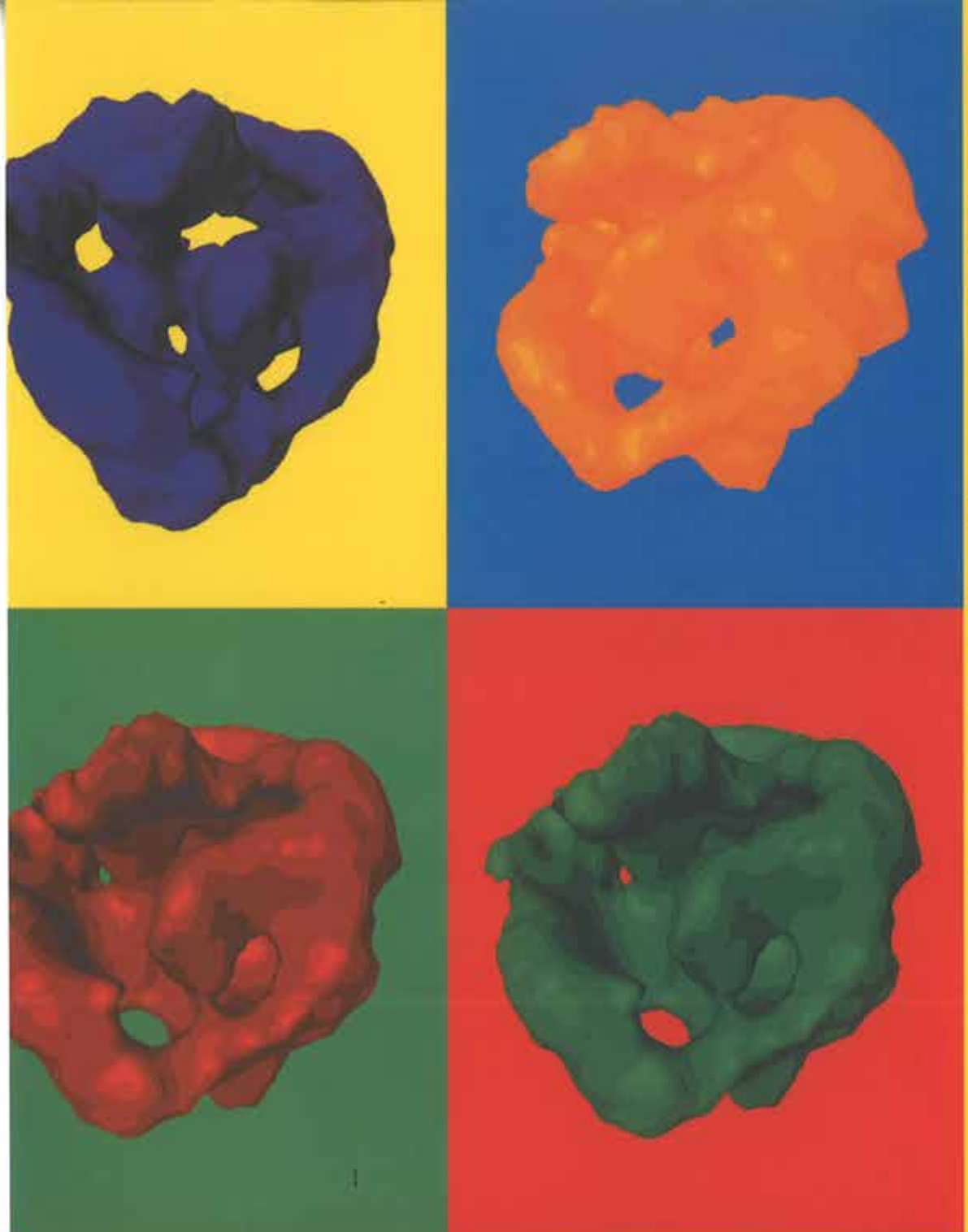

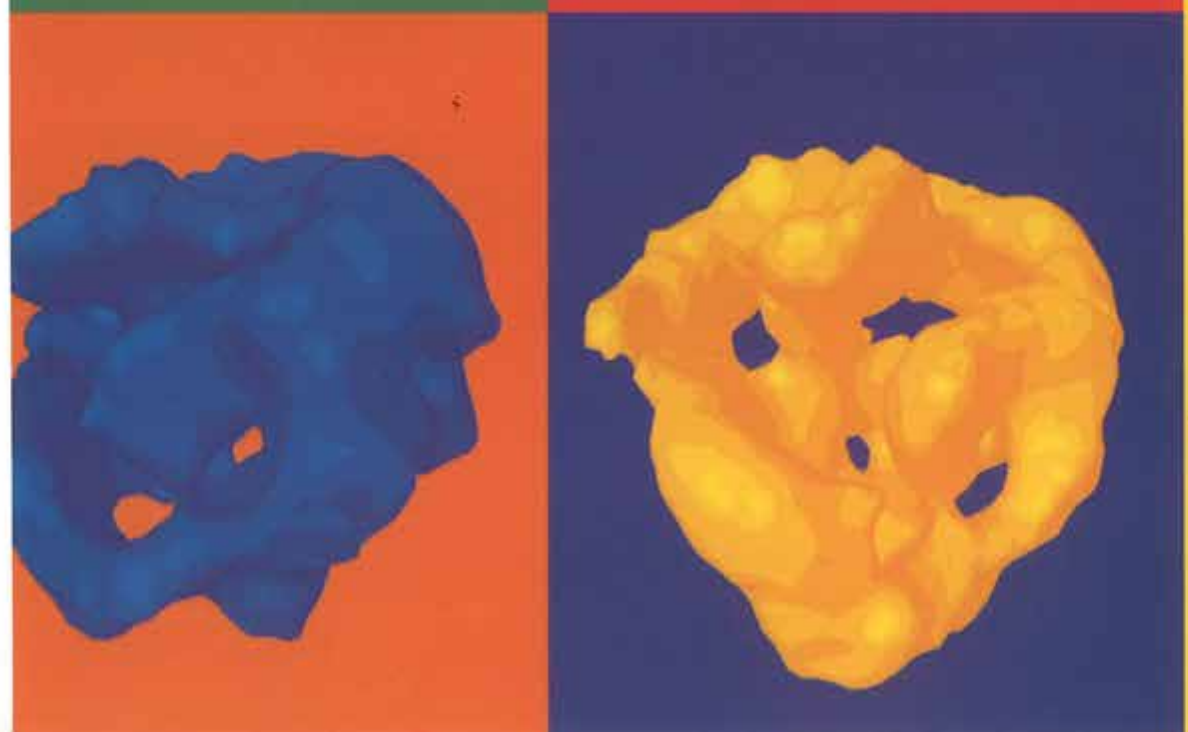

Research Institute of Molecular Pathology
VIENNA BIOCENTER

I.M.P.
2001

Boehringer Ingelheim

The past year has been yet another remarkable one for the IMP. Our science has undoubtedly now moved into a yet higher gear; major discoveries were made at the IMP in the areas of chromatin biology, chromosome segregation, neuronal guidance and stem cell biology.

Our success in recruiting young leaders of the highest quality and potential was confirmed by the fact that EMBO selected four IMP group leaders for its newly established Young Investigator Programme. In addition, Jan-Michael Peters was awarded both the Austrian Novartis Prize and the Roche Prize for cell biology 2001. This year's Wittgenstein Prize (the highest and most generously endowed Austrian award for science) went to Meinrad Busslinger, who was the third IMP recipient of the award in 6 years. Speaking of prizes, we are very proud and happy that Tim Hunt, who has been a member of our Scientific Advisory Board for the past seven years, was awarded this year's Nobel Prize in Physiology & Medicine for his discovery of cyclins.

In a successful search for new young group leaders we were fortunate to be able to recruit Christine Hartmann from Harvard and Anton Wutz from the Whitehead Institute. Both Christine and Anton have now arrived and taken up their work at the IMP. In the course of this turn-around phase, Gerhard Christofori left the IMP for the University of Basel, where he has taken a Chair in Biochemistry. We wish him all the best with this new and challenging position.

The IMP's support services continue to be one of our greatest strengths: micro arrays are now well underway and, together with the other established scientific support facilities, we are able to provide a fantastic range of core services for IMP research groups. The BioInformatics Department continues to make major contributions to research at the IMP, and it is our great hope that we will soon be able to make Structural Biology an integral part of our research environment.

Everyone at the IMP, from Diploma Student through to Group Leader, including all of our fantastic support staff, should be immensely proud of the excellent research to which each and every one of them has contributed. Maintaining the present standard is going to be our greatest challenge for the future!

Kim Nasmyth
December 2001

Research Institute of Molecular Pathology (I.M.P.)
Dr. Bohr-Gasse 7
A-1030 Vienna
Austria

Phone + 43/1/797 30
Fax + 43/1/798 71 53
http://www.imp.univie.ac.at

For a copy of this report please contact Heidemarie Hurtl
at the IMP Public Relations Department
e-mail hurtl@imp.univie.ac.at

Contents

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Hartmut BEUG | Senior Scientist

Stefan GRÜNERT | Postdoc
Andrea KOLBUS | Postdoc
Richard MORIGGL | Postdoc
Cathleen SCHULTE | Postdoc
Thomas WÄRNER | Postdoc
Sebastian CAROTTA | PhD Student (with P. Steinlein)
Martin JECHLINGER | PhD Student
Uwe SCHMIDT | PhD Student
Martina SYKORA | PhD Student
Sandra PILAT | Diploma Student
Astrid STEINMAIR | Diploma Student
Eva Maria DEINER | Technician
Gabi LITOS | Technician
Andreas SOMMER | Technician

Developmental plasticity and its deregulation in oncogenesis

Distinct families of oncogenes and tumor suppressor genes stimulate proliferation/renewal and inhibit differentiation/ apoptosis of primitive progenitor cells in experimental systems analysed by our group (erythroleukemia and breast carcinoma). Interestingly, unmutated genes of these families involved in neoplasia regulate normal processes, i.e. expansion of erythroid progenitors during stress or disease and epithelial/ mesenchymal transitions during embryonic development and wound healing.

Stress-induced alteration of proliferation control in hematopoietic progenitors: a process important in leukemogenesis.

Renewal (proliferation without differentiation) in hematopoietic progenitors can be regulated by the cooperation of plasma membrane- and nuclear receptors, such as receptor tyrosine kinases (RTKs) and cytokine receptors synergizing with class I/II nuclear receptors (NRs, steroid-, thyroid / retinoid acid receptors). Mutated RTKs, NRs and downstream transcriptional regulators function as oncoproteins in avian, murine and human leukemias. Stress erythropoiesis caused by anemia/hypoxia involves excessive renewal of spleen erythroid progenitors *in vivo*, driven by elevated erythropoietin (Epo) and glucocorticoid receptor (GR) ligands. This is mimicked *in vitro* by sustained proliferation of primary murine and human erythroblasts in response to activation of the Epo receptor by Epo, of the RTK c-Kit by stem cell factor/SCF and GR activation by dexamethasone/Dex. Erythroblast renewal requires signal transduction via Stat5 (EpoR driven) and PI3K (c-Kit-driven), shown in normal wt and Stat5 -/- erythroid progenitors and by specific low MW inhibitors. The oncogene v-ErbB and the epidermal growth factor receptor activate both Stat5 and PI3K on their own. Thus leukemic oncogenes utilize the same signalling pathways as employed by normal receptors regulating stress erythropoiesis. During differentiation, Epo upregulates the antiapoptotic effector Bcl-X_L. Exogenous expression of Bcl-X_L in primary mouse erythroblasts allows terminal differentiation in the complete absence of Epo/insulin, showing that erythroid differentiation is a default programme if the cells are protected from apoptosis by Epo via Bcl-X_L (Fig.1).

Current research activities involve: polysome-bound mRNA profiling on Affymetrix chips, focussing on Epo/SCF induced genes and their modulation by GR activation, and genes regulated during terminal differentiation. About 20% of these genes were exclusively regulated by translational control. Within an EU network, we also analyse the EpoR/c-Kit signalosome, using receptor-activating magnetic beads. Finally, multipotent murine progenitors (expanded in specific cytokine/hormone mixes) are used to analyse leukemia oncogenes (v-Ski, MLL/ENL) cooperating with c-Kit, as well as potential hematopoietic commitment/differentiation defects in mice lacking the histone methyltransferases SUV39H1 and 2

Signal transduction by the TGFβ-receptor: required for tumor cell invasiveness and metastasis

During carcinoma progression, epithelial cells lose their polarity required for the protective barrier function of epithelia. We identified epithelial-mesenchymal transition (EMT) as an *in vitro* correlate of local

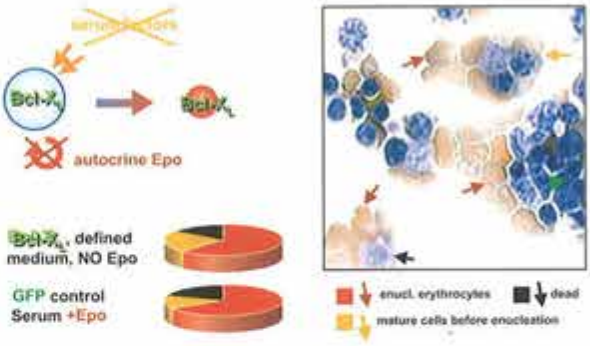


Figure 1: Erythroid differentiation is a default programme in cells protected from apoptosis by the Epo target gene BclXL.
Primary erythroid progenitors upregulate the antiapoptotic protein BclX$_L$ in a strictly Epo-dependent fashion. If BclX$_L$ is expressed in primary mouse fetal liver erythroblasts via retroviral vectors, the cells terminally differentiate in the complete absence of Epo/insulin, using a fully defined medium plus Epo-neutralizing antibody to rule out unknown serum/protein factors and an autocrine Epo loop (top left). >75% enucleated red cells are formed (top right, red arrows, nuclei after enucleation, green arrowheads). Quantitative evaluation of cytospins (bottom) shows that Bcl-X expressing cells without any factors differentiate equally well as control cells plus Epo under optimal conditions (serum)

invasion and metastasis. During EMT, cells acquire mesenchymal charactistics and become metastatic, a process also occurring during embryogenesis, tissue remodelling, inflammation and wound healing. In fully polarized mammary epithelial cells, EMT is caused by cooperation of oncogenic Ras with endogenous transforming growth factor β receptor (TGFβ R). This cooperation protects the cells from TGFβ-induced cell cycle arrest and apoptosis, causes transcriptional loss of epithelial markers plus de novo expression of mesenchymal markers and induces a TGFβ-autocrine loop stabilizing the EMT phenotype. Using Ras effector mutants signalling, preferabely via the MAPK- and PI3K pathways, as well as specific Mek1- or PI3K inhibitors, cooperation of a hyper-active MAPK pathway with TGFβ signalling was shown to be required for EMT *in vivo* and metastasis, while PI3K- plus TGFβ signalling caused enhanced proliferation, reversible induction of a mesenchymal phenotype without gene expression changes (»scattering«) and tumors, but not metastasis. We performed polysome-bound mRNA profiling of multiple EpH4-based cell pairs showing EMT with or without metastasis, scattering plus or minus tumors or apoptosis protection. Cluster analysis identified small groups of genes typical for EMT/metastasis, scattering or Ras oncogene function, identifying signalling pathways, transcription factors, tumor suppressors and enzymes relevant for these processes. Functional characterization of one upregulated pathway revealed that TGFβ induces a PDGF-PDGFR autocrine loop playing distinct, essential roles in the induction and maintenance of EMT. (Fig 2).

