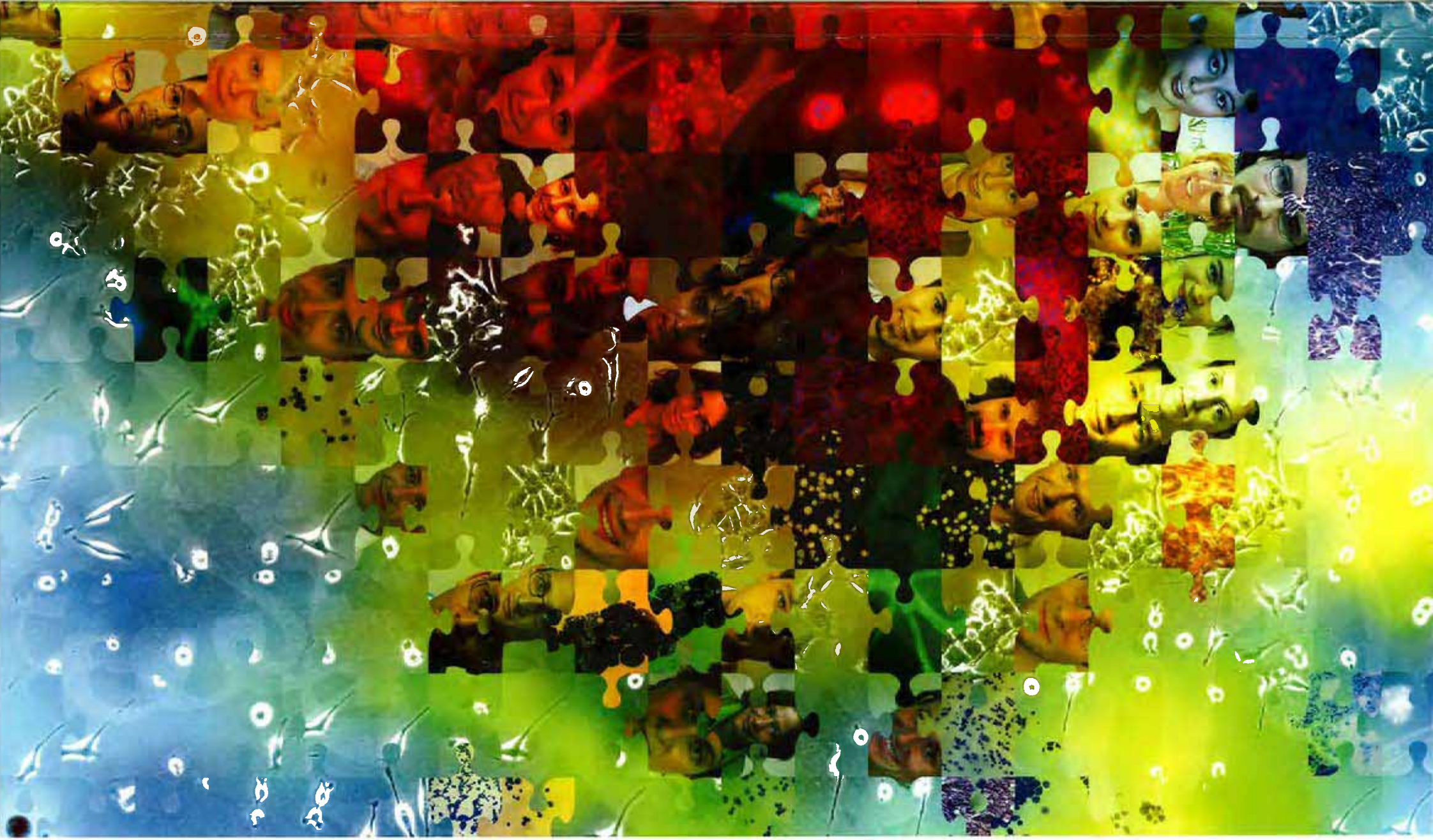

Boehringer Ingelheim

IMP 2004

Research Institute of Molecular Pathology

VIENNA BIOCENTER

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

Contents

Introduction

This has been another successful year for the IMP. We are, as usual, bursting at the seams, temporarily housing two IMBA groups as well as our own but relief is on the horizon. IMBA's new building into which our mice will be moving should be ready by this time next year. The departure of our mice and the IMBA group leaders will create valuable new space for new IMP group leaders and for accommodating new infrastructure. Our longstanding administrative director Nikolaus Zacherl retired this year after long and much valued service at the IMP. We are indebted not only to Boehringer Ingelheim but also to Nikolaus who ensured that our finances are in their current healthy shape. Nikolaus' successor Harald Isemann has now been with us for nearly one year and his energy and intelligence have already made an important impact, especially in completing our cooperation agreement with IMBA.

The Cooperation is meanwhile functioning smoothly, with our scientific services being shared by the IMP and IMBA. The services go from strength to strength and are coping admirably with the extra work load. Two very large projects under the EU sixth framework programme coordinated by IMP group leaders were funded by the EU; these being a network of excellence on epigenomics coordinated by Thomas Jenuwein and an integrated project on mitosis coordinated by Jan-Michael Peters. Anton Wutz was the most recent IMP group leader to be named a new member of EMBO's Young Investigator Programme. The IMP will also enter into an important cooperation with the Christian Doppler society to develop new technologies in mass spectrometry and, with our new state of the art super computer, bioinformatics at the IMP goes from strength to strength and has inspired the University of Vienna to establish a new chair in this area.

As ever, we are extremely grateful to the members of our Scientific Advisory Board, from which Herbert Jäckle stepped down after six years of very valuable service. We welcomed several new members to our Board in 2004, namely Gregory Petsko from Brandeis University, Titia de Lange from the Rockefeller, and Rudi Jaenisch from the Whitehead Institute. Towards the end of the year our PhD students organized another wonderful symposium, this time on Evolution.

Lastly, the forthcoming year will be my last at the IMP as I shall be moving to the University of Oxford in January 2006. The nomination of my successor will be one of next year's key events. We are very fortunate to have a high powered search committee under the chairmanship of Piet Borst from the Netherlands Cancer Institute. The fact that Andreas Barner, Deputy Head of the Board of Directors of BI, is also a member of this committee is surely a sign of how much the company values the IMP.

Kim Nasmyth
December 2004

"When one looks at what happened to major discoveries, and considers the realities of our everyday environment, it can be seen that nearly all technologies, products and achievements which have led to economic and commercial success and/or concrete improvements to the quality of life are based on basic research . . .."
(European Commission, 2004)

By a clear commitment to the principle of discovery oriented basic research Boehringer Ingelheim (BI) has enabled the IMP to evolve into a world-class research institute. However, what holds true for living organisms is just as important for companies and institutions: constant change is crucial for successful development. Thus, a lot of things have again changed at the IMP during the last year, and further new developments can be expected for the coming year.

I took up the position of Administrative Director at the IMP in February 2004. The first major challenge was to work out the details of the research cooperation that BI, the Austrian Academy of Sciences, IMP and IMBA have agreed upon. The completion of a contract on a joint service concept represents an important cornerstone of this cooperation. Highly skilled core services that support the scientific groups in a most efficient way have been one of the key success factors of the IMP. IMBA, which aims to build on the IMP's expertise, will therefore have access to all existing administrative and scientific services in-house. On the one hand, this joint service concept will further increase the capacity and scope as well as the quality of the services offered, on the other hand it will generate synergies that benefit both institutes.

In addition to generous sponsoring from BI, IMP scientists succeeded in acquiring numerous research grants. The most important external financing source is the European Commission. The IMP participates in nine different projects within the 6th Framework Programme of the EU. Two of these - one "Integrated Project" and one "Network of Excellence" - are coordinated at the IMP. We are also very grateful for the continuous support by Austrian funding organizations on a national and local level. These include the Austrian Science Fund (FWF), the Austrian Research Promotion Agency (FFG), GENAU, WWFF, ZIT and in the future also the Christian Doppler Society.

The year 2005 will see some major conversions of the IMP building: the Cafeteria will be extended and new seminar rooms built on the 3rd floor. After the completion of the IMBA building in autumn the animal house and some service facilities will be relocated and the currently somewhat tight space situation will relax.

I would like to thank all IMP members for their remarkable dedication to the institute and their fantastic support in my first year at the institute.

Harald Isemann
December 2004

IMP Ski-outing, April 2004, Schladming.

The IMP and its surroundings

The Research Institute of Molecular Pathology - the 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 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.

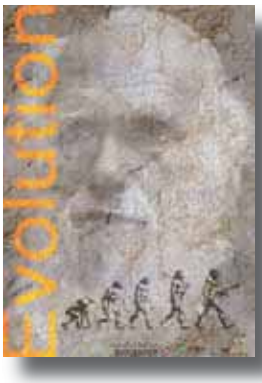
Opened in 1988 close to the city center, the IMP triggered the development of its neighbourhood into a teeming biotechnology hub. Today's "Campus Vienna Biocenter" (VBC) is also home to several institutes of the University and the Medical University of Vienna, two institutes of the Austrian Academy of Sciences (IMBA and GMI), seven biotech-companies, a University of Applied Sciences, a PR-agency and a non-profit scientific society.

Close to 1000 people from 40 different nations currently work at the Campus VBC, 200 of them at the IMP. 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 lecture series, are open to all.

For those whose interests stretch beyond, 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 still felt today. A world-famous opera house, legendary orchestras, unique art-collections and the medieval city center are but a few attractions.

Art, music, and literature are certainly Vienna's major "obsessions" but there is also 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 Neusiedlersee or watching rare birds in the Seewinkel. Even within the borders of Vienna, you can enjoy a hike through dense forest, go canoeing in a national park, climb impressive limestone cliffs or stroll through rolling vineyards. With more than 50% of Vienna's surface area covered by vegetation, you, too, will find your favorite spot.



PhD symposium – Evolution *5-6 November 2004*

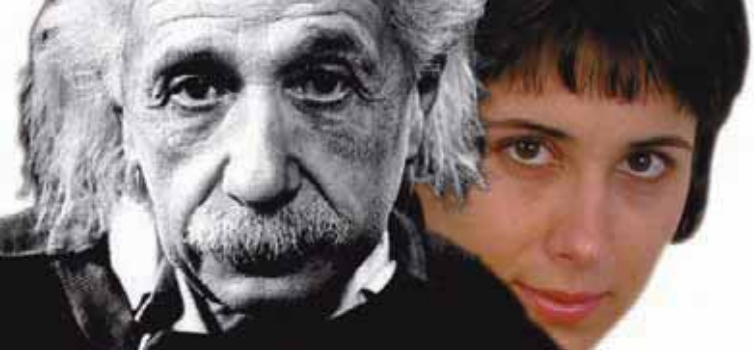
After an intense selection process of possible topics, only the fittest survived, so the 3rd Vienna Biocenter PhD symposium focused on the topic of Evolution. The study of evolution is relevant to anyone interested in biological phenomena, for as Theodore Dobjansky put it "nothing in biology makes sense except in the light of evolution". We invited 16 distinguished and enthusiastic speakers from the diverse areas of this broad field, some of whom were self-proclaimed fossils while others were more recent emergents on the evolutionary timescale. Each speaker generously opened with a general introduction to their particular field of interest, leading one to quip "it's the first meeting of my peers I've attended where I understood all the talks". They then proceeded to share their recent findings and insights on topics ranging from evolution of processes such as nitrogen metabolism and drug resistance, through evolution of complex networks such as the immune and nervous systems, to evolution of cultural traits, including language and even lice! An audience comprised of students, postdocs and group leaders from within the Biocenter and throughout Europe ensured that each talk was followed by a lively discussion. After a year's preparation we were delighted that the meeting was well received by speakers and participants alike. Organising this meeting was a great experience for all involved and we hope it will become a regular feature of the Vienna Biocenter PhD Program. More information about the PhD programme is available at *www.univie.ac.at/vbc/PhD*.

"It was indeed, superb, one of the very best (= interactive and intellectually stimulating) meetings I've been to ever."
Prof. Bruce R. Levin Emory University, Atlanta, USA

"Thanks very much for inviting me - I had a great time. (. . .) You guys did a great job organising things - and I think all the speakers really enjoyed themselves..."
Prof. Alan Cooper Oxford University, United Kingdom

"Thanks to all of you. I can tell you that all of the invitees that I spoke to (and these were many if not all) really enjoyed it immensely."
Prof. Sebastian Bonhoeffer ETH Zürich, Switzerland

"Just a quick note to compliment you on the organisation of a truly first-class symposium. The two-day programme far exceeded the quality of many professionally organised (and priced!) conferences. I thoroughly enjoyed my visit and will be recommending my PhD students to keep a close eye on the programme for the next symposium organised by the IMP PhD students!"
Dr. Robin C. May Hubrecht Laboratory, Utrecht, The Netherlands



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 "flavour" of the IMP lies in its stimulating and focussed atmosphere where science always comes first but social activities are not neglected. Informal interaction 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 Programme, run jointly with the University and our partner-institute IMBA. The doctoral degree is awarded by the University of Vienna. Selection of the students takes place twice a year; PhD contracts typically last 3-4 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 organises a large international conference every other year and smaller workshops in-between. Students are successfully organising 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 for the science in the first place. We do, however, appreciate your private needs and try to make relocation as smooth as possible. For newcomers, there are several apartments in-house 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 such as day-care. For school-age children, Vienna offers a large range of different types of schooling, from public to private, German or foreign speaking, traditional or with more experimental concepts.

Many of our new employees are accompanied by spouses who are themselves looking for a qualified position according to their training. The IMP certainly is aware of that fact and can, in some cases, help with securing a job. We also support your efforts to learn German and offer free courses at 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*

IMP alumni remember...

"For me, the thrill of joining the IMP has not only been a scientific one. When I came to Erwin Wagner's lab at the IMP in early 1989, Vienna used to be at the outskirts of the West, right next to the Iron Curtain. To witness first-hand the fall of the latter was an enthusing experience. Over the following years, it became exciting to see the IMP develop into the premier scientific melting pot between Eastern and Western Europe. In those years the IMP was undoubtedly the most international institution in the entire city of Vienna! I then moved to Switzerland, which is a great place – but can sometimes be very, well..., very "Swiss". I look back at my years in Vienna as extremely enriching from both a scientific and a broadly cultural viewpoint."

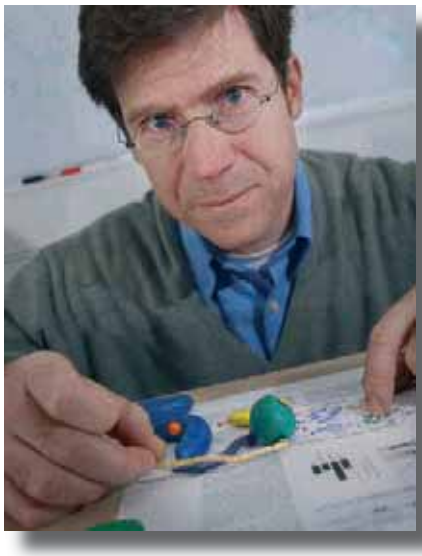
Prof. Adriano Aguzzi
Director, Institute of Neuropathology, University Hospital of Zurich

"The IMP is now one of Europe's elite research Institutions. To be in at the beginning, was for me an unforgettable experience. When I joined back in 1987, IMP's temporary offices were still in the 1st District and the labs in Dr. Bohr-Gasse were barely built. Although we were upbeat, no one could be sure if all this would work out. Would postdocs, group leaders etc. come to what was then the Eastern extremity of Western Europe? Would the parent companies continue to see the importance of having a foot in the basic research camp? Could the IMP compete scientifically? Well, Europe changed, the companies saw what was good for them and, most relevant of all, IMP research was consistently top class. The IMP has self-evidently "arrived" and I for one am proud to be an alumnus."

