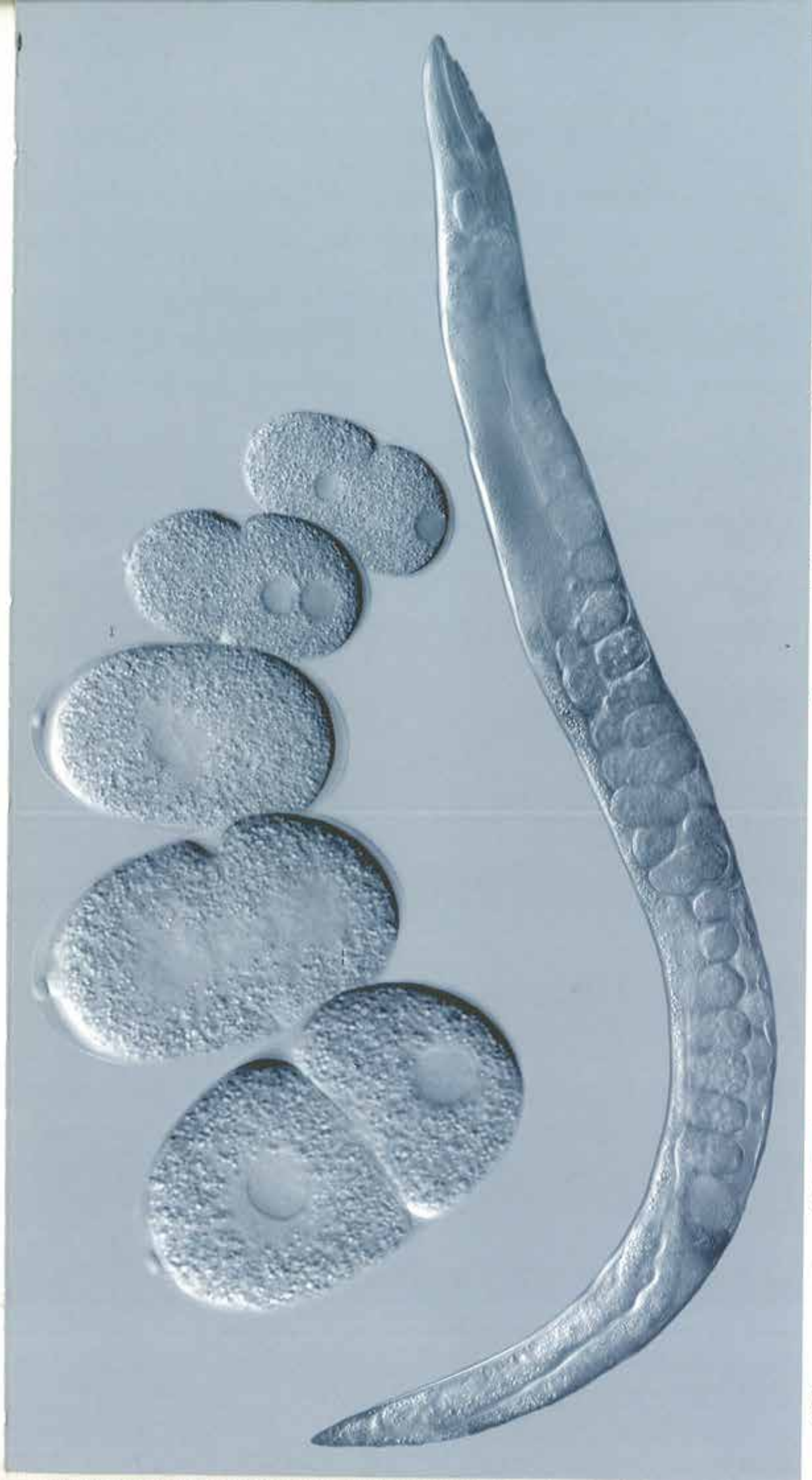

Research Institute of Molecular Pathology
VIENNA BIOCENTER

I.M.P. '99

Boehringer Ingelheim

The IMP is bulging at the seams as the new group leaders who have joined us in recent years build up their groups. Even our latest recruits, Barry Dixon and Annette Neubüser, have labs full of students beavering away at all hours and on all days of the week. This has also been an exceptional year for important discoveries. Of these, possibly the most unexpected were Stephen Nutt's finding that pax5 deficient cells retain the potential to differentiate into many different hematopoietic cell types and Frank Uhlmann's finding that sister chromatid separation in yeast depends on the proteolytic cleavage of a cohesin subunit.
The IMP's scientific reputation continues to grow and this has been recognized by the award of a large EU grant for Postdocs training and a second Wittgenstein prize [Austria's top scientific award]. The latter will be used to set up yet another young group leader, which will doubtless make the institute feel more crowded than ever before.

One of the IMP's great strengths has always been our communal services. As usual, we are indebted to Gotthold Shaffner's group for churning out DNA sequences and oligonucleotides at an ever accelerating rate, to Andreas Bichl's group for tending our mouse colony and caring for our chicken flock, to Christian Theussl for the transgenic mouse service, to Iris Killisch for the EM facility, and to Peter Steinlein for BioOptics. This year has seen three major developments in these crucial services. First, Peter Steinlein and an expanded biooptics department have established microarray equipment that will enable the measurement of expression profiles of thousands of genes simultaneously. Second, Karl Mechtler has made great strides in using mass spectrometry to identify polypeptides. Thanks to our co-operation with the University of Vienna, Karl will soon have access to top of the line MALDI and Q-TOF mass spectrometers and will be in charge of a new peptide synthesis and identification service. Finally, we welcome Frank Eisenhaber as a group leader in charge of a considerably expanded bioinformatics department. Frank and his colleagues will hopefully help us to make sense of the increased flow of information provided by microarray data and access to ever more complete genome sequences. Despite the deluge of biological information now flowing out of databases, we still see the IMP's role not merely as that of collecting data, which seems to be the mania of the moment, but also as making sense of complex data sets, by reducing them to simple concepts that can be grasped by the human mind. It is only simple concepts that are easily falsifiable, which is the crucial criterion that distinguishes innovative science from fact finding.

All these developments promise to be dwarfed by our next challenge: to establish a sister institute on our doorstep, to be funded by the Austrian Academy. Through studying the molecular and cellular biology underlying human disease, the new institute, whose provisional name is IMBA [Institute of Molecular and Cellular Bioinformatics] will complement the IMP's current efforts to study fundamental biological mechanisms. IMBA will have about eight groups and its career structure will be the same as the IMP. It will also share the IMP's scientific advisory board, administration, cafeteria, and scientific services. The goal will therefore be to create a pair of institutes that feel as if they are a single intellectual community. For the successful completion of our negotiations with the Academy and the Ministry of Science, the IMP is hugely indebted to our administrative director Nicholas Zacherl. That the Austrian Academy, the Ministry of Science, and the city of Vienna, who will pay for IMBA's construction, chose the IMP as a partner in this joint venture is a great tribute to our success in recent years, not only in making major discoveries but also in providing opportunities for young investigators of international calibre. We have become a major player on the European scene and now is the time to expand our successful formula.

Kim Nasmyth

Vienna, December 1999

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Developmental plasticity: deregulation in oncogenesis

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

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

Proliferation control of many types of hematopoietic progenitors is regulated by the cooperation of plasma membrane- and nuclear receptors. The first receptor family comprises receptor tyrosine kinases [RTKs], receptor serine kinases [TGFβR family] and cytokine receptors, while the second includes steroid receptors as well as the thyroid/retinoid acid receptor family. Mutated receptor versions, as well as associated corepressors and chromatin regulators, function as oncoproteins in avian, murine and human leukemias. In primary murine and human erythroblasts, sustained proliferation is induced upon activation of the erythropoietin receptor [EpoR], the RTK c-Kit and the glucocorticoid receptor [GR] by their respective ligands [Epo, stem cell factor/SCF and dexamethasone/Dex]. Normal or oncogenic versions of the epidermal growth factor [EGF] receptor [c- / v-ErbB] can substitute for the signals provided by EpoR plus c-Kit. Using genetically modified mice, we showed that the GR is dispensable for standard erythropoiesis, but required for enhanced erythroid progenitor proliferation in stress erythropoiesis [anemia/hypoxia]. The same approach [see Report 1998] revealed that STAT5 and complete EpoR signalling are dispensable for steady state erythropoiesis, but required for erythroid progenitor proliferation in culture. Mortal and immortalized, but otherwise normal erythroid progenitors [from wt and p53-/- mice, respectively; see Report 1997] are currently used to analyse signal transduction downstream of the EpoR and c-Kit and the involvement of STAT5 in signalling and target gene transcription. We already found that pathways used by the EpoR and c-Kit are distinct and that c-ErbB acts by combined signalling along EpoR and c-Kit pathways [Figure 1].
Gene expression profiling of translated genes using polysome-bound versus free mRNA was initiated in the erythroid system, showing that a surprisingly large proportion of genes regulated during erythroid differentiation are also subject to translational control. Applying this approach to Affymetrix chips, we will analyse the cooperation between the EpoR and c-Kit, their cooperation with the GR and their replacement by c- and v-ErbB. In addition, avian and human leukemia oncogenes [v-Ski, MLL/ENL] cooperating with c-Kit in avian multipotent cells will yield further interesting systems for gene expression profiling, after establishing these systems in mouse cells.

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

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

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

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

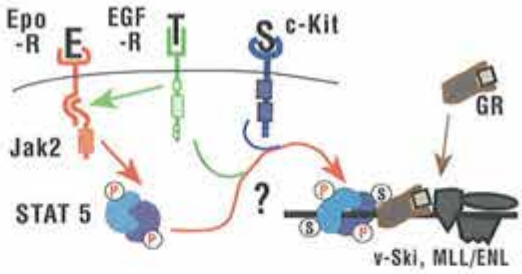
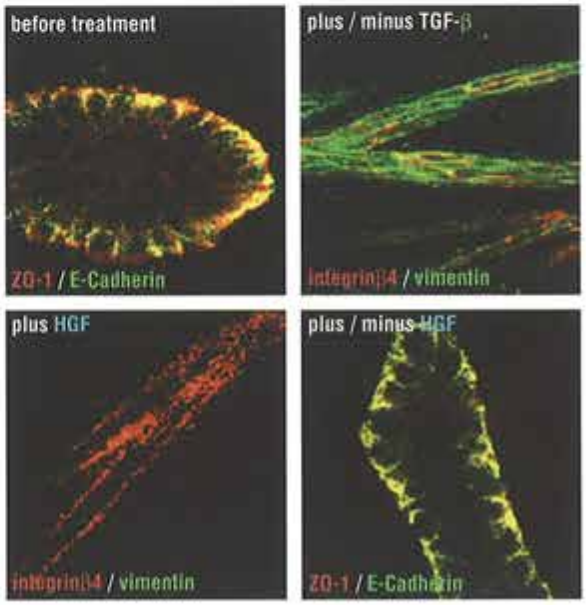


Figure 2



Growth factors implicated in human carcinogenesis: Scattering versus EMT

A new technique, *in situ* antibody staining of three dimensional structures formed by epithelial cells in collagen gels followed by confocal microscopy, allows us to distinguish between bona fide EMT and reversible scattering. Control Ras-transformed mammary epithelial cells form tubular structures with basolateral expression of epithelial markers [E-cadherin/ZO-1, top left]. TGFβ addition induces the cell to invade the gel, lose E cadherin [not shown] and gain the mesenchymal marker vimentin [top right]. In contrast, treatment with HGF/SF causes invasion of the gel but no upregulation of vimentin [bottom left]. When HGF/SF is removed, the cells again form tubular structures expressing epithelial markers [bottom right], while structures formed in TGFβ do not change after TGFβ removal [not shown].

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

We demonstrated that the cooperation of TGFβ-receptor [TβR] signalling with RTKs [HER2] or downstream signal transduction intermediates [Ras; MapK pathway] is required for normal mammary gland morphogenesis and is subverted in EMT, as well as in late processes during carcinogenesis. Interference with TβR signalling reverts EMT and prevents *in vitro* invasiveness and metastasis in numerous murine and human tumor models [see Report 1998]. Furthermore, Ras- or Mek-1 inhibitors abolished EMT, reverting the cells to an epithelial phenotype and restoring sensitivity to TGFβ-induced cell cycle arrest and apoptosis. Thus, EMT requires the sustained activity of two cooperating pathways, Ras- and TGFβ receptor signalling.

EMT has been postulated to occur in other systems, particularly after ligand activation of the receptors for fibroblast growth factors [FGFs] or hepatocyte growth factor/scatter factor [HGF/SF]. These factors have been strongly implicated in human carcinogenesis. However, FGF-R and HGF-R fail to induce EMT in Ras-transformed epithelial cells, instead inducing reversible morphological and migratory changes not accompanied by gene expression changes typical for EMT [loss of epithelial markers, gain of mesenchymal markers; Figure 2].

Analysis of signal transduction pathways downstream of Ras, using specific inhibitors and Ras effector-specific mutants selectively activating the Mek/MapK or PI3K/Akt pathways, revealed that Mek-1/MapK signalling is required for EMT, while the PI3K pathway is required for protection from TGFβ-induced apoptosis. We are now analysing whether both pathways are also required during tumorigenesis and metastasis *in vivo*. Furthermore, we have initiated gene expression profiling of translated genes on Affymetrix chips to find markers and key players involved in EMT. Besides analysing the first, interesting candidates, we will investigate the role of TGFβR signalling in EMT, focussing on the role of SMAD2 and SMAD3 in EMT in combination with Mek-1 signalling.

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Pax gene function in brain development, hematopoiesis and disease

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

Midbrain development

The midbrain and cerebellum develop from an organizing center which is located at the midbrain-hindbrain boundary [mhb] of the vertebrate embryo. We have previously demonstrated that the transcription factors Pax2 and Pax5 are critically involved in the development of this midbrain-hindbrain boundary region. One goal of our laboratory is to unravel the molecular mechanism by which Pax2 and Pax5 regulate midbrain and cerebellum development. Gene targeting and transgenic analysis [Figure 1] have indicated that Pax2 is essential for the initiation of *Pax5* expression at the mhb of the mouse embryo in agreement with the fact that *Pax2* expression precedes that of *Pax5* in this brain region. Apart from this cross-regulatory interaction, *Pax2* and *Pax5* have partially redundant functions in mhb development, as suggested by the analysis of heterozygous *Pax2/Pax5* double-mutant embryos. This hypothesis has been directly tested by replacing the coding sequences of *Pax2* with those of *Pax5* in the mouse germline. The brain phenotype [loss of midbrain and cerebellum] of the *Pax2* mutation is indeed rescued in these knock-in mice, indicating that Pax5 can compensate for the loss of Pax2 function. To date, relatively few genes are known which are expressed in the embryonic mhb region. To search for novel genes expressed in this brain area, we are currently screening mouse cDNA microarrays [containing 9 000 different genes] by hybridization with probes prepared from micro-dissected mhb regions of mouse embryos. Several new genes could be identified which are now being characterized also with regard to their regulation by Pax2 and Pax5. Hence, different experimental approaches are being used to identify upstream regulators and downstream targets of *Pax2* and *Pax5* in midbrain development.

B-lymphopoiesis

Pax5 plays an essential role in early B-lymphopoiesis, as it is required for progression beyond an early progenitor [pro-B] cell stage. The pro-B cells from the bone marrow of Pax5 [-/-] mice can be cultured *ex vivo* on stromal cells in the presence of IL-7. *In vitro* differentiation experiments demonstrated that these early pro-B cells are in fact uncommitted hematopoietic progenitor cells which can differentiate, upon stimulation with appropriate cytokines, to natural killer [NK] cells, T-lymphocytes and different cell types of the myeloid lineage [Figure 2]. However, B cell development is observed only if *Pax5* expression is restored by retrovirus-mediated gene transfer.

