
IMP
Research Institute of Molecular Pathology
VIENNA BIOCENTER
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Boehringer
Ingelheim

Contents

This year *year has heralded many changes for the IMP. The new building for our sister institute IMBA is on the verge of completion and this time next year a lot of people currently working within the IMP will be working next door. These include not only many scientists from IMBA, who have been very welcome guests at the IMP for the past two years, but also all IMP personnel currently engaged with glassware, media preparation, public relations, DNA sequencing, mass spectrometry, and animal husbandry. This will free up large swathes of space for existing group leaders to expand modestly and for new group leaders. The IMBA building looks like a jewel and appears to contain unlimited space but I am sure that within five years it, too, will be bursting at the seams. Another key architectural development that should be completed next spring is a major expansion of our cafeteria, which will be situated between the IMP and IMBA and should provide enough space to accommodate all visitors from the University and the rest of the Campus Vienna Biocenter, as well all IMP and IMBA employees. The cafeteria staff have worked miracles feeding us with their existing facilities and they have justly deserved their new kitchens.*

Another key change is that Barry Dickson takes over from me as director of the IMP at the end of this year. Barry moved to Vienna as a young group leader seven years ago and moved to IMBA as a senior scientist two years ago. The IMP is very fortunate to have found such a talented scientist who is prepared to take on the extra responsibilities and we are all very grateful to the search committee under the direction of Piet Borst for having searched the world and found the ideal candidate eventually on our doorstep. I have been working at the IMP for eighteen years now, ten as a group leader and eight as director. This period has been both fascinating and rewarding. I have been very fortunate to have had wonderful support from a very able administration, first under Nikolaus Zacherl and, more recently, under Harald Isemann, as well as from my scientific colleagues without whom it would not have been possible to have continued to lead an active research group while being director. I was also very fortunate to inherit from Max Birnstiel, my predecessor, an institute that was already running very smoothly.

The IMP has grown enormously in many ways during the past eight years. Our budget has increased by about 50% in real terms and most of this increase has come from external grants. This has been due to the collective efforts of all my colleagues and I would like to congratulate them. The head count has meanwhile risen by over 40 %. Crucially, the services that make the IMP such a convenient and effective place to work in have greatly expanded. Biooptics under Peter Steinlein has gone from strength to strength, DNA sequencing under Gotthold Schaffner now produces sequences over the weekend, and mass spectrometry under Karl Mechtler has grown from nothing to a major operation. The mice under Andreas Bichl have multiplied so fast that we needed a new mouse house in the basement of the IMBA building. Erwin Wagner pioneered mouse genetics at the IMP and this has had enormous repercussions. IMBA's arrival has enabled us to set up new services like Histology. One other area that seems to have gone from strength to strength has been our PhD program under Meinrad Busslinger's very able management.

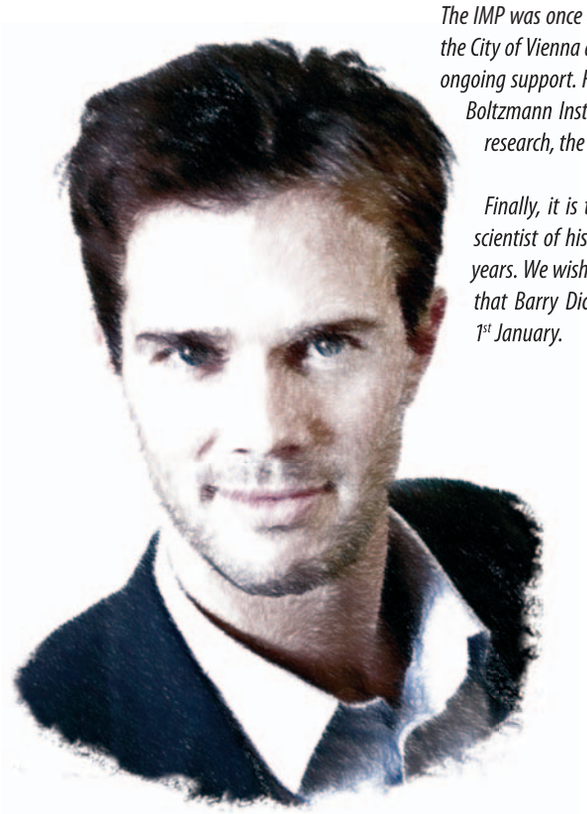
These years have seen many wonderful discoveries that have been recognized by no fewer than four Wittgenstein prizes, which is a remarkable achievement. Barry has already instigated a major new initiative, namely a program to offer independent positions to fellows directly after they have completed their PhDs. Very exciting times lie ahead. There is lots of new space in the IMP, IMBA is about to take off, and both institutes seem to be attracting some of the best candidates on the market as young group leaders. I shall miss the IMP but look forward to coming back and seeing how it develops further in the future.

Kim Nasmyth
December 2005

This has been *a rather turbulent year for the IMP. The start of the construction of our new cafeteria and the new seminar floor, together with a delay in the completion of the new IMBA building, have caused some inconvenience to all of us. Our technical service staff is doing an exceptional job by maintaining all operations at the IMP and setting up the new facilities at the IMBA building at the same time. With the relocation of the mouse house and several service units to the IMBA building we will gain a lot of urgently needed new lab and office space at the IMP. The new cafeteria will open its doors in April and will then serve as the central meeting place for the whole Campus Vienna Biocenter. We know that this market place for information, and food, is of tremendous importance for our scientific development. We have also reached an agreement with the City of Vienna to convert the current IMP parking area into a pleasant, green space which will significantly improve the appearance of the Campus.*

Cooperation with IMBA is developing very well and the increased and combined resources gave us the opportunity to set up two new scientific service facilities: Histology and Electron Microscopy. In August, we appointed Thomas Marlovits – who comes from Yale - as the first joint IMP – IMBA Group Leader.

Moreover, the IMP is in the process of recruiting two new Group Leaders who will start at the institute in the middle of next year. Together with the new fellows, the IMP will then be back to its full group capacity. The IMP was once again very successful in acquiring various research grants and we are very grateful to the City of Vienna and the federal funding organizations as well as to the European Commission for their ongoing support. For the first time we are also participating as a partner in two newly founded Ludwig Boltzmann Institutes which were selected through a very competitive procedure - one is for cancer research, the other for fly genomics.



Finally, it is time to bid farewell to Kim Nasmyth. The IMP has been extremely fortunate that a scientist of his quality and vision has shaped the scientific performance of the institute over many years. We wish him all the best for his new challenges in Oxford. At the same time, we are very proud that Barry Dickson, another fantastic scientist, will take over as scientific director of the IMP on 1st January.

Harald Isemann
December 2005

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 (University and Medical University of Vienna), the Institute of Molecular Biotechnology IMBA and the Gregor Mendel Institute GMI (Austrian Academy of Sciences), a University of Applied Sciences, eight biotech companies, a PR agency and a non-profit scientific society.

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

Vienna – a City of Many Facets

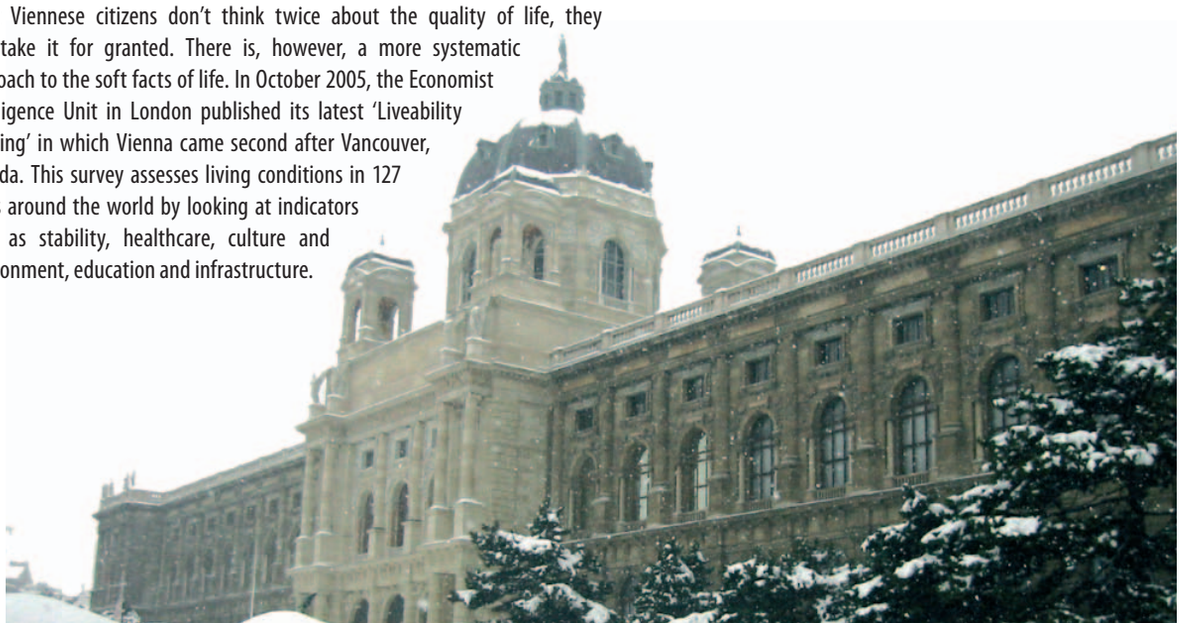
For those whose interests stretch beyond science, Vienna also has a lot to offer. Home to about 1.5 million people, the city is the political and cultural center of the country and its gateway to the east. Once the heart of the largest European empire, Vienna draws on a rich cultural heritage which is reflected in splendid buildings and unique art collections. But Vienna is a city of many facets. Modern architecture, splendid galleries and stylish shops are as much a part of everyday life as the famous concert halls, the big museums and the nostalgic little streets. As any European capital, Vienna also offers a vibrant nightlife, with a rich selection of restaurants, cozy bars, and trendy clubs.

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

And if your brain needs to be refreshed, there is always the call of nature. A day away from the lab may take you skiing to the nearby Alps, windsurfing at Lake Neusiedl or watching rare birds in the Seewinkel. Even within the borders of Vienna, you can enjoy a hike through dense woods, go canoeing in a National Park, climb impressive limestone cliffs or stroll through rolling vineyards. In fact, more than 50% of Vienna's surface area is covered by vegetation.

Quality of Life

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



Your Career at the IMP

The IMP offers exciting positions at all levels of your research training and career. If you consider joining the IMP, you will find first class research and state-of-the-art scientific services. As a member of one of the scientific groups, you will be part of a young, international team, using English as a working language. The unique "flavor" of the IMP lies in its stimulating and focused atmosphere, where science always comes first but social activities are not neglected. Informal inter-action is strongly encouraged as a vital prerequisite for intellectual exchange. Not least, our famous cafeteria offers well-deserved breaks away from the lab.

Graduate students join the IMP through the Vienna Biocenter International PhD Program, run jointly with the University, the Medical University, IMBA and GMI. A call for applications goes out twice a year, with contracts typically lasting 3-4 years. At present, about 50 students are working towards their PhD at our institute.

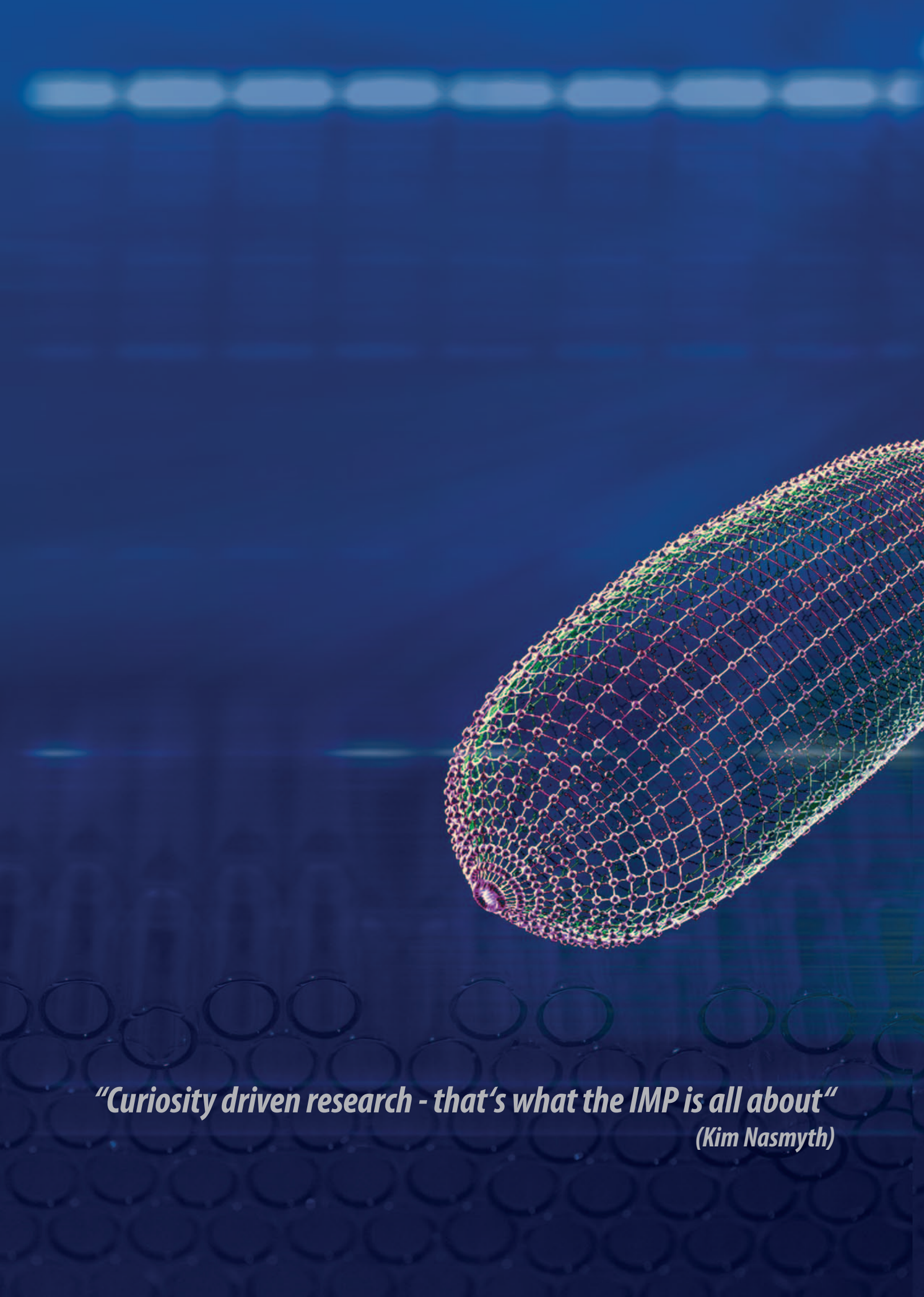
A new kind of position has been created this year: IMP fellowships offer young scientists the unique possibility of conducting creative and independent research at a very early stage in their career. IMP fellows join the IMP within a year of obtaining their PhD degree, and are given a four-year contract. They are granted their own budget and travel allowance and can take full advantage of the scientific infrastructure of the institute. A mentoring committee monitors progress and ensures independence. IMP fellows will be considered for promotion to a group leader position within the first three years.

The IMP research groups are well funded to support a number of pre- and postdoctoral positions. Apart from in-house fellowships, IMP scientists are very successful in securing external funding. A substantial travel budget allows scientists to take part in meetings, conferences and courses. The IMP organizes a large-scale international conference every other year and smaller workshops in between. Students are successfully organizing their own symposia, backed by a generous budget from the institute. An intensive seminar program brings internationally renowned scientists to the IMP at least once a week.

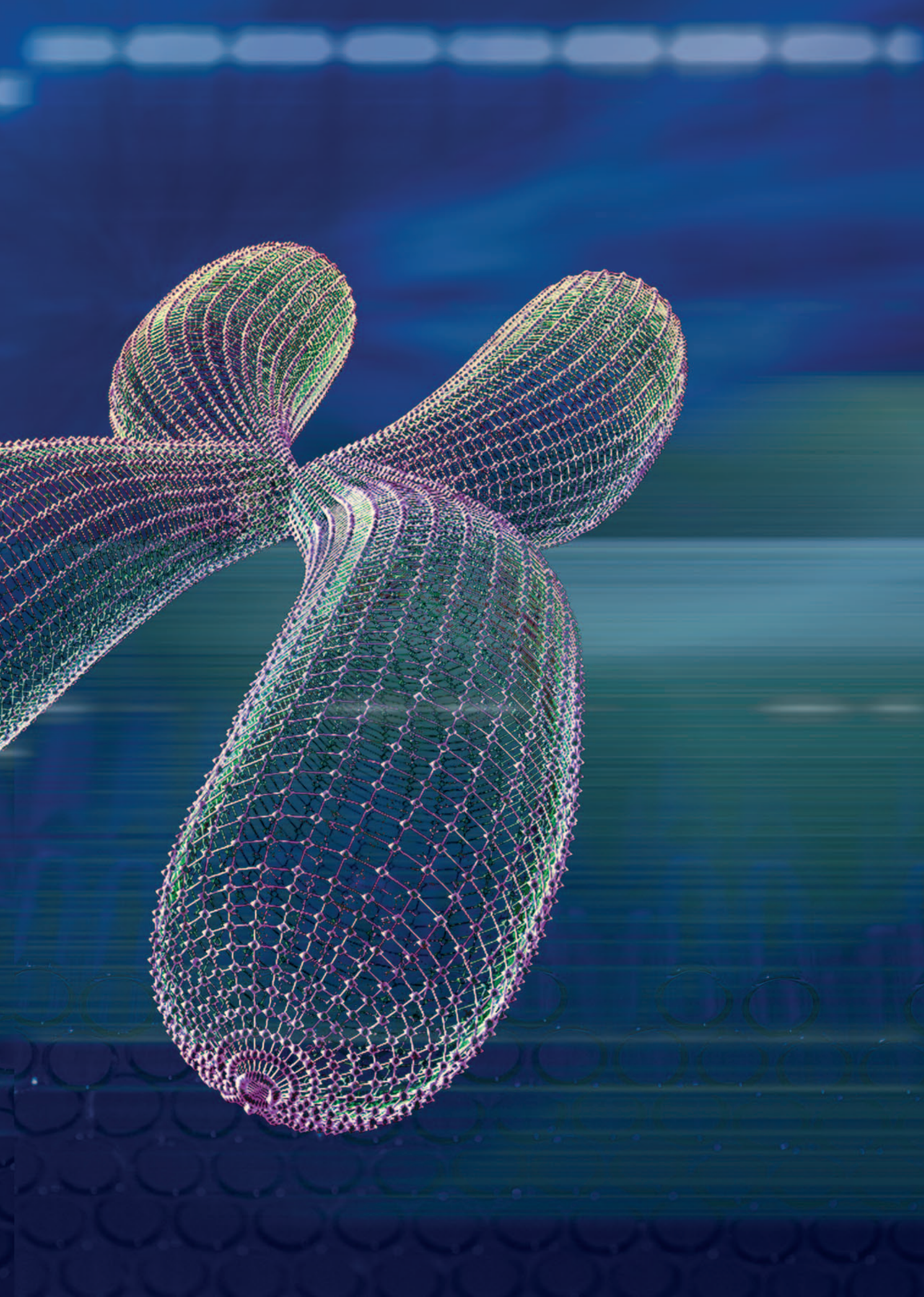
If you come to work at the IMP, you'll obviously come in the first place for the science. We do, however, appreciate your private needs and try to make relocation as smooth as possible. For newcomers, there are several in-house apartments to bridge the time until they have found a place of their own. Our administrative staff is helpful in finding housing and our personnel department will take care of your legal requirements including visas, registration, health insurance and family matters. For parents with young children, two day-care facilities are available in the immediate neighborhood: a municipal kindergarten offers admission from baby-age, and a privately run nursery is also just around the corner. For school-age children, Vienna offers a large range of different types of schooling, from public to private, German- or foreign language-speaking, traditional or with more experimental concepts.