Prof. Adrian Bird
The Wellcome Trust Centre for Cell Biology
University of Edinburgh

"The first years at the IMP were exciting and hilarious. There were few groups and the groups were small. So everybody was interested in everybody else's research. After talks, there were always a lot of questions and remarks, often very critical. Kim Nasmyth and Adrian Bird were always there with their inevitable "clever bastard" type of questions, turning everything upside down. Social life was intense. Most students gathered daily at 5 pm in Joe's disco to watch the "Falcon Crest" soap opera, then back to the lab until 9 or 10 pm and then hit town. There was pioneering feeling. The IMP was an island in one of the most unlikely districts of Vienna, next to broken down industrial buildings and abandoned slaughterhouses. We had to stick together to survive. We had a lot of fun."

Prof. Giulio Superti-Furga
Chief Executive Officer & Scientific Director of CeMM - Center for Molecular Medicine of the Austrian Academy of Sciences



"My time at the IMP was a fantastic experience. The interactions within the institute made all the difference, amazing people at all levels. We shared so much, and not only the thrill of each others' scientific questions..."

Dr. Frank Uhlmann
Head of the Chromosome Segregation Laboratory at the Cancer Research UK London Research Institute

Oncogenesis: abnormal developmental plasticity?

In leukemia and carcinoma development, cooperation of oncogenic receptors/signal transducers with (sometimes mutated) transcriptional regulators causes abnormal proliferation, survival and developmental plasticity. We use novel in vitro cell culture systems and genetically modified mice to investigate the mechanisms by which such effector combinations drive self-renewal of committed hematopoietic progenitors, and plasticity/ trans-differentiation of carcinoma cells during metastasis.

Mechanisms of hematopoietic progenitor renewal and leukemogenesis

Self-renewal of primary erythroid progenitors can be induced *in vitro* by cooperation of the EpoR, c-Kit and the glucocorticoid receptor (GR). This induction reflects a physiological response to stress erythropoiesis and enables large-scale expansion of immature erythroblasts in culture. Recently, we used this approach to expand erythroid progenitors from murine ES-cell-derived embryoid bodies (ESEPs), which are diploid and genetically stable, have an unlimited lifespan in culture and allow to study erythropoiesis in early embryonic lethal mouse mutants lacking hematopoiesis. For instance, ESEPs from Flk-1-/- ES cells failed to undergo long-term erythroblast renewal due to enhanced differentiation (Figure 1). This defect could be rescued by exposure to fibroblast growth factor (FGF)-1, which also induced progenitor plasticity resembling a transformed phenotype, suggesting the suitability of ESEPs as leukemia models. With collaborators, we will also use the ESEP system to analyse the composition/function of the

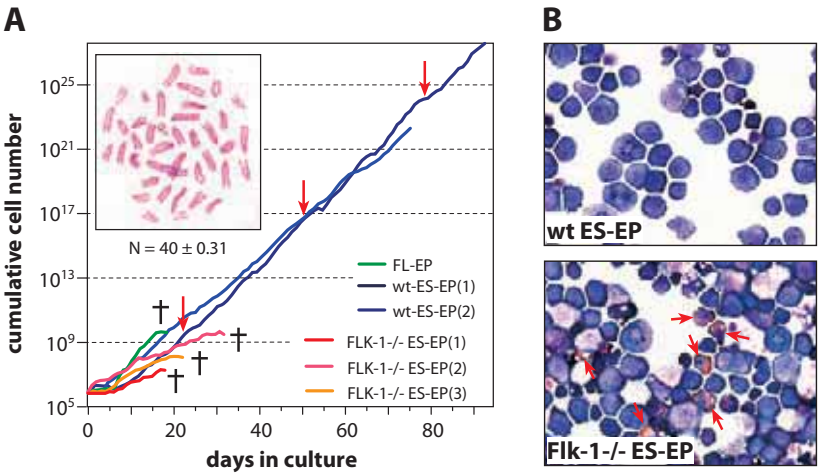


Figure 1: Expansion of wt and mutant ESEPs. (A) wt ESEPs undergo unlimited exponential proliferation (> 110 generations, not shown) while ESEPs from mice lacking the endothelial receptor tyrosine kinase FlK-1 (lethal at ED 7.5) cease to proliferate after 20-30 population doublings, similar to control cells from wt fetal livers. Cells remain diploid (inset, > 40 metaphases counted from 86 day-old cells) and maintain wild-type p53 (analysed at the times indicated by arrows). (B) Cytospins from 25 day-old wt- and Flk1-/- ESEPs stained for hemoglobin (brown) plus histological dyes. Note increased proportion of maturing erythroid cells in the Flk-1-/- cultures (red arrows).

Hartmut Beug

Hartmut Beug / Senior Scientist

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Sabine Maschler / Postdoc
Richard Moriggl / Postdoc
Thomas Waerner[1] / Postdoc
Christoph Gebeshuber[1] / PhD Student
Andreas Mairhofer / PhD Student
Sandra Pilat / PhD Student
Martina Sykora / PhD Student
Memetcan Alacakaptan / Diploma Student
Marc Kerenyi / Diploma Student

Gabi Litos / Technician
Andreas Sommer / Technician
Ido Tamir / Technician

[1] *with BI*

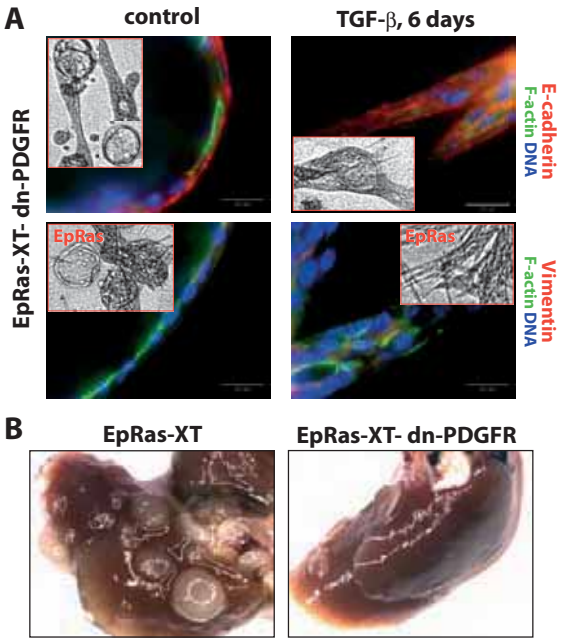

Figure 2: PDGF-receptor signaling is essential for EMT and metastasis. (A) Expression of a dominant-negative PDGF-receptor in EpRas cells after EMT (EpRas-XT) reverts the cells to hollow structures (inset) of polarized epithelial cells (top left, basolateral expression of E-cadherin, red; no vimentin expression, bottom left). These cells depolarize but fail to undergo EMT in response to TGFß (compact, E-cadherin positive structures, right panels/inset). Control EpRas cells before and after TGFß-induced EMT are shown in the bottom left and right insets. (B) Tail vein injection of EpRasXT cells expressing dnPDGFREpH4 cells fail to induce lung metastasis (right) while EpRas-XT control cells cause massive lung metastasis (left).

EpoR/c-Kit-signalosome, its crosstalk with STATs and the GR, and the impact of leukemogenic receptor tyrosine kinases.

In parallel, we analyse the JAK/ STAT pathway in renewing/differentiating erythroblasts from respective mutant mice (EpoR-/-, Jak2-/- STAT5ab^{null}). In collaborations with other groups, the ESEP model will be used to functionally characterize new genes identified from polysome-bound mRNA expression profiling (i.e. the novel type of proapoptotic proteins p12), and their expected functions in self-renewal, differentiation and GR/STAT5 crosstalk.

Genes and signaling pathways essential for epithelial plasticity and metastasis

Epithelial/mesenchymal transition (EMT) and metastasis of polarized mammary epithelial cells (EpH4) expressing oncogenic Ras (EpRas) mirror late stage cancer progression and require a hyperactive Ras-MapK pathway, in cooperation with TGFß-signaling. Polysome-bound mRNA expression profiling identified approximately 30 EMT-specific genes, several of which had already been known to be important for EMT and metastasis, i.e. deltaEF-1, proteins activating NFκB and the PDGF-receptor pathway (Figure 2). Currently, we focus on ILEI, a translationally regulated, secreted protein with no sequence homology in the database. Over-expression of ILEI in EpH4- and unrelated NMuMG cell lines showed that ILEI was sufficient to induce EMT in 3D cultures (collagen gels; Figure 3A). Injections of EpH4-ILEI cells into nude mice resulted in lung metastasis greatly enhanced by co-expressing the anti-apoptotic protein Bcl-2 (Figure 3B). Recently, bioactive, recombinant ILEI could be purified from mammalian cells. Knock-down of endogenous ILEI by RNAi in mesenchymal, metastatic EpRas-XT cells caused them to revert to an epitheloid phenotype and lose metastatic ability, suggesting that ILEI is necessary for EMT and metastasis. Future work will focus on recombinant ILEI. We will analyse ILEI-induced signaling pathways, test whether ILEI acts *via* TGFßR signaling or independently and try to identify the ILEI receptor. We will also analyse ILEI-expression in human tumors and try to generate neutralizing antibodies to ILEI, which should prevent ILEI-induced EMT and might be able to interfere with tumor-growth or metastasis in mice. Finally, ILEI-transgenic and ILEI-knock-out mouse models are being generated with collaborators to study ILEI function *in vivo*.

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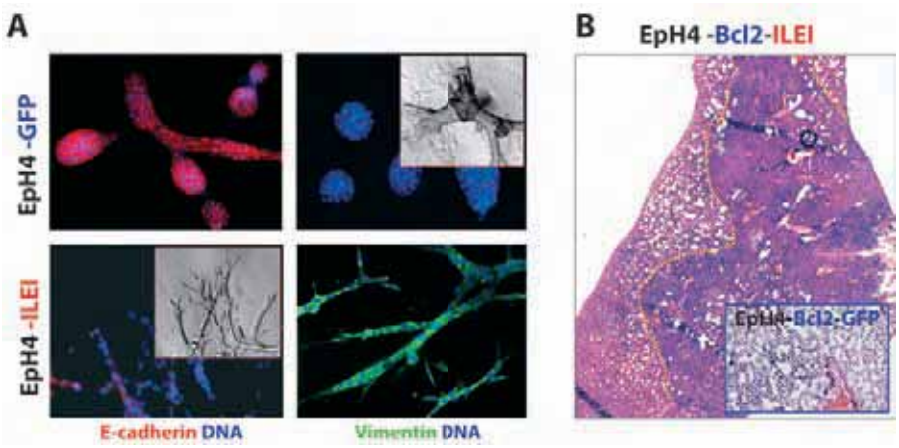

Figure 3: ILEI is sufficient for EMT of EpH4 cells and causes lung metastasis in EpH4-Bcl2 cells. (A) EpH4 cells stably expressing and secreting ILEI (EpH4-ILEI) form disordered structures (inset bottom left) of mesenchymal cells lacking E cadherin (red, bottom left) and express vimentin (green, bottom right) while empty-vector control cells (EpH4-GFP) form compact, E-cadherin positive, vimentin-negative structures (top panels/inset), indicating that EpH4-ILEI cells undergo stable EMT in collagen gels. (B) Histology of a large lung metastasis formed in nude mice after tail-vein injection of EpH4-Bcl2-ILEI cells. Empty vector control EpH4-Bcl2 cells are non-metastatic (inset).

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 a layer of 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 encodes 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.

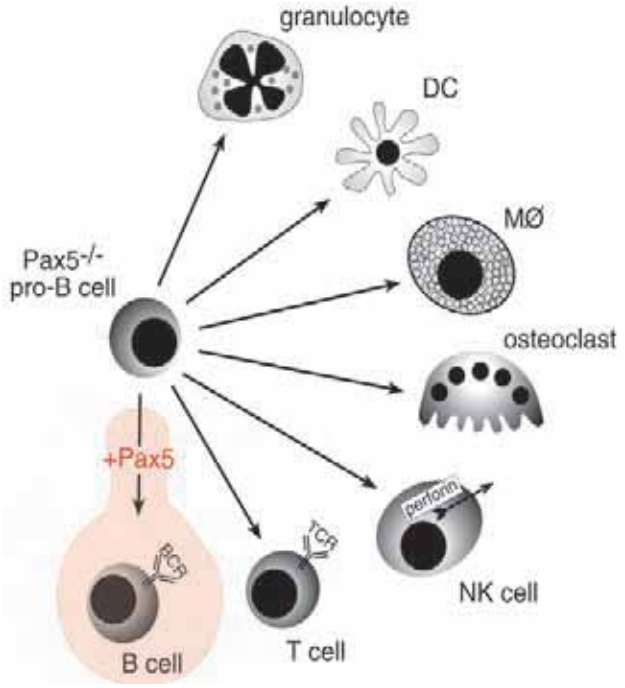

Figure 1: B-lineage commitment by Pax5. Pax5$^{-/-}$ pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.

Meinrad Busslinger / Senior Scientist

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Thorsten Decker / PhD Student
Alessio Delogu / PhD Student
Martin Fuxa / PhD Student
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Giorgia Salvagiotto / PhD Student
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Qiong Sun / Technician

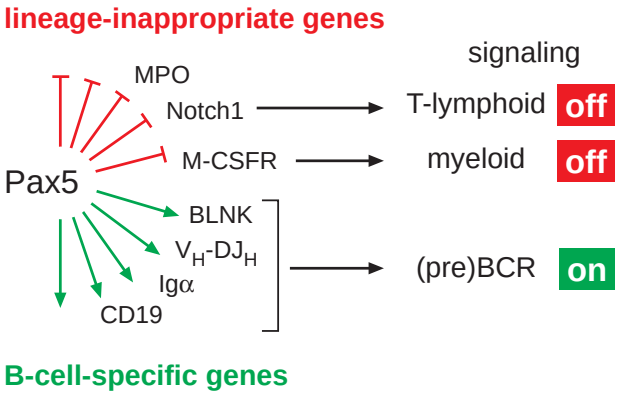

Figure 2: Dual role of Pax5 in B-lymphopoiesis. Pax5 activates B-lymphoid genes (green) and simultaneously represses lineage-inappropriate genes (red).

The immunoglobulin heavy-chain (*IgH*) locus contains ~200 V_H genes, which are spread over a 2.4-Mb region. The question therefore arises 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 the nucleus of Pax5-deficient pro-B cells, the distal V_H genes are separated from the proximal *IgH* region by a large distance, whereas all *IgH* gene segments are colocalized in Pax5-expressing wild-type pro-B cells (Figure 3). Looping of individual sub-domains is responsible for the contraction of the *IgH* locus, which facilitates 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.