Figure 1

Requirement of a functional Pax-binding site in the midbrain-specific enhancer of *Pax5*. β-Galactosidase staining of mouse E11.5 embryos carrying a *Pax5-lacZ* transgene. Mut: mutation of the Pax-binding site identified in the 435-bp *Pax5* enhancer.

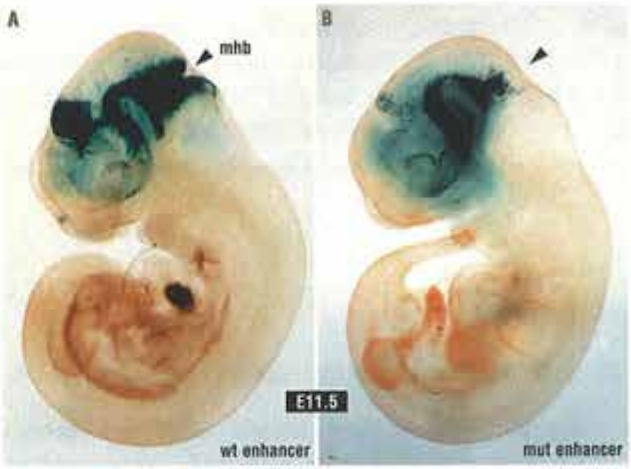
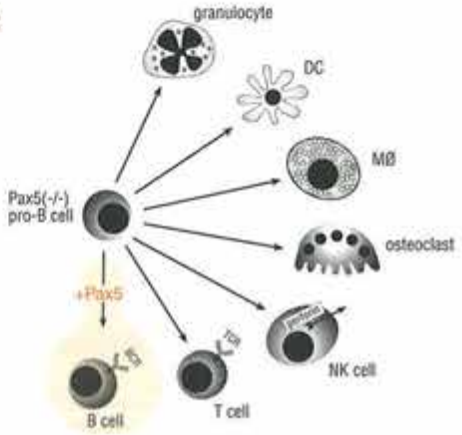
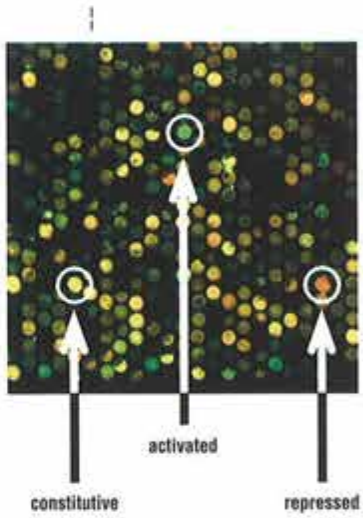


Figure 2



B-lineage commitment by Pax5. *Pax5* [-/-] pro-B cells represent hematopoietic progenitor cells which can differentiate along the indicated hematopoietic lineages with the exception of the B cell pathway. DC, dendritic cell; MØ, macrophage.

Figure 3



Identification of Pax5-regulated genes by cDNA microarray analysis. 9 000 mouse ESTs, spotted on glass slides, were simultaneously hybridized with Cy3 [green]-labelled cDNA from wild-type pro-B cells and Cy5 [red]-labelled cDNA from Pax5 [-/-] pro-B cells. Green and red signals identify genes which are activated or repressed by Pax5, respectively.

Hence, these experiments identified Pax5 as the critical B-lineage commitment factor which restricts the development potential of progenitor cells to the B cell pathway. Moreover, Pax5 also plays an essential role at late stages of B-lymphopoiesis, as shown by conditional gene inactivation experiments. At the molecular level, Pax5 exerts its function either as a transcriptional activator or as a repressor, depending on the specific regulatory sequence context. In order to gain insight into the early and late function of Pax5, we identify target genes by DNA microarray hybridization using cDNA probes from wild-type and Pax5 [-/-] pro-B cells [Figure 3]. Yeast 2-hybrid screens have furthermore led to the isolation of interacting proteins which function either as co-activators or as a co-repressor of Pax5. Together, these experiments will lead to a better understanding of the transcriptional control of B-lymphopoiesis.

Human disease

An unusual feature of mammalian *Pax* genes is their haploinsufficiency, which results in the frequent association of heterozygous *Pax* gene mutations with human disease syndromes and mouse development mutants. Our recent finding that *Pax5* is predominantly transcribed from only one of its two alleles in individual B-lymphocytes suggests that the haploinsufficient phenotypes of *Pax* genes may be caused by their monoallelic expression pattern. *PAX* genes can also be recruited as oncogenes by gain-of-function mutations in human tumors. *PAX5* has thus been implicated as an oncogene in the genesis of medulloblastoma and in non-Hodgkin's lymphomas carrying a specific translocation which brings the *PAX5* gene under the transcriptional control of the immunoglobulin heavy-chain locus. We are currently targeting *Pax5* expression in transgenic mice to the developing cerebellum [medulloblastoma] or to terminally differentiated plasma cells [non-Hodgkin's lymphoma] in order to gain further insight into the oncogenic role of *Pax5*.

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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 determine causal connections between the expression of a particular gene and tumor progression* in vivo. *One of the mouse models [Rip1Tag2] expresses SV40 large T antigen under the control of the rat insulin promoter leading to pancreatic β cell tumors in a multistage tumor progression pathway.*

Tumor cell survival

Our recent work has demonstrated that insulin-like growth factors [IGFs] and their receptor exert a critical survival function for tumor cells. Recently, we have investigated the communication pathway between IGF survival factors and the apoptotic machinery, i.e. the signalling pathway that suppresses the execution of tumor cell apoptosis. Our results indicate that, in tumor cells, IGF-mediated signal transduction can differ from the pathway described in non-transformed cells, such as fibroblasts and neurons. In particular, IGF-mediated survival signalling in tumor cells may not involve the activation of PI3-kinase and protein kinase B/c-Akt [Burtscher et al., 1999].

Tumor angiogenesis

Members of the fibroblast growth factor family [FGFs], in particular FGF-1 and FGF-2, are known to be highly angiogenic growth factors. However, their contribution to angiogenesis and tumor angiogenesis *in vivo* remains elusive. To interfere with FGF activity, we have generated recombinant adenoviruses that express soluble versions of FGF receptors. Expression of soluble FGF receptors inhibited angiogenesis in various bioassays *in vitro* as well as tumor-associated angiogenesis *in vivo*.

Notably, tumor growth in xenograft tumor transplantation experiments and the growth of β cell tumors in Rip1Tag2 transgenic mice were significantly repressed by the inhibition of FGF function [Compagni et al., submitted].

Many cellular and molecular events that are involved in the sprouting and branching of endothelial cells during angiogenesis are shared by the sprouting and branching of epithelial cells during Drosophila trachea development. For example, Drosophila genetics have revealed that FGFs and FGF receptor play an important role in trachea development and, more recently, an antagonist of FGF function has been identified, named Sprouty. We subsequently isolated cDNAs encoding for four different mouse Sprouty proteins and investigated their role in the regulation of angiogenesis. We found that Sprouty not only inhibits FGF-induced and VEGF-induced endothelial cell proliferation and differentiation but is also a target of the signalling cascade elicited by these growth factors [Figure 1]. Currently, we are investigating the mechanism by which Sprouty inhibits growth factor-induced angiogenesis in particular and tyrosine kinase receptor-mediated signal transduction in general. [Impagnatiello et al., submitted]

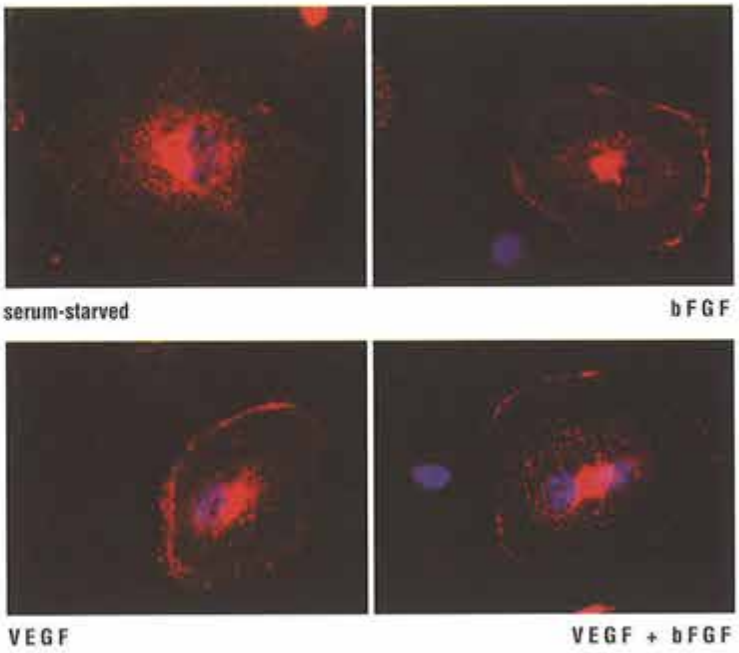

Figure 1

Sprouty-1 is recruited to the plasma membrane upon growth factor stimulation. Subcellular localization of mouse Sprouty-1 was visualized by immunofluorescent staining with affinity-purified antibodies against mouse Sprouty-1. Endothelial cells were serum-starved before addition of either fibroblast growth factor [FGF], vascular endothelial growth factor [VEGF] or the combination of both [FGF + VEGF].

Tumor cell invasion and metastasis

[in collaboration with the laboratory of H. Semb, Umeå University, Umeå, Sweden]

The molecular events involved in the transition from benign tumors to malignant tumors and metastasis are a major focus of our laboratory. Previously, we demonstrated that the loss of E-cadherin-mediated cell-cell adhesion is causally involved in the transition from well-differentiated adenoma to invasive carcinoma [Perl et al., 1998]. A major component of the E-cadherin cell adhesion complex, β-catenin, is also a central player in the Wnt-signalling pathway [Figure 2]. We have now investigated the possibility that changes in E-cadherin-mediated cell-cell adhesion modulate the Wnt-signalling pathway and thus gene expression. Our results indicate that E-cadherin antagonizes the Wnt-signalling pathway by sequestering β-catenin in the cell adhesion complex. Hence, two events are simultaneously required to modulate β-catenin/TCF-mediated transcription: the loss of E-cadherin function and the activation of the Wnt-signalling cascade [Herzig et al., submitted]. During the development of many human cancers, expression of neural cell adhesion molecule [N-CAM] changes from the 120 kd isoform in normal tissue to the 140/180 kd isoforms in tumor tissue. Recently, we have demonstrated that a similar switch of N-CAM isoforms occurs during tumor progression in Rip1Tag2 transgenic mice and that the loss of N-CAM function results in the metastatic dissemination of β tumor cells in Rip1Tag2 transgenic mice [Perl et al., 1999]. Currently, we are investigating the molecular mechanisms that are responsible for the induction of tumor metastasis by the loss of N-CAM function.

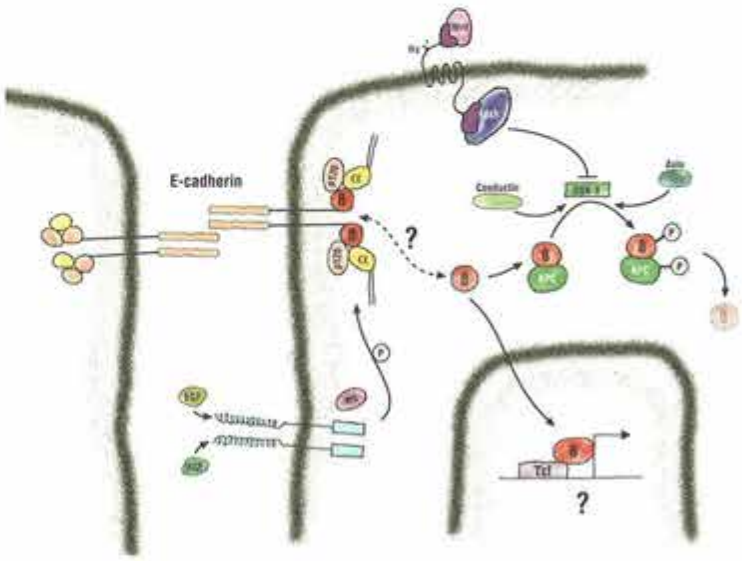

Figure 2

The link between E-cadherin-mediated cell-cell adhesion and the Wnt-signalling pathway. Two major questions for future research are indicated: [1] Does E-cadherin-mediated cell-cell adhesion directly modulate the Wnt-signalling pathway? [2] What are the TCF/β-catenin target genes that are relevant for tumor progression from adenoma to carcinoma?

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Adenovirus and CELO virus biology

The topics in our research group center around understanding how adenovirus and the related CELO virus interact with their host cells, both during productive infection and during transformation. These studies are important for unravelling cell growth control mechanisms, as these viruses must usurp important cell control pathways in order to replicate, and for developing gene delivery and vaccine reagents, as these viruses can be readily manipulated for these tasks.

Recombinant vectors based on the avian adenovirus CELO

The avian adenovirus CELO is being developed as a recombinant viral gene transfer tool. Transduction of avian cells occurs at 10-100 fold greater efficiency [per virus particle] than with an Ad5-based vector carrying the same expression cassette. Most important for gene transfer applications, the CELO vector transduces mammalian cells as efficiently as an Ad5 vector. These CELO vectors are sturdy, easy to modify, cheap to produce in chicken eggs and provide a useful alternative to Ad5-based vectors [Michou et al., 1999].

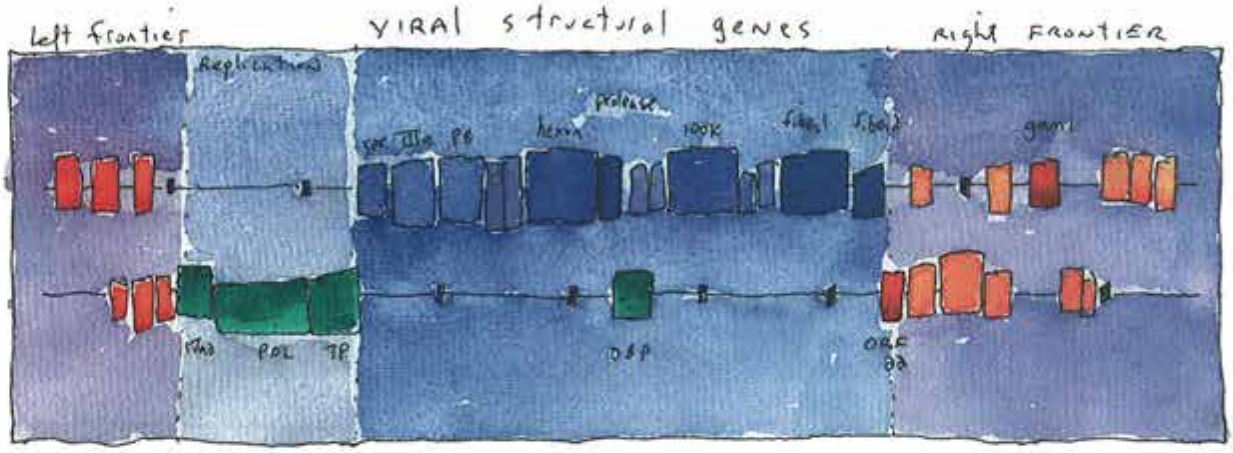
Activation of heat shock response by an adenovirus protein is essential for virus replication

Cellular heat shock responses occur during the replication of many viruses. Heat shock response could be a protective cellular defence against the virus or it could be deliberately activated by the virus to promote replication. We have found that expression of Gam1, an anti-apoptotic protein encoded by the CELO virus, elevates the cellular levels of heat shock protein 70 and 40. A Gam1-negative CELO virus is very defective for replication, however the Gam1 function can be replaced by heat shock. Thus, the essential function of Gam1 during virus replication is to activate the host heat shock response.
We are now seeking to understand the mechanism used by Gam1 to activate this heat shock response.