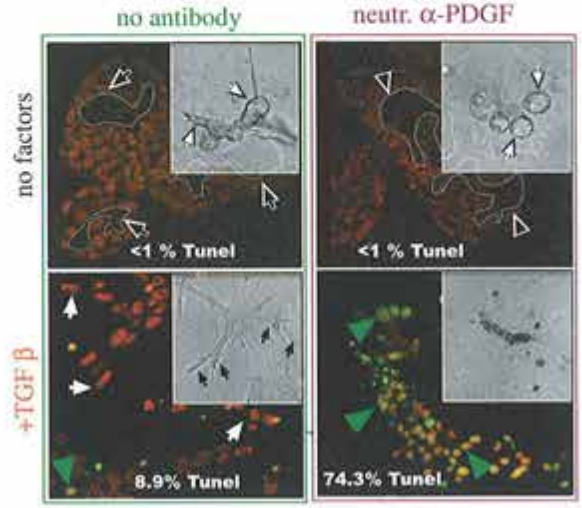


Fig 2. TGFβ-induced PDGF autocrine loop required to protect EpRas from apoptosis during EMT. EpRas cells form hollow structures in collagen gels (top left, shadowed arrows), but are induced to undergo EMT in response to TGFβ, forming unordered structures of mesenchymal-like cells invading the gel (bottom left, simple arrows). Neutralizing PDGF antibodies (neutr. α-PDGF) alone have no effect (top right), but cause massive apoptosis (TUNEL-positive, apoptotic nuclei marked by green arrowheads) in the presence of TGFβ (bottom left)

Current research activities include expression profiling of epithelial cells in which we reconstituted inducible Ras signalling by coexpression of a ligand-inducible Raf-ER plus a PI3K-specific Ras effector mutant, suitable for kinetic analysis of EMT-regulated gene expression. We also investigate the role of TGFβ R signalling in EMT, focussing on the role of SMAD2 and SMAD3 in EMT, in combination with Mek/MAPK signalling. Since this pathway does not inhibit nuclear translocation of Smads, as claimed by others, we focus on transcriptional complexes assembled by Smads, possible modulation of such transcription factor complexes by Mek/MAPK signalling and cooperation of Smads with β-catenin/Wnt signalling through interaction with TCF/LEF1.

Contact: beug@nt.imp.univie.ac.at

Meinrad BUSSLINGER | Senior Scientist

Maxime BOUCHARD | Staff Scientist
Cesar COBALEDA | Postdoc
Dirk EBERHARD[1] | Postdoc
Maud FORSBERG | Postdoc
Caroline HUTTER[2] | Postdoc
Ingvild MIKKOLA[3] | Postdoc
Alessio DELOGU | PhD Student
Martin FUXA | PhD Student
Barry HEAVEY | PhD Student
Marina PASCA DI MAGLIANO | PhD Student
Michael SCHEBESTA | PhD Student
Christof CHARALAMBOUS | Diploma Student
Abdallah SOUABNI | Technician
Qiong SUN | Technician

[1] until Aug. 2001; [2] since Oct. 2001; [3] until May 2001

Stem cell commitment in hematopoiesis and organogenesis

Tissue-restricted stem cells give rise to the different cell types of an organ by undergoing commitment to and subsequent differentiation along distinct lineages. By using a combination of mouse transgenic, cell biological and molecular approaches, we investigate the mechanisms by which the paired box-containing transcription factors of the Pax protein family control the commitment processes involved in B cell, kidney and midbrain development.

Hematopoiesis

A fundamental question in hematopoiesis is how stem cells and early progenitors become committed to a single developmental pathway and then differentiate into mature cell types of the selected lineage. By analyzing the transcription factor Pax5, we have gained insight into the molecular mechanisms controlling commitment to the B-lymphoid lineage. *Pax5* is essential for the progression of B cell development beyond an early progenitor (pro-B) cell stage. Surprisingly, Pax5-deficient pro-B cells are uncommitted hematopoietic progenitor cells, as they can still develop into natural killer (NK) cells, T-lymphocytes and various myeloid cell types (Figure 1). B cell development is, however, only observed upon retroviral restoration of *Pax5* expression. These experiments identified *Pax5* as the B-lineage commitment factor, which restricts the developmental potential of progenitor cells to the B cell pathway. Conditional gene inactivation demonstrated that *Pax5* expression is continuously required to maintain B-lineage commitment in early B cell development and to control the identity of mature B cells in late B-lymphopoiesis. 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 have screened a pro-B cell cDNA microarray to identify novel Pax5 target genes (Figure 2). Using transgenic approaches, we have mapped the B-cell-specific enhancer of *Pax5*, whose analysis will identify the upstream regulators of this gene. Finally, precocious expression of *Pax5* in hematopoietic stem cells and progenitors results in a massive increase of B cell development at the expense of other hematopoietic lineages. Hence, Pax5 is both necessary and sufficient to promote B cell development. Together, these experiments will lead to a better understanding of the transcriptional control of B-lineage commitment.

Midbrain and kidney development

The midbrain and cerebellum develop from an organizing center at the midbrain-hindbrain boundary (MHB), which secretes the signalling molecule Fgf8. The three transcription factors of the Pax2/5/8 family are co-expressed in this embryonic brain region (Figure 3). One goal of our laboratory is to unravel the molecular mechanisms by which Pax proteins regulate midbrain and cerebellum development. Gene targeting and transgenic analyses indicated that Pax2 is essential not only for *Pax5* and *Pax8* expression, but

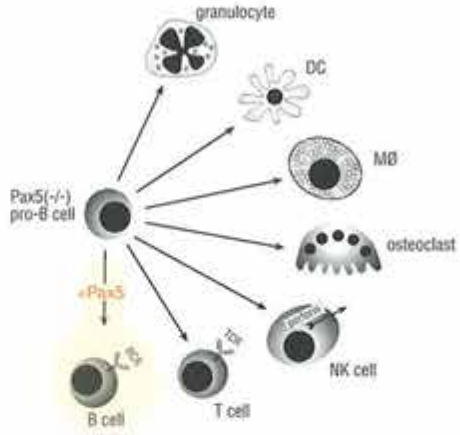


Figure 1: B-lineage commitment by Pax5. *Pax5*$^{-/-}$ pro-B cells are early progenitor cells which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway.

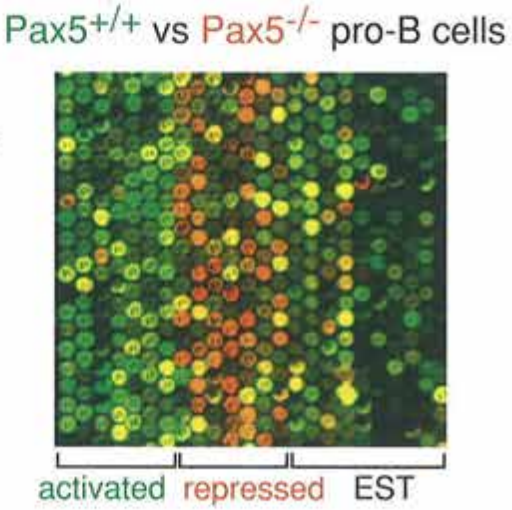


Figure 2: Identification of Pax5-regulated genes. Libraries enriched for activated or repressed genes were generated by cDNA subtraction between wild-type and *Pax5*$^{-/-}$ pro-B cells, and 5'000 clones of each library were spotted together with known ESTs on glass slides. This pro-B cell chip was hybridized with Cy3 (green)-labelled cDNA from wild-type pro-B cells and Cy5 (red)-labelled cDNA from *Pax5*$^{-/-}$ pro-B cells.

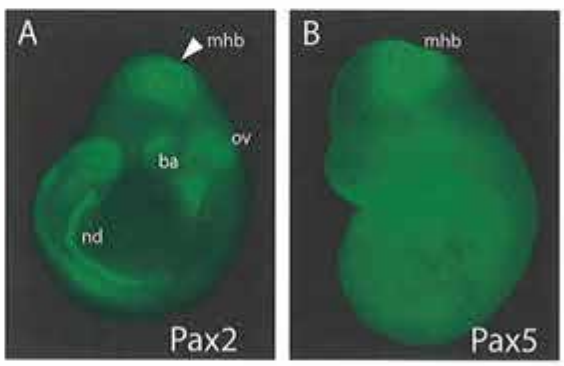


Figure 3: Expression of *Pax2* and *Pax5* BAC transgenes. The green fluorescent protein (GFP) gene was inserted in frame into exon 2 of the mouse *Pax2* or *Pax5* gene. Transgenic embryos at day 9.5 express GFP in all known expression domains of *Pax2* (A) or *Pax5* (B). Mhb, mid-hindbrain boundary; ov, otic vesicle; ba, branchial arches; nd, nephric duct.

also for activation of the organizer signal Fgf8 itself. As Pax2 is the earliest regulator of MHB development, we have subjected this gene to a thorough BAC transgenic analysis, which resulted in the characterization of the early *Pax2* enhancer and identification of its upstream regulator Oct4/Pou2 (Figure 3). Pax2, together with Pax8, is also the earliest marker of kidney development. Moreover, Pax2 and Pax8 are essential for the commitment of intermediate mesoderm cells to the nephric lineage, as *Pax2, Pax8* double mutant embryos fail to form even the earliest stage of kidney development. We are now using cDNA microarray analysis to identify MHB- and kidney-specific Pax target genes.

Human disease

The haploinsufficient *PAX* genes are frequently associated through loss-of-function mutations with human disease syndromes, while they can also be recruited as oncogenes by gain-of-function mutations in human tumors. *PAX5* has been implicated as an oncogene in the genesis of non-Hodgkin's lymphomas carrying a specific t(9;14) translocation, which brings the *PAX5* gene under the transcriptional control of the immunoglobulin heavy-chain 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. Hence, inappropriate expression of the B cell identity gene *Pax5* in the related T-lymphoid lineage results in tumor formation. We are currently investigating the molecular mechanisms responsible for Pax5-mediated lymphomagenesis.

Contact: busslinger@nt.imp.univie.ac.at

Gerhard CHRISTOFORI[1] | Group Leader

Ugo CAVALLARO[1] | Postdoc
Ivana CRNIC[3] | PhD Student
Eva FARRELL[3] | PhD Student
Grainne GANNON | PhD Student
Michaela HERZIG[3] | PhD Student
Birgit SCHAFFHAUSER[3] | PhD Student
Fabio SAVARESE[2] | Diploma Student
Medyiha SALTIK[2] | Technical Assistant

[1] until Aug. 2001; [2] until Sept. 2001; [3] until Nov. 2001

Molecular Mechanisms of Multistage Tumor Development

The major objective of our research has been the identification and characterization of molecular events involved in multistage tumorigenesis. In addition to tumor cell lines in vitro, we employed transgenic mouse models of tumorigenesis to determine causal connections between the expression of a particular gene and tumor progression in vivo. This year our group's stay at the IMP has come to an end and we have moved to the University of Basel. We are extremely grateful for the support lent us by the IMP and by Boehringer Ingelheim during the past years.

Tumor angiogenesis

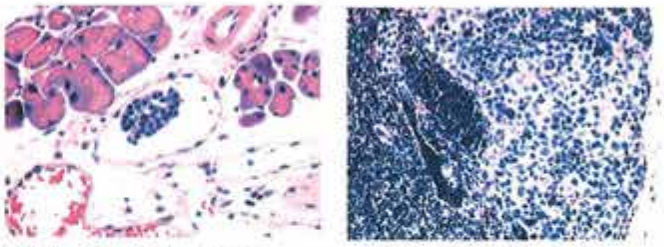
While the pivotal role of vascular endothelial growth factor (VEGF-A) in the onset of tumor angiogenesis is well established, the functional role of other angiogenic factors, in particular fibroblast growth factor-1 and 2 (FGF-1 and 2), has remained elusive. To interfere with FGF and VEGF-A activity *in vivo,* we have generated recombinant adenoviruses that express soluble versions of FGF receptors and VEGF receptors. The expression of soluble FGF or VEGF receptors in xenograft tumor transplantation experiments and in a transgenic mouse model of β cell carcinogenesis (Rip1Tag2) repressed tumor angiogenesis, and thus tumor growth, with comparable efficiencies (Compagni et al., 2000). Together, the results indicate that FGFs, like VEGF-A, are required for the onset and maintenance of tumor angiogenesis.

Conversely, to study the effect of VEGF expression on tumor progression, in collaboration with the laboratories of Michael Pepper (University of Geneva) and Kari Alitalo (University of Helsinki) we have overexpressed VEGF-A and VEGF-C during β cell tumorigenesis in Rip1Tag2 transgenic mice. While forced expression of VEGF-A resulted in an earlier onset of tumor angiogenesis and accelerated tumor growth, expression of VEGF-C resulted in increased lymphangiogenesis and the formation of lymph node metastasis (Mandriota et al., 2001; Gannon et al., 2002).

Recently, an antagonist of FGF function named Sprouty has been identified in *Drosophila* development. Subsequently, we have isolated cDNAs, encoding four different mouse Sprouty proteins and investigated their role in the regulation of angiogenesis. We have found that Sproutys inhibit FGF- and VEGF-induced endothelial cell proliferation and differentiation by repressing the activation of the mitogen-activated protein kinase (MAPK) pathway. Sproutys are anchored to membranes by palmitoylation and are themselves also a target of the MAPK signalling cascade, for example by regulation of their subcellular localization and by phosphorylation (Impagnatiello et al., 2001). Currently, we are investigating the mechanism by which Sproutys intersect tyrosine kinase receptor-mediated signal transduction.

Tumor cell invasion and metastasis

Previously, we demonstrated that the loss of E-cadherin-mediated cell-cell adhesion is causally involved in the transition from adenoma to carcinoma



Intravascular tumor cells Lymph node metastasis

Figure 1: Expression of the lymphangiogenic factor VEGF-C during β cell tumorigenesis in Rip1Tag2 transgenic mice results in upregulated lymphangiogenesis in developing tumors. In these mice, circulating clusters of tumor cells are detected within lymphatic vessels (left panel), subsequently leading to the formation of lymph node metastasis (right panel).

(Perl et al., 1998). A major component of the E-cadherin cell adhesion complex, β-catenin, is also a central player in the Wnt-signalling pathway, and the role of β-catenin/TCF-mediated transcription in the transition from adenoma to carcinoma in Rip1Tag2 transgenic mice was investigated by two experimental approaches: 1) forced expression of constitutive-active β-catenin during Rip1Tag2 tumor progression (in collaboration with Dr. Henrik Semb, Gothenburg University); 2) crossing Rip1Tag2 mice with TCF-1 knock-out mice (generously provided by Dr. Hans Clevers, Utrecht University), since β tumor cells exclusively express TCF-1 as the only member of the TCF/LEF-1 gene family. In both approaches, Rip1Tag2 tumor progression, in particular the transition from adenoma to carcinoma, was not altered.