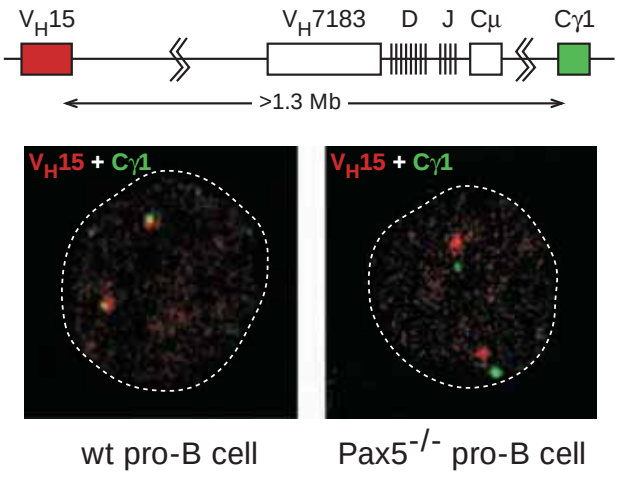

Figure 3: Pax5-dependent contraction of the IgH locus. A schematic diagram of the IgH locus (top) indicates the positions of the different gene segments and DNA probes. Two-color DNA-FISH analysis (bottom) revealed the nuclear positions of the V_H15 and Cγ1 gene segments on a confocal plane containing 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 into immature CD4$^+$CD8$^+$ T cells. Notch-stimulated *Pax5*$^{-/-}$ progenitors rapidly down-regulate the expression of B-cell-specific genes, consistent with a role of Notch1 in preventing B-lymphopoiesis in the thymus. At the same time, Notch signaling induces expression of the transcription factor genes *GATA3* and *Tcf1*, indicating that Notch1 acts upstream of *GATA3* and *Tcf1* in early T cell development. We are currently performing cDNA microarray experiments to better define the role of Notch1 in T cell commitment.

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 to investigate 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 interactions 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 homologues 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 staggered association of two trimeric rings and represents a novel type of a cage-forming protein (Figure 2). The six protease domains construct 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 the Clp proteins.

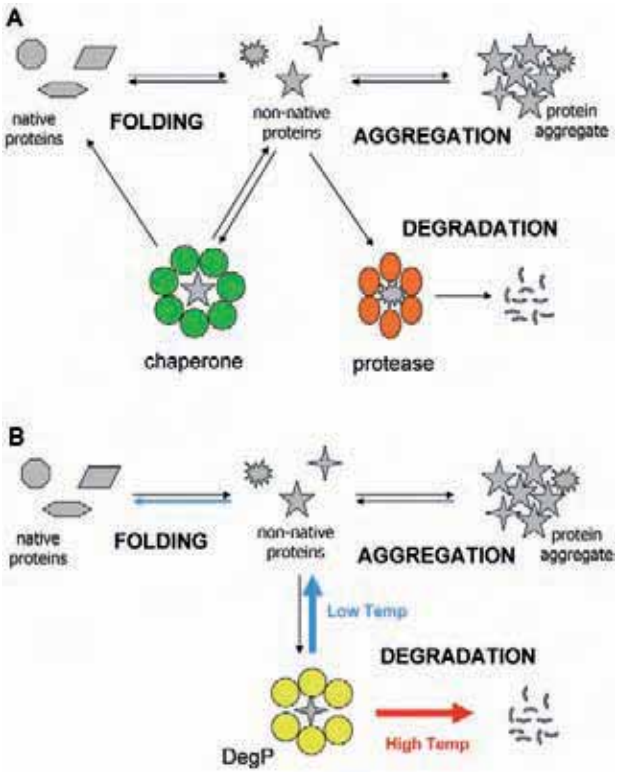

Figure 1: Protein quality control. (A) The scheme illustrates the different fates of misfolded non-native proteins. According to the "kinetic partitioning model" of Gottesman and coworker, aggregate formation is counteracted by the action of proteases and chaperones. (B) DegP combines the antagonistic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

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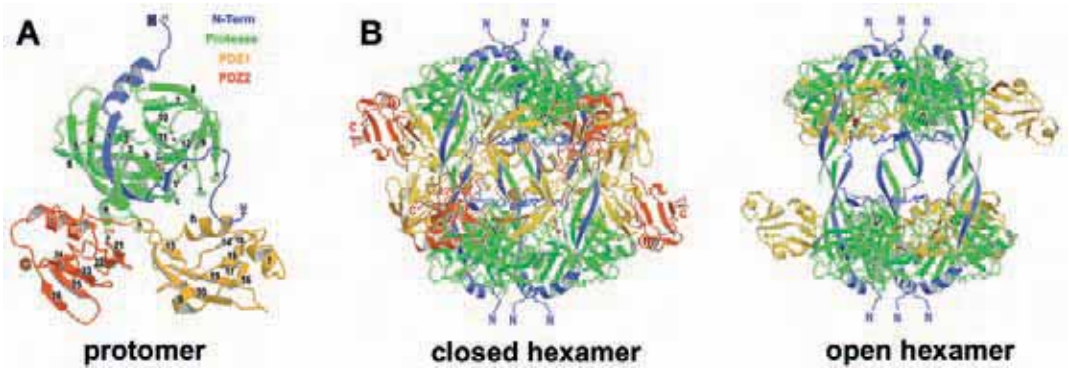

Figure 2: Structure of DegP. (A) Ribbon presentation of the protomer, in which the individual domains are colored differently. Residues of the catalytic triad are shown in a ball-and-stick model. The nomenclature of secondary structure elements, the termini of the protein and flexible regions are indicated.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

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

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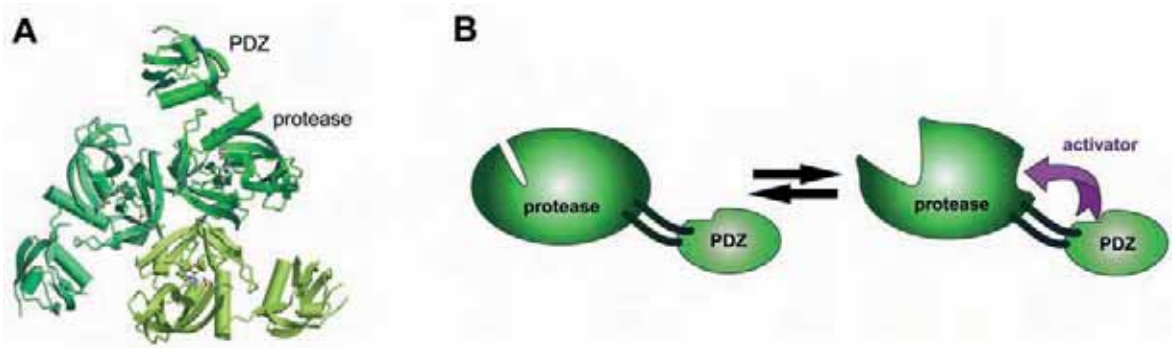

Figure 3: Structure of DegS. (A) Ribbon presentation of the DegS trimer (top view) with each subunit colored differently. (B) Scheme of DegS activation. In DegS, the PDZ domain obtains a regulatory function, offering a binding site for an allosteric activator.

Understanding molecular mechanisms via biomolecular sequence analysis

High-throughput experimental technologies in Life Sciences produce large amounts of uniform data such as biomolecular sequences and mRNA expression values that lack, however, a direct link to biological functions. The combined application of quantitative theoretical concepts and of biological database studies can often provide hints that help to bridge this gap.

Cooperation projects with experimental groups

The mission of the IMP bioinformatics division covers collaborative research activities with experimental groups at the IMP and interacting institutions as well as genuine bioinformatics research on genome text interpretation. The first aspect of our work includes mainly biomolecular sequence analysis at both, protein and nucleic acid levels, but also support in (i) 3D protein structural studies, (ii) general statistical data analysis, (iii) the WWW presentation of results and (iv) database and software development for large-scale experiments. Furthermore, our bioinformatics infrastructure extends a hand to other services that require massive bioinformatics support, such as mass-spectrometry and biooptics groups. The creation of an efficient environment for using biological databases and sequence analysis software in applied projects is the most important technical achievement of the bioinformatics unit. A number of services are available through the internet node (*http://mendel.imp.univie.ac.at*). Our collaboration potential rests on three cornerstones: (a) specialists in sequence

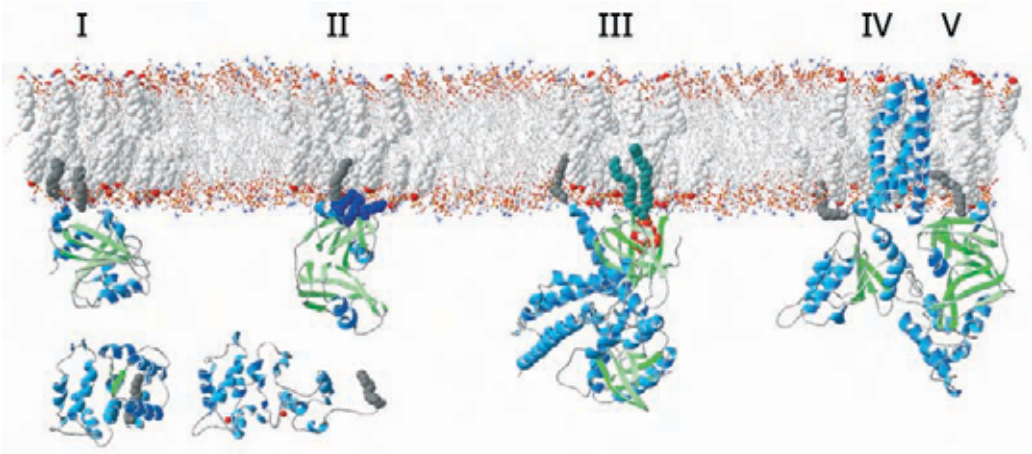

Figure 1: Membrane attachment factors frequently co-occurring with myristoyl-anchors (coMAFs). Subcellular localization, determination and membrane attachment of proteins after their myristoylation depends on several sequence factors. We have analyzed their distribution within sets of known and predicted myristoylated proteins. Five major classes can be distinguished. Subsequent cysteine palmitoylation (class I) and clusters of positive charges (class II) appear to occur in comparable amounts in both known and predicted subsets, while the remaining classes (III – PIP_2-specific binding domain, IV – transmembrane segments, V – other, for example protein-protein interaction) are more densely populated among the new predictions. There are also cases of myristoyl anchors that are hidden in a protein complex and get exposed after conformational changes (lower part).

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Figure 2: Sequence features of query proteins as detected by the ANNOTATOR. Dysferlin is a human membrane repair protein. Disfunction of dysferlin is associated with late-onset muscle dystrophies. This figure shows the sequence architectural detail of human dysferlin. After having executed a large set of sequence-analytic tools over a query protein sequence, the ANNOTATOR presents the positive findings in a concise manner. Sequentially overlapping hits from various methods are easily recognized with the help of rulers. Mouse-over pop-up windows inform about significance measures. For example, the seventh C2 domain of dysferlin (marked with red rulers in the figure) has been found with IMPALA, PFAM (in the global search mode), RPS-BLAST and SMART domain searches. This domain has an insertion in the C-terminal half, illustrated as the interrupted green band, representing two PFAM hits in the local search mode.

and biological data analysis that act upon request from the collaborating groups, (b) ready-to-use sequence analysis workspace for guest scientists that are looked after by our group members and (c) time-efficient protein sequence analyses based on the "ANNOTATOR" suite (see below).

Many genetic screens and cDNA chip studies generate sequences of functionally uncharacterized biomolecules. In such situations, sensitive sequence analyses may produce crucial insights. Hundreds of 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 collaborators.

Protein function prediction is currently the area of computational biology with the highest creative impact on life sciences. Among the results published last year, the GACKIX and the ProflAP domain discoveries were especially notable as well as multiple function predictions of the new cell division and chromatin-associated protein targets. Re-annotation of the CELO virus genome led to a new functional insight into previously uncharacterized genes in the left and right genome parts including a predicted ADP-ribosyltransferase, the first viral example of this enzyme class.

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

The IMP Bioinformatics group is well known for developing highly accurate predictors of lipid post-translational modifications and of peroxisomal localization. Phylogenetic classifications of myristoylated proteins have shown several classes of additional membrane attachment factors (Figure 1). During the last year, predictors of GPI lipid anchor modifications have been extended in their taxonomic range to fungal and plant queries and prototypes of new predictors have been created for farnesylation and geranylgeranylation, two prenyl modifications of proteins.

The ANNOTATOR/NAVIGATOR suite has reached maturity and has become the major tool for protein sequence analysis within the group and among collaborators (Figure 2). More than 30 sequence analytic tools are bundled in the ANNOTATOR so that they can be automatically executed over queries, if suitable, and the results are processed and presented to the researcher in a condensed manner. The NAVIGATOR function allows retrieving information relevant to the projects from the diverse biological databases.

Computer usage and networking within the IMP

Following the wishes of different IMP 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 supported. After new investments, it is now possible to maintain failsafe, 24-hour central services. Routine tasks such as oligonucleotide ordering, stock keeping and trouble ticketing are increasingly supported by specialized software tools.

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The mechanism of cytokinesis

The cell division is not completed 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. The entire process - assembly of the contractile ring and its constriction - requires typically ten minutes. Thus, cytokinesis is a dynamic and spatially regulated process. We are using the nematode *Caenorhabditis elegans* (*C. elegans*) and cultured human cells as model systems to dissect this process. Worm embryos are extremely well suited for real time microscopic analysis (Figure 1). Furthermore, this system can be molecularly dissected using forward and reverse genetics. Human cells are well suited for biochemical analysis and can also be genetically manipulated, using RNAi. We are addressing the following yet unsolved problems: How is the cleavage furrow positioned? How do the contractile ring and the central spindle assemble and function? How is completion of cytokinesis achieved?

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 have 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. We have recently found that mitotic phosphorylation of the ZEN-4 subunit prevents central spindle assembly until the onset of anaphase.

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.

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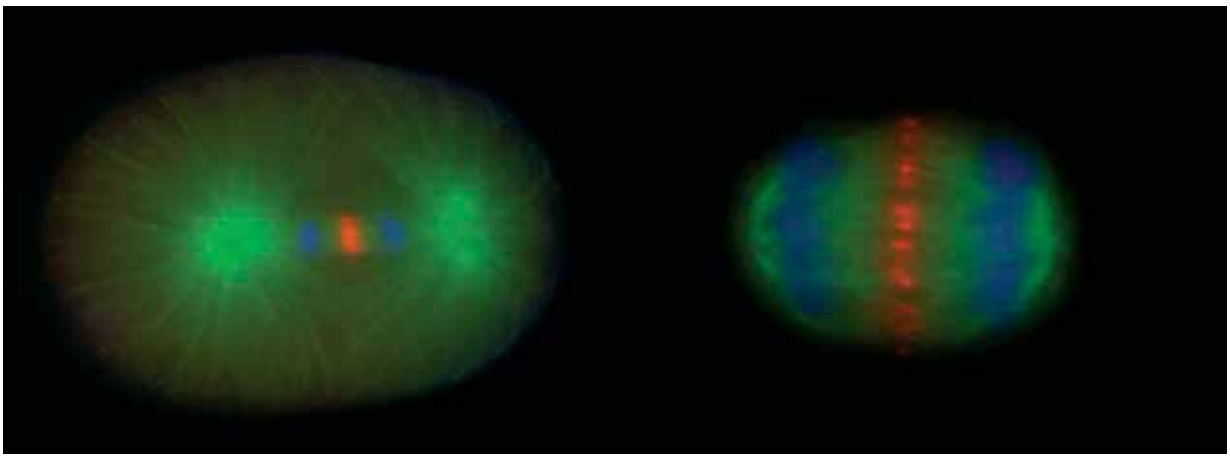

Figure 2: Centralspindlin localizes to the spindle midzone in anaphase. A C. elegans embryo (left) and a mammalian cell have been stained for ZEN-4/MKLP-1 (red), tubulin (green) and DNA (blue).