Many of our new employees are accompanied by spouses who are themselves looking for a qualified position in line with their training. The IMP is certainly aware of this fact and can, in some cases, help with securing a job. We also support your efforts to learn German and sponsor language courses run by one of Vienna's best language schools. In addition to caring for the intellect, the IMP features a soccer club and subsidizes regular physical activities for its members.

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



"Curiosity driven research - that's what the IMP is all about"
(Kim Nasmyth)



Oncogenesis: Abnormal Developmental Plasticity?

In leukemia and carcinoma development, the cooperation of oncogenic receptors/signal transducers with signal-activated, sometimes mutated transcriptional regulators causes abnormal proliferation, survival and developmental plasticity. Genetically modified mice and novel cell culture models derived thereof are combined to investigate the mechanisms as how such oncogene combinations regulate self-renewal versus differentiation of hematopoietic progenitors and epithelial plasticity/trans-differentiation of carcinoma cells during metastasis.

Mechanisms of Hematopoietic Progenitor Renewal and Leukemogenesis

Primary erythroid progenitors undergo self renewal *in vitro* through cooperation of the EpoR, c-Kit and glucocorticoid receptor (GR), reflecting a physiological response to stress erythropoiesis. An optimal cell model to analyze progenitor renewal are erythroblasts from murine ES-cell-derived embryoid bodies (ESEPs). These cells show *in vivo*-like responses to renewal and differentiation factors, are diploid and genetically stable, have an unlimited lifespan in culture and allow analysis of erythropoiesis in early embryonic lethal mouse mutants (e.g. Flk-1-/- mice). Flk-1-/- ESEPs fail to undergo long-term erythroblast renewal due to enhanced differentiation, a defect rescued by FGF-1. Clonal multipotent progenitors can likewise be expanded from ES-cells, particularly if expressing exogenous HoxB4 (Figure 1A, B). In mice, such cells cause a myeloproliferative disease (Figure 1C). In these models, we will analyze the function of STAT-1, -3, -5, the GR and cytokine-driven upstream signaling pathways (e.g. Jak2),

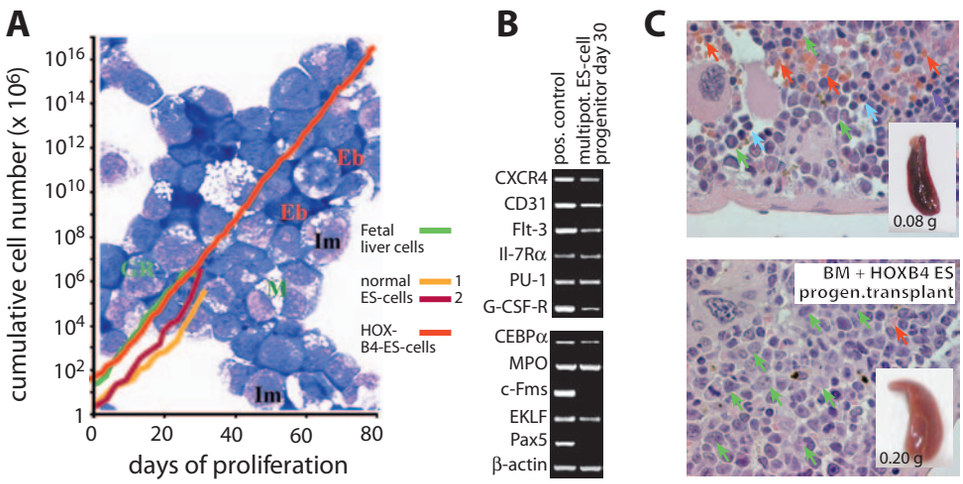


Figure 1: HOXB4 promotes long-term renewal of ES-cell-derived multipotent progenitors. (A). Multipotent progenitors from ES-cell-derived embryoid bodies (day 8) expressing HOX-B4 (red) show long-term expansion in multipotent medium mix (SCM), while fetal liver cells (green) and uninfected ES-derived cells (yellow, brown) show medium-term expansion. Cytospins show immature cells (Im), erythroid (Eb) and various myeloid progenitors (M, GR). (B). HOX-B4 multipotent progenitor clones express markers of erythroid, myeloid and lymphoid lineages (RT-PCR). (C). Lethally irradiated NOD/SCID mice transplanted with HOX-B4-multipotent ES-derived cells plus wt bone marrow develop a myeloproliferative disease in the spleen, showing excessive production of myeloid cells (green arrows) at the expense of erythroid and lymphoid progenitors (red and blue arrows).

Hartmut Beug

Hartmut Beug / Senior Scientist

Agnes Csiszar / Postdoc
Annamaria Gaal / Postdoc
Sabine Maschler / Postdoc
Christoph Gebeshuber[1] / PhD Student
Boris Kovacic / PhD Student
Andreas Mairhofer / PhD Student
Memetcan Alacakaptan / Diploma Student
Jasmin Speil / Diploma Student

Eva Maria Deiner[2] / Technician
Andrea Deutsch[2] / Technician
Gabi Litos[2] / Technician

[1] *with BI*
[2] *part-time*

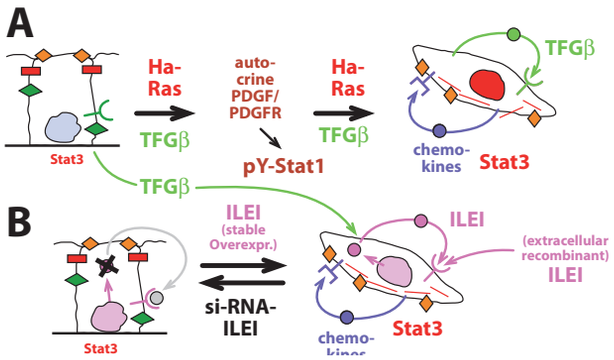


Figure 2: Diagram illustrating molecular events during Ras/TGFβ- and ILEI-dependent EMT. (A). Expression of oncogenic Ha-Ras plus exposure to TGFβ induces EMT in EpH4 cells, involving upregulation of an autocrine PDGF/PDGF-receptor loop, as well as induction of chemokine/ receptor autocrine loops that contribute to EMT as shown by receptor antagonists. These autocrinely acting chemokines may be responsible for upregulation of STAT3 during EMT, the latter beeing stabilized by a TGFβ-autocrine loop. (B). Stable expression of exogenous ILEI, addition of recombinant ILEI or TGFβ-induced upregulation of endogenous ILEI cause EMT in EpH4 cells and derivatives. Stable ILEI expression also induces autocrine chemokines important for EMT and STAT3 upregulation. All these processes are inhibited or reversed by RNAi-mediated knockdown of ILEI.

employing or newly generating respective, homozygously defective ES cells. These models will also serve to study human leukemia oncogenes, e.g. mutated receptors/signal transducers (Flt3/c-Kit/Jak2) cooperating with transcription factors/chromatin regulators (ca-STAT5, RAR/PML, AML-ETO). Polysome-bound mRNA expression profiling of these models will help to identify new key genes regulating the balance between self-renewal and differentiation (with collaborators).

ILEI, a Novel Secreted Protein Essential for Epithelial Plasticity and Metastasis

Epithelial/mesenchymal transition (EMT) and metastasis of polarized mamary epithelial cells (EpH4) – caused by oncogenic Ras (EpRas) plus TGFβ – mirrors late stage cancer progression and requires a hyper-active Ras-MapK pathway. Polysome-bound mRNA expression profiling identified ≈30 EMT-specific genes, many of which contribute to EMT and metastasis (e.g. ΔEF-1, annexin-A1, NFκB and PDGF-receptor signaling; Figure 2A). The most exiting EMT-specific gene identified was the interleukin-like EMT inducer (ILEI), a translationally regulated, secreted protein lacking sequence homology to known genes. Stably over-expressed ILEI was sufficient to induce EMT, slow tumor growth and aspects of metastasis in EpH4- and NMuMG cell lines, enhanced by co-expression of Bcl-2. Bioactive, recombinant ILEI purified from transiently transfected mammalian cells also induced EMT. Endogenous ILEI was essential for both EMT and metastasis, as shown by RNAi (Figure 2B). Besides generating cell lines stably producing correctly processed, bioactive ILEI, we will focus on chemokines induced by stably expressed ILEI. These chemokines play a role in Ras/TGFβ- and ILEI-dependent EMT and induce upregulation of nonphosphorylated STAT3 (Figure 2B), apparently inducing cancer-related genes via unknown mechanisms which we want to investigate. ILEI expression is restricted to apical, Golgi-like vesicles in normal epithelia, but shows delocalized cytoplasmic expression in human tumors, correlated with EMT at invasion fronts (Figure 3A, B), metastasis and bad prognosis in human breast cancer (Figure 3B, C). We will thus investigate whether abnormal vesicle transport/secretion of ILEI is essential for human tumor progression. This may involve Annexin-A1, which causes EMT and metastasis when knocked down via RNAi in normal EpH4 cells.

Contact: beug@imp.univie.ac.at

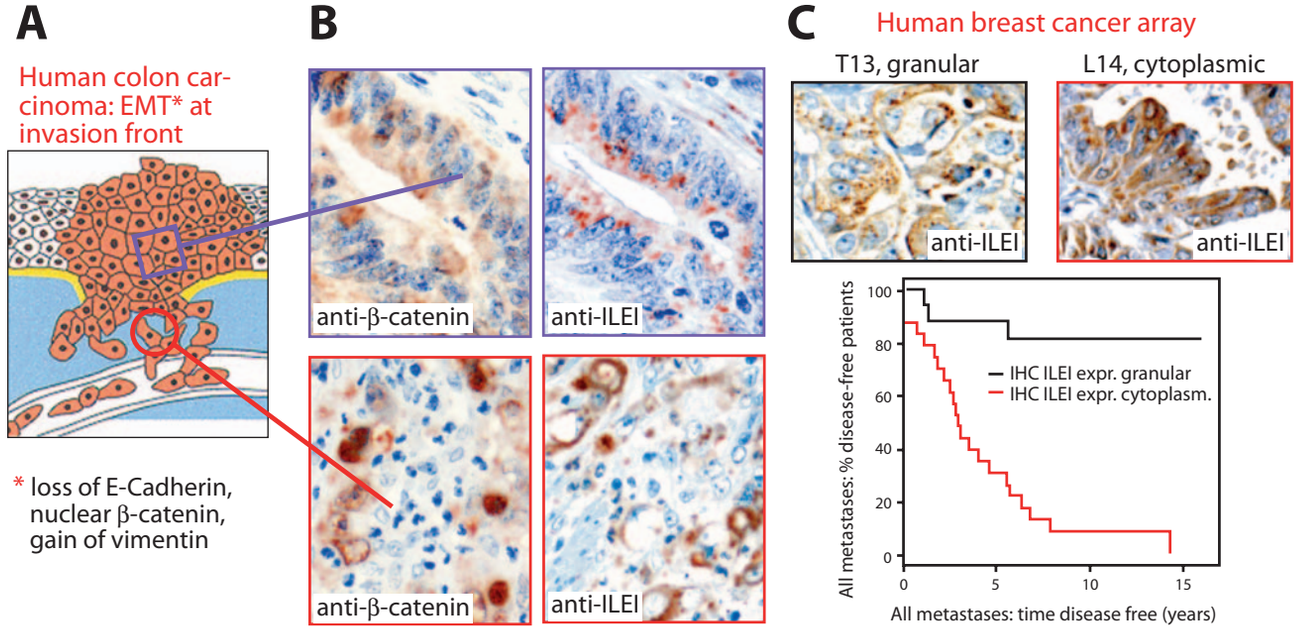


Figure 3: Delocalization of vesicular LEI correlates with EMT and metastasis in human carcinomas. (A, B). ILEI is expressed in apical vesicles in colon carcinoma primary tumor cells (A, blue square) that express cytoplasmic β-catenin (B) and membrane-localized E cadherin. Tumor cells at the invasion front (A, red circle) that have undergone EMT as shown by nuclear β-catenin (B), loss of E-cadherin and gain of vimentin show strong, cytoplasmic ILEI expression (B). (C). Correlation of vesicular (top left) and cytoplasmic ILEI staining (top right) in a large human breast cancer array with patient histories recorded over 15 years shows that cytoplasmic ILEI expression predicts high metastasis incidence (bottom panel) and bad prognosis (not shown).

Stem Cell Commitment in Hematopoiesis

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.

Meinrad Busslinger

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. Conditional gene inactivation revealed that *Pax5* expression is continuously required to maintain B-lineage commitment, as its loss converts committed B-lymphocytes into early hematopoietic progenitors with multilineage potential. Pax5 therefore controls the identity of B-lymphocytes throughout B cell development. We are now identifying the control regions and DNA-binding factors that determine the B cell-specific expression of *Pax5*.

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. To systematically analyze the transcriptional function of Pax5, we used cDNA microarray screening to identify a multitude of novel Pax5-regulated genes. One of the activated Pax5 target genes codes for the central adaptor protein BLNK, which couples signaling from the (pre)B cell receptor to transcriptional changes in the nucleus (Figure 2). A large part of the identified genes are, however, repressed by Pax5. These genes are normally expressed during erythroid, myeloid or T-lymphoid differentiation, demonstrating that the Pax5-deficient progenitors promiscuously express genes from different hematopoietic lineages. We are testing the hypothesis that this promiscuous gene expression is responsible for the developmental plasticity of early progenitors.

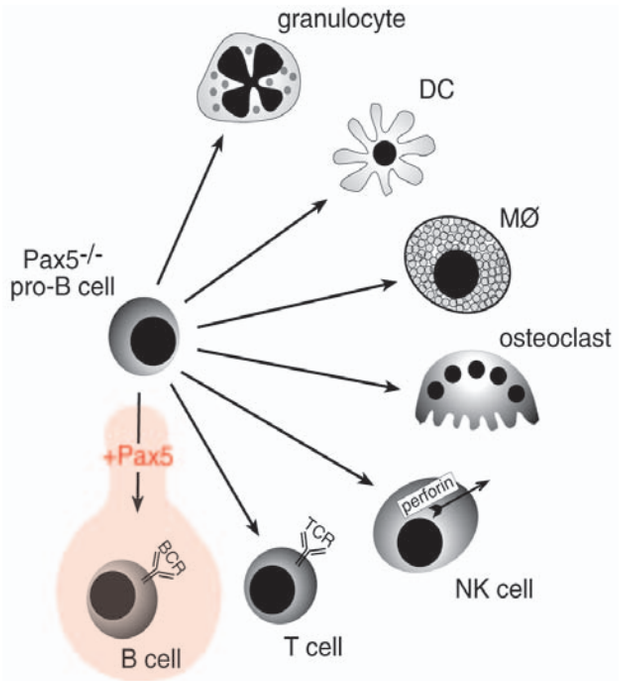


Figure 1: B-lineage commitment by Pax5. Pax5$^{-/-}$ pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.

Meinrad Busslinger / Senior Scientist

Cesar Cobaleda / Postdoc
Alessio Delogu[1] / Postdoc
Anja Ebert / Postdoc
Martin Fuxa[2] / Postdoc
Ramiro Gisler / Postdoc
Kamala Kesavan / Postdoc
Kyongrim Kwon / Postdoc
Stephen Malin / Postdoc
Thorsten Decker / PhD Student
Heike Dorninger / PhD Student
Shane McManus / PhD Student
Giorgia Salvagiotto / PhD Student
Alexandra Schebesta / PhD Student
Abdallah Souabni / Research Assistant
Sonja Höflinger[3,4] / Technician
Qiong Sun / Technician
Jasmin Steininger / Animal Technician

[1] until July 2005, [2] until August 2005, [3] since September 2005, [4] part-time

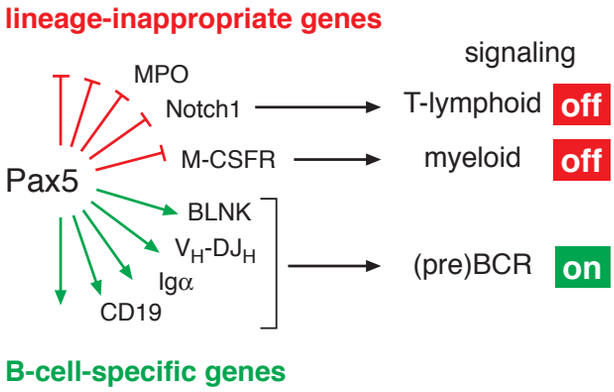


Figure 2: Dual role of Pax5 in B-lymphopoiesis. Pax5 activates B-lymphoid genes (green) and simultaneously represses lineage-inappropriate genes (red).

The immunoglobulin heavy-chain (*IgH*) locus contains ~200 V_H genes, which are spread over a 2.4-Mb region. The question therefore arises of how the distal V_H genes are able to pair with the proximal D_H segments over such a large distance to undergo V_H-DJ_H recombination during pro-B cell development. Recently, we demonstrated that Pax5 promotes the juxtaposition of distal V_H genes next to the proximal *IgH* domain. We refer to this phenomenon as locus contraction. In Pax5-deficient pro-B cells, the distal V_H genes are separated from the proximal *IgH* region by a large distance in the nucleus, whereas all *IgH* gene segments are colocalized in Pax5-expressing wild-type pro-B cells (Figure 3). The looping of individual subdomains is responsible for the contraction of the *IgH* locus, which facilitates the V_H-DJ_H recombination of distal V_H genes. We will next investigate the molecular mechanism by which Pax5 controls the contraction of the *IgH* locus.

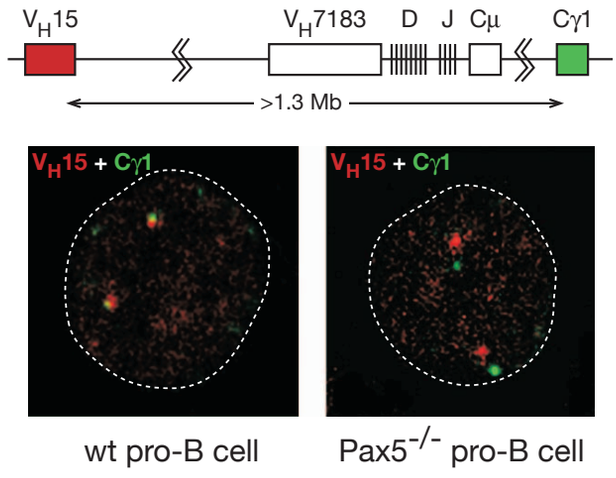


Figure 3: Pax5-dependent contraction of the IgH locus. A schematic diagram of the IgH locus (top) indicates the positions of the different gene segments and DNA probes used. Two-color DNA-FISH analysis (bottom) revealed the nuclear positions of the V_H15 and Cγ1 gene segments on a confocal plane containing the two IgH alleles of a wild-type or Pax5$^{-/-}$ pro-B cell. The contour of the nucleus is shown by a broken line.

