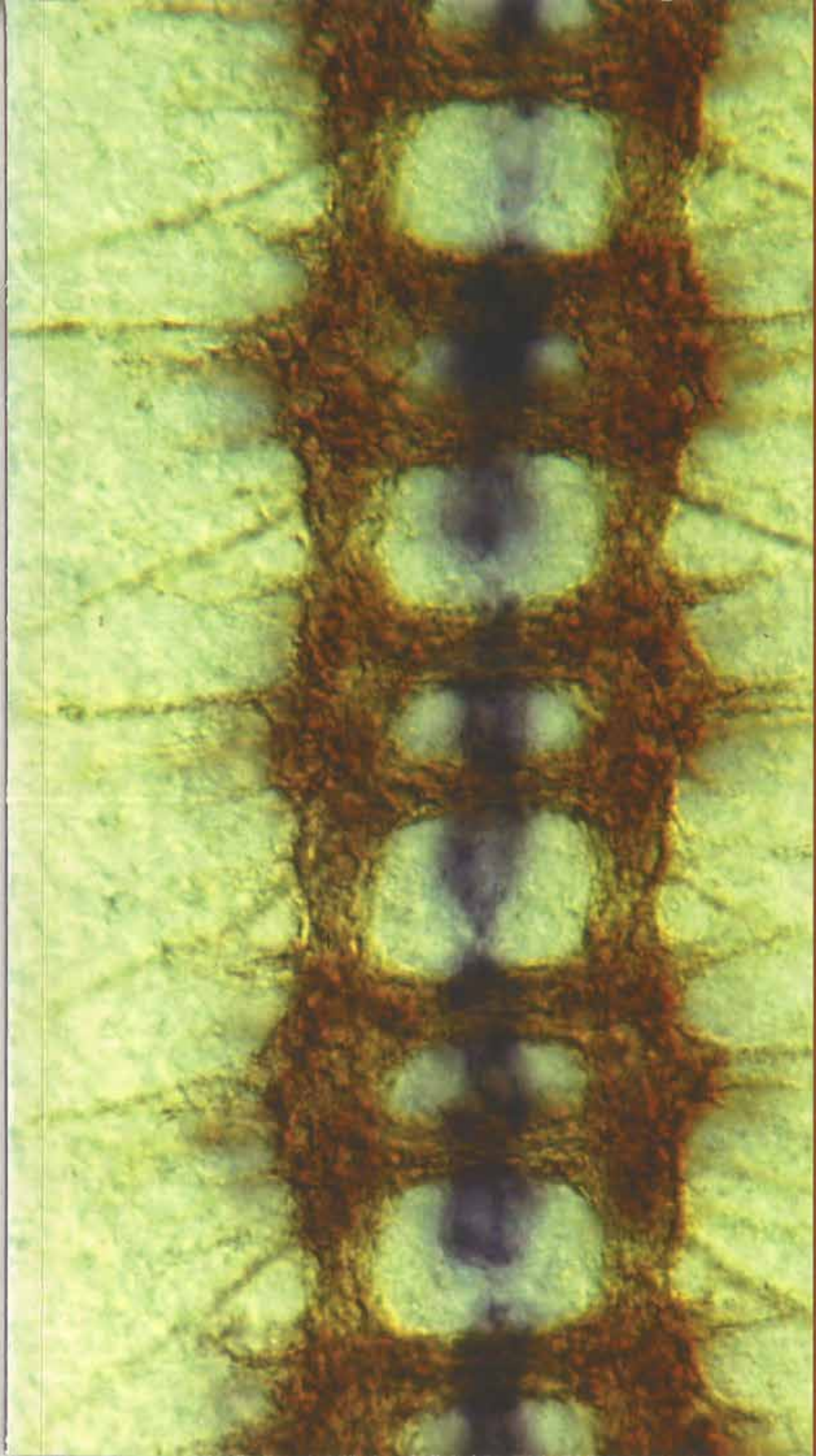

Research Institute of Molecular Pathology
VIENNA BIOCENTER

I.M.P. '98

Boehringer Ingelheim

The IMP is a basic research institute supported by the research oriented international pharmaceutical company Boehringer Ingelheim [BI]. The IMP's goals are twofold: to conduct innovative basic research and to use this experience in advising BI about potential new drug discovery programs.
The IMP was founded, planned, and subsequently directed for ten years by Max Birnstiel, who retired at the end of 1996. The institute was conceived in 1985 and opened its doors in 1988. It was joined four years later by five biological institutes of the University of Vienna, with whom the IMP shares an international PhD program and both library and lecture hall facilities. The IMP and these five university institutes now form what is known as the Vienna Biocenter. The institute contains thirteen research groups, four run by senior scientists with longterm contracts and nine run by young group leaders on shorter ones. All research at the IMP is driven by our group leaders' curiosity and not by BI's requirements. Our progress and proposed future directions are assessed annually by an international Scientific Advisory Board [SAB] whose chairman is presently Michael Bishop. The IMP has a staff of 159, among them 27 technicians, 42 PhD students [1 external], and 34 Postdocs [5 with external stipends]. More than 20 different nationalities are represented at the IMP. Our working language is English, and our annual budget [including external grants] is about US $ 19 million.

Change is a key aspect of the IMP's distinctive character. PhD students and Postdocs come and go with the seasons in most institutes, but only in some institutes do most group leaders only stay for a limited period [usually eight years in the case of the IMP]. This turnover ensures the continual flow of new blood and prevents an intellectual atherosclerosis. This year has been no exception. We are delighted to welcome two new group leaders: Barry Dickson and Annette Neubüser who heads the 'Wittgenstein' group.
Both bring totally new areas of expertise into the IMP. Barry is a Drosophila neurobiologist who studies how sensory neurons in retina make the correct connections with neurons in the brain, whereas Annette studies cranio-facial development in chicken and mice. With their arrival, Developmental biology now becomes a major focus within the IMP. There have also been several changes in our Scientific Advisory Board.
We welcome Janet Rossant and sadly said goodbye to Jeff Schatz and Tada Taniguchi. Jeff had been on the Board since the IMP's founding, whereas Tada had been with us for five years. Both have been the source of much valuable advice. We also welcome Bernd Wetzel, who replaces Christoph Hohbach.

Another major change at the IMP this year has been the establishment of a suite of offices for several group leaders on the top floor and an adjacent 'communication area'. This so-called 'glass palace' has not only enabled us to convert old offices into much needed lab space but also, by bringing together people whose offices had been scattered around the building, created a focal point for the institute. We also nearly completed the expansion of the animal house, the building of a fly kitchen and a new lab for Gotthold Schaffner's service group, all of which was masterminded by Alex Chlup, who managed simultaneously to keep the rest of the building running smoothly. Next year, we hope to improve the institute's Cafeteria.

This year also witnessed our first contact with members of the Boehringer family who collectively visited the institute and were entertained by explanations of the awesome power of genetic analysis in the worm and the mouse. The Austrian Minister of Science and Transport, Caspar Einem, also visited the IMP and we attempted to persuade him that the IMP might be a good model for future initiatives in basic research in Austria.

As head of R&D worldwide, Christoph Hohbach was largely responsible for establishing and strengthening the connections between the IMP and Boehringer. This is a process that continues to evolve. Thanks to Lukas Huber, we have initiated a series of meetings at Gmunden in Upper Austria, at which scientists from the IMP and BI exchange, in an informal and relaxing setting, ideas about potential new drug discovery projects. Furthermore, we are about to initiate a series of informal meetings every month or two with our colleagues from Bender and Biberach. Such meetings are not intended to replace attendance at each other's annual reviews but rather to provide a relaxed forum for intellectual exchange. We will miss Christoph but look forward to further developing our relationship with the company with Bernd Wetzel, his successor.

One of the scientific highlights of the year was the discovery by Anne-Karina Perl in Gerhard Christofori's lab that cell adhesion really does have an important role in blocking the transition from benign adenoma to malignant carcinoma. The diversity of subjects studied at the IMP has greatly increased in the last three years. We now have groups studying all the major model genetic systems, including yeast, worms, flies, frogs, and mice as well as strong Cell biology. Where do we go from here? It is clear that genetic analysis will continue to have an important role in identifying new players for some time to come. Genetics provides the 'opening moves' but the end game cannot be won without biochemistry and ultimately Structural biology. The possible arrival of a new department of Structual biology at the university will be a welcome addition to the Biocenter site. Another direction in which genetic studies in model systems can and should be followed up is in their application to human biology and pathology. As estimates for partial completion of the human genome creep ever nearer, a new era of Human genetics beckons. The era of wallowing around without an accurate map of the genome is drawing to a close and there will be unprecedented opportunities for mapping human traits. How and whether the Vienna Biocenter, and with it the IMP, grasps these opportunities will undoubtedly affect the job opportunities of many of the PhD students flowing out of our doors.
The founding of Intercell by Alex von Gabain and Max Birnstiel will hopefully be only the first of many biotech ventures planning to capitalize on expertise within the Vienna Biocenter. Our challenge for the future is to ensure that not only BI but also our local community best exploits the truly excellent science being conducted at the IMP.

Prof. Kim Nasmyth, F.R.S.

Vienna, December 1998

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Developmental plasticity: important for oncogenesis?

Among other functions, oncoproteins deregulate the balance between proliferation, differentiation and/or apoptosis of progenitor cells in various tissues. In our two systems [erythroleukemia and breast carcinoma], developmental plasticity of normal progenitors and abnormal behavior of respective cancer cells involve molecular players derived from the same gene families. The expansion of erythroid progenitors during stress erythropoiesis and epithelial/mesenchymal transitions during embryonic development are paradigms of normal progenitor plasticity crucial for tumorigenesis.

Expansion of erythroid progenitors during stress erythropoiesis: a process subverted in erythroleukemia?

In human, murine and avian erythroblasts, the receptor tyrosine kinase [RTK] c-Kit [using stem cell factor [SCF] as a ligand] cooperates with the erythropoietin [Epo] receptor to enhance proliferation of differentiating pro-erythroblasts and thus increase erythrocyte numbers. These two plasma membrane receptors cooperate with a member of the nuclear hormone receptor [NR] family, the glucocoorticoid receptor [GR], to induce sustained cell proliferation and differentiation arrest of erythroid progenitors. These normal cells are indistinguishable from erythroleukemic cells expressing v-ErbB and v-ErbA. The RTK-oncoprotein v-ErbB is a ligand-independent, constitutively active EGF-receptor. The NR family member v-ErbA is a mutated thyroid hormone receptor [TRα/c-ErbA] unable to bind ligand and 'frozen' in a constitutively repressing state [see report 1997].
Data from genetically modified mice show that EpoR and c-Kit are required for erythropoiesis *in vivo*. No such role was obvious for the GR. Using GR-deficient mice, we showed that the GR is essential in stress erythropoiesis, caused by experimentally induced anemia or oxygen deprivation [equivalent to 5000 m]. In wild-type mice, these treatments strongly upregulated spleen erythroid progenitors and caused increased levels of erythrocytes and hemoglobin to occur in peripheral blood. Similarly treated GR-deficient mice showed no respective responses. Thus, EpoR, c-Kit and the GR expand the erythroid progenitor compartment during stress erythropoiesis *in vivo*, corresponding to the abnormal self renewal of v-ErbA/v-ErbB transformed erythroleukemic cells [Figure 1].
Recently, we started to screen for target genes regulated by the ER, GR and nonliganded c-ErbA, alone or in coooperation with RTKs [EGFR, c-Kit] or relevant downstream signal transducers [e.g. Stat 5b]. For this, we used a new method for differential screening of mRNA preparations. As a prerequisite for using this technology, v-ErbA, v-ErbB and the respective protooncogenes c-ErbB and TRα/c-ErbA are currently introduced into immortal, but otherwise 'normal' erythroblasts from p53-/- mice. In addition, we try to identify the *in vivo* functions of c-ErbB [embryonic erythropoiesis?] and TRα/c-ErbA, which substitutes for the function of the GR in cultured avian cells, when overexpressed and deprived of ligand [see report 1997].

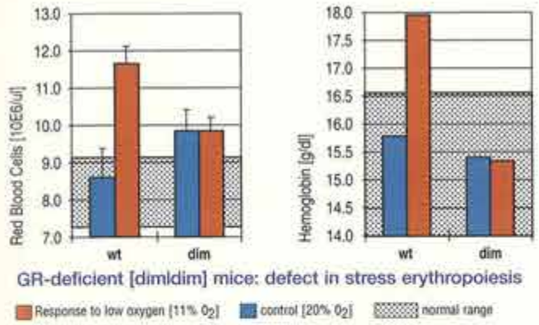
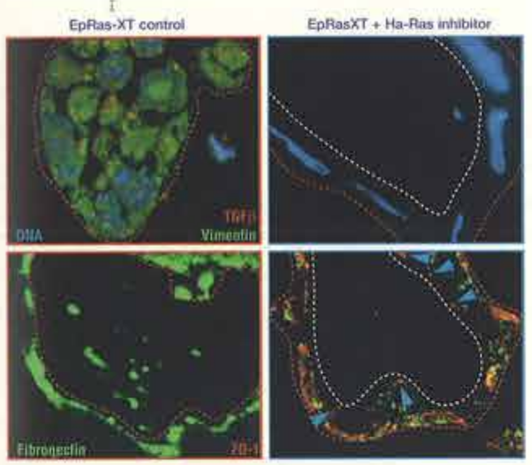


Figure 1
***In vivo* function of the GR in stress erythropoiesis**
The GR plays a major role in stress-induced phenomena. If wild-type [wt] mice are subjected to stress erythropoiesis at low oxygen concentrations [11%, equiv. to 5000m above sea level], erythrocyte count and blood hemoglobin levels are strongly upregulated. Mice carrying a knocked-in, mutant GR defective for transactivation but not transrepression [dim] do not respond to oxygen stress, thus being defective for stress erythropoiesis.

Figure 2
Ha-Ras is required for maintenance of TGFβ-induced EMT
Ras-transformed mammary epithelial cells [Ep-Ras] undergo EMT and establish an autocrine TGFβ loop after *in vitro* treatment with TGFβ or in tumors. To analyse if Ras is essential for maintaining this phenotype, mesenchymal cells isolated from EpRas-induced tumors [EpRasXT] were cultivated in collagen gels and treated [right panels] or not treated [left panels] with a specific Ha-Ras farnesylation inhibitor [ExBW26]. Semithin frozen sections from structures grown in collagen gels are shown. Left panels: untreated cells; solid cords outlined in red. Right panels: inhibitor-treated cells; hollow tubules, red outline; lumen, white line. Top panels: sections stained with antibodies to vimentin [green], TGFβ [red] and cell nuclei [DNA, blue]. Bottom panels: sections stained with anti-fibronectin [green] and the epithelial marker ZO-1 [red]. Note that control EpRasXT cells coexpress vimentin, and TGFβ [top left, red or yellow color]. The inhibitor-treated cells have lost these markers and exhibit lateral ZO-1 staining instead [blue arrowheads].



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

In carcinomas, epithelial cells lose the strict polarity required for the protective barrier function of epithelia. They may even acquire attributes of mesenchymal cells, particularly when colonizing distant organs during metastasis. This process, referred to as epithelial-mesenchymal transition [EMT], also occurs during normal embryogenesis, tissue remodeling and wound healing. In both cases, polarized epithelial cells transiently or permanently aquire molecular markers and functional aspects of motile, mesenchymal cells.

Normal epithelial cells require minute amounts of TGFβ for morphogenesis *in vivo* and *in vitro*, while higher doses of TGFβ induce cell cycle arrest and apoptosis. After transformation by oncogenic v-Ha-Ras or constitutively activated EGFR2 [HER2*], the cells become insensitive to growth arrest by TGFβ, undergo EMT *in vitro* and cause tumors in mice. EMT is maintained in these cells by an autocrine loop involving TGFβ. Using a dominant-negative, kinase-dead version of the TGFβ-receptor subunit II [TGFβRII-dn], we show that cell-autonomous TGFβR signalling is required for EMT, tumorigenesis and metastasis. Overexpression of TGFβRII-dn in various TGFβ-resistant murine and human tumor cells caused reversion of EMT, prevented invasiveness *in vitro* and inhibited tumor growth and metastasis *in vivo*. This even occurred when green fluorescent protein [GFP]-labelled, TGFβRII-dn expressing cells were mixed with unmodified tumor cells, demonstrating the cell-autonomous nature of TGFβR signalling in late stage carcinogenesis [see report 97].
In an attempt to analyse which signal transduction path-ways crosstalk with TGFβR signalling during EMT induction, we showed that Ras- or Mek-1 inhibitors abolished EMT, reverting the cells to an epithelial phenotype. Ras inhibition also restored sensitivity to cell cycle arrest and apoptosis induced by TGFβ. When cooperating with Bcl-2 instead of Ras, TGFβ induced initial stages of EMT [motility] but failed to induce stable EMT. Thus, TGFβ alone can induce transient morphological changes, but Ras is required to suppress apoptosis as well as to induce complete, stable EMT [autocrine loop] [Figure 2]. Future research will focus [I] on further elucidation of the interplay between RTK- and TGFβ-R signal transduction intermediates and [II] on identifying genes involved in EMT induction by differential screening, using the differential screening approach described above.

