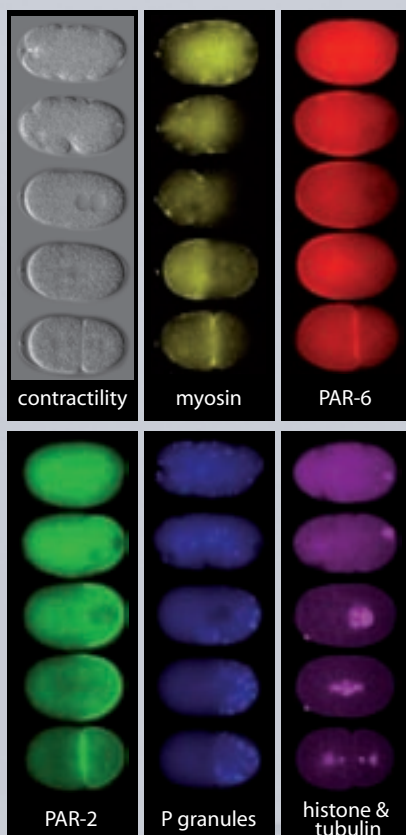
### What is the Polarity Establishment Signal?

Using a combination of genetic mutants and laser ablation, we have demonstrated that the establishment of polarity depends on the centrosome. The most prominent role of centrosomes in most cell types is microtubule nucleation, but the centrosome does not need microtubules to induce polarity in *C. elegans* embryos. The requirement for the centrosome is transitory: once polarity has been initiated, the centrosome is dispensable for polarization. Centrosomes appear to provide a signal to break the symmetry of one-cell embryos. We would like to identify the molecular mechanism of the centrosomal signal.

### What Tells the Embryo when and where to Polarize?

Timing of centrosome assembly correlates with the timing of polarity establishment, and defects in timing of centrosome assembly lead to a failure in polarity establishment. The centrosome lies very close to the cortex at the time of

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**Figure 1:** Polarity establishment in one-cell embryos. About 30 minutes after fertilization, the symmetry of a one-cell embryo is broken when the anterior-posterior axis is established. Polarity establishment involves an asymmetric distribution of the acto-myosin network (yellow) and contractility (gray), which facilitate the segregation of PAR proteins (red and green) into two distinct domains. The PAR proteins determine the segregation of fate determinants (blue) and posterior displacement of the mitotic spindle (pink). The images are from the time of polarity establishment to cytokinesis, a period of only 20 minutes.

**Figure 2:** Centrosomes provide a signal to establish polarity. Centrosomes (black dots) are adjacent to the cell cortex at the time of polarity establishment. The posterior domain (green) expands from the centrosome position until the posterior domain occupies half the embryo; the anterior domain (red) shrinks at the same time.

**Figure 3:** Segregation of polarity during cell division. The anterior-posterior polarity of one-cell embryos is reiterated over several cell divisions during early embryogenesis, similar to stem cell propagation. The exclusive segregation of polarity requires that PAR polarity is precisely matched to the position of cell division. Coordination of domain size with cleavage requires significant changes in the size of the anterior and posterior domains, best observed by the diminishing size of the posterior (green) domain during development.

polarization, and the posterior domain expands uniformly from the centrosome position (Figure 2). We are investigating how the centrosome position influences the spatial regulation of polarity establishment using mechanical manipulations and mutant analysis.

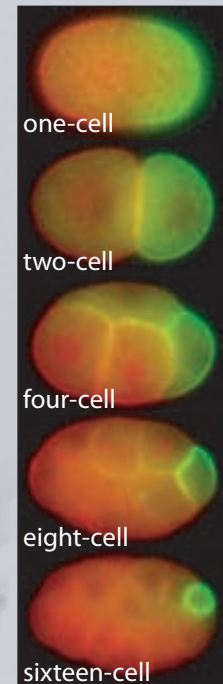
### How does the Polarity Signal make a Cortical Domain?

The establishment of anterior and posterior PAR protein domains relies on differences in the contractility of the acto-myosin cortex: the anterior cortex ruffles but the posterior cortex is smooth. The establishment of anterior-posterior contractile polarity relies on the centrosome. Thus, the centrosome signal must integrate into pathways that regulate the acto-myosin cortex. What is the molecular connection between the centrosome and the cortex? We have used RNAi-based phenotypic screens to identify genes that affect the establishment of acto-myosin contractile polarity. We would like to determine how these molecules interact with the centrosome signal using genetics and biochemistry.

### How is Cell Polarity Segregated during Development?

In *C. elegans* embryos during cell division, the anterior and posterior PAR domains are inherited exclusively by the anterior daughter cell and the posterior PAR proteins are inherited exclusively by the posterior daughter cell (Figure 3). This exclusive segregation of polarity requires that the position of the PAR domain boundary is coordinated with the position of the cell cleavage. We have uncovered a PAR domain correction mechanism acting at anaphase that ensures the PAR domain boundary is precisely matched to the cytokinesis furrow. The anterior and posterior domains shrink or expand to match mispositioned cytokinesis furrows. The correction mechanism depends on molecules required for positioning the furrow in response to spatial information provided by the spindle poles/centrosomes, suggesting that centrosomal signals are required for PAR domain correction. We are using genetics and mechanical manipulations to understand how spindle poles/centrosomes rearrange the PAR domains during cell division.

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## BARRY DICKSON

### Genes, Circuits and Behaviour

barry.dickson@imp.ac.at

Barry Dickson / Senior Scientist and Director

Sheetal Bhalerao / Postdoc

Young-Joon Kim / Postdoc

Carlos Ribeiro / Postdoc

Frank Schnorrrer / Postdoc

Laszlo Tirian / Postdoc

Eleftheria Vrontou / Postdoc

Alexandre Widmer / Postdoc

Kai Feng / PhD Student

Martin Häsemeyer / PhD Student

Sabrina Jörschel / PhD Student

Christian Schusterreiter / PhD Student

Bettina Spitzweck / PhD Student

Nilay Yapici / PhD Student

Jai Yu / PhD Student

Milica Markovic / Diploma Student

Christopher Masser / Diploma Student

Katarina Bartalska / Lab Manager

Ruth Fuchs / Technician

Kerstin Postmann / Technician

Katharina Schernhuber / Technician

*We are using molecular genetic techniques in *Drosophila* to try to understand the genetic and neural basis of animal behaviour. The main focus of our research is on the fly's mating behaviour – a robust and ancient animal instinct that is particularly amenable to genetic analysis.*

### Genes

In the 1960's, Seymour Benzer's work in *Drosophila* pioneered the field of neurogenetics – based on the simple but powerful premise that a genetic mutation could be used as a "microsurgical tool" to pick apart the inner workings of the brain [1]. One fascinating gene studied by Benzer and others is the fruitless (*fru*) gene, which we recently showed to act as a "master gene" for sexual behaviour in the fly (Figure 1). The *fru* gene encodes a set of transcription factors, some of which are sex-specifically expressed. We are now trying to find out when and where the different *fru* isoforms act, which genes they regulate, and what these target genes do.

The classical genetic approach to behaviour that Benzer pioneered has one major limitation: most genes have a variety of functions in different tissues, many of them unrelated to behaviour. Animals entirely mutant for a given gene may die during development, precluding any analysis of adult behaviour. Even if they do survive, mutant animals might misbehave for reasons that have nothing to do with impaired neural function.

To overcome these problems, we have developed a genome-wide transgenic RNAi library in *Drosophila* [2]. With this library, we can now target gene disruption specifically to the cells or tissues of interest – either the entire nervous or just the relevant neurons. We are now using this library to systematically identify and characterise other genes involved in either male or female mating behaviour.

### Circuits

Genes impact behaviour by directing the assembly, function, and plasticity of specific neural circuits. Both male and female mating behaviours appear to be regulated by the subset of sensory, central and motor neurons that express *fru*. These neurons are thought to form a circuit that governs sexually-dimorphic behaviour.

We are currently developing tools to gain genetic access to distinct subsets of the ~3000 neurons that express *fru* (Figure 2). This should ultimately allow us to study the anatomy and physiology of the *fru* circuit at single-cell resolution.

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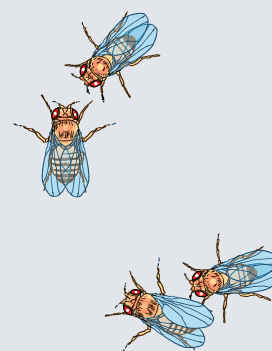
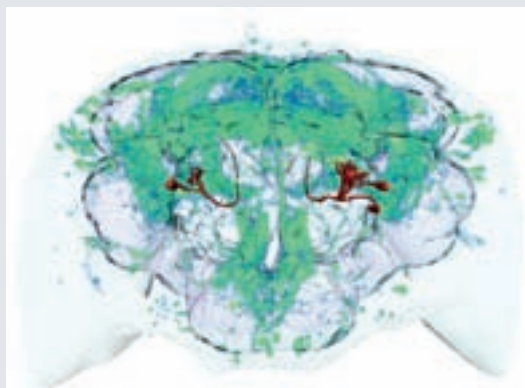


Figure 1: A female fly doing what a female normally doesn't – courting another female. This aberrant behaviour results from expression of the male-specific fru proteins in the female nervous system.

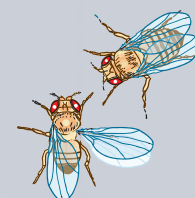
Figure 2: Surface rendering of fru neurons in the fly's brain (green), with specific olfactory projection neurons genetically highlighted (red).

We would like to know what signals each type of neuron response to, how the signals are processed, where the relevant sexual dimorphisms lie, and how all of this contributes to the behavioural output.

One important class of sensory neurons that express fru is the olfactory receptor neurons (ORNs) that detect pheromones. We have recently found that one class of fru+ ORNs expresses the odorant receptor Or67d and responds to a male sex pheromone [3]. This pheromone elicits different behavioural responses in males and females: it suppresses male mating behaviour but promotes female mating. Another class of fru+ ORNs express a different receptor, Or47b, and detects a pheromone that is produced by both sexes. For males, this pheromone is a potent aphrodisiac. By comparing signals detected by these two different types of ORN, a fly can determine the sex of another – something the fly clearly has to get right for successful mating.

## Behaviour

Any complex behaviour, such as courtship, can be described as a series of behavioural states and the transitions between these states. Analysing such behaviours is greatly facilitated by video tracking systems and computational tools that automatically recognise and monitor these states and transitions. We are currently developing such automated systems for the analysis of fly mating behaviours. These systems will allow us to study these behaviours more objectively, quantitatively and efficiently, and with much higher spatial and temporal resolution. Moreover, only through a detailed quantitative understanding of the fly's behavioural patterns can we hope to figure out just what those neural circuits are actually computing.



## FURTHER READING

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# FRANK EISENHABER

## Discovering Biomolecular Mechanisms with Computational Biology and Protein Biochemistry

frank.eisenhaber@imp.ac.at



Frank Eisenhaber / Group Leader

Georg Schneider / Software Architect

Birgit Eisenhaber / Postdoc

Manfred Koranda / Postdoc

Sun Tian / PhD Student

Wolfgang Benetka / PhD Student

Nedim Mujezinovic / PhD Student

Michaela Sammer / Diploma Student

Thomas Burkard / PhD Student

*In-depth theoretical analysis of biomolecular sequence information and of other high-throughput experimental data is a productive approach to gain mechanistic insight into biological processes at the molecular and cellular levels. Particularly protein sequence analytic methods can reveal considerable information. If the functional predictions are combined with targeted verification experiments either in collaborating groups or within the individual biochemical lab, breakthroughs become possible.*

Our mission has involved both collaborative research activities with experimental groups at the IMP and interacting institutions, as well as the realization of genuine bioinformatics research projects in the field of genome text interpretation. Since the foundation of the group in 1999, the group's success stories include (i) discoveries of new biological mechanisms with theoretical methods, (ii) the development of algorithms and software for protein sequence analysis and protein mass spectrometry and (iii) the refinement of experimental assays for lipid posttranslational modifications and the biochemical verification of a variety of predicted protein functions.

### Discovering new biological mechanisms with computational biology

During its history at the IMP, the group has been instrumental in a number of critical discoveries, a few of which are listed here. At the beginning, the prediction of SET domain methyltransferase activity paved the way for the discovery of the suv39h-dependent histone methylation and the start of biochemical epigenetics. The discovery of ATGL, the enzyme at the surface of lipid drops for hydrolysis of triglycerides, closed the gap in the mammalian fat catabolism pathway. The discovery of the kleisin protein family linked mechanistically chromatin organization processes in chromosome segregation, condensation and DNA repair. Our sequence-analytic work gave the critical hints for the discovery of the docking mechanism for substrate-specific subunits of the APC complex. These and dozens of other cases of successful collaborations reside on two cornerstones: (a) on qualified and tightly interacting specialists for sequence and biological data analysis that operate upon request besides their own topic-oriented work and (b) on time-efficient protein sequence analyses based on the "ANNOTATOR" suite.