Using recombinant CYK-4 and ZEN-4 we have reconstituted centralspindlin-mediated microtubule bundling *in vitro* and are using this system to investigate how this kinesin-like protein functions at the molecular level and how its function is regulated in space and time. We have found that ZEN-4 supports motility of microtubules even though it is a structurally divergent kinesin. We are conducting a detailed structure-function analysis of the ZEN-4 motor and the ZEN-4/CYK-4 interaction domains. These biochemical studies will complement ongoing structural collaborations.

Although the central spindle plays a critical role in furrow formation in other systems, *C. elegans* embryos lacking the central spindle are competent to form cleavage furrows. We have discovered that two parallel pathways for furrow formation exist in *C. elegans* embryos (Figure 3). 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.

CYK-4 contains a GAP domain that stimulates GTP hydrolysis by Rho-family GTPases. CYK-4 may promote completion of cytokinesis by its ability to enhance GTP hydrolysis by RhoA. To test this possibility, we have generated human cells and worm embryos in which we can specifically inactivate CYK-4's GAP activity. We aim to determine which hydrolysis defective Rho GTPase causes the same phenotype as the specific deficiency in the GAP activity of CYK-4.

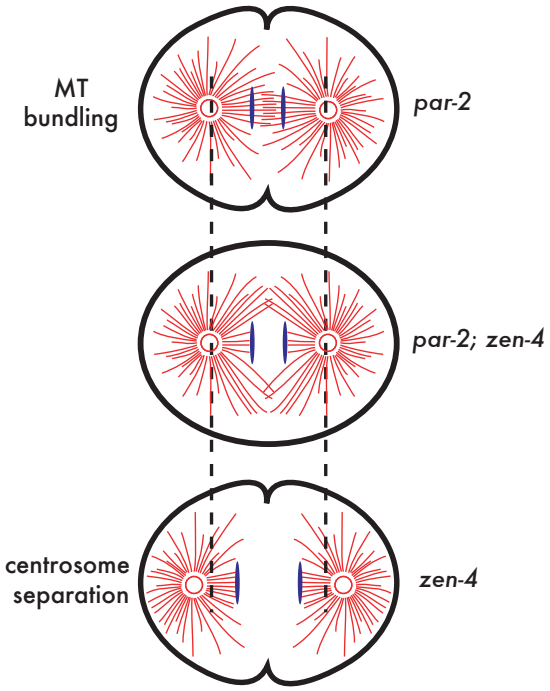

Figure 3: Parallel pathways contribute to furrow positioning. Cleavage furrow formation occurs in embryos that fail to assemble a central spindle, e.g. in zen-4 mutants. Mutant embryos, such as par-2, which show reduced spindle elongation, are also able to initiate and complete cytokinesis. However, zen-4; par-2 double mutant embryos do not form cleavage furrows, demonstrating that these two pathways act redundantly to induce furrow formation.

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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 mouse and 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.

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 mouse, only the loss-of Wnt5a causes skeletal defects. Some of the Wnt-genes expression patterns overlap and it is likely that there exists some redundancy 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, ß-catenin. The resulting knock-out phenotype clearly revealed a role of canonical Wnt-pathway in chondrocyte and osteoblast differentiation, and in the maintenance of joints (Figure 1). Interestingly, all three, chondrocytes, osteoblasts and joint cells share the same mesodermal origin (Figure 2), suggesting that the observed alterations upon loss-of ß-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.

The various skeletal elements that make up 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: Wnt9a controls the expression of a central regulator of chondrocyte maturation in a spatiotemporal 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

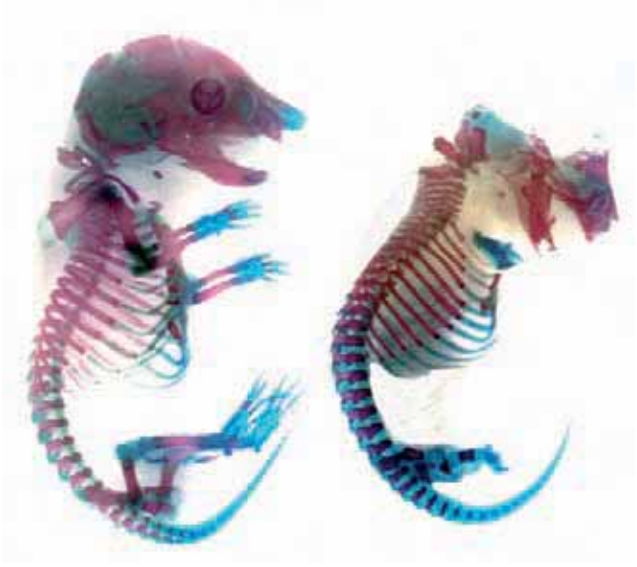

Figure 1: Skeletal preparations of a wild-type (left) and conditional ß-catenin knock-out mouse (right). Cartilaginous regions are stained blue; mineralized areas are stained red.

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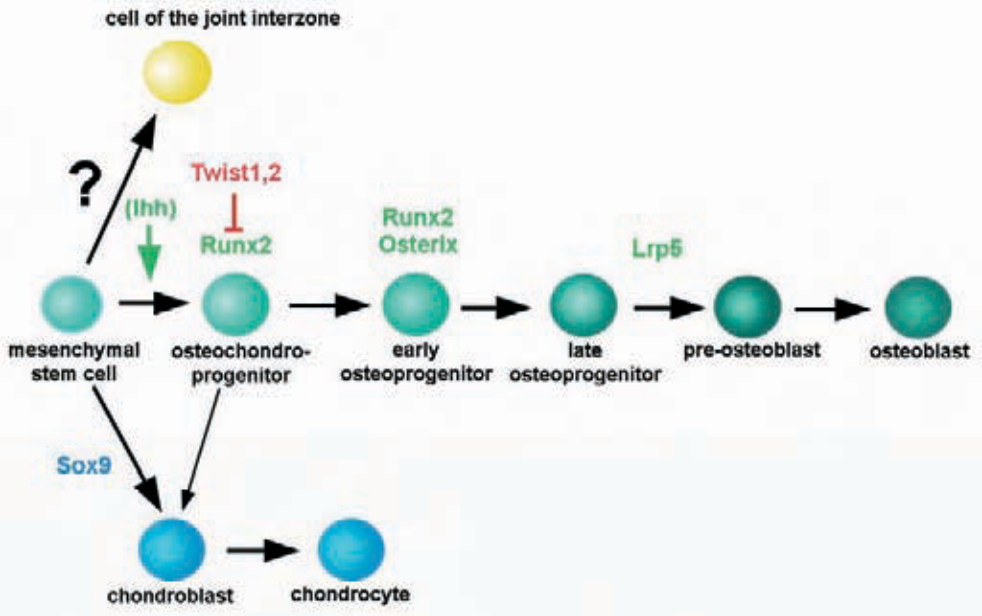

Figure 2: Mesenchymal cells can differentiate into cells of the joint, osteoblasts or chondrocytes. While it has been shown that the transcription factors Runx2 and Osterix are essential for differentiation into osteoblasts and that the transcription factor Sox9 is required for differentiation into chondrocytes, the key-regulators of differentiation into joint cells are so far unknown.

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 in the cells of the joint capsule (Figure 3). Interestingly, the analysis of the Wnt9a knock-out mouse uncovered a role of Wnt9a in maintaining joint integrity of one particular joint. However, loss-of Wnt9a activity alone did not lead to any defects in 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 are addressing this possibility by generating double-Wnt-mutant mice.

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

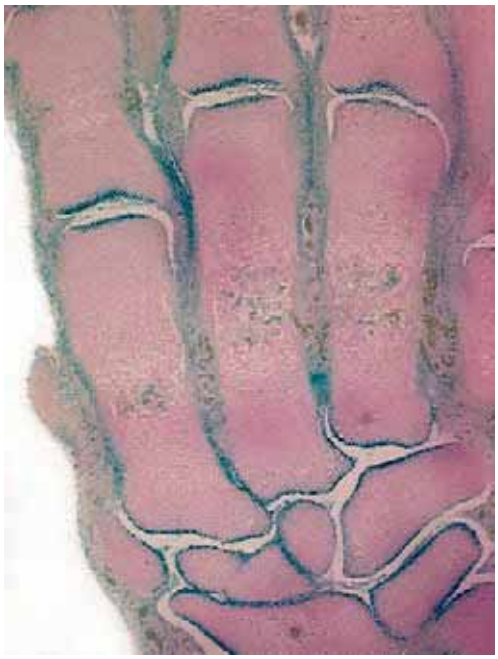

Figure 3: Wnt9a expression in mature synovial joints of the hand visualized by ß-galactosidase staining (green).

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Epigenetic control by histone methylation

In eukaryotes, epigenetic control of gene regulation and the functional organization of chromosomes depend on alterations of the chromatin structure. Recent characterization of histone methyltransferases (HMTases) strongly established histone lysine methylation as a central epigenetic modification of eukaryotic chromatin with far-reaching implications for proliferation, cell-type differentiation, gene expression, genome stability and cancer.

The indexing potential of histone lysine methylation

Histone lysine methylation has been linked to constitutive heterochromatin formation, X inactivation, Polycomb-group (Pc-G) dependent repression and epigenetic gene regulation at euchromatic positions (Figure 1). Each methylatable lysine positioned within the histone N-termini can exist in a mono-, di- or tri-methylated state, which expands the coding potential of this particular modification. We have examined all possible methylation states of histone H3 lysine 9 (H3-K9), histone H3 lysine 27 (H3-K27) and histone H4 lysine 20 (H4-K20) in mammalian chromatin. Using specific methyl-lysine histone antibodies and quantitative mass-spectrometry we showed that pericentric heterochromatin is selectively enriched for H3-K9 tri- and H4-K20 trimethylation. This profile depends on the activity of 'heterochromatic'

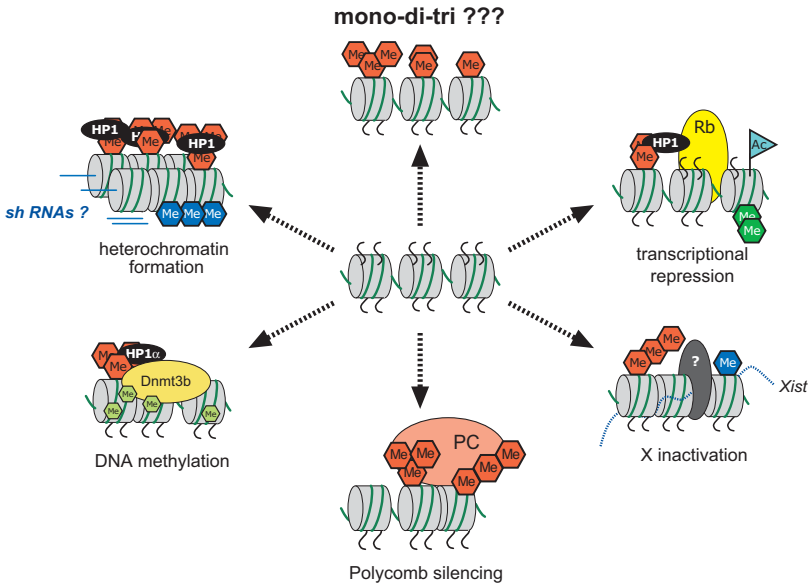

Figure 1: The many faces of histone lysine methylation. The figure summarizes the roles of histone lysine methylation in major epigenetic paradigms. Distinct lysine positions are shown as colour-coded hexagons to indicate H3-K4 (green), H3-K9 (red triangular), H3-K27 (red diagonal) and H4-K20 (dark blue). DNA methylation is depicted by small orange hexagons.

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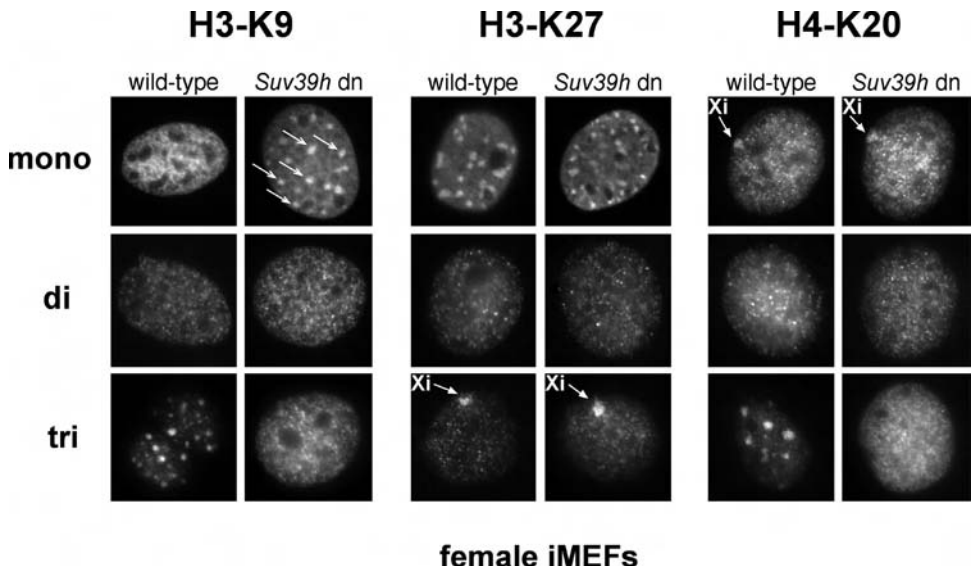

Figure 2: Distinct methylation states as epigenetic landmarks in mouse interphase chromatin. Female wild-type and Suv39h dn iMEFs were stained with methyl-lysine histone antibodies that discriminate mono-, di- and tri-methylation of H3-K9, H3-K27 and H4-K20. Foci of pericentric heterochromatin are decorated by H3-K9 tri-, H3-K27 mono- and H4-K20 tri-methylation in wild-type nuclei. By contrast, the inactive X chromosome (Xi) is enriched for H3-K27 tri- and H4-K20 mono-methylation. H3-K9 mono-methylation of pericentric heterochromatin in Suv39h dn nuclei is indicated by arrows.

Suv39h HMTases and novel Suv4-20h enzymes. The Suv4-20h HMTases are nucleosome-specific and, to be recruited to heterochromatin, require a pre-existing H3-K9 tri-methylation, thus revealing a silencing pathway for the induction of combinatorial histone lysine methylation imprints. Intriguingly, facultative heterochromatin of the inactive X chromosome (Xi) acquires a different methylation pattern (H3-K27 tri- and H4-K20 mono-methylation; Figure 2) that is mediated by the Pc-G enzyme Ezh2 and another HMTase. Our data underscore the combinatorial coding potential of histone lysine methylation as epigenetic landmarks in eukaryotic chromatin.

Plasticity of histone lysine methylation

Murine Suv39h HMTases are encoded by two loci, *Suv39h1* and *Suv39h2*, both of which are widely expressed during embryogenesis. In mature mice, expression of *Suv39h2* is down-regulated in all tissues with the exception of testes. Although single *Suv39h1* and *Suv39h2* null mice are viable, double Suv39h-deficient mice are born at around 30% of the expected Mendelian ratios and display genome instabilities culminating in an increased risk for B-cell lymphomas. In the absence of the Suv39h enzymes, both pericentric and telomeric heterochromatin are severely impaired and attract aberrant methylation imprints (e.g. H3-K9 mono-methylation at pericentric heterochromatin; Figure 2). Similarly, although H3-K9 and H4-K20 tri-methylation appear relatively stable in constitutive heterochromatin of wild-type cells, these and other repressive methylation patterns are under-represented in both quiescent and pluripotent cells. Plasticity of histone lysine methylation is further observed in regenerating cells (selective reduction of tri-methyl states) and in aging and 'stressed' cells (accumulation of perturbed methylation patterns). These data suggest that distinct histone lysine methylation profiles contribute to the differences in the epigenetic 'make-up' of stem cells and of committed cells. To test this hypothesis, we are screening chemical libraries to identify inhibitors of HMTases (collaboration with Boehringer Ingelheim, Ridgefield) that would allow us to modulate histone lysine methylation imprints in cells with various developmental potentials.

