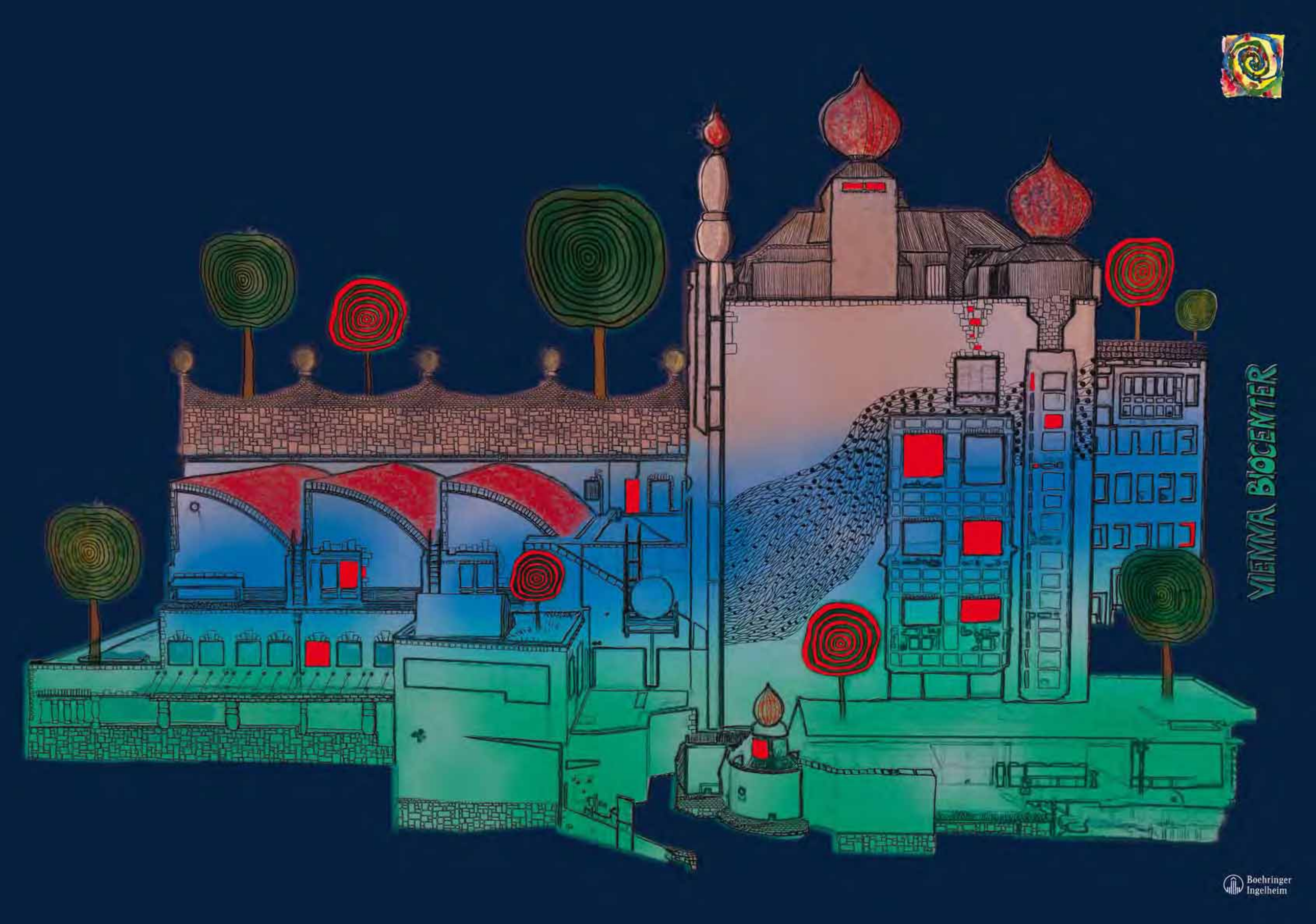

Boehringer Ingelheim

Contents

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

It is time to reflect as another year reaches its end. The cover of the IMP Research Report 2003 shows the IMP somewhat transformed. The IMP reached its 15th birthday this year. It has changed considerably since it opened its doors as an initiative of Boehringer Ingelheim and Genentech. The IMP forms with the neighbouring University of Vienna the core of a campus that has become known as the Vienna Biocenter, which is a dynamic and growing assembly of scientific institutions and Biotech start-up companies.

Three events formed the highlights of the past year's changes. A groundbreaking ceremony took place in June with the start of construction of a brand new research building, adjacent to the IMP at Dr. Bohr-Gasse. This will host two recently established initiatives of the Austrian Academy of Sciences, IMBA (Institute of Molecular Biotechnology) and the GMI (Gregor Mendel Institute of Molecular Plant Biology). The IMP and IMBA have already established a close collaboration and will share much infrastructure, including a common mouse house.

The Vienna Biocenter Campus held "Days of Open Doors" in October, which included a celebration of the 10th anniversary of the University of Vienna's presence at Dr. Bohr-Gasse. This event attracted over 800 participants from schools and the local neighbourhood. Its success demonstrated a growing interest in research among the general population and confirmed the Biocenter status as a distinct and permanent landmark in contemporary Vienna.

Annette Neubüser, our first Wittgenstein team leader, left the IMP in October to take a professor position at the University of Freiburg. Annette has been a pioneering group leader, who introduced developmental biology to the IMP research interests.

The event that perhaps most poignantly caused us all to reflect was the 70th birthday of Prof. Max Birnstiel, the founding director of the IMP. A celebration of Max's birthday in November included a mini symposium "Genes to Genomes" organized by Meinrad Busslinger, who had been a PhD student in Max's lab. The event gathered many renowned scientists from around the world.

Strength of the IMP stems from the efforts of all of its members, from students to group leaders, all the support groups and the administration. I am enormously grateful for their outstanding efforts and remarkable performance. What will 2004 bring? The relationship with IMBA should continue to develop and will soon change our scientific landscape. I am confident that the IMP will benefit from the opportunities that this offers and will become an even more exciting and stimulating place to push back the frontiers of biomedical knowledge.

Kim Nasmyth
December 2003

The IMP and the Campus Vienna Biocenter

The Research Institute of Molecular Pathology (IMP) is a basic biomedical research center in Vienna, 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.

The IMP opened its doors at the present site in 1988. Since then, it has become a major player in the biological sciences, added a new dimension to Austria's research scene and changed its entire neighborhood by attracting more research-driven institutions to the premises. Located next to the IMP are four institutes of the University of Vienna's Faculties of Science and Medicine (from 2004: Medical University of Vienna). For those seeking a more applied approach to science, a "Fachhochschule" offers curricula in Bioengineering and Biotechnology. Also next-door, one finds seven small and middle-sized biotech-companies. Together with the IMP, these institutions form the "Campus Vienna Biocenter".

Three further additions to the Campus are still under construction. In June 2003, the first sod was turned for two new institutes of the Austrian Academy of Sciences: GMI (Gregor Mendel Institute of Molecular Plant Biology) and IMBA (Institute of Molecular Biotechnology), both of which will be located adjacent to the IMP and work in close collaboration. Another building on the site, which will offer lab and office space for rent, is nearing completion.

Currently, more than 900 people from 40 different nations work at the Campus Vienna Biocenter, 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. In October 2003, the Campus members joined forces to organize two Open Days for the public, which were received with great interest. About 800 visitors came to learn about research projects, to tour the labs and to obtain information about training programs available at the Campus.

BACK

Vienna / Austria

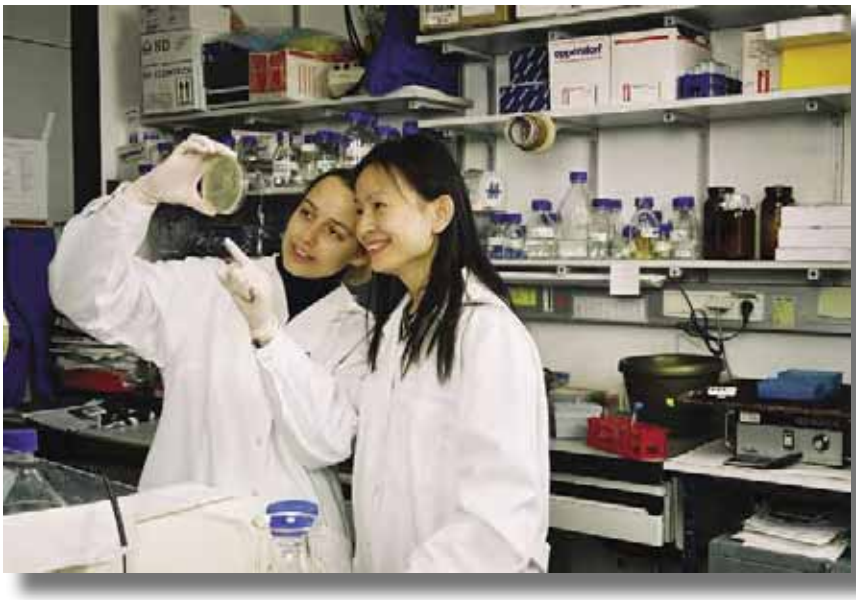
Vienna is the capital city of Austria and home to about 1.5 million people. It is the administrative, political, and cultural centre of the country. Geographically, Vienna's location reflects its historic role at the heart of the large Habsburg empire. Today, with Eastern European countries about to join the European Union, Vienna recognises its chance to become an economic and cultural hub for Central Europe.

Located between the eastern slopes of the Alps and the fertile plains of the river Danube, Vienna enjoys astounding diversity in scenery and activities. One-hour drive can take you to alpine country with opportunities for hiking, climbing or skiing. Travelling in a different direction, you will find yourself at the shores of the lake Neusiedlersee with excellent conditions for sailing and windsurfing in summer and ice-skating in winter. Change your route again and you'll reach the serene "Waldviertel", a densely forested, sparsely populated region with ponds, creeks and moors to explore.

Partly within the city's boundaries is the "Donauauen" National Park, a floodplain forest that extends all the way to the Slovak border and features rare populations of deer, heron, and beaver. Also among Vienna's unique curiosities: it is the only capital city with a substantial wine production on its grounds. Rolling hills rich in vineyards add to the beauty of the scenery surrounding the city.

If you are interested in culture, art and history, Vienna has more to offer than you can possibly manage to see and do. Opera, concerts and theatre, although performed by world-famous ensembles, are not seen as elitist entertainment but are affordable enough to be enjoyed by everyone.

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 one in Austria, offering some 130 types of degree programs. 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. So, if you plan to settle down here for a few years, be prepared for numerous visits by friends and relatives.



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 excellent scientific services. At our institute, you'll be part of a young, international team that uses English as a working language. You will experience a stimulating and focused atmosphere where science always comes first but social activities are not neglected.

Graduate students join the IMP through the Vienna Biocenter International PhD Program. 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. At present, 39 postdocs from 18 different nations work at the IMP. 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 and symposia in-between. 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 you have found your own place. When looking for a flat, a staff member will help you negotiate, deal with brokers and do the paperwork until the deal is made. Speaking of paperwork, our personnel department will take care of your legal requirements including visas, registration, health insurance and family matters e.g. advising on types and availability of day care centers, kindergartens and schools for your children.

If you come from outside the German speaking world, you will find that English is sufficient to get along in Vienna. For most locals, English has become part of their daily lives, both at work and during leisure-time. However, if you want to learn German you will be able to attend courses at the IMP's expense. Apart from sponsoring your language skills, the institute subsidises sporting activities for its members.

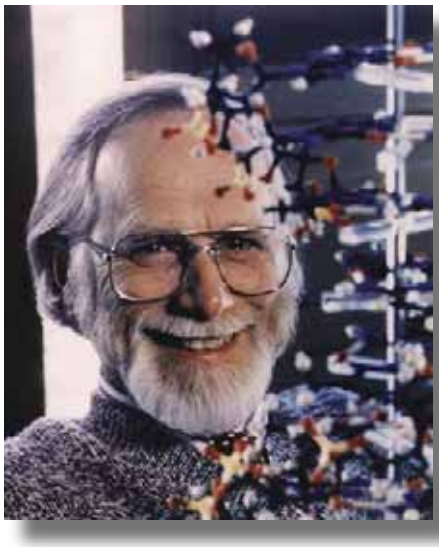
More information about career opportunities at the IMP is available at: www.imp.univie.ac.at

BACK

Impressions from outside

"I'm a huge fan of the IMP, and used to look forward tremendously each year to the recess where, without fail, year after year, exciting and important new results were always announced. The atmosphere of the place is exceptional, not only because of the people who work there, but also because it's a manageable and I would say human size. I also enjoyed the mixture of topics and approaches, ranging from Kim Nasmyth's work on the yeast cell cycle to Hartmut Beug's investigations of the epithelial-mesenchymal transition, with plenty in between about the control of transcription in higher eukaryotes. Always food for thought, always a thriving (if not buzzing) intellectual community. And pleasing to see, more often than not, that a young diploma student had made one of the most important discoveries of the year!"

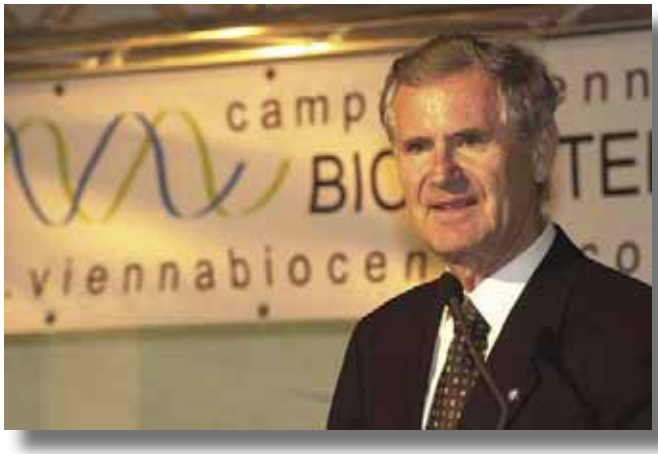
Tim Hunt
ICRF Clare Hall Laboratories, UK
Nobel Prize in Physiology or Medicine 2001



"The IMP is a remarkable institution, a dramatization of the adage that 'small can be beautiful.' It achieved international eminence in record time and has sustained that eminence ever since. It is a lean but not mean research machine. Young scientists thrive there, which is my premier criterion for judging a research institute. Person for person, and euro/shilling/dollar for euro/shilling/dollar, there is hardly a better place. As a result, it has revitalized biomedical research in Vienna and had a beneficial impact on such research throughout Europe."

Mike Bishop
University of California School of Medicine, San Francisco, USA
Nobel Prize in Physiology or Medicine 1989

"The IMP has shown that Vienna can be a place where top-notch biology happens. What a good message for the world - and for Vienna!"



Jeff Schatz
Swiss Science and Technology Council,
Bern, CH

Oncogenesis: Abnormal Developmental Plasticity?

In the development of cancer, e.g. leukemias and breast carcinomas, combinations of oncogenic and normal receptors/signal transducers are crucially important as they stimulate proliferation/renewal and inhibit differentiation/apoptosis of primitive progenitors. We use novel in vitro cell culture systems and genetically modified mice to address the role of such signal combinations in leukemic and stress-induced renewal of hematopoietic progenitors, and in epithelial/mesenchymal transitions during metastasis and tissue remodeling.

Stress-induced alterations of renewal in hematopoietic progenitors: a process important in leukemogenesis

Renewal (i.e. sustained progenitor proliferation without differentiation) of primary erythroid progenitors reflects their physiological response to stress erythropoiesis. EpoR, c-Kit and the glucocorticoid receptor (GR) cooperate in renewal and require signaling through the Stat5 and PI3K pathways. In erythroleukemia, oncogenic receptor tyrosine kinases (RTks; v-ErbB, v-Sea) substitute for stress-induced signaling *via* EpoR/c-Kit, by activating both Stat5 and PI3K on their own. Erythroblasts from mutant mice lacking components of signaling complexes driving renewal (GR-/-, Stat5-/-, Raf-/-, Btk/Tec-/-) showed interesting defects in renewal and/or terminal differentiation (Figure 1). In addition, red cell differentiation proceeded as a cell-autonomous default program if apoptosis was prevented by the Epo-target gene Bcl-XL. Finally, the human leukemia oncogene MLL (related to the *Drosophila* chromatin modifier *trithorax*) required cooperation with c-Kit to transform lymphohematopoietic cells and to cause multilineage leukemia.

We increasingly focus on investigating multipotent progenitor cell systems, and will further analyze interesting erythroid cell defects from respective knockout mice. We have begun to explore hematopoietic progenitors from murine embryonic stem (ES) cells, a system suitable for characterization of possible hematopoietic defects, also in early embryonic lethal mouse mutants. We will address (also by RNAi) the function of a novel proapoptotic protein (p12) in the mitochondrial apoptosis pathway and its role in hematopoietic cells. We will also analyze cooperation between the GR and c-Kit/EpoR by expression profiling and in mice lacking Stat5, GR or both, and the composition/function of the EpoR/c-Kit- signalosome (Figure 1) in oncogene transformed cells.