### Development of new algorithms and software packages for protein sequence analysis and prediction of function

Our group is known for its highly accurate predictors for lipid posttranslational modifications (GPI lipid anchoring, myristoylation, farnesylation, geranylgeranylation), for PKA phosphorylation sites, protein convertase cleavage sites and for peroxisomal localization translocation signals. Large-scale database studies



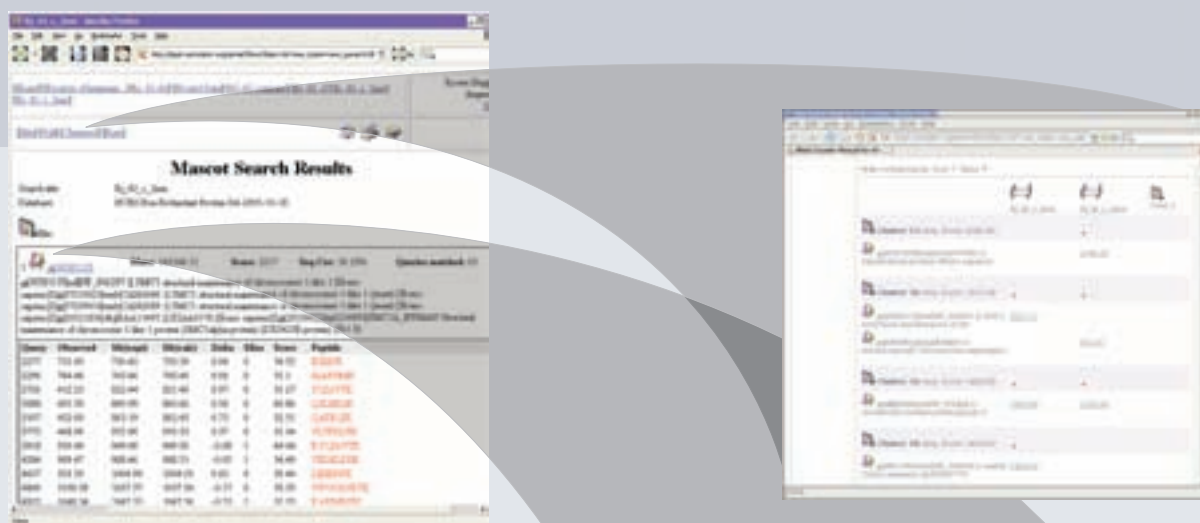


Figure: MS-ANNOTATOR: From protein hit lists to complex composition and to protein function analysis

The MS-ANNOTATOR is a software environment for the efficient analysis of mass spectrometry results for protein identification. The pipeline uploads raw data from the instruments, performs the necessary format transformations, and executes the MS Cleaner for noise removal and MASCOT for the interpretation of the remaining spectra. The final results are presented as a protein list for each measurement (left insert) and as protein complex information (upper insert; essentially identical sequences are clustered and protein contaminants are suppressed). By mouse clicking, the whole sequence analytic repertoire of methods is available and the analysis results are presented together with the peptides (in yellow) that were found in the MS/MS experiment (lower insert).

together with our experimental efforts support the notion of hidden signals for translocation and posttranslational modifications; i.e., targets with in vivo functional sequence signals that remain unused due to the inappropriate biological context in normal physiology.

The ANNOTATOR/NAVIGATOR suite has reached maturity and has become the major tool for protein sequence analysis within the group and collaborators. More than 30 sequence analytic tools as well as composed algorithms are bundled in the ANNOTATOR so that they are automatically executed over queries if suitable, where the results are processed and presented in a condensed manner to the researcher. Even the automated collection of homologous protein families containing tens of thousands of members and their analysis is now possible within the ANNOTATOR framework. The NAVIGATOR function allows retrieval of information relevant to the project from diverse biological databases. A number of these services are also available through the internet node (<http://mendel.imp.ac.at> and <http://annotator.org>).

### Software for protein mass spectrometry

A typical protein identification experiment with the MS/MS methodology leads to approx. 5000 spectra each with approx. 300 peaks. For the final interpretation, just 1% of the spectra play a role and, maybe, 30 peaks per spectrum support the interpretation. The program MS Cleaner is a valuable shortcut for noise removal; it recognizes 50-65% of the spectra as non-relevant for peptide recognition and reduces the number of peaks in the remaining spectra by about one fourth. The MS-ANNOTATOR has now integrated the workflow from raw protein tandem mass spectrometry data over background removal, data interpretation and de novo sequence annotation of protein hits (see Figure).

### Request-based biochemical studies for verifying sequence-based predictions

Sequence-analytic predictions for proteins typically involve molecular-functional properties such as enzymatic activities, ligand-binding properties or posttranslational modifications. We set up biochemical assays for these purposes. The recently developed Benetka-Koranda method for testing protein prenylation was further developed for other lipid modifications and applied on predicted protein targets.



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## CHRISTINE HARTMANN

### Formation and Patterning of the Vertebrate Skeleton

christine.hartmann@imp.ac.at



Christine Hartmann / Group Leader

Sayumi Fujimori / Postdoc  
 Daniela Kostanova-Poliakova <sup>1</sup> / Postdoc  
 Mehran Rafigh / PhD Student  
 Natalia Lyashenko / PhD Student  
 Richard Latham / Technician  
 Martina Weissenböck <sup>2</sup> / Technician  
 Fabienne Lampert <sup>3</sup> / Diploma Student

<sup>1</sup> since November

<sup>2</sup> since February

<sup>3</sup> until March

*The skeleton is essential for the vertebrate organism; it supports the body, provides the mechanical framework for physical movements, and protects internal organs. During embryonic development, it has to be determined where the future skeletal elements (bones) are formed, the size of the skeletal elements has to be controlled and the articulations (joints) between adjoining elements have to form. At the beginning, most of the skeletal elements are formed as cartilaginous templates and therefore need to be remodeled into bony tissue to provide strength to the skeleton. We use mouse and chick as model organisms to gain insight into how these different processes are regulated during embryonic and postnatal development. In particular, we investigate the role of Wnt-signaling in skeletogenesis.*

### Regulation of cell lineage differentiation

The different cell types building up the appendicular skeleton of the limbs, the chondrocytes, osteoblasts and the cells contributing to the future synovial joints are of mesenchymal origin. Over the past years, we have demonstrated, using a genetic approach, that the canonical Wnt-signaling pathway, which is mediated through  $\beta$ -catenin, plays a very essential role in the development of the mouse skeleton (see Figure 1). The Wnt-gene family in vertebrates comprises 19 members, all of which encode secreted signaling molecules. At least nine Wnt genes are expressed during skeletal development in chondrocytes, osteoblasts, and/or in the joint region. Using conditional gene targeting either deleting or stabilizing  $\beta$ -catenin, we have shown that too much  $\beta$ -catenin results in a complete loss of the chondrogenic and osteogenic lineages. In contrast, loss-of  $\beta$ -catenin does not affect the differentiation of mesenchymal cells into chondrocytes, however, no mature osteoblasts differentiate from mesenchymal cells lacking  $\beta$ -catenin. Instead, osteoblast precursors, the osteochondroprogenitor cells, differentiate into chondrocytes (Hill et al., 2005). Furthermore, we have recently shown that the canonical Wnt/ $\beta$ -catenin pathway is also required to suppress the chondrogenic potential of cells in the joint interzone. Using *ex vivo* cell culture systems, we are currently investigating the underlying molecular changes in order to identify target genes of the canonical Wnt-signaling pathway that affect chondrogenesis and osteoblastogenesis.

### Differentiation and Maturation of Chondrocytes

The various skeletal elements that make up 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 controls the expression of a central regulator of chondrocyte maturation in a spatio-temporal manner.



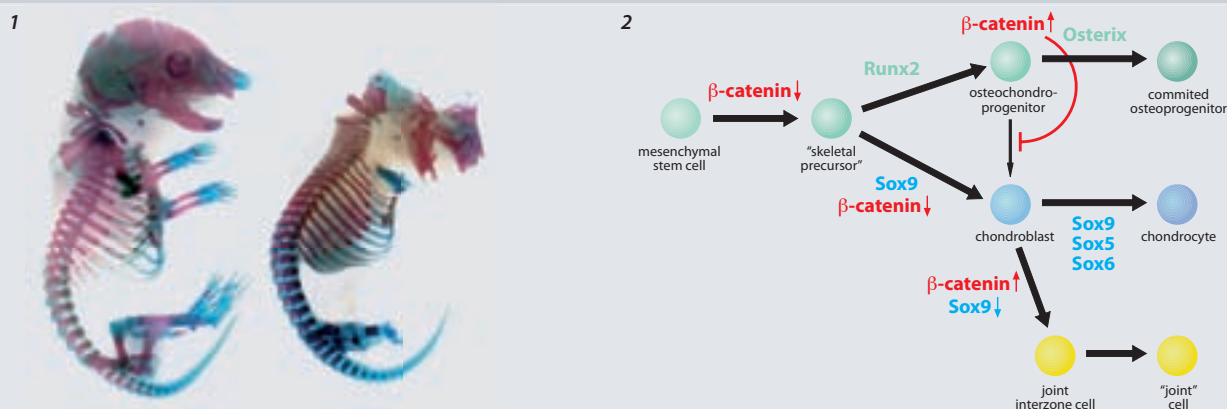


Figure 1: Skeletal preparations of a wild-type (left) and conditional  $\beta$ -catenin knock-out mouse (right). Cartilaginous regions are stained blue; mineralized areas are stained red, the latter include bony tissue and mineralized cartilage.

Figure 2:  $\beta$ -catenin levels need to be modulated during skeletal lineage differentiation: only mesenchymal cells expressing low levels of  $\beta$ -catenin can differentiate into a skeletal precursor which will give rise to osteoblasts, chondrocytes, and cells of the joint. Within the osteoblast and joint lineage,  $\beta$ -catenin levels need to be increased to enable the differentiation into osteoblasts and cells of the joint, respectively. While within the chondrocyte lineage,  $\beta$ -catenin levels need to be maintained at a low level.

Figure 3: Wnt9a expression in mature synovial joints of the hand visualized by  $\beta$ -galactosidase staining (green).

Thus, sequential activation, or temporal recruitment of regulatory molecules, which themselves, like Wnt9a, control a central regulator of chondrocyte maturation, are likely to be involved in fine-tuning the skeletal element size. Preliminary results suggest that Wnt9a might employ two different intracellular signaling pathways. We are currently investigating this possibility further, using genetic, biochemical and cellular approaches. Furthermore, we uncovered that Wnt4 is up-regulated in the Wnt9a mutants and consequently double mutants of Wnt9a and Wnt4 show an augmented chondrocyte maturation defect.

## 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 the 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 (Figure 3). 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. However, loss-of Wnt9a activity did not lead to any defects in early joint formation. Thus, the phenotype points at a role for Wnt9a in the maintenance of joint integrity. 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.

Conditional deletion of Wnt9a, specifically in the limb mesenchyme, allows the overcoming of the perinatal lethality of the complete knock-out. Interestingly, these limb-specific knock-out mice develop progressive changes in their elbow joints, which resemble hallmarks of osteoarthritis, and in addition, these mice show ectopic mineralization within joint ligaments.

In summary, our analysis has shown that Wnt-signaling plays important roles 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.

3







# THOMAS JENUWEIN

## Epigenetic Control by Histone Methylation

thomas.jenuwein@imp.ac.at



Thomas Jenuwein / Senior Scientist

Barna Fodor / Postdoc  
Gunnar Schotta / Postdoc  
Masato Yonezawa / Postdoc  
Borbala Gerle / PhD Student  
Johannes Popow / PhD Student  
Mario Richter / PhD Student  
Manuela Scaranaro / PhD Student  
Georg Winter / Diploma Student  
Monika Kauer / Technician  
Susanne Opravil / Technician  
Michaela Pagani / Technician

Andras Aszodi / Head Of GEN-AU Bioinformatics  
Ido Tamir / GEN-AU Bioinformatician

Dörthe Nickel / NoE Administrator  
Ylva Linderson / NoE Research Manager

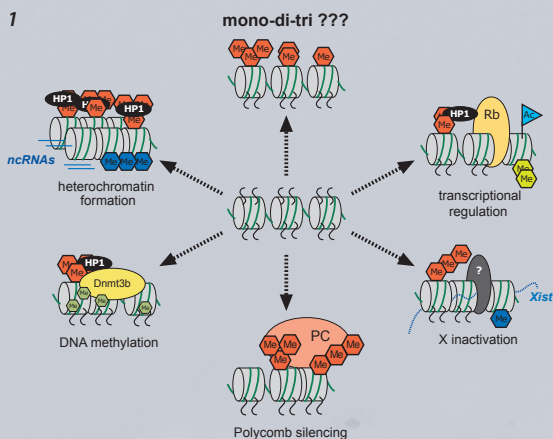
*Epigenetic mechanisms, such as histone modifications, control eukaryotic development beyond DNA-stored information. The characterization of histone methyltransferases (HMTases) strongly established histone lysine methylation as a central epigenetic modification for the organization of eukaryotic chromatin 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 to 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



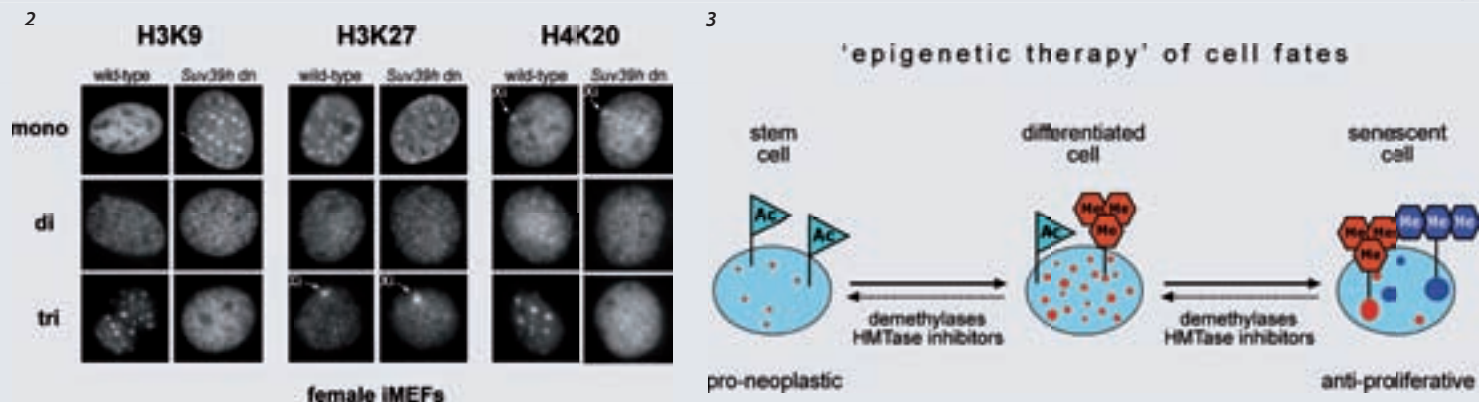


Figure 1: The many faces of histone lysine methylation. The figure summarizes described roles of histone lysine methylation in major epigenetic paradigms. Distinct lysine positions are shown as colour-coded hexagons to indicate H3K4 (green), H3K9 (red triangular), H3K27 (red diagonal) and H4K20 (dark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