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$^+$CD8$^+$ T cells (Figure 4). Notch-stimulated *Pax5*$^{-/-}$ progenitors rapidly downregulate 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. We are currently performing cDNA microarray experiments to better define the role of Notch1 in T cell commitment.

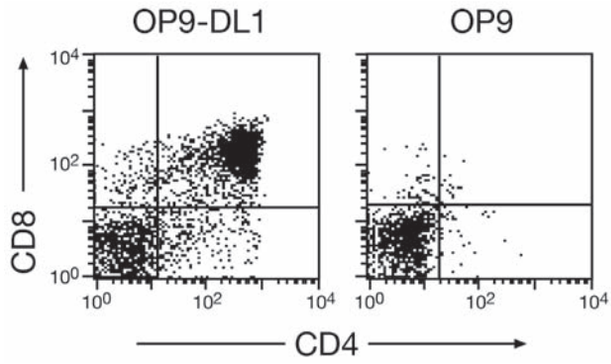


Figure 4: In vitro T cell differentiation of Pax5-deficient progenitors. A single Pax5-deficient pro-B cell is able to differentiate into a colony of CD4$^+$CD8$^+$ T cells within 18 days of co-culture on OP9 cells expressing the Notch ligand Delta-like-1 (OP9-DL1). On control OP9 cells, the Pax5-deficient cell fails to undergo T cell development.

Contact: busslinger@imp.univie.ac.at

Molecular Mechanisms of Protein Quality Control and Stress Response

The misfolding and aggregation of protein molecules is a major threat to all living organisms. Cells have therefore evolved a sophisticated network of molecular chaperones and proteases to prevent protein aggregation (Figure 1A), a process that is regulated by multiple stress response pathways. We perform a structure-function analysis of several of these factors in order to better understand how cells deal with folding stress.

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

A combination of chaperone and protease function in a single protein could provide a direct and rapid response to protein folding problems. The heat shock protein DegP (HtrA) can switch between these dual functionalities in a temperature-dependent manner (Figure 1B) and thus offers unique possibilities for investigating how cells distinguish between proteins that can be refolded and "hopeless" cases that need to be degraded. DegP consists of a protease and two PDZ domains, which are prominent protein-protein interaction motifs. It is a widely conserved protein found in most organisms. Prokaryotic DegP has been attributed to the tolerance against various folding stresses as well as to pathogenity. Human homologs are believed to be involved in arthritis, cell growth, unfolded protein response, and apoptosis.

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.

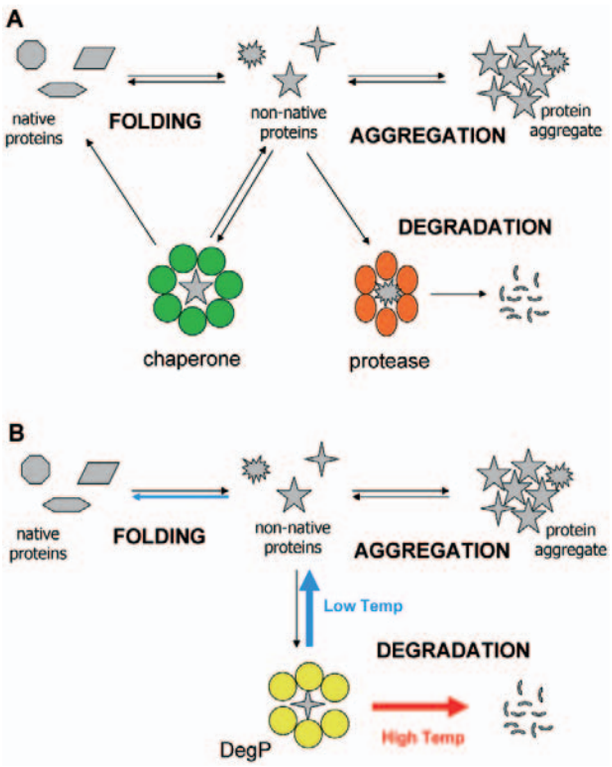


Figure 1: Protein quality control. (A). The diagram illustrates the different fates of misfolded non-native proteins. According to the "kinetic partitioning model" of Gottesman and coworker, aggregate formation is counteracted by the action of proteases and chaperones. (B). DegP combines the antagonistic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

Tim Clausen

Tim Clausen / Group Leader

Hanna Hasselblatt[1] / Postdoc
Rebecca Kirk[2] / Postdoc
Tobias Krojer / Postdoc
Boril Boyanov / PhD Student
Jacob Fuhrmann[2] / PhD Student
Justyna Sawa / PhD Student
Corinna Wilken / PhD Student
Cornelia Gänger / Diploma Student
Linn Gazda[3] / Diploma Student
Robert Kurzbauer / Technical

[1]part-time, [2] since December 2005, [3] since October 2005

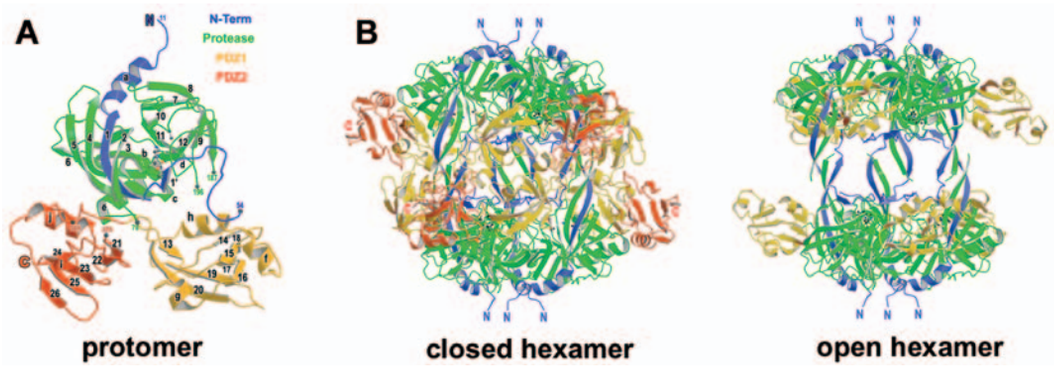


Figure 2: Structure of DegP. (A). Ribbon presentation of the protomer, in which the individual domains are colored differently. Residues of the catalytic triad are shown in a ball-and-stick model. The nomenclature of secondary structure elements and the termini of the protein and flexible regions are 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.

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.

Stress Response by Regulated Intramembrane Proteolysis

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

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

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

Contact: clausen@imp.univie.ac.at

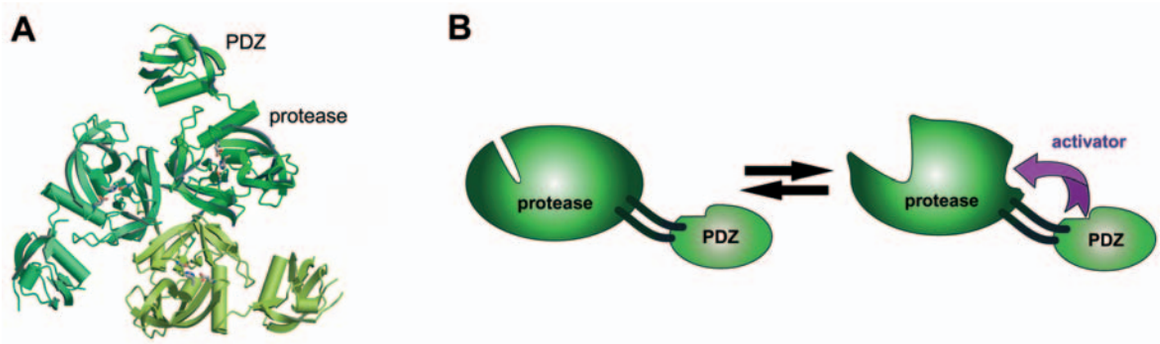


Figure 3: Structure of DegS. (A). Ribbon presentation of the DegS trimer (top view) with each subunit colored differently. (B). Diagram of DegS activation. In DegS, the PDZ domain obtains a regulatory function, offering a binding site for an allosteric activator.

Understanding Molecular Mechanisms with Biomolecular Sequence Analysis and Protein Biochemistry

High-throughput experimental technologies in Life Sciences produce large amounts of uniform data such as biomolecular sequences and mRNA expression values without a direct link to biological function. The combined application of quantitative theoretical concepts and biological database studies can often find hints that help to bridge this gap. Typically, the predictions involve biochemical properties of target proteins. Verification in a biochemical lab is the next step towards a molecular mechanism.

Cooperation Projects with Experimental Groups

The mission of the IMP Bioinformatics Division involves 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. Collaborations include (i) biomolecular sequence analysis and support (ii) in 3D protein structural studies, (iii) in general statistical data analysis, (iv) in the WWW representation of results and (v) in database and software development for large-scale experiments. Additionally, the mass-spectrometry team and the biooptics group are massively supported by our bioinformatics infrastructure. A number of these services are also available through the internet node (http://mendel.imp.univie.ac.at). Our collaboration potential resides on three cornerstones: (a) specialists for sequence and biological data analysis that act upon request from a collaborating group, (b) ready-for-use sequence analysis working places for guest scientists that are looked after by group members and (c) time-efficient protein sequence analyses based on the "ANNOTATOR" suite (see below).

Hundreds of functionally uncharacterized genes and protein families have been studied in great detail during the past year, some of them repeatedly, to elucidate structural and molecular functional features of the gene products or associated genomic regulatory regions. Such investigations have been launched, as a rule, on requests of IMP researchers and their Austrian and international collaborators.

Protein function prediction is currently the area of computational biology with the highest creative impact for life sciences. Among the published results of last year, the discovery of ATGL, a protein that catalyzes the initial step of hydrolysis of triacylglycerides at the surface of lipid droplets in adipocytes, is especially notable. It is surprising that the fundamental activity of this key enzyme escaped from attention so far (Figure).

Development of New Algorithms and Software Packages for Protein Sequence Analysis and Prediction of Function

The IMP Bioinformatics Group is known for its highly accurate predictors for lipid posttranslational modifications (GPI lipid anchoring, myristoylation, farnesylation, geranylgeranylation, and for peroxisomal localization). The ANNOTATOR/NAVIGATOR suite has reached maturity and has become the major tool for protein sequence analysis within the group and its collaborators. More than 30 sequence analytic tools as well as composed algorithms are bundled in the ANNOTATOR so that they are automatically executed via queries and, if suitable, the results are processed and presented in a condensed manner to the researcher. Even the automated collection

Frank Eisenhaber

Frank Eisenhaber / Group Leader

Bioinformatics Group
Georg Schneider / Software Architect
Michael Wildpaner[1] / Software Architect
Georg Kraml[1,2] / Programmer
Birgit Eisenhaber[1,2] / Postdoc
Manfred Koranda[1] / Postdoc
Sebastian Maurer-Stroh[1,2] / Postdoc
Maria Novatchkova[1,2] / Postdoc
Alexander Schleiffer / Postdoc
Georg Neuberger / PhD Student
Sun Tian[1] / PhD Student
Wolfgang Benetka[1,2] / Diploma Student
Florian Leitner[2] / Diploma Student

IMP/IMBA Computer & Network Group
Christian Brandstätter[6] / IT System Manager
Werner Kubina / IT System Manager
Andreas Riepl / IT System Manager
Herlind Wurth / IT System Manager

Long-term Visiting Scientists
Miklos Cserzo[2,3] / Postdoc
Thomas Burkard[4] / PhD Student
Nedim Mujezinovic[5] / PhD Student

[1] *financed by grants or license income,*
[2] *part of the year or part-time,*
[3] *Gen-AU Epigenetics,* [4] *Gen-AU BIN, TU Graz,*
[5] *Gen-AU Proteomics,* [6] *since October 2005*

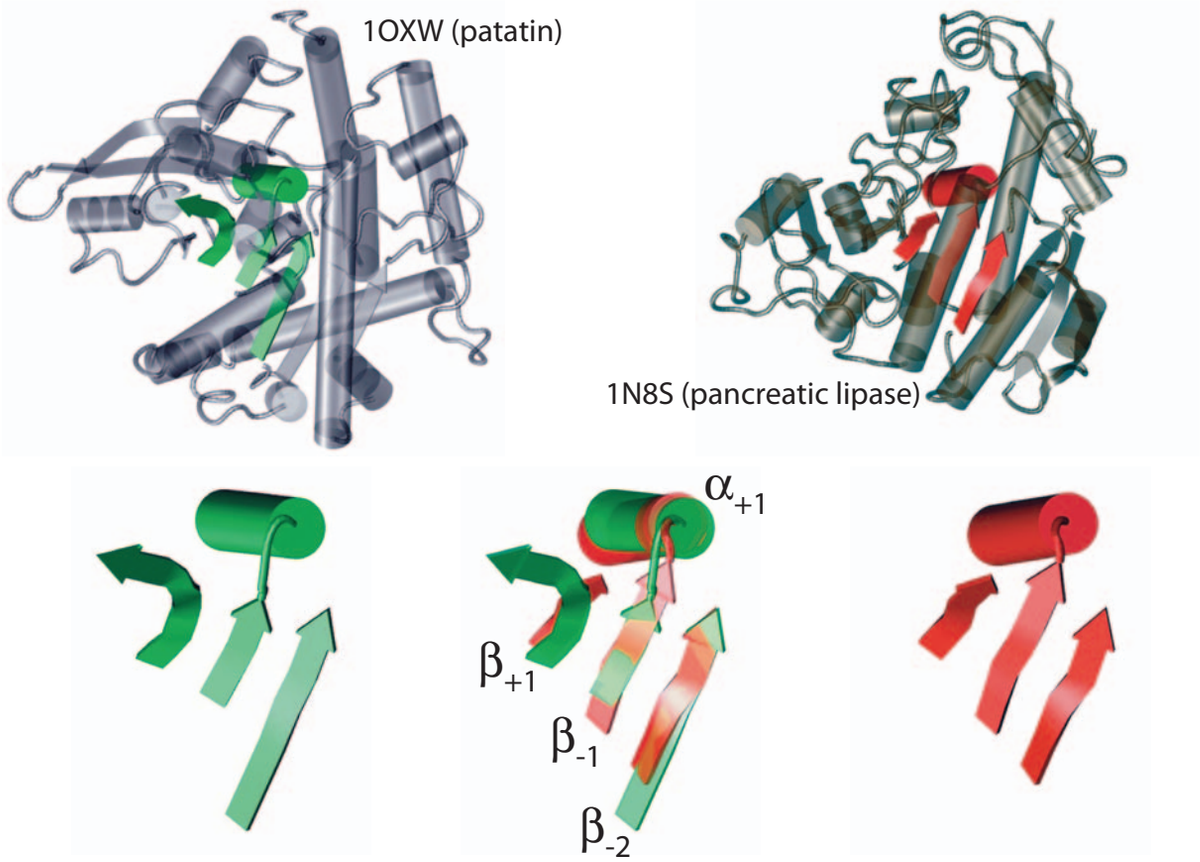


Figure: Structural superposition of the ancestral core in pancreatic lipase and patatin. Although classical lipases and patatin/ATGL have no obvious sequence similarity and differ with regard to fold and catalytic mechanism, they are evolutionarily related. The conserved sequence parts can be narrowed down to an ancestral core module consisting of three β-strands, one α-helix and a turn containing the typical nucleophilic serine. Moreover, this ancestral module also appears in numerous enzymes with various substrate specificities, but that critically relies on nucleophilic attack mechanisms. 3D structures of the conserved core module from pancreatic lipase 1N8S (A, segments 68-77, 144-166, 170-176), patatin 10XW (C, segments 28-37, 70-87, 148-158) and a superposition of both (B). The module starts with a β-strand (β_{-2}) that is followed by another β-strand (β_{-1}), an α-helix (α_{+1}) and a β-strand (β_{+1}). Numbers in parentheses indicate the relative positions to the nucleophilic elbow in the protein sequence. This loop is crucial for catalytic activity and typically contains a catalytic serine. It is located between the adjacent secondary structure elements β_{-1} and α_{+1}. The connections to the other β-strands (β_{-2} and β_{+1}) are not direct and can include longer stretches of additional secondary structure.

of homologous protein families containing tens of thousands of members is possible within the ANNOTATOR framework. For example, we could show the evolutionary relationship
between pancreatic lipase and patatin-like hydrolases such as ATGL (Figure). The NAVIGATOR function allows retrieving information relevant for the project from diverse biological databases. The 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.

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. Small collaborating groups, in particular, have difficulties in following up sequence-based predictions that are relevant for their research. We are gradually accumulating a set of specialized biochemical methods for this purpose. The invention of the efficient Benetka-Koranda method for testing protein prenylation was a major result during the past year.

Computer Usage and Networking Within the IMP

Following the wishes of researchers and taking into account the requirements caused by various scientific activities, a heterogeneous network of Apple Macintosh computers, Windows-based PCs and Unix machines is being supported. It is now possible to maintain failsafe, 24-hour central services. Routine operations of laboratories and administration (for example, oligonucleotide ordering, stock-keeping, trouble ticketing) are increasingly being supported by specialized software tools. The expansion of the IT operation into the integrated IMP/IMBA activities is the major current challenge for the IT group in 2006.

Contact: frank.eisenhaber@imp.univie.ac.at

The Mechanism of Cytokinesis

Cell division is not complete until the replicated chromosomes and cytoplasmic organelles have been partitioned into two daughter cells. Our laboratory is striving to define the molecular mechanism of cytokinesis in animal cells.

Cytokinesis is executed by an actin-based contractile ring that is attached to the overlying cell membrane. The ring assembles in the cell cortex at a site that is positioned midway between the two poles of the mitotic spindle, ensuring that the two separated sets of chromosomes are equally partitioned into the two daughter cells. Assembly of the contractile must therefore be exquisitely regulated in space and time. We are using the nematode *Caenorhabditis elegans (C. elegans)* (Figure 1) and cultured human cells as model systems to dissect this process. We are addressing the following as yet unsolved problems: How is the cleavage furrow positioned? How do the contractile ring and the central spindle assemble and function?

We are particularly interested in the assembly and function of the central spindle, which arises from a subset of the microtubules that make up the mitotic spindle. Central spindle assembly begins at the metaphase to anaphase transition, when chromosomes move polewards on shrinking kinetochore microtubules. At this time, spindle microtubules become bundled to form the central spindle (Figure 2). We discovered an evolutionarily conserved protein complex, centralspindlin, consisting of a Rho family GAP, CYK-4, and a kinesin protein, ZEN-4, that is directly involved in central spindle assembly. Embryos deficient for CYK-4 or ZEN-4 are defective in both central spindle assembly and cytokinesis. Mitotic phosphorylation of the kinesin subunit of centralspindlin delays central spindle assembly until the onset of anaphase.
The central spindle plays important roles throughout the process of cytokinesis. It regulates the initiation of cytokinesis and is essential for its completion. We discovered that two parallel pathways for furrow formation exist in *C. elegans* embryos. One pathway depends on the central spindle, and the other involves a local minimum of microtubule density at the prospective furrowing site. We are using live cell imaging of cleavage furrow components to dissect the molecular basis of both pathways for furrow formation.