Cooperation of RTK- and TGFβ-receptor signaling in tumor cell invasiveness and metastasis

Cooperation of a hyperactive Ras-MapK pathway with TGFβ-signaling causes epithelial/mesenchymal transition (EMT) in polarized mammary epithelial cells (EpH4), a truthful *in vitro* correlate of carcinoma cell metastasis. TGFβ induces Ras-transformed EpH4 cells (EpRas) to undergo EMT, i.e. to acquire fibroblastoid morphology/increased migration and to undergo major reprogramming of gene expression towards mesenchymal cells. TGFβ plus Ras-activated PI3K

Hartmut Beug

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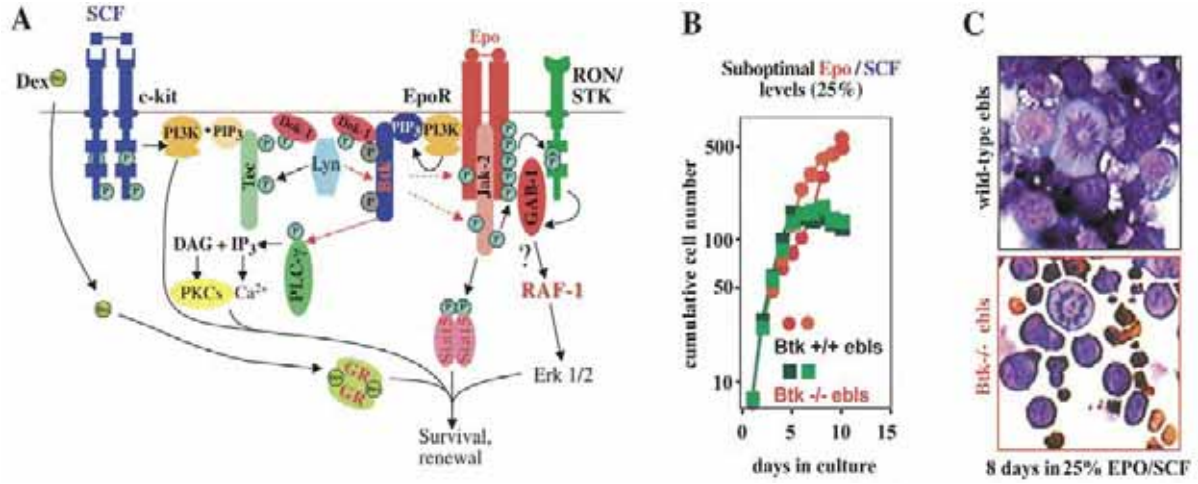

Figure 1: The EpoR / c-Kit signalosome: functional analysis in cells lacking specific signal transducers. (A) Highly simplified scheme of the EpoR/c-Kit signaling complex is shown, consisting of the EpoR, the RTKs Ron and c-Kit, numerous scaffolds (Gab1, Gab2, Doc) and PH-domain proteins e.g. the cytoplasmic tyrosine kinases Btk and Doc, plus further downstream signaling pathways. (B, C) Erythroblasts lacking Btk (Btk -/-) show premature loss of proliferative potential in sub optimal (physiological) concentrations of Epo and SCF (B), caused by premature differentiation (small, brownish erythrocytes) under renewal conditions (C).

signaling induces "scattering" (transient migratory phenotype without reprogramming of gene expression) *in vitro*, and causes tumor development, but not EMT or metastasis *in vivo*. Ras-dependent hyperproliferation of tumor cells *in vivo* could be recapitulated in 3D collagen cultures and was shown to require PI3K signaling (Figure 2). By expression profiling, we have identified multiple genes and pathways as candidates for having a crucial role in EMT/metastatis; e.g. we have found that TGFβ induces a PDGF-autocrine loop which is required for both, induction and maintenance of EMT.

We will increase our efforts to employ *in vivo* mouse models for mammary carcinoma progression and metastasis. Crossbreeding of transgenic MMTV-HER2- and MMTV-TGFβ1-transgenic mice already revealed strongly enhanced lung metastasis as compared to MMTV-HER2 mice, and EMT in the respective mammary tumors. Conversely, crosses between MMTV-HER2- and -dnTGFβRII transgenic mice are being examined for suppression of metastasis. This analysis will be extended to available knock-out mice lacking verified candidate genes from expression profiling (e.g. the E-cadherin repressor ΔEF-1, or the TGFβ co-receptor endoglin/CD105). Functional characterization of other EMT-specific genes, initially based on the RNAi approach, is also in progress. The kinetics of EMT in EpRas cells will be analyzed by expression profiling, after developing suitable methods. Furthermore, we are currently analyzing the possible cross talk between TGFβ- and Wnt/β-catenin signaling, since Smad2 mutants defective for the Smad/Lef-1 interaction abrogate both EMT and metastasis. Finally we will address recently uncovered cooperations between different TGFβRI family members (Alk-1/Alk-5), and ask whether cooperation between TGFβ and BMP signaling is relevant for EMT/metastasis.

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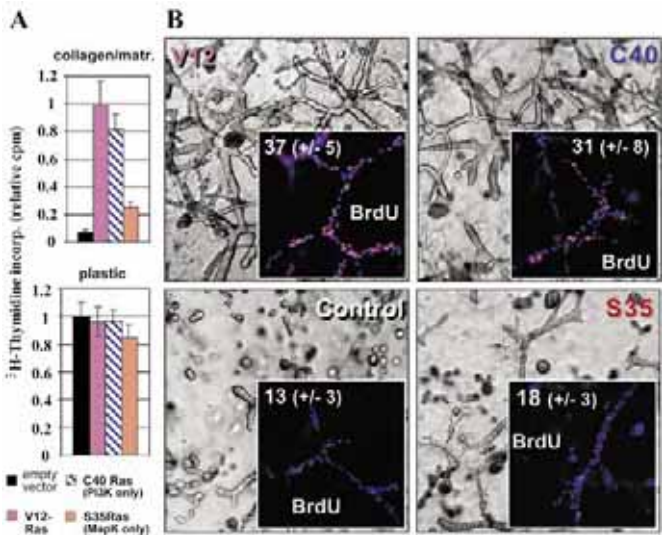

Figure 2: Hyperproliferation of Ras-transformed carcinoma cells driven by PI3K signaling in 3D cultures. (A) EpH4 cells expressing unmutated oncogenic Ras (V12Ras), effector-specific Ras mutants selectively activating either the MapK pathway (S35-Ras) or the PI3K pathway (C40-Ras) and empty vector control cells were seeded in serum-free collagen gels (3D cultures, top) or on plastic (bottom) and analyzed for [3H]-thymidine incorporation. Ras-driven hyperproliferation (as in tumors in vivo) is seen only in collagen gels and is dependent on Ras-driven, hyperactive PI3K signaling. (B) Photographs of collagen gel structures from the same cells as in A, before (bright field) or after staining for incorporated BrdU (insets, pink nuclei), showing again PI3K-dependent enhanced proliferation.

BACK

Stem Cell Commitment in Hematopoiesis

Tissue-restricted stem cells give rise to 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 control the commitment of early hematopoietic progenitors to the B cell lineage.

Meinrad Busslinger

A fundamental question in hematopoiesis is how multipotent stem cells and early progenitors become committed to a single developmental pathway and then differentiate into the mature cell type of the selected lineage. By analyzing the properties of 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, raising the interesting question of the upstream transcription factors regulating *Pax5* expression. Using transgenic approaches, we have mapped the B-cell-specific enhancer of the *Pax5* locus, the analysis of which should now facilitate identification of the upstream regulatory factors.

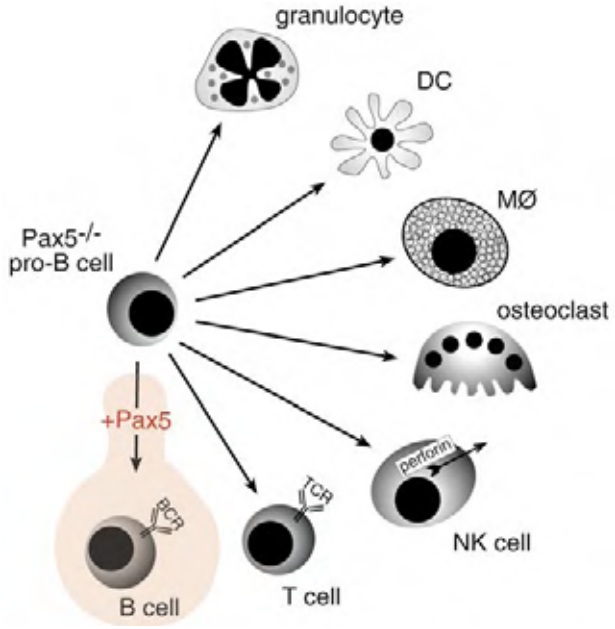

Figure 1: B-lineage commitment by Pax5. Pax5$^{-/-}$ pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.

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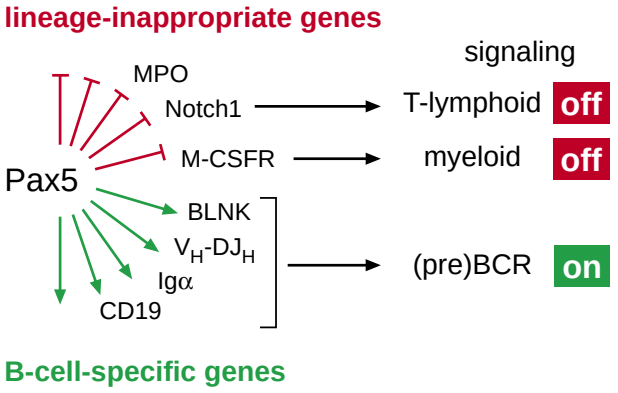

Figure 2: Dual role of Pax5 in B-lymphopoiesis. Pax5 activates B-lymphoid genes (green) and simultaneously represses lineage-inappropriate genes (red).

At the molecular level, Pax5 fulfils a dual role by activating the expression of B-cell-specific genes and by repressing the transcription of lineage-inappropriate genes. To systematically analyze the transcriptional function of Pax5, we used cDNA microarray screening to identify a multitude of novel Pax5-regulated genes. One of the activated Pax5 target genes codes for the central adaptor protein BLNK, which couples signaling from the (pre)B cell receptor to transcriptional changes in the nucleus (Figure 2). The majority 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 currently testing the hypothesis that this promiscuous gene expression is responsible for the developmental plasticity of early progenitors.

We have also investigated the lineage commitment function of Pax5 by forcing its expression in hematopoietic stem cells and early progenitors. Pan-hematopoietic *Pax5* expression strongly promoted B cell development at the expense of T-lymphopoiesis, whereas myeloid and erythroid development was only minimally affected. Pax5 thereby interfered with T-lineage commitment and early thymocyte development by directly repressing the transcription of the T cell specification gene *Notch1* (Figure 2). This ectopic Pax5 expression system allows us now to study the role of Pax5 in controlling V(D)J rearrangements at the immunoglobulin heavy-chain locus in heterologous T cells.

PAX5 has been implicated as an oncogene in the genesis of non-Hodgkin's lymphomas carrying a specific t(9;14) translocation that brings the *PAX5* gene under the transcriptional control of the immunoglobulin heavy-chain (*IgH*) locus. We have reconstructed this translocation in the mouse by inserting a *Pax5* minigene into the *IgH* locus, which is transcriptionally active in both B and T cells. All homozygous knock-in mice die within a few months as they develop aggressive T cell lymphomas (Figure 3). Hence, inappropriate expression of the B cell identity gene *Pax5* in the related T-lymphoid lineage results in tumor formation. The t(9;14) translocation in human patients arises, however, in germinal center B cells where it causes small lymphocytic lymphomas of the plasmacytoid subtype (B-SLL). Therefore, to generate a more relevant tumor model, we have created a second knock-in mouse, in which *Pax5* expression can be conditionally activated in germinal center B cells. These experiments should allow us to test whether forced *Pax5* expression interferes with plasma cell differentiation and thereby contributes to lymphoma formation.

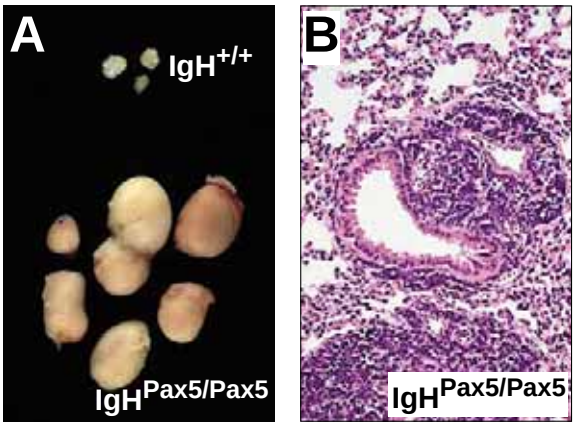

Figure 3: Lymphoma formation in $IgH^{Pax5/Pax5}$ knock-in mice. (A) Enlargement of the lymph nodes from diseased $IgH^{Pax5/Pax5}$ mice compared to wild-type littermates. (B) Massive infiltration of the lung of $IgH^{Pax5/Pax5}$ mice by lymphoma cells.

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BACK

Molecular Mechanisms of Protein Quality Control

Cells precisely monitor the concentration and functionality of each protein for optimal performance. This quality control is achieved by a sophisticated interplay of proteases and molecular chaperones. We are performing a structure-function analysis of the heat-shock protein DegP (HtrA), which combines both refolding and digesting activities and thus offers unique possibilities to investigate how cells distinguish non-native proteins that can be refolded from hopeless cases that have to be digested.

Damaged proteins represent a serious hazard to the cell as they might accumulate as large aggregates, a process associated with prion and other amyloid diseases. *In vivo*, aggregate formation is a highly favorable process due to the extremely high intracellular protein concentrations (100-150 mg/ml). DegP is a protease-chaperone that aims to reduce the amount of unfolded protein. This heat-shock protein was initially identified in *Escherichia coli* by two phenotypes of corresponding null mutants and named accordingly. Mutants either did not grow at elevated temperatures (HtrA, High temperature requirement) or failed to digest misfolded proteins in the periplasm (DegP, Degradation). The defining feature of the DegP family is the combination of a trypsin-like protease domain with at least one C-terminal PDZ domain, a prominent protein-protein interaction motif. Prokaryotic DegP has been attributed to the tolerance against various folding stresses as well as to pathogenicity. Human homologues are believed to be involved in arthritis, cell growth regulation, unfolded protein response and programmed cell death. Beside this physiological impact, the protein itself has unique mechanistic properties suggesting that DegP represents a novel protease-chaperone system.

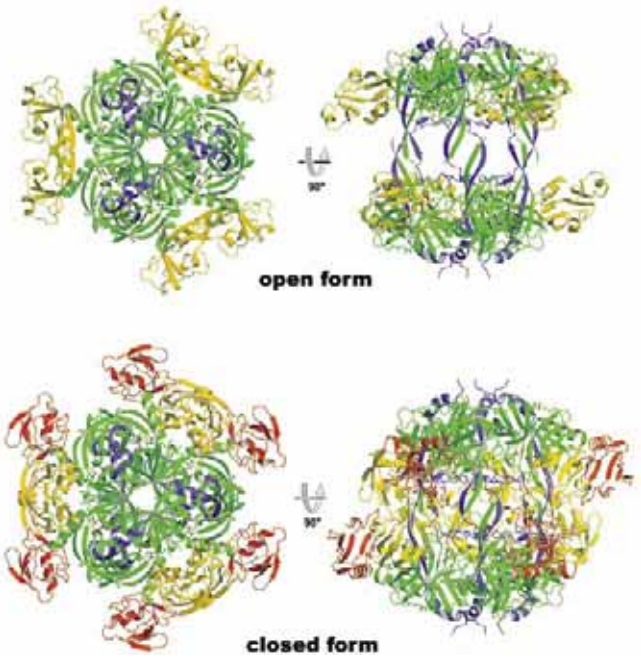

Figure 1: Top and side views of the two hexamers observed in the DegP crystals representing the open and closed form of the molecule. The individual domains are colored differently with the N-terminal domain in blue, the protease in green and the PDZ domains in yellow and red, respectively. Both hexamers are approximately equal in size having a height of 105 Å and a diameter of 120 Å.

Tim Clausen

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[2] *from January 2004*

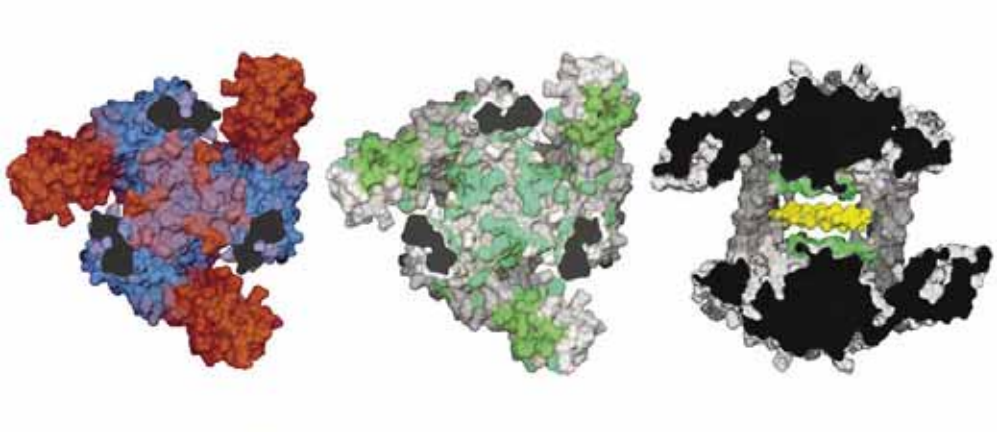

Figure 2: The internal cavity of DegP. For better illustration half cut figures of the molecular surface were prepared from both top and side views. The cutting area is shown in black. The chaperone-like features of the inner cavity are the following: (Left) Plasticity. Flexible portions are in red, whereas rigid areas are colored blue. (Middle) Hydrophobicity. The inner cavity is mainly constructed by hydrophobic residues, which are highlighted in cyan and green. (Right) Size exclusion. To illustrate the geometric restriction of the molecular compactor, a single α-helix (yellow) was modeled into the cavity.

