



Research Institute of Molecular Pathology
VIENNA BIOCENTER

I.M.P.
2000



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Introduction

This has been yet another extra-ordinary year for the IMP. We have published key papers in a wide variety of areas, ranging from chromatin structure, responses of host cells to viral infection, cancer biology, immunology, development, and chromosome segregation, all in a variety of different model organisms. There can be few institutes in the world where the »specific activity« has been as high as at the IMP at the moment and it will be a major challenge to maintain this standard in the coming years. Achievements lead to greater expectations, which in turn lead to yet greater achievements, creating a virtuous circle. Long may this last! Few of these scientific achievements would have been possible without the enthusiastic and highly professional support of our remarkable service departments, which continue to make the institute such an attractive place to work. Nor would they have been possible without the dedication and commitment of some of the finest students, postdocs, and technicians in Europe. This year has also witnessed the re-birth of our cafeteria, thanks largely to the unstinting efforts of Thomas Jenuwein, and major progress in establishing our sister institute IMBA. We have a wonderful architectural design for the new building, have green lights from the City of Vienna, the Austrian Academy of Sciences, the Austrian Government, and the EU, and are currently negotiating with a potential director.

Kim Nasmyth
Vienna, December 2000



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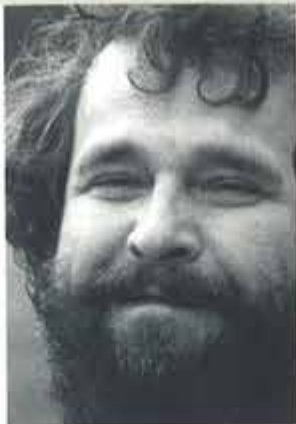
Picture Gallery

Publications 2000

The Scientific Advisory Board

Seminar Speakers at the IMP 2000

Impressum



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Developmental plasticity: deregulation in oncogenesis

Distinct families of oncogenes and tumor suppressor genes affect the balance between proliferation, differentiation and/or apoptosis of progenitor cells in many tissues, including our two experimental systems (erythroleukemia and breast carcinoma). Unmutated members of the same gene families active in neoplasia regulate normal processes such as expansion of erythroid progenitors during stress or disease and epithelial/mesenchymal transitions during embryonic development and wound healing.

Altered proliferation control of hematopoietic progenitors in stress or disease: a process important in leukemia?

Proliferation control of many types of hematopoietic progenitors is regulated by the cooperation of plasma membrane- and nuclear receptors. The first receptor family comprises receptor tyrosine kinases (RTKs), receptor serine kinases (TGF β R family) and cytokine receptors, while the second includes class I and class II nuclear receptors (NRs, steroid- and thyroid / retinoid acid receptors). Mutated receptors and associated corepressors and chromatin regulators function as oncoproteins in avian, murine and human leukemias. In primary murine and human erythroblasts, sustained proliferation is induced upon activation of the erythropoietin receptor (EpoR), the RTK c-Kit and the glucocorticoid receptor (GR) by their respective ligands (Epo, stem cell factor/SCF and dexamethasone/Dex). Normal or oncogenic epidermal growth factor (EGF) receptor (c-v-ErbB) substitutes for the signals provided by EpoR plus c-Kit. Using genetically modified mice, we showed that the GR is dispensible for standard erythropoiesis, but required for enhanced erythroid progenitor proliferation in stress erythropoiesis (anemia/hypoxia). Both STAT5 and complete EpoR signalling are dispensible for steady state erythropoiesis, but required for erythroid progenitor proliferation in culture. Mortal and immortalized, but otherwise normal

erythroid progenitors from wt and p53^{-/-} mice, respectively, are currently used to analyse signal transduction downstream of the EpoR and c-Kit and the involvement of STAT5 in signalling and target gene transcription. We already found that pathways used by the EpoR and c-Kit are distinct and that c-ErbB acts by combined signalling along EpoR and c-Kit pathways (Figure 1). Gene expression profiling of translated genes using polysome-bound versus free mRNA has been initiated in the erythroid system, showing that a surprisingly large proportion of genes regulated during erythroid differentiation are also subject to translational control. Applying this approach to Affymetrix chips, we are currently analysing the cooperation between EpoR and c-Kit, their cooperation with the GR and their replacement by c- and v-ErbB. In addition, avian and human leukemia oncogenes (v-Ski, MLL/ENL) cooperating with c-Kit in avian multipotent cells will yield further interesting systems for gene expression profiling, after establishing these systems in mouse cells.

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

In carcinomas, epithelial cells lose the strict polarity required for the protective barrier function of epithelia and progress to cells with mesenchymal characteristics,

Figure 1: Erythroid progenitor renewal: signalling through plasma membrane- and nuclear receptors

Scheme depicting our current view of which molecular players are essential for erythroid progenitor renewal and how they may talk to each other. Data from genetically modified mice imply the EpoR, c-Kit, Stat5 and GR in erythroid proliferation control during (steady state and/or stress) erythropoiesis. EpoR and c-Kit contribute to renewal via distinct pathways (red and blue arrows), c/v-ErbB signal along pathways employed by the EpoR and c-Kit (green arrows). GR- function can be substituted by corepressors/chromatin regulator oncogenes (v-Ski / MLL/ENL; black) interacting with nuclear receptors, a role also proposed for Stat5.

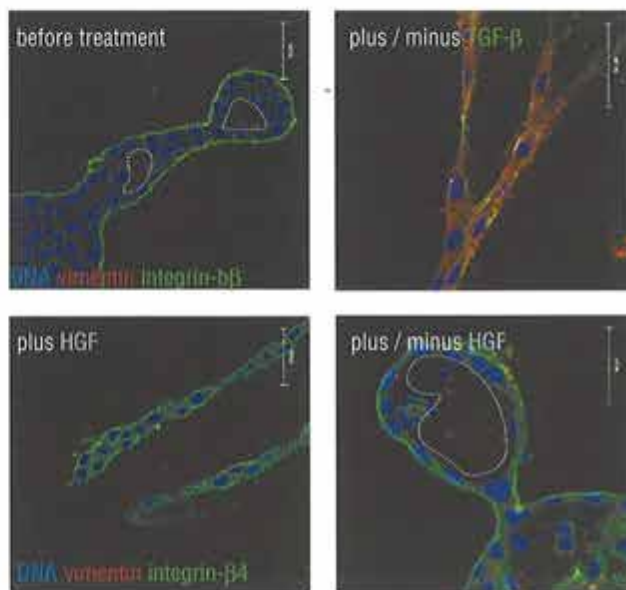
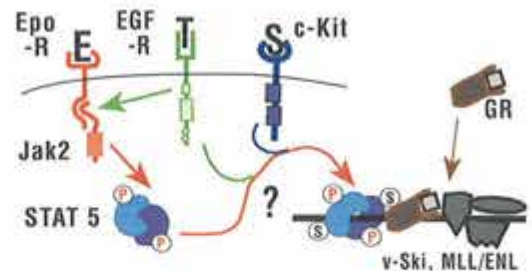


Figure 2: Growth factors implicated in human carcinogenesis: scattering versus EMT

In situ antibody staining of three dimensional structures formed by epithelial cells in collagen gels followed by confocal microscopy allows to distinguish between bona fide EMT and reversible scattering. Control Ras-transformed mammary epithelial cells form tubular structures with basal expression of beta 4 integrin (lumen; white dotted line, top left). TGF β addition induces the cell to invade the gel, lose beta 4 integrin and gain the mesenchymal marker vimentin (red, top right). In contrast, treatment with HGF/SF causes invasion of the gel, but no upregulation of vimentin (bottom left). When HGF/SF is removed, the cells again form tubular structures (bottom right), while structures formed in TGF β do not change after TGF β removal (not shown).

particularly during metastasis. This process of epithelial-mesenchymal transition [EMT] also occurs when epithelial cells acquire migratory and mesenchymal properties during embryogenesis, tissue remodeling and wound healing.

Normal mammary gland morphogenesis requires cooperation between TGF β -receptor (T β R) signalling with RTKs (HER2) or downstream signal transduction intermediates (Ras; MapK pathway). This

cooperation is subverted in EMT as well as in late processes during carcinogenesis. Interference with T β R signalling reverts EMT and prevents *in vitro* invasiveness and metastasis in numerous murine and human tumor models. Furthermore, Ras- or Mek-1 inhibitors abolished EMT, reverting the cells to an epithelial phenotype and restoring sensitivity to TGF β -induced cell cycle arrest and apoptosis. Thus, EMT requires the sustained activity of two cooperating pathways, Ras- and TGF β receptor signalling. For instance EMT was claimed to occur after ligand activation of the fibroblast growth factor (FGF) or hepatocyte growth factor (HGF/SF) receptors, both of which have been strongly implicated in human carcinogenesis. However, FGF-R and HGF-R failed to induce EMT in Ras-transformed epithelial cells, inducing reversible morphological and migratory changes not accompanied by gene expression changes which are typical for EMT (loss of epithelial markers, gain of mesenchymal markers; Figure 2).

Using specific inhibitors and Ras effector-specific mutants selectively activating Ras-downstream signalling, we investigated the role of various pathways in EMT and TGF β -induced apoptosis.

Furthermore, we have performed gene expression profiling of translated genes on Affymetrix chips. Both EMT markers and potential key players involved in EMT have been found and are currently analysed. Finally, we investigate the role of TGF β R signalling in EMT.



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Pax gene function in brain development, hematopoiesis and disease

The development and organogenesis of the mammalian embryo is controlled by a network of differentially expressed transcription factors including the paired box-containing Pax proteins. By using a combination of mouse transgenic, cell biological and molecular approaches, we are investigating the mechanisms by which Pax transcription factors regulate brain patterning, B-lymphopoiesis and, under pathological conditions, the development of disease.

Midbrain development

The midbrain and cerebellum develop from an organizing center, which is located at the midbrain-hind-brain boundary (mhb) of the vertebrate embryo. The three transcription factors of the Pax2/5/8 family are co-expressed in this embryonic brain region, although only Pax2 and Pax5 were so far shown to be essential for mhb development. Our recent analysis of Pax5, Pax8 double-mutant embryos has also identified Pax8 as a critical regulator of the mhb region. One goal of our laboratory is to unravel the molecular mechanisms by which Pax proteins regulate mid-brain and cerebellum development. Gene targeting and transgenic analyses have indicated that Pax2 is essential for the initiation of Pax5 expression at the mhb of the mouse embryo. Apart from this cross-regulatory interaction, Pax2 and Pax5 have partially redundant functions in mhb development, which was confirmed by the generation of a knock-in (ki) mouse carrying a Pax5 minigene under the control of the Pax2 locus. The brain phenotype (loss of midbrain and cerebellum) of the Pax2 mutation was entirely rescued in this knock-in mouse, indicating that Pax5 can compensate for the loss of Pax2 function (Figure 1). To date, relatively few genes are known which are expressed in the embryonic mhb region. To search for

novel genes expressed in this brain area, we have screened mouse cDNA microarrays by hybridization with probes prepared from micro-dissected brain regions of the mouse embryo. Novel mhb-specific genes have been identified, which are now being characterized, also with regard to their regulation by Pax proteins. Different experimental approaches are thus used to identify upstream regulators and downstream targets of Pax genes in midbrain development.

B-lymphopoiesis

Pax5 plays an essential role in B cell development, as it is required for progression beyond an early progenitor (pro-B) cell stage. Pax5-deficient pro-B cells can be cultured *ex vivo* on stromal cells in the presence of IL-7. Surprisingly, these pro-B cells are uncommitted hematopoietic progenitor cells, as they can develop, upon stimulation with appropriate cytokines, into natural killer (NK) cells, T-lymphocytes and various myeloid cell types (Figure 2). B cell development is, however, only observed upon restoration of Pax5 expression by retrovirus-mediated gene transfer. 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 furthermore revealed that Pax5 is

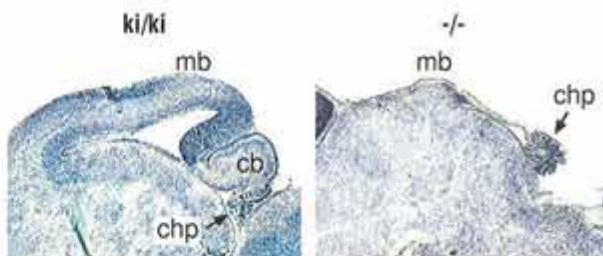


Figure 1: Functional equivalence of Pax2 and Pax5. The midbrain (mb) and cerebellum (cb) develop normally in *Pax2^{ki/ki}* mice in contrast to *Pax2^{-/-}* mice, as revealed by sagittal brain sections of 18.5-day-old embryos. chp, choroid plexus.