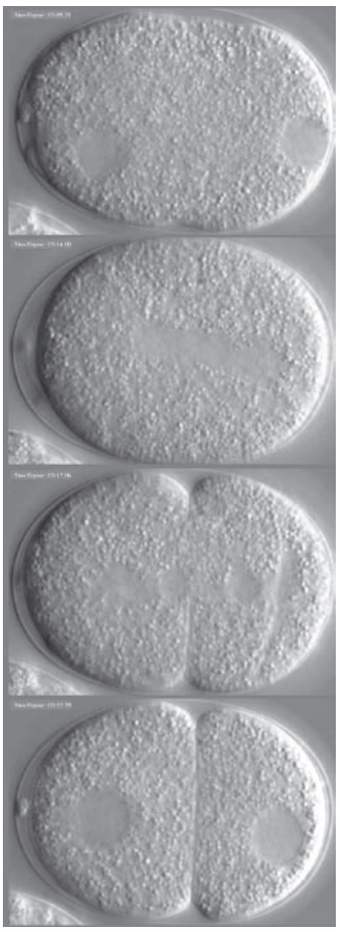
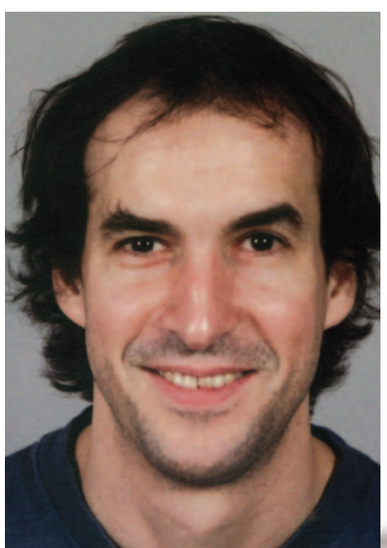


Figure 1: First division of a wild-type C.elegans embryo. Spindle assembly and progression of cytokinesis can be readily observed in living embryos by light microscopy.

Michael Glotzer



Michael Glotzer / Group Leader

Yael Feinstein / Postdoc
Masanori Mishima / Postdoc
Alisa Piekny / Postdoc
Annika Guse / PhD Student
Visnja Pavicic / PhD Student
Michael Werner / PhD Student
Özlem Yüce / PhD Student
Katarina Bartalska / Technician

The Glotzer laboratory relocated in July 2005 to the Department of Molecular Genetics and Cell Biology at the University of Chicago.

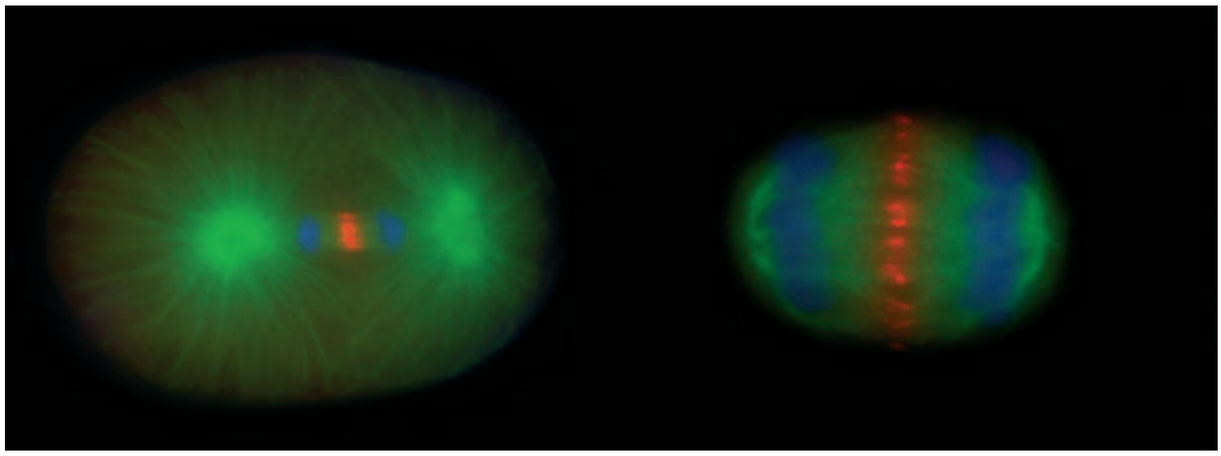


Figure 2: Centralspindlin localizes to the spindle midzone in anaphase. A mammalian cell in anaphase has been stained for MKLP-1 (the mammalian ZEN-4 ortholog; green), tubulin (red) and DNA (blue)

We have recently developed a live probe, based on the *C. elegans* Rho protein, that concentrates at the equatorial cell cortex of human cells prior to furrow ingression (Figure 3). There are several indications that cortical recruitment of this probe reflects activation of RhoA; in particular it requires a guanine nucleotide exchange factor that is essential for cytokinesis, ECT2. ECT2 is recruited to the central spindle by binding to the CYK-4 subunit of the centralspindlin complex. Recruitment of ECT2 to the central spindle enhances the formation of a narrow zone of active RhoA. This analysis indicates that multiple pathways regulate furrow positioning in human cells as in *C. elegans* embryos.

The centralspindlin complex not only mediates RhoA localization, but our results indicate that, in human cells, the CYK-4 subunit of centralspindlin is required for RhoA activation, probably by directly activating ECT2. Mitotic phosphorylation events inhibit CYK-4 binding to ECT2. Thus, multiple inhibitory phosphorylation events inhibit cytokinesis during metaphase.

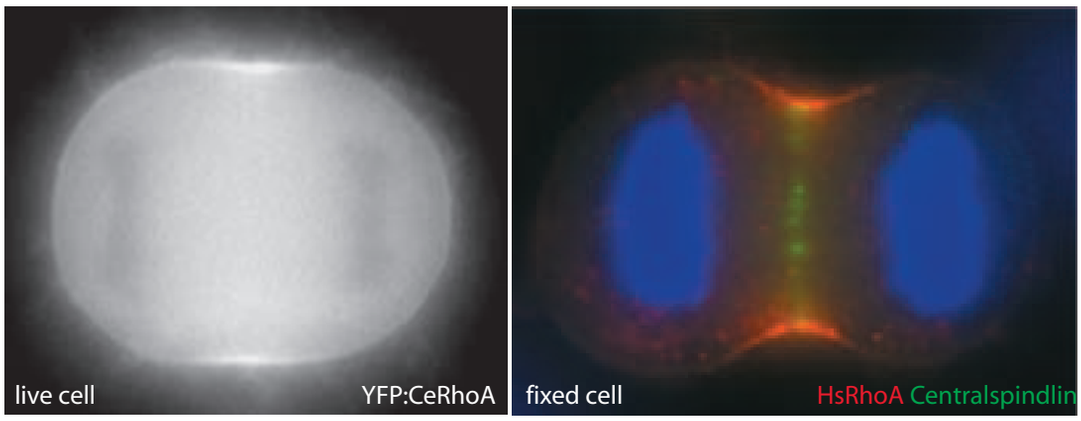


Figure 3: RhoA localizes to the cell cortex prior to furrow ingression and concentrates adjacent to the central spindle. RhoA has been visualized at this site in live cells, using a fluorescent protein fused to C. elegans RhoA and fixed cells, by immunofluorescence.

Contact: mglotzer@uchicago.edu

Formation and Patterning of the Vertebrate Skeleton

The skeleton is an important structure of the vertebrate organism; it supports the body, provides the mechanical framework for physical movements, and protects internal organs. To perform these vital functions, bone and cartilage must form in an exact pattern, with each skeletal element attaining its proper relative length and shape, and each articulation forming precisely between adjoining elements. We use the mouse and the chick as model organisms to gain insight into how these different processes are regulated during both embryonic and postnatal development. In particular, we investigate the role of Wnt-signaling in skeletogenesis.

Christine Hartmann

Regulation of Chondrocyte Maturation and Bone Homeostasis

The Wnt-gene family comprises 19 members, all of which encode secreted signaling molecules. Wnts utilize three different intracellular signaling pathways. At least nine Wnt genes are expressed during skeletal development in chondrocytes, osteoblasts, and in the joint region. However, although many of the Wnt-genes have been knocked out in the mouse, only the loss-of Wnt5a causes skeletal defects. Some of the Wnt-genes expression patterns overlap and it is likely that some redundancy exists in their activities, justifying the observed lack-of a phenotype in the single knock-outs. To investigate Wnts function in the context of the likely redundancy we decided to ask a broader question, namely, whether canonical Wnt-signaling is required for skeletogenesis. Using a conditional gene targeting approach we deleted the key component of the canonical Wnt-pathway, β-catenin. The resulting knock-out phenotype clearly revealed a role of canonical Wnt-pathway in both chondrocyte and osteoblast differentiation, and in the maintenance of joints (Figure 1). In particular, mature osteoblasts fail to differentiate from mesenchymal cells lacking β-catenin. Their precursors, osteochondroprogenitor cells, differentiate into chondrocytes instead (Hill et al., 2005). The three cell types - chondrocytes, osteoblasts and joint cells - share the same mesodermal origin (Figure 2), suggesting that the observed alterations upon loss-of β-catenin activity could be caused by a common mechanism. Using cell culture systems we are currently investigating the underlying molecular clues and plan to identify target genes of the canonical Wnt-signaling pathway that affect chondrogenesis and osteoblastogenesis.

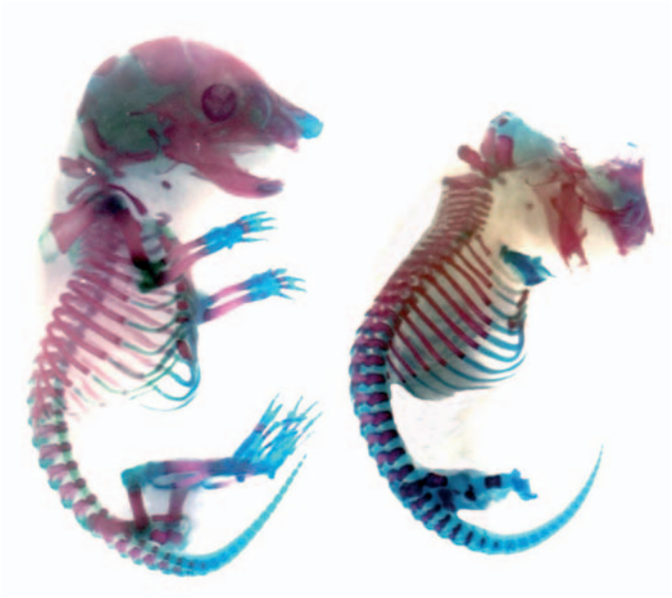


Figure 1: Skeletal preparations of a wild-type (left) and conditional β-catenin knock-out mouse (right). Cartilaginous regions are stained blue; mineralized areas are stained red.

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. However, alterations of chondrocyte maturation often lead to changes in the size of skeletal elements. We uncovered a very specific requirement for one of the Wnt-ligands, Wnt9a, during long-bone development:

Christine Hartmann / Group Leader

Theo Hill / PhD Student
Natalia Lyashenko[1] / PhD Student
Mehran Rafigh / PhD Student
Daniela Später / PhD Student
Richard Latham[1] / Technician

[1] *since November 2005*

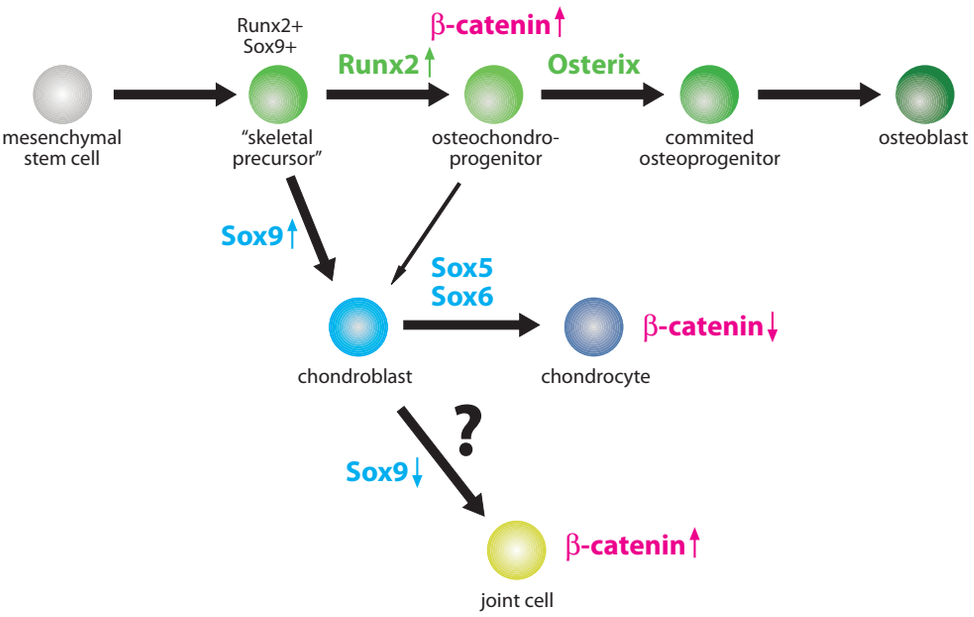


Figure 2: Mesenchymal cells can differentiate into cells of the joint, osteoblasts or chondrocytes. While it has been shown that the transcription factors Runx2 and Osterix are essential for differentiation into osteoblasts and that the transcription factor Sox9 is required for differentiation into chondrocytes, the key regulators of differentiation into joint cells are so far unknown. β-catenin levels have to be raised during osteoblast lineage differentiation, have to be decreased for chondrocyte differentiation and maintained at a high level for joint formation.

Wnt9a controls the expression of a central regulator of chondrocyte maturation in a spatio-temporal manner. It is possible that sequential activation, or temporal recruitment of regulatory molecules, which themselves, like Wnt9a, control a central regulator of chondrocyte maturation, fine-tune the size of skeletal elements. Preliminary results suggest that Wnt9a employs two different intracellular signaling pathways. We are currently investigating this possibility further, using genetic, biochemical and cellular approaches.

Synovial Joint Development

Our gain-of-function analysis of the role of Wnt9a in chick skeletogenesis identified Wnt9a as a major player in the induction of synovial joint development (Hartmann and Tabin, 2001). Wnt9a is expressed early in the joint interzone and, after the formation of the synovial joint, in the articular cartilage and the cells of the joint capsule (Figure 3). The analysis of the Wnt9a knock-out mouse uncovered a role of Wnt9a in maintaining joint integrity of one particular joint, the humeral-radial joint. However, loss-of Wnt9a activity alone did not lead to any defects in early joint formation. Since at least two other Wnt-genes are expressed in the joint regions, it is likely that the activity of other Wnts substitute for the lack-of Wnt9a. We therefore generated Wnt4; Wnt9a double mutant mice, which display defects in additional, but not all joints.

Our long-term goal is to identify regulators of Wnt9a as well as target genes downstream of the Wnt9a signaling pathway. Using a transgenic approach in combination with searching for evolutionarily highly conserved genomic regions within the Wnt9a locus, we aim to identify regulatory elements responsible for Wnt9a expression in the early joint interzone. Identification of such a joint-specific element would provide a useful tool to screen

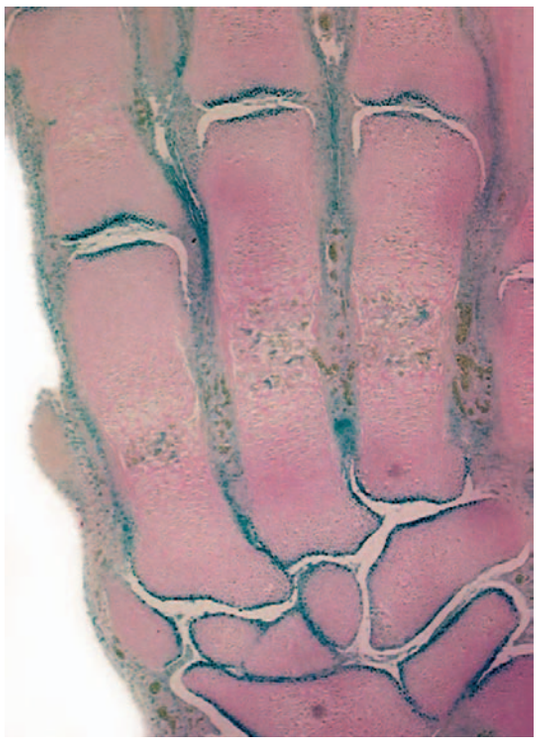


Figure 3: Wnt9a expression in mature synovial joints of the hand visualized by β-galactosidase staining (green).

for factors that are necessary for the initiation and regulation of Wnt9a expression in the joint-forming region and will inevitably allow us to better understand how the skeleton is patterned.

Contact: hartmann@imp.univie.ac.at

Epigenetic Control by Histone Methylation

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

The Indexing Potential of Histone Lysine Methylation

Histone lysine methylation has been linked with constitutive heterochromatin formation, X inactivation, Polycomb-Group (PcG) dependent repression and epigenetic gene regulation at euchromatic positions (Figure 1). Each methylatable lysine residue in the histone N-termini can exist in a mono-, di- or tri-methylated state, thereby extending the coding potential of this particular histone modification. We have examined all possible methylation states for histone H3 lysine 9 (H3K9), H3 lysine 27 (H3K27) and H4 lysine 20 (H4K20) in mammalian chromatin. Using highly specific methyl-lysine histone antibodies together with quantitative mass-spectrometry, pericentric heterochromatin is selectively enriched for H3K9me3 and H4K20me3. This profile is dependent on the 'heterochromatic' Suv39h

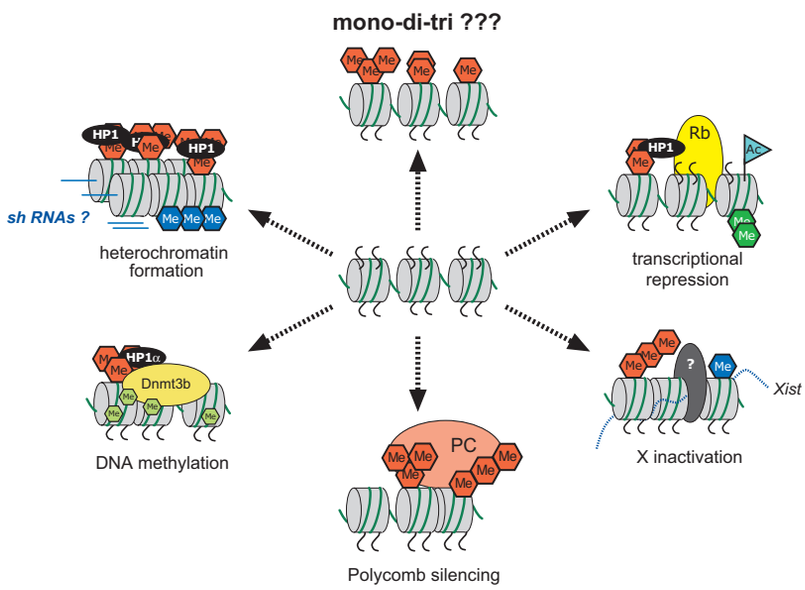


Figure 1: The many faces of histone lysine methylation. The figure summarizes the described roles of histone lysine methylation in major epigenetic paradigms. Distinct lysine positions are shown as color-coded hexagons to indicate H3K4 (green), H3K9 (red triangular), H3K27 (red diagonal) and H4K20 (dark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

Thomas Jenuwein

Thomas Jenuwein / Senior Scientist

Barni Fodor[1] / Postdoc
Ylva Linderson / Postdoc
Joost Martens[2] / Postdoc
Gunnar Schotta[1] / Postdoc
Masato Yonezawa / Postdoc
Alexander Kohlmaier / Phd Student
Stefan Kubicek[3] / Phd Student
Laura Perez-Burgos[3] / Phd Student
Roddy O'Sullivan / Phd Student
Roopsha Sengupta / Phd Student
Susanne Opravil / Technician
Michaela Pagani / Technician

Dörthe Nickel / Noe Manager

[1] personal Marie-Curie, [2] EMBO fellow, [3] GEN-AU

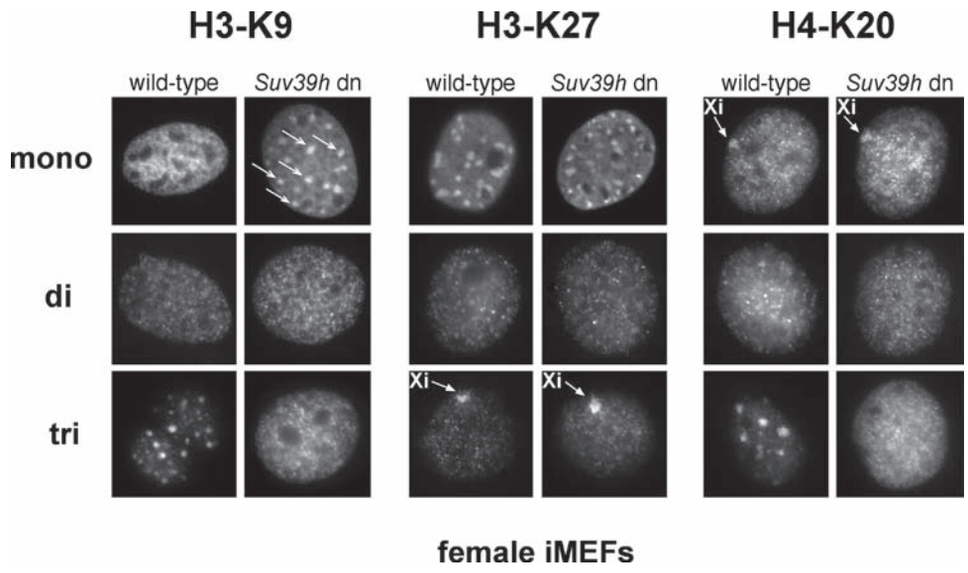


Figure 2: Distinct methylation states are epigenetic landmarks in mouse interphase chromatin. Female wild-type and Suv39h dn iMEFs were stained with methyl-lysine histone antibodies that discriminate mono-, di- and tri-methylation of H3K9, H3K27 and H4K20. Foci of pericentric heterochromatin that were 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.