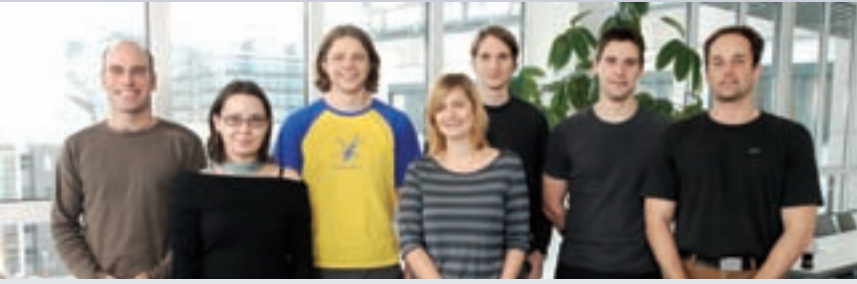
jumonjiC-containing proteins that represent demethylases with the potential to remove repressive H3K9me3 marks. Together, these approaches promise to yield new insights into the plasticity of cell fate decisions and may offer novel strategies for exploratory research to modulate tissue regeneration and 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](http://www.gen-au.at)) and the European Network of Excellence (NoE) 'The Epigenome' ([www.epigenome-noe.net](http://www.epigenome-noe.net)), we have performed large-scale analysis of epigenetic transitions in defined chromatin regions and along entire mouse chromosomes. In particular, we applied chromatin-immunoprecipitation (ChIP) with material from mouse ES cells, differentiated erythroblasts and MEFs (fibroblasts) to profile a high-resolution (using NimbleGen micro-arrays) epigenetic map of all single-copy sequences (coding and non-coding) of mouse chromosome 17 (Chr 17). The data indicate that the majority of histone modifications reflect the organization of transcription units, whereas Suv39h-dependent H3K9me3 accumulates at intergenic repeats (primarily LINEs and LTRs) and defines 'heterochromatic islands' that are highly variable in chromatin of different cell types. We propose that these heterochromatic islands facilitate the partitioning of transcription units vs. intergenic regions and are important for the architecture and identity of mammalian chromosomes.

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## LUDGER KLEIN T Cell Tolerance

ludger.klein@imp.ac.at

Ludger Klein / Group Leader

Christian Spona / Technician

Jan Emmerich<sup>1</sup> / Postdoc

Martin Aichinger / PhD Student

Maria Hinterberger / PhD Student

Jelena Nedjic / PhD Student

Gerald Wirnsberger / PhD Student

Florian Mair / Diploma Student

<sup>1</sup> until September

*Tolerance to "self" is a fundamental property of the immune system, and its breakdown can lead to autoimmune diseases such as multiple sclerosis and diabetes. Our aim is to understand how selection processes during T cell development in the thymus contribute to the generation of a self-tolerant T cell repertoire through removal of potentially dangerous T cells, but as well through the induction of so-called suppressor T cells.*

### Suppressor T Cells

It is well established that the encounter of self-antigen during intrathymic development can lead to the "suicide" of potentially dangerous, autoreactive T cells. However, some T cells with specificity for self-antigens are spared from deletion and instead differentiate into so-called regulatory or suppressor T cells ( $T_s$  cells). The parameters that influence the choice between these mechanisms of tolerance are not understood. One of our goals is to elucidate the developmental cues (stromal interaction partner, signal strength, maturation state) that determine whether an autoreactive T cell is removed from the repertoire (negative selection) or differentiates into a suppressor T cell. In particular, we are trying to dissect the respective role of the cortical versus medullary compartments of the thymus using a combination of T cell receptor and antigen transgenic mice, whereby the fate of "self-specific" T cells in various experimental conditions can be followed.

### "Promiscuous" Expression of Self-antigens in the Thymus

Deletion or re-programming of T cells upon encounter of self-antigens during intrathymic development is a cornerstone of immunological self-tolerance. However, it has remained questionable whether these mechanisms cover the entire spectrum of self-antigens, e.g., in how far such mechanisms could operate for tightly regulated tissue-specific proteins. We found that the range of self-antigens expressed in the thymus is surprisingly broad. This so-called "promiscuous" intrathymic expression of otherwise strictly tissue-specific proteins is confined to medullary epithelial cells (mTEC).



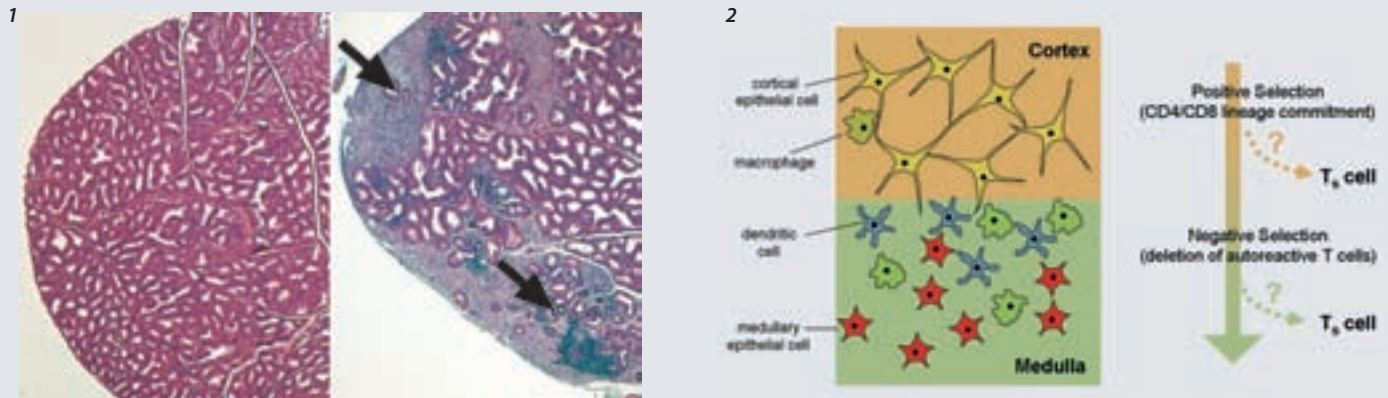
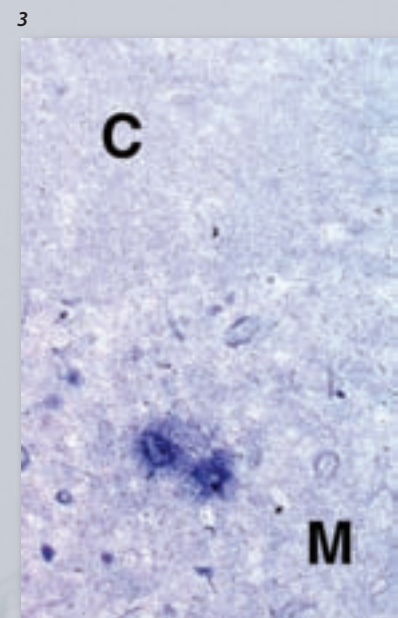


Figure 1: Autoimmunity as a consequence of altered T cell selection in the thymus. The left panel shows the normal histological appearance of the thymic gland in wild-type animals. In the right panel, the thymic gland of an animal carrying a genetic lesion in thymic epithelium that affects T cell selection is shown. Autoimmune T lymphocytes have entered the organ, leading to tissue-destruction (arrows).

Figure 2: During their maturation in the thymus, developing T cells migrate from the outer cortex to the medulla in a temporally and spatially highly ordered manner. While positive selection for self-MHC restriction occurs upon interaction with cortical epithelial cells, negative selection (removal of autoreactive T cells) is thought to be largely confined to the medulla as a consequence of interactions with dendritic cells or medullary epithelial cells. The developmental stage at which the CD25+ lineage of suppressor T cells (Ts) branches off from "mainstream" T cell development remains to be elucidated. We are currently developing transgenic model systems to address this issue.

Figure 3: Visualization by in situ hybridization of two cells expressing a "liver-specific" antigen in a medullary region of the thymus (C = cortex; M = medulla).

The mechanistic basis for this phenomenon (e.g. specific induction versus de-repression of particular genes) is only poorly understood. It was shown that the *Autoimmune Regulator (aire)* gene, a putative transcription factor that is specifically expressed in a not yet characterized subset of mTEC, is involved in "promiscuous" gene expression. Targeted disruption of *aire* leads to reduced expression of numerous self-antigens in mTEC, and *aire*<sup>-/-</sup> mice develop spontaneous autoimmunity. We have initiated a project which aims (i) to identify, isolate and characterize by flow-cytometry mTEC that express *aire*, and (ii) to study the consequences of antigen-expression in *aire*-expressing cells (deletion versus induction of anergy/suppressor function) by crossing these animals to T cell receptor transgenic animals



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# THOMAS MARLOVITS

## Design and Function of Molecular Machines

thomas.marlovits@imp.ac.at

Thomas C. Marlovits / joint IMP-IMBA Group Leader

Oliver Schraidt / PhD Student

Jesus Fernandez Rodriguez / PhD Student

Lisa Königsmaier / PhD Student

Matthias Brunner / Diploma Student

Wolfgang Weber / Lab Manager / Technical Assistant

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

### Host-Pathogen-Interaction

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

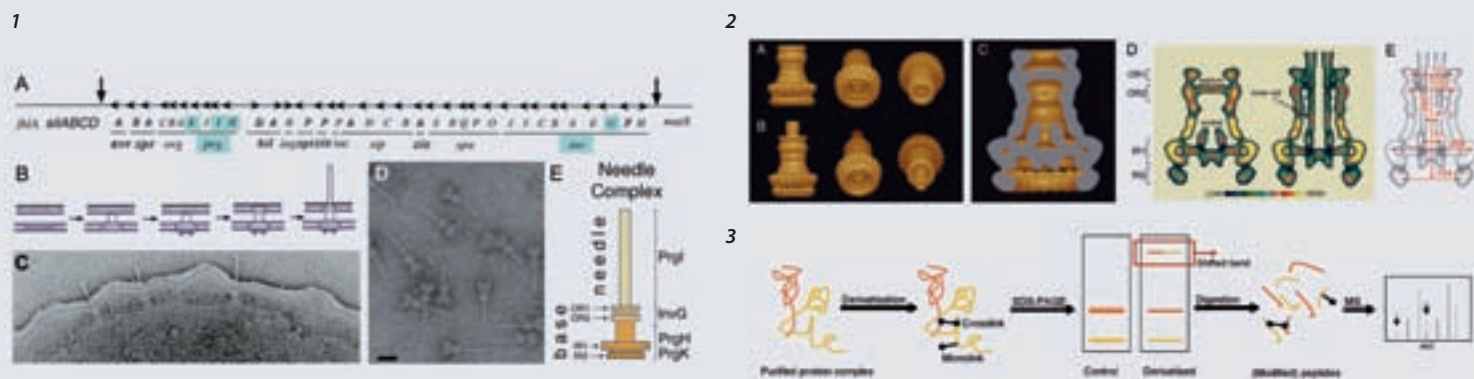
### The Molecular Design

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

The most prominent substructure of the TTSS is known as the “needle complex”, a cylindrical, needle-shaped and membrane-embedded organelle protruding from the bacterial envelope (Figure 1). The needle complex is believed to serve as a conduit for a safe transport of virulence factors from the bacterial cytoplasm through a number of natural barriers into eukaryotic cells. In *Salmonella typhimurium*, which serves as our model for bacterial delivery systems, this complex is formed by multiple copies of only five proteins: PrgH, PrgK, and InvG build up the membrane associated base-structure, PrgJ, the inner rod, and PrgI, the needle filament extending into the extracellular environment (Figure 1E and 2D).

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





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

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

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

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

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

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## JAN-MICHAEL PETERS

### Mitosis

jan-michael.peters@imp.ac.at



Jan-Michael Peters / Senior Scientist

Venugopal Bhaskara <sup>1</sup> / Postdoc  
 Franz Herzog / Postdoc  
 James Hutchins / Postdoc  
 Peter Lénárt / Postdoc  
 Mark Petronczki <sup>2</sup> / Postdoc  
 Kristina Uzunova <sup>3</sup> / Postdoc  
 Erwan Watrin / Postdoc  
 Kerstin Wendt / Postdoc  
 Gordana Wutz / Postdoc  
 Bettina Buschhorn / PhD Student  
 Björn Hegemann / PhD Student  
 Ivana Primorac / PhD Student  
 Erika Schirghuber / PhD Student  
 Julia Schmitz / PhD Student  
 Hanna Schutz / PhD Student  
 Antonio Tedeschi / PhD Student  
 Marta Galova / Research assistant  
 Birgit Koch <sup>4</sup> / Research assistant  
 Martina Sykora / Research assistant  
 Yan Sun / MitoCheck Project Manager

<sup>1</sup> since May

<sup>2</sup> until June,

<sup>3</sup> since February,

<sup>4</sup> until September

*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 biorientation 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 relies 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.

### 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 phosphorylation and binding of the co-activator Cdc20, but its ability to ubiquitinate substrates is largely suppressed by the spindle assembly checkpoint until all chromosomes have become bioriented 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 assembly 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 analyze the structure of APC/C.



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: A 3D model of human APC/C obtained by angular reconstitution of negatively stained cryo-electron microscopy preparations. Courtesy of Holger Stark.

Figure 3: Human cells treated with the Plk1 inhibitor BI 2536 arrest with monopolar spindles (microtubules are stained in green and chromosomes in blue). Courtesy of Peter Lénárt.