How CELO modulates pRb/E2f and p53

Like several of the human adenovirus serotypes, the avian adenovirus CELO is capable of transforming some mammalian cell types yet lacks sequence homology with the transforming genes of human adenoviruses. Transformation by other DNA tumor viruses requires an alteration of the retinoblastoma protein [pRb]/E2F interactions in the target cell as well as eliminating p53 functions. We have found that CELO Gam1 and Orf22 cooperate in activating the E2F pathway. Both proteins bind pRb, with Gam1 interacting with pRb regions outside the pocket domain and Orf22 binding to the pocket domain, similar to other DNA tumor virus proteins.
The motif in Orf22 responsible for the pRb pocket

Figure 1



The organization of open reading frames in the genome of the avian adenovirus CELO. The viral structural genes encoding capsid proteins and the DNA replication proteins are shown in blue and green. These sequences are homologous to Mastadenovirus sequences. The open reading frames novel to CELO are in orange or, for Orf22 and Gam1, in red.

domain interactions is essential for Orf22-mediated E2F activation, yet it is remarkably unlike the E1A LxCxD and may represent a novel form of pRb-binding peptide [Lehrmann and Cotten, 1999]. We have also discovered that CELO virus gene expression results in the proteosome-dependent loss of p53 protein from the host cell and we are well on our way to identifying the CELO proteins required for this process.

Microtubule-independent motility and nuclear targeting of adenoviruses revealed by novel fluorescent viral technology

A novel adenovirus system for analyzing the adenovirus entry pathway has been developed that contains GFP bound to the encapsidated viral DNA [AdLite]. AdLite enters host cells, accumulates around the nuclei, and near the microtubule organizing centers [Figure 2]. In live cells, individual AdLite particles are observed trafficking both towards and away from the nucleus. Depolymerization of microtubules during infection does not affect AdLite motility, nor does it alter the infection process or gene expression from adenovirus-derived vectors. These experiments demonstrate that microtubules are dispensable for the nuclear targeting by adenovirus and call into question the popular model of adenovirus entry.

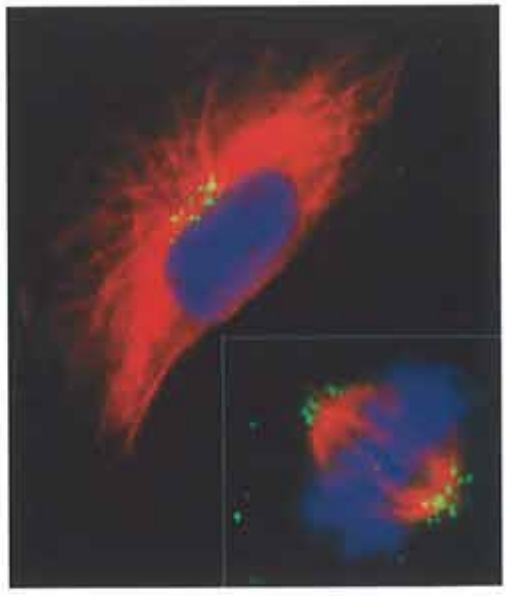


Figure 2

In non-mitotic cells, AdLite [adenovirus with GFP-marked genome] accumulates around the nucleus and microtubule organizing center within 1 hour after infection. In mitotic cells [inset] AdLite accumulates around spindle poles. AdLite – green/yellow, microtubules – red, DNA – blue.

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Axon guidance in Drosophila

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

Axon guidance in the CNS

In bilaterally symmetric organisms, such as flies and humans, many neurons in the central nervous system [CNS] send out axons that cross the midline to make connections on the opposite side of the body. Other axons do not cross the midline, but instead make connections on the same side of the body. Axons that cross the midline form the commissures that connect the two halves of the CNS. These commissural axons then join the non-crossing axons to form the longitudinal nerve fibres that run the length of the body [Fig 1]. The midline, in both flies and humans, secretes proteins that either attract axons towards the midline to form commissures, or repel them away from the midline to keep them in the longitudinal pathways. These guidance cues are members of the Netrin and Slit families, respectively, and act through receptors of the Frazzled/DCC and Roundabout [Robo] families. In Drosophila, axons that cross the midline transiently downregulate Robo levels, so that they are no longer sensitive to the midline repellent Slit. This downregulation of Robo requires a transmembrane protein, Commissureless [Comm] that is expressed by the midline cells.

We have identified two additional members of both the *robo* and *comm* gene families in Drosophila. We are currently investigating how the interactions between the various Robo and Comm proteins control the decision to cross or not to cross the midline. The three Robo receptors also appear to control pathway choices within the longitudinal tracts. Axons expressing all three Robos are strongly repelled by the midline and stay in the most lateral regions of the CNS. Axons expressing Robo3 and Robo1, but not Robo2, seem to be less strongly repelled by the midline and occupy an intermediate zone within the longitudinal pathways. Axons that express only Robo1 stay closest to the midline. We therefore propose that secretion from the midline establishes a gradient of the repellent Slit, and axons seek high, intermediate, or low points on this gradient, according to the combination of receptors they express [Fig 1].

We are currently testing this model by altering the 'Robo code' of individual axons to see if they follow the predicted pathways.

Axon guidance in the visual system

Vision requires very precise targeting of photoreceptor axons to the visual centres of the brain [Fig 2]. We have developed a novel genetic technique to identify genes required for photoreceptor axon targeting in Drosophila. In a saturation mutagenesis of over 32,000 mutant lines, we recovered some 200 mutations representing approximately 30 distinct genes.

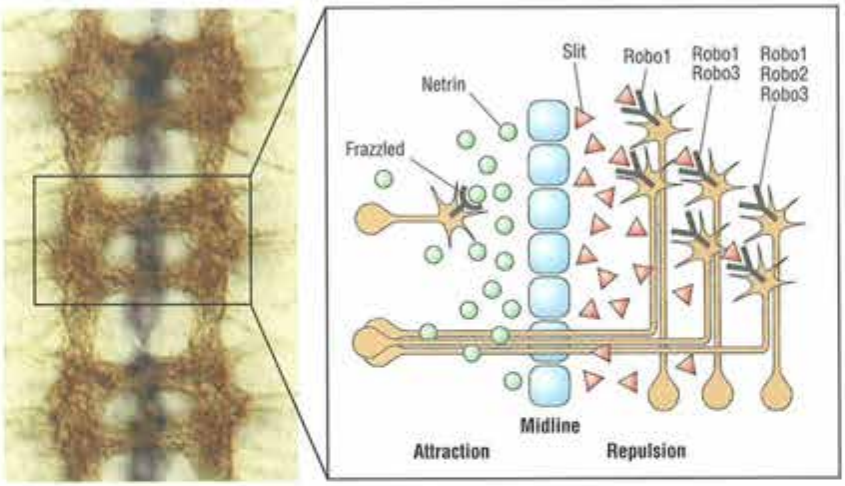


Figure 1

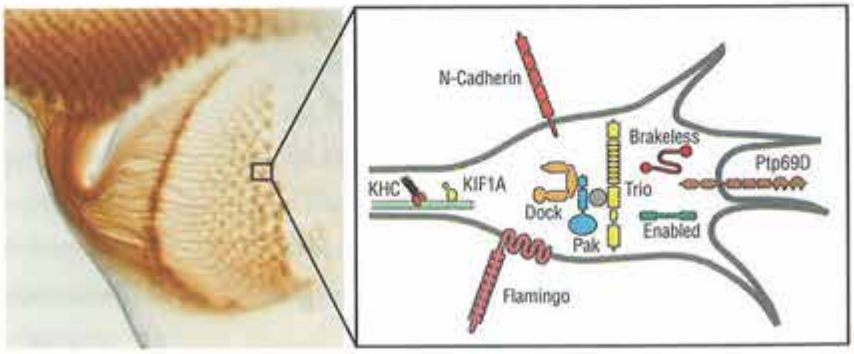
In the embryonic CNS, axons form two longitudinal tracts, one each side of the midline, that are connected in each segment by two commissures. The commissures are formed by axons that first cross the midline before turning into the opposite longitudinal tract. Axons are stained brown and the midline cells purple. The schematic diagram illustrates the signalling mechanisms that pattern these axonal projections. Attractive signals are shown to the left of the midline, repulsive signals to the right. The CNS is, of course, bilaterally symmetric, and in reality both mechanisms act on both sides of the midline, so that each axon shown would have a contralateral homologue, making a mirror image projection.

Ten of these have now been identified [Fig 2]. These genes have been found to encode either transmembrane proteins [PTP69D, N-Cadherin and Flamingo], cytoplasmic signalling components [Dock, Pak, Trio, Enabled and Brakeless], and mediators of axonal transport [KHC and KIF1A]. Most of the remaining 20 genes have now been mapped down to regions of around 100-200kb, and the molecular characterisation of these genes will now be greatly facilitated by the availability of the complete genome sequence of Drosophila. Meanwhile, we continue to examine in detail the functions of the three genes that have been cloned in our laboratory: *trio*, *brakeless* and *KIF1A*.

Trio is a guanine nucleotide exchange factor [GEF]. GEFs stimulate the release of GDP from small GTPases, allowing them to take up GTP and thus switch from the inactive GDP-bound form to the active GTP-bound form. In the active GTP-bound state, these GTPases then associate with and activate a variety of downstream effectors that mediate their distinct cellular responses. We have identified the substrates of Trio as the two Rac GTPases, Rac1 and Rac2, and a novel GTPase that we have named Mtl. Rac1 and Rac2 in turn activate the serine/threonine kinase Pak, which is recruited to the membrane via its association with the SH2-SH3 adaptor Dock. Pak regulates the actin cytoskeleton, possibly via the LIM and myosin light chain kinases, so we propose that localized activation of Trio in growing axons could lead to localized changes in the cytoskeleton that alter the direction of axon outgrowth. Mtl does not activate Pak and must therefore act through different signalling pathways. We are currently using both molecular and genetic approaches to identify additional components of this signalling pathway.

Figure 2

In the developing visual system, photoreceptors in the eye imaginal disc project their axons to specific targets in the brain. Ten proteins required to establish these connections have now been identified. Another twenty await further characterisation.



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The perspective of bioinformatics research at the IMP

The essence of bioinformatics consists in the computer-based meta-analysis of available experimental biological data [at present, mostly biomacromolecular sequences] aimed at the generation of hypotheses on the function of genes and proteins. This new research group focusing on the development of bioinformatics methods and their application on problems of eukaryotic cell division and differentiation started working in August 1999.

Traditionally, observation and, later, wet experimentation were the major sources of new knowledge in biology. It has always been more difficult to obtain new biological data than to analyze it. Recently, this situation has been qualitatively changed due to the development of high-throughput experimental technologies [HTT]. The explosive growth of biomacromolecular sequence and structure databases, of databases with genetic, cell-physiological [RNA and protein expression], and clinical data, e.g. on inherited diseases, etc., as well as of electronic sources of scientific literature make it a compelling necessity to apply sophisticated theoretical methods for the analysis of biological data, as well as for the principal planning of new biological experiments *in vitro* and *in vivo*. The integration of biological data as well as the generation of new biological insight in the form of models offered for subsequent experimental verifications is the task of ***bioinformatics***.

Two primary tasks in the IMP's bioinformatics research

- The computer-based analysis of biomacromolecular sequences and the generation of hypotheses on their native structure and biological function was at the beginning and still is the core of bioinformatics. After the accumulation of small technological advances during the past two decades, the first major breakthrough in HTT was achieved in the field of DNA sequencing. Today, we face the close perspective [months and a few years] that all genomes of medically, agriculturally, and industrially, as well as many evolutionarily important organisms will be known. This also includes the complete human genome and many of its individual variations. The creation of a sequence analysis environment composed of our own developments and the available academic software will be the first task of the bioinformatics group at the IMP. We want to analyze the domain structure, molecular function and evolution of proteins involved in eukaryotic cell division and differentiation.
  We can rely on extensive previous experience. For example, we have successfully developed a new approach for the characterization and prediction of Glycosylphosphatidylinositol sequence motifs in proprotein sequences [see Figure 1].

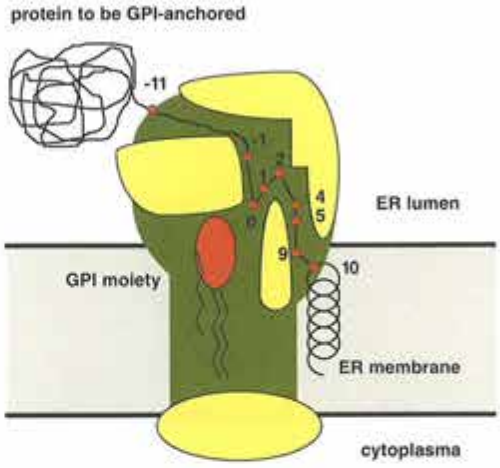


Figure 1

Glycosylphosphatidylinositol [GPI] anchoring is a common posttranslational modification of extracellular eukaryotic proteins. Attachment of the GPI moiety to the carboxyl terminus [ω-site] of the polypeptide occurs after proteolytic cleavage of a C-terminal propeptide. The protein sequence pattern for GPI-modification has been analyzed in terms of physical amino acid properties based on a database analysis of annotated proprotein sequences.
The variations of polypeptide substrates were exploited to suggest a model of the polypeptide binding site of the putative transamidase, the enzyme catalyzing the GPI-modification. The putative transamidase is thought to be a protein with a large membrane domain and another endoplasmic reticulum domain. The diagram represents a section through the enzyme. Inside surfaces of cavities and invaginations are colored dark, the faces of the section are yellow. Important residues of the substrate protein are shown with red circles and are numbered with respect to the ω-site.
The volume of the active site cleft accommodating the four residues ω-1...ω+2 appears at about 540Å^3. This location has to communicate with the GPI-moiety binding site. The channel between the substrate protein in the endoplasmic reticulum [ER] lumen and the catalytic site is occupied by the flexible polypeptide segment ω-11...ω-1. The spacer ω+3...ω+9 [with a possible special binding site for ω+4...ω+5] links the residues in the catalytic cleft with the hydrophobic tail [possibly forming an α-helix] embedded into the ER membrane.

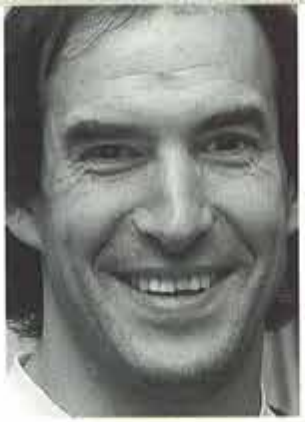
- The knowledge of genome sequences is becoming complemented with transcriptome [mRNA expression] and proteome [protein synthesis] data in specific physiological conditions for cell lines and *in vivo*. The second immediate task of the group consists in developing a software environment for analyzing such data streams and for delineating biological effects. Robert Hoffmann and Anton Beyer have already developed a JAVA-based system for visualizing cDNA chip data and for the selection of co-expressed genes.