HMTases and on novel 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. Intriguingly, 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 30 distinct modifications have been described in the N-termini of the four core histones. While most of these represent transient marks at transcriptionally permissive chromatin, some (in particular, repressive histone lysine tri-methyl states) appear more robust at non-coding regions, where they index silent epigenetic domains. Intriguingly, there is a severe 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. 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 and have screened chemical libraries (in collaboration with Boehringer Ingelheim, Ridgefield U.S.A.) to identify small molecule inhibitors against HMTases. Together, these approaches promise to yield new insights into the plasticity of cell fate decisions and may offer novel strategies for exploratory research to modulate tissue regeneration and to revert aberrant development.

An Epigenetic Map of the Mouse Genome

Alterations in the chromatin structure represent the key epigenetic principle to organize the information stored in the genome. In the context of the Austrian GEN-AU initiative (*www.gen-au.at*) and the European Network of Excellence (NoE) *'The Epigenome'* (*www.epigenome-noe.net*), we have initiated the large-scale analysis of epigenetic transitions in defined chromatin regions and along entire mouse chromosomes. Using chromatin-immunoprecipitation (ChIP) from mouse ES cells on custom-made genomic tiling arrays (ChIP-on-chip), we have analyzed the non-random distribution of repressive histone modifications. The data indicate the selective enrichment of H3K9, H3K27 and H4K20 methylation states at diverse classes of repeat sequences (satellites, interspersed repeats and transposons). In a subsequent step, this ChIP-on-chip approach has been extended to a high-resolution (at 100 bp intervals, using *NimbleGen* micro-arrays) and detailed analysis of coding vs. non-coding regions of mouse chromosome 17. We will continue this epigenetic landscaping with chromatin from ES and hematopoietic cells in wild-type and mutant mouse backgrounds, and by comparing stem cells with differentiated and tumor cells. This project will ultimately lead to the establishment of epigenetic maps of the entire mouse genome, as it is organized under distinct developmental conditions.

Contact: jenuwein@imp.univie.ac.at

T Cell Tolerance

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 also through the induction of so-called suppressor T cells.

Suppressor T Cells

It is well established that 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.

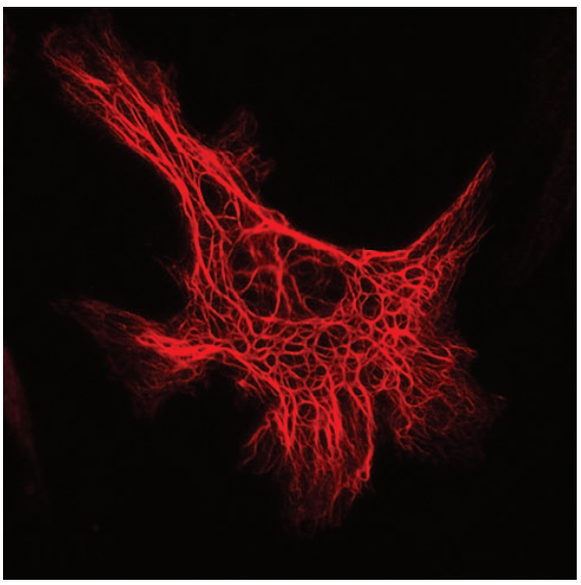


Figure 1: Immunofluorescence of an immortalized thymic epithelial cell, stained for cytokeratin, a typical marker of epithelial cells.

"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). 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

Ludger Klein

Ludger Klein / Group Leader

Katharina Aschenbrenner / PhD Student
Louise D'Cruz / PhD Student
Jan Emmerich / PhD Student
Jelena Nedjic[1] / PhD Student
Martin Aichinger[2] / Diploma Student
Elisabeth Vollmann / Diploma Student
Christian Spona / Technician

[1] *since June 2005,* [2] *since September 2005*

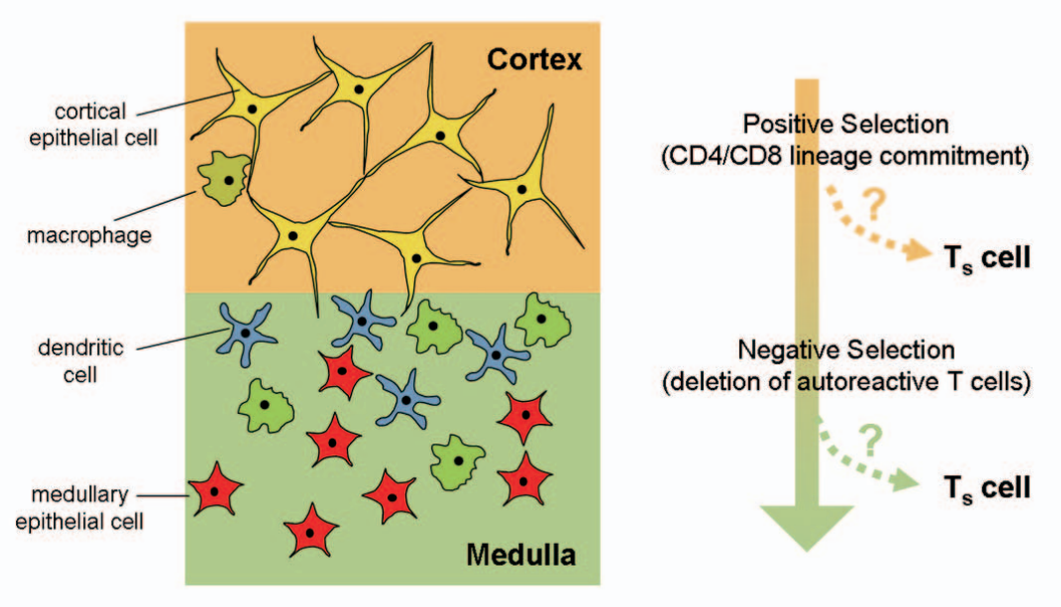


Figure 2: During their maturation in the thymus, developing T cells migrate from the outer cortex to the medulla in a temporally and spatially highly ordered manner. While positive selection for self-MHC restriction occurs upon interaction with cortical epithelial cells, negative selection (removal of 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.

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-/-* mice develop spontaneous autoimmunity. We have initiated a project that 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.

Contact: klein@imp.univie.ac.at

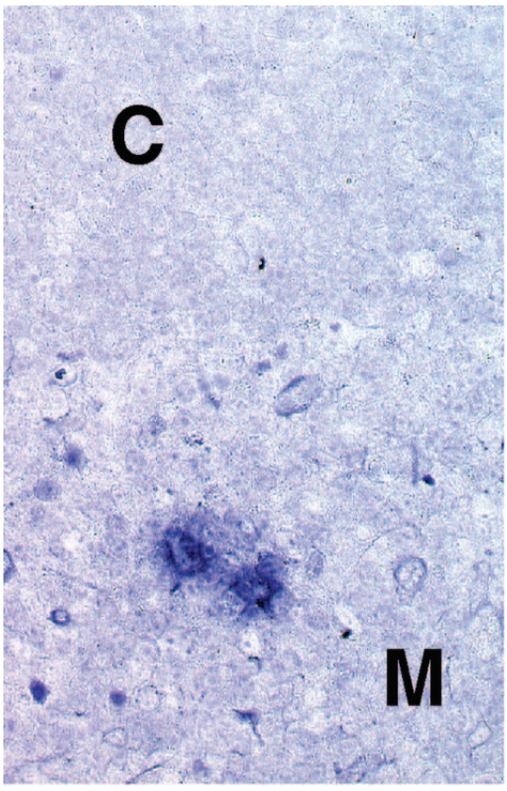


Figure 3: Visualization by in situ hybridization of two cells expressing a "liver-specific" antigen in a medullary region of the thymus (C = cortex; M = medulla).

Design and Function of Molecular Machines

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

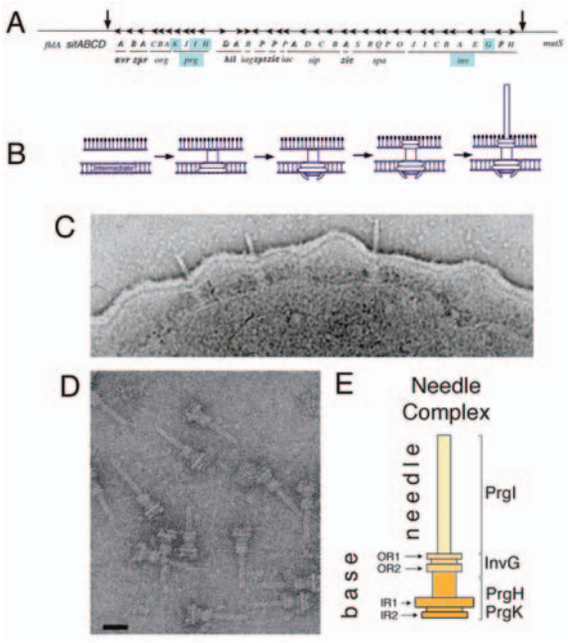


Figure 1: The type III secretion system (A) The type III secretion system is located on the bacterial chromosome but encoded on a specific pathogenicity island. Structural proteins of the needle complex are indicated in blue. (B) Formation of stable intermediate substructures during assembly. (C) Electron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

Host-Pathogen Interaction

A fundamental question in microbial pathogenicity is how bacterial toxins enter eukaryotic host cells. Once inside, they can trigger an entire infection cycle.
Gram negative pathogens, such as *Salmonella, Yersinia*, and *Shigella*, use the type III secretion system (TTSS), a complex macromolecular system to mediate the unidirectional transport of specific effector proteins – often also called virulence factors or toxins - between organisms. These systems are essential for a successful infection resulting in well-known clinical symptoms ranging from mild headaches and diarrhea to even life-threatening diseases such as typhoid fever or bubonic plague.

The Assembly

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

Thomas Marlovits[1,2] / Group Leader

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

Thomas Marlovits

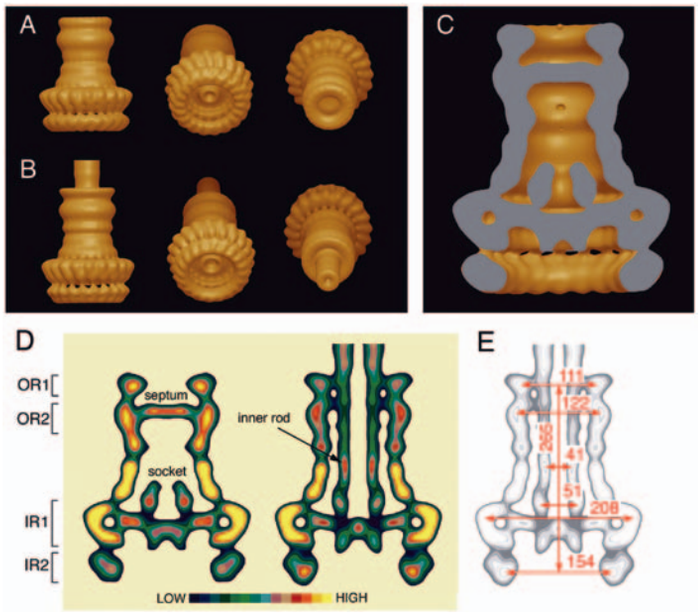


Figure 2: The structure of the base and the needle complex surface renderings of the base (A) and the needle complex (B) show that their overall shape is quite similar. However, tilted views indicate that during the assembly individual domains must undergo large structural rearrangements. (C) The inside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

The Molecular Design

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

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

Recently, we were able to purifiy sufficient amounts of the entire 'needle complex' and its precursor, the 'base', by a combination of detergent extraction and size separation by velocity gradient centrifugation. Our biochemical analysis using quantitative amino acid analysis revealed that the membrane-associated base proteins are present in equimolar amounts (PrgH:PrgK:InvG = 1:1:1). On a structural level, this suggests that the three proteins are likely to share the same rotational symmetry of the cylindrically shaped base. We were, however, intrigued to discover that the membrane-anchored base can adopt different sizes. A detailed structural analysis by three-dimensional electron cryo microscopy and single particle analysis finally revealed that several rotational symmetries or oligomeric states are present in the population of the 'needle complex' and the 'base'. Whether all of these complexes have a physiological role remains an open question.

In addition, our analysis identified a new structural component, the inner rod, present right in the center of the needle complex (Figure 2). It not only extends the secretion path from the base into the needle filament, but also serves as an anchor to stably connect the needle filament into the base. During assembly, the inner rod and the needle filament are added as new structural components to the base. As a consequence, it must undergo large conformational rearrangements, which demonstrates the flexible but also stable property of the base. Functionally, this dynamic behavior is a crucial event during the assembly process, which reprograms the secretion machine so that it becomes competent for the secretion of virulence factors only after the growth of the needle filament is complete.

The Future

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

Contact: marlovits@imp.univie.ac.at

Chromosome Segregation During Mitosis and Meiosis

The simultaneous separation of 46 pairs of sister chromatids at the metaphase to anaphase transition is one of the most dramatic events of the human cell cycle. Already in 1879, Flemming had noticed that, "the impetus causing nuclear threads to split longitudinally acts simultaneously on all of them". What is Flemming's "impetus" triggering loss of cohesion between sister chromatids? What holds sisters together before they separate? How do cells ensure that sister kinetochores attach to microtubules with opposite polarity and that sister separation never occurs before all pairs of chromatids have been aligned on the metaphase plate? How can loss of sister chromatid cohesion between chromosome arms and centromeres take place at different times? Such questions are equally pertinent to mitosis and meiosis, and are at the core of our group's interest.

In budding yeast *Saccharomyces cerevisiae* a multi-subunit complex called cohesin is essential for holding sister chromatids together from the time of DNA replication until the onset of anaphase. Cohesin ensures that sister chromatids attach to microtubules with opposite orientations, known as bi-orientation - a precondition for their traction towards opposite poles of the cell. Once chromosomes have bi-oriented, cohesin resists the tendency for sister chromatids to be split apart by microtubules until a cysteine protease called separase cleaves cohesin's Scc1 subunit, thus triggering the movement of sisters to opposite poles. To dissect the molecular mechanism of cohesion, we have investigated cohesin's assembly from its four subunits (Smc1, Smc3, Scc1, and Scc3). Using EM, biochemistry and crystallography we have shown that cohesin's Smc protomers fold up individually into rod-shaped molecules. A 45 nm intra-molecular coiled coil separates a dimerization region from an ABC-like ATPase "head" domain. Smc1 and Smc3 bind to each other via heterotypic interactions between their dimerization domains to form a V-shaped heterodimer. The two heads of this Smc1/3 heterodimer are connected by the opposite ends of the cleavable Scc1 subunit. Cohesin therefore forms a large tripartite proteinaceous ring, within which DNA molecules might be trapped (Figure 1a). Consistently, proteolytic severance of Smc3's coiled coil causes both dissociation of cohesin from chromosomes and the loss of sister chromatid cohesion. We have recently confirmed a key prediction of this hypothesis, namely cohesin's association with a small mini-chromosome is abolished by linearization with a restriction enzyme, which permits cohesin to slide off the ends of chromatin fibres.

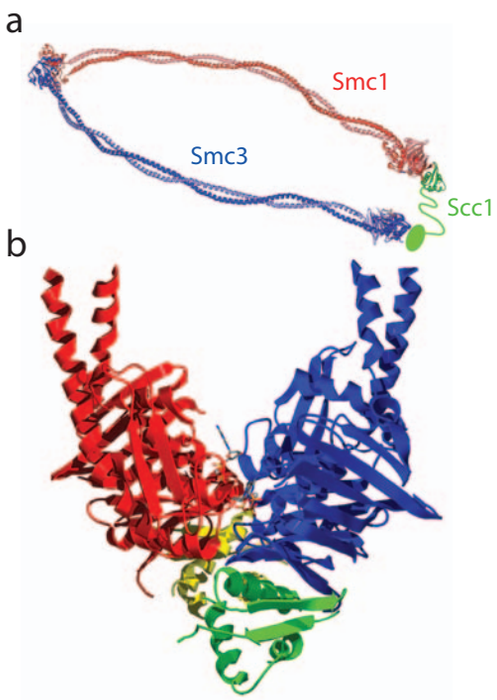


Figure 1: Ribbon models of the yeast Smc1 head homodimer complex with C-termini of Scc1 and of the cohesin ring. (A). Scc1 connects Smc1´s and Smc3´s head domains to form a tripartite ring structure. (B). Two Smc1 head domains (red, blue) dimerize by sandwiching two molecules of ATP analogues in between their interaction surfaces. Scc1's winged helix domain (green, yellow) binds tightly to two β-strands of Smc1.

Kim Nasmyth

Kim Nasmyth / Senior Scientist and Director

Prakash Arumugam / Postdoc
Juraj Gregan / Postdoc
Stephan Gruber / Postdoc
Pejman Hanifi-Moghaddam / Postdoc
Dmitri Ivanov / Postdoc
Nobuaki Kudo / Postdoc
Tatsuya Nishino / Postdoc
Bülent Cetin / PhD Student
Ana-Maria Farcas / PhD Student
Barry Mc Guinness / PhD Student
Ajay Mishra / PhD Student
Andrea Pauli / PhD Student
Christian Riedel / PhD Student

Marta Galova / Technician
Wolfgang Helmhart / Technician
Hiromi Kudo[1] / Technician
[1]*part-time*

Kim Nasmyth and his laboratory moved to the Department of Biochemistry at the University of Oxford at the end of 2005.