## Mitotic kinases

Initiation of mitosis and virtually all steps during mitosis and cytokinesis are controlled by a small set of protein kinases, such as Cdk1, Plk1 and Aurora A and B. We identified the small molecule Hesperadin as an inhibitor of Aurora B and found that this kinase is required for correcting syntely, a type of attachment in which both sister kinetochores of a chromosome become erroneously attached to one spindle pole. We discovered that Aurora B is also required for the spindle assembly checkpoint, for dissociation of heterochromatin protein 1 from centromeres and for association of the condensin I complex with mitotic chromosomes. In collaboration with Boehringer Ingelheim, we are using the small-molecule inhibitor BI 2536 to study functions of Plk1 in mitotic entry, spindle assembly and cytokinesis and to evaluate Plk1 as a potential drug target in cancer therapy.



### MitoCheck

Although the central importance of protein kinases in controlling mitosis has long been recognized, 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 Aurora B and Plk1.

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## SIMON RUMPEL

### Synaptic and Circuit Mechanisms of Memory Formation

simon.rumpel@imp.ac.at



Simon Rumpel / Group Leader

Annerose Kuras / PhD Student

Juliane Tinter / Diploma Student

Jiss John / Technician

*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.*

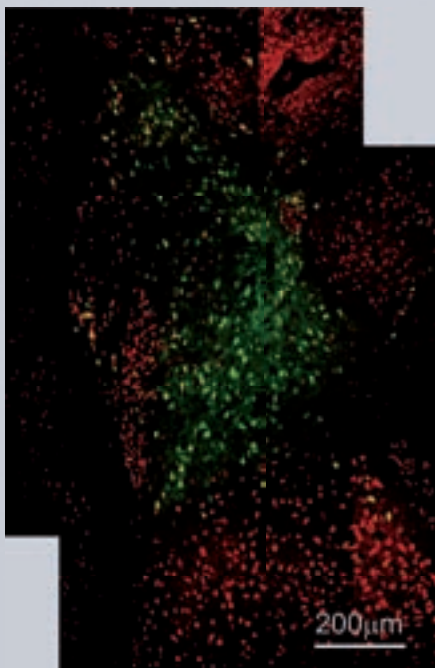
#### Trafficking of Synaptic Receptors during Memory Formation

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.

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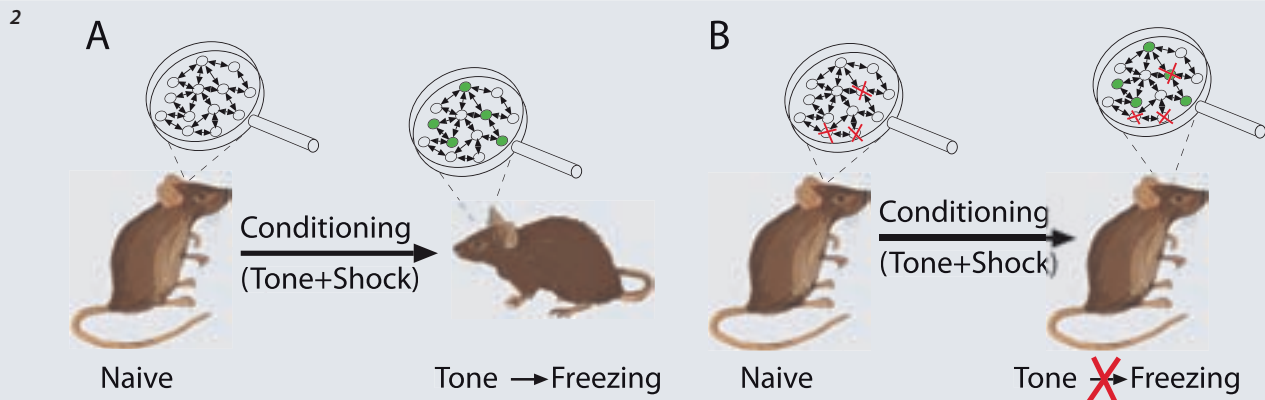


Figure 1: Virus-mediated gene transfer *in vivo* allows specific manipulation of neuronal function during behavior. Neurons in the nucleus of the lateral amygdala express green fluorescent protein after *in vivo* infection. Red counterstain against neuronal marker NeuN.

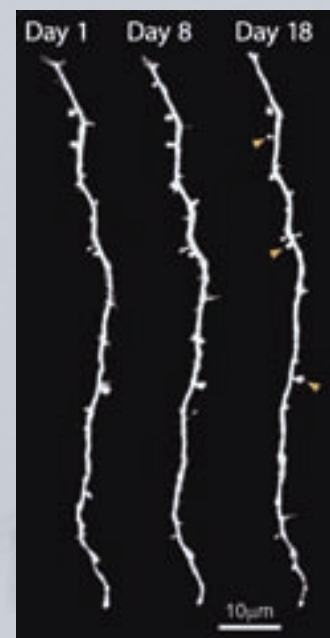
Figure 2: Memory traces in the lateral amygdala appear to be distributed, but highly sensitive to perturbations of synaptic plasticity. (A) Rats show freezing behavior when presented to a tone that has been previously paired with a shock during a conditioning session. This behavior indicates that an 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 3: Chronic imaging of individual neurons and synapses. Two-photon laser scanning microscopy allows imaging of fluorescent neurons in the brain: Images of an individual neuronal process with synaptic contacts (so called spines, indicated by orange arrows) taken at different days. The connectivity of the neuron can be monitored by following the appearance, disappearance and size of synaptic spines.

## 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.

3



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## PEGGY STOLT-BERGNER

### Structure-Function Analysis of Membrane Proteins and their Interaction Partners

peggy.stolt-bergner@imp.ac.at

Peggy Stolt-Bergner / Independent Postdoctoral Fellow

Barbara Nussbaumer / Diploma Student

Elisabeth Malle <sup>1</sup> / Diploma Student

<sup>1</sup> since December

*All cells are surrounded by at least one impermeable lipid bilayer, and have developed numerous classes of molecules to allow them to interact with their environment across this membrane. These molecules include integral membrane proteins, such as G protein-coupled receptors, channels, and transporters, as well as single-pass transmembrane receptors with large extra-cellular or intracellular regions. We are interested in the structural mechanisms these proteins use to accomplish their tasks, mainly focusing on secondary active transporters.*

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 these functions due to the challenges inherent in working with such hydrophobic proteins.

Currently, we are focusing on the structural characterization of secondary active transporters, which drive the transport of substrates against their concentration gradient by coupling this transport to an energetically favorable process. While recent work has begun to uncover some of the details underlying secondary transport mechanisms, there are still only a handful of individual structures available, and many important questions remain unresolved, such as how substrate and proton or ion translocation are coupled, and what conformational changes are necessary for transport to occur. In addition, due to the small number of available structures, it is likely that many transport mechanisms remain uncharacterized. Additional structures of diverse types of transporters and structures of different conformational states will most likely help to address these questions.

Structural studies on membrane proteins are challenging primarily due to the instability of these proteins when outside of the native membrane environment. In addition, the conformational heterogeneity of membrane transporters can contribute to their instability (see Figure 1). One approach to facilitate the crystallization of transporters, and membrane proteins in general, is to identify more stable forms of the protein of interest.

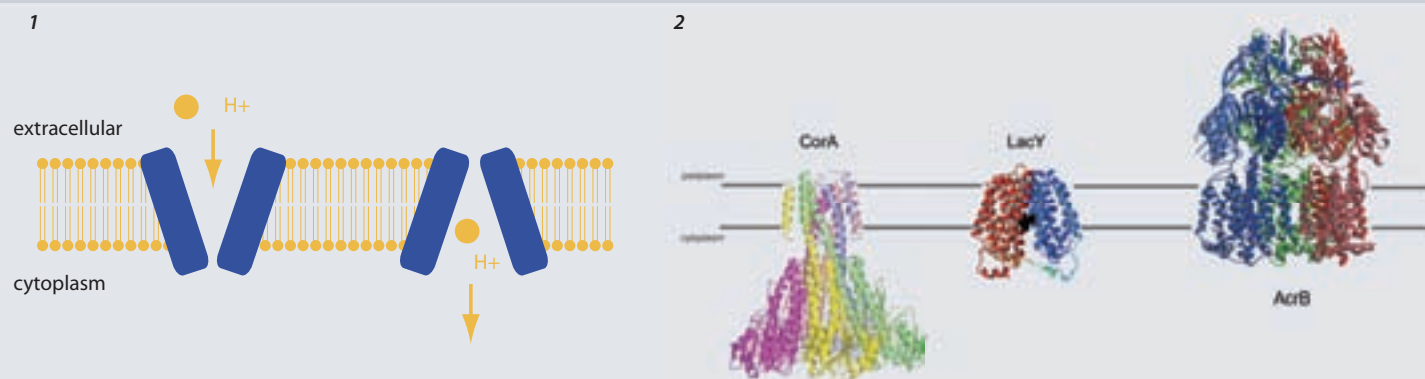


Figure 1: The alternating access hypothesis of membrane transport. Secondary transporters have long been thought to function by alternating between at least two conformations. In one conformation, the substrate-binding site is open to the outside of the cell, and upon substrate binding, the protein undergoes conformational changes that open the substrate-binding cavity to the inside of the cell. While there is some structural evidence to support this model, it does not apply to all transporters, as alternative transport mechanisms have also been characterized (see Figure 2).

Figure 2: Current transporter structures reveal diverse types of transport mechanisms. Recent structural work has uncovered evidence for multiple transport mechanisms. The *E. coli* lactose permease LacY adopts the alternating access model, which most likely involves large conformational changes (Abramson et al., 2003 Science 301:610; 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 (Lunin et al 2006 Nature 440:833; Eshaghi et al 2006 Science 313:354; figure adapted from Eshaghi et al). The trimeric *E. coli* multi-drug efflux pump AcrB utilizes an alternating binding site mechanism, which involves more subtle conformational changes (Murakami et al 2006 Nature 443:173; Seeger et al Science 313:1295; figure adapted from Murakami et al 2002 Nature 419:587). These diverse structures and mechanisms emphasize the need for structural characterization of a larger number of transporters, and for structures in different conformational states in order to generate deeper insights into these and other as yet uncharacterized transport mechanisms.

This approach has already proven successful for transporters; LacY, the lactose permease from *E. coli*, was first successfully crystallized using a mutant transporter that shows very little transport activity but is still able to bind its substrate (Abramson et al., 2003 Science 301:610).

Given the potential utility of this approach in increasing the number of transporter structures, we are interested in systematically identifying mutants that will prove amenable to crystallization, and thereby help produce novel structures as well as structures in different conformations of the transport cycle. Stable mutant transporters identified through this process will be used as tools for crystallization and in vitro functional characterization, to increase our understanding of transport mechanisms.

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## ERWIN WAGNER

### Gene Function in Mammalian Development and Disease

erwin.wagner@imp.ac.at

Erwin F. Wagner / Senior Scientist and Deputy Director

Latifa Bakiri / Postdoc  
 Juan Guinea / Postdoc  
 Lionel Gresh / Postdoc  
 Peter Hasselblatt / Postdoc  
 Aline Bozec / Postdoc  
 Lijian Hui / Postdoc  
 Domenico Migliorini / Postdoc  
 Alok Mishra / Postdoc  
 Guillaume Beranger<sup>5</sup> / Postdoc  
 Helia Schönthaler / Postdoc  
 Ewa Stepniak<sup>2</sup> / Postdoc  
 Ivana Cusic<sup>1</sup> / PhD Student  
 Iris Uras<sup>3</sup> / Diploma Student  
 Maria Helena Idarraga-Amado / Technician  
 Uta Möhle-Steinlein / Technician  
 Martina Rath / Technician  
 Harald Scheuch / Technician  
 Hiromi Kudo<sup>6</sup> / Technician  
 Stefan Sladeczek<sup>4</sup> / Technician

<sup>1</sup> PhD Student jointly with Hartmut Beug

<sup>2</sup> until May,

<sup>3</sup> until April

<sup>4</sup> March-September

<sup>5</sup> since April

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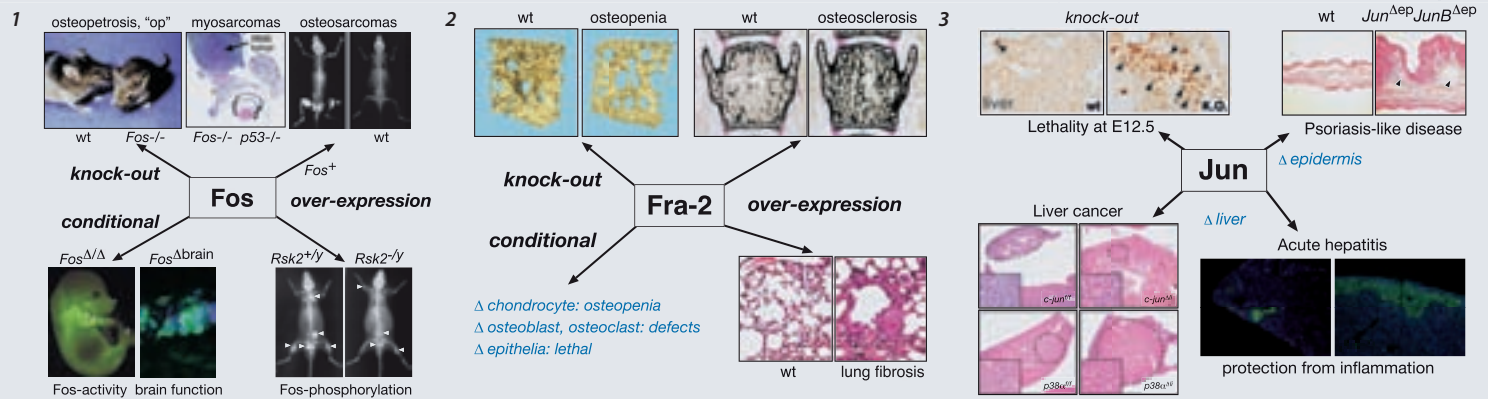
*The major focus of our studies is the analysis of gene function in normal and pathological conditions, e.g. in tumor development, using the mouse as a model organism. Specifically, the functions of AP-1 in regulating cell proliferation, differentiation and cell death are investigated. Our studies revealed that the AP-1 proteins Fos and Jun play pivotal roles in bone, liver, skin and hematopoietic 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 tumors, 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 osteopenia, a low bone mass disease. On the other hand, transgenic mice over-expressing Fra-1 develop an osteoblastic bone disease termed osteosclerosis. Recently, we analyzed 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 osteopenia 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 c-Fos in bone development and pathogenesis. The ERK-dependent kinase RSK2 can phosphorylate c-Fos on serines 362 and 374 and the RSK2 kinase is essential for the development of c-Fos-dependent osteosarcomas *in vivo* (Figure 1). To further study c-Fos phosphorylation *in vivo*, c-Fos mutant knock-in mice were generated in which the endogenous wild-type c-Fos allele was replaced by a point mutant allele with serines 362 and 374 mutated to alanines (Fos<sup>AA</sup>). Recent results show that c-Fos C-terminal phosphorylation is dispensable for skeletogenesis but required for osteoclastogenesis *in vitro* and for normal bone homeostasis in adult mice.