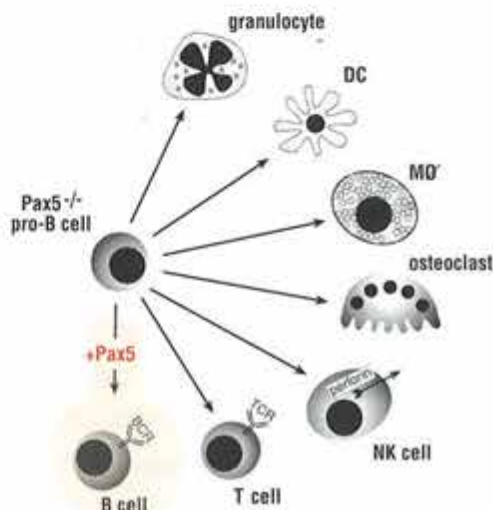


Figure 2: B-lineage commitment by Pax5. *Pax5^{-/-}* pro-B cells are early progenitor cells, which can differentiate along the indicated hematopoietic lineages with the exception of the B-cell pathway.

Pax5^{+/+} vs Pax5^{-/-} pro-B cells

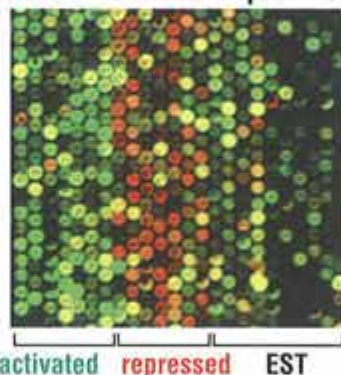


Figure 3: Identification of Pax5-regulated genes. Libraries enriched for activated or repressed genes were generated by cDNA subtraction between wild-type and *Pax5^{-/-}* pro-B cells, and 5'000 clones of each library were spotted together with known ESTs on glass slides. This pro-B cell chip was hybridized with Cy3 (green)-labelled cDNA from wild-type pro-B cells and Cy5 (red)-labelled cDNA from *Pax5^{-/-}* pro-B cells.

also essential for maintaining 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 through the recruitment of specific coactivators or Groucho corepressors, respectively. To systematically analyze the transcriptional function of Pax5, we have generated a pro-B cell microarray by cDNA subtraction between wild-type and Pax5-deficient pro-B cells. Expression profiling of this pro-B cell chip is currently under way to identify novel Pax5 target genes. The functional characterization of these genes will provide important insight into the role of Pax5 in early and late B-lymphopoiesis. Using transgenic approaches, we have mapped the B-cell-specific enhancer at a distant location from the Pax5 promoter. Characterization of this enhancer should identify the upstream regulators controlling Pax5 transcription. Together, these experiments will lead to a better understanding of the transcriptional hierarchy and control of B-lymphopoiesis.

Human disease

An unusual feature of mammalian Pax genes is their haploinsufficiency, which results in the frequent association of heterozygous Pax gene mutations with human disease syndromes and mouse developmental mutants. PAX genes are also recruited as oncogenes by gain-of-function mutations in certain 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.



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The major molecular mechanisms of multistage tumor development

The objective of our research is the identification and characterization of molecular events involved in multistage tumorigenesis. In addition to tumor cell lines in vitro, we employ transgenic mouse models of tumorigenesis to determine causal connections between the expression of a particular gene and tumor progression in vivo.

One of the mouse models (Rip1Tag2) expresses SV40 large T antigen under the control of the rat insulin promoter leading to pancreatic β cell tumors in a multistage tumor progression pathway.

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, and expression of soluble FGF or VEGF receptors in xenograft tumor transplantation experiments and in Rip1Tag2 transgenic mice 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.

Recently, an antagonist of FGF function has been identified in *Drosophila* development, named Sprouty. 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 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., 2000). Currently, we are investigating the mechanism by which Sproutys intersect tyrosine kinase receptor-mediated signal transduction.

Tumor cell invasion and metastasis

Previously, we have demonstrated that the loss of E-cadherin-mediated cell-cell adhesion is causally involved in the transition from adenoma to carcinoma (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 (Fig. 1), 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

β-CATENIN SIGNALLING

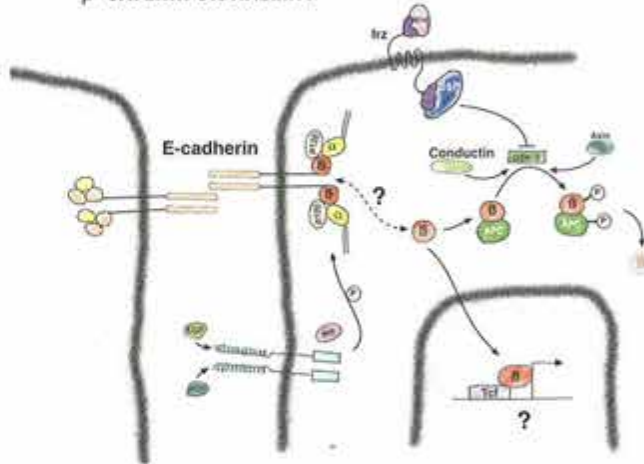


Figure 1: The link between E-cadherin-mediated cell-cell adhesion and the Wnt-signalling pathway. Two major questions of our current research are indicated: (1) Does E-cadherin-mediated cell-cell adhesion directly modulate the Wnt-signalling pathway? (2) Are changes in TCF/β-catenin target gene expression relevant for tumor progression from adenoma to carcinoma?

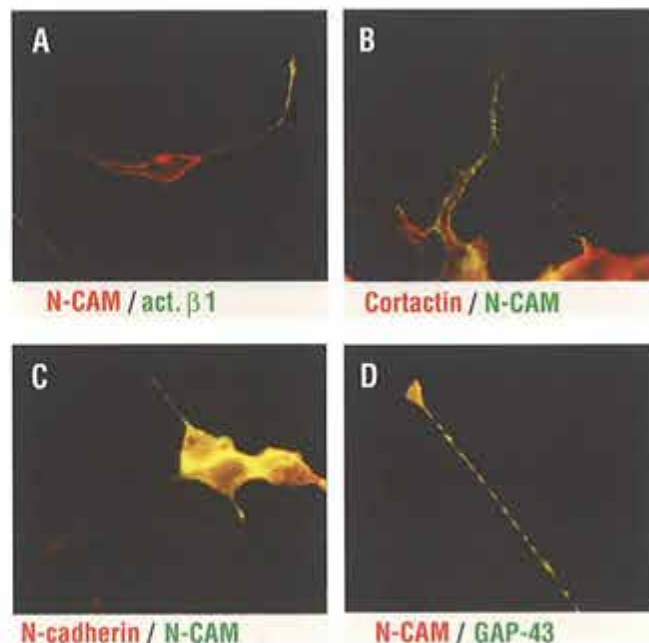


Figure 2: The N-CAM/N-cadherin/FGF receptor complex in neurites of N-CAM expressing β tumor cells. Co-localization of cortactin, N-cadherin, GAP-43, and activated β1 integrin with N-CAM was determined by double-immunofluorescence analysis in N-CAM-induced neurites of β tumor cells as indicated.

of the TCF/LEF-1 gene family. In both approaches, Rip1Tag2 tumor progression, in particular the transition from adenoma to carcinoma, was not altered. 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.

During the development of many human cancers, expression of neural cell adhesion molecule (N-CAM) is downregulated concomitant with a progression to tumor malignancy. Recently, we have 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). Notably, N-CAM-deficient tumors 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. Components of the FGFR signal transduction pathways, such as pp60^{src}, FRS2, PLCγ, cortactin and also GAP-43 are found to associate with N-CAM, N-cadherin and FGFR-4. N-CAM and some of these factors specifically co-localize with activated β1 integrin in neurites of N-CAM expressing cells (Figure 2). Conversely, dominant-negative FGFR-4 and specific inhibitors of FGFR signalling repress N-CAM-mediated neurite outgrowth (Cavallaro et al., 2000). The results indicate that N-CAM induces cell-matrix adhesion by activating FGFR signalling, a potential mechanism for modulating tumor metastasis.



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Axon guidance in *Drosophila*

*How does the nervous system get wired up? What rules underlie the intricate patterns of connectivity in the nervous system? To seek answers to such questions, we have turned to the fruit fly, *Drosophila melanogaster*. As a model system, *Drosophila* offers both complex patterns of neuronal connectivity and powerful methods for genetic analysis. While the final patterns of connectivity in our own nervous system are far more complex than those of the fly, the developmental mechanisms that establish these connections are remarkably similar.*

During development of the nervous system, each neuron projects an axon that seeks out and connects with one or more specific target cells. Axons follow very specific routes to their targets, ignoring paths that other axons might take, and ignoring targets that other axons might choose. How does each axon recognise its own specific route and destination? Car drivers face exactly the same problem, and perhaps not surprisingly, drivers and axons have found similar solutions. Just like cars, axons are also equipped with a motor and a steering mechanism - these reside in the growth cone at the tip of the axon. Drivers navigate by looking out for the specific signposts that will lead them to their destination. In the same way, axons use receptors in the growth cone to look for guidance cues in the extracellular environment that will direct them to their targets. We want to know the nature of these cues, the receptors that recognise them, and how the motor and steering machinery in the growth cone work. Our approach is to try to create havoc on the neural highways: to remove the 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 disrupt the engine or 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 face a number of important decisions: to cross or not to cross the midline; to select a specific »lane« on the highway running from the brain to the tip of the nerve cord; and when and where they should exit the highway in search of their final targets. We are now beginning to understand how many of these decisions are controlled. The major guidance cue here is Slit, a repellent that is secreted by cells at the midline. The decision to cross or not to cross is regulated by the receptors Robo1 and Robo2. Axons expressing high levels of Robo1 and Robo2 are repelled by Slit and do not cross the midline; only axons expressing low levels of both receptors can cross. Robo1 and Robo2 levels are in turn controlled by the proteins Comm1 and Comm2, which therefore indirectly determine which axons cross and which do not. We are currently trying to figure out how the Comms regulate the Robos. Slit also determines which lane an axon chooses as it turns into the major highway running up and down the nerve cord. Slit does this by signalling through both Robo1 and Robo2, as well as a third receptor unimaginatively called Robo3.

By altering the »Robo code« of specific axons, we

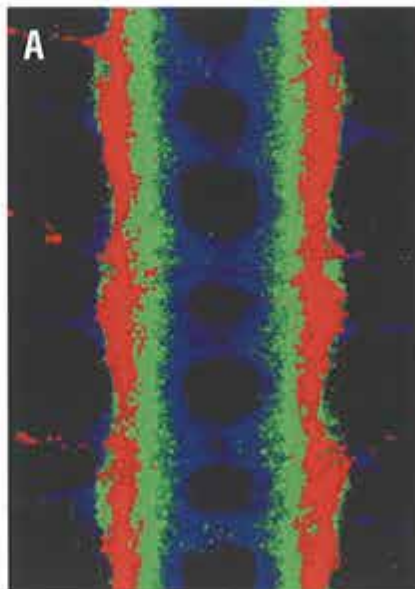


Figure 1: Lanes on the axonal highways. (A) In the CNS of a *Drosophila* embryo, axons in the longitudinal tracts are sorted into three lanes. Axons expressing Robo1 choose the inner lane (blue), axons expressing Robo1 and Robo3 take the middle lane (green), and axons expressing all three Robos take the outer lane (red). The longitudinal axon tracts are connected in each segment by two commissures (blue), formed by axons that cross the midline. (B) These pathways are patterned by a combination of attractive and repulsive guidance cues. In this schema, the attractive cue (Netrin) is shown on the left and the repulsive cue (Slit) on the right. In reality, the CNS is bilaterally symmetrical, and both mechanisms operate on both sides of the midline.