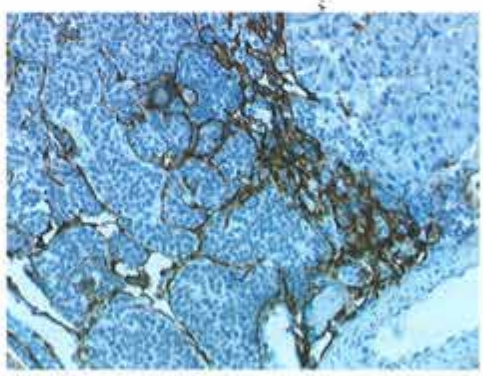


Figure 2: N-CAM-deficient tumors exhibit increased lymphangiogenesis as manifested by a dramatic increase in lymphatic vessel density. An immunohistochemical staining with antibodies against the lymphatic vessel-specific marker LYVE-1 (a kind gift from Dr. D. Jackson, University of Oxford, UK) is shown.

Hence, β-catenin/TCF-mediated transcription is not involved in late stage tumor progression, and our future experimentation will focus on alternative signalling pathways that are activated by the loss of E-cadherin-mediated cell-cell adhesion. In the course of these experiments, we have discovered novel TCF target genes. The results indicate that TCF-1 is not only a repressor but also an activator of transcription.

During the development of many human cancers, expression of neural cell adhesion molecule (N-CAM) is downregulated concomitant with progression to tumor malignancy. Recently, we demonstrated that the loss of N-CAM function results in the metastatic dissemination of β tumor cells in Rip1Tag2 transgenic mice (Perl et al., 1999). N-CAM-deficient tumors notably exhibit dramatic tissue disaggregation, and in subsequent experiments with cell lines established from these tumors we found that N-CAM-deficient β tumor cells are defective in adhesion to extracellular matrix. Biochemical analysis revealed that N-CAM associates with N-cadherin and fibroblast growth factor receptor-4 (FGFR-4), resulting in the activation of FGFR-4 signalling and increased cell-matrix adhesion (Cavallaro et al., 2001). The results indicate that N-CAM induces cell-matrix adhesion by activating FGFR signalling, a potential mechanism for modulating tumor metastasis. In addition to defects in cell matrix adhesion, we also found that the lack of N-CAM function results in an upregulation of lymphangiogenesis (Figure 2). The molecular mechanisms connecting N-CAM function and the regulation of lymphangiogenesis will be a major research focus in the future.

Contact: gerhard.christofori@unibas.ch
(New address: Institute of Biochemistry,
University of Basel, Vesalgasse 1,
CH - 4051 Basel)

Barry DICKSON | Group Leader

Fabian FEIGUIN | Postdoc
Satoko SUZUKI | Postdoc
Takashi SUZUKI | Postdoc
Jürg BERGER | PhD Student
Georg DIETZL | PhD Student
Srikanth RAJAGOPALAN | PhD Student
Kirsten-André SENTI | PhD Student
Krystyna KELEMAN | Research Assistant
Svetla DIMITROVA | Technical Assistant
Angela GRAF | Technical Assistant

Axon guidance and target specificity

Extraordinarily intricate patterns of neuronal connectivity are essential for nervous system functioning. How are these connections specified during development? How do axons find their way to the target tissue? And how do individual axons select specific target cells within that tissue? To seek answers to such questions, we turned to the fruit fly, Drosophila melanogaster. While the fly's nervous system is far less complex than our own, the molecular mechanisms of axon guidance and target specificity appear to be remarkably similar.

During the development of the nervous system, each neuron extends an axon that seeks out and connects with one or more specific target cells. Axons follow specific routes to their targets, ignoring paths that others might follow, and ignoring targets that other axons might select. How does each axon recognise its own specific route and destination? The problem is not unlike that facing the motorist. Axons, like cars, are equipped with both a motor and a steering mechanism. These reside in the growth cone at the tip of the axon. Drivers navigate by looking out for specific signposts that will lead them to their destination. In the same way, axons use receptors in their growth cone to sense cues in the extracellular environment that direct them to their targets. We want to know the nature of these cues, the receptors that recognise them, and how they control growth cone motility and steering. Our approach is to create havoc on the neural highways: to remove signposts or put them in the wrong place, to make axons blind to signals they should follow or follow signals they should ignore, and to interfere with the motility and steering mechanisms in the growth cone. Our favourite playgrounds are the embryonic CNS and the adult visual system.

Axon guidance in the CNS

In bilaterally symmetric nervous systems, such as our own and that of the fly, axons in the CNS must decide whether to grow across the midline, extend parallel to it, or grow away from it and out into the periphery (Figure 1). In *Drosophila*, these decisions are controlled by two guidance cues secreted by midline cells: Netrin and Slit. Netrin signals through the receptor Frazzled to attract some axons towards the midline, and through the receptor Unc5 to repel other axons away from the midline. Slit acts as a repellent through receptors of the Robo family: Robo, Robo2 and Robo3. Acting together, Robo and Robo2 determine which axons cross the midline and which do not. All three Robos also contribute to the »Robo code« that specifies which pathway an axon follows as it runs parallel to the midline. We are now trying to understand how Frazzled, Unc5, and Robo family receptors control the growth cone cytoskeleton in order to steer the growth cone. Another major focus

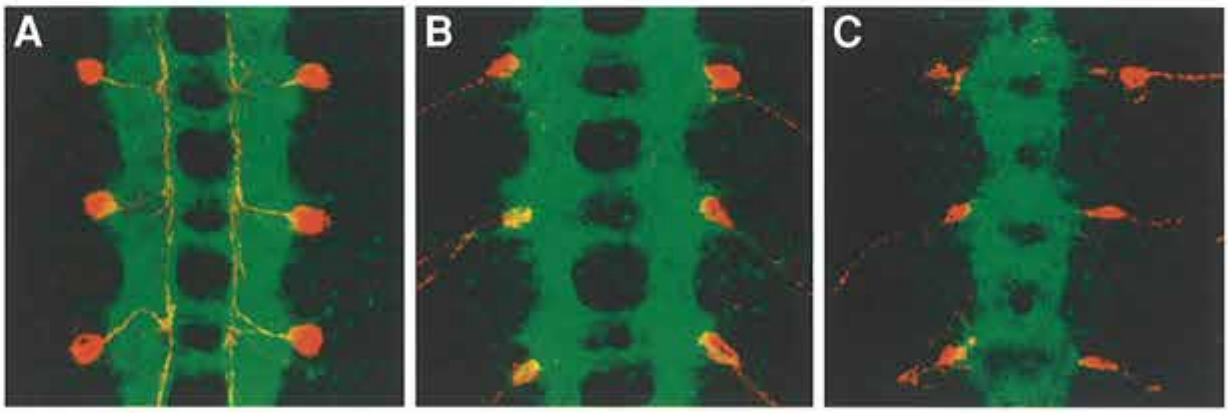


Figure 1: Inverting the CNS
(A) The ventral nerve cord (»spinal cord«) of a *Drosophila* embryo. Axons are stained green. They form two main longitudinal tracts that run each side of the midline, and a series of commissures that cross the midline to connect the two symmetric halves of the nervous system. One neuron in each hemisegment is stained red. Its axon grows towards, but not across, the midline, before turning to run alongside it.
(B) If the red axon is forced to express the Unc5 receptor, it is repelled by Netrin and now grows away from the midline and out into the periphery.
(C) If the Robo receptor is removed from all axons, the green axons are no longer repelled by Slit, and are thus diverted from the longitudinal pathways into the commissural pathways. The red axon expressing Unc5 still grows away from the midline, demonstrating that midline repulsion by Netrin and Unc5 operates independently of repulsion by Slit and Robo.

is to find out how expression of Robo receptors is regulated, since this ultimately controls the decision to cross or not to cross the midline.

Axon guidance in the visual system

Vision requires the establishment of precise axonal connections between photoreceptors in the eye and their target neurons in the brain. As a first step in determining how these connections are established in *Drosophila*, we recently performed a genetic screen in which over 32,000 mutant lines were examined histologically to identify those with abnormal connectivity patterns. The mutations recovered in this screen define some 60 different genes that are required for photoreceptor axons to connect to their appropriate targets in the brain. So far, 22 of these genes have been identified. One of them encodes the LAR receptor tyrosine phosphatase, which we found capable of signalling in both the »forward« and »reverse« directions to allow R7 photoreceptor axons to select targets in a specific layer of the brain (Figure 2). We continue to work towards the identification of the remaining genes, and elucidating the molecular mechanisms by which they dictate the pattern of neuronal connections between the eye and the brain.

Contact: dickson@nt.imp.univie.ac.at

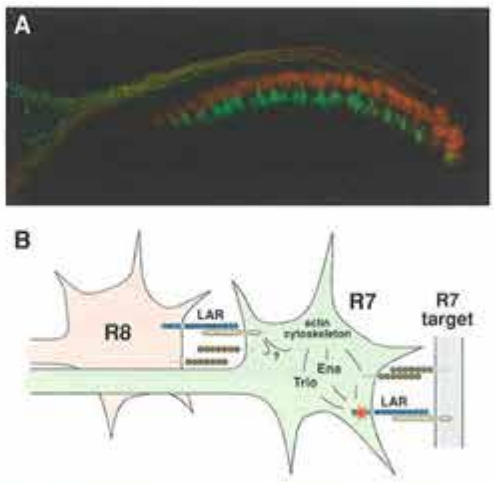


Figure 2: Layer-specific targeting of R7 photoreceptor axons
(A) Photoreceptor axons innervating the developing brain. R8 photoreceptor axons are labelled red, R7 axons are green. They target distinct layers in the brain.
(B) A model for how the LAR receptor tyrosine phosphatase controls R7 target selection.

Frank EISENHABER

Bioinformatics group
Anton BEYER | Scientist
Georg SCHNEIDER | Software Architect[1]
Birgit EISENHABER | Postdoc[1*]
Alexander SCHLEIFFER | Postdoc
Sebastian MAURER-STROH | PhD Student[1*]
Maria NOVATCHKOVA | PhD Student
Michael WILDPANER | PhD Student[*]
Georg NEUBERGER | Diploma Studen

IMP Computer & Network group
Jörg ERDEI | System Manager
Andreas RIEPL | System Manager

[1]part-time occupation or only part of the year
[*]external funding

Understanding molecular mechanisms with biomolecular sequence analysis

High-throughput experimental technologies in Life Sciences produce large amounts of uniform data such as biomolecular sequences and mRNA expression values without a direct link to biological function. The combined application of quantitative theoretical concepts (e.g. sequence evolution and pattern description models) and biological database studies can often find hints that help to bridge this gap.

Application projects in cooperation with experimental groups

The creation of an efficient environment for using biological databases and sequence analysis software in applied projects is the most important technical achievement of the bioinformatics unit. Automatic updating procedures ensure that the most recent versions of important databases and academic software packages are downloaded, installed and prepared for immediate use. All major types of biomolecular sequence analyses including sophisticated database searches can be carried out locally on IMP computers. A number of these services are available not only via command line within the bioinformatics group net, but also through the local intranet and internet node (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 produce crucial insights. More than 100 gene or protein families have been studied in great detail during the past year, some of them repeatedly, to elucidate structural and molecular functional features of the gene products or associated genomic regulatory regions. Such investigations have been launched, as a rule, upon requests of IMP researchers but also as co-operations with the University of Vienna and the Austrian Academy of Sciences. Often, the predictions opened new directions for subsequent experimentation or allowed principally new interpretations of biological data.

For example, we analyzed ~50 sequences of genes found to be transcriptionally regulated in endothelium under angiogenic stimuli (Fig. 1). We found that most of them play a role in extracellular matrix breakdown and deposition, in cellular migration (cytoskeletal remodelling), and in adhesion as well as in endo- and exocytosis. Only ~20% of the targets appear to play a role in regulation at more general levels (G-protein signalling, perception of thyroid hormone levels, transcription regulation, local tissue signalling).

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

Genuine bioinformatics research is oriented to the creation of new methods or integrative theories, but scientifically relevant directions of such efforts are determined by interaction with experimental life sciences. Our methodical research was grouped around two main lines:

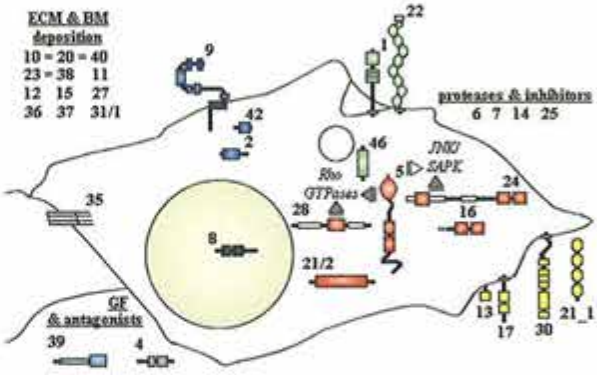


Fig. 1: The cellular context of proteins derived from transcriptionally regulated genes in angiogenic endothelium. We conclude that the transcripts (http://mendel.imp.univie.ac.at/SEQUENCES/TEMS/) found to be up-regulated in angiogenesis are involved in extracellular matrix remodelling, cellular migration, adhesion, and cell-cell communication, rather than in angiogenesis initiation or integrative control. Cellular localization and putative cellular function of angiogenic endothelial marker proteins has been schematically illustrated (numbers in the extracellular space - ECM deposition and remodelling, blue – general signalling, yellow - adhesion, green - endo-/exocytosis, dark-grey - transcription factor, red - cytoskeleton remodelling). The numbering follows the list of TEMs in http://www.sagenet.org/angio/table2.htm. Interactions with other signalling molecules are shown with grey triangles for proven and open triangles for hypothetical relationships.

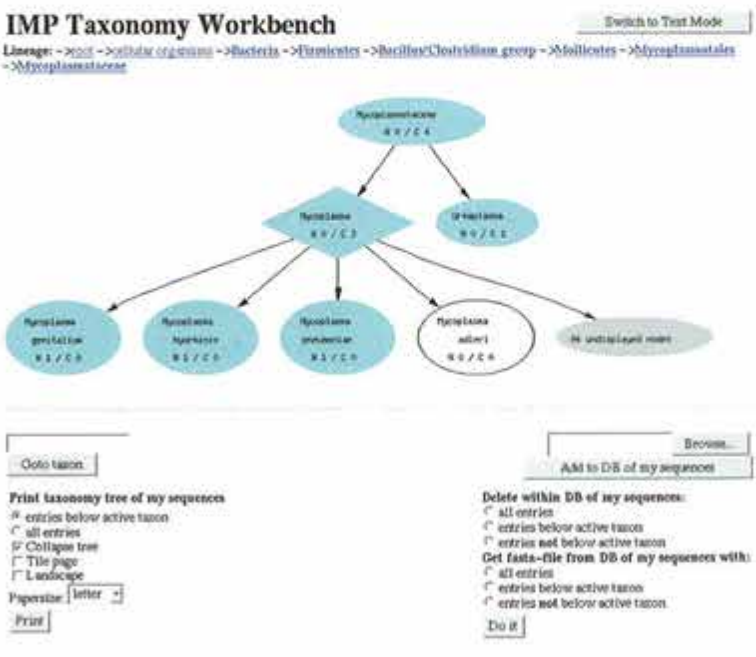
1. Recognition of posttranslational modifications in protein sequences. We developed a myristoylation predictor.
2. Integration of diverse sequence analysis methods in a higher order framework ("automatic sequence analyzer") for applications in largescale protein sequence annotation, a joint project with Boehringer-Ingelheim Austria. The taxonomy workbench is a part of this software development (Fig. 2).