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Pax gene function in brain development, hematopoiesis and disease

The mammalian Pax [paired box] genes code for sequence-specific transcription factors which are essential for embryonic pattern formation and organogenesis. How these important regulators exert their developmental function at the molecular level is, however, still largely unknown. In our group we are interested in the role of Pax-5 [BSAP] and its related family members Pax-2 and Pax-8 during embryogenesis, B-lymphopoiesis and human disease. A combination of transgenic, cell biological and biochemical approaches is used to investigate the molecular function of these Pax proteins in the regulatory cascades underlying midbrain and B cell development.

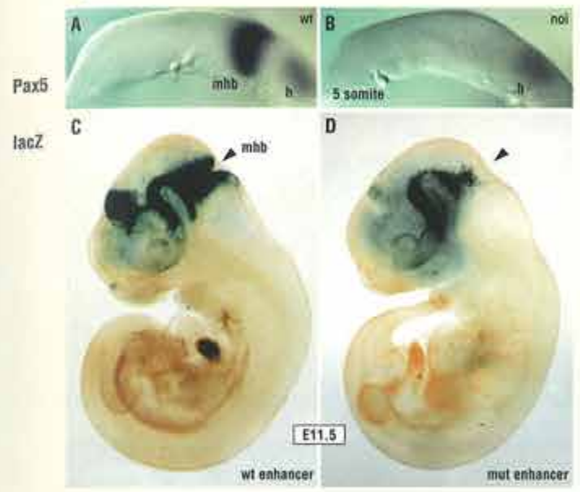
Midbrain development

The midbrain and cerebellum develop from an organizing center which is located at the midbrain-hindbrain boundary [mhb] of the vertebrate embryo. This organizer depends on the function of several transcriptional regulators [Pax-2, Pax-5, En-1, En-2, Otx-2, Gbx-2] and secreted factors [Wnt-1, Fgf-8]. How these factors interact in the same pathway is, however, difficult to determine genetically, as they show similar loss-of-function phenotypes. To gain further insight into this regulatory cascade, we are characterizing the midbrain-specific enhancers of *Pax-2* and *Pax-5*. Recently, we demonstrated that the expression of *Pax-5* at the mhb requires the *Pax-2* [noi] function in the zebra-fish embryo [Figure 1A, B]. In agreement with this result, a functional Pax-binding site was identified in the midbrain-specific enhancer of mouse *Pax-5* [Figure 1C, D], indicating that this gene is under direct cross- and auto-regulatory control during brain development. Transcription factors binding to other critical enhancer elements are currently under investigation. *Pax-2* and *Pax-5* appear to have partially redundant functions in mhb development, as suggested by gene targeting and expression analyses. To test this hypothesis, the coding sequences of *Pax-2* have been replaced with those of *Pax-5* in the mouse germline. Most aspects of the Pax-2 phenotype are rescued in homozygous knock-in mice, indicating that Pax-5 is able to compensate for the Pax-2 function during brain development. A search for neuronal Pax target genes has been initiated by analyzing neuroepithelial precursor cells derived from the mhb region of wild-type and mutant embryos. Hence, loss-of-function, transgenic and cell culture experiments are currently used to identify upstream regulators and downstream targets of *Pax-2* and *Pax-5* in midbrain development.

B-lymphopoiesis

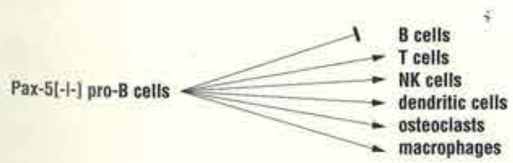
Pax-5 plays an essential role in early B-lymphopoiesis, as it is required for progression beyond an early progenitor [pro-B] cell stage. The pro-B cells from the bone marrow of *Pax-5* [-/-] mice can be cultured *ex vivo* on stromal cells in the presence of IL-7. *In vitro* differentiation experiments demonstrated that these early 'pro-B cells' are in fact non-committed hematopoietic progenitors which are able to differentiate upon appropriate stimulation to T cells, natural killer [NK] cells and different cell types of the myeloid lineage [Figure 2]. Hence, *Pax-5* expression restricts the developmental potential to the B-lymphoid lineage, and its absence allows the *ex vivo* expansion of an early hematopoietic progenitor cell which is characterized by the promiscuous expression of genes specific for different lineages. *Pax-5* is expressed throughout B cell development and plays an essential role also at late stages of B-lymphopoiesis, as shown by conditional gene inactivation using the Cre-loxP system. A major effort is therefore devoted to the identification of Pax-5 target genes which contribute to B-lineage commitment and/or the late function of Pax-5.
The transcription factor Pax-5 is able to activate or repress gene expression, depending on the specific regulatory sequence context. In agreement with this observation, yeast 2-hybrid screens have led to the identification of interacting proteins which function either as co-activators or co-repressors of Pax-5.

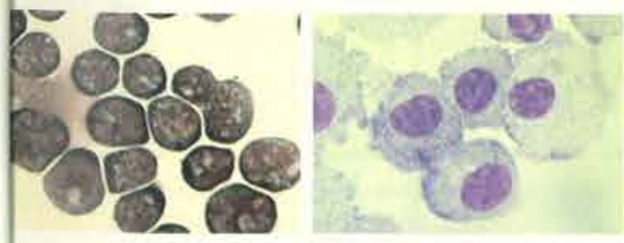
Figure 1



Cross-regulation of Pax genes during midbrain-hindbrain boundary [mhb] development. [A, B] Loss of Pax-5 mRNA expression in Pax-2$^{-/-}$ [noi] mutant embryos of zebrafish compared to wild-type [wt] embryos at the 5-somite stage [lateral view]. [C, D] Functional requirement of a Pax-binding site in the midbrain-specific enhancer of Pax-5. LacZ staining of mouse E11.5 embryos carrying a Pax-5-lacZ transgene. Mut: mutation of the identified Pax-binding site in the Pax-5 enhancer.

Figure 2
B-lineage commitment by Pax-5 [BSAP]. Pax-5 [-/-] pro-B cells represent hematopoietic progenitor cells which are blocked in their B-lymphoid developmental potential, but which are able to differentiate along the indicated hematopoietic lineages. The morphology of Pax-5 [-/-] pro-B cells [left] and macrophages [right] is shown.





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. Last year, we were able to demonstrate that mutations in the human *PAX-8* gene are responsible for congenital hypothyroidism. Our finding that *Pax-5* is predominantly transcribed from only one of its two alleles in individual B-lymphocytes suggests that the haploinsufficient phenotypes of Pax genes may be caused by their monoallelic expression pattern.
PAX genes can also be recruited as oncogenes by gain-of-function mutations in human tumours. P*AX-5* has been implicated as an oncogene in the genesis of a subset of non-Hodgkin's lymphomas containing the t[9;13][p13;q32] translocation which brought the *PAX-5* gene under the transcriptional control of the immunoglobulin heavy-chain *[IgH]* locus. To gain further insight into the oncogenic role and allele-specific regulation of *Pax-5*, we are using gene targeting and transgenic techniques to overexpress *Pax-5* either from its own locus or from the endogenous *IgH* locus, thus mimicking the translocation present in non-Hodgkin's lymphomas.

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Molecular mechanisms of multistage tumor development

The major 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 prove 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 and develops pancreatic ß cell tumors in a multistage tumor progression pathway.*

Tumor cell survival

Our recent work demonstrated that insulin-like growth factors [IGFs] and their receptor exert a critical survival function for tumor cells [Christofori et al., 1994]. Moreover, we have assessed the importance of survival factor function for tumor growth and for tumor therapy. A novel fluorescence-activated cell sorting [FACS] method [Lamm et al., 1997] was developed to show that the impairment of IGF survival signalling potentiates conventional chemotherapeutic treatment of tumor cells [Lamm and Christofori, 1998]. Currently, we are investigating the communication pathway between IGF survival factors and the apoptotic machinery, i.e. the signalling pathway that suppresses the execution of tumor cell apoptosis. Our results indicate that in tumor cells IGF-mediated signal transduction differs from the pathway described in non-transformed cells, such as fibroblasts and neurons. In particular, IGF-mediated survival signalling in β tumor cells does not involve the activation of PI3-kinase and protein kinase B.

Tumor angiogenesis

During our studies on tumor angiogenesis we developed a particular interest in the functional role of FGF family members in tumor progression. Previously, we had documented that, despite the lack of a signal sequence for secretion, acidic and basic fibroblast growth factors [FGF-1 and FGF-2, respectively] are exported by highly angiogenic tumor cell lines but not by their normal counterparts [Christofori and Luef, 1997]. However, the functional contribution of FGFs to tumor progression and/or tumor angiogenesis still remains unknown. To interfere with FGF activity, we have generated transgenic mice that express dominant-negative versions of FGF receptors under the control of the tetracycline-inducible system. Currently, we are using these mice to determine the functional role of FGFs during embryonic development and tumorigenesis *in vivo*.

Tumor cell invasion and metastasis

[in collaboration with the laboratory of H. Semb, Umeå University, Umeå, Sweden]
The molecular events that are involved in the transition from benign tumors to malignant tumors and metastasis are a major focus in our laboratory. Previously, we had demonstrated that the loss of E-cadherin-mediated cell-cell adhesion is causally involved in the transition from well-differentiated adenoma to invasive carcinoma [Perl et al., 1998a].

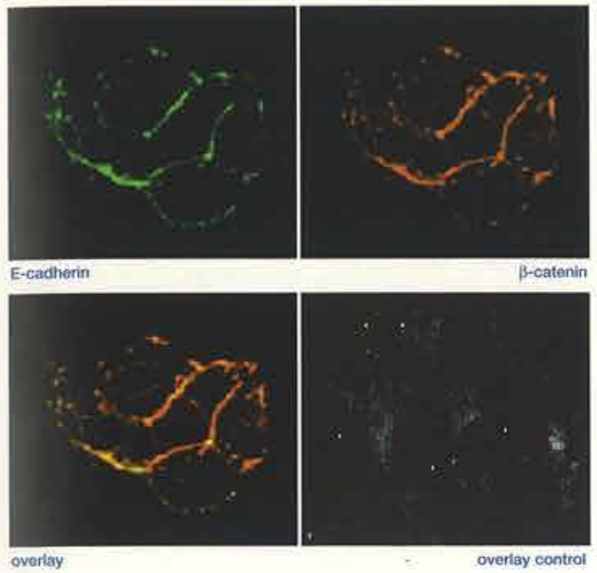
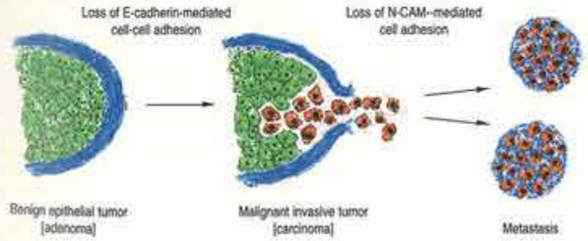


Figure 1
Subcellular localization of E-cadherin [green] and β-catenin [red] in β tumor cell lines. Note that β-catenin co-localizes with E-cadherin in E-cadherin expressing cells [overlay; yellow], whereas in the absence of E-cadherin β-catenin is readily degraded by the APC/proteasome pathway and is thus not detectable [overlay control].

Figure 2
In Rip1Tag2 transgenic mice, loss of E-cadherin-mediated cell-cell adhesion is causally involved in the transition from adenoma to carcinoma. In contrast, loss of N-CAM cell adhesion is a rate-limiting event in the actual metastatic dissemination of β tumor cells.

Progression to tumor malignancy



The results indicate that the loss of E-cadherin-mediated cell-cell adhesion activates downstream signalling pathways. A major component of the E-cadherin cell adhesion complex is β-catenin, a central player in the Wnt-signalling pathway. Hence, we are currently investigating the possibility that changes in E-cadherin-mediated cell-cell adhesion modulate the Wnt-signalling pathway and thus gene expression.

During the development of many human cancers, expression of neural cell adhesion molecule [N-CAM] changes from the 120 kd isoform in normal tissue to the 140/180 kd isoforms in tumor tissue. However, the functional contribution of N-CAM to tumor progression remains unknown. We have demonstrated that a similar switch of N-CAM isoforms occurs during tumor progression in Rip1Tag2 transgenic mice, and we have employed a two-pronged approach to determine the functional role of N-CAM during tumor progression: loss of N-CAM function by crossing Rip1Tag2 transgenic mice with N-CAM knock-out mice, and gain of function by maintaining the expression of N-CAM 120 in β tumor cells of Rip1Tag2 transgenic mice. The results indicate that downregulation of N-CAM expression is a rate-limiting event in the actual metastatic dissemination of tumor cells [Perl et al., 1998b].

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Adenovirus and DNA delivery

The research efforts in our group are concentrated on adenovirus and DNA delivery into higher eukaryotic cells. The adenovirus topics include an analysis of viral/host cell interactions, especially in the area of viral entry and host responses to viral entry, and an analysis of the avian adenovirus CELO early gene functions. A second area of research involves the development of gene transfer systems, with special emphasis on large DNA delivery and on the development of CELO virus-based recombinant vectors.

Characterization of the novel sequences in CELO

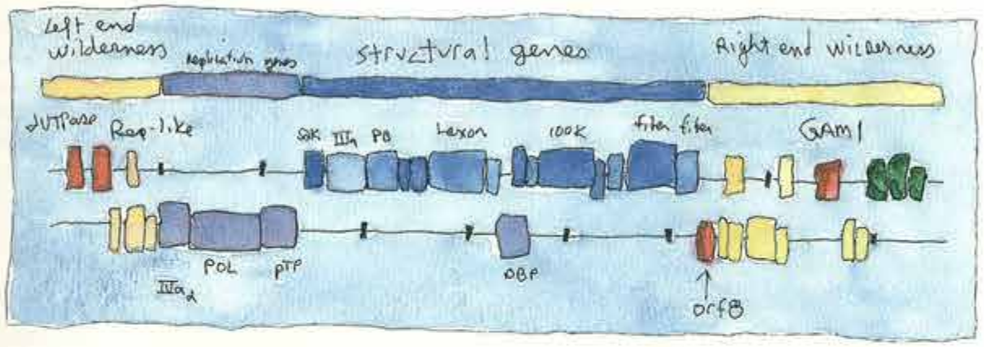
The genomic organization of CELO differs from other adenoviruses, especially in the non-capsid genes [Figure 1]. Among these peculiar genes we have identified an anti-apoptotic gene [GAM-1], a novel gene with E2F activating/pRb binding function [orf8], an essential gene that is related to the parvovirus Rep genes, and a gene encoding a protein that interacts with p300. These gene products are being further characterized.

Development of CELO virus as a gene transfer vector

We have generated a method of screening the CELO genome to determine the essential, non-essential, and complementable sequences. This effort has provided a functional map of the CELO genome, useful for the development of CELO vectors. A CELO variant was created which is replication competent and bears an expression cassette for novel sequences. Vectors based on this variant can be grown to high yield in embryonated chicken eggs. CELO-based vectors transduce both avian and mammalian cells with efficiencies comparable to the current adenovirus type 5 vectors.

These vectors will be developed both as gene delivery tools for molecular biology and as vaccine reagents.

Figure 1



The organization of open reading frames in the genome of the avian adenovirus CELO. Capsid, and E2 sequences homologous to Mastadenovirus genes are shown in blue, open reading frames novel to CELO are in yellow or red, and dispensable open reading frames are shown in green.

Analysis of the adenovirus entry pathway

Despite years of application in molecular biology, the entry pathway of adenovirus into the cell nucleus is still poorly understood. A variety of methods, including immunofluorescence [Figure 2], and GFP marking, are being used to clarify the intracellular route, the required host structures and the kinetics of the virus movement in infected cells.

Large DNA delivery

A major problem in gene delivery to mammalian cells is the maintenance of expression of the introduced genes. Genes carried on non-integrated DNA molecules are lost upon cell division, while genes that integrate into the host chromosome are frequently silenced. In efforts to solve these difficulties we are developing large DNA vectors [>150 kb] that maintain gene expression, exist in a non-integrated form and are reliably transferred to daughter cells during mitosis.

Figure 2
Distribution of adenovirus particles in human cells 1 hour after infection, visualized by anti-capsid staining [green/yellow] and microtubule staining [red]. The virus is seen to accumulate at the nuclear membrane before entry into the nucleus.

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Axon guidance in Drosophila

Electrical engineers lay out the wiring instructions for digital computers in the two-dimensional form of electronic circuit diagrams. Evolution has encoded the wiring instructions for nature's computers in the one-dimensional form of a genetic program. Our goal is to unravel such genetic circuit diagrams. We have chosen Drosophila as a model system, as it offers both a sufficiently complex nervous system and powerful methods for genetic analysis.