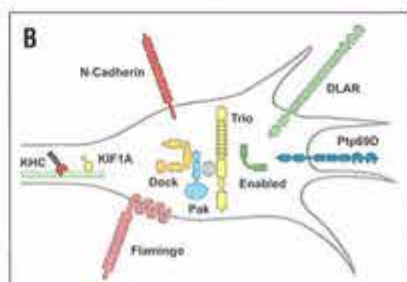
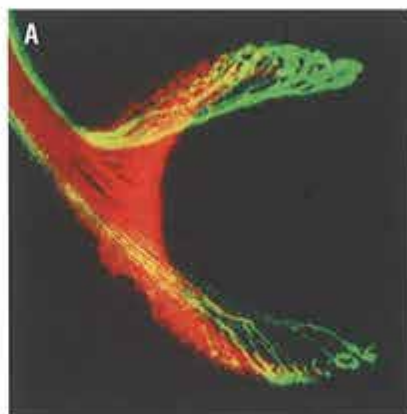
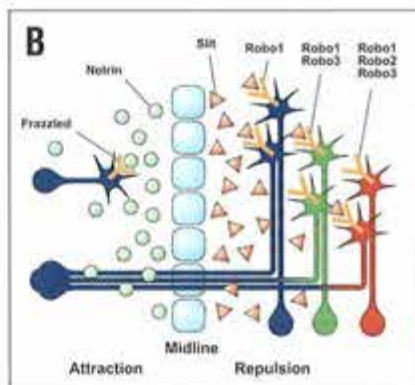


Figure 2: Photoreceptor axons innervating the brain. (A) Projections are retinotopic, so that dorsal and ventral axons (green) project to the dorsal and ventral regions of the optic lobe. (B) Our genetic screen is beginning to reveal the path-finding machinery inside photoreceptor growth cones.

have discovered a simple logic to lane selection: axons expressing only Robo1 stay in the lane nearest the midline, axons expressing Robo1 and Robo3 take the middle lane, and axons expressing all three Robos take the outside lane. The three Robos identify these lanes by reading positional values on a Slit gradient.

Axon guidance in the visual system

Vision requires the precise targeting of photoreceptor axons to the visual centres of the brain. These axons make »retinotopic maps« that project spatial information directly into the brain. Photoreceptors tuned to specific colours project their maps into different target layers. How do these axons find their way into the brain? How do they read the coordinates of the retinotopic map? How do they know which target layer they should choose? To find out, we have recently performed a saturation genetic screen, examining over 32,000 mutant lines to identify those in which one or more of these decisions goes wrong. We recovered nearly 200 such mutants, and have now assigned each to one of approximately 40 different genes. In most of these mutants, axons make a very specific guidance error. For example, in some mutants axons choose the wrong target layer. In others, they project to the wrong location on the retinotopic map. Our task is now to identify each of these genes so that we can then examine the molecular basis of each decision. So far, we have identified 15 of them. The proteins they encode include cell surface receptors that sense specific guidance cues, and molecules that direct growth cone extension in response to these cues. In the coming year, we will continue to examine in detail the functions of several of these genes. In parallel, we are also taking advantage of the recently completed *Drosophila* genome sequence to develop high-throughput high-resolution mapping techniques in order to identify most of the remaining 25 genes.



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Understanding gene function with biomolecular sequence analysis and cDNA chip data studies

High-throughput experimental technologies in Life Sciences (for example, DNA sequencing and the cDNA array technology) 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 past first year. 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 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. More than 100 protein families have been studied with sensitive sequence analysis methods in great detail during the past year, some of them repeatedly, to elucidate structural and molecular functional features of the gene products or associated genomic regulatory regions. Such investigations have been launched, as a rule, on requests of IMP researchers but also as cooperations with the University of Vienna and the Austrian Academy of Sciences.

For example, the putative histone methyltransferase activity of su(var) proteins has been predicted by a distant but yet significant homology of their SET domain segment with plant methyltransferases. Subsequent experiments carried out by the Jenuwein group verified this hypothesis successfully. This finding opens new possibilities for studying epigenetic control of chromatin structure on a molecular level, taking into account histone modifications via methylation, acetylation, and phosphorylation.

Development of new methods, algorithms and software packages for sequence and DNA chip data analysis

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

1. Recognition of posttranslational modifications in proprotein sequences (see Fig. 1)
2. Integration of sequence analysis methods in a shell for applications in large scale protein sequence annotation, a common project with BI Austria
3. The Java microarray information management system JMIMS (see Fig. 2)

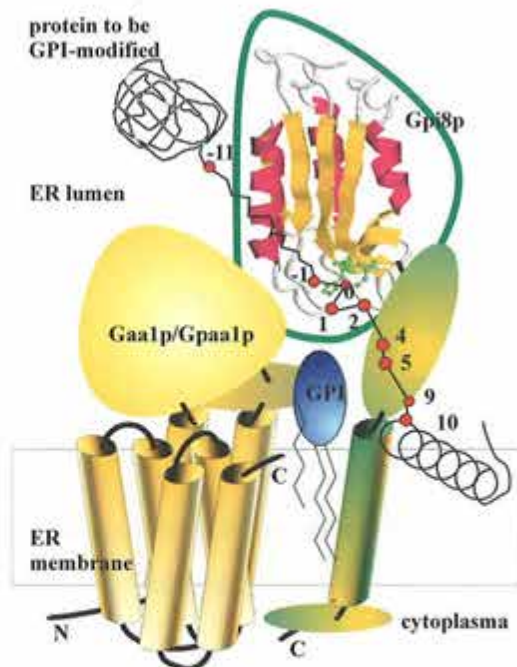


Figure 1: Model of the transamidase complex for transfer of the glycosylphosphatidyl-lipid anchor to substrate proteins in eukaryotes

After cleavage of the signal peptide, the transamidase Gpi8p (shown mainly in green) is located in the ER lumen but attached to its membrane by a single transmembrane region. The N-terminal endopeptidase domain is distantly related to caspases and ginsipain R in accordance with sequence similarity and secondary structure predictions. Among all residues with functional side chains, only a histidine and a cysteine (ca. 40 residues apart) and a few small residues nearby are strictly conserved in an alignment with other C13 proteases. These two catalytic residues (shown as ball-and-stick in green) form the catalytic dyad.

The Gaa1p/Gpa1p protein (shown in yellow) is a seven-transmembrane region protein. The N-terminus of the polypeptide chain is in the cytoplasm, the C-terminus is located in the ER lumen. The domains located in the ER activate the (acylated) GPI anchor (shown in blue) and supply it to the Gpi8 protein and to the cleaved substrate polypeptide.

The substrate protein (shown as continuous black line with a few residues emphasized by red circles) is recognized by the transamidase alone. An unstructured region (ω -11... ω -1) connects the globule of the substrate protein with the catalytic cavity comprising residues ω -1... ω +2 followed by a moderately polar region. The mainly hydrophobic residues from ω +9 or ω +10 to the C-terminal appear to be bound by a hydrophobic pocket of Gpi8p and/or to dive into the membrane.

This knowledge is incorporated into the big-IT software for GPI-anchor site prediction available also as WWW-service from the IMP (http://mendel.imp.univie.ac.at/gpi_server.html).

Computer use 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, a heterogeneous network of Apple Macintosh computers, WindowsNT PCs and Unix machines is supported. The concept of the computer & network group has started to change gradually from mainly individual user-service contacts to generalized problem solutions for the whole institute.

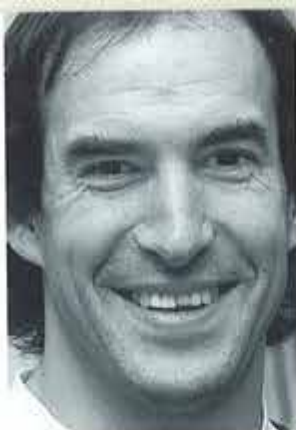
The last year has seen an unprecedented technical rearmament of the core facilities: new central file and mail servers with a 10fold increased disk capacity have been installed. The central parts of the IMP network are equipped with new switches/routers, the core connections have changed to optical cabling (with ~1 Gbit/second transmission). Most laboratories are now equipped with 100 Mbit links to the central switches. Both planning and installation of the new network design has been realized without outside support. Training in PC operating systems and major software packages is offered to IMP employees.

The IMP system managers have started to analyze their ways of spending working time for different tasks in order to find methods for improving performance. Currently, 46% of all manpower is still used for finding solutions to individual problems.



Figure 2: JMIMS – the Java Microarray Information Management System

The development of in-house production and application of cDNA chips required a corresponding software solution. Object-oriented Analysis and Design (OOA&D) on the domain of production of cDNA microarrays and their subsequent use in hybridizations, scannings and image analyses resulted in a class schema (persistent object model). This class schema was implemented using the Java binding of the Object Data Standard version 3.0, as defined by the Object Data Management Group (ODMG). We used the pure Object Database Management System (ODBMS) POET OSS as the back-end. A web-based user-interface to this database was implemented using the Java2 Enterprise Edition (J2EE) technologies Java Server Pages (JSP) and Java Servlets. The software interface allows technicians and scientists to handle chip-associated data at all stages of chip production and use.



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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 is focussed on understanding this process of cytokinesis in molecular detail.

Cytokinesis is mediated by an actin-based contractile ring that is attached to the overlying cell membrane. The ring assembles in the cell cortex at a site that is positioned midway between the two poles of the mitotic spindle, thus ensuring that the two separate sets of chromosomes are equally partitioned into the two daughter cells. The entire process – the assembly of the contractile ring, its constriction and the separation of the two nascent cells – usually requires ten minutes. Thus, cytokinesis is a dynamic and spatially regulated process. The nematode *C. elegans* is an excellent model system to dissect this complex process since worm embryos are extremely well suited for real time microscopic analysis. 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 (Figure 2). We are studying the *cyk-4* gene, which is essential for the formation of the central spindle and for cytokinesis. In *cyk-4* mutant embryos, chromo-

somes 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 embryo becomes multinucleate. Surprisingly, CYK-4 contains a GAP domain that stimulates GTP hydrolysis by Rho-family GTPases. Central spindle assembly does not require the Rho GTPase and we have indications that CYK-4 mediates central spindle assembly by associating with a kinesin-like protein known as ZEN-4/CeMKLP1. Embryos lacking ZEN-4 have essentially the same defects as embryos lacking CYK-4. CYK-4 and ZEN-4 are thus each necessary for central spindle assembly. We hope to determine next if these two proteins are sufficient for this process, by attempting to reconstitute antiparallel microtubule bundling using recombinant CYK-4 and ZEN-4. It is likely 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.

A second protein that localizes to the central spindle is the Aurora-like kinase AIR-2. In the past year we studied the function of Incenp, a protein that binds to chromosome arms in prometaphase, the inner centromeric region in metaphase, and the spindle midzone during cytokinesis. We discovered that



Figure 1:
The division of
a worm embryo

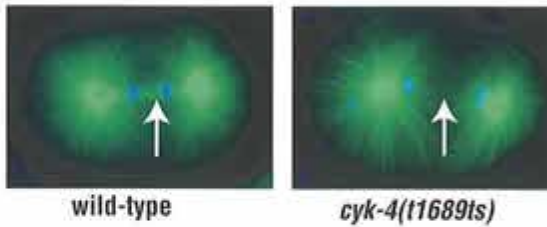


Figure 2: *cyk-4* mutants are defective in central spindle assembly

A wild-type and a *cyk-4* mutant embryo in early anaphase were fixed and stained with anti-tubulin antibodies. The central spindle is clearly visible in wild-type embryos but not in *cyk-4* mutant embryos.

ICP-1 (the nematode Incenp) binds to AIR-2 and is required for the localization of this kinase. Embryos lacking AIR-2 and ICP-1 are defective in both chromosome segregation and cytokinesis. By following the distribution of ZEN-4::GFP in real time, we have been able to show that at least one way in which ICP-1 and AIR-2 promote cytokinesis is by promoting the stable localization of ZEN-4 to the central spindle. We now plan to investigate in more detail if ZEN-4 or CYK-4 are direct substrates of the AIR-2 kinase. Interestingly, ICP-1 does not only function to promote cytokinesis, it also is required for chromosome segregation. This function also requires AIR-2 and we are investigating if ICP-1 regulates AIR-2 or only serves to localize this kinase.