The perspective of computing at the IMP

The bioinformatics group as the most extensive computer user has been merged with the previously existing computer group. It is thought to reshape the forms of providing central computing and networking services by focusing on major tasks. We aim at improving standards under the conditions of a growing institute, of the increasing necessity of computer usage in experimental labs and also of short cycles of the renewal of computer hardware and software.

Figure 2

X-ray structure of HIV-1 reverse transcriptase [HIV-1 RT] bound to DNA, which is shown as a ball and stick model.
HIV-1 RT is a key target for drug development in AIDS research. Inhibition of this protein blocks the replication of the HIV-1 virus.



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The mechanism of cytokinesis

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

Cytokinesis is accomplished by an actin-based contractile ring that is attached to the overlying cell membrane [Figure 1]. The ring assembles in the cortex midway between the two poles of the mitotic spindle so that the two separated sets of chromosomes are equally partitioned into the two daughter cells. The entire process, the assembly of the contractile ring, its constriction and the separation of the two nascent, cells typically requires about ten minutes. Thus, cytokinesis is a dynamic and spatially regulated process and analysis of this process therefore requires the use of techniques that provide spatial and temporal resolution. Early embryos of the nematode *C. elegans* are an excellent model system to dissect this complex process since this system is extremely well suited for the microscopic analysis of intact embryos. Furthermore, this system can be molecularly dissected using forward and reverse genetics.

The basic approach we are pursuing is to use forward genetics to identify mutations that cause specific defects in cytokinesis and then use molecular techniques to identify the affected genes. Subsequently, we use biochemical and cell biological analysis to understand the function of these novel proteins and to investigate whether they may regulate the function of any of the previously characterized components. Our recent analysis of one such mutant can illustrate this approach.

As mentioned above, the contractile ring is an actin-based structure. Like many actin-containing cellular structures, the rho family of GTPases plays an important role in its assembly and regulation. One of the mutants we have characterized in the past year encodes a GTPase activating protein [GAP] for rho GTPases family. This protein seems to play a crucial role in the later stages of cytokinesis. Interestingly, we find that this protein is crucial for proper reorganization of the spindle during anaphase. Thus, this protein provides a link between the mitotic spindle and the contractile ring. We have evidence that this GAP protein is functionally associated with a microtubule-based molecular motor, which may, in part, explain how it can regulate the mitotic spindle. We are now using biochemical and genetic techniques to discover additional new proteins with which this GAP protein may interact.

The nematode *C. elegans* is also highly amenable to reverse genetics. The entire genome has been sequenced and there are a number of genes which may be implicated in cytokinesis for a variety of reasons. We have made extensive use of the technique of RNA-mediated interference [RNAi] to facilitate the cloning of the genes affected in the mutant strains.

This technique is also useful for exploring whether genes implicated in cytokinesis in other systems – such as in yeast or plants – which cleave by somewhat different mechanisms than animal cells, are likewise required for cytokinesis in the nematode. In plant cells, for example, a member of the syntaxin gene family is required for cytokinesis. Since plant cells have a rigid cell wall, they make extensive use of membrane fusion machinery to divide after mitosis. We investigated whether animal cells also require any components of this membrane fusion machinery for cytokinesis. By using RNAi to individually inactivate each of the eight syntaxin genes that could be found in the *C. elegans* genome, we identified one such syntaxin that we demonstrated as being essential for cytokinesis [Figure 2].

A key goal for the next year will be to screen for additional cytokinesis-defective mutations. As there is evidence that several of the factors that control cytokinesis are also required for the viability and fertility of an adult worm, we have chosen to screen for conditional mutations that cause cytokinesis defects. We hope to assemble a collection of temperature-sensitive embryonic lethal mutations and will then use a battery of secondary assays to determine which of these mutations are defective in 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. During the past year we reconstituted the nucleotide-dependent polymerization of a recombinant septin and demonstrated that this novel cytoskeletal filament is a polar structure [Figure 3]. We hope to now investigate the dynamic properties of these filaments in intact cells during cytokinesis and to identify factors that specifically interact with filamentous septins.

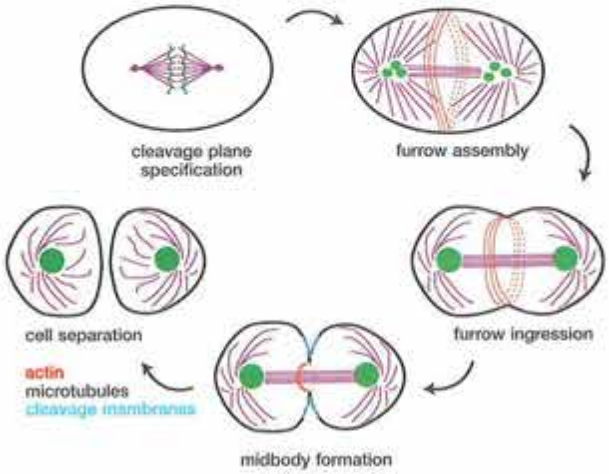

Figure 1

A schematic view of the stages of cytokinesis

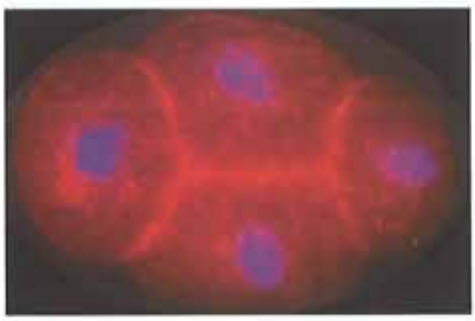

Figure 2

The distribution of the syntaxin Syn-4 [red] and DNA [blue] in a 4-cell *C. elegans* embryo

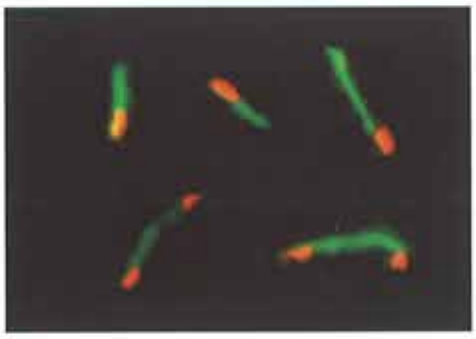

Figure 3

Polarized assembly of septin filaments polymerized *in vitro*

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Epithelial polarity

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

One major tool we used was well characterized cell systems that display both epithelial polarity and its disruption in cancer in an *in vivo*-like fashion. We primarily used a mouse mammary gland epithelial cell line [EpH4] expressing an estrogen-inducible c-JunER fusion protein, which allows the analysis of reversible loss of epithelial polarity in a fashion superior to existing approaches. This hormone-induced disruption of epithelial polarity in JunER cells entailed the loss of transepithelial resistance, the redistribution of both apical and basolateral proteins over the entire plasma membrane and the destabilization of junctional complexes [Fialka et al., J. Cell Biol 1996]. Using this cell system, we aimed at identifying genes and proteins that were differentially expressed during loss of epithelial polarity. Two different approaches were taken to study this problem. Firstly, we screened for genes differentially expressed during loss of epithelial polarity, using Differential Display techniques. Secondly, we analyzed differentially expressed proteins of the intracellular protein transport and sorting machinery, analyzing purified subcellular organelles by 2D gel technology and microsequencing.

TIS7/PC4, a new regulator of wnt-signalling in epithelial cells

Last year we reported that TIS7/PC4 was upregulated during c-Jun-induced transient loss of epithelial polarity, as well as being detached from the plasma membrane and translocated to the cytoplasm or nucleus [Figure 1]. Thereafter, TIS7/PC4 interacts with components of the wnt-signalling pathway, selectively squelching gene activation by beta-catenin. TIS7/PC4 could thus be a co-repressor of beta-catenin/TCF/LEF-regulated transcription, necessary for cell fate decisions of epithelial cells during transient loss of cellular polarity [Vietor et al., submitted].

p14, a Golgi-adaptor for mitogen activated protein kinases

We discovered a novel, highly conserved protein of about 14 kD [p14] that localized to the Golgi apparatus and to the intermediate compartment between ER and Golgi [ERGIC 53]. Using yeast two-hybrid screening, we identified MP1 [MEK Partner 1] as a specific binding partner of p14. MP1 was proposed to serve as a scaffold protein of the MAP kinase cascade in higher eukaryotic systems and the protein seems to selectively associate with MEK1 and ERK1 [Schaeffer et al., Science, 1998]. We also observed that phosphorylated MEK1 was recruited to the Golgi [Figure 2] when disassembly starts during prophase in mitosis [Fialka et al., manuscript in preparation].

Molecular interactions in lipid rafts of epithelial cells

CD44, the major cell-surface receptor for hyaluronic acid [HA], was shown to localize to lipid rafts, i.e. detergent-resistant, cholesterol-rich microdomains, in fibroblasts and blood cells. We have investigated the molecular environment of CD44 within the plane of the basolateral membrane of polarized mammary epithelial cells and our data indicate that the vast majority of CD44 in mammary epithelial cells interacts with annexin II in lipid rafts in a cholesterol-dependent manner. These CD44-containing lipid microdomains interact with the underlying actin cytoskeleton. [Oliferenko et al., J. Cell Biol. 1999].

Technology development: Organelle-PROTEOMICS

In the context of our 'protein transport and sorting' projects we developed several new techniques that have already proven their usefulness for cell biologists. We adapted flow cytometry to sort and analyze intracellular organelles, combining conventional subcellular fractionation techniques with high speed organelle sorting in a flow cytometer [fluorescent activated organelle sorting, FAOS; Fialka et al., Elecetrophoresis 1999; Fialka, Steinlein et al., J. Biol. Chem., 1999]. These highly purified membrane fractions required specialized techniques for subsequent biochemical analysis. We established a novel procedure which enabled us to resolve purified membrane proteins on high-resolution 2D gels at both the analytical and preparative level [for examples see articles in Electrophoresis Vol 18, 1997, No. 14, special issue: Vesicular Traffic].

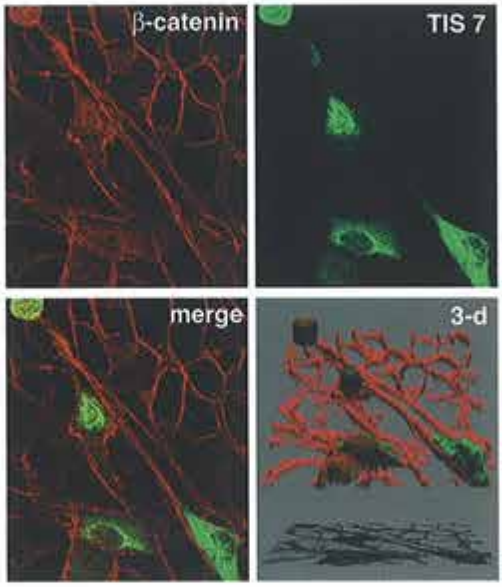

Figure 1: TIS 7 overexpression re-distributes β-catenin .

Confluent mammary epithelial cells were infected with the recombinant CELO virus expressing Myc-tagged TIS7 and cultured for a further 48 hrs. Cells were fixed, permeabilized and visualized by double-immunofluorescence labelling with the affinity-purified polyclonal anti-Myc antibody [green] and monoclonal antibody against β-catenin [red]. Extended focus images of serial sections are shown in two upper panels and their merge is shown in the bottom left panel. 3D-reconstruction of those sections is shown in the bottom right panel. Two days after infection, TIS 7 localized predominantly in the cytoplasm and also in the nucleus of overexpressing cells. These cells lost the typical polarized epithelial phenotype and formed elongated processes. β-catenin re-distributed into the cytoplasm of overexpressing cells. Subsequently, TIS 7 overexpressing cells rounded up and protruded from the monolayer [merge and 3D reconstruction of the same field after isosurface calculation]. Cells infected with a CELO-EGFP genotypic control virus showed no such effects [data not shown].

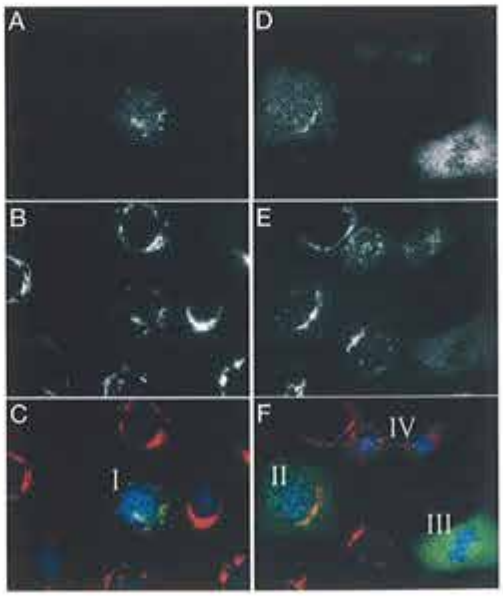

Figure 2: Activated MEK1/2 localizes to the Golgi in early prophase

Mammary epithelial grown in 5% FCS were fixed and activated MEK1/2 were detected using phospho[Ser217/221]-specific antibodies [A,D], together with antibodies to giantin [B,E] to highlight the Golgi apparatus. Note the localization of activated kinases to the Golgi early in prophase [I], the accumulation in metaphase [II, III] and the rapid deactivation towards cytokinesis [IV] in C and F [merged images].

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Mammalian higher order chromatin

In eukaryotic model systems, like yeasts and flies, epigenetic control of gene regulation and the functional organization of chromosomes depends on higher order chromatin. Particularly for the high complexity of mammalian development, deregulated inheritance of gene expression patterns ['transcriptional memory'] results in perturbed differentiation and proliferation [cancer], and compromised centromere activity induces mis-segregation of chromosomes and genomic instability [aneuploidies]. To analyze components and functions of mammalian higher order chromatin, we have isolated homologues of the Drosophila Polycomb-group [Pc-G] gene Enhancer of zeste [E(z)] [designated Ezh1 and Ezh2 (Laible et al., 1997; Laible et al., 1999)] and of the Drosophila PEV modifier Su[var]3-9 [designated Suv39h1 and Suv39h2 (Aagaard et al., 1999; O'Carroll et al., submitted)]. Our data reveal that Ezh genes can confer 'transcriptional memory' by modulating repressive chromatin domains and that Suv39h genes encode novel components of higher order chromatin at mammalian centromeres.

Ezh and conserved gene silencing

Transcriptionally restrictive chromatin domains – for example at centromeric positions in *Drosophila* or at *S.cerevisiae* telomeres – repress gene activity in a gene non-specific manner. To demonstrate the function of the mammalian *Ezh* and *Suv39h* homologues, we examined their potential to silence gene activity in both of these model systems. Extra gene copies of human *EZH2* in transgenic flies enhance the suppression of pericentromeric PEV [position effect variegation] alleles of e.g. the *white* gene, and the over-expression of *EZH2* or murine *Ezh1* restores gene repression in *S.cerevisiae* mutants that are impaired in telomeric silencing [Laible et al., 1997]. These data provided a direct functional link between *Polycomb*-dependent gene repression and inactive chromatin domains [Jenuwein et al., 1998], and indicate that silencing mechanism[s] may be broadly conserved in eukaryotes. A similar modification of PEV has also been shown with transgenic flies that carry an extra gene copy of human *SUV39H1* [Aagaard et al., 1999].

