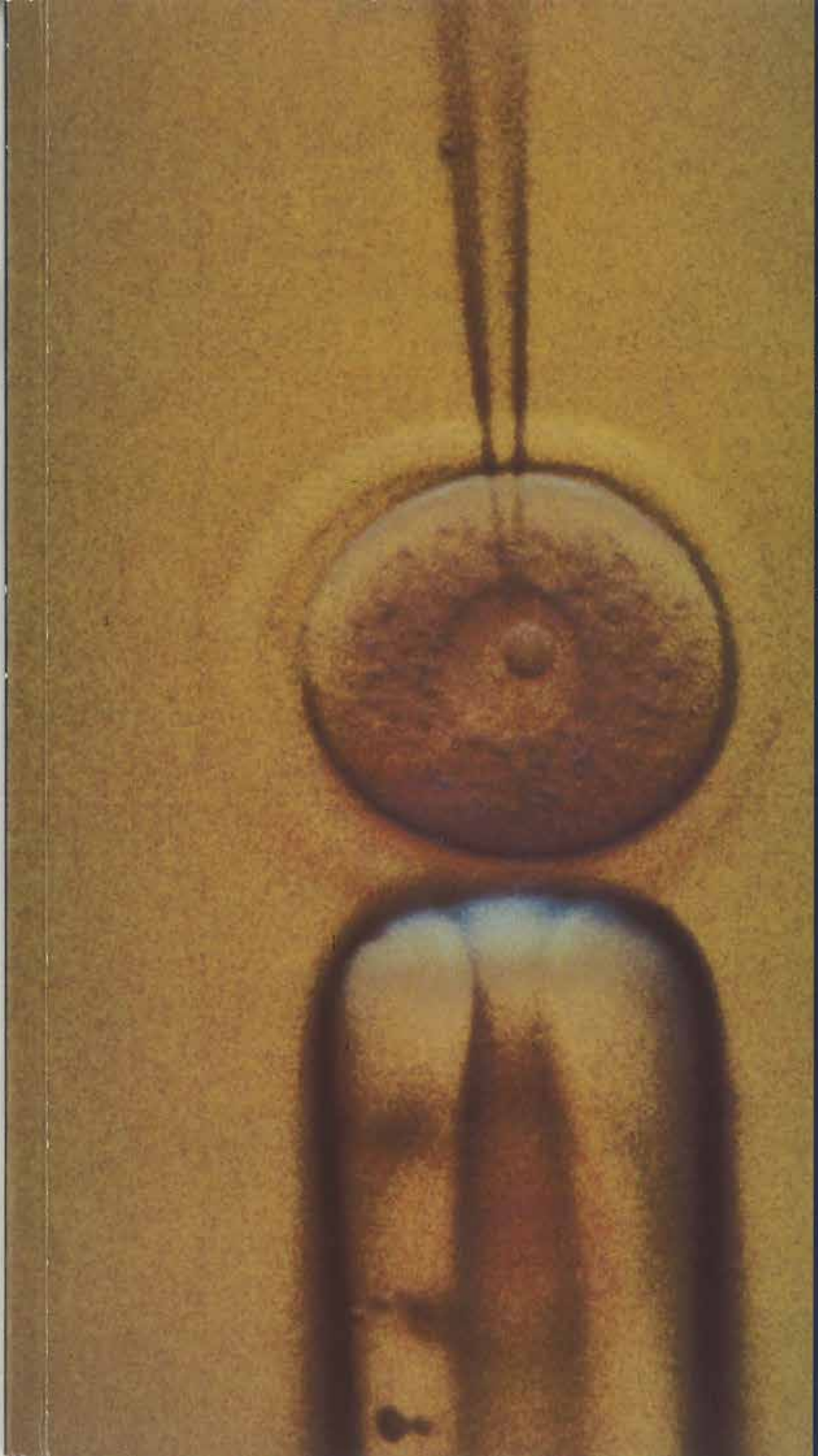

Research Institute of Molecular Pathology
VIENNA BIOCENTER

I.M.P. '97

BOEHRINGER INGELHEIM

The IMP is a basic research institute supported by the research oriented German pharmaceutical firm 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 programmes. 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 programme and both library and lecture hall facilities. The IMP and these five university institutes now form what is known as the Vienna Biocenter.

The IMP was founded, planned, and subsequently directed for ten years by Max Birnstiel, who retired at the end of 1996. The institute contains a dozen research groups, four run by senior scientists with longterm contracts and eight 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 about 130, among them 25 technicians, 37 PhD students, and 31 postdocs (11 with external stipends). 20 different nationalities are represented at the IMP, our working language is English, and our annual budget (including external grants) is about US $ 15 million.

There have been several changes at the IMP this year: I took over from Max Birnstiel as the new director, Erwin Wagner became deputy director, two new group leaders (Michael Glotzer and Jürgen Knoblich) joined us, and Andreas Weith left us for a post as head of a genomics group with BI. Meanwhile, the constellation of our SAB was changed in that Bob Weinberg, who had been with us from the very beginning, retired from the board. In-house changes this year include the establishment of a new 'biooptics' service run by Peter Steinlein. This group will deal with cell sorting and fluorescence microscopy, whilst Iris Killisch will run the newly-formed electron microscopy service. Both of these additions reflect our increasing demand for state of the art cell biological techniques.

The molecular biological sciences are currently at the crossroads between a past that was almost entirely 'problem' oriented and a future in which an increasing amount of information will be collected by 'systematic' means. Genomic sequencing is a good example of the new 'systematic' approach to biology. It is fast being joined by the systematic description of gene expression patterns and by the description of phenotypes produced by systematic gene 'knock outs'. This does not mean, however, that problem oriented research is on the way out. Far from it! Just as the systematic description of species facilitated experimental and evolutionary biology during the 19th century, so will the sequencing of genomes and description of their expression patterns help but not replace experimental studies in the 21st century. The 'devil will remain in the details'. I suspect therefore that research at the IMP will remain largely experimental in nature for some time to come.

One of the revelations of the last decade has been the realization of how conserved biological mechanisms are within eukaryotic cells. For this reason, processes that occur in human cells can sometimes best be studied by analysing mice, chicken, frogs, flies, worms, or even yeast. We now have groups studying all these 'model' organisms. The IMP's skills with major experimental model systems therefore complement those of research programmes within BI, which are directly concerned with drug discovery and the alleviation of human disease. The quest to unravel how human beings develop from fertilized eggs and fend off disease for three score years and ten is still in its early phases, but tools of unparalleled power are now available to those curious to discover how it all works. Exciting times lie ahead.

Prof. Kim Nasmyth, F.R.S. Vienna, December 1997

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Cellular malfunction in oncogenesis

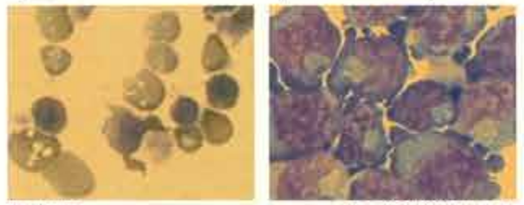
In tumors, oncoproteins cooperate to upset the balance between cell proliferation, differentiation and/or programmed cell death. In avian leukemia, we analyse mechanisms how endogenous or oncogenic receptors for growth factors/ cytokines and steroid hormones cooperate to regulate or disturb this balance. In breast carcinoma, we try to understand, how co-signaling from two different membrane receptor families alters epithelial cell polarity and -plasticity, both in metastasis and normal mammary gland development.

Proliferation versus differentiation in erythroleukemia and normal erythropoiesis

Two oncoproteins (v-ErbB, v-ErbA) cooperate to induce fatal erythroleukemia in chicks. V-ErbB represent a mutated, constitutively active receptor tyrosine kinase (EGF-receptor). V-ErbA is a mutated, ligand-independent thyroid hormone receptor (TRα/c-ErbA) that is 'frozen' in a constitutively repressing state. Members of the same two gene families, i.e. receptor tyrosine kinases and nuclear hormone receptors, regulate the balance between proliferation and differentiation in normal erythropoiesis. In human, murine and avian erythroblasts, the stem cell factor (SCF) receptor (c-Kit) cooperates with the erythropoietin (Epo) receptor to enhance progenitor proliferation without affecting differentiation, increasing the number of erythrocytes produced per progenitor. When c-Kit and EpoR cooperate with a member of the nuclear hormone receptor family, the glucocoorticoid receptor (GR), cells proliferate and are arrested in differentiation (Figure 1).

In chicks, these 'self-renewing' normal proerythroblasts are similar to leukemic cells, when exposed to all ligands (Epo, SCF and Dex), but differentiate (or die) upon ligand removal. In contrast, the leukemic cells are rendered factor independent by their constitutively active oncoproteins. Both v-ErbA and overexpressed TRα/c-ErbA in the absence of any ligand substitute for the GR to stimulate proliferation and arrest differentiation. Both proteins also require cooperation with c-Kit for biological activity (Figure 2). We currently try to identify molecular mechanisms relevant for this cooperation between receptor tyrosine kinases and nuclear hormone receptors, such as relevant signal transduction pathways (Stat 5b ?), differential cell cycle regulation in proliferating/ leukemic and differentiating primary erythroblasts (e.g. loss or retainment of cdk4/D-cyclin function ?) and target genes regulated by the GR, v-ErbA and nonliganded c-ErbA (e.g. CAII, c-myb, screening for unknown ones). We want to assess the *in vivo* relevance of the above (proto)oncogene cooperations, preferably by using genetically modified mice. Finally, we study the function and cooperation of murine and human leukemia oncoproteins, using primary avian cells as model systems.

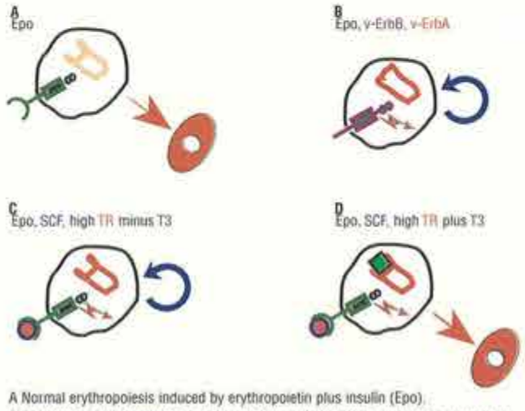
Figure 1 p53 -/- mouse erythroblasts



Epo, Insulin | Epo, IGF-1, SCF, Dexameth.

Figure 2

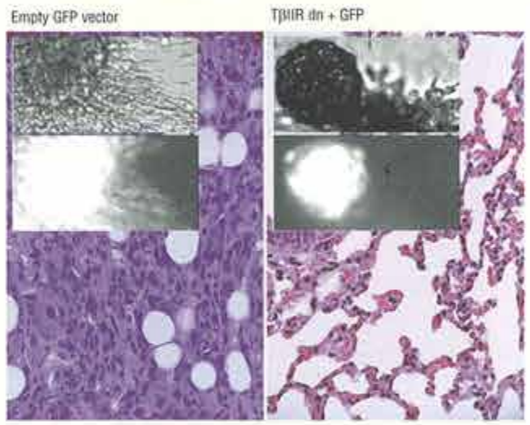
TRα/c-ErbA: A molecular switch regulating proliferation versus differentiation



A Normal erythropoiesis induced by erythropoietin plus insulin (Epo).
B Proliferation and differentiation arrest (blue circular arrow) caused by cooperation of v-ErbB(pink) and v-ErbA (red).
C Proliferation plus differentiation arrest in normal cells: cooperation of liganded c-Kit and overexpressed TRα/c-ErbA minus ligand (T3).
D Minute levels of T3 (10-11M) stop proliferation and induce differentiation in cells identical to those in C.