An epigenetic map of the mouse genome

In the context of the Austrian GEN-AU initiative (*www.gen-au.at*), we have initiated the large-scale analysis of epigenetic transitions in defined chromatin regions. Using chromatin-immunopecipitation (ChIP) on custom-made genomic microarrays, we have examined the non-random distribution of repressive histone modifications. Our data reveal a selective enrichment of H3-K9 tri-methylation in almost all repeat sequences (tandem satellites, interspersed repeats, DNA and retro-transposons). Subsequently, we will analyze the histone lysine methylation patterns of coding vs. non-coding regions, ultimately establishing an epigenetic map of the entire mouse genome. In addition, we are using a candidate approach to characterize novel HMTases from about 50 SET-domain genes that are present in the mouse genome, and a genetic screen to map all known *Su(var)* genes (around 60) in Drosophila (collaboration with Gunter Reuter in Halle/S., Germany). A deeper understanding of histone lysine methylation systems promises to yield new insights into the mechanisms of the plasticity of cell fate decisions and may offer novel strategies for the reversion of aberrant development.

Grants and other activities

Coordinator of GEN-AU network "*Epigenetic plasticity of the mammalian genome*"(*www.gen-au.at*), combining five Austrian research groups (Barlow, Busslinger, Jenuwein, Seiser, Wutz) to map developmentally regulated chromatin modifications along entire mouse chromosomes.

Coordinator of Network of Excellence (NoE) "*The Epigenome*" (*www.epigenome-noe.net*), combining > 25 European research groups to establish a coherent platform for epigenetic research in Europe.

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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 as well as through the induction of so-called suppressor T cells.

Suppressor T cells

It is well established that encounter of self-antigens 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 (Ts cells). The parameters that influence the choice between these mechanisms of tolerance are not understood. One of our goals is to find out how interactions between T cells and different thymic stromal cell types affect this decision. We are using T cell receptor and antigen transgenic mice in order to be able to follow the fate of "self-specific" T cells in various experimental conditions.

We are also interested in the mode of action of Ts cells *in vivo*. Results from Ts cells characterization *in vitro* indicate that these cells are anergic and that suppression of co-cultured conventional T cells is not mediated by soluble factors. It is unclear how faithfully these characteristics reflect the behavior of Ts cells *in vivo*. Using adoptive transfer of antigen specific Ts cells we have started to characterize their behavior *in vivo*. Unexpected from their *in vitro* characteristics, we found that these cells readily proliferate upon antigen encounter *in vivo*. A co-transferred population of "conventional" T cells of identical specificity was overgrown by these cells. These data reveal a surprisingly dynamic behavior of Ts cells *in vivo* and suggest a mode of action of these cells that may be based on competition for growth factors in an antigen exposed microenvironment.

Another focus of our lab 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 transgenic approaches and chimaeric thymus organ reaggregates.

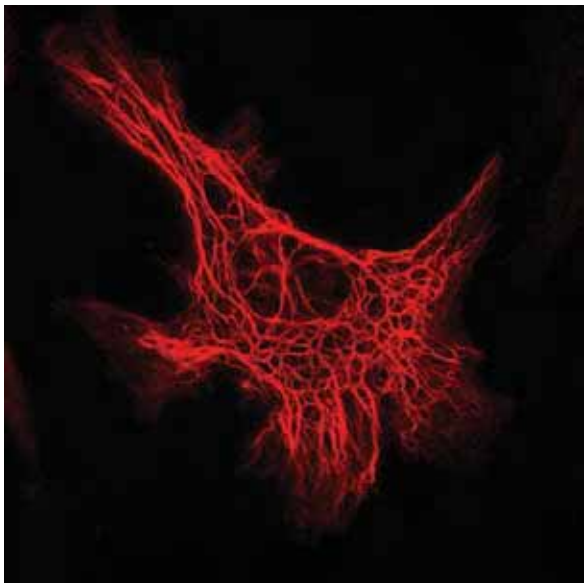

Figure 1: Immunofluorescence of an immortalized thymic epithelial cell, stained for cytokeratin, a typical marker of epithelial cells.

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Ludger Klein / Group Leader

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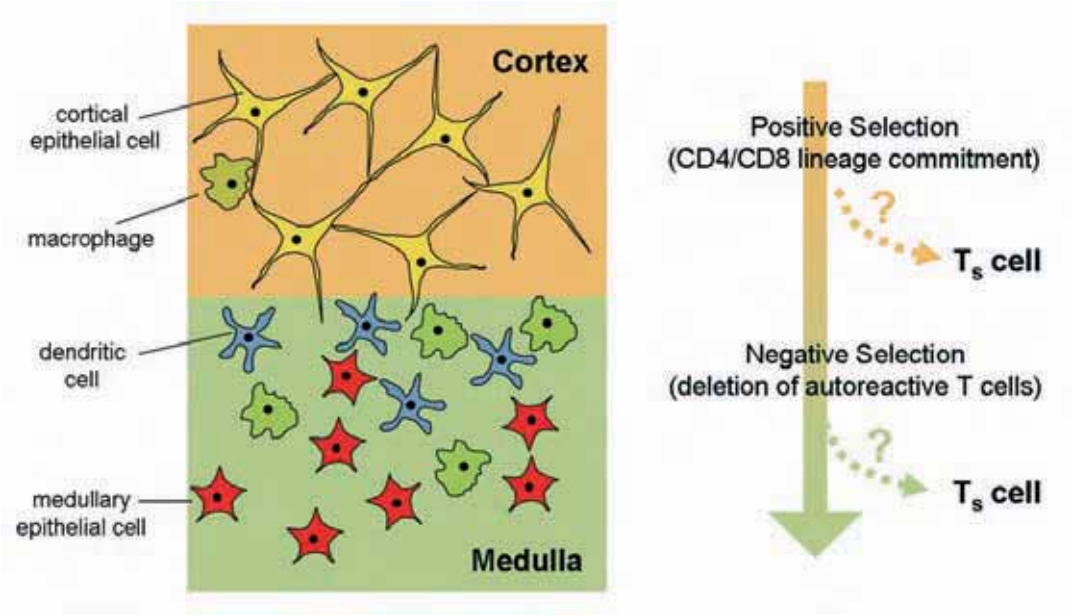

Figure 2: During their maturation in the thymus, developing T cells migrate from the outer cortex to the medulla in a temporally and spatially highly ordered manner. While positive selection for self-MHC restriction occurs upon interaction with cortical epithelial cells, negative selection (removal of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

"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 remains questionable whether these mechanisms are sufficient to cover the entire spectrum of self-antigens, e.g., whether and to what extent these mechanisms operate in case of 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 of this phenomenon, e.g. specific induction versus de-repression of particular genes, is only poorly understood. It was shown that the Autoimmune Regulator (*aire*) gene, a putative transcription factor that is specifically expressed in a not yet characterized subset of mTEC, is involved in "promiscuous" gene expression. Targeted disruption of *aire* leads to reduced expression of numerous self-antigens in mTEC, and *aire*-/- mice develop spontaneous autoimmunity. We have initiated a project that aims (i) to identify, isolate and characterize mTEC that express *aire*, and, (ii) to study the consequences of antigen-expression in *aire*-expressing cells (deletion versus induction of anergy/suppressor function).

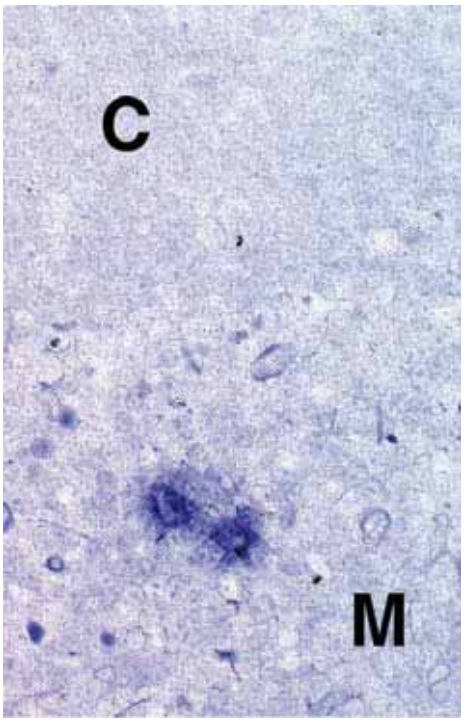

Figure 3: Visualization by in situ hybridization of two cells expressing a „liver-specific" antigen in a medullary region of the thymus (C = cortex; M = medulla).

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Asymmetric cell division in Drosophila

While most cells divide into two identical daughter cells, some cell divisions are asymmetric and give rise to two different daughter cells. To achieve this, cell fate determinants localize asymmetrically during mitosis and segregate into only one of the two daughter cells. Asymmetric cell divisions are best understood in insects and in worms, yet they contribute to the development of the mammalian brain as well.

Numb belongs to a group of proteins that act as segregating determinants during the development of the *Drosophila* nervous system. Numb is a membrane-associated protein. It localizes asymmetrically during mitosis and segregates into one of the two daughter cells, thus establishing its fate to differentiate into a cell type different from its sister's (Figure 1). When Numb is absent (*numb* mutants) or overexpressed, both daughter cells become identical. Numb is conserved in vertebrates. It seems to play a similar role in asymmetric cell divisions that take place during the mouse brain development. Our goal is to understand how Numb and other cell fate determinants localize asymmetrically in the parental cells, and how they influence the fate of one of the daughters.

Asymmetric localization of Numb, and of several other cell fate determinants, requires the conserved Par-protein complex. This complex contains the protein kinase aPKC and two PDZ domain proteins, Par-3 and Par-6. Before mitosis, the Par-complex localizes to the cell cortex at the site opposite to where Numb will accumulate. Using preparative immunoprecipitation and mass spectrometry, we identified an additional component of the Par-protein complex, the cytoskeletal protein Lgl (Figure 2A). Lgl is active at one side of the cell where it allows the recruitment of cell fate determinants to the cell cortex. At the other side of the cell, Lgl is phosphorylated by aPKC. Phosphorylation inactivates Lgl and blocks its association with the cortical actin cytoskeleton. Deletion analysis revealed that the C-terminus of Lgl associates with

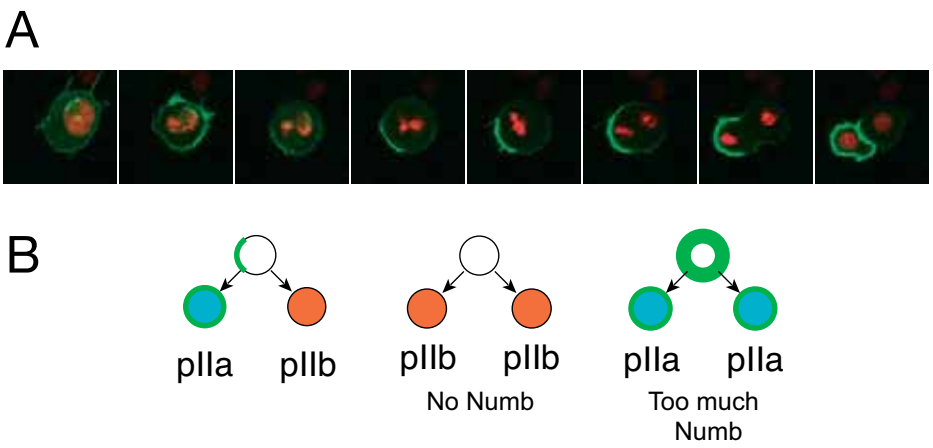

Figure 1: How cells divide asymmetrically. (A) Stills from a movie of a neural precursor cell (SOP) undergoing asymmetric cell division in a Drosophila pupa. Neural precursors are visible as they specifically express an RFP fusion to histone (in red, to visualize DNA) and a GFP fusion to Pon (Partner of Numb, green). Pon is a Numb-binding partner that colocalizes with Numb thus enabling its indirect visualization. (B) In wild-type animals, the two daughter cells of an asymmetric cell division assume different fate. However, in numb mutants or when Numb is overexpressed, both daughter cells become identical.

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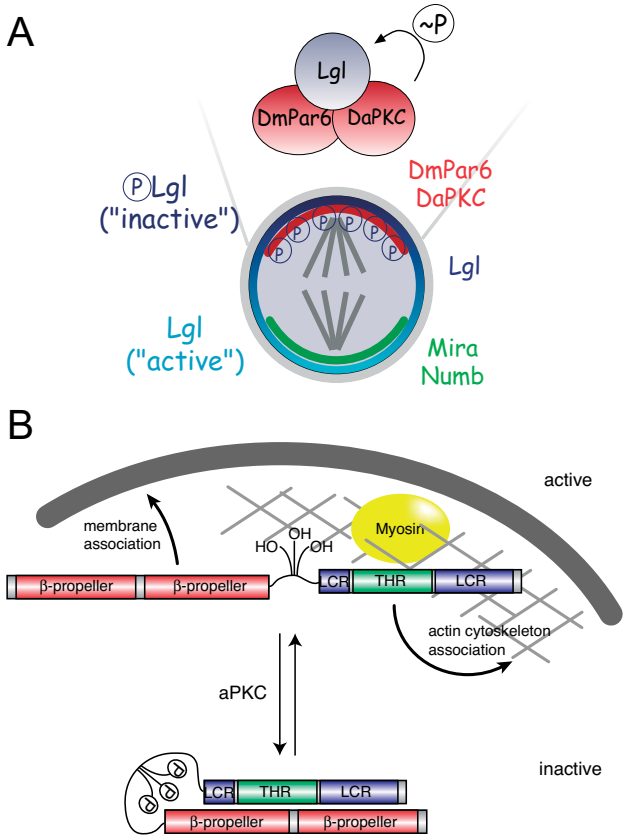

Figure 2: How Lgl directs asymmetric cell division. (A) In neural precursor cells, the Par-protein complex localizes asymmetrically and phosphorylates the cytoskeletal protein Lgl on one side of the cell. Phosphorylation inactivates Lgl. On the opposite side, however, non-phosphorylated Lgl is active and allows localization of cell fate determinants to the cell cortex . (B) In the active form, the C-terminus of Lgl interacts with myosin. Upon phosphorylation, however, the N-terminus binds to the C-terminus abolishing its interaction with myosin. In consequence, Lgl becomes inactive and translocates into the cytosol.

cytoplasmic myosin II. Upon phosphorylation, the N-terminus of Lgl binds to the C-terminus thus blocking myosin interaction (Figure 2B). Hence, Lgl activity is regulated by phosphorylation-induced auto-inhibition. We are using mass spectrometry to identify binding partners of the various functional domains of the Lgl to gain a better understanding of how this protein mediates the localization of cell fate determinants to the cell cortex.

How does Numb establish a particular cell fate? Genetic experiments revealed that Numb represses the activity of the transmembrane receptor Notch. In asymmetric cell divisions, Notch and its ligand Delta are present in both daughter cells. However, Notch is active only in one cell. In the other cell, Notch activity is abolished by Numb-induced endocytosis of Notch and/or of other members of the Notch pathway. Numb triggers this reaction by binding to α-Adaptin, a protein involved in receptor-mediated endocytosis. In a genetic screen for mutations that cause a phenotype similar to *numb*, we identified alleles of *α-adaptin* that specifically disrupted this interaction and displayed defects in the development of the nervous system, consistent with Numb loosing its ability to suppress Notch. Like Numb, α-Adaptin is asymmetrically localized during asymmetric cell division. Numb may therefore act by polarizing the key components of the endocytic machinery. We are currently investigating the subcellular distribution of the major endocytic compartments and analyzing whether other endocytic proteins show an asymmetric distribution as well.