Suv39h1, a novel centromere-associated protein

To analyze the chromatin association of endogenous Suv39h1 and EZH2 proteins, we generated affinity-purified, rabbit polyclonal anti-sera. In contrast to the diffuse distribution of Ezh2/EZH2 proteins, immunodetection of endogenous Suv39h1/SUV39H1 in mammalian cells indicates enriched localization at heterochromatic foci during interphase and accumulation at centromeric positions on metaphase chromosomes [Figure 1][Aagaard et al., 1999]. However, forced expression results in abundant associations with chromatin, suggesting additional role[s] for Suv39h proteins in the structural organization of chromosomes. Using stably transfected cell lines, we identified several interacting proteins that specifically co-precipitate with overexpressed [myc]3-tagged human SUV39H1. Based on these interactions, we are currently purifying the first mammalian SUVAR complex.

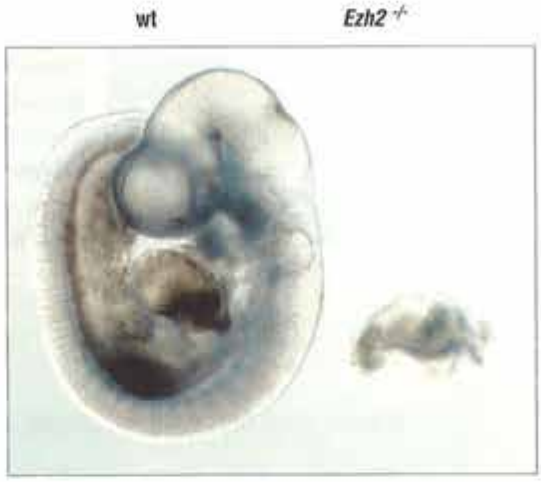

Figure 2

Ezh2 is required for normal mouse development. Whole-mount preparation of wild-type [wt] and *Ezh2*-/- litter-fetuses at embryonic day 9.5. *Ezh2* null fetuses stop progressing beyond day 8.5 of gestation, indicating an essential function for the *Ezh2* gene during mammalian development.

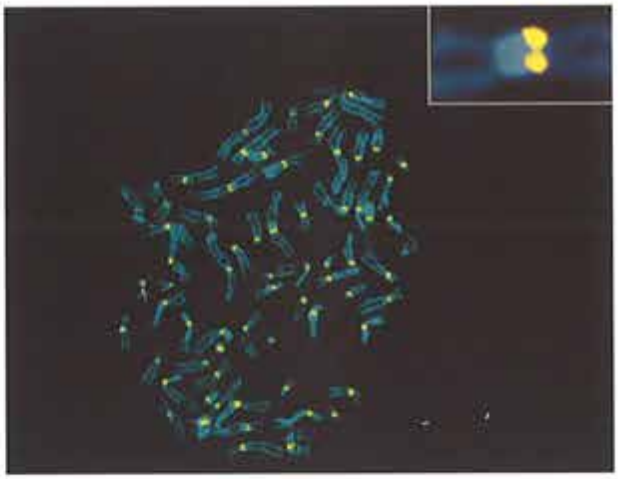

Figure 1

Specific association of SUV39H1 with centromeric positions at human metaphase spreads. HeLa cells were enriched for metaphase arrest with colcemid, and unfixed metaphase chromosomes were processed for indirect immunofluorescence with α-Suv39h1 antibodies. The DNA was counterstained with DAPI. The enlarged insert documents specific, two-dotted co-localization with the centromeres of the sister chromatids of human chromosome 1.

Higher order chromatin and growth control

To generate gain-of-function mutations *in vivo*, we established transgenic mice for human *EZH2* and *SUV39H1*. Whereas several of the *EZH2* lines display only low to moderate expression levels, two mouse lines were identified that overexpress [myc]3-tagged SUV39H1. Surprisingly, *SUV39H1*-transgenic mice display smaller body mass at birth and exhibit skeletal malformations in the A-P axis, suggesting that high SUV39H1 protein levels are incompatible with normal growth control and development. Consistent with such a function, *Suv39h1*$^{-/-}$ mice gain larger body mass with increasing age and *ex vivo Suv39h1*$^{-/-}$ cells are also shifted in their proliferative potential [unpublished]. We are currently investigating whether this altered proliferation can be correlated with compromised chromosomal stability – a phenotype that has been suggested to underlie the etiology of many human cancers. Finally, we have disrupted both *Ezh* loci in the mouse germline. Whereas *Ezh1* null mice are viable, disruption of the more embryonically expressed *Ezh2* gene results in lethality during early gestation [Figure 2] [O'Carroll et al., in prep.]. These data define an essential function for the *Ezh2* gene during mammalian development.

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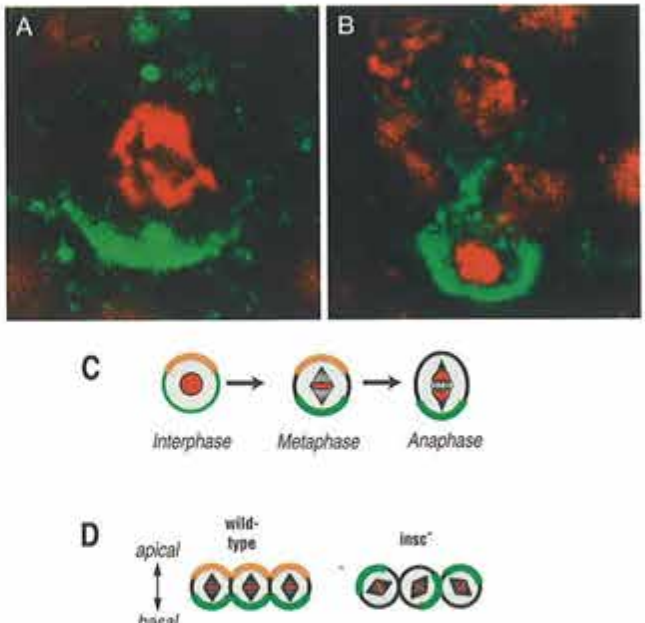
Asymmetric cell division during *Drosophila* nervous system development

To generate the many different cell types in a multicellular organism, cells must be able to divide 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 1A, B]. In the absence of Numb, this daughter cell is transformed into its sister cell, whereas the overexpression of *numb* leads to the opposite cell fate transformation. Thus, Numb acts as a segregating determinant during the development of the *Drosophila* nervous system.
Correct asymmetric segregation of Numb requires the protein Inscuteable. Like Numb, Inscuteable is asymmetrically localized in dividing neural precursor cells. However, Inscuteable localizes already in interphase – before Numb – and it localizes to the opposite side of the cell [Figure 1C]. In the absence of Inscuteable, Numb either fails to localize asymmetrically or the Numb crescents form at random positions around the cell [Figure 1D]. Inscuteable is also required for the correct orientation of the mitotic spindle, which determines the division plane. Neuroblasts which normally divide along the apical-basal axis [Figure 1D] divide with random division planes in *inscuteable* mutants.
Conversely, ectopic expression of Inscuteable in epithelial cells which normally divide parallel to the epithelial surface leads to reorientation of the mitotic spindle and cell division perpendicularly to the surface. Thus, Inscuteable directs and coordinates several events during asymmetric cell divisions.

To ensure that the right cell types are generated at the correct positions, asymmetric cell divisions have to be coordinated with the general body plan.
During the past year, we were able to characterize the mechanism that orients asymmetric cell divisions in *Drosophila* neuroblasts [Markus Schober, Matthias Schaefer]. These neuroblasts arise by delamination from polarized epithelial cells in the ventral neuroectoderm. In these epithelial cells, the PDZ domain protein Bazooka localizes to the apical cell cortex [asterisks in Figure 2A] and is required for apical-basal polarity. Apical Bazooka localization is maintained when neuroblasts delaminate from the epithelium and the expression of Inscuteable starts [arrowheads in Figure 2A]. Inscuteable binds to Bazooka *in vitro* and *in vivo*, and in the absence of Bazooka, Inscutea-

Figure 1

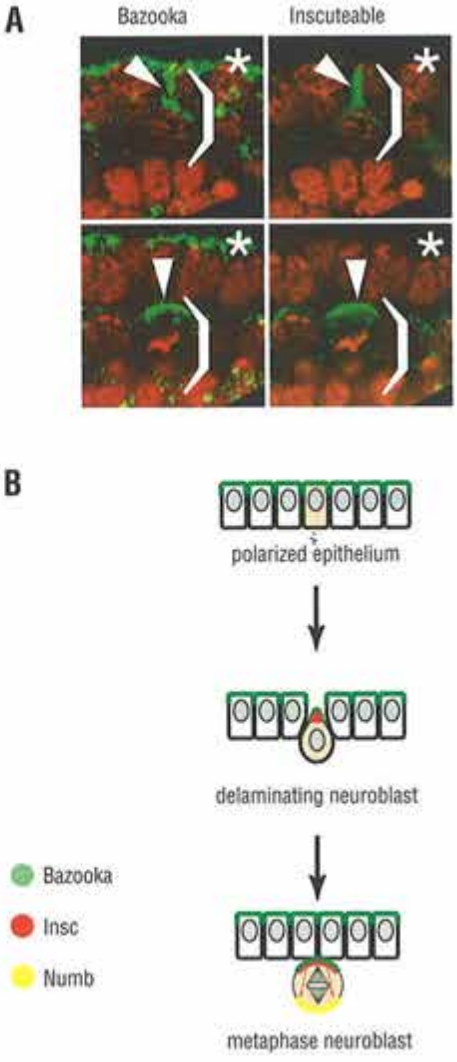


Asymmetric cell division in Drosophila neuroblasts

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

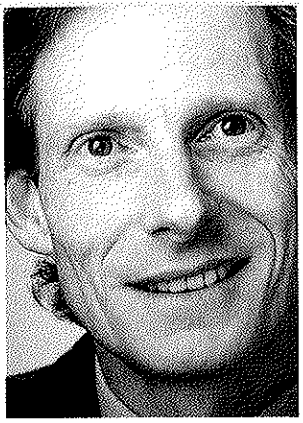
ble fails to localize asymmetrically and is found in the cytoplasm instead. Thus, direct binding to Bazooka mediates the asymmetric localization of Inscuteable and Bazooka and Inscuteable cooperate in translating epithelial polarity into asymmetry during neuroblast division [Figure 2B].

Figure 2



The events that occur downstream of Inscuteable are less well understood. In a two-hybrid screen for Inscuteable interacting proteins, we identified the microtubule binding coiled-coil protein Cornetto [Silvia Bulgheresi]. Like Inscuteable, Cornetto localizes asymmetrically in neuroblasts. Cornetto localization is Inscuteable dependent and the characterization of *cornetto* mutants will hopefully tell us whether Cornetto is involved in Inscuteable dependent spindle orientation. In addition, we have started a large scale genetic screen for mutations affecting asymmetric cell division [Tibor Török, Daniela Berdnik]. So far, we have identified at least eight new genes that are required for making certain cell divisions in the *Drosophila* nervous system asymmetric. We hope that the characterization of the corresponding proteins will help us to understand the molecular machineries that cells utilize to divide asymmetrically.

[A] Localization of Bazooka [green, left panels] and Inscuteable [green, right panels] in epithelial cells [asterisks] and neuroblasts [arrowheads]. Epithelial cells express Bazooka, but not Inscuteable. In delaminating neuroblasts [top panels] Bazooka and Inscuteable co-localize in a stalk that is left behind in the epithelial cell layer. In metaphase neuroblasts [bottom panels], both proteins co-localize at the apical cell cortex. [B] Bazooka and Inscuteable cooperate to orient neuroblast divisions. Apically localized Bazooka protein [green] is inherited by delaminating neuroblasts. During delamination, Inscuteable [red] binds to Bazooka. Both proteins co-localize at the apical cell cortex and during mitosis, Inscuteable directs spindle orientation and asymmetric localization of Numb.



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Chromosome segregation during mitosis and meiosis

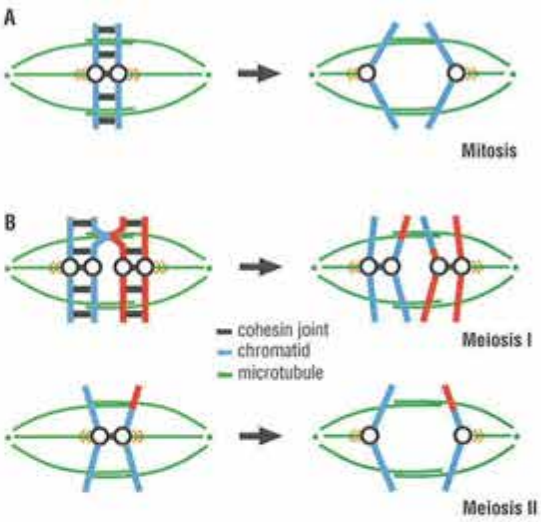
The simultaneous separation of 46 pairs of sister chromatids at the metaphase to anaphase transition is one of the most dramatic events of the human cell cycle. Even as long ago as 1879, Flemming noticed that 'the impetus causing nuclear threads to split longitudinally acts simultaneously on all of them'. Chromosome splitting is an irreversible event and must therefore be highly regulated. Once sister chromatids separate from one another, damage to the genome cannot easily be repaired using recombination nor can mistakes in chromosome alignment be corrected.

Sister chromatids are pulled to opposite halves of the cell by microtubules emanating from spindle poles at opposite sides of the cell [Fig. 1A]. One set of microtubules interdigitates with others emanating from the opposite pole. Their role is to keep [and drive] the two poles apart. Meanwhile, a second set of microtubules attaches to chromosomes via specialized structures called kinetochores and pulls them towards the poles. Sister chromatids segregate away from each other because their kinetochores attach to microtubules emanating from opposite poles. Chromosomes are not mere passengers during this process. During metaphase, the tendency of microtubules to move sisters apart is counteracted by cohesion holding sisters together. Cohesion therefore generates the tension by which cells align sister chromatids on the metaphase plate. Were sisters to separate before spindle formation, it is difficult to imagine how cells could distinguish sisters from chromatids that were merely homologous. The sudden loss of cohesion, rather than an increase in the exertion of microtubules, is thought to trigger sister separation during anaphase.

What holds sister chromatids together after chromosome replication? What is Flemming's impetus that triggers loss of cohesion? How do cells ensure that sister separation never occurs before all pairs of sister chromatids have been aligned on the metaphase plate? Such questions are equally pertinent to meiosis, where loss of sister chromatid cohesion within chromosome arms and centromeres must take place at different times [Fig. 1B].