During development of the nervous system, each individual neuron sends out an axon, which seeks out and then forms a synapse with one or more specific target cells – either other neurons or muscle cells. These target cells may be located in very distant parts of the embryo, but axons nevertheless follow direct and reproducible pathways in order to reach their own specific target cells, ignoring many other potential target cells *en route* – cells that may themselves be the targets of other axons. Axonal traffic is controlled by guidance cues in the extracellular environment. These cues are read by a specialized structure, called the growth cone, at the tip of each axon. Since the number of neuronal connections in the nervous system exceeds the number of genes in the genome by many orders of magnitude, it is clear that there cannot be specific cues for each axon any more than there can be specific signposts for every driver on the highway. Rather, there must be a system of general signposts and each axon [or driver] decides which signs to follow and which to ignore. Our approach to studying the control of axonal traffic is to try to create havoc on the neural highways: we try to remove the signposts, or put them in the wrong place, and to make axons blind to signals they should follow or follow signals they should ignore.

Photoreceptors in the developing compound eye of Drosophila project their axons into the optic lobes of the brain [Figure 1]. We have used a novel genetic strategy to screen for mutations that lead to abnormal photoreceptor axon projections. We created genetic mosaics in which the photoreceptors of the eye are mutant, while the brain itself is normal. We then looked for mutations that disrupt the pathfinding abilities of mutant photoreceptor axons in a normal brain [Figure 1]. After screening over 32,000 mutant lines, we identified some 200 mutations that disrupt genes required for photoreceptor axon guidance. Complementation and mapping indicate that these mutations represent a total of approximately 20 different genes. We have thus far identified 5 of these genes. Three encode cell surface molecules: two cadherins [calcium-dependent cell adhesion molecules], and a receptor tyrosine phosphatase. The other two genes characterized so far encode a previously identified adaptor protein [called dreadlocks], consisting of SH2 and SH3 domains, and a newly discovered member of the guanine nucleotide exchange factor [GEF] family. GEF proteins catalyze the exchange of GDP for GTP on the small GTPase proteins, molecular switches that are active in the GTP-bound form and inactive in the GDP-bound form. The new GEF we have identified is predicted to be

Figure 1
Photoreceptors project axons [labelled brown] from the developing eye into the brain [left]. Photoreceptor axons lacking the protein tyrosine phosphatase Ptp69D [centre] or a Rho-subfamily GEF [right] make various pathfinding errors in the brain.

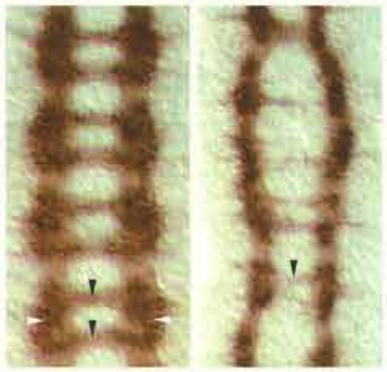
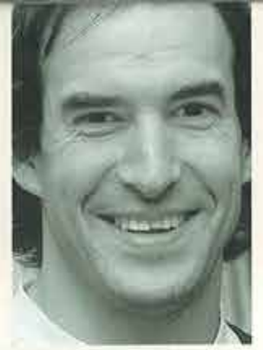


Figure 2
In the embryonic CNS [left], axons form two major longitudinal tracts [white arrowheads] connected by two commissures [black arrowheads] in each segment. The commissures are formed by axons that cross the midline before turning into one of the longitudinal tracts. If axons express high levels of a roundabout*-like gene [right], they are prevented from crossing the midline, resulting in a CNS with greatly reduced commissures.*

specific for the Rho subfamily of GTPases, which are known to control the actin cytoskeleton. So this GEF provides a potential link between cell surface receptors that recognize extracellular guidance cues, and the Rho family GTPases that then regulate the actin cytoskeleton to alter the direction of axon outgrowth. Ongoing research is aimed at exploring the possible links between the receptors and signal transduction components we have thus far identified, and characterizing the 15 or so genes that still await molecular analysis.

In the embryo, axons of the CNS form two main longitudinal bundles, one on each side of the CNS midline. Before joining these longitudinal pathways, some, but not all, axons first cross the midline. These axons form the commissures that connect the two longitudinal tracts like the rungs on a ladder [Figure 2]. Why do some axons cross the midline and not others, and what guides them first towards and then away from the midline? The midline secretes both chemoattractants, netrins, and as yet unidentified chemorepellents that CNS axons sense through the receptors frazzled and roundabout respectively. In order to determine how CNS axons respond to these two cues, and why commissural and longitudinal axons respond differently, we are currently trying to identify mutations that interact genetically with mutations in either the *frazzled* or *roundabout* genes. Such mutations are likely to lead to the identification of other components in this pathway, such as the signal transduction molecules downstream of frazzled and/or roundabout.

In parallel to these loss-of-function approaches, we are also taking a gain-of-function approach by looking for genes that, when misexpressed, can redirect axons to inappropriate targets. In this approach we take advantage of a recently developed method for placing random genes under the control of defined regulatory elements. This is achieved by placing such regulatory elements on a transposable element [the P element] that is then mobilized to allow it to insert at random sites in the genome. In a pilot screen we identified one new gene, related to *roundabout*, that is capable of redirecting axonal projections in both the embryonic CNS [Figure 2] and in the visual system. Ectopic expression of this gene in the CNS causes most commissural axons to follow longitudinal pathways, exactly the opposite phenotype to that seen in loss-of-function mutations in the *roundabout* gene itself. We are presently investigating the normal function of this *roundabout*-like gene, while simultaneously preparing to conduct such misexpression screening on a much larger scale.



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The mechanism of cytokinesis

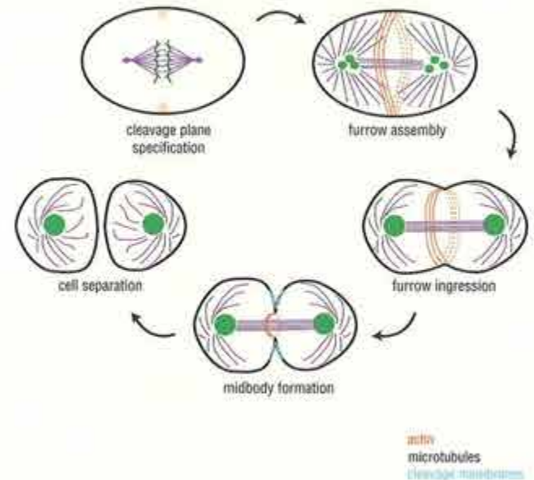
Cytokinesis leads to the formation of two daughter cells from a single progenitor. The division process is spatially and temporally regulated so that each daughter cell receives a full complement of chromosomes and other essential organelles.

In animal cells, cytokinesis can be subdivided into five subprocesses: cleavage plane specification, furrow assembly, furrow ingression, midbody formation, and cell separation [Figure 1]. In anaphase, the mitotic spindle specifies the site at which the cleavage furrow will form. A contractile ring containing actin and myosin assembles. The ring contracts, furrowing the overlying plasma membrane. A transient structure, the midbody, forms when the furrow reaches the remnants of the mitotic spindle. Finally, the common membrane that surrounds the two nascent cells is divided so that the cells may separate.

All of these subprocesses are poorly understood; moreover, it is likely that much of the machinery that participates in cytokinesis remains to be identified. Although several proteins are known to be required for cytokinesis (tubulin, actin, myosin, and various actin-binding proteins), most of these proteins are required for a diverse variety of cellular functions and it is likely that there are specific regulatory molecules that coordinate them during cytokinesis.
Few proteins of this category have been identified. Naturally, our understanding of the molecular mechanisms of cytokinesis lags behind the inventory of the molecules involved.

Thus, a major goal will be to identify proteins that control and participate in cytokinesis. Genetic analysis is needed to identify such molecules. As we are particularly interested in animal cell cytokinesis, the model organism that we have selected for this analysis is the nematode *C. elegans*. This organism is particularly well-suited for this analysis because one can easily follow the progress of the early divisions by high resolution microscopy. The *C. elegans* genome project is almost completed and this wealth of sequence data will greatly facilitate both forward and reverse genetics. We will identify and characterize mutations in which cytokinesis is impaired, but in which other events such as pronuclear migration, spindle assembly, spindle orientation, and anaphase appear normal. We have already identified several mutants with these properties by screening a mutant collection assembled by the Schnabel laboratory. One mutant has a defect in the early stages of cytokinesis, prior to furrow ingression while, others fail at later stages. The mutant phenotypes will be characterized genetically and cytologically and some of the corresponding genes will be cloned. We will then study the localization and biochemical properties of the encoded proteins.

A schematic view of cytokinesis Figure 1 The stages of cytokinesis



We are already in the process of studying, on the biochemical level, a family of proteins that are required for cytokinesis in budding yeast and in *Drosophila*, the septins. Using *Xenopus* embryos and extracts prepared from *Xenopus* eggs, we have shown that the septins are required for furrow ingression, they bind to both actin filaments and microtubules, and they can be purified from *Xenopus* extracts in a filamentous state. We will be primarily concerned with three questions, which will be addressed biochemically using *Xenopus* extracts.

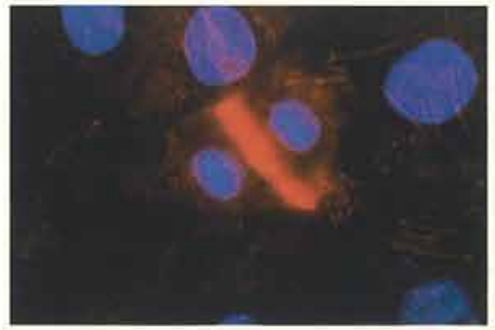
- How do septins assemble into filaments?
- Do septins associate directly with actin filaments and with microtubules? How does septin binding affect the organization of actin filaments and microtubules?
- How do septins promote furrow ingression? Is their ability to associate with actin filaments and with microtubules essential for their function in cytokinesis?

One other project is to explore the role of membrane insertion in cytokinesis. During the course of cytokinesis of *Xenopus* embryos, membrane is inserted specifically into the furrow region. Interestingly, inhibition of cytokinesis by perturbing actin-containing structures does not affect insertion of these membranes. Thus, insertion of cleavage membranes is independent of the contractile events that occur during furrow ingression. Interestingly, one of the few similarities between cytokinesis in plant and animal cells is the insertion of membranes. Thus, characterization of cleavage membrane insertion may provide insight into an evolutionarily conserved, and hence fundamental, feature of cytokinesis. This project would initially focus on identifying the machinery that mediates this fusion event. Once the machinery is known, it will be possible to inactivate this process so that we can assess the role of membrane insertion in cytokinesis.

Figure 2

The first cytokinesis of a C. elegans embryo

Figure 3



The distribution of septins in a dividing cell

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Epithelial polarity

The central aim of our laboratory is to understand how the complex three-dimensional organization of polarized epithelial cells is lost during early stages of carcinogenesis. As our main model system, we use a mouse mammary gland epithelial cell line, expressing an estrogen-inducible c-JunER fusion protein, in which reversible loss of epithelial polarity can be studied.

Our results show that this transient loss of polarity has to be regulated by a fine-tuned interplay of intracellular signalling systems, rearrangement of extracellular contacts and the cytoskeletal and protein transport machineries involved, and that these processes might be much more tightly interconnected than previously appreciated. Our major research focus will continue to be on:

- Gene expression during loss of epithelial polarity, and
- Protein transport and sorting.

Gene expression during loss of epithelial polarity

Last year we reported the identification of the immediate early gene product TIS7/PC4 as a JunER target gene by PCR-based differential display [DD]. The protein localizes at the inner leaflet of the basolateral plasma membrane in the vicinity of adherens junctions. Upon hormone-induced loss of polarity in c-JunER cells, the protein is upregulated, translocates to the cytoplasm and then accumulates in the nucleus.

In addition, the TIS7/PC4 gene product interacts with components of the Wnt-signalling pathway. We are now generating inducible cell lines expressing wild-type and mutated TIS7/PC4 to study if this protein could be a co-repressor of mesenchymal genes, necessary for the correct expression of the differentiated phenotype of an epithelial cell.

In a related project we observed that c-JunER activation results in a reversible loss of tight junctions accompanied by a redistribution of occludin and ZO-1 from the cell membrane to the cytoplasm. Treatment of polarized monolayers with occludin loop peptides has a similar effect [Figure 1] but additionally induces transcription of ZO-1 and E-cadherin, and leads to the formation of multi-layered cell clusters. This indicates a cross-talk between adherens and tight junctions during loss of epithelial polarity. We initiated studies with polyArg-tagged anti-ZO-1 antibodies as well as with antisense probes to functionally interfere with tight junction formation in epithelial cultures.

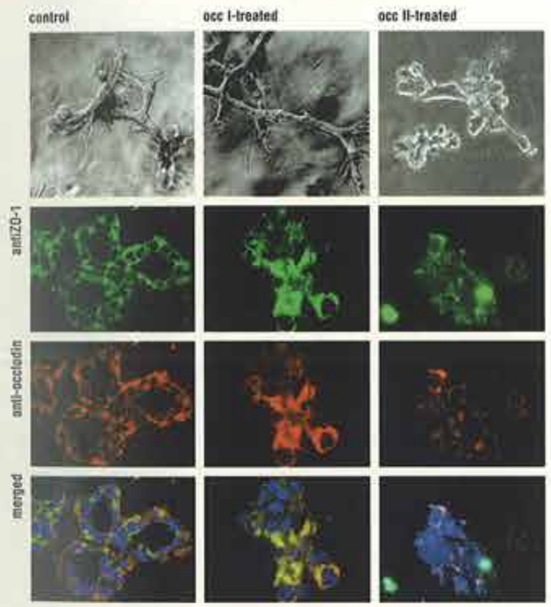
Protein transport and sorting

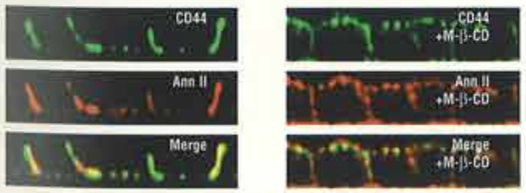
After subcellular fractionation and 2-D gel-analysis of organelle-associated proteins we discovered and microsequenced a novel protein of about 14 kD. Following cDNA cloning and generation of antibodies we were able to localize the protein to the membrane of the Golgi apparatus and additional vesicular structures by immunofluorescence and electron microscopy. The protein is extractable by mild detergent treatments before fixation, indicative as an association with the cytoplasmic phase of the respective membranes, where most of the regulatory complexes in protein transport are assembled.

We started a yeast two-hybrid screen to identify interacting molecules. We are presently establishing epithelial lines with tet-inducible p14 which will be instrumental for functional protein transport assays with GFP-tagged, viral reporter molecules specific for apical vs. basolateral sorting.

In another project we were interested in the sorting of proteins within the plane of the plasma membrane. We found that CD44 can act as an integral transmembrane protein in cholesterol-enriched microdomains [lipid rafts] of the basolateral plasma membrane [Figure 2]. These CD44-lipid raft complexes are stabilized by an interaction of annexin-II with the underlying actin cytoskeleton. We will now investigate which physiological stimuli [e.g., ligand binding] would regulate CD44's entry into or loss from lipid rafts, with a possible extension of the project to compare normal and metastatic tumor cells.

Figure 1
Examples of a morphology assay. In-collagen cultures of mouse mammary epithelial cells develop into a branched tubular network. Normal epithelial cells are fully polarized with the apical surface towards the lumen. Treatment with extracellular loop I or loop II peptides of the transmembrane protein occludin result in the loss of epithelial polarity where no lumen is formed. Loop I-treated cells form long solid tubules, while loop II-treated cells develop multiple branched cell clusters on short solid tubules. Shown on the top is the overall morphology of controls, loop I- or loop II-treated cells in collagen, respectively. Below are typical thin sections of each condition. The immunofluorescence features ZO-1 [green], occludin [red] and the merged pictures including cell nuclei [blue].





The basolateral proteins CD44 and annexin II re-distribute all over the cell, including apical plasma membrane, upon depletion of membrane cholesterol.

Figure 2
Vertical sections of control [left] and cholesterol-depleted [right] cells are shown. Both CD44 and annexin II colocalize to the basolateral plasma membrane domain in mammary epithelial cell line EpH4. Upon depletion of membrane cholesterol with cholesterol-sequestering agents, such as digitonin methyl-β-cyclodextrin, both proteins can be found all over the plasma membrane; however, they do not colocalize any more, which indicates that their interaction is not direct and most possibly is mediated by lipids.

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Mammalian higher order chromatin

Higher order chromatin has been implicated in epigenetic gene control [e.g. 'transcriptional memory' mediated by Polycomb-group genes] and in regulating chromosomal architecture [e.g. centromere activity, and mitotic and meiotic chromosome segregation]. To start analyzing components and functions of mammalian higher order chromatin, we recently isolated homologues of the Drosophila Polycomb-group gene Enhancer of zeste and of the dominant Drosophila suppressor of position-effect-variegation [PEV] Su(var)3-9. Both of these mouse [designated Ezh1, Ezh2 and Suv39h1, Suv39h2], and the corresponding human homologues are represented by two loci in the mammalian genome [Laible et al., 1997; O'Carroll et al., 1998].