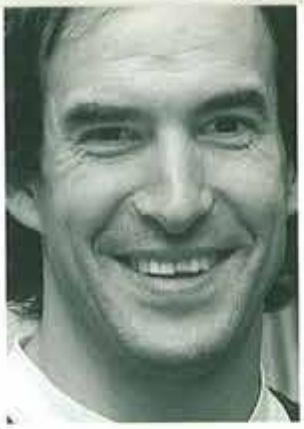
Computer usage and networking within the IMP

Modern experimental biological research, as well as efficient administration and maintenance of the institute, is impossible without powerful computer & network services. Following the wishes of different IMP researchers and taking into account the requirements necessitated by various scientific activities, a heterogeneous network of Apple Macintosh computers, WindowsNT PCs and Unix machines is supported.

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

Fig. 2: The WWW taxonomy workbench. At advanced stages of working with user-defined protein and gene sequence collections, it is frequently necessary to link these data to the taxonomic tree and to extract subsets in accordance with taxonomic considerations. Our taxonomy workbench allows us to process sequence sets, to map these sets onto the taxonomic tree, to collect taxonomic subsets from them and to print the whole tree or some part of it. An implementation of the taxonomy workbench is accessible for public use as a WWW-service at http://mendel.imp.univie.ac.at/taxonomy/. Navigation within the tree is possible with mouse clicks on graphically shown nodes, as well as on axon names in the lineage (part of the tree above the active node).





Michael GLOTZER | Group Leader

Masanori MISHIMA | Postdoc
Reinhard DECHANT | PhD Student
Susanne KAITNA | PhD Student
Ivica KRASCENICOVA | PhD Student
Manuel MENDOZA | PhD Student
Alper ROMANO | PhD Student
Gabriele GAHMON | Technician

The mechanism of cytokinesis

The ultimate task of the cell division cycle is to partition the replicated chromosomes and cytoplasmic organelles into two cells. Our laboratory focusses on understanding this process, cytokinesis, in molecular detail.

Cytokinesis is mediated by an actin-based contractile ring that is attached to the overlying cell membrane. Following the onset of anaphase, the contractile 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. Thus, cytokinesis is regulated in both time and space. The nematode C. elegans is an excellent model system to dissect this complex process, since worm embryos are well suited for real time microscopic analysis (Fig. 1). Furthermore, this system can be molecularly dissected using forward and reverse genetics.

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 the shrinking kinetochore microtubules. At this time, the non-kinetochore microtubules become bundled to form the central spindle (Fig. 2). A few years ago, we discovered the *cyk-4* gene, which is essential for the formation of the central spindle and for cytokinesis. CYK-4 contains a RhoGAP domain and an N-terminal domain that binds to the kinesin-like protein known as ZEN-4/CeMKLP1, thus forming a complex we have termed »centralspindlin« (Fig 3). In both *cyk-4* mutant embryos and *zen-4* mutant embryos, chromosomes segregate normally, but the central spindle does not form. Subsequently, the cleavage furrow assembles and ingresses to near completion, but a late stage of cytokinesis is blocked and the embryos become multinucleate. We have found that these two proteins are sufficient to induce antiparallel microtubule bundling. We think that CYK-4 acts not only to build the central spindle, but also to promote the completion of cytokinesis by virtue of its ability to promote GTP hydrolysis by Rho. We would also like to obtain structural insight into the centralspindlin complex.

A second protein complex, the ABI complex, also localizes to the central spindle. This complex contains the Aurora-B kinase AIR-2, the survivin homolog BIR-1, and the Incenp homolog ICP-1. Embryos lacking AIR-2 and ICP-1 are defective in both chromosome segregation and cytokinesis. We previously showed that the ABI complex is required for the stable localization of centralspindlin and will now use our *in vitro* assay to study if and how the ABI complex regulates centralspindlin. In the past year, we investigated the mechanism by which the ABI complex

Figure 1: The first division of a worm embryo

regulates meiotic and mitotic chromosome segregation, and have evidence that the ABI functions differently in these two related, but distinct, processes.

Although these protein complexes have, for the first time, given us the ability to reduce cytokinesis into some subreactions that can be reconstituted *in vitro*, there are still many burning questions that we want to address. These include: how is the division plane positioned? How is the ultimate splitting of the two daughter cells achieved?
To identify additional factors required for cytokinesis, we are using a forward genetics to identify mutants specifically defective in cytokinesis and then to identify the affected genes by molecular techniques. Using this strategy, we recently identified a novel component of the ABI pathway and are studying another mutant that forms ectopic cleavage furrows.

Contact: glotzer@nt.imp.univie.ac.at

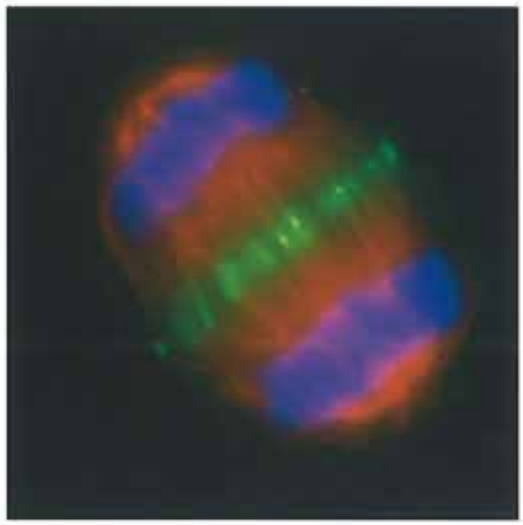


Figure 2: Centralspindlin localizes to the spindle midzone in anaphase. A mammalian cell in anaphase has been stained for MKLP-1 (green), tubulin (red) and DNA (blue).

Figure 3: A schematic model of the centralspindlin complex
The interacting domains of CYK-4 (blue) and ZEN-4 (red) have been defined and the nature of the complex studied with biochemical techniques. Based on these data, we proposed this working model of centralspindlin bound to a microtubule (green).

Lukas HUBER I Group Leader

Ilja VIETOR I External Postdoc, FWF
Niki WICK[1] I Postdoc
Bernhard HAMPOELZ I PhD Student
Santosh KUMAR I PhD Student
David TEIS I PhD Student
Winfried WUNDERLICH[1] I PhD Student
Astrid HAASE I MD Student
Angelika AUER I Laboratory Technician
Robert KURZBAUER I Laboratory Technician

[1] *until July 2001*

Epithelial biology

The central aim of our research group at the IMP is to understand how the complex three-dimensional organization of polarized epithelial cells is lost during early stages of carcinogenesis and how these changes influence the gene expression programme of mammary epithelial cells.

One major tool we used were well-characterized cell systems that display both epithelial polarity and its disruption in cancer in an *in vivo*-like fashion. We primarily used a mouse mammary gland epithelial cell line (EpH4) expressing an estrogen-inducible c-JunER fusion protein, which allows the analysis of reversible loss of epithelial polarity in a fashion superior to existing approaches. This hormone-induced disruption of epithelial polarity in JunER cells entailed loss of trans-epithelial resistance, redistribution of both apical and basolateral proteins over the entire plasma membrane and destabilization of junctional complexes (Fialka et al., J. Cell Biol. 1996). Using this cell system, we aimed to identify genes and proteins that were differentially expressed during loss of epithelial polarity. Two different approaches to studying this problem were taken. Firstly, we screened for genes differentially expressed during loss of epithelial polarity. Secondly, we studied differentially expressed proteins on subcellular organelles by proteomics techniques (Fialka et al., J. Biol. Chem.1999; Fialka et al., Electrophoresis 1999; Wunderlich et al., J. Cell Biol, 2001).

During the past two years we focused our research on the analysis of targets derived from those screens, namely (1) a MAP kinase signalling complex on endosomes and (2) the transcriptional co-repressor TIS7.

P14 is a positive regulator of MAP kinase signalling on late endosomes

We have identified a novel, highly conserved protein of 14 kD that is peripherally associated with the cytoplasmic faces of late endosomes and multivesicular bodies (Figure 1A). We identified MP1 (Mek1 partner), a catalytic scaffold protein of the MAPK pathway, as an interacting protein (Wunderlich et al., J Cell Biol. 2001). We can now demonstrate the existence of a trimeric protein complex consisting of p14/MP1 and Mek1 or Erk1 on late endosomes (Figure 1B) and are characterizing this scaffolding complex in more detail to identify other proteins. Using an ELK-dependent reporter assay, we can now demonstrate that properly localized p14 and MP1 together have a positive co-operative effect on MAPK-signalling.

In a reversed genetics approach, we are investigating this possible role of p14 in *Drosophila melanogaster*. Using an imprecise excision screen, we deleted the p14 locus. This mutant interferes genetically with the Sevenless pathway that is activated during photoreceptor development in the compound eye. The p14 deletion is capable of suppressing the rough eye phenotype caused by a constitutive active Raf. Furthermore, the p14 deletion causes synthetic lethality of a Raf hypomorph. In agreement with our data

from mammalian cells, these preliminary genetic interactions group p14 as a positive regulator of MAPK signalling.

The transcriptional co-repressor TIS7 associates with the SIN3 histone deacetylase complex

Last year we reported on the transcriptional co-repressor function of TIS7. During this year we have gained more insight into the underlying molecular mechanism. In a two-hybrid screen, using TIS7 as bait, we found several interacting proteins that participate in the histone deacetylation reaction, thereby regulating transcription. TIS7 co-imunoprecipitated in a multiprotein complex, together with the linker molecule mSIN3B and histone deacetylase HDAC1. The co-imunoprecipitated TIS7 complex also shows enzymatic HDAC activity (Vietor et al., submitted). cDNA microarray analysis of cells overexpressing TIS7 reveals selective repression of transcription of a set of genes (Figure 2). Analyses of the upstream regulatory regions reveal a binding site for the transcription module C/EBPα-Sp1, which was common to these downregulated genes (Wick et al., in preparation).

We are presently working on the functional characterization of a gene highly related to TIS7. We recently cloned mSKMC15, which has 88% homology with TIS7 at the amino acid level. The TIS7 knockout mice are under investigation. We have also embarked on the knockout of the mSKMC15 gene and the respective double knockout.

Contact: huber@nt.imp.univie.ac.at

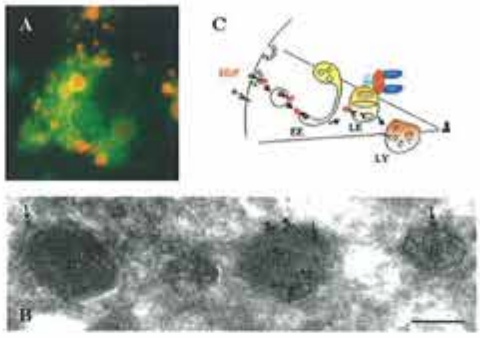
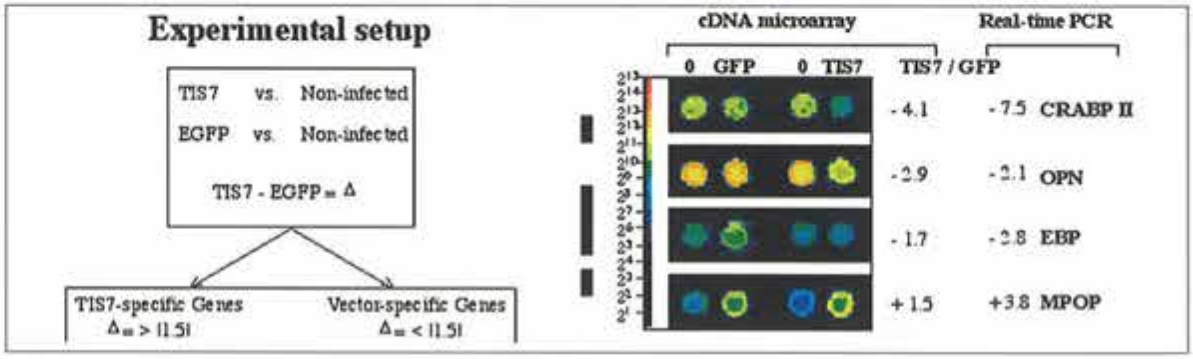


Figure 1:
A: EGF-rhodamine was internalized for 24 hrs in Caco-2 cells, stably transfected with EGFP-p14. Living cells were observed under a confocal microscope. After 24 hrs internalization, the EGFP-p14 containing compartment was filled with EGF-rhodamine.
B: Caco-2 cells were fixed and processed for frozen sectioning. Sections were double labelled for p14 (detected with 15nm gold labelled goat anti-rabbit antibodies) and lysobisphosphatidic acid (LBPA, detected with 10nm gold labelled goat anti-mouse antibodies). Specific labelling for EGFP-p14 (large gold; arrows in B-D) is associated with large multivesicular late endosomes, which are labelled for LBPA, (arrowheads, all panels). In addition to the labelling of late endosomes, lower but specific labelling is associated with putative lysosomes, which are electron dense and LBPA-negative ('L') and with putative endosome carrier vesicles ('E'). Bar 200nm.
C: Schematic representation of the intracellular routes taken by the activated EGF-receptor and the p14/MP1/MAP kinase complex on late endosomes.

Figure 2: Identification of TIS7 downstream target genes by cDNA microarray analysis (Incyte mouse chip): genes regulated differentially by TIS7 or the vector CELO, and vector-associated alterations only. Raw and normalized data were stored in a relational database, and secondary data were analyzed using a web-based software tool (MicroarrayViewer) All expression data can be viewed and downloaded via the web-browser at »http://www.imp.univie.ac.at/lh/chip_10_5_01/ index.html«. On the left side, the intensities of hybridization signals from control (0), Celo-eGFP and Celo-TIS7 infected cells, respectively, are shown. On the right side, the ratio between the signal in Celo-TIS7 and Celo-eGFP samples is shown. The results were confirmed using »Light cycler« real-time PCR analysis. CRABP II, cellular retionic acid binding protein; OPN, osteopontin; EBP, emopamil binding protein; MPOP, myelin protein 0 precusor.



Thomas JENUWEIN | Group Leader

Antoine PETERS | Postdoc
Monika LACHNER | PhD Student
Stephen REA | PhD Student
Alexander KOHLMAIER | Diploma Student
Stephan SAUER | Diploma Student
Susanne OPRAVIL | Research Assistant
Michaela PAGANI | Research Assistant

Epigenetic control by mammalian histone methyl transferases

In eukaryotes, epigenetic control of gene regulation and the functional organisation of chromosomes depends on higher-order chromatin. Particularly for the high complexity of mammalian development, deregulated inheritance of gene expression patterns ('transcriptional memory') results in perturbed differentiation and proliferation (cancer). Moreover, compromised centromere activity induces mis-segregation of chromosomes and genomic instability (aneuploidies). To analyse components and functions of mammalian higher-order chromatin, we have isolated homologues of Drosophila *chromatin regulators comprising the evolutionarily conserved SET domain. Our data reveal that SET domain-containing genes are linked with methyltransferase activities that appear intrinsically involved in the structural organisation of higher-order chromatin. Disruption of these genes in the mouse germ line induces severe developmental defects and genomic instabilities, offering new therapeutic avenues for combating cancer.*

Histone methylation and the biochemistry of heterochromatin

Higher-order chromatin has been proposed to be nucleated by the covalent modification of histone N-termini and the subsequent establishment of chromosomal subdomains by non-histone modifier factors. We recently identified mouse *(Suv39h1)* and human *(SUV39H1)* genes that encode novel histone methyltransferases (Suv39h HMTases) which selectively methylate histone H3 at lysine 9 (H3-K9). Notably, histone H3-K9 methylation by the Suv39h enzymes generates a heterochromatic affinity for HP1 proteins, thus defining the SUV39H1-HP1 methylation system as an important regulator for the propagation of chromosomal subdomains. Because the Suv39h HMTases are enriched at heterochromatin and also transiently accumulate at centromeres, these results provide biochemical evidence that Suv39h-mediated H3-K9 methylation represents an important epigenetic signal towards the induction and assembly of mammalian higher-order chromatin (see Figure 1).