Our goal is to refine our understanding of how the proteins mentioned above act to promote cytokinesis. We will rely extensively on both biochemical and genetic tools to achieve this goal. However, these are probably only a small subset of the factors that mediate cytokinesis, many additional aspects are entirely uncharacterized. For example, 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 conducted a screen for mutants defective in this process. This screen was designed to allow us to identify both temperature sensitive and non-conditional mutations that cause homozygous mutant worms to be viable but to produce only dead embryos – so-called maternal effect embryonic lethal mutations. This screen allowed us to isolate several mutants that have inte-

resting defects in the early embryo, including one that is cytokinesis defective. We are using single nucleotide polymorphisms (SNPs) to aid the mapping and cloning of the affected genes. Further rounds of screening will enable us to identify critical proteins that are required for the successful completion of cytokinesis.

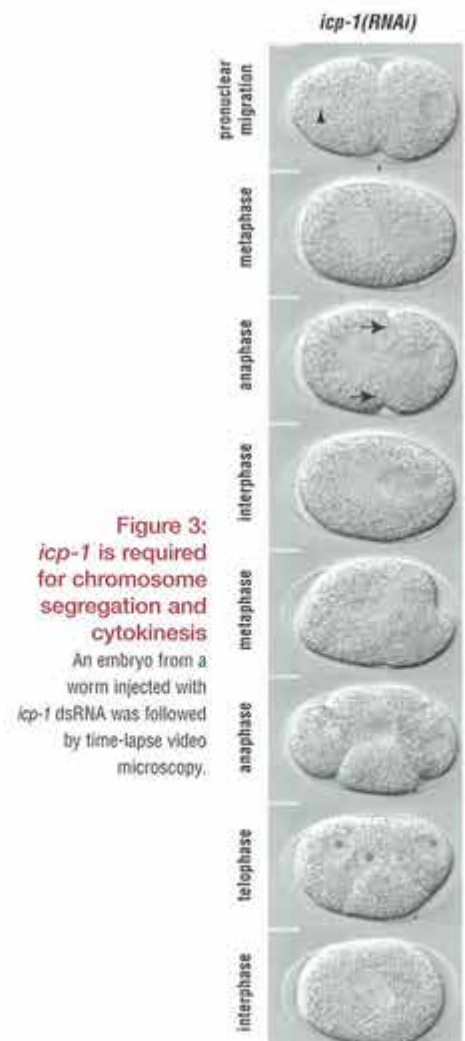


Figure 3:
icp-1 is required
for chromosome
segregation and
cytokinesis

An embryo from a worm injected with *icp-1* dsRNA was followed by time-lapse video microscopy.



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Epithelial biology

The central aim of my 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 program of epithelial cells.

One major tool we used was well-characterized cell systems that display both epithelial polarity and its disruption in cancer in an *in vivo*-like fashion. Primarily, we used a mouse mammary gland epithelial cell line (EpH4) expressing an estrogen-inducible c-JunER fusion protein, which allows analyzing 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 at identifying 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, using Differential Display techniques. Secondly, we analyzed differentially expressed proteins of the intracellular protein transport and sorting machinery, analyzing purified subcellular organelles by 2D gel technology and microsequencing (Fialka et al., J. Biol. Chem. 1999, Fialka et al., Electrophoresis 1999).

TIS7/PC4, a new a transcriptional co-regulator in epithelial cells

Last year we reported that TIS7/PC4 was up regulated during c-Jun-induced transient loss of epithelial pola-

city, as well as detached from the plasma membrane and translocated to the cytoplasm or nucleus. Thereafter, TIS7/PC4 interacts with components of the wnt-signalling pathway, selectively squelching gene activation by beta-catenin. TIS7/PC4 could thus be a co-repressor of beta-catenin/TCF/LEF-regulated transcription, necessary for cell fate decisions of epithelial cells during transient loss of cellular polarity (Vietor et al., submitted).

p14, scaffold or regulator for mitogen activated protein kinases?

We discovered a novel, highly conserved protein of about 14 kD (p14) that localized to the late endosome/lysosome (Figure 1). Using yeast two-hybrid screening we identified MP1 (MEK Partner 1) as specific binding partner of p14. MP1 has been proposed to serve as a scaffold protein of the MAP kinase cascade in higher eukaryotic systems and the protein seems to selectively associate with MEK1 and ERK1 (Schaeffer et al., 1998). We can immunoprecipitate the Mp1/MEK1/ERK1 complex together with p14 and can reconstitute an activateable complex *in vitro* (Wunderlich and Fialka et al., J. Cell Biol. 2001, in press). We will analyze now the influence of p14 on the function of this signalling module.

CD44 in lipid rafts is the plasma membrane transmitter of HA »guidance cues«

CD44, the major cell-surface receptor for hyaluronic acid (HA), was shown to localize to detergent-resistant cholesterol-rich microdomains, called lipid rafts, in fibroblasts and blood cells. These CD44-containing lipid microdomains interact with the underlying actin cytoskeleton (Oliferenko et al. J. Cell Biol., 1999). Both CD44 and HA are thought to be involved in several processes ultimately requiring cytoskeleton rearrangements. In continuation of our work we could also show that the small guanine nucleotide (GTP)-binding protein Rac1 could be activated upon HA binding to CD44. When applied locally to a passive cell edge, HA promoted the formation of lamellipodial protrusions in the direction of stimulus (Figure 2). This process was inhibited by the prior injection of cells with dominant-negative N17Rac recombinant protein or by pretreatment of cells with monoclonal anti-CD44 antibodies, interfering with HA binding, implying the direct involvement of CD44 in signalling to Rac1. The observation we describe here would suggest the possibility of the direct involvement of CD44 and HA in cell guidance (Oliferenko et al., J. Cell Biol. 2000). We would like to propose that HA might serve as an external stimulus influencing cell orientation, with CD44 mediating signalling to the actin cytoskeleton.

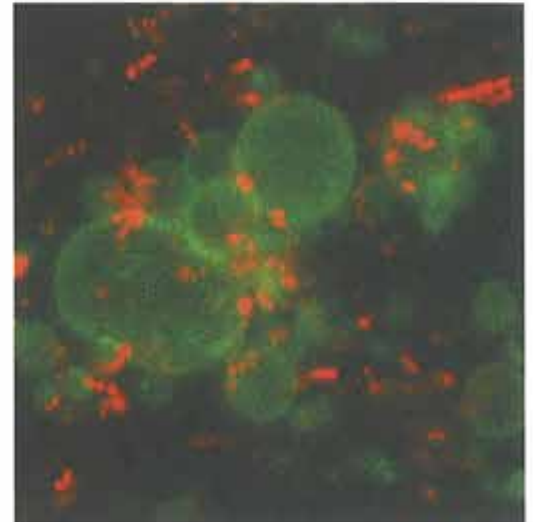
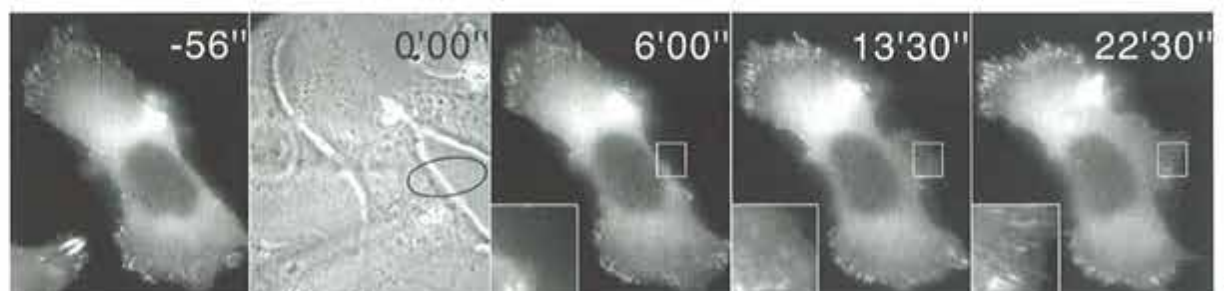


Figure 1: Projection of a live microscopical stack of the intestinal epithelial cell line Caco-2 expressing EGFP-p14(green) after 30min internalisation of EGF-Rhodamine(red)

Figure 2: Lamellipodial outgrowth is spatially restricted and confined to the region of local HA application.

When oligosaccharide HA (40 µg/ml) was supplied through the microinjection needle to the defined region of the cell, we could observe quite rapid lamellipodia formation exclusively underneath the micropipette. The lamellipodia formed exhibited normal focal contact formation and growth, as shown here for the EGFP-zyxin-expressing cell. High molecular weight HA (40 µg/ml) was equally potent in inducing local lamellipodial outgrowth.





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Regulation of mammalian higher-order chromatin

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 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 the combat of cancer.

Suv39h genes encode novel histone H3 methyltransferases

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 have recently isolated mouse (*Suv39h1*) and human (*SUV39H1*) genes that encode novel histone H3 lysine 9-selective methyltransferases (*Suv39h* HMTases). The catalytic motif was mapped to the conserved SET domain which requires adjacent cysteine-rich regions to confer HMTase activity. Notably, methylation of lysine 9 (Lys9) interferes with phosphorylation of serine 10, a known histone modification required for chromosome segregation. By contrast, *Suv39h*-dependent methylation is also influenced by pre-existing phosphorylation and acetylation in the H3 N-terminus. These data provide experimental evidence for the «histone code» hypothesis, which proposes that distinct modifications of histone N-termini would regulate different chromatin-based outputs, like transcriptional activation, mitotic

chromosome condensation or the marking of heterochromatic domains at centromeres and telomeres. Because *Suv39h* proteins are enriched at heterochromatin and also transiently accumulate at centromeres, our results further suggest that *Suv39h*-mediated Lys9 methylation of the H3 N-terminus represents an important epigenetic signal towards the induction and assembly of mammalian higher-order chromatin (see Figure 1).

Genomic instabilities in *Suv39h*-deficient mice

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 ~ 20-25% of the expected ratios, are growth retarded and display hypogonadism in males. By contrast, analyses of primary mouse fibroblasts (PMEFs) and of *ex vivo* fetal liver cultures indicate accelerated proliferation that is accompanied by higher indices of

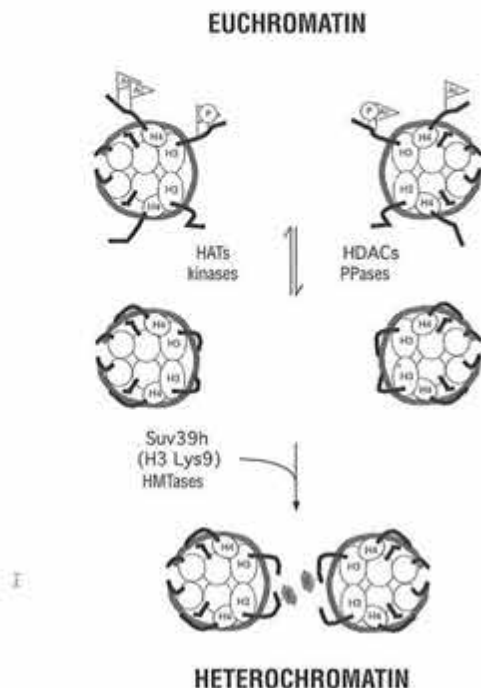


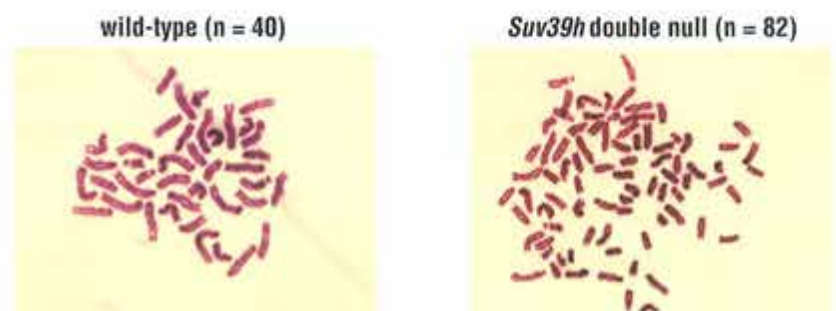
Figure 1: Model for the establishment of heterochromatin by the Suv39h HMTases. Activated regions of chromatin (euchromatin) are represented by acetylated (Ac) and phosphorylated (P) N-termini (thick black lines) of histones H3 and H4 – modifications that have been correlated with enhanced transcriptional activity and which will impair substrate recognition by the Suv39h HMTases. For clarity, modifications are only shown at one pair of histone H3 and H4 per nucleosome. In chromosomal regions devoid of transcriptional activity (e.g. centromeres and telomeres) or under conditions where histone H3 N-termini are largely unmodified (e.g. after replication or because of reduced transcriptional activity), the Suv39h HMTases methylate Lys9 in H3 (Me) which is proposed to generate a binding site for heterochromatin-associated proteins (not shown). Although this model centers on H3, it does not exclude H4 modifications in participating in the formation of chromosomal subdomains.

aneuploidies in the mutant cells (see Figure 2). We are currently investigating whether this compromised chromosomal stability can be correlated with an increased risk for tumorigenesis, as it has been suggested for the etiology of many human cancers. These *in vivo* data would characterise *Suv39h1* and *Suv39h2* as potential tumor-suppressor genes and suggest that *Suv39h*-mediated higher-order packaging of chromatin could provide a «protective» function for mammalian chromosomes.