Figure 3

Dominant negative TGFβ receptor: Inhibition of in vitro invasiveness and metastasis



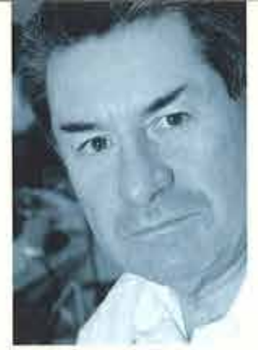
Mouse colon carcinoma cells (CT3) were infected with green fluorescent protein (GFP) expressing retroviruses containing no extra gene (empty vector) or dominant-negative TGFβ-receptor II (TβRIIdn) and seeded into collagen gels (insets). Note that the TβRIIdn expressing cells fail to invade the collagen gel. The same cell preparations were sorted for GFP fluorescence and 1x10⁶ cells injected into mice. Control cells form metastases within 4 days (left panel) whereas TβRIIdn expressing cells are unable to form metatstases even after several months (right panel).

Molecules inducing loss of epithelial polarity and causing epithelial-mesenchymal transition.

Carcinomas (≈80% of all human neoplasms) are solid epithelial cell tumors. Epithelial cells are stricty polarized, accounting for the protective barrier function of epithelia. In tumors, epithelial cells may lose polarity and show aspects of mesenchymal cells, for instance during metastasis. These complex alterations are generated by cooperation of multiple dominant oncogenes and tumor suppressor genes. We focus on oncogene-induced epithelial-mesenchymal transition (EMT) i.e.polarized epithelial cells aquiring behavioral and molecular aspects of motile, mesenchymal cells.

In our mouse tumor model, activated Ras cooperates with the endogenous TGFβ receptor, inducing polarized mammary epithelial cells to lose epithelial polarity and undergo EMT. After EMT, the fibroblastoid tumor cells produce their own TGFβ, maintaining EMT via an autocrine loop. On its own, Ha-Ras has little effect on epithelial polarity. The liganded TGFβR is required for mammary gland development, but causes growth arrest and apoptosis at high ligand concentrations.

Using a dominant-negative, kinase-dead version of the TGFβ-receptor subunit II (TβRIIdn), we show that TGFβ-activated signals are required for EMT, tumorigenesis and metastasis. Overexpression of TβRIIdn in various murine and human tumor cells prevented invasiveness in vitro and caused reversion of EMT. In vivo, expression of TβRIIdn retarded tumor growth and prevented metastasis (Figure 3). Present and future studies in this system will concentrate on signal tranduction pathways utilized by the TGFβR to induce EMT and how such pathways may interlock with Ras-MapK-pathways. In addition, we plan to analyse the contribution of tumor suppressor genes and steroid hormone receptors to mammary carcinogenesis, preferentially by using genetically modified mice.



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Chemically defined, cell-free, generic tumor vaccines

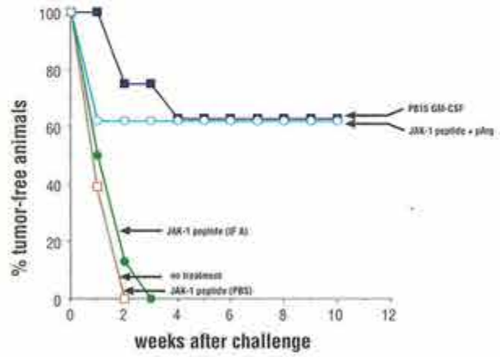
Novel therapies are needed for cancer. It is estimated that in 1997 more than 560,000 patients will die from cancer in the USA alone. Despite tremendous efforts to improve cancer treatment, the overall mortality rate of cancer has increased from 1973-1994 by 5.4% in the USA (SEER cancer report). It is thus not surprising that the National Cancer Institute of the USA predicts that cancer may very soon become the leading fatal illness in the USA.

Recent molecular and immunological investigations have revealed the existence of tumor antigens in several tumor types and this has lead to the conclusion, that perhaps all, but at least most, tumors are indeed immunogenic, but that the response of the immune system to tumor antigens is inadequate and in need of enhancement if immunotherapy of cancer is to be attempted.

We and others have shown that first generation tumor vaccines consisting of cytokine gene-modified, autologous whole tumor cells are exceptionally effective in relevant animal models. Here, the autologous cells serve as the source of tumor antigens whereas the cytokine locally secreted after transfection induces potent immune responses. Such a first generation vaccine was used in a Boehringer Ingelheim phase I clinical trial lead by Professor G. Stingl, Vienna and Professor G. Bröcker, Würzburg. Despite the very much advanced disease of the patients treated, it appeared that encouraging immune responses against parental cancer cells or in a few cases even transient stabilization of the disease was observed. The major drawback of this type of vaccine, however, was that autologous (the patient's own cells) needed to be prepared and gene modified. It soon became evident that for several reasons functional vaccine could only be produced for 2/3 of the patients and that vaccine production was cumbersome, expensive and virtually impossible to standardize.

To circumvent the problems associated with first generation cancer vaccines we sought to identify potent immune modulators (adjuvants) allowing vaccination with synthetic tumor antigen derived peptides or recombinant tumor antigen(s) themselves. The steadily increasing number of newly discovered tumor antigens, and the short petides recognized by CTLs contained within the tumor antigen protein sequence, has made chemically defined, peptide-based cell-free vaccines a possible alternative to cellular vaccines. However, these short peptides administered on their own do not induce adequate immune responses and standard adjuvants commonly used in vaccination procedures are not effective.

Figure 1
Naive DBA/2 mice were vaccinated three times at weekly intervals followed by a challenge with 2 x 10⁴ viable P815 tumor cells one week after the last vaccination. For immunizations the peptide SYFPEITHI derived form the tyrosine kinase Jak-1 (100 µgs per mouse) was injected either dissolved in PBS, emulsified with incomplete Freund's adjuvant (IFA) or in conjunction with polyarginine (pArg).



Peptide vaccination protects animals from tumor growth.

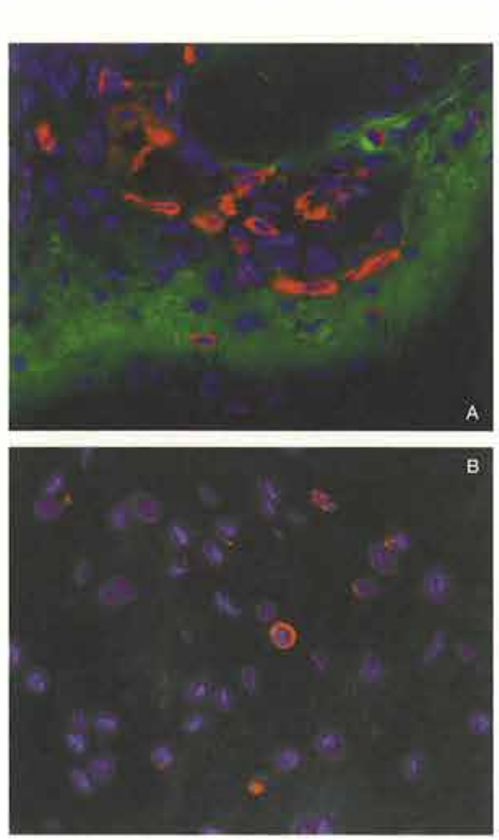


Figure 2
Photomicographs of injection sites. DBA/2 mice were injected with a mixture of a fluorescein-tagged, MHC class I binding peptide (GYKDGNEYI; =green signal) and polyarginine (A) or with fluorescein-labelled peptide alone (B). Three days after administration of peptides injection sites were excised and fixed cryosections stained with an antibody recognizing MHC class II (=red signal). Nuclei were counterstained with DAPI (blue signal)

Antigen presenting cells heavily infiltrate the peptide vaccine following vaccination.

Our current research led to the discovery of very potent polycationic adjuvants including polyarginine and polylysine which in conjunction with tumor antigen derived peptides induce very potent immune responses leading to tumor rejection in relevant animal models, such as the P815 mastocytoma model (Figure 1). Injection of the peptides alone or together with standard adjuvants was ineffective. Our results indicate that by means of the new polycationic compounds a deposit of peptide or protein antigens is created at the vaccination site when injected subcutaneously and that these antigens are finally taken up at enhanced rates by a very important cell type of the immune system, the antigen presenting cell (APC) (Figure 2). APCs in turn activate cytotoxic T lymphocytes which destroy tumor cells expressing the peptide/protein.

Future experiments will address in more detail the mechanisms of how anti-cancer immunity following vaccination with tumor antigen peptides is generated. We will also modify the adjuvants in order to further improve vaccine efficiency, because in some, but not all models, the peptide vaccine is not yet quite as effective as cytokine transfected cellular vaccines. One potential disadvantage of peptide vaccines is their HLA restriction. We will address this problem by vaccinating with multiple peptides or tumor antigen proteins themselves together with the above adjuvants. The ultimate goal is to develop generic, chemically defined, cell-free vaccines for the treatment of human cancer.

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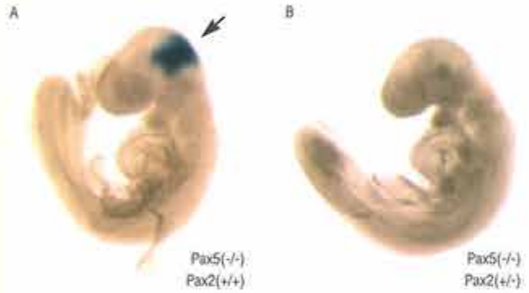
The role of Pax transcription factors in midbrain development, B cell differentiation and human disease

The mammalian Pax genes code for transcription factors with important functions in embryonic patterning and differentiation processes. A hallmark of these developmental regulators is the conserved paired box which harbours a potent and versatile DNA-binding function. In our group we are studying the role of Pax-5 (BSAP) and its related family members Pax-2 and Pax-8 in early development, B-lymphopoiesis and human disease by using a combination of transgenic, cell biological and biochemical approaches. The aim of our research is to contribute to the elucidation of the regulatory cascades underlying midbrain and B cell development.

The midbrain and cerebellum develops from an organizing center which is located at the midbrain-hindbrain boundary (mhb) of the vertebrate embryo. Pax-2 and Pax-5 are expressed in an overlapping manner in this brain region and are both required for the formation of the organizing center, as their combined inactivation in the mouse germline results in the loss of midbrain and cerebellum development (Figure 1). Similar phenotypes are observed in mice lacking the Wnt-1, Fgf-8 or En genes, indicating a role for all of these genes in the same regulatory pathway. To define the position of Pax-2 and Pax-5 in this regulatory cascade, we are characterizing the midbrain-specific enhancers of these genes and complement the transgenic experiments by establishing in vitro culture conditions for the propagation of neuroepithelial precursor cells derived from the mhb region. The loss-of-function, transgenic and cell culture experiments should allow the identification of upstream regulators and downstream targets of Pax-2 and Pax-5 in midbrain development.

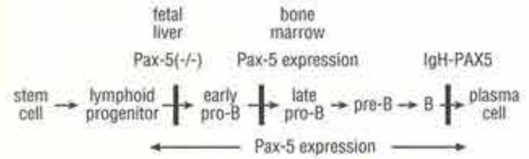
B-lymphopoiesis is an ideal experimental system for studying the molecular mechanisms underlying differentiation processes, as the developmental pathway leading to mature B cells and ultimately to immunoglobulin-secreting plasma cells has been dissected into several distinct stages according to the differential expression of cell surface markers and the sequential rearrangements of immunoglobulin genes. The Pax-5 gene is expressed throughout B cell development and codes for the transcription factor BSAP which is required for B-lineage commitment in the fetal liver and for progression beyond the early pro-B cell stage in adult bone marrow (Figure 2).

Figure 1
Cooperation of Pax-2 and Pax-5 in midbrain and cerebellum development. β-Galactosidase staining indicates an early loss of cells in the midbrain-hindbrain boundary region of double mutant embryos.



midbrain development

Figure 2
Schematic diagram of B cell development indicating the developmental blocks observed in Pax-5 (-/-) mice and human lymphoma patients with a IgH-PAX5 translocation.