The many faces of H3-K9 methylation

In addition to its function in constitutive heterochromatin formation at pericentric regions, the SUV39H1 HMTase is also involved in local gene repression and is targeted to specific cell cycle genes through the tumor suppressor Rb. Further, H3-K9 methylation also occurs at facultative heterochromatin of the inactive X chromosome in female mammals. H3-K9 methylation is retained through mitosis, indicating that it could provide an epigenetic imprint for the maintenance of the inactive state. Disruption of Suv39h HMTase activities abolishes staining at constitutive heterochromatin but reveals persistent H3-K9 methylation of the inactive X chromosome which, however, fails to localise the heterochromatin-associated HP1 proteins. These data reveal the existence of a Suv39h-HP1 independent pathway in regulating H3-K9 methylation at facultative heterochromatin that may reinforce the specialised chromatin structure of the inactive X chromosome.

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 with the exception of testes. Although single *Suv39h1* and *Suv39h2* null mice are viable, double *Suv39h*-deficient mice are born at only ≈ 30% of the expected Mendelian ratios, are growth-retarded and display hypogonadism in males. Notably, *Suv39h*-deficient mice display genome instabilities that culminate in an increased tumor risk for B-cell lymphomas and perturbed chromosome interactions during male meiosis. These *in vivo* data characterise the *Suv39h1* and *Suv39h2* HMTases as potential tumor-suppressor genes and suggest that *Suv39h*-mediated H3-K9 methylation could provide a 'protective' function for mammalian chromosomes.

Heterochromatin was first described cytologically more than 70 years ago. Because of its stable appearance in the cell nucleus, it has been proposed to have crucial roles in chromosome segregation and the inheritance of cell type identities. Our results have important implications for basic and applied research on higher-order chromatin biology in mammalian systems, ranging from a function of HMTases in centromere identity, chromatid cohesion and genome stability, to their influence in epigenetic gene regulation, X inactivation, imprinting, cell lineage plasticity and nuclear reprogramming. Thus, although our knowledge of SUV39H1 provides a good entry point into SET domain-containing MTases, plenty of exciting surprises will no doubt surface from further investigations on histone lysine methylation and its role in higher-order chromatin organisation. The impact for human biology and disease, including cancer and aging, are far-reaching.

Contact: jenuwein@nt.imp.univie.ac.at

Figure 1: Artistic view of the establishment of heterochromatin by the Suv39h HMTases. Nucleosomes (blue spheres) are cross-linked by the heterochromatin-associated HP1 proteins (golden chains). The affinity of HP1 for heterochromatin is generated by Suv39h-dependent H3-K9 methylation (metal hook). Artwork by Hannes Tkadletz (IMP).

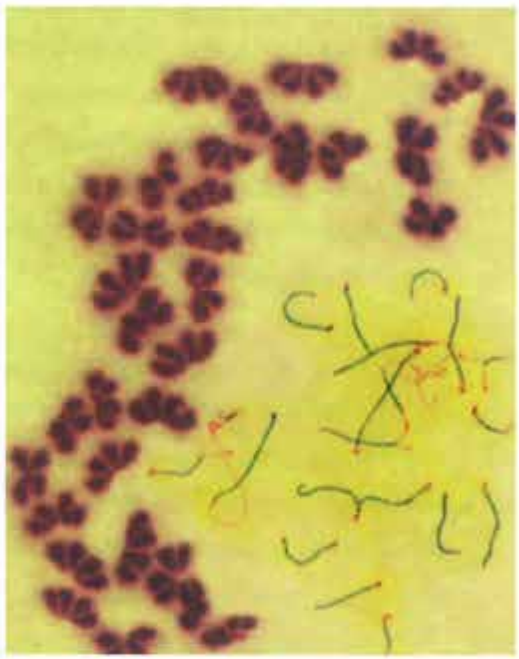


Figure 2: Suv39h deficiency impairs genome stability in mitosis and meiosis. Loss of both *Suv39h* genes in the mouse germ line results in genomic instability that is associated with an increased tumor risk and complete spermatogenic failure. Shown are 'butterfly' chromosomes present in B-cell lymphomas and illegitimate interactions between meiotic chromosomes observed in *Suv39h* dn spermatocytes.

Jürgen KNOBLICH | Group Leader

Dara DUNICAN | Postdoc
Daniela BERDNIK | PhD Student
Joerg BETSCHINGER | PhD Student
Sheetal BHALERAO | PhD Student
Ciara GALLAGHER | PhD Student
Matthias SCHÄFER | PhD Student
Oliver HÖLLER | Diploma Student
Elke KLEINER | Technician

Asymmetric cell division in *Drosophila*

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

In *Drosophila*, asymmetric cell divisions are involved in the development of both the central and the peripheral nervous system. In both tissues, the protein Numb plays an important role during these asymmetric cell divisions. Numb is a membrane associated protein which localizes asymmetrically in mitotic neural precursor cells and segregates into one of their two daughter cells (Fig. 1A, B). In the absence of Numb, this daughter cell is transformed into its sister cell, whereas the overexpression of *numb* leads to the opposite cell fate transformation. Thus, Numb acts as a segregating protein determinant during the development of the *Drosophila* nervous system.

A general concept for the establishment of asymmetric cell divisions is illustrated in Fig. 1C. Before mitosis, an axis of polarity is established which serves as a reference point for asymmetric cell division. During mitosis, they orient the mitotic spindle and localize determinants asymmetrically along this axis so that cytokinesis creates two daughter cells that contain unequal amounts and ultimately assume different cell fates. What is the molecular nature of this axis of polarity?

The *Drosophila* protein Inscuteable is a key player in the establishment of this axis. Like Numb, Inscuteable localizes asymmetrically in neural precursors. Unlike Numb, however, it already localizes before mitosis and accumulates at the opposite side (Fig. 1D). Without Inscuteable, mitotic spindles do not orient properly and Numb does not localize to the right side, indicating that Inscuteable is required for setting up the axis of polarity. How does Inscuteable do this? To answer this question, we have taken a biochemical approach to identify Inscuteable binding partners. Using preparative immunoprecipitation and mass-spectroscopy, we have identified two proteins that are associated with Inscuteable *in vivo*. One of them is the 70kD protein Pins, while the other turned out to be a heterotrimeric G-protein α-subunit, Gα i. Several observations indicate that these two proteins are crucial for the establishment of asymmetric cell division: Like Inscuteable, both proteins localize asymmetrically before and during mitosis in cells that undergo asymmetric division (Fig. 2A). In *pins* mutants or in mutants that disrupt G-protein function, we observe defects in asymmetric division similar to the ones observed in *inscuteable*. Thus, the

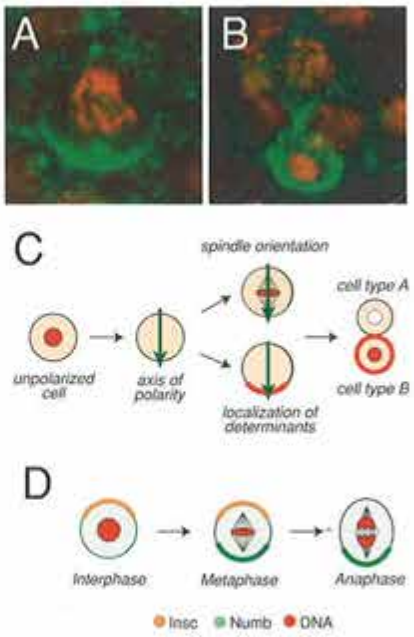


Figure 1: Asymmetric cell division in Drosophila neuroblasts. (A, B) Numb protein (green), DNA (red) and centrosomes (green, cen) in dividing *Drosophila* neuroblasts. Numb localizes asymmetrically in anaphase cells (A) and segregates into one daughter cell in telophase (B). (C) During interphase, an axis of polarity is established that provides positional information both for the orientation of the mitotic spindle and for asymmetric protein localization during mitosis. Both processes have to be coordinated to ensure asymmetric segregation of determinants and to create two different cell types. (D) While Numb localization (green) occurs in metaphase and is maintained through anaphase, Inscuteable (orange) localizes asymmetrically to the opposite side in interphase and metaphase, but disappears in anaphase.

asymmetric recruitment of Pins and Gα i by Inscuteable seems to set up the axis of polarity.

Heterotrimeric G-proteins serve important functions in transducing signals that are received from the outside of the cell via so-called G-protein coupled receptors. Usually, ligand binding to these receptors activates the G-proteins by triggering the release of the βγ-subunit (Fig. 2B). During asymmetric cell division, however, a different mechanism seems to be used. In its C-terminus, the Pins protein contains three so-called GoLoco domains. These domains bind to Gαi and can cause the release of the βγ -subunit, even in the absence of any receptor or extracellular signal. We can mimic this effect by small peptides corresponding to one of the GoLoco domains, suggesting that it can form the basis for a search for small-molecule G-protein activators. Thus, the axis of polarity seems to be established by the activation of heterotrimeric G-proteins through Pins on one side of a cell.

We are continuing to use biochemistry to search for proteins that act downstream of the G-proteins and connect them to the machineries for spindle orientation and asymmetric protein localization. In addition, we have carried out a large-scale genetic screen for mutations affecting asymmetric cell division (Daniela Berdnik, Tibor Török, Andrea Hutterer). We have identified at least 20 genes that are required for different aspects of asymmetric cell division. Some of them are necessary for proteins like Numb to localize asymmetrically and we hope that their identity will help us resolve this very unusual type of subcellular protein localization.

Contact: knoblich@nt.imp.univie.ac.at

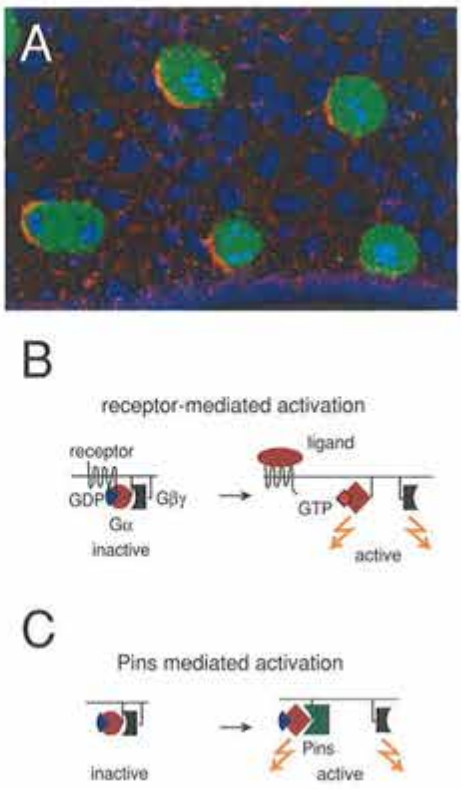


Figure 2: (A) Heterotrimeric G-proteins (red) localize asymmetrically in cells that undergo asymmetric cell division. Shown are sensory organ precursor cells (marked by expression of asense, green) the progenitors of the *Drosophila* peripheral nervous system. DNA is in blue. (B) Conventional activation of G-proteins. Ligand binding to the receptor causes GDP/GTP exchange and release of the βγ -subunit. (C) Pins can cause the release of the βγ -subunit without the need for any receptor or ligand suggesting it triggers a cell-autonomous activation of G-protein signalling.

Kim NASMYTH | Senior Scientist and Director

Tomoyuki TANAKA[1] | Staff Scientist
Prakash ARUMUGAM[2] | Postdoc
Rosemary CLYNE | Postdoc
Dmitri IVANOV | Postdoc
Vito KATIS | Postdoc
Nobuaki KUDO | Postdoc
Camilla SJÖGREN[3] | Postdoc
Koichi TANAKA[4] | Postdoc
Karin WIRTH | Postdoc

Gabriela ALEXANDRU[5] | PhD Student
Sara BUONOMO | PhD Student
Christian HÄRING | PhD Student
Silvia PANIZZA | PhD Student
Mark PETRONCZKI | PhD Student
Kirsten-Peter RABITSCH | PhD Student
Maria SIOMOS | PhD Student
Stefan GRUBER | Diploma Student
Marta GALOVA | Technical Assistant
Wolfgang HELMHART[6] | Technical Assistant

[1] until June 01; [2] from Jan. 02; [3]until Feb. 01; [4] from Aug. 01; [5] until Aug. 01; [6] from April 01

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. Even as early as 1879, Flemming noticed that »the impetus causing nuclear threads to split longitudinally acts simultaneously on all of them«. Chromosome splitting is an irreversible event and must therefore be highly regulated. Once sister chromatids separate from one another, damage to the genome cannot easily be repaired using recombination nor can mistakes in chromosome alignment be corrected. Missegregation of chromosomes during mitosis is responsible for the abnormal karyotypes of many malignant tumor cells, whereas mistakes during meiosis give rise to Down's syndrome and contribute to infertility and miscarriages.

Sister chromatids are pulled to opposite halves of the cell by microtubules emanating from spindle poles at opposite sides of the cell. Sister chromatids segregate away from each other because their kinetochores attach to microtubules emanating from opposite poles, which is called bi-orientation. Chromosomes are not mere passengers during this process. During metaphase, the tendency of microtubules to move sisters apart is counteracted by cohesion holding sisters together. Cohesion therefore generates the tension by which cells align sister chromatids on the metaphase plate. Were sisters to separate before spindle formation, it is difficult to imagine how cells could distinguish sisters from chromatids that were merely homologous. The sudden loss of cohesion, rather than an increase in the exertion of microtubules, is thought to trigger sister separation during anaphase.

What holds sister chromatids together after chromosome replication, what is Flemming's impetus that triggers loss of cohesion, and how do cells ensure that sister kinetochores attach to microtubules with opposite polarity and that sister separation never occurs before all pairs of sister chromatids have been aligned on the metaphase plate? Such questions are equally pertinent to meiosis, where loss of sister chromatid cohesion within chromosome arms and centromeres must take place at different times.