The Polycomb-group gene *Ezh2* is required for early mouse development

Among the ~ 15 known Polycomb-group (Pc-G) genes, the *E(z)*-related gene family contains the evolutionarily conserved SET domain which has recently been shown to confer methyltransferase (MTase) activity (see above). Murine *E(z)* genes are encoded by two loci, *Ezh1* and *Ezh2*, of which *Ezh2* is predominantly expressed during early mouse development. Although *Ezh* proteins have not (yet) been associated with direct MTase activity, *Ezh2* is a member of a histone deacetylase (HDAC) complex. To investigate the *in vivo* function of mammalian *Ezh* genes, we disrupted both loci in the mouse germline. Whereas *Ezh1* null mice are viable, *Ezh2*-deficient mice die around day E7.5, with lethality also observed before implantation. Moreover, *Ezh2*-deficient blastocysts display impaired outgrowth potential, preventing the establishment of *Ezh2* null ES cells. Together, these data define an essential role for *Ezh2* during mouse embryogenesis and phenotypically link *Ezh2* with *eed* and *YY1*, the only other Pc-G genes that are crucial for early mouse development.

Figure 2: Increased aneuploidies in *Suv39h* double null PMEFs. Karyotype analysis of passage 3 primary mouse fibroblasts (PMEFs) from wild-type (wt; n = 40) and *Suv39h* double null cells, indicating a hyper-tetraploid (n = 82) set of mouse chromosomes.





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Asymmetric cell division in *Drosophila*

*While most cell divisions are symmetric and produce two identical daughter cells, some cells can divide asymmetrically into two different daughter cells. Such asymmetric cell divisions can be generated by the segregation of protein determinants into one daughter cell, which then make this cell different from its sister. We are using the fruitfly *Drosophila melanogaster* as a model system to understand how determinants can be segregated during mitosis and how asymmetric cell divisions are coordinated with the general body plan to ensure that the right cell types are generated at the correct positions.*

In *Drosophila*, asymmetric cell divisions are involved in the development of both the central and 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 (Figure 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 determinant during the development of the *Drosophila* nervous system.

Correct asymmetric segregation of Numb requires the protein Inscuteable. Like Numb, Inscuteable is asymmetrically localized in dividing neural precursor cells. However, Inscuteable localizes already in interphase -before Numb- and it localizes to the opposite side of the cell (Figure 1C). In the absence of Inscuteable, Numb either fails to localize asymmetrically or the Numb crescents form at random positions around the cell (Figure 1D). Inscuteable is also required for the correct orientation of the mitotic spindle, which determines the division plane. Neuroblasts which normally divide along the apical-basal axis (Figure 1D) divide with random division planes in *inscuteable* mutants. Conversely, ectopic expression

of Inscuteable in epithelial cells which normally divide parallel to the epithelial surface leads to reorientation of the mitotic spindle and cell division perpendicularly to the surface. Thus, Inscuteable directs and coordinates several events during asymmetric cell divisions. Over the past year, we could show that a complex containing the PDZ domain proteins Bazooka and DmPar-6 directs the apical localization of Inscuteable (Markus Schober, Matthias Schaefer, Mark Petronczki). Neuroblasts, the precursors of the fly CNS, arise from polarized epithelial cells. In these epithelial cells, the PDZ domain proteins Bazooka and DmPar-6 localize to the apical cell cortex (asterisks in Figure 2A) and are required for apical-basal polarity. When neuroblasts delaminate, these proteins remain apically localized (arrowhead in Figure 2A). Inscuteable binds to Bazooka and in neuroblasts, both Bazooka and DmPar-6 are required for the apical localization of Inscuteable. Thus, Bazooka and DmPar-6 cooperate in translating epithelial polarity into asymmetry during neuroblast division (Figure 2B) and to make sure that neuroblasts always divide along the apical-basal axis. To determine how apically localized Inscuteable protein orients asymmetric cell division, we have used preparative immunoprecipitation and mass-spectroscopy (Matthias Schaefer in collaboration with Anna and Andrej Shevchenko, EMBL Heidelberg) to iden-

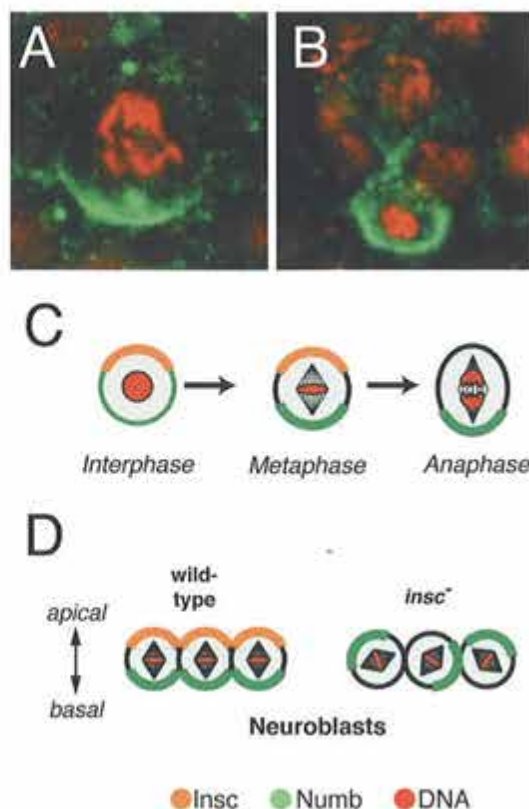


Figure 1: Asymmetric cell division in *Drosophila* neuroblasts. (A, B) Numb protein (green), DNA (red) and centrosomes (green, cen) in dividing *Drosophila* neuroblasts. Numb localizes asymmetrically in anaphase cells (A) and segregates into one daughter cell in telophase (B). (C) While Numb localization (green) occurs in metaphase, Inscuteable (orange) is already asymmetrically localized in interphase neuroblasts. (D) In wild-type neuroblasts, the mitotic spindle is oriented along the apical basal axis. Numb forms a basal crescent, whereas the Inscuteable crescent is located apically. In *inscutable* mutants, spindle orientation and Numb localization become random and are no longer coordinated.

tify the Inscuteable binding protein Pins (Partner of Inscuteable). Pins colocalizes with Inscuteable in neuroblasts (Fig. 2A, B) and Inscuteable is required and sufficient for Pins localization. Pins mutants have an Inscuteable-like phenotype suggesting that Inscuteable functions – at least in part – through Pins. Pins contains three so-called GoLoco domains which bind heterotrimeric G-proteins and indeed, we also find a heterotrimeric G-protein alpha subunit in a complex with Inscuteable. Heterotrimeric G-proteins have been implicated in cell polarity and vertebrate homologs of Pins can activate heterotrimeric G-protein signalling. We are currently testing the exciting hypothesis that Inscuteable functions by activating heterotrimeric G-protein signalling at the apical (but not basal) neuroblast cell cortex (Matthias Schaefer, Mark Petronczki, Daniela Dorner). 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. All complementation groups are mapped to narrow intervals in the genome and we are in the process of cloning and characterising some of them. Taken together, we believe that these experiments will ultimately tell us how the two daughter cells of asymmetric cell divisions can become different.

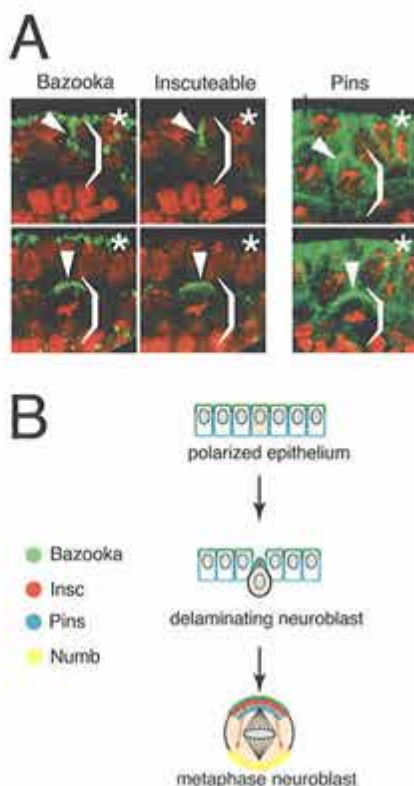


Figure 2: (A) Localization of Bazooka (green, left panels), Inscuteable (green, middle panels) and Pins (green, right panels) in epithelial cells (asterisks) and neuroblasts (arrowheads). Epithelial cells express Bazooka and Pins, but not Inscuteable. In contrast to Bazooka, Pins is not asymmetrically localized in epithelial cells. In delaminating neuroblasts (top panels) Bazooka, Pins and Inscuteable colocalize in a stalk that is left behind in the epithelial cell layer. In metaphase neuroblasts (bottom panels), all three proteins colocalize at the apical cell cortex. (B) Graphical representation of the protein localization data shown in (A). Inscuteable (red) forms a molecular link between Bazooka (green) and Pins (blue) and recruits Pins to the apical cell cortex to orient asymmetric cell divisions in neuroblasts, and ensure the proper segregation of Numb (yellow) into the basal daughter cell.



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Chromosome segregation during mitosis and meiosis

The simultaneous separation of 46 pairs of sister chromatids at the metaphase to anaphase transition is one of the most dramatic events of the human cell cycle. Even as long ago 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.

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. 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 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 (also known as Esp1p or separin) induces the proteolytic cleavage of the Scc1p cohesin 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 separin 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.

Loss of sister chromatid cohesion along chromosome arms is essential for chromosome segregation during meiosis I. Meanwhile, however, 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 (see Fig. 2) and are currently studying how Rec8 in the vicinity of centromeres is protected from separase until the second meiotic division. Lastly, we have identified, using a «functional genomics» screen, 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) (see Fig. 3). Our next goal is to identify Mam1 homologues in other eukaryotic organisms and to study the molecular mechanism of mono-orientation.

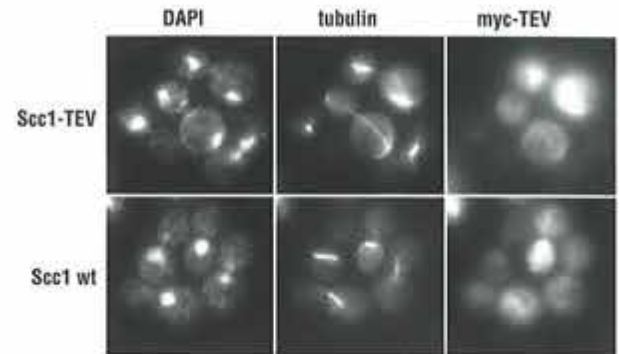


Figure 1: Triggering the segregation of sister chromatids using the foreign TEV protease. The top panels show DNA stained by DAPI, microtubules stained with tubulin-specific antibodies, and TEV expression using antibodies specific for a myc tag in cells that express a version of Scc1 in which one of 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.

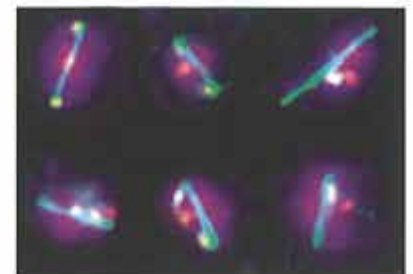


Figure 2: Rec8 cleavage is required for the resolution of chiasmata and for the first meiotic division. Shown are DNA (blue), spindles (green), centromeres (yellow) and telomeres (red) in cells that express non-degradable Rec8. Centromeres are pulled to the poles but persistent sister chromatid cohesion in chromosome arms prevents the resolution of chiasmata. As a consequence, telomeric sequences from maternal and paternal chromosomes cannot be separated from each other.