Genetic and biochemical studies on the budding yeast *Saccharomyces cerevisiae* have identified a multi-sub unit complex called cohesin that is essential for holding sister chromatids together from DNA replication until the onset of anaphase [Fig. 2]. A related complex exists in human cells [see Peters]. In yeast, cohesin is loaded onto chromosomes during late G1 with the aid of a cohesin loading factor [CLF]. Our working hypothesis is that connections between sister chromatids [mediated by cohesin] are established at replication forks with the aid of a protein called Eco1p. They persist until the onset of anaphase, whereupon activation of a 'separin' protein [Esp1p] induces the proteolytic cleavage of the Scc1p cohesin subunit,

Figure 1

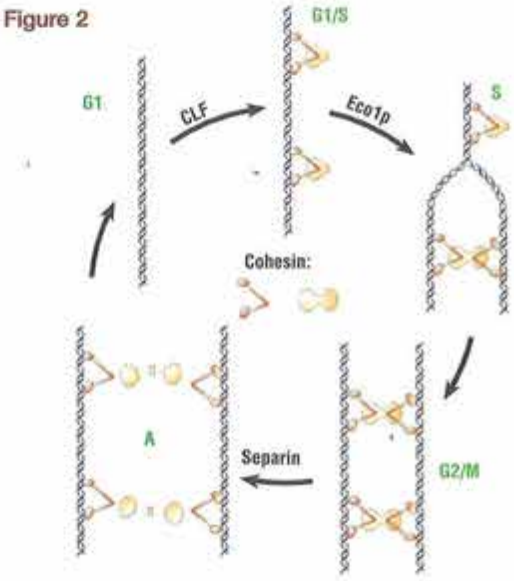


Chromosome segregation during mitosis and meiosis

which may be the trigger for sister chromatid separation. Separin is kept inactive from S phase till the onset of anaphase by its association with a securin protein [Pds1p]. The liberation of separin from its securin is mediated by a multisubunit ubiquitin protein ligase called the Anaphase Promoting Complex or cyclosome [APC/C], which promotes the ubiquitination and hence proteolysis of securin. We are currently studying how CLF mediates the loading of cohesin onto chromosomes, what sort of structures are formed during this process, and how these structures are modified with the help of Eco1p during the passage of replication forks. We are also trying to establish whether separin is the protease responsible for cleaving Scc1p and are investigating how Scc1p cleavage remains tightly cell cycle regulated in mutants that lack securins.

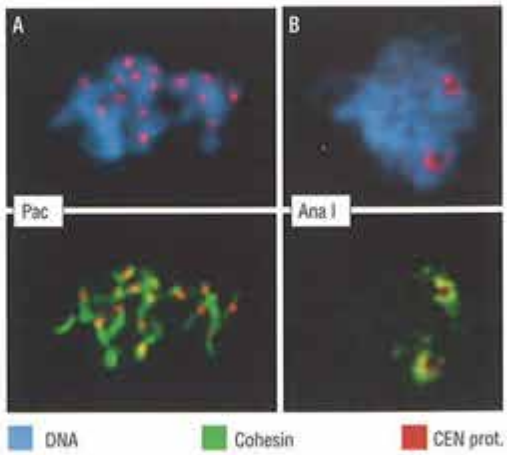
Loss of sister chromatid cohesion along chromosome arms is essential for chromosome segregation during meiosis I. Meanwhile, however, cohesion between sister centromeres persists so that it can later be used to align sisters on the meiosis II metaphase plate. The different timing of sister chromatid cohesion loss between chromosome arms and centromeres is therefore a crucial aspect of meiosis [Fig. 1B]. The budding yeast genome encodes a second Scc1-like protein called Rec8p, which is needed for preventing precocious separation of sister chromatids during meiosis. Rec8p and other cohesin subunits are found all along the longitudinal axis of chromosomes during pachytene. They disappear from chromosome arms during the first meiotic division but persist in the neighbourhood of centromeres until metaphase II [Fig.3]. We are currently investigating whether the removal of Rec8p from chromosomes is mediated by proteolytic cleavage induced by separin. We are also interested in understanding what prevents the removal of Rec8p from sequences close to centromeres until the second meiotic division.

Figure 2



The cohesin cycle

Figure 3



Rec8p distributed all along chromosomes during pachytene and persisting at centromeres until the second meiotic division.

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Patterning and early morphogenesis of the vertebrate face

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

Development of the midfacial region begins with the appearance of the nasal placodes, bilateral ectodermal thickenings at the ventro-lateral sides of the forebrain that will give rise to the olfactory epithelium [Fig.1]. Subsequently, tissue around the nasal placodes and the primitive mouth starts to grow out to form distinct buds, the facial primordia, and continued outgrowth depends on epithelial mesenchymal interactions. How the early facial region is patterned and how the areas of mesenchymal outgrowth are established is not understood. In particular, it is not clear what role the surface ectoderm, the nasal placodes and the underlying forebrain play for patterning of the neural crest derived mesenchyme of this region. We are using the mouse and the chick as experimental organisms to address these questions – the chick to study the morphological and molecular consequences of micro-surgical manipulations of the early face in ovo, and the mouse for a genetic approach towards facial development.

The function of the nasal placodes for facial development

In a series of transplantation experiments we demonstrated that the nasal placodes are required for normal facial mesenchyme development [Fig.2]. Preliminary data suggests that the nasal placodes may pattern the facial mesenchyme indirectly via the induction of signalling molecules at the boundary between the placode and the surface ectoderm which in turn direct mesenchyme development. However, a direct interaction of the placode and the underlying mesenchyme has not yet been ruled out. To further address this issue, we are currently studying how placode ablation experiments and other micro-surgical manipulations of the face affect the expression of marker genes. In addition, we are also studying facial development in mouse embryos homozygous for a point mutation in the gene encoding the paired box transcription factor Pax6 which fail to form a nasal placode and display severe facial abnormalities at later stages.

FGF8 function during facial development

The gene encoding Fibroblast Growth Factor 8 [FGF8] has a complex, dynamic expression pattern during facial development [Fig. 3]. Embryos in which this gene has been inactivated in the facial region are born with severe facial defects. Such embryos display midfacial clefts and most derivatives of the first branchial arch are severely reduced or absent. Defects first become morphologically apparent around E9.0 as a reduction in the size of the facial primordia. This reduction in size can at least in part be accounted for by a dramatic increase in cell death in early facial mesenchyme in the absence of *Fgf8* expression in the overlying surface ectoderm. FGF8 therefore

Figure 1

The nasal placodes [np], thickenings of the facial ectoderm, are the first morphologically distinct structures to form in the prospective midfacial region. By E10.5, the mesenchyme around the placodes has started to grow out to form the medial [mnp] and lateral [lnp] nasal processes and the placodes have now come to lie in shallow depressions, the nasal pits [np, the future nasal cavities], between the nasal processes.

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

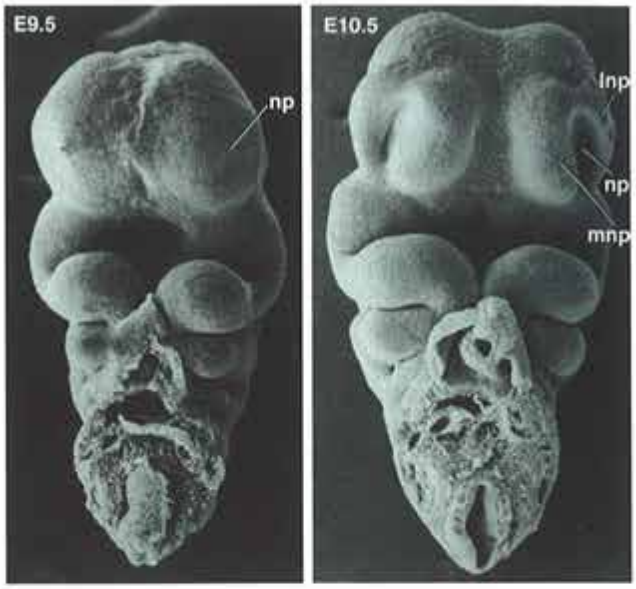
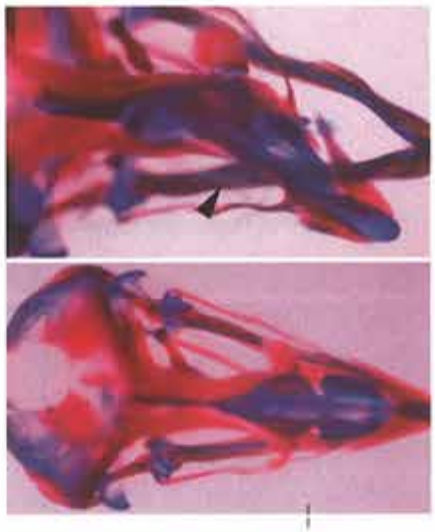


Figure 2

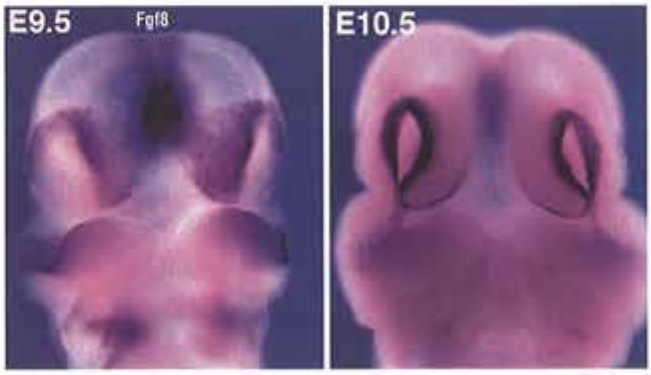
Top: Top view of the beak of a chick embryo after surgical removal of the right nasal placode at an early stage of development.
Note that on the operated side [arrow] a variety of skeletal elements are missing.
Bottom: Removal of ectoderm between the two nasal placodes does not affect skeletal development.



Ablation of the nasal placode affects development of the facial skeleton

Figure 3

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



seems to act as a survival factor for early facial mesenchyme. In addition, patterning in the remaining tissue is affected as judged by the analysis of the expression of a small set of marker genes. A detailed phenotypic and molecular analysis of these mutants is in progress and will help to better define the function of *Fgf8* for patterning of the facial mesenchyme.

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

In order to understand how FGF8 controls development of the facial mesenchyme, it is essential to identify the genes induced or repressed in response to FGF8 signalling. We are using an *in vitro* explant culture system in which facial mesenchyme is culture in contact with facial ectoderm, in isolation or in contact with polymeric beads soaked in FGF8 protein to identify such genes. Using a candidate approach, we have so far shown that the expression of the transcription factors *Pax3, Tbx2, Erm* and *Pea3* in facial mesenchyme requires signals from the overlying ectoderm and that FGF8 protein is sufficient for induction of their expression. To systematically screen for genes regulated by FGF signalling, we will use facial mesenchyme cultured in the presence or absence of FGF8 as starting material to generate a subtracted library enriched for such genes. This library will then be used to generate a customized DNA micro-array, which will be probed with cDNA derived from mesenchyme cultured with or without FGF8.

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Regulation of mitosis by the anaphase-promoting complex

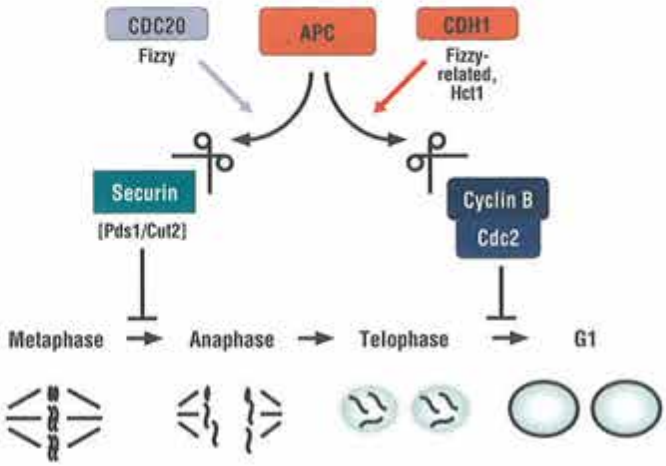
The propagation of genetic information during cell proliferation requires the accurate replication and subsequent segregation of chromosomal DNA. Both these events are controlled by ubiquitin-dependent proteolysis, a regulatory mechanism that is ideally suited to generate directionality in the cell cycle due to its irreversible nature. We are studying how ubiquitin-dependent proteolysis mediated by the anaphase-promoting complex controls the separation of sister chromatids in anaphase and the subsequent exit from mitosis in vertebrate cells.

The initiation of sister chromatid separation at the metaphase-anaphase transition is a 'point of no return' during the eukaryotic cell cycle. High fidelity and proper timing of this event are essential to ensure equal segregation of the duplicated genome to the forming daughter cells and are thus required to maintain genomic stability during cell proliferation. Defects in sister chromatid separation can cause aneuploidy and may therefore contribute to human diseases such as congenital trisomies and cancer.
The segregation of replicated DNA during mitosis is achieved by the formation of a mitotic spindle apparatus with bilateral symmetry. This apparatus can only be formed if duplicated DNA molecules ['sisters'] are physically held together following replication, a phenomenon called sister chromatid cohesion. To allow the separation of sisters in anaphase, cohesion needs to be dissolved. Recent evidence suggests that in eukaryotes from yeast to man this process is initiated by activation of a multi-subunit ubiquitination complex called the anaphase-promoting complex [APC] or cyclosome. We first discovered the APC as the cell cycle regulated component of an enzymatic pathway that ubiquitinates cyclin B at the end of mitosis and thus targets this protein for destruction by the 26S proteasome. Subsequently, the APC has also been found to ubiquitinate numerous other mitotic proteins, including protein kinases, spindle proteins and inhibitors of DNA replication. However, how the APC catalyzes the assembly of poly-ubiquitin chains on substrate proteins, how its activity is regulated during the cell cycle and how its activation initiates the separation of sister chromatids is little understood. We are using *Xenopus* eggs and human cells to address these questions by biochemical, molecular and cytologic approaches.

The vertebrate APC is composed of eleven subunits that are part of the complex throughout the cell cycle, whereas two activator proteins, called CDC20 and CDH1, bind to the APC in a cell cycle-regulated manner. Genetic and biochemical data suggest that the ordered activation of the APC by CDC20 in metaphase and by CDH1 in telophase is essential for anaphase and for exit from mitosis, respectively. We have recently shown that CDC20 can only bind to the

Figure 1
Micrograph of a human cell in prometaphase. At this stage of mitosis the chromosomes [in the left panel] are condensing to allow subsequent separation of their sister chromatids in anaphase. The APC activator protein CDC20 [stained in the right panel] is partly soluble [seen as a diffuse background staining] and partly associated with kinetochores [seen as pairs of dots], the structures on sister chromatids that will be captured by spindle microtubules until metaphase. Why CDC20 associates with kinetochores is not understood, but an interesting possibility is that its association with these structures is required to monitor the binding of microtubules to kinetochores. Earlier work has shown that APC and CDC20 can only initiate anaphase once the two kinetochores of every chromosome in the mitotic cell are attached to microtubules emanating from the two different spindle poles. This spindle assembly checkpoint mechanism helps to avoid the missegregation of sister chromatids that could result from premature anaphase initiation. Micrograph by B. Peters and E. Kramer.

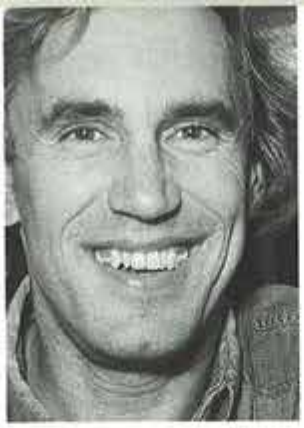
Figure 2
Ubiquitin-dependent proteolysis mediated by the APC regulates two important transitions in mitosis. Anaphase inhibitors such as securin need to be degraded to initiate the separation of sister chromatids at the metaphase-anaphase transition. Destruction of cyclin B by the same pathway is an important mechanism that helps to inactivate the protein kinase CDC2. Inactivation of this kinase is essential for forming daughter cells to exit from mitosis into the subsequent interphase. One of two activator proteins, called CDC20 and CDH1, has to associate with the APC to activate these ubiquitination reactions.



APC once the complex has been phosphorylated by mitotic kinases which explains why the activity of APC^{CDC20} is restricted to mitotic cells. In contrast, the interaction of CDH1 with the APC is inhibited by phosphorylation of CDH1 during the S and G2 phase and most of the mitosis, restricting the existence of APC^{CDH1} complexes to G1 where CDH1 is not phosphorylated. The antagonistic effects of phosphorylation reactions on APC^{CDC20} and APC^{CDH1} help to explain the temporal order of APC activation by CDC20 and CDH1 and may ensure that exit from mitosis is not initiated before anaphase has occurred.