To better understand its mode of action we have started a structural analysis of the well-characterized *E. coli* DegP protein. Central questions of interest include the ATP-independent mechanism of protease and refolding activities, analysis of the reversible, temperature-dependent switch from chaperone to protease, the function of the PDZ domains and the structural determinants of substrate specificity. The crystal structure of the chaperone form of DegP, which was recently determined in our laboratory, is a start point in dissecting the molecular mechanism of this quality control factor, providing the required stereochemical framework for further genetic, biochemical, and biophysical studies.

The DegP hexamer is formed by staggered association of trimeric rings (Figure 1). The proteolytic sites are located in a central cavity that is only accessible laterally. The mobile sidewalls are constructed by twelve PDZ domains, which mediate the opening and closing of the particle and probably the initial interaction with substrate. Further binding sites for misfolded proteins are located within the inner cavity (Figure 2). Due to the geometric constraints of this chamber, substrates must be at least partially unfolded to enter. As in other chaperones of known structure, the DegP cavity is lined by hydrophobic residues. These residues form two binding platforms, which have a pronounced structural flexibility as judged from their high thermal motion factors. This plasticity should allow binding of diverse polypeptides.

Cage-forming proteases and chaperones can be energy-dependent or energy-independent. In the former group, ATPase activity is important for recognition of target proteins, their dissociation and unfolding, their translocation within the complex and various gating mechanisms. The present crystal structure indicates why these functions are not relevant for DegP. DegP preferably degrades substrates, which are *per se* partially unfolded and which might accumulate under extreme conditions. Alternatively, threading of substrate through the inner chamber could promote unfolding into an extended conformation. Removal of higher order structural elements would allow the substrate to reinitiate folding after exit from DegP. Recruitment of PDZ domains for the gating mechanism should permit a direct coupling of substrate binding and translocation within the DegP particle. Accordingly, the PDZ domains may function as tentacular arms capturing substrates and transferring them into the inner cavity. By binding to the C-terminus or a β-hairpin loop of a protein, the PDZ domains could properly position the substrate for threading it into the central cavity. After accessing this chamber, the fate of the unfolded protein depends on the interplay of several active site loops (loops LA, L1, L2, L3 using the protease nomenclature), which regulate proteolytic activity.

Future studies will concentrate on the characterization of the protease form of DegP. We will aim to determine high-resolution crystal structures with substrates and inhibitors and extend the approach to the related DegQ and DegS proteases. The search for additional physiological substrates and for cellular effectors that either inhibit or activate members of the DegP family will contribute to our understanding of the fascinating network controlling protein composition, which is undoubtedly one of the key metabolic pathways of each cell.

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Axon Guidance and Target Recognition

It is a fascinating but daunting problem: How do just a few thousand genes direct the assembly of neuronal circuits with such staggering complexity as those of the human brain? We chose to tackle the fly first. It's not that the fly's nervous system is any less challenging, but at least we have a powerful set of genetic tools to work with. And what we learn about the development of the fly's nervous system may provide new insights into our own. After all, it seems that there's a bit of a fly in all of us.

Neuronal circuits are formed as each neuron sends out axons and dendrites to find, recognize and connect with the appropriate target cells. Finding the target is the task of the growth cone, a highly motile and exquisitely sensitive structure at the tip of each axon and dendrite. Growth cones detect various guidance cues in the extracellular environment, and somehow manage to extract from this information the correct route towards their target. By studying this process in Drosophila, we hope to find out what these cues are, how growth cones detect them, and how each growth cone knows which cues it should follow and which it should ignore. Genetics is a powerful tool in this endeavor. Mutations that disrupt neuronal connectivity can lead us to the genes that encode the guidance cues themselves, or to the molecules that growth cones use to detect and respond to these cues. And, by examining how these molecules work *in vitro* and *in vivo*, we can begin to explore the molecular, cellular and developmental mechanisms that ensure that each growth cone responds to the right cues at the right time, and ignores the rest. We are focussing on two different systems: the ventral nerve cord of the embryo, and the adult visual system. Some of our recent findings and ongoing projects are highlighted here.

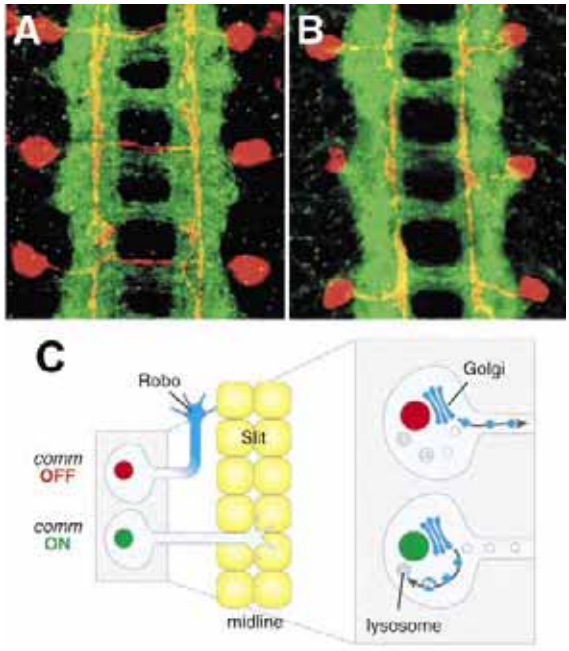

Figure 1: Crossing the midline. In the Drosophila CNS, some axons cross the midline (like the red axons in A), but others do not (B). Those that do cross, called commissural axons, cross only once. Comm functions as a switch to control this decision (C). Comm is ON in commissural neurons as they cross, but OFF in ipsilateral neurons and post-crossing commissural neurons. Comm regulates the sensitivity of axons to the midline repellent Slit (yellow in C). It does this by controlling the intracellular trafficking of Robo (blue), the Slit receptor.

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Axon guidance in the ventral nerve cord

In bilaterally symmetric nervous systems, such as our own and the fly's, axons in the CNS must decide whether or not to grow across the midline. Our recent work has revealed how this decision is controlled in the Drosophila nerve cord (Figure 1). Crossing and non-crossing axons differ in their sensitivity to the midline repellent Slit. Both commissural axons (which cross) and ipsilateral axons (which don't) express the Slit receptor Roundabout (Robo for short). In ipsilateral axons, Robo is inserted into the growth cone, making them sensitive to the repulsive activity of Slit. But in commissural neurons, Robo is not delivered to the growth cone until *after* it has crossed the midline. So these axons are able to cross, but only once. Before crossing, an intracellular sorting receptor called Commissureless (Comm) collects the newly synthesized Robo protein at the Golgi and delivers it to lysosomes, where it is degraded. Comm is normally active only in commissural neurons as their axons first grow toward and across the midline. If it is made inactive (by a mutation), no axon can cross the midline, resulting in the commissureless phenotype from which the gene got its name. In ipsilateral neurons, and post-crossing commissural neurons, Comm is normally inactive. But by activating Comm in these neurons, we can force them to cross (or recross). This defines Comm as a simple genetic switch to control midline crossing (Figure 1). We are currently trying to find out how this switch is turned ON and OFF, and how Comm selects Robo and sorts it to lysosomes.

Axon guidance in the visual system

Flies have excellent vision. This rests in part on the extraordinarily precise connections established between photoreceptors in the eye and their targets in the brain. As a first step in determining how these connections are established, we screened through more than 32,000 mutant lines to find some 200 mutants with abnormal connectivity patterns. These mutations define about 50 different genes, 31 of which we have now identified. These genes encode cell surface receptors and signaling molecules, as well as factors involved in gene transcription, axonal transport, and membrane trafficking. One of them encodes a 7-transmembrane cadherin called Flamingo. Flamingo is expressed on many different photoreceptor axons and their target cells in the brain (Figure 2). It is needed for one class of photoreceptors (R8s) to select their correct targets in a specific layer of the brain. We continue to look for the remaining genes, and are beginning to piece together the molecular pathways and processes that underlie this exceptional example of neuronal engineering.

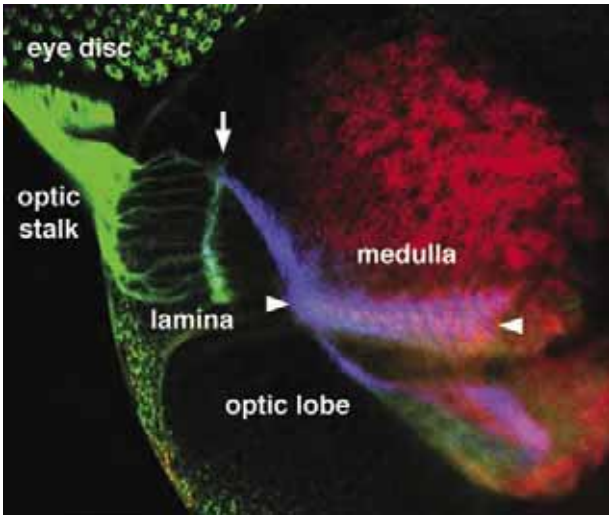

Figure 2: Photoreceptor axon targeting. Photoreceptor axons (green) extend from the eye disc, through the optic stalk, and into the optic lobe. Some axons terminate in the lamina (arrow). Others, including R8 axons, terminate in the medulla (arrowhead), where they form synapses with medulla neurons (red). The Flamingo protein (blue) is required for correct targeting of R8 axons.

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Understanding Molecular Mechanisms through Biomolecular Sequence Analysis

High-throughput experimental technologies in Life Sciences produce large amounts of homogeneous 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.

Application projects in cooperation with experimental groups

The establishment 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 these services are available not only *via* the command line within the bioinformatics group net but also through the local intranet and internet nodes (http://mendel.imp.univie.ac.at).

Many genetic screens and cDNA chip studies end up in sequences of functionally uncharacterized biomolecules. In such situations, sensitive sequence analyses may provide crucial insights. During the past year more than 200 gene and protein families have been studied in great detail, 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 request of IMP researchers and their Austrian and international collaborators. For example, using sequence-analytic arguments, we have shown that a number of known chromatin-related proteins as well as many hypothetical translations constitute a protein family of so-called kleisins. Members of the kleisin family act as ubiquitous partners for SMC molecules in the formation of protein rings that topologically fix chromatin fibers (Figure 1). These SMC kleisin/rings have been postulated to organize chromatin during DNA condensation (in the condensin complex), chromosome segregation (in the cohesin complex) and in DNA repair. Subsequent experiments carried out by the Glotzer, Nasmyth and Peters groups have successfully verified many predictions derived from these hypotheses.

Development of new methods, algorithms and software packages for bioinformatics research

Genuine bioinformatics research is oriented towards the creation of new methods and integrative theories. However, the scientifically relevant directions of such efforts are determined by interactions with experimental life sciences. Our methodical research is grouped into two main approaches:

1. Recognition of posttranslational modification signals and targeting signals in protein sequences. For example, we have developed predictors for many lipid posttranslational modifications and for the PTS1 peroxisomal signal (Figure 2).

2. Integration of diverse sequence analysis methods in a higher order shell ("automatic sequence analyzer") for applications in the large-scale protein sequence annotation; a project joint with Boehringer-Ingelheim Austria.

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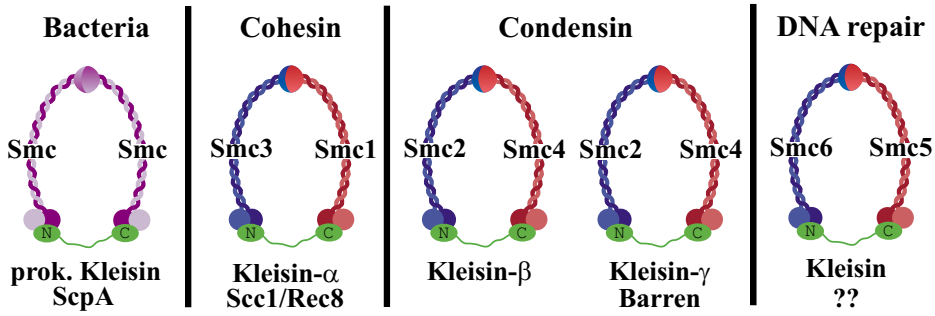

Figure 1: The kleisin family and the SMC/kleisin complexes. Kleisins have globular N- and C-terminal domains interconnected by a significantly less structured linker region. The SMC molecules consist of terminal and central globular domains and intermittent helical regions. The latter form intramolecular coiled coils so that the terminal regions are brought together and form a functional ATPase with the classical Walker box motif. Two SMC molecules tightly interact via their central globular domains, the hinge region. A kleisin molecule and a SMC dimer form a protein ring that holds chromatin fibers bundled topologically. In prokaryotes, there is only one SMC molecule type that forms homodimers and interacts with a ScpAp-homologue, the prokaryote kleisin. In eukaryotes, there are six different SMCs (numbered from 1 to 6) that form heterodimers and interact with various kleisins (Scc1p/Rec8p homologues of cohesin complexes, Barren homologues of condensin complexes and yet non-identified kleisins in DNA repair complexes).

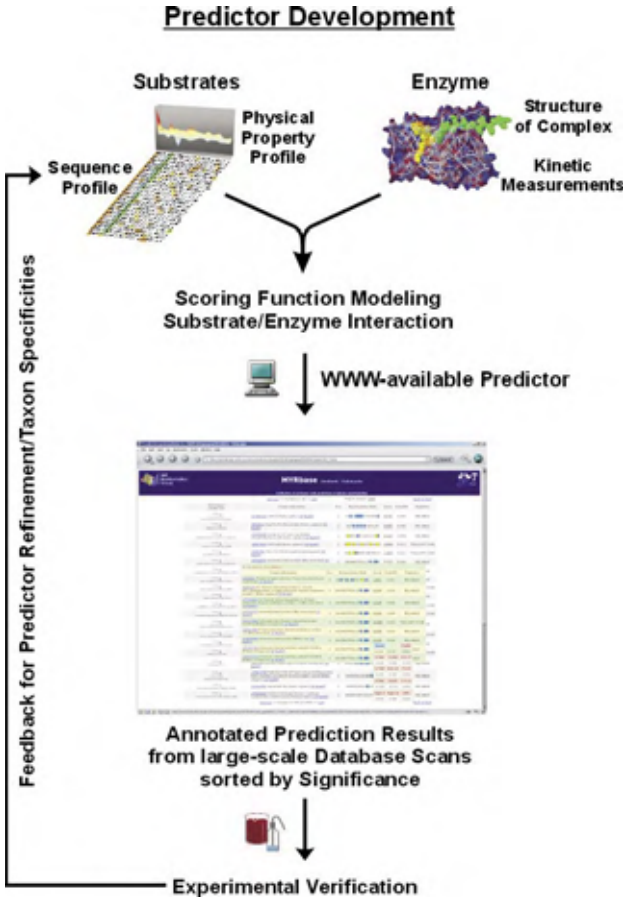

Figure 2: The derivation of a new predictor for a posttranslational modification illustrated by the example of the myristoylation predictor. The starting point is the analysis of sequences of known substrate proteins that are aligned with respect to the modification site (here, the N-terminal glycine). Both, a classical sequence profile characterization, and a description in terms of preferred physical properties, is derived from this alignment. Special attention is given to correlations among alignment positions with respect to physical properties or amino acid types. The physical property pattern is augmented by insights derived from analysis of the structure of the enzyme executing the modification, if available. All this knowledge is incorporated into a model of substrate/enzyme interactions and formulated into mathematical expressions that evaluate the concordance of query protein segments with the model. After a new predictor has been developed, it is applied in large-scale database scans. The protein entries predicted for the given type of modification can be grouped into homologous families for further investigation. Subsequent experimental analysis of especially interesting examples can lead to an enlargement of the known set of verified substrates and, consequently, to an improvement of the predictor.

Computer usage and networking within the IMP

Modern experimental biological research as well as efficient administration and maintenance of the institute are impossible without powerful computer and network services, including the internet connections. 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.

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BACK

Cytokinesis in Animal Cells

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

Cytokinesis is mediated 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, its constriction, and the separation of the two nascent cells - typically requires ten minutes. Thus, cytokinesis is a dynamic and spatially regulated process. We are using the nematode *Caenorhabditis elegans (C. elegans)* as a model system to dissect this complex process since 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 to address the following unsolved problems: How is the cleavage furrow positioned? How does the contractile ring assemble and function? How does the central spindle assemble and function? How is completion of cytokinesis achieved?