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 DNA replication until the onset of anaphase. A related complex exists in human cells (see Peters). Our working hypothesis is that connections between sister chromatids (mediated by cohesin) are established at replication forks with the aid of a protein called Eco1p. They persist until the onset of anaphase, whereupon activation of a cysteine protease called separase induces the proteolytic cleavage of the Scc1p cohesin

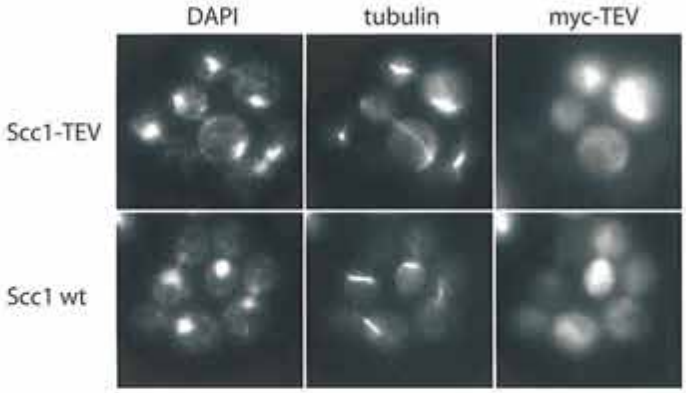


Figure 1: Triggering the segregation of sister chromatids using the foreign TEV protease. The top panels show DNA stained by DAPI, microtubules stained with tubulin-specific antibodies, and TEV expression using antibodies specific for a myc tag in cells that express a version of Scc1 in which one of the two separase cleavage sites has been replaced by that for the TEV protease. Cells were arrested in metaphase by inactivating the APC activator protein Cdc20 and TEV was then induced from the GAL promoter. The bottom panels show cells with wild- type Scc1 protein treated in the same way.

subunit, which is the trigger for sister chromatid separation (Fig. 1). Separase is kept inactive from S phase till the onset of anaphase by its association with a securin protein (Pds1p). The liberation of separase from its securin is mediated by a multisubunit ubiquitin protein ligase called the Anaphase Promoting Complex or cyclosome (APC/C), which promotes the ubiquitination, and hence proteolysis, of securin. We have succeeded in reconstructing the yeast cohesin complex, using baculoviruses engineered to express all four yeast cohesin subunits in insect cells. We are currently studying the interactions between subunits and their structure using electron microscopy. Our goal is to understand how cohesin mediates bridges between sister chromatids and how these bridges are destroyed by separase. One hypothesis based on our current work is depicted in Figure 2.

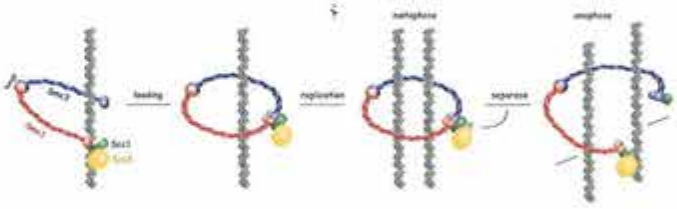


Figure 2: A working hypothesis for how cohesin could connect sister chromatids and how separase might sever this. Cohesin's Smc1 and Smc3 subunits each form long coiled coils which interact with each other by virtue of heterotypical interactions between their hinge domains. This creates a large V-shaped molecule with globular domains at each extremity. Cohesin's Scc1 subunit links the two globular ends of the V together, thereby creating a proteinaceous circle with a diameter of more than 30 nm. We suggest that cohesin binds to chromosomes by closing its jaws around a chromatin fibre and that Scc1 locks its jaws shut. If so, cohesion could be generated by DNA replication through this proteinaceous circle and be destroyed due to Scc1's cleavage by separase.

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, which 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 in the vicinity of centromeres is protected from separase until the second meiotic division. Lastly, using a »functional genomics« screen, we have identified a meiosis-specific protein called Mam1, which is essential for ensuring that sister kinetochores attach to spindles from a single pole during meiosis I (mono-orientation). Our next goal is to identify partners of Mam1 in budding yeast and homologues of these proteins in other eukaryotic organisms and, finally, to study the molecular mechanism of mono-orientation.

Contact: nasmyth@nt.imp.univie.ac.at

Annette NEUBÜSER | Group Leader – Wittgenstein Group

Monika BACHLER | PhD Student
Nicole FIRNBERG[1] | PhD Student
Andreas LEIBBRANDT | PhD Student
Markus MANDLER | PhD Student
Vera WAGNER[2] | Diploma Student
Johannes WERZOWA[3] | Diploma Student
Marion REPITZ | Technician

[1] since May 2001; [2] until July 2001; [3] until Oct. 2001

Patterning and early morphogenesis of the vertebrate face

The face is one of the most intricately shaped parts of the vertebrate body and in humans it is frequently affected in congenital malformation syndromes. We are using the mouse and the chick as model organisms to study how development of this complex structure is regulated during embryogenesis.

The vertebrate face develops from buds of tissue, the facial primordia that surround the primitive mouth (Fig.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 they become morphologically apparent, the mesenchyme around them starts to grow out to form the nasal processes. Continued outgrowth depends on interaction 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 the focus of our interest.

FGF8 function during facial development

FGF8 is a member of the fibroblast growth factor family of signalling 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 (Fig. 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 (Fig. 2 C,D). Defects first become morphologically apparent around E9.5 as a reduction in the size of the facial primordia. This size reduction can be accounted for by a dramatic increase in cell death and a reduction in cell proliferation in early facial mesenchyme in the absence of *Fgf8*. FGF8 therefore seems to act as a survival factor for early facial mesenchyme and to promote its proliferation. In addition, patterning in the remaining tissue is affected, in particular in the midfacial area at E9.5, as judged by the analysis of the expression of marker genes. At E10.5, six other members of the FGF family are expressed in partially overlapping domains, with *Fgf8* around the nasal placodes, and may partially compensate for the loss of FGF8. In addition to the mesenchymal defects, development of the nasal placode is also abnormal in *Fgf8* mutant embryos and this defect is currently being characterized in more detail. In order to further study the function of FGF signalling, we are using avian retroviruses for the over-expression of soluble dominant negative versions of the FGF receptors and of Sprouty-2, an antagonist of FGF signalling, to interfere with signalling by several FGF family members at the same time.

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

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 signalling. 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. Using a candidate approach, we have shown that FGF signalling 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 micro-array. This micro-array 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 signalling and we have begun to characterize some of them. We believe that this analysis will ultimately help to understand the function of FGF signalling during development on a molecular level.

Regulation of *Fgf8* expression

We are also interested in understanding how the complex, dynamic expression pattern of *Fgf8* is established during facial development. Using an *in vitro* culture system in which various regions of the face can be co-cultured, we have shown that tissue interactions are involved in the regulation of *Fgf8* expression. These experiments also revealed that the early midfacial expression and the later expression around the nasal placodes are established by different mechanisms. Experiments to characterize the underlying molecular mechanisms are in progress.

Contact: neubueser@nt.imp.univie.ac.at

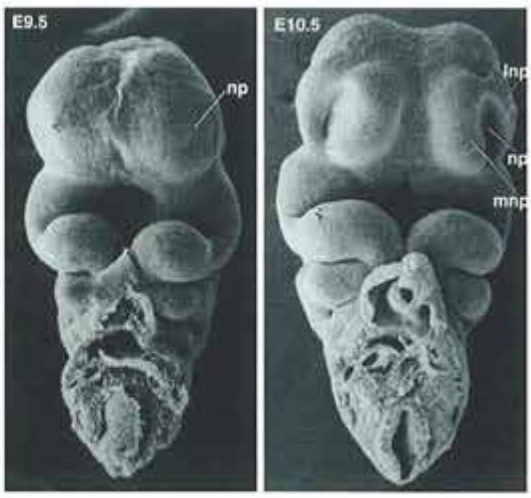


Figure 1: Scanning electron micrographs of the facial region of mouse embryos at E9.5 and E10.5 The nasal placodes (np), thickenings of the facial ectoderm, are the first morphologically distinct structures to form in the prospective midfacial region. By E10.5, the mesenchyme around the placodes has 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.

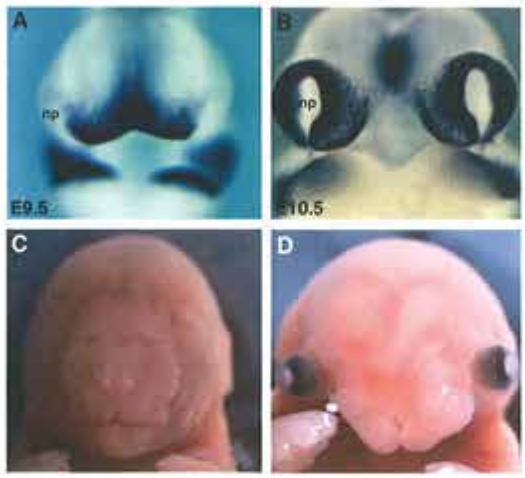


Figure 2: Tissue-specific inactivation of Fgf8 in the facial area results in severe facial defects. Facial expression of Fgf8 at E9.5 (A) and at E10.5 (B). The face of a wild-type (C) and an *Fgf8* mutant embryo (D) at E16.5. Embryos in which *Fgf8* has been inactivated in the facial area develop a midfacial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

Jan-Michael PETERS | Group Leader

Christian GIEFFERS | Postdoc
Silke HAUF | Postdoc
Irene WAIZENEGGER | Postdoc (Boehringer Ingelheim)
Juan Francisco GIMÉNEZ-ABIÁN | Guest Scientist
Claudine KRAFT | PhD Student
Izabela SUMARA | PhD Student
Hartmut VODERMAIER | PhD Student
Elisabeth VORLAUFER | PhD Student
Christine ZIMMER | PhD Student
Franz HERZOG | Diploma Student
Florine DUPEUX | Technical Assistant
Beate PETERS | Technical Assistant (part-time)

Regulation of mitosis in vertebrate cells

The initiation of sister chromatid separation at the metaphase-anaphase transition is a »point of no return« during the eukaryotic cell cycle (Figure 1). In eukaryotes from yeast to man, this event is initiated by activation of a ubiquitin ligase called the anaphase-promoting complex (APC) or cyclosome. The APC was first discovered as a multi-subunit complex that ubiquitinates cyclin B at the end of mitosis and thus targets this protein for destruction by the 26S proteasome. Subsequently, the APC has also been found to initiate anaphase by activating separase, a protease distantly related to caspases. We are using human cells and eggs of the Xenopus *frog to address the following topics:*

Regulation of sister chromatid separation in vertebrate cells

In yeast, the separation of sister chromatids depends on the cleavage of the chromosomal cohesin complex by separase at the onset of anaphase. We found that, in vertebrates, cohesin begins to dissociate from chromosome arms in prophase and from centromeres at the onset of anaphase. We showed that the latter event coincides with cleavage of cohesin by separase and that this cleavage reaction is essential for anaphase, also in human cells (Figure 2). We discovered that cohesin dissociation in prophase depends on Polo-like kinase, but not on separase, and is mediated at least in part by cohesin phosphorylation. To understand the function of cohesin dissociation in prophase, we will map and mutate cohesin phosphorylation sites, hoping that this will yield »non-dissociatable« mutants, whose effects on chromatid condensation and separation can be analyzed *in vivo*.

Mechanisms and regulation of human APC and separase

Separase is activated by ubiquitin-dependent proteolysis of its inhibitor securin, which is mediated by APC. To understand at the molecular level how APC ubiquitinates substrates and how its activity is regulated, we are reconstituting human APC biochemically and are collaborating with crystallography and electron microscopy groups to analyze APC's structure. We discovered that APC has a complex asymmetric structure, in which an outer protein wall surrounds a large inner cavity. Although the function of this cavity is unknown, it is possible that it represents a reaction chamber in which ubiquitination reactions take place (Figure 3). We are also using biochemical and structural approaches to analyze separase. We could show that securin binding does not only inhibit separase but is also required for its activation. We further discovered that securin degradation allows autocatalytic cleavage of separase into a mature form, which then cleaves cohesin complexes.

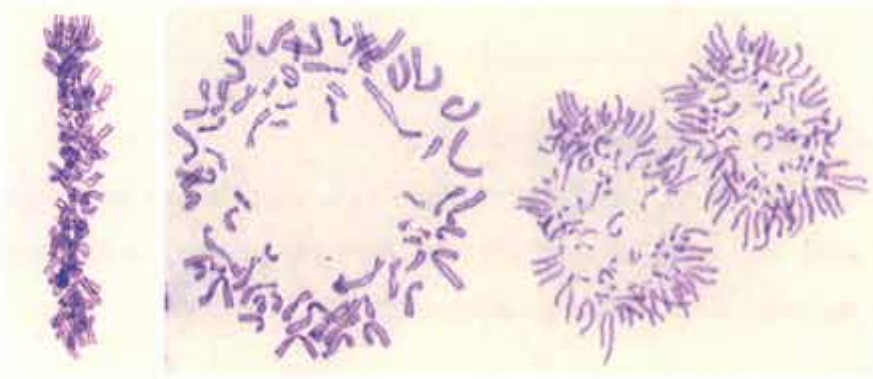
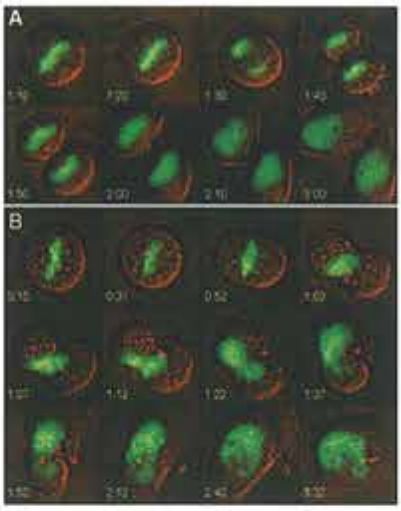


Figure 1: Chromosomes are split into their two sister chromatids at the transition from metaphase to anaphase. The micrographs show spread chromosomes from a human cancer cell line (HeLa; Giemsa staining). On the left and in the middle, respectively, side and polar views of chromosomes in metaphase plates can be seen. On the right, chromatids that have been separated from each other in anaphase are shown. Courtesy of Juan Fancisco Giménez-Abián.

Figure 2: Video microscopy reveals that human cells expressing a mutant version of cohesin that cannot be cleaved by separase fail to undergo anaphase and cytokinesis (B). Cells that express wild-type cohesin undergo anaphase and cytokinesis normally (A). Cell lines were stably transfected with histone H2B-GFP to visualize chromatin. For further details see Hauf et al., Science 293, 1320-1323, 2001.



Analysis of mitosis through chemical biology

Specific small molecule inhibitors can represent valuable tools to analyze rapid-onset phenotypes in cells. In collaboration with Boehringer Ingelheim, we are characterizing compounds that inhibit mitosis, but whose targets are unknown. We have identified a candidate target for one of these inhibitors and will try to validate our hypothesis through RNAi phenocopy experiments, by establishing structure-activity relationships and through screening for a compound-resistant mutant of the candidate enzyme. This approach may reveal novel insight into mitotic regulation and could also identify pharmaceutically interesting drug targets.