We discovered recently that APC^{CDH1} does not only exist in proliferating cells but also in differentiated cells such as postmitotic neurons. This observation raises the unexpected possibility that the functions of APC^{CDH1} may not be restricted to controlling the cell cycle but that the APC may also ubiquitinate yet unknown substrates in differentiated cells.

To initiate the separation of sister chromatids, the APC has to ubiquitinate an inhibitor of anaphase, called securin. In yeast, securin proteolysis activates a pathway that initiates sister separation by removing cohesion proteins [cohesins] from chromosomes, thus liberating sister chromatids for poleward movement in anaphase. A 14S complex containing homologues of yeast cohesins also exists in *Xenopus* but, unlike in yeast, this complex dissociates from chromatin in prophase, long before sisters separate. In vertebrates, it is therefore not known how sister chromatids are held together between pro- and anaphase, and it is also not known whether the solubilization of cohesins in prophase depends on activation of the APC, as it does in yeast. We have recently shown that the dissociation of vertebrate cohesins from chromatin in prophase does not depend on the APC, and in the future we want to identify the pathway that is responsible for this event. We would further like to identify proteins that maintain sister chromatid cohesion between pro- and anaphase and want to study if and how these proteins are regulated by the APC pathway.



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Gene function in mammalian development and oncogenesis

The mouse is used as a model organism for the analysis of gene function in normal and pathological development. One major focus is the analysis of AP-1 proteins such as Fos and Jun and their role as regulators of proliferation, differentiation and cell death. Furthermore, we are aiming to define the specific function of VEGF/Flk-1 receptor signalling in bone and endothelial cells.

Fos proteins in bone cell differentiation

c-Fos is a key regulator of bone development. Transgenic mice expressing exogenous Fos develop bone tumors, whereas mice lacking c-Fos are osteopetrotic due to a differentiation block in bone resorbing osteoclasts. We are interested in the molecular mechanisms by which c-Fos and its related protein Fra-1, which is essential for mouse development, control osteoclast differentiation. Fra-1, a c-Fos target gene is a potent inducer of osteoclast differentiation and transgenic mice overexpressing Fra-1 develop the bone disease osteosclerosis due to increased bone formation [Fig. 1]. These systems are being used to identify c-Fos and Fra-1 target genes during bone cell differentiation.

c-Jun and Jun kinases controlling cell differentiation, proliferation and apoptosis

To overcome the embryonic lethality of *c-jun* knockout mice and to analyze the function of c-Jun at later stages of development, we have used the *cre/loxP* recombination system to investigate the role of c-Jun in hepatocytes and chondrocytes. Deletion of *c-jun* in the liver of adult mice revealed that c-Jun is dispensable for postnatal liver function, but is essential for liver regeneration. Chondrocyte-specific inactivation of the floxed *c-jun* allele using a collagen2a-cre transgene results in severe scoliosis caused by failure of intervertebral disc formation and abnormal vertebral arch development, suggesting that c-Jun is a novel regulator of sklerotomal differentiation.

An important mechanism regulating c-Jun activity is phosphorylation of c-Jun at serine 63 and 73 within its N-terminus [JNP] by the c-Jun amino-terminal kinases [JNKs]. To study the function of JNK signalling *in vivo*, we have generated null mutations in the *jnk1* and *jnk2* genes [in collaboration with M. Karin] and mice carrying a *c-jun* allele mutated in the JNK phosphoacceptor sites *[junAA]*. *Jnk1-/-*, *jnk2-/-* and *junAA* mice are healthy and fertile, but the absence of *jnk1* and JNP results in growth retardation and fibroblasts from these mice show proliferation defects. *Jnk1-/- jnk2-/-* double mutants develop brain defects due to deregulated apoptosis [Fig. 2] and *jnk2-/-* and *junAA* thymocytes are resistant to CD3-induced apoptosis. Moreover, Jnk1 and c-Jun phosphorylation appear to be required for efficient osteoclast differentiation. Therefore, JNK signalling and JNP differentially regulate cell proliferation, differentiation and apoptosis in different biological processes.

JunB – a negative regulator of cell proliferation and differentiation

JunB plays a key role in mouse development since fetuses lacking JunB die during embryogenesis. In contrast, constitutive JunB overexpression in transgenic mice does not lead to an obvious phenotype.

Figure 1

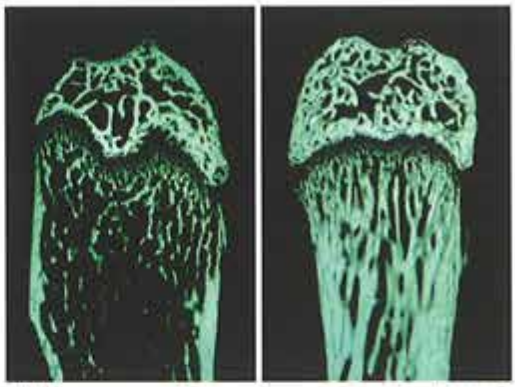


Figure 1: Fra-1 causes osteosclerosis due to increased bone formation in transgenic mice

Figure 2

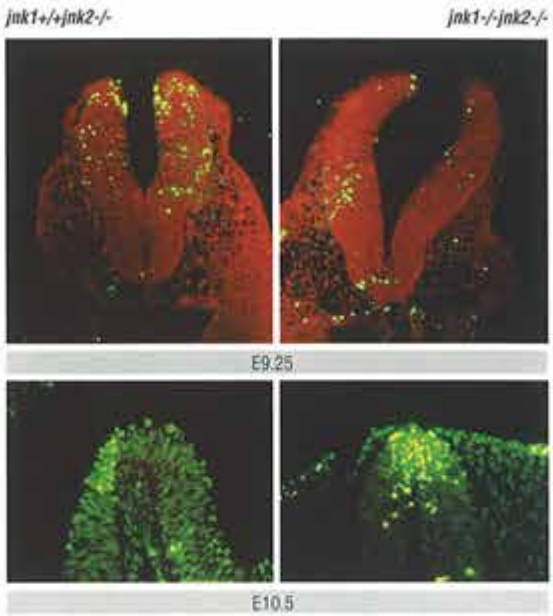
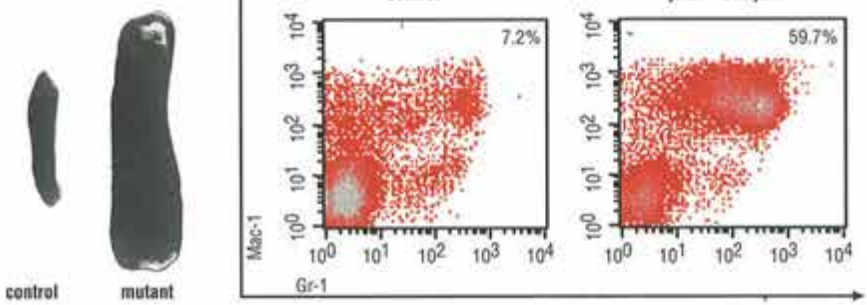


Figure 2: JNK-1 and JNK-2 are essential for regulated apoptosis in the developing hindbrain [apoptotic cells are labelled in yellow]

Figure 3

Splenomegaly and increased myeloid cell differentiation in *jun*B -/- JunB transgenic mice



However, fibroblasts derived from these mice exhibit a strong proliferation defect. We recently demonstrated that JunB suppresses fibroblast proliferation by transcriptional activation of the CDK inhibitor p16/INK4a and may therefore act as a tumor suppressor gene. Further support for a tumor suppressor function of JunB stems from findings that the absence of JunB in the myeloid lineage leads to the development of chronic myeloid leukemia resembling the human disease [Figure 3]. Present studies aim to define the molecular mechanisms by which JunB controls myeloid cell proliferation and differentiation.

Blood vessel development and vascular tumors

A conditional allele of the endothelial-specific growth factor receptor Flk-1 known to be essential for endothelial cell formation was generated to test whether Flk-1 is essential for the action of PymT oncogene. PymT transforms endothelial cells and causes vascular tumors. We found that PymT can functionally replace Flk-1 and endothelial cells can be formed in its absence *in vitro*. Present studies address the role of Flk-1 in tumor angiogenesis and we will determine whether the cytoplasmic tyrosine kinase Fps/Fes that potentially acts downstream of VEGF signalling can elicit similar tumors in the absence of Flk-1.

The functional importance of VEGF-A expression in developing chondrogenic tissues is being analyzed with a conditional VEGF-A allele using collagen2a1-Cre transgenic lines. Deletion of a single VEGF-A allele results in embryonic lethality [at E10.5] characterized by the aberrant development of the dorsal aorta and intersomatic blood vessels along with defects in the heart. Surviving E17.5 mutant mice show aberrant endochondral bone formation and develop a heart phenotype resembling a dilated form of ischemic cardiomyopathy.

Gotthold SCHAFFNER | Scientist

Elisabeth AIGNER | Technician
Herbert AUER | Technician *[till October]*
Ivan BOTTO | Technician
Markus HOHL | Technician *[since October]*
Karl MECHTLER | Technician
Gabriele BOTTO | Technician Media Kitchen
Christa CZAPKA | Technician Media Kitchen

The Service Department offers a variety of high quality and rapid services to IMP scientists. The majority of our effort involves DNA sequencing, oligonucleotide synthesis and peptide synthesis.

Our Media Kitchen staff prepare substantial quantities of reagent quality solutions and media for cell culture, flies and worms. We also prepare many selected reagents such as DNA molecular weight markers, enzymes and a variety of transformation-competent *E.coli* strains and maintain a stock of cloning vectors, primers and other cloning reagents.

Oligonucleotide synthesis

We started in 1988 with about 500 DNA oligonucleotides and last year prepared approximately 3650 with an average size of 25 bases. During the first 9 months of 1999 we produced more than 4200 oligos, an increase of almost 100% compared to 1997. In order to meet the demands for PCR oligos [25 to 40 bases] and long oligos [70 bases] for mutation/gene disruption experiments, we invested in an additional Oliogosynthesizer [PerSeptive Expedite].

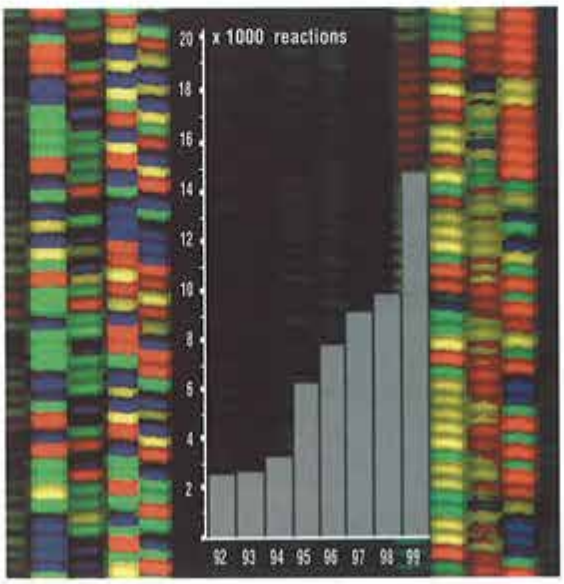


Figure 1

A sequencing run on ABI 377 PRISM and number of reactions done with dye deoxy terminators [scale 0 to 20'000] from 1992 to 1999.

Peptide synthesis, protein sequencing by mass spectroscopy and monoclonal antibodies

Synthetic peptides are still of great importance to IMP research activities. This year we synthesized a great variety of oligopeptides, mainly coupled to PEG or KLH for immunization purposes. The production of monoclonal antibodies in collaboration with IMP groups is of increasing importance and needs more of our capacity. We also continued, to a lesser extent, to isolate and HPLC-purify monoclonal antibodies from established hybridoma cell lines. In the spring we started to sequence proteins isolated from Coomassie stained gels with the Electro-/Nanospray Mass Spec equipment we have been using for quality control of synthesized peptides. Mass Spec has become a standard method for identifying unknown proteins.

Sequencing and DNA isolation

With the two ABI PRISM 377 DNA sequencers, we sequenced approximately 12000 samples in the first 9 months of this year [an increase of more than 50% as compared to 1998] with an average reading length of 700-800 base pairs for 'good' DNA. Sequencing of BACS and other artificial chromosomes is now established. We are saving time by using an easy and fast clean-up protocol using small Sephadex columns on 96-well microtiter plate format. The protocol is readily adaptable to our robotic system.

Peter STEINLEIN | Staff Scientist
Karin PAIHA | Technician

BioOptics at the IMP

The BioOptics Department offers a wide variety of services in the field of microscopy, image processing/analysis and flow cytometry. The major goal of the department is the development and implementation of new technologies in close collaboration with the research groups at the IMP.

Microscopy

To meet the constantly increasing demand for advanced microscopy, both equipment and services have been improved. Together with N. Wick [Group Huber], time lapse video microscopy of living cells over extended periods of time [> 4 days] has been implemented. This technique can be combined with microinjection of fluorescent probes or expression plasmids coding for fluorescent proteins and allows tracking of living cells in different experimental settings.

In collaboration with M. Glotzer and W. Wunderlich [Groups Glotzer and Huber, respectively], time lapse imaging of organelle movement and cytokinesis in living cells expressing GFP-fusion proteins using a high performance, intensified CCD-camera was established. This setup enables us to monitor fast intracellular processes with extremely high sensitivity.

The Confocal Laser Scanning Microscope [CLSM] was equipped with a near-infrared, pulsed laser enabling the visualization of UV-excitable dyes and GFP, using multiphoton-excitation. Thus, laser-scanning microscopy of living cells stained with supravital dyes or expressing BFP and its fusion proteins is now possible without UV-induced damage to the cells.

Image processing/analysis

Image processing of both CLSM and widefield images has become a standard application over the past year. Deconvolution, analysis of co-localization, and 3D reconstruction of volume stacks are now performed on a routine basis. Currently, an image database system for many types of image data is being set up thus allowing efficient management of primary image data and processed images.

Flow Cytometry

During the past year, the demand for flow cytometric applications continued to increase. Single cell sorting for either further cultivation of cells or for analysis by single cell PCR and organelle sorting [Fluorescence activated organelle sorting, FAOS] has been established as a routine technique.