### Tumor suppression by JunB and Fos

JunB is a transcriptional activator of the cyclin-dependent kinase inhibitor p16/INK4a and functions as a negative regulator of cell proliferation in fibroblasts. Using conditional gene ablation we could show that JunB acts as a tumor suppressor *in vivo*. JunB absence in the myeloid lineage led to a transplantable myeloproliferative stem cell disease resembling human chronic myeloid leukemia. Initially identified as a *bona fide* oncogene, the Fos protein was found to have an unexpected function as a tumor suppressor in the muscle lineage. Loss of both, Fos and p53 resulted in the formation of rhabdomyosarcomas with a frequency



greater than 90% (Figure 1). Re-expression of Fos in double mutant muscle tumor-derived cell lines induced apoptosis, indicative of a novel mechanism of tumor suppression by Fos.

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

## Jun/AP-1 - 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 tumor initiation as well as in progression (Figure 3). We were able to show that for efficient liver regeneration, a p53/p21 and p38-MAPK-dependent pathway is essential. Analogous genetic strategies are employed to investigate the functions of Jun/AP-1 proteins in liver cancer and inflammatory disease. 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 analyzed. Moreover, c-Jun is highly expressed in colitis-associated intestinal tumors, 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. Deletion of *jun* in the epidermis of the skin causes an eye closure defect and affects proliferation of keratinocytes *in vitro* and skin tumor development *in vivo* likely through down-regulation of HB-EGF and EGFR. When *junB* was 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 are able to demonstrate in patient's 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 was inactivated in the epidermis in a constitutive manner, mutant pups died due to a cytokine storm, which involves deregulation of TNF-TIMP3 expression.

## VEGF, VEGF-R2/Flk-1 and EGF-R and p38

Conditional alleles of VEGF, Flk-1 and EGF-R were employed to study the role of VEGF and Flk-1 in the development of endothelial and hematopoietic stem cells. In collaboration with Erwin Tschachler and Maria Sibilio from the Medical University of Vienna, we are investigating the functional importance of VEGF-A in skin biology and the role of EGF-R in normal and skin tumor development. In addition, we have employed conditional alleles of the MAPK p38 to study their functions in hematopoietic cells, specifically in erythroblast and macrophage differentiation as well as in muscle cell biology (coll. Pura Munoz, CRG, Barcelona).





# STEFAN WESTERMANN

## Molecular Mechanisms of Kinetochore Function

stefan.westermann@imp.ac.at



Stefan Westermann / Group Leader

Eva Kiermaier / PhD Student

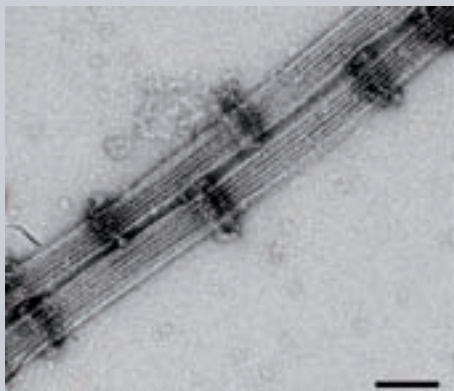
Tomasz Zimniak / PhD Student

Peter Hornung / Diploma Student

Katharina Stengl / Technician

*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.*

1



### Organization of the Budding Yeast Kinetochore

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 organization are conserved throughout evolution. A challenge for the future is to understand the precise molecular functions of individual kinetochore complexes, to reassemble them into a functional kinetochore *in vitro* and to analyze how their function is regulated by posttranslational modifications.

### Formation of a Dynamic Kinetochore Interface

We have started to reconstitute individual kinetochore complexes by co-expressing their subunits in insect cells or bacteria to analyze their structures, biochemical activities and interactions. Electron microscopy has revealed that the 10-protein Dam1 complex, a key microtubule-binding element of the budding yeast kinetochore, oligomerizes into a 16-fold ring around a microtubule *in vitro* (Figure 1). 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. Single particle analysis and helical reconstruction have been used to gain insights into the architecture of the ring complex (Figure 2). A protrusion domain extending towards the surface of the microtubule is a major landmark of the ring complex and is involved in regulating the interaction with the microtubule.

Many questions about the kinetochore ring complex remain unanswered:



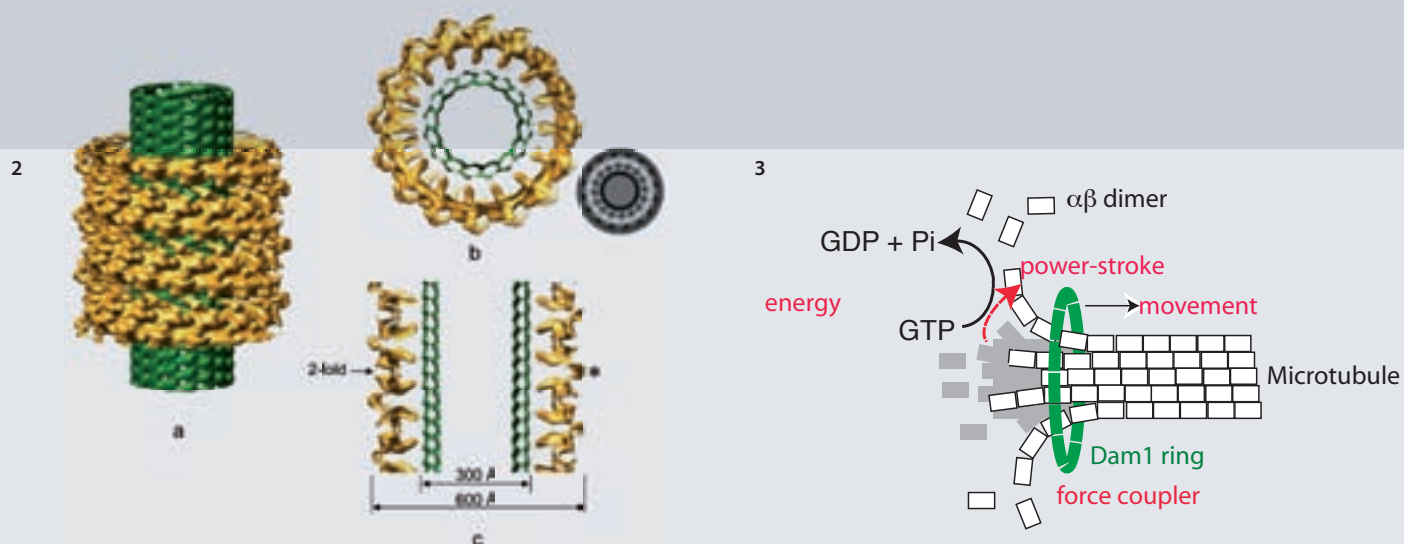


Figure 1: The 10-protein Dam1 complex oligomerizes into a ring around the microtubule *in vitro*. Negative stain electron microscopy of Dam1 rings decorating taxol-stabilized microtubules. Bar: 25 nm.

Figure 2: Structural analysis of the Dam1 ring complex. (a) Reconstruction of Dam1 complex-microtubule helical assembly showing two anti-parallel Dam1 spirals (golden) around a 16-protofilament microtubule (green). End-on (b) and central (c) cross-sections of the reconstruction showing the bridge density towards microtubule surface extending from the inside surface of the Dam1 spiral. This bridge density corresponds to the thin layer of density present in the inner surface of the rings as shown in a 16-fold averaged ring (inset of b). One of the two-fold axes is indicated in c.

Figure 3: Principle of the Dam1 ring complex as a novel biological motor: Upon GTP-hydrolysis, microtubule protofilaments curve and push the Dam1 ring over the microtubule lattice. This mechanism can connect the kinetochore to a depolymerizing microtubule end.

How are 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?

## Mechanisms of Force Generation

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 depolymerization 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 *in vitro* microscopy assay, we could directly visualize 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 (Figure 3). This constitutes a new mechanism for microtubule-based motility that uses the energy of GTP hydrolysis by the microtubule and converts it into a processive movement. We will continue to develop microscopy assays with dynamic microtubules to uncover further aspects of kinetochore motility.

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## ANTON WUTZ

### Mammalian X-Chromosome Inactivation

anton.wutz@imp.ac.at

Anton Wutz / Group Leader

Aditya Sengupta / Postdoc

Ruben Agrelo / Postdoc

Hiroiyuki Kishimoto / Postdoc

Tatsuya Ohata / Postdoc

Dieter Pullirsch <sup>1</sup> / PhD Student

Karen Ng / PhD Student

Martin Leeb / PhD Student

Renate Härtl <sup>2</sup> / Diploma Student

Mediyha Saltik / Technician

<sup>1</sup> until May

<sup>2</sup> since 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 (Ng et al., 2007). The long non-coding *Xist* RNA is critical for initiation of this silencing process and associates physically along the entire chromosome (Figure 1).

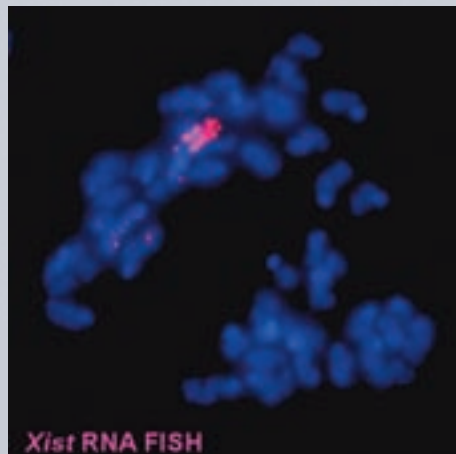
### The function of Polycomb group proteins at the initiation of X-inactivation

Polycomb group (PcG) complexes are recruited by *Xist* at an early stage in X inactivation and mediate chromosome-wide modifications of histones. We have shown that recruitment of Polycomb repressor complex 1 (PRC1) and PRC2 is strictly dependent on *Xist* RNA at all stages of differentiation. *Xist* can initiate chromosome-wide silencing in ES cells lacking PRC2 activity by a mutation in *Eed*. Notably, PRC1 can be recruited by *Xist* independent of PRC2 and mediates ubiquitination of histone H2A. In order to investigate the function of PRC1 in X inactivation we have disrupted *Ring1B*. We find that several PRC1 proteins are lost in *Ring1B*-deficient ES cells (Leeb and Wutz, 2007). This leads to a derepression of developmental control genes and aberrant ES cell differentiation (Figure 2). Ubiquitination of H2A is abolished, but *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 have both essential roles in embryogenesis and can be recruited by *Xist* independent of each other, we propose that they might act redundantly during X inactivation. Future experiments aim for the establishment and characterization of ES cell lines deficient in both PRC1 and PRC2, to define Polycomb function in X inactivation.

### Turning off chromosomes by *Xist* during development

Early in embryogenesis *Xist* expression triggers the inactivation of one of the two female X chromosomes. 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

1



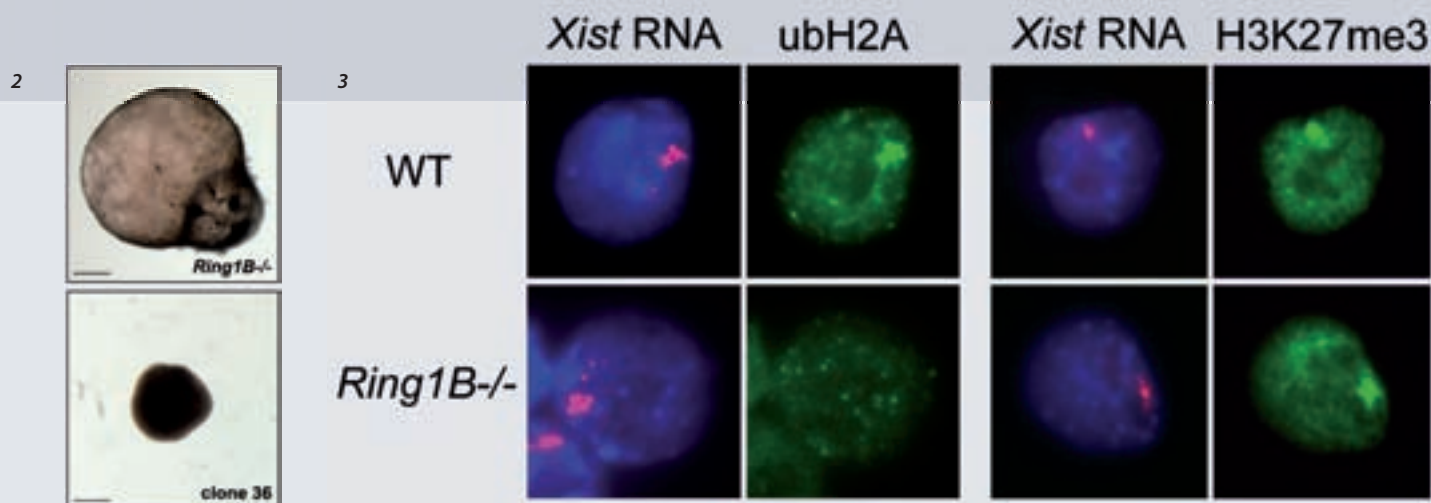


Figure 1: *Xist* RNA association with chromatin revealed by RNA FISH. Mouse chromosome spreads were prepared from ES cells expressing *Xist* from a transgene on chromosome 11 and hybridized with a fluorescent probe detecting *Xist* RNA (red). DNA was counter stained with DAPI (blue).