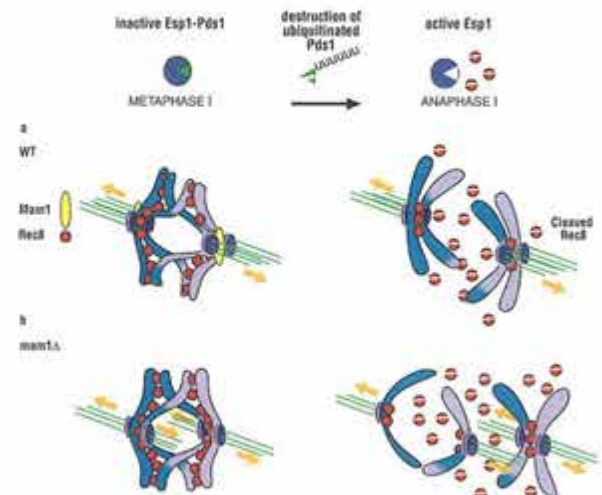


Figure 3: A meiosis-specific protein called Mam1 is necessary for mono-orientation of sister kinetochores. In Mam1's absence, sister kinetochores bi-orient and attach to microtubules from opposite poles. However, cohesion persisting around centromeres prevents sister chromatid separation.



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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 interactions between the epithelium covering these processes and the underlying mesenchyme. How the areas of mesenchymal outgrowth are established and how the early facial region is patterned is not well understood and is at the focus of our interests.

The function of the nasal placodes for facial development

Through microsurgical experiments in which one of the nasal placodes of a chicken embryo was removed or transplanted into other areas of the face *in ovo* we have found that the nasal placodes are required for normal development of the facial mesenchyme. When a nasal placode was removed, the nasal capsule and the nasal bone failed to form on the operated side, whereas an ectopic nasal capsule formed around transplanted placodes. In addition, mouse embryos homozygous for a point mutation in the gene encoding the paired box transcription factor Pax6 (*Small eye* mutants), that fail to form a nasal placode, also lack

nasal capsules and nasal bones at later stages of development. Molecular analysis revealed that one of the functions of the nasal placode is the induction of signalling molecules in the surrounding ectoderm. This induction does not require the underlying mesenchyme and is mediated by a diffusible signal from the placode. The molecular identification of this signal is one of our long-term goals.

FGF8 function during facial development.

Fibroblast growth factor 8 (Fgf8) is one of the genes regulated by the nasal placode signal. *Fgf8* is widely expressed in the ectoderm covering the midfacial area at early stages but becomes restricted to a horseshoe shaped domain of expression around the nasal placodes at later stages of development. Mouse embryos in which this gene has been inactivated in the facial region develop severe facial defects (Fig. 2). Such embryos display midfacial clefts and most derivatives of the first branchial arch are severely reduced or absent. Defects first become morphologically apparent around E9.0 as a reduction in the size of the facial primordia. This size reduction can at least in part be accounted for by a dramatic increase in cell death in early facial mesenchyme in the absence of *Fgf8* expression in the overlying surface ectoderm. FGF8 therefore seems to act as a survival factor for early facial mesenchyme. In addition, patterning in the remaining tissue is affected as judged

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.

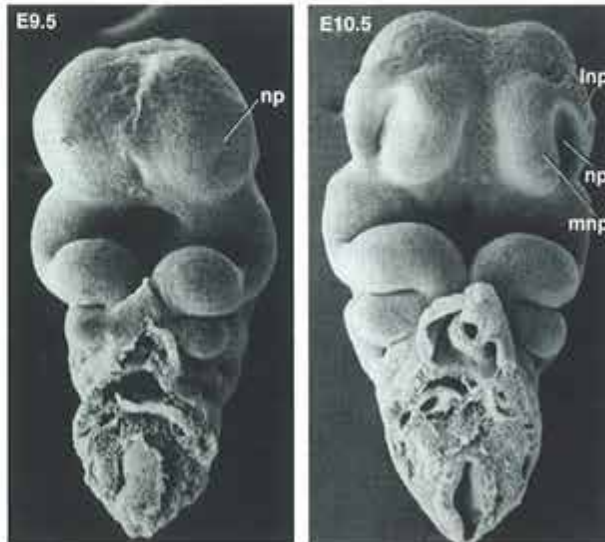


Figure 2 : Tissue specific inactivation of *Fgf8* in the facial area results in severe facial defects

The face of a wildtype (A) and an *Fgf8* mutant embryo (B) at E16.5. Embryos in which *Fgf8* has been inactivated in the facial area develop a midfacial cleft and show a severe reduction of the lower jaw and peri-ocular tissue.

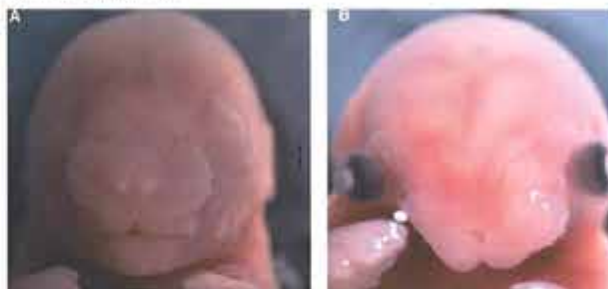


Figure 3 : *Tbx2* is a downstream target of FGF signalling in the facial mesenchyme.

Tbx2 expression in facial explants consisting of ectoderm and mesenchyme (A), mesenchyme (B), and mesenchyme cultured in the presence of beads soaked in recombinant FGF8 (C) after 24 hours of culture *in vitro*. *Tbx2* expression is maintained in the presence of the FGF8 producing ectoderm (A) but is lost in mesenchyme culture in isolation (B) and is induced in a halo around beads soaked in recombinant FGF8 protein (C).



by the analysis of the expression of marker genes. However, six other members of the FGF family are expressed in partially overlapping domains with *Fgf8* during craniofacial development and may serve redundant functions. In order to further characterize 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-1, 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 so far shown that FGF signalling induces the expression of the transcription factors *Pax3*, *Tbx2*, *Erm* and *Pea3* in facial mesenchyme (Fig. 3). 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 and we are currently analyzing clones that show differential hybridization. We hope that the characterization of the genes induced by FGF signalling will ultimately help to understand the function of FGF signalling for facial development on a molecular level.



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Regulation of mitosis by the anaphase-promoting complex

The propagation of genetic information during cell proliferation requires the accurate replication and subsequent segregation of chromosomal DNA. Both of these events are controlled by ubiquitin-dependent proteolysis, a regulatory mechanism that is ideally suited to generate directionality in the cell cycle due to the irreversible nature of protein degradation. We are studying how ubiquitin-dependent proteolysis mediated by the anaphase-promoting complex controls the separation of sister chromatids in anaphase and the subsequent exit from 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. High fidelity and proper timing of this event are essential to ensure equal segregation of the duplicated genome to the forming daughter cells and are thus required to maintain genomic stability during cell proliferation. Defects in sister chromatid separation can cause aneuploidy and may contribute to human diseases such as congenital trisomies and cancer.

In presumably all eukaryotes from yeast to man, sister chromatid separation is initiated by activation of a multi-subunit ubiquitin-protein ligase, called the anaphase-promoting complex (APC) or cyclosome. We first discovered the APC as the cell cycle-regulated component of an enzymatic pathway 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 ubiquitinate numerous other mitotic proteins, including several protein kinases, spindle-associated proteins, inhibitors of DNA replication and its own activator CDC20/Fizzy. We are using *Xenopus* eggs and human cells to address the following questions:

How is the activity of the APC regulated?

The vertebrate APC is composed of at least eleven subunits that are part of the complex throughout the

cell cycle, whereas two activator proteins, called CDC20 and CDH1, bind to the APC in a cell cycle-regulated manner and thereby activate it specifically in mitosis and G1. We have shown that CDC20 can only bind to the mitotically phosphorylated APC, explaining why the activity of APC^{CDC20} is restricted to mitotic cells. In contrast, the interaction of CDH1 with the APC is inhibited by phosphorylation of CDH1 during S and G2 phase and most of mitosis, restricting the existence of APC^{CDH1} complexes to the G1 phase. These antagonistic effects of phosphorylation reactions on APC^{CDC20} and APC^{CDH1} help to explain the temporal order of APC activation by CDC20 and CDH1. Earlier work in yeast suggested that CDC20 and CDH1 may target different sets of substrates to the APC. To test this hypothesis we compared the ability of APC^{CDC20} and APC^{CDH1} to ubiquitinate eight different substrates *in vitro*. We found that APC^{CDC20} can efficiently ubiquitinate most and APC^{CDH1} all substrates tested. In vertebrates, CDC20 and CDH1 may therefore predominantly differ in their temporal regulation but only partly in their ability to recognize different substrates.

What is the mechanism of the APC?

It is unclear how the APC mediates the assembly of multiubiquitin chains on substrate proteins and why it requires about a dozen subunits to catalyze these

reactions. To address this problem we first reinvestigated the subunit composition of the human APC. In addition to eight subunits that were originally described we identified three additional subunits, called APC10/DOC1, CDC26 and APC11 (orthologs of which had previously been found in yeast). By expressing individual APC subunits in *E. coli* we discovered that the 10 kDa RING-H2 finger protein APC11 alone is sufficient to mediate the ubiquitination of APC substrates, although this subunit represents only about 1% of the total mass of the holo-APC. APC11 may therefore play a central part in the catalysis of ubiquitination reactions mediated by holo-APC, whereas the majority of other APC subunits may have yet uncharacterized structural or regulatory roles.

How does the APC initiate anaphase?

In budding yeast APC^{CDC20} initiates anaphase by mediating the ubiquitin-dependent proteolysis of the securin Pds1p, a protein that inhibits the separase (separin) Esp1p. Following Pds1p proteolysis, Esp1p removes a cohesin complex from chromosomes by cleaving its subunit Scc1p/Mcd1p, thereby presumably liberating sister chromatids for poleward movement in anaphase (see report of Kim Nasmyth's group). It has remained unclear how activation of APC^{CDC20} initiates anaphase in other eukaryotes because in vertebrates the cohesin complex dissociates from chromatin already during prophase, i.e. long before sisters separate. By reconstituting this process in mitotic *Xenopus* extracts we found that most cohesin

Figure 1: When cells are arrested in metaphase by drug treatment, sister chromatid arms separate but sisters remain attached at centromeres. This immunofluorescence micrograph shows that in such cells a tagged version of the cohesin subunit SCC1 (in red) can be detected at centromeres but not on chromosome arms. Kinetochores, flanking the centromeric region, were labelled with human CREST serum (in green) and DNA is shown in blue.

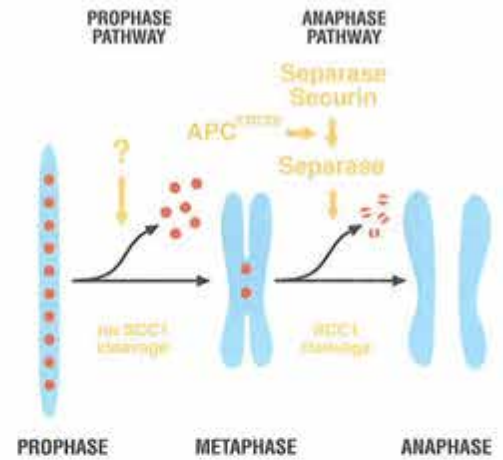
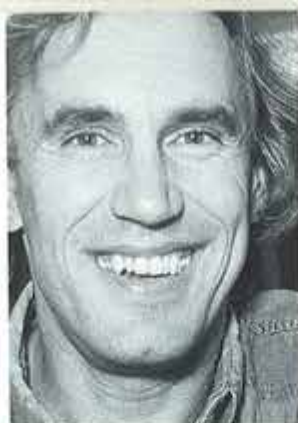


Figure 2: A two-step model for the sequential loss of sister chromatid cohesion in animal cells. The bulk of cohesin (red dots) is removed from condensing chromosomes during prophase by an APC-independent pathway which might involve mitotic kinases. APC^{CDC20} then initiates anaphase by activating separase through ubiquitin-dependent proteolysis of the separase inhibitor securin. Once activated, separase cleaves residual cohesin complexes that remain at centromeres and may thereby liberate sister chromatids for poleward movement in anaphase.

complexes dissociate from chromatin in a reaction that does not depend on the APC and does not involve cleavage of SCC1, the ortholog of yeast Scc1/Mcd1. To address how sister chromatid cohesion is maintained between prophase and anaphase we reinvestigated the subcellular distribution of SCC1 in human mitotic cells. We observed that a small amount of SCC1 remains associated with metaphase chromosomes where it is preferentially located at centromeres until it disappears in anaphase. We further discovered that a similarly small amount of SCC1 is cleaved *in vivo*, specifically in anaphase, but not in pro- or metaphase. Reconstitution of this reaction *in vitro* showed that immunopurified human separase is sufficient to cleave the SCC1 subunit of purified cohesin, provided that separase is activated by APC-dependent proteolysis of its bound inhibitor securin. Our results suggest that vertebrate cohesin complexes are regulated by two different pathways that affect cohesin in a spatially and temporally distinct manner: in prophase a cleavage-independent pathway removes the bulk of cohesin from the arms of condensing chromosomes, whereas at the metaphase-anaphase transition an APC- and separase-dependent pathway removes centromere-bound cohesin complexes by cleaving their subunit SCC1. In the future we will analyze the roles of these pathways in chromosome condensation and sister chromatid separation.