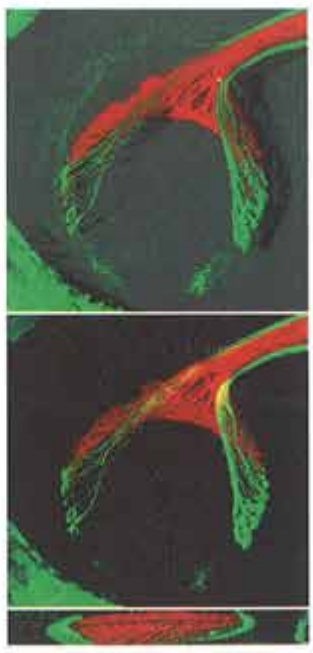


Figure 1

D. melanogaster photoreceptor axons innervating the optic lobes of a 3rd instar larvae. Late axons are labeled by mAb 24B10 [red], polar axons are labeled by an anti-LacZ antibody directed against an omb-enhancer trap driving lacZ [green]. A 64-plane image stack was taken using a Leica TCS NT confocal laser scanning microscope, objective magnification was 20x. The total dimensions are 500x500x23µm. [A] shows a shadow projection of the 3D reconstruction; [B] shows the extended focus projection and [C] a lateral cross-section of the optic lobes. The images were processed using Imaris 2.7 [Bitplane, CH]. [Primary data by T. Newsome, Group Dickson]

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

Norma HOWELLS | Consultant

Beata BALUCINSKA | Technician
Mijo DEZIC | Technician
Erika KILIGAN | Technician, *on maternity leave*
Dominik MAYR | Technician
Svetlana PEKEZ | Technician
Katja STEPANEK | Technician
Esther ZWICKELSDORFER | Technician

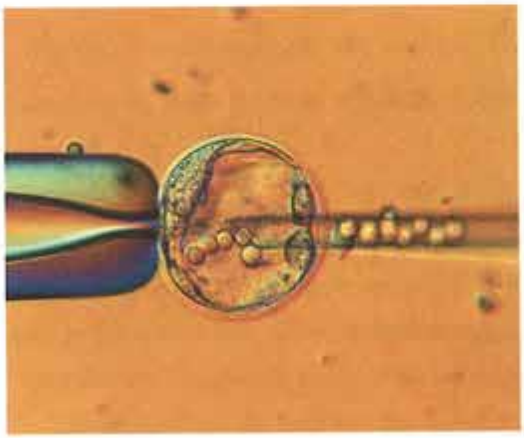
Animal house

The animal house provides technical support for the various research groups. It is divided into three main areas and contains the following species: mice, chicken and Xenopus. The largest and still expanding area is the mouse section. To cope with the increasing demand during the past two years the animal house has been refurbished and expanded [completion of the annex, adaption of existing rooms into mouse rooms]. By the end of this year refurbishment of the last room will be finished. The animal house will then hold more than 10,000 mice. Breeding colonies, stock and experimental animals are maintained by the animal house staff. They include transgenic and knock-out mouse lines. To provide a constant supply of mice for the various projects, 21 standard strains are routinely bred in-house. In addition, eighty eggs are produced each week from our 'closed-colony' chicken flocks.

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

The following groups benefit most from the animal house services:

- Mouse studies: Hartmut Beug, Meinrad Busslinger, Gerhard Christofori, Lukas Huber, Thomas Jenuwein, Kim Nasmyth, Annette Neubüser and Erwin Wagner, service department, mouse service department.
- Chicken studies: Hartmut Beug and Annette Neubüser.
- Xenopus studies: Jan Peters.
- Drosophila studies: Barry Dickson and Jürgen Knoblich.



Hans-Christian THEUSSL | Technician

Mouse service department

The Mouse Service Department was set up at the beginning of 1998 to cope with the increasing demand for mouse studies and generation of transgenics.

The main duties of this service unit are the injection of ES cells into blastocysts [also tetraploid] and of DNA into the pronucleus of fertilized mouse eggs. This service also provides the transfer of 'clean' embryos into our animal house, the freezing of embryos for preservation of specified mouse strains and the teaching of basic embryological techniques to the IMP staff. During this year initial experiments for freezing mouse sperm were performed and a mouse strain data base is being generated. About 30 different ES cell clones and several DNA constructs are being successfully injected per year, mainly for the groups of Meinrad Busslinger, Gerhard Christofori, Thomas Jenuwein and Erwin Wagner. The activities of this department are governed by an Animal User Committee, which meets monthly to set priorities and coordinate the duties. At present it is chaired by Erwin Wagner.

Members of the Scientific Advisory Board

Prof. Michael Bishop
Dept. of Microbiology and Immunology, Univ. of California, San Francisco

Prof. Nick Hastie
MRC Human Genetics Unit, Western General Hospital, Edinburgh

Prof. Tim Hunt
ICRF Clare Hall Laboratories, South Mimms

Prof. Herbert Jäckle
MPI für Biophysikalische Chemie, Göttingen

Prof. David Lane
Dept. of Biochemistry, Univ. of Dundee

Prof. Martin Raff
MRC Laboratory for Molecular Cell Biology, University College London

Prof. Janet Rossant
Samuel Lunenfeld Research Institute, Toronto

Prof. Peter Swetly
Boehringer Ingelheim Austria, Vienna

Prof. Bernd Wetzel
Boehringer Ingelheim GmbH, Ingelheim

The Scientific Advisory Board

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

First time visitors to the IMP invariably notice two things: how young and how international the institute feels. Both factors don't come about by chance. By assigning temporary contracts, the IMP assures a high turnover rate among its scientific staff and a constant influx of new ideas. Even group leaders are only hired on a 5-year basis, with the option to extend their stay for a further three years after positive review by the SAB.

The multi-cultural character of the institute – 25 nations are represented at the IMP – is mainly due to the large number of students from all continents. Every autumn, an international PhD Program is advertised in a leading journal, prompting a large number of applications. Following a tough selection process and a round of interviews, approximately 10 to 15 students are admitted to the program each year. The PhD Program is carried out jointly with the University of Vienna, which has had close ties with the IMP right from the start. Since 1992, five institutes of the Faculties of Science and Medicine have been located in a neighbouring building. Together with the IMP, they constitute the 'Vienna Bio Center', which has become a recognized center of excellence in the biological sciences and something of a role model for other academic institutions in Austria. By bringing the two institutions together, a 'critical mass' of resources has been generated which has led to a number of further spin-off projects. Among them is 'Intercell', a young and ambitious company initiated by Max Birnstiel and focused on innovative vaccine development.

With support from the city of Vienna, the IMP's neigbourhood is gradually being developed into a biotech campus, combining university- and industry-based institutes, start-up companies and a new branch of the Austrian Academy of Sciences. The 'Institute for Molecular and Cellular Bioinformatics [IMBA]' will be set up next door and connected to the IMP. It will reflect the IMP's structure and complement its research. The two units will collaborate under the provisional name 'IMP/IMBA Genome Research Center'.

Interaction and communication are key elements in building successful research structures. They are strongly encouraged, both on an informal basis and as organized events. On the social side, employees meet over lunch at the IMP's cafeteria, relax at monthly 'beer hours', join forces as a soccer team or on volleyball nights, enjoy occasional parties in 'Joe's disco' and organize lab-outings to the countryside.
On the scientific calendar, the 'IMP Spring Conference' ranks among the top events of the year. In 2000, it will take place from May 11-13 under the title 'From Development to Molecular Medicine'.
Throughout the year, the IMP hosts a series of 'Thursday seminars' and 'Impromptu seminars' where top-ranking scientists are invited to lecture on their latest findings. These seminars generate lively interactions as well as introduce the IMP scientists to other fields of research.
Students have organized their own weekly journal club; they are expected to present their work annually to their colleagues at one of the regular Monday seminars and are encouraged to participate in international conferences. Students' progress is monitored by a PhD committee which meets annually. Upon graduation, they receive their PhD degree from the University of Vienna. Equipped with an excellent research background, close contacts to the scientific community and first publications in internationally recognized journals, most of those who leave the IMP don't find it hard to move up to the next step in their career, although they might find it hard to part with colleagues who have become friends.

About 500 scientists attended the 10th IMP Spring Conference in May 1999. This year's presentations focused on 'The Cell Biology of Cell Division'. The conference was once again held at the Imperial Palace in the heart of the city, a venue favoured by most visitors for its beauty and central location.

In July, Kim Nasmyth's outstanding research on the regulation of cell division was recognized by the awarding of the Wittgenstein Prize, Austria's premier scientific honor. Austria's Minister of Science Caspar Einem and Federal Chancellor Viktor Klima were among the first to congratulate him.

A new type of collaboration is being established between the IMP/Boehringer Ingelheim and the Austrian Academy of Sciences. Following months of intense negotiations, a contract was formally signed on September 29 which paved the way for the new 'Institute for Molecular and Cellular Bioinformatics'.

Nobel laureate Michael Bishop, who had been a member of the SAB for 7 years, retired from this position in 1999. His advice and experience had considerably helped to shape the institute's structure and its role within the scientific landscape. On his farewell in October, he received a high distinction from the Ministry of Science, the 'Österreichisches Ehrenkreuz für Wissenschaft und Kunst 1. Klasse'.

At the IMP fancydress party, the creativity and hidden artistic talents of the institute's employees are challenged.

The IMP skiing-trip, now an annual event, is a highlight for all employees who take time out to enjoy the slopes, cross-country skiing or leisurely walks in the snow. An enthusiasm for the mountains seems to unite most scientists at the IMP. Could it be that the nearby Austrian Alps add to the IMP's attraction?

Once a year, the young and the very young get together to celebrate. At the IMP kids' Christmas party, the main lecture hall is turned upside down. Children and parents are then in for a few surprises. A well kept secret: who will play Santa Claus this year?

publications 1999

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seminar speakers at the IMP 1999

January
12. Hannah MONYER – *MPI, Heidelberg*
18. Francois TRONCHE – *DKFZ, Heidelberg*
22. Roger LIPPE – *EMBL, Heidelberg*
27. Wilfried ELLMEIER – *Skirball Institute, NY*

February
16. Allan BRADLEY – *Baylor Collage, Houston*
25. Jürgen BEHRENS – *MDC, Berlin*
26. Klaus LINDPAINTNER – *Roche, Basel*

March
04. Anton STÜTZ – *Novartis, Vienna*
08. Lars ROGGE – *Roche, Milan*
10. Tony KOUZARIDES – *Wellcome/CRC, Cambridge*
11. Klaus PALME – *Max Delbrück Laboratory, Cologne*
12. Amanda FISHER – *Hammersmith Hospital, London*
    Moshe YANIV – *Institut Pasteur, Paris*
    Peter HERRLICH – *Forschungszentrum Karlsruhe*
15. Georg HALDER – *R.M.Bock Laboratories, Madison*
    Minoo RASSOULZADEGAN – *Univ. Nice*
16. Gordon PETERS – *ICRF, London*
18. Christine FARNIOK – *Boehringer Ingelheim Austria*
25. Ulrich HARTL – *MPI, Martinsried*
29. Daniel FINLEY – *Harvard Med. School, Boston*
30. Charles SHERR – *St. Jude Children's Res. Hospital, Memphis*

April
08. Dinshaw PATEL – *Memorial Sloan-Kettering Cancer Center, NY*
12. Denise BARLOW – *Netherlands Cancer Institute, Amsterdam*
13. Richard ANDERSON – *Univ. Texas, Dallas*
15. Frank GROSVELD – *Erasmus Univ., Rotterdam*
16. Paul DUPREE – *Univ. Cambridge*
19. Ingvild MIKKOLA – *Univ. Tromso*
    Christian FORST – *Univ. Illinois*
20. Laurence HURST – *Univ. Bath*
21. Michael JENSEN – *National Cancer Inst., Bethesda*
22. Haruo SAITO – *Dana Farber Cancer Inst., Boston*
23. Frank EISENHABER – *EMBL, Heidelberg*
    Jürg OTT – *Rockefeller Univ., NY*
29. Kees MURRE – *UC San Diego*

May
06. Angus LAMOND – *ICRF, London*
12. Gero MIESENBOECK – *Memorial Sloan-Kettering Cancer Center, NY*
17. Randy STRICH – *Fox Chase Cancer Center, Philadelphia*
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20. Stephen K. BURLEY – *Rockefeller Univ., HHMI, NY*
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02. Anne DEBANT – *CNRS, Montpellier*
08. David SACKS – *Harvard Medical School, Boston*
10. Stephen DILWORTH – *Hammersmith Hosp., London*
11. Jonathan PETTITT – *Univ. Aberdeen*
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July
05. Roberto DI LAURO – *Stazione Zoologica Anton Dohrn, Naples*
13. Clemencia COLMENARES – *The Cleveland Clinic Foundation*
22. Walter KÖLCH – *CRC Beatson Labs, Glasgow*
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16. Peter GRUSS – *MPI, Göttingen*
27. Liquin LUO – *Stanford University*
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03. Sven PETTERSON – *Karolinska Inst., Stockholm*
06. Philippe SORIANO – *Fred Hutchinson Cancer Res.Center, Seattle*
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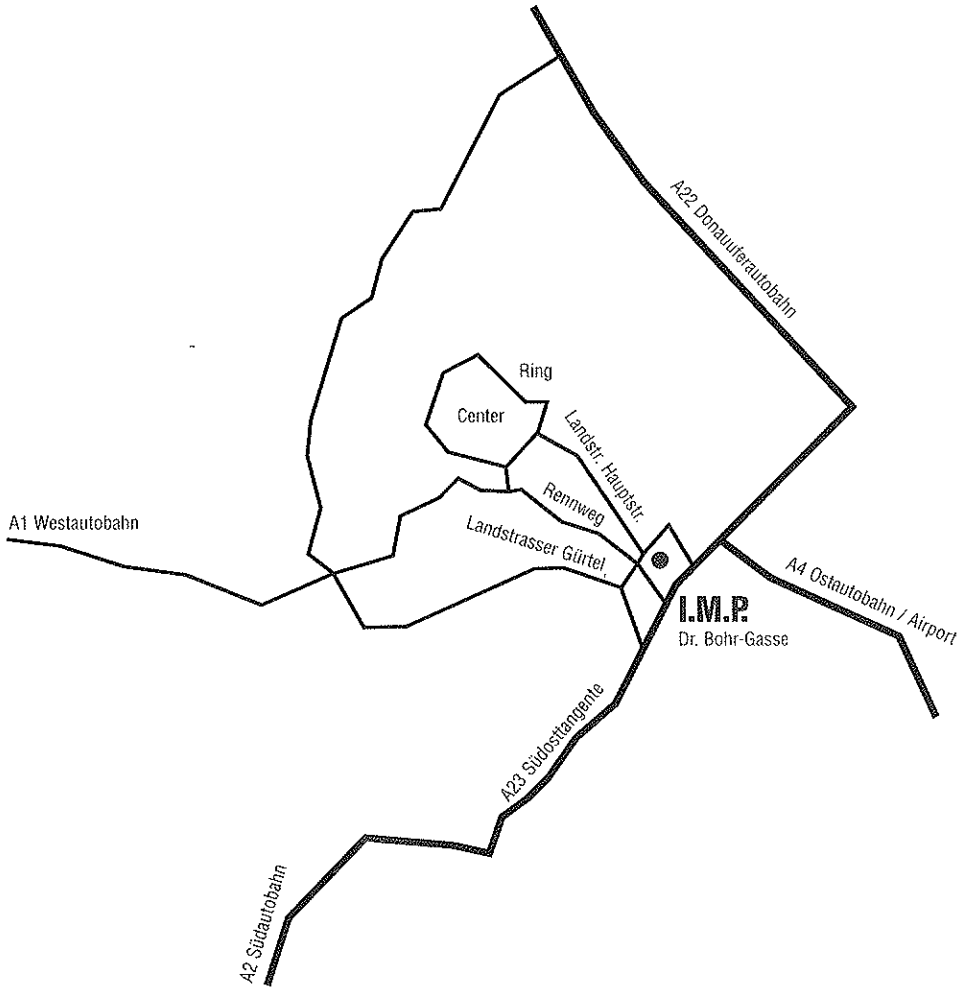
22. Susann SCHWEIGER – *MPG Berlin*
23. Masanori MISHIMA – *Kyoto University*

December
07. Richard MORIGGL – *St. Jude Children's Research Hospital, Memphis*

Where we are



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Cover
An adult *C. elegans* hermaphrodite.
This transparent worm is about 1 mm long and consists of 959 somatic cells.
Each hermaphrodite produces about 300 progeny
some of which are visible in the center of the worm.
Also shown is a series of pictures of an embryo undergoing its first cell division.
[Pictures courtesy of Alper Romano and Susi Kaitna of the Glotzer group].

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