Figure 2: Aberrant differentiation of ES cells lacking *Ring1B*. Embryoid bodies derived from *Ring1B* deficient ES cells show striking hollow sphere morphology compared to 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.

gene repression remains an open question. Using an inducible *Xist* allele in mice we found that the potential of *Xist* to initiate silencing is lost during embryogenesis in a gradual and cell type-specific manner. In adult male mice, ectopic expression of *Xist* causes an anemia 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. This suggests that *Xist* can function in cells of the adult organism and point to epigenetic differences among specific differentiation stages (Wutz, 2007). 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.

## Identification of the mechanism of chromosomal silencing by *Xist* RNA

To gain insight into the molecular mechanism by which *Xist* causes transcriptional repression, we focus on the isolation of proteins that interact with the repeat A of *Xist* RNA, a crucial element for initiation of silencing. In collaboration with Karl Mechtler from the IMP protein sequencing facility we have identified proteins from nuclear extracts that specifically interact with repeat A RNA. Ongoing experiments focus on the functional analysis of candidate proteins and the identification of a potential pathway for *Xist*-mediated transcriptional 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-chromatinizing 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

*Discoveries in biosciences are often stimulated by the invention of new scientific tools. We like to push single-molecule fluorescence techniques beyond the classic spatial and typical temporal resolution limits. Such noninvasive 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 contribution to solving biological or medical problems focuses on the development and adaptive modification of such single molecule-sensitive fluorescence techniques, particularly on answer neurobiological questions.

### Quantifying protein diffusion and binding constants in live cells: Fluorescence fluctuation techniques highlight molecular dynamics

Complementary to the very popular, however relatively 'slow' fluorescence imaging techniques, there are ultra-fast spectroscopic techniques, such as confocal Fluorescence Correlation Spectroscopy (FCS), with temporal resolution typically in the  $\mu$ s-s range. FCS is an ideal tool to study intracellular diffusion, binding, and local molecular concentrations in real time. Within a confocal setup, FCS analyzes minute fluorescence intensity fluctuations in the equilibrium of a small ensemble ( $<10^3$ ) of molecules to derive dynamic constants. These fluctuations act like a 'fingerprint' of a molecular species detected when entering and leaving a femtoliter-sized, optically defined observation volume.

In my former lab, we developed several cell-friendly, multi-color versions based on two-photon excitation and FCS which allowed us to determine the  $\text{Ca}^{2+}$  dependent intracellular dissociation constants of the signaling molecules Calmodulin (CaM) and  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase II (CKII). The dynamics of these molecules are assumed to be the key to understanding one of the principal mechanisms of learning and memory. Recently, a flexible two-photon FCS platform has been started up in my lab at the IMP. One of our goals is to utilize this technique and setup to monitor real-time binding and mobility of signaling molecules in dendritic spines. Moreover, we plan to develop a respective two-photon 'FCS array', which would allow for simultaneous monitoring of mobility and binding characteristics at different locations within a cell for a time-resolved 'mapping' of molecular behavior.

### Having the closest look: Fluorescence Imaging in 3D with nanometer precision

A major goal in neurobiology in recent years has been to gain insight into the molecular machinery that mediates synaptic transmission. So far, we have a very limited understanding of what makes up a synapse. What is the molecular composition of a synapse? Where are specific synaptic proteins localized in a set of synapses? Which proteins actually target

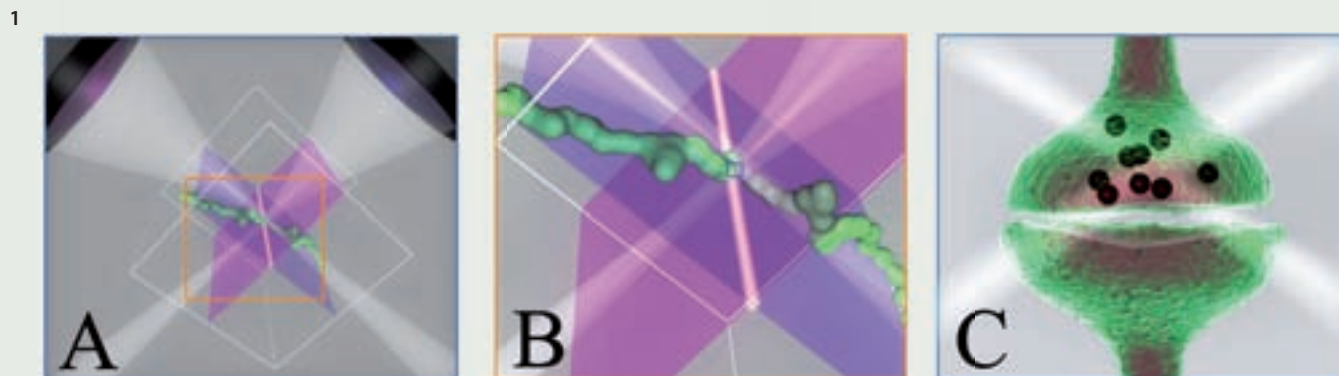


Figure 1: Schematic of the dual axis setup of the nanoscope: Two objective lenses image a cube of brain tissue containing neurons (A). After selecting an area of interest (B), subsets of images are recorded at a dendrite with synapses (B, prolated structure (green)). A two-photon activation line scan along the intersection (light pink) of the two perpendicular focal planes (light and dark purple) defines the area where molecules will be excited and detected for the given subset. Finally the position information is assembled in a 3D reconstruction of an image (C).

specific subcellular compartments and how does the molecular composition of a synapse correlate to its lifetimes?

In collaboration with the group of Simon Rumpel, we plan to pinpoint the localization of various synaptic proteins (e.g. AMPA-type glutamate receptors, NMDA receptors) in a set of synapses to answer these questions.

Unfortunately, today's optical microscopy tools prevent a good estimation of the spatial 3D distribution of synaptic molecules as the typical size of a central synapse (~300 nm Ø, thickness of the postsynaptic density (PSD) ~30-50 nm) is significantly smaller than the accessible optical resolution limit.

Thus, we are currently working to develop a single molecule 'nanoscope' for optical 3D imaging of synapses based on PhotoActivatable Fluorescent Proteins (PA-FPs) and a dual imaging axis setup (Figure 1). The hypothesis to test is whether specific synaptic proteins define subsets of synapses. We suppose that excitatory synapses on pyramidal neurons in the neocortex of mice represent a heterogeneous population that can be classified by their qualitative composition of postsynaptic proteins.

## FURTHER READING

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Betzig, E., Patterson, G.H., Sougrat, R., Lindwasser, O.W., Olenych, S., Bonifacino, J.S., Davidson, M.W., Lippincott-Schwartz, J., and Hess, H.F. (2006). Imaging intracellular fluorescent proteins at nanometer resolution. *Science* 313(5793):1642-1645.



# VIENNA DROSOPHILA RNAi CENTER (VDRC)

## Krystyna Keleman, Dirk Holste, Peter Duchek

office@vdrc.at

Krystyna Keleman / Head of the VDRC

### **Maintenance and Distribution of the RNAi Library**

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

### **Research**

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

Nadege Minois / Postdoc  
 Luiza Deszcz / Technician

### **Keleman Group**

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

### **Holste Group**

Dirk Holste / Staff Scientist  
 Ralf Bortfeldt<sup>1</sup> / PhD Student  
 Martin Pohl<sup>1</sup> / Diploma Student  
 Doris Chen<sup>2</sup> / Postdoc

### **Duchek Group**

Peter Duchek / Staff Scientist  
 Sabine Brynda / Technician  
 Ciara Gallagher<sup>3</sup> / Technician

<sup>1</sup> co-supervised with Prof. Stefan Schuster, Friedrich-Schiller University Jena

<sup>2</sup> co-supervised with Prof. Renee Schröder (2006-2007)

<sup>3</sup> January to June 2007

## Genome-wide RNAi in *Drosophila*

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

The creation of a genome-wide transgenic RNAi library [1] has revolutionized *Drosophila* genetics. The VDRC, a joint IMP-IMBA initiative, maintains and distributes this library. In just its first seven months of operation, the VDRC has delivered over 25,000 lines to more than 800 registered users world-wide. This has been made possible in part through core funding provided by the city of Vienna and the federal government, with the rest of the costs covered by user fees. We continue to further develop both the library and the service, and should soon also be able to host external researchers who wish to conduct their RNAi screens on-site. The VDRC already provides such support for researchers in-house, having delivered over 145,000 lines to IMBA and IMP groups in 2007. The VDRC is also home to the Keleman, Holste and Duchek Research Groups, supported entirely by external funds.

## Krystyna Keleman

### **Learning and Memory**

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

We are trying to understand the molecular and cellular mechanisms that underlie this robust and powerful form of long-term memory. We have recently demonstrated that the CPEB protein Orb2 – a regulator of mRNA translation – plays a critical role in this process [2]. We found that Orb2 function is required in a specific set of mushroom body neurons during or shortly after training (Figure 1). Without Orb2, or more specifically without its intriguing glutamine-rich domain, a memory initially forms but decays within just a few hours. We are now trying to identify target mRNAs that are regulated by Orb2 in these neurons, and to better define the cues – probably pheromones – that the male is learning to discriminate. Meanwhile, we are also using the transgenic RNAi library in unbiased approaches to find other factors involved in long-term memory formation.

We do also commercially exploit the *Drosophila* transgenic RNAi library in collaborative project with the biotech company to identify novel drug targets.



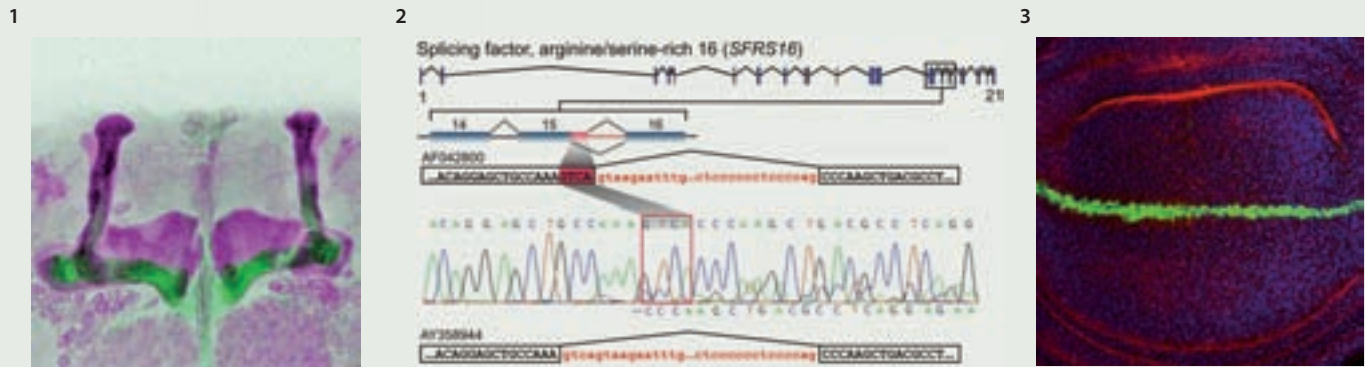


Figure 1. Mushroom body neurons in the fly's brain – Orb2 is required in these neurons for courtship conditioning.

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

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

## Dirk Holste

### Computational Biology of post-transcriptional Gene Expression

Gene expression is ubiquitous and controlled on different several cellular levels under diverse contexts. In metazoans, where the majority of genes are transcribed as pre-mRNAs, the splicing of precursors to mRNAs constitutes an essential step for the genetic regulation of gene expression at the level of RNA processing. Many pre-mRNAs show variable splicing patterns: different splice sites may be used as alternatives, giving rise to multiple alternatively spliced isoforms, and thus producing mature mRNAs and ultimately polypeptides (highly similar or markedly different) originating from the same locus (Figure 2). We use computational approaches to investigate observed patterns and underlying mechanisms of splicing, using genome analysis and mathematical-statistical models incorporating various types of data, which can be experimentally verified [3]. A unifying goal of our research is to shift biology from case studies toward a global approach, by the dissection of circuits between functional cis-regulatory sequence elements and trans-factors governing the regulation of gene expression ("RNA code") during the development of organisms.