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⁵ since June 2000; ⁶ since Oct. 2000; ⁷ since March 2000; ⁸ since July 2000;

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.

One 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 osteoblastic/chondrogenic bone tumors, whereas mice lacking *c-fos* are osteopetrotic due to a differentiation block in bone resorbing osteoclasts. The Fos-related protein Fra-1, itself a c-Fos target gene, is essential for mouse development (Fig. 1). 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. Interestingly, gene replacement of *c-fos* by *fra-1* using a knock-in approach showed functional equivalence of these two proteins. To better understand the mechanisms by which c-Fos and Fra-1 control osteoblast and osteoclast differentiation, we have been employing cDNA microarray technology to identify common and specific target genes.

c-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 *c-jun* in the liver of adult mice showed that c-Jun is dispensable for postnatal liver function, but essential for liver regene-

ration. Chondrocyte-specific inactivation using a collagen2a1-cre transgene results in severe scoliosis caused by failure of intervertebral disc formation and abnormal vertebral arch development, suggesting that c-Jun is a novel regulator of sklerotomal differentiation. Interestingly, gene replacement strategies indicated that JunB substitutes for c-Jun during embryonic development but not during adulthood. This rescue experiment implies that JunB overexpression can fully restore AP-1-dependent transcriptional regulation, which is impaired in mice lacking *c-jun*. An important mechanism regulating c-Jun activity is phosphorylation of c-Jun at serine 63 and 73 within its N-terminus through the c-Jun amino-terminal kinases (JNKs). To study the function of JNK signaling *in vivo* we have generated null mutations in the *jnk1* and *jnk2* genes (in collaboration with M. Karin) and mice carrying a *c-jun* allele mutated in the JNK phosphoacceptor sites (*junAA*). *Jnk1*^{-/-}, *jnk2*^{-/-} and *junAA* mice are healthy and fertile, but the absence 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 due to deregulated apoptosis and *jnk2*^{-/-} and *junAA* thymocytes are resistant to CD3-induced apoptosis. Moreover, *Jnk1* and c-Jun phosphorylation appear to be required for effi-

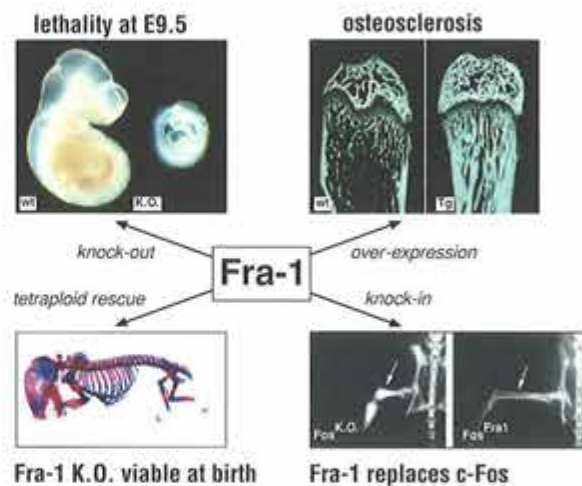


Figure 1: Functional analysis of Fra-1

cient 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

The analysis of cells derived from transgenic mice overexpressing JunB demonstrated that 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 recently found that the absence of JunB expression in the myeloid lineage results in a transplantable myeloproliferative disease, which eventually progresses to blast crisis thereby resembling human chronic myeloid leukemia. 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 (Fig. 2).

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

The VEGF/Fik-1 signalling system is essential for the development of endothelial and hematopoietic cells.

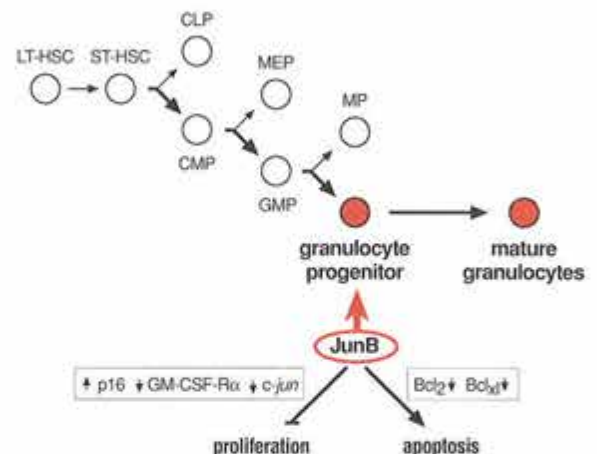


Figure 2: Model of JunB functions during myeloid differentiation

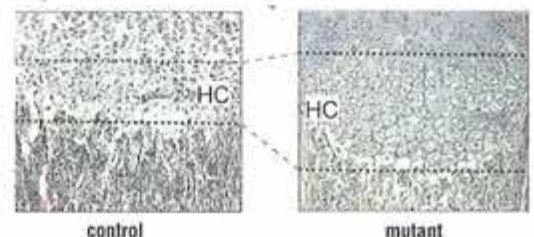


Figure 3: The role of VEGF in the growth plate of bone

A conditional allele of Fik-1 was generated to test its role in adult mice and in tumor angiogenesis following its deletion with an endothelial-specific Tie-2 CreER transgenic line. The functional importance of VEGF-A in developing chondrogenic tissues was analysed with a conditional allele using collagen2a1-Cre transgenic lines. Deletion of a single VEGF-A allele results in embryonic lethality at E10.5, whereas surviving E17.5 mutant mice show aberrant endochondral bone formation (Fig. 3) and develop a heart phenotype.

Finally, conditional and mutated EGF-Receptor alleles were used to study the role of EGF-R in skin tumor development (in collaboration with M. Sibilio, Univ. Vienna). We found that EGF-R plays a central role as a survival factor in oncogenic transformation.



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Gabriele BOTTO | Technician Media Kitchen

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Ulrike WINDHOLZ | Technician Media Kitchen (since June 2000)

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

Oligonucleotide synthesis

We started in 1988 with about 500 DNA oligonucleotides and last year we prepared 6100 with an average size of approximately 30 bases. From July until December last year the Nasmyth group did many mutation/gene disruption experiments. For this reason, up to 25% of the synthesized oligonucleotides were about 70 bases long.

In the first 9 months this year we again produced more than 4100 oligos, an increase of almost 100% compared to 1997.

Production of antibodies

The production of monoclonal antibodies in collaboration with IMP groups and organizing the production

of polyclonal antibodies with an outside company is of increasing importance and needs part of our capacity. We also continued, to a lesser extent, to isolate and HPLC purify monoclonal antibodies from established hybridoma cell lines.

Peptide synthesis and mass spectroscopy are no longer part of the service department group.

Sequencing and DNA isolation

With the two ABI PRISM 377 DNA sequencers, we sequenced approximately 16000 samples in the first 9 months of this year (an increase of 100 % compared to 1998) with an average reading length of 700-900 base pairs for »good« DNA. We are saving time by using an easy and fast clean-up protocol using small Sephadex columns on 96-well microtiter plate format. Since the full sequence information of several scientifically important organisms is available, much more screening experiments are going on. Thus, we will need a reliable and even faster sequencing capacity. We plan to develop capillary sequencing capacity for PCR products (e.g. with ABI PRISM 3100, 3700 or Pharmacia MegaBace) in the next months.

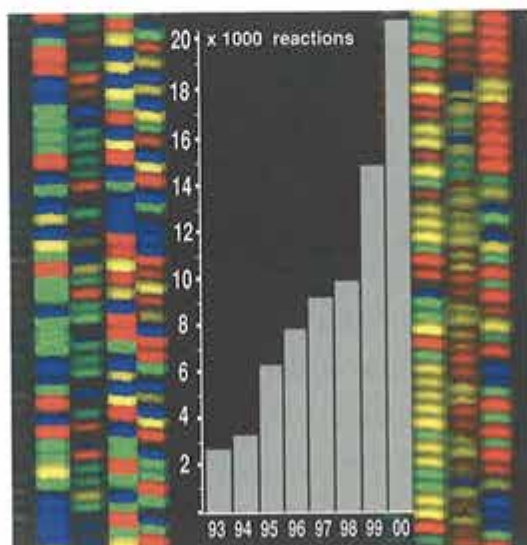


Figure 1: A sequencing run on an ABI 377 PRISM and number of reactions done with dye deoxy terminators (scale 0 to 20'000) in the years 1993 to 2000.



Peter STEINLEIN | Staff Scientist

Iris GRÜNERT | Postdoc (Electron microscopy)

Sebastian CAROTTA | PhD Student (joint project with H. Beug)

Herbert AUER | Technician (Micro-arrays)

Karin PAIHA | Technician (Flow Cytometry, Microscopy and Imaging)

BioOptics Department

In late 1999, our department extended its services by setting up a micro-array facility for production of cDNA chips. Initially, we will focus on the production and analysis of arrays of murine genes using either commercially available clones representing known genes or clones from cDNA-libraries produced by researchers at the IMP. Currently, the main application of micro-arrays is the search for target genes. Having established more advanced image analysis and statistical methods, comparative analyses (e.g. time courses) will be offered.

Flow cytometry

During the past year, we still noticed a steady increase in demand for flow cytometric applications covering apoptosis assays, analysis of cell cycle, multicolor analyses and sorting of antibody-stained and/or fluorescent protein expressing cells.

Microscopy and image analysis

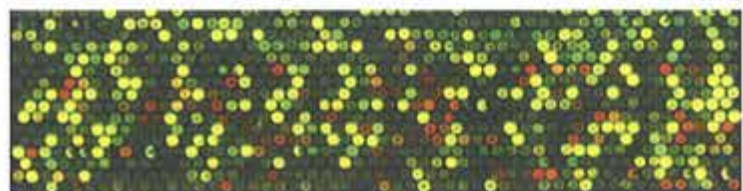
To satisfy the increasing demand for laser scanning microscopy, an additional laser scanning microscope (Zeiss LSM 510) was set up allowing high resolution, multidimensional imaging. Two existing, motorized fluorescence microscopes were equipped with high speed, high resolution CCD-cameras and adapted for the automated acquisition of multidimensional, time-lapse image series of living cells. To keep track of the dramatically increasing amount of image data (>350 Gbyte in 2000 vs. ~100 Gbyte in 1999), databases for the users, as well as a backup service, have been established.

Micro-arrays

In November 1999, a facility for the production of cDNA-Micro-arrays was installed and became opera-

tional in January 2000. The equipment consists of a micro-array printer and scanner (Promedia Associates, Larchmont, NY) allowing the production of up to 230 arrays per batch each having >40.000 features. The scanner is a laser-based system scanning two wavelengths simultaneously at a resolution of 10 µm. Currently, our clone stocks consist of >6000 sequence verified clones from the I.M.A.G.E. consortium, >11000 clones from the Brain Molecular Anatomy Project (both purchased from Research Genetics, Groningen, NL) and >45000 clones from different cDNA-libraries produced at the IMP. The service comprises storage, amplification and spotting of clones as well as scanning and analysis of hybridized arrays. Together with Gerald Löffler (IMP Bioinformatics Department) a micro-array information management system was set up to monitor all steps involved in the logistics and management of clones, PCR-products, printed and hybridized micro-arrays and data thereof. In the future, this system will also include more advanced statistical analysis methods (in collaboration with Susanna Lüdemann, IMP Bioinformatics Department).