Ezh and conserved gene silencing

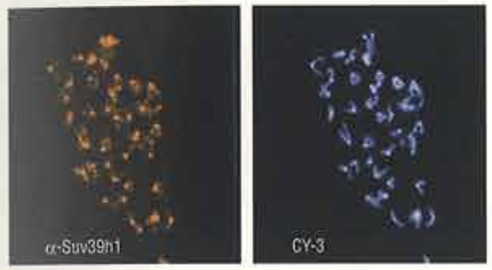
Condensed chromatin regions [heterochromatin], for example at centromeric positions in *Drosophila* or at *S.cerevisiae* telomeres, repress transcriptional activity in a gene non-specific manner. To demonstrate function of the mammalian *Ezh* and *Suv39h* homologues, we examined their potential to silence gene activity in both of these model systems. Extra gene copies of human *EZH2* in transgenic flies enhance suppression of pericentromeric PEV alleles of e.g. the *white* gene, and over-expression of *EZH2* or murine *Ezh1* restores gene repression in *S.cerevisiae* mutants that are impaired in telomeric silencing [reviewed in Jenuwein et al., 1998]. These data provide a direct functional link between *Polycomb*-dependent gene repression and inactive chromatin domains, and indicate that silencing mechanism[s] may be broadly conserved in eukaryotes. A similar modification of PEV has also been shown with transgenic flies that carry an extra gene copy of human *SUV39H1* [Aagaard et al., 1998].

Suv39h1, a novel centromere-associated protein

To analyze chromatin association of the endogenous Suv39h1 and EZH2 proteins, we generated affinity-purified, rabbit polyclonal anti-sera. In contrast to the diffuse distribution of Ezh2/EZH2 proteins, immunodetection of endogenous Suv39h1/SUV39H1 in mammalian cells indicates enriched localization at heterochromatic foci during interphase and accumulation at centromeric positions on metaphase chromosomes [Figure 1] [Aagaard et al., 1998]. Moreover, ectopic expression results in abundant associations with mitotic chromatin, suggesting a dynamic role[s] for Suv39h proteins in the structural organization of chromosomes. Using stably transfected cell lines, we identified several interacting proteins that specifically co-precipitate with over-expressed $[myc]_3$-tagged human SUV39H1. Based on these interactions, we are currently purifying the first mammalian SUVAR complex.

Immunolocalization of endogenous Suv39h1 at mouse metaphase spreads

Figure 1
Mouse PD31 pre-B cells were treated with colcemid, resulting in metaphase arrest of approximately 20% of the cells. Cells were hypotonically swollen, broken up by centrifugation and stained prior to fixation with an affinity-purified rabbit anti-Suv39h1 polyclonal antibody, followed by a secondary goat anti-rabbit antibody that had been conjugated to CY-3. The DNA has been stained with DAPI.

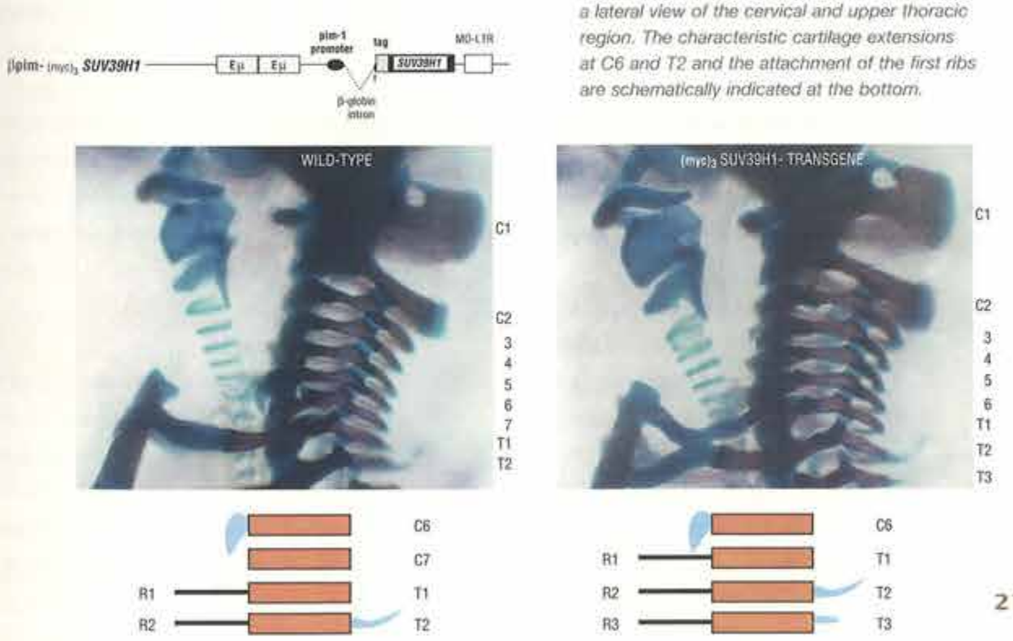


Modulation of higher order chromatin *in vivo*

To generate gain-of-function mutations *in vivo*, we established transgenic mice for human *EZH2* and *SUV39H1*. Whereas several of the *EZH2* lines display only low to moderate expression levels, two mouse lines were identified that overexpress $[myc]_3$-tagged SUV39H1 and which, surprisingly, exhibit posterior transformations of vertebral identities [Figure 2]. Since ectopic SUV39H1 is gradually lost upon clonal expansion of stably transfected cells and is only detected as mosaic expression in these transgenic mouse lines, the data suggest that high SUV39H1 protein levels are incompatible with normal growth control. Consistent with such a function, cells derived from *Suv39h1*$^{-/-}$ mice are also shifted in their proliferative and differentiation potential. Finally, we disrupted both *Ezh* loci in the mouse germline. Whereas *Ezh1* null mice appear phenotypically normal, disruption of the more embryonically expressed *Ezh2* gene results in lethality during early gestation. These mutant embryos will be used to analyze possible perturbed patterns of gene silencing.

Overexpression of human $[myc]_3$-tagged *SUV39H1* in transgenic mice induces posterior transformations of vertebral identities

Figure 2
The skeletons of wild-type and transgenic day 18 embryos were cleared and stained for bone [alizarin red] and cartilage [alcian blue]. Shown is a lateral view of the cervical and upper thoracic region. The characteristic cartilage extensions at C6 and T2 and the attachment of the first ribs are schematically indicated at the bottom.



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Asymmetric cell division during *Drosophila* development

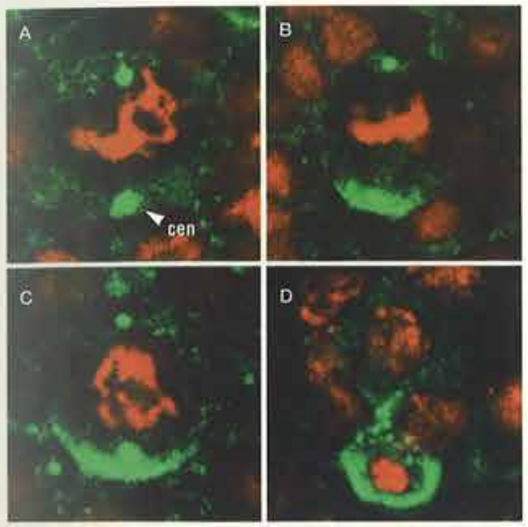
While most cell divisions are symmetrical and generate two identical daughter cells, some cells are capable of dividing asymmetrically into two different daughter cells. One way to generate asymmetric cell divisions is the segregation of protein determinants into one of the two daughter cells, which makes this cell different from its sister cell.
We are using the fruitfly Drosophila melanogaster *as a model system to understand the molecular mechanisms that generate and orient asymmetric cell divisions.*

Asymmetric cell divisions are involved in the development of both the central and peripheral nervous systems of *Drosophila*. 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 1]. 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 2A]. In the absence of Inscuteable, Numb either fails to localize asymmetrically or the Numb crescents form at random positions around the cell [Figure 2B]. Inscuteable is also required for the correct orientation of the mitotic spindle, which determines the division plane. In the absence of Inscuteable, Neuroblasts which normally divide along the apical-basal axis divide with random division planes. 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 [Figure 2C]. Thus, Inscuteable directs and coordinates several events during asymmetric cell divisions.

We are using Inscuteable as a molecular entry point to identify components of the cellular machinery that directs asymmetric cell divisions. Proteins that associate with Inscuteable are candidate components of this machinery. As an initial step towards identifying Inscuteable associated proteins, we have narrowed down the functional domains of the protein.

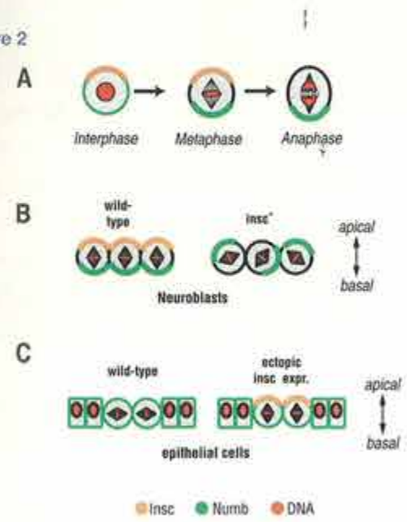
Figure 1



Asymmetric localization of Numb during mitosis

Confocal cross-sections through *Drosophila* neuroblasts in various stages of mitosis. DNA is shown in red, the Numb protein and the centrosomes (cen) are shown in green. Prophase neuroblasts (A) show no signs of asymmetric localization, but in meta- (B) and anaphase cells (C), Numb localizes asymmetrically. In telophase (D), the protein segregates into one of the two daughter cells.

Figure 2



Summary of Inscuteable localization and function

We have generated serial deletions of Inscuteable and expressed them in transgenic flies. This deletion analysis has identified a central 364 aa domain in the Inscuteable protein, which we call the asymmetry domain. This domain is required and sufficient for membrane localization and asymmetric localization of Inscuteable. When expressed by itself or fused to *lacZ*, the asymmetry domain is sufficient to cause a reorientation of the mitotic spindle in epithelial cells. When expressed in *inscuteable* mutants, the same domain can rescue the Numb localization-defect. Thus, most of the known functions of Inscuteable map to the same domain of the protein.

Using the yeast two-hybrid system, we have identified a number of proteins that specifically interact with this domain and we are currently analyzing the function of these molecules, using the excessive genetic and cell biological tools available in *Drosophila*. We are also searching for other proteins involved in asymmetric cell division using biochemical and genetic methods, and we hope that identifying these molecules will help us to understand the molecular mechanisms of asymmetric cell division.

A Asymmetric localization of Numb and Inscuteable.
B 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 *inscuteable* mutants, spindle orientation and Numb localization become random and are no longer coordinated.
C Epithelial cells of the prospective epidermis normally divide parallel to the epithelial surface, but after ectopic expression of *inscuteable*, cells reorient their mitotic spindle and divide perpendicularly to the surface. Note, however, that *inscuteable* is not sufficient to induce the asymmetric localization of Numb.

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Cell division and differentiation in yeast

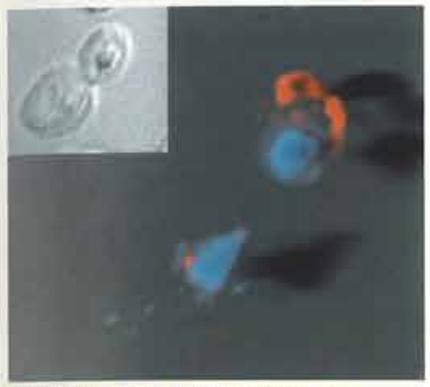
An unbroken series of cell divisions connect us to the common ancestor of all life on this planet. Cell division is the basis of all life. Furthermore, it plays an important part in many human diseases. A refusal of somatic cells to continue dividing contributes to senescence, while the unregulated proliferation of tumor cells is a cause of cancer. Meiosis, a variation on the mitotic theme, generates progeny with different characteristics (sexual reproduction), which enables multicellular organisms to evade their parasites.

Cell division involves two sorts of processes: those that duplicate the cell's contents and those that segregate them equally between two daughter cells. Sometimes, the unequal segregation of very specific constituents, known as 'determinants', ensures that the two daughter cells adopt different patterns of gene expression, which is the basis for cell differentiation and the development of multicellular organisms. One of the revelations of the last decade of Cell biology has been the recognition that all existing eukaryotic cells use mechanisms that clearly existed in their common ancestor. This means that the mechanisms used by human cells are remarkably similar to those used by invertebrate as well as fungal or plant cells. Thus, much of our knowledge about the workings of human cells can be, and indeed has been, gleaned from work on 'model' organisms, which are easier to study. One of the favorite models for studying cell division has been the budding yeast Saccharomyces cerevisiae. Most work in my laboratory is conducted on this organism on the premiss that its powerful genetic and physiological techniques can be exploited to tell us more about ourselves.

Because most constituents of cells are synthesized under instructions from their genomes, they take extraordinary care in duplicating and segregating their chromosomes. Chromosome duplication during S phase produces sister chromatid pairs, which are held together by specific chromosomal proteins called Cohesins. The subsequent separation of sister chromatids is mediated by microtubules that connect sister kinetochores to opposite poles of the mitotic spindle. A 'tug of war' between the splitting force exerted by microtubules and cohesive forces due to Cohesins is responsible for the alignment of sister chromatid pairs on the metaphase plate.
The eventual segregation of sister chromatids to opposite poles of the cell (anaphase) is thought to be due primarily to a loss of cohesion between sister chromatids. The transition from metaphase to anaphase must be very tightly regulated, because it is difficult or impossible for cells to correct DNA damage or misaligned chromosomes after the commencement of anaphase.
The properties of the cohesin Scc1p (also known as Mcd1p) have recently shed insight into the mechanism by which sister chromatid cohesion might be lost. Scc1p binds to chromosomes as part of a complex with proteins of the SMC family; it is required to prevent premature separation of sister chromatids, and it dissociates from chromosomes at the metaphase to anaphase transition. A key question is what causes the sudden dissociation from chromosomes of Scc1p. This event depends on inactivation of a regulatory protein called Pds1p, which, like mitotic cyclins, is proteolyzed as a consequence of its prior ubiquitination by a large ubiquitination machine called the Anaphase promoting complex (APC).
Our work suggests that Pds1p blocks the activity of a protein called Esp1p, which is required for the dissociation of Scc1p and the loss of sister cohesion. We are currently interested in what determines the timing of Pds1p destruction by the APC, how surveillance mechanisms called Checkpoints regulate this process, what, in addition to Pds1p, controls the activity of Esp1p, and how Esp1p causes the dissociation of Scc1p from chromosomes. Most, if not all, of these yeast proteins have homologues in human cells, suggesting that the mechanism by which cells separate sister chromatids is conserved in all eukaryotes.

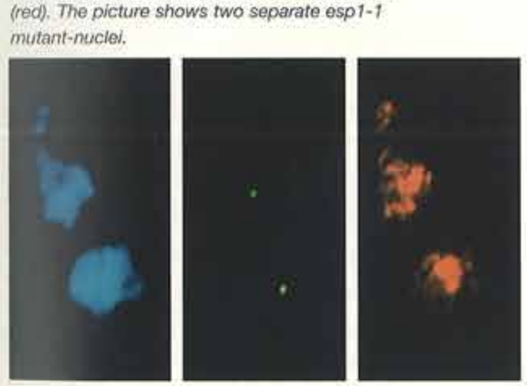
In haploid yeast cells, cell division gives rise to a mother cell that expresses the HO gene and switches its mating type, and a daughter cell that fails to do so because of the preferential accumulation within its nucleus of a repressor protein called Ash1p.
ASH1's mRNA is made from both sister chromatids during anaphase and is transported by a type V myosin (Myo4) along actin cables to the distal tip of buds, where it is anchored and translated.
The asymmetric segregation of mRNA 'determinants' plays an important role in the development of multicellular organisms. My lab is interested in the mechanism by which ASH1 mRNA is transported along actin cables by Myo4, how it is subsequently anchored at the distal tip of buds, and how its translation is regulated during this process.

Figure 1
Asymmetric localization of ASH1 mRNA in yeast. The messenger RNA of the transcriptional regulator Ash1p is localized during mitosis to the tip of the daughter cell. Shown is a three-dimensional image (shadow projection) of ASH1 mRNA (red) and the two nuclei (blue) derived from mRNA in situ hybridization and DAPI staining, respectively. Inset: Reduced size bright field image of the same mother/daughter cell pair.



Asymmetric ASH1 mRNA localization

Figure 2
Esp1p is required for separation of cenV sequences marked by GFP (green) and for the dissociation from chromatin (blue) of the cohesin Scc1p (red). The picture shows two separate esp1-1 mutant-nuclei.