Contact: peters@nt.imp.univie.ac.at

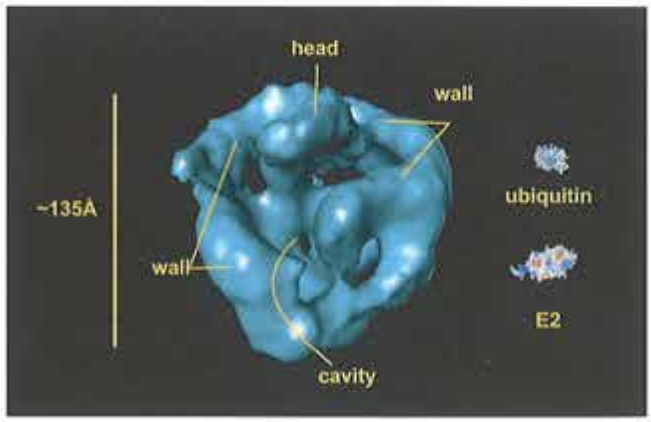
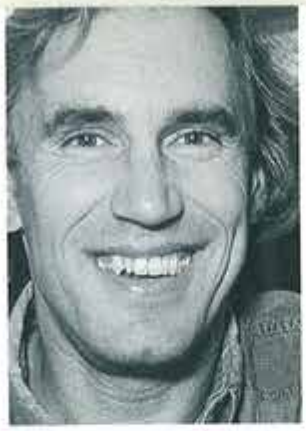


Figure 3: 3D model of the human anaphase-promoting complex (APC) at a resolution of 24 Å obtained by cryo-electron microscopy. APC has a complex asymmetric structure, 140 x 140 x 135 Å in size, in which an outer protein wall surrounds a large inner cavity. The structures of ubiquitin and a ubiquitin-conjugating enzyme (E2) are shown for comparison. For further details, see Gieffers and Dube et al., Mol. Cell 7, 907-913, 2001.



Erwin F. WAGNER | Senior Scientist and Deputy Director

Koichi MATSUO[1] | Staff Scientist
Latifa BAKIRI[2] | Postdoc
Jean-Pierre DAVID | Postdoc
Robert EFERL | Postdoc
Astrid HOEBERTZ[3] | Postdoc
Lukas KENNER[4] | Postdoc
Arabella MEIXNER | Postdoc
Romeo RICCI | Postdoc
Rainer ZENZ | Postdoc
Agnieszka SZREMSKA | PhD Student
Maria-Grazia RUOCCO[5] | Diploma Student
Martina AUSTRUP | Technician
Maria H. IDARRAGA-AMADO | Technician (60%)
Uta MÖHLE-STEINLEIN | Technician (50%)
Martin RADOLF | Technician

[1] until April 01; [2] since Sept. 01; [3] since Nov. 01; [4] since May 01; [5] until Oct. 01

Gene function in mammalian development and oncogenesis

The mouse is used as a model organism for the analysis of gene function in normal and pathological development. The major focus is the analysis of AP-1 proteins such as Fos and Jun and their role as regulators of proliferation, differentiation and cell death. We are also studying the specific functions of VEGF, Flk-1 and EGF-Receptor in bone, epithelial and endothelial cells.

Fos proteins in bone cell differentiation

Fos proteins are key regulators of bone development. Transgenic mice expressing *c-fos* develop bone tumors, whereas mice lacking *c-fos* are osteopetrotic and lack bone-resorbing osteoclasts. The Fos-related protein Fra-1, itself a c-Fos target gene, is essential for mouse development. While Fra-1 is a potent inducer of osteoclast differentiation *in vitro*, transgenic mice overexpressing Fra-1 develop an osteoblastic bone disease, osteosclerosis due to increased bone formation (Fig. 1). Interestingly, gene replacement of *c-fos* by *fra-1* showed functional equivalence of these two proteins (Fig. 1). To better understand the mechanisms by which c-Fos and Fra-1 control osteoblast and osteoclast differentiation, we have 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. The rescued mice were viable and showed no severe bone phenotype (Fig. 1). The conditional allele of *c-fos* allows us to monitor expression of c-Fos during development, as well as to study c-Fos function in the CNS (Fig. 2).

Jun and Jun kinases controlling cell differentiation, proliferation and apoptosis

We have used the *cre/loxP* recombination system, knock-in strategies and transgenic rescue experiments to investigate the specific functions of Jun family members. Specific deletion of *jun* in the liver of adult mice showed that Jun is dispensable for postnatal liver function, but essential for liver regeneration (Fig. 3). Moreover, we found that Jun is required during liver tumor development. Deletion of *jun* in the skin did not affect proliferation of keratinocytes in adult mice, but lead to an eye closure defect during embryonic development. Chondrocyte-specific deletion results in severe scoliosis caused by failure of intervertebral disc formation, suggesting that Jun is a novel regulator of sklerotomal differentiation (Fig. 3). Interestingly, replacing JunB for Jun indicates that JunB can 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 through the Jun amino-terminal kinases (JNKs). To study the function of JNK signalling, *in vivo* null mutations in the *Jnk1* and *Jnk2* genes (with M. Karin) and mice carrying a *jun* allele mutated in the JNK phosphoacceptor sites were generated *(JunAA). Jnk1-/-, Jnk2-/-* and *JunAA* mice are healthy and fertile, but the ab-

sence of *Jnk1* and Jun-N-terminal phosphorylation (JNP) results in growth retardation and fibroblasts from these mice show proliferation defects. *Jnk1-/- Jnk2-/-* double mutants develop brain defects and *Jnk2-/-* and *JunAA* thymocytes are resistant to CD3-induced apoptosis. Moreover, Jnk1 and Jun phosphorylation appear to be required for efficient osteoclast differentiation. Therefore, JNK signalling and JNP differentially regulate cell proliferation, differentiation and apoptosis in different biological processes.

Investigating the tumor suppressive function of JunB

JunB is a transcriptional activator of the cyclin-dependent kinase inhibitor p16/INK4a and functions as a negative regulator of cell proliferation. Using different *in vivo* approaches, we found that the absence of JunB expression in the myeloid lineage results in a myeloproliferative disease resembling human chronic myeloid leukemia. Furthermore, JunB was identified as a key transcriptional regulator of myelopoiesis which controls the numbers of granulocyte progenitors through inhibition of proliferation and promotion of apoptosis. We are currently investigating the function of JunB in human myeloid leukemia.

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

The VEGF/Flk-1 signalling system is essential for the development of endothelial and hematopoietic cells. The conditional allele of Flk-1 is being used to test its role in adult mice and in tumor angiogenesis, whereas the conditional allele of VEGF was used to analyse the functional importance of VEGF-A in developing chondrogenic tissues. Moreover, this conditional allele is also being used to study the role of VEGF-A in skin biology (with E. Tschachler, Univ. Vienna). In collaboration with M. Sibilia, Univ. Vienna, we found that EGF-R plays a central role as a survival factor in oncogenic transformation and its role in liver regeneration is being investigated.

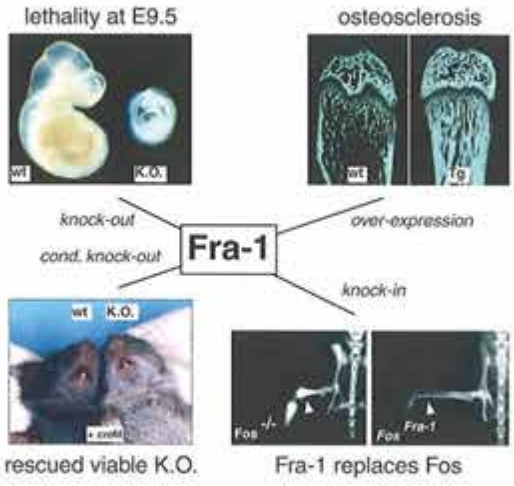


Figure 1: Functional analysis of Fra-1

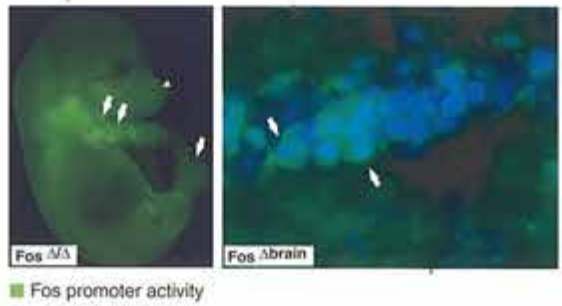


Figure 2: Expression of Fos in bone development and the CNS

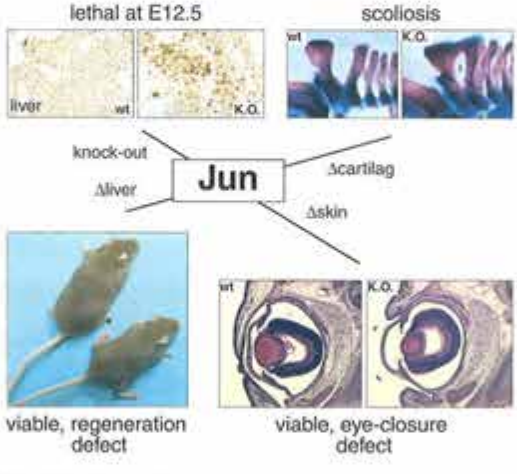


Figure 3: Functional analysis of Jun

Contact: wagner@nt.imp.univie.ac.at

Anton WUTZ[1] | Group Leader

Fabio SAVARESE[1] | PhD Student
Stefan SCHÖFTNER[1] | PhD Student
Mediyha SALTIK[1] | Technician
[1] *from October 1st, 2001*

Mammalian X-chromosome inactivation

For successful development, the information coded in 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.

X-chromosome inactivation is an epigenetic event that results in the transcriptional inactivation of one of the two X chromosomes in female mammals. Thereby, compensation for the dosage imbalance arising from the unequal genetic constitution of XY males and XX females is achieved. The *Xist* gene is specifically transcribed from the inactive X chromosome and has been shown to be required for X-inactivation. *Xist* expression precedes silencing and Xist RNA associates with chromatin of the inactive X chromosome. X inactivation is initiated early in mouse embryogenesis. The entire process that converts an active X chromosome into a heterochromatic structure can be studied during the differentiation of mouse embryonic stem (ES) cells. To study the function of *Xist*, we have previously constructed a mouse *Xist* cDNA and expressed it in male ES cells, using an inducible expression system (Fig. 1). Xist RNA produced from the tetracycline inducible transgene was sufficient for initiation of chromosome-wide silencing in undifferentiated ES cells. All steps of X inactivation were recapitulated by the transgene during ES cell differentiation. However, in undifferentiated ES cells silencing was reversible, Xist-dependent and independent of chromosomal modifications known to correlate with the inactive X at later stages in cell differentiation (Fig. 2).

The characterisation of the initiation events of X inactivation

Initiation of X-inactivation has to occur in a developmental window in which silencing is reversible and Xist-dependent, demonstrating that reversible silencing is part of the normal process of X-inactivation in female cells. We are using the inducible *Xist* expression system in mouse ES cells to study the initiation phase of Xist-mediated silencing by characterisation of the timing of specific histone modifications and chromosomal transformations in early ES cell differentiation.

We are further interested in understanding the mechanism by which Xist RNA associates in *cis* with chromatin and mediates transcriptional repression. To this end, we initiated experiments aimed at the biochemical purification of proteins that interact with Xist RNA, using affinity purification and chromatin isolation methods as competing and complementary approaches. By using ES cells, we hope to specifically identify factors that interact with Xist RNA in the initiation of chromosome-wide silencing, avoiding complications that result from the progressive heterochromatinisation of the inactive chromosome in cellular differentiation. Our experiments will shed light on the pathway by which Xist localises in *cis* to chromatin and initiates silencing.

Controlled *Xist* expression in mouse ES cells

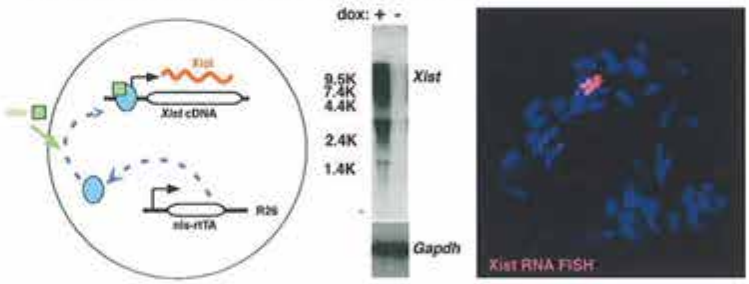


Fig. 1: Inducible Xist expression in mouse ES cells. The tetracycline inducible system was set up in ES cells by targeted insertion of the cDNA of the nls-rtTA doxycylcine-responsive transactivator into the ubiquitously expressed ROSA26 locus. Xist RNA was expressed from cDNA transgenes under control of an inducible promoter in the presence of doxycylcine, as shown by Northern analysis. RNA FISH demonstrates that transgenic Xist RNA localises to chromatin of a condensed metaphase chromosome.

Xist-mediated silencing in mice

We have previously shown in ES cells that initiation of Xist-mediated silencing has to occur early in differentiation and conversely in differentiated somatic cells. *Xist* expression in general no longer leads to silencing, despite the ability of Xist RNA to localise to chromatin. We have generated an inducible *Xist* allele in mice to study the initiation of silencing in development. We are especially focusing our studies on the germline stem cells, tumor cells and somatic stem cells. We expect to be able to epigenetically classify cells in the developing mouse as Xist-responsive or non-responsive and thereby visualise this epigenetic transition *in vivo*.

Functional studies of X inactivation in mice and in ES cells will provide insight into the epigenetic regulation of gene expression in mammals. It appears that *Xist*-mediated silencing is a paradigm for a powerful epigenetic system that is capable of hetero-chromatinising an entire chromosome and determining its specific nuclear localisation. Experiments are underway to clarify the role of the Xist RNA in this process and to define functional regions along the RNA, which are candidates for interactions with chromatin. It is expected that similar interactions underlie the regulation of other genes - however, with less dramatic consequences. The involvement of an RNA in chromatin formation is novel, and might also pinpoint a general process by which transcription feeds back on chromatin structure.

Contact: wutz@nt.imp.univie.ac.at

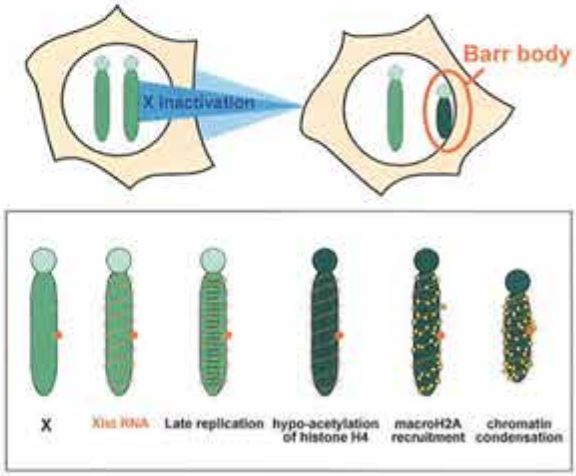


Fig. 2: Chromosomal modification of the inactive X in cellular differentiation. *Xist* expression initiates X-inactivation early in development. The heterochromatisation of the chromosome is a multistep process which encompasses late chromosomal replication, histone H4 hypoacetylation, and recruitment of histone macroH2A leading to the formation of a condensed structure in the periphery of the cell nucleus commonly referred to as the Barr body.

Gotthold SCHAFFNER | Scientist

Elisabeth AIGNER | Technician
Ivan BOTTO | Technician
Markus HOHL | Technician
Gabriele BOTTO | Technician Media Kitchen
Ulrike WINDHOLZ | Technician Media Kitchen

Service Department

The Service Department offers a variety of high-quality and rapid services to IMP scientists. The majority of our effort involves DNA sequencing, oligonucleotide synthesis and preparation of various media and solutions.
Our Media Kitchen staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies (approximately 200,000 bottles and tubes per year) and worms. We also prepare many selected reagents such as DNA molecular weight markers, enzymes and a variety of transformation-competent E. coli strains and maintain a stock of cloning vectors, primers and other cloning reagents.