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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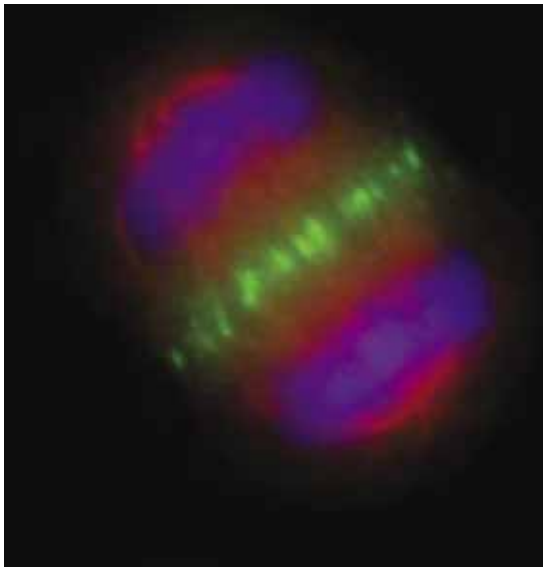

Figure 2: Centralspindlin localizes to the spindle midzone in anaphase. A mammalian cell in anaphase has been stained for MKLP-1 (the mammalian ZEN-4 ortholog; green), tubulin (red) and DNA (blue).

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, non-kinetochore spindle microtubules become bundled to form the central spindle (Figure 2). We have found an evolutionarily conserved protein complex, centralspindlin, consisting of a Rho family GAP, CYK-4, and a kinesin like 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. CYK-4 contains a GAP domain that stimulates GTP hydrolysis by Rho-family GTPases. CYK-4 may promote completion of cytokinesis by virtue of its ability to promote GTP hydrolysis by RhoA.

Using recombinant CYK-4 and ZEN-4 we have reconstituted centralspindlin-mediated microtubule bundling *in vitro* and are using this system to understand how this kinesin-like protein functions at the molecular level and how its function is regulated in space and time. In particular, we are investigating the regulation of centralspindlin by mitotic kinases. AIR-2 (aurora B) kinase, and its activating and targeting subunits ICP-1 (Incenp), BIR-1 (Survivin), and CSC-1 promote the stable localization of ZEN-4 to the central spindle. We are investigating whether ZEN-4 or CYK-4 is a direct substrate of the AIR-2 kinase and if so, how phosphorylation affects the activity of the centralspindlin complex.

C. elegans embryos lacking the central spindle are competent to form cleavage furrows, thus the central spindle is not required for the early stages of cytokinesis. However, the central spindle is essential for furrow formation in other systems, such as Drosophila. We have recently gained insight into this apparent paradox, by manipulating the extent of spindle elongation. We discovered that when the extent of spindle elongation is reduced, the central spindle is essential for furrow initiation in *C. elegans* embryos (Figure 3). These results support a new model of furrow positioning in which a local minimum of microtubules leads to activation of furrowing. We are now developing tools that will allow us to dissect the molecular basis of furrow positioning.

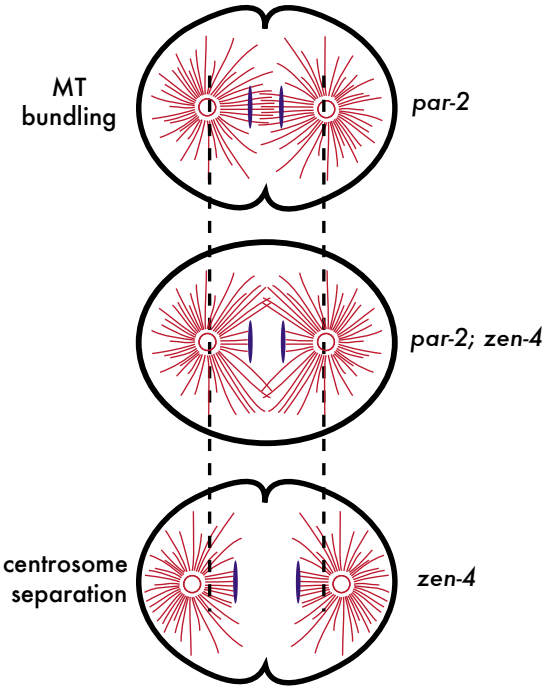

Figure 3: Parallel pathways contribute to furrow positioning. Cleavage furrow formation occurs in embryos that fail to assemble a central spindle, such as zen-4 mutant embryos. Embryos, such as par-2 mutant embryos, that show reduced spindle elongation are able to initiate and complete cytokinesis. However, 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

Christine Hartmann

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 are using mouse and chick as model organisms to gain insight into how these different processes are regulated and coordinated during embryonic and postnatal development.

Regulation of chondrocyte maturation and bone homeostasis

Using a gain of function approach we have shown, that three Wnt genes, Wnt4, Wnt5a and Wnt5b are involved in regulating chondrocyte maturation in chick. While both, Wnt5a and Wnt5b serve as negative signals, Wnt4 acts as a positive signal in chondrocyte maturation and, in addition, in bone collar differentiation (Hartmann and Tabin, 2000; unpublished observation). How is this specificity achieved? We have obtained evidence that the different Wnts utilize distinct intracellular signaling pathways; Wnt4 signals through the canonical Wnt pathway, while Wnt5a/5b do not. We are currently investigating which pathway is utilized by Wnt5a/5b. Our long-term interest is to understand the fine tuning mechanisms enabling coordinated growth of adjacent skeletal elements. We would like to uncover cross talk between the different regulatory signals known to control chondrocyte maturation. Therefore, currently we are also investigating other factors affecting chondrogenesis.

Although all, Wnt4, Wnt5a and Wnt5b are expressed in chondrogenic regions in mouse, changes in cartilage maturation have only been observed in *Wnt5a* loss-of-function mutants. Interestingly, in the mouse the expression domains of Wnt4, Wnt5a, and Wnt5b overlap in the growth plates of long bones. The expression patterns of these Wnts in skeletal elements of the mouse are somewhat different from those observed in chick. Rather unexpectedly, the analysis of various double mutant combinations has not revealed any obvious defects in skeletal maturation (work done in collaboration with Dr. McMahon's lab at

Figure 1: Skeleton of a newborn mouse. Cartilagenous regions are stained blue; bone is stained red.

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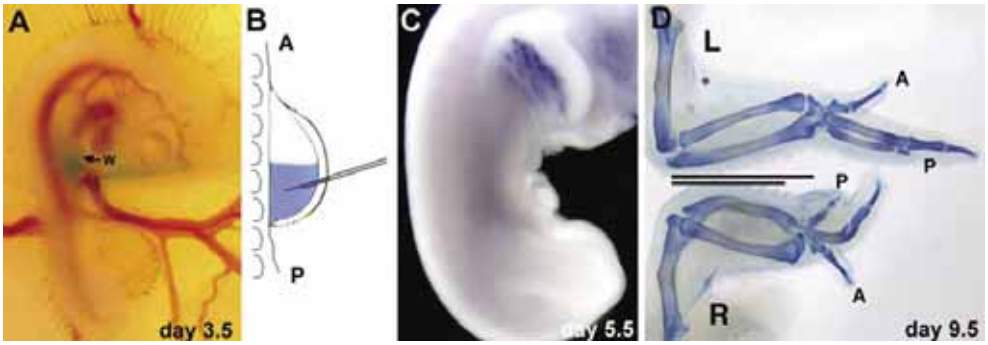

Figure 2: In ovo gain-of-function experiment with chick using retroviral injections to investigate the potential role of different factors in skeletogenesis. (A) Injections of retroviral particles are performed at day 3.5 of chick embryonic development into the posterior half anlage of the future wing (w). (B) Schematic drawing of the injected limb bud (injected region is colored blue). (C) Visualization of the infection two days after the injection, shown by the blue staining. (D) Visualization of morphological changes six days after the injection, showing the effects of the gain-of-function experiment on the skeletal elements of the right (R) wing while the left (L) wing is unaffected (cartilage elements are stained blue).

Harvard University). Consequently, we are now addressing whether the canonical Wnt-pathway plays a role in mouse skeletogenesis at all using a conditional gene targeting approach.

Recent reports have implicated Wnt-signaling in the control of bone development and maintenance (Hartmann and Tabin, 2000; Kato et al., 2002). In particular, we are interested in analyzing potential roles of Wnt4 and Wnt9a (formerly known as Wnt14) in bone homeostasis using both, chick and mouse model systems.

Synovial joint development

Our recent gain-of-function analysis of the role of Wnt9a in skeletogenesis has identified Wnt9a as a major player in the induction process of synovial joint development in chick (Hartmann and Tabin, 2001). However, in various cell culture systems Wnt9a is unable to induce the same responses as *in ovo*. Thus, we are trying now to establish an *in vitro* co-culture system that would allow us to identify co-factors required for the induction of markers characteristic of the early joint interzone. Furthermore, we are investigating whether Wnt9a is also necessary for joint development in the mouse model system. In addition, since Wnt9a continues to be expressed in synoviocytes of the mature joint we are interested to determine whether Wnt9a plays a role in maintaining joint integrity. This late expression of Wnt9a is very interesting in light of joint diseases associated with alterations of the synovium, such as rheumatoid- or osteoarthritis. We are currently addressing potential later functions of Wnt9a in the joint by gain- and loss-of-function experiments.

Our long-term goal is to identify regulators as well as target genes of Wnt9a. Using a transgenic approach in combination with searching for evolutionary highly conserved genomic regions within the Wnt9a locus we are attempting to identify regulatory elements responsible for Wnt9a expression in the early joint interzone. The identification of such a joint specific element would provide a useful tool in investigating which other factors are necessary for initiation and regulation of Wnt9a expression in the joint forming region and will inevitably allow us to understand how the skeleton is patterned.

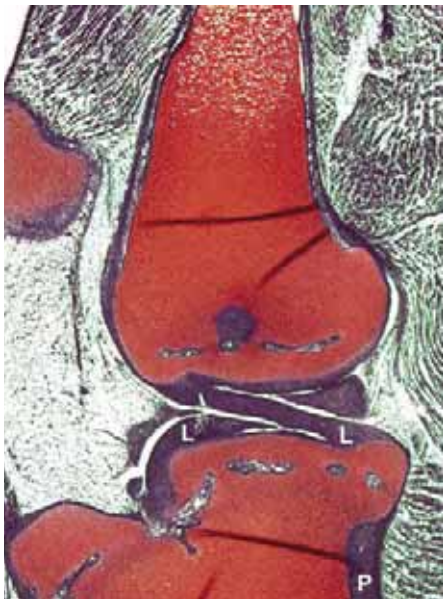

Figure 3: Section through the joint of a chicken knee. Cartilage is stained in red, soft tissue is stained in greenish, perichondrium (P) and ligaments (L) in the joint are stained in dark blue.

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Epigenetic Control by Histone Methylation

In eukaryotes, both epigenetic control of gene regulation and the functional organization of chromosomes depend on higher-order chromatin organization. Within the last three years, great progress has been made in understanding the functional implications of histone methylation, in particular through the characterization of histone methyltransferases (HMTases) that direct the site-specific methylation of lysines located within the histone H3 N-terminus. Thus, histone lysine methylation has emerged as a central epigenetic modification in the organization of eukaryotic chromatin with far-reaching implications for the regulation of cell proliferation, cell-type differentiation, overall development, gene expression, genome stability, and the genesis of cancer.

Thomas Jenuwein

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, thereby multiplying the coding potential of this particular histone modification. We have examined all possible methylation states of histone H3 lysine 9 (H3-K9) and histone H3 lysine 27 (H3-K27) in mammalian chromatin, and showed that selective combinations of distinct H3-K9 and H3-K27 methylation patterns can discriminate between heterochromatic and euchromatic subdomains. Using highly specific methyl-lysine antibodies together with quantitative mass-spectrometry, we showed that pericentric heterochromatin is selectively enriched for H3-K27 mono-methylation and H3-K9 tri-methylation. This pericentric histone lysine methylation profile is dependent on the activity of the 'heterochromatic' Suv39h HMTases but not the 'euchromatic' G9a HMTase. Intriguingly, in the absence of the Suv39h enzymes, pericentric heterochromatin undergoes alternative methylation patterns and accumulates H3-K27 tri-methylation and H3-K9 mono-methylation. Our data underscore the combinatorial coding potential of histone lysine methylation as epigenetic landmarks of eukaryotic chromatin and reveal a surprising plasticity in propagating distinct histone lysine methylation patterns.

Heterochromatin and genome stability

Murine *Suv39h* genes are encoded by two loci, both of which are widely expressed during embryogenesis, whereas 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 only approximately 30% of the expected Mendelian ratios, are growth retarded, and display hypogonadism in males. Notably, *Suv39h*-deficient mice display genome instabilities that culminate in the development of B-cell lymphomas and perturbed chromosome interactions during male meiosis. Recently, it was shown that H3-K9 methylation could direct DNA methylation in *N. crassa* and *A. thaliana*. To investigate whether a similar mechanistic link exists also in mammals, we generated *Suv39h* dn mouse embryonic stem (ES) cells. In these dn ES cells, we detect an enhanced interaction between the DNA-methyltransferase 3b (Dnmt3b) and HP1α, both of which, however, fail

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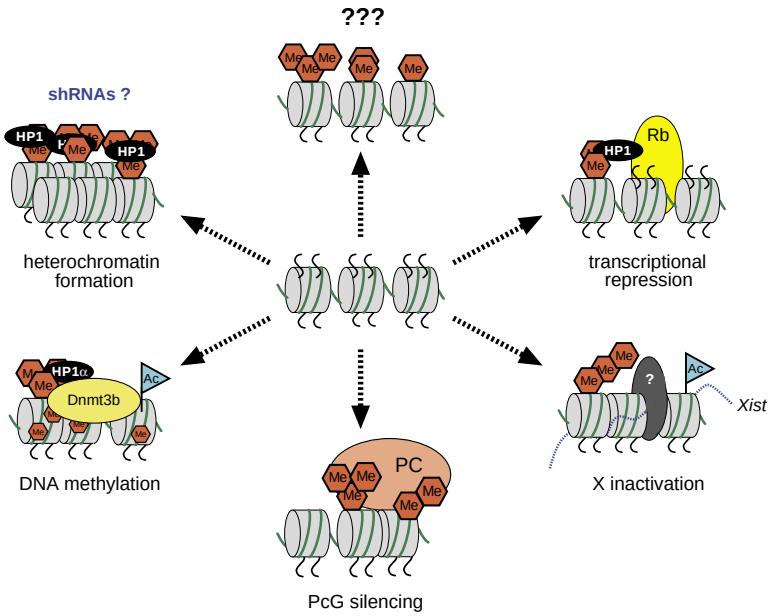

Figure 1: The many faces of histone lysine methylation. The figure summarizes the roles of H3-K9 and H3-K27 methylation in major epigenetic paradigms. Although available data have largely focused on lysine di-methylation, it remains to be explored how mono- and tri-methylation (see example on top) influence downstream events. H3-K9 methylation is depicted by vertical, H3-K27 methylation by diagonal, and DNA methylation by small hexagons (Me).

to localize to pericentric heterochromatin. In addition, there is significant DNA demethylation of major satellite repeats present at pericentric heterochromatin. By contrast, *Suv39h*-dependent H3-K9 methylation persists in DNMT-deficient ES cells. These data demonstrate an evolutionarily conserved link between histone H3-K9 methylation and DNA methylation in mammals. While the *Suv39h* HMTases are required to direct H3-K9 tri-methylation and Dnmt3b-dependent DNA methylation at pericentric repeats, DNA methylation at centromeric repeats occurs independent of *Suv39h* function. Thus, our data also indicate a more complex relationship between histone and DNA methylation systems in mammals, which is likely to be important to reinforce the stability of heterochromatic subdomains, thereby protecting genome integrity.

Epigenetic landscaping of mouse chromosomes

Alterations of the chromatin structure represent the key epigenetic mechanism organizing the information stored in the genome. In the context of the Austrian GEN-AU initiative (http://www.gen-au.at), we have started the large-scale analysis of epigenetic transitions in defined chromatin regions. Using chromatin-immunopecipitation (ChIP) on custom-made genomic microarrays (ChIP-on-chip), we have analyzed the non-random distribution of repressive histone modifications. Our data indicate that H3-K9 tri-methylation is selectively enriched within almost all repeat sequences (satellites, interspersed repeats and transposons). This analysis will be extended to the detailed analysis of one entire mouse chromosome, ultimately leading to the establishment of an epigenetic map of the mammalian genome. In addition, we are using a candidate screen to characterize novel HMTases from the pool of around 50 SET-domain genes that are present in the mouse genome. A more detailed understanding of chromosome-wide histone lysine methylation patterns and of their associated HMTase networks promises to yield new insights into our understanding of the plasticity of cell fate decisions and may offer novel strategies for the reversion of aberrant development.

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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 not only through the removal of potentially dangerous T cells, but also through the induction of so-called suppressor T cells.