Cell cycle regulated association of Scc1p with chromosomes

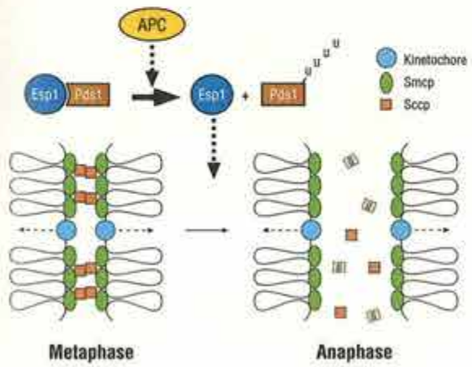


Figure 3
The APC triggers sister separation by liberating the 'separin' Esp1 from inhibition by Pds1

Annette NEUBÜSER | Group Leader – Wittgenstein Group

Group Member
Marion REPITZ | Technician

Patterning and early morphogenesis of the vertebrate face

The face is one of the most intricately shaped parts of the adult 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 the development of this complex structure is regulated during embryogenesis.

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 [Figure 1]. Subsequently, tissue around the nasal placodes and the primitive mouth starts to grow out to form distinct buds, the facial primordia, and continued outgrowth depends on epithelial-mesenchymal interactions. How the early facial region is patterned and how the areas of mesenchymal outgrowth are established is not understood. In particular, it is not clear what role the surface ectoderm, the nasal placodes and the underlying forebrain play for patterning of the neural crest-derived mesenchyme of this region. We are using both the mouse and the chick as experimental organisms to address these questions, the chick to study the morphological and molecular consequences of micro-surgical manipulations of the early face in ovo, and the mouse for a genetic approach towards facial development.

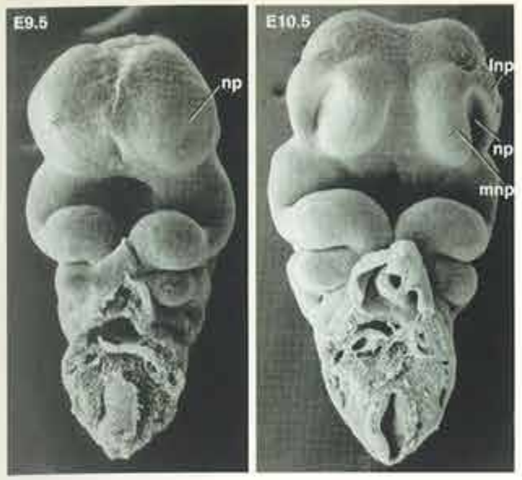
The function of the nasal placodes for facial development

In a series of ablation experiments we demonstrated that the nasal placodes are required for normal facial mesenchyme development [Figure 2].

Preliminary data suggests that the nasal placode may pattern the facial mesenchyme indirectly via the induction of signalling molecules at the boundary between the placode and the surface ectoderm which in turn direct mesenchyme development. However, a direct interaction of the placode and the underlying mesenchyme has not yet been ruled out. To further address this issue, we are currently studying how placode ablation experiments and other micro-surgical manipulations of the face affect the expression of marker genes. In addition, we are also studying facial development in mouse embryos homozygous for a mutation in the gene encoding the paired box transcription factor Pax6 which fail to form a nasal placode and display severe facial abnormalities at later stages.

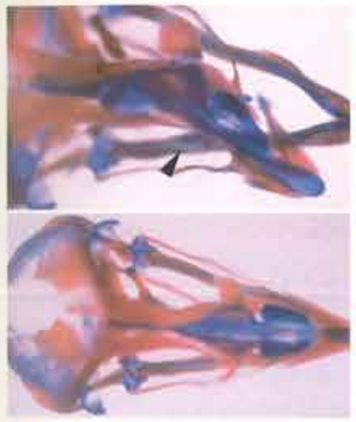
FGF8 function during facial development

Partial loss of function experiments in the mouse from Gail Martin's laboratory have recently demonstrated that *Fgf8* is required for normal facial development, but the phenotype of those embryos varies and is therefore difficult to analyze. Embryos homozygous for a null allele of *Fgf8* die at gastrulation.



Scanning electron micrographs of the facial region of mouse embryos at E9.5 and E10.5

Figure 1
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.



Ablation of the nasal placode affects development of the facial skleton

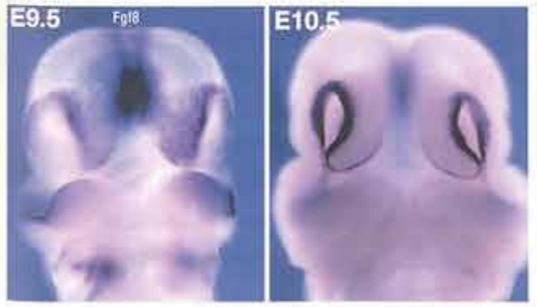
Figure 2
Top: Top view of the beak of a chick embryo after surgical removal of the right nasal placode at an early stage of development. Note that on the operated side [arrow] a variety of skeletal elements are missing.
Bottom: Removal of ectoderm between the two nasal placodes does not affect skeletal development.

As a first step towards a tissue specific inactivation of *Fgf8* in facial ectoderm, we used a mouse line in which the Cre-recombinase was 'knocked into' the gene encoding the transcription factor BF1 to produce embryos in which *Fgf8* is 'knocked out' only in certain regions of the embryo.
These embryos were born with severe facial defects and a morphological and molecular analysis of their phenotype will serve as a starting point to address the function of *Fgf8* during facial development and to identify downstream targets of *Fgf8*.

Regulation of *Fgf8* expression

Fgf8 has a complex, dynamic expression pattern during facial development [Figure 3]. To learn more about the mechanisms involved in patterning the facial ectoderm, we are studying how the expression pattern of *Fgf8* in facial ectoderm is set up.
A major goal is to identify tissue interactions that are involved in patterning of the facial ectoderm and that regulate *Fgf8* expression. For this purpose we are using an in vitro culture system in which various regions of the face can be co-cultured. This culture system also allows us to directly test the effect on *Fgf8* expression of growth factors and signalling molecules suspected to be involved in facial patterning.

Figure 3
Expression pattern of Fgf8 in mouse embryos at E9.5 and E10.5



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Beate PETERS | Technical Assistant [50%]

Cell cycle control by ubiquitin-dependent proteolysis

Progression through the cell cycle depends on the activity of two ubiquitination complexes: the Skp1-cullin-F box protein complex [SCF] controls the initiation of DNA replication at the G1-S transition, whereas activation of the anaphase-promoting complex/cyclosome [APC] is essential for the initiation of anaphase and exit from mitosis.

The APC ubiquitinates proteins whose subsequent degradation by the 26S proteasome is essential for the initiation of sister chromatid separation at the metaphase-anaphase transition. Later, in anaphase and telophase, the APC promotes the inactivation of the mitotic protein kinase Cdc2 by ubiquitinating its activating subunit cyclin B. The APC also mediates the ubiquitin-dependent proteolysis of several other mitotic regulators, including Polo-like kinases, spindle-associated proteins and inhibitors of DNA replication. We are interested in understanding how the APC is regulated and how activation of the APC initiates the separation of sister chromatids in vertebrate cells.

Purification of the APC from Xenopus eggs and human cells showed that the complex is composed of at least ten subunits, nine of which are related to subunits of the budding yeast APC.

The subunit APC2 is related to the SCF subunit Cdc53, suggesting that these ubiquitination complexes are evolutionary related and utilize similar reaction mechanisms. Whereas the ten APC subunits identified by biochemical purification are constitutively associated with the complex, we found that two other proteins called CDC20 and CDH1 bind to the APC in a cell cycle-regulated manner. Previous genetic experiments in yeasts and flies and our biochemical experiments suggest that activation of the APC at the metaphase-anaphase transition requires its association with CDC20, whereas APC activity is maintained during exit from mitosis and throughout G1 by association with CDH1. The binding of CDC20 and CDH1 appears to be regulated by phosphorylation, but how CDC20 and CDH1 activate the APC is presently unknown. In yeast, CDC20 and CDH1 activate the APC in a substrate-specific manner, suggesting that these proteins may function as specific substrate recognition factors of the APC.

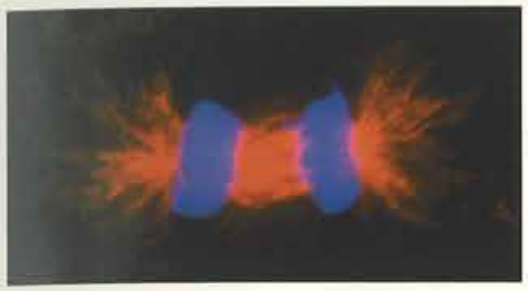
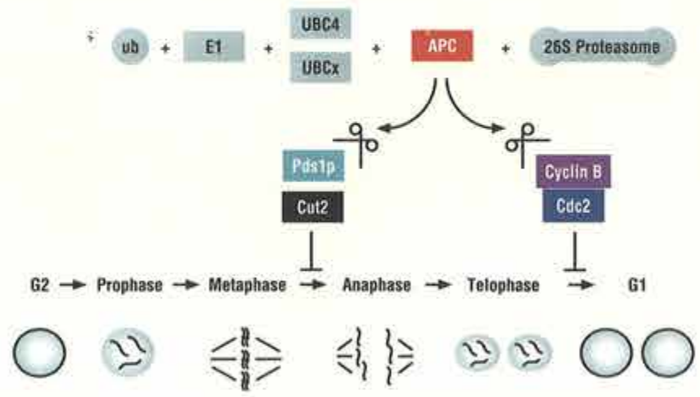
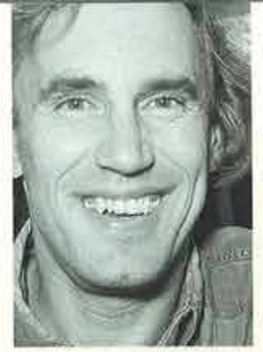


Figure 1
Micrograph of an isolated human anaphase spindle [stained in red] with attached chromosomes [stained in blue; micrograph by I. Waizenegger]. The onset of anaphase is controlled by the APC, as is discussed in the text.

How activation of the APC results in the initiation of anaphase is not understood in animal cells. Yeast genetics have identified the Cut1/Esp1 protein as an activator of anaphase which triggers dissociation of cohesin proteins from sister chromatids. APC is believed to activate this process by ubiquitinating an anaphase inhibitor bound to Cut1/Esp1, called Cut2/Pds1. To obtain insight into anaphase regulation in vertebrate cells, we have begun to characterize Xenopus and human homologs of Cut1/Esp1 and are searching for Cut2/Pds1-like proteins associated with them. We have also identified a chromatin-binding multi-subunit complex that contains homologs of yeast cohesins. We are analyzing the role of vertebrate Cut1/Esp1, cohesins and APC in anaphase control by cytological experiments in human cells and by using biochemical assays in Xenopus extracts which recapitulate mitotic events such as chromosome condensation, dissociation of cohesins from chromatin and sister chromatid separation.

Figure 2
Ubiquitin-dependent proteolysis mediated by the APC regulates two important transitions in mitosis. Proteins such as Pds1 in budding yeast and Cut2 in fission yeast need to be degraded to initiate the separation of sister chromatids at the metaphase-anaphase transition. Destruction of cyclin B by the same pathway is an important mechanism that helps to inactivate the protein kinase Cdc2, which is a prerequisite for forming daughter cells to exit from mitosis into the next interphase.





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Uta MÖHLE-STEINLEIN | Technician [5

[1] *until June;* [2] *since June*

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 Fos and Jun proteins and their roles as regulators of proliferation, differentiation and cell death in bone and hematopoietic development. Furthermore, we are aiming to define the specific function of EGF-receptor [EGFR] in neural and epithelial tissues and of VEGF/Flk-1 receptor signalling in bone and endothelial cells.

Fos proteins in bone cell differentiation

c-Fos is a key regulator of bone development. Transgenic mice expressing exogenous Fos develop bone tumors, whereas mice lacking c-Fos are osteopetrotic due to a differentiation block in bone resorbing osteoclasts. We are interested in the molecular mechanisms by which c-Fos and its related protein Fra-1, which is c-Fos inducible, control osteoblast proliferation and osteoclast differentiation. Fra-1 is an essential gene for mouse development and transgenic mice overexpressing Fra-1 develop the bone disease osteosclerosis, possibly due to increased osteoblast proliferation. However, Fra-1 can also induce osteoclast differentiation in the absence of c-Fos *in vitro* and *in vivo* [Figure 1]. This system is being used to identify c-Fos target genes and to better characterize the role of Fra-1 and NFAT transcription factors in controlling bone cell development and differentiation.

Jun proteins controlling cell proliferation and apoptosis

Both c-Jun and JunB proteins play key roles in development since inactivation of these genes in mice causes embryonic lethality. Mice lacking *jun*B die during early embryogenesis, mainly due to extra-embryonic defects, whereas c-*jun*-/- fetuses die at midgestation and exhibit liver defects.

To investigate the function of c-Jun in hepatocytes, c-*jun* was deleted in both the fetal and the adult liver, using the cre/loxP system. Surprisingly, c-*jun* does not seem to be required for liver development or function, but appears to be essential for liver regeneration. N-terminal phosphorylation by the c-Jun N-terminal kinases [JNKs] is an important mechanism regulating the activity of c-Jun. To define the function of c-Jun N-terminal phosphorylation [JNP] *in vivo*, we have generated mice carrying null mutations of JNK-1 and -2 [in collaboration with Michael Karin], as well as mice harbouring a mutant allele of c-*jun* lacking the JNK phosphoacceptor sites [*junAA*]. JNK1-/-, JNK2-/- and *junAA* mice are viable and fertile. However, both JNK1-/- and *junAA* mice are growth retarded, and fibroblasts from these mice show proliferation defects. JNK2-/- and *junAA* thymocytes are resistant to anti-CD3-induced cell death and mice lacking JNP are protected from kainate-induced epileptic seizures and neuronal apoptosis [Figure 2]. Thus, JNP mediated by distinct JNKs differentially regulates cell proliferation and death in different biological processes. Analysis of fibroblasts lacking c-*jun* revealed that c-Jun acts as a positive regulator of the cell cycle by suppressing the p53 pathway [in collaboration with Peter Angel]. In contrast, analysis of fibroblasts overexpressing *jun*B demonstrated that JunB is a negative regulator of proliferation by controlling the level of the CDK inhibitor $p16^{INK4a}$. Through genetic complementation experiments we discovered that JunB plays a key role in myeloid cell proliferation/differentiation since *jun*B null mice rescued by a *jun*B transgene develop chronic myeloid leukemia, probably due to the absence of *jun*B expression. A causal relationship between *jun*B expression, p16 levels and the deve-lopment of myeloid leukemias is presently being investigated in mouse and human cells.

Figure 1
Fra-1 induces tooth eruption and osteoclast formation in c-Fos-/- mice

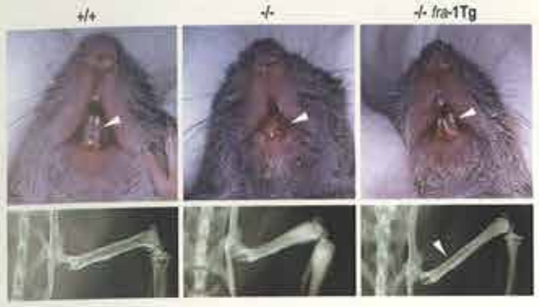

Figure 2
JNP is required for kainate-induced neuronal apoptosis [neurons are labelled in red, apoptosis is detected in green].

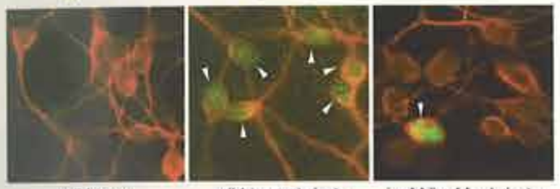

Figure 3
The K5-Sos transgene [right] rescues the viability and hair growth defects of EGF-Receptor -/- mice

EGF-receptor signalling in mice

Depending on their genetic background, EGFR null mice die at midgestation, birth or can live up to postnatal day 20 and display defects in brain and epithelial tissues such as skin. Different genetic approaches are being employed to dissect the downstream EGFR signalling pathways and to understand the molecular mechanisms responsible for these tissue-specific defects:

• The 'knock-in' of a wild-type 'floxed' human EGFR cDNA completely rescues the brain phenotype, thus prolonging the lifespan, but leads to the development of a heart hypertrophy, which is most likely caused by misexpression of the 'floxed' allele. • Expressing an activated form of Ras in postmitotic neurons also rescues neuronal apoptosis, but not the viability of EGFR knock-out mice. • Transgenic mice expressing an activated form of the human Son of Sevenless [hSOS] gene in the skin develop severe epidermal hyperplasias and papillomas. Overexpression of hSOS can partially rescue the skin defects, restore hair growth and prolong the lifespan of EGFR mutant mice [Figure 3]. Interestingly, the presence of a mutant EGFR allele attenuates the development of hSOS-dependent skin tumors. These results provide genetic evidence for cooperation between EGFR and SOS, not only during skin development but also during tumor formation.