B cell development

Figure 3
Allele-specific transcription of the Pax-5 gene in B-lymphocytes. The genomic DNA (in green) and the precursor RNA (in red) of Pax-5 are detected in the nucleus of monoallelically (mono) and biallelically (bi) expressing cells by fluorescence in situ hybridization (FISH).

monoallelic expression

Importantly, the pro-B cells from Pax-5 (-/-) bone marrow can be cultured in vitro, thus providing a convenient system for the identification of BSAP target genes. To gain insight into the role of Pax-5 in pro-B cell development, we have therefore initiated a systematic screen for BSAP-regulated genes by using Pax-5 (-/-) pro-B cells expressing a BSAP induction system. Pax-5 is likely to play an important role also in late B cell differentiation. To study these late functions, we have used the Cre-loxP system to generate a novel mouse strain in which the Pax-5 gene can be conditionally inactivated by Cre-mediated deletion at late stages of B cell development.

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. Our recent observation that Pax-5 is predominantly transcribed from only one of its two alleles in individual B-lymphocytes (Figure 3) suggests that monoallelic expression of Pax genes may cause their haploinsufficient phenotypes. PAX-5 has also been implicated as an oncogene in the genesis of a subset of non-Hodgkin lymphoma by gain-of-function mutations whereby the translocated PAX-5 gene has been brought under the transcriptional control of the immunoglobulin heavy-chain (IgH) locus (Figure 2). Transgenic mice containing a Pax-5 gene insertion in the IgH locus are currently being generated to test the hypothesis that the IgH-PAX5 translocation prevents plasma cell differentiation and may be causally involved in tumor formation. Together these experiments should help to provide insight into the mechanisms underlying midbrain and B cell development under normal and pathological conditions.

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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 Proliferation

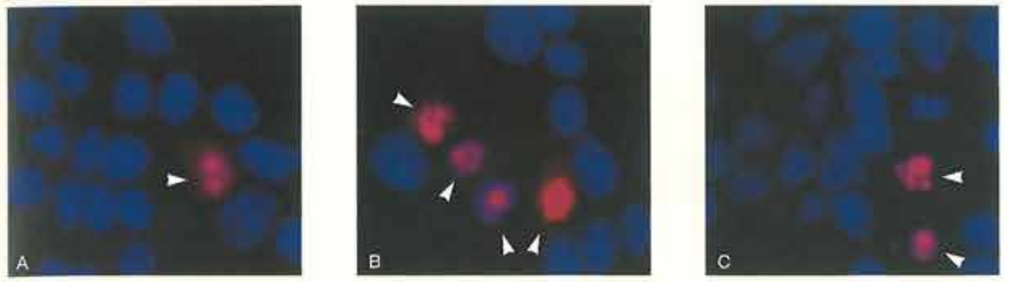
It is thought that SV40 large T antigen transforms cells by sequestration and inactivation of the tumor suppressor proteins p53 and retinoblastoma (pRb). However, in contrast to the expected acceleration of tumor development, tumor volumes were significantly reduced when Rip1Tag2 transgenic mice were intercrossed with p53-deficient mice. The mitotic index was significantly reduced in p53-deficient tumor cells, whereas the incidence of tumor cell apoptosis was unaffected. Notably, in the absence of p53, a reduction in steady state levels of T antigen coincided with decreased E2F activity. Biochemical analysis revealed that T antigen was stabilized by p53 to quantitatively sequester and inactivate members of the retinoblastoma gene family resulting in unrestricted E2F activity and high tumor cell proliferation. Thus, in Rip1Tag2 mice the oncogenic potential of SV40 T antigen is higher in the presence of p53 than in the absence of p53.

Tumor Cell Survival

Recently, we have shown that in the absence of survival factors, such as insulin-like growth factor II (IGF-II), ß tumor cells proliferate at a high rate, yet at the same time they undergo programmed cell death (Christofori et al. 1994; Nature 369, 414-418). We have now demonstrated that IGF-II acts as a survival factor for ß tumor cells by counteracting apoptosis that has been induced, for example, by staurosporine (Figure 1). Thereby, communication of the survival factor IGF-II with the apoptotic machinery appears to involve distinct, tumor cell-specific signaling pathways. Finally, we have characterized some of the factors that are involved in the execution of ß tumor cell apoptosis. We have cloned novel isoforms of the cysteine protease caspase 2 which appear to exhibit differential activity in tumor cells versus non-transformed cells.

Tumor Angiogenesis

Another apparent secondary event in the development of ß cell tumors is the control of tumor angiogenesis. Several angiogenic factors are released by the tumor cells in order to induce the formation of new blood vessels. One of these factors, acidic fibroblast growth factor (FGF-1), is expressed in all stages of tumor development. However, although FGF-1 does not have a classical signal sequence for secretion, it is nevertheless released by a novel export pathway from ß tumor cells. Our current studies attempt to unravel the mechanism of FGF export and its regulation. In addition, we employ transgenic complementation experiments to study the functional role of FGFs in tumorigenesis in vivo.



Recombinant IGF-II blocks staurosporine-induced apoptosis of IGF-II-deficient β tumor cells.

Figure 1
Immunofluorescence micrographs of apoptotic IGF-II-deficient β tumor cells detected by Cy5-CTP and the TUNEL reaction (red). Nuclei of all cells are visualized by staining with DAPI (blue). Cells were treated in the absence or presence of recombinant IGF-II (50 ng/ml) with staurosporine (400ng/ml) (panels B and C, respectively). Untreated control tumor cells grown under normal high serum conditions are shown in panel A. Arrowheads indicate apoptotic nuclei. Magnification: 200x.

Transition from ß cell adenoma to ß cell carcinoma in Rip1Tag2 transgenic mice.

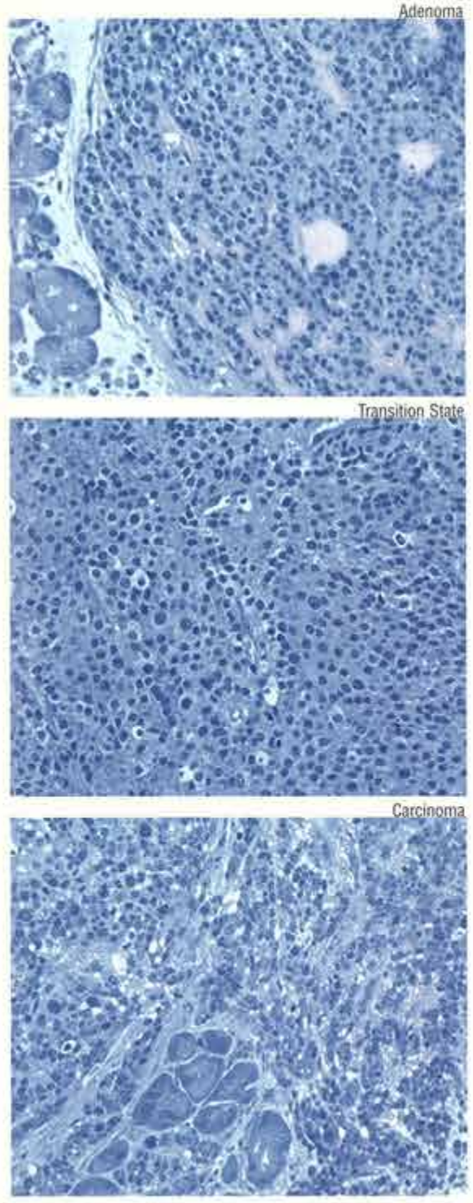


Figure 2
Histolopathological analysis of ß cell tumor development by hematoxylin/eosin staining. Note the focal transition from benign, well-differentiated adenoma to invasive carcinoma (transition state). Magnification: 100x

Tumor Cell Invasion and Metastasis

(in collaboration with H. Semb and U. Dahl, Umeå University, Umeå, Sweden)

During the terminal stages of carcinogenesis, the transition of well-differentiated adenoma to invasive carcinoma and metastasis is frequently accompanied by the loss of E-cadherin expression. To assess whether loss of E-cadherin-mediated cell adhesion is a cause or a consequence of tumor progression in vivo we have intercrossed Rip1Tag2 mice with transgenic mice that express in the pancreatic ß cells either wildtype E-cadherin or a dominant-negative form of E-cadherin. Maintenance of E-cadherin expression during ß cell tumorigenesis results in arrest of tumor development at the adenoma stage. In contrast, expression of dominant-negative E-cadherin induces early invasion and metastasis. The results demonstrate that loss of E-cadherin-mediated cell adhesion is required and sufficient for the progression from adenoma to carcinoma in vivo.

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Introduction

The research efforts in our group concern two topics: An analysis of viral/host cell interaction, especially in the area of viral entry and host responses to viral entry, and more recently with the analysis of CELO early gene functions, virus interactions with the pRB/E2F and apoptosis pathways. A second area of research involves the practical development of gene transfer systems, with a special emphasis on large DNA delivery.

Further studies on Gam-1

Our studies on the inflammatory and apoptotic responses of adenovirus entry and our characterization of the chicken adenovirus CELO led to the identification of GAM-1, a novel anti-apoptotic gene. GAM-1 probably fulfils the function of the E1B-19K gene in slowing the host cell response to virus infection, blocking both apoptosis and interfering with NF-κB signalling (Chiocca et al., 1997). Gam-1 functions by a mechanism distinct from Bcl-2 family members. It is now clear that Gam-1 activates transcription from a variety of promoters, probably through interactions with the cellular factors such as YY1 as well as with proteins that interact with the TATA box element.

Activation of E2F by CELO

Further studies are in progress to identify the early genes of CELO. A function screen has identified a CELO gene (orf 8) that can activate the cellular transcription factor E2F, by directly interacting with pRb. Orf 8 contains the motif LxCxD similar to many viral and cellular proteins that bind pRB yet shares no further homology with these proteins.
Orf 8 activation of the E2F pathway is further stimulated by other CELO genes and experiments are underway to characterize the complete mechanism.

CELO vectors

The characterization of CELO early genes has been aided by our development of technology to generate recombinant CELO virus vectors and to introduce modifications in the genome. These methods use an infectious, plasmid-borne copy of the CELO genome which can be manipulated by homologous recombination in the appropriate bacterial strains. We are currently identifying regions of the CELO genome that are dispensable for tissue culture growth of the virus and can be replaced with marker or therapeutic genes.

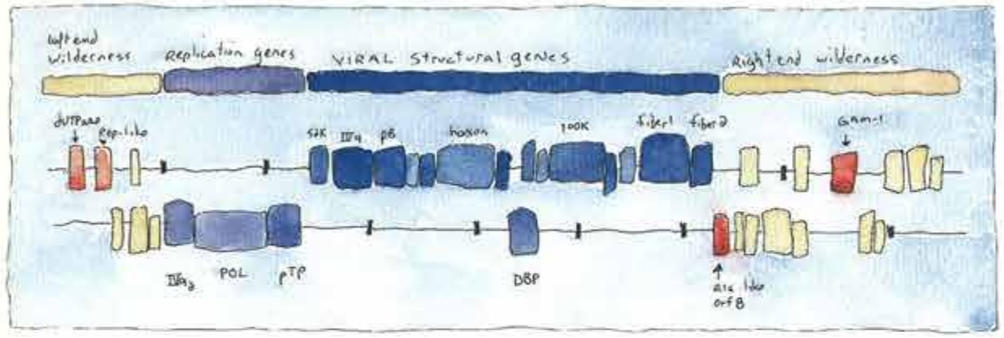
Cellular responses to PRRS

With the arrival of Professor Michael Murtaugh we have initated a study of the pathogen PRRS virus (porcine respiratory and reproductive syndrome virus). This virus emerged as a novel pathogen of the pork industry in 1987 and very little information is available about this arterivirus.
In particular, the cellular apoptotic and inflammatory responses to individual PRRS open reading frames will be examined.