Suppressor T cells

It is well established that encounter of self-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 and instead differentiate into so-called regulatory or suppressor T cells (Ts cells). The parameters that influence the choice between these two 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. Thus, we are using Tcell receptor and antigen transgenic mice, to be able to follow the fate of "self-specific" T cells under various experimental conditions. Furthermore, we are interested in gaining an insight into the mode of action of Ts cells *in vivo*. Ts cells have been mostly characterized *in vitro* and it has been suggested that these cells are anergic and that the 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 the behavior of Ts cells *in vivo*. Unexpectedly, compared to their characteristics *in vitro*, we found that Ts 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 an unexpectedly dynamic behavior of Ts cells *in vivo* and suggest that the mode of action of these cells may be based on competition for growth factors in an antigen exposed microenvironment.

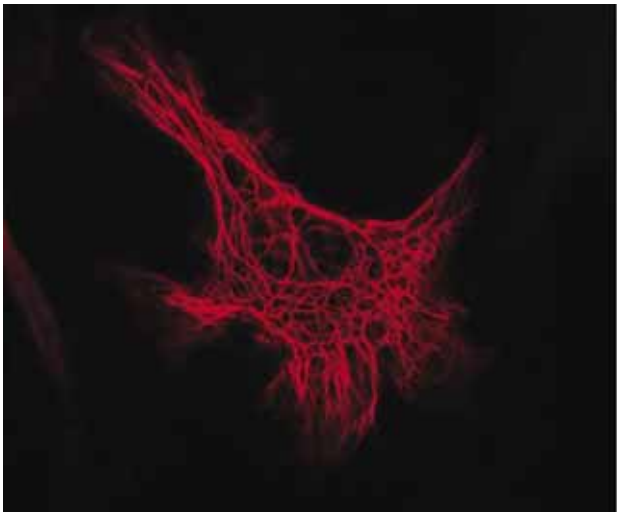

Figure 1: Immunofluorescence of an immortalized thymic epithelial cell, stained for cytokeratin, a typical marker of epithelial cells.

Ludger Klein

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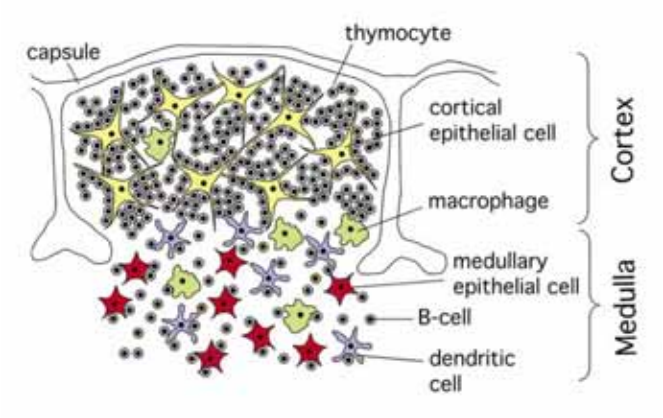

Figure 2: During their maturation in the thymus, developing T cells migrate from the outer cortex to the medulla. On their way, they can make contact with various types of thymic stromal cells. We want to understand how these interaction partners and the differentiation stage at which self-antigen is encountered determine the developmental fate of autoreactive T cells.

"Promiscuous" expression of self-antigens in the thymus

Deletion or re-programming of T cells upon encounter of self-antigens during intrathymic development is a cornerstone of immunological self-tolerance. However, it remains questionable whether these mechanisms apply to the entire spectrum of self-antigens, e.g., whether such mechanisms could operate for tightly regulated tissue-specific proteins as well. We found that the range of self-antigens expressed in the thymus is surprisingly broad. This so-called "promiscuous" intrathymic expression of otherwise strictly tissue-specific proteins is confined to medullary epithelial cells (mTEC). The mechanistic basis for this phenomenon (e.g. specific induction versus de-repression of particular genes) is only poorly understood. It has been 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* by flow-cytometry, and (ii) to study the consequences of antigen-expression in *aire*-expressing cells (deletion versus induction of anergy/suppressor function).

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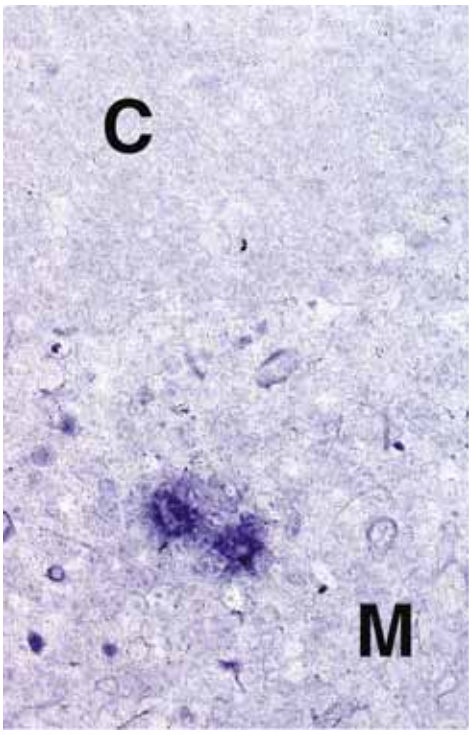

Figure 3: Visualization by in situ hybridization of two cells expressing a „liver-specific" antigen in a medullary region of the thymus (C = cortex; M = medulla).

BACK

Asymmetric Cell Division in Drosophila

To generate the many different cell types one can encounter in a multicellular organism, some cells divide asymmetrically into two different daughter cells. To achieve this, protein determinants localize asymmetrically during mitosis and segregate into one of the two daughter cells making this cell different from its sister. We are using the fruitfly Drosophila melanogaster *as a model system to understand the molecular mechanisms of such asymmetric cell divisions.*

Jürgen Knoblich

In *Drosophila,* like in vertebrates, asymmetric cell divisions play an important role in the development of the nervous system. The conserved protein Numb plays a crucial role during these asymmetric cell divisions. Numb is a membrane-associated protein that localizes asymmetrically in mitotic neural precursor cells and segregates into one of their two daughter cells (Figure 1A, B). In *numb* mutants, the daughter cell that normally inherits Numb is transformed into its sister cell, and conversely, overexpression of *numb* causes the opposite cell fate transformation. Thus, Numb acts as a segregating determinant during the development of the *Drosophila* nervous system. How Numb localizes asymmetrically and how it induces a particular cell fate are the key questions we are trying to answer.

How are determinants localized?

To study the asymmetric localization of determinants like Numb, we use neuroblasts, the precursors of the *Drosophila* central nervous system. These cells divide asymmetrically, much like stem cells, into an apical daughter cell that continues to proliferate and a basal daughter cell that divides only once more giving rise to two differentiating neurons. During neuroblast division, several proteins including Numb, but also a transcription factor called Prospero and an adaptor protein called Miranda, localize asymmetrically and segregate into the basal daughter cell. Asymmetric localization of all of these proteins requires a conserved protein complex that assembles within the apical cell cortex before mitosis. When this complex is absent, neither Numb nor Miranda localize asymmetrically and mitotic spindles are misoriented. Thus, in interphase, the apical complex establishes an axis of polarity that is used in mitosis to direct cell fate determinants to the opposite, basal side of the cell (Figure 1C). How is this achieved?

Besides two PDZ domain proteins called Par-6 and Par-3, the apical complex contains a protein kinase called atypical protein kinase C (aPKC). Using preparative immunoprecipitation and mass spectrometry, we have identified the cytoskeletal protein Lethal (2) giant larvae (Lgl) as a crucial substrate of this protein kinase (Figure 1D). Lgl is localized around the whole neuroblast cortex. On the apical side, it is transiently recruited into the Par-protein complex and phosphorylated by aPKC. Phosphorylation inactivates Lgl by releasing the protein from its association with the cytoskeleton. Although the precise molecular function of Lgl is unclear, we know that it is essential for recruiting cell fate determinants or their adaptors (like Miranda) to the cell cortex. Since Lgl is inactive on the apical side, these determinants and adaptors are excluded from this area and can only localize to the opposite, basal side of the cell cortex. Thus, the apical complex directs determinants to the basal side by inhibiting their localization to the apical side of the cell cortex.

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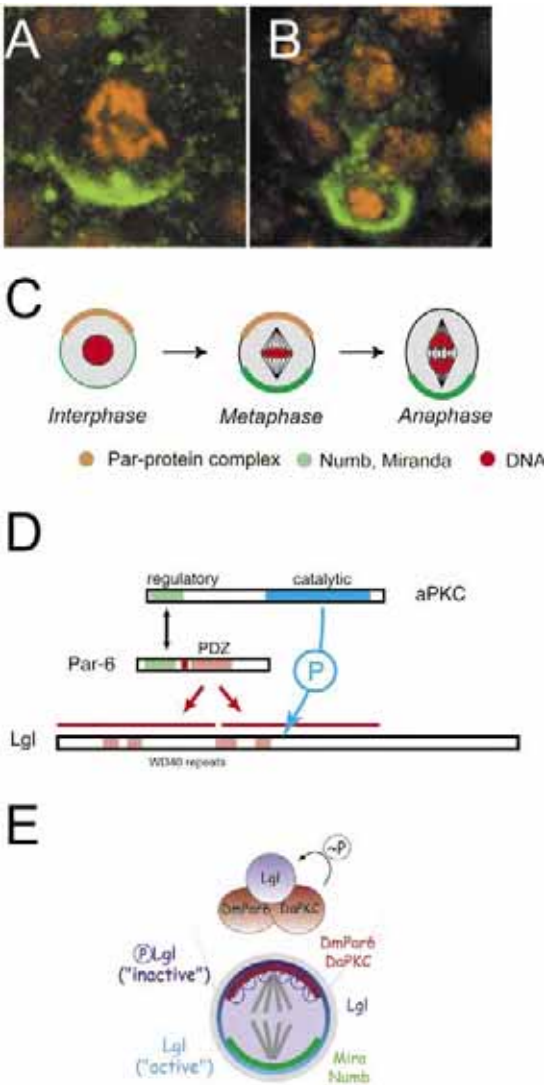

Figure 1: Asymmetric cell division in Drosophila neuroblasts. (A, B) Numb protein (green), DNA (red) and centrosomes (green) in dividing Drosophila neuroblasts. Numb localizes asymmetrically in anaphase cells (A) and segregates into one daughter cell in telophase (B). (C) Numb and Miranda (green) localize to the basal cell cortex in metaphase and anaphase. Their basal localization requires a protein complex (in orange) that assembles already in interphase and localizes to the opposite, apical, cell cortex. (D) Schematic representation of proteins in the apical complex. (E) The apical complex phosphorylates and inactivates Lgl, a cytoskeletal protein required for cortical localization of Miranda.

How do determinants influence cell fate?

Numb segregates into one of the two daughter cells and makes this cell different from its sister. How does Numb do it? Previous experiments had shown that Numb somehow represses signal transduction through the transmembrane receptor Notch. To understand, how Numb inhibits Notch, we have carried out a large-scale genetic screen for mutants that affect asymmetric cell divisions in external sensory (ES) organs. Defects in Numb function and localization lead to characteristic morphological phenotypes in ES organs because their development involves a series of asymmetric cell divisions during which Numb acts as the segregating determinant (Figure 2A, B). One of the almost 100 mutants we have identified, has a phenotype very similar to *numb*: All asymmetric cell divisions during ES organ development become symmetric (Figure 2 C). However, Numb is present and normally localized in this mutant, indicating that the affected gene acts somewhere downstream of *numb* in establishing a particular cell fate. The mutation affects α-Adaptin, a protein involved in receptor-mediated endocytosis. We could show that Numb binds to α-Adaptin and that this interaction causes asymmetric localization of α-Adaptin (Figure 2D) and its preferential segregation into one of the two daughter cells. Thus, the function of Numb is to polarize components of the endocytic machinery. One of the targets of Numb / α-Adaptin mediated endocytosis is the receptor Notch and we are currently investigating how Numb mediated endocytosis affects this important signal transduction pathway.

Asymmetric cell division is crucial for the development of all multicellular organisms. Recent experiments have demonstrated that vertebrate neural stem cells can asymmetrically segregate proteins related to Numb. To understand how asymmetric cell divisions contribute to the cell fate specification in vertebrates, we have recently begun to generate mouse knock-outs of the vertebrate homologues of the genes that we have identified in *Drosophila*. We hope that these experiments will tell us to what extent asymmetric cell divisions contribute to the development of the vertebrate, and ultimately our own body.

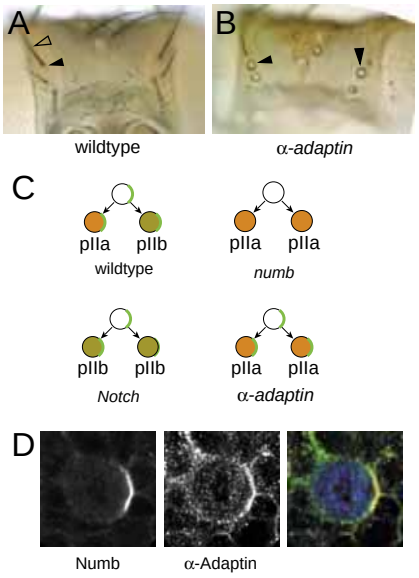

Figure 2: (A) Wild type Drosophila head. Each external sensory organ has one hair (open arrowhead) and one socket (filled arrowhead). (B) In α-adaptin mutants, external sensory organs are abnormal: Hairs are missing and replaced by extra socket cells (arrowheads). (C) Asymmetric division of sensory organ precursor cells in wild type and numb, Notch or α-adaptin mutant flies. (D) Numb binds to α-Adaptin and induces its asymmetric localization. Right: Overlay. Green: α–Adaptin, red: Numb, blue: Asense (marker of sensory organ precursor cells).

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BACK

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.

Genetic and biochemical studies on the budding yeast *Saccharomyces cerevisiae* have identified a multi-subunit complex called cohesin that is essential for holding sister chromatids together from the time of DNA replication until the onset of anaphase. Cohesin is essential for ensuring that sister chromatids attach to microtubules with opposite orientations (known as bi-orientation), which is 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 Scc1p subunit, thus triggering the movement of sisters to opposite poles.

How does cohesin bind to chromosomes? How does it hold sister DNA molecules together? How does cleavage of Scc1 break the linkage between sisters? To address these questions, we have investigated cohesin's assembly from its four subunits (Smc1, Smc3, Scc1, and Scc3). The crystal structure of a bacterially expressed SMC "hinge" region along with EM studies and biochemical experiments 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 different 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. As predicted by this hypothesis, severance of Smc3's coiled coil with the TEV protease causes both dissociation of cohesin from chromosomes and the loss of sister chromatid cohesion. We are currently testing whether cleavage of a circular DNA also causes release of DNA from the cohesin's ring.

The similarity to other ABC-like ATPases suggests that ATP bound to Smc1's head will be contacted by signature motifs within Smc3's head and *vice versa*. 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. Mutations

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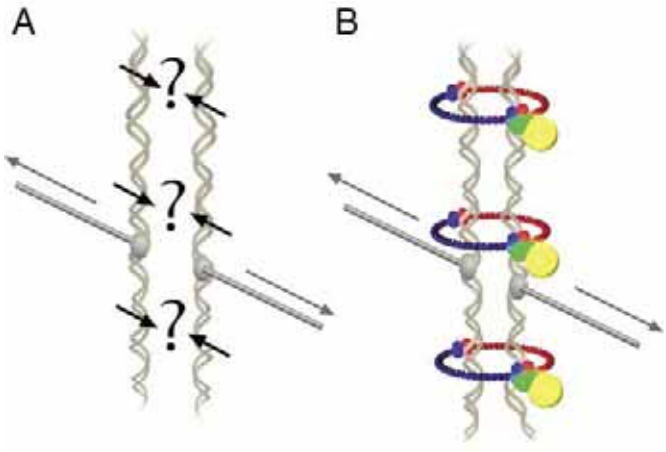

Figure 1: (A) How sister chromatids are held together has long been elusive. (B) Our current model suggests that the cohesin complex connects sister chromatids by forming a ring around them.

predicted to abolish binding of ATP to Smc1's head abolished the binding of Scc1's C-terminus to Smc1 whereas mutations affecting hydrolysis of ATP bound to either Smc1's or Smc3's head had no effect on the formation of tripartite rings. However, the latter mutations abolished cohesin's loading onto chromosomes raising the possibility that ATP hydrolysis is required for the entry of DNA into cohesin's ring. If DNA is to enter the ring, one of the three contacts between Smc1, Smc3, and Scc1 must become transiently broken. Whether this is actually the case is currently under investigation.

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 Rec8p that is needed for preventing precocious separation of sister chromatids during meiosis. Rec8p and other cohesin subunits are found all along the longitudinal axis of chromosomes during pachytene. They disappear from chromosome arms during the first meiotic division but persist in the neighbourhood of centromeres until metaphase II. We have recently 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* has recently identified a highly conserved protein necessary for protecting centromeric cohesion. We are also investigating whether cleavage of Rec8 is necessary for the first meiotic division during spermatogenesis and oogenesis in mice.