## Peter Duchek

### Cell Signaling and Morphogenesis

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

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

## FURTHER READING

[1] Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblaue, S., Couto, A., Marra, V., Keleman, K., Dickson, B.J. (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*. (12)1587-93

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



## BIOOPTICS DEPARTMENT

Peter Steinlein / Head of Facility

Karin Aumayr / Microscopy and Image Analysis

Carmen Czepe<sup>1</sup> / Micro Arrays

Andreas Mairhofer<sup>2</sup> / Micro Arrays

Pawel Pasierbek / Microscopy

Martin Radolf / Micro Arrays

Gabriele Stengl / Flow Cytometry

<sup>1</sup> until June,

<sup>2</sup> since August

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

### Current activities

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

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

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

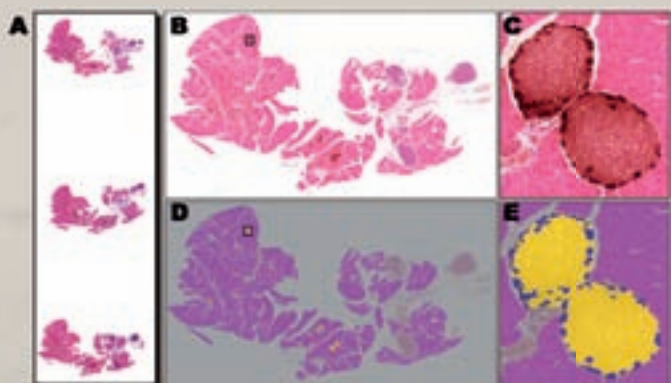


Figure: (A.) shows the scan of a slide containing 3 sections of a mouse pancreas immunohistochemically-labelled with an anti-Glucagon antibody and counterstained with Eosin. (B.) and (D.) show one of the sections with the blow up of two of the islets of Langerhans containing the Glucagon-labeled cell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





## ELECTRON MICROSCOPY

guenter.resch@imba.oeaw.ac.at

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

Günter Resch / Head of Electron Microscopy Facility

Marlene Brandstetter / Technical Assistant  
Pedro Serrano Drozdowskij / Programmer

### Sample Preparation

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

### Microscopy

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

While the Morgagni is an excellent tool for conventional TEM, more advanced applications will be the domain of the FEI TF30 Polara. This 300 kV TEM, unique in Austria and equipped with the most advanced imaging systems, was funded by a Vienna Spot of Excellence grant. The installation (Figure) was started in March 2007 and by Autumn, the microscope was up and running except for its most advanced functions. Primarily, it will be used for cryo-TEM of molecules and cells, electron tomography, as well as analytical applications.

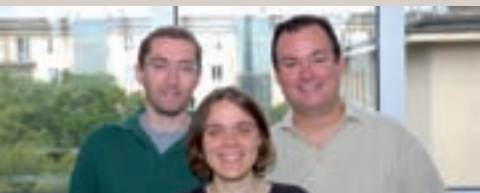
### Data Management and Image Processing

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



Figure: The TF30 Polara coming to life.





# BIOINFORMATICS

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 analytic services, scientific data mining, hardware and software infrastructure, and training in bioinformatics.*

## Sequence Analysis

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

## Large Scale Data Analysis

Additional demands arise from the investigation of large functional genomics or high-throughput biological datasets. We engage in custom software and database development, and design computational and mathematical solutions that can cope with the higher load and memory requirements. In the past year, we have set up a data warehouse system based on the InterMine project to integrate data from various biological databases and formats. The IMP-IMBA FlyMine cross-links extensive public genome information with in-house fly

screen results and custom annotations. To perform complex sequence analytic tasks we also maintain the IMP ANNOTATOR, a user-friendly web application and high throughput protein annotation system.

## Training

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

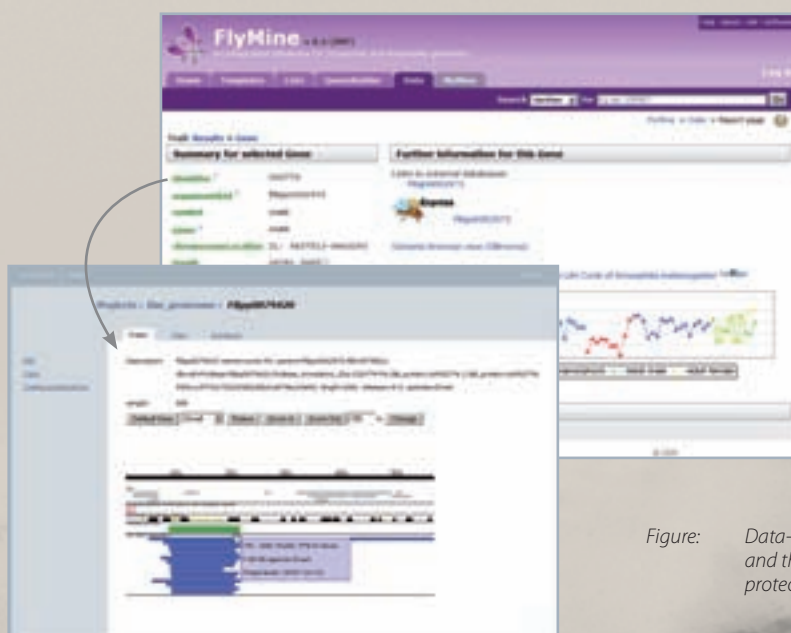


Figure: Data-mining using our in-house FLYMINE installation and the annotation of the *Drosophila melanogaster* proteome with the IMP ANNOTATOR.



## PROTEIN CHEMISTRY FACILITY

karl.mechtler@imp.ac.at

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

### Improvement in Phosphorylation Analysis

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

### Analysis of protein complexes and Enrichment of phosphopeptides from complex mixtures

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

### The Christian Doppler Laboratory for Proteome Analysis

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

### Peptide Synthesis and Antibody Purification

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

*Figure: Silver-stained gels of protein complexes subjected to phospho-site mapping. Following tryptic digestion and enrichment of phosphopeptides on TiO<sub>2</sub> tips, it was possible to isolate and identify a significant number of phosphorylation sites. A number of these originated from proteins in gel regions where silver staining indicated no proteins were present (marked with asterisk).*

Karl Mechtler / Head of Facility

Karin Grosstessner-Hain / PhD Student

Gabriela Krssakova / Technician

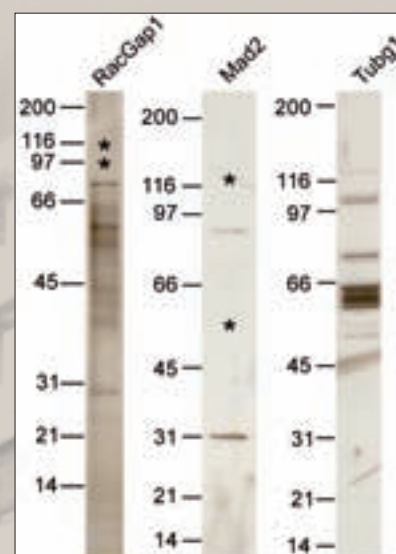
Goran Mitulovic / Technician

Mathias Madalinski / Technician

Elisabeth Roitinger / Technician

Ines Steinmacher / Technician

Christoph Stingl / Technician





## HISTOLOGY DEPARTMENT

vukoslav.komnenovic@imba.oeaw.ac.at

Vukoslav Komnenovic / Head of Facility

Mihaela Grivej / Technician

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

### Histology Services

The histology services include the embedding of tissues, assistance with the sectioning of paraffin and cryo-preserved tissues, and preparation of slides for standard H&E stains, as well as specialized stainings such as PAS, Alcian blue, Cab, Gomori, 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](http://www.mta-labor.info)).

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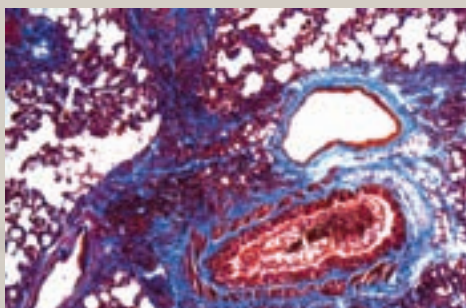


Figure 1: Trichrom blue staining. The picture shows 4 different signals, light blue for Lung fibrosis, red for Mast cells and Neutrophils, pink for Myeloid cells and dark blue for the Nuclei.

2

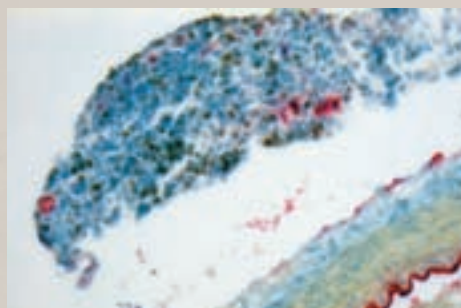


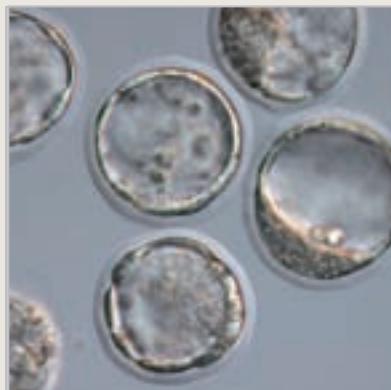
Figure 2: Double Staining with Ki67 and von Willebrand Factor. Ki67 turns out to be brown whereas vWF shows a red signal. The counter staining was done with Hematoxylin (blue).



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 attendants - provides husbandry of animals and services for the various research groups.*

### Husbandry:

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

### Animal House Services:

Veterinary services, such as monitoring of the facility's health-status (sentinel-program etc.), experimental procedures in animals such as collection of blood, implantation of tumor cells and administration of substances. All procedures are performed to a high standard under appropriate anaesthetic regimes and in conjunction with the necessary project licenses. Animal procurement, such as ordering of mice from external breeding companies, organizing and handling of incoming and outgoing mouse-shipments per year.

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

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

The main duties of this service unit are the injection of ES cells into blastocysts (also tetraploid and 8-cell) and of DNA into the pronucleus of fertilized mouse eggs. This service also provides for the transfer of 'clean' embryos into our Animal House, the freezing of embryos for the preservation of specified mouse strains and the teaching of basic embryological techniques to the IMP and IMBA staff.

Many different ES cell clones and DNA/BAC constructs are being injected per year. The activities of this department are overseen by an Animal User Committee, which meets bimonthly to set priorities and to coordinate the duties. At present, it is chaired by Erwin F. Wagner.

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

Figure 2: Mouse blastocysts.



## SERVICE DEPARTMENT

gotthold.schaffner@imp.ac.at

Gotthold Schaffner / Scientist

Ivan Botto / Technician

Markus Hohl / Technician

Shahryar Taghybeeglu / Technician

Gabriele Botto / Technician Media Kitchen

Christa Detz-Jaderny / Technician Media Kitchen

Ulrike Windholz / Technician Media Kitchen

Sabine Jungwirth / Technician Fly Food Preparation

Franziska Stransky / Technician Fly Food Preparation

Anna Windholz / Technician Fly Food Preparation

Oliver Botto / Help Fly Food Preparation

Thomas Haydn / Help Fly Food Preparation

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

Our Media Kitchen and Fly Food staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies (more than 1,200,000 bottles and tubes per year) and worms. The Fly Food staff will soon move back to the IMP building where we have more space to create better and more convenient working conditions for preparing fly food.

We also prepare many selected reagents such as DNA molecular weight markers, enzymes, a variety of transformation-competent *E.coli* strains and we maintain a stock of cloning vectors, sequencing primers and other cloning reagents.

### Production of antibodies

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

### Sequencing and DNA isolation

The 16 capillary ABI 3100 Genetic Analyzer is rarely used at the moment. The 48 capillary ABI 3730 DNA Analyzer is our workhorse. We sequenced approximately 45,000 samples in the first 10 months of this year. This increased demand due to a number of new customers, but also due to many screening projects and to new groups at the IMBA as well as at the IMP. We primarily use the 3730 DNA Analyzer because of its sensitivity and lower running costs. The average read-length is 700-900 bases for standard DNA samples, with both Genetic Analyzers equipped either with 80 cm capillaries of ABI 3100 or 50 cm capillaries of ABI 3730.

DNA sample quality even from sophisticated Kits like Qiagen Midi- or Maxipreps is still a problem, as are wrong primersets and not sufficiently documented plasmid constructs from outside sources. The clean-up protocol with Sephadex G50 superfine columns on 96-well microtiter plate format with optimized sephadex consistency and centrifugation conditions is still working fine and does not produce "dye blobs" with good quality DNA samples.