The key components of the asymmetric cell division machinery are conserved in vertebrates. Mouse Numb segregates asymmetrically during mouse brain development. The Par-complex is involved in mammalian cell polarity and, like in *Drosophila*, acts by phosphorylating the Lgl homolog. We have just begun to analyze mammalian homologues of genes involved in asymmetric cell division in *Drosophila*: We are examining their subcellular distribution, and have begun to generate knock-out mouse strains to study their respective mutant phenotypes. These experiments should provide insight into how asymmetric cell divisions contribute to mammalian development and what role they play in stem cells. Ultimately, we hope to understand to what extent asymmetric cell divisions contribute to the development of our own body.

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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 to 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 sister DNA molecules might be entrapped and thereby held together (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 are currently testing whether cleavage of a circular DNA can also release the DNA from the cohesin's ring.

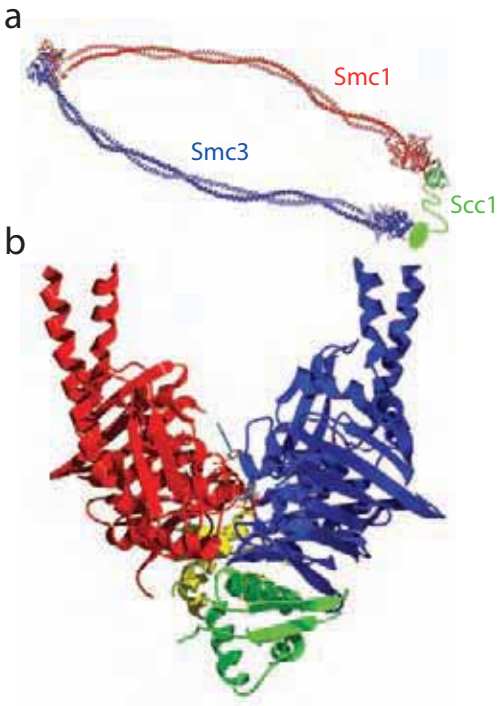

Figure 1: Ribbon models of the yeast Smc1 head homodimer complex with C-termini of Scc1 and of the cohesin ring. (a) Scc1 connects Smc1´s and Smc3´s head domains to form a tripartite ring structure. (b) Two Smc1 head domains (red, blue) dimerize by sandwiching two molecules of ATP analogues in-between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

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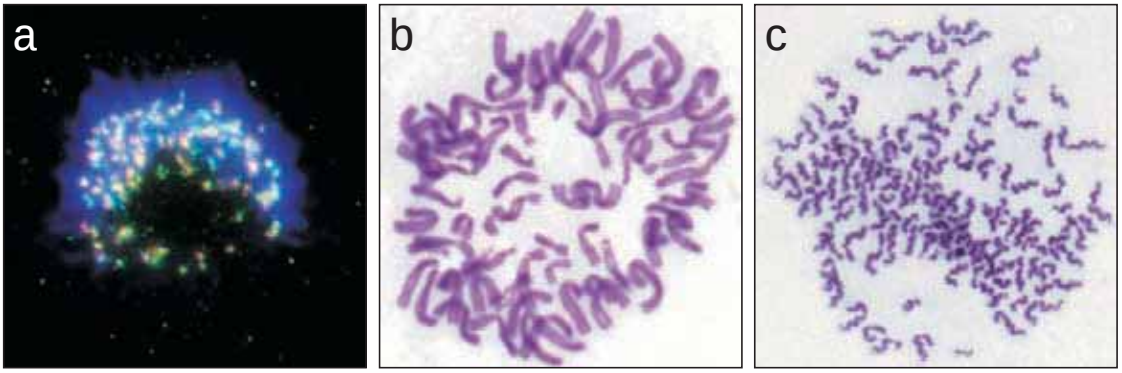

Figure 2: Sgo1 protects centromeric cohesin during mammalian mitosis. (A) Human Sgo1 (green) associates with centromeres (labelled with CREST antiserum shown in red) during mitosis (DNA stained with DAPI shown in blue). (B) Giemsa stained metaphase HeLa chromosome spread. Sister chromatids are resolved 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.

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 hydrolyse 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 ATPγS, a slowly hydrolysable 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 ATPγS 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 goal is to reconstruct the entire cohesin complex from its constituents and to coax it into interacting with DNA *in vitro*.

Loss of sister chromatid cohesion along chromosome arms is essential 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 and are currently studying how Rec8 located in the vicinity of centromeres is protected from separase until the second meiotic division. 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. 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). Future experiments will address how Sgo1 protects cohesin.

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Mitosis

To pass the genome from one cell generation to the next, mitotic cells must package replicated DNA into chromosomes, attach all chromosomes to both poles of the mitotic spindle, and separate each chromosome into its 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. The resulting cohesion between sister chromatids is essential to achieve the bi-polar attachment of chromosomes to the spindle. Subsequently, however, cohesin must be removed from chromosomes to allow 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 and on the mitotic kinases Plk1 and Aurora B. However, cohesin at centromeres is protected from this pathway by the Mei-S332/shugoshin protein Sgo1. Sgo1 is essential to maintain cohesion at centromeres until all chromosomes have been attached to both poles of the spindle in metaphase. At this stage, the protease separase is activated, cleaves centromeric cohesin and initiates sister chromatid separation. We are interested in understanding how SA2 phosphorylation leads to unloading of cohesin from chromosomes, how centromeric cohesin is protected from this mechanism, and how separase activity is regulated.

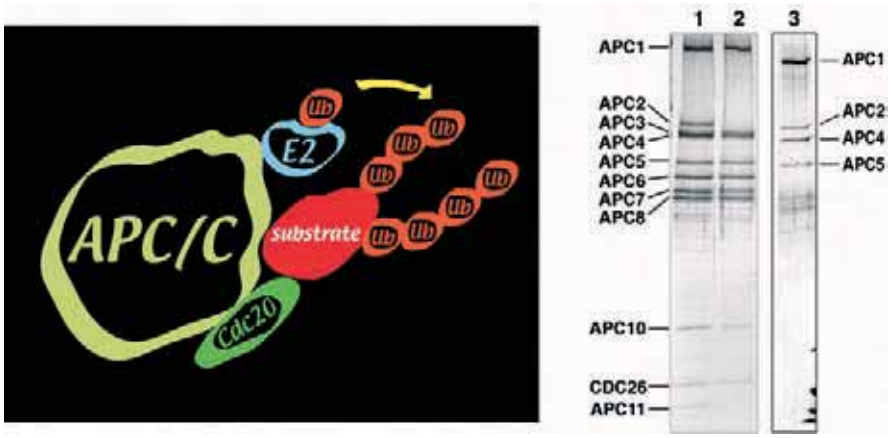

Figure 1: The anaphase promoting complex/cyclosome (APC/C). Left, cartoon view of how the APC/C, E2 enzymes and the Cdc20 activator might assemble polyubiquitin chains (Ub) on substrate proteins. Right, subunit composition of the APC/C (lane 1) and APC/C subcomplexes (lanes 2 and 3) as revealed by SDS-PAGE and silver staining. We are analyzing the roles of individual subunits through the analysis of APC/C subcomplexes. For further details, see Vodermaier et al., Curr Biol. 13, 1459-1468, 2003.

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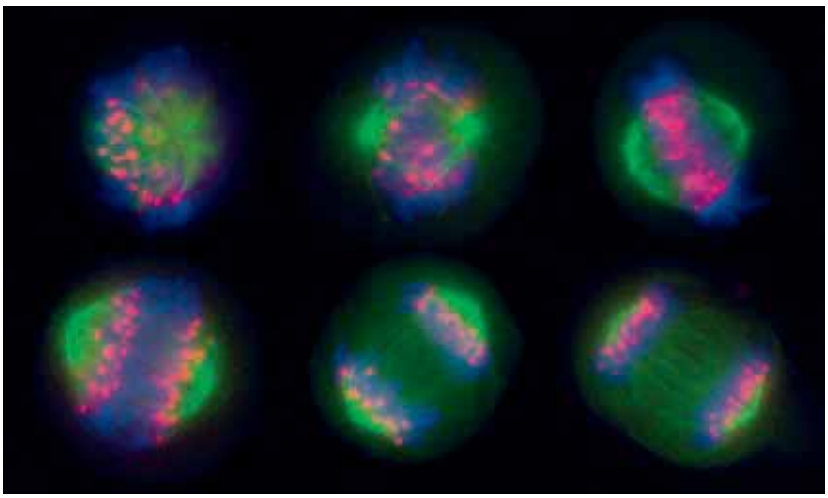

Figure 2: Microscopic images of human cells at different stages of mitosis. Fixed HeLa cells were stained for DNA (blue), microtubules (green) and kinetochores (red). Courtesy of Toru Hirota.

Chromosome condensation

While cohesin is removed from chromosome arms during prophase and prometaphase, two complexes related to cohesin, called condensin I and condensin II, associate with axial domains of sister chromatids: The nuclear condensin I complex binds to chromosomes in prophase, whereas the cytoplasmic condensin II complex associates with chromosomes after nuclear envelope breakdown in prometaphase. In the absence of condensins chromosomes are able to condense, but condensation is delayed and the chromosomes become less rigid than those that contain condensins. We would like to find out how condensins confer rigidity to chromosomes, and which other molecules contribute to chromosome condensation.

Chromosome bi-orientation

While chromosomes condense, kinetochores assemble on centromeric DNA, become captured by spindle microtubules and bi-orient all chromosomes on the mitotic spindle. A surveillance mechanism, called the spindle checkpoint, ensures that anaphase does not begin until all chromosomes became bi-oriented. Both, Aurora B and Plk1 have been implicated in this process and we began to analyze the underlying mechanisms by using RNA interference, chemical inhibitors and video microscopy. We identified Hesperadin as an inhibitor of Aurora B and revealed that this kinase is required to correct syntely - an erroneous attachment of both sister kinetochores of a chromosome to one spindle pole. Aurora B's correction function is also required for proper functioning of the spindle checkpoint. In contrast, Plk1 appears to function in the assembly of spindles that generate pulling forces, required to stabilize microtubule-kinetochore interactions, and in silencing of the spindle checkpoint. In the future we would like to identify Aurora B and Plk1 substrates controlling chromosome bi-orientation (see MitoCheck project below).

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 phosphorylation and binding of the activating subunit 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. To analyze the structure of APC/C we are collaborating with crystallography and electron microscopy groups.

MitoCheck

Protein kinases have long been recognized to play a central role in mitosis control. However, their substrates and the effect of phosphorylation on their activities remain elusive. We have founded an Integrated Project called MitoCheck (*www.mitocheck.org*) within which eleven European institutes will co-develop approaches to systematically study mitosis. MitoCheck is funded through the 6th Framework Programme of the European Union and its main goals are: (i) to identify human proteins required for mitosis by using genome-wide RNA interference screens, (ii) to systematically map mitotic phosphorylation sites on these proteins by using mass spectrometry, and (iii) to determine which kinases are needed for the generation of which phospho-sites by using chemical biology approaches.

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AP-1 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 *cre*M, 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. Preliminary data suggest that Fra-2 is essential for postnatal mouse development and maintenance of proper bone mass. Moreover, transgenic mice over-expressing Fra-2 have increased bone mass. We are currently investigating the underlying molecular mechanisms leading to the bone disease.

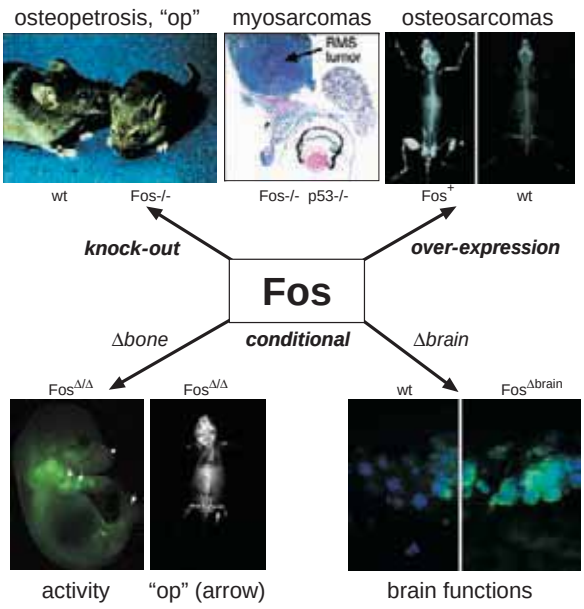

Figure 1: Functional analysis of Fos in bone and CNS development, and the dual role of Fos as an oncogene and an anti-oncogene.

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.

Erwin F. Wagner

Erwin F. Wagner / Senior Scientist and Deputy Director

Robert Eferl / Staff Scientist
Latifa Bakiri[1] / Postdoc
Juan Guinea[2] / Postdoc
Peter Hasselblatt[3] / Postdoc
Astrid Hoebertz[4] / Postdoc
Lijian Hui[1] / Postdoc
Lukas Kenner[5] / Postdoc
Domenico Migliorini[6] / Postdoc
James Witowsky / Postdoc
Ewa Stepniak / PhD Student
Florian Karreth[7] / Diploma Student

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[1] *EMBO fellowship*
[2] *P3 +3 CNIC grant; since November 2004*
[3] *DFG grant; since July 2004*
[4] *Marie Curie grant; until August 2004*
[5] *until March 2004*
[6] *RTN-EU grant; since March 2004*
[7] *until February2004*
[8] *part-time*

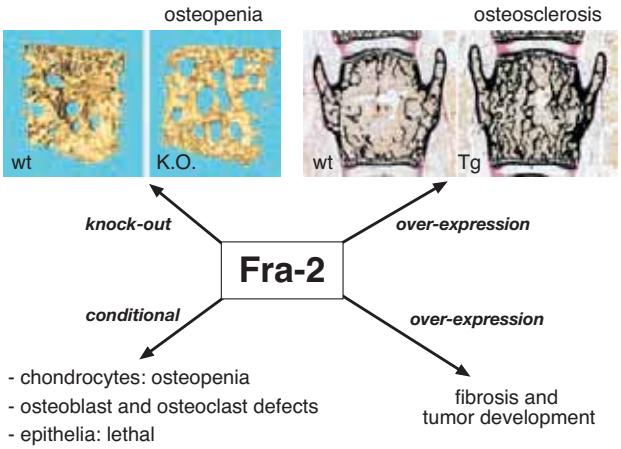

Figure 2: Functional analysis of Fra-2 in bone development and disease.

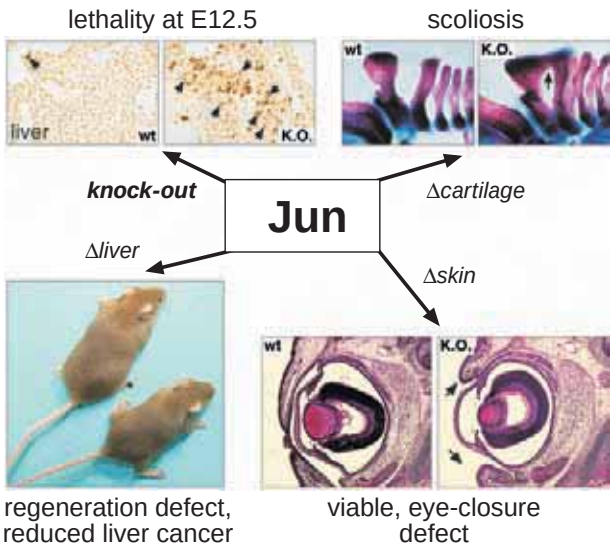

Figure 3: Functional analysis of Jun in development and in disease.