Big DNA delivery

We have tested the DNA size limit for gene transfer systems using a series of bacterial artificial chromosomes (BACs) and have obtained efficient delivery of 170 kb BACs using a modified adenovirus/polyethylenimine (PEI) system (Baker and Cotten, 1997). Transfected large DNA molecules provide a surprising stability of gene expression in the absence of selection and vectors based on these large DNA molecules have been developed as molecular biology tools. Furthermore, this efficient large DNA delivery is now being exploited to characterize large sequence elements important for centromere function and chromosome stability.

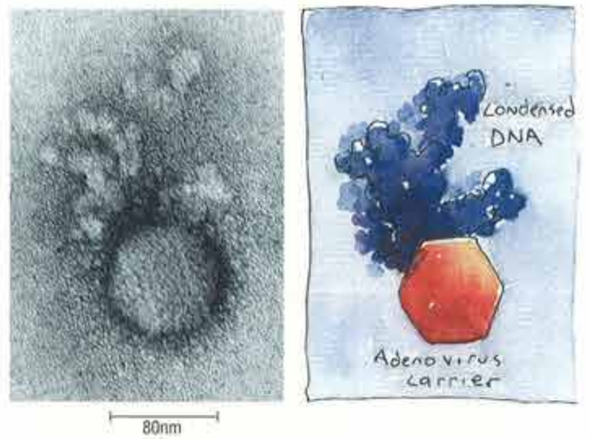
Figure 1

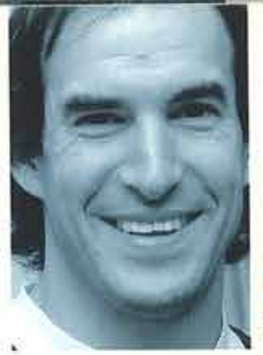


The organization of open reading frames in the genome of the vian adenovirus CELO.

Figure 2
Electron microscopy image (from Iris Killisch) of an adenovirus/PEI/DNA transfection complex showing the association of condensed DNA with the virion. See Baker et a., 1997 for details.

Adenovirus/PEI/DNA complex





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The Mechanism of Cytokinesis

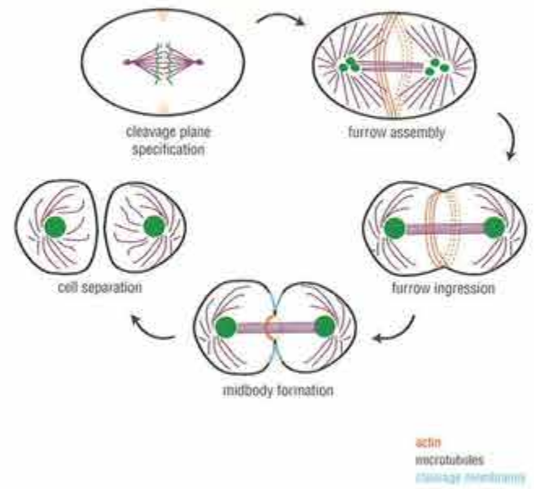
Cytokinesis leads to the formation of two daughter cells from a single progenitor. The division process is spatially and temporally regulated so that each daughter cell receives a receives a full complement of chromosomes and other essential organelles.

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 nearly 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 already have 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

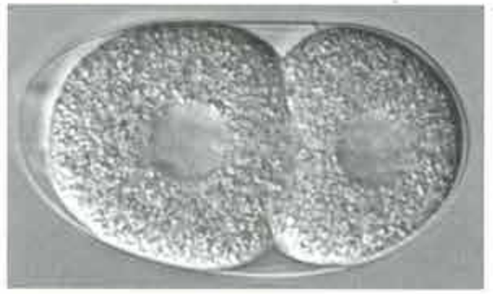


We are already in the process of studying, on the biochemical level, a family of proteins that are required for cytokinesis in budding yeast and in Drosophila, the septins. Using Xenopus embryos and extracts prepared from Xenopus eggs, we have shown that the septins are required for furrow ingression,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

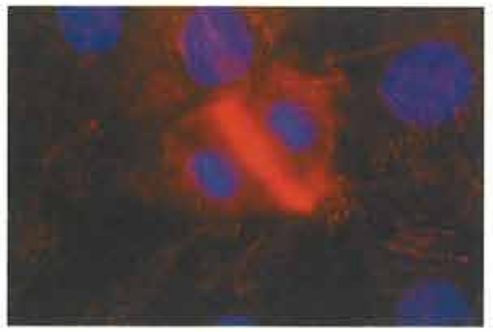
One other project is to explore the role of membrane insertion in cytokinesis. During the course of cytokinesis of Xenopus embryos, membrane is inserted specifically into the furrow region. Interestingly, inhibition of cytokinesis by pertubing actin-containing structures does not affect insertion of 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 would be possible to inactivate this process so that we could assess the role of membrane insertion in cytokinesis.

Figure 2



The first cytokinesis of a C. elegans embryo

Figure 3



The distibution of septins in a dividing cell

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Epithelial polarity and cancer

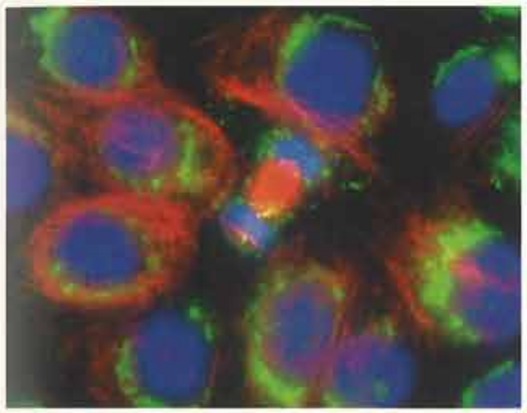
Cell polarity is the ultimate reflection of complex mechanisms that establish and maintain functionally specialized domains in the plasma membrane and the cytoplasm. To understand how the complex three-dimensional organization of polarized epithelial cells is established and maintained is the central question in our laboratory.

Whereas normal cells form highly polarized monolayers, oncogene transformed cells display an unpolarized phenotype, detaching from the substratum and developing multilayers. If the cells are derived from malignant transformations they break through the basement membrane, invade the underlying mesenchyme and metastasize to different sites of the body. Of the many components involved in these processes, only a few molecules have been identified so far.

We are using a well established mouse mammary epithelial cell system, expressing an estrogen-inducible c-JunER fusion protein (Fialka et al.,1996). These cells form three-dimensional and branching tubular structures in collagen-I gels, which closely resemble the ducts of mammary glands in vivo. After treatment with estrogen, however, the cells change their behaviour and grow in unpolarized multilayers. Upon withdrawal of the hormone, epithelial polarity and junctional integrity can be fully restored within a few days.

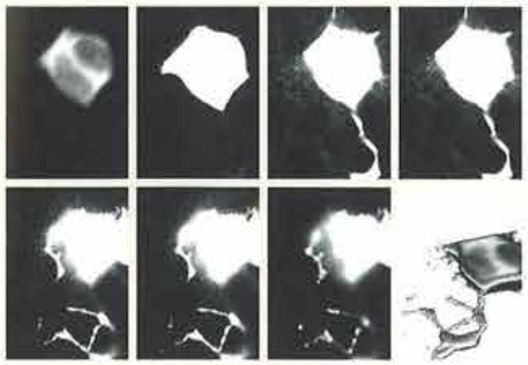
One major strategy we follow is the molecular characterization of protein traffic in c-JunER cells . We postulate that intracellular protein transport and sorting is an early target of transformation. We have identified differentially expressed proteins of the intracellular protein transport and sorting machinery in purified subcellular organelles derived from normal versus oncogenic transformed epithelial cells (for an example see Figure 1). We are also interested in the molecular organization of polarized microdomains in the plasma membrane. We are studying the role and molecular interactions of ZO-1, a member of the membrane-associated guanylate kinase protein family (MAGUK), during the disassembly of tight junctions. This protein is extracted from junctions in c-JunER induced cells and redistributes to the cytosol where it is found in a protein complex. In a collaboration with Ursula Guenthert (Basel Institute for Immunology, Switzerland) we are investigating the altered expression and redistribution of CD44 and CD44 variants. We found that CD44 can act as an integral transmembrane in cholesterol-enriched microdomains of the basolateral plasma membrane. These CD44 complexes are kept in the plane of the basolateral membrane by an interaction of annexin II with the underlying actin cytoskeleton.

Figure 1
After subcellular fractionation and two-dimensional gel analysis of organelle-associated proteins we identified and microsequenced a protein whose cDNA sequence was subsequently found as EST-sequence in the databases on WWW. Peptide antibodies against the highly conserved protein were raised and used for indirect immunofluorescence (green). Sparsely grown mammary epithelial cells (EpH4) were co-stained for tubulin (red). The novel protein of unknown function localizes to the Golgi which fragments during mitosis (indicated by the mitotic spindle). Nuclei were counterstained with DAPI (blue).



A newly identified protein associates with the Golgi

Figure 2
EpH4 c-JunER mouse mammary gland epithelial cells were grown on filter inserts and transiently transfected with the pcDNA 3.1(-) HisMyc TIS7 plasmid construct.
5 days later TIS7 protein expression was detected by indirect immunofluorescence with a confocal laser scanning microscope. Representative optical sections (µm) vertical slices. 3 dimensional reconstruction as shown in the bottom right pannel was made by the Imaris software.



Ectopic TIS 7 Expression

In a second screen that complements our biochemical approaches described above, we have applied PCR-based differential display (DD) to enrich for genes that are altered in polarized versus unpolarized cells from the same cell type.
This screen has independently lead to the identification of several DD RT-PCR products which were known to be involved in regulation of organelle movement, gene expression, and signal transduction, respectively, during oncogenic transformation.
One particular interesting protein that we found is the immediate early gene product TIS7 . The protein localizes at the inner leaflet of the basolateral plasma membrane in the vincinity of adherens junctions. Upon induced loss of polarity the protein is upregulated and translocates the cytoplasm and is then accumulating in the nucleus. Transient overexpression of TIS7 in confluent monolayers leads to a disruption of epithelial polarity and cells develop irregularly outgrowing processes (Figure 2).
These findings suggest a possible role for TIS7 in a signal transduction pathway linking the junctional region of the basolateral plasma membrane of epithelial cells with their nucleus.

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Transcriptional memory in mammalian chromatin

The maintenance of gene expression patterns ('transcriptional memory') is fundamental for inheriting cell type identities in eukaryotes and has been correlated with the organization of chromatin domains that modulate gene activities. Paradigms in Drosophila for stabilizing repressed transcriptional states – presumably by regulating higher order chromatin – include Polycomb-dependent restriction of expression boundaries of the homeotic selector genes (HOM-C) and Su(var)-dependent suppression of gene silencing at heterochromatic rearrangements (so-called position-effect-variegation [PEV]). To start analyzing components and mechanisms of transcriptional memory in mammalian chromatin, we have recently isolated mouse and human homologues of the Drosophila Polycomb-group gene Enhancer of zeste (designated Ezh1 and EZH2) and of the strongest PEV suppressor gene Su(var)3-9 (designated Suv39h, Suv39h2 and SUV39H.).

EZH2 and repressive chromatin domains

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 *E(z)* and *Su(var)3-9* 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 *Ezh1* restores gene repression in *S.cerevisiae* mutants that are impaired in telomeric silencing [2]. These data provide a direct functional link between Pc-G *(EZH2)*-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 *SUV39H [4].*

Suv39h is a mitotically stable chromatin regulator

To analyze chromatin association of the endogenous Suv39h and EZH2 proteins, we generated affinity-purified, rabbit polyclonal anti-sera. Whereas the two anti-sera detect nuclear interphase staining for both proteins, Suv39h (but not EZH2) remains stably associated with mitotic chromatin and displays enriched binding sites at heterochromatic regions (Figure 1). Together with the ubiquitous expression profile of *Suv39h* during mouse development [4], these data implicate a role for Suv39h in the structural organization of mammalian chromatin and provide a paradigm for a chromatin regulator to propagate distinct transcriptional states during cell divisions [4]. Using stably transfected cell lines, we also identified several interacting proteins that specifically co-precipitate with overexpressed $(myc)_3$-tagged SUV39H. Based on these interactions, we started to purify the first mammalian SUVAR complex.