Oligonucleotide synthesis

We started in 1988 with about 500 DNA oligonucleotides. After reaching a yearly production of about 6000 oligos, we started to outsource our oligoproduction in March this year. We still produce oligos for urgent needs or for small sequencing projects (»primer walking«) in order to save time and speed up the project (up to 3x). The free capacity is invested in sequencing.

Production of antibodies

The production of monoclonal antibodies in hybridomas in collaboration with IMP group members, the production of polyclonal antibodies by immunizing mice in our animal house facilities and organizing the antibody production in rabbits with an outside company is of increasing importance and uses part of our capacity.

Sequencing and DNA isolation

With the two ABI PRISM 377s and the new ABI 3100 Genetic Analyzer, a capillary sequencer, we sequenced approximately 32,000 samples in the first 9 months of this year (a 4x increase compared to 1998) with an average reading length of 700-900 bases for »good« DNA on the 377s and 600-800 on the 3100. We are saving time by using an easy and fast clean-up protocol with small Sephadex columns or magnetic MagneSil beads (PROMEGA) on 96-well microtiter plate format.
Since the full sequence information of several scientifically important organisms is available, many more screening experiments (e.g. SNPs) are going on. Thus, we needed a reliable and even faster sequencing capacity. We successfully introduced a capillary sequencer (ABI 3100 Genetic Analyzer) and, so far, have analyzed approximately 20,000 samples with 50 cm capillaries.

Contact: schaffner@nt.imp.univie.ac.at

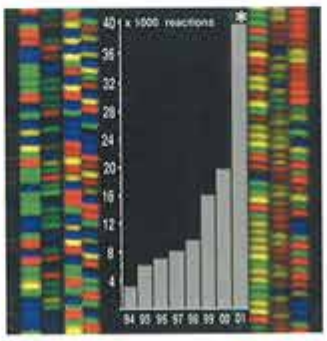


Figure 1: A sequencing run on an ABI 377 PRISM and number of reactions done with dye deoxy terminators (scale 0 to 40,000) during the years 1994 to 2000.
*calculated from January 2001 to September 2001 data

Peter STEINLEIN | Staff Scientist

Sebastian CAROTTA | PhD Student (joint project with H .Beug)
Volker LEIDL | Software Developer
Herbert AUER | Technician (micro arrays)
Karin PAIHA | Technician (microscopy and image analysis)
Gabriele STENGL | Technician (flow cytometry and microscopy)

Biooptics Department

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

Flow cytometry

In order to satisfy the increased demand for multicolor flow cytometry, a Becton Dickinson LSR analytical flow cytometer equipped with three lasers was purchased. We can now offer the analysis of intracellular processes such as Ca^{2+}-flux or the simultaneous measurement of cell cycle status and antigen-expression in a fast and reliable way.

Microscopy and image analysis

Besides standard techniques in light and fluorescence microscopy (both wide field and confocal), several more advanced techniques such as FRAP (**F**luorescence **R**ecovery **A**fter **P**hotobleaching) and multidimensional time lapse microscopy of fluorescently labelled cells have been established and are now routinely used.

Micro arrays

During the past year, more than 250 micro array experiments were performed by IMP researchers and their collaborators. The experiments performed at the IMP cover the whole range of gene expression profiling, from target gene identification to time course studies in development and differentiation. Major improvements have been achieved in labelling techniques and it is now possible to use RNA from as little as 50,000 cells for micro array experiments, thus allowing gene expression profiling in experimental systems where only very limited quantities of cells or tissue are available.

Currently, we offer more than 10 different custom array types for mouse, human, and chicken, with up to 25,000 spots per array. The total number of cDNA clones available for the production of arrays is almost 100,000 (>71,000 mouse, >13,000 human and >13,000 chicken cDNAs). The identity of approximately 40% of these clones is known.

The functionality of the micro array information management system developed in our group (JMIMS) has been enhanced; it now offers basic analysis features via a web front-end. In addition, a second database has been set up that automatically gathers publicly available gene annotation information from databases such as Unigene, Locuslink, Gene Ontologies, Interpro, and others, for clones with a known identity. All available annotation information is directly linked to and accessible from JMIMS.

Contact: steinlein@nt.imp.univie.ac.at

The Biooptics team (from left to right): Gabriele Stengl, Sebastian Carotta, Herbert Auer, Peter Steinlein, Volker Leidl, Karin Paiha

Andreas BICHL | Head, Veterinarian
Erwin F. WAGNER | Scientific Coordinator

Norma HOWELLS | Consultant

Lina Mabel BONILLA[1] | Technician
Mijo DEZIC | Technician
Katja FLAHNDORFER-STEPANEK | Technician
Erika KILIGAN | Technician
Dominik MAYR | Technician
Svjetlana PEKEZ-NICOLIC | Technician
Esther RAUSCHER[2] | Technician

[1] until May 2001; [2] on maternity leave

Animal house

The animal house group provides husbandry of animals and services for the various research groups:

Husbandry:

The husbandry is divided into three main areas and contains 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, and include 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-programme etc.), experimental procedures in animals, such as collection of blood, implantation of tumor cells and administration of other substances by various routes, e.g. intravenous, intraperitoneal and subcutanous injections etc). 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 includes updating and record-keeping of laboratory animal statistics, specific documentation and recording of laboratory animal experiments.
Contact: bichl@nt.imp.univie.ac.at

Mouse service department

Hans-Christian THEUSSL (Technician)

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) were performed and the mouse strain data base is being established. About 30 different ES cell clones and several DNA constructs are being successfully injected per year, mainly for the groups of Meinrad Busslinger, Lukas Huber, Kim Nasmyth and Erwin Wagner. The activities of this department are overseen by an Animal User Committee, which meets bimonthly to set priorities and coordinate the duties. At present, it is chaired by Erwin Wagner.

Karl MECHTLER I Head of Facility

Jan-Michael PETERS I Scientific Coordinator
Ines STEINMACHER I Technician

Protein chemistry facility

Mass spectroscopy is a powerful tool to solve problems in protein identification and post-translational modifications.

Mass spectrometry allows the determination of molecular masses of peptides and of many other molecules of biological interest with high accuracy. For this purpose, the mass to charge ratio (m/z) of ionized molecules is measured during separation of the sample in vacuum. Only femtomole amounts are required for this measurement, making mass spectrometry a particularly sensitive analytical method. In recent years, mass spectrometry has also been used to determine the sequence of peptides and to identify their posttranslational modifications such as phosphorylation. Due to these applications, mass spectrometry has become an invaluable tool in molecular biology.

The IMP mass spectrometry facility is operated together with the Institutes of the Vienna Biocenter and the CISTEM Biotechnologies company.

Currently, we are using the following instruments:

a) MALDI-TOF (Reflex III from Bruker)

b) ESI-IonTrap (LCQ Classic from ThermoQuest)

c) ESI-Ion Trap (LCQ-Deca XP from ThermoQuest)

d) ESI-Qtof (Qstar from Applied Biosystems)

All ESI Instruments are equipped with a Nano-HPLC from LC-Packings.

The most sensitive method in mass spectrometry is MALDI (matrix assisted laser desorption ionization). We adapted a new »anchor target« sample preparation method for identifying proteins in the low femtomol range, which can be used even to identify bands isolated from silver-stained gels.

If database searches with the resulting data are ambiguous, additional sequence information is obtained by acquiring post source decay spectra (PSD).

For samples which cannot be found in protein databases, we use de novo sequencing with the classic Nanospray approach on a Q-TOF machine.

Protein phosphorylation

Phosphorylation of amino acid residues in proteins plays a major role in many biological processes. Phosphorylation/dephosphorylation acts as a molecular switch that controls the behaviour of many proteins, for example during cell division, signal transduction etc. Therefore, the identification of phosphoamino acid residues in proteins is an important task in protein analysis.

Peptide synthesis and antibody purification

We are synthesizing about 150 peptides per year, including an increasing number of peptides bearing acetylated, phosphorylated or methylated amino acid residues. We employ a special protocol for affinity purification of antibodies under mild conditions.

Contact: mechtler@nt.imp.univie.ac.at

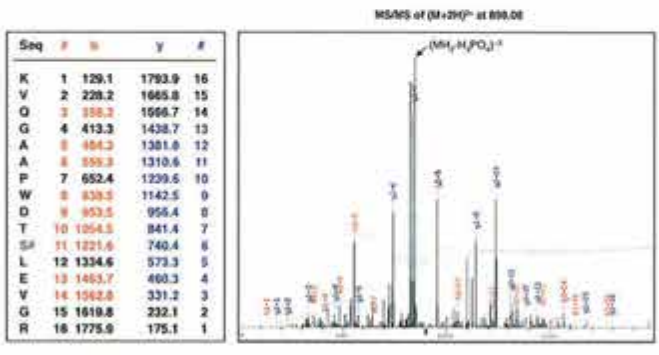


Figure 1: Tandem mass spectrum of a phospho-peptide of the yeast cohesin subunit Scc1 and analysis of the fragmentation series to confirm the identity of the peptide and the exact phosphorylation site. The spectrum also shows a peak at m/z=849.3 generated by loss of H_3PO_4 from 898.08,$(M+H)^{2+}$ of phosphorylated peptide (Alexandru et al., 2001)

The IMP is located in Vienna's third district, halfway between the international airport and the heart of the city. During the fourteen years since its establishment, the institute has seen considerable changes in its neighbourhood. From a solitary research building, the IMP turned into the nucleus of a rapidly developing biomed-campus. Today, four university institutes and eight start-up companies are situated on the campus. Two biology institutes of the Austrian Academy of Sciences are under construction. Combining academic and commercial aspects of research, the »Vienna Bio Center« – as it is now known – has become Vienna's most innovative and exciting hub in biotechnology. Shared facilities, such as the »Max Perutz Library«, create synergies and bring scientists from the different units together.

The IMP itself has grown, too. At present, 189 scientists and a support staff of 23 – representing in total 32 nationalities – work at the institute. Due to the international character of the IMP, English is used for communication and publications. For those who are interested in a more authentic experience of Vienna and the Viennese, taking German lessons is encouraged and sponsored by the IMP.

From the very beginning, the IMP has had strong links to the University of Vienna. Apart from joint seminars, lecture courses and collaborations, the international PhD Programme is the major common activity. Through this programme, PhD students are selected from among highly-qualified applicants from all over the world and are given the opportunity for training and work in different areas at the forefront of modern biology. Emphasis is placed on academic and technical excellence. The PhD salaries are at an internationally competitive level and are granted for up to four years. Upon graduation, the PhD degree is obtained from the University of Vienna.

In a Europe that is gradually growing together, Vienna is located in the very centre. The city's century-long heritage as an imperial capital is still evident and attracts millions of visitors every year. But nowadays Vienna is a very lively cultural centre, famous for classical as well as for modern music, exhibitions, theatre, opera, and café life, to mention but a few. Since Vienna is home to many international organizations, its cultural life is also very enjoyable for non-German speaking people (e.g. English cinemas, theatre, international festivals).

The geographical location of Vienna makes it ideal for many kinds of outdoor activities, such as skiing, hiking, climbing, cycling and water sports. In addition, the proximity of several other European countries allows weekend escapes abroad.

As far as quality of life is concerned, Vienna is one of the safest capitals in the world. Public transport is extremely efficient, but for those who prefer, it is also possible to get everywhere by bike.

Publications 2001

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(* equal contributions)

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Awards and honours in 2001

Meinrad Busslinger
Wittgenstein Prize by the Austrian Federal Ministry of Education, Science and Culture (Vienna, June 27)

Barry Dickson
Michael Glotzer
Jürgen Knoblich
Jan-Michael Peters
Young Investigators Program (YIP) Award of the European Molecular Biology Organisation EMBO (Heidelberg, July 4)

Jürgen Knoblich
FEBS (Federation of European Biochemical Societies) Anniversary Prize (Lisbon, July 5)

Kim Nasmyth
Golden Medal of the Faculty of Natural Sciences of the Charles University, Prague (Prague, August 26)

Anton Beyer
NRCT (National Research Council of Thailand) Award 2001 (Bangkok, September 20)

Irene Waizenegger
ÖGGGT (Austrian Society for Genetics and Gene Technology) Research Prize 2001 (Vienna, September 25)

Nikolaus Zacherl
Honorary title of »Professor« by the Austrian Federal Government (Vienna, October 24)

Jan-Michael Peters
Roche Molecular Biochemicals Research Prize for Cell Biology (Strasbourg, November 8)

Jan-Michael Peters
Novartis Prize 2001 for Biology (Vienna, January 25, 2002)

Seminar speakers at the IMP 2001

January
11. RANDY SCHEKMAN – *UC Berkeley*
19. FRANCIS STEWART – *EMBL*
25. TODD STUKENBERG – *Virginia Univ.*

February
12. DIMITRIS KIOUSSIS – *NIMR, London*
15. TED YOUNG – *Washington Univ.*
26. HERMANN SCHINDELIN – *SUNY Stonybrook*

March
01. KALERVO HILTUNEN – *Univ. Oulu*
02. THANOS HALEZONETIS – *Wistar Institute, Philadelphia*
12. CLAUDE ANTONY – *Inst. Curie*
14. ASTRID HOEBERTZ – *Univ. College, London*
15. THOMAS SÜDHOF – *Univ. Texas, Dallas*
16. CAROLINE HUTTER – *ICRF*
19. STYLIANOS ANTONARAKIS – *Univ. Geneva*
22. MARTIN HUYNEN – *EMBL*
26. RON McKAY – *NIH, Bethesda*
27. ALAIN MAUVIEL – *INSERM, Paris*
28. GIANNO CAZZANIGA – *University of Milan*
29. NICK TONKS – *Cold Spring Harbor*
30. GERTRAUD BURGER – *Univ. Montreal*

April
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20. CRAIG P. HUNTER – *Harvard Univ.*
25. HOLGER PUCHTA – *IPK Gatersleben*
26. JUERGEN WEHLAND – *Braunschweig*
27. JIM HAGMAN – *Denver*
30. HERMANN BUJARD – *ZMBH, Heidelberg*

May
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03. JEFF STOCK – *Princeton Univ.*
08. JAMES ROTHMAN – *Sloan-Kettering Institute*
09. JUAN F. GIMÉNEZ-ABIÁN – *Centro de Investigationes*
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17. TOM OWEN-HUGHES – *Univ. Dundee*
18. DENISE ZICKLER – *Univ. Paris-Sud*
23. CAROLINE KISKER – *SUNY, New York*
28. TERENCE PARTRIDGE – *Imperial College School of Medicine, London*
31. PAUL BURGOYNE – *NIMR, London*

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05. OLLI KALLIONIEMI – *NIH, Bethesda*
07. RANDALL MOON – *Univ. Washington*
08. MARLA SOKOLOWSKI – *Univ. Toronto*
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24. YOSHINORI WATANABE – *Univ. Tokyo*
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16. HARINDER SINGH – *University of Chicago*
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November
09. CLAUDE SARDET – *Montpellier*
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