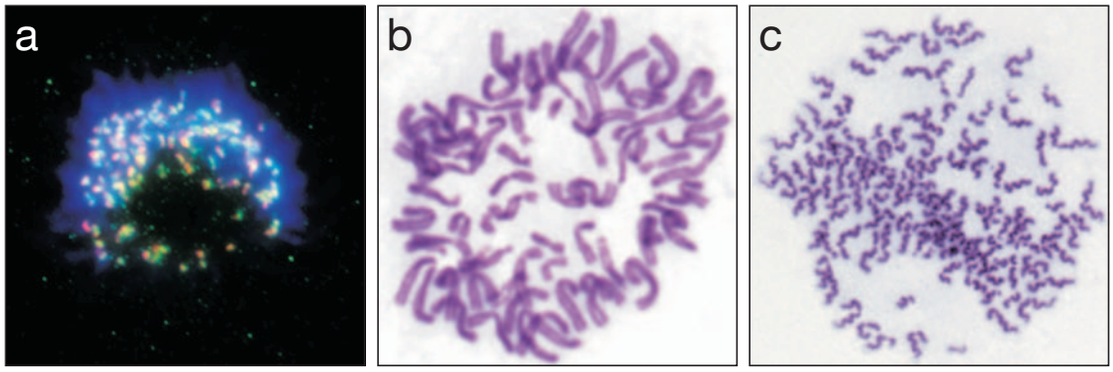


Figure 2: Sgo1 protects centromeric cohesin during mammalian mitosis. (A). Human Sgo1 (green) associates with centromeres (labeled with CREST antiserum shown in red) during mitosis (DNA stained with DAPI shown in blue). (B). Giemsa stained metaphase HeLa chromosome spread. Sister chromatids are resolved along arms, but remain tightly associated at centromeres. (C). Chromosome spread of Sgo1-depleted HeLa cell. Sister chromatids have separated before anaphase entry, leading to cell cycle arrest and hypercondensation of separated chromatids.

Sister chromatid cohesion could be due either to the entrapment of sister DNAs inside a single cohesin ring or association between two separate cohesin rings that have each trapped a sister DNA. We aim to distinguish these possibilities by developing techniques to measure cohesion between small circular sister chromatids *in vitro*.

The similarity to other ABC-like ATPases suggests that ATP bound to Smc1's head interacts with signature motifs within Smc3's head. This class of ATPases is thought to hydrolyze ATP only after the two heads have formed a heterodimer containing a pair of ATP molecules sandwiched between them. We have purified a complex formed between Scc1's highly conserved C-terminal domain and Smc1's ATPase head and in collaboration with Jan Löwe in Cambridge solved the structure of crystals formed in the presence of ATPgS, a slowly hydrolyzable ATP analogue. This reveals a twofold rotationally symmetrical structure in which two Smc1 heads each bound to Scc1 interact with each other by sandwiching a pair of ATPgS molecules in between their contact surfaces (Figure 1b). Presumably a similar structure forms between Smc1 and Smc3 heads in native cohesin complexes and executes the ATP hydrolysis necessary for cohesin to associate with chromosomes. Scc1 binds to the Smc1 head via a winged helix motif normally associated with DNA binding proteins. Mutation of Scc1 or Smc1 residues involved in their interaction prevents complex formation between Scc1 and Smc1/3 heterodimers and abolishes cohesin function *in vivo*. Our current goal is to reconstruct the entire cohesin complex along with the Scc2/Scc4 loading complex from their constituents and to coax cohesin into trapping DNA *in vitro*.

Loss of sister chromatid cohesion along chromosome arms is essential for the resolution of chiasmata and for chromosome segregation during meiosis I. Meanwhile, cohesion between sister centromeres persists so that it can later be used to align sisters on the meiosis II metaphase plate. The different timing of sister chromatid cohesion loss between chromosome arms and centromeres is therefore a crucial aspect of meiosis. The budding yeast genome encodes a second Scc1-like protein called Rec8 that prevents precocious separation of sister chromatids during meiosis. Rec8 and other cohesin subunits localize along the entire chromosome length during pachytene, disappear from chromosome arms during the first meiotic division, but persist at the centromeres until metaphase II. We have shown that separase triggers the first meiotic division by cleaving Rec8 along chromosome arms, which resolves chiasmata. Recent work in our lab has shown that the proteolytic activity of separase is essential for resolving chiasmata also in mouse oocytes.

A screen involving the knocking out of meiosis-specific genes in the fission yeast *S. pombe* identified a conserved protein called Sgo1 that is necessary for protecting centromeric cohesion during meiosis I. The human orthologue of Sgo1 associates with centromeres during mitosis (Figure 2a) and is necessary for preventing precocious dissociation of cohesin and the loss of sister chromatid cohesion before cells can initiate anaphase (Figure 2b, c). Recent experiments suggest that Sgo1 protects Rec8 at centromeres in both budding and fission yeast by recruiting phosphatase PP2A. We therefore suggest cleavage of Rec8 by separase depends on cohesin's phosphorylation, which is prevented at centromeres by PP2A. Finally, we have over the years defined several subunits of a yeast monopolin complex that prevents bi-orientation of sister kinetochores and thereby ensures that maternal and paternal kinetochore pairs are pulled in opposite directions at meiosis I (known as mono-orientation of sister kinetchores). Till now none of monopolin's subunits appear conserved but we have recently shown that monopolin recruits to meiosis I kinetochores a highly conserved protein kinase whose activity is also essential for mono-orientation.

Contact: Kim.Nasmyth@bioch.ox.ac.uk

Mitosis

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

During S-phase, replicated DNA molecules (sister chromatids) become physically connected by cohesin complexes. This cohesion is essential to allow the bipolar attachment of sister chromatid pairs to the spindle, whereas the subsequent removal of cohesin from chromosomes is required for sister chromatid separation in anaphase. In vertebrates, the bulk of cohesin is removed from chromosome arms already in prophase and prometaphase by a mechanism that depends on phosphorylation of the cohesin subunit SA2, on the mitotic kinases Plk1 and Aurora B, and on the protein complex condensin I. Cohesin at centromeres is protected from this "prophase pathway" by a protein called Sgo1. Sgo1 is essential to maintaining cohesion at centromeres until metaphase when 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 how cohesin is loaded onto chromatin, how SA2 phosphorylation results in the unloading of cohesin from chromosomes in mitosis, and how separase activity is regulated.

Chromosome Condensation

While cohesin is removed from chromosome arms during prophase and prometaphase, two related complexes, called condensin I and II, bind to axial domains in sister chromatids. Condensin I is a nuclear complex and binds to chromosomes in prophase, whereas condensin II is cytoplasmic and associates with chromosomes only after nuclear envelope breakdown in prometaphase. In the absence of condensins,

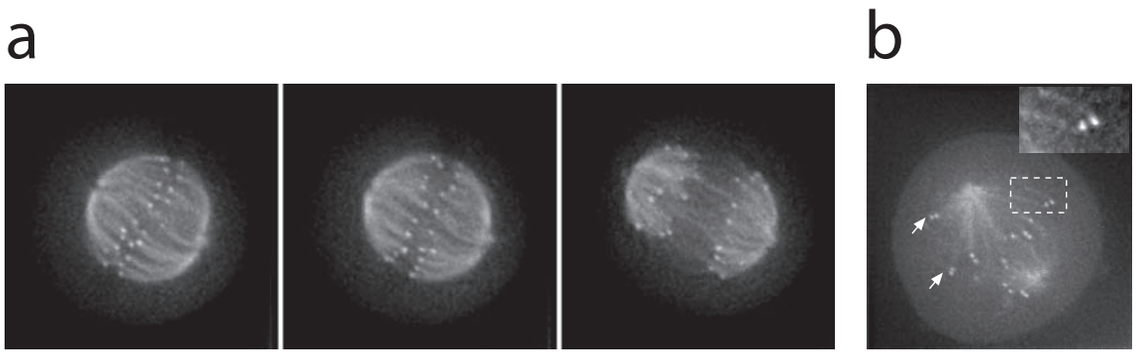


Figure 1: Aurora B kinase controls chromosome biorientation. (A). Microscopic images of a human HeLa cell which is undergoing anaphase. The mitotic spindle and kinetochores on chromosomes were labeled by stable expression of EGFP-α-tubulin and EGFP-CENP-A, respectively. At anaphase onset, sister kinetochores are moved towards opposite poles of the mitotic spindle. (B). Microscopic image of a HeLa cell in which Aurora B has been depleted by RNA interference. Note that many chromosomes are only attached to one spindle pole, and that some of these are "syntelically" attached with both kinetochores to microtubules from the same pole (one example is magnified in the insert). Courtesy of Jesse Lipp.

Jan-Michael Peters

Jan-Michael Peters / Senior Scientist

Toru Hirota[1] / Postdoc
James Hutchins / Postdoc
Claudine Kraft[2] / Postdoc
Peter Lenart / Postdoc
Mark Petronsky[3] / Postdoc
Erwan Watrin / Postdoc
Kerstin Wendt / Postdoc
Gordana Wutz / Postdoc
Bettina Buschhorn / PhD Student
Björn Hegemann / PhD Student
Franz Herzog / PhD Student
Stephanie Küng / PhD Student
Ivana Primorac[4] / PhD Student

Julia Schmitz[5] / PhD Student
Jesse Lipp / Diploma Student
Stephan Zettl[6] / Diploma Student
Birgit Koch / Technical Assistant
Beate Peters[7] / Technical Assistant
Martina Sykora[5] / Technical Assistant

Yan Sun / MitoCheck Project Manager

[1] until September 2005, [2] until November 2005, [3] since December 2005, [4] since August 2005, [5] since November 2005, [6] since June 2005, [7] part-time, until October 2005

chromosomes are able to condense, but condensation is delayed and the resulting chromosomes are less rigid than chromosomes that contain condensins. We are interested in understanding how condensins associate with chromosomes and confer rigidity to them, and which other molecules contribute to chromosome condensation.

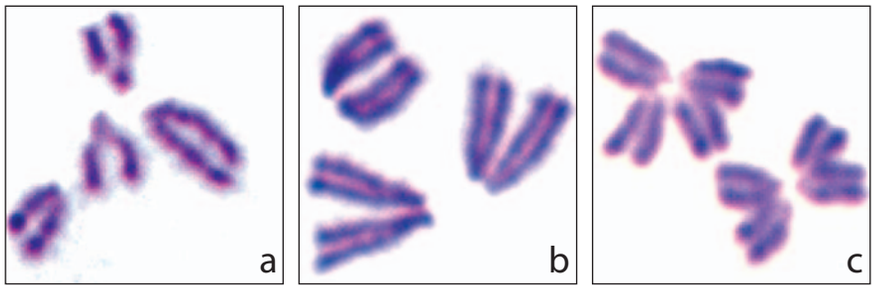


Figure 2: Separase is essential for sister chromatid separation but not for progression through the cell cycle. Mouse fibroblasts whose separase genes were deleted by recombination were stimulated to enter the cell cycle, and chromosomes were analyzed by Giemsa staining and light microscopy in the first (A), second (B) or third (C) mitosis. Note that chromosomes in the first mitosis are composed of two sister chromatids, but that cells that have progressed through either one or two additional cell cycles in the absence of separase contain four and eight chromatids, respectively. This and other observations (see Wurth et al., J. Cell Biol. 2006) indicate that cells lacking separase fail to separate sister chromatids in anaphase but can exit from mitosis and re-replicate their DNA. Courtesy of Gordana Wutz.

Chromosome Biorientation

During chromosome condensation, kinetochores assemble on centromeric DNA and are subsequently captured by spindle microtubules, resulting eventually in the biorientation of all chromosomes on the mitotic spindle. A surveillance mechanism, called the spindle checkpoint, ensures that anaphase is not initiated until all chromosomes have achieved this state. We are using RNA interference, chemical inhibitors and video microscopy to analyze how Aurora B and Plk1 control chromosome biorientation. 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. Our work implies that Aurora B's correction function is also required for proper functioning of the spindle checkpoint. In contrast, Plk1 appears to be required for the assembly of spindles that are able to generate pulling forces, which in turn are needed to stabilize microtubule-kinetochore interactions and to silence the spindle checkpoint. In the future, we would like to identify Aurora B and Plk1 substrates that control chromosome biorientation (see "MitoCheck" project below).

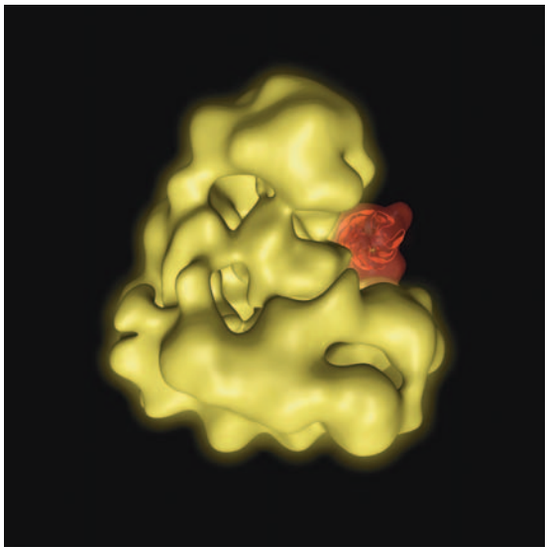


Figure 3: 3D model of the Xenopus APC/C bound to its co-activator Cdh1. Cdh1 associates with APC/C during mitotic exit and G1 phase of the cell cycle and activates APC/C by recruiting substrates. The 3D model of APC/C^{Cdh1} was obtained by angular reconstitution of negatively stained cryo-electron microscopy preparations. A WD40 domain that is found in Cdh1 has been modeled into the density that has been attributed to Cdh1 (colored in red). For details, see Kraft et al., Mol Cell, 2005; Dube et al., Mol. Cell, 2005. Courtesy of Holger Stark.

The Anaphase Promoting Complex/Cyclosome (APC/C)

Separase is activated by ubiquitin-dependent proteolysis of its inhibitor securin. This process is mediated by the ubiquitin ligase APC/C, a 1.5 MDa complex that is composed of a dozen different subunits. APC/C is activated early in mitosis by the phosphorylation and binding of the co-activator Cdc20, but its ability to ubiquitinate securin and other substrates such as B-type cyclins is suppressed by the spindle checkpoint until metaphase. To understand how the APC/C ubiquitinates substrates and how its activity is regulated we are using biochemical approaches in human cells, Xenopus egg extracts and yeast, and are collaborating with crystallography and electron microscopy groups to analyze the structure of APC/C.

"MitoCheck"

The central importance of protein kinases in controlling mitosis has long been recognized, but 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 will together develop approaches to studying mitosis in an "Integrated Project" 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 map mitotic phosphorylation sites on these proteins, and to use chemical biology approaches to understand which kinases are needed for the generation of which phospho-sites.

Contact: peters@imp.univie.ac.at

Gene Function in Mammalian Development and Disease

The major focus of our studies is to analyze 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, heart, skin, hematopoietic and neuronal development.

Fos/Jun/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. Transgenic mice over-expressing Fra-1 develop an osteoblastic bone disease termed osteosclerosis. Interestingly, gene replacement of c-fos with fra-1 revealed functional equivalence of these two proteins (Figure 1). We generated conditional alleles of c-fos and fra-1 to investigate how c-Fos and Fra-1 control osteoblast and osteoclast differentiation. The embryonic lethality of fra-1 knock-out mice was rescued by a conditional allele of fra-1 using More-cre, a Cre mouse line that conditionally deletes specifically in the embryo. The mutant mice were viable but developed a low bone mass disease called osteopenia (Figure 2). Recently, we started to analyze the properties of the Fos-related protein Fra-2 using analogous loss and gain of function approaches. We found that Fra-2 is essential for postnatal mouse development and maintenance of proper bone mass, since mutant newborns die with severe osteopenia. 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 bone and lung disease.

We have also started to investigate the relevance of post-translational modifications of c-Fos in bone development and pathogenesis. The ERK-dependent kinase RSK2 can phosphorylate c-Fos on serine 362 and 374. We were able to show that RSK2 is essential for the development of c-Fos dependent osteosarcomas *in vivo* due to reduced c-Fos protein caused by lack of phosphorylation on serine 362 (Figure 1). To further study the physiological role of c-Fos phosphorylation *in vivo*, we have generated c-Fos knock-in mice in which the endogenous wild-type c-Fos allele was replaced by a point mutant allele with serines 362

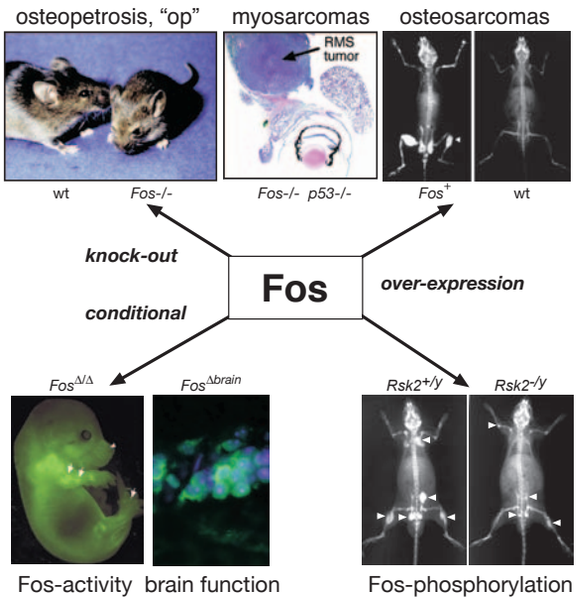


Figure 1: Functional analysis of Fos in bone and CNS development, and the dual role of Fos as an oncogene and anti-oncogene.

Erwin F. Wagner / Senior Scientist, Deputy Director

Robert Eferl[1] / Staff Scientist
Latifa Bakiri / Postdoc
Aline Bozec[2] / Postdoc
Lionel Gresh[3] / Postdoc
Juan Guinea[4] / Postdoc
Peter Hasselblatt[5] / Postdoc
Lijian Hui[6] / Postdoc
Domenico Migliorini[7] / Postdoc
James Witowsky[8] / Postdoc
Rainer Zenz[8] / Postdoc
Ewa Stepniak / PhD Student

Maria Helena Idarraga-Amado[9] / Technician
Uta Möhle-Steinlein[9] / Technician
Martina Rath[9] / Technician
Harald Scheuch / Technician

[1] until June 2005, [2] EU-STREP, [3] EMBO fellowship, [4] P3 +3 CNIC grant, [5] DFG grant [6] EMBO fellowship and personal Marie-Curie, [7] RTN-EU grant, [8] FWF-grant, [9] part-time

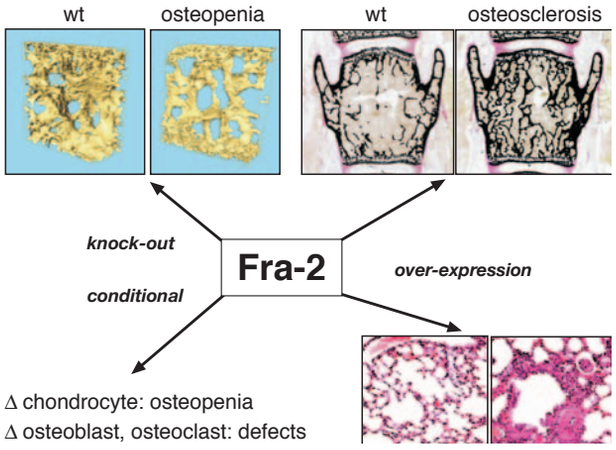


Figure 2: Functional analysis of Fra-2 in bone development and disease.

and 374 mutated to alanines. Preliminary 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.
The functions of Jun proteins in bone development are less well defined. Chondrocyte-specific inactivation of c-Jun causes severe scoliosis due to a failure in intervertebral disc formation (Figure 3). JunB acts also as a positive regulator of bone remodeling, since mice lacking JunB are severely osteopenic. Interestingly, replacement of Jun with JunB revealed functional redundancy during embryogenesis but not during adulthood.