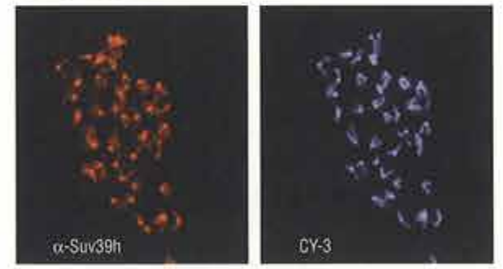
Modulation of higher order chromatin in vivo

To generate gain-of-function mutations *in vivo*, we established transgenic mice for human *EZH2* and *SUV39H*. Whereas several of the *EZH2* lines display only low to moderate expression levels, three mouse lines were identified that overexpress $(myc)_3$-tagged *SUV39H* and which, surprisingly, exhibit posterior transformations of vertebral identities (Figure 2). Although the transformations are subject to variable degrees of penetrance (10-30%), these data suggest that the mammalian *Su(var)3-9* homologue participates in specifying the antero-posterior body axis. This interpretation is supported by disrupting the X-linked *Suv39h* locus in the mouse (in collaboration with Maria Sibilia and Erwin F. Wagner). 129/Sv male chimeric mice carrying a *Suv39h* null allele exhibit pronounced anterior transformations of the axial skeleton. Together, these complementary phenotypes are reminiscent of defects observed upon misregulation of several *Hox* genes and suggest a model in which gene expression within 'complex loci' (i.e. the *Hox* gene clusters) is particularly sensitive to the modulation of chromatin domains [3]. However, since *Suv39h* null F2 males (predominantly of C57/Bl6 origin) do not display skeletal abnormalities, Suv39h function appears to severely depend upon genetic background effects or may be rescued by maternal *Suv39h* transcripts.

Finally, we have also generated gene disruptions for *Ezh1* and *Ezh2*, and are in the process of targeting the second *Suv39h2* locus. Together, these studies aim at the generation of double null mice that contain compromised higher order chromatin.

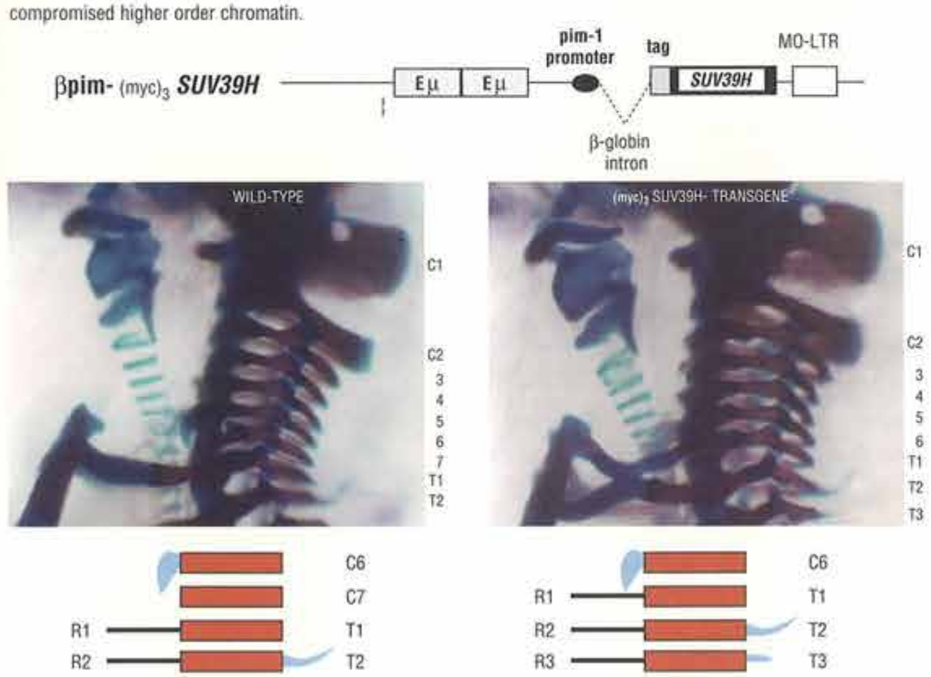
Immunolocalization of endogenous Suv39h at mouse metaphase spreads

Figure 1
Mouse PD31 pre-B cells were treated with colcemid, resulting in metaphase arrest of approximately 20% of the cells. Cells were hypotonically swelled, broken up by centrifugation and stained prior to fixation with an affinity-purified rabbit anti-Suv39h polyclonal antibody, followed by a secondary goat anti-rabbit antibody that had been conjugated to CY-3. The DNA has been stained with DAPI.



Overexpression of human $(myc)_3$-tagged *SUV39H* 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 S) and cartilage (alcian blue 8GS). 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 is schematically indicated at the bottom.



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Asymmetric cell division during *Drosophila* development

While most cell divisions are symmetric 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 make 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.

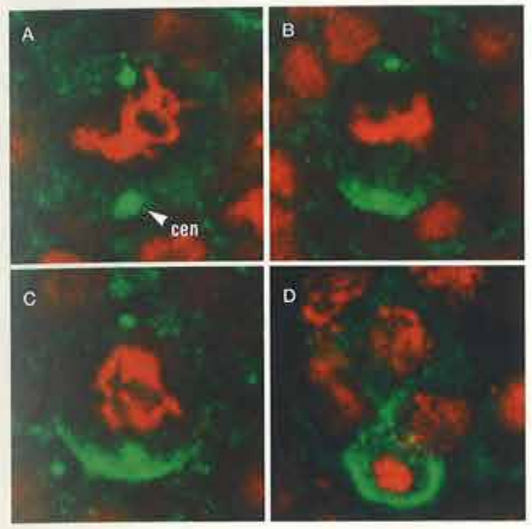
Correct asymmetric segregation of Numb requires the protein Inscuteable. Like Numb, Inscuteable is asymmetrically localized in dividing neural precursor cells. However, Inscuteable localizes already in interphase – before Numb – and it localizes to the opposite side of the cell (Figure 2A). In the absence 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.

To identify domains in the Inscuteable protein that mediate the different functions of the protein, we have generated a series of deletion constructs and expressed them in transgenic flies. Surprisingly, only a central 350aa domain is required for asymmetric localization and reorientation of the mitotic spindle, while deletions outside this domain do not affect the function of Inscuteable. When expressed by itself or fused to *lacZ*, these 350aa are sufficient for asymmetric localization and spindle reorientation, while a smaller 150aa fragment can localize to the cell membrane but fails to localize asymmetrically or reorient the mitotic spindle. Preliminary results indicate that the same 350aa of Inscuteable are also responsible for directing the asymmetric localization of Numb to the opposite side of the cell. Thus, most functions of Inscuteable map to the same domain of the protein.

In a two-hybrid screen for proteins that associate with the central 350aa domain of Inscuteable we have identified at least one interesting new protein. The sequence of this protein predicts a domain with a high degree of homology to ankyrin and therefore suggests a cytoskeletal function. Consistent with a role in asymmetric cell division, the corresponding gene is expressed in cells that require the function of Inscuteable. We are currently in the process of generating a mutant in this gene and creating antibodies to analyze its subcellular distribution.

To better understand how cells orient their mitotic spindle and analyze the role of Inscuteable in this process, we want to produce time lapse recordings of mitotic spindles in vivo. For these experiments, transgenic flies will be generated that express a tubulin-GFP fusion protein and therefore have fluorescent mitotic spindles. In other organisms astral microtubules which extend from the centrosome towards the cell cortex are thought to pull this centrosome towards a cortical site and thus orient the mitotic spindle. Inscuteable might be directly involved in the attachment of astral microtubules to the cell cortex or exert its influence on spindle orientation in a more indirect way. Analyzing astral microtubules and the movement of the mitotic spindle in vivo in wild-type and *inscuteable* mutant *Drosophila* embryos will help to distinguish between these possibilities.

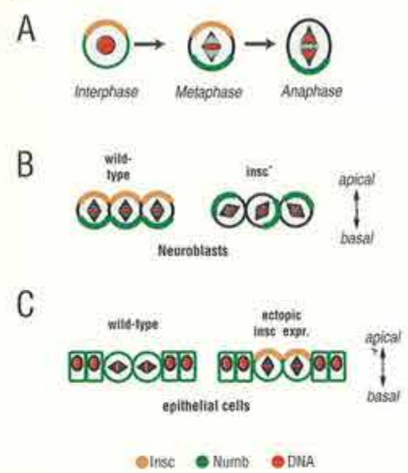
Figure 1



Asymmetric localization of Numb during mitosis

The figure shows 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

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 tumour 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 about ourselves.

Because most constituents of cells are synthesised 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 dificult 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) has 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 proteolysed as a consequence of its prior ubiquitination by a large ubiquitination machine called the Anaphase promoting complex (APC).

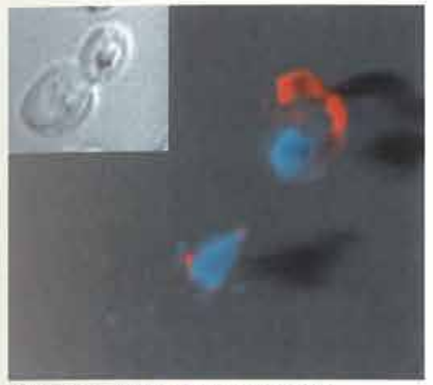
Our work suggests that Pds1p blocks the activity of a protein called Esp1p, which is required for the dissociation of Scc1p and the loss of sister cohesion. We are currently interested in what determines the timing of Pds1p destruction by the APC, how surveillance mechanisms called Checkpoints regulate 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 expesses 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.

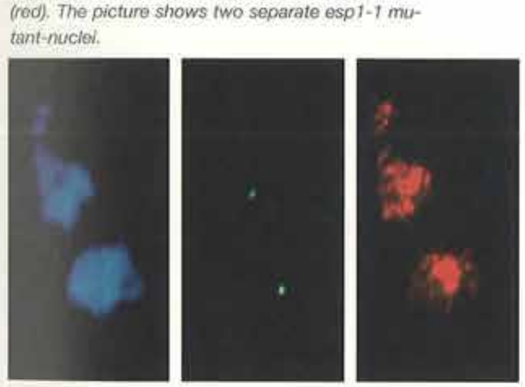
The asymmetric segregation of mRNA 'determinants' plays an important role in the development of multicellular organisms. My lab is interested in the mechanism by which ASH1 mRNA is transported along actin cables by Myo4, how it is subsequently anchored at the distal tip of buds, and how its translation 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

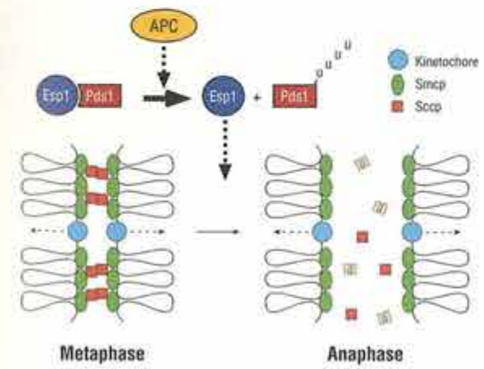


Figure 3
The APC triggers sister separation by liberating the 'separin' Esp1 from inhibition by Pds1

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Cell Cycle Control by Ubiquitin-Dependent Proteolysis

The propagation of genetic information during cell proliferation requires the accurate replication and subsequent segregation of chromosomal DNA. To ensure that genetic information is neither lost nor inappropriately multiplied during this process, each part of the genome has to be replicated once and only once during S-phase, and each pair of sister chromatids has to be segregated equally during mitosis. Defects in either of these processes could lead to the formation of aneuploid daughter cells.