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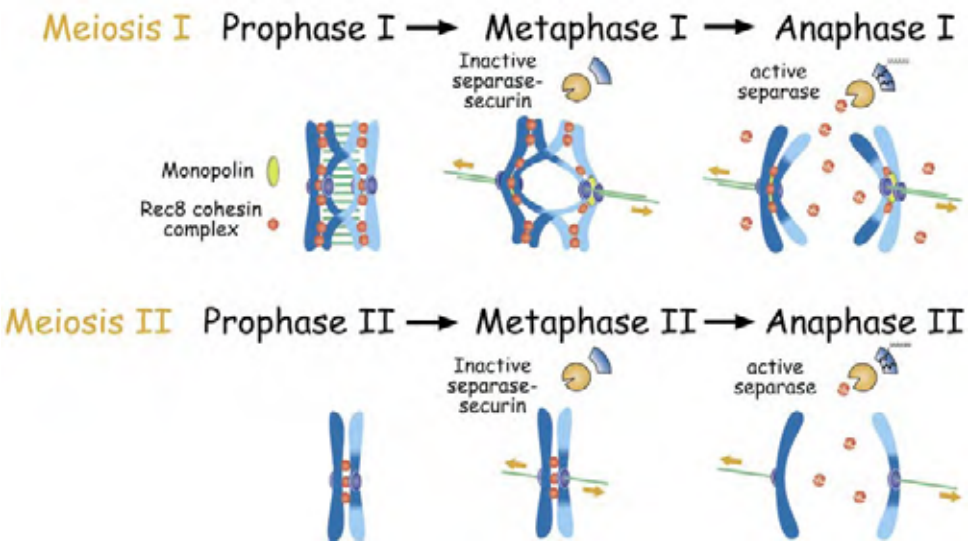

Figure 2: Chromosome segregation during meiosis.

Molecular Mechanisms of Vertebrate Development

The body of all vertebrates is essentially composed of a large number of highly specialized cells. These cells are not randomly scattered but are organized in defined spatial arrangements to give rise to functional units such as organs or body parts. We are using the mouse and chick as model organisms to study the molecular mechanisms that control the development of organized structures from initially very simple groups of cells. Our research focuses on the vertebrate face - a structure that is frequently affected in congenital malformation syndromes in humans.

Annette Neubüser

The vertebrate face develops from buds of tissue, the facial primordia, which surround the primitive mouth (Figure 1). Development of the midfacial region begins with the appearance of the nasal placodes - bilateral ectodermal thickenings at the ventro-lateral sides of the forebrain - that will give rise to the olfactory epithelium. Shortly after the placodes become morphologically apparent the mesenchyme around them starts to grow out to form the nasal processes. Continued outgrowth depends on interactions between the epithelium covering these processes and the underlying mesenchyme. How the areas of mesenchymal outgrowth are established and how the early facial region is patterned is not well understood and is at the focus of our interests.

Figure 1: Scanning electron micrographs of the facial region of mouse embryos at E9.5 and E10.5. The nasal placodes (np), thickenings of the facial ectoderm, are the first morphologically distinct structures to form in the prospective midfacial region. By E10.5, the mesenchyme around the placodes has started to grow out to form the medial (mnp) and lateral (lnp) nasal processes and the placodes have now come to lie in shallow depressions, the nasal pits (np, the future nasal cavities), between the nasal processes.

FGF8 function during facial development

FGF8 is a member of the fibroblast growth factor family of signaling molecules. *Fgf8* is widely expressed in the ectoderm covering the midfacial area at early stages of facial development but becomes restricted to a horseshoe shaped domain of expression around the nasal placodes at later stages (Figure 2 A, B). Mouse embryos in which this gene has been inactivated in the facial region develop severe facial defects. Such embryos display midfacial clefts and most derivatives of the first branchial arch are severely reduced or absent (Figure 2 C, D). In early mutant facial mesenchyme the amount of cell death is increased and cell proliferation is reduced. Patterning in the remaining tissue is also affected, in particular in the midfacial area at E9.5 as

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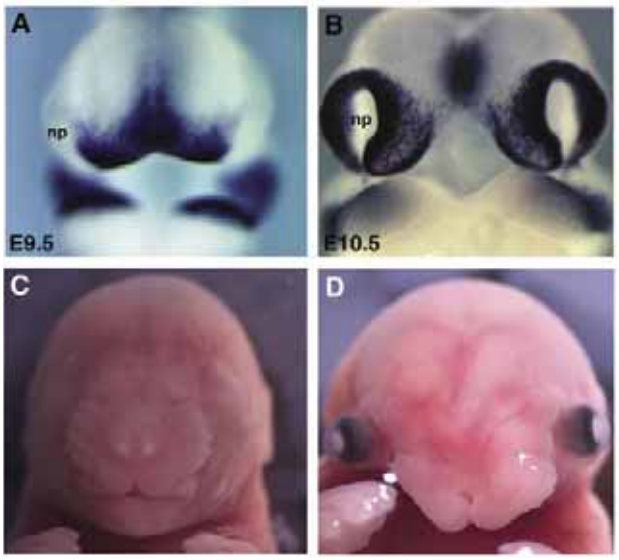

Figure 2: Tissue specific inactivation of Fgf8 in the facial area results in severe facial defects. Facial expression of Fgf8 at E9.5 (A) and E10.5 (B). The face of a wildtype (C) and an Fgf8 mutant embryo (D) at E16.5. Embryos in which Fgf8 has been inactivated in the facial area develop a midfacial cleft and show a severe reduction of the lower jaw and peri-ocular tissue.

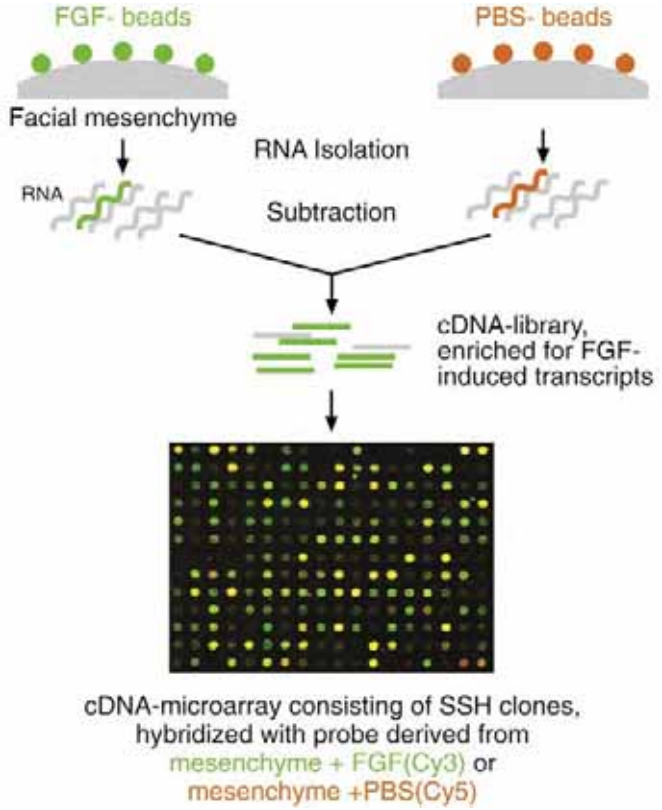

Figure 3: Identification of FGF-inducible genes. Facial mesenchyme was cultured in vitro with FGF or PBS soaked beads. RNA was isolated from these explants and used to generate a subtracted (SSH) cDNA-library, enriched for FGF inducible clones. The inserts of 4400 clones from this library were then arrayed on glass slides. The resulting microarray was hybridized with probe derived from RNA isolated from facial mesenchyme cultured with FGF or PBS soaked beads, labeled with a green or red fluorescent dye (Cy3 or Cy5), respectively.

judged by the analysis of the expression of marker genes. In addition to the mesenchymal defects, also the development of the nasal placodes and the surrounding ectoderm is abnormal in *Fgf8* mutant embryos. This includes changes in the expression patterns of ectodermal signaling molecules. Therefore, altered signaling between the mutant ectoderm and the underlying mesenchyme is likely to contribute to the defects observed at later stages.

Identification of genes transcriptionally regulated in facial mesenchyme in response to FGF signaling

In order to understand how FGF8 controls development of the facial mesenchyme it is important to identify the genes induced or repressed in response to FGF8 signaling. We are using an *in vitro* explant culture system in which facial mesenchyme is cultured in contact with facial ectoderm, in isolation or in contact with polymeric beads soaked in FGF8 protein, to identify such genes (Figure 3).

Using a candidate approach, we have shown that FGF signaling induces the expression of the transcription factors *Pax3, Tbx2, Erm* and *Pea3* in facial mesenchyme. To systematically screen for FGF inducible genes, we have generated a subtracted cDNA-library from facial mesenchyme cultured in the presence or absence of FGF and have used this library to produce a customized DNA microarray. This microarray was probed with cDNA derived from mesenchyme cultured with or without FGF. The expression pattern of 200 clones with the strongest differential hybridization was then analyzed by whole mount *in situ* hybridization and inducibility by FGF8 was confirmed. Through this screen we have identified more than 50 genes that are induced in the facial mesenchyme in response to FGF signaling and we have begun to characterize some of them. We believe that this analysis will ultimately help us to understand the function of FGF signaling during development at the molecular level.

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BACK

Mitosis

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

Jan-Michael Peters

Chromosome cohesion and condensation

During S-phase, replicated DNA molecules (sister chromatids) become physically connected by cohesin complexes. This cohesion is essential to allow the bipolar attachment of sister chromatid pairs to the spindle, which in turn is an essential prerequisite for the symmetrical segregation of the chromatids into daughter cells during mitosis. Because cohesin connects sister chromatids, these complexes have to 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 the activity of Polo-like kinase Plk1. At the same time, protein complexes related to cohesin, called condensins, bind to chromosomes and contribute to their condensation. Throughout the early stages of mitosis, small amounts of cohesin persist at centromeres where they maintain sister chromatid cohesion until all chromosomes have been attached to both poles of the mitotic spindle, i.e. until metaphase. At this stage, the protease separase becomes activated and cleaves centromeric cohesin, thereby initiating sister chromatid separation. We are interested in understanding how Plk1 unloads cohesin from chromosomes, how centromeric cohesin is protected from this mechanism, and how cohesin unloading and condensin loading contribute to the structural organization of the chromosome.

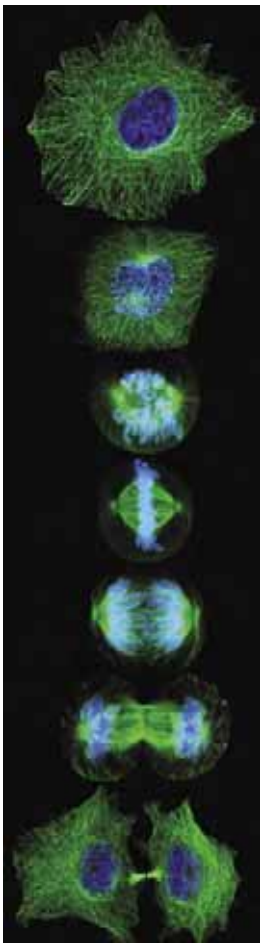

Figure 1: Mitosis in human cells. Courtesy of Toru Hirota.

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[1] until October 2003, [2] until February 2003, [3] since June 2003, [4] until April 2003, [5] since January 2003, [6] maternity leave since June 2003, [7] until August 2003, [8] since November 2003, [9] until May 2003, [10] until June 2003, [11] since July 2003, [12] part-time, [13] since September 2003

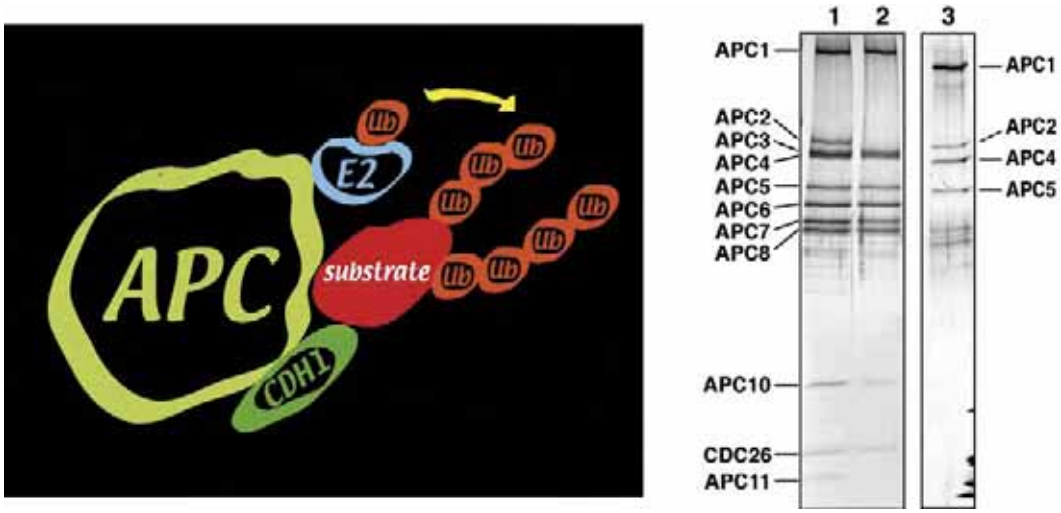

Figure 2: The Anaphase-Promoting Complex (APC). Left, cartoon view of how the APC, E2 enzymes and the CDH1 activator might assemble polyubiquitin chains (Ub) on substrate proteins. Right, subunit composition of the APC (lane 1) and APC sub-complexes (lanes 2 and 3) as revealed by SDS-PAGE and silver staining. Through the analysis of APC sub-complexes we are analyzing the roles of individual subunits. For further details, see Vodermaier et al., Curr Biol. 13, 1459-1468, 2003.

Chromosome attachment to the spindle

During chromosome condensation, kinetochores assemble on centromeric DNA and are subsequently captured by spindle microtubules, resulting eventually in the attachment of all chromosomes to both poles of the mitotic spindle. A special surveillance mechanism, called the spindle checkpoint, ensures that anaphase is not initiated until all chromosomes have achieved this bipolar state. We are using RNA interference, chemical inhibitors and video microscopy to analyze how mitotic kinases control chromosome attachment to the spindle. We recently identified the small molecule Hesperadin as an inhibitor of the mitotic kinase Aurora B and found that the activity of this kinase is required for correcting syntely, a type of attachment in which both sister kinetochores of a chromosome become erroneously attached to microtubules from one spindle pole. Our work implies that Aurora B's correction function at the kinetochore is also required for proper functioning of the spindle checkpoint.

The Anaphase-Promoting Complex (APC)

Separase is activated by ubiquitin-dependent proteolysis of its inhibitor securin, a process that is mediated by the multi-subunit ubiquitin ligase APC. APC 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 ubiquitinates substrates and how its activity is regulated we are dissecting the APC biochemically and we are collaborating with crystallography and electron microscopy groups to analyze APC's structure. We identified numerous phosphorylation sites on the APC, found that a subset of these can be phosphorylated by cyclin-dependent kinase 1 (Cdk1), and that Cdk1 activity is sufficient for Cdc20 binding and APC activation. On the APC, we also identified candidate receptors for Cdc20 and the related activator protein Cdh1. In the future, we will try to understand how the binding of these proteins activates the APC and how the spindle checkpoint suppresses this activation.

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Gene Function in Mammalian Development and Oncogenesis

The major focus of our studies is to analyze gene functions in normal and pathological, e.g. tumor development, using mouse as a model organism. Specifically, the functions of AP-1 proteins such as Fos and Jun, in regulating cell proliferation, differentiation, and cell death in bone, liver, heart, and skin, and in hematopoietic and neuronal development, are investigated. In addition, specific functions of VEGF, Flk-1, and EGF-Receptor are also studied in bone, epithelial and endothelial cells.

The role of Fos/AP-1 proteins in bone cell differentiation

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, itself a c-Fos target gene, is essential for mouse development, and transgenic mice over-expressing Fra-1 develop an osteoblastic bone disease, osteosclerosis (Figure 2). Interestingly, gene replacement of c-*fos* by *fra*-1 revealed functional equivalence of these two proteins (Figure 2). To investigate how c-Fos and Fra-1 control osteoblast and osteoclast differentiation, we generated conditional alleles of *c-fos* and *fra-1*. The embryonic lethality of the *fra-1* knock-out mice was rescued with a conditional allele of *fra-1* using *cre*M mice, however, the mutant mice developed osteopenia (Figure 2). The conditional allele of c-*fos* was also used to monitor expression of c-Fos during development and to study c-Fos function in the CNS (Figure 1).

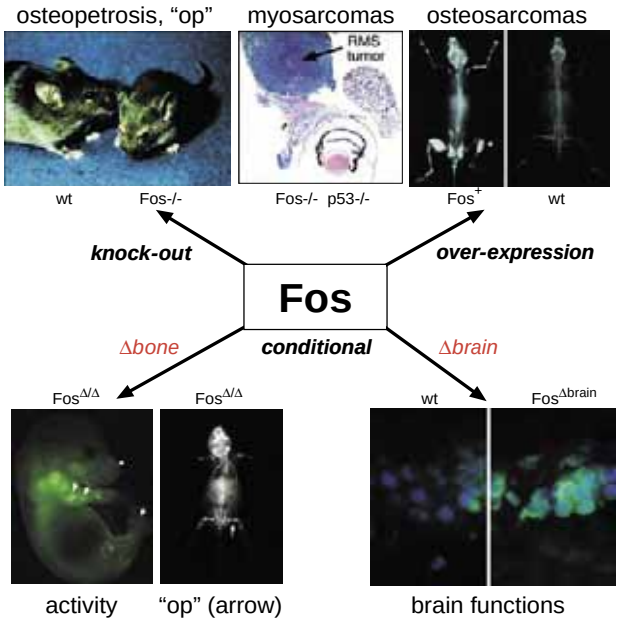

Figure 1: Functional analysis of Fos in bone and CNS development, and the dual role of Fos as oncogene and anti-oncogene.