Signalling in endothelial cells by VEGF/Flk-1 and PymT

The specific activity of the Polyoma middle T [PymT] oncogene to transform endothelial cells and to cause vascular tumors is being exploited to analyze the underlying growth control mechanisms. We have generated a conditional 'floxed' allele of the endothelial-specific growth factor receptor Flk-1 known to be essential for endothelial cell formation [vasculogenesis] to test whether Flk-1 is essential for the action of PymT oncogene. It appears that PymT can functionally replace Flk-1 and endothelial cells can be formed in its absence *in vitro*. Present studies will address the role of Flk-1 in tumor angiogenesis and in hematopoiesis, using conditional mouse model systems.

Anton BEYER | Scientist

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Gerald LÖFFLER | Postdoc
Bianca HABERMANN | PhD Student
Robert HOFFMANN | Diploma Student

Protein modelling

The main focus of our work concerns protein sequence and structure function relationships.

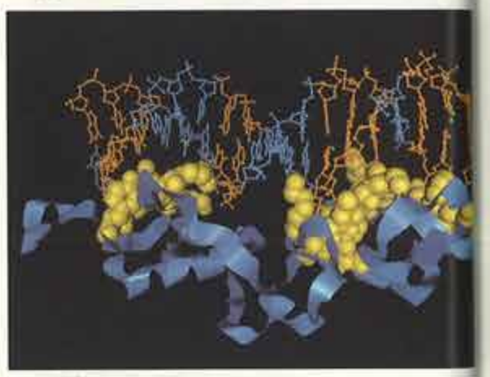
Electrostatic properties of proteins

Gerald Löffler solved the Poisson-Boltzmann equation for a system consisting of a low dielectric cavity [the protein] and a high dielectric environment for the surroundings. He has developed an algorithm to calculate the dielectric constant of a protein based on MD simulation results

Analysis of the interaction code for C4-zinc finger proteins and DNA

There are several DNA-binding motifs in proteins which have been characterized up to now, including helix-turn-helix motifs, basic region leucine zippers, and different classes of zinc fingers.
Random mutagenesis is used as a tool to study the protein/DNA-interaction of C4-zinc finger proteins. Using mutant variants of the Grlucocorticoid receptor [GR], the DNA-binding helix of the GR is randomly mutated, leaving out residues which are conserved throughout the family of the Nuclear Hormone Receptors. A functional assay in yeast, using beta-galactosidase and the green flourescent protein [gfp] as reporter genes is used to look for functional mutants in the pool of the protein library.

Figure 1
Side chains of the protein involved in DNA binding are shown in spacefill (yellow). The base pairs contacted by the protein are coloured in orange.



Three dimensional structure of the glucocorticoid receptor DNA-binding domain complexed with its response element.

Correlated mutations in the protein and the nucleic acid point toward physical interaction between the two binding partners.
In a second project, Bianca Habermann is developing statistical methods fot the analysis of gene expression experiments.
Robert Hoffmann has started to perform a statistical analysis of the amino acid composition of short pieces of sequences in relation to their structural preference in the whole protein.

Gotthold SCHAFFNER | Scientist

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Herbert AUER | Technician
Ivan BOTTO | Technician
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Karl MECHTLER | Technician
Christa CZAPKA | Technician Media Kitchen
Gabriele BOTTO | Technician Media Kitchen

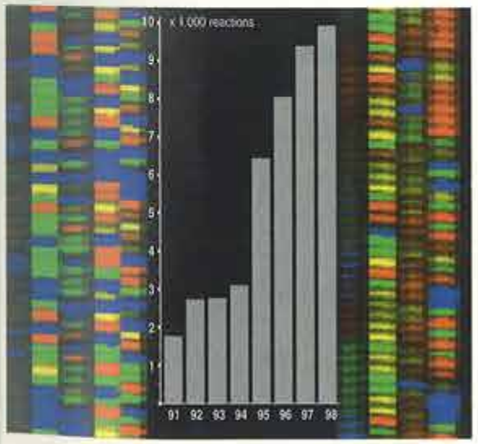
The Service Department offers a variety of high quality and rapid services to IMP scientists.

The bulk of our efforts involves DNA sequencing, oligonucleotide synthesis and peptide synthesis.

Our Media Kitchen staff prepare substantial quantities of reagent quality solutions and media for cell culture, which will expand this year to include media for 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 per year and last year we prepared approximately 2650 with an average size of 25 bases. In the first 9 months of 1998 we produced more than 2900 oligos, an increase of almost 50% as compared to 1997.
At the same time, the average length increased because of a much higher proportion of oligos >50 bases.



Peptide synthesis and production of monoclonal antibodies

Synthetic peptides have increased in importance to IMP research activities. This year we synthesized a great variety of oligopeptides, mainly coupled to PEG or KLH for immunization purposes.
This year we initiated the production service of monoclonal antibodies with fusion and selection in collaboration with IMP groups. We also continued to isolate and HPLC purify monoclonal antibodies from established hybridoma cell lines.

Sequencing and DNA isolation

With the two ABI PRISM 377 DNA sequencers, we sequenced approximately 7300 samples in the first 9 months of this year with an average reading length of 700-800 base pairs for 'good' DNA. This increase is mainly due to reproducible gel quality and the new ABI/Perkin-Elmer dye-deoxy-terminator [big-dye] kits and we are now saving time by introducing an easy and fast clean-up protocol for the sequencing reactions, using columns on 96-well microtiter plate format.
The protocol is readily adaptable to our robotic system. Sequencing of BACS and other artifical chromosomes is now established.

Figure 1
A sequencing run on ABI 377 PRISM and number of reactions done with dye deoxy terminators [scale 0 to 10'000] from 1991 to 1998.

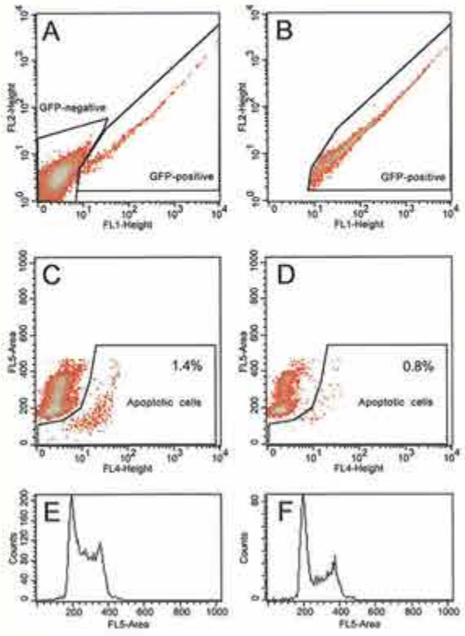
Group Members
Peter STEINLEIN | Staff Scientist
Karin PAIHA | Technician

The BioOptics department was founded in 1998. This unit provides various services in the field of flow cytometry, microscopy, imaging, and biosensor applications to scientists at the IMP.
Over the past years, research at the IMP showed increasing demands for flow-cytometric and microscopic applications. Many of these applications require profound knowledge of underlying principles and a thorough training for users. To facilitate access of scientists to these techniques, a service group was created which provides this expertise to all scientists at the institute. Besides training of users and maintenance of the equipment, development and implementation of novel techniques is a major goal of the unit.

Flow cytometry

The Flow Cytometry facility is equipped with two analytical flow cytometers and one preparative cytometer. The analytical machines are used by IMP members mainly for cell cycle analysis and immunophenotyping. Applications involving the use of the FACS Vantage are performed by staff members. A wide variety of applications has been established by our group including:

- Cell and organelle sorting
- Analysis of binding and internalization
- Analysis of apoptosis, especially of cells expressing GFP



Microscopy and imaging

Besides one micro-injection/time-lapse video microscope, three fluorescence microscopes equipped with cooled CCD-cameras [including one motorized microscope to obtain optical sections for deconvolution and 3D-reconstruction] and one Leica TCS NT laser scanning microscope are managed by the department. The microscopes are used by all scientists at the IMP after training provided by the BioOptics staff. Besides basic image processing, the BioOptics group provides deconvolution, 3D-reconstruction and fluorescence colocalization of microscopic images.

Biosensor applications

Two BIACore biosensors are currently used for the determination of rate constants of DNA-binding enzymes [in collaboration with G. C. M. Smith and S. P. Jackson, Wellcome/CRC, Cambridge, U. K.] and for the analysis of antibody-antigen interactions [in collaboration with C. Koch and H. Stockinger, University of Vienna].

Figure 1 TUNEL-assay of GFP-expressing cells
Panel A shows mouse erythroblasts co-transfected with a cdk4/6-inhibitor and EGFP. EGFP-positive cells used for analysis of apoptosis are shown in panel B. Panels C and D show DAPI staining of DNA [Y-axis] and Cy5-dCTP-staining of apoptotic cells [X-axis] for EGFP-negative cells [C] and EGFP-positive cells [D]. The cell cycle profiles of EGFP-negative cells [F] and EGFP-positive cells [E] exhibit a significant decrease of cells in S-phase of the cell cycle in the case of EGFP-expressing cells. Arrest of cells in G1/G0 results in a slightly reduced apoptotic index [in collaboration with J. Hofmann, Group Beug].

Group Member
Iris KILLISCH | Postdoc

EM facility at the IMP

The recently established electron microscopy service makes general EM techniques and specialized applications [e.g. EM-immunohistochemistry of frozen sections] available to groups at the IMP and Boehringer Ingelheim.

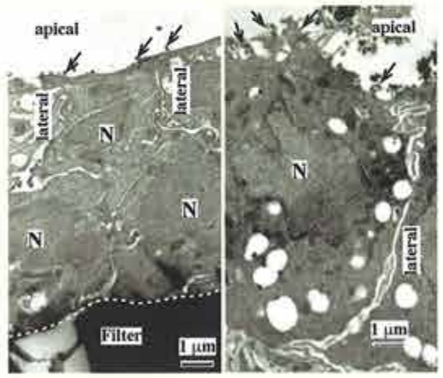
Service features

The EM facility offers its services via a cooperative and interactive approach. Projects and the necessary experimental approaches, as well as the input required from the group are discussed and determined with the scientists involved. Besides standard EM work, we establish new methods and design equipment not commercially available. Interested members of IMP groups may also be individually trained with respect to EM methods, in order to speed up project progress.

Figure 1
EM micrographs of cryo-sectioned polarized epithelial cells grown on filters [A], or in collagen gels [B]. Nuclei are indicated with N, Microvilli on the apical domain with arrows, apical and lateral membrane compartments are labelled accordingly. The white stipled line in [A] denotes the border of the filter.

EM projects: from yeast to animal cells

At the beginning of this year, projects in yeast [S. cerevisiae], worms [C. elegans], mice and animal cells were initiated. One major focus of the EM service was to analyze epithelial polarity and its loss in carcinogenesis. EM analysis showed that tight junctions formed by epithelial cells were disrupted after oncogenic transformation and could be restored by inhibiting the function of this oncogene. Furthermore, light-microscopical colocalization of two epithelial cell-regulatory proteins at the cytoplasmic face of the plasma membrane could be confirmed at the EM level by immunogold techniques. In all these cases, newly developed methods to obtain and process frozen sections from epithelial cells grown under *in vivo*-like conditions [porous membrane supports, collagen gels] were instrumental [Figure 1].



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Erwin F. WAGNER | Scientific Coordinator

Norma HOWELLS | Consultant

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Svetlana PEKEZ | Technician
Esther ZWICKELSDORFER | Technician

Animal house

The animal house provides technical support for the various research groups. It is divided into three main areas and contains the following species: mice, chicken and Xenopus. The largest and still expanding area is the mouse section. To cope with this expansion, during the summer of this year construction of an annex was completed, providing us with two rooms for housing chickens, a room for Drosophila, a multi-purpose room and an office. This enabled us to adapt the existing chicken rooms, which are in the main animal house facility, into mouse rooms, which will hold a further 3,500 mice in addition to the 10,000 already in-house. Breeding colonies, stock and experimental animals are maintained by the animal house staff. They include transgenic and knock-out mouse lines. To provide a constant supply of mice for the various projects, nineteen standard strains are routinely bred in-house. In addition, eighty eggs are produced each week from our 'closed-colony' chicken flocks.

The animal house staff also perform technical procedures for the research groups, such as collection of blood, implantation of tumors, and administration of substances by various routes, e.g. intravenous, intraperitoneal and subcutaneous injections. All procedures are performed to a high standard under appropriate anaesthetic regime and in conjunction with the necessary project licence.

The major research groups using animals are:

- Mouse studies: Hartmut Beug, Max Birnstiel, Meinrad Busslinger, Gerhard Christofori, Thomas Jenuwein, Annette Neubüser and Erwin Wagner
- Chicken studies: Hartmut Beug and Annette Neubüser
- *Xenopus* studies: Jan Peters
- Drosophila studies: Barry Dickson and Jürgen Knoblich

Hans-Christian THEUSSL | Technician

Mouse service department

The mouse service department was set up at the beginning of 1998 to cope with the increasing demand for mouse studies and the generation of transgenics. To enhance the use and production of this service, an additional injection facility was established. The main duties of this service unit are the injection of ES cells into blastocysts and of DNA into the pronucleus of fertilized mouse eggs. In addition, this service also provides the transfer of 'clean' embryos into our animal house as well as the freezing of embryos for preservation of specified mouse strains. To date, 30 different ES cell clones and several DNA constructs have been successfully injected for the four main research groups led by Meinrad Busslinger, Gerhard Christofori, Thomas Jenuwein and Erwin Wagner. The activities of this department are governed by an Animal User Committee, which meets monthly to set priorities and coordinate the duties. At present it is chaired by Erwin Wagner.

Members of the Scientific Advisory Board

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Dept. of Microbiology and Immunology, Univ. of California, San Francisco

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The Scientific Advisory Board

The SAB consists of internationally recognized scientists who are active in basic medical and biological research. They meet once a year and, together with the IMP scientists, discuss the quality and the significance of research findings as well as the main focus of future work at the IMP.

The IMP's working environment is characterized by a young and dynamic atmosphere. In accordance with the institute's philosophy, there is a high turnover rate among the scientific staff, including group leaders. As an employer, the IMP is in a position to offer young scientists just embarking on an independent research career the unique opportunity of setting up a lab without the administrative hurdles they usually have to face at this stage of their careers. Group Leaders' contracts are initially for 5 years but they are usually extended for a further 3 years to a total of 8 following review by the Scientific Advisory Board which meets annually.

Strong collaborative links have been established, both internationally and within Austria. From the very beginning of the IMP's existence, links to the University of Vienna have been especially close. 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 Biocenter', which is a recognized center of excellence in the biological sciences. The IMP also shares some of its facilities, such as the lecture hall, the library and an electron microscope, with the University. A joint international PhD Program has been established, which allows 10-15 students per year to participate and receive generous stipends. The aim of this program is to equip the students with all the necessary skills required for a career in molecular biology. Emphasis is not only placed on academic and technical excellence but also on communication. Students have organized their own weekly journal club and once a year present their data to the institute at one of the regular 'Monday seminars'. Presentation of research outside the institute is also encouraged and students are given the opportunity to participate in international conferences. The PhD program is advertised in a leading journal each autumn and students who apply are required to undergo a selection process. Following evaluation of the applications by a committee, 20 to 30 candidates are invited for interviews in February, after which the final decisions are made. This selection process guarantees high scientific standards and optimum training conditions. The IMP is particularly interested in maintaining the international and multicultural nature of the institute. At present, more than 20 nationalities from all continents are represented at the IMP.

Every year, the IMP organizes a large international conference which has already established itself as a highlight of the European scientific calendar. In 1999, the '10th IMP Spring Conference' will be held once again at Vienna's magnificent Imperial Palace. It will take place from 27th to 29th May and will focus on 'The Cell Biology of Cell Division'. Apart from this international conference, which attracts around 500 scientists, the IMP hosts a series of 'Thursday seminars' and 'Impromptu seminars' where top-ranking scientists are invited to present their latest data. These seminars generate lively interactions as well as introducing the IMP scientists to other fields which stimulate their own research.

The scientific staff is supported by a highly professional team of administrative and technical personnel. The collaborative and interactive nature of the IMP members has been encouraged from the beginning, and newcomers are rapidly integrated. Popular events in the social calendar include the bi-annual IMP skiing trip and joint hiking tours ['Wandertage']. Spontaneous lab outings to the nearby Alps are organised by skiing/climbing enthusiasts, which the IMP seems to attract. Other physical activities include regular games of soccer and volleyball, and the IMP also sponsors private sports initiatives.

Popular daily meeting places are the cafeteria, where members of staff exchange ideas over lunch or coffee, and 'Joe´s Disco', where various social events take place. There are always many reasons to celebrate during the year. Christmas parties are a highlight for IMP staff and their families, and the legendary fancy dress parties bring forth all the creativity and artistic talent of IMP members. Coupled with the special atmosphere of Vienna and the beautiful Alps, scientists can get the best of both worlds – work and pleasure.