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 specific 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.

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 development (Figure 3). Analogous genetic strategies are now employed to investigate the functions of other Jun/AP-1 proteins in liver cancer and inflammatory disease. 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*. These phenotypes are likely caused by down

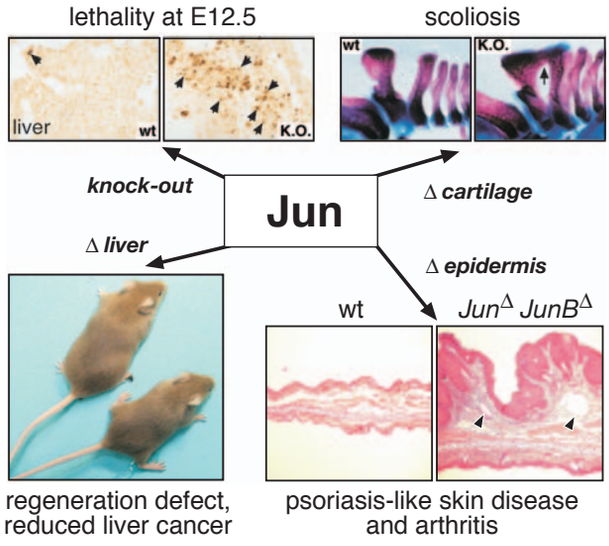


Figure 3: Functional analysis of Jun in development and disease.

regulation of HB-EGF and EGFR. When junB is inactivated in the epidermis, mice are born healthy, but develop a multiorgan disease likely due to deregulated cytokine expression. Moreover, we are able to demonstrate in patients´samples and employing an inducible mouse model that down regulation of Jun proteins in keratinocytes can cause psoriasis, a common chronic disease affecting skin and joints.
Jun activity is regulated by phosphorylation of serines 63 and 73 by the Jun amino-terminal kinases (JNKs). Jnk1-/-, Jnk2-/- and JunAA (a jun allele mutated at the JNK phosphoacceptor sites) mice are healthy and fertile. However, the absence of both Jnk1 and Jun-N-terminal phosphorylation (JNP) causes growth retardation, fibroblast proliferation defects and inefficient osteoclast differentiation. Thus, JNK signaling and JNP distinctively regulate cell proliferation, differentiation and apoptosis in diverse biological processes.

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

Conditional alleles of VEGF, Flk-1 and EGF-R were generated 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 Sibilia from the Dermatology Department, 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 function in postnatal development and cancer.

Contact: wagner@imp.univie.ac.at

Mammalian X-chromosome Inactivation

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. The long non-coding *Xist* RNA is critical for initiation of this silencing process and associates physically along the entire chromosome (Figure 1).

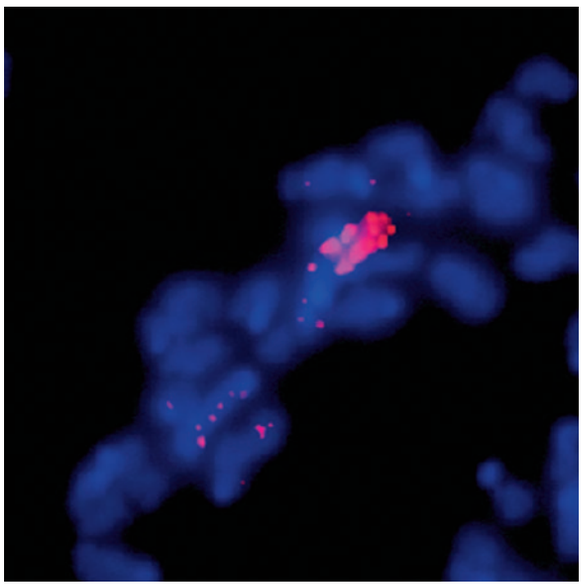


Figure 1: Xist RNA association with chromatin revealed by RNA FISH. Mouse chromosome spreads were prepared from ES cells expressing Xist from a transgene on chromosome 11 and hybridised with a fluorescent probe detecting Xist RNA (red). DNA was counter stained with DAPI (blue).

The Function of Polycomb Group Proteins at the Initiation of X Inactivation

X inactivation is associated with the establishment of histone modifications along the inactive X-chromosome. Tri-methylation of histone H3 lysine 27 (H3K27me3), mono-methylation of histone H4 lysine 20 (H4K20m1), and ubiquitination of histone H2A (H2AK119ub1) follow *Xist* localization with rapid kinetics. H3K27me3 and H2AK119ub1 are catalyzed by complexes containing the conserved Polycomb proteins Ezh2 and Ring1b, respectively. In a collaborative effort with Thomas Jenuwein´s group, we have elucidated the kinetics of H3K27me3 during X inactivation, using an inducible *Xist* expression system in mouse ES cells. In this system, the ability of *Xist* to trigger silencing is restricted to an early window in ES cell differentiation. This window is also important for establishing methylation patterns on the potential Xi (Figure 2). By immunofluorescence and chromatin immunoprecipitation experiments, histone H3 lysine 27 tri-methylation (H3K27m3) and H4 lysine 20 mono-methylation (H4K20m1) are associated with *Xist* expression in undifferentiated ES cells and mark the initiation of X inactivation. Both marks depend on *Xist* RNA localization but are independent of silencing. Induction of *Xist* expression after the initiation window leads to a markedly reduced ability to induce H3K27m3, whereas expression before the restrictive time point allows efficient H3K27m3 establishment. *Xist* expression early in ES cell differentiation establishes a chromosomal memory, which is maintained in the absence of silencing. One consequence of this memory is the ability to introduce H3K27m3 efficiently after the restrictive time point on the chromosome that has expressed *Xist* early.

Anton Wutz

Anton Wutz / Group Leader

Ruben Agrelo[1] / Postdoc
Aditya Sengupta / Postdoc
Karen Ng / PhD Student
Dieter Pullirsch / PhD Student
Fabio Savarese / PhD Student
Stefan Schöftner / PhD Student
Martin Leeb / Diploma Student
Mediyha Saltik / Technician

[1]*since April 2005*

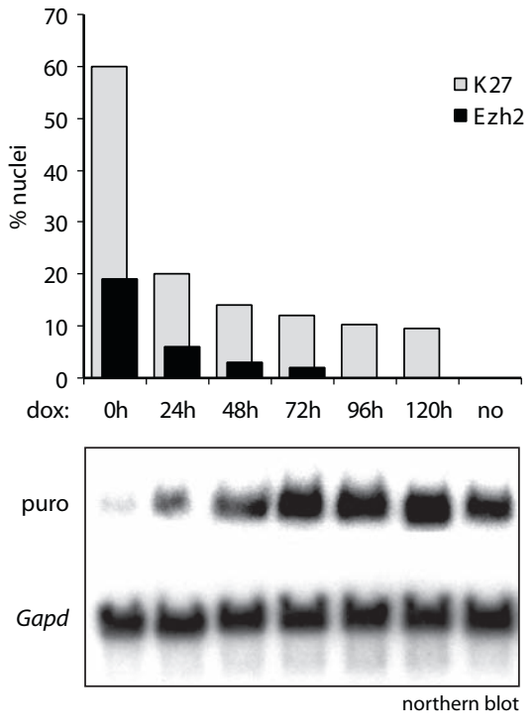


Figure 2: Restriction of efficient H3K27m3 and initiation of transcriptional repression coincide in ES cell differentiation. Transgenic Xist expression was induced at various timepoints in differentiating ES cells and the levels of histone methylation and effect on a puromycin marker gene genetically linked to the Xist transgene were determined at day 8 of differentiation. High histone methylation levels and gene silencing are initiated by Xist only in early differentiation.

Ongoing efforts focus on the function of these histone modifications during X inactivation. To address this question, we generate loss-of-function mutations in the chromatin modifying enzymes by gene targeting in ES cells. This approach will allow us to understand the mechanism of a Polycomb mediated transcriptional memory during X inactivation.

Turning off Chromosomes by *Xist* During Development

Initiation of silencing in response to *Xist* RNA expression is restricted to a specific time interval at the onset of cellular differentiation and could be potentially regarded as a feature of stem cells. To characterize the function of *Xist* during development and in adult mice we have used an inducible *Xist* allele allowing experimental manipulation of *Xist* expression in mice. Our results indicate that the potential of *Xist* to initiate silencing is lost during embryogenesis in a gradual and probably cell type-specific manner. In adult male mice, ectopic expression of *Xist* in the haematopoietic lineages causes severe anaemia that can eventually lead to lethality. Further analysis of the defect shows that cell loss occurs in multiple haematopoietic lineages at a stage that includes early progenitors, but not in the c-kit+ Sca+ lin- stem cell compartment. These results suggest that *Xist* can function in cells of the adult organism and point to epigenetic differences among specific stages of the haematopoietic differentiation cascade. Further work is directed towards understanding the molecular basis for this epigenetic difference and the investigation of whether cell types that respond to *Xist* expression with gene silencing are also part of tumors of the haematopoietic system.

Identification of Proteins that Interact with *Xist* RNA in Chromosomal Silencing

To gain insight into the molecular mechanism by which *Xist* causes transcriptional repression, we aim at the isolation of proteins that interact with the repeat A of *Xist* RNA, a crucial element for the initiation of silencing. We have isolated proteins from nuclear extracts that specifically interact with the sense but not the antisense repeat A RNA (Figure 3). In collaboration with Karl Mechtler from the IMP protein sequencing facility we have identified candidate proteins, all of which contain RNA binding motifs. 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 in mice and ES cells will provide insight into the epigenetic regulation of gene expression in mammals. It appears that *Xist* mediated silencing is a paradigm for a powerful epigenetic system that is capable of hetero-chromatinising an entire chromosome and determines its specific nuclear localization. It is expected that similar interactions underlie the regulation of other genes - however, with less dramatic consequences. The involvement of long non-coding RNAs in regulation of chromatin is of central interest to contemporary epigenetic research, and might also pinpoint a general process by which transcription feeds back onto chromatin structure.

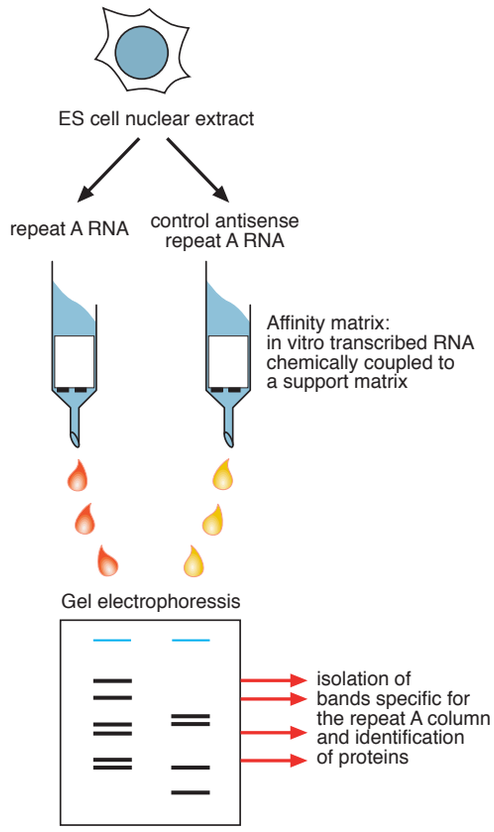


Figure 3: Biochemical purification of proteins that bind Xist repeat A. Repeat A on the 5´-end of Xist RNA is crucial for gene silencing. Proteins are isolated by affinity chromatography on a matrix consisting of in vitro transcribed repeat A RNA or antisense control RNA. Bound proteins are eluted and analyzed by gel electrophoresis. Proteins specific for repeat A RNA are identified by mass spectrometry.

Contact: wutz@imp.univie.ac.at

Service Department

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

Preparation of Media and Other Cloning Reagents

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

Production of Antibodies

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

Sequencing and DNA Isolation

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

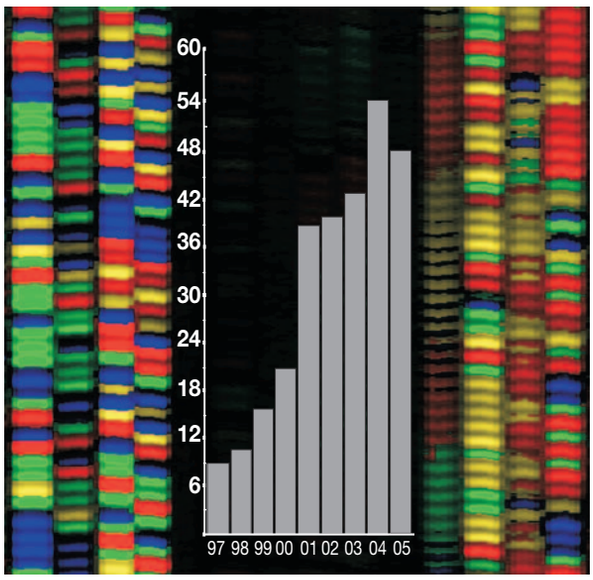


Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (1997 - 2001), on ABI 3100 (2001 - 2005) and on ABI 3730 (2004 - 2005) done with dye deoxy terminators (v3.0 since 2001) from 1998 to 2005 (scale 0 to 60'000).
**calculated from data for January 2005 to October 2005*

Contact: schaffner@imp.univie.ac.at

Gotthold Schaffner / Head of Facility

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

Biooptics Department

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

Current Research Activities

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

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

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

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

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

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

Contact: steinlein@imp.univie.ac.at

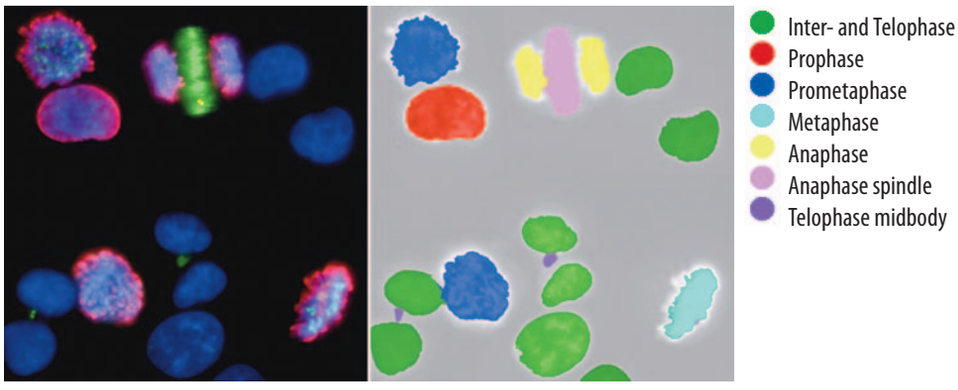


Figure: Left: Input image of cells in various phases of mitosis stained with DAPI (dsDNA) in blue, Anti-phospho(Ser10) Histone H3 in red and anti-Aurora-B in green (40x magnification; data provided by Jan-Michel Peters Group). Right: Output image after fully automated classification.

Peter Steinlein / Head of Facility

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

[1]*until April 2005*

Animal House

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

Husbandry

The husbandry is divided into two main areas containing the following species: mice and *Xenopus*. The larger area is the mouse section, where more than 10,000 mice are kept. These comprise breeding colonies, stock, and experimental animals including many transgenic and knock-out mouse lines. To provide a constant supply of mice for the various projects, 20 standard strains are routinely bred in-house.

Animal House Services

Veterinary services, such as monitoring of the facility's health-status (sentinel program etc.), experimental procedures in animals such as the collection of blood, implantation of tumor cells and administration of substances by iv, ip or sc injections. All procedures are performed to a high standard under appropriate anaesthetic regimes and in conjunction with the necessary project licenses.

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

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

Contact: bichl@imp.univie.ac.at

Mouse Service

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

The main duties of this service unit are the injection of ES cells into blastocysts [also tetraploid] and of DNA into the pronucleus of fertilized mouse eggs. This service also provides for the transfer of 'clean' embryos into our Animal House, the freezing of embryos for the preservation of specified mouse strains and the teaching of basic embryological techniques to the IMP and IMBA staff. *In vitro* fertilization experiments (IVF) are performed and the mouse strain database is kept up-to-date. About 30 different ES cell clones and several DNA constructs are being successfully injected *per* year. The activities of this department are overseen by an Animal User Committee, which meets bimonthly to set priorities and to coordinate the duties. At present, it is chaired by Erwin F. Wagner.

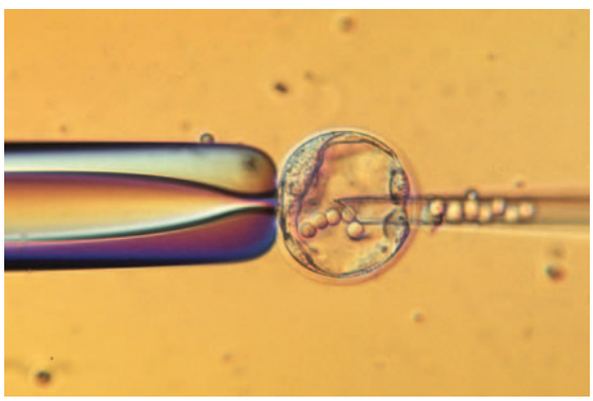


Figure: Injection of embryonic stem cells into mouse blastocytes.

Contact: theussl@imp.univie.ac.at

Animal House

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

Mouse Service Department

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

[1] *since October 2005*

Protein Chemistry Facility

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

Improvement in Phosphorylation Analysis

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

A. Analysis of protein complexes

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

B. Enrichment of phosphopeptides from complex mixtures

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

Doppler Lab for Proteome Analysis

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

Proteome Bioinformatics

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

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

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

Peptide Synthesis and Antibody Purification

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

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

Karl Mechtler / Head of Facility

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

Histology Service Department

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

Histology Services

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

Sectioning of Paraffin and Frozen Tissues

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

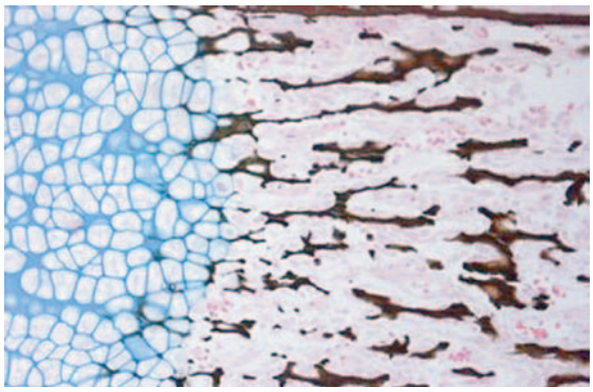


Figure 1: Van Kossa staining. This picture shows 3 different signals: dark for bone mineralization, blue for bone tissues and red for red blood cells (erythrocytes).