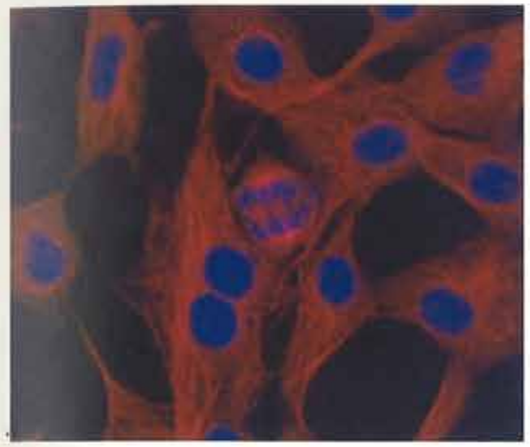
Depending on how genetic information had been missegregated, such cells could either be unviable, or worse, they could gain a growth advantage and become tumorigenic in a metazoan organism. Therefore, both DNA replication and chromosome segregation need to be tightly controlled. This is in part achieved by ubiquitin-dependent proteolysis, a mechanism by which proteins are targeted for proteasome-mediated degradation by covalent attachment of poly-ubiquitin chains. The initiation of DNA replication at S-phase and the onset of chromosome segregation at anaphase are regulated by distinct mutli-subunit ubiquitination complexes, the S-phase promoting complex (SPC) and the anaphase promoting complex (APC, also called cyclosome), respectively.

We are interested in understanding how the APC controls the proper timing of anaphase and thus ensures that chromosomes are equally segregated. The initiation of chromosome segregation is thought to depend on the activation of the APC.

Once activated, this complex ubiquitinates proteins such as Pds1p in budding yeast and Cut2p in fission yeast whose proteolysis is essential for chromosome segregation. Furthermore, the APC promotes exit from mitosis by causing the destruction of cyclin B, the activating subunit of the mitotic protein kinase Cdc2, and is likely to ubiquitinate a number of other regulatory proteins.

How the activity of the APC pathway is regulated is poorly understood. We are using the embryonic cell cycle of the frog Xenopus as one model system to approach this question. We have found that the Cdc2-cyclin B kinase is able to phosphorylate and stimulate the ubiquitination activity of APC in mitosis. In addition, the difference between the ubiquitination activity of interphase and mitotic APC is further enhanced by an inhibitor that suppresses the low activity of interphase APC but not the activity of mitotic APC. These results suggest that during

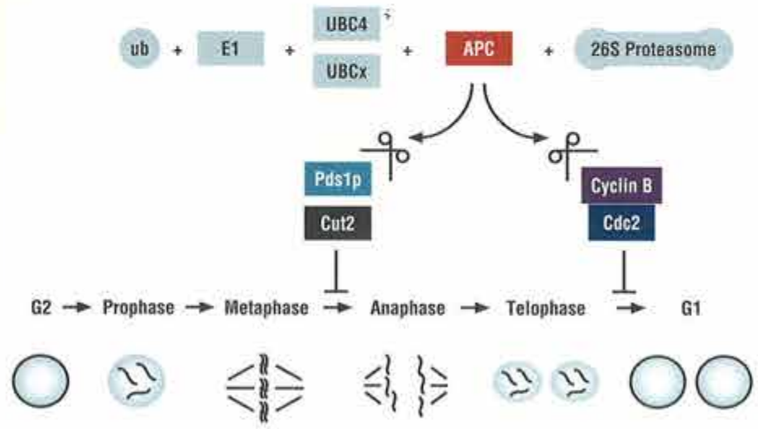
Figure 1
Micrograph of RAT1 cells in which the DNA is stained in blue and microtubules are stained in red (image by B. Peters). The cell in the center has recently initiated anaphase and is in the process of separating its sister chromatids. The onset of this event appears to be controlled by the anaphase promoting complex (see text and Figure 2).

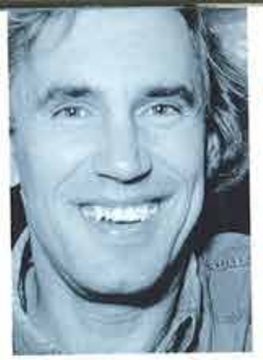


early embryonic cell cycles, where no distinct G1 and G2 phases exist, APC is regulated by mitosis-specific phosphorylation. However, this model cannot explain the observation that in somatic cells APC-dependent cyclin B proteolysis continues until the end of the G1-phase where mitotic kinases are inactive. Nevertheless, we find that the APC pathway is also regulated at the level of APC in cultured somatic mammalian cells, although the underlying mechanisms are presently unknown.

Proteolysis mediated by the APC pathway is subject to an additional level of control in situations that require cells to arrest at metaphase, namely in the cytostatic factor (CSF)-induced metaphase II arrest of vertebrate eggs, and in the spindle assembly checkpoint of somatic cells. The first arrest is maintained until eggs are fertilized and thus allows synchronisation of the egg and the sperm cell cycle. The second mechanism delays entry into anaphase if the mitotic spindle has not been properly assembled and thus reduces the risk of unequal chromosome segregation. In both cases anaphase is thought to be prevented by inhibition of the APC pathway, although the precise mechanisms remain obscure. We have found that activation of the MAP kinase pathway inhibits APC-dependent proteolysis in Xenopus eggs. This inhibiton is likely to contribute to the CSF arrest and its characterization may therefore provide important insight into meiotic and mitotic checkpoint mechanisms.

Figure 2
Ubiquitin-dependent proteolysis mediated by the anaphase promoting complex (APC) regulates two important transitions in mitosis. Proteins such as Pds1p in budding yeast and Cut2 in fission yeast need to be degraded to initiate the separation of sister chromatids at the metaphase to anaphase transition. Destruction of cyclin B by the same pathway is an important mechanism that inactivates the Cdc2 kinase, which is a prerequisite for forming daughter cells to exit from mitosis into the next interphase.





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[1] since November; [2] since July; [3] until June

Gene Function in Mammalian Development and Oncogenesis

Transgenic mouse models are being applied for the analysis of gene function in normal and pathological development. One major focus is the analysis of Fos and Jun proteins and their role as regulators of cell differentiation/proliferation in bone and liver development. Furthermore, we are aiming to define the specific function of the EGF-receptor in neural and epithelial tissues and of VEGF/Flk-1 receptor signalling in 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 control osteoblast transformation and osteoclast differentiation. We are employing an *in vitro* co-culture system where osteoclast differentiation is strictly dependent on the newly introduced Fos proteins (Figure 1). This system will allow us to identify functional domains but also Fos-regulated target genes which are critically involved in the normal genetic program for the development of osteoclasts.

Jun Proteins controlling cell proliferation

Both c-Jun and JunB play key roles in development since inactivation of these genes in mice causes embryonic lethality. Mice lacking c-Jun most likely die of liver dysfunction whereas mice lacking JunB exhibit extraembryonic defects. Interestingly, JunB null mice rescued by a JunB transgene develop chronic myeloid leukemia probably due to lack of expression of the transgene in these cells. As these two proteins are closely related, we are interested in determining whether they have overlapping functions. Transgenic complementation experiments demonstrate that only JunB can partially substitute for c-Jun *in vivo*. Fibroblasts lacking c-Jun have a proliferation defect, which can also be rescued by JunB. Molecular analysis reveals that c-Jun acts as a positive regulator of cell cycle by suppressing p53 (in collaboration with Peter Angel, Heidelberg) and that JunB in the absence of c-Jun can downregulate the p53 pathway and therefore rescue the proliferation defect.

The activity of c-Jun is also regulated by phosphorylation, which is mediated by the c-Jun N-terminal kinases (JNKs). In order to define the function of c-Jun phosphorylation *in vivo*, we have generated mice carrying null mutations of JNK-1 and -2 (in collaboration with Michael Karin, UCSD) as well as mice harbouring a *c-jun* allele mutated in the JNK phosphoacceptor sites (Figure 2).

In parallel, conditional 'floxed' c-Jun alleles have been generated to study the role of c-Jun in adult liver cell function (Figure 2).

Understanding signalling of epidermal growth factor receptor (EGFR)

Depending on the genetic background EGFR mutant mice die at midgestation (129/Sv), at birth (C57BL/6) or can live up to postnatal day 20 (MF1 or C3H).

Figure 1
c-Fos dependent osteoclast formation (right, red). No osteoclasts differentiate without the c-fos gene transfer (left).

Figure 2
Tissue-specific deletion of a 'floxed' c-jun allele and the 'knock-in' of a phosphoacceptor mutant of c-jun. These mutant alleles are used to understand the role of c-Jun and c-Jun phosphorylation in vivo.

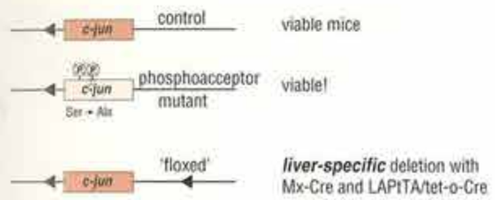
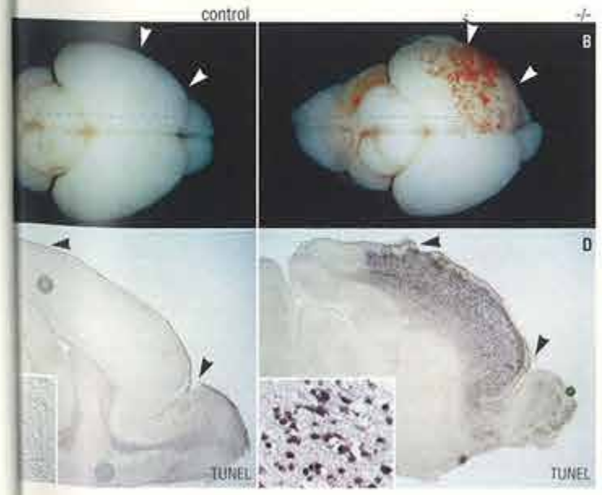


Figure 3
Massive apoptosis in EGFR mutant brains at postnatal day 5. (A, B) Dorsal view of the brain of control (A) and EGFR-/- mice (B) showing hemorrhagic lesions on the surface of the mutant forebrain (arrows). (C, D) As evidenced by TUNEL staining, in correspondence to the hemorrhages, the region of the cortex between the arrows is undergoing massive apoptosis in the mutants (D, inset), whereas no apoptotic cells can be detected in the control (C, inset).



The multiple phenotypes are manifested in defects in the placenta, the skin, the lung and also in the brain. A neurodegenerative disease characterised by massive apoptosis starts around postnatal day 5 in various brain regions (Figure 3). To understand the molecular mechanisms responsible for these defects, we have generated various conditional mutant EGFR alleles. The 'knock-in' of a wild-type 'floxed' human EGFR cDNA completely rescues the brain phenotype and prolongs the lifespan of the mutant mice. These mice develop a heart disease which is most likely caused by misexpression of the 'floxed' allele in this organ.

In an alternative approach we are performing a genetic complementation test with the goal of identifying downstream components of the EGFR pathway in skin. Transgenic mice that express the human Son of Sevenless (hSOS) gene under the control of the keratin-5 promoter develop skin tumors. Genetic experiments reveal that overexpression of hSOS can partially rescue the skin defects of EGFR mutant mice. We are currently in the process of investigating which downstream pathways are responsible for 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 analyse the underlying growth control mechanisms. We have generated a conditional 'floxed' allele of the endothelial specific growth factor receptor Flk-1 to test whether an autocrine growth stimulation loop is causal to the action of the PymT oncogene.

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Neuroblastoma candidate genes

Cancerogenesis involves a multistep genetic scenario that reveals two major types of gene rearrangements: gain and loss of function. Genes that contribute to tumorigenesis upon their functional loss are generally referred to as tumor suppressor genes. Most of these genes were shown to have essential functions either in cell cycle control or in cellular differentiation. Many tumor suppressor genes have been identified by positional cloning, since their location in the genome is often pinpointed by significant chromosomal aberrations. Our group has focused on the cloning of a neuroblastoma susceptibility gene located in the chromosomal region 1p36, as this band is affected by allelic deletions or translocations in a significant number of tumors.