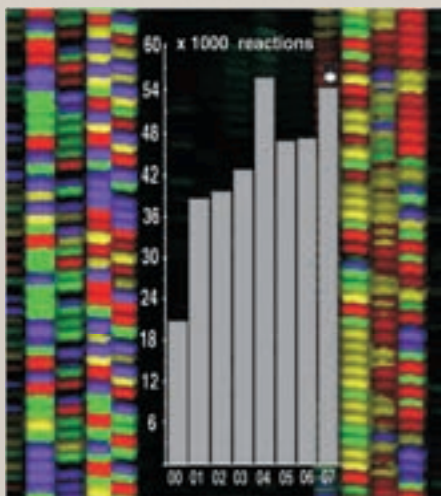


Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (- 2001), on ABI 3100 (since 2001) and on ABI 3730 (since June, 2004) done with dye deoxy terminators (v3.0 since 2001) in the years 2000 to 2007 (scale 0 to 60,000).  
\*calculated from January 2006 to October 2007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

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



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## MOUSE SERVICE

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## Awards

### Agnes Csiszar

Elizabeth Hay Poster Prize Award founded by TEMTIA (The EMT International Association) (September 2007)

### Thomas Jenuwein

Erwin Schrödinger Prize of the Austrian Academy of Sciences (October 2007)

### Amina Kurtovic

VBC PhD Award (November 2007)

### Jan-Michael Peters

Binder Innovation Prize by the German Society for Cell Biology (March 2007)

The Malvin & Eleanor Mayer Lecture in the Life Sciences hosted by the Massachusetts Institute of Technology (November 2007)

## Seminar Speakers

### JANUARY

08. January Thomas Sternsdorf  
*Salk Institute San Diego*  
Artificial oncogenes for understanding leukemogenesis in Acute Promyelocytic Leukaemia (APL)
11. January Richard A. Rachubinski  
*Department of Cell Biology, University of Alberta, Edmonton*  
Global Analysis of Kinase and Phosphatase Action in Peroxisome Biogenesis in *Saccharomyces cerevisiae*
12. January Georg Schett  
*Dept. of Internal Medicine, University of Erlangen*  
Wnt signaling in murine and human joint disease
18. January Julian Downward  
*Signal Transduction Laboratory, ICRF, London*  
Use of functional genomics to study Ras signaling networks in oncogenesis
24. January Wolfgang Zachariae  
*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden*  
How sisters stay together in meiosis
29. January Donal O'Carroll  
*Lymphocyte Signaling, Rockefeller University, New York*  
Genetic insights into the mechanism of microRNA mediated gene silencing in mice

### FEBRUARY 2007

01. February Philipp Khaitovich  
*CAS-MPG Partner Institute for Computational Biology, Shanghai*  
Gene Expression in Human Evolution
02. February Conrad Bleul  
*Max-Planck-Institute for Immunobiology, Freiburg*  
Multipotent hematopoietic precursors in the mouse thymus
07. February Yusuke Nakamura  
*Human Genome Center, Institute of Medical Science, University of Tokyo*  
From cancer genomics to cancer therapy
13. February Walter Lerchner  
*Division of Biology, California Institute of Technology*  
Neurons Turning Silent - Mice Turning Quietly
15. February Christine Mézard  
*Institut Jean-Pierre Bourgin, Institut National de la Recherche Agronomique (INRA), Versailles*  
Meiotic recombination in *Arabidopsis thaliana*: sex and interference
16. February Andrea Musacchio  
*European Institute of Oncology, Milan*  
Kinetochore-microtubule attachment and the spindle assembly checkpoint

22. February Susan Gasser  
*Friedrich Miescher Institute for Biomedical Research*  
A novel function for Orc in cohesion

28. February Steve Blacklow  
*Department of Pathology, Harvard Medical School*  
Structural and biochemical insights into Notch restraint and activation

### MARCH 2007

02. March Wouter de Laat  
*Dept. of Cell Biology and Genetics, Erasmus MC, Rotterdam*  
Chromatin architecture uncovered by 4C technology
08. March Edith Heard  
*Mammalian Developmental Epigenetics Group, Curie Institute, Paris*  
The nuclear dynamics and epigenetic plasticity of X-chromosome inactivation
14. March Jean-Christophe Marine  
*University of Ghent (DMBR)*  
Studying the p53 tumor suppressor pathway using the mouse as a model
15. March Yves Barral  
*Institute for Biochemistry, ETH-Hoenggerberg, Zurich*  
Temporal coordination of late mitotic events
22. March Tada Taniguchi  
*Medical University Tokyo*  
Nucleic acid sensing and activation of IRFs in the immune system
22. March Allan Mowat  
*Division of Immunology, Infection and Inflammation, Glasgow Biomedical Research Centre*  
Regulation of intestinal immunity and tolerance by dendritic cells

### APRIL 2007

02. April Elizabeth Winzeler  
*The Scripps Research Institute, La Jolla, California*  
Systems biology of malaria
04. April Gian Paolo Dotto  
*University of Lausanne*  
Notch1 is a p53 target gene involved in human keratinocyte tumor suppression
04. April Stefan Hoppler  
*Institute of Medical Sciences, Foresterhill, University of Aberdeen*  
Wnt signalling in *Xenopus* development: mechanisms of tissue-specific Wnt signalling and Wnt6 function in heart organogenesis
12. April Brian Johnstone  
*Oregon Health and Science University, Portland*  
Skeletal tissue regeneration with mesenchymal stem cells
18. April Thomas Mueller-Reichert  
*Max Planck Institute for Molecular Cell Biology & Genetics, Dresden*  
Spindle organization in three dimensions
20. April Philipp Selenko  
*Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology*  
High-resolution in-cell NMR spectroscopy

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

26. April Pernille Rørth  
*Cell Biology and Biophysics Unit, EMBL, Heidelberg*  
Guiding migration: one cell cluster, multiple signaling mechanisms

## MAY 2007

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

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

09. May Ka Wan Li  
*Research Institute of Neurosciences, Amsterdam*  
Quantitative proteomics analysis of brain synapses

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

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

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

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

24. May Abby Dernburg  
*Lawrence Berkeley National Labs, Berkeley, California*  
Chromosome choreography during meiosis: It takes two to tango

25. May David Tuveson  
*CRUK Cambridge Institute*  
Modeling Malignancy in Mice

31. May Bas Van Steensel  
*Netherlands Cancer Institute, Amsterdam*  
Chromatin genomics in flies and humans

## JUNE 2007

05. June Peter L. Pfeffer  
*Agresearch Ruakura, Hamilton, New Zealand*  
Elf5 in placental development and embryonic patterning

06. June Martin W. Hetzer  
*The Salk Institute, La Jolla*  
Biogenesis of the cell nucleus

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

13. June Karin Loser  
*Westfälische Wilhelms University, Münster*  
Effects of regulatory T cells on cutaneous immune responses

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

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

21. June Wendell A. Lim  
*UCSF*  
The Modular Logic of Cell Signaling Systems

27. June Kim Nasmyth  
*University of Oxford Department of Biochemistry*  
Does the cohesin ring really trap sister DNAs?

28. June Chi-Chung Hui  
*Hospital for Sick Children, Toronto*  
Conserved and divergent features of Hedgehog signal transduction

29. June Roger Kornberg  
*Stanford University Medical School*  
The Molecular basis of eucaryotic transcription

29. June Richard Roberts  
*New England Biolabs, Ipswich, USA*  
New ways to find restriction enzymes using bioinformatics

## JULY 2007

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

10. July Stefan Strack  
*University of Iowa, Carver College of Medicine*  
Kinases and phosphatases in mitochondrial morphogenesis and neuronal death

11. July Peter Murray  
*St. Jude Children's Research Hospital, Memphis*  
Signal transduction from the IL-10 receptor to the anti-inflammatory response

12. July John Mattick  
*Institute of Molecular Biosciences, University of Queensland*  
The human genome as an RNA machine

17. July Rainer Pepperkok  
*EMBL Heidelberg, Advanced Light Microscopy Core Facility*  
Illuminating the secretory pathway

19. July Bernd Bukau  
*ZMBH, Heidelberg*  
Mechanisms of molecular chaperones



## AUGUST 2007

24. August Marius Wernig  
*Whitehead Institute for Biomedical Research, Cambridge*  
Induction of pluripotency in mouse fibroblasts

## SEPTEMBER 2007

13. September Narry V. Kim  
*Institute of Molecular Biology and Genetics, Seoul National University*  
MicroRNA biogenesis
18. September Jeremy Green  
*Department of Craniofacial Development, Kings College London*  
PAR polarity, Wnt signalling and neurogenesis in *Xenopus* development
20. September Christopher E. Rudd  
*Head of the Cell Signalling Section, Department of Pathology, Cambridge University*  
Co-receptors, adaptors and T-cell adhesion
20. September Peter Becker  
*Adolf-Butenandt-Institute, Munich*  
Dosage compensation in *Drosophila*: fine tuning transcription through chromatin structure
21. September Kristin Scott  
*Molecular and Cell Biology Department, University of California, Berkeley*  
Taste recognition in *Drosophila*
27. September So Iwata  
*Imperial College London*  
Towards structural determination of membrane proteins

## OCTOBER 2007

08. October Alexander Fleischmann  
*Columbia University, New York*  
Neuronal Diversity and Odor Perception in Mice
18. October Rodney Phillips  
*Nuffield Department of Clinical Medicine, Oxford*  
HIV Evolution
25. October Daniel Huber  
*HHMI, Janelia Farm Research Center, Virginia*  
Probing cortical circuits with optical microstimulation

## NOVEMBER 2007

05. November Judy Lieberman  
*CBR Institute for Biomedical Research, Boston*  
let-7 regulates self-renewal and tumorigenicity of breast cancer stem cells
06. November Oliver Muehlemann  
*Institute of Cell Biology, University of Berne*  
Quality control of gene expression: mechanisms to recognize and eliminate expression of nonsense mRNA
19. November Maria Leptin  
*Institut für Genetik, Universität zu Köln*  
A gene hierarchy from morphogen to morphogenesis: functions of conserved and fast evolving genes in the *Drosophila* embryo
29. November Craig S. Pikaard  
*Biology Department, Washington University*  
Roles of the Arabidopsis RNA Polymerase IV-dependent siRNA pathway in chromatin organization and repetitive gene silencing
30. November Irmgard Irminger  
*University Hospitals Geneva*  
BARD1 isoforms: the Trojan horse in gynecological cancers

## DECEMBER 2007

06. December Michael Dickinson  
*California Institute of Technology, Pasadena*  
How Flies Fly
07. December Niels Galjart  
*Department of Cell Biology and Genetics, Erasmus MC*  
Functional analysis of mouse CTCF



## Spotlight on 2007

### IMP-IMBA Mini Recess

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

### Dragon Boat Cup

*Venimus Vidimus Vicimus...from Vienna to Velden*

For the first time ever, a team of 25 people from the IMP took part in the Dragon Boat Cup 2007, rowing full speed ahead as they found their rhythm and raced over a distance of 250 meters against time and other boats. The early round in Vienna took place on June 1. The IMP "IMPerfectos" reached the final and won this day's race, competing against 24 other teams. The prize was a ticket to the all-Austrian final in Velden am Wörthersee, Carinthia from July, 6-8. Supported by a great number of loyal fans, the IMPerfectos, who changed into intimidating black racing outfits, raced against the 10 best dragon boat teams from all over Austria. At the end of the day, the IMP team came third out of 110 participating teams in the Dragon Boat Cup 2007. Congratulations IMPerfectos!

### Joint Retreat of Viennese and German PhD Students

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

### Vienna Biocenter PhD Retreat

One of the most important events in the PhD calendar is the annual VBC PhD Retreat. Now in its second year, many students from the program took part in this year's retreat in Litschau, Lower Austria. The PhD Retreat provides a great opportunity

for students from different institutes and at different stages of their PhDs to get to know each other and their science, away from the lab.

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

### EDRC 2007 - 20th European Drosophila Research Conference Vienna

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

### IMP-IMBA Recess

From October 3-5, IMP scientists met with members of the Scientific Advisory Board (SAB) to present their work and discuss their research. The SAB members, consisting of internationally recognized scientists, were once more impressed by the scientific performance and high standards of the research presented. IMP SAB members: page 53 in this booklet

### Symposium "Molecules to Mind"

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



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## Project Management

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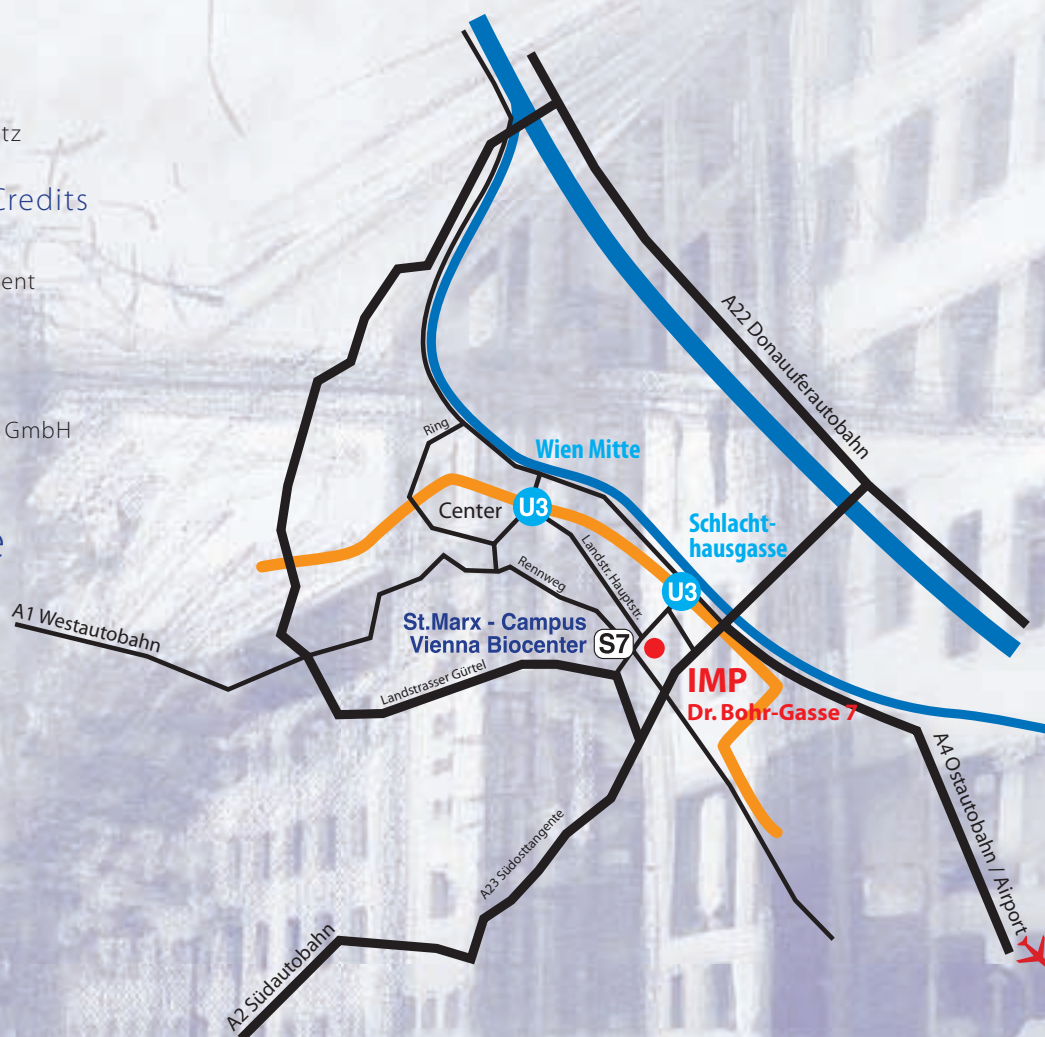
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## Where We Are



## 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

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