Vienna is located along the river Danube, between the eastern edge of the Alps to the west and the plains of Central Europe to the east. This transitional character is reflected not only in its climate and vegetation, but also in the mentality of its inhabitants and its cultural life. In the past, Vienna was the capital city, not only of the Austrians, but also of the Czechs, Slovaks, Poles, Ruthenians, Ukrainians, Hungarians, Romanians, Serbs, Croats, Slovenians and Italians. During the 18th and 19th centuries it was the centre of the Habsburg dynasty, which controlled much of Central Europe for over 600 years. Vienna's cosmopolitan nature attracted great minds from all over Europe and it developed into a 'mecca of the arts'.

In this century, however, Vienna has had a tough time, deprived of its hinterland by World War I, then trapped on the edges of western Europe by the arbitrary divisions that followed the last war. The loss of many great artists and scientists left a gap which was painfully felt for a long time. However tradition-bound Austrians may be, they slowly developed a new awareness which derived from the country's identity as a neutral and independent republic. During the Seventies and Eighties a refreshingly inventive cultural scene established itself in Vienna. The collapse of the Iron Curtain all of a sudden placed the city back in the heart of Europe.

Today, Vienna has a population of just over 1.5 million and is Austria's political, economic, cultural and administrative center. It has acquired a role in world affairs as a neutral meeting place and is seen as a gateway to the East by many companies. Apart from its reputation as a popular venue for congresses, Vienna is the seat of a number of United Nations bodies. The presence of large international organizations has added a colorful, multicultural aspect to the city. American bookstores and exotic restaurants, bilingual schools and English radio programs are appreciated by Austrians and foreigners alike.

Following a long tradition, Vienna is also home to numerous scientific institutions. Within the city's boundaries are eight universities, of which the 'University of Vienna' is the biggest. Founded in 1365, it is the oldest university in the German-speaking world. The tradition-rich academic environment is complemented by the presence of a vibrant youth culture, largely attributed to the high number of student enrolments – about 130,000 in total were recorded in Vienna for 1997.

Tradition, culture and vitality are factors which contribute to the city's attractive lifestyle, with a high degree of social and moral freedom. The fact that Vienna ranks as one of the safest metropolitan areas in the world is a quality which is immediately apparent to visitors, though it is something the locals have long taken for granted.

Those who want to escape from city life are faced with many choices. A short drive or a somewhat longer boat-ride upstream will bring you to the Wachau – a romantic stretch of Danube between vine-bearing hills. Drive for a couple of hours and you are in the middle of the Austrian Alps, where glacier-carved lakes and craggy peaks offer a full range of summer and winter sports. An hour's drive south, bordering on Hungary, is the vast Neusiedl lake where the Viennese love to spend their summer-weekends surfing and sailing. Those with a distaste for crowds may want to turn north and explore the Waldviertel with its dense forests and hidden moors. The abundance of choice and opportunity in and around Vienna caters for every taste.

publications 1998

Group Beug

Bauer, A. / Mikulits, W. / Lagger, G. / Stengl, G. / Brosch, G. and Beug, H. [1998]. The thyroid hormone receptor functions as a ligand-operated, developmental switch between proliferation and differentiation of erythroid progenitors. EMBO J. 17 [15] 4291-4303.

Ciana, P. / Braliou, G.G. / Demay, F.G. / von Lindern, M. / Barettino, D. / Beug, H. and Stunnenberg, H.G. [1998]. Leukemic transformation by the v-ErbA oncoprotein entails constitutive binding to and repression of an erythroid enhancer *in vivo*. EMBO J., in press.

Dahl, R. / Kieslinger, M. / Beug, H. and Hayman, M.J. [1998]. Transformation of hematopoietic cells by the Ski oncoprotein involves repression of retinoic acid receptor signaling. Proc. Natl. Acad. Sci. USA, 95, 11187-11192.

Fuchs, B. / Wagner, T. / Rossel, N. / Antoine, M. / Beug, H. and Niessing, J. [1997]. Structure and erythroid cell-restricted expression of a chicken cDNA encoding a novel zinc finger protein of the Cys + His class. Gene 195, 277-284.

Mikulits, W. / Schranzhofer, M. / Bauer, A. / Dolznig, H. / Lobmayer, L. / Beug, H. and Müllner, E.W. [1998]. Translational Repression of Ferritin H subunit mRNA in primary erythroid progenitors: switch to iron-dependent ferritin biosynthesis by the v-ErbA oncoprotein. [Submitted].

Oft, M. / Heidler, K.H. and Beug, H. [1998]. TGFβ signalling is essential for carcinoma cell invasiveness and metastasis. Current Biology, in press.

Pereira, R. / Tran-Quang, C. / Lesault, I. / Dolznig, H. / Beug, H. and Ghysdael, J. [1998]. FLI-1 inhibits differentiation and induces proliferation of primary erythroblasts. Oncogene, in press.

Reichardt, H.M. / Kaestner, K.H. / Tuckermann, J. / Kretz, O. / Wessely, O. / Bock, R. / Gass, P. / Schmid, W. / Herrlich, P. / Angel, P. and Schütz, G. [1998]. DNA binding of the glucocorticoid receptor is not essential for survival. Cell 93, 531-41.

Schroeder, C. / Beug, H. and Müller-Esterl, W. [1997]. Cloning and functional characterization of the ornithokinin receptor. Recognition of the major kinin receptor antagonist, HOE140, as a full agonist. J. Biol. Chem. 272, 12475-12481.

van't Hof, R.J. / von Lindern, M. / Nijweide, P.J. and Beug, H. [1997]. Stem cell factor stimulates chicken osteoclast activity in vitro. Faseb J. 11, 287-293.

von Lindern, M. / Boer, L. / Wessely, O. / Parker, M. and Beug, H. [1998]. The AF-2 domain but not the DNA binding domain of the estrogen receptor is required to inhibit differentiation of avian erythroid progenitors. Mol. Endocrin. / 12 [2]: 263-277.

von Lindern, M. / Zauner, W. / Steinlein, P. / Mellitzer, G. / Fritsch, G. / Huber, K. / Löwenberg, B. and Beug, H. [1998]. Human erythropoiesis: The glucocorticoid receptor cooperates with the Epo-receptor and c-Kit to induce long-term proliferation of erythroid progenitors. [Submitted].

Wessely, O. / Bauer, A. / Tran Quang, C. / Deiner, E.M. / von Lindern, M. / Steinlein, P. / Ghysdael, J. and Beug, H. [1998]. A novel way to induce erythroid progenitor self renewal: cooperation of c-Kit with the erythropoietin receptor. Biol. Chem. [Hoppe-Seyler], in press.

Wessely, O. / Deiner, E.M. / Lim, K.C. / Mellitzer, G. / Steinlein, P. and Beug, H. [1998]. Mammalian GM-CSF receptor expressed in primary avian hematopoietic progenitors: Lineage-specific regulation of proliferation and differentiation. J. Cell. Biol. 141, 1041-51.

Group Busslinger

Busslinger, M. and Nutt, S.L. [1998]. Role of the transcription factor BSAP [Pax-5] in B-cell development. In Molecular Biology of B-Cell and T-Cell Development, [J.G. Monroe and E.V. Rothenberg, eds.]. Humana Press Inc. / Totowa, NJ, pp. 83-110.

Eberhard, D. and Busslinger, M. [1998]. The partial homeodomain of the transcription factor Pax-5 [BSAP] is an interaction motif for the retinoblastoma and TATA-binding protein. Cancer Res., in press.

Kozmik, Z. / Czerny, T. and Busslinger, M. [1997]. Alternative spliced insertions in the paired domain restrict the DNA sequence specificity of Pax-6 and Pax-8. EMBO J. 16, 6793-6803.

Macchia, P.E. / Lapi, P. / Krude, H. / Pirro, M.T. / Missero, C. / Chiovato, L. / Souabni, A. / Baserga, M. / Tassi, V. / Pinchera, A. / Fenzi, G. / Grüters, A. / Busslinger, M. and Di Lauro, R. [1998]. *PAX8* mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nature Genet. 19, 83-86.

Morrison, A.M. / Jäger, U. / Chott, A. / Schebesta, M. / Haas, O.A. and Busslinger, M. [1998]. Deregulated *PAX-5* transcription from a translocated *IgH* promoter in marignal zone lymphoma. Blood 92, 3865-3878.

Morrison, A.M. / Nutt, S.L. / Thévenin, C. / Rolink, A. and Busslinger, M. [1998]. Loss- and gain-of-function mutations reveal an important role of BSAP [Pax-5] at the start and end of B cell differentiation. Sem. Immunol. 10, 133-142.

Nutt, S.L. / Morrison, A.M. / Dörfler, P. / Rolink, A. and Busslinger, M. [1998]. Identification of BSAP [Pax-5] target genes in early B-cell development by loss- and gain-of-function experiments. EMBO J. 17, 2319-2333.

Nutt, S.L. / Vambrie, S. / Steinlein, P. / Rolink, A. / Weith, A. and Busslinger, M. [1998]. Allele-specific regulation of *Pax-5* [BSAP] during B cell development. [Submitted].

Pfeffer, P.L. / Gerster, T. / Lun, K. / Brand, M. and Busslinger, M. [1998]. Characterization of three novel members of the zebrafish *Pax2/5/8* family: dependency of *Pax5* and *Pax8* expression on the *Pax2.1* [noi] function. Development 125, 3063-3074.

Schwarz, M. / Alvarez-Bolado, G. / Urbánek, P. / Busslinger, M. and Gruss, P. [1997]. Conserved biological function between *Pax-2* and *Pax-5* in midbrain and cerebellum development: Evidence from targeted mutation. Proc. Natl. Acad. Sci. USA 94, 14518-14523.

Thévenin, C. / Nutt, S.L. and Busslinger, M. [1998]. Early function of Pax5 [BSAP] prior to the pre-B cell receptor stage of B-lymphopoiesis. J. Exp. Med. 188, 735-744.

Group Christofori

Burtscher, I. / Lamm, G. and Christofori, G. [1999]. A PI3-kinase-independent survival signaling pathway in β tumor cells. [Submitted].

Burtscher, I. and Christofori, G. [1999]. The IGF/IGF-1 receptor signaling pathway as potential target for cancer therapy [Review]. Drug Res. Update, in press.

Christofori, G. [1998]. The implications of angiogenesis on tumor invasiveness [News and Views]. Angiogenesis. 2, 21-23.

Christofori, G. and Semb, H. [1999]. The functional role of the cell adhesion miolecule E-cadherin as a tumor suppressor [Review]. Trends Biochem. Sci., in press.

Esni, F. / Täljedal, I.-B. / Perl, A.-K. / Cremer, H. / Christofori, G. and Semb, H. [1999]. Neural cell adhesion molecule [N-CAM] is required for cell type segregation in pancreatic islets of Langerhans. [Submitted].

Herzig, M. and Christofori, G. [1999]. An unexpected role for p53 in SV 40 T antigen-mediated transformation. Biol. Chem., in press.

Lamm, G.M. and Christofori, G. [1998]. Impairment of survival factor function potentiates chemotherapy-induced apoptosis in tumor cells. Cancer Res. 58, 801-807.

Lamm, G.M. / Steinlein, P. / Cotten, M. and Christofori, G. [1997]. A rapid, quantitative and inexpensive method for detecting apoptosis by flow cytometry in transiently transfected cells. Nucleic Acid Res. 25, 4855-4857.

Perl, A.-K. / Wilgenbus, P. / Dahl, U. / Semb, H. and Christofori, G. [1998]. A causal role for E-cadherin in the transition from adenoma to carcinoma. Nature 392, 190-193.

Perl, A.K. / Dahl, U. / Wilgenbus, P. / Cremer, H. / Semb, H. and Christofori, G. [1999]. Neural cell adhesion molecule [N-CAM] modulates the metastatic dissemination of pancreatic β tumor cells. [Submitted].

Semb, H. and Christofori, G. [1998]. The tumor suppressor function of E-cadherin [Review]. Am. J. Hum. Genet., in press.

Vlodavsky, I. and Christofori, G. [1998]. Fibroblast growth factors in tumor progression and angiogenesis. In: Angiogenic Agents in Cancer Therapy, [Teicher, B.A., ed.]. Humana Press Inc. / Totowa, NJ., in press.

Group Cotten

Baker, A. / Steinlein, P. and Cotten, M. [1998]. Stable gene expression in mammalian cells from large circular BACs bearing viral chromosome tethering sequences. [Manuscript submitted].

Cotten, M. and Wagner, E. [1998]. Receptor-mediated gene delivery strategies, in *The Development of Human Gene Therapy* [T. Friedmann,ed.]. Cold Spring Harbor Press, in press.

Cotten, M. / Saltik, M. and Baker, A. [1998]. Transfection complexes generated with adenovirus and polyethylenimine-condensed DNA. Methods in Molecular Biology; 21, 295-307. *Adenovirus Methods and Protocols*. [W.S.M. Wold, ed]. Humana Press Inc. / Totowa, N.J.

Diebold, S. / Lehmann, H. / Kursa, M. / Wagner, E. / Cotten, M. and Zenke, M. [1998]. Efficient Gene Delivery into Human Dendritic Cells by Adenovirus/Polyethylenimine [Ad/PEI] and Mannose/Polyethylenimine [ManPEI] transfection. [Manuscript submitted].

Glotzer, J. / Chiocca, S. / Michou, A.I. / Moseley, P. and Cotten, M. [1998]. The CELO virus anti-apoptotic protein Gam-1 modulates hsp70 expression and localization. [Manuscript submitted].

Lehrmann, H. and Cotten, M. [1998]. The avian adenovirus CELO encodes an Rb-binding, E2F-activating protein that is clearly distinct from the mastadenovirus E1a protein. [Manuscript submitted].

Michou, A.I. / Lehrmann, H. / Saltik, M. and Cotten, M. [1998]. A mutational analysis of the avian adenovirus CELO providing the basis for gene delivery vectors. Journal of Virology, in press.

Group Dickson

Dickson, B.J. [1998]. Photoreceptor development: Breaking down the barriers. Current Biology 8, R90-R92.

Dickson, B.J. [1998]. A roundabout way of avoiding the midline [News and Views]. Nature 391, 4412-4413.

Group Glotzer

Glotzer, M. [1997]. The mechanism and control of cytokinesis. Current Opinion in Cell Biology, 9, 815-824.

Group Huber

Böck, G. / Steinlein, P. and Huber, L.A. [1997]. Cell biologists sort things out: analysis and purification of intracellular organelles by flow cytometry. Trends in Cell Biol., 7, 499-503.

Böck, G. / Steinlein, P. / Haberfellner, M. / Gruenberg, J. and Huber, L.A. [1998]. Fluorescence activated sorting of endocytic organelles. In: Cell Biology: a laboratory handbook. [J.E. Celis, ed.]. Academic Press, San Diego, CA, 2nd Edition, Vol. 2, 63-69.

Fialka, I. / Oft, M. / Reichmann, E. / Huber, L.A. and Beug, H. [1998]. Three-dimensional organotypic growth of epithelial cells in reconstituted extracellular matrix. In: Cell Biology: a laboratory handbook. [J.E. Celis, ed.]. Academic Press, San Diego, CA, 2nd Edition, Vol. 1, 107-112.

Fialka, I. / Pasquali, C. / Kurzbauer, R. / Lottspeich, F. and Huber, L.A. [1998]. Loss of epithelial polarity is accompanied by differential association of proteins with intracellular membranes. Electrophoresis, in press.

Fialka, I. / Pasquali, C. / Lottspeich, F. / Ahorn, H. and Huber, L.A. [1997]. Subcellular fractionation of polarized epithelial cells and identification of organelle-specific proteins by two-dimensional gel electrophoresis. Electrophoresis, 18, 2582-2590.

Huber, L.A. and Simons, K. [1998]. Preparation and purification of post-golgi vesicles from perforated Madin-Darby Canine Kidney Cells. In: Cell Biology: a laboratory handbook. [J.E. Celis, ed.]. Academic Press, San Diego, CA, 2nd Edition, Vol. 1, 56-62.

Mosleh, I. / Huber, L.A. / Steinlein, P. / Pasquali, C. / Günther, D. and Meyer, T.F. [1999]. Neisseria gonorrhoeae porin modulates phagosome maturation, J. Biol. Chem., in press.

Pasquali, C. / Fialka, I. and Huber, L.A. [1997]. Preparative two-dimensional electrophoresis of membrane proteins. Electrophoresis 18, 2573-2581.

Pasquali, C. / Fialka, I. and Huber, L.A. [1998]. Subcellular fractionation, electromigration analysis and mapping of organelles. J. Chromatography B, in press

Scianimanico S. / Pasquali, C. / Lavoie, J. / Huber, L.A. / Gorvel, J.P. and Desjardins, M. [1997]. Two-dimensional gel electrophoresis analysis of endovacuolar organelles. Electrophoresis 18, 2566-2572.