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 AP-1 proteins in liver cancer. Deletion of *jun* in 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-regulation of transcriptional targets of Jun, HB-EGF and EGFR. We are currently investigating the role of Jun proteins in human skin diseases such as psoriasis.

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 and EGF-R

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. We also investigate the functional importance of VEGF-A in developing chondrogenic tissues and in skin biology in collaboration with Erwin Tschachler, Medical University of Vienna and the role of EGF-R in normal and tumor skin development in collaboration with Maria Sibilia, Medical University of Vienna.

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Mammalian X-chromosome inactivation

For successful development the information stored in the genome needs to be precisely tuned. During differentiation each individual cell uses an everchanging 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 between the sexes in X-linked genes. This is achieved by transcriptional silencing of one of the two X-chromosomes in female cells. *Xist* is the critical regulator of this silencing process. The gene encodes a large non-coding RNA that is transcribed from the inactive X and associates physically with the chromosome along its entire length triggering gene silencing.

Functional analysis of histone methylation patterns at the initiation of X-inactivation

In a collaborative effort with the group of Thomas Jenuwein we have begun to elucidate the kinetics of histone methylation during X inactivation using an inducible *Xist* expression system in mouse embryonic stem (ES) cells (Figure 1). In this system the ability of *Xist* to trigger silencing is restricted to an early time window during ES cell differentiation. This window is also important for establishing methylation patterns on the potential Xi. Immunofluorescence and chromatin immunoprecipitation experiments revealed that both, 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 methylation events depend on *Xist* RNA localisation 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 (Figure 2). Our data show that *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.

Ectopic initiation of X-inactivation 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 characterise 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 a severe anaemia that eventually can 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.

Anton Wutz

Anton Wutz / Group Leader

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Karen Ng / PhD Student
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Fabio Savarese / PhD Student
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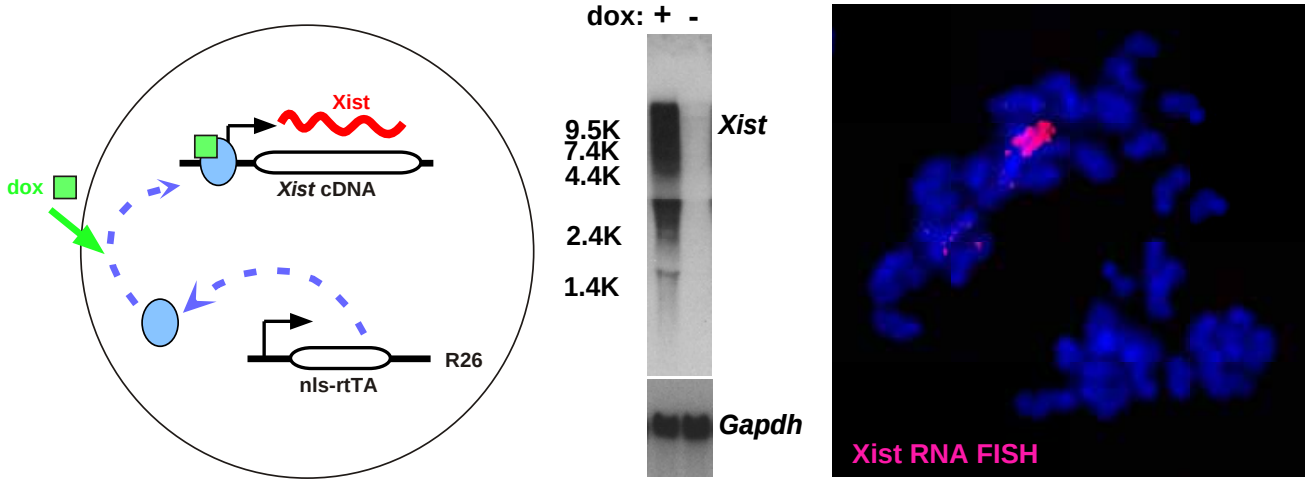

Figure 1: Inducible Xist expression in mouse ES cells. Using a tetracycline inducible promoter Xist RNA can be expressed from cDNA transgenes integrated at different positions in the genome. RNA FISH demonstrates that transgenic Xist RNA localises specifically to the chromosome from which it is transcribed. Mouse metaphase 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 counterstained with DAPI (blue).

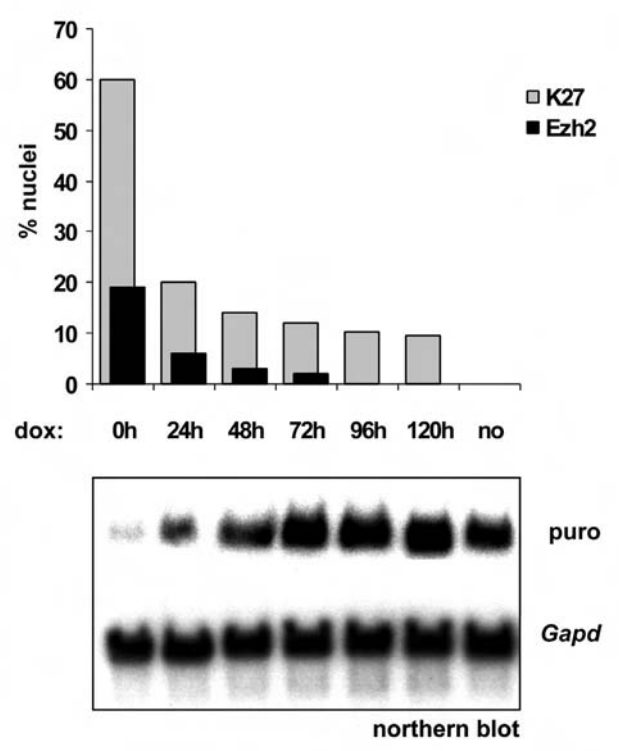

Figure 2: Restriction of efficient H3K27m3 and initiation of transcriptional repression coincide in time during ES cell differentiation. Transgenic Xist expression was induced at various time points in differentiating ES cells. The levels of histone methylation and the effect on the expression of a puromycin 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.

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 to isolate and characterise proteins that interact with the repeat A of *Xist* RNA, a crucial element for initiation of silencing. We have isolated proteins from nuclear extracts that specifically interact with the sense but not with the antisense repeat A RNA (Figure 1). In collaboration with Karl Mechtler form the IMP Protein Chemistry Facility we have identified candidate proteins, all of which contain RNA binding motifs. Ongoing experiments focus on the functional analysis of the candidate proteins and on the identification of the pathway of *Xist* mediated transcriptional silencing.

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Service Department

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

Sequencing and DNA isolation

Using two ABI 3100 Genetic Analyzer capillary sequencers, and, since June, an additional ABI 3730 DNA Analyzer, we sequenced approximately 41'000 samples in the first 9 months of this year (see figure). This is a substantial increase as compared with 2003. The increase has been caused not only by the acquisition of "new customers" from IMBA but also by generally higher number of requests and "genetic screens" as compared with the previous years. The average read-length on both capillary sequencers, the 3100 Genetic Analyzers equipped with the 80 cm capillaries, and the 3730 DNA Analyzer equipped with the 50 cm capillaries, is comparable (700-900 bases) for standard DNA samples. However, the 3730 DNA Analyzer is more sensitive and requires smaller amounts of expensive reagents, and of DNA. For both platforms we employ the same easy and fast clean-up protocol with Sephadex columns on a 96-well microtiter plate.

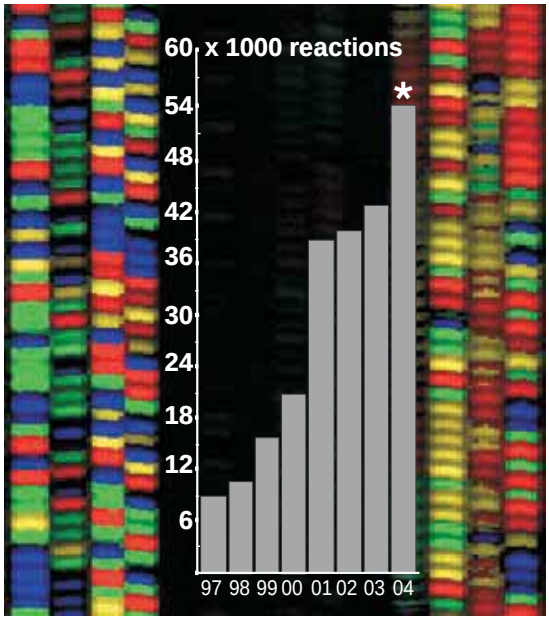

Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (1997 - 2001), on ABI 3100 (2001 - 2004) and on ABI 3730 (since June 2004) done with dye deoxy terminators (v3.1) in the years 1997 to 2004 (scale 0 to 60'000).
**calculated from January 2004 to September 2004 data*

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Production of antibodies

Our department is also responsible for the production and isolation of continuously increasing amounts and varieties of monoclonal antibodies in hybridomas, and for organizing the antibody production in rabbits with an outside company.

Preparation of solutions and media

Our Media Kitchen prepares substantial quantities of reagent quality solutions and media for cell culture, *D. melanogaster* (approximately 500'000 bottles and tubes per year) and *C. elegans*. 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.

Service Department

Gotthold Schaffner / Scientist

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

Biooptics Department

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

Flow cytometry

This year, in response to increasing demands for multicolor flow cytometric analyses, a new FACSCanto flow cytometer was installed allowing acquisition of data from cells simultaneously labelled with up to six different fluorochromes.

Microscopy and image analysis

The major accomplishment of the microscopy unit in 2004 was the establishments of a variety of 4D-technologies that utilize laser scanning and spinning disk confocal microcopy, wide-field fluorescence microscopy and deconvolution technology. As the number of users and the amount of generated images steadily increases, an institute-wide database system for image management is currently being implemented.

Microarrays

This year we were able to dramatically improve the quality of our cDNA-microarrays by reengineering almost all of the steps of the array production. The generation and purification of the PCR-products is now highly automated, and, by changing the buffer conditions the printing of arrays is independent of environmental conditions such as temperature and humidity. The improved quality of the arrays allows faster and more reliable analysis of the data. An additional benefit of the reengineering is an approx. 50% reduction of the costs *per* array.

Together with the group of Thomas Jenuwein, we are building up the infrastructure and technology to generate a high-resolution epigenetic map of the mouse chromosome 17 using the ChIP-on-chip technology. So far we have produced microarrays enabling us to map the non-repetitive sequences with an average resolution of approximately 15kb. Next year we plan to increase the resolution to 5kb. To refine our methodology we are yet optimizing array production, sample preparation and downstream analysis. In addition, in order to visualize the data, a web-based epigenome-browser is being developed in collaboration with Insilico Software, Vienna. The browser will be inter-connected with other tools and databases available in our department.

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Figure: Example of a ChIP-on-chip experiment. First generation genomic array is hybridized with input DNA (red) and anti-Trimethyl-H3-K4 (green) from mouse embryo fibroblasts. (Data provided by Joost Martens, Group Jenuwein).

Peter Steinlein / Staff Scientist

Sebastian Carotta[1] / Postdoc
Volker Leidl / Software Architect
Karin Paiha / Microscopy and Imaging
Pawel Pasierbek[2] / Microscopy and Imaging
Martin Radolf / Microarrays
Gabriele Stengl / Flow Cytometry

[1] *until August 2004*
[2] *since September 2004*

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 three main areas containing the following species: mice, chicken and *Xenopus*. The largest 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 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 ordering of mice from external breeding companies, 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.

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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.

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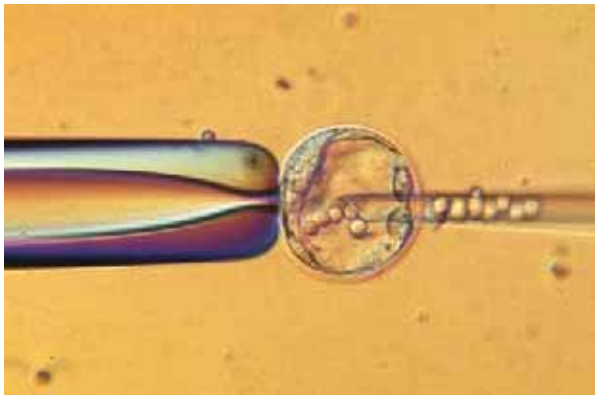

Animal House

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

Mouse Service Department

Hans-Christian Theussl / Technician

Protein Chemistry Facility

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

Shotgun 2D Proteomics

Tandem mass spectrometry (MS/MS) experiments generate short stretches of sequence information but in most cases only a small fraction of all generated peptides can be recovered and analyzed. To overcome this problem we developed a "shotgun" approach: Protein complexes are purified by immunoprecipitation and digested by different enzymes, the generated peptides are separated by multi-dimensional liquid chromatography and analyzed by MS/MS. By combining the high sensitivity and the resolution of nanoscale multi-dimensional liquid chromatography with the precise structural specificity of MS/MS spectral data the sites and types of modifications are identified in large portions of the sequence of the protein complexes. This approach is particularly important for analyzing the structure of multi-subunit protein complexes. In addition we designed a web-based program called "Mascot Protein Extractor" (see figure) for rapid merging and comparing of sequence search engine results from multiple LC-MS/MS peptide analysis (*http://extractor.imp.univie.ac.at*).

Figure: Mascot Extractor - software for merging and comparing mass spectrometry data.

Post-translational modifications

Protein phosphorylation is the most important reversible post-translational modification. Thus, analysis of phosphorylated proteins and identification of the phosphorylation sites helps us to understand their biological functions. Our group develops strategies to improve the sensitivity and selectivity of phosphorylation analysis techniques such as:

- Immobilized metal affinity chromatography (IMAC)
- Beta-Elimination of the phosphate group and Michael addition with 2-aminoethanethiol
- Neutral loss and precursor ion scan with our new QTRAP 4000 mass spectrometer.

Post-translational modifications of histones, e.g. methylation, can modulate transcription according to the 'histone code' hypothesis. Mass spectrometry (MS) has proven a valuable tool to identify and quantify changes in histone methylation patterns. In quantitative MS, it is essential to correct for different efficiencies of ionization and detection of differentially modified peptides. These efficiencies are measured using isotopically labeled synthetic peptides as external standards. The labeled peptides are excellent tools for proteomics and are used in applications ranging from absolute quantification of protein abundance and modifications to determination of complex stoichiometry.

Peptides and antibodies

We synthesize about 150 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.

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Protein Chemistry Facility

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Nedim Mujezinovic / PhD Student
Richard Imre / Technician
Gabriela Krssakova / Technician
Mathias Madalinski / Technician
Goran Mitulovic / Technician
Michael Schutzbier / Technician
Ines Steinmacher / Technician
Christoph Stingl / Technician
Sebastian Schmittner / Summer Student
Reinhard Wohlfart / Summer Student

MANAGEMENT

Kim NASMYTH	Managing Director
Erwin F. WAGNER	Deputy Director
Harald ISEMANN	Administrative Director

MANAGEMENT ASSISTANTS

Diane TURNER	Executive Assistant
Gerti KÖLBL	Assistant to the Administrative Director

The following services are part of the joint service concept between the IMP and IMBA (employment status indicated in brackets). For detailed description of shared scientific services see pp. 34-37.