Figure 1: Detail of the 25k-micro-array hybridized with RNA from Pax5^{-/-} B-cells (red) and wild-type B-cells (green) (Data provided by Alexandra Schebesta, Group Busslinger).





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Animal house

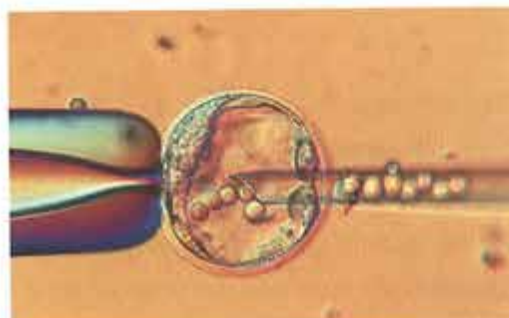
The animal house group provides husbandry of animals and services for the various research groups:

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 held. 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, 21 standard strains are routinely bred in-house. In addition, eighty eggs are produced each week for specified projects from our »closed-colony« chicken flocks.

Veterinary services, such as monitoring of the facility's health-status (sentinel-program etc.), experimental procedures in animals such as collection of blood, implantation of tumor cells and administration of other substances by various routes, e.g. intravenous, intraperitoneal and subcutaneous 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.



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 the transfer of »clean« embryos into our animal house, the freezing of embryos for 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 coordinates the duties. At present it is chaired by Erwin Wagner.



Karl MECHTLER | Head of Facility

Jan-Michael PETERS | Scientific Coordinator

Irmina GORNY | Technician (50%) employed by Intercell

The main activities of the Protein Chemistry Facility at the IMP are to synthesize peptides, purify antibodies and analyze proteins by mass spectrometry.

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.

At the IMP we established a new mass spectrometry facility at the beginning of the year 2000. This facility presently uses a matrix-assisted laser desorption ionization (MALDI-TOF) and electrospray quadrupole time of flight (ESI-Q-TOF) instruments. In the future, both of these instruments will be located in the new Intercell building (Rennweg 95b) and will be operated together with the company Intercell and with the Institutes of the Vienna Biocenter.

Mass Spectrometry

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 to identify even 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 Nano-spray approach on a Q-TOF machine.

Posttranslational modifications and very heterogeneous samples are analyzed by Nano-HPLC coupled to an Ion Trap Mass Spectrometer adapted with a Nano-spray source.

To avoid common keratin contamination problems we developed a modified protocol for the preparation of Coomassie blue-stained samples.

Peptide Synthesis and antibody purification

We are synthesizing about 200 peptides per year, including an increasing number of peptides bearing acetylated, phosphorylated or methylated amino acid residues. We further developed a new protocol for the affinity purification of acid-sensitive peptide antibodies at high flow rates up to 7.5 ml/min.

Life at the IMP continues to be dynamic and challenging. As new groups and facilities are set up, labs and offices are being restructured or relocated. A constant turnover of the scientific staff is achieved by assigning temporary contracts to Group Leaders, Postdocs, PhD- and Diploma Students. At the beginning of the year, employees working at the IMP totalled 175 plus 9 scientists with external grants.

Apart from being a very young institute – the average age is 35 years – the IMP is also truly international in character. Today, more than 25 nationalities are represented at our institute, the working language is English. An international PhD-Program appeals to students from all continents. Every year, the program is advertised in a leading journal, prompting numerous applications. Following a competitive selection process and several interviews, about 10 to 15 students are finally accepted into the program annually. The PhD-Program is carried out jointly with the University of Vienna, which has had close ties to the IMP right from the start. Since 1992, five institutes of the Faculties of Science and Medicine have been located in a neighbouring building. Together with the IMP, they constitute the »Vienna Bio Center«, which has become recognised as a center of excellence in the biological sciences and a role model for other academic institutions in Austria. By bringing the two institutions together, a »critical mass« of resources has been generated which, in turn, has led to a number of further spin-off projects. In an ambitious effort, with the support of the city of Vienna and the Austrian government, the IMP's neighbourhood is rapidly developing into a veritable biotech-campus, combining university- and industry-based institutes, start-up companies and a new branch of the Austrian Academy of Sciences. This »Institute of Molecular and Cellular Bioinformatics (IMBA)« will be set up next to the IMP, building upon the IMP's expertise and complementing its research. The two units will collaborate under the name »IMP/IMBA Genome Research Center«. To design the new building, an international architecture competition was held. In September, the winner Boris Podrecca presented his design to the public. Construction on the new institute will start in 2001, its completion is expected to be in the year 2003.





Interaction and communication are key elements in establishing successful research structures. They are highly encouraged, both on an informal basis and as organized events. On the social side, employees meet over lunch at the IMP's cafeteria, relax at monthly »beer hours«, play soccer or volleyball together, enjoy occasional parties in »Joe's Disco«, organize lab-outings to the nearby Alps and go on an annual skiing-trip.

Among the scientific events, the »IMP Spring Conference« is the largest and most ambitious. In 2000, it was held for the 11th time under the title »From Development to Molecular Medicine«. In the future, the conference will take place every other year, starting from 2002.

Throughout the year, the IMP hosts a series of »Thursday seminars« and »Impromptu seminars«, where top-ranking scientists are invited to lecture on their latest findings. These seminars, which are also open to the public, generate lively interactions and introduce the IMP-scientists to other fields of research.

On Mondays, the IMP's own progress is presented and discussed. Students are expected to prepare a seminar once a year and expose their work to the critical minds of their colleagues. Weekly lab-meetings, a journal club and the advice of the PhD committee ensure regular interaction and constant monitoring of students' progress. After three to four years of work and the completion of a thesis, students graduate with a PhD from the University of Vienna. With the experience they have gathered, the international contacts they have established and their first papers published, they are well equipped for a career in science.





▲ The architect's vision of the new IMBA building adjacent ►
to the IMP (by courtesy of architect Boris Podrecca)



▲ IMP scientists take time off from lab work to savour the fresh mountain air.



▲ The annual IMP Christmas Party



Poster discussion at the IMP recess meeting ▲



▲ The IMP Spring Conference 2000 – artistically presented (poster by Matt Cotten)

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seminar speakers at the IMP 2000

January

20. THOMAS BÖHM – *MPI Freiburg*

February

04. MALCOLM BRENNER – *Center for Cell and Gene Therapy, Houston*
 07. GARY RUVKUN – *Mass. General Hospital, Boston*
 17. BEN SCHERES – *Univ. Utrecht*
 22. RICHARD STANLEY – *Albert Einstein College of Medicine, NY*
 23. SHAMIL SUNYAEV – *EMBL*
 24. CHRISTIAN STEINKÜHLER – *IRBM, Pomezia*
 25. VLADIMIR BOTCHKAREV – *Boston Univ.*
 25. DAVID NAOR – *Lautenberg Center, Jerusalem*

March

02. CHRIS PONTING – *MRC, Oxford*
 03. MICHAEL J. DUNN – *Harefield Hospital*
 07. GARETH WILLIAMS – *Cambridge Univ.*
 09. CARL-HENRIK HELDIN – *Ludwig Inst. Cancer Res., Uppsala*
 16. LAURIE GLIMCHER – *Harvard School of Public Health, Boston*
 27. PIERRE A. COULOMBE – *Johns Hopkins Univ., Baltimore*
 28. MIGUEL ANDRADE – *EMBL*
 29. SAKAE TANAKA – *Univ. Tokyo*
 30. WENDY BICKMORE – *MRC, Edinburgh*

April

05. TATSUO SUDA – *Showa University, Tokyo*
 07. JONAS FRISEN – *Karolinska Institute*
 12. WALTER GEHRING – *Biozentrum Basel*
 13. MIKLOS CSERZO – *Univ. of Birmingham*
 17. HARALD HUTTER – *MPI Heidelberg*
 26. SOPHIE LELIÈVRE – *Lawrence Berkeley Lab.*
 27. HANS BOHNERT – *Univ. Arizona, Tucson*
 28. ANDREW MATUS – *Friedrich Miescher Inst., Basel*

May

04. STEVE WEST – *ICRF*
 05. JEAN-PIERE CHERVET – *LC Packings, Amsterdam*
 08. ALEXANDER TARAKHOVSKY – *Univ. Cologne*
 18. PAUL WASSARMAN – *Mount Sinai Medical Center, NY*
 19. MANUEL SERRANO – *Nat. Center of Biotechn., Madrid*
 23. DANIEL SUTER – *Yale University*
 24. BENEDIKT BERNINGER – *UC San Diego*
 25. ED PALMER – *Basel Institute of Immunology*
 26. BERNHARD HERRMANN – *MPI Freiburg*
 29. FRANCOIS SCHWEISGUTH – *Ecole Normale Supérieure, Paris*
 30. CATHRIN BRISKEN – *Whitehead Institute*
 31. HIDDE PLOEGH – *Harvard Medical School*

June

06. ANDREAS IVESSA – *Princeton University*
 08. NEIL BROCKDORFF – *Hammersmith Hospital*
 09. WOLFRAM BODE – *MPI, Martinsried*
 14. JONATHAN PINES – *CRC, Cambridge*
 15. ULRIKE HEBERLEIN – *UCSF*
 16. THOMAS JENTSCH – *ZMNH, Hamburg*
 20. CAROLINE HILL – *ICRF*
 23. CESAR COBALEDA – *Univ. Salamanca*
 30. LAZLO TORA – *IBGMC, Strasbourg*

seminar speakers at the IMP 2000

July

- 03. JO MILNER – *Univ. of York*
- 07. DAVID TALMAGE – *Columbia Univ.*
- 13. ANNE RIDLEY – *Ludwig Inst. f. Cancer Research, London*
- 25. HANSGEORG SCHINDLER – *Inst. Biophysik, Linz*
- 27. JAN LOWE – *MRC, Cambridge*
- 27. MICHAEL WEBER – *Univ. Virginia, Charlottesville*
- 28. THOMAS BRANDT – *TU Braunschweig*

August

- 02. DAVID L.VAN VACTOR – *Harvard Medical School*
- 10. BRIAN BURKE – *Univ. Calgary*
- 11. ARIE OTTE – *Univ. Amsterdam*
- 17. HANS VAN DAM – *Leiden Univ.*
- 31. PETER DUESBERG – *UC Berkeley*

September

- 07. MARKUS AEBI – *ETH Zurich*
- 14. ANTONIUS ROLINK – *Basel Inst. of Immunology*
- 15. PIER PAOLO DI FIORE – *Europ. Inst. of Oncology, Milan*
- 19. MARTIN HETZER – *EMBL*
- 21. ROBIN ALLSHIRE – *MRC, Edinburgh*
- 25. JORDAN RAFF – *Wellcome / CRC*

October

- 02. HISAO MASAI – *Tokyo Univ.*
- 12. PAMELA SILVER – *Dana Farber Cancer Inst., Boston*
- 13. LEE NISWANDER – *Sloan-Kettering Institute, NY*
- 13. GARY KARPEN – *Salk Institute*
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- 19. MAXIMILIAN MUENKE – *NIH, Bethesda*
- 20. STEPHEN COHEN – *EMBL*
- 20. ALAN COLMAN – *PPL Therapeutics*

November

- 02. MATTHEW FREEMAN – *MRC Cambridge*
- 03. WALTER GUENZBURG – *Univ. of Veterinary Sciences, Vienna*
- 03. AXEL ULLRICH – *MPI Munich*
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- 23. RONALD PLASTERK – *Netherlands Cancer Inst.*
- 24. STEPHEN NUTT – *Wellcome / CRC*
- 30. MARIANN BIENZ – *MRC Cambridge*

December

- 05. MANFRED EIGEN – *MPI Göttingen*
- 11. DAVID LIVINGSTON – *Harvard Univ.*
- 14. JEAN-PIERRE CHANGEUX – *Inst. Pasteur, Paris*

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Histological analysis of the growth plate of a murine humerus at day 17.5 of development.

The image shows the expanded hypertrophic cartilage zone in a VEGF mutant fetus.

(Picture provided by Jody Haigh and Erwin Wagner, see also Haigh et al.

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