Loss of heterozygosity studies have previously marked a consensus deletion of approximately 10 Mbp in chromosome 1p36.3. Subsequent mapping of long insert clones (YACs, PACs, BACs) by fluorescent in situ hybridisation (FISH) revealed very small interstitial deletions in a subset of neuroblastomas leading to the determination of a 2-3 Mbp consensus deletion. We could also localize the breakpoints of translocations found in two independent tumor cell lines (NGP and SK-N-MC) to this consensus deletion. These translocations appeared to be reciprocal, hence we suspected that the translocation breakpoints would directly point at the location of the gene(s) of interest. We, therefore, focused our efforts towards mapping and cloning of the two breakpoints.

FISH mapping first revealed that the two translocation breakpoints were not matching. The two breakpoints are at least 600 kbp apart from each other. This indicated either that one or both breakpoints may be located in an irrelevant genomic region or that two independent important genes may be affected. Notably, one of the tumor cell lines, SK-N-MC, has previously been re-characterized as a Ewing sarcoma due to the presence of the Ewing-specific EWS-FLI1 gene translocation, despite the clear neuroblastoma resemblance of this cell line.

We mapped and cloned the SK-N-MC breakpoint in a contig of cosmid clones derived either from a chromosome 1-specific cosmid library or from mapped YAC clones. Long range genomic sequencing and transcription analysis eventually led to the identification of a gene located across the translocation breakpoint. A DNA sequence database search revealed a high homology to a family of mouse and rat genes involved in neuronal differentiation and maintenance. Sequence determination at the SK-N-MC breakpoint showed that the open reading frame of this gene is disrupted between two functionally important motifs. Presently, further mutation analyses address the question of mutations in other neuroblastoma tumors as well as in a number of other neuroectodermal tumors. Whilst the SK-N-MC translocation represents a

DIRVISH mapping of the NGP translocation breakpoint.

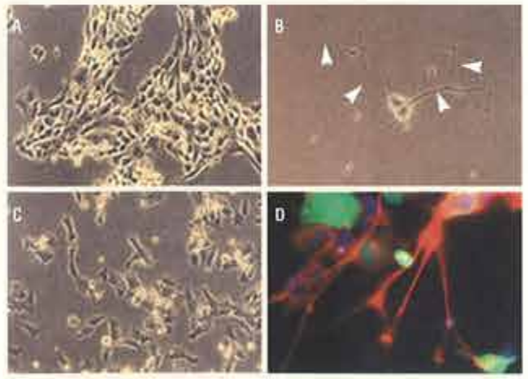
Figure 1
Double color hybridization of a PAC clone distal of the breakpoint (red label) and a PAC clone that reveals a triple signal in FISH experiments (green label). Owing to its FISH pattern the latter clone was previously mapped either to the trisomic region proximal of the breakpoint or directly onto it. The overlap with a distal clone (yellow dots) clearly localizes this clone to the breakpoint.

clearly reciprocal case, the NGP translocation resembles a more complex rearrangement, involving a partial trisomy immediately proximal of the consensus deletion-specific breakpoint. This rendered the mapping of the important breakpoint region more difficult. Applying DIRVISH (direct visualization of in situ hybridization) with long insert clones we eventually identified two overlapping PAC clones, one of which was previously mapped proximal of the breakpoint and one distal of it (Figure 1). Subcloning and exon trapping methods led to the identification of a novel gene which is located directly across the breakpoint. We discovered by hybridization mapping that the open reading frame of this gene is disrupted by the translocation in NGP. Furthermore we could show that approximately 40% of neuroblastoma tumor cell lines display structural rearrangements in the gene, some of the rearrangements being homozygous. We could also show that a BAC clone containing the breakpoint gene confers a post-mitotic, terminally differentiated neuronal phenotype to two different neuroblastoma tumor cell lines upon transfection (Figures. 2). Further work will address questions such as the identity of this gene and its involvement in neuronal differentiation and tumorigenesis.

This group will discontinue working by the end of 1997. We wish to express our gratitude to Max L. Birnstiel for the opportunity to work in this exceptionally inspiring environment and for his continuous support throughout the seven years that we could spend at the I.M.P. Thanks to the team leaders and to all I.M.P. members as well for the great time we had.

Figure 2
All cells are derived from the SK-N-AS neuroblastoma cell line and are shown after 14 day culture in serum-free medium. Parallel experiments using the NGP cell line yield comparable results.
A non-transfected SK-N-AS cells.
B SK-N-AS cell clone containing the breakpoint BAC. Cells have stopped cycling and clearly display neurite outgrowth.
C SK-N-AS cell clone after transfection with a chromosome 10-specific BAC clone as a negative control. The cellular phenotype remained unchanged as compared to non-transfected cells.
D immunocytochemical characterization of cells shown in B. A monoclonal antibody against microtubule associated proteins (MAP5, red label) clearly displays the neurite identity of the protrusions. Green fluorescence: GFP labelling of BAC bearing clones, blue fluorescence: nuclear staining with DAPI.

Transfections using a BAC clone containing the NGP breakpoint region and the beakpoint gene induce neuronal differentiation.



Anton BEYER | Scientist

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Gerald LÖFFLER | Postdoc
Bianca HABERMANN | PhD Student

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

Electrostatic properties of proteins

Gerald Löffler, Anton Beyer

Gerald Löffler is mainly interested in describing electrostatic interactions in proteins. The established method to calculate the electrostatic potential of a protein is based on solving the Poisson-Boltzmann equation for a system consisting of a low dielectric cavity (the protein) and a high-dielectric environment for the surrounding. Gerald Löffler has developed an algorithm to calculate the dielectric constant based on MD simulation results.
(Springer Verlag, Lecture Notes in Computer Science, in press; J. Mol. Biol., in press).

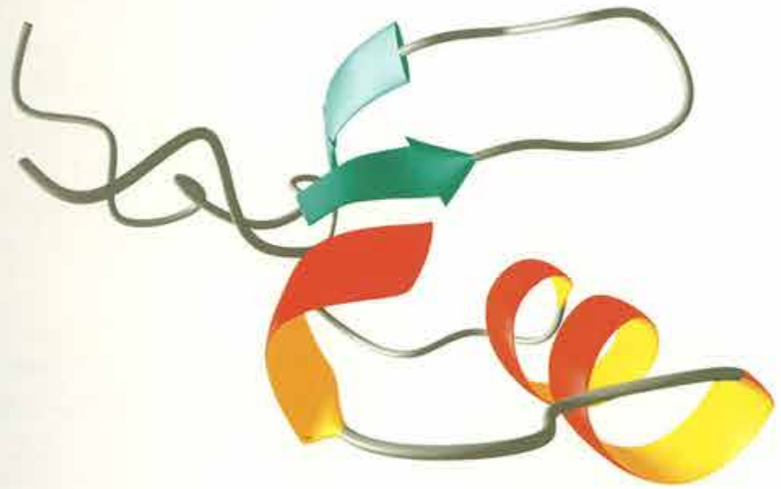
Analysis of the Interaction Code for C4-Zinc Finger Proteins

Bianca Habermann, Anton Beyer, Hartmut Beug

Specific interaction of a protein with DNA depends on the physical properties of the side chains involved in binding the DNA and the base pair sequence of the DNA-element recognised by the protein.
Most DNA-binding proteins have small domains that are responsible for the binding to the nucleic acid. Several DNA-binding motifs have been characterised up to now. Examples are helix-turn-helix motifs, leucine zippers and three different classes of zinc finger proteins.

The superfamily of the nuclear hormone receptors belongs to the class of C4-zinc finger proteins. They are ligand inducible regulators of transcription and are involved in the regulation of tissue- and developmentally- specific genes. They include the steroid and thyroid hormone receptors, the retinoc acid and retinoic X receptors, the vitamin D receptor and a large number of receptors, whose ligands have not been found yet, which are called the orphan receptors. Upon ligand binding, the nuclear hormone receptors bind to their response elements as dimers, whereby formation of homo-, as well as hetero-dimers is possible and induce gene expression.
The nature of their cognate DNA element, which is generally referred to as Hormone Response Element (HRE), is palindromic, whereby inverted, everted and tandem repeats occur.
The DNA-binding domain of the nuclear hormone receptors consists of two zinc fingers. Each zinc ion is coordinated by four cysteines. The DNA-binding helix, as seen in the crystal structure, lies at the end of the first zinc finger, including the last four residues of the first zinc finger, the P-box.

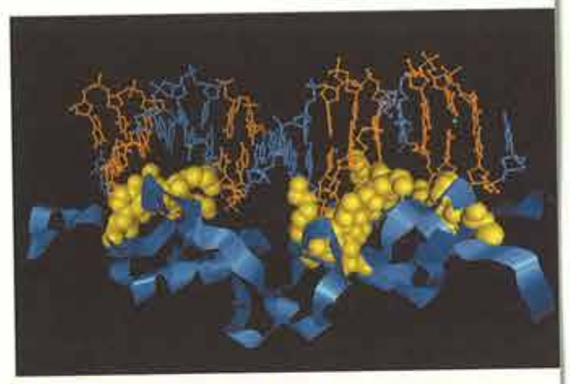
Figure 1



The DNA-binding domain of H1 protein is one of our model systems for studying biophysical properties of proteins by theoretical methods.

Correlated mutations are easiest to detect using statistics as a tool of analysis. One may for instance perform correlation analysis of the data, matching each mutated position on the response element with each mutated position of the recognition helix.
There is, however, a major disadvantage using classical statistics to evaluate data like these, since they are not random. There are two major constraints on sequence data obtained from an approach like the random mutagenesis. Randomised mutagenesis of seven positions in the recognition helix results in a sequence space of 1,28 x 109 different possible mutated proteins. One constraint results from the structure, since the resulting mutant proteins have to adapt the correct fold, restricting the available sequence space. The second constraint comes from the function, since the screening system is based on a functional protein. At the end, a very small fraction of the sequence space is left, Correlated mutations will therefore be evaluated using Bayes Theorem, which deals with conditional probabilities.
This project is a joint cooperation with the group of Harmut Beug at the IMP.

Figure 2
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.

Gotthold SCHAFFNER | Scientist

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Introduction

The Service Department offers a variety of services to IMP scientists. The majority of our effort involves DNA sequencing, oligonucleotide synthesis and peptide synthesis. These services continue to be of high quality and rapid. In addition, we produce monoclonal antibodies in hybridoma cell lines and isolate the antibodies by HPLC. We also provide assistance in designing immunization protocols, organize rabbit immunization and antisera production by an outside company. Space used for preparing diverse reagents and cell culture media meets now the requirements for the hugh quantities of various high quality solutions prepared by our Media Kitchen staff. This is vital for the continuous success of cell culture and other exacting work done in the institute. 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 etc..

Oligonucleotide Synthesis

We started in 1988 with about 500 DNA oligonucleotides and last year we prepared about 2650+ with an average size of 25 bases. In this year we will again reach this level, but we had a greater demand for oligonucleotides > 40 bases. 10% of all oligonucleotides in the first 8 month 1997 were in this class.

Peptide Synthesis and production of monoclonal antibodies

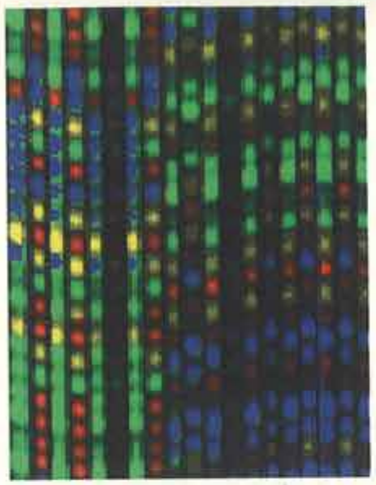
Synthetic peptides became important in IMP research activities. We synthesized a large quantities Yeast alpha-factor, a great variety of peptides coupled to PEG or KLH for immunization purposes, and many peptides attached to Biotin or FITC have been synthesized this year.