GENERAL ADMINISTRATION

Engelbert BERGER	Head of Controlling (IMP)
Birgit GRUBER	Secretary (part-time; IMP)
Larissa KAHR	Grants Manager (IMP)
Werner LEITNER	Head of Personnel Department (IMP)
Sabine SVOBODA	Personnel Officer (part-time; IMP)
Brigitte WEISER	Chief Accountant (IMP)
Anita SOMMER	Assistant Accountant (IMBA)
Eva-Maria RUDLOF	Secretary (part-time; IMP)
Renate BICHLER	Travel Agent (IMP; until March)
Robert LASETZKY	General Assistant / Driver (IMP)

SCIENTIFIC SECRETARIAT

Diane TURNER	Executive Assistant (IMP)
Virginia SALVA MILLAN	Assistant to the Senior Scientists (IMP; until August)
Christopher ROBINSON	Assistant to the Senior Scientists (IMP; since August)

PUBLIC RELATIONS

Heidemarie HURTL	Public Relations Officer (part-time; IMP)

PURCHASE DEPARTMENT

Friedrich KUNTNER	Head of Purchase Department (IMP)
Kashinath MITRA	Store Manager (IMP)
Angela GANGLBERGER	Secretary (IMP)
Brigitte LENZ	Secretary (IMBA)
Nikolaus KUNTNER	Warehouseman (part-time; IMBA)

LIBRARY

Susanne VRBA	Chief Librarian (part-time; IMP)
Wolfgang GÖSCHL	Assistant Librarian (part-time; IMP)

BIOINFORMATICS SERVICE

(provided by the IMP group of Frank Eisenhaber)

IT SERVICE DEPARTMENT

Andreas RIEPL	IT System Manager (IMP)
Werner KUBINA	IT System Manager (IMP)
Herlind WURTH	IT System Manager (IMBA)

GRAPHICS DEPARTMENT

Hannes TKADLETZ	Graphics Service (IMP)
Jola GLOTZER	Web Maestra (part-time; IMP)

TECHNICAL DEPARTMENT

Alexander CHLUP	Chief Engineer (IMP)
Martin COLOMBINI	Mechanical Workshop (IMP)
Nadir AYKUT	House Maintenance (IMP)
Christian DAVID	House Maintenance (IMP)
Vladimir KOLCAR	House Maintenance (IMP)
David KUMMERER	Technical Maintenance (IMBA)
Gerhard MÜLLER	Technical Maintenance (IMP)
Martin ROJDL	House Maintenance (IMBA)
Grete KOCINA	Receptionist (part-time; IMP)

HISTO LAB

Vukoslav KOMNENOVIC	Head of the Histo Lab (IMBA)

WASHING KITCHEN

Nuray KILIC	Laboratory Technician (IMP)
Erika KLEIN	Laboratory Technician (IMP)
Renate STIX	Laboratory Technician (IMP)
Renate WÖHRER	Laboratory Technician (IMP)

CAFETERIA

Michael ENZBERGER	Chef de Cuisine (IMP)
Markus GIGLER	Junior Chef (IMP)
Helga MEHOFER	Buffet (IMP)
Sabine SMALCL	Buffet (part-time; IMP)
Güler CICOS	Washing up (part-time; IMP)
Selma YILDIRIM	Washing up (part-time, IMBA)

Publications

GROUP BEUG

Bakker, W. J., Blazquez-Domingo, M., Kolbus, A., Besooyen, J., Steinlein, P., Beug, H., Coffer, P. J., Lowenberg, B., Von Lindern, M., and Van Dijk, T. B. (2004). FoxO3a regulates erythroid differentiation and induces BTG1, an activator of protein arginine methyl transferase 1. J. Cell Biol. 164, 175-84.

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PROTEIN CHEMISTRY FACILITY

Hauf, S., Roitinger, E., Koch, B., Dittrich, C., Mechtler, K., and Peters, J. M. (2005). Dissociation of cohesin from chromosome arms and loss of arm cohesion during prophase and prometaphase depends on phosphorylation of SA2. PloS Biology, in press.

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Publications

Awards and honours

Richard Moriggl
Received the second prize in the Aventis "Otto-Kraupp-Preis 2003" for the best habilitation in medicine (August 2004).

Joerg Betschinger
Received the award from the Austrian Society for Biochemistry and Molecular Biology for his PhD thesis (September 2004).

Karl Mechtler
Received a science prize of the federal state of Lower Austria for his research in protein chemistry (September 2004).

Frank Eisenhaber
Was awarded the Glasser Visiting Professor Award by the University of the Sciences in Philadelphia, USA (October 2004).

Michael Glotzer
Was elected EMBO Member (October 2004).
Was named Keith R. Porter Fellow (December 2004).

Anton Wutz
Was selected for the EMBO-Young Investigator's Programme 2004 (November 2004).

Maria Novatchkova
Mark Petronczki
Received Campus Vienna Biocenter PhD Award (December 2004).

Seminar speakers

JANUARY

08.01.04	PETER STALLER (ETH Zürich)
13.01.04	MASAHIKO HARATA (Grad. School of Agric. Sci., Tohoku Univ., Japan)
15.01.04	NIKOLAUS PFANNER (Univ. of Freiburg, Germany)
16.01.04	RON HAY (Univ. of St. Andrews, Scotland)
20.01.04	SEBASTIAN FUGMANN (Yale School of Med.)
22.01.04	GLORIA LUCIANI (Univ. of Dundee)
22.01.04	IAIN MATTAJ (EMBL, Heidelberg)
27.01.04	JOHANNA JOYCE (UCSF)
28.01.04	MIKE VAN DEN BOSCH (Univ. of Galway, Ireland)
29.01.04	DIEGO LOAYZA (Rockefeller Univ.)

FEBRUARY

05.02.04	LUK VAN PARIJS (Center for Cancer Res., Cambridge, MA)
05.02.04	MARIA PIA POSTIGLIONE (Stazione Zoologica, Naples, Italy)
17.02.04	DANIEL TENEN (Harvard Instit. of Med., Boston)
19.02.04	GERALD H. POLLACK (Univ. of Washington, Seattle)

MARCH

04.03.04	NICK BROWN (Univ. of Cambridge)
11.03.04	SUSAN GASSER (Univ. of Geneva)
12.03.04	JOMUNA V. CHOUDHURI (Bielefeld Univ., Germany)
16.03.04	ANDREAS KUNGL (Univ. of Graz)
17.03.04	STEPHANIE BENESCH
18.03.04	PETER PARKER (Lincolns Inn Fields Lab., London)
19.03.04	KOICHI MATSUO (Keio Univ., Tokyo)
22.03.04	TADATSUGU TANIGUCHI (Univ. of Tokyo)
23.03.04	JONATHAN WEISSMANN (UCSF)
24.03.04	ROY DRISSEN (Univ. of Rotterdam)
25.03.04	HARALD VON BOEHMER (Harvard Univ.)
26.03.04	CONSTANZE BONIFER (Univ. of Leeds)
30.03.04	KATRIEN NEIRYNCK (Ghent Univ.)

APRIL

06.04.04	JOAN MASSAGUE (Memorial Sloan-Kettering Cancer Center, NY)
13.04.04	MICHAEL KIEBLER (MPI Tuebingen)
15.04.04	PHIL BEACHY (The Johns Hopkins Univ., Baltimore)
16.04.04	ALBERT SICKMANN (Rudolf-Virchow-Center for Exper. Biomed., Wurzburg)
19.04.04	ULRICH CORTES (IARC, France)
20.04.04	REBECCA HEALD (Univ. of California, Berkeley)
22.04.04	MARK VAN BREUGEL (MPI Dresden)
23.04.04	ANDRE MOELLER (MPI Goettingen)
27.04.04	CHRISTOPHER BAUM (MHH Hannover)
30.04.04	PETER LENART (EMBL Heidelberg)

MAY

03.05.04	MARIANO BARBACID (CNIO, Madrid)
06.05.04	JUAN CARLOS ZÚÑIGA-PFLÜCKER (Sunnybrook & Women's College Health Sciences Centre, Toronto)
06.05.04	THOMAS KIEFHABER (Univ. of Basel)
11.05.04	FRANK LYKO (DKFZ, Heidelberg)
12.05.04	TOM LUEDDE (Medical Univ., Hannover)
13.05.04	COLIN STEWART (NCI-FCRDC, Maryland)
18.05.04	CAROL PRIVES (Columbia Univ., New York)
19.05.04	JIM MANLEY (Columbia Univ., New York)
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27.05.04	DAVID VIRSHUP (Univ. of Utah)
28.05.04	JOHN CONDEELIS (Albert Einstein College of Med., New York)
28.05.04	DIRK SCHUEBELER (FMI, Basel)

JUNE

03.06.04	FRANK McKEON (Harvard Med. School)
14.06.04	GORDON KELLER (Mount Sinai School of Med., New York)
18.06.04	ELI GILBOA (Duke Univ. Med. Centre, North Carolina) (SPECIAL LECTURE IM MEMORIAM LAURA STINGL)
22.06.04	TOM ROBERTS (Harvard Med. School)
24.06.04	GERD JÜRGENS (Univ. of Tuebingen)
29.06.04	I. KING JORDAN (NCBI, Bethesda)
30.06.04	ANDREA MUSACCHIO (Europ. Inst. of Oncology, Milan)

JULY

01.07.04	CORNELIS J.M. MELIEF (Leiden Univ. Med. Center, The Netherlands)
05.07.04	DAVE GILBERT (SUNY Upstate Med. Univ., Syracuse, N.Y)
06.07.04	HENRY ROEHL (Sheffield Univ.)
09.07.04	DOMINIQUE FERRANDON (IBMC du CNRS, Strasbourg, France)
15.07.04	JIM SMITH (Univ. of Cambridge, UK)
16.07.04	GERRY RUBIN (HHMI)
26.07.04	DOUGLAS HANAHAN (UCSF)
29.07.04	RALF ERDMANN (Inst. of Physiol. Chem., Bochum, Germany)
30.07.04	ANTON MEINHART (Gene Center, Univ. of Munich)

AUGUST

16.08.04	ARNDT VON HAESELER (Univ. of Duesseldorf)
19.08.04	STEFAN SCHUSTER (Friedrich Schiller Univ., Jena, Germany)

Seminar speakers

SEPTEMBER

02.09.04	YOSEF SHILOH (Tel Aviv Univ., Israel)
03.09.04	DANIEL GERLICH (EMBL Heidelberg)
06.09.04	SHIGEAKE KATO (Univ. of Tokyo)
09.09.04	DAVID SIDEROVSKI (UNC-Chapel Hill School of Med.)
09.09.04	JERRY WORKMAN (Stowers Inst. for Med. Res., Kansas City)
10.09.04	CHRIS DOE (Inst. of Neurosci./HHMI, Oregon)
15.09.04	JIMENA WEIBEZAHN (ZMBH, Heidelberg)
16.09.04	ALFRED WITTINGHOFER (MPI Dortmund)
17.09.04	JASON D. FONTENOT (Univ. of Washington, Seattle)
23.09.04	TATSUYA HIRANO (CSHL)
30.09.04	CHARLIE BOONE (Banting and Best Dept. of Med. Res., Toronto)

OCTOBER

01.10.04	YOSHINORI YAMASHITA (Dept. of Oncology, Pharm. Res. Centre of Kyowa Hakko Kogyo Co. Ltd.)
12.10.04	JUERG BAEHLER (The Wellcome Trust Sanger Inst., Cambridge)
18.10.04	HALLGUIR RUI (Georgetown Univ., Washington DC)
19.10.04	MARC REHMSMEIER (Univ. of Bielefeld)
20.10.04	GRAHAM ANDERSON (Dept. of Anatomy, Inst. for Biomed. Res., Univ. of Birmingham)
20.10.04	LAURE STROCHLIC (Univ. of Cambridge)
22.10.04	PHILIPP SELENKO (Harvard Med. School, Boston)
22.10.04	VINCENT ARCHAMBAULT (Rockefeller Univ.)
28.10.04	HANS SCHOELER (MPI Muenster)
29.10.04	WOLFGANG FISCHLE (Lab. of Chromatin Biol., The Rockefeller Univ., New York)

NOVEMBER

03.11.04	JIRI FRIML (ZMBP, Univ. of Tuebingen)
04.11.04	STEFAN UHLIG (Leibniz Center for Med. and Bioscience)
08.11.04	DONALD F. HUNT (Depts. of Chem. and Path., Univ. of Virginia, Charlottesville, Virginia)
12.11.04	JEROEN DOBBELAERE (Inst. of Biochem., Zürich)
12.11.04	INKA PAWLITZKY (Univ. of Massachusetts, Boston)
16.11.04	TOM A. RAPOPORT (Harvard Med. School, Dept. of Cell Biol.)
18.11.04	JOHN SCOTT (Howard Hughes Med. Inst., Portland, USA)
23.11.04	TON ROLINK (Univ. of Basel)
25.11.04	ALEXANDER JOHNSON (UCSF)
26.11.04	PATRICK MATTHIAS (FMI, Basel)

DECEMBER

02.12.04	JAMES MUSSER (Baylor College of Med., Texas, USA)
03.12.04	MATTHEW POLLI
06.12.04	MONIKA PRÜNSTER (Innsbruck Med. Univ.)
10.12.04	PETER F. AMBROS (Children's Cancer Res. Inst., St. Anna Kinderspital, Vienna)
14.12.04	KAY-UWE WAGNER (Eppley Inst. for Res. in Cancer and Allied Diseases)
15.12.04	MICHAEL LEITGES (MPI for Exper. Endocr., Hannover)

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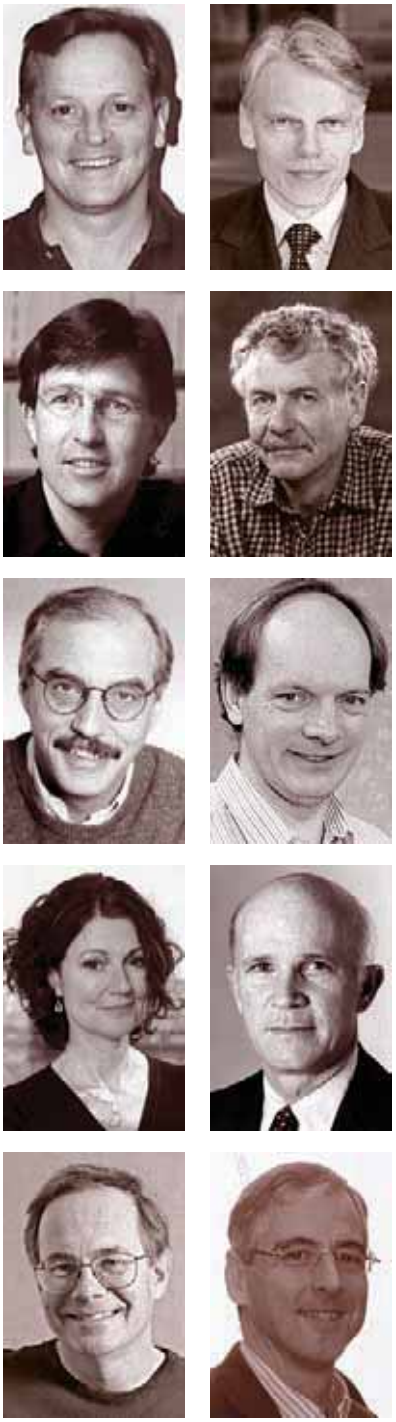

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