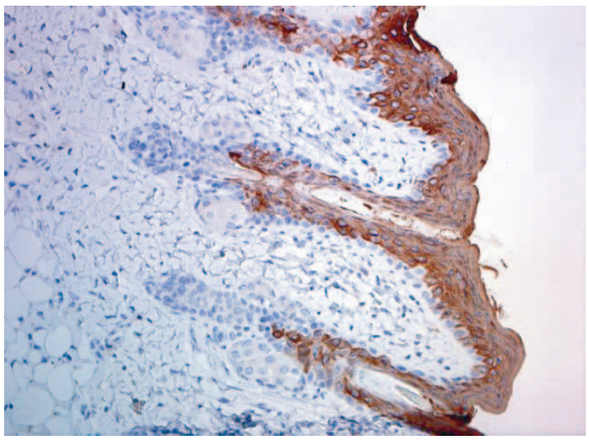


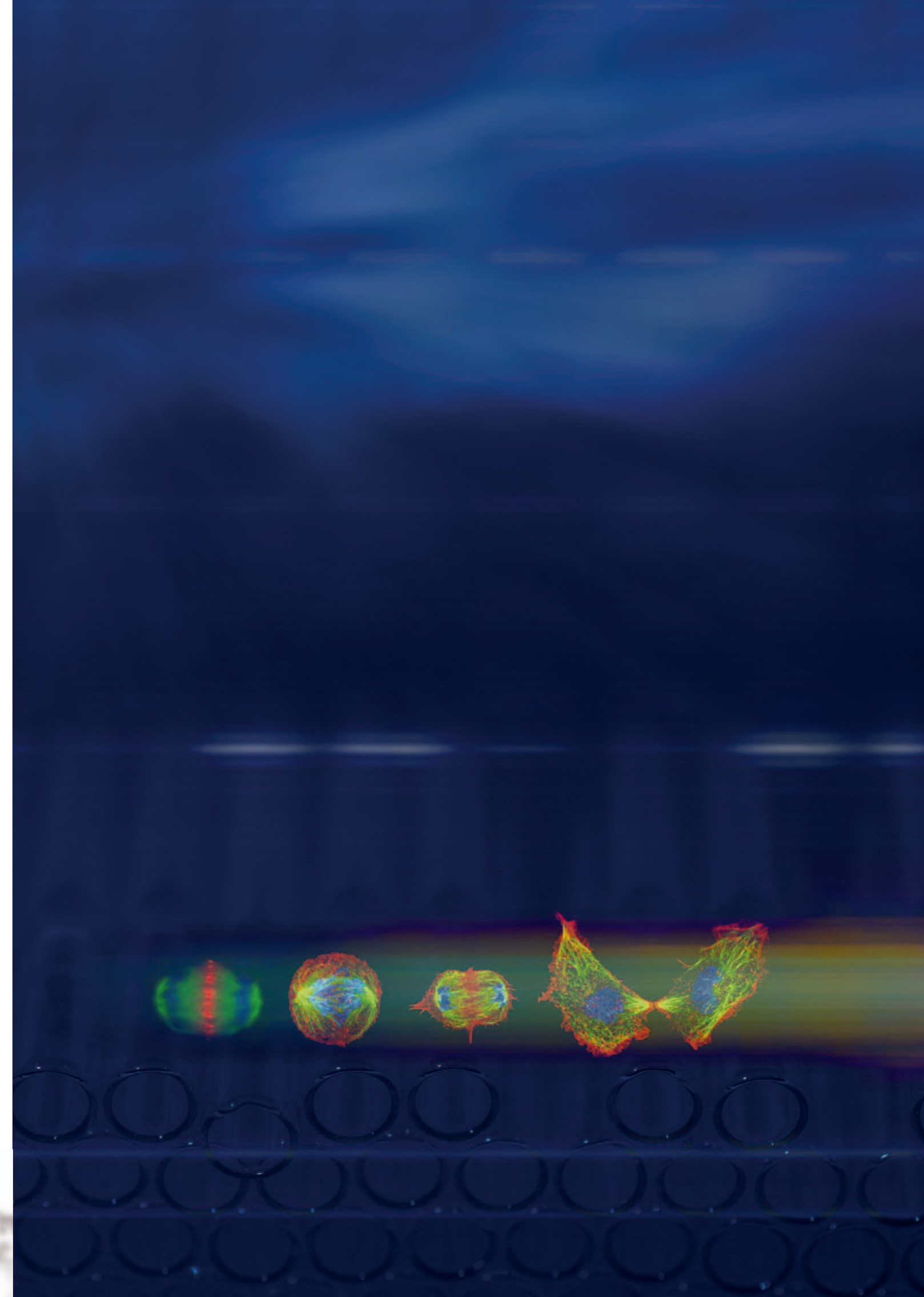
Figure 2: Keratin 10 Staining. The brown signal shows the suprabasal, cornified and granular layers of the epidermis. (skin from c-fos f/f mouse after TPA treatment), Courtsey of Juan Guinea.

Immunohistochemistry

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

Contact: komnenovic@imp.univie.ac.at

Vukoslav Komnenovic / Head of Facility



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HISTOLOGY SERVICE DEPARTMENT

Jones, H. D., Nakashima, T., Sanchez, O., Kozieradzki, I., Komarova, S. V, Sarosi, I., Morony, S., Rubin, E., Sarao, R., Hojilla, C. V., Komnenovic, V., Kong, Y.-Y., Schreiber, M., Dixon, F. J., Sims, S. M., Khokha, R., Wada, T., and Penninger, J. M. (2006). Chemotactic regulation of epithelial tumor cell migration and bone metastasis by RANKL. Nature. In press.

Seminar Speakers

JANUARY

04/01/05 KONRAD HOCHEDLINGER (Whitehead Institute, Cambridge, USA)
05/01/05 EMAMANUELLE PASSEGUE (Stanford University School of Medicine, USA)
18/01/05 ULRICH JAEGER (Medical University of Vienna, Austria)
25/01/05 THOMAS LECUIT (IBDM/LGPD, Marseilles, France)
26/01/05 ERHARD HOHENESTER (Imperial College London, UK)
28/01/05 ANDREAS TRUMPP (ISREC, Lausanne, Switzerland)

FEBRUARY

03/02/05 JIM WOODGETT (University of Toronto, Canada)
08/02/05 CLIFF TABIN (Harvard Medical School, Boston, USA)
08/02/05 GUNTER REUTER (Martin Luther University, Halle-Wittenberg, Germany)
10/02/05 MARIO DE BONO (MRC Cambridge, UK)
11/02/05 PURA MUNOZ CANOVES (CRG, Barcelona, Spain)

MARCH

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

APRIL

07/04/05 KATSUHIKO SHIRAHIGE (Tokyo Institute of Technology, Japan)
08/04/05 GEORG KROHNE (University of Würzburg, Germany)
12/04/05 PETER MEYER (University of Leeds, UK)
13/04/05 ERIC WIESCHAUS (Princeton University, USA)
15/04/05 MICHEL NUSSENZWEIG (Rockefeller University, New York, USA)
19/04/05 STAN COHEN (Stanford University, California, USA)
22/04/05 NOBORU MIZUSHIMA (Tokyo Metropolitan Institute of Medical Science, Japan)
25/04/05 ZHAO-QI WANG (IARC, Lyon, France)

MAY

02/05/05 KATZUFUMI MOCHIZUKI (University of Rochester, USA)
02/05/05 LESLIE LEINWAND (University of Colorado, USA)
03/05/05 HIDEYUKI SAYA (Kumamoto University, Japan)
04/05/05 INGRID MECKLENBRAEUKER (Rockefeller University, New York, USA)
04/05/05 ORLY REINER (Weizmann Institute of Science, Rehovot, Israel)
06/05/05 DIRK HOLSTE (Institute of Technology, Massachusetts, USA)
09/05/05 CHIUOU-FEN CHUANG (Rockefeller University, New York, USA)
10/05/05 HEINRICH JASPER (University of Rochester Medical Center, USA)
11/05/05 MARK BIX (University of Washington, USA)
12/05/05 JOHN F. MARKO (University of Illinois, Chicago, USA)
17/05/05 ROBIN HIESINGER (Howard Hughes Medical Institute, USA)
18/05/05 LEONIE RINGROSE (University of Heidelberg, Germany)
18/05/05 WALTER NEUPERT (University of Munich, Germany)
20/05/05 CHARLES SAMUEL (University of California, USA)
23/05/05 TILL MARQUARDT (Salk Institute for Biological Studies, La Jolla, CA, USA)
24/05/05 GUIDO KROEMER (Gustave-Roussy Institute, Villejuif, France)
25/05/05 GEORG BORNKAMM (GSF, Munich, Germany)
30/05/05 BENJAMIN KAUPP (Research Centre Jülich, Germany)
31/05/05 MARTIN HÜLSKAMP (University of Cologne, Germany)

JUNE

01/06/05 YUN-CAI LIU (La Jolla Institute for Allergy and Immunology, USA)
02/06/05 KAMI AHMAD (Harvard Medical School, Boston, USA)
02/06/05 TOM KORNBERG (University of California, San Francisco, USA)
07/06/05 HIROSHI TAKAYANAGI (Tokyo Medical and Dental University, Japan)
08/06/05 PAUL SCHULZE-LEFERT (MPI, Cologne, Germany)
10/06/05 THIERRY BOON (Ludwig Institute for Cancer Research, Brussels, Belgium)
13/06/05 RANDALL MOON (University of Washington, USA)
14/06/05 GERRY GRAHAM (University of Glasgow, UK)
14/06/05 MONICA GOTTA (ETH-Hoenggerberg, Zurich, Switzerland)
14/06/05 MANFRED S. WEISS (EMBL Hamburg Outstation, Germany)
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23/06/05 GERARD KARSENTY (Baylor College of Medicine, Texas, USA)
24/06/05 CRIS KUHLEMEIER (Berne University, Switzerland)
30/06/05 MICHAEL NEUBERGER (MRC Cambridge, UK)

JULY

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08/07/05 JUAN JOSÉ CAZZULO (University of Buenos Aires, Argentina)
13/07/05 JIRI FRIML (ZMBP, University of Tübingen, Germany)
14/07/05 CONSTANZE BREITHAUPT (MPI Biochemistry, Martinsried, Germany)
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15/07/05 RALF JAUCH (MPI Biophysical Chemistry, Goettingen, Germany)
20/07/05 PAPAI GABOR (Hungarian Academy of Sciences, Szeged, Hungary)
29/07/05 MICHAEL J. PANKRATZ (Forschungszentrum Karlsruhe, Germany)

AUGUST

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18/08/05 STEFANO PICCOLO (University of Padua, Italy)
19/08/05 CHOZHAN RATHINAM (Hannover Medical School, Germany)
23/08/05 ROWENA LEWIS (Deakin University, Melbourne, Australia)

Seminar Speakers

SEPTEMBER

01/09/05 ANGELIKA AMON (Howard Hughes Medical Institute, USA)
09/09/05 HERGEN SPITS (University of Amsterdam, Holland)
09/09/05 MITSUHIRO YANAGIDA (Kyoto University, Japan)
15/09/05 MICHAEL KARIN (University of California, San Diego, USA)
22/09/05 STEFFEN JUNKER (University of Aarhus, Denmark)
23/09/05 MARK KRASNOW (Stanford University School of Medicine, USA)
26/09/05 PETER WALTER (HHMI/UCSF, USA)
29/09/05 WILHELM KREK (ETH Zurich, Switzerland)

OCTOBER

12/10/05 DAVID COREY (Harvard Medical School, Boston, USA)
18/10/05 BERND GRONER (Georg-Speyer-Haus, Germany)
18/10/05 STEPHEN NUTT (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia)
21/10/05 THOMAS GINGERAS (Affymetrix Research Laboratories, Santa Clara, California, USA)
27/10/05 WOLF REIK (Babraham Institute, Cambridge, UK)

NOVEMBER

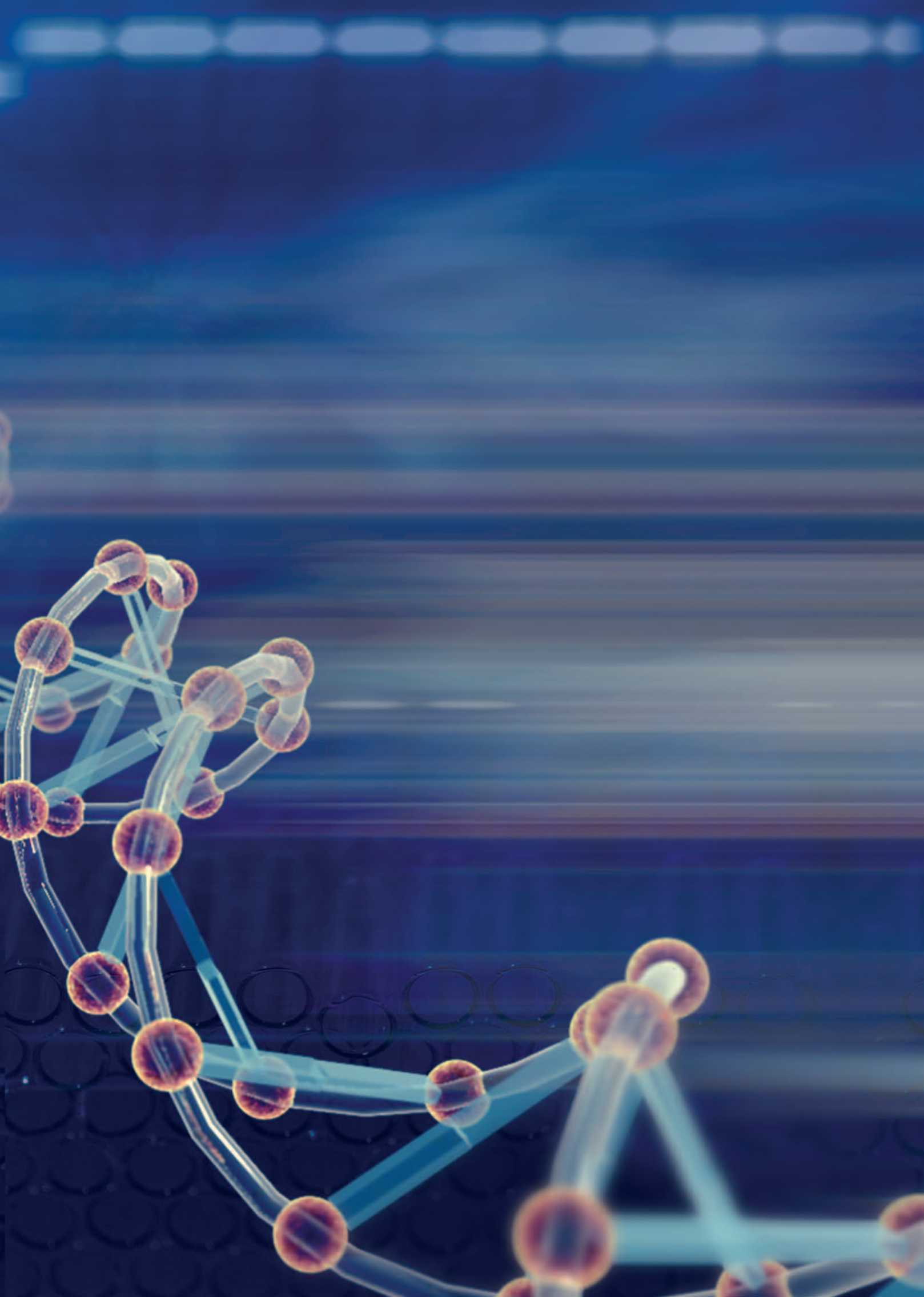
02/11/05 MARC K. JENKINS (University of Minnesota Medical School, USA)
04/11/05 PIERRE GOENCZY (ISREC, Lausanne, Switzerland)
09/11/05 OLIVIER POURQUIÉ (Stowers Institute for Medical Research, Kansas, USA)

DECEMBER

01/12/05 RYUZO YANAGIMACHI (University of Hawaii, Mānoa)
02/12/05 JOHN O'SHEA (University of Cincinnati, Maryland, USA)
13/12/05 ANNE-KARINA PERL (Cincinnati Children's Hospital, USA)
14/12/05 GEORGE WEINSTOCK (Baylor College of Medicine, USA)
16/12/05 KATHRIN BREUKER (Center for Molecular Biosciences, Innsbruck, Austria)

“If the IMP didn‘t already exist, one would have to invent it“

(Jeff Schatz)



Awards and Honors

Jan-Michael Peters
Received the Boehringer Ingelheim R&D Award 2005 (February 2005).

Thomas Jenuwein
Received the Sir Hans Krebs Medal of the Federation of European Biochemical Societies (Budapest, July 2005).

Tim Clausen
Was selected for the EMBO Young Investigators´ Program YIP (October 2005).

Stephan Gruber
Received the Campus Vienna Biocenter PhD Award (December 2005).

Claudine Kraft
Received the Campus Vienna Biocenter PhD Award (December 2005).

Spotlight on 2005

IMP-IMBA Spring Conference

The 13th IMP Spring Conference, held from May 19-21, was at the same time the Inaugural Conference for IMBA, the Institute of Molecular Biotechnology. The scientific meeting, which was organized by Barry Dickson and Josef Penninger, drew an international audience to the historic Hofburg-palace in the heart of Vienna.

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

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

IMP-IMBA Recess

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

Open Space for Open Minds

To create opportunities for young students to get in touch with science, Austria's Federal Ministry for Transport, Innovation and Technology organized a pilot project called "Open Space for Open Minds". In November 2005, 100 pupils aged around 17 had the chance to meet a scientist and get a glimpse of his or her working environment. At the IMP, Ludger Klein welcomed a group of students to his lab where they spent an interesting afternoon and were encouraged to ask all the questions that came to mind.

Long Night of Research

On October 1st, research-institutions in Vienna, Linz and Innsbruck opened their doors till midnight to let visitors experience science on a first-hand basis. The Austrian Council for Research and Technology development and three ministries had initiated the first "Long Night of Research" under the motto "safety". At the Campus Vienna Biocenter, about 1300 curious minds were attracted that night. The IMP participated in the event by setting up a display on "DNA packaging". Young scientists from the Epigenome NoE demonstrated how long strands of DNA are wrapped up within the nucleus and how a cell's function is influenced by the packaging.

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Erwin F. WAGNER	Deputy Director
Harald ISEMANN	Administrative Director

MANAGEMENT ASSISTANTS

Diane TURNER	Executive Assistant
Gerti KÖLBL	Assistant to the Administrative Director

SCIENTIFIC SECRETARIAT

Christopher ROBINSON	Assistant to the Senior Scientists

The following services are part of the joint service concept between the IMP and IMBA (employment status indicated in brackets).
For a detailed description of shared scientific services see pp. 34-38.

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Martin ROJDL	House Maintenance (IMBA)
Bettina ERLACH	Receptionist (part-time; IMBA)
Grete KOCINA	Receptionist (part-time; IMP)
Christian LAUFER	Receptionist (part-time; IMBA)

Scientific Advisory Board

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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Prof. Dr. Georg Stingl
Austrian Academy of Sciences,
Vienna, Austria

Where We Are

Where We Are

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IMP - Research Institute of Molecular Pathology

Dr. Bohr-Gasse 7
1030 Vienna
Austria

Phone: +43(1)797 30
Fax: +43(1)798 71 53
office@imp.univie.ac.at
www.imp.univie.ac.at

The IMP is a basic research institute within the Boehringer Ingelheim group of companies.
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