For the 'Tumour-Vaccines-Project' we synthesized several peptides to be tested in peptide-'Transloading'-experiments (see report of Birnstiel group).
Last year we started to produce, and subsequently purify by HPLC, a variety of monoclonal antibodies from hybridoma cell-lines. Many of these antibodies are essential for cell depletion studies in mice.
Others were used for in situ hybridization and immunoprecipitations to investigate subcellular processes.
This activities of our group has resulted in considerable financial saving for the institute, but at the same time we had to invest in new equipment like an additional HPLC system (PerSeptive Vision Workstation) and a peptide synthesis robot (SYRO II, MultiSynTech, Bochum, Germany). This robot enables us to increase peptide synthesis capacity substantially.

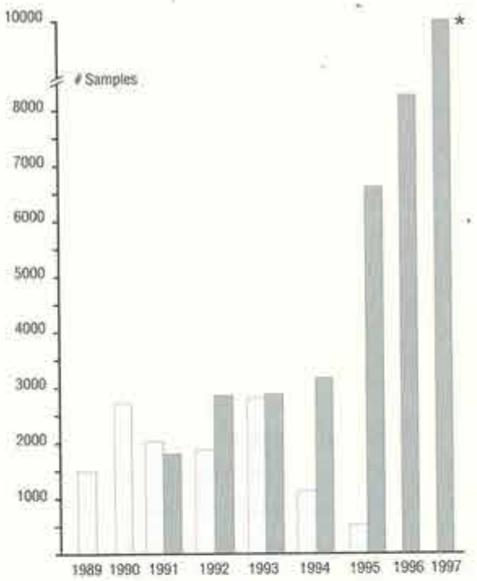
Sequencing and DNA isolation

The ABI PRISM 377 DNA sequencer accounts to about 60% of the 8200 samples analyzed last year. We begun to use sequencing kits with fluorescently labelled didesoxynucleotides which have an altered spectral emission and more homogenous incorporation with Taq polymerase. Thus, we can not use the ABI 373A any longer because of its filter based detection system. We invested therefore in a second ABI PRISM 377 for next year. With the new kits we will have better 'basecalling' too: instead of reading up to 650 bases we obtain routinely 700 to 800.
At the same time we will increase the capacity, this is necessary as we have already analyzed 6800 samples in the first 8 month this year.
Our robotic system for preparing DNA from plasmids grown in bacteria works successfully since the end of August. The DNA prepared is of high quality as judged in a transfection assay. The robot starts with the bacterial cultures shaking for a given time on its working area, and then transfers the bacteria to 24-well filterplates, where the bacteria are lysed.
In the following steps the DNA samples are isolated with ion-exchange and activated glass filter columns arranged in a standard 96-well microtiterplate format.

Figure 1



A sequencing run on ABI 377 prism sequencer



Total numbers of samples sequenced in 1989 - 1997

Figure 2
The grey bars represent number of samples sequenced with conventional Sanger protocoll with radioactive dATP (33 and 32 P). The darker gray bars represents numbers of automated sequencing reactions done on both ABI 373A (1991 to 1997) and 377 (1995 to 1997) DNA sequencers with fluorescently labelled dideoxynucleotides.

** Calculated from data to end of October.*

The IMP is an open institute with a high turmover rate among the scientific staff, including the group leaders. The IMP is therefore 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 usually encountered at this stage of their careers. The IMP provides its group leaders with salaries for co-workers as well as generous budgets for consumables and material investments. Group Leaders' contracts are initially for 5 years but they are usually extended for a further 3 years (total of 8) following review by our Scientific Advisory Board which meets annually.

Strong collaborative links habe been stablished, both internationally and within Austria and 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 centre 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 a generous stipend. The purpose of this PhD-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 journal club and once a year present their data to the institute at one of the weekly '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.

After a initial selection 20-30 candidates are invited for interview which are held in Febuary. This selection process guarantees a high scientific standard and optimum training conditions.
The IMP is particularly interested in maintaining the international and multicultural nature of the institute (20 nationalities are represented at the IMP at this time).

Every year, the IMP organises a large international conference which has already established itself as a highlight of the European scientific calendar. In 1998 the '9th IMP Spring Conference' will be held in a magnificent location, the imperial palace. It will take place from 28 – 30 May, and will focus on 'Cell Biology of Cancer'. Besides 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 introduce the IMP-scientists to other fields and 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 relations between IMP members has been encouraged and supported from the beginning and newcomers are rapidly integrated and become part of the crowd. Popular events in the social calendar include the bi-annual IMP ski-trip or 'Wandertag'. A popular daily meeting place is the cafeteria, where members of staff meet over lunch or coffee or in 'Joe´s Disco' where various social events take place. But as if the lab work were not enough to keep the staff busy, a lot of joint sports, cultural or other activities take place, after all Vienna and the beautiful Alps offer opprtunities one can hardly resist.

Vienna´s golden years as the cultural centre of Europe were in the 18th and 19th centuries when the city was the centre of the Habsburg dynasty which had controlled much of central Europe for over 600 years. The dynasty had a supranational identity and its capital epitomized the cosmopolitan nature of the empire, attracting great minds from all over central Europe. This was reflected in the cultural sphere; the work of Freud, Klimt, Schiele and Schoenberg is as impressive today as it was centuries ago. But in this century 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. Fortunately, the collapse of the iron curtain all of a sudden placed Austria back in the heart of Europe.

There is, however, more to Vienna than a glorious past: a strong, home-grown, youthful culture, coupled with new influences from previously Iron Curtain-bound neighbours is making it once again a city right at the heart of contemporary European cultural life. Vienna is no longer a city dominated by old ladies in furs sipping coffe in a 'Kaffeehaus' – even though the latter still constitutes an integral part of Viennese life. The city now has much more to offer: tradition, culture and vitality make a heady combination and results in a increasingly attractive lifestyle with a high degree of social and moral freedom. Worth a mention: Vienna ranks as one of the safest metropolitan areas in the world.

Those who want to take a rest from city life only have to drive less than an hour to find themselves in the Wachau, a romantic stretch of Danube between vine-bearing hills. Drive for a couple of hours and you are in the middle of Austrian Alps, where glacier-carved lakes and craggy peaks offer a full range of summer and winter sports: the lakes in offer swimming, fishing, sailing and windsurfing. Thousands of km of well-signposted hiking trails invite you to explore the Alps; paragliding and mountain biking are both popular. In winter, skiing and snowboarding are the order of the day, although those with a distaste for crowds may find cross-country skiing more rewarding.

The scientific advisory board

Once every year the research at the IMP is scrutinized. This is when the scientific advisory board (SAB) meets. It consists of internationally recognised scientists, who are themselves active in basic medical and biological research. Together with the IMP researchers, they discuss the quality and the significance of research findings as well as the main focus of future work. Thus, they exert a decisive influence on the IMP's fate.

Members of the scientific advisory board

Prof. Michael Bishop
Dept. of Microbiology and Immunology; University of California

Prof. Nick Hastie
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seminar speakers at the imp 1997

January
09. Giulio DRAETTA – *Milano*
10. Matthias W. HENTZE – *EMBL, Heidelberg*
23. Aron MARCHLER-BAUER – *NIH, Bethesda*
    William COOKSON – *Univ. of Oxford*
28. Matthias MANN – *EMBL*
30. Mary WEISS – *Institut Pasteur, Paris*

February
13. Jeff FRIEDMAN – *Rockefeller Univ.*
20. Peter SARNOW – *Stanford*
26. Georgii GEORGIEV – *Biogen, Moscow*
27. Paolo SASSONE-CORSI – *Strasbourg*

March
05. Friedemann KIEFER – *Mount Sinai Hospital*
11. Martin WELSCHOF – *Univ. Heidelberg*
13. Axel ULLRICH – *Martinsried*
20. Martin SCHEFFNER – *DKFZ, Heidelberg*
21. Karin MÖLLING – *Zurich*

April
02. Ernst REICHMANN – *ISREC, Epalignes*
03. Stephen COHEN – *EMBL, Heidelberg*
04. Jozsef ZAKANY – *Univ. Geneva*
10. Björn VENNSTRÖM – *Karolinska Institute, Stockholm*
15. Winfried WAGNER-REDEKER – *Spectronex AG*
18. James C. SMITH – *NIMR, London*
24. Mike GUSTIN – *Rice University, Houston*

May
15. Alan LAMBOWITZ – *Ohio State University*
26. Tony PAWSON – *Mount Sinai Hospital,*
27. Hamilton SMITH – *Johns Hopkins University*

June
02. Carlos DOTTI – *EMBL Heidelberg*
04. Jean-Pierre DAVID – *Yale University*
05. Tom A. RAPOPORT – *Harvard Medical School*
09. Alan P. WOLFFE – *NIH Bethesda*
10. Marc KIRSCHNER – *Harvard Med. School*
11. Rochelle E. ESPOSITO
12. Philip BENFEY – *New York University*
13. Werner RISAU – *MPI, Bad Nauheim*
17. Christoph LENGAUER – *Johns Hopkins Oncology Center*
19. Terry ORR-WEAVER – *Whitehead Institute, Cambridge*
26. John F.X. DIFFLEY – *ICRF, Hertsfordshire*

July
01. Peter KRAMMER – *DKFZ Heidelberg*
03. Peter JACKSON – *Standford Univ.*
08. Fuyuki ISHIKAWA – *Tokyo Inst. of Technology*
10. Austin SMITH – *Univ. of Edinburgh*
11. Doug HANAHAN – *UCSF*
17. Katherine WILSON – *John Hopkins Univ.*
24. Stefan JENTSCH – *ZMBH Heidelberg*
29. Hartmut LAND – *ICRF*
30. Martin SCHWARZ – *MPI Göttingen*

August
07. Raymond J. DESHAIES – *Caltech, Pasadena*
14. Ruth S. NUSSENZWEIG – *NYU Medical Center*
22. Erwin G. van MEIR – *Lausanne*
27. Geoffrey WAHL – *Salk Institute, La Jolla*

September
04. Alex J. van der EB – *Leiden University*
08. Eli GILBOA – *Duke University*
09. Max PERUTZ – *MRC, Cambridge*
10. Ed HARLOW – *Mass. Gen. Hospital*
11. Roy PARKER – *Univ. Arizona*
15. Jonathan JONES – *The Sainsbury Laboratory*
16. Bertrand NADEL – *Scripps Research Inst., La Jolla*
18. Alfred L. GOLDBERG – *Harvard Medical School*
25. Kiyoshi NAGAI – *MRC, Cambridge*

October
02. Wolfgang BAUMEISTER – *Max Planck Institut*
06. Dirk GÖRLICH – *ZMBH Heidelberg*
07. Gerard KARSENTY – *Anderson Cancer Center, Houston*
09. Andrew LUMSDEN – *MRC, London*
10. Philip BEACHY – *Johns Hopkins University*
    Lorraine PILLUS – *Boulder Univ.*
16. Lionel WHIGHTMAN – *St. Thomas's Hospital, London*
22. Julian DAVIS – *Univ. of British Columbia*
23. Gail MARTIN – *UCSF*
30. Michael G. ROSSMANN – *Purdue University, Indiana*
31. Gisou van der GOOT – *Univ. Geneva*

November
04. Prem PONKA – *McGill Univ., Montreal*
10. Yasushi MATSUI – *University of Tokyo*
13. Lukas KÜHN – *ISREC*
18. Henk STUNNENBERG – *Univ. of Nijmegen*
21. Ursula GÜNTHERT – *Basel Inst. of Immunology*
27. Gerd JÜRGENS – *Univ. Tübingen*
28. Michael NAUMANN – *MPI, Berlin*

December
03. Peter B. BECKER – *EMBL Heidelberg*
04. Richard KOLESNICK – *Mem. Sloan Kettering Cancer Center*
12. Manuel ROJO – *Univ. Geneva*
16. Nathaniel HEINTZ – *Rockefeller Univ.*
18. Tim CHAMBERS – *St. George's Hospital, London*

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