Erwin F. Wagner

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[2]*Marie Curie Grant*
[3]*since December 2003*
[4]*until June 2003*
[5]*until January 2003*
[6]*FWF Grant*
[7]*part-time*

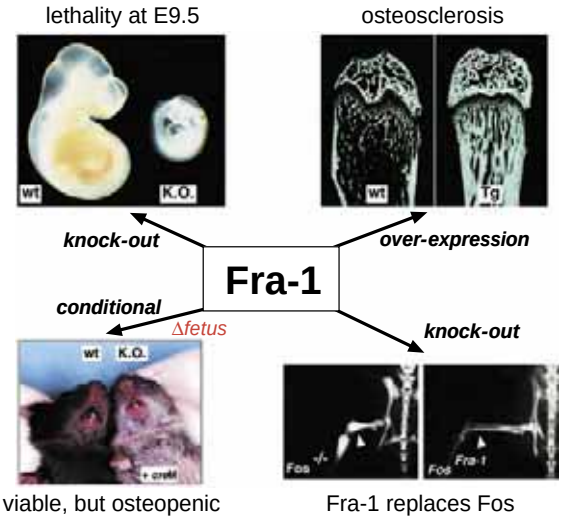

Figure 2: Functional analysis of Fra-1.

Tumor suppressive function of 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 different in vivo approaches including conditional inactivation, we showed that the absence of JunB expression in the myeloid lineage results in a transplantable myeloproliferative disease, which eventually progresses to blast crisis thereby resembling human chronic myeloid leukemia. On the other hand, JunB was also shown to be a positive regulator of bone remodeling, since mice lacking JunB are severely osteopenic. When the fos-/- osteopetrotic mice were crossed into the p53 background, double mutant mice developed rhabdomyosarcomas (Figure 1). Re-expression of Fos in double mutant muscle tumor-derived cell lines induced apoptosis implying a novel, totally unexpected function of the proto-oncogene Fos as a potential tumor suppressor in the muscle lineage.

Jun/AP-1 function in cell proliferation, differentiation, and apoptosis

The functions of the Jun family members are analyzed by conditional mutagenesis, knock-in strategies, and transgenic rescue experiments. Deletion of *jun* in adult mouse liver revealed that Jun is dispensable for postnatal liver function, however it is essential for liver regeneration (Figure 3). Moreover, Jun is required as a survival factor during liver tumor development. Deletion of *jun* in skin did not affect proliferation of keratinocytes in adult mice, but led to an eye closure defect during embryonic development and affected skin tumor development. These phenotypes are likely caused by down-regulation of HB-EGF and EGFR, which was found to be a transcriptional target of Jun. Chondrocyte-specific inactivation resulted in severe scoliosis caused by failure of intervertebral disc formation suggesting that Jun is a novel regulator of sklerotomal differentiation (Figure 3). Interestingly, replacing Jun with JunB showed that JunB could substitute for Jun during embryonic development but not during adulthood.

An important mechanism regulating Jun activity is phosphorylation of Jun at serine 63 and 73 by the Jun amino-terminal kinases (JNKs). Null alleles of *Jnk1* and *Jnk2* genes (collaboration with M. Karin, UCSD) as well as a *jun* allele mutated at the JNK phosphoacceptor sites (*JunAA)* were generated. *Jnk1*-/-, *Jnk2*-/- and *JunAA* mice are healthy and fertile, but the absence of *Jnk1* and Jun-N-terminal phosphorylation (JNP) causes growth retardation and fibroblast proliferation defects. Moreover, Jnk1 and Jun phosphorylation are required for efficient osteoclast differentiation. Therefore, JNK signaling and JNP differentially regulate cell proliferation, differentiation and apoptosis in diverse biological processes.

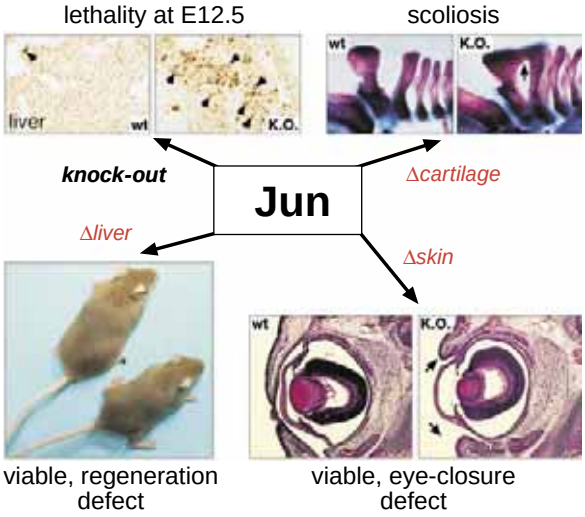

Figure 3: Functional analysis of Jun in development and disease.

Functional studies of VEGF, VEGF-R2/Flk-1, and EGF-R

The VEGF/Flk-1 signaling system is essential for the development of endothelial and hematopoietic cells. To study Flk-1's role in adult mice and in tumor angiogenesis a conditional allele of Flk-1 was generated. A conditional allele of VEGF was employed to analyze the functional importance of VEGF-A in developing chondrogenic tissues and in skin biology (collaboration with E. Tschachler, General Hospital, University of Vienna). In addition, conditional and mutated human EGF-Receptor alleles were created to study the role of EGF-R in normal and tumor development of the skin (collaboration with M. Sibilia, Department of Dermatology, University of Vienna).

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BACK

Mammalian X-chromosome Inactivation

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

The mammalian *Xist* gene has been shown to be responsible for the initiation of X-inactivation. *Xist* RNA spreads in *cis* from its site of transcription over the entire X-chromosome and mediates the transcriptional silencing of one of the two X-chromosomes in female cells. Thereby, compensation for the dosage difference of X-linked genes between XY males and XX females is achieved. X-inactivation begins early in cell differentiation and initially transcriptional silencing is reversible and dependent on continuous *Xist* expression. Later in differentiation, the silent state becomes irreversible and independent of *Xist*. Our previous studies have shown that the localization of *Xist* and silencing can be separated by specific mutations. Deletion of a repeat element on the 5´-end of *Xist*, the repeat A, results in production of an RNA that localizes in *cis* and spreads over the chromosome, but does not cause transcriptional repression.

In collaborative work with the group of Thomas Jenuwein, we have shown that *Xist* localization independent of silencing can trigger chromosomal modifications such as histone H3 lysine 27 tri-methylation. Detailed measurements of the kinetics and efficiency of histone methylation also reveal a novel chromosomal memory that is triggered by *Xist* RNA localization independent of silencing (Kohlmaier, et al. submitted). This finding has implications for the mechanism of X-inactivation, as previous models have implicated the existence of self-perpetuating heterochromatin structures and hence gene silencing as the principal memory of the inactive state at later phases of X-inactivation. Future work is directed at the identification of the molecular basis of this form of chromosomal memory and its specific function in the process of X-inactivation.

Identification of proteins that interact with *Xist* RNA in chromosomal silencing

To gain insight into the molecular mechanism by which *Xist* affects transcriptional repression we aim to isolate proteins that interact with the repeat A of *Xist* RNA - the crucial element involved in initiation of silencing. From nuclear extracts, we have isolated proteins that specifically interact with the sense but not with the antisense repeat A RNA (Figure 1). In collaboration with Karl Mechtler at 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 in order to elucidate the potential pathway for *Xist* mediated transcriptional silencing. Experimental approaches will include generation of specific antibodies and analysis of loss-of-function mutations created by gene targeting in ES cells and mice.

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[1] *since April 2002*
[2] *since October 2003*
[3] *until April 2003*

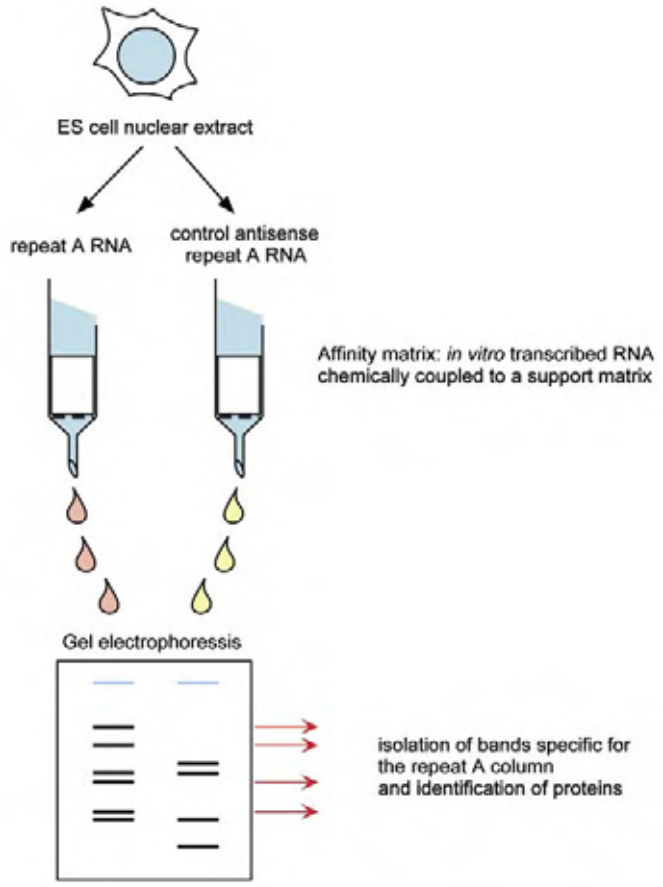

Figure 1: Biochemical purification of proteins that bind Xist repeat A. Repeat A at the 5´-end of Xist RNA is crucial for gene silencing. Proteins are isolated by affinity chromatography on a matrix consisting of in vitro transcribed repeat A RNA or antisense control RNA. Bound proteins are eluted and analysed by gel electrophoresis. Proteins specific for binding to the repeat A RNA are identified by mass spectrometry.

Xist-mediated silencing in mice

Experiments performed in mouse ES cells demonstrate that initiation of *Xist*-mediated silencing is restricted to an early state of differentiation. Thus, the cellular context in which *Xist* effects gene silencing is closely linked to, and could be potentially regarded as a feature of stem cells. In differentiated cells *Xist* expression does not lead to initiation of transcriptional silencing even in cases where the RNA properly localizes to the chromatin. To study the initiation of silencing in mammalian development we have generated an inducible *Xist* allele in mice. Preliminary analysis shows that chromosome wide silencing on the X can be achieved by ectopic expression of *Xist* in mouse embryos. Induction of *Xist* expression leads to histone H3 Lysine 27 methylation, transcriptional repression of X-linked genes and induces cell death in male embryos. In adult mice ectopic *Xist* expression from the single male X-chromosome leads to a severe haematopoietic defect. Currently, we are characterising the mutant phenotype in greater detail focusing on the haematopoietic stem cell compartment.

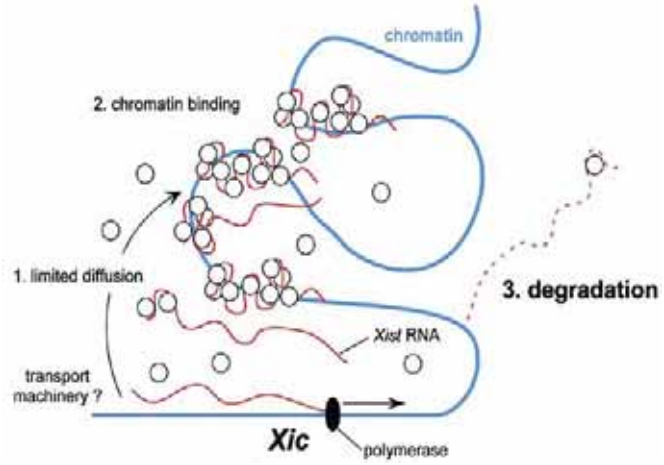

Figure 2: A model for Xist RNA localisation to chromatin in cis. Different stages (1 to 3) are shown to clarify important features. (1) Xist RNA is transcribed from the Xic and associates with binding proteins. The complexes formed are unstable due to the presence of only low affinity binding sites. (2) When the concentration of Xist is sufficiently high a cooperative binding mechanism ensures complex stability. One of the factors in the cooperative binding process is the existence of docking sites on chromatin and Xist preferentially localizes to chromatin in proximity to its site of synthesis (which potentially could also include regions on other chromosomes which are by chance in close proximity to the Xic). Complexes that fail to associate with chromatin diffuse away and are degraded. (3) Xist-mediated silencing is initially reversible and upon cell division inadvertently silenced parts of autosomes become reactivated, because they are unlinked to the Xic and are therefore unlikely to remain at the close distance. This adjusts the range of the inactivation before it becomes irreversible and explains the precise pattern normally found in cells.

Functional studies of X inactivation in mice and ES cells should provide insight into the epigenetic regulation of gene expression in mammals. It appears that *Xist* mediated silencing is a paradigm for a powerful epigenetic system that is capable of hetero-chromatinising an entire chromosome and determines its specific nuclear localisation (Figure 2). It is expected that similar interactions underlie the regulation of other genes - however, with less dramatic consequences.

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BACK

Service Department

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

Our Media Kitchen staff prepares substantial quantities of reagent quality solutions and media for cell culture, flies (*D. melanogaster*; approximately 300'000 bottles and tubes per year) and worms (*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.

Production of antibodies

In collaboration with various IMP Research Groups, the Service Department produces monoclonal antibodies in hybridomas and organizes the antibody production in rabbits with an outside company.

Sequencing and DNA isolation

With the two ABI 3100 Genetic Analyzer capillary sequencers we sequenced approximately 34'000 samples in the first 9 months of this year. This is a slight increase as compared to 2002. Next year we expect a substantial increase of our sequencing output due to the acquisition of many IMBA members as "customers". The average read-length on the 3100 Genetic Analyzers equipped with the 80 cm capillaries is 700-900 bases for standard DNA samples. We are saving both, time, by using an optimized and fast clean-up protocol with small Sephadex G50 superfine columns on 96-well microtiter plates, and money, by reducing the amount of sequencing reagents for plasmid and PCR DNA samples.

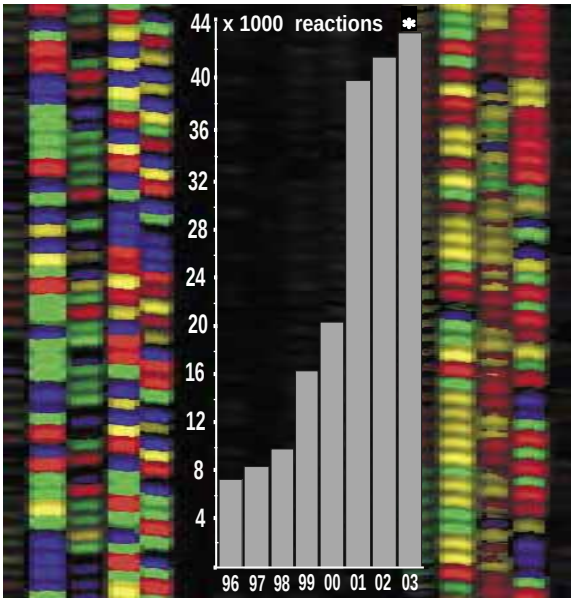

Figure: A sequencing run on an ABI 377 PRISM and number of reactions analyzed on ABI 377 (1996 - 2001) and on ABI 3100 (2001 - 2003) done with dye deoxy terminators (v3.1 since 2002) in the years 1996 to 2003 (scale 0 to 44'000).

**calculated from January 2003 to September 2003 data*

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Biooptics Department

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

Flow cytometry

The year 2003 saw the installation of a new flow cytometer (FACSAria, Becton Dickinson) allowing us to perform multicolor sorts at high speed (routinely about 35,000 events/second). Currently, we are establishing protocols for sorting rare cells (as few as 0.005% of the total cell population) at speeds >500,000 cells/second using more than seven colors simultaneously.

Microarrays

During the last year, major improvements of the microarray experimentation were achieved with establishment of new labeling techniques for RNA samples. By introducing a linear amplification protocol for mRNA, the amount of total RNA needed for a microarray experiment could be reduced to less than 2µg. In combination with an indirect labeling method using Alexa-dyes, the reproducibility of the experiments was greatly improved. Furthermore, easy to use statistical analysis and normalization methods were included in our microarray information management system.

Microscopy and Image Analysis

Following major reconstruction works at the IMP all microscopes were transferred to new facilities. All microscopes (currently 12 different ones including two laser scanning confocal microscopes and one spinning disk confocal microscope) are now located in the close vicinity of researchers, facilitating a much faster and efficient support of our users. In the past year, a web based booking system has been implemented, allowing each user the on-line booking of microscopes, FACS-machines, analysis workstations, and array-lab instrumentation. On request, this service has been extended to the booking of seminar rooms, guest apartments, and other instrumentation. The backbone of the system is a complex database (BOSS), which includes, among others, equipment and user administration databases.