Taylor, R.S. / Fialka, I. / Huber, L.A. and Howell, K.E. [1997]. 2D mapping of the endogenous proteins of the rat hepatocyte golgi complex cleared of proteins in transit. Electrophoresis 18, 2601-2612.

Vietor, I. and Huber, L.A. [1997]. In search of differentially expressed genes and proteins. Biochim. Biophys. Acta 1359, 187-199.

Group Jenuwein

Aagaard, L. / Laible, G. / Selenko, P. / Wolf, A. / Dorn, R. / Schotta, G. / Kuhfittig, S. / Lebersorger, A. / Reuter, G. and Jenuwein, T. [1998]. Functional mammalian homologues of the *Drosophila* PEV modifier *Su(var)3-9* encode centromere-associated proteins that can spread into chromosomal regions. EMBO J. [Submitted].

Jenuwein, T. / Laible, G. / Dorn, R. and Reuter, G. [1998]. SET domain proteins modulate chromatin domains in eu- and heterochromatin. Cell. Mol. Life Sci. 54, 80-93.

Laible, G. / Haynes, A. / Lebersorger, A. / O'Carroll, D. / Mattei, M.G. / Denny, P. / Brown, S.D.M. and Jenuwein, T. [1998]. The murine *Polycomb*-group genes *Ezh1* and *Ezh2* map close to *Hox* gene clusters on mouse chromosomes 16 and 6. Mamm. Genome, in press.

O'Carroll, D. / Lebersorger, A. / Laible, G. / Haynes, A. / Mattei, M.G. / Denny, P. / Brown, S.D.M. and Jenuwein, T. [1998]. Isolation and characterisation of *Suv39h2*, a second murine homologue of the *Drosophila* PEV modifier *Su(var)3-9*, in preparation.

Group Knoblich

Knoblich, J.A. [1997]. Mechanisms of asymmetric cell division during animal development. Curr Opin Cell Biol 9, 833-841.

Knoblich, J.A. / Jan, L.Y. and Jan, Y.N. [1997]. Asymmetric segregation of the *Drosophila* numb protein during mitosis: facts and speculations. Cold Spring Harb Symp Quant Biol 62, 71-77.

Knoblich, J.A. / Jan, L.Y. and Jan, Y.N. [1997]. The N terminus of the *Drosophila* Numb protein directs membrane association and actin-dependent asymmetric localization. Proc. Natl. Acad. Sci. USA 94, 13005-13010.

Shen, C.P. / Knoblich, J.A. / Chan, Y.M. / Jiang, M.M. / Jan, L.Y. and Jan, Y.N. [1998]. Miranda as a multidomain adapter linking apically localized inscuteable and basally localized Staufen and Prospero during asymmetric cell division in *Drosophila*. Genes Dev 12, 1837-1846.

Group Nasmyth

Ciosk, R. / Zachariae, W. / Michaelis, C. / Shevchenko, A. / Mann, M. and Nasmyth, K. [1998]. An Esp1/Pds1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell, 93, 1067-1076.

Shirayama, M. / Zachariae, W. and Nasmyth, K. [1998] The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae. EMBO J. 17, 1336-1349.

Tanaka, T. and Nasmyth, K. [1998]. Association of RPA with chromosomal replication origins requires an Mcm protein, and is regulated by Rad53, and cyclin- and Dbf4-dependent kinases. EMBO J., 17, 5182-5191.

Uhlmann, F. and Nasmyth, K. [1998]. Cohesion between sister chromatids must be established during DNA replication. Current Biology, 8, 1095-1101.

Zachariae, W. / Shevchenko, A. / Andrews, P.D. / Galova, M. / Stark, M.J.R. / Mann, M. and Nasmyth, K. [1998]. Mass spectrometric analysis of the anaphase promoting complex from yeast: Identification of a subunit related to cullins. Science 279, 1216-1219.

Zachariae, W. / Schwab, M. / Nasmyth, K. and Seufert, W. [1998]. Control of cyclin ubiquitination by CDK-regulated binding of HCT1/CDH1 to the anaphase promoting complex. Science, in press.

Group Neubüser

Neubüser, A. / Peters, H. / Balling, R. and Martin, G.R. [1997]. Antagonistic interactions between FGF and BMP signaling pathways: A mechanism for positioning the sites of tooth formation. Cell 90, 247-255.

Peters, H. / Neubüser, A. / Kratochwil, K. and Balling, R. [1998]. *Pax-9* deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. Genes Dev. 12, 2735-2747.

Group Peters

Grossberger, R. / Gieffers, C. / Zachariae, W. / Schleiffer, A. / Nasmyth, K. and Peters, J.-M. [1998]. Characterization of the APC10/DOC1 subunit of the yeast and the human anaphase-promoting complex. [Submitted].

Kramer, E.R. / Gieffers, C. / Hölzl, G. / Hengstschläger, M. and Peters, J.-M. [1998]. Activation of the human anaphase promoting complex by proteins of the CDC20/Fizzy family. Current Biology 8, 1207-1210.

Peters, J.-M. [1998]. SCF and APC: the Yin and Yang of cell cycle regulated proteolysis. Curr. Op. Cell Biol. 10, 759-768 [Review].

Peters, J.-M. / King, R.W. and Deshaies, R.J. [1998]. Cell cycle control by ubiquitin-dependent proteolysis. In Peters, J.-M. / Harris, J.R. and Finley, D. [ed.]. Ubiquitin and the biology of the cell, Plenum Press, 345-387 [Book chapter].

Vorlaufer, E. and Peters, J.-M. [1998]. Regulation of the cyclin B degradation system by an inhibitor of mitotic proteolysis. Mol. Biol. Cell 9, 1817-1831.

Yu, H. / Peters, J.-M. / King, R.W. / Page, A.M. / Hieter, P. and Kirschner, M.W. [1998]. Identification of a cullin homology region in a subunit of the anaphase-promoting complex. Science 279, 1219-1222.

Group Wagner

Behrens, A. / Sibilia, M. and Wagner, E.F. [1998]. N-terminal phosphorylation of c-Jun regulates neuronal apoptosis and cellular proliferation. [Submitted].

Bennet, D.C. / Trayner, I.D. / Piao, X. / Easty, D.J. / Klüppel, M. / Alexander, W.S. / Wagner, E.F. and Bernstein, A. [1998]. Recessive spotting: a linked locus that interacts with W/kit but is not allelic. Genes to Cells 3, 235-244.

Burkart, V. / Wang, Z.Q. / Radons, J. / Heller, B. / Stingl, L. / Wagner, E.F. and Kolb, H. [1998]. Mice lacking the poly[ADP-ribose] polymerase gene are resistant to diabetes development. [Submitted].

De Graeve F. / Bahr, A. / Sabapathy, K. / Wagner, E.F. Hauss, C. / Kedinger, C. and Chatton, B. [1998]. Role of the ATFa/JNK2 complex in Jun activation. [Submitted].

Matsuo, K. / Owens. J.M. / Tonko, M. / Elliot C. / Chambers, T.J. and Wagner, E.F. [1998]. Osteoclast differentiation by the c-Fos target gene Fra-1. [Submitted].

Morrison, C. / Smith, G.C. / Stingl, L. / Jackson, S.P. / Wagner, E.F. and Wang, Z.Q. [1997]. Genetic interaction between PARP and DNA-PK in V[D]J recombination and tumorigenesis. Nature Genetics 17, 479-482.

Olmsted, E.A. / Gannon, F.H. / Wang, Z.Q. / Grigoriadis, A.E. / Wagner, E.F. / Zasloff, M.A. / Shore, E.M. and Kaplan, F.S. [1998]. Embryonic Over-Expression of the c-Fos Proto-Oncogene: A Murine Stem Cell Chimera Applicable to the Study of Fibrodysplasia Ossificans Progressive in Humans. Clinical Orthopaedics and Related Research 346, 81-94.

Owens, J.M. / Matsuo, K. / Nicholson, G.C. / Wagner, E.F. and Chambers, T.J. [1998]. Fra-1 stimulates osteoclastic differentiation and function in bipotential osteoclast-macrophage precursors, under revision.

Sabapathy, K. / Hu, Y. / Kallunki, T. / Schreiber, M. / David, J.P. / Wagner, E.F. and Karin, M. [1998]. JNK2 is required for efficient T cell activation but not for normal lymphocyte development. [Submitted].

Schorpp-Kistner, M. / Wang, Z.Q. / Angel, P. and Wagner, E.F. [1998]. JunB is Essential for Mammalian Placentation, EMBO J. in press.

Schreiber, M. / Kolbus, A. / Piu, F. / Tian, J. / Möhle-Steinlein, U. / Karin, M. / Angel, P. and Wagner, E.F. [1998]. Control of cell cycle progression by c-Jun is p53 dependent, under revision.

Sibilia, M. / Steinbach, J.P. / Stingl, L. / Aguzzi, A. and Wagner, E.F. [1998]. A strain-independent postnatal neurodegeneration in mice lacking EGF-receptor. EMBO J. 17, 719-731.

Tropepe, V. / Sibilia, M. / Ciruna, B.G. / Rossant, J. / Wagner, E.F. and van der Kooy, D. [1998]. Distinct Neural Stem Cells Proliferate in Response to EGF and FGF2 in the Developing Mouse Telencephalon, Dev. Biology. [Submitted].

Wagner, E.F. [1998]. Oncogene Function in Normal Development and Disease. OECD Proceedings, 201-207.

Computer Group

Löffler, G. [1998]. A Multithreaded Java Framework for Solving Linear Elliptic Partial Differential Equations in 3D, Lecture Notes in Computer Science 1343, 121-128.

Service Department

Breiteneder-Geleff, S. / Matsui, K. / Soleiman, A. / Meraner, P. Poczewski, H. / Kalt, R. / Schaffner, G. and Kerjaschki, D. [1997]. Podoplanin, novel 43-kd membrane protein of glomerular epithelial cells, is down-regulated in puromycin nephrosis. Am. J. Pathol. 151, 1141-1152.

Buschle, M. / Schmidt, W. / Berger, M. / Schaffner, G. / Kurzbauer, R. / Killisch, I. / Tiedemann, J.-K. / Trska, B. / Kirlappos, H. / Mechtler, K. / Schilcher, F. / Gabler, C. and Birnstiel, M.L. [1998]. Chemically defined, cell-free cancer vaccines: use of tumor antigen-derived peptides or polyepitope proteins for vaccination. Gene Ther. Mol. Biol. 1, 309-321.

Mayr, B. / Reifinger, M. / Alton, K. and Schaffner, G. [1998]. Novel p53 tumour suppressor mutations in cases of a spindle cell sarcoma, pleomorphic sarcoma and fibrosarcoma in cats. Vet.Res.Communications 22, 249-255.

Ogris, M. / Steinlein, P. / Kursa, M. / Mechtler, K. / Kircheis, R. and Wagner, E. [1998]. The size of DNA/transferrin-PEI complexes is an important factor for gene expression in cultured cells. Gene Therapy 5, 1425-1433.

Onyango, P. / Lubyova, B. / Gardellin, P. / Kurzbauer, R. and Weith, A. [1998]. Molecular cloning and expression analysis of five novel genes in chromosome 1p36. Genomics 50, 187-198.

Richardson, V.J. / Botto, I. and Scriba, M. [1997]. Reinfection of guinea pigs with herpes simplex virus type2: failure to establish a second latent ganglionic infection. Can. J. Microbiol. 33, 679-683.

seminar speakers at the IMP 1998

January
12. Norbert KRAUT – *Seattle*
13. Annette NEUBÜSER – *UCSF*
19. Barry DICKSON – *Univ. Zürich*
26. Antonio IAVARONE – *Mem. Sloan-Kettering*
27. Elly TANAKA – *Ludwig Inst. f. Cancer Res.*
28. Elisa IZZAURALDE – *Geneva*
29. Alain VERREAULT – *Cold Spring Harbor, NY*

February
11. Anindya DUTTA – *Harvard Medical School*
12. Peter MOMBAERTS – *Rockefeller Univ.*

March
02. Isabella GRAEF – *Stanford Med. School*
03. Dirk GÖRLICH – *ZMBH, Heidelberg*
04. Zsuzsanna IZSVAK – *Netherlands Cancer Inst.*
09. Andreas TRUMPP – *UCSF*
10. Marta MUZIO – *Mario Negri Inst., Milano*
11. Marek MLODZIK – *EMBL*
12. David ALLIS – *Univ. Rochester*
19. Charles STREULI – *Univ. Manchester*
26. Susan GASSER – *ISREC*

April
02. Elisabeth GEORGES-LABOUESSE – *Strasbourg*
14. Andrew MURRAY – *USCF*
15. Tom JESSELL – *Columbia Univ.*
16. Brenda BASS – *Univ. Utah*
21. Gerard KARSENTY – *Houston*
23. Wilhelm KREK – *Friedrich Miescher Institut, Basel*
23. Keith ROBERTS – *John Innes Institute, Norwich*
24. Reinhard KOFLER – *Univ. Innsbruck*

May
07. Hiroshi YOSHIKAWA – *Nara Inst. of Science & Technology*
11. Selina CHEN-KIANG – *Cornell Univ.*
13. Denis DUBOULE – *Univ. Geneva*
15. Aron MARCHLER-BAUER – *NCBI, Bethesda*

June
04. Warren GRAHAM – *ICRF, London*
08. Toshio SUDA – *Kumamoto Univ.*
10. Joachim HERZ – *Univ. Texas, Dallas*
18. Wolfgang WURST – *MPI Munich*
23. Guido BOEHMELT – *Univ. Toronto*
26. Willy HOFSTETTER – *Univ. Bern*

July
02. Heiner WESTPHAL – *NIH, Bethesda*
03. Wongi MIN – *Taejon, Korea*
06. YONGWON CHOI – *Rockefeller Univ.*
07. Michelle DESJARDINS – *Univ. Montreal*
14. Gordon KELLER – *National Jewish Center, Denver*
21. Mercedes RINCON – *Univ. of Vermont*

August
07. Sasha TARAKHOVSKY – *Univ. Köln*

September
04. David CAPELLEN – *CNRS, Paris*
10. Larry GERACE – *Scripps Res. Inst.*
21. Napoleone FERRARA – *Genentech, Inc.*
22. Mike SYVANEN – *Univ. California, Davis*
24. Nic JONES – *ICRF*

October
01. Joel BELASCO – *Skirball Institute, NY*
06. Giora SIMCHEN – *Hebrew Univ., Jerusalem*
    Titia DE LANGE – *Rockefeller Univ.*
15. Ari HELENIUS – *ETH Zurich*
16. Olivier GANDRILLON – *Lyon*
    Andrea MUSACCHIO – *Harvard Univ., Boston*
20. Pope MOSELEY – *Univ. of New Mexico*
    Doris WEDLICH – *Univ. Ulm*
22. Katia GEORGOPOULOS – *Mass. Gen. Hospital*
23. James IHLE – *St. Jude's Children's Research Hospital*
27. Julian DOWNWARD – *ICRF, London*
28. Robert KINGSTON – *Mass. General Hospital*

November
02. Michael McKAY – *Univ. Melbourne*
06. Pierre GONCZY – *EMBL*
11. Markus HENGSTSCHLÄGER – *AKH, Vienna*
12. Pascale COSSART – *Inst. Pasteur*
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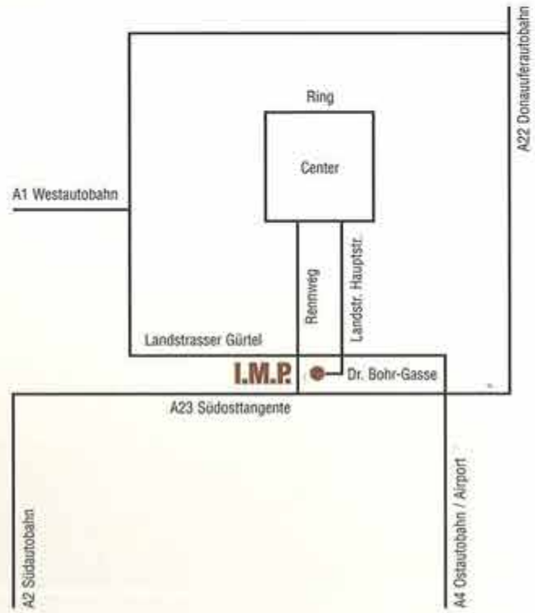
24. Randall JOHNSON – *San Diego*
26. Christoph ENGLERT – *Karlsruhe*

December
03. Karl O. STETTER – *Univ. Regensburg*
04. Thomas HAAF – *MPI, Berlin*
10. David M. LIVINGSTON – *Dana Farber Cancer Inst.*
11. Alain PROCHIANTZ – *CNRS, Paris*
17. Tamas KISS – *CNRS, Toulouse*

Where we are



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Netrins (purple) guide commissural axons towards the midline of the Drosophila central nervous system, forming the rungs in the ladder-like structure of nerve fibres (brown).
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