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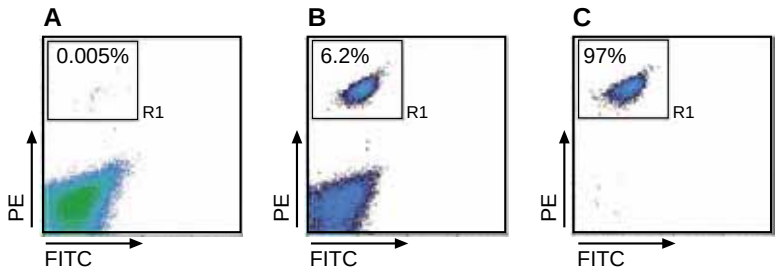

Figure: High speed sorting of rare cells. 5x10^8 murine spleen cells containing a population of 0.005% PE-stained target cells (A) were first sorted at a speed of 900,000 cells/sec. The enriched population (B, 6.2% positive cells) was then sorted under high purity condition, resulting in a population with 97% purity (C) and a yield of 75%. The total time to perform this procedure was approximately 15 minutes.

Peter Steinlein / *Staff Scientist*

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Volker Leidl / *Software Architect*
Karin Paiha / *Microscopy and Imaging*
Martin Radolf / *Microarrays*
Gabriele Stengl / *Flow Cytometry*

Animal House

The animal house group provides husbandry of animals and services for the various research groups at the IMP.

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 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 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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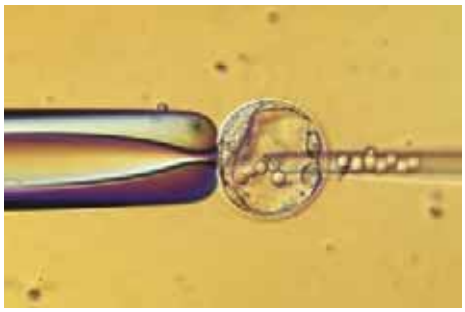

Animal House

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Erwin F. Wagner / *Scientific Coordinator*
Norma Howells / *Consultant*
Mijo Dezic / *Technician*
Katja Flahndorfer-Stepanek / *Technician*
Sabine Häckl / *Technician*
Sabine Jungwirth / *Technician*
Erika Kiligan / *Technician*
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Maja Tumpej[1] / *Technician*

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Mouse Service Department

Hans-Christian Theussl / *Technician*

Protein Chemistry Facility

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

The IMP mass spectrometry facility operates together with the Institutes of the Vienna Biocenter and the CISTEM Biotechnologies Company. Further collaborations were established with Applied Biosystems and Dionex to develop novel methods for analysis and quantification of post-translational modifications.

Quantitative mass spectrometry of histone 3 methylation states

This year we have started a challenging project, namely the identification and quantification of post-translational modifications of histone H3 N-terminus (collaboration with the Jenuwein group). Histone H3 N-terminus contains several lysine (K) residues that can occur either in a mono-, di-, or trimethylated form. A prerequisite for any comparative analysis by mass spectrometry is that similar peptides containing either the H3-K9 or H3-K27 residues can be efficiently cleaved from H3 molecules. We therefore developed a novel peptide cleavage method in which isolated H3 molecules are first chemically modified at every susceptible (i.e. unmodified or mono-methylated) lysine residue by propionylation. Subsequent digestion with trypsin results in efficient cleavage after each arginine position, thereby enabling quantitative analyses of similar peptide fragments by nano-HPLC using electrospray ionization coupled to an ion-trap mass spectrometer.

Peptide synthesis and antibody purification

We are synthesizing 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. We employ a special protocol for affinity purification of antibodies under mild conditions.

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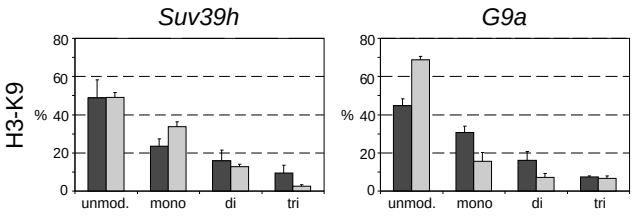

Figure: Quantitative mass spectrometry of H3-K9 methylation states. Comparative analysis of H3-K9 mono-, di-, and tri-methylation levels in H3 (aa 9-17) peptide fragments isolated from Suv39h dn and G9a-deficient ES cells. The relative level of a particular H3-K9 methylation state is indicated with respect to the sum of all H3-K9 modifications present in the H3 (aa 9-17) peptide population. Levels were normalized for different ionization potentials as determined by measuring the relative recovery of known amounts of chemically synthesized and modified peptide standards.

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BACK

Publications 2003

Group Beug

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Group Busslinger

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Awards and honours

Erwin F. Wagner
Charles Rodolphe Brupbacher Prize for Cancer Research
(Zürich, March 13, 2003)

Kim Nasmyth
Boveri Award for Molecular Cancer Genetics
(Würzburg, March 27, 2003)

Tim Clausen
Heinz Maier-Leibnitz-Preis of the "Deutsche Forschungsgemeinschaft"
(Bonn, May 15, 2003)

Kim Nasmyth
Honorary degree of Doctor from the University of York
(York, UK, July 9, 2003)

Jürgen Knoblich
ELSO Early Career Award
(Dresden, September 23, 2003)

Stephan Gruber and Christian H. Haering
Cell Cycle Publication Prize to young cell cycle researchers in Austria
(Innsbruck, November 13, 2003)

Seminar speakers at the IMP

January

03.01.03	KONRAD HOCHEDLINGER (Whitehead Inst., Cambridge)
10.01.03	MAGDALENA GOETZ (MPI of Neurobiol., Munich)
14.01.03	KERSTIN WENDT (MPI of Biochem., Martinsried)
24.01.03	KYONGRIM KWON (IGBMC, France)
31.01.03	CENK SUMEN (Stanford Univ., California)

February

06.02.03	NADIA ROSENTHAL (EMBL, Monterotondo)
07.02.03	INGRID ZEELENBERG (The Netherlands Cancer Inst.)
25.02.03	DARIA GRAPHODATSKAYA (ETH, Zürich)
27.02.03	MAJORI MATZKE (Univ. of Salzburg)
28.02.03	ARIEL RUIZ I ALTABA (NYU School of Med.)
28.02.03	ZEN KOUCHI (Tokyo Women's Med. Univ.)

March

06.03.03	ANTONINA ROLL-MECAK (Rockefeller Univ.)
26.03.03	ANDREAS STRASSER (WEHI, Melbourne)
27.03.03	STEVE BROWN (MRC Harwell, U.K.)
28.03.03	WILLIAM B. WOOD (Univ. of Colorado, Boulder)

April

03.04.03	ROBERT HUBER (MPI of Biochem., Martinsried)
10.04.03	JONATHAN HODGKIN (Oxford Univ.)
11.04.03	MARIANNE BRONNER-FRASER (Calif. Inst. of Technol.)
11.04.03	ERWAN WATRIN (Universite de Rennes I, France)
22.04.03	JOANNE ATTEMA (Australian Nat. Univ., Canberra)

May

02.05.03	RAMIRO GISLER (Lund Univ., Sweden)
05.05.03	PETER PAPATHANASIOU (The Austral. Nat. Univ., Canberra)
08.05.03	EDWARD TRIFONOV (Genome Diversity Center, Univ. of Haifa, Israel)
14.05.03	WILLIAM KAELIN (Dana-Farber Cancer Inst., Boston)
15.05.03	JUERGEN HESCHELER (Inst. of Neurophysiol, Univ. of Cologne)
22.05.03	TOM MISTELI (NIH, Bethesda)
23.05.03	NIKLAS FINNBERG (Karolinska Inst., Stockholm)

June

02.06.03	MATTHIAS BOCHTLER (Internat. Inst. of Mol. and Cell Biol., Warsaw)
03.06.03	TODD PRAY (San Francisco)
04.06.03	HORACE FREELAND JUDSON (The George Washington Univ.)
05.06.03	JUERG KOHLI (Univ. of Berne)
11.06.03	MATTHIAS HEBROK (Univ. of California, San Francisco)
12.06.03	JAMES WANG (Harvard Univ.)
12.06.03	KRISZTINA NAGY (Eötvös Loránd Univ., Budapest)
16.06.03	PETER HASSELBLATT (Med. Univ. Freiburg)
18.06.03	TERRY HASSOLD (Case Western Reserve Univ., Ohio)
20.06.03	AMY LAWS (Univ. of Massachusetts)
23.06.03	KENTARO NAKAGAWA (Univ. of Tokyo)
24.06.03	GERO MIESENBÖCK (Memorial Sloan-Kettering Cancer Center, NY)
25.06.03	MARC KIRSCHNER (Harvard Med. School, Boston)
25.06.03	MARTINA LUTTEROVA (Inst. of Preventive and Clin. Med. Bratislava)
26.06.03	MICHAEL YOUNG (Rockefeller Univ.)
27.06.03	AMBER ALSOP (Cambridge Univ.)

July

03.07.03	JONATHON HOWARD (MPI, Dresden)
08.07.03	LOTAR HENNIGHAUSEN (MPI of Biochem., Martinsried)
10.07.03	ALISON BAKER (Univ. of Leeds, UK)
16.07.03	TADASHI UEMURA (Inst. for Virus Research, Kyoto Univ.)
17.07.03	PATRICK SUNG (Univ. of Texas Health Science Center, San Antonio)
21.07.03	YLVA LINDERSON (Karolinska Inst., Sweden)
24.07.03	JOCHEM WITTBRODT (EMBL, Heidelberg)
28.07.03	CHRIS NELSON (Univ. of British Columbia, Vancouver)
31.07.03	DAVID MORGAN (UCSF)

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August

07.08.03	ANGEL NEBREDA (EMBL Heidelberg)
13.08.03	OLIVIERO CARUGO (ICGEB Trieste and Univ. Pavia, Italy)
14.08.03	JERRY CRABTREE (Stanford Univ.)
14.08.03	MICHAEL MANNS (Medizinische Hochschule, Hannover)
20.08.03	ALAIN MAUVIEL (Hopital Saint-Louis, Paris)
21.08.03	ADRIANO AGUZZI (Univ. of Zurich)
26.08.03	CHRISTOF LENZ (Applied Biosystems, Darmstadt)
28.08.03	KAY DAVIES (Univ. of Oxford)

September

05.09.03	TOBIAS JUNT (ETH Zurich)
08.09.03	HANS VAN DAM (Leiden Univ., The Netherlands)
08.09.03	ANDREAS FÖRSTER (Univ. of Utah)
08.09.03	CHRISTELLE BOURGEOIS (NIH, Bethesda)
11.09.03	YOSHINORI WATANABE (Univ. of Tokyo)
11.09.03	SHIV GREWAL (Nat. Cancer Inst., Bethesda)
12.09.03	SHOICHIRO ONO (Emory Univ., Atlanta, Georgia)
12.09.03	ANTONIO LANZAVECCHIA (Bellinzona, Switzerland)
15.09.03	ALEXEY RUZOV (Univ. of Edinburgh)
18.09.03	ALEXANDER TARAKHOVSKY (The Rockefeller Univ.)
19.09.03	PETER JACKSON (Stanford Univ. School of Med., California)
23.09.03	OLIVER HOBERT (Columbia Univ.)
25.09.03	PATRICK CRAMER (Univ. of Munich)

October

06.10.03	FODOR BARNA (Biol. Res. Center, Hung. Academy of Sciences)
07.10.03	LORI PASSMORE (Instit. of Cancer Research, London)
08.10.03	DAVID SPECTOR (Cold Spring Harbor Laboratory)
15.10.03	STEPHANIE NOTTROTT (MPI, Goetingen)
16.10.03	FRANK VAN KUPPEVELD (Nijmegen Center for Mol. Life Sciences, The Netherlands)
17.10.03	"GENES TO GENOMES" Symposium to honour Max Birnstiel on his 70th birthday
20.10.03	PAUL KINCADE (Oklahoma Medical Res. Found.)
23.10.03	ROBERT GOLDMAN (Feinberg School of Med., Chicago)
24.10.03	FRAUKE MELCHIOR (MPI for Biochem., Munich)
28.10.03	JAMES HUTCHINS (Univ. of Dundee)
29.10.03	FRANCESC POSAS (Univ. Pompeu Fabra, Barcelona)
30.10.03	WOLFGANG BAUMEISTER (MPI of Biochem., Martinsried)

November

04.11.03	AXEL BEHRENS (Lincoln's Inn Fields Laboratories, London)
06.11.03	MICHAEL ROSBASH (Brandeis Univ., Waltham, MA)
14.11.03	MAREK MLODZIK (Mount Sinai School of Med., New York)
21.11.03	STEPHEN MALIN (Microbiol. and Tumour Biol. Center, Karolinska Institutet, Stockholm)
24.11.03	JUAN GUINEA (Clinica Puerta de Hierro, Madrid)
27.11.03	CHRISTINE HOLT (Univ. of Cambridge)

December

04.12.03	ROBERT KRUMLAUF (Stowers Inst. for Med. Res., Kansas City)
05.12.03	JAREMA MALICKI (Harvard Med. School)
11.12.03	DETLEF WEIGEL (MPI for Dev. Biol., Tübingen)
17.12.03	DOMENICO MIGLIORINI (Europ. Inst. of Onc., Milan)
17.12.03	BRIAN MCCABE (Univ. of California, Berkeley)

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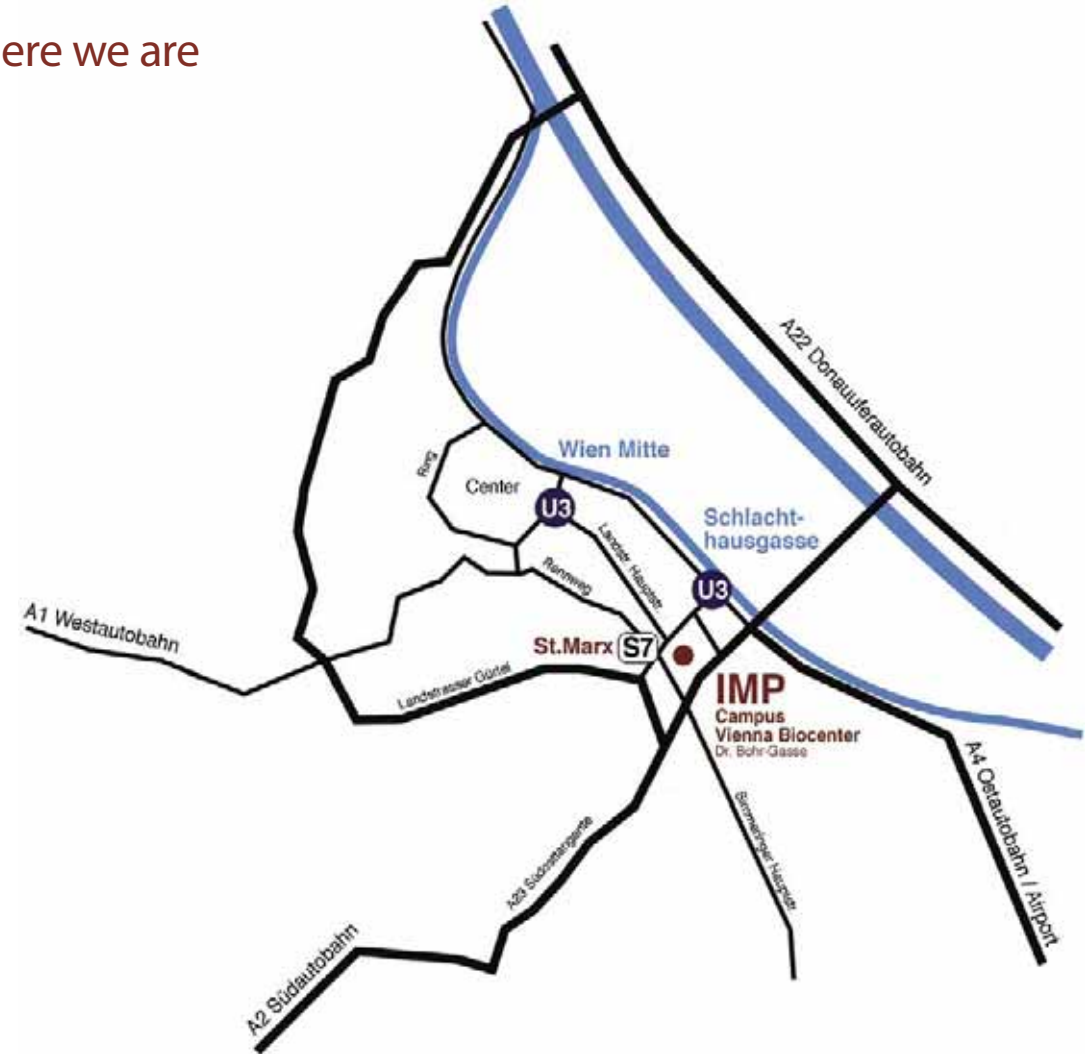

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